UNIVERSIDADE FEDERAL DE CIÊNCIAS DA SAÚDE DE PORTO ALEGRE - UFCSPA
PROGRAMA DE PÓS-GRADUAÇÃO DE CIÊNCIAS DA SAÚDE

Mayara Cristina Batista da Silva

Análise integrativa de variantes de genes relacionados aos sistemas dopaminérgico e serotoninérgico com ingestão alimentar em crianças

Porto Alegre
2022

Mayara Cristina Batista da Silva

Análise integrativa de variantes de genes relacionados aos sistemas dopaminérgico e serotoninérgico com ingestão alimentar em crianças

Dissertação apresentada ao Programa de Pós-Graduação em Ciências da Saúde, da Fundação Universidade Federal de Ciências da Saúde de Porto Alegre, como requisito para a obtenção do título de Mestre em Ciências da Saúde.

Orientador: Prof. Drª. Silvana de Almeida
Coorientador: Dr. Edson Cordeiro do Valle
Coorientadora: Drª. Vanessa Feistauer

Porto Alegre

2022

Catalogação na Publicação

Silva, Mayara Cristina Batista da
Análise integrativa de variantes de genes relacionados aos sistemas dopaminérgico e serotoninérgico com ingestão alimentar em crianças / Mayara Cristina Batista da Silva. -- 2022.
84 p. : il., tab. ; 30 cm.

Dissertação (mestrado) -- Universidade Federal de Ciências da Saúde de Porto Alegre, Programa de Pós-Graduação em Ciências da Saúde, 2022.

Orientador(a): Profa. Dra. Silvana de Almeida ; coorientador(a): Dr. Edson Cordeiro do Valle, Dra. Vanessa Feistauer.

1. Ingestão alimentar. 2. Variantes genéticas . 3. Dopamina. 4. Serotonina. 5. Obesidade. I. Título.

Sistema de Geração de Ficha Catalográfica da UFCSPA com os dados fornecidos pelo(a) autor(a).

AGRADECIMENTOS

Aos meus orientadores, principalmente minha orientadora, Prof.ª Drª Silvana de Almeida pela força em todos os momentos. Obrigada por ter me proporcionado a oportunidade de realizar este trabalho, por ter confiado em mim, por ter dedicado seu tempo a mim, mesmo com tantos percalços. Muito obrigada!

Agradeço à minha família por nunca se queixarem dos momentos em que deixei de dar-lhes atenção. Agradeço principalmente ao meu marido Filipe por todo suporte e paciência, principalmente na reta final. Amo vocês!

Aos meus amigos e colegas de trabalho do Hospital Geral de Novo Hamburgo, que acompanharam minha jornada desde o início e sempre me ajudaram.

Agradeço a todos os professores da Universidade Federal de Ciências da Saúde de Porto Alegre e do PPG Ciências da Saúde, e a todos os professores que passaram pela minha vida por todo o conhecimento compartilhado. Foi esse conhecimento que vocês ajudaram a construir que me proporcionou chegar onde estou hoje.

Enfim, agradeço a todos que contribuíram de alguma forma para este trabalho e que me acompanharam nesta etapa, muito obrigada!

RESUMO

A obesidade é uma condição que confere alto risco para o desenvolvimento de muitas doenças crônicas e tem se tornado cada vez mais prevalente em todo o mundo. O principal tratamento para a obesidade é a mudança do estilo de vida, principalmente no que se refere à ingestão alimentar. Porém, para muitos pacientes esta é uma tarefa praticamente impossível de ser realizada, principalmente para os que estão inseridos em um ambiente obesogênico, no qual predomina a fome hedônica que é mediada pelas vias dopaminérgica e serotoninérgica. Fatores genéticos também podem influenciar nestas vias, uma vez que nem todos os indivíduos expostos a um ambiente obesogênico desenvolvem a obesidade. Diversos estudos que analisam polimorfismos, individualmente, associaram variantes em genes relacionados à dopamina e à serotonina com ingestão alimentar, obesidade e outros fenótipos correlatos, enquanto estudos de genoma amplo explicam uma baixa proporção da variabilidade. Entretanto, tais estudos não são capazes de capturar o efeito de interações mais complexas como as interações gene-gene. Portanto, novos métodos de análise dos dados se fazem necessários. O objetivo do presente estudo foi analisar o efeito em conjunto de variantes de genes das vias dopaminérgica e serotoninérgica, já analisados, de forma individual, pelo nosso grupo de pesquisa, na ingestão alimentar de crianças em três diferentes etapas do desenvolvimento, utilizando regressão PLS (partial least squares). Para isso foram gerados modelos utilizando dados de variantes genéticas como variáveis preditoras e de ingestão alimentar das crianças aos 12 meses, 3 anos e 6 anos de idade como desfechos. Na nossa amostra, foram obtidos modelos que explicaram de 65,2% a 83% da ingestão energética diária média. Também foram realizadas simulações para predizer a ingestão energética, utilizando as variáveis preditoras, e os valores simulados foram comparados com os valores reais de ingestão energética diária média nas três idades. Não houve diferenças estatisticamente significantes entre os valores reais e os valores simulados, o que indica boa precisão dos modelos. O método utilizado tem potencial aplicação para predição de desfechos quantitativos de variantes genéticas previamente associadas com características complexas. Não foram encontrados na literatura outros estudos utilizando o mesmo método para análise do efeito em conjunto de variantes de genes dopaminérgicos e serotoninérgicos na ingestão alimentar de crianças. Novas análises com outras variantes genéticas e outros desfechos são

necessárias para melhor avaliação da regressão PLS na análise conjunta de variantes genéticas com fenótipos complexos.

Palavras-chave: Dopamina. Ingestão alimentar. Variantes genéticas. Obesidade. Serotonina.

ABSTRACT

Obesity is a condition that confers high risk for the development of many chronic diseases and has become increasingly prevalent throughout the world. The main treatment for obesity is lifestyle change, especially with regard to food intake. However, for many patients this is a practically impossible task to be performed, especially for those who are inserted in an obesogenic environment, in which hedonic hunger predominates, which is mediated by dopaminergic and serotonergic pathways. Genetic factors can also influence these pathways, since not all individuals exposed to an obesogenic environment develop obesity. Several studies that analyze individual genes have found association between variants in dopamine and serotonin-related genes and food intake, obesity, and other related phenotypes, while genome-wide studies explain a low proportion of the variability. However, such studies are not able to capture the effect of more complex interactions such as gene-gene interactions. Therefore, new methods of data analysis are necessary. The aim of the present study was to analyze the effect of gene variants of the dopaminergic and serotonergic pathways, already analyzed individually by our research group, on food intake of children at three different stages of development, using PLS (partial least squares) regression. To do so, models were generated using data from genetic variants as predictors and food intake of children at 12 months, 3 years and 6 years of age as outcomes. In our sample, we obtained models that explained 65.2% to 83% of the average daily energy intake. Simulations were also performed using the predictor variables and the simulated values were compared with the real values of average daily energy intake at the three ages. There were no statistically significant differences between the real values and the simulated values, which indicates good accuracy of the models. This method has potential application for predicting quantitative outcomes of genetic variants previously associated with complex traits. No other studies were found in the literature using the same method to analyze the combined effect of variants of dopaminergic and serotonergic genes on food intake in children. New studies with other genetic variants and other outcomes are needed to better evaluate PLS regression in the joint analysis of genetic variants with complex phenotypes.

Keywords: Dopamine. Energy intake. Genetic variation. Obesity. Serotonin.

SUMÁRIO

1 INTRODUÇÃO

As doenças complexas compreendem uma gama de condições clínicas cujo desenvolvimento é o resultado da predisposição genética, mediada por múltiplos genes, em conjunto com fatores ambientais. São doenças comuns na população, mas apresentam alto grau de morbidade e mortalidade, como doenças autoimunes, doença de Alzheimer, obesidade, entre outras (MURAMATSU; OKUDA; OKAZAKI, 2014).

Ao contrário das doenças mendelianas, as doenças complexas não seguem um padrão de herança específico e pouco se sabe acerca dos mecanismos genéticos envolvidos na predisposição para a maioria deste tipo de doenças. Entretanto, sabe-se que é necessária a contribuição simultânea de diversos genes polimórficos, em um contexto em que cada variante genética envolvida contribui com uma baixa porcentagem de risco e que, individualmente, exerceriam pouco efeito para o seu desenvolvimento (BURGHES, 2001; PRICE; SPENCER; DONNELLY, 2015).

O fenômeno da obesidade tem se agravado nas últimas décadas, causando um impacto desfavorável na saúde e qualidade de vida da população mundial, em todas as faixas etárias, a ponto de se tornar uma epidemia (JAMES, 2008; MITCHELL et al., 2011; YANOVSKI, 2015). Pessoas com obesidade tem maior chance de desenvolver outras comorbidades como diabetes tipo II, doenças cardiovasculares, doença hepática gordurosa não alcoólica, câncer, entre outras (ANDOLFI; FISICHELLA, 2018; APOVIAN, 2016). Mais recentemente, com o surgimento do SARS-CoV-2, foi demonstrado que a obesidade exerce um papel importante no risco de desenvolvimento de formas severas de COVID-19 (DE LEEUW et al., 2021).

De acordo com a Organização Mundial de Saúde (OMS), a prevalência da obesidade quase triplicou desde 1975, resultando em mais de 650 milhões de adultos obesos no mundo em 2016, cerca de 13% da população mundial. Também houve um aumento na prevalência da obesidade e sobrepeso em crianças e adolescentes (5 a 19 anos de idade): de 4% em 1975 para 18% em 2016, o que representa mais de 340 milhões de indivíduos. Já em crianças abaixo dos 5 anos, a estimativa é de que 39 milhões eram obesas ou tinham sobrepeso em 2020 (WHO, 2021).

No Brasil, dados do IBGE de 2008-2009 demonstram que, dentre crianças de 5 a 9 anos de idade, 34,8% dos meninos e 32% das meninas apresentaram excesso de peso. Destes, 16,6% e 11,8% eram obesos, respectivamente. Em adolescentes de 10 a 19 anos de idade, a prevalência do excesso de peso foi de 20,5%, sendo 4,9% obesos. Entre adultos, 50,1% dos homens e 48% das mulheres foram diagnosticados com excesso de peso. Destes, a frequência de obesos foi de 12,5% e 16,9%, respectivamente (IBGE, 2010). Já em 2017, dados da Pesquisa de Vigilância de Fatores de Risco e Proteção para Doenças Crônicas por Inquérito Telefônico – Vigitel Brasil 2017 – mostram que, no conjunto das capitais brasileiras e Distrito Federal, a frequência de excesso de peso e obesidade em adultos foi de 54% e 18,9%, respectivamente (BRASIL, 2018).

A obesidade é uma doença altamente heterogênea e complexa, portanto vários fatores devem ser considerados para se compreender as causas de seu desenvolvimento, como idade, sexo, cultura, padrão socioeconômico, etnia, comportamento, escolaridade, entre outros (GONZÁLEZ-MUNIESA et al., 2017). De uma forma simplista, é necessário que haja um balanço energético positivo, ou seja, a quantidade de calorias ingeridas deve ser maior que a quantidade utilizada com a manutenção do metabolismo basal e atividade física. O estilo de vida contemporâneo contribui para o balanço energético positivo pois, com a industrialização e o rápido avanço tecnológico, é necessário cada vez menos energia para a realização de tarefas rotineiras, como deslocamento e trabalhos domésticos, além de que, os alimentos industrializados, altamente palatáveis, ricos em açúcares e lipídios, são os mais acessíveis e consumidos em grandes porções (HILL; WYATT; MELANSON, 2000; MITCHELL et al., 2011). Esse estilo de vida também facilita o desenvolvimento do estresse, o qual está associado à obesidade tanto pela hiperatividade do eixo hipotalâmico-hipofisário, quanto pelo aumento da ingestão alimentar (HEWAGALAMULAGE et al., 2016).

A sensação de recompensa proveniente da ingestão de alimentos altamente palatáveis pode desencadear a fome hedônica, na qual a ingestão alimentar tem como objetivo a própria sensação de recompensa, e não o suprimento da demanda metabólica (ZIAUDDEEN et al., 2015). Dopamina e serotonina, entre outros neurotransmissores, estão intimamente ligados ao mecanismo de recompensa

alimentar. Acredita-se que alterações na sinalização dopaminérgica e serotoninérgica favorecem o consumo elevado de alimentos palatáveis através do aumento da busca por recompensa mediado pelo sistema dopaminérgico, e da diminuição do *feedback* homeostático mediado pelo sistema serotoninérgico (STICE et al., 2013; VAN GALEN et al., 2018; YABUT et al., 2019). Através da ativação deste mecanismo, o indivíduo passa a dar preferência a tais alimentos, em detrimento de alimentos menos saborosos, o que eleva a quantidade de calorias ingeridas (STICE et al., 2013; ZIAUDDEEN et al., 2015). Desse modo, muitos indivíduos podem desenvolver um comportamento alimentar disfuncional, como a hiperfagia e, consequentemente, chegar à obesidade (LEIGH; MORRIS, 2018).

Entretanto, nem todos os indivíduos expostos a um ambiente obesogênico desenvolvem obesidade, o que indica a presença de um componente genético que interfere na predisposição individual à obesidade (ALBUQUERQUE et al., 2017). Em estudos de avaliação de herdabilidade realizados com gêmeos e com famílias, foi demonstrado que o fator genético exerce grande contribuição na variação do índice de massa corpórea (IMC) (SILVENTOINEN et al., 2010; WARDLE et al., 2008). De acordo com revisão sistemática e meta-análise, a herdabilidade estimada para o IMC em estudos com gêmeos foi de 47 a 90% e de estudos com famílias foi de 24 a 81%, sendo maior na infância que na fase adulta (ELKS et al., 2012). Em relação à ingestão alimentar, vários estudos demonstram a influência do componente genético nas preferências alimentares, com moderada herdabilidade em crianças (BREEN; PLOMIN; WARDLE, 2006). Em adolescentes, a influência genética persiste e se torna mais evidente devido à redução da influência do ambiente familiar compartilhado (SMITH et al., 2016), o que tende a se estender até a vida adulta (KESKITALO et al., 2008). Para a ingestão calórica em adultos, de 32 a 48% da variância pode ser atribuída a fatores genéticos (HASSELBALCH et al., 2008; LIU et al., 2013).

A publicação da primeira versão do genoma humano e seu mapeamento, além do desenvolvimento de técnicas de genotipagem em larga escala, permitiram o advento do *Genome-Wide Association Studies* (GWAS). O GWAS é um método exploratório que busca, por meio da análise simultânea dos genótipos de milhares de variantes nos genomas de um grande número de indivíduos, encontrar variantes que possam estar associadas a um fenótipo específico (DEHGHAN, 2018).

Em 2007, Frayling e colaboradores realizaram um GWAS com o objetivo de encontrar variantes de susceptibilidade para o diabetes tipo 2. Foram comparados 490.032 SNPs (*single nucleotide polymorphisms*) autossômicos de 1.924 pacientes portadores de diabetes tipo 2 com 2.938 indivíduos controle. Os autores encontraram uma forte associação de variantes presentes no gene *FTO (fat mass and obesity-associated)* com diabetes tipo 2, que é mediada através do IMC (FRAYLING et al., 2007). Desde então, muitos GWAS para obesidade foram realizados e com amostras cada vez maiores, resultando em mais de 900 variantes associadas à obesidade (YENGO et al., 2018). Dentre todos os loci identificados, o gene *FTO* explica a maior, mas ainda muito pequena, parte da variação genética do IMC: cerca de 0,34% (SPELIOTES et al., 2010). Todas as variantes associadas com o IMC em conjunto explicam por volta de apenas 6% da herdabilidade estimada (YENGO et al., 2018). Variantes no gene *FTO* também foram reportadas em GWAS de ingestão alimentar, cuja presença do alelo menos frequente foi associada a um maior consumo de proteínas (TANAKA et al., 2013). Entretanto, a herdabilidade da ingestão de macronutrientes explicada por GWAS é ainda mais baixa que a herdabilidade do IMC: 3,9% para carboidratos, 3,3% para lipídios e 3,2% para proteínas (MERINO et al., 2019).

A diferença entre a herdabilidade estimada por meio de estudos com famílias e a herdabilidade explicada por genotipagem em estudos de associação com doenças e características complexas é comumente chamada de herdabilidade perdida (GÉNIN, 2019). Várias hipóteses foram propostas na tentativa de explicar a herdabilidade perdida (EICHLER et al., 2010). Uma delas é a interação gene-gene e seu efeito em conjunto, principalmente quando estes genes estão correlacionados e/ou participam da mesma via associada ao fenótipo (TYLER et al., 2009). Para a determinação do seu efeito e o quanto isso influencia na herdabilidade, é necessário um modelo estatístico que inclua as variáveis de correlação e interação entre as variantes, o que é negligenciado em modelos de regressão tradicionais (MOORE; ASSELBERGS; WILLIAMS, 2010).

Apesar do modelo "*hypothesis-free*" do GWAS ter possibilitado novas associações de diversas variantes e vias biológicas com doenças e traços complexos (KITSIOS; ZINTZARAS, 2009), existem inúmeros trabalhos de associação na

literatura que investigam hipóteses funcionais a priori. A análise conjunta de variantes em genes relacionados aos neurotransmissores que proporcionam sensação de recompensa após a ingesta de alimentos palatáveis e aqueles relacionados com a redução do *feedback* homeostático pode ser considerada uma análise de genes da mesma rota com hipótese funcional. Considerando essa hipótese funcional, tais estudos baseiam-se em proteínas envolvidas diretamente no metabolismo e função da dopamina e serotonina, como enzimas e receptores, e seus respectivos genes.

A dopamina é sintetizada no sistema nervoso central e periférico a partir da tirosina por intermédio das enzimas tirosina hidroxilase, que converte a tirosina em L-diidroxifenilalanina (L-DOPA), e L-aminoácido aromático descarboxilase (DOPA-descarboxilase) que, por sua vez, converte a L-DOPA em dopamina (PURVES et al., 2001). Já a serotonina é derivada do aminoácido essencial triptofano, cuja principal fonte é proveniente da ingestão de proteínas. O transportador de aminoácidos neutros e catiônicos SLC6A14 tem sido relacionado à sinalização para liberação de hormônios reguladores do apetite. O SLC6A14 é expresso no íleo, intestino grosso e em células pancreáticas secretoras de polipeptídio pancreático e sua deficiência aumentou o risco de obesidade em ratos com dieta com alto teor de gorduras comparado com ratos selvagens (SIVAPRAKASAM et al., 2021). A hipótese é de que a presença de aminoácidos neutros e catiônicos, como o triptofano, ativariam as células que expressam o SLC6A14, provocando a liberação de hormônios anorexígenos (BHUTIA et al., 2022).

