UNIVERSIDADE FEDERAL DE MINAS GERAIS
Instituto de Ciências Biológicas
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CONTRIBUIÇÃO DE POLIMORFISMOS FUNCIONAIS EM GENES DO SISTEMA MONOAMINÉRGICO PARA A ANSIEDADE MATEMÁTICA EM CRIANÇAS EM IDADE ESCOLAR

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"Contribuição de polimorfismos funcionais em genes do sistema monoaminérgico para a Ansiedade Matemática em crianças em idade escolar"

Fernanda Caroline dos Santos

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Às crianças com dificuldade de aprendizagem e às suas famílias

Dedico.

“Nothing in life is to be feared, it is only to be understood. Now is the time to understand more so that we may fear less.”

- Marie Skłodowska-Curie

“Don't let anyone rob you of your imagination, your creativity, or your curiosity. It's your place in the world; it's your life. Go on and do all you can with it, and make it the life you want to live.”

- Mae Jemison

"Courage is like — it’s a habitus, a habit, a virtue: you get it by courageous acts. It’s like you learn to swim by swimming. You learn courage by couraging."

- Marie Daly

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Sumário

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Lista de abreviaturas, siglas e unidades de medida

5'-UTR: 5'untranslated region

5-HT: Serotonina

5HTT: Transportador de serotonina

5-HTTLPR: *Serotonin transporter-linked polymorphic region*

ALSPAC: *Avon Longitudinal Study of Parents and Children*

AM: ansiedade matemática

AMAS: *Abbreviated Math Anxiety Scale*

AP2: Fator de transcrição AP2

APA: *American Psychiatric Association*

bp: *Base pairs*

CID: Código Internacional de doenças

CNV: *Copy number variation*

COMT: *Catechol-O-Methyltransferase*

D1: Receptores dopaminérgicos tipo D1

DAM: Dificuldade de aprendizagem na Matemática

DAME: dificuldade de aprendizagem de matemática e escrita

DAT: Transportador de dopamina

DAT1: *Dopamine Transporter 1 gene*

DCDC2: *Doublecortin domain-containing protein 2*

DNAH5: *Dynein Axonemal Heavy Chain 5*

DRD1: *Dopamine Receptor 1*

DRD2: *Dopamine Receptor 2*

DRD3: *Dopamine Receptor 3*

DRD4: *Dopamine Receptor 4*

DSM: Manual Diagnóstico e Estatístico de Transtornos Mentais

DYX1C1: *Dyslexia susceptibility 1 candidate gene 1 protein*

DZ: Dizigóticos

FAM43A: *Family With Sequence Similarity 43 Member A*

FMR1: *Fragile X Mental Retardation 1*

GRIK1: *Glutamate receptor, ionotropic, kainate 1*

GWAS: Genome-wide association study

INDEL: polimorfismo de inserção/deleção

L: *Long* - alelos de 16 repetições do locus 5-HTTLPR

LCR: Low copy repeat region

MA: math anxiety

MAI: *Mathematics Anxiety Interview*

MAOA: *Monoamine Oxidase A*

MAOA-LPR: *MAOA-linked polymorphic region*

MAOB: *Monoamine Oxidase B*

MAQ: *Math Anxiety Questionnarie*

MAQ A: *Math Anxiety Questionnarie Subscale A*

MAQ B: *Math Anxiety Questionnarie Subscale B*

MAQ C: *Math Anxiety Questionnarie Subscale C*

MAQ D: *Math Anxiety Questionnarie Subscale D*

MARS: *Mathematics Anxiety Rating Scale*

MARS-E: adaptação do questionário MARS para pré-adolescentes

Met: Metionina

mRNA: RNA mensageiro

MT: Memória de Trabalho

MYO18B: *Myosin XVIIIB*

MMP7: Matrix metalloproteinase-7

OECD: *Organization for Economic Co-operation and Development*

pb: Pares de bases

PEA: *phenethylamine*

PISA: *Programme for International Student Assessment*

RMARS: *Revised Mathematics Anxiety Rating Scale*

S: *Short* - alelos de 14 repetiçõesno locus 5-HTTLPR

SERT: Transportador de serotonina

SPOCK1: Gene *SPOCK 1*

SNC: sistema nervoso central

SNP: *single nucleotide polymorphism*

TDAH: Transtorno do déficit de atenção e hiperatividade:

TEDS: *Twins Early Development Study*

WTCCC2: *Wellcome Trust Case-Control Consortium 2*

Val: Valina

VMAT: *Vesicular monoamine transporter*

VNTR: *Variable number of tandem repeats*

XL: *Extra-long* – alelo com mais de 16 repetições no locus 5-HTTLPR

Resumo

A ansiedade matemática (AM) é um subtipo específico de ansiedade que afeta as habilidades matemáticas. Ela se manifesta como sintomas de desconforto, tensão, e medo em relação a atividades matemáticas interferindo na manipulação de números e na solução de problemas aritméticos. A AM pode afetar, portanto, o desempenho do indivíduo tanto no ambiente escolar quanto em situações do cotidiano. A AM é uma condição que se manifesta no início da vida escolar e pode persistir até a vida adulta, ocasionando, dentre outros desfechos, baixo desempenho, dificuldade de administrar recursos financeiros e a evasão de carreiras nas ciências e exatas. A AM é um fenótipo multifatorial, dessa forma, fatores ambientais como a má qualidade do ensino, professores ansiosos com relação à matemática e ambiente de aprendizagem desfavorável contribuem para o surgimento e agravamento da AM. Esses e outros fatores ambientais vêm sendo amplamente investigados ao longo dos últimos 50 anos. Entretanto, sabe-se que fatores genéticos também contribuem para uma parcela considerável da variância fenotípica da AM. Apesar disso, até o momento, apenas um estudo se propôs a identificar as causas genético-moleculares dessa característica, através de um estudo de associação de um polimorfismo funcional em um gene candidato. Diante da ausência de estudos genéticos para a AM, das evidências neurobiológicas e quantitativas da existência de componentes genéticos e do sucesso do primeiro estudo em propor como candidato, um gene envolvido no metabolismo de neurotransmissores importantes para a cognição matemática e para o processamento de emoções, o presente estudo se propôs a investigar outros genes candidatos para a AM. Dessa forma, o objetivo desse estudo foi investigar a contribuição de polimorfismos funcionais em três genes responsáveis pela modulação do sistema monoaminérgico (*SLC6A4*, *MAOA* e *MAOB*) para a AM em uma amostra de crianças em idade escolar. Como resultado, a heterose no locus 5-HTTLPR no gene *SLC6A4* se mostrou um fator de risco para níveis elevados de AM. Diferentemente, a heterozigose no locus MAOA-LPR no gene *MAOA*, em mulheres, foi um fator protetivo para a AM, sendo esse efeito restrito ao sexo feminino. Já para o locus rs1799836 no gene *MAOB*, o efeito foi mais pronunciado em indivíduos do sexo masculino e uma interação entre o sexo e o genótipo G/G em mulheres e G em homens foi descrita. Este é o segundo estudo a identificar genes associados à AM. Os resultados aqui descritos reforçam a existência de fatores genéticos contribuindo para a AM e revela que os efeitos de substituição alélica nos loci investigados variam de acordo com o sexo do indivíduo, podendo inclusive se restringir a apenas um dos sexos, ou apresentar efeitos opostos. Além disso, os resultados sugerem que em estudos de associação para polimorfismos do sistema monoaminérgico, deve-se testar diferentes modelos genotípicos de acordo com o sexo.

Palavras-Chave: Dificuldade de Aprendizagem na Matemática, Ansiedade Matemátca, Atitudes com relação à Matemática, MAOA, MAOB, SLC6A4, 5-HTTLPR

Abstract

Math anxiety (MA) is a specific subtype of anxiety that affects mathematical skills. It manifests as symptoms of discomfort, tension, and fear of activities involving math and it can interfere with number manipulation and in solving arithmetic problems. Therefore, MA can affect the individual's performance both in the school environment and in everyday life. MA is a condition that manifests at the beginning of school life and it can persist until adulthood, causing, among other outcomes, poor performance, difficulty in managing financial resources and the avoidance of careers in STEM. MA is a multifactorial phenotype, therefore environmental factors such as poor teaching quality, math anxious teachers and adverse learning environment can contribute to the emergence and worsening of MA. These and other environmental factors have been extensively investigated over the last 50 years. However, it is known that genetic factors also contribute to a considerable portion of the phenotypic variance of MA. Nevertheless, to date, only one study has proposed to identify the genetic-molecular causes of this trait using a candidate gene association approach. Given the absence of genetic studies for MA, the neurobiological and quantitative evidence of the existence of genetic components and the success of the first study to propose as a candidate, a gene involved in neurotransmitter metabolism important for math cognition and emotion processing, the present study aimed at investigating other candidate genes for MA. Therefore, the aim of this study was to investigate the contribution of functional polymorphisms in three genes responsible for the modulation of the monoaminergic system (*SLC6A4*, *MAOA* and *MAOB*) to MA in a sample of school-age children. As a result, heterosis at the 5-HTTLPR locus in the *SLC6A4* gene were found to be a risk factor for high levels of MA. In contrast, heterozygosity at the MAOA-LPR locus in the *MAOA* gene in girls was a protective factor for MA, and this effect was restricted to females. For the locus rs1799836 in the *MAOB* gene, the effect was more pronounced in boys and an interaction between sex and G/G genotype in girls and G genotype in boys was described herein. This is the second study to identify genes associated with MA. The results described herein reinforce the existence of genetic factors contributing to MA and reveal that the effects of allelic substitution in the investigated loci vary according to sex, and can be restricted to only one sex, or have opposite effects regarding sex. Furthermore, the results suggest that in association studies for monoaminergic system polymorphisms, different genotypic models should be tested for each sex.

Keywords: Math Learning Disability, Math Anxiety, Attitudes Towards Math, MAOA, MAOB, SLC6A4, 5-HTTLPR

1 Introdução Geral

1.1 Ansiedade Matemática

Nem toda dificuldade de aprendizado da matemática resulta de dificuldades cognitivas (A. Dowker, Sarkar, & Looi, 2016). Componentes afetivos são importantes durante o processo de aprendizagem, pois dentre outras coisas, as emoções determinam o comportamento do indivíduo com relação ao objeto de aprendizagem, tendo um papel crucial em fatores como engajamento e motivação para aprender (Pajares & Graham, 1999). Nesse aspecto, a ansiedade matemática tem o potencial de afetar severamente o aprendizado e o desempenho de um indivíduo. Aspectos mais específicos desse construto serão abordados nos tópicos que se seguem.

1.1.1 Conceito

A ansiedade matemática (AM) é um subtipo específico de ansiedade que afeta as habilidades matemáticas. Ela pode ser definida como sintomas de desconforto, tensão, e medo em relação a atividades que envolvam matemática. Esse conjunto de sensações interfere na manipulação de números e na solução de problemas envolvendo a matemática, afetando o indivíduo tanto no ambiente escolar quanto em situações do contidiano. É uma condição que se manifesta no início da vida escolar, podendo persistir até a vida adulta (Richardson & Suinn, 1972). Hembree e colaboradores (1990) sugerem que a AM se assemelha e tem correlação média com outros tipos de ansiedade específica, como a ansiedade na realização de testes (r=0,3-0,5) (Hembree, 1990). Entretanto, AM é claramente um contruto distinto, não podendo ser confundido com ansiedade generalizada ou com outros tipos específicos de ansiedade, apesar de comorbidade não ser um fato incomum (Emma Carey, Devine, Hill, & Szűcs, 2017).

Os prejuízos causados pela AM são diversos e vão além do desempenho acadêmico. Indivíduos afetados pela AM estão mais susceptíveis a desenvolver atitudes ruins com relação à matemática, como evitar jogos e brincadeiras que envolvam números, evitar deveres de casa ou atividades que se proponham a consolidar o aprendizado da sala de aula, evitar profissões e cursos superiores em que o uso da matemática seja obrigatório, dentre outras (Hoffman, 2010; Meece, Wigfield, & Eccles, 1990). Além dessas atitudes serem prejudiciais para o

aprendizado da matemática, elas podem ainda comprometer a vida do indivíduo por conduzí-lo a situações de emprego com salários mais baixos, dificuldades no planejamento financeiro tanto individual, quanto familiar, baixo status socio-econômico e maior vulnerabilidade a psicopatologias (McKenna & Nickols, 1988; S. S. Wu, Willcutt, Escovar, & Menon, 2014).

1.1.2 Prevalência

A AM é um problema universal que afeta indivíduos de todas as idades. Estimativas de prevalência não são comuns, visto que a AM ainda não está inclusa no Manual Diagnóstico e Estatístico dos Transtornos Mentais (DSM-5) e por isso ainda não possui uma definição diagnóstica clara e universal. Entretanto, algumas estimativas foram divulgadas nos últimos anos para alguns países. Richardson and Suin (1972) descreveram que em meio aos estudantes universitários investigados, cerca de 11% se sentiam ansiosos o suficiente com relação amatemática para solicitar aconselhamento pedagógico. Nos Estados Unidos, cerca de 93% dos adultos reportam já terem experimentado ansiedade matemática em algum nível, enquanto 17% da população afirma conviver com altos níveis de ansiedade matemática (S. Luttenberger, Wimmer, & Paechter, 2018). Em uma amostra de estagiários adolescentes do Reino Unido, cerca de 30% dos indivíduos alegou sentir altos níveis de ansiedade matemática, enquanto 18% alegou sentir a ansiedade e os prejuízos a ela associados (Johnston-Wilder, Brindley, & Dent, 2014). Em meio aos adolescentes, 2-6% dos alunos do ensino secundário investigados por Chinn e colaboradores (2009) apresentaram altos níveis de ansiedade matemática (Chinn, 2009).

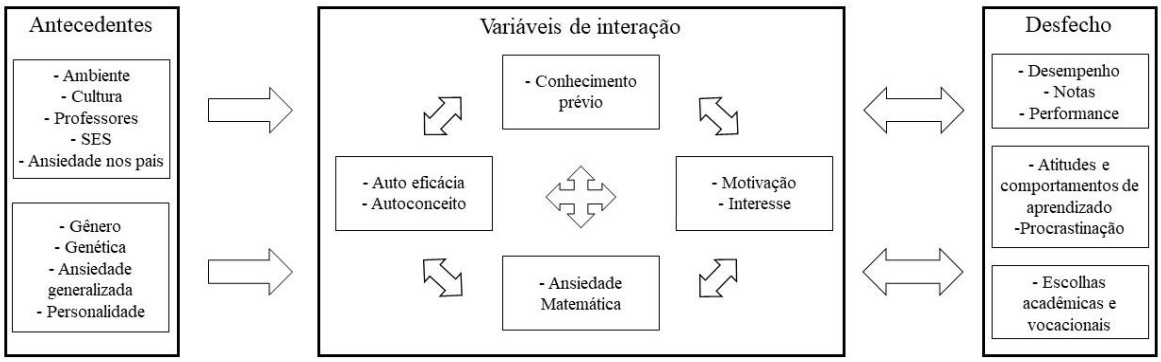
A estimativa mais abrangente, entretanto, vem do Programa Internacional de Avaliação de estudantes (PISA). Em 2012, ao avaliar estudantes de 15-16 anos, de 34 países (incluindo o Brasil), revelou-se que 31% dos alunos ficam muito ansiosos ao resolver problemas de matemática, 33% sentem tensão ao fazer para casa de matemática e 59% se preocupam ao pensar que as aulas de matemática serão difíceis (S. Luttenberger et al., 2018).

1.1.3 Etiologia

Por se tratar de um tipo específico de ansiedade, a AM pode ser caracterizada como um fenótipo complexo de origem multifatorial, em que tanto fatores genéticos, individuais e ambientais interagem e contribuem para o estabelecimento do fenótipo. Recentemente,

Luttenberger e colaboradores (2018) descreveram um fluxograma para a compreensão da AM. Uma versão adaptada do fluxograma é apresentada na Figura 3.

Figura 1. Fluxograma para compreensão da Ansiedade Matemática. Ele expõe os fatores antecedentes, de interação e de desfecho para a ansiedade matemática. Adaptado de Luttenberger et al., 2018.



Segundo Luttenberger e colaboradores (2018), os fatores envolvidos na AM podem ser subdivididos em fatores antescendentes, fatores de interação e de desfecho. Dentre os fatores antescendentes, dois grupos se destacam: os fatores individuais e os fatores ambientais. Fatores individuais são todas aquelas características próprias do indivíduo, como o gênero, características da personalidade e fatores de predisposição genética, por exemplo. Fatores ambientais envolvem cultura, status sócio-econômico, ambiente escolar ou de aprendizagem e interação com outros indivíduos. Todos esses fatores contribuem para a AM, para aquisição do conhecimento, para a construção do auto-conceito e para a auto-eficácia, além de modularem os níveis de motivação e interesse da criança para a matemática. Durante o aprendizado, todos esses fatores interagem de forma a culminar em baixo desempenho, atitudes ruins com relação à matemática e influenciar nas decisões vocacionais ou acadêmicas. Estes desfechos podem ainda, à longo prazo, ter efeito sobre os fatores de interação, reforçando os sintomas de AM e os seus efeitos.

Não existe, portanto, uma causa única para a AM, mas múltiplos fatores, que ao interagirem contribuem para o surgimento, manutenção e evolução do fenótipo. A forma com que cada fator contribui para a AM será melhor abordada no tópico dessa introdução que abora os "Fatores que influenciam a AM".

1.1.4 Neurobiologia da Ansiedade Matemática

Apesar de a etiologia da AM ainda não estar completamente elucidada, os efeitos fisiológicos estão bem descritos. Aumento da frequência cardíaca, suor nas mãos, esquecimentos de conteúdo ou de procedimentos, e náusea são as principais queixas de indivíduos que sofrem de AM (Faust, 1994). Sintomas intermediários veem sendo investigados, com o objetivo de melhor compreender esse fenótipo. Níveis de cortisol, um hormônio associado ao estresse e à ansiedade, foram investigados em indivíduos com altos e baixos níveis de AM enquanto faziam tarefas de matemática. Em seguida o desempenho de cada grupo foi associado aos níveis de cortisol e ao desempenho nos testes. Entretanto, a relação encontrada entre AM, cortisol e desempenho não foi direta, mas pareceu ser mediada por diferenças individuais na memória de trabalho (Mattarella-Micke, Mateo, Kozak, Foster, & Beilock, 2011).

Estudar os correlatos neurais da AM pode auxiliar na compreensão e na investigação de processos subjacentes a esse fenótipo. A literatura ainda não é muito consistente com relação a este assunto, mas de forma geral, sabe-se que a AM aumenta a ativação de estruturas cerebrais envolvidas nas vias de dor e emoção, como a amígdala, o núcleo accumbens, o giro cingular anterior e a ínsula; e reduz a ativação de regiões cerebrais envolvidas no processamento de estímulos e informações, como, o córtex frontal dorsolateral, diminuindo a eficiência do indivíduo em atividades que envolvam cálculos (Artemenko, Daroczy, & Nuerk, 2015; Lyons & Beilock, 2012; Young, Wu, & Menon, 2012). Maior conectividade entre a amígdala e o córtex pré-frontal ventromedial, e conectividade reduzida entre a amígdala e o lobo parietal superior (bilateral) também foram descritos em indivíduos com altos níveis de AM. Segundo os autores, essas conecções aberrantes estariam envolvidas em mecanismos compensatórios, nem sempre tão bem-sucedidos, já que déficits no desempenho desses indivíduos são comuns (Young et al., 2012). O circuito da dor no cérebro foi relacionado à AM antecedendo o teste/atividade em adultos, enquanto que o circuito do medo, mostrou ativação diferencial em crianças, durante a execução de tarefas e testes matemáticos. Esses resultados podem indicar que a ativação de diferentes circuitos é idade-dependente ou um fator individual, que pode variar entre os sujeitos. É importante ressaltar, que a ausência de estudos utilizando os mesmos paradigmas e amostras similares, dificulta a extrapolação de que circuitos diferentes seriam ativados em idades diferentes (Artemenko et al., 2015).

Os padrões de ativação e conectividade descritos, conjuntamente com a evidência de que os níveis de cortisol aumentados não conduzem necessariamente à baixa performance, reforçam as teorias de Rubinsten and Tannock (2010) e Pletzer e colaboradores (2015) (Pletzer, Kronbichler, Nuerk, & Kerschbaum, 2015; Rubinsten & Tannock, 2010). Essas teorias afirmam que emoções negativas que acompanham a AM precisam ser reguladas e processadas por indivíduos que apresentam altos níveis da mesma. O processamento e a regulação dessas emoções seriam realizados pelo córtex frontal dorsolateral, o qual dividiria recursos para inibir os efeitos das emoções, direcionar a atenção para a tarefa e ainda atuar diretamente na execução da tarefa. Aparentemente, uma parcela dos indivíduos com altos níveis de AM, conseguem compensar a sobrecarga dessa região cerebral com maior conectividade e ativação da amígdala e do córtex frontal. Entretanto, uma outra parcela não desenvolve esses mecanismos compensatórios, e consumindo uma boa parcela dos recursos cognitivos que deveriam estar direcionados à tarefa com preocupações e processamento dos inputs recebidos das vias de emoção e dor no sistema nervoso central. Caracterizando baixa eficiência de processamento e consequente baixo desempenho. O mesmo não ocorre em indivíduos que desenvolvem mecanismos compensatórios eficientes. É importante ressaltar que, independentemente do desfecho, indivíduos com altos níveis de AM despendem maiores esforços cognitivos do que indivíduos com baixos níveis de AM para alcançar desempenho semelhante (Artemenko et al., 2015). Fatores individuais como a variabilidade genética poderiam explicar diferenças individuais na eficácia de mecanismos de compensação desenvolvidos por esses indivíduos.

1.1.5 Métodos para mensurar a AM

Diversas ferrametas têm sido aplicadas, tanto na área da educação quanto na pesquisa, para a identificação e mensuração dos sintomas da AM em todas as idades. A maioria dessas ferramentas são questionários compostos por escalas de classificação.

Os dois métodos mais utilizados em adultos são o MARS (“Mathematics Anxiety Rating Scale”) e a sua versão reduzida, o RMARS (“Revised Mathematics Anxiety Rating Scale”). Ambos, são compostos por itens que descrevem diferentes situações de utilização da matemática, onde os indivíduos devem apontar em uma escala de Likert o quanto aquela situação o deixa ansioso. A ampla utilização desses métodos pode ser atribuída a sua alta eficiência atestada em diversos testes de validação, mas também às suas capacidades de

acessar diferentes facetas da AM, como ansiedade de provas, ansiedade em cursos e disciplinas, ansiedade computacional, ansiedade para aplicar a matemática em situações do cotidiano e medo de professores de matemática, por exemplo (Plake & Parker, 1982; Richardson & Suinn, 1972). Questionários menos abrangentes, como o AMAS ("Abbreviated Math Anxiety Scale"), focam apenas em ansiedade em provas e ansiedade de números (Hopko, Mahadevan, Bare, & Hunt, 2003).

MARS-E é uma adaptação do questionário MARS para pré-adolescentes e adolescentes, podendo ser aplicado em indivíduos a partir dos 10 anos de idade. Similarmente ao questionário de origem, o MARS-E utiliza o mesmo método para acessar os níveis de ansiedade, entretanto as situações descritas são adaptadas para cobrir experiências escolares e cotidianas de indivíduos dessa idade (Suinn, Taylor, & Edwards, 1988).

Em crianças menores, mais adaptações são feitas nos questionários, para reduzir o impacto de aspectos do desenvolvimento da criança, como a habilidade de leitura. Geralmente, as adaptações aplicadas envolvem o uso de figuras e desenhos que representam de forma lúdica o sentimento da criança com relação à situação relatada no teste (na escala Likert), ou até mesmo as situações em que a criança lida com a matemática podem ser apresentadas à criança em forma de desenho. O MAI (Mathematics Anxiety Interview) é um exemplo de questionário que apresenta as situações em forma de desenho. Ele pode ser aplicado para crianças de 7-10 anos de idade. O MAI é capaz de mensurar não apenas o nível de ansiedade da criança, mas também o quanto ela se sente empolgada, preocupada, seus batimentos cardíacos e ainda a vontade da criança de escapar da situação representada nas figuras (Kohn et al., 2013).

O questionário de ansiedade de Matemática, Math Anxiety Questionnarie (MAQ), desenvolvido por Thomas e Dowker (2000) e adaptado em uma versão na língua portuguesa pela equipe do LND (Haase et al., 2012; Wood et al., 2012) é a ferramenta utilizada no presente trabalho. Ele é composto por quatro subescalas de 6 itens cada, em que o indivíduo avalia situações em relação ao seu desempenho em Matemática (auto-percepção) (MAQ-A), suas atitudes em relação à problemas Matemáticos (MAQ-B), como se sente em relação à problemas matemáticos (feliz ou triste) (MAQ-C) e o quanto se preocupa quando tem problemas na Matemática (Wood et al., 2012). Perguntas na subescala MAQ A iniciam sempre com "Quão bom você é em ...", em MAQ-B, "Quanto você gosta de ...", em MAQ-C,

“Quão feliz ou triste você fica quando tem problemas com ...”, e em MAQ-D, “ Quão preocupado você fica quanto tem problemas com...”. Os seis itens em cada subescala, contemplam sempre os itens seguintes: 1) matemática em geral, 2) cálculos fáceis, 3) cálculos difíceis, 4) cálculos escritos, 5) cálculos mentais e 6) dever de casa de matemática. As opções de respostas são organizadas em uma escala visual lúdica (Leikert), apresentada à criança após cada pergunta. Nela, a criança deve apontar as respostas para cada item perguntado. Os pontos são dados em uma escala de 1 a 5 para cada item, sendo 5 sempre o pior resultado (muito ruim em perguntas de MAQ-A, detesto em perguntas de MAQ-B, muito triste em perguntas de MAQ-C e muito preocupado em perguntas de MAQ-D). Ao final do teste, um escore para cada subescala de MAQ é calculado a partir da soma das respostas dos seis itens (Wood et al., 2012). O MAQ pode ser aplicado para crianças de 6 a 12 anos, entretanto, sua eficiência em idades abaixo de 8 anos, pode ser questionada.

Trabalhos anteriores do grupo de pesquisa demonstraram que a ansiedade matemática acessada pelo MAQ não se correlaciona com ansiedade geral (r=0,1). As subescalas MAQ-A e MAQ-B possuem correlação média-alta e medem componentes cognitivos da AM, enquanto que MAQ-C e MAQ-D também possuem correlação média-alta e medem componentes afetivos da ansiedade matemática. Além disso, os mesmos trabalhos demonstraram que os escores das MAQs se correlacionam negativamente com habilidades matemática (r = -0,37) (Haase et al., 2012; Wood et al., 2012).

1.1.6 Fatores que influenciam a AM

Nesse tópico, os diversos fatores ambientais e individuais que afetam os níveis de AM serão abordados.

1.1.6.1 Sexo

Um dos fatores que contribuem para os níveis de AM mais investigados até o momento é o sexo. Indivíduos do sexo feminino tendem a apresentar maiores níveis de AM, quando comparados a indivíduos do sexo masculino, mesmo em países que garantem educação igual para ambos os sexos (Spelke, 2005). Além disso, mulheres tendem a se considerar piores em matemática (pior autoconceito), mesmo quando apresentam desempenho igual ou superior aos homens (Devine, Soltesz, Nobes, Goswami, & Szucs, 2013). Alguns autores afirmam que

essas diferenças surgem ou são mais pronunciadas durante a adolescência (Ann Dowker, Bennett, & Smith, 2012; Harari, Vukovic, & Bailey, 2013; S. Wu, Amin, Barth, Malcarne, & Menon, 2012), entretanto, Dowker e colaboradores (2012) observaram esse tipo de comportamento também em crianças de 7 a 9 anos.

Estereótipo de gênero, influência ou transferência de ansiedade de pais e professores ansiosos, e características afetivas e de personalidade são apontadas como as principais causas dessa diferença de auto-conceito entre homens e mulheres (Beilock, Gunderson, Ramirez, & Levine, 2010; A. Dowker et al., 2016).

Estereótipos são definidos como as suposições que as pessoas fazem sobre as habilidades e características dos membros de um determinado grupo, e as premissas sobre como os membros do grupo estereotipado geralmente se comportam. Estereótipos de gênero envolvem, portanto, suposições e premissas atrabuídas a homens e mulheres, em decorrência unicamente do sexo. Na matemática, o estereótipo mais relevante é de que homens lidam melhor com a matemática, possuem melhor desempenho e possuem maior tendência a seguir carreiras que envolvam números, quando comparados às mulheres (Bieg, Goetz, Wolter, & Hall, 2015). Segundo Casad e colaboradores (2015), o endosso de estereótipos de gênero prediz os níveis de ansiedade matemática, no que diz respeito ao autoconceito e à autoeficácia. O efeito do estereótipo de gênero é ainda mais importante quando pais e professores o manifestam (Jakobsson, Levin, & Kotsadam, 2013; Maloney, Ramirez, Gunderson, Levine, & Beilock, 2015). Segundo Goetz e colaboradores (2013), indivíduos do sexo feminino nem sempre apresentam níveis de ansiedade muito maiores do que homens, entretanto, devido aos estereótipos de gênero, elas criam expectativas de apresentar mais ansiedade e isso faz com que elas se sintam desencorajadas a seguir carreiras ou realizar cursos e atividades que envolvam matemática. Esse fenômeno é denominado ameaça de estereótipo, sendo observado predominantemente em adolescentes e adultos (Goetz, Bieg, Ludtke, Pekrun, & Hall, 2013).

Pais e professores ansiosos em relação à matemática também podem transferir essa fobia para seus filhos e alunos, ou promoverem experiências negativas ao ensinarem matemática. Apesar desse fenômeno não se restringir a um sexo específico, os efeitos de transferência são mais pronunciados nas interações entre indivíduos do mesmo sexo (professoras com alunas ou mães com alunas, por exemplo) (Casad, Hale, & Wachs, 2015). Esse dado é ainda mais relevante se considerarmos que a maior parte dos professores nos anos iniciais de educação

são do sexo feminino e que frequentemente são as mães que orientam os filhos durante a execução do dever de casa. Dessa forma, a transferência de ansiedade também é um fenômeno importante na compreensão dos efeitos de sexo sobre a ansiedade matemática.

Finalmente, fatores de personalidade e características afetivas, como níveis de neuroticismo e ansiedade generalizada, também contribuem para a diferença de AM entre os sexos. O neuroticismo é um dos cinco traços de personalidade que compõem a teoria da personalidade revisada de Costa e McCrae (2008). Indivíduos com altos níveis de neuroticismo tendem a experimentar mais ansiedade, preocupação, medo, raiva, frustração e humor depressivo. Além disso, eles tendem a apresentar respostas piores a estressores e maior risco de desenvolver transtornos afetivos como a ansiedade generalizada ou específica. Níveis mais altos de neuroticismo e ansiedade generealizada são frequentemente encontrados no sexo feminino, contribuindo para o aumento da ansiedade matemática nesse grupo (Costa Jr & McCrae, 2008).

1.1.6.2 Idade

Idade também é um fator importante para a predição dos níveis de AM. Casos severos de AM não são comuns em crianças de pouca idade, entretanto casos de AM ocorrem em crianças até mesmo do nível primário (S. Wu et al., 2012). Na infância, a AM tende a aumentar com a idade e as atitudes com relação à matemática também tendem a deteriorar com a idade. Vários fatores podem contribuir para o aumento da AM ao longo da idade. Alguns exemplos são alterações no conteúdo da matemática, maior exposição a experiências negativas e até mesmo comparação entre alunos nas escolas.

Ao longo dos anos escolares, a complexidade do conteúdo de matemática aumenta, exigindo da criança a aplicação de conteúdos e procedimentos aprendidos ao longo dos anos escolares além do aprendizado dos novos. Esse fator pode contribuir para o aumento da ansiedade, principalmente em crianças que já apresentaram dificuldade de aprendizado ou performance ruim nos anos anteriores. Soma-se ao aumento da complexidade, as sucessivas experiências negativas com a matemática como notas baixas, contato com professores ansiosos ou que utilizam metodologias de ensino antiquadas. Além disso, ao longo dos anos escolares, os alunos começam a experimentar um aumento na intolerância e na incerteza nas crianças. Além disso, as crianças apresentam um aumento da percepção de comparações sociais

(comparação entre alunos) e aumenta-se também a necessidade de corresponder as expectativas da sociedade sobre o seu desempenho em matemática. Todos esses fatores podem contribuir para o aumento da ansiedade em com o aumento da idade (A. Dowker et al., 2016).

1.1.6.3 Cultura, nacionalidade e status sócio-econômico

Aspectos envolvendo a matemática diferem consideravelmente entre países. Diferenças de desempenho, atitudes com relação à matemática, atribuição do desempenho na matemática à habilidade ou esforço e até mesmo a relevância atribuída à matemática são aspectos de cultura e nacionalidade que influenciam os níveis de AM (Askew, Hodgen, Hossain, & Bretscher, 2010). Em países de alto desempenho na matemática, a existência de crianças com baixos níveis de AM pode refletir o bom desempenho desses indivíduos, mas o bom desempenho também pode ser uma consequência dos baixos níveis de AM. Entretanto, em países com alto desempenho que atribuem grande importância à matemática e ao desempenho acadêmico, os indivíduos tendem a se comparar mais com colegas e a sentir mais medo de falhar, aumentando assim os níveis de AM. Um exemplo seriam os altos níveis de AM observados na Coréia e no Japão, países onde a pressão para se ter bom desempenho é consideravelmente maior. Já em países da Europa ocidental como a Finlândia, a Holanda e a Suíça, apesar dos altos desempenhos na matemática, os níveis de AM são consideravelmente inferiores do que nos países da Ásia citados (J. Lee, 2009; Tan & Yates, 2011).

Estudos sugerem que diferenças étnicas também influenciam na AM. Nos Estados Unidos e no Reino Unido minorias étnicas reportam melhores atitudes com relação à matemática do que caucasianos e essa diferença não está necessariamente atrelada a diferenças de performance (A. Dowker et al., 2016; Lubienski, 2002).

Apesar do status sócio-econômico dos indivíduos, assim como a posição econômica do país, ter grande influência sobre o engajamento e o desempenho na matemática, Jazdzewski e colaboradores (2012) sugerem que estas variáveis não tenham um efeito muito relevante sobre a AM. Entretanto, poucos estudos tentaram investigar essas questões até o momento (Jazdzewski, 2012).

1.1.6.4 Escola, Ensino e experiências negativas

Um dos fatores ambientais que mais contribuem para a AM é o conjunto de experiências envolvendo matemática que foram vivenciadas pelo indivíduo. Uma boa parte dessas experiências são vivenciadas no ambiente escolar. Segundo Radisic e colaboradores (2015), apenas 6% da variância encontrada para a AM pode ser explicada por diferenças entre escolas, sendo os fatores mais importantes a rigidez e a disciplina da escola. Os autores explicam que esses fatores, quando em excesso, criam um ambiente desfavorável para o aluno, em que eles se sentem pressionados a atender as expectativas dos colegas e da própria escola, contribuindo para o aumento da ansiedade (Radišić, Videnović, & Baucal, 2015). O restante da variância seria explicado por outros fatores, alguns dos quais já foram citados nesta introdução, mas também por diferenças dentro da própria escola, como turmas, grupos de estudos e professores (Teodorović, 2011).

Diversos estudos têm demonstrado que as experiências dentro de sala de aula contribuem consideravelmente para elevar os níveis de ansiedade matemática (ex. (Silke Luttenberger, Paechter, & Ertl, 2019; O'Leary, Fitzpatrick, & Hallett, 2017; Ramirez, Hooper, Kersting, Ferguson, & Yeager, 2018). Níveis mais altos de disciplina dentro da sala de aula, por exemplo, contribuem para a redução dos níveis de AM, por promoverem um ambiente mais organizado e propício para a aprendizagem do conteúdo (Radišić et al., 2015). Já as experiências negativas com professores parecem ser o fator que mais contribui para o aumento dos níveis de AM. Professores em treinamento (estagiários) acometidos por alta AM, quando questionados sobre as suas experiências em sala de aula como estudante, reportaram que seus professores de matemática se comportavam de forma hostil, insensível, impaciente e crítica, envergonhando alunos na frente da turma através de comentários negativos e da divulgação dos erros cometidos, respondendo agressivamente às dúvidas e falta de compreensão quando os alunos apresentavam dificuldade de assimilar conceitos (Jackson & Leffingwell, 1999). Métodos de ensino inadequados também foram apontados como fatores que contribuíram para o aumento da AM em professores em treinamento. Dentre estes, destacam-se a velocidade de apresentação do conteúdo e sanar dúvidas de forma a fazer o aluno se sentir menos inteligente por não ter compreendido os conceitos ensinados. Neste mesmo estudo, os autores avaliaram o impacto dessas experiências negativas sobre a carreira

destes estagiários e constataram que elas contribuíram para que eles se sintam pouco confiantes ao ensinar matemática (Brady & Bowd, 2005).

O'Leary e colaboradores (2018) avaliaram uma amostra de 131 estudantes de graduação de vários cursos, sendo 73% da amostra composta por mulheres. Diferentemente dos estudos anteriormente citados, participaram deste estudo alunos com alto e baixo nível de ansiedade matemática. Os autores então investigaram a relação entre as experiências negativas envolvendo a matemática a que esses estudantes foram expostos ao longo de toda a vida escolar e os níveis de AM desenvolvidas por eles. Em resumo, neste estudo a presença de altos níveis de AM foi relacionada à ausência de apoio e auxílio por parte dos professores e dos pais, aos métodos de ensino e ao desempenho. Para os dois primeiros itens, o período escolar que mais teve influência foi o ensino médio, já para o último, as experiências vividas tanto no ensino fundamental quanto no ensino médio se mostraram igualmente importantes.