Após absorção, o triptofano é transportado para o tecido nervoso, onde sofre a ação da triptofano hidroxilase para produzir 5-hidroxitriptofano que, por fim, é convertido em serotonina pela L-aminoácido aromático descarboxilase. Após a síntese, os neurotransmissores são armazenados em vesículas. Na presença de um potencial de ação, há um influxo de Ca^{2+} que leva à fusão das vesículas à membrana pré-sináptica. Os neurotransmissores são liberados na fenda sináptica por exocitose, onde se ligarão a receptores específicos (PURVES et al., 2001).

Os receptores de dopamina pertencem à superfamília de receptores acoplados à proteína G e são classificados em dois grupos de acordo com sua estrutura, propriedades bioquímicas e farmacológicas: a classe de receptores D1, que engloba os receptores D1 e D5, encontrados exclusivamente na membrana pós-sináptica; e a

classe D2, que integra os receptores D2, D3 e D4, que podem ser encontrados tanto na membrana pós-sináptica como na membrana pré-sináptica (BEAULIEU; GAINETDINOV, 2011). A grande maioria dos receptores de serotonina também pertence à superfamília de receptores acoplados à proteína G e existem, ao todo, 14 subtipos de receptores de serotonina descritos, distribuídos em sete famílias (5-HT_{1-7}). À exceção do receptor 5-HT_{5B}, todos os demais receptores de serotonina são expressos no SNC. O subtipo 5-HT_{2B} também pode ser encontrado em outros tecidos, como fígado, rins e coração (MCCORVY; ROTH, 2015).

Os receptores de dopamina e serotonina estão envolvidos em diversas funções no SNC, incluindo a regulação da ingestão alimentar, que é mediada principalmente pelos receptores D2 e 5-HT_{2C}. Estudos realizados em humanos mostram que pessoas obesas possuem uma menor quantidade de receptores D2 no corpo estriado, o que também contribui para um aumento do consumo alimentar, e diversos estudos com animais também demonstram a correlação entre o ganho de peso e menor quantidade de receptores D2 (STICE et al., 2010a). Já a função do receptor 5-HT_{2C} de regulação do balanço energético é bem reconhecida, sendo este alvo de muitos fármacos com efeitos anorexígenos, como a lorcaserina, um agonista do receptor 5-HT_{2C} (SMITH et al., 2010). Além disso, como o receptor 5-HT_{2C} é coexpresso em neurônios dopaminérgicos na área tegmental ventral (área do mesencéfalo envolvida no circuito de recompensa), há evidências de que também esteja envolvido no comportamento alimentar hedônico (YAO et al., 2021).

A atividade sináptica da dopamina e serotonina é finalizada através da recaptação destes neurotransmissores pelo neurônio terminal, por meio do transportador de dopamina (DAT) nos neurônios dopaminérgicos e do transportador de serotonina (5-HTT) nos neurônios serotoninérgicos. Ambos os transportadores agem de forma Na^+/Cl^- dependente e regulam a concentração destes neurotransmissores na fenda sináptica. Após a recaptação, os neurotransmissores sofrem degradação principalmente pelas enzimas monoaminooxigenase (MAO), que atuam no catabolismo tanto da dopamina quanto da serotonina, e pela catecol-O-metiltransferase (COMT), que está envolvida na inativação da dopamina (BU, 2021; SIEGEL et al., 1999).

A MAO é expressa na membrana externa das mitocôndrias dos neurônios e células da glia, mas também em células de outros tecidos, como fígado, rins e intestino delgado (JONES; ANN, 2021). Duas isoformas da MAO foram identificadas e podem ser diferenciadas por sua especificidade a diferentes substratos. A MAO-A age principalmente no catabolismo da norepinefrina e serotonina, enquanto que a maior parte do catabolismo da dopamina é realizada pela MAO-B (MEISER; WEINDL; HILLER, 2013; SIEGEL et al., 1999). COMT também possui duas isoformas: a forma solúvel S-COMT, que é localizada principalmente nos tecidos periféricos, e M-COMT, encontrada nas membranas do retículo endoplasmático rugoso das células do SNC (MEISER; WEINDL; HILLER, 2013; SIEGEL et al., 1999). As proteínas envolvidas nas sinalizações dopaminérgica e serotoninérgica, com seus respectivos genes e variantes de interesse deste trabalho, estão representadas respectivamente nas Figuras 1 e 2.

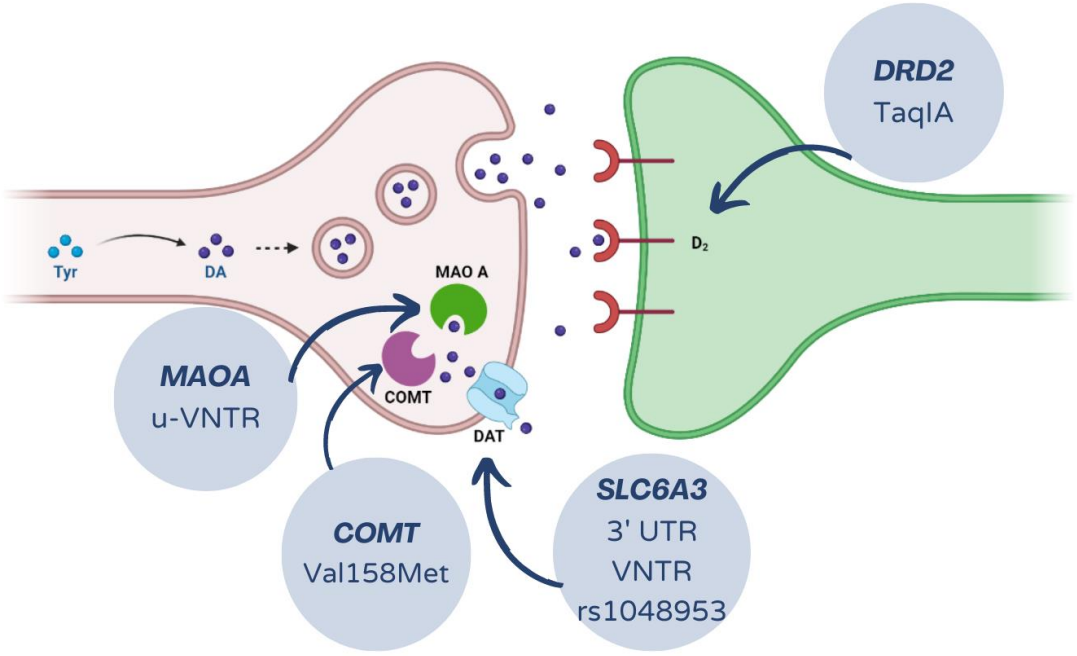

Figura 1: Representação da sinalização dopaminérgica, demonstrando enzimas e receptores que participam desta via, com seus respectivos genes e variantes estudadas neste trabalho.

A relação destas proteínas com ingestão alimentar também é evidenciada através de estudos moleculares e de associação com variantes de seus respectivos genes. Dentre as variantes descritas na literatura, destacam-se: *MAOA* u-VNTR,

COMT Val158Met; *SLC6A3* 3' UTR VNTR, *SLC6A3* rs1048953, *SLC6A4* HTTLPR5, *DRD2/ANKK1 Taq*IA, *HTR2C* rs3813928 (−997G/A), *HTR2C* rs3813929 (–759C/T), *SLC6A14* rs2071877, *SLC6A14* rs12391221 e *SLC6A14* rs2312054.

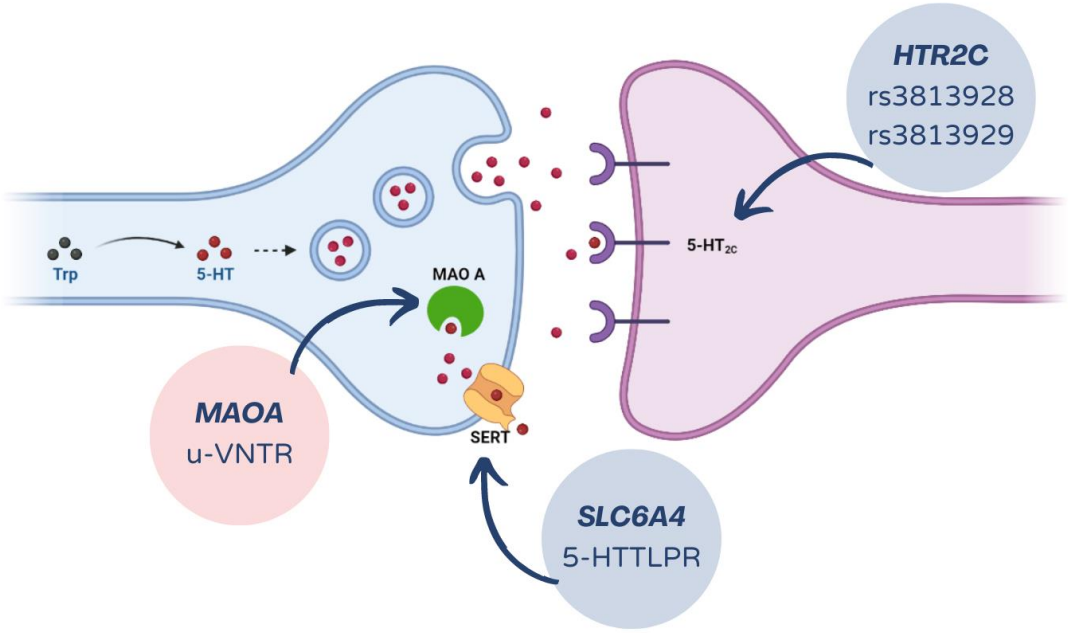

Figura 2: Representação da sinalização serotoninérgica, demonstrando enzimas e receptores que participam desta via, com seus respectivos genes e variantes estudadas neste trabalho.

Em 1998, Sabol e colaboradores identificaram uma repetição em tandem de número variável (VNTR – *variable number tandem repeats*) a 1,2 quilobases (kb) de distância acima da sequência codificante de *MAOA*, que mais tarde foi nomeada *MAOA* u-VNTR. A sequência repetitiva desta variante funcional possui 30 pares de base (pb) e os alelos podem conter 3, 3,5, 4 ou 5 repetições. Em estudo utilizando transfecção gênica em linhagens de células de neuroblastoma, os mesmos autores demonstraram que os alelos com 3,5 ou 4 cópias (alelos de alta atividade) foram expressos em níveis 2 a 10 vezes maiores que os alelos com 3 ou 5 cópias (alelos de baixa atividade) (SABOL; HU; HAMER, 1998). Em estudo conduzido com indivíduos depressivos, portadores de um dos alelos de baixa atividade e com sintomas depressivos de alta categoria consumiram mais alimentos não-adocicados hipercalóricos que os demais (AGURS-COLLINS; FUEMMELER, 2011). Diversos estudos também relatam a associação entre os alelos de baixa atividade com IMC e

obesidade (CAMARENA et al., 2004; DUCCI et al., 2006; FUEMMELER et al., 2008; NEED et al., 2006; WALLMEIER et al., 2013).

COMT rs4680, também relatado na literatura como *COMT* Val158Met, é uma variante não sinônima presente no exon 4 do gene, com alteração de uma guanina (G) para uma adenina (A). Esta variante refletiu na quantidade e atividade enzimática, sem alterar os níveis de mRNA, em estudo utilizando amostras de tecidos de córtex pré-frontal coletados de 108 indivíduos post-mortem. Indivíduos com genótipo Met/Met apresentaram menor atividade de COMT que os indivíduos com genótipo Val/Val, e indivíduos Val/Met apresentaram fenótipo intermediário, o que sugere que o alelo Val pode ter efeitos relativamente negativos na função pré-frontal, devido à maior degradação e, consequentemente, menor disponibilidade de dopamina (CHEN et al., 2004). Esta relação também foi demonstrada em estudos de associação com ingestão alimentar. De acordo com resultados do nosso grupo de pesquisa, em uma coorte de 354 crianças, houve um maior consumo de alimentos ricos em lipídios em crianças portadoras do alelo Val comparado com homozigotas Met/Met (GALVÃO et al., 2012). Outro estudo relatou maior desejabilidade por alimentos em geral e por alimentos considerados não saudáveis em indivíduos homozigotos Val/Val (WALLACE et al., 2015).

O gene do transportador DAT, *SLC6A3*, possui um VNTR na região 3' UTR que comumente apresenta 9 (9R) ou 10 (10R) repetições de 40 pb, apesar de alelos de 3 a 11 repetições já terem sido relatados (VANDENBERGH; PERSICO; UHL, 1992). O genótipo 10R/10R já foi associado com maior ingestão de alimentos ricos em lipídios e energeticamente densos em crianças de 3 a 4 anos de idade (FONTANA et al., 2015), com maior consumo de alimentos adocicados hipercalóricos em mulheres com sintomas depressivos de alta categoria (AGURS-COLLINS; FUEMMELER, 2011), e com maior IMC (AZZATO et al., 2009; EPSTEIN et al., 2002; GONZÁLEZ-GIRALDO; TRUJILLO; FORERO, 2018). Outra variante presente no gene *SLC6A3*, a rs1048953, estudada pelo nosso grupo de pesquisa, também foi associada ao consumo alimentar: houve maior ingestão energética diária em crianças de 3 a 4 anos idade portadoras do genótipo T/T que as demais (FONTANA et al., 2015).

O SERT também possui uma variante na região promotora em seu gene, *SLC6A4*, que interfere na transcrição. O *SLC6A4* 5-HTTLPR está localizado a cerca

de 1,5 kb acima do sítio de iniciação da transcrição e consiste em uma inserção/deleção de 44 pb, denominados alelos longo (L) e curto (S), respectivamente (HEILS et al., 1996). A expressão do gene foi estudada utilizando linhagens celulares de linfoblastos e descobriu-se que células portadoras do genótipo L/L produziram 1,4 a 1,7 vezes mais mRNA que as demais, o que também se refletiu ao nível de proteína e atividade: houve uma maior captação de serotonina em células L/L que as demais (LESCH et al., 1996). Apesar de resultados controversos, diversos estudos reportaram associação desta variante com sobrepeso/obesidade, IMC e comportamento alimentar (BONNET et al., 2017; BORKOWSKA et al., 2015; DIAS et al., 2016; FUEMMELER et al., 2008; LAN et al., 2009; MARKUS; CAPELLO, 2012; MIRANDA et al., 2017; SOOKOIAN et al., 2008; VAN STRIEN et al., 2016).

A *Taq*IA é uma variante de nucleotídeo único presente no exon 8 do gene *ANKK1*, região localizada a 10 kb abaixo do gene *DRD2*. O alelo C desta variante contém um sítio de restrição da enzima *Taq* I e é denominado alelo A2; já o alelo T é denominado alelo A1. Estudos realizados *in vivo* com indivíduos saudáveis demonstraram que a presença do alelo A1 está associada a uma menor disponibilidade do receptor D2 no corpo estriado (JÖNSSON et al., 1999; THOMPSON et al., 1997). Utilizando técnicas de neuroimagem funcional, portadores do alelo A1 apresentaram menor resposta em diversas áreas cerebrais à exposição a alimentos palatáveis (FELSTED et al., 2010; STICE et al., 2008, 2010b), além de que vários relatos na literatura evidenciam a associação do alelo A1 com maior ingestão de carboidratos, lipídios e alimentos hipercalóricos (AGURS-COLLINS; FUEMMELER, 2011; BARNARD et al., 2009; CAMERON et al., 2013; FEISTAUER et al., 2018; NOBLE et al., 1994).

Ratos deficientes do gene do receptor 2C de serotonina, *HTR2C*, apresentaram descontrole do comportamento alimentar com consequente aumento de peso em estudo realizado por Tecott e colaboradores (TECOTT et al., 1995). A expressão de *HTR2C* demonstrou ser afetada por duas variantes funcionais presentes na região promotora do gene: rs3813929 (–759C/T) e rs3813928 (−997G/A), sendo que as células contendo os alelos C e A demonstraram menor atividade transcricional que as demais (BUCKLAND et al., 2005). Ambos os polimorfismos já foram associados com ganho de peso induzido por antipsicóticos (BAH et al., 2010; GODLEWSKA et al.,

2009; OPGEN-RHEIN et al., 2010; POOLEY et al., 2004; VIMALESWARAN et al., 2010; YUAN et al., 2000). Nosso grupo de pesquisa também identificou uma associação entre o alelo A da variante rs3813928 e do alelo T da variante rs3813929 com maior média de ingestão energética diária, maior consumo de alimentos ricos em lipídios e maior média de ingestão energética diária por quilograma em crianças de 3 a 4 anos de idade (MIRANDA et al., 2015), .

O gene *SLC6A14* está localizado em uma região que já foi previamente associada com obesidade em estudo de ligação realizado com famílias finlandesas (ÖHMAN et al., 2000). O mesmo grupo de pesquisa também encontrou associação de variantes neste gene com obesidade (SUVIOLAHTI et al., 2003). Mais recentemente, foi demonstrado que ratos deficientes de *SLC6A14* ganharam mais peso que ratos normais quando expostos a uma dieta rica em lipídios (SIVAPRAKASAM et al., 2021). Três variantes intrônicas no gene *SLC6A14* foram estudadas pelo nosso grupo de pesquisa. Meninos de 7 a 8 anos portadores do alelo C da variante rs2071877 tiveram maior soma das dobras cutâneas subescapular e triciptal (MIRANDA et al., 2015); o mesmo alelo também foi associado com obesidade e ingestão alimentar em adultos de origem francesa (DURAND et al., 2004). Já em relação às variantes rs2312054 e rs12391221, vários parâmetros de ingestão alimentar foram maiores em crianças de 7 a 8 anos de idade portadoras do alelo A de ambas as variantes, como maior consumo energético diário, maior consumo de alimentos ricos em lipídios e maior consumo de alimentos energeticamente densos (MIRANDA et al., 2015).

2 JUSTIFICATIVA

A obesidade é, atualmente, um dos principais problemas de saúde no mundo todo. É um fator de risco importante para o desenvolvimento de várias doenças, gerando prejuízo na qualidade de vida do paciente e na saúde pública, uma vez que os custos para tratamento de comorbidades associadas à obesidade são elevados.

Apesar de existir tratamento para a obesidade, que consiste principalmente na mudança do estilo de vida, o resultado na maioria das vezes não é satisfatório. Controlar a alimentação e realizar atividade física, em um ambiente obesogênico, demanda muito mais do esforço cognitivo do paciente do que seu próprio metabolismo. Desse modo, tanto a perda, quanto a manutenção do peso, tornam-se um trabalho árduo e, muitas vezes, praticamente impossível de ser realizado.