Além das experiências negativas anteriormente citadas, outras experiências dentro e fora da sala de aula foram também apontadas como razões pelas quais os indivíduos desenvolveram AM. Relação professor-aluno baseada em desrespeito, humilhação e medo, relacionamento abusivo com os pais e lar conflituoso; grandes eventos e transições na vida da criança, perfeccionismo do aluno, dos pais e dos professores, cultura e estereótipos de gênero são outros fatores citados (Silke Luttenberger et al., 2019; O'Leary et al., 2017; P. G. Schmidt, 2005). Além destes, podemos ressaltar ainda os diversos estudos que relacionam o baixo desempenho na matemática com o auto-conceito e os níveis de ansiedade do aluno (Ashcraft & Kirk, 2001; Ashcraft & Moore, 2009; E. Carey, Hill, Devine, & Szucs, 2015; Dew, Galassi, & Galassi, 1984; Foley et al., 2017; Meece et al., 1990). Ressaltmos também a transferência de ansiedade de pais e professores que expressam sua ansiedade quando estão ensinando matemática para os seus filhos ou alunos (Beilock et al., 2010; Maloney et al., 2015).

1.1.6.5 Fatores genéticos

Poucos estudos investigaram os fatores genéticos envolvidos na AM. Segundo Wang e colaboradores (2014) AM tem herdabilidade moderada, sendo 40% da variância atribuída a fatores genéticos. A amostra do estudo contou com 514 pares de gêmeos de 12 anos de idade e a maior parte da variância não atribuída a fatores genéticos foi explicada por fatores ambientais não compartilhados. Resultados similares foram encontrados por Malachini e

colaboradores (2017) em uma amostra de gêmeos monozigóticos e dizigóticos com idade variando entre 18-21 anos (Malanchini et al., 2017; Wang et al., 2014).

Dowker e colaboradores (2016) propuseram que os fatores genéticos envolvidos na AM poderiam ser variantes genéticas que conferem predisposição tanto para déficits na cognição matemática quanto para a ansiedade generalizada. Outras variantes, específicas para AM ou associadas a características de personalidade ou de resiliência também podem estar relacionadas à predisposição para a AM (A. Dowker et al., 2016).

O primeiro estudo de associação para a AM foi publicado este ano, pelo grupo da Profa. Maria Raquel Carvalho e do Prof. Vitor Haase. Nele, o polimorfismo Val158Met no gene da *COMT*, um gene amplamente estudado por estar envolvido em aspectos comportamentais e cognitivos, foi investigado em uma amostra de 389 crianças brasileiras em idade escolar que tiveram os dados de AM mesurados através do MAQ. Os resultados mostraram um efeito de genótipo dependente do sexo, em que indivíduos heterozigotos, quando pertencentes ao sexo feminino, apresentaram os menores níveis de AM, enquanto em indivíduos do sexo masculino, a heterozigose apresentou os níveis mais elevados de AM. Homozigotos do sexo masculino apresentaram os níveis mais baixos de AM, enquanto esse mesmo genótipo no sexo feminino foi associado aos níveis mais altos de ansiedade da amostra (Julio-Costa et al., 2019). Além de características cognitivas e comportamentais, o gene da *COMT* também foi associado à cognição matemática e à memória de trabalho (essencial para a aprenzdizagem matemática) (Júlio-Costa et al., 2014; Julio-Costa et al., 2013), confirmando a ideia proposta por Dowker e colaboradores (2016), de que fatores genéticos envolvidos na cognição matemática poderiam também contribuir para AM.

1.1.7 Intervenções

A falta de informações sobre os mecanismos envolvidos na AM dificulta a proposição de intervenções. Como os mecanismos ambientais estão melhor esclarecidos na literatura, intervenções precoces baseadas nesses fatores têm sido propostas para prevenir que o ciclo vicioso ansiedade matemática-baixo desempenho se estabeleça.

Atitudes simples de pais e professores como demonstrar atitudes positivas e evitar atitudes negativas com relação à AM podem contribuir para a redução dos níveis de AM. Já as

intervenções terapêuticas podem envolver desde aulas de reforço ou métodos de ensino alternativos que visem a sanar as dificuldades da criança e a aumentar a sua auto-confiança, até tratamentos psicológicos específicos para a ansiedade como a dessensibilização sistemática ou a terapia cognitivo-comportamental. Estudos demonstraram que a ressignificação cognitiva da situação estressora pode contribuir para a redução dos níveis de AM e até mesmo para a melhora na performance dos indivíduos (Jamieson, Peters, Greenwood, & Altose, 2016; Johns, Inzlicht, & Schmader, 2008; Johns, Schmader, & Martens, 2005). Redigir sobre os sentimentos negativos e as preocupações parece ser uma prática eficaz na redução da AM, pois além de fazer o aluno se questionar sobre necessidade de ter medo ou preocupação, escrever sobre pode aliviar a memória de trabalho (Park, Ramirez, & Beilock, 2014; Ramirez & Beilock, 2011). Tutoria cognitiva baseada na exposição continuada (supervisionada por um psicólogo) aos estímulos matemáticos que geram ansiedade, também se mostrou eficiente como intervenção para a AM (Supekar, Iuculano, Chen, & Menon, 2015).

Dowker e colaboradores (2016) citam a estimulação cerebral não invasiva como uma possível intervenção, baseado nos resultados de estudos como o de Sarkar e colaboradores (2014), no qual a estimulação transcraniana aplicada ao córtex pré-frontal dorsolateral foi capaz de amenizar os sintomas afetivos de estresse e medo e melhorar a performance de indivíduos com AM (Sarkar, Dowker, & Kadosh, 2014). Entretanto, apesar de eficiente, a utilização de estimulação cerebral deve ser avaliada com cautela, afim de estabelecer se esta técnica seria viável e traria benefícios reais quando utilizada como uma intervenção.

1.2 Polimorfismos funcionais

Estima-se que o genoma humano possui aproximadamente 3.1 milhões de SNPs. Além das variações de base única, existem ainda outras variações polimórficas a nível de sequência do DNA, como VNTRs (repetições de número variado em tandem) e CNVs (número variado de cópias), por exemplo. Entretanto nem todas as variações polimórficas na sequência do DNA são classificadas como funcionais (Haraksingh & Snyder, 2013).

Polimorfismos funcionais são variações na sequência do genoma com frequência alélica mínima (MAF) superior a 1% e que promovem alteração na função de um ou mais genes ou de seus produtos gênicos (Haraksingh & Snyder, 2013). Albert (2011) propôs uma

classificação funcional de quatro classes para polimorfismos. Na classe 0 estariam os polimorfismos com função indeterminada ou com função predita apenas *in silico*, mas não experimentalmente demonstrada. Na classe 1, estariam polimorfismos funcionais *in vitro*, cujo efeito tenha sido demonstrado experimentalmente, entretanto, o impacto da substituição alélica no polimorfismo sobre a expressão do gene endógeno ou *in vivo* seria desconhecida. Na classe 2 estariam os polimorfismos funcionais in vivo, cujo efeito funcional tenha sido testado também em ensaios com modelos celulares e cujo impacto na expressão gênica seja então comprovado nesses modelos, seja a nível celular ou tecidual. Finalmente, na classe 3, estariam polimorfismos cujo efeito funcional tenha sido confirmado em modelos animais ou tenha sido correlacionado com alterações de função em tecidos humanos (Albert, 2011). Na presente tese, consideramos como funcionais, polimorfismos que se enquadrem nas classes 2 e 3, segundo a classificação de Albert (2011).

A investigação de polimorfismos funcionais em estudos de associação de características complexas tem sido efetivo, pois permite que sejam avaliadas hipóteses específicas sobre a relevância de vias metabólicas sobre características complexas. Nesse contexto, na presente tese, investigamos o efeito de polimorfismos funcionais no sistema monoaminérgico, (sistema sabidamente envolvido na cognição e no comportamento), sobre a ansiedade matemática.

1.2 Sistema Monoaminérgico

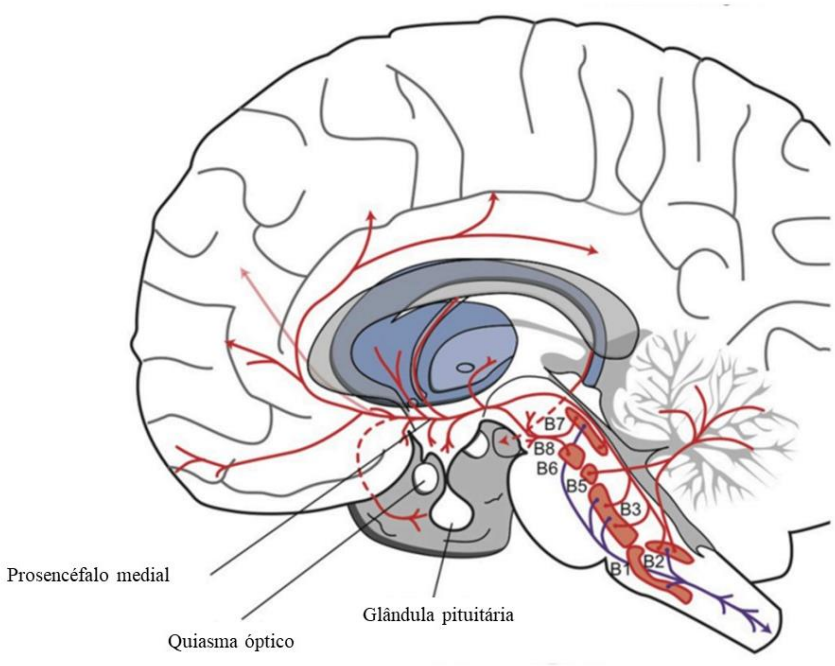
Sistema monoaminérgico é o nome dado ao conjunto de neurônios que produz neurotransmissores e neuromodulares derivados de aminas e denominados aminas biogênicas. As principais aminas biogênicas são as catecolaminas (dopamina, noradrenalina e adrenalina), serotonina e as histaminas. As aminas biogênicas são produzidas a partir da conversão de aminoácidos como a tirosina, no caso das catecolaminas, e do triptofano, no caso da serotonina, catalisadas por enzimas específicas. Os neurônios que sintetizam monoaminas se originam principalmente no tronco encefálico e se ramificam para diversas regiões do cérebro e da medula espinhal para desempenhar as mais diversas funções. Controle motor, cognição, processamento de memória, modulação endócrina e comportamento são exemplos de funções moduladas por monoaminas biogênicas. Disfunções na neurotransmissão monoaminérgica, particularmente dopaminérgica e serotonérgica, têm sido associadas em vários transtornos

neurológicos, neuropsiquiátricos (Kandel et al., 2000; Libersat & Pflueger, 2004). Nos tópicos seguintes, revisaremos os sistemas serotonérgico, dopaminérgico e noradrenérgico e as suas relações com os polimorfismos investigados na presente tese.

1.2.1 Sitema Serotonérgico

A via serotonérgica no sistema nervoso central (SNC) é uma via com componentes ascendentes e descendentes, sendo boa parte dos neurônios que alimentam esta via localizados nos núcleos da rafe na formação reticular do tronco encefálico. A via descendente é responsável pelo controle da dor através das vias analgésicas e a via ascendente se ramifica em diversas outras, projetando-se para quase todas as estruturas do prosencéfalo e para o cerebelo, incluindo estruturas como córtex, tálamo, hipotálamo, núcleo caudado e sistema límbico. Dessa forma o Sistema Serotonérgico é responsável por funções essenciais à vida, como o controle do ciclo sono-vigília, ao promover a ativação cortical; termorregulação e digestão através do tálamo; controle do movimento, através das projeções para os núcleos da base; e modulação de comportamentos afetivos através das projeções para estruturas do sistema límbico, como o giro do cíngulo, a amígdala e o Núcleo acumbens (Figura 4) (Kandel et al., 2000). Devido a isso, as vias serotonérgicas vêm sendo frequentemente estudadas em transtornos comportamentais e psiquiátricos como o transtorno de ansiedade generalizada (Sen, Burmeister, & Ghosh, 2004), Transtorno do Déficit de Atenção e Hiperatividade (Fowler et al., 2009; Kent et al., 2002), depressão (Zammit & Owen, 2006), comportamento e ideações suicidas (Caspi et al., 2003) e estresse pós-traumático (Xie et al., 2009). Entretanto, as vias serotonérgicas vêm sendo associadas também a fenótipos cognitivos como funções e performances cognitivas (Meneses & Liy-Salmeron, 2012) e prejuízos na aprendizagem e na memória episódica verbal (Mendelsohn, Riedel, & Sambeth, 2009).

Figura 2. Vias serotoninérgicas no Sistema nervoso central. Fonte: Adaptado de Loonen et al., 2016 (Loonen & Ivanova, 2016).



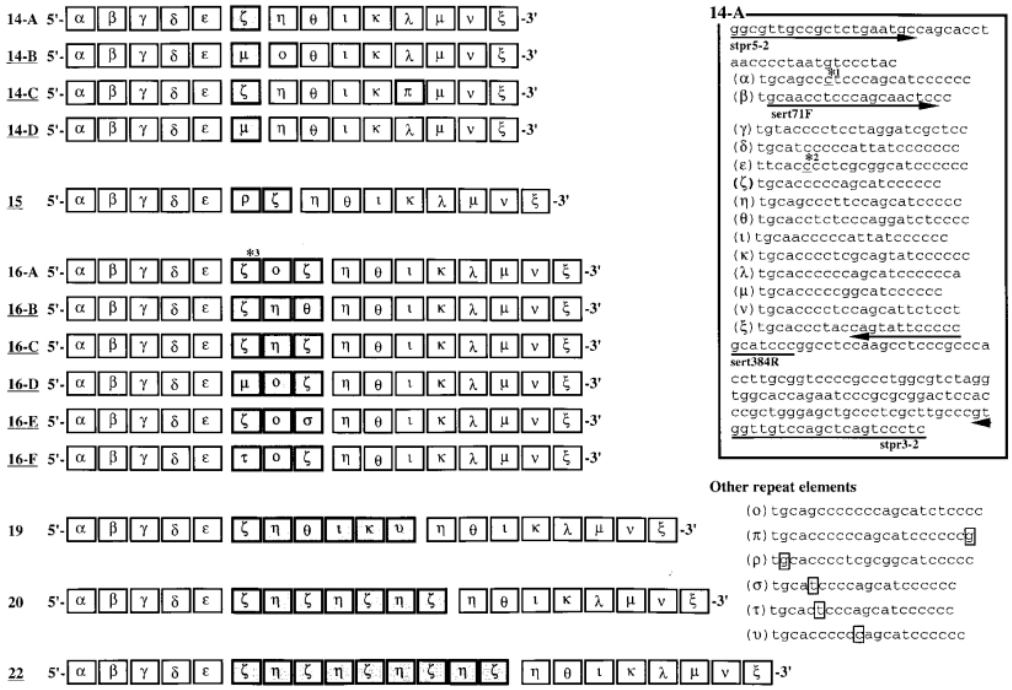
Um dos principais moduladores da via serotonérgica é o transportador de serotonina 5-HTT, também conhecido como SERT, produto do gene *SLC6A4* localizado no braço longo do cromossomo humano 17 (17q11.2). Após sintetizado, o 5-HTT é transportado para a membrana plasmática dos botões sinápticos em neurônios pré-sinápticos e de células da glia onde exercerá a sua função. O 5-HTT é responsável pela recaptação da serotonina (5-HT) liberada na fenda sináptica. Dessa forma, o 5-HTT tem a capacidade de modular, a ativação de receptores pós-sinápticos para esse neurotransmissor e, portanto, modular a resposta à serotonina no neurônio pós-sináptico. Ao ser recaptado pelo neurônio pré-sináptico, a serotonina pode ter dois destinos: a degradação, via MAO-A, ou a integração à uma vesícula pré-sináptica via transportador vesicular do tipo VMAT, podendo ser liberada novamente na fenda sináptica quando houver o próximo potencial de ação. Quando na fenda sináptica, a serotonina pode se ligar a um autoreceptor, no neurônio pré-sináptico, o qual gerará um sinal inibitório sobre a exocitose de vesículas no terminal pré-sináptico, ou a serotonina pode se ligar a receptores pós-sinápticos, promovendo diferentes respostas, a depender do tipo de receptor ativado. As respostas mais comuns são a excitabilidade neuronal, ativação de cascatas que geram moléculas de neuroproteção, ativação de canais iônicos, produção de fatores de transcrição que modulam a expressão gênica e plasticidade sináptica, este último

um mecanismo amplamente associado à aprendizagem e à formação de memória. A modulação da quantidade de serotonina disponível na fenda sináptica é essencial para o controle das respostas acima citadas, e ela é executada, basicamente, através do controle da quantidade e atividade do transportador 5-HTT, da enzima MAO-A e do autoreceptor inibitório 5-HT_{1B} (Kandel et al., 2000).

Dois polimorfismos no gene *SLC6A4* têm sido alvo de investigação nos estudos funcionais e de associação: o 5-HTTLPR (Serotonin transporter-linked polymorphic region) e o rs25531. Ambos estão localizados na região promotora do gene *SLC6A4* e possuem alelos com efeito funcional sobre a transcrição deste gene. O 5-HTTLPR é um polimorfismo de repetição (VNTR) cujo monômero tem tamanho (~20pb) e sequência variáveis. Esse polimorfismo apresenta mais cerca de 18 tipos de alelos descritos, sendo dois deles comuns e os demais raros. Os alelos podem variar de 14 a 24 monômeros (Avula, Rand, Black, & O'Kane, 2011; Ehli, Hu, Lengyel-Nelson, Hudziak, & Davies, 2012; Nakamura, Ueno, Sano, & Tanabe, 2000), sendo que os mais comuns são os alelos de 14 repetições, denominados *Short* (S) e os alelos de 16 repetições, denominados de *Long* (L), por essa razão, esse polimorfismo vem sendo chamado também de um polimorfismo de inserção/deleção (INDEL), nesse caso, considerando o fragmento de ~43pb (dois monômeros, um de 20pb e um de 23pb) como um fragmento inserido no caso do alelo L, e deletado no caso do alelo S.

A maioria dos estudos aqui revisados trabalham apenas com os alelos L e S. Quando encontrados, indivíduos contendo alelos maiores do que L são excluídos da amostra ou analisados como um grupo de alelos denominado XL. Entretanto, estudos que optaram por trabalhar com os alelos maiores não conseguiram detectar efeito significativo nos fenótipos estudados (Avula et al., 2011; Ehli et al., 2012). Os alelos L e S apresentam ainda, 4 formas alélicas, no caso do alelo S, e 6 formas alélicas, no caso do alelo L. Essas formas alélicas se diferenciam entre si pelos tipos de monômeros que as compõem (Figura 5) (Nakamura et al., 2000).

Figura 3. Formas alélicas descritas para o Locus 5-HTTLRP. Cada letra grega representa um tipo diferente de monômero, cuja sequência está descrita no quadro à direita. Destaques em negrito representam monômeros variáveis entre as diferentes formas alélicas. Fonte: Nakamura e colaboradores (2000)



Evidências obtidas através de estudos funcionais, indicam que o alelo S promove a redução em até 2 vezes do número de transcritos do gene *SLC6A4* quando comparado aos níveis de transcritos codificados pelo alelo L. De forma que indivíduos homozigotos para o alelo L expressam mais 5-HTT do que indivíduos homozigotos para o alelo S. Indivíduos heterozigotos apresentaram níveis intermediários de 5-HTT nesses mesmos estudos (K.-P. Lesch et al., 1996; Murphy, Hollander, Rodrigues, Kremer, & Schatzberg, 2004). Além disso, alterações de conectividade funcional e menor ativação de áreas cerebrais específicas foram observadas em portadores do alelo S. Hamberg e Lesch (2011) demonstraram ainda que indivíduos S/S apresentam redução de massa cinzenta no giro pós-central e no pré-cuneos (Homberg & Lesch, 2011).

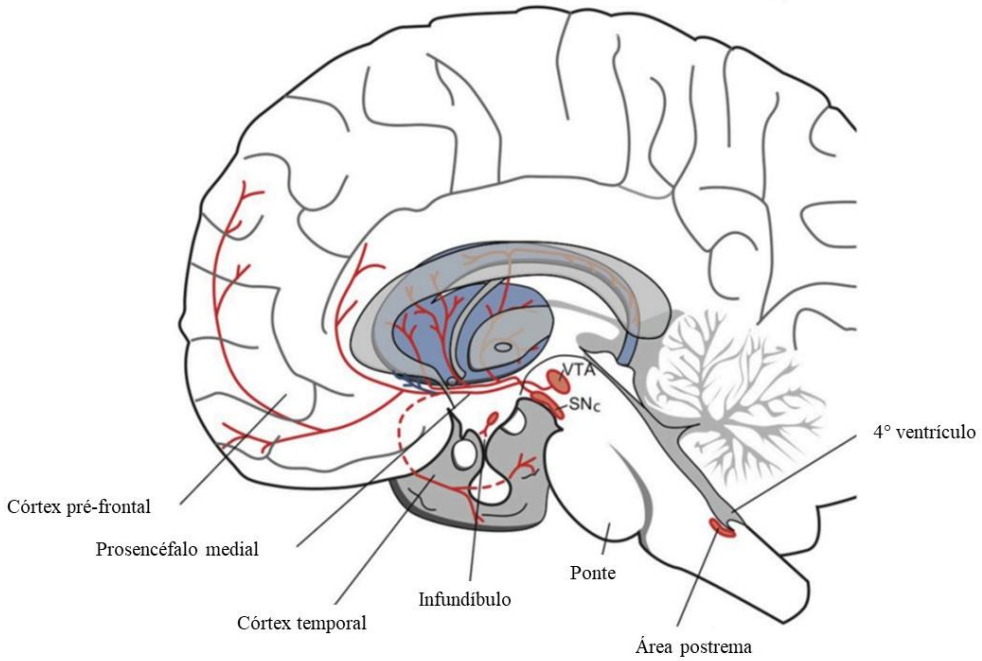
O polimorfismo rs25531, localizado dentro do polimorfismo 5-HTTLPR (na unidade de repetição não-variável μ; Figura 5), é um SNP em que ocorre a substituição de uma adenina por uma guanina. Essa variação tem sido investigada em conjunto com a 5-HTTLPR sob a justificativa de que juntos os dois *loci* formam um *locus* tri-alélico, em que a presença de um

alelo G em fase com o alelo L poderia modificar o seu efeito de expressão, através da criação de um sítio de ligação para o fator de transcrição AP2, o qual promoveria a supressão da transcrição do gene *SCL6A4*, sendo o efeito observado similar ao efeito de um alelo S (Ehli et al., 2012). Entretanto, a maioria dos estudos de associação optam por investigar apenas o locus 5-HTTLPR, visto que os seus efeitos funcionais estão melhor estabelecidos na literatura.

1.2.2 Sistema Dopaminérgico

A dopamina é a catecolamina mais abundante no sistema nervoso. Os neurônios que sintetizam dopamina são encontrados no mesencéfalo, diencéfalo e no telencéfalo. No sistema nervoso central, quatro vias dopaminérgicas compõem o sistema dopaminérgico: a via nigro-estriatal, relacionada ao controle do movimento, a via mesolímbica, responsável pelo controle das emoções, a via túbero-infundibular, envolvida no controle hormonal, e a via mesocortical, envolvida no controle de aspectos cognitivos e outras funções (Figura 6).

Figura 4. Ramificações dopaminérgicas no Sistema nervoso central. Fonte: Adaptado de Loonen et al., 2016.



Estudos têm associado o sistema dopaminérgico com cognição, aprendizagem e memória de trabalho (Júlio-Costa et al., 2014; Puig, Rose, Schmidt, & Freund, 2014). Outros ainda, têm

demonstrado a importância desse sistema para o desenvolvimento e o tratamento de transtornos afetivos e comportamentais, como a depressão, a dependência ao álcool e o TDAH, por exemplo (Bobb et al., 2005; Morgese & Trabace, 2019; K. Schmidt et al., 2001). Os níveis da dopamina são críticos para a modulação das funções desempenhadas pelo sistema dopaminérgico, pois a ativação de receptores pós-sinápticos e a consequente transdução de sinal é altamente dependente da concentração de dopamina na fenda sináptica. Evidências sugerem, por exemplo, que no córtex pré-frontal, níveis intermediários de dopamina estão associados ao melhor desempenho da memória de trabalho, enquanto que tanto o aumento ou a redução desses níveis provocariam queda no desempenho (Cools & D'Esposito, 2011).

O controle da disponibilidade de dopamina na fenda depende da atividade de degradação das enzimas Catecol O-Metiltransferase (COMT) e Monoamina oxidases A e B (MAO-A e MAO-B), e da atividade de recaptação do transportador de membrana DAT (Bloom, 2010). Polimorfismos funcionais nos genes que codificam essas proteínas têm sido investigados por alterar os níveis transcricionais desses genes ou até mesmo a função dessas enzimas. Esses polimorfismos estão associados, portanto, à modulação dos níveis de dopamina nas sinapses dopaminérgicas e aos mais diversos fenótipos modulados por este sistema (Tunbridge et al., 2019).

O SNP Val158Met no gene da *COMT* é o polimorfismo mais investigado na literatura. Nele uma substituição não-sinônima codifica a substituição de uma valina por uma metionina na posição 158 da proteína. As repercussões funcionais dessa substituição são o aumento do nível de metilação, e a redução nos níveis de expressão, atividade e estabilidade da proteína. No gene *DAT*, dois polimorfismos do tipo VNTR na região promotora (rs28363170 e rs3836790) têm sido associados à atividade do promotor, aos níveis de transcrição e à capacidade de ligação à dopamina para transportar da sinapse pra dentro do neurônio. Uma VNTR no gene *MAOA* (MAOA-LPR) possui 5 alelos bem descritos na literatura (2R,3R,3.5R, 4R, 5R), cujos alelos mais frequentes são 4R e 3R. Os alelos 4R e 3.5R têm sido relacionados à maior atividade do promotor e da enzima quando comparados ao alelo 3R. No gene *MAOB*, um SNP no íntron 13 em que ocorre a substituição de uma adenina por uma guanina, os estudos são discordantes quanto a qual alelo ocasiona o efeito, mas o locus pode estar relacionado à redução da atividade da enzima MAO-B. Com exceção, do Val158Met, os

demais efeitos funcionais aqui descritos tiveram resultados inconclusivos na metanálise realizada por Tundbridge e colaboradores (2019). Entretanto, a maioria dos estudos parece apontar que os alelos 3R no locus MAOA-LPR, Met no locus Val158Met, e 9R no locus rs28363170 estão associados com níveis elevados de dopamina no córtex e no estriado.

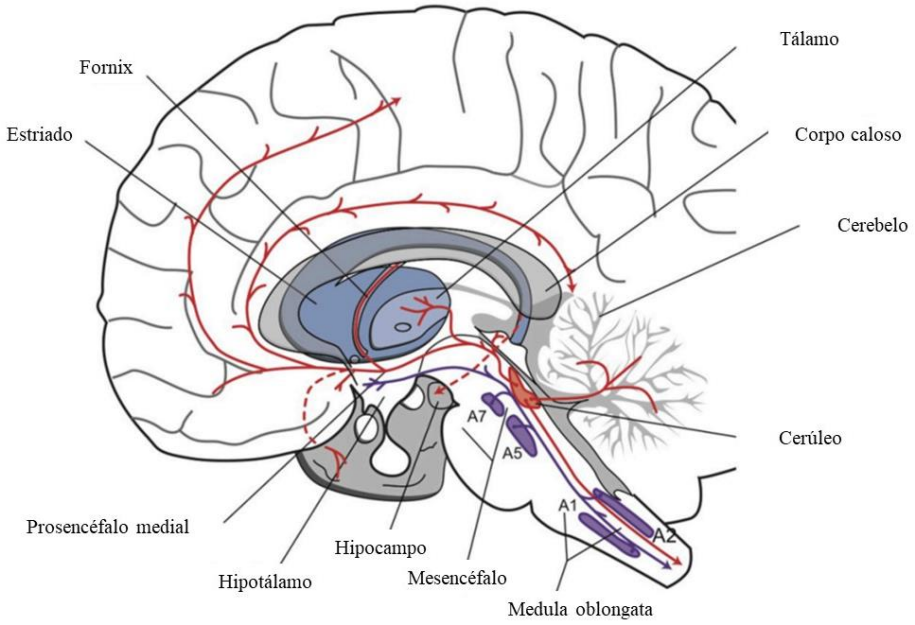
Por este motivo, diversos estudos vêm investigando esses polimorfismos funcionais em estudos de associação de genes candidatos com fenótipos de interesse. A presente tese utiliza esta abordagem para investigar os mecanismos genéticos e moleculares envolvidos na ansiedade matemática.

1.2.3. Sistema Noradrenérgico

Os neurônios noradrenérgicos produzem massivamente noradrenalina como neurotransmissor. Os neurônios noradrenérgicos se originam na medula e na ponte, sendo organizados em três grupos principais: o complexo do locus cerúleo, o sistema tegmental lateral e o sistema medular dorsal (Kandel et al., 2000). As projeções neuronais se difundem por todo o cérebro e cerebelo, formando sinapses no córtex dorsolateral, medial e límbico (Figura 7). A via noradrenergica dorsal se projeta anteriormente e dorsalmente, inervando quase todo o telencéfalo, o diencéfalo (tálamo e hipotálamo), o cerebelo e medula espinhal. A via tegmentar lateral se projeta de forma descendente para atuar no sistema periférico simpático, e se projeta ventralmente para inervar o tálamo. Dessa forma, essa via modula funções do sistema periférico simpático autônomo. O sistema medular dorsal envia projeções para o núcleo solitário, onde modula funções parassimpáticas executadas pelos nervos cranianos que se originam nesse núcleo (Kandel et al., 2000; Szabadi, 2013).

Assim como a dopamina, a noradrenalina está envolvida em um amplo espectro de funções fisiológicas e comportamentais, dentre as quais podemos destacar o controle do ciclo sono-vigília e o processamento de estímulos sensoriais. Através do processamento de estímulos sensoriais o sistema noradrenérgico é capaz de reduzir a responsividade a estímulos fracos e aumentar a responsividade a estímulos fortes controlando assim respostas somáticas como hiperventilação, palpitação e transpiração, a fatores estressores (Sullivan, Coplan, Kent, & Gorman, 1999).

Figura 5. Ramificações noradrenérgicas no sistema nervoso central. Fonte: Adaptado de Loonen et al., 2016.



A degradação da norepinefrina é realizada pelas enzimas MAO-A e COMT e a recaptação é realizada pelo transportador de membrana NET. Assim como nos demais sistemas, a ação dessas proteínas modula a concentração de noradrenalida nas sinapses e no ambiente extracelular, já que esse neurotransmissor funciona também como um neuromodulador, através do efeito de transmissão por volume (Szabadi, 2013). Fenótipos como medo, ansiedade generalizada e fobias específicas têm sido relacionados com a modulação dos níveis de noradrenalina (Sullivan et al., 1999). Como a AM é considerada por alguns autores como uma fobia específica relacionada ao fator estressor matemática, trabalhos que visem a investigar o impacto de polimorfismos funcionais em genes que modulam os níveis de noradrenalina em estruturas como o córtex pré-frontal e o sistema límbico, podem auxiliar na elucidação dos mecanismos genético-moleculares envolvidos na AM.

1 Justificativa, objetivos e design do estudo

A humanidade se baseia em números. Acordar e olhar as horas, contar moedas para comprar o pão da manhã, calcular tempo e planejar uma agenda, pegar o ônibus para ir para o trabalho, calculando o tempo para não chegar atrasado. Ao chegar na empresa, seguir um protocolo em etapas enumeradas, ranquear prioridades, sair para o almoço e dividir a conta entre os colegas, calcular os gastos do dia, separar o dinheiro do ônibus para voltar para casa. E ao chegar em casa, auxiliar o filho no dever de casa de matemática, enquanto duplica uma receita para fazer o jantar. Ao se deitar, planejar o dia seguinte, conferir o horário do ônibus, acertar o alarme no relógio e tomar um remédio cuja administração deve ser feita de 8 em 8 horas. Quantas situações no dia a dia envolvem o uso e a aplicação de conceitos matemáticos? Quantas profissões envolvem cálculos matemáticos e/ou um raciocínio rápido na matemática?

A realidade é que a matemática é a base de quase tudo o que vivenciamos hoje, desde tarefas simples como as descritas acima, até o complexo sistema que permite o desenvolvimento e manutenção de um sistema de computadores ou a existência da internet. Em uma sociedade que se baseia em números, ter dificuldades de manipular ou sentir sintomas de ansiedade e aversão à matemática é um problema de extrema relevância, principalmente se avaliarmos o potencial impacto individual e social disso. Sendo a AM uma fobia específica, um medo irracional, que impacta tanto a vida acadêmica quanto o cotidiano de milhares de pessoas ao redor do mundo, promovendo tanto sintomas somáticos como sintomas psicológicos (falta de motivação, incapacidade, depressão); dada a relevância do tema para a escolha de carreiras e profissões; e o possível impacto social e econômico de formar indivíduos que evitem situações que envolvam números, como as ciências, ou até mesmo o aprendizado econômico e financeiro; torna-se relevante compreender os fatores que geram predisposição para a AM, para que possíveis mecanismos de intervenção sejam propostos.

Intervenções educacionais e psicológicas vêm sendo propostas e aplicadas na clínica. Entretanto, sabe-se que a AM é um traço complexo, influenciado por fatores genéticos e moleculares que promovem susceptibilidade ao traço. Dowker e colaboradores (2016) ao revisar a literatura da AM, propuseram que os fatores genéticos associados a AM seriam fatores que explicassem os sintomas similares a ansiedade generalizada e/ou déficits na cognição matemática. A autora do presente trabalho vai ainda além e propõe que fatores genéticos envolvidos na neurofisiologia da motivação e dos mecanismos de resiliência e

resposta ao estresse, também seriam candidatos para a investigação das bases genético-moleculares da AM. Nesse contexto, o sistema monoaminérgico se destaca; por ser um sistema amplamente associado a modulação dos traços aqui citados.

Dessa forma, **o presente estudo se propôs a investigar se polimorfismos funcionais em genes do sistema monoaminérgico estariam associados à ansiedade matemática em uma amostra de crianças em idade escolar**. Para atingir este objetivo, selecionamos três polimorfismos funcionais amplamente estudados e realizamos um estudo de associação para cada um deles individualmente, avaliando também a contribuição de fenótipos cognitivos para a AM.

Os polimorfismos investigados na presente tese foram:

1) 5-HTTLPR no gene *SLC6A4*;
2) MAOA-LPR no gene *MAOA*; e
3) rs1799836 no gene *MAOB.*

Os resultados da presente tese serão apresentados na forma de capítulos contendo os artigos em fase final de redação, sendo:

Capítulo 1 – *"A FUNCTIONAL POLYMORPHISM (5-HTTLPR VNTR) IN SLC6A4 GENE IS ASSOCIATED WITH COGNITIVE AND AFFECTIVE ASPECTS OF MATH ANXIETY IN CHILDREN"*. O objetivo deste capítulo foi investigar a contribuição da VNTR funcional no promotor do gene *SLC6A4* para os níveis de Ansiedade Matemática em uma amostra de crianças de idade escolar.

Capítulo 2 - *"FUNCTIONAL POLYMORPHISMS IN MAOA AND MAOB GENES AFFECT CHILDREN SUSCEPTIBILITY TO MATH ANXIETY, WITH SEX DIFFERENCES"*. O objetivo deste capítulo foi investigar a influência dos polimorfismos MAOALPR e rs1799836 para os níveis de Ansiedade Matemática em uma amostra de crianças em idade escolar.

Os produtos gerados durante o período de doutoramento estão listados em anexo (ANEXO VI).

3 Capítulo 1 – A functional polymorphism (5-HTTLPR VNTR) in *SLC6A4* gene is associated with cognitive and affective aspects of math anxiety in children

O objetivo do presente estudo foi investigar a associação entre o locus 5-HTTLPR e a ansiedade matemática em crianças em idade escolar. Para este fim, no âmbito dos projetos Endofenótipos da dificuldade da aprendizagem e Discalculia do Desenvolvimento, 577 crianças com idade entre 8 e 12 anos, frequentando do terceiro ao sétimo anos do ensino fundamental, foram avaliadas. Dentre outros dados neuropsicológicos e de cognição matemática, dados dos níveis de AM foram também coletados através do Questionário de Ansiedade Matemática (MAQ). Amostras biológicas foram também coletadas para extração de DNA genômico e os genótipos para o locus 5-HTTLPR foram obtidos para cada criança através de PCR e eletroforese em gel de poliacrilamida. Após a obtenção dos genótipos, as amostras foram agrupadas de acordo com o genótipo, sendo classificados como L/L; indivíduos que possuíam dois alelos do tipo longo (16 repetições), S/S; indivíduos portando dois alelos do tipo curto (14 repetições), L/S; indivíduos portando um alelo longo e um curto, finalmente, classificamos indivíduos portadores do alelo extra longo (>16 repetições) em XL/L; quando acompanhado por um alelo longo, ou XL/S; quando acompanhados por um alelo curto. Os grupos genotípicos foram caracterizados em função de frequência genotípica e o locus foi avaliado em função da aderência ao equilíbrio de Hardy-Weinberg e homogeneidade entre as populações de diferentes origens. Após a exclusão dos genótipos menos frequentes (XL/L e XL/S).

Testes de associação foram realizados utilizando regressões lineares para cada subescala MAQ e também para o MAQ Total Score, uma variável criada no presente estudo para representar ambas as dimensões cognitiva e afetiva da AM. Os modelos lineares incluíram diferentes modelos genéticos de acordo com o sexo (dominância, recessividade, codominância e heterose) e covariáveis, como sexo, cidade de origem, inteligência, transcodificação e desempenho em matemática e em ortografia. Os resultados obtidos indicam que indivíduos heterozigotos para o locus 5-HTTLPR tendem a apresentar pior autopercepção sobre suas habilidades matemáticas (MAQ-A). Indivíduos com genótipos L/S e S/S tendem a apresentar maior ansiedade matemática (MAQ-D), quando comparados a indivíduos L/L.