Conhecer como as variantes genéticas, associadas ao sistema de recompensa alimentar, influenciam na ingestão alimentar e como atuam em conjunto é importante para que se possa entender mais a fundo a relação desta via com a obesidade e embasar novos tratamentos. Estas variantes e seus efeitos, analisados de forma conjunta, podem contribuir na determinação de parte da herdabilidade perdida da obesidade, visto que são descritos em estudos de associação, porém não foram relatadas em GWAS. Alguns fatores tentam explicar este fato: as amostragens utilizadas, talvez, ainda não tenham sido consideráveis o suficiente para alcançar um nível descritivo significante dessas variantes, algumas variantes possuem alelos com baixas frequências nas populações estudadas ou, ainda, que os desfechos clínicos abordados não sejam os ideais ou tenham sido coletados com a precisão necessária para detectar o efeito.

Portanto, uma análise integrativa dos resultados já obtidos em estudos com hipóteses funcionais, tanto pelo nosso grupo de pesquisa, como por outros grupos que realizaram estudos de associação do genoma completo, demonstra-se necessária e, possivelmente essencial, para a maior compreensão dos mecanismos relacionados à ingestão alimentar e, consequentemente, à obesidade.

3 OBJETIVOS

3.1 OBJETIVO GERAL

Analisar o efeito em conjunto de variantes genéticas relacionadas aos sistemas dopaminérgico e serotoninérgico, *SLC6A14* rs2071877, rs12391221 e rs2312054; *SLC6A4* 5-HTTLPR; *SLC6A3* 3' UTR VNTR e rs1048953; *HTR2C* rs3813928 e rs3813929; *DRD2/ANKK1 Taq*IA; *COMT* Val158Met; *MAOA* u-VNTR, na ingestão alimentar de duas coortes de crianças analisadas aos 12 meses, 3 anos e 6 anos de idade.

3.2 OBJETIVOS ESPECÍFICOS

– Gerar modelos de regressão de mínimos quadrados parciais (PLS – *partial least squares*) para avaliar o efeito em conjunto das variantes genéticas, com sexo, etnia e grupo de estudo original (controle/intervenção), na ingestão energética diária média de crianças aos 12 meses, 3 anos e 6 anos de idade e na ingestão de alimentos com alta densidade de açúcar (ADA) e gordura (ADG) aos 3 e 6 anos de idade;

– Gerar modelos de regressão PLS para avaliar o efeito em conjunto somente das variantes genéticas na ingestão energética diária média de crianças aos 12 meses, 3 anos e 6 anos de idade e na ingestão de ADA e ADG aos 3 e 6 anos de idade;

– Simular os valores de ingestão energética diária média das crianças cujos dados não foram utilizados para gerar os modelos (grupo de teste);

– Comparar os valores reais de ingestão energética diária média com os valores simulados pelos modelos.

4 ARTIGO CIENTÍFICO

O manuscrito intitulado ***The joint effect of variants of genes related to dopamine and serotonin action is associated with food intake in children*** foi submetido ao periódico *Appetite*, ISSN: 0195-6663, fator de impacto 5.016 (2021).

The joint effect of variants of genes related to dopamine and serotonin action is associated with food intake in children

Mayara Cristina Batista da Silva[1] mayaracbatista@hotmail.com
Márcia Regina Vitolo[1] vitolo@ufcspa.edu.br
Paula Dal Bó Campagnolo[2] pcampagnolo@unisinos.br
Vanessa Suñé Mattevi[3] vmattevi@ufcspa.edu.br
Vanessa Feistauer[3] vfeistauer@gmail.com
Edson Cordeiro do Valle[4] edsoncv@ufrgs.br
Silvana de Almeida[1,3] salmeida@ufcspa.edu.br

[1]Health Sciences Graduate Program, Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA), Rua Sarmento Leite, 245, Prédio 1, sala 309; CEP 90050-170, Porto Alegre, Rio Grande do Sul, Brazil.

[2]School of Health, Universidade do Vale do Rio dos Sinos (UNISINOS), Av. Unisinos, 950, Caixa Postal 275; CEP 93022-000, São Leopoldo, RS, Brazil.

[3]Biosciences Graduate Program, Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA), Rua Sarmento Leite, 245, Prédio 1, sala 309; CEP 90050-170, Porto Alegre, Rio Grande do Sul, Brazil.

[4]Department of Chemical Engineering, Universidade Federal do Rio Grande do Sul (UFRGS), Rua Engenheiro Luiz Englert, s/nº, Prédio 12.204, 90040-040, Porto Alegre, Rio Grande do Sul, Brazil.

Corresponding author: Silvana Almeida. Universidade Federal de Ciências da Saúde de Porto Alegre, Programa de Pós-graduação em Biociências, Rua Sarmento Leite, 245, Prédio 1, sala 309, 90050-170 Porto Alegre, RS, Brazil. E-mail: salmeida@ufcspa.edu.br. ORCID ID: 0000-0001-7959-3723.

ABSTRACT

Obesity is one of the major health issues worldwide and can lead to high morbidity and mortality. One of the causes for the high prevalence of obesity is the exposure to an obesogenic environment, leading to overconsumption of high palatable food and decreased physical activities. However, not everyone exposed to an obesogenic environment develops obesity, which means that a genetic background may be involved. Dopamine and serotonin are neurotransmitters intimately involved in hedonic eating and related genetic variants have been previously associated with obesity and food intake. Yet, most of these studies only focus on the individual effect of the variant, which tends to be very little in complex traits like obesity and food intake, and fail on capturing more complex interactions like gene-gene. In this study we used partial least squares (PLS) regression to analyze the joint effect of 11 dopamine and/or serotonin related genetic variants on average daily energy intake, sugar-dense and lipid-dense food in two cohorts of children followed until 6 years old and evaluated at 12 months, 3 years, and 6 years old. In our sample, the explained variability for daily energy intake models ranged from 65.2 to 83%. Our results showed not only that these dopamine and serotonin related variants are associated with daily energy of food intake, but also demonstrate the contribution of the joint effect of the variants to the outcome. To our knowledge, this is the first study using PLS regression to investigate the influence of genetic variants on food intake in children.

Keywords: Dopamine. Energy intake. Genetic variation. Obesity. Serotonin.

LIST OF ABBREVIATIONS

COVID-19 - Coronavirus Disease 2019
WHO - World Health Organization
VS - Ventral striatum
BOLD - Blood oxygenation level dependent
fMRI - Functional magnetic resonance imaging
DNA - Deoxyribonucleic acid
GRS - Genetic risk score
LDF - lipid-dense food
PLS - Partial least squares
PCA - Principal component analysis
SVD - Single Value Decomposition
NIPALS - Nonlinear Iterative Partial Least Squares
SLC6A14 - Solute carrier family 6 (amino acid transporter) member 14 gene
SLC6A4 - Solute carrier family 6 (neurotransmitter transporter) member 4 gene
5-HTTLPR - Serotonin transporter linked polymorphic region
SLC6A3 - Solute carrier family 6 (neurotransmitter transporter) member 3 gene
SDF - sugar-dense food
3' UTR - Three prime untranslated region
VNTR - Variable number tandem repeat
HTR2C - 5-hydroxytriptamine (serotonin) receptor 2C G protein-coupled gene
DRD2/ANKK1 - Dopamine receptor D2 and Ankyrin repeat and kinase domain containing 1 genes
*Taq*IA - *Taq*I A restriction fragment length polymorphism
COMT - Cathecol-O-methyltransferase gene
MAOA - Monoamine oxidase A gene
u-VNTR - Upstream variable number tandem repeat region
RS - Rio Grande do Sul
RFLP - Restriction fragment length polymorphism
PCR - Polymerase chain reaction
GWAS - Genome-wide association studies

PRS - Polygenic risk scores

BMI - Body mass index

1 INTRODUCTION

Obesity is a serious health condition associated with low quality of life and the development of several diseases, like type 2 diabetes, hypertension, coronary artery disease, and even some types of cancers (González-Muniesa et al., 2017). More recently, obesity has been shown to play a role in infections as well, increasing the risk of severe forms of COVID-19 (Cummings et al., 2020). In the last decades, obesity has become one of the biggest problems of public health worldwide. According to the World Health Organization (WHO), about 39% of the world's adult population were overweight and 13% were obese in 2016. That represents 1,9 billion overweight and 650 million obese adults, almost the triple observed in 1975. In the same year, more than 340 million children over the age of 5 and adolescents under the age of 19 were overweight or obese, and in 2020 about 39 million children under the age of 5 were obese (World Health Organization, 2021). In Brazil, the picture is even worse: more than a half (60.3%) of adult Brazilians were overweight, in 2019, and 25.9% were obese (Brasil/IBGE & Coordenação de trabalho e rendimento, 2020). In a systematic review of the nutritional status of Brazilian children and adolescents, 17.1% and 10.7% of children and adolescents between ages 6 and 18 years old were overweight and obese, respectively, and in those studies that reported just an excess of weight, 25% were overweight/obese (Pitanga et al., 2021).

Several factors contribute for the high prevalence of overweight and obesity, such as increased consumption of energy-dense food, low physical activity, socioeconomic factors, gender, age, and genetic factors. Easy access to inexpensive highly palatable energy-dense food and less energy expenditure are the basis of the obesogenic environment. Individuals exposed to an obesogenic environment can be induced to follow a hedonistic diet, that can result in overweight and obesity by overconsumption (Lee & Dixon, 2017; Ziauddeen et al., 2015). However, the literature review suggests that individual genetic background may also be involved with susceptibility for hedonistic diet habits (Jacob et al., 2018).

Dopamine and serotonin are neurotransmitters intimately involved in the process of hedonic eating. Association studies have shown evidence that genetic variants in dopamine and serotonin-related genes influence food response (Agurs-

Collins & Fuemmeler, 2011; Sardahaee et al., 2017; Stice et al., 2008; Wallace et al., 2015), body mass index (BMI) (Carr et al., 2013; Sookoian et al., 2007; Vimaleswaran et al., 2010), and obesity (Azzato et al., 2009; Durand et al., 2004; Fuemmeler et al., 2008; Need et al., 2008). Our research group has also found associations between those variants with food intake and adiposity parameters in children (Feistauer et al., 2018; Fontana et al., 2015; Galvão et al., 2012; Miranda et al., 2015, 2017).

Most of the association studies only focus on the individual effect of one genetic variant, which tends to be very little in complex traits like obesity or food intake behavior. Despite the effort that has been made to increase statistical power by increasing the sample sizes on association studies, it is still challenging to assess complex interactions such as gene-gene and gene-environment (Tam et al., 2019). Some studies have been conducted to assess the joint effect of variants on food intake. Nikolova et al. (2011) evaluated the impact of five dopamine related genetic variants on the reward-related ventral striatum (VS) reactivity measured with blood oxygenation level dependent (BOLD) imaging and functional magnetic resonance imaging (fMRI) in adults, which corresponded to 10.9% of the inter-individual variability (Nikolova et al., 2011). In a similar study, Stice et al. (2012) found that adolescents with a higher number of dopamine related variants showed a higher BOLD signal in DNA-based reward regions in response to palatable food receipt (Stice et al., 2012). In other study carried out in a sample of 1,757 adolescents, Sardahaee et al. (2017) calculated a genetic risk score (GRS) to assess the joint effect of obesity susceptibility variants on disordered eating and found that the GRS was associated to uncontrolled appetite/overeating in the total sample, and with overeating in 13 to 15 years old adolescents (Sardahaee et al., 2017).

To assess more complex interactions, partial least squares (PLS) method is briefly described. PLS is a multivariate regression technique which combines principal component analysis (PCA) and multivariate regression to build a mathematical model that predicts the value of a dependent variable, also known as output variables, based on values of independent variables, also known as input variables (Varmuza & Filzmoser, 2009). The main advantage in PLS is that it tries to remove the multicollinearity between the input variables before the regression itself, resulting in a

regression using a reduced space of independent variables, which results in models that are numerically more robust (less influenced by measurement errors).

A recent literature review carried out by us did not find previous association studies with the PLS regression technique for the evaluation of genetic susceptibility of average daily energy intake in children. Therefore, in this study, we evaluated the association of the joint effects of *SLC6A14* rs2071877, rs12391221, and rs2312054, *SLC6A4* 5-HTTLPR, *SLC6A3* 3' UTR VNTR and rs1048953, *HTR2C* rs3813928 and rs3813929, *DRD2/ANKK1* TaqIA, *COMT* Val158Met, and *MAOA* u-VNTR genetic variants on food intake of children at 12 months, 3 years, and 6 years old using this integrative method.

2 METHODS

2.1 Samples:

The total sample was composed of 1,074 children of which we had information about genetic variants (*SLC6A14* rs2071877, rs12391221, and rs2312054, *SLC6A4* 5-HTTLPR, *SLC6A3* 3' UTR VNTR and rs1048953, *HTR2C* rs3813928 and rs3813929, *DRD2/ANKK1* TaqIA, *COMT* Val158Met, and *MAOA* u-VNTR) and food intake data of them at three different life stages (12 months, 3 and 6 years old). The data were derived from two similar studies of our research group (Ferreira et al., 2019; Vitolo et al., 2008).

The data of sample 1 comprise information about 359 children from São Leopoldo-RS/Brazil, which were included in a randomized intervention, realized between October 2001 and July 2002 (Vitolo et al., 2005). The authors aimed to evaluate the impact of dietetical counseling on breastfeeding and the children's diet at the first years of age. The children's food intake data were collected at 12 months, 3 and 6 years old. More details about the sample can be found at Louzada et al. (2012) and Vitolo et al. (2010).

The sample 2 has information about 715 children of a cohort derived from a cluster randomized field trial, realized between April 2008 and September 2009 (Bernardi et al., 2011). The aim of this research was to evaluate the impact of health workers training about breastfeeding practice. The authors of this study recruited 715

mother-child pairs at basic health units from Porto Alegre – RS/Brazil. The children's biological sample and information about food intake were collected at 12 months, 3 and 6 years old (Chaffee et al., 2014; Sangalli et al., 2021).

Ethical approval to undertake this study was obtained from the Research Ethics Committees of the institutions involved. All mothers of the children included in the cohort signed an informed consent form when they were invited to participate of this study.

2.2 Genetic variants analyses:

Genetic variant analyses of the sample 1 were performed in previous studies. The genotypes were determined by conventional PCR followed by electrophoresis for *SLC6A4 5-HTTLPR* (Miranda et al., 2017), *SLC6A3* 3'UTR VNTR (Fontana et al., 2015), and *MAOAu-VNTR* (Galvão et al., 2012) polymorphisms; RFLP-PCR was used for *DRD2 Taq*IA rs1800497 (Feistauer et al., 2018), and *COMT* rs4680 (Galvão et al., 2012) polymorphisms and real-time PCR using TaqMan© system was used for *SLC6A14* (rs2071877, rs12391221, rs2312054) and *HTR2C* (rs3813328, rs3813329) polymorphisms (Miranda et al., 2015). The aim of these studies was to identify individual associations between the genetic variants with food intake and anthropometric parameters of children at three different stages of development. For sample 2, similar analyses were conducted, but the results of the individual associations were not published yet.

2.3 Outcome and independent variables:

For sample 1, we used average daily energy intake at the three ages, sugar-dense food (SDF - 50% or more sugar per 100 g in the composition, e.g., soda, Jell-O, candies and artificial juice) intake at 3 and 6 years old and lipid-dense food (LDF - 30% fat per 100 g, e.g., fried pastries, cookies with fillings, cold cuts and sausages, fried foods and chocolate) intake at 3 and 6 years old as outcomes, because of lack of information about SDF and LDF of the children at 12 months. For sample 2, we used average daily energy, SDF and LDF intake at the three ages as outcome. Information regarding food intake was collected using two 24-hours dietary recalls at an interval of 15 to 30 days. The interviewers asked the mothers about all food and drink consumed

by the children in the previous day, including details like food types, quantities, brands and preparation methods. Food consumption mean of the two 24-hours dietary recalls was used to estimate the average daily energy, SDF and LDF intake. Original study group (control/intervention), sex, ethnicity, and genetic variants were used as independent variables.

2.4 Data structuring and cleaning:

The data structuring and cleaning were performed using R software (R Core Team, 2015). To optimize the sample size, subsets were created and correspond to each outcome at the different life stages. Individuals with missing data of any independent variable or outcome were excluded from the analysis. Individuals that had SDF and/or LDF intake value equal to zero, that means, that did not ingest SDF or LDF food on the original studies, were also excluded from the analysis. The subsets have different sample sizes because of information losses throughout the original studies.

Also, for each subset, the sample 1 was randomly split in two groups: the training group (75%) and the test group (25%), defined arbitrarily according with final sample size of each subset. The training group sample was used for regression model construction and the sample of test group was used for evaluation of the algorithm generated from the PLS regression model. The sample 2 was entirely used for a novel training model with the objective of replicating the findings of the sample 1. This sample was not split in the training and test group because of the reduction of the final sample size after data cleaning step.

2.5 Statistical analyses:

PLS regression models of average daily energy intake, SDF and LDF intake were generated for each training group. These models were produced with data of average daily energy, SDF and LDF intake of the three/two collections of each sample. To evaluate the effect of the genetic variants alone on the outcome, we also generated PLS regression models using only the genetic variants as independent variables of each training group. The PLS regressions models and simulations with the test group were performed using VRAnalyst software (Finkler et al., 2006).

To evaluate whether there was difference between the real values of average energy intake and those predicted by the algorithm generated from the models in PLS regressions, we performed a paired sample T-test using the test group of sample 1. For this comparison, we used the real values of the average daily energy, SDF and LDF intake of each individual and their predicted values for the model with all independent variables and the model with genetic variants alone. These analyses were performed using SPSS software (SPSS, Chicago, IL, USA). A p value of <0.05 was considered significant.

3 RESULTS

To evaluate the joint effects of the independent variables on the average daily energy, SDF and LDF intake, we generated PLS regression models using information about food intake and genetic variants of children at three different stages of development (12 months, 3 years old and 6 years old). After data structuring and exclusion of individuals with missing genotypic data, for the average daily energy intake outcome, the total individuals of sample 1 subsets were 214 children at 12 months, 226 children at 3 years old and 199 children at 6 years old. Subsets of sample 2 consisted of a total of 141, 153 and 146 individuals at 12 months, 3 years old and 6 years old, respectively.

For sample 1, the models explained 68.9% (n = 214) of the average daily energy intake variability of children at 12 months, 79.3% (n = 226) at 3 years old, and 83% (n = 199) at 6 years old. When we evaluated the sample 2, the explained variability was 74% of the average daily energy intake of children at 12 months (n = 141), 81.7% (n = 153) at 3 years old and 80.9% (n = 146) at 6 years old. We also generated models to assess the joint effect of the genetic variants alone for the same outcome, the explained variability was 65.2% at 12 months, 77.1% at 3 years old and 80.3% at 6 years old for sample 1, and 68.7% at 12 months, 77.8% at 3 years old and 76.1% at 6 years old for sample 2 (Table 1).

Table 1: Explained variability, mean error and number of components of average daily energy intake at the three ages of children on both samples.

	Subsets	n	Explained variability		Mean error (kcal)		Components	
			AIV	OGV	AIV	OGV	AIV	OGV
Sample 1	12 months	214*	68.9%	65.2%	250	287	9	5
	3 years old	226*	79.3%	77.1%	252	287	9	6
	6 years old	199*	83%	80.3%	214	245	9	5
Sample 2	12 months	141	74%	68.7%	214	275	8	4
	3 years old	153	81.7%	77.8%	222	265	7	6
	6 years old	146	80.9%	76.1%	282	359	9	6

n: sample size; AIV: all independent variables; OGV: only genetic variants; *the sample size corresponds to the training group size, which is 75% of sample 1.

The explained variability of the models generated for SDF and LDF intake using all independent variables and only genetic variants as independent variables, on the two samples at each age, can be found at supplementary tables 1 and 2.

The comparison between real values of average daily energy, SDF and LDF intake and the simulated values of these outcomes for each child resulted on no significant difference between the real and the simulated values. The mean difference of real and simulated values of all variables model and only genetic variants model of average daily energy intake were, respectively: -73.93 and -24.29 kcal for 12 months, -65.27 and -42.84 kcal for 3 years old, and 32.17 and 84.29 kcal for 6 years old, which represents a percentage difference from the mean of the real values of average daily energy intake of -8.23% and -2.71% for 12 months, -4.44% and 2.91% for 3 years old, 2.02% and 5.29% for 6 years old (Table 2). For SDF and LDF intake, the results of the comparisons can be found at supplementary tables 3 and 4.

Table 2: Comparison between real and simulated values of average daily energy intake of sample 1 test group by paired sample t-test.

		n	Mean (kcal)*	SE Mean (kcal)	%**	*p*
12 months	All independent variables	72	-73.93	51.19	-8.23%	0.153
	Only genetic variants		-24.29	52.56	-2.71%	0.645
3 years old	All independent variables	76	-65.27	50.39	-4.44%	0.199
	Only genetic variants		-42.84	44.99	-2.91%	0.344
6 years old	All independent variables	67	32.17	53.60	2.02%	0.550
	Only genetic variants		84.29	55.68	5.29%	0.135

n: sample size; SE: standard error; *mean difference between real values and simulated values; ** percentage of the difference from the mean of the real values of average daily energy intake.

4 DISCUSSION

In the present study, we used PLS regression as an integrative approach to investigate the joint effects of those 11 dopamine and/or serotonin related genetic variants on average daily energy intake in two cohorts of children at 12 months, 3 years, and 6 years old. Some genetic variants analyzed by us have already been reported on individual association studies with outcomes related to food intake. In a cross-sectional study with 1,551 individuals, it has been found that males homozygous for *DRD2/ANKK1 Taq*IA*A2 allele consumed less of high-calorie non-sweet food than heterozygous and A1 homozygous (Agurs-Collins & Fuemmeler, 2011). The *DRD2/ANKK1 Taq*IA*A1 allele has also been associated with brain response at fMRI to high palatable food (Felsted et al., 2010; Stice et al., 2008, 2010). More recently, a cross-sectional study, conducted with 531 women from Iran, found that the group of *DRD2/ANKK1 Taq*IA *A1 allele carriers had higher BMI and hedonic hunger than A2 homozygous group (Aliasghari et al., 2021). Regarding to *COMT* rs4680 (Val158Met), *Val allele has been associated with higher desirability of “unhealthy” food (Wallace et al., 2015). In addition, several studies reported that *SLC6A4 5-HTTLPR*, *DRD2/ANKK1 Taq*IA, *MAOAu-VNTR*, *SLC6A14* rs2071877, *SLC6A3 3' UTR VNTR, 5-HTR2C* rs3813929, and *5-HTR2C* rs3813929 have also been associated to obesity/overweight, weight gain and BMI (Azzato et al., 2009; Bah et al., 2010; Carpenter et al., 2013; Ducci et al., 2006; Fuemmeler et al., 2008; González-Giraldo et al., 2018; Kring et al., 2009; Lan et al., 2009; Markus & Capello, 2012; Miller et al., 2005; Need et al., 2008; Noble et al., 1994; Pooley et al., 2004; Reynolds et al., 2003; Roth et al., 2013; Ryu et al., 2007; Schepers & Markus, 2017; Sookoian et al., 2007; Spitz et al., 2000; Suviolahti et al., 2003; Templeman et al., 2005; Winkler et al., 2012).

In a GWAS meta-analysis of macronutrient intake conducted with more than 30,000 individuals, Chu et al. (2013) found an explained variance for carbohydrate, protein and fat intake of 6.6%, 8% and 7.3% respectively (Chu et al., 2013). In another GWAS of dietary patterns of almost 450,000 individuals, the largest explained variance was 6.65% (Cole et al., 2020). A meta-analysis of GWAS for height and BMI in approximately 700,000 individuals found an explained variance of approximately

24.6% and 6.0% for height and BMI, respectively (Yengo et al., 2018). In the largest GWAS conducted to date, the authors have found an explained variance of 2.6% in 1,331,010 individuals with insomnia (Jansen et al., 2019). Although GWAS have been successfully associated more than 60,000 loci to many diseases and traits (MacArthur et al., 2017), which is a huge contribution to the understanding of genetic variation of common traits and genetic susceptibility of common diseases, it is still difficult to explain most of the variance of common diseases and traits. Using GWAS summary statistics, polygenic risk scores (PRS) are a widely used approach to estimate the genetic risk of complex diseases and traits (Khera et al., 2018, 2019; Mavaddat et al., 2019; Natarajan et al., 2017; Palla & Dudbridge, 2015; Song et al., 2021). PRSs are calculated as the sum of the risk alleles carried by an individual for a certain phenotype, weighted by their effect sizes (Konuma & Okada, 2021). However, since PRS are based in additive genetic models and assume that the genetic variants are independent, they don't consider gene-gene or gene-environment interactions (Choi et al., 2020; Lewis & Vassos, 2020). Alternative statistical methods are necessary to investigate those interactions.

To our knowledge, this is the first study using PLS regression to investigate the influence of genetic variants on food intake in children. With this method, we were able to obtain an explained variability ranging from 65.2% to 83% for average daily energy intake, which is a large proportion compared with other results reported for other multivariate linear regression models. This result can be explained by the difference in how the collinear independent variables are handled in PLS regression compared with multivariate regression.

A general multivariate linear model is presented by $y = b_0 + b_1.x_1 + b_2.x_2 + \cdots + + b_j.x_j + \cdots + b_m.x_m + e$ (Equation 1); where y: is the independent variable (also known as output variables), x_0 to x_m: are the independent variables (also known as input variables), b_0 to b_m: are the regression coefficients, m: the number of variables, e: the residual (error term). The residual, e, is defined by $e = y - \hat{y}$ (Equation 2); where $\hat{y}$ is the prediction of y by the model. The key point of the multivariate regression is to estimate the b_m coefficients of Equation 1 using a data set of observed values of x and y either by using analytical or by numerical methods (Konishi, 2014). The estimate of b_m coefficients is carried out minimizing the residuals (e, presented in Equation 2),

which is the difference between the observed output variables, y and the output predicted by the model, $\hat{y}$. Since in the multivariate regression a set of x and y values are used, Equation 1 is usually written in its matrix form: $\boldsymbol{Y} = \boldsymbol{b}.\boldsymbol{X} + \boldsymbol{e}$ (Equation 3); where $\boldsymbol{Y}$: is the column vector of the output values' observations of size l; $\boldsymbol{b}$: is the column vector of the coefficients of size m; $\boldsymbol{X}$: is the matrix of the independent variable values' observations of size $m \times l$; $\boldsymbol{e}$: is the vector of the residual (errors) of the model. After the regression the model of predicted $\mathbf{Y}$, can be presented by $\widehat{\boldsymbol{Y}} = \boldsymbol{X}.\boldsymbol{b}$ (Equation 4). The most common and analytical form of estimating the vector $\mathbf{b}$, is to solve using the least squares method in the matrix form. The first step to this approach is to multiply both sides of the Equation 4 by the transpose of matrix $\boldsymbol{X}$, as presented $\boldsymbol{X^T}.\widehat{\boldsymbol{Y}} = (\boldsymbol{X^T}.\boldsymbol{X}).\boldsymbol{b}$ (Equation 5). Then, multiplying both sides of Equation 5 by $(\boldsymbol{X^T}.\mathbf{X})^{-1}$, where the exponent "-1" denotes the operation of matrix inversion. The solution of Equation 5 is $\boldsymbol{b} = (\boldsymbol{X^T}.\mathbf{X})^{-1}.\boldsymbol{X^T}.\widehat{\mathbf{Y}}$ (Equation 6). Estimating the coefficients of a linear model using a linear regression have some limitations. When data from the independent variables are highly correlated, the matrix $(\boldsymbol{X^T}.\mathbf{X})^{-1}$ may be difficult or impossible to compute. The aforementioned problem may occur also when numerical methods are used to compute the coefficients of the model. Also, even when the data from independent variables are correlated and the inverse matrix $(\boldsymbol{X^T}.\mathbf{X})^{-1}$, can be calculated, the model estimation problem become very sensitive to errors in input variables data. This means that within the resulting model, small measurement errors in independent variables results in a wide range of model parameters, which degenerates the model robustness for output variables' prediction capability (Varmuza & Filzmoser, 2009).

To avoid the problem of regression with correlated input variables, several methods can be applied. The PLS method (Höskuldsson, 1988) is an effective and mathematically robust method to deal with the aforementioned problem. The main idea of the PLS method is transform the independent variables data in a new space where these data are not correlated using the PCA method. In a practical language, with the application of the PCA method, if a data set of two or more independent variables are correlated, they will be transformed in new data set of independent variables where the resulting variables are not correlated. A graphical representation of the PCA

transformation is presented at Figure 1, where a data set of three correlated variables is transformed in a 1-dimension component. Then with the resulted uncorrelated data, the estimation of the models' parameter can be carried out using regression techniques, e.g. estimating the parameters of the model, $\mathbf{b}$, that minimizes the difference between y and $\hat{y}$.

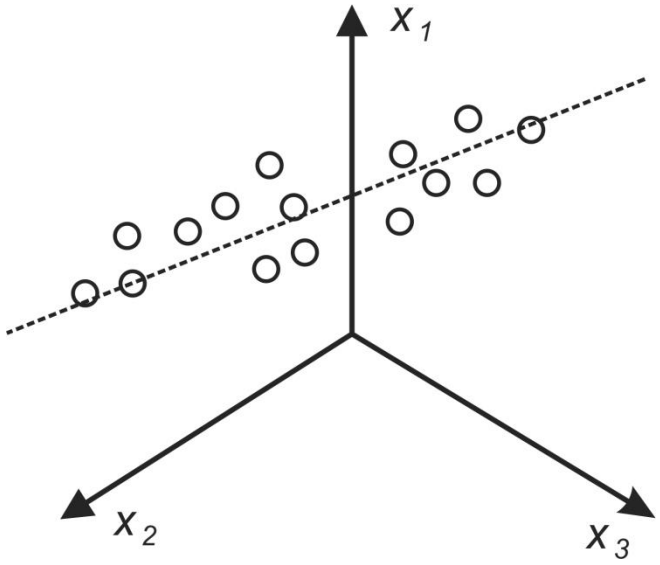

Figure 1: PCA method applied to a 3 variable data set resulting in a 1 component dimension.

Mathematically, the PLS regression is very simple. First the PCA technique is applied to the $\boldsymbol{X}$ matrix decomposing it into the scores matrix, $\boldsymbol{T}$, and the loadings matrix, $\boldsymbol{P}$, as presented $\boldsymbol{X} = \boldsymbol{T}.\boldsymbol{P}^{\boldsymbol{T}} + \boldsymbol{E}$ (Equation 7). The $\boldsymbol{T}$ matrix have a very powerful mathematical property since they are orthogonal to each other, so any two score vectors are uncorrelated. For this reason, it contains the maximum amount of information of $\boldsymbol{X}$ among all matrices of the data of independent variables. The next step for the PLS regression is the substitution of the $\boldsymbol{X}$ matrix from Equation 4, resulting in $\boldsymbol{Y} = (\boldsymbol{T}.\boldsymbol{P}^{\boldsymbol{T}}).\boldsymbol{b} + \boldsymbol{E}_{\boldsymbol{t}} = \boldsymbol{T}.\boldsymbol{g} + \boldsymbol{E}_{\boldsymbol{t}}$ (Equation 8)**;** where $\boldsymbol{g} = (\boldsymbol{P}^{\boldsymbol{T}}).\boldsymbol{b}$ is the new coefficient vector of Equation 8 that need to be estimated. Similarly, as Equation 6, the $\mathbf{g}$ vector can be estimated using $\boldsymbol{g} = (\boldsymbol{T}^{\boldsymbol{T}}.\boldsymbol{T})^{-1}.\boldsymbol{T}^{\boldsymbol{T}}.\widehat{\boldsymbol{Y}}$ (Equation 9). However, the vector $\boldsymbol{g}$, is not the original coefficient vector, and to obtain the original $\boldsymbol{b}$ vector it is necessary to carry out the transformation $\boldsymbol{b} = \boldsymbol{P}.\boldsymbol{g}$ (Equation 10)**.** It is important to notice that if the independent variable the data is correlated, the vector b, presented in Equation 10 will not be same as vector $\boldsymbol{b}$ calculated from Equation 6, but using the $\boldsymbol{b}$

calculated using Equation 9 and Equation 10 will result in a model with more robustness.

Although the mathematical approach to PLS regression is simple, the main issue in this method is the decomposition of the $\boldsymbol{X}$ matrix into the scores matrix, $\boldsymbol{T}$. It is also important to choose the minimum number of the dimension that eliminates the correlation between the data, but also avoid the loss of information within the data. Several methods are presented by the literature to obtain the number of dimensions and the $\boldsymbol{T}$ matrix appropriately, such as Jacobi Rotation, Single Value Decomposition (SVD) and Nonlinear Iterative Partial Least Squares algorithm (NIPALS) (Varmuza & Filzmoser, 2009).

In this study we evaluated genetic variants of related pathways, that have been previously associated with either food intake, BMI or other related phenotypes, in an integrative method to evaluate the joint effect of those variants on food intake. To do so, we used PLS regression, which is a method that reduces dimensionality while deals with multicollinearity. These may justify the explained variability obtained on our average daily energy intake models. Our method also allowed us to simulate the average daily energy, SDF and LDF intake from the algorithm generated with the PLS models, using the genotypic data of the test group. The simulated values had no statistically significant difference from the real values, with a difference from the mean of the real values ranging from -8.23% to 5.29% for average daily energy intake. For SDF and LDF intake outcomes of sample 1, the models with all independent variables and only genetic variants explained from 43.1% to 59.3% of the variability of children at 3 years and 6 years old, that was less than the average daily energy intake at the three ages. This can be elucidated by the possibility that the subjects, under parents control, might not have access to food freely, especially the ones that are known to be “unhealthy”. For sample 2, the explained variability of SDF and LDF intake ranged from 50.1% to 96.1%. Although the effect of dopamine and serotonin related genetic variants could be expected to be higher for palatable food intake outcomes, such as SDF and LDF intake, there’s a limitation that must be taken to account. As subjects that did not ingest any kind of SDF or LDF food were excluded from the analyses, the sample size of SDF and LDF intake of sample 2 resulted to be small. The highest explained variability of SDF and LDF intake of sample 2 were exactly of children at 12

450 months, which had the smallest sample size. In addition, the overall sample size was
451 limited, so the results must be interpreted with caution. We recommend further work
452 with larger sample sizes, other genes, and other outcomes to better evaluate the
453 reproducibility of this method in association studies of genetic variants with complex
454 traits or diseases.
455 Our study showed that serotonin and dopamine related genetic variants are
456 associated with daily energy food intake in our samples and strengthens the idea of
457 the contribution of the joint effects of these variants to the extent of the variation. These
458 findings help to increase our knowledge on the genetic influence on obesity
459 mechanism, mediated by complex interactions of genetic variants that can modulate
460 the food intake pattern. This knowledge can be further widened and applied in early
461 interventions to prevent obesity in individuals showing a genetic risk of dysregulated
462 feeding behavior, besides the possibility of new personalized therapies as
463 psychological managements and the implementation of health food intake environment
464 for the children. Therefore, we highlight the importance of future investigation towards
465 the understanding of the complex genetic interactions not only of obesity development,
466 but also other related complex phenotypes.
467
468 **ACKNOWLEDGEMENTS:** We are grateful to Crisciele Fontana, Raquel Krüger
469 Miranda, Silvia B. Vetter, Ananda Santos Galvão (*in memoriam*), and Mariana Eick for
470 laboratorial work in genetic analysis available in the research group's database.
471
472 **Author Contributions:**
473 The contribution of each author to the manuscript was as follows: M.C.B.S.,
474 E.C.V. and S.A.: designed research and conceived the project; P.D.B.C., V.F. and
475 M.R.V: performed the data collection and laboratory analyzes; M.C.B.S, and S.A.:
476 carried out the research; M.C.B.S and E.C.V.: performed literature search and
477 analyzed data; M.C.B.S. and S.A.: performed data interpretation and wrote the
478 manuscript; S.A.: had the primary responsibility for final content. All authors were
479 involved in writing the paper and had final approval of the submitted and published
480 versions.
481

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

REFERENCES

Agurs-Collins, T., & Fuemmeler, B. F. (2011). Dopamine polymorphisms and depressive symptoms predict foods intake. Results from a nationally representative sample. *Appetite*, *57*(2), 339–348. https://doi.org/10.1016/j.appet.2011.05.325

Aliasghari, F., Nazm, S. A., Yasari, S., Mahdavi, R., & Bonyadi, M. (2021). Associations of the ANKK1 and DRD2 gene polymorphisms with overweight, obesity and hedonic hunger among women from the Northwest of Iran. *Eating and Weight Disorders - Studies on Anorexia, Bulimia and Obesity*, *26*(1), 305–312. https://doi.org/10.1007/s40519-020-00851-5

Azzato, E. M., Morton, L. M., Bergen, A. W., Wang, S. S., Chatterjee, N., Kvale, P., Yeager, M., Hayes, R. B., Chanock, S. J., & Caporaso, N. E. (2009). SLC6A3and body mass index in the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial. *BMC Medical Genetics*, *10*(1), 9. https://doi.org/10.1186/1471-2350-10-9

Bah, J., Westberg, L., Baghaei, F., Henningsson, S., Rosmond, R., Melke, J., Holm, G., & Eriksson, E. (2010). Further exploration of the possible influence of polymorphisms in HTR2C and 5HTT on body weight. *Metabolism*, *59*(8), 1156–1163. https://doi.org/10.1016/j.metabol.2009.11.007

Bernardi, J. R., Gama, C. M., & Vitolo, M. R. (2011). Impacto de um programa de atualização em alimentação infantil em unidades de saúde na prática do aleitamento materno e na ocorrência de morbidade. *Cadernos de Saúde Pública*, *27*(6), 1213–1222. https://doi.org/10.1590/S0102-311X2011000600018

Brasil/IBGE, & Coordenação de trabalho e rendimento. (2020). *Pesquisa nacional de saúde 2019: atenção primária à saúde e informações antropométricas* (IBGE (ed.)).