Além disso, heterozigotos para esse locus também apresentam em média maior MAQ Total Score. A associação da presença do alelo S com a Ansiedade Matemática, descrita aqui pela primeira vez, tem duas implicações principais: 1) reforça a evidência de um componente biológico na Ansiedade Matemática; 2) níveis mais elevados ou intermediários de serotonina nas sinapses estão associados à Ansiedade Matemática. Resultados similares foram descritos para outros fenótipos relacionados a ansiedade e a baixa resiliência, fatores que podem estar envolvidos na resposta ao estímulo matemático (fator estressor).

Ver artigo em Anexo I

4 Capítulo 2 - Functional polymorphisms in *MAOA* and *MAOB* genes affect children susceptibility to math anxiety, with sex differences

Neste capítulo, apresentamos um estudo de associação, no qual investigamos a contribuição de dois polimorfismos funcionais em dois genes do sistema monoaminérgico, conhecidos por modular o metabolismo das catecolaminas: MAOA-LPR VNTR localizado no promotor do gene *MAOA* e rs1799836, um polimorfismo de nucleotídeo único no 13º íntron do gene *MAOB*. Em ambos os loci, a substituição alélica promove um efeito funcional de redução de síntese e atividade das enzimas MAO-A e MAO-B, promovendo alterações na disponibilidade de monoaminas na fenda sináptica. Neste estudo, 577 crianças com idade entre 8 e 12 anos foram avaliados quanto à inteligência, desempenho aritmético e de escrita, capacidade de transcodificação verbal-arábica e Ansiedade Matemática através do questionário MAQ. Amostras biológicas de cada criança foram coletadas para a obtenção de DNA genômico. A genotipagem para o locus MAOA-LPR foi feita através de PCR seguida por análise de fragmento em sequenciador, com confirmação em gel de poliacrilamida, quando necessário. A genotipagem para o locus *MAOB* rs1799836 foi feita através da técnica high resolution melting, sendo a calibragem da técnica feita a partir do sequenciamento (Sanger) de amostras para obtenção de controles positivos de cada genótipo. As amostras foram subdivididididas em grupos genotípicos para cada locus. Alelos raros foram excluídos para o locus MAOA-LPR para a realização dos testes de associação.

Testes de associação foram realizados considerando os genótipos em cada locus e as pontuações em cada subescala de Ansiedade Matemática, avaliadas por meio do MAQ. Os testes de associação foram realizados separadamente para meninos e meninas, permitindo que diferentes modelos genotípicos fossem testados para cada sexo. A existência de interações entre sexo e genótipo também foi interrogada. Como resultado, os dois loci mostraram efeito sobre a Ansiedade Matemática, sendo este efeito dependente de genótipo, de sexo e restrito ao sexo feminino no locus MAOA-LPR. A heterozigose de MAOA-LPR em meninas mostrou um efeito protetor para autopercepção negativa sobre suas próprias habilidades matemáticas (MAQ A). Um efeito protetor semelhante foi encontrado para o locus do *MAOB* rs1799836, mas apenas para meninos portadores de G, os quais apresentaram os níveis mais baixos de Ansiedade Matemática afetiva, como infelicidade (MAQ-C) e preocupações (MAQ-D)

relacionadas à presença de dificuldades na matemática. Ao considerar os domínios cognitivo e afetivo da Ansiedade Matemática em uma pontuação única (MAQ Total Score), os meninos portadores do alelo G em rs1799836 apresentaram os níveis mais baixos de Ansiedade Matemática, enquanto as meninas G/G apresentaram os níveis mais altos de AM em comparação às meninas A/A e A/G e meninos de qualquer genótipo. Este é o primeiro estudo a mostrar a contribuição de polimorfismos funcionais nos genes *MAOA* e *MAOB* para a Ansiedade Matemática e a prever um efeito protetor dependente de sexo nesses loci para a Ansiedade Matemática.

Ver artigo em Anexo II

5 Discussão Geral

A Ansiedade Matemática é um subtipo específico de ansiedade, considerada por alguns autores como uma fobia, que causa desconforto, sintomas de ansiedade ou medo e pode ainda causar impactos consideráveis ao processo de aprendizagem, e ao desempenho do indivíduo na Matemática. Indivíduos com altos níveis de AM tendem a evitar situações do cotidiano e carreiras que envolvam a Matemática. Em nossa sociedade, atualmente, poucas profissiões ou atividades não dependem de habilidades numéricas, dessa forma, a Ansiedade Matemática além de trazer prejuízos individuais, pode promover também problemas sociais e econômicos para um país. A alta frequência de AM no mundo, é ainda um fator agravante, principalmente considerando que ela pode ser persistente e que o fenômeno de transferência de ansiedade é algo importante para a propagação da AM, principalmente no contato entre pais e filhos ou alunos e professores. Dessa forma, compreender os fatores envolvidos no surgimento e no estabelecimento da AM é um passo indispensável para que sejam propostas intervenções que visem a diminuir os sintomas da AM, reduzir o impacto da AM sobre a vida do indivíduo e impedir, que crianças, ao possuírem predisposição, não desenvolvam um quadro de Ansiedade Matemática.

Dentre os fatores que conferem predisposição à Ansiedade Matemática, podemos citar: fatores de personalidade, resiliência, estratégias de *coping*, motivação e ainda fatores cognitivos associados ao aprendizado da matemática ou ainda fatores de predisposição para transtornos de ansiedade e fobias específicas (Douglas & LeFevre, 2018; Marušić & Matić, 2017). Nesse contexto, estudos que visem a identificar fatores de predisposição genética a esses fenótipos intermediários associados à AM, podem auxiliar na elucidação dos mecanismos envolvidos no surgimento e no estabelecimento da AM.

O sistema monoaminérgico é um sistema que engloba o metabolismo das catecolaminas e da serotonina, dentre outras aminas biogênicas. Essas moléculas são conhecidas por suas ações como neurotransmissores e neuromoduladores no sistema nervoso central, ao atuar em processos como cognição, controle das emoções e modulação do comportamento (Libersat & Pflueger, 2004). Genes do sistema monoaminérgico têm sido implicados em diversos fenótipos descritos que conferem predisposição à AM, como: maior neuroticismo como um dos componentes da personalidade, baixa resiliência, escolha de mecanismos de coping ativos

vs passivo, baixa motivação e reação alterada diante de estímulos estressores, por exemplo (K. P. Lesch, 2003; Limson et al., 1991).

Nesse contexto, a presente tese teve como objetivo investigar a contribuição dos polimorfismos 5-HTTLPR, MAOA-LPR e rs1799836 para a Ansiedade Matemática, em uma amostra de crianças em idade escolar. O polimorfismo 5-HTTLPR é uma VNTR no promotor do gene *SLC6A4*. Alelos longos (L) estão associados à maior taxa de transcrição, enquanto alelos curtos (S) estão associados à menor taxa de transcrição e, portanto, menos transportadores de serotonina na membrana e menor recaptação de serotonina nas sinapses (Avula et al., 2011). O excesso de serotonina nas sinapses tem sido associado à maior reatividade da amigdala, e indivíduos portadores do alelo S, mostraram maior ativação da amígdala direita em resposta a imagens que causam medo (Hariri & Holmes, 2006). Além disso, portadores do alelo curto, mostraram mais traços de neuroticismo na personalidade e maior sensibilidade a estímulos afetivos (Beevers, Ellis, Wells, & McGeary, 2010; Sen et al., 2004). De forma similar, no presente trabalho, indivíduos portadores do alelo S apresentaram níveis mais altos de AM. Entretanto, curiosamente, a média das MAQs de indivíduos heterozigotos é ainda mais alta do que a média de homozigotos para o alelo S. Este resultado mostra que níveis intermediários de serotonina nas sinapses, conferem maior suceptibilidade ao desenvolvimento da AM em crianças. As possíveis razões para esse resultado nunca ter sido reportado antes são: 1) a maioria dos estudos de associação não testam modelos genéticos diferentes, como a heterose, mas apenas o modelo de codominância ou, em alguns casos, o aditivo; 2) além disso, a presença do polimorfirmo rs25531 (A→G) pode alterar o efeito funcional do alelo L nesses indivíduos. Segundo a literatura, o haplótipo LG teria um efeito semelhante ao alelo S, entretanto, poucos estudos avaliaram os efeitos desse haplótipo. Dessa forma, efeitos adicionais desse segundo locus poderiam estar conferindo ao genótipo L/S um efeito funcional ainda mais significativo do que a presença de dois alelos curtos em homozigose; 3) outro ponto importante seria que níveis intermediários de serotonina na fenda podem realmente ser desfavoráveis no contexto da Ansiedade Matemática. Um estudo recente, investigando o impacto do polimorfismo Val158Met no gene da COMT sobre os níveis de AM, demonstrou que a heterozigose é desvantajosa em meninos, mas vantajosa em meninas. Demonstrando que níveis intermediários de catecolaminas nas sinapses também podem ter um efeito pior do que a homozigose do alelo considerado de risco nesse locus (Met/Met). 4) Por fim, a interação com outros loci no genoma podem atuar em conjunto

contribuindo para o fenótipo e modificando a tendência esperada para esse locus, já que vimos ao longo da presente tese que vários genes são responsáveis pelo metabolismo e modulação de monoaminas no cérebro. Estes resultados foram apresentados no capítulo 1 da presente tese.

No segundo capítulo desta tese, apresentamos os resultados da investigação sobre a contribuição dos polimorfismos MAOA-LPR e rs1799836, nos genes *MAOA* e *MAOB*, respectivamente, para a AM. Os dois loci apresentaram efeito sobre esse fenótipo, sendo este efeito diferente de acordo com o genótipo e o sexo da criança. Além disso, a contribuição do locus MAOA-LPR foi restrita ao sexo feminino, enquanto a contribuição do locus rs1799836 foi mais pronunciada no sexo masculino. Este resultado é interessante, pois efeitos similares já foram observados em estudos anteriores. Alelos que codificam alta atividade para a enzima MAO-A (4R e 3.5R), foram associados depressão maior apenas em mulheres (Schulze et al., 2000) e a distúrbios do sono e suicídios apenas em homens (Du, Bakish, Ravindran, & Hrdina, 2004; Du et al., 2002), por exemplo, demonstrando que esse locus parece sofrer um forte efeito de sexo. Outra evidência de efeito funcional dependente de sexo são os estudos de Philibert e colaboradores (2008 e 2011), em que os autores observaram maior efeito de fatores ambientais como álcool, tabaco e maus tratos na infância sobre a metilação do promotor do gene *MAOA*. Outro resultado apresentado no capítulo 2 foi o efeito protetor do genótipo heterozigo em meninas (Philibert, Gunter, Beach, Brody, & Madan, 2008; Philibert et al., 2011). Indivíduos do sexo feminino, sabidamente apresentam níveis mais altos de Ansiedade Matemática, entretanto, a média de AM das heterozigotas não foi diferente da média de AM dos indivíduos do sexo masculino no presente trabalho. O locus MAOA-LPR se localiza no cromossoma X em um locus que sofre inativação completa e aleatória. Portanto, homens são hemizigotos e mulheres, quando heterozigotas, podem expressar diferentes alelos em diferentes grupos celulares. Devido a isso, mulheres heretozigotas para o locus MAOA-LPR expressam tanto enzimas de alta atividade quanto enzimas de baixa atividade. Esse fato pode conferir alguma vantagem em relação a predisposição para a AM. Um efeito protetor semelhante foi encontrado para o locus do *MAOB* rs1799836, mas apenas para meninos portadores do alelo G, os quais apresentaram os níveis mais baixos de Ansiedade Matemática afetiva, como infelicidade (MAQ-C) e preocupações (MAQ-D) relacionadas a dificuldades matemáticas. Além disso, meninas G/G apresentaram os níveis mais altos de AM. Portanto, alelos que codificam enzimas de baixa atividade são mais vantajosos para indivíduos do sexo

masculino, entretanto, em mulheres, ter alelos de baixa atividade parece ser desfavorável, estando associada a maior predisposição de desenvolver AM.

MAO-A e MAO-B são duas enzimas responsáveis pela degradação de monoaminas. MAO-A é essencial para a degradação de serotonina e noradrenalina, e MAO-B é responsável pela degradação de uma monoamina neuromoduladora chamada PEA (phenethylamine), que atua como um estimulante do sistema nervoso central, estimulando, inclusive, a liberação de neurotransmissores como a serotonina, a noradrenalina e a dopamina. Ambas as enzimas degradam dopamina. A função de degradação é essencial para a modulação da concentração de monoaminas nas sinapses e portanto, da ativação pós sináptica (Kalgutkar, Dalvie, Castagnoli, & Taylor, 2001). Nesse contexto, os resultados encontrados na presente tese, indicam que alelos de baixa atividade de MAO-B, ou seja, maior concentração de PEA, dopamina dentre outros, estão associados a menores níveis de AM, em homens, mas não em mulheres. Nas mulheres, a expressão de enzimas com alta e baixa atividade, está associada a menores níveis de AM. Mais estudos investigando o efeito funcional da heterozigose no cromossomo X são necessários para melhor compreendermos os mecanismos envolvidos na vantagem da presença dos dois alelos. Provavelmente, a presença dos dois alelos permite maior variabilidade fenotípica e, portanto, maior adaptabilidade.

6 Considerações Finais

Este é o primeiro estudo a mostrar a contribuição de polimorfismos funcionais nos genes *5HTT*, *MAOA* e *MAOB* para a Ansiedade Matemática e a prever um efeito protetor dependente de sexo, nos dois últimos loci, para a Ansiedade Matemática. Além disso, este é o segundo estudo de associação genética para a Ansiedade Matemática. O presente trabalho reforça os estudos que propõem a presença de um componente genético para a Ansiedade Matemática. Ao longo de 10 anos, o nosso grupo vem investigando as bases genéticas da dificuldade da matemática, seus endofenótipos e agora agrega estudos sobre a genética da Ansiedade Matemática. Os resultados da presente tese agregam informação a um estudo anterior do grupo que teve o objetivo de investigar a contribuição de um polimorfismo no gene da *COMT* para a Ansiedade Matemática.

Como perspectivas, nosso grupo está investigando a contribuição de outros polimorfismos em genes do sistema dopaminérgico para fenótipos relacionados à cognição matemática e a AM. Além disso, seria interessante avaliar outros loci nos genes aqui reportados, interrogando se a presença de diferentes alelos modifica a média predita para o grupo genotípico avaliado. Nesse contexto seria interessante avaliar o locus rs25531. Estudos visando avaliar o nível de metilação dos promotores, também agregariam informação ao presente estudo, visto que esses genes têm sido relacionados a modificações epigenéticas, com efeito funcional sexo-dependente e, portanto, esses fatores podem ser confundidores no presente estudo.

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8 ANEXOS

8.1 Anexo I – Artigo 1

A FUNCTIONAL POLYMORPHISM (5-HTTLPR VNTR) IN *SLC6A4* GENE IS ASSOCIATED WITH COGNITIVE AND AFFECTIVE ASPECTS OF MATH ANXIETY IN CHILDREN

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ABSTRACT

Math anxiety represents a specific type of anxiety evoked by number processing and math and presenting with increasing prevalence from early school age to adolescence. It can be persistent and have a significant impact on academic achievement and professional prospects, because affected individuals usually tend to avoid situations involving math. As a multifactorial phenotype, math anxiety is influenced by environmental factors and individual factors, such as genetics. To date, only few studies investigated genetic factors contributing to math anxiety. The *SLC6A4* gene encodes the protein 5-HTT or SERT, a serotonin transporter, responsible for serotonin reuptake from the synaptic cleft, modulating the serotonin response in postsynaptic neurons in many parts of the brain, including regions responsible for emotion and cognition such as the limbic system and frontal cortex, respectively. A VNTR polymorphism (5-HTTLPR) in the *SLC6A4* promoter modulates the expression of 5-HTT. Individuals, who are homozygous for the long allele (L/L), produce more 5-HTT than those homozygous for the short allele (S/S). Heterozygous individuals present intermediary 5-HTT levels. This polymorphism has been associated with general anxiety, ADHD, depression, suicide ideation, and post-traumatic stress, among other phenotypes. The aim of the present study was to investigate the association of 5-HTTLPR VNTR genotype with math anxiety in school-age children. We assessed math anxiety in 577 children (aged 8 - 12 years) by the Mathematical Anxiety Questionnaire (MAQ) and genotypes for the 5-HTTLPR locus were obtained for each child. Association tests were performed for each MAQ subscale and for MAQ Total Score. Models included different genotypic models and covariates such as sex, city of origin, intelligence, verbal to Arabic transcoding performance as well as math and spelling performances. Results indicated that, 5-HTTLPR VNTR heterozygous individuals tended to present lower self-perception about their math abilities (MAQ-A) as well as a higher MAQ Total Score, a measure that accounts for both cognitive and affective MA dimensions. Additionally, L/S and S/S carriers had higher math anxiety scores (MAQ-D). The specific association of S-carrier genotypes with math anxiety, described here for the first time, has two main implications. First, it substantiates evidence for a genetic component of math anxiety. Second, it suggests that higher levels of serotonin in the synaptic cleft seem to be associated with higher scores on math anxiety, similarly to what has been described for other anxiety-related phenotypes and lower resilience to stressful events.

KEY-WORDS: Math anxiety, Attitudes towards math, 5-HTT, 5-HTTLPR, SLC6A4

INTRODUCTION

Math learning difficulties may result from or be aggravated not only by cognitive impairments, but also by emotional influences (Ashcraft & Krause, 2007). In this vein, a substantial number of children and adults with typical cognitive functions experience Math Anxiety (MA), a specific type of anxiety evoked by number processing and mathematics. It is a condition that typically emerges at the beginning of school life and is characterized by feelings of tension, discomfort, phobia, and even fear in relation to activities involving numbers and mathematics (A. Dowker, Sarkar, & Looi, 2016; Haase, Guimarães, & Wood, 2019; Richardson & Suinn, 1972). MA can severely affect math learning and performance, by causing affected children to avoid math related situations and activities (e.g., Ashcraft & Ridley, 2005; Dew, Galassi, & Galassi, 1984) and/or by overloading working memory during math tasks (e.g., Ashcraft & Krause, 2007). Moreover, MA can be persistent and has the potential to severely affect many aspects of the individual's life such as handling money, evaluating sales prices, dividing workloads, balancing banking accounts or defining career paths (Dew et al., 1984; Richardson & Suinn, 1972). It is unclear, however, whether MA directly causes math learning difficulties or whether low math performance associated with the emotional impact of failures in math causes MA (A. Dowker et al., 2016). Most probably, both phenomena co-occur. Nevertheless, whether an individual loves or fears math usually has considerable influence on his or her (school) career and may even impact later income and social status (Luttenberger, Wimmer, & Paechter, 2018; M. T. Wang & Degol, 2017).

Although MA was observed to be associated with general anxiety in several studies, including a meta-analysis (Hembree, 1990), MA is clearly a distinct construct, which seems more closely related to test anxiety, than to general anxiety (Malanchini et al., 2017; Pizzie & Kraemer, 2018). However, this relationship can change with age and according to methodological aspects of the respective studies such as assessment methods or sample characteristics. As there is no general agreement on criteria to consider people as math anxious, few studies on MA reported on its prevalence. According to Richardson and Suinn (1972), 11% of university students are anxious enough towards math to seek counseling and Chinn et al (2009) reported that 2 to 6% of secondary school students in England reported experiencing high levels of MA.

Similar to other types of anxiety, MA is a complex phenotype, influenced by individual differences such as genetic factors (Z. Wang et al., 2014) and sex (Goetz, Bieg, Ludtke, Pekrun, & Hall, 2013), as well as environmental factors such as nationality, culture, socio-economic status (SES), and school quality (Askew, Hodgen, Hossain, & Bretscher, 2010; Chiu & Xihua, 2008; Lee, 2009). However, only few studies have addressed the genetic causes of MA. Among them, a twin study reported that 40% of the variance in MA can be attributed to genetic factors (Z. Wang et al., 2014). Similar results were observed in a second study with older twins (18-21 yo) (Malanchini et al., 2017). Nevertheless, these genetic factors could be specific for MA or predisposing genetic risk factors associated with math cognition and or general anxiety.

To date, only one study in the literature has investigated the effect of a specific gene to MA. Julio-Costa and co-workers (2019) investigated the influence of the dopaminergic system on MA, using the VAL158MET polymorphism in the *COMT* gene. This gene encodes an enzyme also known as COMT (catechol-O-methyltransferase) that degrades dopamine in the prefrontal cortex. This polymorphism induces an amino acid substitution in the protein, which impacts COMT activity. The wild-type allele contains a valine in position 158 and presents higher enzymatic activity, whereas the mutated allele presents a methionine in this position, and consequently, less activity. Genotypes in autosomal loci are composed of two alleles. Therefore, individuals may present three genotypes: Val/Val, Val/Met or Met/Met. It has long been suggested that different genotypes in COMT would associate with behavioral differences. To investigate the effects of COMT in MA, the authors contrasted different genotypic models.

Genotypic models allow to investigate the interaction between the alleles in a gene. Most studies investigate just the additive model, which supposes that allele substitution effects change gradual- and similarly. For example, considering a locus with alleles 1 and 2, under an additive model, genotype effects would be 1/1<1/2<2/2 *or* 1/1>1/2>2/2. Alternatively, there may be a dominance relation between the alleles of a locus. In the 1-dominant genotypic model, genotypes 1/1 and 1/2 would have similar effects, differing from 2/2. In the 2-dominant genotypic model, 2/2 and 1/2 genotypes would have similar effects, differing from 1/1. A far less investigated genotypic model is heterosis. In this model, homozygous (1/1 and 2/2) genotypes present similar effects, differing from the heterozygous genotype (1/2).

By contrasting genotypic models, Júlio-Costa and coworkers (2019) reported differential effects of the VAL158MET homozygous genotype depending on sex: homozygous boys showed lower math anxiety (below the population mean) than homozygous girls (above the population mean), whereas heterozygous children present MA results around the mean, regardless of their sex. As such, an important result of that study was that heterosis was the best model to account for genetic differences in MA in this locus.

Another interesting result obtained by Julio-Costa and coworkers (2019) was the dissociation between the sexes. The same COMT VAL158MET genotypes were associated with completely opposite outcomes in girls and boys (lower MA in homozygous boys and higher MA in homozygous girls), suggesting that genotype effects are modulated by sex. Sex or gender is an important source of individual differences in MA. Higher MA levels in females persist in spite of improvement in opportunities for women and absence of average sex-differences in math achievement (Lindberg, Hyde, Petersen, & Linn, 2010; Wai, Cacchio, Putallaz, & Makel, 2010). One line of reasoning attributes the higher MA levels in females to gender stereotype threat. Evidence indicates that female attitudes, feelings towards and performance in math are vulnerable to the effects of negative stereotypes (e.g., Krendl, Richeson, Kelley, & Heatherton, 2008; see however, Stoet & Geary, 2012). Girls may also be especially vulnerable to social transmission of MA by female teachers (Beilock, Gunderson, Ramirez, & Levine, 2010).

Under equal opportunities and similar school achievement in math, females at all ages usually experience greater MA and tend to rate themselves as worse than males in math (Beilock et al., 2010). Among the possible causes for this sex differences are gender stereotypes and the influence and social transmission of anxiety by anxious female teachers (Beilock et al., 2010). Additionally, MA might be related to the usually higher rates of general anxiety and neuroticism in females (Chapman, Duberstein, Sörensen, & Lyness, 2007; Costa Jr, Terracciano, & McCrae, 2001; Feingold, 1994) as well as to the fact that males usually show more self-confidence and rate themselves better in math when compared to females (Jakobsson, Levin, & Kotsadam, 2013). However, biological and genetic differences may also be implicated in the higher vulnerability to MA in females. It has been suggested that lower MA levels in boys are mediated by better visuospatial processing abilities, possibly related to higher fetal testosterone levels (Maloney, Waechter, Risko, & Fugelsang, 2012). Finally, the

results of Júlio-Costa and coworkers (2019) indicate that genetic variability may be associated with MA in complex ways that differ between the sexes. The interaction of sex and genotypes shown by Julio-Costa and co-workers (2019) and additional evidence for sex hormones modulating neurotransmitter responses suggest that gender may play an even more complex role for MA manifestation and should be investigated in more depth.

MA is a complex construct (e.g., Krinzinger, Kaufmann, & Willmes, 2009). MA may be defined at different levels: cognitive (negative attitudes, worrisome rumination, feelings of helplessness, low self-esteem, self-efficacy, etc.); affective (dysphoria); behavioral (avoidance, hurry-up to finish math tasks, etc.), and physiological (sweating, trembling, high pulse rate, etc.). Definitions of MA may also focus on performance or on the self (Chinn, 2009). MA is usually assessed with self-report questionnaires of the cognitive and affective dimensions. These two dimensions are well validated but neglect other dimensions of the phenotype (Haase et al., 2019).

The cognitive dimension accounts for the worry and concerns about their own performance and the consequences of failure in math, while the affective dimension reflects the associated emotionality such as nervousness and tension in testing situations and the reactions of the autonomic nervous system derived from it (Ho et al., 2000). For assessing MA and its subdimensions different instruments have been proposed, predominantly questionnaires and rating scales, primarily made for assessing MA in adolescents and adults, but also for children (Ann Dowker, Bennett, & Smith, 2012; Dreger & Aiken Jr, 1957; Fennema & Sherman, 1976; Krinzinger et al., 2007; Richardson & Suinn, 1972; Thomas & Dowker, 2000). All methods developed to assess MA so far, showed good reliability (A. Dowker et al., 2016).

The Mathematics Anxiety Questionnaire (MAQ) is an instrument used to evaluate children's perceptions of their own skills and feelings, when performing math activities or having difficulties in performing them (Krinzinger et al., 2009; Thomas & Dowker, 2000; Wood et al., 2012). MAQ is composed by four subscales: MAQ-A evaluates self-perception about math performance; MAQ-B evaluates the attitudes towards math, as liking or not liking it; MAQ-C evaluates how the individual feels about having difficulties or failing in mathematical tasks; and, MAQ-D evaluates individual concerns by solving mathematical tasks. MAQ scores correlate negatively with math skills (r=-0.37) (Haase, Júlio-Costa, et al.,

2012; Wood et al., 2012). As referred above, it is difficult to establish the contribution of MA to math learning difficulties (MLD) and vice-versa.

The investigation of the biological components can help understanding MA. The genotypic model presented in Júlio-Costa and co-workers' study explains less than 10% of the genetic variance, and consequently, most of the heritability estimated for MA remains unexplained. Júlio-Costa and coworkers (2019) study, together with other studies presenting evidence of the neurobiological aspects of MA (Klados, Pandria, Micheloyannis, Margulies, & Bamidis, 2017; Moustafa et al., 2017; Suarez-Pellicioni, Nunez-Pena, & Colome, 2016), suggest that, in addition to *COMT*, other genes involved in the regulation of neurotransmitter systems might also influence MA levels.

The serotonergic pathways have been frequently studied in behavioral and psychiatric disorders such as general anxiety in adults (Sen, Burmeister, & Ghosh, 2004), attention deficit and hyperactivity disorder in children (Fowler et al., 2009; Kent et al., 2002), depression (Zammit & Owen, 2006), suicidal behavior and ideation (Caspi et al., 2003), and post-traumatic stress disorder (Navarro-Mateu, Escámez, Koenen, Alonso, & Sánchez-Meca, 2013). In addition, the serotonergic pathways have been also associated with cognitive functions and performances (Meneses & Liy-Salmeron, 2012) and impairments in learning and in verbal episodic memory (Mendelsohn, Riedel, & Sambeth, 2009). One of the major modulators of the serotonergic pathway is 5-HTT or SERT. This protein is encoded by *SLC6A4*, a gene located on the long arm of the human chromosome 17 (17q11.2). 5-HTT is located in the presynaptic neurons and glial cells, where it is responsible for the reuptake of the neurotransmitter serotonin released in the synaptic cleft. Therefore, 5-HTT modulates the activation of postsynaptic receptors, and thus, the postsynaptic response to serotonin. When reuptaken by the presynaptic neuron, serotonin follows two destinations: degradation by the enzymes monoamine oxidase A (MAO-A) and monoamine oxidase B (MAO-B) or inclusion into the presynaptic vesicles by the VMAT-type vesicular transporter, to be released again into the synaptic cleft after an action potential. When released in the synaptic cleft, serotonin can bind to an auto-receptor in the presynaptic neuron, which will generate an inhibitory signal on the exocytosis of vesicles in the presynaptic terminal or it can bind to postsynaptic receptors, promoting different responses depending on the type of receptor activated. The most common answers to serotonin receptor activation are neuronal excitability, activation of

cascades that generate neuroprotection, activation of ion channels, production of transcription factors that modulate gene expression and synaptic plasticity. The modulation of the amount of serotonin available in the synaptic cleft is essential for the control of these responses. It is implemented, basically, by controlling the quantity and activity of the 5-HTT and MAO-A, and by the activation of the auto-receptor 5-HT1B, which inhibits the release of serotonin (Jacobs & Azmitia, 1992; D. L. Murphy & Lesch, 2008).

A functional polymorphism in the *SLC6A4* gene has often been investigated: 5-HTTLPR (Serotonin transporter linked polymorphic region). 5-HTTLPR is a repeat polymorphism (VNTR) that encompasses a repetitive region of the gene promoter. The repeat monomer has variable size (~20bp) and variable sequence. Consequently, more than 18 different alleles have already been described, two of them are common and the others appear rarely in the studies published so far. Alleles may vary from 14 to 24 monomers (Avula, Rand, Black, & O'Kane, 2011; Ehli, Hu, Lengyel-Nelson, Hudziak, & Davies, 2012; Nakamura, Ueno, Sano, & Tanabe, 2000). Most common are the 14-repeat alleles, known Short (S), and the 16-repeat alleles, known as Long (L). Most studies published, decided to work only with the L and S alleles, and individuals containing alleles larger than L are excluded from the samples or grouped as Extra Long alleles (XL). However, studies working with the XL alleles failed to detect significant effects, probably due to its low frequency in samples (Avula et al., 2011; Ehli et al., 2012). The S and L alleles have also multiple allelic forms, four for the S allele and six for the L allele were described so far.

Evidence obtained through functional studies (K.-P. Lesch et al., 1996; G. M. Murphy, Hollander, Rodrigues, Kremer, & Schatzberg, 2004), indicate that the S allele promotes a reduction in the amount of transcripts of the *SLC6A4* gene when compared to the L allele. Therefore, L/L homozygous individuals would express more 5-HTT than S/S homozygous individuals, while L/S heterozygous individuals would present intermediate levels of 5-HTT. In addition, neuroimage studies have described functional brain connectivity differences and less activation of specific brain areas, such as the amygdala, in S-carriers individuals (L/S or S/S). 5-HTTLPR S allele has been previously associated to anxiety and fear related phenotypes, including specific phobias, such as social anxiety (Furmark et al., 2004; Garpenstrand, Annas, Ekblom, Oreland, & Fredrikson, 2001). Additionally, it has been associated with personality aspects such as higher neuroticism, a predisposing factor to MA

(K. P. Lesch, 2003; Marušić & Matić, 2017). Added to that, the involvement of 5-HTT in the modulation of amygdala activation (Hariri & Holmes, 2006), and its importance for stress response mechanisms such as coping (Puglisi-Allegra & Andolina, 2015), contribute to the emergence of the 5-HTT polymorphism as a possible candidate for MA.

In the present study, we aimed at investigating the contribution of the locus 5-HTTLPR in the *SLC6A4* gene to Math Anxiety. To achieve this goal, we genotyped the 5-HTTLPR VNTR in a group of demographically recruited school-age children from Brazil, showing intelligence above the PR15. In addition to assessing MA through a self-report questionnaire, we also investigated the participants' performance in math, spelling and their Verbal to Arabic transcoding performance, to interrogate if these variables, together with 5-HTTLPR genotype could help to explain a portion of MA variance. After evaluating the contribution of each variable for MA, different genotypic models were tested in the association analysis, to investigate the possible interallelic interactions between the alleles in the locus (codominance, heterosis, L-dominant model, or S-dominant model), following the criteria described in Julio-Costa et al. (2019). To test the hypothesis that different genotypic models could be taking place in girls and boys for this locus (as observed for COMT VAL158MET locus in Julio-Costa et al., 2019), linear regressions separated by each sex were fitted using the most probable genotypic model in each sex. Herein, the most probable genotypic models to be tested were chosen based on mean differences and similarities among genotypic groups. To the best of our knowledge, this is the first study to investigate the contribution of a polymorphism in the serotonergic system to MA, and the second to investigate the molecular-genetic underpinnings of MA.

MATERIAL AND METHODS

Ethical consideration

The study complied with current bioethical research standards and was approved by the Ethics in Research Committee of the Federal University of Minas Gerais (COEP-MG) under the numbers ETIC 42/08 and CAAE 15070013.1.0000.5149. Participation was conditioned to obtaining written informed consent from parents or surrogates and orally from children.

Participants

722 children were recruited from state-runned and private schools in Belo Horizonte, Minas Gerais State, Brazil (n=601) and Porto Alegre, Rio Grande do Sul State, Brazil (n=121). The recruitment included children attending 2nd to 7th grade, corresponding to 7 to 12 years of age, with normal intelligence (PR>15). Children attending 1st and 2nd grade were excluded from the sample due to difficulties in understanding MAQ task. Moreover, children who had the 5-HTTLPR VNTR XL alleles were also excluded from the final sample, due to low allelic frequency. Finally, only children with no missing data were considered in the analyses. This made a sample of 577 children, 482 from Belo Horizonte and 95 from Porto Alegre. The final sample included children attending 3rd to 7th grade, corresponding to 8 to 12 years of age (mean age= 9.23; sd=1.05; 55% female).

Constructs and Instruments

General intelligence was assessed using the Raven's Coloured Progressive Matrices - CPM (Angelini, Alves, Custódio, Duarte, & Duarte, 1999).

School achievement was assessed using the Arithmetic and Spelling subtests of the Brazilian School Achievement Test (TDE, Ferreira et al., 2012; Stein, 1994). The Arithmetic subtest comprises of three orally presented word problems and 45 written arithmetic problems of different complexity levels. The Spelling subtest requires children to write by dictation 34 real words.

A number writing task was used to assess children's verbal to Arabic number transcoding performance (Moura et al., 2013).

In order to evaluate MA, the Brazilian version of the Math Anxiety Questionnaire (MAQ) was used (Haase, Costa, Antunes, & Alves, 2012; Wood et al., 2012). The MAQ consists of four subscales containing six questions each, in which children have to answer questions regarding his/her performance in Math (self-perception) (MAQ-A), his/her attitudes towards mathematics (MAQ-B), how he/she feels about having problems with mathematics (happy or sad) (MAQ-C) and his/her anxiety level related to having problems with mathematics (MAQ-D) (Wood et al., 2012). Questions in the subscale MAQ-A always start with "How good are you in ..."; in MAQ-B, "How much do you like ..."; in MAQ-C, "How happy or sad do you

get when you have problems with... ", and in MAQ-D, "How worried do you get when you have problems with ... ". The six questions in each subscale, always address the following aspects: 1) mathematics in general; 2) easy calculations; 3) difficult calculations; 4) written calculations; 5) mental calculations; and, 6) math homework. The alternatives to answer these questions are organized on a Likert scale with pictures, which is presented to the child after each question. The child should point to the picture that better represents his or her feelings regarding each question asked. Scores are given on a scale of 1 to 5 for each item, 5 being always the more severe result ("very bad" answers in MAQ-A; "I hate" answers in MAQ-B, "very sad" in MAQ-C, and "very worried" in MAQ-D). At the end of the test, a total score for each MAQ subscale is generated from the sum of the responses of the six items. Reliability coefficients (Cronbach's α) of MAQ scales ranged from 0.74 to 0.88 (Wood et al., 2012).

MAQ Total Score

A new variable was created to represent a general math anxiety construct, covering all MAQ scales. To create this new variable, a Principal Component Analysis (PCA) was performed using the correlation matrix of the z-scores of MAQ-A, MAQ-B, MAQ-C, and MAQ-D. The first component explained 54% of the variance of the MAQ subscales. Then, we generated principal component scores by multiplying the loadings in the first component by the MAQ subscales matrix. This new, PCA-based variable was named MAQ Total Score and showed high and positive correlation with all MAQ subscales: MAQ-A (r=0.71), MAQ-B (r=0.72), MAQ-C (r=0.79) and MAQ-D (r=0.71).

Procedure

Data collection was conducted at the participant's schools. Data for the current analyses were obtained from two different projects. In both projects, children participated in groups of up to eight in testing of intelligence (Raven's CPM) and school Achievement (TDE Arithmetic and Spelling). Data from the Brazilian Education Quality Index (IDEB) was obtained for each school, to be used as a measure of school quality (http://ideb.inep.gov.br/). In a subsequent individual session, children completed the MAQ and a saliva sample was collected.

Sample collection and genotyping

Saliva samples were collected from each participant and stored in a tube containing 500μL of 0.5M EDTA. Total DNA extraction was performed using an in-house adapted protocol of DNA extraction using proteinase K and saline precipitation (adapted from (S. Miller, Dykes, & Polesky, 1988), and Aidar & Line, 2007). DNA amount and purity were assessed through spectrophotometry in a Nanodrop.

The 5-HTTLPR VNTR was genotyped through PCR and gel electrophoresis. PCR primers were designed using Primer3 software in NCBI (Untergasser et al., 2012) and the specificity of the primers was tested using the in silico PCR tool in UCSC Genome Browser (Rhead et al., 2010) (Supplementary Table 1). PCR reactions were set in a 25μL total volume and consisted of 5 μL of 5x Buffer, 2 μL of DMSO 100%, 20 mM dNTP, 25pmol of each primer, 2 μg of Taq DNA polymerase, 100ng of total DNA, and water milliQ qsp. PCR cycling was composed of an initial 5 min denaturation at 94ºC, followed by 25 cycles of 94ºC for 30s, 60°C for 30s and 72° C for 30s, and a final extension step of 72°C for 5min. PCR fragments were visualized by electrophoresis in 8% polyacrylamide gels. PCR fragments were classified as long (L), when they presented an approximate size of 488bp, and as short (S), when they presented an approximate size of 445bp. Larger alleles were classified as extra-long (XL).