Carpenter, C. L., Wong, A. M., Li, Z., Noble, E. P., & Heber, D. (2013). Association of dopamine D 2 receptor and leptin receptor genes with clinically severe obesity. *Obesity*, *21*(9), E467–E473. https://doi.org/10.1002/oby.20202

514 Carr, K. A., Lin, H., Fletcher, K. D., Sucheston, L., Singh, P. K., Salis, R. J., Erbe, R.
515 W., Faith, M. S., Allison, D. B., Stice, E., & Epstein, L. H. (2013). Two functional
516 serotonin polymorphisms moderate the effect of food reinforcement on BMI.
517 *Behavioral Neuroscience*, *127*(3), 387–399. https://doi.org/10.1037/a0032026
518 Chaffee, B. W., Vítolo, M. R., & Feldens, C. A. (2014). The Porto Alegre Early Life
519 Nutrition and Health Study. *Revista Brasileira de Epidemiologia*, *17*(4), 1015–
520 1018. https://doi.org/10.1590/1809-4503201400040018
521 Choi, S. W., Mak, T. S.-H., & O'Reilly, P. F. (2020). Tutorial: a guide to performing
522 polygenic risk score analyses. *Nature Protocols*, *15*(9), 2759–2772.
523 https://doi.org/10.1038/s41596-020-0353-1
524 Chu, A. Y., Workalemahu, T., Paynter, N. P., Rose, L. M., Giulianini, F., Tanaka, T.,
525 Ngwa, J. S., Qi, Q., Curhan, G. C., Rimm, E. B., Hunter, D. J., Pasquale, L. R.,
526 Ridker, P. M., Hu, F. B., Chasman, D. I., & Qi, L. (2013). Novel locus including
527 FGF21 is associated with dietary macronutrient intake. *Human Molecular*
528 *Genetics*, *22*(9), 1895–1902. https://doi.org/10.1093/hmg/ddt032
529 Cole, J. B., Florez, J. C., & Hirschhorn, J. N. (2020). Comprehensive genomic analysis
530 of dietary habits in UK Biobank identifies hundreds of genetic associations. *Nature*
531 *Communications*, *11*(1), 1467. https://doi.org/10.1038/s41467-020-15193-0
532 Cummings, M. J., Baldwin, M. R., Abrams, D., Jacobson, S. D., Meyer, B. J., Balough,
533 E. M., Aaron, J. G., Claassen, J., Rabbani, L. E., Hastie, J., Hochman, B. R.,
534 Salazar-Schicchi, J., Yip, N. H., Brodie, D., & O'Donnell, M. R. (2020).
535 Epidemiology, clinical course, and outcomes of critically ill adults with COVID-19
536 in New York City: a prospective cohort study. *The Lancet*, *395*(10239), 1763–
537 1770. https://doi.org/10.1016/S0140-6736(20)31189-2
538 Ducci, F., Newman, T. K., Funt, S., Brown, G. L., Virkkunen, M., & Goldman, D. (2006).
539 A functional polymorphism in the MAOA gene promoter (MAOA-LPR) predicts
540 central dopamine function and body mass index. *Molecular Psychiatry*, *11*(9),
541 858–866. https://doi.org/10.1038/sj.mp.4001856
542 Durand, E., Boutin, P., Meyre, D., Charles, M. A., Clement, K., Dina, C., & Froguel, P.
543 (2004). Polymorphisms in the Amino Acid Transporter Solute Carrier Family 6
544 (Neurotransmitter Transporter) Member 14 Gene Contribute to Polygenic Obesity

in French Caucasians. *Diabetes*, *53*(9), 2483–2486. https://doi.org/10.2337/diabetes.53.9.2483

Feistauer, V., Vitolo, M. R., Campagnolo, P. D. B., Mattevi, V. S., & Almeida, S. (2018). Evaluation of association of DRD2 TaqIA and -141C InsDel polymorphisms with food intake and anthropometric data in children at the first stages of development. *Genetics and Molecular Biology*, *41*(3), 562–569. https://doi.org/10.1590/1678-4685-gmb-2017-0202

Felsted, J. A., Ren, X., Chouinard-Decorte, F., & Small, D. M. (2010). Genetically Determined Differences in Brain Response to a Primary Food Reward. *Journal of Neuroscience*, *30*(7), 2428–2432. https://doi.org/10.1523/JNEUROSCI.5483-09.2010

Ferreira, V. R., Sangalli, C. N., Leffa, P. S., Rauber, F., & Vitolo, M. R. (2019). The impact of a primary health care intervention on infant feeding practices: a cluster randomised controlled trial in Brazil. *Journal of Human Nutrition and Dietetics*, *32*(1), 21–30. https://doi.org/10.1111/jhn.12595

Finkler, T. F., Soares, R. D. P., & Valle, E. C. D. (2006). *VRAnalyst*. VRTech Industrial Technologies.

Fontana, C., Vitolo, M. R., Campagnolo, P. D. B., Mattevi, V. S., Genro, J. P., & Almeida, S. (2015). DRD4 and SLC6A3 gene polymorphisms are associated with food intake and nutritional status in children in early stages of development. *The Journal of Nutritional Biochemistry*, *26*(12), 1607–1612. https://doi.org/10.1016/j.jnutbio.2015.07.030

Fuemmeler, B. F., Agurs-Collins, T. D., McClernon, F. J., Kollins, S. H., Kail, M. E., Bergen, A. W., & Ashley-Koch, A. E. (2008). Genes Implicated in Serotonergic and Dopaminergic Functioning Predict BMI Categories. *Obesity*, *16*(2), 348–355. https://doi.org/10.1038/oby.2007.65

Galvão, A. C. S., Krüger, R. C., Campagnolo, P. D. B., Mattevi, V. S., Vitolo, M. R., & Almeida, S. (2012). Association of MAOA and COMT gene polymorphisms with palatable food intake in children. *The Journal of Nutritional Biochemistry*, *23*(3), 272–277. https://doi.org/10.1016/j.jnutbio.2010.12.004

González-Giraldo, Y., Trujillo, M. L., & Forero, D. A. (2018). Two dopaminergic genes, DRD4 and SLC6A3 , are associated with body mass index in a Colombian sample

577 of young adults. *Archives of Physiology and Biochemistry*, *124*(4), 330–334.
578 https://doi.org/10.1080/13813455.2017.1401643
579 González-Muniesa, P., Mártinez-González, M.-A., Hu, F. B., Després, J.-P.,
580 Matsuzawa, Y., Loos, R. J. F., Moreno, L. A., Bray, G. A., & Martinez, J. A. (2017).
581 Obesity. *Nature Reviews Disease Primers*, *3*(1), 17034.
582 https://doi.org/10.1038/nrdp.2017.34
583 Höskuldsson, A. (1988). PLS regression methods. *Journal of Chemometrics*, *2*(3),
584 211–228.
585 Jacob, R., Drapeau, V., Tremblay, A., Provencher, V., Bouchard, C., & Pérusse, L.
586 (2018). The role of eating behavior traits in mediating genetic susceptibility to
587 obesity. *The American Journal of Clinical Nutrition*, *108*(3), 445–452.
588 https://doi.org/10.1093/ajcn/nqy130
589 Jansen, P. R., Watanabe, K., Stringer, S., Skene, N., Bryois, J., Hammerschlag, A. R.,
590 de Leeuw, C. A., Benjamins, J. S., Muñoz-Manchado, A. B., Nagel, M., Savage,
591 J. E., Tiemeier, H., White, T., Tung, J. Y., Hinds, D. A., Vacic, V., Wang, X.,
592 Sullivan, P. F., van der Sluis, S., … Posthuma, D. (2019). Genome-wide analysis
593 of insomnia in 1,331,010 individuals identifies new risk loci and functional
594 pathways. *Nature Genetics*, *51*(3), 394–403. https://doi.org/10.1038/s41588-018-
595 0333-3
596 Khera, A. V., Chaffin, M., Aragam, K. G., Haas, M. E., Roselli, C., Choi, S. H.,
597 Natarajan, P., Lander, E. S., Lubitz, S. A., Ellinor, P. T., & Kathiresan, S. (2018).
598 Genome-wide polygenic scores for common diseases identify individuals with risk
599 equivalent to monogenic mutations. *Nature Genetics*, *50*(9), 1219–1224.
600 https://doi.org/10.1038/s41588-018-0183-z
601 Khera, A. V., Chaffin, M., Wade, K. H., Zahid, S., Brancale, J., Xia, R., Distefano, M.,
602 Senol-Cosar, O., Haas, M. E., Bick, A., Aragam, K. G., Lander, E. S., Smith, G.
603 D., Mason-Suares, H., Fornage, M., Lebo, M., Timpson, N. J., Kaplan, L. M., &
604 Kathiresan, S. (2019). Polygenic Prediction of Weight and Obesity Trajectories
605 from Birth to Adulthood. *Cell*, *177*(3), 587-596.e9.
606 https://doi.org/10.1016/j.cell.2019.03.028
607 Konishi, S. (2014). *Introduction to Multivariate Analysis: Linear and Nonlinear*
608 *Modeling*. Chapman and Hall/CRC. https://doi.org/10.1201/b17077

609 Konuma, T., & Okada, Y. (2021). Statistical genetics and polygenic risk score for
610 precision medicine. *Inflammation and Regeneration*, *41*(1), 18.
611 https://doi.org/10.1186/s41232-021-00172-9
612 Kring, S. I. I., Werge, T., Holst, C., Toubro, S., Astrup, A., Hansen, T., Pedersen, O.,
613 & Sørensen, T. I. A. (2009). Polymorphisms of Serotonin Receptor 2A and 2C
614 Genes and COMT in Relation to Obesity and Type 2 Diabetes. *PLoS ONE*, *4*(8),
615 e6696. https://doi.org/10.1371/journal.pone.0006696
616 Lan, M. Y., Chang, Y. Y., Chen, W. H., Kao, Y. F., Lin, H. S., & Liu, J. S. (2009).
617 Serotonin transporter gene promoter polymorphism is associated with body mass
618 index and obesity in non-elderly stroke patients. *Journal of Endocrinological*
619 *Investigation*, *32*(2), 119–122. https://doi.org/10.1007/BF03345699
620 Lee, P. C., & Dixon, J. B. (2017). Food for Thought: Reward Mechanisms and Hedonic
621 Overeating in Obesity. *Current Obesity Reports*, *6*(4), 353–361.
622 https://doi.org/10.1007/s13679-017-0280-9
623 Lewis, C. M., & Vassos, E. (2020). Polygenic risk scores: from research tools to clinical
624 instruments. *Genome Medicine*, *12*(1), 44. https://doi.org/10.1186/s13073-020-
625 00742-5
626 Louzada, M. L. D. C., Campagnolo, P. D. B., Rauber, F., & Vitolo, M. R. (2012). Long-
627 term Effectiveness of Maternal Dietary Counseling in a Low-Income Population: A
628 Randomized Field Trial. *Pediatrics*, *129*(6), e1477–e1484.
629 https://doi.org/10.1542/peds.2011-3063
630 MacArthur, J., Bowler, E., Cerezo, M., Gil, L., Hall, P., Hastings, E., Junkins, H.,
631 McMahon, A., Milano, A., Morales, J., Pendlington, Z. M., Welter, D., Burdett, T.,
632 Hindorff, L., Flicek, P., Cunningham, F., & Parkinson, H. (2017). The new NHGRI-
633 EBI Catalog of published genome-wide association studies (GWAS Catalog).
634 *Nucleic Acids Research*, *45*(D1), D896–D901.
635 https://doi.org/10.1093/nar/gkw1133
636 Markus, C. R., & Capello, A. E. M. (2012). Contribution of the 5-HTTLPR gene by
637 neuroticism on weight gain in male and female participants. *Psychiatric Genetics*,
638 *22*(6), 279–285. https://doi.org/10.1097/YPG.0b013e328358632a
639 Mavaddat, N., Michailidou, K., Dennis, J., Lush, M., Fachal, L., Lee, A., Tyrer, J. P.,
640 Chen, T.-H., Wang, Q., Bolla, M. K., Yang, X., Adank, M. A., Ahearn, T., Aittomäki,

641 K., Allen, J., Andrulis, I. L., Anton-Culver, H., Antonenkova, N. N., Arndt, V., …
642 Easton, D. F. (2019). Polygenic Risk Scores for Prediction of Breast Cancer and
643 Breast Cancer Subtypes. *The American Journal of Human Genetics*, *104*(1), 21–
644 34. https://doi.org/10.1016/j.ajhg.2018.11.002
645 Miller, D. D., Ellingrod, V. L., Holman, T. L., Buckley, P. F., & Arndt, S. (2005).
646 Clozapine-induced weight gain associated with the 5HT2C receptor ?759C/T
647 polymorphism. *American Journal of Medical Genetics Part B: Neuropsychiatric*
648 *Genetics*, *133B*(1), 97–100. https://doi.org/10.1002/ajmg.b.30115
649 Miranda, R. C. K., Genro, J. P., Campagnolo, P. D. B., Mattevi, V. S., Vitolo, M. R., &
650 Almeida, S. (2017). Biallelic and triallelic approaches of 5-HTTLPR polymorphism
651 are associated with food intake and nutritional status in childhood. *The Journal of*
652 *Nutritional Biochemistry*, *43*, 47–52. https://doi.org/10.1016/j.jnutbio.2017.01.015
653 Miranda, R. C. K., Vetter, S. B., Genro, J. P., Campagnolo, P. D. B., Mattevi, V. S.,
654 Vitolo, M. R., & Almeida, S. (2015). SLC6A14 and 5-HTR2C polymorphisms are
655 associated with food intake and nutritional status in children. *Clinical Biochemistry*,
656 *48*(18), 1277–1282. https://doi.org/10.1016/j.clinbiochem.2015.07.003
657 Natarajan, P., Young, R., Stitziel, N. O., Padmanabhan, S., Baber, U., Mehran, R.,
658 Sartori, S., Fuster, V., Reilly, D. F., Butterworth, A., Rader, D. J., Ford, I., Sattar,
659 N., & Kathiresan, S. (2017). Polygenic Risk Score Identifies Subgroup With Higher
660 Burden of Atherosclerosis and Greater Relative Benefit From Statin Therapy in
661 the Primary Prevention Setting. *Circulation*, *135*(22), 2091–2101.
662 https://doi.org/10.1161/CIRCULATIONAHA.116.024436
663 Need, A. C., Ahmadi, K. R., Spector, T. D., & Goldstein, D. B. (2008). Obesity is
664 Associated with Genetic Variants That Alter Dopamine Availability. *Annals of*
665 *Human Genetics*, *70*(3), 293–303. https://doi.org/10.1111/j.1529-
666 8817.2005.00228.x
667 Nikolova, Y. S., Ferrell, R. E., Manuck, S. B., & Hariri, A. R. (2011). Multilocus Genetic
668 Profile for Dopamine Signaling Predicts Ventral Striatum Reactivity.
669 *Neuropsychopharmacology*, *36*(9), 1940–1947.
670 https://doi.org/10.1038/npp.2011.82
671 Noble, E. P., Noble, R. E., Ritchie, T., Syndulko, K., Bohlman, M. C., Noble, L. A.,
672 Zhang, Y., Sparkes, R. S., & Grandy, D. K. (1994). D2 dopamine receptor gene

673 and obesity. *International Journal of Eating Disorders*, *15*(3), 205–217.
674 https://doi.org/10.1002/1098-108X(199404)15:3<205::AID-
675 EAT2260150303>3.0.CO;2-P
676 Palla, L., & Dudbridge, F. (2015). A Fast Method that Uses Polygenic Scores to
677 Estimate the Variance Explained by Genome-wide Marker Panels and the
678 Proportion of Variants Affecting a Trait. *The American Journal of Human Genetics*,
679 *97*(2), 250–259. https://doi.org/10.1016/j.ajhg.2015.06.005
680 Pitanga, F. H., Souza, A. da S., Batista, G. D. S., & Rocha, R. E. R. da. (2021).
681 Nutritional status of children and adolescents in brazil: a systematic literature
682 review. *Brazilian Journal of Development*, 7(5), 46676–46695.
683 https://doi.org/10.34117/bjdv7n5-194
684 Pooley, E. C., Fairburn, C. G., Cooper, Z., Sodhi, M. S., Cowen, P. J., & Harrison, P.
685 J. (2004). A 5-HT2C receptor promoter polymorphism (HTR2C ? 759C/T) is
686 associated with obesity in women, and with resistance to weight loss in
687 heterozygotes. *American Journal of Medical Genetics*, *126B*(1), 124–127.
688 https://doi.org/10.1002/ajmg.b.20143
689 R Core Team. (2015). *R: A language environment for statistical computing.* R
690 Foundation for Statistical Computing. https://www.r-project.org/
691 Reynolds, G. P., Zhang, Z., & Zhang, X. (2003). Polymorphism of the Promoter Region
692 of the Serotonin 5-HT 2C Receptor Gene and Clozapine-Induced Weight Gain.
693 *American Journal of Psychiatry*, *160*(4), 677–679.
694 https://doi.org/10.1176/appi.ajp.160.4.677
695 Roth, C. L., Hinney, A., Schur, E. A., Elfers, C. T., & Reinehr, T. (2013). Association
696 analyses for dopamine receptor gene polymorphisms and weight status in a
697 longitudinal analysis in obese children before and after lifestyle intervention. *BMC*
698 *Pediatrics*, *13*(1), 197. https://doi.org/10.1186/1471-2431-13-197
699 Ryu, S., Cho, E. Y., Park, T., Oh, S., Jang, W.-S., Kim, S.-K., Lee, D., & Hong, K. S.
700 (2007). −759 C/T polymorphism of 5-HT2C receptor gene and early phase weight
701 gain associated with antipsychotic drug treatment. *Progress in Neuro-*
702 *Psychopharmacology and Biological Psychiatry*, *31*(3), 673–677.
703 https://doi.org/10.1016/j.pnpbp.2006.12.021