Statistical Analyses

Statistical analysis were performed in four main steps: 1) First, allelic and genotypic frequencies were described and compared between the two cities where samples were collected, to ensure that both samples could be analyzed as a unique sample; 2) Univariate analysis of neuropsychological variables were used to perform a description of the data and to determine the tests to be used in the bivariate and multivariate analyses; 3) then, bivariate analyses of neuropsychological data among variables were performed to define which variables contribute to explain the variance of each MAQ and also to determine the presence of multicollinearity among them; 4) finally, MAQ subscales and MAQ Total Score means were compared among genotype groups to define the most probable genotypic model to be used in the association analyses. After the definition of the most probable genotypic model for each MAQ subscale according to sex, linear models with genotype and covariables were fitted for each MAQ subscale. Details about how each step of this workflow was performed and the tools used are described in the following paragraphs.

- *Allelic and genotypic descriptives*

Allelic and genotypic frequencies were calculated in R (Gentleman, Ihaka, & Bates, 2009). The fit to the Equilibrium of Hardy Weinberg was tested using the package *HWxtest* implemented in R (Engels, 2016). This package provides a valid test for Hardy-Weinberg expectancies for virtually any set of genotype counts, being applicable to multiallelic loci as 5-HTTLPR. After excluding the XL alleles from sample, allelic and genotypic differentiation between populations (Belo Horizonte and Porto Alegre) were assessed through an exact G test in GenePop version 4.2 (Rousset, 2008).

- *Univariate analyses of neuropsychological data*

Neuropsychological variables were described in terms of minimum, maximum, mean, median, and standard deviation using the package fBasics in R. The distribution of each variable was evaluated for the Total Sample and also for each city of origin using histograms and the adherence to normality was tested through Shapiro-Wilk test (alpha=0.05). Some of the neuropsychological variables investigated in the present study vary with age. Therefore, z-score standardization by age in years was used to correct for age effects.

- *Bivariate analyses of neuropsychological data*

Bivariate descriptive analyses were performed using correlations (SPSS v.20 IBM Corp., 2011). Additionally, to correlation, general linear regressions, assuming gamma distribution, were used to evaluate if intelligence, verbal to Arabic transcoding performance, math and spelling performances or IDEB individually contribute to explain the variance of each MAQ subscale and MAQ Total Score. In this first step, an alpha≤0.20 was used to determine which variables should be included in the association regressions together with genotype.

- *Genotypic model definition and association analyses*

A first evaluation of the genotypic model was conducted by comparing means for the different genotypes/genotype groupings, meaning, additive model (L/L *vs.* L/S *vs.* S/S), L-dominant model (L/L + L/S *vs.* S/S), S-dominant model (L/L *vs.* L/S + S/S), and heterosis model (L/L + S/S *vs.* L/S). The comparisons were done for each variable, considering each sex separately when an unique genotype model did not seem to apply for both sexes. After the

definition of the best model for each MAQ variable and sex, aiming to investigate the contribution of 5-HTTLPR to MA, association tests were performed using linear regression models. In these models, MAQs were defined as dependent variables and genotype as independent variables. Sex, spell and math achievement (TDE-spelling and TDE-arithmetic), verbal to Arabic transcoding performance, intelligence, and IDEB were included as covariables, according to results of the bivariate analyses. The decision about which variables should be kept in the final model was made based on the results of a stepwise regression (car package in R) (Fox et al., 2012). To ensure that collinearity among the variables kept in the model was not inflating the models, the function vif() in the package car in R was used (Fox et al., 2012). Inflation factor values smaller than 5 were considered adequate. Finally, to assess the minimum sample necessary to obtain a predictive power of 80%, considering an alpha of 0.05 and a 3% effect size was calculated using G*Power (Faul, Erdfelder, Buchner, & Lang, 2009).

RESULTS

In the present study, we aimed at evaluating the contribution of the 5-HTTLPR polymorphism to MA in a sample of Brazilian school-age children. In addition to genotype, the contribution of other measures as intelligence, verbal to Arabic transcoding ability and the children's performances in arithmetic and spelling, was also evaluated.

In this section, we will present the results obtained, first reporting the allelic and genotypic frequencies for each sample, the comparison between observed and expected frequencies under the HWE hypothesis, and the comparison of frequencies according to city of origin. Then a brief description about each neuropsychological data assessed will be presented, focusing on their distribution and whether they differ among genotypic groups. Then, we will present the comparison of MAQ means among genotypic groups, focusing in which genotypic models are more probable. Finally, we will present the linear regressions for each MAQ subscale and for MAQ Total Score, highlighting the contribution of each variable tested and in whether or not the 5-HTTLPR alleles are associated to MA.

Allelic and genotypic descriptive statistics

5HTTLPR allele and genotype frequencies are presented in Table 1. As expected, the XL allele showed low frequency and, therefore, was excluded from subsequent analysis. Testing Hardy-Weinberg Equilibrium hypothesis, we aimed to evaluate whether evolutionary factors would be affecting 5HTTLPR. No significant evidence of departure from HWE expectancies were detected in the Total sample, BH or PA samples (Table 1). After excluding the XL-carriers, no significant differences in genotypic frequencies were detected between sexes (χ^2= 1.2, df = 2, p= 0.546), and means of school grades (*F*=0.29, df=2, p=0.75), and age in years (*F*=1.13, df=2, p=0.32). Additionally, no differences in allelic and genotypic frequencies were observed due to the city of origin, suggesting that both samples could be analyzed as a unique sample in the following analyses (p=0.35; p=0.36, respectively).

Table 1. 5HTTLPR genotypes and allele frequencies in Total, Belo Horizonte and Porto Alegre samples

Genotype or Allele	**Total (n=722, %)**	**BH (n=601, %)**	**PA (n=121, %)**
L/L	254 (0.352)	215 (0.358)	39 (0.322)
L/S	333 (0.461)	275 (0.478)	58 (0.479)
S/S	122 (0.169)	99 (0.165)	23 (0.19)
XL/L	11 (0.015)	10 (0.017)	1 (0.008)
XL/S	2 (0.003)	2 (0.003)	0 (0)
L	852 (0.590)	715 (0.595)	137 (0.566)
S	579 (0.401)	475 (0.395)	104 (0.430)
XL	13 (0.009)	12 (0.010)	1 (0.004)
HWE p-value	0.18	0.25	0.92
Het. Excess p-value	0.27	0.27	0.45

Abbreviations: BH – Belo Horizonte; PA - Porto Alegre;

HWE – Hardy-Weinberg Equilibrium (p-value probability);

Het. Excess – Heterozygosis excess (p-value U test).

Sex and genotypic group effects on neuropsychological performance

Univariate analyses were conducted to investigate whether neuropsychological differences were associated with age, sex and genotypic group effects. Most of the neuropsychological variables investigated here presented age-effects. Consequently, they were transformed using z-scores by age. Range, mean, and standard deviations for each neuropsychological variable in z-scores are presented in Table 2 (for variables in raw scores, see Supplementary Table 2). Only TDE-arithmetic and MAQ Total Score presented normal distribution (Table 2). Between sex differences were observed in all variables, except for TDE arithmetic, MAQ-B, and MAQ-C.

Table 2. Descriptive statistics of variables in z-score calculated by age

Variable	Sample	N	min	max	mean	median	SD	Deviation from normality (W)	Sex diff (U)
Intelligence	all	577	-1	2.61	0.8	0.86	0.72	***	-
	female	320	-1	2.44	0.74	0.82	0.71	**	*
	male	257	-0.97	2.61	0.87	0.97	0.72	*	
TDE-Spell	all	577	-2.6	2.11	0.48	0.63	0.79	***	-
	female	320	-2.6	2.11	0.54	0.68	0.77	***	*
	male	257	-2.28	1.87	0.39	0.48	0.81	***	
TDE-Arithmetic	all	577	-3.07	3.12	0.36	0.43	1.02	no	-
	female	320	-2.76	3.12	0.36	0.28	1.06	no	no
	male	257	-3.07	3.12	0.36	0.43	0.97	.	
Transcoding	all	577	-5.45	1.07	0	0.41	1	***	-
	female	320	-5.37	1.07	-0.08	0.36	1.04	***	**
	male	257	-5.45	1.07	0.11	0.43	0.94	*	
MAQ A - Self-perceived Performance	all	577	-2.04	4.18	-0.01	-0.04	1	***	-
	female	320	-2.04	4.09	0.10	0.09	1	***	**
	male	257	-2.04	4.18	-0.15	-0.18	0.99	***	
MAQ-B - Attitudes Toward Mathematics	all	577	-1.93	3.55	-0.01	-0.14	1	***	-
	female	320	-1.81	3.32	0.01	-0.14	0.98	**	
	male	257	-1.93	3.55	-0.03	-0.18	1.03	***	no
MAQ-C - Unhappiness About Mathematics	all	577	-2.28	2.38	-0.01	-0.02	1	***	-
	female	320	-2.28	2.38	0.02	0.05	1.06	**	
	male	257	-2.10	2.06	-0.04	-0.09	0.92	*	no
MAQ-D - Anxiety Toward Mathematics	all	577	-2.5	2.35	-0.01	-0.06	1	***	-
	female	320	-2.5	2.24	0.08	0.09	1.04	**	
	male	257	-2.18	2.35	-0.12	-0.10	0.93	*	*
MAQ Total Score	all	577	-3.82	5.20	-0.02	0.01	1.47	no	-
	female	320	-3.70	4.21	0.10	0.14	1.52	no	*
	male	257	-3.82	5.20	-0.17	-0.10	1.39	no	

‘.’ P<0.1; ‘*’ P<0.05; ‘**’ P<0.005; ‘***’ P<0.0005

Abbreviations: N – Sample size; min – minimum; max – maximum; SD – Standard Deviation

The genotypic groups were homogeneous regarding intelligence (F=0.81, df=2, p=0.45), verbal to Arabic transcoding performance (F=2.35, df=2, p=0.09), spelling (*F*=2.05, df=2, p=0.36), and math achievement (F=0.82, df=2, p=0.44). Therefore, differences among genotypic groups were not due to heterogeneous sample regarding abilities and cognition. In Table 3, we present the demographic data of children divided according to sex and genotype for MAQ subscales.

MAQ-C and MAQ-D means were lower in PA, compared to BH (p-value=4.1e-14 and p-value=3.1e-11, respectively), probably reflecting that PA sample did not include 5th-graders.

Table 3. Demographic data of children divided according to sex and 5-HTTLPR genotype

		Total N (%)	**L/L N (%)**	**L/S N (%)**	**S/S N (%)**
Sample distribution	Girls	320 (55.5)	118 (36.9)	143 (44.7)	59 (18.4)
	Boys	257 (44.5)	92 (35.8)	125 (48.6)	40 (15.6)
		Mean (SD)	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**
Grade	-	3.99 (0.82)	4.01 (0.76)	3.97 (0.88)	3.95 (0.81)
Age (years)	Girls	9.27 (0.95)	9.17 (0.88)	9.40 (1.01)	9.15 (0.93)
	Boys	9.19 (0.97)	9.25 (0.93)	9.17 (1.04)	9.10 (0.87)
Intelligence (z-score)	Girls	0.74 (0.71)	0.77 (0.71)	0.73 (0.71)	0.70 (0.73)
	Boys	0.87 (0.72)	0.92 (0.69)	0.88 (0.76)	0.75 (0.67)
Transcoding (z-score)	Girls	-0.08 (1.04)	0.08 (0.83)	-0.24 (1.20)	-0.02 (0.92)
	Boys	0.11 (0.94)	0.12 (0.86)	0.07 (0.99)	0.17 (0.94)
TDE Arit. - Arithmetic achievement (z-score)	Girls	0.36 (1.05)	0.41 (1.12)	0.29 (1.02)	0.46 (1.01)
	Boys	0.36(0.97)	0.37 (0.90)	0.33 (1.04)	0.43 (0.94)
MAQ A - Self-perceived Performance (z-score)	Girls	0.10 (1.00)	-0.02 (0.99)	0.23 (1.00)	0.02 (0.99)
	Boys	-0.15 (0.99)	-0.14 (0.92)	-0.07 (1.04)	-0.39 (0.97)
MAQ B - Attitudes Toward Mathematics (z-score)	Girls	0.01 (0.98)	-0.05 (0.98)	0.03 (0.98)	0.06 (1.00)
	Boys	-0.03 (1.03)	-0.11 (0.95)	0.09 (1.07)	-0.19 (1.04)
MAQ C - Unhappiness About Mathematics (z-score)	Girls	0.02 (1.06)	0.00 (1.09)	0.08 (1.04)	-0.09 (1.03)
	Boys	-0.04 (0.92)	-0.04 (0.94)	0.02 (0.89)	-0.24 (0.98)
MAQ D - Anxiety Toward Mathematics (z-score)	Girls	0.08 (1.04)	0.02 (1.07)	0.10 (1.01)	0.17 (1.05)
	Boys	-0.12 (0.93)	-0.24 (0.90)	-0.03 (0.95)	-0.16 (0.97)
MAQ Total Score	Girls	0.10 (1.52)	-0.03 (1.48)	0.22 (1.57)	0.07 (1.48)

	Boys	-0.17 (1.39)	-0.26 (1.35)	0.01 (1.39)	-0.49 (1.43)

Abbreviations: N – Sample size; SD – Standard Deviation; TDE Arit. – TDE Arithmetic

Neuropsychological and genetic correlates of math anxiety

Gamma-distribution-based general linear models were used to investigate the relationship among variables assessed herein, and their isolated contribution for each MAQ subscales and to MAQ Total Score (Table 4). MAQ-A was negatively correlated with intelligence, verbal to Arabic transcoding performance, achievement in spelling, and achievement in math. MAQ-B showed negative relation with verbal to Arabic transcoding performance and achievement in arithmetic. Although achievement in spelling did not show a significant relationship with MAQ-B, it was also tested in the final model, as $p \leq 0.20$ was our inclusion criteria. MAQ-C did not present significant relationship with any other quantitative variable evaluated herein. MAQ-D showed only a negative correlation with achievement in math. Achievement in spelling did not show a significant relationship with MAQ-D, however, it was also included in the final model, as the criteria for inclusion was satisfied. MAQ Total Score showed a suggestive negative correlation with intelligence, which was included in the final model. Additionally, verbal to Arabic transcoding performance, achievement in spelling, and achievement in math were also negatively associated with MAQ Total Score (Table 4). Pearson correlations among all variables were also assessed to check for collinearity among covariables (Supplementary Table 3), however only weak correlations were observed among them.

Table 4. General linear model (gamma distribution) bivariate

Variable		**Intelligence N=577**	**Transcoding N=577**	**TDE-Spell N=577**	**TDE-Arit. N=577**	**IDEB N=577**
MAQ-A	Coef	0.94	0.92	0.91	0.90	0.98
	(CI 95%)	(0.90-0.97)	(0.89-0.94)	(0.88-0.94)	(0.88-0.92)	(0.94-1.02)
	p-value	0.0008***	7.3e-11***	9e-09***	<2e-16***	0.34
MAQ-B	Coef	0.97	0.94	0.96	0.91	1.04
	(CI 95%)	(0.92-1.03)	(0.90-0.98)	(0.91-1.01)	(0.88-0.95)	(0.98-1.10)
	p-value	0.34	0.003**	0.10 .	4.6e-06***	0.25
MAQ-C	Coef	1.01	1.00	1.02	0.98	1.01
	(CI 95%)	(0.97-1.04)	(0.97-1.03)	(0.98-1.05)	(0.96-1.01)	(0.96-1.05)
	p-value	0.89	0.98	0.36	0.24	0.74
MAQ-D	Coef	0.98	0.99	0.97	0.96	0.99
	(CI 95%)	(0.94-1.02)	(0.96-1.01)	(0.94-1.01)	(0.94-0.98)	(0.95-1.04)
	p-value	0.24	0.28	0.14	0.002**	0.79
MAQ Total Score	Coef	0.98	0.95	0.95	0.92	1.00
	(CI 95%)	(0.94-1.02)	(0.92-0.98)	(0.92-0.99)	(0.89-0.95)	(0.96-1.05)
	p-value	0.08 .	0.0004***	0.01*	1.6e-08***	0.92

'.' P<0.1; '*' P<0.05; '**' P<0.005; '***' P<0.0005

Abbreviations: N – Sample size; Coef – Coefficient; CI – Confidence interval; TDE Arit. – TDE Arithmetic; MAQ A - Self-perceived Performance; MAQ B - Attitudes Toward Mathematics; MAQ C - Unhappiness About Mathematics; MAQ D - Anxiety Toward Mathematics

After defining the bivariate relationships between quantitative variables, and therefore which variables should be included in the association models, we performed comparisons of MAQ means among genotypic groups to choose the most probable genotypic models to be tested together with the covariables in the association analyses. As a first approach to the genotype effects on MA, we compared sex and genotype effects on means, SE and SD for the MAQ scales (Figure 1 and Supplementary Figure 3). For MAQ-A, different genotypic models were suggested when means were compared separately in each sex. In the girls, both L/L and S/S differed from L/S, suggesting a heterosis model (Supplementary Table 4). In the boys, no differences were observed between L/L and L/S, while both L/L and L/S genotypes differed from SS, suggesting an L-dominant genotype model (Supplementary Table 4). The lowest MAQ-A mean was observed in S/S boys (0.39 SD below the mean) and no difference was observed between L/L and L/S boys (p=0.41). In girls, the highest MAQ-A mean was observed for heterozygous girls (0.23 SD above the mean) and no difference was observed between L/L and S/S (p=0.49). The greater difference of MAQ-A means observed occurred between boys and girls L/S-carriers (0.30 SD, p=0.005). For MAQ-B (Supplementary Table 5) and MAQ-C (Supplementary Table 6), no significant differences were detected when comparing genotype means in each sex. Therefore, no genotypic model was suggested by the analysis of sex in separate. In MAQ-C, S/S boys showed the lowest mean (0.24 SD under the mean) and the highest mean was observed for heterozygous girls (0.08 SD above the mean), characterizing a difference of 0.3 SD among the two genotypes. However, no significant differences were observed among other genotypes in boys or girls. For MAQ-D, differences among genotypes were not significant in the girls. In the boys, the only significant difference was detected between the means of the L/L and L/S. Therefore, no specific genotypic model is suggested by the analysis of the genotype means separated by sex. The lowest mean was observed in L/L boys (0.24 SD below the mean) and no difference was observed between S/S and L/S boys (p=0.20). The highest MAQ-D mean was observed for S/S girls (0.17 SD above the mean), but no difference was observed among genotypes in girls (p=0.49) (Supplementary Table 7). The comparison of the means suggested different genotypic model according to the sex, however no significant differences were observed in the girls. For MAQ Total Score, the only difference detected in the means by genotype and sex was observed in the L/L *vs.* L/S in the boys. Means differed significantly for MAQ Total Score, the lowest mean was observed in S/S boys (0.49 SD below the mean) and no difference was observed between L/L and L/S

boys (p=0.13). The highest MAQ Total Score mean was observed for L/S girls (0.22 SD above the mean), however no difference was observed among genotypes in girls (p=0.46) (Supplementary Table 8). Therefore, a sex specific pattern emerges for MAQ-A, MAQ-D, and MAQ Total Score. In the boys, the mean of the heterozygous group is higher and closer to the sample mean, whereas the homozygous groups presented MA lower means. In the girls, the means of the homozygous groups are closer to the mean of the total sample, whereas the mean of the heterozygous group is higher. Therefore, for both sexes, being heterozygous for 5-HTTLPR means higher MA levels.

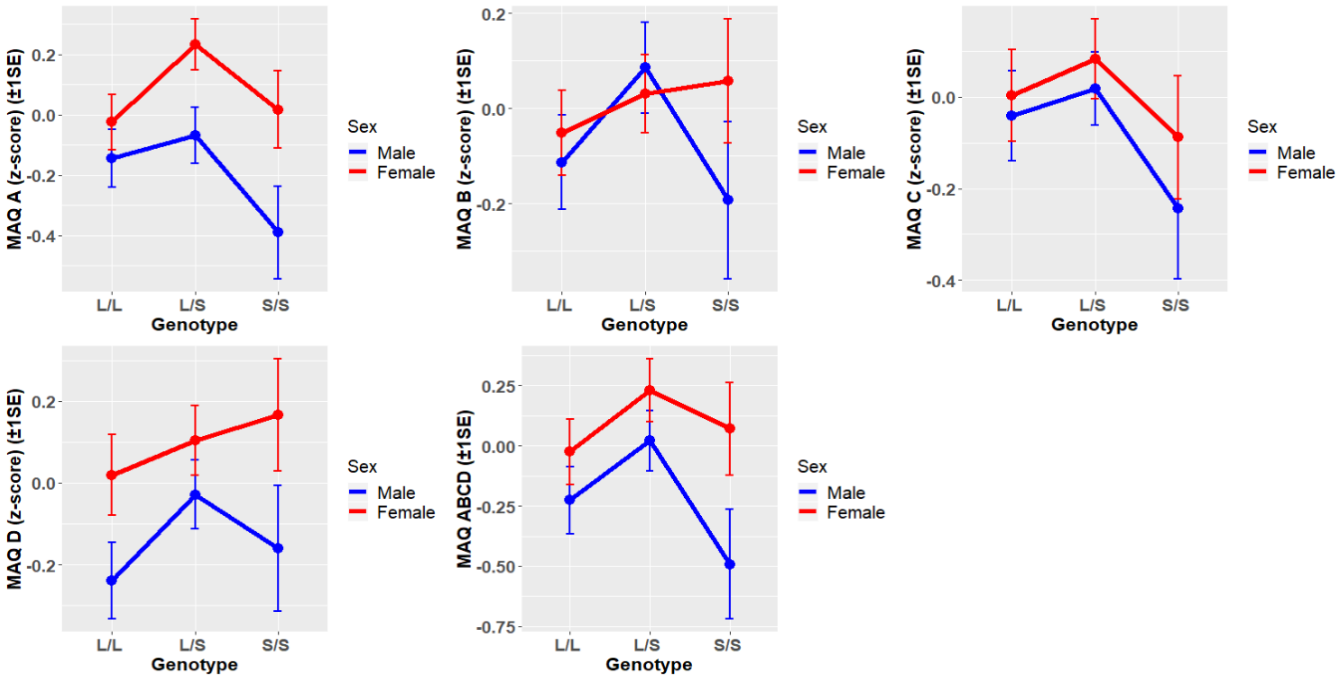

Figure 1. MAQ subscales and MAQ Total Score (MAQ ABCD) means organized by sex and 5-HTTLPR genotype. Vertical bars represent 1 standard error. N total=577; N Girls: L/L=118, L/S=143, S/S=59; N boys: L/L=92, L/S=125, S/S=40.

Finally, association analyses were performed to evaluate if a portion of the MA variance assessed through MAQ subscales and MAQ Total Score can be explained by 5-HTTLPR genotypes together with the covariables previously defined for each MAQ. Results of each regression will be presented for each MAQ subscale separately. The final models for each MAQ subscale were fitted using linear regression including as independent variables all these

that presented a p≤0.20 in the bivariate analysis (Table 4), sex, and city of origin. Genotype (under specific genotypic models for each MAQ subscale and MAQ Total Score) and sex interactions were tested but they were not significant. The genetic model was chosen based on the comparisons of means among the genotypic groups. The genotypic models tested were heterosis for MAQ-A, MAQ-B, and MAQ Total Score; L-dominant for MAQ-C; and, S-dominant for MAQ-D. Only models with significant or suggestive genotypic effect are presented in details herein.

MAQ-A

Self-perceived performance in Mathematics (MAQ-A) showed a suggestive association with the heterosis genotypic model, indicating that heterozygous children show an increase of 0.59 points in the mean raw score of MAQ-A score (Table 5). Additionally, sex was associated to MAQ-A, in which females tend to present an increase of 0.87 points in the mean raw score of MAQ-A, corresponding to 0.22 of a SD. Verbal to Arabic transcoding performance and achievement in arithmetic were also associated with MAQ-A, for both transcoding and achievement in arithmetic, higher achievement in the tasks is associated with a reduction in the mean raw score of MAQ-A (coef = -0.59 and coef = -1.03, respectively). Without the covariables, and evaluating both sexes together, the difference between homozygous and heterozygous genotype groups is significant (p=0.02; Adj. R-squared=0.01). In the girls, the heterosis model is significant (p=0.03; Adj. R-squared=0.01). In the boys, the L-dominant model approaches significance (p=0.09; Adj. R-squared=0.01). However, these models explain much less, than the models in which covariables were included (Table 5).

Table 5. Linear Regression MAQ-A – Self-perceived performance in Mathematics

	Coefficient	**Prob. (>|T|)**	**Coefficient (Raw Score)**	**Adj. R-squared**	**p-value (F-statistics)**
Intercept	-0.11	0.14	-0.43	0.146	<2.2e-16 ***
Heterosis	0.15	0.06 .	0.59		
Sex (female)	0.22	0.005 **	0.87		
Transcoding	-0.15	0.0004 ***	-0.59		
TDE-Arithmetic	-0.26	5.9e-10 ***	-1.03		

‘.’ P<0.1; ‘*’ P<0.05; ‘**’ P<0.005; ‘***’ P<0.0005
Abbreviations: Adj. – Adjusted; Prob. – Probability

MAQ-D

Considering both sexes together in the model, anxiety towards Mathematics (MAQ-D) showed a suggestive association under the S-dominant genetic model: L/S or S/S children show an increase of 0.58 points in the mean MAQ-D raw score (or 0.15 of a SD) (Table 6). This result could be attributed basically to boys, who presented the larger difference between heterozygous and homozygous genotype groups (Supplementary Table 7). Indeed, no differences among the means of the genotype groups were observed in the girls, suggesting that the genotype effect may be restricted to boys. Additionally, sex was associated to MAQ-D, in which females tend to present an increase 0.77 points (or 0.20 of a SD) in the mean raw score of MAQ-D. Achievement in arithmetic was also associated with MAQ-D, and higher achievement in the task was associated with a reduction in the mean raw score of MAQ-D score (coef = -0.42, in raw score, and -0.11 in DP). IDEB, a Brazilian measure of school quality, was also associated with Anxiety towards Mathematics, showing that in better classified schools, children tend to present a reduction in the mean of anxiety towards mathematics (coef = -0.50, in raw score, and -0.13 in DP). MAQ-D was significantly lower for the sample collected in Porto Alegre (Table 6). Due to sample size limitations, we did not investigate the hypothesis of two different models according to sex, additive in girls and heterosis in boys.

Considering the effects of the genotypes isolated, S-dominant model is non-significant in the whole sample (p=0.12; Adj. R-squared=0.002), nor in the girls (p=0.39; Adj. R-squared=0.00), neither in the boys (p=0.11; Adj. R-squared=0.01).

Table 6. Linear Regression MAQ-D – Anxiety towards Mathematics

	Coefficient	**Prob. (>|T|)**	**Coefficient (Raw Score)**	**Adj. R-squared**	**p-value (F-statistics)**
Intercept	0.88	0.04 *	3.39	0.105	2.4e-13 ***
S-dominant	0.15	0.07 .	0.58		
Sex (female)	0.20	0.01 *	0.77		
City (PA)	-0.81	1.4e-12 ***	-3.12		
IDEB	-0.13	0.03 *	-0.50		
TDE-Arithmetic	-0.11	0.005 **	-0.42		

‘.’ P<0.1; ‘*’ P<0.05; ‘**’ P<0.005; ‘***’ P<0.0005

Abbreviations: PA – Porto Alegre; Adj. – Adjusted; Prob. – Probability; IDEB - Basic Education Development Index

MAQ Total Score

For MAQ Total Score, the group presenting the genotype L/S present a significant increase in the mean score of MAQ Total Score (coef = 0.25) (Table 7). Girls tend to present an increase of 0.28 points in the mean score of MAQ Total Score. The achievement in arithmetic is also a factor significantly associated with MAQ Total Score, in which higher achievement is associated with a reduction in the mean score of this measure (coef = -0.33). Additionally, MAQ Total Score was also significantly lower for the sample collected in Porto Alegre (Table 7).

Table 7. Linear Regression MAQ-Total Score – Total Mathematics Anxiety

	Coefficient	**Prob. (>|T|)**	**Coefficient (Raw Score)**	**Adj. R-squared**	**p-value (F-statistics)**
Intercept	-0.05	0.66	-0.05	0.098	6.4e-13 ***
Heterosis	0.25	0.03 *	0.25		
Sex (female)	0.28	0.02 *	0.28		
City (PA)	-0.75	2.2e-06 ***	-0.75		
TDE-Arithmetic	-0.33	1.95e-08 ***	-0.33		

'.' P<0.1; '*' P<0.05; '**' P<0.005; '***' P<0.0005
Abbreviations: PA – Porto Alegre; Adj. – Adjusted; Prob. – Probability

Attitudes towards Mathematics (MAQ-B) and Unhappiness about Mathematics (MAQ-C) did not show any association with the genotypic models tested (prob(>|T|)=0.18; and prob(>|T|)=0.13, respectively) (Supplementary Tables 4 and 5). For MAQ-B, IDEB approached significance and TDE-Arithmetic was significant. For MAQ-C, IDEB and city were significant.

The minimum sample necessary to obtain a predictive power of 80%, considering an alpha of 0.05 and a 3% effect size was 284 children. As our sample comprises 577 children, it had sufficient power to detect allelic substitution effects of 3% or more. Therefore, based on this knowledge, we can affirm that 5-HTTLPR heterosis model was associated to MAQ Total Score in the present study. However, as MAQ-A and MAQ-D showed only suggestive associations even with this sample size, probably the effect of this locus over these subscales is very small (<3%) to be detected with this sample size.

DISCUSSION

In the present study, we investigated the contribution of the 5HTTLPR VNTR alleles for math anxiety in school-aged children. Genotypic and allelic frequencies did not deviate from HWE and were similar in the two cities where the data was collected. Consistently, the heterozygous genotype showed the highest levels of MA in boys for all MAQs and also in girls for MAQ-A, MAQ-C and MAQ Total Score, suggesting that this genotype may be a predisposing factor for higher levels of math anxiety in children. Even though heterosis shows a tendency to be the best model to describe MA in all scales, linear regressions fitted with covariables, showed that 5-HTTLPR allelic substitution effects were significant only for MAQ Total Score, and suggestive for MAQ-A and MAQ-D. In this section, we will discuss the MA measures evaluated in this study, specifically addressing their relationships with age, differences found between sex and math and spelling achievement, intelligence, verbal to Arabic transcoding performance, and school quality (IDEB). Then, we will discuss the 5-HTTLPR VNTR association with MA and how its relationship with serotonergic system modulation could be involved in this phenotype. Finally, we will discuss some limitations of the present work.

Math anxiety and cognition

Cognitive MAQ subscales (MAQ-A: r=0.01, p=0.74; MAQ-B: r=0.02, p=0.57) did not correlate with age, whereas the affective MAQ subscales (MAQ-C: r=0.13, p=0.002; MAQ-D: r=0.11, p=0.009; n=577 for all tests) did correlate with age. This small positive correlation with age is in accordance with previous studies indicating that MA tends to increase with age (A. Dowker et al., 2016). Unlike previous results, we found no increase of cognitive MA with age. In general, studies suggest that elementary school children are positive regarding math, but attitudes towards math tend to deteriorate with age (Ma & Kishor, 1997). Nonetheless, there are some exceptions, represented by children who show high levels of MA since the beginning of the school life (Jameson, 2013; Jameson & Fusco, 2014). As children at this age have little previous experience with formal math teaching, MA at this early age could be more related to inherent individual characteristics and home numeracy experiences (Batchelor, Gilmore, & Inglis, 2017). In some studies, even 5-yo children show high levels of MA (Petronzi, Staples, Sheffield, Hunt, & Fitton-Wilde, 2018). However, different studies have given conflicting results about MA and age correlation (Haase, Costa, et al., 2012; Newstead,

1998; Ramirez, Gunderson, Levine, & Beilock, 2013; Vukovic, Kieffer, Bailey, & Harari, 2013; Wu, Amin, Barth, Malcarne, & Menon, 2012). In our sample, only children 8 to 12-yo were evaluated, which characterizes a sample of junior primary school children, and little or no correlation of MA raw scores and age was found. Dowker (2016) suggested that most of the studies reporting deterioration of attitudes towards math with age were performed in USA samples and may reflect cultural aspects. To avoid age effects, all variables used in the present study were transformed using z-scores for age in years.

Our results also indicated that a worse self-perceived performance (MAQ-A) in math was negatively correlated with intelligence, verbal to Arabic transcoding performance, and performance in math and spelling. However, in the final model, only performance in math (TDE-Arithmetic) and verbal to Arabic transcoding performance remained as relevant skills for MAQ-A. MAQ-A represents one of the cognitive measures of MA, so it can reflect cognitive abilities and school achievement (Douglas & LeFevre, 2018), even though in some cases, gender stereotypes and low self-confidence also contribute to a worse child's self-perception about math ability, these aspects will be discussed in details later. MAQ-A has been shown to be the best predictor of performance (Ann Dowker, Cheriton, Horton, & Mark, 2019). Whether MA affects performance or performance affects MA, it is not completely clear (A. Dowker et al., 2016). Two main and non-exclusionary hypotheses regarding MA causing worse performance have been proposed. According to one of them, MA would lead the individual to avoid math related activities, which would lead to less practice and consequent low performance (Ashcraft, 2002). According to the other, higher MA levels, similarly to clinical anxiety, would lead to mental rumination and worrying thoughts, consuming working memory resources, that would be needed for math activities (Eysenck & Calvo, 1992). Arguments reinforcing this second hypothesis would be the neurophysiological evidence that math anxious individuals show higher levels of activity in the right amygdala, associated with emotional arousal, and reduced activity in posterior parietal cortex and prefrontal cortex, regions related to math cognition and working memory processes (Young, Wu, & Menon, 2012). Interestingly, these differences were found in the cue that preceded the math task, indicating that rumination and negative thoughts regarding the future exposure to math could be responsible for lower working memory activity in these children during the math task (Young et al., 2012). However, as a self-concept, the increase in MAQ-A scores could also reflect previous failures and negative experiences regarding math. In this situation,

children would present low performance in math due to cognitive deficits, learning disabilities or even poor education access and these results would culminate in a bad but realistic self-concept about their abilities to perform math (Ma & Xu, 2004). Dowker et al. (2016) concluded in a review that both routes are possible and in many situations a vicious cycle is established in which, independently of what came in first place, MA or bad performance, both reinforce each other, thus creating the correlation observed in many studies and confirmed herein (Ashcraft & Moore, 2009; Braham & Libertus, 2018; Carey, Hill, Devine, & Szucs, 2015). Evidence from longitudinal studies comparing both routes diverge in whether MA has a higher impact on performance (Cargnelutti, Tomasetto, & Passolunghi, 2017) or performance on MA (Ma & Xu, 2004). Herein, low performance in math negatively correlated with not only self-perceived math performance (MAQ-A), but also with enjoyment in math (MAQ-B) and math anxiety (MAQ-D). In addition, MAQ Total Score is also influenced by math and spelling performances, and also by verbal to Arabic transcoding performance.

School quality, math achievement and Math Anxiety

School quality was assessed herein through a Brazilian Basic Education Quality Index (IDEB), which is a governmental index obtained by the product between the Brazilian children's progression in the school system and their literacy and numeracy performance (Villani & Oliveira, 2018). These measures are part of an Educational Developmental Plan, proposed by the government in 2007, which establish goals and strategies to improve educational access and quality in Brazil. In our sample, the vast majority of children attend good state-runned schools (IDEB median=7.1; mean=6.87; sd=0.69) (Supplementary Table 2), and only 49 out of the 577 in our sample attend to schools showing IDEB lower than the nationwide IDEB mean, which was 5.8 in 2017 (INEP, 2018). Therefore, large differences in MA regarding school quality, as measured by IDEB, were not expected. This was confirmed in the bivariate analyses, where no MAQ subscales showed association to IDEB measures. However, when considered in the linear regressions, the inclusion of IDEB increased the ability of the model to predict two MAQ subscales and showed significant association with them. According to these results, higher IDEB measures were associated with a reduction in the mean math anxiety (MAQ-D in Table 6) and in the mean feeling of unhappiness when

failing in math (MAQ-C in Supplementary Table 6) in our sample. Radišić, Videnović, & Baucal (2015) evaluated the PISA results from 2003 from many countries, including Brazil, with a focus on understanding the relationship of socio-economic and school quality measures, MA and performance. According to this study, approximately 6% of the total variance of math anxiety is explained by differences among schools and 94% could be explained by differences within the school. Accordingly, Teodorović (2011) also affirms that most of the variance in MA and math achievement were more attributable to individual differences than to student groups or schools to which them attend. In spite of the small contribution of school to MA, some aspects of school quality should be taken into account, since they significantly change the mean anxiety level of children. For example, more discipline in class and good school atmosphere are related to lower levels of MA, while firm school discipline, in the institution level, on the contrary, is related to higher levels of MA. These results could indicate that the more rigid and strict the school atmosphere is, the more the students worry about their performance or fear scoring badly in math. However, organization and discipline inside the classroom, contribute for a better atmosphere and for a healthy learning process, hence reducing anxiety level of students regarding math (Radišić, Videnović, & Baucal, 2015). Still in this same study, socioeconomic status contributes to MA, in a manner that students attending schools of higher average socioeconomic status, also show higher levels of anxiety. This finding could be related to the existence of a more rigid atmosphere in these schools, or even to anxiety feelings regarding as a consequence of pressure to reach the ideal performance demanded directly by the institution or indirectly by their peers. However, PISA is a more complex evaluation than IDEB, what allows all the conclusions obtained in Radišić, Videnović, & Baucal (2014). IDEB is still a recent school quality index, which present some weaknesses that does not allow this method to embrace all the complexity represented by the Brazilian educational system (Neri & Buchmann, 2008). Moreover, our sample does not represent equally all the IDEB range observed in Brazilian schools, what could also be the reason for the lack of contribution of this variable to MA in the bivariate analyses performed in the present work.

Sex and Math Anxiety

Sex was also a variable considered in the present study, since MA is usually higher in females than in males, independently of performance (Devine, Soltesz, Nobes, Goswami, & Szucs,

2013; Spelke, 2005). Self-perceived math performance (MAQ-A) and math anxiety (MAQ-D) were significantly higher in females when compared to males. No sex differences were observed for Attitudes as (dis)liking math and (un)happiness as assessed through MAQ-B and MAQ-C, respectively. It is already well documented in the literature that girls, tend to rate themselves lower than males regarding math performance, even in countries where educational access is equally provided for both sexes and low or no difference in performance is observed (Devine et al., 2013; A. Dowker et al., 2016; Else-Quest, Hyde, & Linn, 2010; Spelke, 2005). According to Hyde (2005), such differences are not huge, however they become more prominent during adolescence (Ann Dowker et al., 2012; Harari, Vukovic, & Bailey, 2013). Among the possible causes for this gender difference are higher levels of general anxiety and neuroticism in females when compared to males (Chapman et al., 2007; Costa Jr et al., 2001; McLean, Asnaani, Litz, & Hofmann, 2011), exposure to gender stereotypes (Casad, Hale, & Wachs, 2015), the social transmission of anxiety by female teachers who are themselves anxious regarding math (Beilock et al., 2010). Additionally, males tend to show more confidence and rate themselves higher in a number of domains when compared to females, and this could be a socio-cultural factor (Beyer, 1990; Beyer & Bowden, 1997; Jakobsson et al., 2013). Although girls are more math anxious than males, Hembree et al. (1990) found that MA is more negatively correlated with performance in males than in females. Additionally, the way that MA correlates with math performance also seems to be dependent on gender. MA seems to be more related to basic math difficulties in males. In females, MA seems to be more correlated to applied math (H. Miller & Bichsel, 2004). Gender differences seem to be more related to environmental aspects than to cognitive differences between the sexes. However, Ganley and Vasilyeva (2014) reported some relationship between MA and working memory capacity differences attributed to gender. Nonetheless, as boys and girls do not differ in enjoying or feeling unhappy after failure experiences in math, but differ in self rating and worry feelings, the stereotype threat has been proposed as a reasonable explanation of why girls are usually more affected. Stereotype threat is the name given to feeling at risk of confirming a negative stereotype about a group they belong. Usually, females are frequently reminded of the stereotype that males perform better than females in math or that females show more anxiety regarding math. This idea may discourage women from pursuing math activities and courses, leading to less training, and consequently, worse performance (Bieg, Goetz, Wolter, & Hall, 2015; Ganley & Vasilyeva,

2014; Goetz et al., 2013). However, the stereotype threat is not always observed in children (Ganley et al., 2013), but it has been reported as an important factor in women's decision about pursuing a degree in Science, Technology, Engineering and Mathematics (STEM), for example. In the other direction, it has recently been argued that more than culture and stereotypes, what is really important for explaining the gap between males and females in math related fields is the women relative advantage in reading (Breda & Napp, 2019).

A critical analysis of the gender stereotype threat hypothesis was advanced by (Stoet & Geary, 2012). These authors reviewed the literature on gender stereotype threat and math and observed that stereotype effects were observed only in those studies which did not control for previous math ability. An alternative explanation for gender differences comes when the extremes of the population distribution is examined (Stoet & Geary, 2013). Mean performance does not differ by gender. However, a literacy advantage for females and a math advantage for males is observed in the extremes of the distribution. The Stoet and Geary (2013) and Brenda and Napp (2019) results add up in suggesting that gender differences could be associated with differences in the extremes of the distribution. This suggests a complex pattern of interactions between gender, math achievement, and math anxiety. Following Júlio-Costa and coworkers (2019), this is the second study investigating sex-differences in MA and simultaneously considering achievement and genetic influences.

Math anxiety and serotonin

Another result that calls our attention, is the association of the heterozygous genotype on the locus 5-HTTLPR VNTR with MAQ Total score, and the suggestive association with self-perceived performance (MAQ-A) in math and math anxiety *per se*. Assumedly, non-anxious children may have experienced similar negative situations as anxious children and, even though, present different responses to these experiences. These different outcomes could be related to a subjective interpretation of such experiences, which could be driven by personality aspects as resilience and self-efficacy, for example. These aspects could be related to the way parents, teachers, siblings, or peers around the student deal with math (Ann Dowker et al., 2019) and/or to intrinsic characteristics as genetic predisposition.

As proposed by Wang et al. (2014), approximately 40% of MA variance is explained by genetic factors. From these 40%, a portion of variance is explained by genetic variations associated to cognitive aspects of MA and learning. Other variants would be associated with emotional aspects, as predisposition to general anxiety, phobias or depression. Still others could even be specific to MA. Julio-Costa et al., (2019) reported the contribution of a functional polymorphism in the *COMT* gene to attitudes towards math (MAQ-B) and math anxiety (MAQ-D). The VAL158MET allele substitution *per se* did not affect math anxiety levels; however, genotype *vs.* sex interaction was detected. Both heterozygous boys and girls presented mean MAQ-D values close to the sample mean. In homozygotes, genotype effects were associated with opposite levels of mean MAQ-D, when comparing boys and girls. Homozygous genotypes in boys were associated with the lowest levels of MA, while homozygous genotypes in girls were associated with the highest levels of MA.

In the present study, no sex *vs*. genotype interactions were observed. However, heterozygosis in the 5-HTTLPR VNTR seems to predispose to higher levels of MA in both males and females (Table 7, p=0.03), while the homozygous genotype for the long allele L/L, is frequently associated with lower levels of math anxiety and a better self-perception of math performance also in both sexes (MAQ-A, p=0.06; MAQ-D, p=0.04; MAQ Total Score, p=0.05). S/S genotype in boys, present an effect similar to the L/L genotype in boys (MAQ-A, p=0.09; MAQ-D, p=0.31; MAQ Total Score, p=0.20), confirming the previously reported idea that heterosis seems to would be a risk model for MA in males.

Several polymorphic loci in *SLC6A4* have been reported to affect 5-HTT expression or its function (Avula et al., 2011; Hranilovic et al., 2004). 5-HTTLPR, investigated in the present study, is a polymorphic site with more than 18 allelic forms, which vary in size and in the sequence of its repeated monomers (Avula et al., 2011; Nakamura et al., 2000). 5-HTTLPR L, the most frequent allele at this locus, is actually a group of several Long alleles, which in general increase 5-HTT transcription levels twice, when compared to the 5-HTTLPR S alleles (Hu et al., 2006). Increased transcription of 5-HTT leads to increased expression of this transporter on serotonergic neurons which promotes higher and rapidly reuptake of serotonin from synaptic clefts, modulating the activation of the serotonin receptors (Ehli et al., 2012; Hu et al., 2006). Therefore, low levels of 5-HTT transcription, as codified by S alleles, culminate in higher levels of serotonin in the synaptic cleft and in the surrounding area.

Several phenotypes have already been associated with the 5-HTTLPR locus. Most studies report the 5-HTTLPR S as a risk allele for cognitive and emotion related phenotypes, as general anxiety, depression, post-traumatic stress, and individual aspects of personality, such as higher levels of neuroticism (Grochans et al., 2013; Olsson et al., 2015; Sen et al., 2004). To the best of our knowledge, there are no studies reporting association of the 5-HTTLPR locus with math anxiety. However, Marusic and Matic (2017) have highlighted the relationship of MA with personality traits as neuroticism, which is associated with higher vulnerability to symptoms of anxiety, depression, hopelessness, somatization, guilt, hostility, and affective temperament (Gonda et al., 2009). Some of these symptoms have already been related to MA in girls (A. Dowker et al., 2016) and all of them are modulated by the serotonin system (Ward, Sreenivas, Read, Saunders, & Rogers, 2017). Moreover, the development of MA can also be associated with the individual's ability to deal with stress situations and with the negative experiences regarding math. In this sense, inherent resilience, emerging from both genetic and environmental influences would be a protective factor in the case of math anxiety-arousing experiences. Coping consists of strategies used by the individual to manage, reduce, minimize or tolerate stress (Folkman & Lazarus, 1985). In humans, coping strategies can be divided into two groups: active coping and passive coping. Active coping implies removal or avoidance of the stressor, while passive coping implies reduction of the emotional impact, what sometimes lead to the feeling of hopelessness (Austenfeld & Stanton, 2004). Both coping strategies are important and have advantages and disadvantages according to the situation experienced. However, depending on some individual characteristics, different

coping strategies may be preferable over the other (Puglisi-Allegra & Andolina, 2015). In general, resilient individuals are the ones who use active coping strategies to deal with stressful life experiences. However, using active coping strategies in an uncontrollable or unavoidable stressful situation can let the individual without energy and resources to deal with other situations. Therefore, flexible coping is probably the healthiest strategy (Austenfeld & Stanton, 2004). Coping styles depend also on genetic predisposition. The capacity to regulate limbic reactivity to stress can be a protective factor to promote resilience (Southwick & Charney, 2012).

Puglisi (2015) proposed a neural circuit controlling the coping responses to stressful situations, based on the activation of serotonergic neurons in the raphe nuclei. According to this system, in situations in which high levels of serotonin are released in the medial prefrontal cortex and in the right amygdala, a response in amygdala will inhibit glutamatergic neurons, which stimulate the release of dopamine in the nucleus accumbens, culminating in a passive coping strategy. On the other hand, low levels of serotonin will promote less activation of the medial prefrontal cortex and lack of inhibition of glutamatergic neurons in the amygdala, which will stimulate the nucleus accumbens to release dopamine and generate an active coping strategy. Therefore, the 5-HTTLPR VNTR genotype could act as one of the factors that influence the amount of serotonin in the synapses in this systems, creating a genetic predisposition for preference for one kind of coping strategies over the other, though increasing the chances of maladaptive responses to stressful situations and bad experiences, which we hypothesize could be related to math anxiety or negative attitudes towards math.

In the literature, the S/S genotype has been associated with neuroticism (Hariri & Holmes, 2006; Talati et al., 2017) and both the L/S and S/S genotypes have been associated with higher anxiety related traits and higher activation of the amygdala in response to stress (Furmark et al., 2004). Growing evidence has driven to the conclusion that S allele carriers present hyperactivation of central and basolateral amygdala under stressful stimuli (Fang et al., 2013; Hariri & Holmes, 2006). We hypothesize that this hyperactivation of the amygdala would produce the stress related physiological responses observed in high math anxious individuals and, simultaneously, would interfere in the selection of coping strategies. Unsuccessful strategies used to deal with stress, together with other predisposing factors, as

higher levels of neuroticism, could culminate in worse self-perception, feelings of helplessness and negative attitudes towards math.

Limitations

Some limitations should be considered when evaluating the conclusions of the present study. Significant association of 5-HTTLPR genotype was found to MAQ Total Score, but only suggestive association ($p<0.1$) was observed for Self-perceived math performance (MAQ-A) and math anxiety (MAQ-D). These results could be related to reduced sample size or to other confounding factors in the sample. Additionally, in some cases, different genotypic models seem to occur in boys and girls. However, due to small differences among models and reduced sample size in the S/S allele group, sex and genotypic interaction was tested, but was not significant at a 5% alpha. Therefore, based on the results herein reported, no sex genotype interactions were observed, however, the authors do not discard the possibility of this phenomena.

General anxiety was not evaluated in the present sample. However, a part of the sample used in the present study (n=146) was evaluated for general anxiety through the Child Behavior Checklist - CBCL (Achenbach, Dumenci, & Rescorla, 2001). These results indicated that correlations between MA and general anxiety were weak. Additionally, MA but not general anxiety explained math achievement (Haase, Júlio-Costa, et al., 2012).

As any multicenter project, this study could present confounding factors due to sample origin. Significantly lower values MAQ-C and MAQ-D means were observed when comparing samples from Porto Alegre and Belo Horizonte. We found no differences in allele and genotype frequencies in 5-HTTLPR locus, which would preclude analyzing the sample of Porto Alegre and Belo Horizonte cities together. Brazilian population is trihybrid, composed of European, African and Amerindian ancestry (Callegari-Jacques et al., 2003; Crispim, Trigueiro, De Sá e Benevides Filho, & Salzano, 1972). There is a large variation in the contribution of the different population components among regions, in this case South Brazil (Porto Alegre) and Southeast Brazil (Belo Horizonte). Therefore, although not differing in 5-HTTLPR, these samples were extracted from populations with different genetic backgrounds with a wide range of possible phenotype repercussions However, these differences may reflect differences in sample collection or even to education differences also.

CONCLUSION

We investigated the contribution of a functional polymorphism in the gene *SLC6A4* in children attending to primary schools in two cities of Brazil. Results of the present study suggest that 5-HTTLPR VNTR heterozygous individuals tend to present a worse self-perception about their math abilities (MAQ-A) and L/S or S/S carriers tend to present higher math anxiety (MAQ-D), when compared to L/L individuals for this locus. Additionally, the results reported here suggest that 5-HTTLPR VNTR heterozygous individuals also present a tendency to higher MAQ Total Score, which could be described as a tendency to present higher levels of anxiety in both cognitive and affective MA dimensions. Moreover, despite sex was found to be a contributing factor for two MAQ subscales scores (Self-perceived math performance and math anxiety), with females presenting the highest anxiety levels, no significant interaction between genotype and sex was observed in the present study. The association of the S allele with math anxiety described here corroborate studies' conclusions that higher levels of serotonin lead to a predisposition to anxiety-related phenotypes and lower resilience to stressful events.

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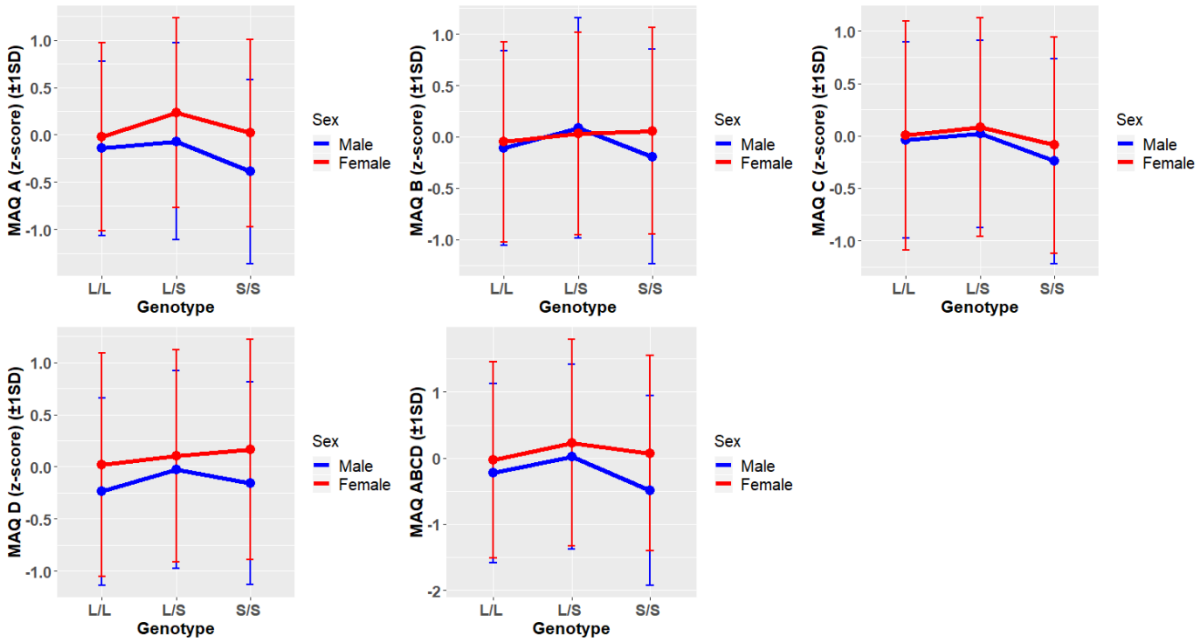
Supplementary material

- Supplementary Figures



Supplementary Figure 1. MAQ subscales and MAQ Total Score means organized by sex and 5-HTTLPR genotype. Vertical bars represent 1 standard deviation. N total=577; N Girls: L/L=118, L/S=143, S/S=59; N boys: L/L=92, L/S=125, S/S=40.

- Supplementary Tables

Supplementary Table 1. Primers used to genotype the 5-HTTLPR VNTR

Primer	Sequence	Tm (ºC)*	Fragment size (bp)
Forward primer 5HTTLPR_INDEL_for	5'-GGTGCCACCTAGACGCC-3'	58.7	455-488 or bigger**
Reverse primer 5HTTLPR_INDEL_rev	5'-TCCCAAGCTTGTTGGGGATT-3'	57.0	

*Tm (50mM NaCl)

** Extra Long alleles (XL) produce fragments bigger than 488bp

Supplementary Table 2. Descriptive statistics of variables (raw scores or percentiles)

Variable	Sample	N	min	max	mean	median	SD
Intelligence percentile	all	577	16	100	74.26	80	20.53
	female	320	16	99	72.80	79	20.94
	male	257	17	100	76.07	83	19.90
TDE-Spell	all	577	0	35	23.11	24	7.55
	female	320	0	35	23.92	25	7.20
	male	257	0	35	22.10	24	7.85
TDE-Arithmetic	all	577	0	32	15.45	15	5.64
	female	320	0	32	15.75	15	5.85
	male	257	0	31	15.07	15	5.36
Transcoding percentile	all	577	0	1	0.85	0.98	0.24
	female	320	0	1	0.84	0.98	0.25
	male	257	0.09	1	0.87	0.98	0.22
MAQ A - Self-perceived Performance	all	577	6	30	13.84	14	3.95
	female	320	6	29	14.26	14	3.96
	male	257	6	30	13.31	13	3.88
MAQ B - Attitudes Toward Mathematics	all	577	6	30	13.87	13	4.66
	female	320	6	28	13.94	13	4.54
	male	257	6	30	13.77	13	4.81
MAQ C - Unhappiness About Mathematics	all	577	6	30	17.55	17	5.53
	female	320	6	30	17.74	18	5.81
	male	257	6	29	17.30	17	5.16
MAQ D - Anxiety Toward Mathematics	all	577	6	30	18.49	18	5.41
	female	320	6	30	19	19	5.62
	male	257	6	30	17.86	17	5.08
IDEB	all	577	3.9	8.4	6.87	7.1	0.69
	female	320	3.9	8.4	6.85	7.0	0.68
	male	257	3.9	8.4	6.88	7.1	0.70

Abbreviations: N – Sample size; min – minimum; max – maximum; SD – Standard Deviation;
IDEB - Basic Education Development Index

Supplementary Table 4. Dunn's pairwise Z test statistic and p-values (italic) for comparisons MAQ A– Self-perceived math performance among genotypes

		Female 5-HTTLPR genotype		**Male 5-HTTLPR genotype**	
		L/L	**L/S**	**L/L**	**L/S**
5-HTTLPR genotype	**L/S**	-1.71 *0.04**	-	-0.23 *0.41*	-
	S/S	0.02 *0.49*	1.39 *0.08 .*	1.56 *0.06 .*	1.79 *0.04**

'.' P<0.1; '*' P<0.05; '**' P<0.005; '***' P<0.0005

Supplementary Table 5. Dunn's pairwise Z test statistic and p-values (italic) for comparisons MAQ B – Attitudes towards math among genotypes

		Female 5-HTTLPR genotype		**Male 5-HTTLPR genotype**	
		L/L	**L/S**	**L/L**	**L/S**
5-HTTLPR genotype	**L/S**	-0.54 *0.30*	-	-1.11 *0.13*	-
	S/S	-0.60 *0.27*	-0.18 *0.43*	0.51 *0.30*	1.38 *0.08 .*

‘.’ P<0.1; ‘*’ P<0.05; ‘**’ P<0.005; ‘***’ P<0.0005

Supplementary Table 6. Dunn's pairwise Z test statistic and p-values (italic) for comparisons MAQ C – Unhappiness About Mathematics among genotypes

		Female 5-HTTLPR genotype		**Male 5-HTTLPR genotype**	
		L/L	**L/S**	**L/L**	**L/S**
5-HTTLPR genotype	**L/S**	-0.67 *0.25*	-	-0.40 *0.34*	-
	S/S	0.44 *0.33*	1.04 *0.16*	1.21 *0.11*	1.57 *0.06 .*

‘.’ P<0.1; ‘*’ P<0.05; ‘**’ P<0.005; ‘***’ P<0.0005

Supplementary Table 7. Dunn's pairwise Z test statistic and p-values (italic) for comparisons MAQ D – Math anxiety among genotypes

		Female 5-HTTLPR genotype		**Male 5-HTTLPR genotype**	
		L/L	**L/S**	**L/L**	**L/S**
5-HTTLPR genotype	**L/S**	-0.83 *0.20*	-	-1.76 *0.04**	-
	S/S	-1.13 *0.13*	-0.50 *0.31*	-0.49 *0.31*	0.83 *0.20*

‘.’ P<0.1; ‘*’ P<0.05; ‘**’ P<0.005; ‘***’ P<0.0005

Supplementary Table 8. Dunn's pairwise Z test statistic and p-values (italic) for comparisons MAQ Total Score – Total Mathematics Anxiety among genotypes

		Female 5-HTTLPR genotype		**Male 5-HTTLPR genotype**	
		L/L	**L/S**	**L/L**	**L/S**
5-HTTLPR genotype	**L/S**	-1.24 *0.11*	-	-1.11 *0.13*	-
	S/S	-0.48 *0.32*	0.51 *0.31*	0.96 *0.17*	1.84 *0.03**

‘.’ P<0.1; ‘*’ P<0.05; ‘**’ P<0.005; ‘***’ P<0.0005

Supplementary Table 9. Linear Regression MAQ-B – Attitudes Towards Mathematics

	Coefficient	**Prob. (>|T|)**	**Coefficient (Raw Score)**	**Adj. R-squared**	**p-value (F-statistics)**
Intercept	-0.70	0.09 .	-3.26	0.038	1.3e-5 ***
Heterosis	0.11	0.18	0.51		
IDEB	0.10	0.09 .	0.47		
TDE-Arithmetic	-0.19	3.04e-06 ***	-0.89		

'.' P<0.1; '*' P<0.05; '**' P<0.005; '***' P<0.0005

Abbreviations: PA – Porto Alegre; Adj. – Adjusted; Prob. – Probability; IDEB - Basic Education Development Index

Supplementary Table 10. Linear Regression MAQ-C – Unhappiness About Mathematics

	Coefficient	**Prob. (>|T|)**	**Coefficient (Raw Score)**	**Adj. R-squared**	**p-value (F-statistics)**
Intercept	0.94	0.03 *	5.20	0.097	2.97e-13 ***
L-dominant	0.16	0.13	0.88		
IDEB	-0.14	0.02 *	-0.77		
City (PA)	-0.89	1.8e-14 ***	-4.92		

'.' P<0.1; '*' P<0.05; '**' P<0.005; '***' P<0.0005

Abbreviations: PA – Porto Alegre; Adj. – Adjusted; Prob. – Probability; IDEB - Basic Education Development Index

8.2 Anexo II – Artigo 2

FUNCTIONAL POLYMORPHISMS IN *MAOA* AND *MAOB* AFFECT CHILDREN SUSCEPTIBILITY TO MATH ANXIETY, WITH SEX DIFFERENCES

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ABSTRACT

Math anxiety is a specific type of anxiety, characterized by feelings of discomfort, tension and worries when facing math activities or difficulties. It is a worldwide issue that affects individuals of all ages and both sexes, being more prevalent among females. Neurofunctional evidence suggest that math anxiety could be caused by impaired activation of brain areas responsible by stress response and math cognition. To date, only one study has investigated the molecular-genetic mechanisms underlying math anxiety. This study found evidence of the involvement of an enzyme of catecholamine metabolism in math anxiety (COMT). Herein, we present a gene candidate association study, investigating the contribution of two functional polymorphisms in the monoaminergic system that also affect the catecholamine metabolism: MAOA-LPR VNTR located in the promoter region of *MAOA* and rs1799836, a single nucleotide polymorphism in the 13th intron of *MAOB*. In both loci, allele substitution reduces the activity of the enzymes, causing changes in the availability of monoamines in the synaptic cleft. 577 children aged 8-12 years, were assessed for intelligence, arithmetic and spelling performance, verbal Arabic transcoding performance and math anxiety. Children were genotyped for both loci. Association tests were performed considering the genotypes in each locus and the scores in each math anxiety subscale, as assessed through the Math Anxiety Questionnaire (MAQ). Association tests were performed separately for each sex, allowing different genetic models to be tested for each sex. Additionally, when evaluated together, sex and genotype interactions were also addressed. As a result, both loci showed an effect on math anxiety. This effect is genotype and sex dependent and restricted to female sex in the MAOA-LPR locus. Results indicate that the MAOA-LPR heterozygosis in girls has a protective effect regarding a negative self-perception about their own math abilities (MAQ-A). A similar protective effect was found for the MAOB rs1799836 locus, but only for G-carrier boys, who presented the lowest levels of affective math anxiety, such as unhappiness (MAQ-C) and worries (MAQ-D) related to math difficulties. When considering both cognitive and affective domains of math anxiety in a unique score, rs1799836 G-carrier boys showed again the lowest levels of math anxiety, while G/G girls presented the highest levels compared to A/A and A/G girls and to boys with any genotype. This is the first study to show the contribution of functional polymorphisms in *MAOA* and *MAOB* genes to math anxiety,

and to predict a sex-dependent protective effect of allelic substitution in these loci for math anxiety.

Key-words: Math anxiety, monoamine oxidase A, monoamine oxidase B, dyscalculia, Math learning

INTRODUCTION

Math anxiety (MA) is a specific type of anxiety defined as feelings of tension and worry that interfere with the manipulation of numbers or in solving math problems in academic situations or in life (Richardson & Suinn, 1972). Math anxiety affects individuals of all ages, worldwide. According to the Program for International Student Assessment (PISA), which assess students in 34 countries, 59% of adolescents (15-16 yo) report being worried about difficulties in math classes, 31% report getting nervous by solving math problems, and 33% experience feelings of tension when doing math homework (Luttenberger, Wimmer, & Paechter, 2018). Richardson and Suinn (1972) reported that 11% of university students in England are math anxious enough to need counseling. Moreover, around 17% of North American adults report having high levels of math anxiety (Ashcraft & Moore, 2009). These numbers are alarming, since MA is a condition that starts in the beginning of school life, causing children to perform badly in subjects and tasks involving math, impacting their school performance and also increasing risks of behavioral problems in childhood and adolescence. Additionally, MA can be persistent, affecting career decisions, socio-economic status, and even contributing for worst incomes (Ann Dowker, Cheriton, Horton, & Mark, 2019).

MA may severely impact math learning (Carey, Hill, Devine, & Szucs, 2015; Ann Dowker et al., 2019; A. Dowker, Sarkar, & Looi, 2016). PISA 2012 reported that in 15-16 years old adolescents, MA explains a considerable portion of math performance variance (Luttenberger et al., 2018). It is still unclear, whether MA causes a bad performance or a bad performance generates MA. Apparently, both ways are possible and, in both cases, a cycle is created, in which bad performance increases math anxiety, and math anxiety per se may difficult learning

and cause avoidance of math training, which culminate in worse performance, failures and bad experiences, then causing even more math anxiety (A. Dowker et al., 2016).

MA is a heterogeneous phenotype. It can be considered as a type of attitude towards math and it can be divided mostly in two dimensions: one cognitive and one affective (Ho et al., 2000; Wigfield & Meece, 1988). The cognitive dimension accounts for concerns and worries about performance, failures in math tests and their consequences. The affective dimension reflects emotional aspects as nervousness and tension when dealing with math tasks, and the autonomous reactions that usually occur in anxious spectrums (A. Dowker et al., 2016; Wigfield & Meece, 1988). MA can be assessed by different instruments, which are predominantly composed by questionnaires and rating scales, which usually are self-report based (Ann Dowker, Bennett, & Smith, 2012; Dreger & Aiken Jr, 1957; Fennema & Sherman, 1976; Krinzinger et al., 2007; Richardson & Suinn, 1972; Thomas & Dowker, 2000). The Mathematics Anxiety Questionnaire (MAQ), proposed by Thomas and Dowker (2000), is a self-report instrument used to evaluate children's perceptions about their own performance, attitudes and feelings when dealing to math difficulties. A Brazilian version of this questionnaire was adapted, having its internal consistency and correlations to other cognitive measures evaluated in a 7-12 yo sample from Belo Horizonte city, Brazil (Haase, Costa, Antunes, & Alves, 2012; Wood et al., 2012). MAQ is composed of four sections, each of them evaluating a different aspect of MA, including self-perceived math performance (MAQ-A), attitude towards math (liking or not) (MAQ-B), unhappiness about having troubles in dealing with math (MAQ-C) and, finally, the math anxiety in terms or preoccupation or worry about failing in math tasks and activities (MAQ-D) (Thomas & Dowker, 2000; Wood et al., 2012). Considering the MAQ quality and consistency, its easiness to be used inside schools, and the availability of a validated version, adapted for Brazilian children, herein, we used MAQ to assess MA.

MA is a complex phenotype, caused by genetic and environmental factors (A. Dowker et al., 2016). Environmental factors include school environment and quality (Chiu & Xihua, 2008), nationality and culture (Askew, Hodgen, Hossain, & Bretscher, 2010; Lee, 2009), math

anxiety transference from parents or teachers who are themselves math anxious (Beilock, Gunderson, Ramirez, & Levine, 2010; Pesu, Aunola, Viljaranta, Hirvonen, & Kiuru, 2018), and experience of failures in math (O'Leary, Fitzpatrick, & Hallett, 2017). Individual aspects as gender, personality, and genetics also play a role on math anxiety levels and predisposition (Malanchini et al., 2017; Marušić & Matić, 2017). Regarding gender, despite similar opportunities and school achievement in math, females still present higher levels of anxiety when compared to males (Goetz, Bieg, Ludtke, Pekrun, & Hall, 2013). Regarding genetics, only three studies have addressed the contribution of genetic factors to MA. Two twin studies reported that around 40% of MA variance can be attributed to genetic factors (Malanchini et al., 2017; Wang et al., 2014). According to Wang and coworkers (2014), a portion of these genetic factors may be shared with general anxiety and math learning difficulties. However, some genetic factors may also be specific to MA (Wang et al., 2014). In the third genetic study, the role of the dopaminergic system on MA was interrogated, through the investigation of the VAL158MET polymorphism in the *COMT* gene and its influence on MA levels (Julio-Costa et al., 2019). This was the first candidate gene association study performed for MA. Their results suggest a role for this variant in MA, with an important sex effect. Interestingly, genotype influence suggested an heterosis model, in which VAL/MET heterozygous children are similarly anxious (around the population mean) regardless of their sex and homozygous girls showed more anxiety (above the population mean) than homozygous boys (below the population mean) (Julio-Costa et al., 2019). This and other studies evidencing neurophysiological aspects of MA (Klados, Pandria, Micheloyannis, Margulies, & Bamidis, 2017; Moustafa et al., 2017; Suarez-Pellicioni, Nunez-Pena, & Colome, 2016) led to the hypothesis that other genes involved in the regulation of neurotransmitter systems, and in the activation of brain structures associated with fear, stimuli processing, learning and motivation, could also contribute to MA levels. Among them, monoamine oxidases emerge as theoretical candidates.

Monoamine oxidases (MAO) are flavin-containing amine oxidoreductase enzymes located in the external membrane of the mitochondria, which are associated to the inactivation metabolism of neurotransmitters such as serotonin, norepinephrine, and dopamine. Consequently, several MAO inhibitors are commercialized as efficient drugs in neurologic

and psychiatric treatments (Finberg & Rabey, 2016). MAO-A and MAO-B are two functional isoforms of MAO, expressed by the transcription of two adjacent genes in the X-chromosome (Xp11.3). These two isoforms show 70% of amino acid similarity; both play critical roles in the functioning and structural development of amygdala, a structure widely related to cognitive and social functions in the brain (Phelps, 2006). Although having similar structures, MAO-A and MAO-B show differences in substrate preferences, tissue expression, and physiological role. While MAO-A degrades mostly dopamine, serotonin, and noradrenaline, and is mostly expressed in the central nervous and gastrointestinal systems, MAO-B metabolizes mostly dopamine, phenethylamine, and benzylamine, being more expressed in platelets, brain and spinal cord and also in many other parts of the body (Kalgutkar, Dalvie, Castagnoli, & Taylor, 2001; HIPED - GeneCards, assessed in September 8, 2019), mostly from adolescence to senescence (Tong et al., 2013). According to data released in the Human Protein Atlas, MAO-A protein levels are low in the cerebral cortex, medium in the hippocampus and in the caudate, and undetectable in the cerebellum. On the other hand, MAO-B protein levels are high in the cerebral cortex, low in the hippocampus and medium in the caudate nucleus and in the cerebellum (Uhlen et al., 2015; https://www.proteinatlas.org/ENSG00000189221-MAOA/tissue; https://www.proteinatlas.org/ENSG00000069535-MAOB/tissue, assessed in August 2019).

A variable number of tandem repeats (VNTR) in the promoter region of *MAOA*, named MAOA-LPR affects gene transcription. Five allelic variants have been described for this locus. They vary from two to five copies of a 30 bp sequence (2R, 3R, 3.5R, 4R, and 5R), being 4R and 3R the most frequent alleles. Due to the low frequency of the other alleles, studies usually investigate only 3R and 4R alleles, being the shorter associated to lower transcription and less function, higher anxiety and aggressive behavior (Deckert et al., 1999; Gottschalk & Domschke, 2016; Guo, Ou, Roettger, & Shih, 2008; Sabol, Hu, & Hamer, 1998). To date, no study has investigated the role of MAOA-LPR in MA.

Despite MAO-A being more investigated in psychiatric and cognitive traits, evidence of the involvement of MAO-B in similar traits are recent and should not be neglected. MAO-B is an essential enzyme for Gamma-Aminobutyric Acid (GABA) synthesis in glial cells (Yoon et al., 2014), which plays an important role in modulating anxiety stimulus response (Nuss,

2015). Additionally, when MAOA is not expressed, MAO-B, as a isoenzyme, participates in the serotonin metabolism (Bortolato, Chen, & Shih, 2008), this role becomes evident in platelets, where MAO-B, as the only isoenzyme expressed, plays an essential role in regulating the plasmatic levels of serotonin (Bortolato et al., 2008).

A SNP in *MAOB* intron 13 (rs1799836), which promotes a substitution of an adenine by a guanine (A644G), modulates the enzymatic activity of MAO-B. The G allele has been associated with low/intermediate enzymatic activity in the brain, compared to the A allele, with tissue specificity (Garpenstrand, Annas, Ekblom, Oreland, & Fredrikson, 2001). Changes in MAO-B activity and expression have been associated with neuropsychiatric disorders such as alcoholism, impulsivity, depression, suicidal behavior, ADHD, ASD, and also neurodegenerative diseases (Adolfsson, Gottfries, Oreland, Wiberg, & Winblad, 1980; Chakraborti et al., 2016; X. Gao et al., 2008; Karmakar et al., 2017; Mann & Chiu, 1978; Nikolac Perkovic et al., 2016; Verma et al., 2014).

To date, no study has investigated the role of MAO-A or MAO-B in math anxiety. Given their important role in modulating the metabolism of many neurotransmitters, including GABA, dopamine and serotonin, which have been previously related to anxiety symptoms and also to cognitive functions implicated in math learning, we hypothesized that they may also contribute to explain genetic variance in math anxiety. Herein, we have investigated the potential association of MAOA-LPR and rs1799836 polymorphisms in math anxiety levels in age-schooled children.

MATERIAL AND METHODS

Ethical consideration

This study was approved by the Ethics in Research Committee of the Federal University of Minas Gerais (COEP-MG) under the numbers ETIC 42/08 and CAAE 15070013.1.0000.5149.

Participants

In the ambit of two populational projects developed in Brazil, with the aim to investigate the neuropsychological and genetic aspects of learning disabilities, a sample of 722 school-age children was selected. The sample included predominantly children from public, but also some from private schools in Belo Horizonte (BH) city in Minas Gerais state (n=601) and Porto Alegre (PA) city in Rio Grande do Sul state (n=121). The sample included children attending 2nd-7th grades, being 7 to 12 years-old and normal intelligence (IQ>PR15). A signed written consent was obtained from their parents and an oral consent was obtained from the children. After exclusion criteria mentioned below, 577 children, 488 from BH and 95 from PA, were investigated in the association analyses. The final sample included children attending to 3rd to 7th grades, being 8 to 12 years-old (mean age= 9.23; SD=1.05; 55% female). The Brazilian Education Quality Index of the schools sampled (IDEB) ranged from 3.9 to 8.4 (mean=6.9; median=7.1; SD=0.69)

Constructs measured and Instruments

The Raven's Coloured Progressive Matrices - CPM was used to assess children's general intelligence (Angelini, Alves, Custódio, Duarte, & Duarte, 1999). School performance was evaluated using the Brazilian School Achievement Test (TDE), which is composed of three subtests: reading, spelling, and arithmetic. However, only the spelling and the arithmetic tasks were completed, because tests were applied in groups of children (see below). The TDE-arithmetic contains three orally presented word problems and 45 written arithmetic calculations of increasing complexity levels (Ferreira et al., 2012; Stein, 1994). A transcoding task was used to assess children's verbal Arabic transcoding performance (Moura et al., 2013). The adapted Brazilian version of the Math Anxiety Questionnaire (MAQ) was used to evaluate children's math anxiety (Haase, Costa, et al., 2012; Wood et al., 2012). MAQ-Consists of four subscales containing six questions each, in which the individual answers questions regarding his/her self-perceived performance in Mathematics (MAQ-A), how much he/she likes math (attitudes) (MAQ-B), how does he/she feel (happy or sad) about performing poorly in math (MAQ-C), and his/her worry level about having trouble in solving math tasks (Wood et al., 2012). Questions in the MAQ-A subscale always start with "How good you are in ..."; in MAQ-B, "How much do you like ..."; in MAQ-C, "How happy or sad do you get, when you have problems with... ", and in MAQ-D, "How worried do you get, when you have problems with ... ". The six questions in each subscale, always address the following items: 1) math in general, 2) easy calculations, 3) difficult calculations, 4) written calculations, 5) mental calculations; and, 6) homework. An illustrated Lickert scale is presented to the child after each question and the child has to point out the answer for each question asked. Scores are given on a scale of 1 to 5 for each item, 5 being always the more severe result ("very bad" answers in MAQ-A; "I hate" answers in MAQ-B, "very sad" in MAQ-C, and "very worried" in MAQ-D). At the end of the test, a total score for each MAQ subscale is generated from the sum of the responses of the six items. MAQ scales present reliability coefficients (Cronbach's α) ranging from 0.74 to 0.88 (Wood et al., 2012).