Sangalli, C. N., Leffa, P. S., Valmórbida, J. L., Lumey, L. H., & Vitolo, M. R. (2021). Impact of promoting healthy infant feeding practices on energy intake and anthropometric measures of children up to 6 years of age: A randomised controlled trial. *Journal of Human Nutrition and Dietetics*, *34*(5), 771–783. https://doi.org/10.1111/jhn.12881

Sardahaee, F. S., Holmen, T. L., Micali, N., & Kvaløy, K. (2017). Effects of single genetic variants and polygenic obesity risk scores on disordered eating in adolescents – The HUNT study. *Appetite*, *118*, 8–16. https://doi.org/10.1016/j.appet.2017.07.003

Schepers, R., & Markus, C. R. (2017). The interaction between 5-HTTLPR genotype and ruminative thinking on BMI. *British Journal of Nutrition*, *118*(8), 629–637. https://doi.org/10.1017/S0007114517002562

Song, N., Liu, Q., Wilson, C. L., Sapkota, Y., Ehrhardt, M. J., Gibson, T. M., Morton, L. M., Chanock, S. J., Neglia, J. P., Arnold, M. A., Michael, J. R., Gout, A. M., Mulder, H. L., Easton, J., Bhatia, S., Armstrong, G. T., Zhang, J., Delaney, A., Hudson, M. M., … Wang, Z. (2021). Polygenic Risk Score Improves Risk Stratification and Prediction of Subsequent Thyroid Cancer after Childhood Cancer. *Cancer Epidemiology, Biomarkers & Prevention*, *30*(11), 2096–2104. https://doi.org/10.1158/1055-9965.EPI-21-0448

Sookoian, S., Gemma, C., García, S. I., Fernández Gianotti, T., Dieuzeide, G., Roussos, A., Tonietti, M., Trifone, L., Kanevsky, D., González, C. D., & Pirola, C. J. (2007). Short Allele of Serotonin Transporter Gene Promoter Is a Risk Factor for Obesity in Adolescents*. *Obesity*, *15*(2), 271–276. https://doi.org/10.1038/oby.2007.519

Spitz, M. R., Detry, M. A., Pillow, P., Hu, Y., Amos, C. I., Hong, W. K., & Wu, X. (2000). Variant alleles of the D2 dopamine receptor gene and obesity. *Nutrition Research*, *20*(3), 371–380. https://doi.org/10.1016/S0271-5317(00)00130-5

Stice, E., Spoor, S., Bohon, C., & Small, D. M. (2008). Relation Between Obesity and Blunted Striatal Response to Food Is Moderated by Taq IA A1 Allele. *Science*, *322*(5900), 449–452. https://doi.org/10.1126/science.1161550

Stice, E., Yokum, S., Burger, K., Epstein, L., & Smolen, A. (2012). Multilocus Genetic Composite Reflecting Dopamine Signaling Capacity Predicts Reward Circuitry

736 Responsivity. *Journal of Neuroscience*, *32*(29), 10093–10100.
737 https://doi.org/10.1523/JNEUROSCI.1506-12.2012
738 Stice, E., Yokum, S., Zald, D., & Dagher, A. (2010). Dopamine-Based Reward Circuitry
739 Responsivity, Genetics, and Overeating. In *Brain Imaging in Behavioral*
740 *Neuroscience* (Issue November 2011, pp. 81–93).
741 https://doi.org/10.1007/7854_2010_89
742 Suviolahti, E., Oksanen, L. J., Öhman, M., Cantor, R. M., Ridderstråle, M., Tuomi, T.,
743 Kaprio, J., Rissanen, A., Mustajoki, P., Jousilahti, P., Vartiainen, E., Silander, K.,
744 Kilpikari, R., Salomaa, V., Groop, L., Kontula, K., Peltonen, L., & Pajukanta, P.
745 (2003). The SLC6A14 gene shows evidence of association with obesity. *Journal*
746 *of Clinical Investigation*, *112*(11), 1762–1772. https://doi.org/10.1172/JCI17491
747 Tam, V., Patel, N., Turcotte, M., Bossé, Y., Paré, G., & Meyre, D. (2019). Benefits and
748 limitations of genome-wide association studies. *Nature Reviews Genetics*, *20*(8),
749 467–484. https://doi.org/10.1038/s41576-019-0127-1
750 Templeman, L. A., Reynolds, G. P., Arranz, B., & San, L. (2005). Polymorphisms of
751 the 5-HT2C receptor and leptin genes are associated with antipsychotic drug-
752 induced weight gain in Caucasian subjects with a first-episode psychosis.
753 *Pharmacogenetics and Genomics*, *15*(4), 195–200.
754 https://doi.org/10.1097/01213011-200504000-00002
755 Varmuza, K., & Filzmoser, P. (2009). *Introduction to Multivariate Statistical Analysis in*
756 *Chemometrics*. CRC Press. https://doi.org/10.1201/9781420059496
757 Vimaleswaran, K. S., Zhao, J. H., Wainwright, N. W., Surtees, P. G., Wareham, N. J.,
758 & Loos, R. J. F. (2010). Association between serotonin 5-HT-2C receptor gene
759 (HTR2C) polymorphisms and obesity- and mental health-related phenotypes in a
760 large population-based cohort. *International Journal of Obesity*, *34*(6), 1028–
761 1033. https://doi.org/10.1038/ijo.2009.292
762 Vitolo, M. R., Bortolini, G. A., Dal Bó Campagnolo, P., & Feldens, C. A. (2008).
763 Effectiveness of a nutrition program in reducing symptoms of respiratory morbidity
764 in children: A randomized field trial. *Preventive Medicine*, *47*(4), 384–388.
765 https://doi.org/10.1016/j.ypmed.2008.07.008
766 Vitolo, M. R., Bortolini, G. A., Feldens, C. A., & Drachler, M. de L. (2005). Impactos da
767 implementação dos dez passos da alimentação saudável para crianças: ensaio

768 de campo randomizado. *Cadernos de Saúde Pública*, *21*(5), 1448–1457.
769 https://doi.org/10.1590/S0102-311X2005000500018
770 Vitolo, M. R., Rauber, F., Campagnolo, P. D. B., Feldens, C. A., & Hoffman, D. J.
771 (2010). Maternal Dietary Counseling in the First Year of Life Is Associated with a
772 Higher Healthy Eating Index in Childhood. *The Journal of Nutrition*, *140*(11),
773 2002–2007. https://doi.org/10.3945/jn.110.125211
774 Wallace, D. L., Aarts, E., d'Oleire Uquillas, F., Dang, L. C., Greer, S. M., Jagust, W. J.,
775 & D'Esposito, M. (2015). Genotype status of the dopamine-related catechol-O-
776 methyltransferase (COMT) gene corresponds with desirability of "unhealthy"
777 foods. *Appetite*, *92*(74–80), 74–80. https://doi.org/10.1016/j.appet.2015.05.004
778 Winkler, J. K., Woehning, A., Schultz, J.-H., Brune, M., Beaton, N., Challa, T. D.,
779 Minkova, S., Roeder, E., Nawroth, P. P., Friederich, H.-C., Wolfrum, C., &
780 Rudofsky, G. (2012). TaqIA polymorphism in dopamine D2 receptor gene
781 complicates weight maintenance in younger obese patients. *Nutrition*, *28*(10),
782 996–1001. https://doi.org/10.1016/j.nut.2011.12.018
783 World Health Organization (WHO). (2021). *Obesity and overweight*.
784 https://www.who.int/en/news-room/fact-sheets/detail/obesity-and-overweight
785 Yengo, L., Sidorenko, J., Kemper, K. E., Zheng, Z., Wood, A. R., Weedon, M. N.,
786 Frayling, T. M., Hirschhorn, J., Yang, J., & Visscher, P. M. (2018). Meta-analysis
787 of genome-wide association studies for height and body mass index in ~700000
788 individuals of European ancestry. *Human Molecular Genetics*, *27*(20), 3641–
789 3649. https://doi.org/10.1093/hmg/ddy271
790 Ziauddeen, H., Alonso-Alonso, M., Hill, J. O., Kelley, M., & Khan, N. A. (2015). Obesity
791 and the Neurocognitive Basis of Food Reward and the Control of Intake. *Advances*
792 *in Nutrition*, *6*(4), 474–486. https://doi.org/10.3945/an.115.008268
793
794

5 DISCUSSÃO

A obesidade é uma condição altamente heterogênea, portanto é necessário compreender cada aspecto para melhor manejo dos pacientes. A ativação do sistema de recompensa é um fator preponderante na regulação da ingestão alimentar, que modera o ganho de peso. Trabalhos recentes têm demonstrado que esta rota exerce influência na obesidade poligênica e também monogênica, uma vez que variantes que contribuem com o risco da obesidade poligênica podem influenciar na expressão de variantes monogênicas (CHAMI et al., 2020; LOOS; YEO, 2021)

Estudos com hipóteses funcionais, que foram realizados anteriormente pelo nosso grupo de pesquisa, investigaram as associações de cada variante genética avaliada neste trabalho, individualmente, com ingestão alimentar e parâmetros de adiposidade em crianças (FEISTAUER et al., 2018; FONTANA et al., 2015; GALVÃO et al., 2012; MIRANDA et al., 2015, 2017). Deste modo, as variantes incluídas no modelo de análise já haviam sido associadas com desfechos relacionados à ingestão alimentar previamente nesta amostra. No entanto, considerando que a ingestão alimentar é uma característica complexa, a avaliação da associação de variações genéticas em conjunto, assim como suas interações, pode representar um panorama mais fidedigno da contribuição genética dos genes relacionados aos sistemas dopaminérgico e serotoninérgico na ingestão alimentar de crianças em diferentes etapas do desenvolvimento. Para esta avaliação, nós aplicamos o método de regressão PLS, uma metodologia de análise ainda não utilizada para avaliação de variantes genéticas com este desfecho.

A regressão PLS foi desenvolvida nos anos 60 com a finalidade de resolver problemas como dados com pouca amostra e multicolinearidade em regressões múltiplas no campo da econometria, sendo nos dias atuais amplamente utilizada pela quimiometria (PIROUZ, 2006). O objetivo da regressão PLS é, principalmente, predizer uma ou mais variáveis dependentes, ou variáveis resposta, a partir de um conjunto de variáveis independentes, ou variáveis preditoras, através da redução das variáveis preditoras a um menor grupo de componentes não correlacionados (ABDI, 2007). Nós utilizamos como variáveis preditoras os dados das variantes genéticas, sexo, etnia e grupo de estudo original (caso/controle) e, como variáveis resposta, a

ingestão energética diária média nas três idades, ingestão de alimentos com alto teor de açúcar (ADA – mais de 50% da composição) e alimentos com alto teor de gordura (ADG – mais de 30% da composição) aos 3 e 6 anos de idade. Também foram gerados os mesmos modelos utilizando somente os dados das variantes genéticas como variáveis preditoras. Em geral, as variáveis preditoras utilizadas permitiram um ajuste de modelo que explicou de 65,2% a 83% da variação da ingestão energética diária média na nossa amostra. Para os demais desfechos, ADA e ADG, os modelos não ficaram bem ajustados, sendo que a variação explicada nos modelos ficou entre 24 e 54,2%.

Alguns fatores devem ser considerados na interpretação dos nossos resultados. A partir de uma revisão na literatura, não localizamos outros trabalhos que tenham utilizado a regressão PLS para investigar o efeito em conjunto de variantes genéticas na ingestão alimentar de crianças. Desta forma, os dados devem ser interpretados com cautela, porém, a simulação dos valores de ingestão energética diária média, a partir dos genótipos das crianças no subgrupo teste, demonstrou que os valores preditos foram similares aos valores reais, pois não houve diferença estatisticamente significante, o que indicaria a boa precisão dos modelos. Esta avaliação evidencia que, para este desfecho, nesta amostra, a regressão PLS foi um bom modelo preditor para análise de múltiplas variantes genéticas em conjunto.

Recentemente, apesar de ainda serem realizados, os estudos de associação, que utilizam hipótese funcional, perderam força diante dos estudos de genoma amplo. No entanto, a partir dos GWAS os dados têm sido gerados em uma velocidade maior do que a capacidade de analisá-los. De fato, os GWAS são de grande relevância, principalmente, para revelar novos loci e novas vias biológicas para o desfecho analisado. Entretanto, as evidências emergidas dos estudos com hipótese funcional não devem ser ignoradas. Se faz cada vez mais necessário o desenvolvimento de novos métodos de análise dos dados já obtidos, tanto com GWAS como estudos com hipótese funcional, para avançarmos no conhecimento dos mecanismos das doenças e características complexas.

O método de análise aplicado, neste estudo, pode ser útil para análise comprobatória, conjunta, de variantes genéticas que já possuem indícios de que estejam associadas a um fenótipo. Essa análise conjunta teria aplicações importantes,

principalmente, nas áreas de estudo em farmacogenética e nutrigenética, para a predição de respostas quantitativas ao efeito conjunto de variantes já conhecidas e associadas previamente. No entanto, ressaltamos que mais estudos, com outros desfechos e conjuntos de variantes, devem ser realizados para avaliar a aplicabilidade da regressão PLS na análise de associação de múltiplas variantes genéticas com características complexas.

6 REFERÊNCIAS

ABDI, H. Partial Least Square Regression. In: SALKIND, N. (Ed.). . **Encyclopedia of Measurement and Statistics**. Thousand Oaks (CA): Sage, 2007.

AGURS-COLLINS, T.; FUEMMELER, B. F. Dopamine polymorphisms and depressive symptoms predict foods intake. Results from a nationally representative sample. **Appetite**, v. 57, n. 2, p. 339–348, out. 2011.

ALBUQUERQUE, D. et al. The contribution of genetics and environment to obesity. **British Medical Bulletin**, v. 123, n. 1, p. 159–173, 1 set. 2017.

ANDOLFI, C.; FISICHELLA, P. M. Epidemiology of Obesity and Associated Comorbidities. **Journal of Laparoendoscopic and Advanced Surgical Techniques**, v. 28, n. 8, p. 919–924, 2018.

APOVIAN, C. M. Obesity: definition, comorbidities, causes, and burden. **The American journal of managed care**, v. 22, n. 7, p. s176–s185, 2016.

AZZATO, E. M. et al. SLC6A3 and body mass index in the prostate, lung, colorectal and ovarian cancer screening trial. **BMC Medical Genetics**, v. 10, p. 1–9, 2009.

BAH, J. et al. Further exploration of the possible influence of polymorphisms in HTR2C and 5HTT on body weight. **Metabolism**, v. 59, n. 8, p. 1156–1163, ago. 2010.

BARNARD, N. D. et al. D2 dopamine receptor Taq1A polymorphism, body weight, and dietary intake in type 2 diabetes. **Nutrition**, v. 25, n. 1, p. 58–65, jan. 2009.

BEAULIEU, J.-M.; GAINETDINOV, R. R. The Physiology, Signaling, and Pharmacology of Dopamine Receptors. **Pharmacological Reviews**, v. 63, n. 1, p. 182–217, mar. 2011.

BHUTIA, Y. D. et al. Unconventional Functions of Amino Acid Transporters: Role in Macropinocytosis (SLC38A5/SLC38A3) and Diet-Induced Obesity/Metabolic Syndrome (SLC6A19/SLC6A14/SLC6A6). **Biomolecules**, v. 12, n. 2, 2022.

BONNET, G. et al. Serotonin-transporter promoter polymorphism modulates the ability to control food intake: Effect on total weight loss. **Molecular Nutrition & Food Research**, v. 61, n. 11, p. 1700494, nov. 2017.

BORKOWSKA, A. et al. Effect of the 5-HTTLPR polymorphism on affective temperament, depression and body mass index in obesity. **Journal of Affective Disorders**, v. 184, p. 193–197, 2015.

BRASIL. MINISTÉRIO DA SAÚDE. **Vigitel Brasil 2017: Vigilância de Fatores de Risco e Proteção para Doenças Crônicas por Inquérito Telefônico**. Brasília: Ministério da Saúde, 2018.

BREEN, F. M.; PLOMIN, R.; WARDLE, J. Heritability of food preferences in young children. **Physiology & behavior**, v. 88, n. 4–5, p. 443–447, jul. 2006.

BU, M. Dynamic control of the dopamine transporter in neurotransmission and homeostasis. **npj Parkinson's Disease**, 2021.

BUCKLAND, P. R. et al. Low Gene Expression Conferred by Association of an Allele of the 5-HT 2C Receptor Gene With Antipsychotic-Induced Weight Gain. **American Journal of Psychiatry**, v. 162, n. 3, p. 613–615, mar. 2005.

BURGHES, A. H. M. GENETICS: The Land Between Mendelian and Multifactorial Inheritance. **Science**, v. 293, n. 5538, p. 2213–2214, 21 set. 2001.

CAMARENA, B. et al. Family-based association study between the monoamine oxidase A gene and obesity: Implications for psychopharmacogenetic studies. **Neuropsychobiology**, v. 49, n. 3, p. 126–129, 2004.

CAMERON, J. D. et al. The TaqIA RFLP is associated with attenuated intervention-induced body weight loss and increased carbohydrate intake in post-menopausal obese women. **Appetite**, v. 60, n. 1, p. 111–116, 2013.

CHAMI, N. et al. The role of polygenic susceptibility to obesity among carriers of pathogenic mutations in MC4R in the UK Biobank population. **PLOS Medicine**, v. 17, n. 7, p. e1003196, 21 jul. 2020.

CHEN, J. et al. Functional analysis of genetic variation in catechol-O-methyltransferase (COMT): Effects on mrna, protein, and enzyme activity in postmortem human brain. **American Journal of Human Genetics**, v. 75, n. 5, p. 807–821, 2004.

DE LEEUW, A. J. M. et al. Obesity and its impact on COVID-19. **Journal of Molecular Medicine**, v. 99, n. 7, p. 899–915, 2021.

DEHGHAN, A. Genome-Wide Association Studies. In: **Methods in Molecular Biology**. [s.l: s.n.]. v. 1793, p. 37–49, 07 jun. 2018.

DIAS, H. et al. Association of polymorphisms in 5-HTT (SLC6A4) and MAOA genes with measures of obesity in young adults of Portuguese origin. **Archives of Physiology and Biochemistry**, v. 122, n. 1, p. 8–13, 2016.