MAQ Total Score

Aiming at creating a variable that could represent a total score of all MAQ subscales, we proposed the creation of a new variable herein named as MAQ Total Score. To create this new variable, a Principal Component Analysis (PCA) was performed using the correlation matrix of MAQ-A, MAQ-B, MAQ-C, and MAQ-D z-scores normalized by age in years. The first component (PC1) explained 54% of the variance of the MAQ subscales. Therefore, we used the first component to obtain the principal component scores, by multiplying PC1 loadings to the MAQ subscales matrix. MAQ Total Score showed high and positive correlation with all MAQ subscales: MAQ-A (r=0.71), MAQ-B (r=0.72), MAQ-C (r=0.79), and MAQ-D (r=0.71). MAQ Total Score accounts simultaneously for cognitive and affective aspects of math anxiety.

Procedure

Phenotypic data collection took place at participant's schools. In the first step, children, in groups of eight, took the intelligence test (Raven's CPM) followed by the Brazilian School Achievement Test and the verbal Arabic transcoding task. In a second moment, biological material was collected for DNA extraction and the children answered to the MAQ individually in a quiet room.

Sample collection and genotyping

Genomic DNA was extracted using a proteinase K/salting out method (Aidar & Line, 2007; Miller, Dykes, & Polesky, 1988). The protocol is available under request. DNA amount and purity were assessed through spectrophotometry in Nanodrop.

The MAOA-LPR VNTR was genotyped using PCR with a FAM-labelled primer followed by a fragment analysis in capillary sequencer. The PCR consisted of 50ng of total DNA, 10pmol of each primer, 2 μg of Taq DNA polymerase, 5 μL of 5x Buffer (Phoneutria Biotechnology, Belo Horizonte, Brazil), 2 μL of DMSO 100%, 20 mM dNTP and milliQ water to a total volume of 25μL. PCR cycling was composed of a 5 minutes initial denaturation step at 94ºC, followed by 25 cycles of 94ºC for 30s, 56°C for 20s, and 72° C for 30s, and a last extension step of 5 minutes at 72°C. Amplicons were analysed in an ABI 3730 capillary sequencer (Thermo Fisher Scientific), using the GeneScan™ 1200 LIZ® Size Standard. Genotypes were obtained in the Applied Biosystems® Sizing Analysis module Peak Scanner software, version 3.0, available online in the Thermo Fisher Cloud. Alleles were classified according to fragments size in 2-repeat (2R), 183 bp; 3-repeat (3R), 213 bp; 3.5-repeat (3.5R), 229 bp; 4-repeat (4R), 243 bp; 5-repeat (5R), 373 bp. Primers sequences were obtained from the literature (Mickey et al., 2008). Primer sequences, PCR condition and amplicon size are informed in Supplementary Table 1.

The MAOB rs1799836 polymorphism was genotyped through a High-Resolution Melting genotyping technique (HRM) using the MeltDoctor™ HRM Reagent Kit from Thermo Fisher, following the manufacturer protocol. HRM primers were designed using uDesign version 1.0, a small amplicon design software (Dwight, Palais, & Wittwer, 2013). Positive controls for each genotype were obtained using Sanger sequence and the primers for sequencing were selected using the Primer3 software in NCBI (Untergasser et al., 2012). The specificity of the primers was tested using the in silico PCR tool in UCSC Genome Browser (Rhead et al., 2010).

Statistical Analyses

Allelic and genotypic frequencies were calculated and the adherence to the Hardy-Weinberg Equilibrium (HWE) was tested using the package HardyWeinberg implemented in R, which can deal with both biallelic and multiallelic loci on X-chromosome (Graffelman & Weir, 2016). Genotypic differentiation between populations was assessed using a Fisher exact test for MAOA-LPR locus and using a Pearson Chi-square test for MAOB rs1799836 locus. Descriptive statistics of the distributions of the neuropsychological variables were obtained using the package *fBasics:* min, max, mean, median, standard deviation, among others. The distribution of each variable was evaluated for the total sample, and Belo Horizonte and Porto Alegre samples, using histograms. Age effects were observed for all neuropsychological variables. Therefore, all variables were age-corrected using z-scores by age in years.

Bivariate descriptive analyses were performed using Pearson correlations in the software SPSS v.20 (Corp., 2011). Modelling was conducted using a two-step approach. Initially, a general linear regression assuming a gamma distribution and an alpha=0.20 was used to determine if intelligence, verbal Arabic transcoding performance, and math and spelling performances explained a portion of the variance of the MAQ subscales, and should, then, be

included in the final models as covariables. Kruskal-Wallis tests, followed by Dunn post-hoc, were performed in R to compare MAQs means in sample stratified by genotype, sex, and city.

To deal with the multiallelic locus MAOA-LPR and the rare alleles in it, only the alleles 4R and 3R were used in the association analyses. Genotypes containing the 2R, 3.5R and 5R alleles were excluded from the analysis.

XWAS software (F. Gao et al., 2015) was used to test if there was more variance in heterozygous compared to homozygous girls; to perform a linear model stratified by sex, and to obtain its Fisher combined p-Value; and finally, to identify different allelic effects between girls and boys. Considering that the allelic frequencies were similar in both sexes (MAO-LPR, p=0.90; MAOB rs1799836, p=0.94), general linear models were fitted in R for each MAQ subscale and MAQ Total Score, adding genotype, sex, city, IDEB, intelligence, verbal Arabic transcoding performance, math and spelling performances as covariables, according to significant results in the bivariate analyses. Linear regressions were fitted for boys and girls separately, and also for total sample, where hemizygous genotypes in boys were grouped with homozygous genotypes in females, as proposed by Clayton (2008) (Clayton, 2008), then sex was included in these models and sex:genotype interaction was tested. The package car in R was used to perform stepwise regressions to determine the co-variables that should be kept in the final model (based in the smaller AIC criteria). The inflation factor of the variables in the model was assessed using the vif() function, also available in the package car. Inflation factors below than 5 were considered ideal.

RESULTS

In the present study, the potential contribution of two functional polymorphisms in *MAOA* and *MAOB* genes for math anxiety was investigated using MAOA-LPR, a 30bp VNTR polymorphism in *MAOA* promoter, and rs1799836, an intronic SNP in *MAOB*, in a sample

composed of 577 children from two Brazilian cities. Behavioral and cognitive parameters were investigated as possible contributors for math anxiety in these children, together with genotype, sex, city, and IDEB.

MAOA and *MAOB* are X-linked genes. Consequently, women, having two X-chromosomes, will have two alleles at each locus with three possible genotypes. For example, in MAOA-LPR locus women will have 3R/3R, 3R/4R, or 4R/4R genotypes. Men, having only one X-chromosome, will have only one allele and, therefore, two possible genotypes, 3R or 4R. Consequently, men are referred to as hemizygotes. Allelic and genotypic frequencies were calculated for both loci, MAOA-LPR and rs1799836 in *MAOA* and *MAOB* genes, respectively (Table 1) using all individuals genotyped for these loci (n=722). A Fisher Exact test was performed to compare allelic frequencies between males and females for MAOA-LPR locus and a Pearson Chi-square test was used for the same purpose for rs1799836. No significant sex differences were found between allele frequencies for both loci (MAOA-LPR p=0.90; MAO-B rs1799836 p=0.94). Additionally, no significant departure from HWE were observed for both loci (MAOA-LPR p=0.227; MAO B rs1799836 p=0.691) (Table 1). No differences were found when comparing genotypic frequencies between the two cities (p=0.90; p=0.79), therefore the following analyses were performed for Total sample only.

Table 1. MAOA-LPR and MAOB rs1799836 genotypes and allele frequencies calculated by sex in Total, Belo Horizonte and Porto Alegre samples

Polymorphism	**Sex**	**Genotype**	**Total (G/B) (n=396/326, %)**	**BH (G/B) (n=330/271, %)**	**PA (G/B) (n=66/55, %)**
MAOA-LPR	**Girls**	2R/3R	3 (0.008)	3 (0.009)	0 (0)
		2R/4R	7 (0.018)	7 (0.021)	0 (0)
		3R/3R	51 (0.129)	44 (0.133)	7 (0.106)
		3R/3.5R	7 (0.018)	7 (0.021)	0 (0)
		3R/4R	176 (0.444)	142 (0.430)	34 (0.515)
		3R/5R	3 (0.008)	2 (0.006)	1 (0.015)
		3.5R/4R	2 (0.005)	1 (0.003)	1 (0.015)
		4R/4R	141 (0.356)	120 (0.364)	21 (0.318)
		4R/5R	5 (0.013)	3 (0.009)	2 (0.030)
		5R/5R	1 (0.003)	1 (0.003)	0 (0)
	Boys	2R	8 (0.025)	8 (0.030)	0 (0)
		3R	122 (0.374)	100 (0.369)	22 (0.400)

		3.5R	3 (0.009)	3 (0.011)	0 (0)
		4R	190 (0.583)	158 (0.583)	32 (0.582)
		5R	3 (0.009)	2 (0.007)	1 (0.018)
	HWE p-value		0.227	0.07 .	0.91
MAOB rs17799836	**Girls**	A/A	82 (0.207)	66 (0.200)	16 (0.242)
		A/G	188 (0.475)	156 (0.473)	32 (0.485)
		G/G	126 (0.318)	108 (0.327)	18 (0.273)
	Boys	A	139 (0.426)	119 (0.439)	20 (0.364)
		G	187 (0.574)	152 (0.561)	35 (0.636)
	HWE p-value		0.691	0.822	0.412

'.' P<0.1; Abbreviations: BH – Belo Horizonte; PA - Porto Alegre; HWE – Hardy-Weinberg Equilibrium (p-value probability); G – Girls; B – Boys.

Descriptive statistics of neuropsychological variables are presented in Table 2 (z-score) and in Supplementary Table 2 (raw scores). Besides math performance and MAQ Total Score, no other variable showed normal distribution, therefore, non-parametric tools were used for mean comparisons. Sample characterization according to sex and genotypes are presented in Tables 3 and 4, for MAOA-LPR (n=544) and *MAOB* rs1799836 (n=577), respectively.

Table 2. Descriptive statistics of variables in z-score calculated by age

Variable	Sample	N	min	max	mean	median	SD	Deviation from normality (W)	Sex diff (U)
Intelligence	all	577	-1	2.61	0.8	0.86	0.72	***	-
	female	320	-1	2.44	0.74	0.82	0.71	**	*
	male	257	-0.97	2.61	0.87	0.97	0.72	*	
TDE-Spell	all	577	-2.6	2.11	0.48	0.63	0.79	***	-
	female	320	-2.6	2.11	0.54	0.68	0.77	***	*
	male	257	-2.28	1.87	0.39	0.48	0.81	***	
TDE-Arithmetic	all	577	-3.07	3.12	0.36	0.43	1.02	no	-
	female	320	-2.76	3.12	0.36	0.28	1.06	no	no
	male	257	-3.07	3.12	0.36	0.43	0.97	.	
Transcoding	all	577	-5.45	1.07	0	0.41	1	***	-
	female	320	-5.37	1.07	-0.08	0.36	1.04	***	**
	male	257	-5.45	1.07	0.11	0.43	0.94	*	
MAQ A - Self-perceived Performance	all	577	-2.04	4.18	-0.01	-0.04	1	***	-
	female	320	-2.04	4.09	0.10	0.09	1	***	**
	male	257	-2.04	4.18	-0.15	-0.18	0.99	***	
MAQ-B - Attitudes Toward Mathematics	all	577	-1.93	3.55	-0.01	-0.14	1	***	-
	female	320	-1.81	3.32	0.01	-0.14	0.98	**	
	male	257	-1.93	3.55	-0.03	-0.18	1.03	***	no
MAQ-C - Unhappiness About Mathematics	all	577	-2.28	2.38	-0.01	-0.02	1	***	-
	female	320	-2.28	2.38	0.02	0.05	1.06	**	
	male	257	-2.10	2.06	-0.04	-0.09	0.92	*	no
MAQ-D - Anxiety Toward Mathematics	all	577	-2.5	2.35	-0.01	-0.06	1	***	-
	female	320	-2.5	2.24	0.08	0.09	1.04	**	
	male	257	-2.18	2.35	-0.12	-0.10	0.93	*	*
MAQ Total Score	all	577	-3.82	5.20	-0.02	0.01	1.47	no	-
	female	320	-3.70	4.21	0.10	0.14	1.52	no	*
	male	257	-3.82	5.20	-0.17	-0.10	1.39	no	

‘.’ P<0.1; ‘*’ P<0.05; ‘**’ P<0.001; ‘***’ P<0.0001

Abbreviations: N – Sample size; min – minimum; max – maximum; SD – Standard Deviation

In Table 3, we present the results of bivariate general linear models for each MAQ subscale, MAQ Total Score, and the covariables evaluated in the present study, as intelligence, verbal Arabic transcoding performance task, math and spelling performances, and IDEB.

Table 3. General linear model (gamma distribution) bivariate

Variable		Intelligence N=577	Transcoding N=577	TDE-Spell N=577	TDE-Arit. N=577	IDEB N=577
MAQ-A	Coef	0.94	0.92	0.91	0.90	0.98
	(CI 95%)	(0.90-0.97)	(0.89-0.94)	(0.88-0.94)	(0.88-0.92)	(0.94-1.02)
	p-value	0.0008***	7.3e-11***	9e-09***	<2e-16***	0.34
MAQ-B	Coef	0.97	0.94	0.96	0.91	1.04
	(CI 95%)	(0.92-1.03)	(0.90-0.98)	(0.91-1.01)	(0.88-0.95)	(0.98-1.10)
	p-value	0.34	0.003**	0.10 .	4.6e-06***	0.25
MAQ-C	Coef	1.01	1.00	1.02	0.98	1.01
	(CI 95%)	(0.97-1.04)	(0.97-1.03)	(0.98-1.05)	(0.96-1.01)	(0.96-1.05)
	p-value	0.89	0.98	0.36	0.24	0.74
MAQ-D	Coef	0.98	0.99	0.97	0.96	0.99
	(CI 95%)	(0.94-1.02)	(0.96-1.01)	(0.94-1.01)	(0.94-0.98)	(0.95-1.04)
	p-value	0.24	0.28	0.14	0.002**	0.79
MAQ Total Score	Coef	0.98	0.95	0.95	0.92	1.00
	(CI 95%)	(0.94-1.02)	(0.92-0.98)	(0.92-0.99)	(0.89-0.95)	(0.96-1.05)
	p-value	0.08 .	0.0004***	0.01*	1.6e-08***	0.92

‘.’ P<0.1; ‘*’ P<0.05; ‘**’ P<0.001; ‘***’ P<0.0001

Abbreviations: N – Sample size; Coef – Coefficient; CI – Confidence interval; TDE Arit. – TDE Arithmetic; MAQ A - Self-perceived Performance; MAQ B - Attitudes Toward Mathematics; MAQ C - Unhappiness About Mathematics; MAQ D - Anxiety Toward Mathematics

Additionally, Pearson correlations between these variables are presented in Supplementary Table 3. As a result, self-perceived math performance (MAQ-A) was negatively correlated to intelligence, verbal Arabic transcoding performance, and to both math and spelling performances. Attitudes towards math (MAQ-B) does not correlate with intelligence or spelling performance and shows a negative correlation with math performance and verbal Arabic transcoding performance, only. Feelings about math difficulties (MAQ-C) is not explained by any variable evaluated in the present study, while math worries (MAQ-D) shows negative correlation with math performance, only. MAQ Total Score showed negative correlations with verbal Arabic transcoding performance and both math and spelling performance. A suggestive negative correlation was also found between MAQ Total Score and intelligence. Additionally, despite not being significant at a 5% alpha, spelling performance passed the threshold established herein to enter the stepwise linear models for

MAQ-B and MAQ-D. IDEB was not correlated with any MAQ measure. Sex differences were observed for MAQ-A, MAQ-D, and MAQ Total Score means only, with boys showing lower means than girls (MAQ-A, p=0.002; MAQ-B, p=0.57; MAQ-C, p=0.50; MAQ-D, p=0.01; MAQ Total Score, p=0.03). City differences were observed for MAQ-C, MAQ-D, and MAQ Total Score, with Porto Alegre means being lower than Belo Horizonte means for these scales (MAQ-A, p=0.14; MAQ-B, p=0.72; MAQ-C, p=4.12e-14; MAQ-D, p= 3.14e-11; MAQ Total Score, p=2.5e-06).

MAOA-LPR

Individuals having genotypes which included the rare MAOA-LPR alleles were excluded and only genotypes containing the alleles 3R or 4R were included in subsequent analyses. As a consequence, a total sample of 544 individuals was evaluated in the association analysis of the MAOA-LPR locus. Table 4 shows the means of MAQ subscales according to genotype and sex.

Homozygous girls for the 3R allele (3R/3R) showed higher means of math anxiety (MAQ-D), when compared to girls having the other genotypes (3R/3R x 4R/3R, p=0.02; 3R/3R x 4R/4R, p=0.04) and boys carrying any hemizygous genotype (3R/3R x 3R, p=0.002; 3R/3R x 4R, p=0.004). This difference was assessed through a Kruskal-Wallis test (p=0.04) and a Dunn post hoc test (alpha=0.05/2) was used in the pairwise comparisons. No significant differences in MAQ-D means were detected in the pair-wise comparison of the genotypes 4R/4R, 4R/3R, 3R, and 4R.

MAQ Total Score means were significantly higher in girls than in boys. When comparing MAQ Total Score means among sex by genotype, only homozygous girls presented higher means when compared to males (3R/3R x 3R, p=0.02; 4R/4R x 4R, p=0.02). MAOA-LPR heterozygous girls showed means as low as males of any genotype. Means of MAQ subscales

and MAQ Total Score according to MAOA-LPR genotypes and sex are shown in Figure 1. No variance heterogeneity between heterozygous and homozygous females was observed for any MAQ subscale nor for MAQ Total Score.

Table 4. Demographic data of children divided according to sex and MAOA-LPR genotype

		Total N (%)	**4R/4R or 4R N (%)**	**4R/3R N (%)**	**3R/3R or 3R N (%)**
Sample distribution	Girls	296 (54.4)	116 (39.2)	144 (48.6)	36 (12.2)
	Boys	248 (45.6)	148 (59.7)	-	100 (40.3)
		Mean (SD)	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**
Grade	-	3.97 (0.81)	4.00 (0.80)	3.92 (0.82)	4.08 (0.84)
Age (years)	Girls	9.25 (0.95)	9.26 (0.91)	9.17 (0.94)	9.53 (1.11)
	Boys	9.16 (0.97)	9.16 (0.94)	-	9.16 (1.01)
Intelligence (z-score)	Girls	0.74 (0.71)	0.77 (0.68)	0.72 (0.74)	0.76 (0.72)
	Boys	0.87 (0.73)	0.91 (0.72)	-	0.82 (0.74)
Transcoding (z-score)	Girls	-0.11 (1.06)	0.06 (0.78)	-0.27 (1.26)	0.04 (0.85)
	Boys	0.10 (0.95)	0.10 (0.92)	-	0.09 (1.01)
TDE Arit. - Arithmetic achievement (z-score)	Girls	0.33 (1.07)	0.32 (1.08)	0.34 (1.04)	0.35 (1.18)
	Boys	0.36 (0.98)	0.40 (1.02)	-	0.30 (0.92)
MAQ-A - Self-perceived Performance (z-score)	Girls	0.13 (1.01)	0.14 (1.03)	0.08 (0.98)	0.26 (1.08)
	Boys	-0.15 (0.99)	-0.17 (0.92)	-	-0.12 (1.08)
MAQ-B - Attitudes Toward Mathematics (z-score)	Girls	0.02 (0.99)	0.13 (1.10)	-0.08 (0.91)	0.06 (0.90)
	Boys	-0.04 (1.03)	-0.07 (1.01)	-	0.01 (1.07)
MAQ-C - Unhappiness About Mathematics (z-score)	Girls	0.01 (1.07)	0.02 (1.15)	-0.05 (1.02)	0.21 (0.94)
	Boys	-0.05 (0.93)	-0.07 (0.89)	-	-0.03 (0.98)
MAQ-D - Anxiety Toward Mathematics (z-score)	Girls	0.07 (1.05)	0.05 (1.07)	0.02 (1.04)	0.37 (1.00)
	Boys	-0.14 (0.94)	-0.11 (0.93)	-	-0.19 (0.97)
MAQ Total Score	Girls	0.11 (1.54)	0.17 (1.66)	-0.02 (1.46)	0.45 (1.45)
	Boys	-0.19 (1.39)	-0.21 (1.34)	-	-0.16 (1.47)

Abbreviations: N – Sample size; SD – Standard Deviation; TDE Arit. – TDE Arithmetic

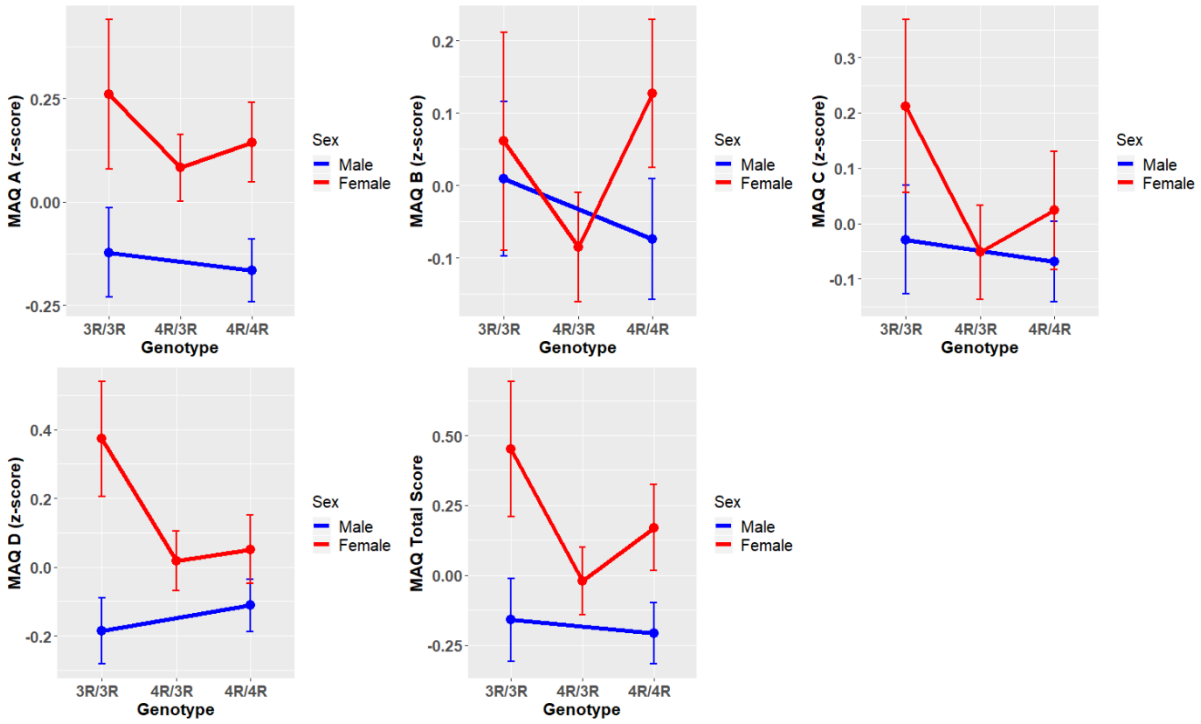

Figure 1. Distribution of means (standardized in z-scores by age) of the MAQ subscales and MAQ Total Score, organized by sex and MAOA-LPR genotype group. Vertical bars represent 1 standard error. Number of females by genotype: 3R/3R=36, 4R/3R=144, 4R/4R=116; Number of males by genotype: 3R=100, 4R=148. (Supplementary Figure 1 shows a similar graph, with vertical bars representing 1 standard deviation).

Linear models including covariables were fitted in R for Total sample and also for each sex separately, to allow for different genotypic models being tested for boys and girls. In boys, due to hemizygosity, only the two genotypes can be compared (3R *vs*. 4R). No significant genotype effects were found for any MAQ subscales nor for MAQ Total Score in boys (Supplementary tables 4-7). In girls, genotype models can be properly applied. Effects approached significance under the heterosis model for MAQ-B and MAQ Total Score, and 4R-dominant for MAQ-D. The model for MAQ-C and genotype was not different from the model containing only the intercept (p=0.18).

MAOB rs1799836

Table 5 shows the MAQ subscales means according to sex and genotype. The means of the MAQ subscales and the MAQ Total Score were compared among genotypic groups using the Kruskal-Wallis test, followed by a Dunn post hoc test (alpha=0.05/2) (Figure 2 and Supplementary Figure 2). According to these results, heterozygous girls present similar means of MAQ-A when compared to A or G genotypes in boys. However, homozygous girls present higher means of MAQ-A (A/A x A, p=0.02; G/G x G, p=0.0004). In MAQ-C, boys presenting the G allele show significantly lower means than boys presenting the A allele and than G/G girls (G x A, p=0.01; G/G x G, p=0.02). The same is true for MAQ-D, where, boys having the G allele show lower math anxiety when compared to A boys (p=0.007) or to any genotype in girls (G x G/G, p=0.0001; G x A/G, p=0.007; G x A/A, p=0.007). For MAQ Total Score, A/A, A/G girls and A boys, show similar means; while G boys present a lower mean when compared to homozygous girls (G x G/G, p=0.0003; G x A/A p=0.02).

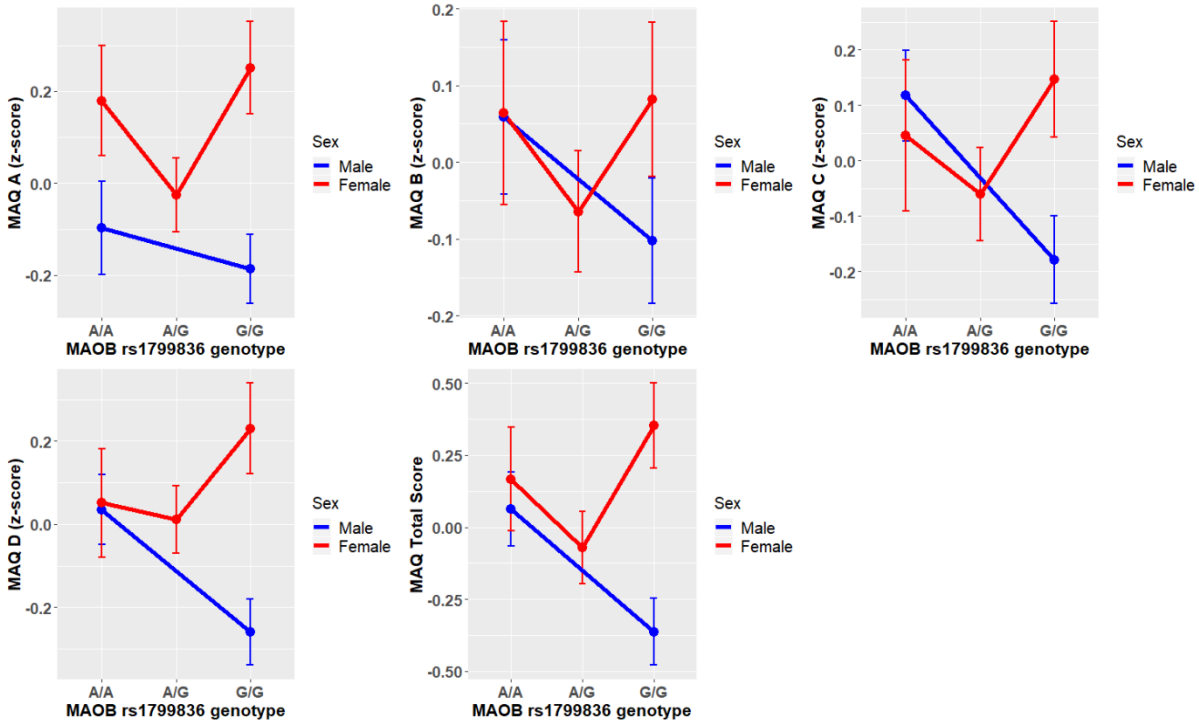

Figure 2. MAQ subscales and MAQ Total Score means organized by sex and MAOB rs1799836 genotype. Number of female samples by genotype: A/A=67, A/G=159, G/G=94; Number of male samples by genotype: A=118, G=139. Herein, A and G means were plotted following the A/A and G/G positions in the X-axis, respectively. Vertical bars represent 1 standard error.

Table 5. Demographic data of children divided according to sex and MAOB rs1799836 genotype

		Total N (%)	**A/A or A N (%)**	**A/G N (%)**	**G/G or G N (%)**
Sample distribution	Girls	320 (55.5)	67 (20.9)	159 (49.7)	94 (29.4)
	Boys	257 (44.5)	118 (45.9)	-	139 (54.1)
		Mean (SD)	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**
Grade	-	3.99 (0.82)	3.98 (0.84)	4.01 (0.86)	3.97 (0.79)
Age (years)	Girls	9.27 (0.95)	9.24 (0.97)	9.30 (0.94)	9.23 (0.98)
	Boys	9.19 (0.97)	9.18 (0.97)	-	9.19 (0.98)
Intelligence (z-score)	Girls	0.74 (0.71)	0.77 (0.61)	0.70 (0.76)	0.79 (0.71)
	Boys	0.87 (0.72)	0.87 (0.71)	-	0.88 (0.71)
Transcoding (z-score)	Girls	-0.08 (1.04)	-0.20 (1.21)	-0.10 (1.04)	0.02 (0.88)
	Boys	0.11 (0.94)	0.04 (0.97)	-	0.18 (0.90)
TDE Arit. - Arithmetic achievement (z-score)	Girls	0.36 (1.05)	0.28 (1.06)	0.42 (1.03)	0.33 (1.09)
	Boys	0.36(0.97)	0.36 (1.02)	-	0.36 (1.02)
MAQ-A - Self-perceived Performance (z-score)	Girls	0.10 (1.00)	0.18 (0.98)	-0.03 (1.01)	0.25 (0.97)
	Boys	-0.15 (0.99)	-0.10 (1.10)	-	-0.19 (0.89)
MAQ-B - Attitudes Toward Mathematics (z-score)	Girls	0.01 (0.98)	0.06 (0.97)	-0.06 (0.99)	0.08 (0.97)
	Boys	-0.03 (1.03)	0.06 (1.09)	-	-0.10 (0.97)
MAQ-C - Unhappiness About Mathematics (z-score)	Girls	0.02 (1.06)	0.04 (1.12)	-0.06 (1.06)	0.15 (1.01)
	Boys	-0.04 (0.92)	0.12 (0.89)	-	-0.18 (0.93)
MAQ-D - Anxiety Toward Mathematics (z-score)	Girls	0.08 (1.04)	0.05 (1.06)	0.01 (1.02)	0.23 (1.06)
	Boys	-0.12 (0.93)	0.04 (0.91)	-	-0.26 (0.94)
MAQ Total Score	Girls	0.10 (1.52)	0.17 (1.48)	-0.07 (1.57)	0.35 (1.43)
	Boys	-0.17 (1.39)	0.06 (1.39)	-	-0.36 (1.36)

Abbreviations: N – Sample size; SD – Standard Deviation; TDE Arit. – TDE Arithmetic

In Figure 2, we present the means and standard errors of the MAQ subscales and Total Score according to MAOB rs1799836 genotypes and sex. No variance differences were found between heterozygous and homozygous girls for any MAQ subscale or for MAQ Total Score. The linear model stratified by sex, performed in XWAS, returned significant differences in MAQ-C, MAQ-D and MAQ Total Score for allele substitution in boys only (p=0.03, p=0.03 and p=0.02, respectively). However, the Fisher combined p-value was significant only for MAQ Total Score (p=0.02) in boys. Therefore, significant differences in allele effect according to sex were confirmed in XWAS for MAQ-C (β_boys = 0.12, β_girls = -0.07, p=0.008), MAQ-D (β_boys = 0.12, β_girls = -0.09, p=0.002) and MAQ Total Score (β_boys = 0.13, β_girls = -0.11, p=0.0006).

Linear regressions, including covariables, were fitted using R to model each MAQ subscale and MAQ Total Score. Similar to the models developed for MAOA-LPR, separate models were fitted for each sex and also for total sample, in which hemizygous males were grouped with homozygous females to test for genotype and sex interactions. Results indicate a heterosis model for this locus in girls (Table 6), in which A/G girls show the lowest levels of MAQ-A, together with boys of any genotype. Therefore, heterozygous girls tend to consider themselves better in math than homozygous girls. MAQ-B was not explained by genotype differences or any other variable evaluated, except for math performance (Supplementary Table 8). Happiness/unhappiness about presenting math difficulties (MAQ-C) was explained only by the rs1799836 genotype in boys (Table 7).

Table 6. Linear Regression MAQ A – Self-perceived math performance and MAOB rs1799836

	Coefficient	**Prob. (>|T|)**	**Coefficient (Total Score)**	**Adj. R-squared**	**p-value (F-statistics)**
Female					
Intercept	0.27	0.0004 ***	1.07	0.14	1.1e-10 ***
Heterosis	-0.23	0.03 *	-0.91		
Transcoding	-0.20	0.0002 ***	-0.79		
TDE-Arithmetic	-0.21	0.0003 ***	-0.83		
Male					

Intercept	0.04	0.65	0.16	0.14	3.5e-09 ***
G genotype	-0.09	0.44	-0.36		
TDE-Arithmetic	-0.38	5.8e-10 ***	-1.50		

‘.’ P<0.1; ‘*’ P<0.05; ‘**’ P<0.005; ‘***’ P<0.0005

Abbreviations: PA – Porto Alegre; Adj. – Adjusted; Prob. – Probability;

The model also showed a significant interaction between GG or G genotypes and the female sex (coef=2.21, p=0.0495). While the substitution of the A by the G allele in boys is related to lower MAQ-C mean, the GG genotype in girls causes no changes in the MAQ-C mean (Table 7). Math anxiety (MAQ-D) variance could be explained by the rs1799836 genotype and math performance. A significant interaction between G/G or G and the female sex was also detected (coef=2.60, p=0.02). In boys, MAQ-D means were lower for the G genotype compared to the A genotype, while in girls, no significant allelic substitution effect was detected (Table 8).

Table 7. Linear Regression MAQ C – Unhappiness about math and MAOB rs1799836

	Coefficient	**Prob. (>|T|)**	**Coefficient (Total Score)**	**Adj. R-squared**	**p-value (F-statistics)**
Female					
Intercept	0.15	0.18	0.83	0.003	0.18
A-dominant	-0.18	0.18	-1.00		
Male					
Intercept	0.12	0.16	0.66	0.022	0.01 *
G genotype	-0.30	0.01 *	-1.66		

‘.’ P<0.1; ‘*’ P<0.05; ‘**’ P<0.005; ‘***’ P<0.0005

Abbreviations: PA – Porto Alegre; Adj. – Adjusted; Prob. – Probability;

Table 8. Linear Regression MAQ D – Math anxiety and MAOB rs1799836

	Coefficient	Prob. (>|T|)	Coefficient (Total Score)	Adj. R-squared	p-value (F-statistics)
Female					
Intercept	0.26	0.02 *	1.41	0.01	0.06
A-dominant	-0.20	0.11	-1.08		
TDE-Arithmetic	-0.10	0.07 .	-0.54		
Male					
Intercept	0.09	0.28	0.49	0.05	0.001 **
G genotype	-0.29	0.01 *	-1.57		
TDE-Arithmetic	-0.16	0.007 **	-0.87		

‘.’ P<0.1; ‘*’ P<0.05; ‘**’ P<0.005; ‘***’ P<0.0005

Abbreviations: PA – Porto Alegre; Adj. – Adjusted; Prob. – Probability;

When evaluating MAQ Total Score and its relationship with MAOB rs1799836, results suggested that part of the variance of this variable is explained by genotype, math performance and verbal Arabic transcoding performance in girls, and only by genotype and math performance in boys (Table 9). Sex and genotype interaction was also relevant for this model (coef=0.66, p=0.02). The linear regression model in the girls only returned similar results; however, the A-dominant model seemed to be the more plausible model in girls, as A/A and A/G girls showed the lowest means compared to G/G girls (Table 9). In boys, the G allele was associated with a reduction in MAQ Total Score mean, when compared to A males or any genotype females (Table 9).

Table 9. Linear Regression MAQ Total Score and MAOB rs1799836

	Coefficient	Prob. (>|T|)	Coefficient (Total Score)	Adj. R-squared	p-value (F-statistics)
Female					
Intercept	0.43	0.01 *	0.43	0.05	0.0002 ***
Heterosis	-0.37	0.04 *	-0.37		
TDE-arithmetic	-0.21	0.02 *	-0.21		
Transcoding	-0.17	0.05 .	-0.17		
Male					
Intercept	0.21	0.09 .	0.21	0.10	6.9e-07 ***
G genotype	-0.43	0.01 *	-0.43		
TDE-arithmetic	-0.41	2.3e-06 ***	-0.41		

‘.’ P<0.1; ‘*’ P<0.05; ‘**’ P<0.005; ‘***’ P<0.0005

Abbreviations: PA – Porto Alegre; Adj. – Adjusted; Prob. – Probability;

As a last question, we investigate the hypothesis of different sex effects on A/A *vs*. A and G/G *vs*. G genotypes. Except for MAQ-A, no significant difference was observed in the comparison of A/A girls *vs*. A boys. In the comparison of G/G girls *vs*. G boys, differences were significant, except for MAQ-B (p=0.07). These results suggest a specific sex effect in G/G and G genotypes, where the means of the girls were always the highest means, whereas the means of the boys were lower than the total sample means. Greater differences between the means of G/G an G genotype groups were observed for MAQ-C, MAQ-D and MAQ Total Score (Table 10).