DUCCI, F. et al. A functional polymorphism in the MAOA gene promoter (MAOA-LPR) predicts central dopamine function and body mass index. **Molecular Psychiatry**, v. 11, n. 9, p. 858–866, 2006.

DURAND, E. et al. Polymorphisms in the amino acid transporter solute carrier family 6 (neurotransmitter transporter) member 14 gene contribute to polygenic obesity in French Caucasians. **Diabetes**, v. 53, n. 9, p. 2483–2486, 2004.

EICHLER, E. E. et al. Missing heritability and strategies for finding the underlying causes of complex disease. **Nature Reviews Genetics**, v. 11, n. 6, p. 446–450, 2010.

ELKS, C. E. et al. Variability in the Heritability of Body Mass Index: A Systematic Review and Meta-Regression. **Frontiers in Endocrinology**, v. 3, 2012.

EPSTEIN, L. H. et al. Dopamine Transporter Genotype as a Risk Factor for Obesity in African-American Smokers. **Obesity Research**, v. 10, n. 12, p. 1232–1240, dez. 2002.

FEISTAUER, V. et al. Evaluation of association of DRD2 taqIA and-141C InsDel polymorphisms with food intake and anthropometric data in children at the first stages of development. **Genetics and Molecular Biology**, v. 41, n. 3, p. 562–569, 2018.

FELSTED, J. A. et al. Genetically determined differences in brain response to a primary food reward. **Journal of Neuroscience**, v. 30, n. 7, p. 2428–2432, 2010.

FONTANA, C. et al. DRD4 and SLC6A3 gene polymorphisms are associated with food intake and nutritional status in children in early stages of development. **Journal of Nutritional Biochemistry**, v. 26, n. 12, p. 1607–1612, 2015.

FRAYLING, T. M. et al. A Common Variant in the FTO Gene Is Associated with Body Mass Index and Predisposes to Childhood and Adult Obesity. **Science**, v. 316, n. 5826, p. 889–894, 11 maio 2007.

FUEMMELER, B. F. et al. Genes implicated in serotonergic and dopaminergic functioning predict BMI categories. **Obesity**, v. 16, n. 2, p. 348–355, 2008.

GALVÃO, A. C. S. et al. Association of MAOA and COMT gene polymorphisms with palatable food intake in children. **Journal of Nutritional Biochemistry**, v. 23, n. 3, p. 272–277, 2012.

GÉNIN, E. Missing heritability of complex diseases: case solved? **Human Genetics**, v. 139, n. 1, p. 103–113, 2019.

GODLEWSKA, B. R. et al. Olanzapine-induced weight gain is associated with the -759C/T and -697G/ C polymorphisms of the HTR2C gene. **Pharmacogenomics Journal**, v. 9, n. 4, p. 234–241, 2009.

GONZÁLEZ-GIRALDO, Y.; TRUJILLO, M. L.; FORERO, D. A. Two dopaminergic genes, DRD4 and SLC6A3, are associated with body mass index in a Colombian sample of young adults. **Archives of Physiology and Biochemistry**, v. 124, n. 4, p. 330–334, 2018.

GONZÁLEZ-MUNIESA, P. et al. Obesity. **Nature Reviews Disease Primers**, v. 3, n. 1, p. 17034, 15 dez. 2017.

HASSELBALCH, A. L. et al. Studies of twins indicate that genetics influence dietary intake. **Journal of Nutrition**, v. 138, n. 12, p. 2406–2412, 2008.

HEILS, A. et al. Allelic variation of human serotonin transporter gene expression. **Journal of Neurochemistry**, v. 66, n. 6, p. 2621–2624, 1996.

HEWAGALAMULAGE, S. D. et al. Stress, cortisol, and obesity: a role for cortisol responsiveness in identifying individuals prone to obesity. **Domestic Animal Endocrinology**, v. 56, p. S112–S120, 2016.

HILL, J. O.; WYATT, H. R.; MELANSON, E. L. GENETIC AND ENVIRONMENTAL CONTRIBUTIONS TO OBESITY. **Medical Clinics of North America**, v. 84, n. 2, p. 333–346, mar. 2000.

IBGE. **Pesquisa de orçamentos familiares 2008-2009: antropometria e estado nutricional de crianças, adolescentes e adultos no Brasil**. Rio de Janeiro: Instituto Brasileiro de Geografia e Estatística, 2010.

JAMES, W. P. T. WHO recognition of the global obesity epidemic. **International Journal of Obesity**, v. 32, p. S120–S126, 2008.

JONES, D. N.; ANN, M. The role of monoamine oxidase enzymes in the pathophysiology of neurological disorders. **Journal of Chemical Neuroanatomy**, v. 114, p. 1-13, jul. 2021.

JÖNSSON, E. G. et al. Polymorphisms in the dopamine D2 receptor gene and their relationships to striatal dopamine receptor density of healthy volunteers. **Molecular Psychiatry**, v. 4, n. 3, p. 290–296, 1 maio 1999.

KESKITALO, K. et al. Genetic and environmental contributions to food use patterns of young adult twins. **Physiology & Behavior**, v. 93, n. 1–2, p. 235–242, jan. 2008.

KITSIOS, G. D.; ZINTZARAS, E. Genome-wide association studies: hypothesis-“free”

or “engaged”? **Translational Research**, v. 154, n. 4, p. 161–164, out. 2009.

LAN, M. Y. et al. Serotonin transporter gene promoter polymorphism is associated with body mass index and obesity in non-elderly stroke patients. **Journal of Endocrinological Investigation**, v. 32, n. 2, p. 119–122, 2009.

LEIGH, S. J.; MORRIS, M. J. The role of reward circuitry and food addiction in the obesity epidemic: An update. **Biological Psychology**, v. 131, p. 31–42, 2018.

LESCH, K.-P. et al. Association of Anxiety-Related Traits with a Polymorphism in the Serotonin Transporter Gene Regulatory Region. **Science**, v. 274, n. 5292, p. 1527–1531, 29 nov. 1996.

LIU, J. et al. Genetic and environmental influences on nutrient intake. **Genes and Nutrition**, v. 8, n. 2, p. 241–252, 2013.

LOOS, R. J. F.; YEO, G. S. H. The genetics of obesity: from discovery to biology. **Nature Reviews Genetics**, v. 0123456789, 2021.

MARKUS, C. R.; CAPELLO, A. E. M. Contribution of the 5-HTTLPR gene by neuroticism on weight gain in male and female participants. **Psychiatric Genetics**, v. 22, n. 6, p. 279–285, 2012.

MCCORVY, J. D.; ROTH, B. L. Structure and function of serotonin G protein-coupled receptors**. Pharmacology and Therapeutics**, v. 150, jun. 2015.

MEISER, J.; WEINDL, D.; HILLER, K. Complexity of dopamine metabolism. Cell Communication and Signaling, v. 11, n. 1, p. 34, 17 dez. 2013.

MERINO, J. et al. Genome-wide meta-analysis of macronutrient intake of 91,114 European ancestry participants from the cohorts for heart and aging research in genomic epidemiology consortium. **Molecular Psychiatry**, v. 24, n. 12, p. 1920–1932, 2019.

MIRANDA, R. C. K. et al. SLC6A14 and 5-HTR2C polymorphisms are associated with food intake and nutritional status in children. **Clinical Biochemistry**, v. 48, n. 18, p.

1277–1282, 2015.

MIRANDA, R. C. K. et al. Biallelic and triallelic approaches of 5-HTTLPR polymorphism are associated with food intake and nutritional status in childhood. **Journal of Nutritional Biochemistry**, v. 43, p. 47–52, 2017.

MITCHELL, N. S. et al. Obesity: Overview of an Epidemic. **Psychiatric Clinics of North America**, v. 34, n. 4, p. 717–732, 17 dez. 2011.

MOORE, J. H.; ASSELBERGS, F. W.; WILLIAMS, S. M. Bioinformatics challenges for genome-wide association studies. **Bioinformatics**, v. 26, n. 4, p. 445–455, 2010.

MURAMATSU, M.; OKUDA, A.; OKAZAKI, Y. Genomic aspects of common diseases. **Biochemical and Biophysical Research Communications**, v. 452, n. 2, p. 211–212, 2014.

NEED, A. C. et al. Obesity is associated with genetic variants that alter dopamine availability. **Annals of Human Genetics**, v. 70, n. 3, p. 293–303, 2006.

NOBLE, E. P. et al. D2 dopamine receptor gene and obesity. **International Journal of Eating Disorders**, v. 15, n. 3, p. 205–217, 1994.

ÖHMAN, M. et al. Genome-wide scan of obesity in finnish sibpairs reveals linkage to chromosome Xq24. **Journal of Clinical Endocrinology and Metabolism**, v. 85, n. 9, p. 3183–3190, 2000.

OPGEN-RHEIN, C. et al. Association of HTR2C, but not LEP or INSIG2, genes with antipsychotic-induced weight gain in a German sample. **Pharmacogenomics**, v. 11, n. 6, p. 773–780, 2010.

PIROUZ, D. M. An Overview of Partial Least Squares. **SSRN Electronic Journal**, v. 4, n. April, p. 1–55, 2006.

POOLEY, E. C. et al. A 5-HT2C Receptor Promoter Polymorphism (HTR2C -759C/T) Is Associated with Obesity in Women, and with Resistance to Weight Loss in Heterozygotes. **American Journal of Medical Genetics - Neuropsychiatric**

Genetics, v. 126 B, n. 1, p. 124–127, 2004.

PRICE, A. L.; SPENCER, C. C. A.; DONNELLY, P. Progress and promise in understanding the genetic basis of common diseases. **Proceedings of the Royal Society B: Biological Sciences**, v. 282, n. 1821, p. 20151684, 22 dez. 2015.

PURVES, D. et al. **Neuroscience. 2nd edition**. 2nd. ed. Sunderland: Sinauer Associates, 2001.

SABOL, S. Z.; HU, S.; HAMER, D. A functional polymorphism in the monoamine oxidase A gene promoter. **Human Genetics**, v. 103, n. 3, p. 273–279, 1998.

SIEGEL, G. J. et al. **Basic Neurochemistry: Molecular, Cellular and Medical Aspects**. 6. ed. Philadelphia: Lippincott-Raven, 1999.

SILVENTOINEN, K. et al. The genetic and environmental influences on childhood obesity: A systematic review of twin and adoption studies. **International Journal of Obesity**, v. 34, n. 1, p. 29–40, 2010.

SIVAPRAKASAM, S. et al. SLC6A14 deficiency is linked to obesity, fatty liver, and metabolic syndrome but only under conditions of a high-fat diet. **Biochimica et Biophysica Acta - Molecular Basis of Disease**, v. 1867, n. 5, p. 1-10, 2021.

SMITH, A. D. et al. Genetic and environmental influences on food preferences in adolescence. **American Journal of Clinical Nutrition**, v. 104, n. 2, p. 446–453, 2016.

SMITH, S. R. et al. Multicenter, Placebo-Controlled Trial of Lorcaserin for Weight Management. **New England Journal of Medicine**, v. 363, n. 3, p. 245–256, 15 jul. 2010.

SOOKOIAN, S. et al. Contribution of the functional 5-HTTLPR variant of the SLC6A4 gene to obesity risk in male adults. **Obesity**, v. 16, n. 2, p. 488–491, 2008.

SPELIOTES, E. K. et al. Association analyses of 249,796 individuals reveal 18 new loci associated with body mass index. **Nature Genetics**, v. 42, n. 11, p. 937–948, 10 nov. 2010.

STICE, E. et al. Relation between obesity and blunted striatal response to food is moderated by TaqIA A1 allele. **Science**, v. 322, n. 5900, p. 449–452, 2008.

STICE, E. et al. Reward circuitry responsivity to food predicts future increases in body mass: Moderating effects of DRD2 and DRD4. **NeuroImage**, v. 50, n. 4, p. 1618–1625, maio 2010.

STICE, E. et al. Dopamine-based reward circuitry responsivity, genetics, and overeating. **Current topics in behavioral neurosciences**, v. 6, p. 81–93, nov. 2011.

STICE, E. et al. The contribution of brain reward circuits to the obesity epidemic. **Neuroscience and Biobehavioral Reviews**, v. 37, n. 9, p. 2047–2058, nov. 2013.

SUVIOLAHTI, E. et al. The SLC6A14 gene shows evidence of association with obesity. **Journal of Clinical Investigation**, v. 112, n. 11, p. 1762–1772, 1 dez. 2003.

TANAKA, T. et al. Genome-wide meta-analysis of observational studies shows common genetic variants associated with macronutrient intake. **American Journal of Clinical Nutrition**, v. 97, n. 6, p. 1395–1402, 2013.

TECOTT, L. H. et al. Eating disorder and epilepsy in mice lacking 5-HT2C serotonin receptors. **Nature**, v. 374, n. 6522, p. 542–546, abr. 1995.

THOMPSON, J. et al. D2 dopamine receptor gene (DRD2) Taql A polymorphism: reduced dopamine D2 receptor binding in the human striatum associated with the A1 allele. **Pharmacogenetics**, v. 7, n. 6, p. 479–484, dez. 1997.

TYLER, A. L. et al. Shadows of complexity: what biological networks reveal about epistasis and pleiotropy. **BioEssays**, v. 31, n. 2, p. 220–227, fev. 2009.

VAN GALEN, K. A. et al. The role of central dopamine and serotonin in human obesity: lessons learned from molecular neuroimaging studies. **Metabolism: Clinical and Experimental**, v. 85, p. 325–339, 2018.

VAN STRIEN, T. et al. Emotional eating as a mediator between depression and weight gain. **Appetite**, v. 100, p. 216–224, 2016.

VANDENBERGH, D. J.; PERSICO, A. M.; UHL, G. R. A human dopamine transporter cDNA predicts reduced glycosylation, displays a novel repetitive element and provides racially-dimorphic TaqI RFLPs. **Molecular Brain Research**, v. 15, n. 1–2, p. 161–166, set. 1992.

VIMALESWARAN, K. S. et al. Association between serotonin 5-HT-2C receptor gene (HTR2C) polymorphisms and obesity-and mental health-related phenotypes in a large population-based cohort. **International Journal of Obesity**, v. 34, n. 6, p. 1028–1033, 2010.

WALLACE, D. L. et al. Genotype status of the dopamine-related catechol-O-methyltransferase (COMT) gene corresponds with desirability of “unhealthy” foods. **Appetite**, v. 92, n. 74–80, 2015.

WALLMEIER, D. et al. Genetic modulation of the serotonergic pathway: Influence on weight reduction and weight maintenance. **Genes and Nutrition**, v. 8, n. 6, p. 601–610, 2013.

WARDLE, J. et al. Evidence for a strong genetic influence on childhood adiposity despite the force of the obesogenic environment. **American Journal of Clinical Nutrition**, v. 87, n. 2, p. 398–404, 2008.

WORLD HEALTH ORGANIZATION (WHO). **Obesity and overweight**. Disponível em: <https://www.who.int/en/news-room/fact-sheets/detail/obesity-and-overweight>. Acesso em: 6 out. 2019.

YABUT, J. M. et al. Emerging Roles for Serotonin in Regulating Metabolism: New Implications for an Ancient Molecule. **Endocrine Reviews**, v. 40, n. 4, p. 1092–1107, 2019.

YANOVSKI, J. A. Pediatric obesity: An introduction. **Appetite**, v. 93, p. 3–12, 1 out. 2015.

YAO, T. et al. Central 5-HTR2C in the Control of Metabolic Homeostasis. **Frontiers in Endocrinology**, v. 12, n. July, p. 1–12, 21 jul. 2021.

YENGO, L. et al. Meta-analysis of genome-wide association studies for height and body mass index in ~700 000 individuals of European ancestry. **Human Molecular Genetics**, v. 27, n. 20, p. 3641–3649, 2018.

YUAN, X. et al. Identification of polymorphic loci in the promoter region of the serotonin 5-HT(2c) receptor gene and their association with obesity and Type II diabetes. **Diabetologia**, v. 43, n. 3, p. 373–376, 2000.

ZIAUDDEEN, H. et al. Obesity and the Neurocognitive Basis of Food Reward and the Control of Intake. **Advances in Nutrition**, v. 6, n. 4, p. 474–486, 15 jul. 2015.

ANEXOS

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Você, na qualidade de pai/mãe ou representante legal, está sendo convidado a permitir a participação de seu filho(a) na pesquisa intitulada: **"Avaliação da associação de variantes genéticas com a ingestão alimentar e o status nutricional, bioquímico e hematológico em crianças".** Os procedimentos previstos nesta pesquisa estão de acordo com orientações nacionais (Resolução 466/12 do CNS/MS) para pesquisas envolvendo seres humanos.

I. Por que realizar essa pesquisa e qual o objetivo dela?

A obesidade, assim como, a falta de nutrientes na infância pode trazer muitas consequências à saúde. A predisposição genética associada à alimentação inadequada pode aumentar o risco destas complicações. O objetivo deste estudo é verificar se determinadas diferenças genéticas estão associadas com a obesidade infantil e anemia.

II. Qual o procedimento que será utilizado?

Para a análise de DNA nós utilizaremos uma parte da amostra de sangue que seu filho já coletou para os outros exames de sangue (bioquímicos e hematológicos) da pesquisa "Impacto nas condições nutricionais e de saúde de crianças na idade de 6-7 anos que participaram de um ensaio de campo randomizado por conglomerados no primeiro ano de vida". Ao final do presente trabalho, as amostras de DNA serão guardadas por 10 anos para, eventualmente, serem utilizadas em futuras pesquisas sobre esse assunto. A utilização em estudos futuros somente será realizada mediante nova aprovação do Comitê de Ética em Pesquisa. Todos os dados que relacionem a identidade de seu filho (a) com os dados obtidos serão separados em diferentes bancos de dados

III. Quais os riscos em participar?

Os riscos em participar da pesquisa são mínimos e estão relacionados à coleta de sangue já autorizada e realizada anteriormente.

IV. Quais os benefícios em participar deste estudo?

Embora esta pesquisa não possa gerar nenhum benefício imediato aos participantes, ela poderá trazer vários benefícios em longo prazo, quando será possível conhecer quais pessoas possuem características genéticas associadas com a obesidade e anemia. Por fim, a sua participação ajudará no desenvolvimento de novos conhecimentos, que poderão eventualmente beneficiar você, seu filho (a) e outras pessoas no futuro.