Table 10. Mean differences (SD), Dunn's pairwise test and Levene' homogeneity variance test for MAQ subscales and MAQ Total Score for MAOB rs1799836 homozygous/hemizygous genotypes

	A/A *vs* A			G/G *vs* G		
	Mean diff.	Dunn test	Levene' homogeneity variance test	Mean diff.	Dunn test	Levene' homogeneity variance test
MAQ-A	-0.28	Z= -2.01 p=0.02 *	F= 0.59 PR(>F)= 0.44	-0.44	Z= -3.35 p=0.0004 ***	F= 1.52 PR(>F)= 0.21
MAQ-B	-0.01	Z= -0.23 p=0.41	F= 0.17 PR(>F)= 0.68	-0.18	Z= -1.48 p=0.07 .	F= 0.01 PR(>F)= 0.93
MAQ-C	-0.16	Z= 0.34 p=0.37	F= 3.89 PR(>F)= 0.05	-0.33	Z= -2.16 p=0.02 *	F= 0.47 PR(>F)= 0.63
MAQ-D	-0.02	Z= 0.03 p=0.49	F= 2.51 PR(>F)= 0.12	-0.49	Z= -3.74 p=0.0001 ***	F= 4.21 PR(>F)= 0.04
MAQ Total Score	-0.10	Z= -0.56 p=0.29	F= 0.77 PR(>F)= 0.38	-0.72	Z= -3.44 p=0.0003 ***	F= 0.23 PR(>F)= 0.63

DISCUSSION

Math anxiety is a specific type of anxiety, characterized by worries and feelings of fear and discomfort when dealing or having troubles involving math (A. Dowker et al., 2016; Richardson & Suinn, 1972). As a complex trait, MA levels can be influenced by environmental and individual factors, such as sex and genetics (Luttenberger et al., 2018). Regarding genetic factors, an architecture composed by many genes contributing each one with a small effect to MA is more plausible (Malanchini et al., 2017; Wang et al., 2014), since MA levels can be predicted by cognitive and behavioral traits, that present this same genetic architecture complexity, as working memory, learning disability, personality and resilience, for example (Haase, Guimarães, & Wood, 2019). Genetic association studies using variants in candidate genes have been a major tool for identifying genes conferring susceptibility to

complex disorders (Lewis & Knight, 2012). In the present study, we developed pair-wise, candidate gene association studies to investigate the contribution of two functional polymorphisms in *MAOA* and *MAOB* genes for MA in school-age children.

MA was assessed in 577 children through a self report Math anxiety questionnaire (MAQ) (Thomas & Dowker, 2000; Wood et al., 2012). A VNTR in *MAOA* gene promoter (MAOA-LPR) and a SNP in *MAOB* 13th intron (rs1799836) were genotyped. Data analysis was performed using XWAS, a program created to consider the specificities of the X-chromosome in association studies. Additionally, linear regressions fitted in R, considering sex, intelligence, verbal Arabic transcoding performance, spelling and math performances, and school quality as covariables. The presence of these variables in the final models was conditioned to passing a threshold of $p \leq 0.20$ in the bivariate general linear models, and in the results of stepwise regression. Linear regressions were fitted separately for males and females. However, considering that sex dependent allele effect has already been observed for *MAOA* and *MAOB* genes and for other genes in the dopaminergic system (Dlugos, Palmer, & de Wit, 2009; Gasso et al., 2008; Julio-Costa et al., 2019; Li et al., 2008; Lin, Davamani, Yang, Lai, & Sun, 2008), we tested for sex and genotype interaction, by fitting linear regressions in which hemizygous males were included in the same class of homozygous females, as proposed elsewhere for loci that undergo X-chromosome inactivation(Clayton, 2008; Konig, Loley, Erdmann, & Ziegler, 2014).

Herein, marginal associations and suggestive sex and genotype interactions were found for the MAOA-LPR locus and math anxiety (MAQ-D). Females, which usually present higher math anxiety levels when compared to males, have proven this true but only for homozygous females (4R/4R and 3R/3R). 4R/3R girls presented MAQ-D means as low as 4R or 3R male carriers, suggesting that the heterozygous genotype in this locus promotes advantage compared to homozygous genotype for math anxiety in girls. Results of association studies are ambiguous. While some studies detect high activity alleles (H), as 4R, associated with higher risk for depression (Rivera et al., 2009; Schulze et al., 2000; Yu et al., 2005), suicidal attempt in women (Ho et al., 2000; Jollant et al., 2007) and less happiness in women (Chen et

al., 2013), another study has found the low activity alleles (L), as 3R, as a risk factor for depression (Brummett et al., 2007). Moreover, risk allele seems to change according to sex in this locus. Low activity alleles (3R) predicted aggressive behavior in males, independently of maltreatment, while in females, aggressive behavior was present only in 4R-carriers that experimented maltreatment (Byrd & Manuck, 2014). Panic disorder was also associated to high activity alleles, but only in females (Reif et al., 2012). In Attention Deficit and Hyperactivity Disorder (ADHD), haplotypes containing the 3R allele were associated with higher transmission rate in ADHD probands, but in boys, only (Guan et al., 2009; Nymberg et al., 2013). Although allelic effect differences regarding sex are commonly described in the literature, to our knowledge, this is the first study reporting advantage for heterozygosis in females in this locus.

Significant associations between *MAOB* rs1799836 and all MAQ subscales, except for MAQ-B, were found in the present study. Self-perceived math performance (MAQ-A) was significantly better in *MAOB* heterozygous girls, compared to *MAOB* homozygous girls and no effect was found for allelic substitution in males. Similar to *MAOA* results, although females usually present a worse self-perception compared to males, this was not true for heterozygous girls. This finding suggests that the heterozygous state in *MAOB* rs1799836 may also be a protective factor for self-perceived math performance.

Unhappiness about math failures (MAQ-C) and preoccupation regarding math activities (MAQ-D) were significantly lower in G-carriers compared to A-carrier boys and all girls independently of genotype. Interaction between G/G or G and sex was observed, indicating that while the substitution of A by G in boys is associated to a reduction in MAQ-C and MAQ-D levels, in girls a different effect is observed. MAQ Total Score showed a similar result regarding sex and GG or G genotype interaction. Additionally, allelic substitution effects were observed not only in boys, but also in females, being the A-dominant model, the most probable model in girls. Similar to MAQ-A results, MAQ Total Score means were significantly lower in heterozygous girls, but also in A/A girls.

In summary, A/G genotype in girls and G genotype in boys seems to have a protective effect regarding math anxiety. Additionally, allelic substitution in *MAOB* rs1799836 has stronger effect in boys when compared to girls. This phenomenon has already been observed in a previous study, in which the G allele was considered a risk factor for schizophrenia only in females (Gasso et al., 2008). Karmakar et al (2017) also reported significant association of other alleles in *MAOA* and *MAOB* loci with ADHD, with these associations being restricted to boys. Recently, Julio-Costa et al. (2019) demonstrated a sex-dependent genotype effect for the Val158Met polymorphism in *COMT*, another important gene involved in the metabolism of monoamines. Altogether, these results suggest that the MAOA and MAOB mechanisms underlying the causes of these traits, could be different in males and females.

Another aspect emerging from this study is a sex effect on a specific genotype. For MAQ-C, MAQ-D and MAQ Total Score, G/G girls were more anxious than the mean of the sample, whereas the boys were less anxious considering the total sample mean.

Math Anxiety and the Monoaminergic System

MAOA and MAOB are two important enzymes responsible for the oxidation of structurally diverse amines in the central nervous system, and also in the periphery. Throughout this oxidative function, MAOA and MAOB plays an important role in the degradation of important monoaminergic neurotransmitters, dopamine (DA), norepinephrine (NE), tyramine, 2-phenylethylamine (PEA), and serotonin. Therefore, MAOA and MAOB control availability of these neurotransmitters and the postsynaptic response to them (Kalgutkar et al., 2001; Sodhi & Sanders-Bush, 2004).

Despite the high similarity between MAOA and MAOB, these isozymes are distinguished by their differences in substrate preferences and tissue expression. Regarding substrate preferences, MAOA degrades mostly 5-HT and NE, while, MAOB degrades selectively PEA and benzylamine, two stimulator molecules in the central nervous system. Dopamine, tryptamine, and tyramine are degraded by both enzymes (Kalgutkar et al., 2001). Regarding tissue expression, both enzymes are expressed in the majority of human tissues, except for platelets, which express mostly MAOB. The highest expression levels of MAOA are observed in placenta, lungs and small intestines, while the highest expression levels of MAOB occur in the myocardium. In the central nervous system, dopaminergic neurons express predominantly MAOA, while serotonergic neurons express more MAOB (Kalgutkar et al., 2001).

In humans, the modulation of a variety of functions in the central nervous system depends on monoamines. Cognition, emotions, memory process and behavior are examples of important brain functions affected by monoamine levels in synapses, which are controlled by important molecular mechanisms as degradation, reuptake and releasing of monoamines in synapses (Barnes, Dean, Nandam, O'Connell, & Bellgrove, 2011). Dysfunctions in monoaminergic transmission, have been implicated in neuropsychiatric and behavioral disorders, which are treated with drugs that act modifying the function of molecules implicated in the mechanisms cited above (Libersat & Pflueger, 2004). Despite of acting in the degradation of dopamine and serotonin, two neurotransmitters widely implicated in behavioral and cognitive functions, MAOA and MAOB also degrade other neuromodulators that induce the release of monoamines in the synapses and the phosphorylation of transporter proteins as the Dopamine transporter (DAT), implicated in the reuptake of dopamine. Besides that, as neuromodulators, the monoamines can act in a broader area than neurotransmitters, by activating receptors in cell membranes linked to G-proteins, and by stimulating signaling cascades, which promote modulation of ion channels and changes in gene transcription (Kalgutkar et al., 2001).

Polymorphisms in genes of the monoaminergic system have been associated to phenotypes such as general anxiety (You, Hu, Chen, & Zhang, 2005), ADHD symptoms (Guan et al., 2009; Li et al., 2007), post-traumatic stress disorder (Svob Strac et al., 2016), mood

(Grochans et al., 2013), personality (Balestri et al., 2017; Dlugos et al., 2009) and depression (Brummett et al., 2007). Additionally, specific endophenotypes associated to math performance as numerical cognition (Julio-Costa et al., 2013) and math anxiety (Julio-Costa et al., 2019) have also been associated with variations in genes involved in the regulation of monoamine degradation. All these phenotypes are sensitive to dopamine, norepinephrine and serotonin fluctuations in the brain, and can though be explained by functional polymorphisms in these genes. Intermediate levels of dopamine in the prefrontal cortex are ideal for higher working memory performance (Arnsten, 1998; Williams & Castner, 2006). However, dopamine levels may be tissue dependent. Therefore, the activity of degrading and reuptaking enzymes may differ among tissues, in a way of guaranteeing ideal levels of these neurotransmitters.

High activity alleles as 4R and 3.5R in MAOA-LPR locus and the A allele in MAOB rs1799836, have been associated with lower levels of dopamine and serotonin in the brain cortex and amygdala (Puglisi-Allegra & Andolina, 2015; Tunbridge et al., 2019). In the present study, heterozygous genotypes in both polymorphisms evaluated were suggestively or significantly associated with lower math anxiety in girls. It's not the first time in which positive heterosis or sex effects are described in the monoaminergic system (Barnett et al., 2007; Costas et al., 2011; Gosso et al., 2008; Julio-Costa et al., 2019). However, *MAOA* and *MAOB* genes are located on the X-chromosome and suffer full and random inactivation in females (Tukiainen et al., 2017). For this reason, considering that only one of the alleles would be active in each cell, in heterozygous females both alleles could be expressed, each one in a different cell and tissue. So we hypothesize that this shuffled expression pattern could then bring advantage for heterozygous girls, by being a mechanism introducing phenotypic diversity to the monoaminergic system regulation in different parts of the brain. Moreover, the high frequencies of the low activity alleles observed in the population, reinforce that heterosis may be advantageous for some MAO-related phenotypes in girls, or even that the low activity allele alone or in homozygosis may be advantageous in some phenotypes. These ideas were first proposed by Julio-Costa et al., (2019), who observed a similar mechanism in COMT Val158Met polymorphism.

In boys, G-carriers were significantly less anxious compared to A-carriers, meaning that lower activity of MAOB may contribute to lower predisposition to develop math anxiety, and to be unhappy about presenting math difficulties. The absence of MAOB has been correlated to increased levels of PEA, but to no changes in 5-HT, DA, or NE degradation, in mouse model (Shih & Chen, 1999). Phenotypically, MAOB-KO mice present higher reactivity to stress situations, but no aggressiveness (Grimsby et al., 1997).

PEA is known as the endogenous amphetamine of the body. It acts as a stimulator of the central nervous system, by stimulating the release of monoamines in the synaptic cleft and inducing the phosphorylation of the monoamine transporter proteins. This phosphorylation makes monoamine transporters to run in reverse, what leads to even more monoamines in synapses. PEA competes with monoamines for reuptake and degradation enzymes, once again contributing for the increase of mainly dopamine, serotonin, and norepinephrine in synapses. PEA also acts as a neuromodulator, binding to receptors in surrounding cells outside of the synapses and inducing changes in gene transcription. All these functions contribute to the idea that PEA works as a neuromodulator of the catecholamine neurotransmission in the central nervous system. PEA is usually present in low concentrations in the central nervous system, with slightly higher concentration in mesolimbic structures, and it undergoes a rapid turnover in healthy conditions (Paterson, Juorio, & Boulton, 1990). MAOB low activity could though increase levels of PEA in central nervous system, inducing hyperactivation of mesolimbic structures involved in the control of anxiety levels and stress response, which could interfere in a child's attitudes towards math difficulty (Malanchini et al., 2017; Moustafa et al., 2017).

Math Anxiety and School performance

It has been a common sense that MA is negatively correlated to math performance, with self-perceived math performance being its best predictor (Ann Dowker et al., 2019; Ramirez, Gunderson, Levine, & Beilock, 2013; Vukovic, Kieffer, Bailey, & Harari, 2013). As a cognitive scale of MA, MAQ-A represents a self-concept about one's abilities and due to this

reason, it could be reflecting real cognitive disabilities, as lower intelligence or realistic bad school achievement, due to specific reasons (Douglas & LeFevre, 2018). However, in some cases, even high achievement individuals show bad scores in MAQ-A, which in these cases could be reflecting a distorted self-perception caused by individual aspects of personality, gender, self-confidence and even external aspects as gender stereotypes (Casad, Hale, & Wachs, 2015; Ann Dowker et al., 2019).

In the present study, low intelligence was a predictor for self-perceived math performance (MAQ-A), only. While, math performance was a predictor for all MAQ subscales with exception to MAQ-C. Given that intelligence show a small but positive correlation with math performance, these results reinforce the idea previously reported that attitudes towards math, do not reflect intelligence (Hembree, 1990; Young, Wu, & Menon, 2012). Alternatively, attitudes towards math could be reflecting specific cognitive abilities which impair math cognition, causing bad performance and then higher anxiety levels regarding math (Wang et al., 2014). However the correlation between math anxiety and math performance could also be explained by another hypothesis, which states that MA is caused by individual predisposition factors to negative emotional response to the stress caused by math activities, which leads to higher anxiety symptoms and then to a worse performance in math (A. Dowker et al., 2016; Wang et al., 2014). Studies have reported that both pathways are possible and probable, and independently of what came first, both pathways feed a cycle, in which bad performance increases anxiety levels and higher anxiety levels, lead to even worse performance (Carey et al., 2015; A. Dowker et al., 2016).

MA can affect performance through different mechanisms. Ashcraft (2002) has proposed that math anxiety may cause avoidance of math activities, less practice and consequent low performance. Eysenck (1992), on the other hand, suggest that higher levels of math anxiety, cause mental ruminations and preoccupying thoughts, which consume working memory resources, essential for math activities. In this case, the bad performance in math would be a consequence of the low cognitive resources applied to solve math tasks and tests but not to low intelligence or low knowledge in math. Neurophysiological evidence, showing higher

levels of amygdala and lower levels of parietal and frontal cortex activations in math anxious individuals preceding math tasks, reinforce Eysenck et al. (1992) theory (Ashcraft, 2002; Eysenck & Calvo, 1992). A longitudinal study performed in school-age children suggested that MA has a worse impact on math performance, than math performance has over math anxiety (Cargnelutti, Tomasetto, & Passolunghi, 2017), however, contrastant results have also been published (Ma & Xu, 2004).

In the current study, we identified a negative effect of low performance in math in self-perceived math performance (MAQ-A), child's enjoyment in math (MAQ-B), and math worries (MAQ-D). MAQ Total Score was influenced by math and spelling performances, but also by verbal Arabic transcoding performance.

Math Anxiety and School Quality

It has been described that approximately 6% of the total variance of math anxiety is explained by differences among schools (Radišić, Videnović, & Baucal, 2015). Most of MA variance and math achievement is explained by individual differences between students and not by differences among groups as classrooms or schools (Teodorović, 2011). Although the school as an institution contributes few for MA variance, some aspects of school quality significantly change the mean anxiety level of children. Analysis of PISA assessment in 34 countries including Brazil, revealed that discipline inside the classroom and a good school environment correlate with lower levels of math anxiety, while a rigid and strict school environment, at institutional level, is associated with higher levels of math anxiety (Radišić et al., 2015), These findings suggest that discipline inside the classroom creates a healthy and organized environment for learning, but inflexibility and higher levels of discipline in the school environment may make children more anxious and create the feeling of obligation in achieving better performances.

Math-related class experiences can severely impact student's anxiety levels regarding math (Luttenberger et al., 2018; O'Leary et al., 2017; Ramirez, Hooper, Kersting, Ferguson, & Yeager, 2018). Elementary teachers, for instance, usually report to experience high levels of math anxiety (Battista, 1986; Bryant, 2009; Hembree, 1990) and consequently, to have severe difficulties to feel efficacious about their teaching responsibilities (Bursal & Paznokas, 2006; Gresham, 2008; Swars, Daane, & Giesen, 2006). The feeling of less efficacy can lead to inflexibility about teaching strategies or even lead to hostile reactions to student's difficulties, creating negative experiences for math students (Jackson & Leffingwell, 1999). Studies have consistently associated MA in adults to negative experiences with elementary school teachers (Chavez & Widmer, 1982; Markovits, 2011). However, this relationship is still true when considering higher level teachers (more specialized in math) (Ramirez et al., 2018). In these cases, summed to teacher's insecurity or inflexible strategies, teacher's beliefs about students abilities and also gender stereotypes can be transferred to students and contribute for higher levels of MA inside the classroom (Beilock et al., 2010).

The Brazilian Basic Education Quality Index (IDEB) is a governmental index obtained by the product between the Brazilian children's literacy and numeracy performance and children flux in the school system (Villani & Oliveira, 2018). IDEB was proposed by the Brazilian government in 2007, as an evaluation tool to be used in the Educational Developmental Plan, which establishes goals and strategies to increase educational access and quality in Brazil (INEP, 2018; Villani & Oliveira, 2018). In the present study, IDEB was used as a measure of school quality. In the last IDEB assessment, the mean IDEB value for schools in Brazil was 5.8 (INEP, 2018). Accordingly, most of the children in our sample attended good state-runned schools, with IDEB estimates higher than the national mean (IDEB median =7.1; mean=6.87; sd=0.69) (Supplementary Table 2). Only 8.5% of our sample attended to schools showing worse IDEB measurements (IDEB<5.8).

The measure assessed in the present study to represent school quality (IDEB) did not show correlation with any math anxiety subscale. The lack of contribution of IDEB for MA could be explained by three main reasons: IDEB does not capture directly teacher-student

relationship or teaching methods quality, factors that explain the major portion of variance in MA inside schools; moreover IDEB is considered to be a recent school quality indicator, which is incapable to embrace all the Brazilian educational system complexity (Neri & Buchmann, 2008; Villani & Oliveira, 2018). Finally, even if IDEB could explain a small proportion of MA anxiety, in our sample, only a small portion of schools in Brazil is represented, being most of them classified as above the national mean, regarding quality. Therefore, our sample may not capture all the variance this variable could present.

Limitations

Some limitations should be considered when evaluating the conclusions of the present study. No significant association of MAOA-LPR genotype was found to any MAQ subscale. However, suggestive association was observed for Self-perceived math performance (MAQ-A). These results could be related to reduced sample size or to other confounding factors in the sample. Different allelic or genotypic effects according to sex seem to be place for both *MAOA* and *MAOB* genes.

General anxiety was not evaluated herein. However, no significant correlation between general anxiety and math anxiety was reported in a previous study of the group, which assessed part of this same sample (n=146) (Haase, Júlio-Costa, et al., 2012).

As any multicenter project, the present study could present confounding factors due to sample origin. Different genetic background, differences in sample collection, or even cultural or education differences could affect results reported herein. Although, no genetic differences were observed for the loci studied herein, other genetic differences over the genome, could contribute to differences in the association of MAOA-LPR or MAOB rs1799836 to MA. Significant difference in mean MAQ-C and MAQ-D were observed in PA however, they did

not cause great impact on total sample means, when normalizing the data by the mean and the standard deviation of the total sample.

CONCLUSIONS

In conclusion, functional polymorphisms in *MAOA* and *MAOB* genes seem to affect math anxiety levels in school-age children, with the effects of allelic substitution being restricted or dependent on sex, according to locus. MAOA-LPR allelic substitution effects were observed only in girls, under a positive heterosis model. The MAOB rs1799836 polymorphism contributes to changes in MAQ-Affective measures, as unhappiness about math difficulties and math worries, but also to math anxiety in general, a new variable introduced in the present study. MAOB rs1799836 allelic substitution effects were more pronounced in males. G-carrier boys show the lower levels of MA in both affective MAQ scales, while in girls, no allelic substitution effect was observed. G/G girls present the higher levels of MA (in general) of all sample, while G boys present the lower levels of MA. This result suggests that lower activity alleles of *MAOB* are associated with a protective effect in boys regarding affective types of math anxiety. However, *MAOB* low activity alleles in girls, when in homozygosis, are associated with higher levels of math anxiety. Results reported herein suggest that alterations in the catecholamine metabolism may impact child's predisposition to math anxiety, and that this impact is dependent on genotype and sex. This is the first study to show the contribution of functional polymorphisms in *MAOA* and *MAOB* genes to math anxiety, and to predict a sex-dependent protective effect of allelic substitution in these loci to math anxiety.

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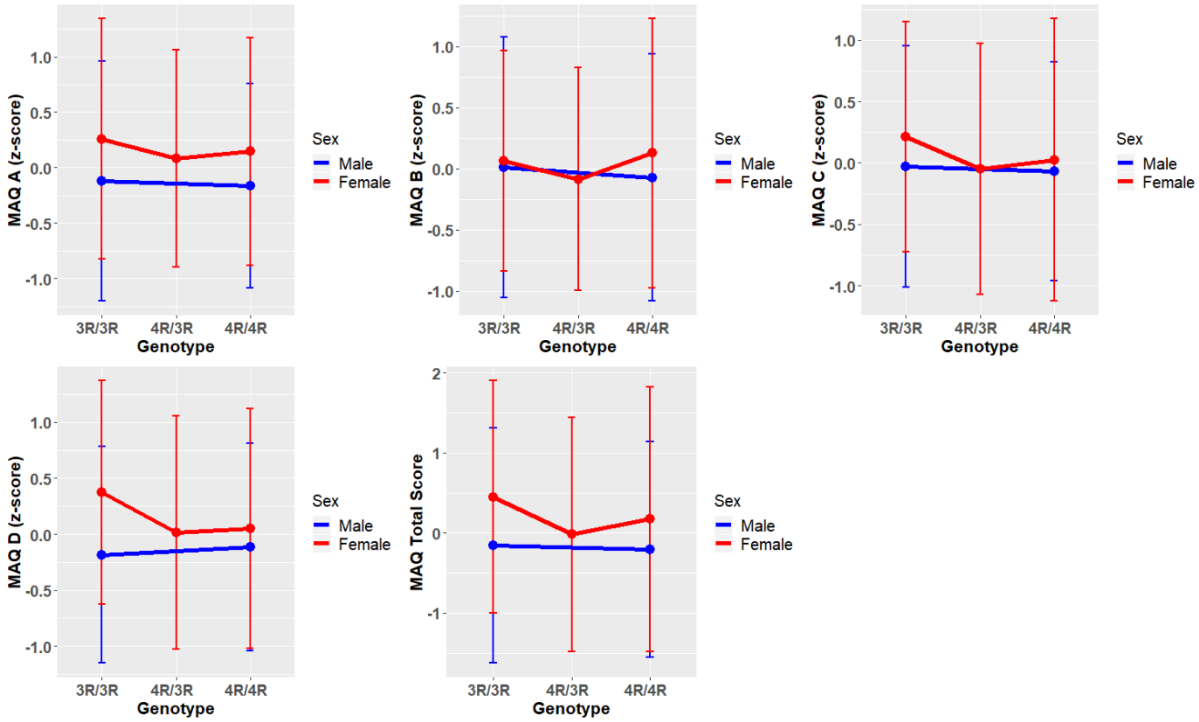
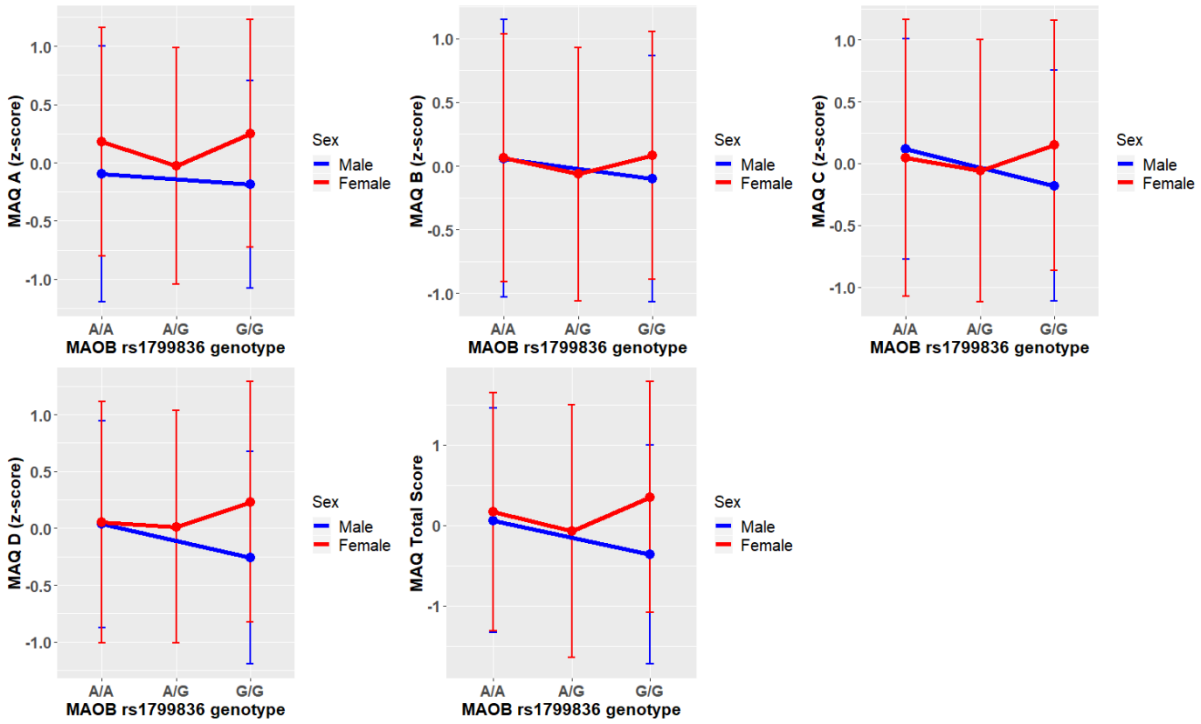
Supplementary Material

- **Supplementary Figures**



Supplementary Figure 1. MAQ subscales and MAQ Total Score means organized by sex and MAOA-LPR genotype. Number of female samples by genotype: 3R/3R=36, 4R/3R=144, 4R/4R=116; Number of male samples by genotype: 3R=100, 4R=148. Herein, 3R and 4R means were plotted following the 3R/3R and 4R/4R positions in the X-axis, respectively. Vertical bars represent 1 standard deviation.



Supplementary Figure 2. MAQ subscales and MAQ Total Score means organized by sex and MAOB rs1799836 genotype. Number of female samples by genotype: A/A=67, A/G=159, G/G=94; Number of male samples by genotype: A=118, G=139. Herein, A and G means were plotted following the A/A and G/G positions in the X-axis, respectively. Vertical bars represent 1 standard deviation.

- **Supplementary Tables**

Supplementary Table 1. Primer pairs

Gene	Primer	Sequence	Tm (°C)	Fragment size (bp)
MAOA*	Forward	5'-/56-FAM/CCCAGGCTGCTCCAGAAACATG-3'	60.6	183-373
	Reverse	5'-GTTCGGGACCTGGGCAGTTGT-3'	62.7	
MAOB (SEQ)	Forward	5'-TGACTGCCAGATTTCATCCTCT-3'	55.9	211
	Reverse	5'-AAAGACCTTTTGGCGCCTC-3'	56.1	
MAOB (HRM)	Forward	5'-ACACACTGGCAAATAGCAAAA-3'	53.3	43
	Reverse	5'-GCAGATTAGAAGAAAGATGGTGT-3'	52.8	

* MAOA primer pair sequence was obtained from Mickey et al. (2008)

Abbreviations: SEQ – Sequencing primer pair; HRM – High Resolution Melting primer pair.

Supplementary Table 2. Descriptive statistics of variables in raw score calculated by age

Variable	Sample	N	min	max	mean	median	SD
Intelligence percentile	all	577	16	100	74.26	80	20.53
	female	320	16	99	72.80	79	20.94
	male	257	17	100	76.07	83	19.90
TDE-Spell	all	577	0	35	23.11	24	7.55
	female	320	0	35	23.92	25	7.20
	male	257	0	35	22.10	24	7.85
TDE-Arithmetic	all	577	0	32	15.45	15	5.64
	female	320	0	32	15.75	15	5.85
	male	257	0	31	15.07	15	5.36
Transcoding percentile	all	577	0	1	0.85	0.98	0.24
	female	320	0	1	0.84	0.98	0.25
	male	257	0.09	1	0.87	0.98	0.22
MAQ-A - Self-perceived Performance	all	577	6	30	13.84	14	3.95
	female	320	6	29	14.26	14	3.96
	male	257	6	30	13.31	13	3.88
MAQ-B - Attitudes Toward Mathematics	all	577	6	30	13.87	13	4.66
	female	320	6	28	13.94	13	4.54
	male	257	6	30	13.77	13	4.81
MAQ-C - Unhappiness About Mathematics	all	577	6	30	17.55	17	5.53
	female	320	6	30	17.74	18	5.81
	male	257	6	29	17.30	17	5.16
MAQ-D - Anxiety Toward Mathematics	all	577	6	30	18.49	18	5.41
	female	320	6	30	19	19	5.62
	male	257	6	30	17.86	17	5.08
IDEB	all	577	3.9	8.4	6.87	7.1	0.69
	female	320	3.9	8.4	6.85	7.0	0.68
	male	257	3.9	8.4	6.88	7.1	0.70

Abbreviations: N – Sample size; min – minimum; max – maximum; SD – Standard Deviation;

IDEB - Basic Education Development Index

Supplementary Table 4. Linear Regression MAQ A – Self-perceived math performance and MAOA-LPR genotype

	Coefficient	Prob. (>|T|)	Coefficient (Total Score)	Adj. R-squared	p-value (F-statistics)
Female					
Intercept	0.25	0.002	0.99	0.13	1.2e-09 ***
Heterosis	-0.16	0.16	-0.63		
TDE-Arithmetic	-0.20	0.0004 ***	-0.79		
Transcoding	-0.22	0.0002 ***	-0.87		
Male					
Intercept	-0.01	0.89	-0.04	0.13	2.12e-08 ***
4R genotype	-0.01	0.95	-0.04		
TDE-Arithmetic	-0.37	3.06e-09 ***	-1.46		

'.' P<0.1; '*' P<0.05; '**' P<0.001; '***' P<0.0001

Abbreviations: Adj. – Adjusted; Prob. – Probability

Supplementary Table 5. Linear Regression MAQ B – Attitudes towards math and MAOA-LPR genotype

	Coefficient	Prob. (>|T|)	Coefficient (Total Score)	Adj. R-squared	p-value (F-statistics)
Female					
Intercept	0.17	0.04 *	0.79	0.04	0.001 **
Heterosis	-0.19	0.09 .	-0.89		
TDE-Arithmetic	-0.17	0.001 **	-0.79		
Male					
Intercept	0.07	0.49	0.33	0.03	0.007 **
4R genotype	-0.06	0.63	-0.28		
TDE-Arithmetic	-0.21	0.002 **	-0.98		

'.' P<0.1; '*' P<0.05; '**' P<0.001; '***' P<0.0001

Abbreviations: Adj. – Adjusted; Prob. – Probability

Supplementary Table 6. Linear Regression MAQ-D – Anxiety towards Mathematics and MAOA-LPR genotype

	Coefficient	Prob. (>|T|)	Coefficient (Total Score)	Adj. R-squared	p-value (F-statistics)
Female					
Intercept	0.44	0.014 *	2.38	0.02	0.04 *
4R dominant	-0.33	0.073 .	-1.79		
TDE-Arithmetic	-0.14	0.071 .	-0.76		
Male					
Intercept	-0.19	0.049 *	-1.03	0.00	0.55
4R genotype	0.07	0.565	0.38		

'.' P<0.1; '*' P<0.05; '**' P<0.001; '***' P<0.0001

Abbreviations: Adj. – Adjusted; Prob. – Probability

Supplementary Table 7. Linear Regression MAQ Total Score and MAOA-LPR genotype

	Coefficient	Prob. (>|T|)	Coefficient (Total Score)	Adj. R-squared	p-value (F-statistics)
Female					
Intercept	0.31	0.01 *	0.31	0.05	0.0004 ***
Heterosis	-0.32	0.07 .	-0.32		
TDE-Arithmetic	-0.21	0.02 *	-0.21		
Transcoding	-0.19	0.04 *	-0.19		
Male					
Intercept	-0.04	0.76	-0.04	0.07	3.8e-05 ***
4R genotype	-0.01	0.96	-0.01		
TDE-Arithmetic	-0.40	6.9e-06 ***	-0.40		

'.' P<0.1; '*' P<0.05; '**' P<0.001; '***' P<0.0001

Abbreviations: Adj. – Adjusted; Prob. – Probability

Supplementary Table 8. Linear Regression MAQ B – Attitudes towards math and MAOB rs1799836

	Coefficient	Prob. (>|T|)	Coefficient (Total Score)	Adj. R-squared	p-value (F-statistics)
Female					
Intercept	0.12	0.11	0.55	0.03	0.003 **
Heterosis	-0.12	0.26	-0.55		
TDE-Arithmetic	-0.16	0.002 **	-0.75		
Male					
Intercept	0.14	0.15	0.65	0.04	0.002 **
G genotype	-0.16	0.20	-0.75		
TDE-Arithmetic	-0.22	0.0008 ***	-1.03		

'.' P<0.1; '*' P<0.05; '**' P<0.005; '***' P<0.0005

Abbreviations: Adj. – Adjusted; Prob. – Probability

8.3 Anexo III – Certificado de aprovação do Projeto 1 pela COEP UFMG

UNIVERSIDADE FEDERAL DE MINAS GERAIS
COMITÊ DE ÉTICA EM PESQUISA - COEP

Parecer nº. ETIC 42/08

Interessado(a): Prof. Vitor Geraldi Haase
Departamento de Psicologia
Faculdade de Filosofia e Ciências Humanas - UFMG

DECISÃO

O Comitê de Ética em Pesquisa da UFMG – COEP aprovou, no dia 16 de maio de 2008, após atendidas as solicitações de diligência, o projeto de pesquisa intitulado **"Discalculia do desenvolvimento em crianças de idade escolar: triagem populacional e caracterização de aspectos cognitivos e genético-moleculares"** bem como o Termo de Consentimento Livre e Esclarecido.

O relatório final ou parcial deverá ser encaminhado ao COEP um ano após o início do projeto.

Profa. Maria Teresa Marques Amaral
Coordenadora do COEP-UFMG

8.4 Anexo IV - Certificado de aprovação do Projeto 2 pela COEP UFMG

UNIVERSIDADE FEDERAL DE MINAS GERAIS

Plataforma Brasil

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Endofenótipos das dificuldades de aprendizagem da matemática
Pesquisador: Vitor Geraldi Haase
Área Temática:
Versão: 4
CAAE: 15070013.1.0000.5149
Instituição Proponente: PRO REITORIA DE PESQUISA
Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 1.160.705
Data da Relatoria: 30/06/2015

Apresentação do Projeto:
Trata-se de um estudo observacional com delineamento transversal que se propõe a investigar os principais mecanismos cognitivos subjacentes aos transtornos de aprendizagem em grupos com dificuldade específica da matemática, dificuldade de leitura, grupo comórbido e crianças controles de idade escolar. Além disso, o estudo de endofenótipos cognitivos poderá facilitar a escolha de possíveis genes candidatos a influenciar os transtornos. A amostra (crianças de escolas públicas, entre 8 e 10 anos) terá caráter pseudo-aleatório, uma vez que convidaremos todas as crianças de 2º ao 4º anos de escolas parceiras, mas avaliaremos apenas aquelas que devolverem o TCLE assinados pelos pais. A coleta de dados ocorrerá em duas sessões, não havendo qualquer tipo de retestagem ou acompanhamento longitudinal.Após o aceite da coordenação da escola, em consenso com os professores, serão enviados aos pais dos alunos o TCLE, juntamente com uma carta convite do grupo de pesquisa. Duas mil crianças regularmente matriculadas em escolas parceiras do projeto serão convidadas a participar de uma primeira fase, em grupo, na qual são aplicados os subtestes de escrita e aritmética do Teste de Desempenho Escolar (Stein, 1994) e o teste de inteligência Matrizes Coloridas Progressivas de Raven (Angelini et al., 1999), além da coleta de material biológico. Os participantes com inteligência normal (percentil > 15 no Raven) participarão de uma IIetapa de avaliação neuropsicológica individual. A avaliação neuropsicológica que ocorre posteriormente nas escolas inclui tarefas de memória de trabalho (Subteste de Dígitos

Endereço: Av. Presidente Antônio Carlos,6627 2º Ad SI 2005
Bairro: Unidade Administrativa II **CEP:** 31.270-901
UF: MG **Município:** BELO HORIZONTE
Telefone: (31)3409-4592 **E-mail:** coep@prpq.ufmg.br

UNIVERSIDADE FEDERAL DE MINAS GERAIS

Considerações sobre os Termos de apresentação obrigatória:

Documentos apresentados: projeto de pesquisa formatado na plataforma Brasil e também em word; folha de rosto devidamente preenchida e assinada pelo Diretor da Faculdade de Filosofia e Ciências Humanas; parecer consubstanciado emitido pelo Departamento de Psicologia da Faculdade de Filosofia e Ciências Humanas; carta de anuência da Diretoria do Instituto de Educação de Minas Gerais; autorização do Biobanco de Materiais do LGHM (Laboratório de Genética Humana e Médica; Biobanco Termo de Consentimento Livre e Esclarecido;TALE; TCLE para os pais; TCLE para professores.

Recomendações:

Recomenda-se a aprovação da emenda ao projeto de pesquisa.

Conclusões ou Pendências e Lista de Inadequações:

Somos favoráveis à aprovação da emenda ao projeto " Endofenótipos das dificuldades de aprendizagem da matemática do Pesquisador Prof. Dr. Vitor Geraldi Haase, com a inclusão de do TCLE para os professores.

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

Considerações Finais a critério do CEP:

Diante do exposto, o Comitê de Ética em Pesquisa da UFMG/ COEP-UFMG, de acordo com as atribuições definidas na Resolução CNS nº 466 de 2012 e na Norma Operacional nº 001 de 2013 do CNS, manifesta-se pela aprovação da emenda proposta ao projeto de pesquisa.

8.5 Anexo V - TCLE – Termo de Consentimento Livre e Esclarecido

Termo de Consentimento Livre e Esclarecido

Título da Pesquisa:

Avaliação de estratégia de diagnóstico neuropsicológico e genético-molecular dos transtornos do desenvolvimento cognitivo (retardo mental)

Prezado (a),

Este Termo de Consentimento Livre e Esclarecido (TCLE) é um convite para vocês participarem do projeto de pesquisa acima. Aqui, queremos fornecer a vocês todas as informações necessárias para vocês entenderem o projeto e qual seria sua participação nele, para poderem decidir se querem participar ou não. A participação é voluntária. Se vocês optarem por não participar, não tem problema. Isto não irá influenciar o seu atendimento ou o da criança pela qual você é responsável.

O objetivo deste projeto de pesquisa é desenvolver e avaliar ferramentas (testes) para o diagnóstico de crianças em idade escolar com transtornos do desenvolvimento cognitivo (retardo mental). Além disto, buscamos avaliar testes para investigar se há uma causa genética para a dificuldade detectada na criança.

Estamos sua à disposição para esclarecer quaisquer dúvidas em relação à pesquisa antes da sua realização e durante a execução da mesma.

Leia as informações abaixo antes de expressar ou não o seu consentimento para participar da pesquisa.

Quando uma criança não se desenvolve como é esperado de acordo com a idade, são necessários testes para esclarecer o que está acontecendo (diagnóstico). Além disto, precisamos saber quão alterado desenvolvimento é e se há outros aspectos do desenvolvimento comprometidos. Para isto são necessários os testes. Os testes têm que ser padronizados, ou seja, crianças são muito diferentes uma das outras. Precisamos saber o que é normal numa determinada idade, para depois

definirmos se o desenvolvimento de uma criança é normal ou não. Não é possível usar dados de outros lugares, pois o desenvolvimento varia conforme o ambiente. Assim, nesta pesquisa estamos desenvolvendo e padronizando testes para uso na população de Minas Gerais. A idéia e obter-se um perfil completo do funcionamento da criança em idade escolar, ou seja, quais as funções estão comprometidas e quais as preservadas, que sirva como referência para orientação do seu atendimento. Também a definição de um modelo de aconselhamento voltado para a reabilitação se tornará viável.

A outra pergunta frequente, quando a família ou o profissional de saúde se depara que um desenvolvimento diferente do esperado, é qual é a causa. Podem ser problemas genéticos (mesmo quando não há outros casos na família), podem ser problemas relacionados à gestação ou ao parto, assim como efeito de drogas ou acidentes. Atualmente, há muitos testes diagnósticos para investigar as causas genéticas das alterações do desenvolvimento cognitivo. O uso destes testes depende da hipótese formulada pelo médico, mas também depende dos custos. Alguns destes testes são baratos e outros muito caros. Alguns destes testes são novos e não estão ainda disponíveis para a nossa população. Assim, é preciso estabelecer critérios para o uso dos testes, levando em conta a freqüência dos diversos tipos de doenças genética no nosso meio, e a ordem de utilização dos testes. Ao longo deste projeto, alguns testes novos passaram a ser oferecidos. Estes testes visam avaliar alterações em alguns genes ou cromossomas ou partes de cromossomas, que quando alterados causam alteração do desenvolvimento. Estes testes são baseados em diversos métodos (MLPA, PCR, qPCR, cariótipo, aGCH, entre outros) e para cada um deles devemos avaliar as vantagens, dificuldades e custos. Esta pesquisa é financiada pelo SUS e queremos obter as informações necessárias para que este órgão disponibilize estes testes à população atendida.

A pesquisa será conduzida pelo Laboratório de Neuropsicologia do Desenvolvimento, do Departamento de Psicologia, da Faculdade de Filosofia e Ciências Humanas (FAFICH), da UFMG em conjunto com o Laboratório de Genética Humana e Médica, do Departamento de Biologia Geral, do Instituto de Ciências Biológicas (ICB), da UFMG.

Caso você autorize a participação de seu (sua) filho (a) na pesquisa, você deverá preencher questionários e participar de uma entrevista para coleta da história da criança em idade escolar. Além disto, haverá a avaliação da criança em idade escolar, que será feita em aproximadamente 4 sessões de 1h e 30min cada. A criança responderá testes que avaliam inteligência, habilidades escolares, atenção, memória, percepção, destreza motora, velocidade de realização das tarefas, linguagem e planejamento. De maneira geral, são tarefas simples e divertidas, feitas com lápis e papel, jogos ou na tela do computador.

Finalmente, haverá um encontro de aproximadamente 1h para a devolução dos resultados.

Caso a pessoa não tenha sido avaliada por um Geneticista Clínico, poderá ser efeito exame clínico-genético, que constará de coleta da história da criança em idade escolar (anamnese), exame clínico e história familiar. Para investigação molecular, será feita uma coleta de material biológico. Este material pode ser obtido uma das seguintes maneiras: 1. sangue (5 ml, de uma veia do braço); saliva (neste caso a criança em idade escolar será convidada a cuspir num tubo de ensaio); 3. raspado de bochecha (a criança abre a boca e o coletador raspa delicadamente sua bochecha com um palito de madeira). A coleta será feita por profissionais de saúde habilitados e com experiência na coleta de sangue ou outros materiais. O material biológico será utilizado para análises genéticas no Departamento de Genética Humana e Médica do Departamento de Biologia Geral do ICB da UFMG, ao qual somente os pesquisadores têm acesso. Todos os procedimentos serão realizados no Serviço de Psicologia Aplicada da UFMG (SPA), o qual dispõe de consultórios apropriados à realização de todo o procedimento. Caso autorize a coleta de material biológico de seu filho, o mesmo poderá ser guardado em um banco de materiais biológicos humanos, podendo vir a ser usado em pesquisas futuras. Neste caso, será coletado outro termo de consentimento livre e esclarecido, especifico para o biobanco de materiais biológicos humanos.

Poderá ser necessária a realização de fotografia do corpo ou de partes do corpo do paciente, assim como filmagem ou gravação da entrevista ou de parte do exame físico ou neuropsicológico. Esta documentação será armazenada em banco de dados digital, sob responsabilidade dos coordenadores da pesquisa. Uma vez que estamos desenvolvendo uma linha de pesquisa em busca de métodos diagnósticos, e que, para muitas doenças, estes ainda não estão disponíveis, estes documentos serão armazenados permanentemente.

As fotografias e filmagens serão usadas apenas em reuniões e publicações científicas. Neste caso, as fotos serão modificadas (cobertas por tarjas, borradas parcialmente ou cortadas, de forma a apresentar apenas o detalhe relevante, de maneira a impedir sua identificação. Em nenhuma forma de publicação serão apresentados dados que permitam sua identificação ou a de seu(s) dependente(s).

Os dados coletados na avaliação neuropsicológica serão armazenados em um banco de dados no Laboratório de Neuropsicologia do Desenvolvimento do Departamento de Psicologia da UFMG, sob responsabilidade do professor Dr. Vitor Geraldi Haase. O material biológico colhido e seus derivados (DNA, por exemplo) serão armazenados em um banco de material biológico sob a responsabilidade da Profa. Dra. Maria Raquel Santos Carvalho. Ambos os bancos de dados são regulamentados pelo Comitê de Ética em Pesquisa da UFMG (COEP/UFMG) e poderão ser utilizados em outras pesquisas. Neste caso, é obrigatório por lei que se peça uma nova autorização ao COEP/UFMG.

É importante mencionar que a participação da criança na pesquisa é voluntária. Dessa forma, você poderá negar o consentimento ou optar, em qualquer momento da pesquisa, pelo encerramento da participação da criança sem sofrer nenhum tipo de prejuízo. Ou seja, você pode continuar a receber atendimento, caso opte por não participar da pesquisa.

Toda a participação na pesquisa é gratuita. Vocês não precisarão pagar pelos exames, avaliações ou consultas de orientação e entrega de resultados em momento algum. Por outro lado, o fato de vocês participarem da pesquisa não implica em compromisso financeiro para as equipes da UFMG ou os órgãos financiadores da pesquisa. Ou seja, não haverá qualquer forma de pagamento, ressarcimento ou indenização, em função da participação da criança em idade escolar e sua família no projeto de pesquisa.

Os resultados da pesquisa poderão ser utilizados em trabalhos científicos publicados ou apresentados oralmente em congressos e palestras, também sem revelar a identidade dos participantes. Os resultados serão apresentados em geral de forma coletiva, por exemplo, quantos pacientes tiveram alterado um determinado exame. Entretanto, se o estudo de uma criança em idade escolar acrescentar novas informações, que auxiliem a compreensão da doença ou dos métodos diagnósticos, seu caso poderá ser publicado isoladamente, sempre se protegendo sua identidade e de sua família.

Quanto aos riscos oferecidos pela pesquisa, assegura-se que são mínimos. A coleta de sangue pode doer um pouco e deixar uma mancha no local, mas, como numa escoriação rotineira, desaparece com o passar do tempo. Os outros métodos não apresentam riscos. Todo o material utilizado para coleta de amteriais biológicos é estéril, descartável, e **não existe nenhum risco de contrair doenças.** Em relação à avaliação neuropsicológica, o risco máximo é de desconforto físico e ansiedade relacionada aos procedimentos de testagem. Todo esforço será feito no sentido de atentar para o bem-estar físico e psicológico dos participantes, interrompendo-se a avaliação aos menores sinais de desconforto, além de se adotar procedimentos de relaxamento e esclarecimento nessas ocasiões.

Como benefício aos participantes será oferecido, em uma entrevista de devolução, um relatório contendo todos os resultados da avaliação neuropsicológica realizada, ressaltando os domínios cognitivos preservados e, porventura, afetados do indivíduo. Além disto, as famílias receberão os resultados dos testes genéticos realizados, sejam eles alterados ou normais. Caso seja identificado algum problema de saúde ou alguma necessidade educacional específica, você e sua família serão orientados e encaminhados para os serviços disponíveis na comunidade com o objetivo de melhorar sua saúde, bem-estar e capacidades de aprendizagem.

Agradecemos sua atenção e valiosa colaboração, e nos colocando a sua disposição para esclarecer dúvidas que apareçam a qualquer tempo, durante o desenvolvimento deste projeto, subscrevendo-nos.

Atenciosamente,

_____

Prof. Dr. Vitor Geraldi Haase
CRM-MG 29960-T
Coordenador da Pesquisa
Professor Associado do Departamento de Psicologia da UFMG
Av. Antônio Carlos, 6627, FAFICH-UFMG, Sala 4060
Laboratório de Neuropsicologia do Desenvolvimento
Tel: (31)34096295, (31)91059589/
E-mail: haase@fafich.ufmg.br

_____

Profa. Dra. Maria Raquel Santos Carvalho
CRM-MG 54.170
Pesquisadora responsável pela parte genética da pesquisa
Professora Associada do Departamento de Biologia Geral da UFMG
Av. Antônio Carlos, 6627, ICB-UFMG
Laboratório de Genética Humana e Médica
Tel: (31)34092598/(31)-91559531
E-mail: mraquel@icb.ufmg.br

Para maiores esclarecimentos:

Comitê de Ética em Pesquisa (COEP/UFMG), na Av. Antônio Carlos, 6627 – Unidade administrativa II, 2º andar/ Campus Pampulha- UFMG
Tel: (31)34094592/ E-mail: coep@prpq.ufmg.br

Responsável pela coleta do termo

Eu, _____, CI n°_____, declaro que coletei este TCLE. Declaro ainda que expliquei seu conteúdo, informando os participantes de sobre todos os aspectos pertinentes, e respondi às perguntas que me foram feitas.

Responsável

Eu, _____, abaixo assinado (a), declaro ter sido informado (a) sobre os procedimentos e propostas da pesquisa *Avaliação de estratégia de diagnóstico neuropsicológico e genético-molecular dos transtornos do desenvolvimento cognitivo retardo mental)* e concordo com a participação voluntária da criança em idade escolar _____

_____ pela qual sou responsável. Declaro que li este termo e por estar de acordo, assino.

Belo Horizonte, _____ de _____de _____

_____

Assinatura

Participante

Eu,_____,
abaixo assinado (a), declaro ter sido informado (a) sobre os procedimentos e propostas da pesquisa *Avaliação de estratégia de diagnóstico neuropsicológico e genético-molecular dos transtornos do desenvolvimento cognitivo (retardo mental)* e concordo com a minha participação voluntária.

Belo Horizonte, _____ de _____ de _____

Assinatura

8.6 Anexo VI – Produções científicas

Produção científica e atividades desenvolvidas durante os quatro anos de Doutorado

Manuscritos publicados em periódicos

1. OLIVEIRA, L. F. S.; JULIO-COSTA, A.; **DOS SANTOS, F.C.**; CARVALHO, M. R. S.; HAASE, V. G.. Numerical processing impairment in 22q11.2 (LCR22-4 to LCR22-5) microdeletion: A cognitive-neuropsychological case study. Frontiers in Psychology, 2018.

2. FONSECA, PABLO A. S. ; LEAL, THIAGO P. ; **SANTOS, FERNANDA C.** ; GOUVEIA, MATEUS H. ; ID-LAHOUCINE, SAMIR ; ROSSE, IZINARA C. ; VENTURA, RICARDO V. ; BRUNELI, FRANK A. T. ; MACHADO, MARCO A. ; PEIXOTO, MARIA GABRIELA C. D. ; TARAZONA-SANTOS, EDUARDO ; CARVALHO, MARIA RAQUEL S. . Reducing cryptic relatedness in genomic data sets via a central node exclusion algorithm. Molecular Ecology Resources, v. 27, p. 1-13, 2018.

3. FONSECA, PABLO AUGUSTO DE SOUZA; **DOS SANTOS, FERNANDA CAROLINE**; LAM, STEPHANIE; SUÁREZ-VEGA, AROA; MIGLIOR, FILIPPO; SCHENKEL, FLAVIO S; DINIZ, LUIZA DE ALMEIDA FERREIRA; ID-LAHOUCINE, SAMIR; CARVALHO, MARIA RAQUEL SANTOS; CÁNOVAS, ANGELA. Genetic mechanisms underlying spermatic and testicular traits within and among cattle breeds: systematic review and prioritization of GWAS results. JOURNAL OF ANIMAL SCIENCE, v. 1, p. 10, 2018.

4. **DOS SANTOS, FERNANDA CAROLINE;** PEIXOTO, MARIA GABRIELA CAMPOLINA DINIZ; FONSECA, PABLO AUGUSTO DE SOUZA; PIRES, MARIA DE FÁTIMA ÁVILA; VENTURA, RICARDO VIEIRA; ROSSE, IZINARA DA CRUZ; BRUNELI, FRANK ANGELO TOMITA; MACHADO, MARCO ANTONIO; CARVALHO, MARIA RAQUEL SANTOS. Identification of Candidate Genes for Reactivity in Guzerat (Bos indicus) Cattle: A Genome-Wide Association Study. Plos One, v. 12, p. e0169163, 2017.

5. BECKER, NATALIA; VASCONCELOS, MAILTON; OLIVEIRA, VANESSA; **DOS SANTOS, FERNANDA CAROLINE**; BIZARRO, LISIANE; DE ALMEIDA, ROSA M.M.; DE SALLES, JERUSA FUMAGALLI; CARVALHO, MARIA RAQUEL SANTOS. Genetic and environmental risk factors for developmental dyslexia in children: systematic review of the last decade. Developmental Neuropsychology, v. 42, p. 1-23, 2017.

6. DE SOUZA FONSECA, PABLO AUGUSTO; **DOS SANTOS, FERNANDA CAROLINE**; ROSSE, IZINARA CRUZ; VENTURA, RICARDO VIEIRA; BRUNELLI, FRANK ÂNGELO TOMITA; PENNA, VÂNIA MALDINI; DA SILVA VERNEQUE, RUI;

MACHADO, MARCO ANTÔNIO; DA SILVA, MARCOS VINÍCIUS GUALBERTO BARBOSA; CARVALHO, MARIA RAQUEL SANTOS; PEIXOTO, MARIA GABRIELA CAMPOLINA DINIZ. Retelling the recent evolution of genetic diversity for Guzerá: Inferences from LD decay, runs of homozygosity and Ne over the generations. Livestock Science (Print), v. 193, p. 110-117, 2016.

7. **SANTOS, F. C**.; SILVA, J. F.; BOELONI, J. N.; TEIXEIRA, E. A.; Turra, E. M.; SERAKIDES, R.; OCARINO, N. M.. Morphological and immunohistochemical characterization of angiogenic and apoptotic factors and the expression of thyroid receptors in the ovary of tilapia Oreochromis niloticus in captivity. Pesquisa Veterinária Brasileira (Online), v. 35, p. 371-376, 2015.

8. **SANTOS, F. C**.; Fonseca, P.A.S; Tavares, M.L.; Moro, L.. TEMAS TRANSVERSAIS ENFOQUE NA ABORDAGEM E DESENVOLVIMENTO DE TEMAS COM ÊNFASE EM DROGAS EM UM COLÉGIO PARTICULAR DE BELO HORIZONTE. Revista de Ensino de Biologia da Associação Brasileira de Ensino de Biologia (SBEnBio), v. 7, p. 2059-2071, 2014.

Manuscritos em preparação

1. HETEROSIS IN 5-HTTLPR VNTR IN SLC6A4 CONTRIBUTE FOR HIGHER MATH ANXIETY IN BOTH COGNITIVE AND AFFECTIVE DIMENSIONS IN CHILDREN

2. FUNCTIONAL POLYMORPHISMS IN MAOA AND MAOB AFFECT CHILDREN SUSCEPTIBILITY TO MATH ANXIETY, WITH SEX DIFFERENCES

Capítulos de Livros

1. CARVALHO, M. R. S; **SANTOS, F. C**.; MARTINS, A. A. S.; Becker, N; HAASE, V. G. Fatores Genéticos na Dislexia do Desenvolvimento In: Dislexias do desenvolvimento e adquiridas.1 ed. São Paulo: Pearson, 2017, v.1, p. 135-168.

Resumos apresentados em congressos e eventos

1. **DOS SANTOS, F. C.**; JORGE, C. D. C. A.; GOMIDES, M. R. A.; PAIVA, G. M.; HAASE, V. G.; CARVALHO, M. R. S.. Evaluation of the impact of functional polymorphisms in the genes slc6a4, mao-a and mao-b to math anxiety in children. In: World Congress on Brain Behavior and Emotions, 2019, Brasília. Proceedings of World Congress on Brain Behavior and Emotions, 2019.

2. Fonseca, P.A.S ; **Dos Santos, F.C.** ; SUÁREZ-VEGA, AROA ; LAM, STEPHANIE ; DINIZ, L. A. F. ; ID-LAHOUCINE, SAMIR ; CARVALHO, M. R. S. ; CÁNOVAS, ANGELA . Identification of functional candidate genes associated with male fertility traits subjected to specie-specific selection pressure in cattle. In: X-meeting 2018 - 14th International Conference of the AB3C, 2018, São Pedro. Proceedings X-meeting 2018, 2018.

3. **Dos Santos, F.C.**; GOMIDES, M. A. ; PAIVA, G. M. ; HAASE, V. G. ; CARVALHO, M. R. S. . The 5-HTTLPR locus in the SLC6A4 gene affects children self-perception about their Mathematics abilities. In: Gene Time Conference 2018, 2018, Belo Horizonte. Proceedings Gene Time Conference 2018, 2018.

4. STALKER, L. ; RUSSELL, S. ; **Dos Santos, F.C.** ; LAMARRE, J. . Identification and Characterization of piRNAs in Male Dogs. In: 51st Annual Meeting of the Society for the Study of Reproduction - SSR2018, 2018, New Orleans. Proceedings of the 51st Annual Meeting of the Society for the Study of Reproduction - SSR2018. New Orleans: SSR, 2018.

5. CARVALHO, M.R.S.; **SANTOS, F.C.**; OLIVEIRA, L.F.S; SALAZAR, G.C.; HAASE, V.G.; Dyscalculia in typical and distal 22q11.2 deletion syndromes: the riddle of genotypic-phenotypic correlations. The origins of numerical abilities - The Royal Society 2017.

6. MARTINS, A. A. S.; JULIO-COSTA, A. ; CASSEMIRO, P. M. ; **DOS SANTOS, FERNANDA CAROLINE** ; ALVES, I. S. ; LOPES-SILVA, J. B. ; HAASE, V. G. ; CARVALHO, M. R. S. . THE ROLE OF COMT VAL158MET AND DAT1 3'-UTR VNTR IN WORKING MEMORY TASKS PERFORMANCES OF SCHOOLAR CHILDREN. In: World Congress on Brain Behavior and Emotions, 2017, Porto Alegre. Proceedings of World Congress on Brain Behavior and Emotions, 2017.

7. OLIVEIRA, G. S. ; LAGE, M. C. G. R. ; **DOS SANTOS, FERNANDA CAROLINE** ; Fonseca, P.A.S ; CARVALHO, M. R. S. . MELANOMA EM EQUINOS DE PELAGEM TORDILHA: PADRONIZAÇÃO DA REAÇÃO DE POLIMERIZAÇÃO EM CADEIA (PCR) PARA O SNP c-KIT c.1960G.A. In: Mostra PEX - PUC Minas - Unidade Betim, 2017, Belo Horizonte. Mostra PEX - PUC Minas - Unidade Betim, 2017.

8. **SANTOS, F. C**.; SALAZAR, G. C. ; CHAMI, A. M. ; MARTINS, A. A. S. ; GONCALVES, R. T. ; AGUIAR, M. J. B. ; CARVALHO, M. R. S. . CASE REPORT: BECKWITH WIEDEMANN SYNDROME AND A COMPLEX CHROMOSOMAL REARRANGEMENT: 11P15 MICRODUPLICATION AND MICRODELETION AND 18Q23 MICRODUPLICATION. In: Genética2016 Brazilian-International Congress of Genetics, 2016, Caxambu. Proceedings of Genética 2016, 2016. v. 62.

9. **SANTOS, F. C.;** Fonseca, P.A.S ; ROSSE, I. C. ; DINIZ, L. A. F. ; CARVALHO, M. R. S. . Metabolic pathways involved in bovine temperament. In: X-meeting 2016, 2016, Belo Horizonte. Proceedings of X-meeting 2016, 2016.

10. MARTINS, A. A. S. ; **DOS SANTOS, FERNANDA CAROLINE** ; SALAZAR, G. C. ; JULIO-COSTA, A. ; MOREIRA, A. ; MOURA, R. ; PAIVA, G. M. ; HAASE, V. G. . DAT1 Gene and Working Memory in school children in Brazil. In: 62° Congresso Brasileiro de Genética, 2016, Caxambu. Genética 2016, 2016.

11. **SANTOS, F.C.**; Fonseca, P.A.S ; Pires, M.F.A ; ROSSE, I. C. ; BRUNELI, F. A. T. ; VENTURA, R. V. ; Peixoto, M.G.C.D. ; CARVALHO, M. R. S. . In silico investigation of the contribution of intergenic variations for a behavioral trait in Guzerá cattle. In: X-Meeting 2015 - 11th International Conference of th AB3C + Brazilian Symposium of Bioinformatics, 2015, São Paulo. Proceedings X-Meeting 2015. São Paulo: AB3C, 2015.

12. Fonseca, P.A.S ; **SANTOS, F.C.** ; ROSSE, I. C. ; VENTURA, R. V. ; BRUNELI, F. A. T. ; PENNA, V. M. ; CARVALHO, M. R. S. ; Peixoto, M.G.C.D. . Impact of recent evolutionary events on the current inbreeding values of Guzerá assessed using a genome wide genotyping approach. In: 61º Congresso Brasileiro de Genética, 2015, Águas de Lindóia. Prossedings 61º Congresso Brasileiro de Genética, 2015.

13. Fonseca, P.A.S ; **SANTOS, F.C.** ; GOUVEIA, M. H. ; LEAL, T. P. ; ROSSE, I. C. ; VENTURA, R. V. ; Machado, M.A, ; SILVA, M. V. G. B. ; Peixoto, M.G.C.D. ; TARAZONA-SANTOS, E. M. ; CARVALHO, M. R. S. . A new, highly efficient strategy for

decomposing population genetic structure and reducing consanguinity in non-random samples through a G-matrix based, centrality approach. 2015. In: 'X-Meeting 2015 - 11th International Conference of th AB3C + Brazilian Symposium of Bioinformatics, 2015, São Paulo. Proceedings X-Meeting 2015. São Paulo: AB3C, 2015.

14. PAIVA, A. E.; Fonseca, P.A.S; **SANTOS, F.C.**; CARVALHO, M. R. S.. Performance of the Illumina Bovine SNP50 V2 Bead Chip for the Guzerá Breed. In: 61Congresso Brasileiro de Genética, 2015, Águas de Lindoia. Proceedings of 61º Congresso Brasileiro de Genética, 2015.

15. PAIVA, A. E.; Fonseca, P.A.S; **SANTOS, F.C.**; ROSSE, I. C.; MOURA, G. S.. Missense mutations in candidate genes for reproductive disorders in a Gir bull identified through whole-genome sequencing. In: 'X-Meeting 2015 - 11th International Conference of th AB3C + Brazilian Symposium of Bioinformatics, 2015, São Paulo. Proceedings X-Meeting 2015, 2015.

Outras produções bibliográficas

1. Fonseca, P.A.S ; MATOSINHO, C. G. R. ; ROSSE, I. C. ; **Fernanda C. Santos** ; Pires, M.F.A ; GAMA, M. A. S. ; LOPES, F. C. F. ; BRUNELI, F. A. T. ; Machado, M.A, ; SILVA, M. V. G. B. ; Peixoto, M.G.C.D. ; CARVALHO, M. R. S. . O Guzerá na era pós-grnômica. Juiz de Fora: Embrapa Gado de Leite, 2018 (Informativo no Livro Anual do PNMGuL - Documentos 218).

2. Fonseca, P.A.S ; ROSSE, I. C. ; **DosSantos, F.C.** ; Assis, J.G. ; OLIVEIRA, F. S. ; LEITE, L. R. ; ARAUJO, F. ; ZERLOTINI, A. ; VOLPINI, A. ; DOMINITINI, A. J. ; Pires, M.F.A ; GAMA, M. A. S. ; LOPES, F. C. F. ; Machado, M.A, ; BRUNELI, F. A. T. ; SILVA, M. V. G. B. ; Oliveira, G. ; Peixoto, M.G.C.D. ; CARVALHO, M. R. S. . O Guzerá na pesquisa genômica. Juiz de Fora: Embrapa Gado de Leite, 2017 (Informativo no Livro Anual do PNMGuL - Documentos 201).

3. Fonseca, P.A.S ; ROSSE, I. C. ; **DosSantos, F.C.** ; Assis, J.G. ; OLIVEIRA, F. S. ; LEITE, L. R. ; ARAUJO, F. ; ZERLOTINI, A. ; VOLPINI, A. ; DOMINITINI, A. J. ; PIRES, MARIA DE FÁTIMA ÁVILA ; GAMA, M. A. S. ; LOPES, F. C. F. ; Machado, M.A, ; BRUNELI, F. A. T. ; SILVA, M. V. G. B. ; Oliveira, G. ; Peixoto, M.G.C.D. ; CARVALHO,

M. R. S. . O Guzerá na pesquisa genômica. Juiz de Fora: Embrapa Gado de Leite, 2016 (Informativo no Livro Anual do PNMGuL - Documentos 188).

4. Fonseca, P.A.S ; ROSSE, I. C. ; **SANTOS, FC** ; Assis, J.G. ; OLIVEIRA, F. S. ; LEITE, L. R. ; ARAUJO, F. ; ZERLOTINI, A. ; VOLPINI, A. ; DOMINITINI, A. J. ; Pires, M.F.A ; GAMA, M. A. S. ; LOPES, F. C. F. ; Machado, M.A, ; BRUNELI, F. A. T. ; SILVA, M. V. G. B. ; Oliveira, G. ; Peixoto, M.G.C.D. ; CARVALHO, M. R. S. . O Guzerá na pesquisa genômica. Juiz de Fora/MG: EMBRAPA Gado de Leite, 2015 (Informativo no Livro Anual do PNMGuL - Documentos 178)

Prêmios

1. Best Poster Award (Session Genes and Genomics), X-Meeting 2018 - 14th International Conference of the AB3C. (2018)

2. Prêmio Sílvio de Almeida Toledo Filho - Melhor trabalho da área de Genética Evolução e Melhoramento Animal, 61º Congresso brasileiro de Genética, Sociedade Brasileira de Genética. (2015)

Entrevistas

1. **DosSantos, F.C.**. Genética, cérebro e discalculia. 2018. (Programa de rádio ou TV/Entrevista). Link: https://www.youtube.com/watch?v=ERArNpSsO6U&t=178s

2. CARVALHO, M. R. S.; **SANTOS, F.C**.. Gênio difícil - Pesquisa do ICB relaciona genes a comportamento arredio de bois da raça guzerá. 2015. (Programa de rádio ou TV/Entrevista). Link: https://www.ufmg.br/online/arquivos/039964.shtml

3. CARVALHO, M. R. S.; **SANTOS, F.C.**; ROSSE, I. C.; Fonseca, P.A.S. Genes da prosperidade. 2015. (Programa de rádio ou TV/Entrevista). Link: https://issuu.com/fapemig/docs/mfc_62_

Orientações e Supervisões

1. Glayce S. Oliveira. Estágio obrigatório em Biologia molecular. 2017. Orientação de outra natureza. (Medicina Veterinária) - Pontifícia Universidade Católica de Minas Gerais. Orientador: Fernanda Caroline dos Santos.

2. Marlon Bernardo Costa. Estágio obrigatório em BIologia molecular. 2016. Orientação de outra natureza. (Ciências Biológicas) - Centro Universitário UNA. Orientador: Fernanda Caroline dos Santos.

Organização de eventos

1. DosSantos, F.C.. 4th Brazilian Student Council Symposium. 2019. (Congresso).
2. DosSantos, F.C.. 3rd Brazilian Student Council Symposium. 2018. (Congresso).

Participação em banca de comissões julgadoras

Concurso Público

1. Banca examinadora de Seleção de Mestrado em Genética 1/2016 - Programa de Pós-graduação em Genética, Universidade Federal de Minas Gerais, 2016.

Trabalho de Conclusão de Curso

1. **DosSantos, F.C.** Banca de Trabalho de Conclusão de Curso – Candidato: Núbia Luíza Mattos Vasconcelos. Investigação da associação entre a VNTR do gene DRD4 e o desempenho em tarefas de Memória de trabalho em uma amostra de crianças em idade escolar. 2019. Curso Ciências Biológicas - Universidade Federal de Minas Gerais.

2. **DosSantos, F.C.**. Oral and Poster presentation judge - 3rd Brazilian Student Council Symposium. 2018. Pontifícia Universidade Católica de Minas Gerais.

3. **DosSantos, F.C.**. Avaliação de trabalhos interdisciplinares das Disciplinas Genética Animal e Histologia Veterinária. 2016. Pontifícia Universidade Católica de Minas Gerais.

4. **DosSantos, F.C..** Avaliação de trabalhos interdisciplinares das Disciplinas Genética Animal e Histologia Veterinária. 2016. Pontifícia Universidade Católica de Minas Gerais.

Outras participações

1. Comissão avaliadora de trabalhos interdisciplinares das Disciplinas de Genética Animal e Histologia Veterinária. Pontifícia Universidade Católica de Minas Gerais, 2017.

2. Comissão avaliadora de trabalhos interdisciplinares das Disciplinas de Genética Animal e Histologia Veterinária. Pontifícia Universidade Católica de Minas Gerais, 2017.

Aulas, palestras e cursos Ministrados

Universidade Federal de Minas Gerais

Disciplinas ministradas:

1. Professora voluntária da disciplina DIG BIG058 GENÉTICA para o curso de Medicina da Universidade Federal de Minas Gerais 2018/2. Carga Horário total da disciplina: 60 horas; Carga horária total ministrada: 30h

2. Professora voluntária da disciplina "Tópicos especiais de Genética e Evolução II: Estudo de Funcionalidade das Mutações" ministrada na Pós-Graduação em Genética da Universidade Federal de Minas Gerais 2018/1. Carga Horário total do semestre: 30 horas

3. Monitora da disciplina “Introdução ao R - Manipulação de dados e aplicações” no Programa de Pós-Graduação em Genética da UFMG. 2018. Carga Horário total do semestre: 30 horas

4. Professora da disciplina de “Genética do Comportamento” no PCND. Programa de Capacitação em Neuropsicologia do Desenvolvimento (PCND) - APAE-BH, FeAPAE de Minas Gerais e UFMG. 2017. Carga Horário total do semestre: 60 horas

5. Professor voluntário no curso preparatório para o mestrado. Ministrou 15 horas do Curso preparatório de Genética para entrada no Processo de Seleção de Mestrado, entrada 2016/1, no período de 25 a 29 de janeiro de 2016. Carga Horário total do semestre: 15 horas

Centro Universitário UNA

1. Palestra Ministrada: Evolução dos programas de melhoramento das raças zebuínas. Palestra ministrada para os alunos de graduação em Medicina Veterinária. PUC Minas – Betim - no dia 14/06/2018.

2. Palestra Ministrada: As bases genéticas da dificuldade de aprendizado - 'Dificuldade em aprender matemática é genético?'. 2015. Palestra ministrada no evento Quintas da saúde no dia 25/10/2015.

3. Palestra Ministrada: O temperamento na raça Guzerá: A genética da reatividade. 2015. Palestra ministrada no evento Caracterização e definição de fenótipos para incorporação nas avaliações genéticas.

Participação em Eventos

1. World Congress on Brain, Behavior and Emotions 2019. Evaluation of the impact of functional polymorphisms in the genes slc6a4, mao-a and mao-b to math anxiety in children. (Congresso).

2. 3rd Brazilian Student Council Symposium. 2018. (Simpósio).

3. Gene Time Conference 2018. The 5-HTTLPR locus in the SLC6A4 gene affects children self-perception about their Mathematics abilities. 2018. (Congresso).

4. II Argentine Meeting on Non Coding RNA Biology. 2018. (Encontro).

5. X-meeting 2018 - 14th International Conference of the AB3C. Identification of functional candidate genes associated with male fertility traits subjected to specie-specific selection pressure in cattle. 2018. (Congresso).

6. VI Seminário do dia Mundial das Doenças Raras de Belo Horizonte. 2017. (Seminário).

7. Genética 2016 Brazilian-International Congress on Genetics. Case report: Beckwith-Wiedemann syndrome and a complex chromosomal rearrangement: 11p15 microduplication and microdeletion and 18q23. 2016. (Congresso).

8. Simpósio Melhoramento Genético de Bovinos de Leite - estado da arte e perspectivas futuras e II TACG s). 2016. (Simpósio).

9. X-meeting 2016. Metabolic pathways involved in bovine temperament. 2016. (Congresso).

10. 61° Congresso Brasileiro de Genética. 2015. (Congresso).

11. Caracterização e definição de fenótipos para incorporação nas avaliações genéticas. O temperamento na raça Guzerá: A genética da reatividade. 2015. (Simpósio).

12. X Meeting/BSB 2015. In silico investigation of the contribution of intergenic variations for a behavioral trait in Guzerá cattle. 2015. (Congresso).