V. Com quem você pode esclarecer suas dúvidas?

Em caso de qualquer dúvida quanto à pesquisa ou para saber sobre os seus direitos, você poderá contatar a qualquer momento durante a pesquisa, a professora Dra. Silvana de Almeida, Doutora em Genética e Biologia Molecular e professora da Universidade Federal de Ciências da Saúde de Porto Alegre, responsável pelo estudo, pelo telefone (51) 3303-8763. Assim como, com o Comitê de Ética em Pesquisa da UFCSPA (51) 3303-8804, Rua Sarmento Leite, 245, CEP 90050-170, Porto Alegre.

VI. Quais são os seus direitos e de seu filho?

A participação de seu filho no estudo é voluntária. Caso você decida não participar, isto não afetará no tratamento normal que você e seu filho têm direito. Além disso, você tem liberdade para abandonar a pesquisa a qualquer momento sem nenhum prejuízo, devendo apenas contatar a responsável pelo estudo (citada logo acima). Os seus registros médicos serão sempre tratados confidencialmente. Os resultados deste estudo poderão ser usados para fins científicos, mas você não será identificado pelo nome. Você receberá uma cópia deste termo de consentimento.

CEP/UFCSPA
TCLE
APROVADO
14/10/14

FORMULÁRIO DE CONSENTIMENTO PARA PARTICIPAR DE UM ESTUDO EM GENÉTICA

Eu, ...(representante legal/pai ou mãe) fui informado dos objetivos da pesquisa acima de maneira clara e detalhada. Recebi informação a respeito do procedimento e esclareci minhas dúvidas. Sei que em qualquer momento poderei solicitar novas informações e terei liberdade de retirar o consentimento de participação de meu filho (a) na pesquisa. O pesquisador (a) certificou-me de que todos os dados desta pesquisa serão confidenciais, bem como o meu tratamento não será modificado em razão desta pesquisa.

Caso tiver novas perguntas sobre este estudo, posso chamar Dra. Silvana de Almeida no telefone (51) 3303-8763. Para qualquer pergunta sobre os meus direitos como participante deste estudo ou se penso que fui prejudicado pela minha participação, posso chamar Dra. Silvana de Almeida no telefone (51) 3303-8763 ou o Comitê de Ética em Pesquisa da UFCSPA (Rua Sarmento Leite, 245,CEP 90050-170, Porto Alegre, telefone (51) 3303-8804).

Declaro que recebi cópia do presente Termo de Consentimento.

_____ _____

Assinatura representante legal **Nome representante legal**

_____ Silvana de Almeida

Assinatura pesquisador responsável **Nome Pesquisador responsável**

_____ ____/____/____

Nome da criança **Data**

Termo de Assentimento para crianças de 7 anos:

Eu, _____, recebi as informações sobre os objetivos e importância desta pesquisa de forma clara e concordo em participar do estudo.

_____

Assinatura da criança

Nome da criança:

Data:

CEP/UFCSPA
TCLE
APROVADO

UNIVERSIDADE FEDERAL DE CIÊNCIAS DA SAÚDE DE PORTO ALEGRE

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: INVESTIGAÇÃO DA CO-ASSOCIAÇÃO DE VARIANTES DE GENES RELACIONADOS AOS SISTEMAS DOPAMINÉRGICO E SEROTONINÉRGICO COM OBESIDADE E FENÓTIPOS RELACIONADOS EM CRIANÇAS E ADULTOS

Pesquisador: Silvana de Almeida

Área Temática: Genética Humana:
(Trata-se de pesquisa envolvendo Genética Humana que não necessita de análise ética por parte da CONEP;);

Versão: 1

CAAE: 30208220.6.0000.5345

Instituição Proponente: Universidade Federal de Ciências da Saúde de Porto Alegre

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 3.977.156

Apresentação do Projeto:

Trata-se de um projeto de mestrado do Programa de Pós-graduação em Ciências de Saúde intitulado INVESTIGAÇÃO DA CO-ASSOCIAÇÃO DE VARIANTES DE GENES RELACIONADOS AOS SISTEMAS DOPAMINÉRGICO E SEROTONINÉRGICO COM OBESIDADE E FENÓTIPOS RELACIONADOS EM CRIANÇAS E ADULTOS

Objetivo da Pesquisa:

Objetivo geral: Analisar de forma integrativa a co-associação de variantes em genes relacionados aos sistemas dopaminérgico e serotoninérgico com obesidade e desfechos associados.

Objetivos específicos: – Analisar de forma integrativa a co-associação de variantes em genes, relacionados aos sistemas dopaminérgico e serotoninérgico, com ingestão alimentar e estado nutricional de crianças, utilizando banco de dados genéticos já analisados individualmente gene a gene pelo grupo de pesquisa. – Avaliar a co-associação de variantes em genes, relacionados aos sistemas dopaminérgicos e serotoninérgicos, com obesidade utilizando bancos de dados públicos de GWAS. – Implementar a metodologia score-based statistics (SBS) através de scripts (sequência lógica de comandos) usando o aplicativo “R”.

Endereço: Rua Sarmento Leite ,245
Bairro: Sarmento **CEP:** 90.050-170
UF: RS **Município:** PORTO ALEGRE
Telefone: (51)3303-8804 **E-mail:** cep@ufcspa.edu.br

UNIVERSIDADE FEDERAL DE CIÊNCIAS DA SAÚDE DE PORTO ALEGRE

Avaliação dos Riscos e Benefícios:

Os riscos são mínimos e estão relacionados à coleta de sangue já autorizada e realizada anteriormente. Os benefícios são indiretos, com a produção de conhecimento sobre identificação das características genéticas associadas à obesidade e anemia.

Comentários e Considerações sobre a Pesquisa:

A pesquisa consiste basicamente em análises estatísticas realizadas em uma base de dados já coletada anteriormente e aprovada pelo CEP. O TCLE indica a possibilidade da utilização dos dados para futuras pesquisas.

Considerações sobre os Termos de apresentação obrigatória:

Folha de rosto devidamente assinada.

Termo de entrega de relatório.

Conclusões ou Pendências e Lista de Inadequações:

Sem pendências

Considerações Finais a critério do CEP:

De acordo com o parecer do Relator.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1526052.pdf	20/03/2020 17:29:54		Aceito
Folha de Rosto	FolhadeRostoAssinada.pdf	20/03/2020 17:28:46	Silvana de Almeida	Aceito
Outros	TermoCompromissoEntregaRelatorioAssinado.pdf	20/03/2020 17:24:53	Silvana de Almeida	Aceito
Projeto Detalhado / Brochura Investigador	Projeto_CEP.docx	13/03/2020 11:30:23	Vanessa Feistauer	Aceito
Parecer Anterior	Parcer_previo_CEP_Amostra.doc	13/03/2020 11:30:13	Vanessa Feistauer	Aceito
Parecer Anterior	Parecer_previo_CEP_analise_molecular.pdf	13/03/2020 11:30:05	Vanessa Feistauer	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_POA7_8anos.PDF	13/03/2020 11:28:25	Vanessa Feistauer	Aceito

Endereço: Rua Sarmento Leite ,245
Bairro: Sarmento **CEP:** 90.050-170
UF: RS **Município:** PORTO ALEGRE
Telefone: (51)3303-8804 **E-mail:** cep@ufcspa.edu.br

UNIVERSIDADE FEDERAL DE CIÊNCIAS DA SAÚDE DE PORTO ALEGRE

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

PORTO ALEGRE, 17 de Abril de 2020

Assinado por:
Fernanda Bordignon Nunes
(Coordenador(a))

Endereço: Rua Sarmento Leite ,245
Bairro: Sarmento **CEP:** 90.050-170
UF: RS **Município:** PORTO ALEGRE
Telefone: (51)3303-8804 **E-mail:** cep@ufcspa.edu.br

APPETITE

AUTHOR INFORMATION PACK

TABLE OF CONTENTS

ISSN: 0195-6663

DESCRIPTION

Appetite is an international research journal specializing in cultural, social, psychological, sensory and physiological influences on the selection and intake of foods and drinks. It covers normal and disordered eating and drinking and welcomes studies of both human and non-human animal behaviour toward food. Appetite publishes research reports, reviews and commentaries. Thematic special issues appear regularly. From time to time the journal carries abstracts from professional meetings. Submissions to Appetite are expected to be based primarily on observations directly related to the selection and intake of foods and drinks; papers that are primarily focused on topics such as nutrition or obesity will not be considered unless they specifically make a novel scientific contribution to the understanding of appetite in line with the journal's aims and scope.

Research areas covered include:

- Psychological, social, sensory and cultural influences on appetite
- Cognitive and behavioural neuroscience of appetite
- Clinical and pre-clinical studies of disordered appetite
- Nutritional influences on appetite
- Food attitudes and consumer behaviour towards food
- Psychology and ethnography of dietary habits
- History of food cultures

Benefits to authors
We also provide many author benefits, such as free PDFs, a liberal copyright policy, special discounts on Elsevier publications and much more. Please click here for more information on our author services .

Please see our Guide for Authors for information on article submission. If you require any further information or help, please visit our Support Center

AUDIENCE

Psychology, Social Research, Neuroscience, Physiology, Nutrition, Sensory Food Science

IMPACT FACTOR

2021: 5.016 © Clarivate Analytics Journal Citation Reports 2022

ABSTRACTING AND INDEXING

Scopus
PubMed/Medline
ISI Web of Knowledge
ISI Science Citation Index

EDITORIAL BOARD

GUIDE FOR AUTHORS

INTRODUCTION

Types of article

Full length papers

Full length papers including empirical reports and theoretical reviews are published. Reviews may be of any length consistent with succinct presentation, subdivided as appropriate to the subject matter.

Special Sections or Issues

Proposals for a themed collection, symposium or commentary should be sent to the Contact Editor and appetite@elsevier.com, listing provisional authors, titles and lengths of papers and suggesting Executive, Advisory or Guest Editors with a timetable for recorded peer-reviewing, revision and transmittal in the format required for publication. The reviews or reports in a special section or issue will be subject to the normal process of peer-review.

Commentary sections

Commentary sections may include a keynote paper, brief comments and reply.

Conference Abstracts

Conference Abstracts in guest-edited sets from international multidisciplinary conferences are sometimes published. All the abstracts in a set must be limited to a total word count of no more than 300 (4 per page) and formatted as a single paragraph with no subheadings. The abstract starts with the title (mostly in lower case), name(s) of author(s) (upper case) and one postal address, complete with postcode and country, followed on the same line by one stand-alone e-mail address. Any acknowledgements or references are included within the paragraph: between the cited author(s) and year can be placed the abbreviated title of the journal, volume and pages. Tables, Figures and footnotes are not allowed. A published abstract should not promise findings or discussion, nor refer to presentation at the meeting. The title of the meeting as the main title, the location and dates as a sub-title must be provided to form the heading of the set of abstracts. Any session titles, special lectures or other material must fit into the format and word count for the abstracts in that set.

Please note that questionnaires and interview protocols (in Figure form) are not published.

Contact details for submission

Authors should submit their articles electronically at: https://www.editorialmanager.com/appetite/Default.aspx

Submission checklist

You can use this list to carry out a final check of your submission before you send it to the journal for review. Please check the relevant section in this Guide for Authors for more details.

Ensure that the following items are present:

One author has been designated as the corresponding author with contact details:
• E-mail address
• Full postal address

All necessary files have been uploaded:
Manuscript:
• Include keywords
• All figures (include relevant captions)
• All tables (including titles, description, footnotes)
• Ensure all figure and table citations in the text match the files provided
• Indicate clearly if color should be used for any figures in print
Graphical Abstracts / Highlights files (where applicable)
Supplemental files (where applicable)

Further considerations
• Manuscript has been 'spell checked' and 'grammar checked'
• All references mentioned in the Reference List are cited in the text, and vice versa

• Permission has been obtained for use of copyrighted material from other sources (including the Internet)
• A competing interests statement is provided, even if the authors have no competing interests to declare
• Journal policies detailed in this guide have been reviewed
• Referee suggestions and contact details provided, based on journal requirements

For further information, visit our Support Center.

BEFORE YOU BEGIN

Ethics in publishing

Please see our information on Ethics in publishing.

Studies in humans and animals

Research involving human participants, human material, or human data, must have been performed in accordance with the Declaration of Helsinki and must have been approved by an appropriate independent ethics committee. A statement detailing the approval, including the name of the ethics committee and the reference number where appropriate, must appear in all manuscripts reporting such research. If an appropriate committee granted an exemption from requiring ethics approval, this should also be detailed in the manuscript, including the name of the ethics committee that granted the exemption. Further information and documentation to support this should be made available to the Editor on request. The manuscript should be in line with the Recommendations for the Conduct, Reporting, Editing and Publication of Scholarly Work in Medical Journals and aim for the inclusion of representative human populations (sex, age and ethnicity) as per those recommendations. The terms sex and gender should be used correctly.

Required disclosures: We require every research article submitted to include a statement in the methods section that the study obtained ethics approval (or a statement that it was not required and why), including the name of the ethics committee(s) or institutional review board(s) and a statement that participants gave informed consent before taking part and a statement about assent for children and youth where appropriate. Wherever possible authors should also insert a specific ethics/approval number. The privacy rights of human subjects must always be observed.

Papers that report studies involving vertebrate animals must conform to the ARRIVE Guidelines summarised at www.nc3rs.org.uk and should be carried out in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments, or the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978) and the authors should clearly indicate in the manuscript that such guidelines have been followed. The sex of animals must be indicated, and where appropriate, the influence (or association) of sex on the results of the study. The Editors will not accept papers that have not been reviewed and approved by an animal experimentation ethics committee or regulatory organisation.

Required disclosures: Where a paper reports studies involving vertebrate animals, authors must state in the Methods section the institutional and national guidelines for the care and use of animals that were followed and that all experimental procedures involving animals were approved by the [insert name of the ethics committee or other approving body; wherever possible authors should also insert a specific ethics/approval number].

Declaration of interest

All authors must disclose any financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work. Examples of potential competing interests include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding. Authors must disclose any interests in two places: 1. A summary declaration of interest statement in the title page file (if double anonymized) or the manuscript file (if single anonymized). If there are no interests to declare then please state this: 'Declarations of interest: none'. 2. Detailed disclosures as part of a separate Declaration of Interest form, which forms part of the journal's official records. It is important for potential interests to be declared in both places and that the information matches. More information.

Data code and availability statements
All papers must include a statement regarding the availability of all data used in the study. Where data was downloaded from the public domain, the source and means of obtaining the data should be stated. Data, or metadata that were newly acquired for the present study should ideally be made available to the community via a suitable open repository, preferably at the time of paper submission. If data is not able to be made openly available then a reasonable rationale should be provided, such as ethics or privacy issues of clinical data, or restrictions imposed by the administering institution. If data is only to be made available via a request to the Authors, then the conditions of such a request, and any restrictions - such as the need for a formal data sharing agreement - should be clearly stated.

Authors are requested to declare if their work has been peer-reviewed previously, and if so they are encouraged to supply along with their manuscript files their responses to previous review comments.

Submission declaration and verification

Submission of an article implies that the work described has not been published previously (except in the form of an abstract, a published lecture or academic thesis, see 'Multiple, redundant or concurrent publication' for more information), that it is not under consideration for publication elsewhere, that its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright-holder. To verify originality, your article may be checked by the originality detection service Crossref Similarity Check.

Preprints
Please note that preprints can be shared anywhere at any time, in line with Elsevier's sharing policy. Sharing your preprints e.g. on a preprint server will not count as prior publication (see 'Multiple, redundant or concurrent publication' for more information).

Use of inclusive language

Inclusive language acknowledges diversity, conveys respect to all people, is sensitive to differences, and promotes equal opportunities. Content should make no assumptions about the beliefs or commitments of any reader; contain nothing which might imply that one individual is superior to another on the grounds of age, gender, race, ethnicity, culture, sexual orientation, disability or health condition; and use inclusive language throughout. Authors should ensure that writing is free from bias, stereotypes, slang, reference to dominant culture and/or cultural assumptions. We advise to seek gender neutrality by using plural nouns ("clinicians, patients/clients") as default/wherever possible to avoid using "he, she," or "he/she." We recommend avoiding the use of descriptors that refer to personal attributes such as age, gender, race, ethnicity, culture, sexual orientation, disability or health condition unless they are relevant and valid. Authors should use person first language when referring people/participants who are living with various conditions e.g. people with obesity (not obese people or people who are obese). These guidelines are meant as a point of reference to help identify appropriate language but are by no means exhaustive or definitive.

Changes to authorship

Authors are expected to consider carefully the list and order of authors **before** submitting their manuscript and provide the definitive list of authors at the time of the original submission. Any addition, deletion or rearrangement of author names in the authorship list should be made only **before** the manuscript has been accepted and only if approved by the journal Editor. To request such a change, the Editor must receive the following from the **corresponding author**: (a) the reason for the change in author list and (b) written confirmation (e-mail, letter) from all authors that they agree with the addition, removal or rearrangement. In the case of addition or removal of authors, this includes confirmation from the author being added or removed.
Only in exceptional circumstances will the Editor consider the addition, deletion or rearrangement of authors **after** the manuscript has been accepted. While the Editor considers the request, publication of the manuscript will be suspended. If the manuscript has already been published in an online issue, any requests approved by the Editor will result in a corrigendum.

Article transfer service
This journal is part of our Article Transfer Service. This means that if the Editor feels your article is more suitable in one of our other participating journals, then you may be asked to consider transferring the article to one of those. If you agree, your article will be transferred automatically on your behalf with no need to reformat. Please note that your article will be reviewed again by the new journal. More information.

Copyright

Upon acceptance of an article, authors will be asked to complete a 'Journal Publishing Agreement' (see more information on this). An e-mail will be sent to the corresponding author confirming receipt of the manuscript together with a 'Journal Publishing Agreement' form or a link to the online version of this agreement.

Subscribers may reproduce tables of contents or prepare lists of articles including abstracts for internal circulation within their institutions. Permission of the Publisher is required for resale or distribution outside the institution and for all other derivative works, including compilations and translations. If excerpts from other copyrighted works are included, the author(s) must obtain written permission from the copyright owners and credit the source(s) in the article. Elsevier has preprinted forms for use by authors in these cases.

For gold open access articles: Upon acceptance of an article, authors will be asked to complete a 'License Agreement' (more information). Permitted third party reuse of gold open access articles is determined by the author's choice of user license.

Author rights
As an author you (or your employer or institution) have certain rights to reuse your work. More information.

Elsevier supports responsible sharing
Find out how you can share your research published in Elsevier journals.

Role of the funding source

You are requested to identify who provided financial support for the conduct of the research and/or preparation of the article and to briefly describe the role of the sponsor(s), if any, in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication. If the funding source(s) had no such involvement, it is recommended to state this.

Open access

Please visit our Open Access page for more information.

Elsevier Researcher Academy
Researcher Academy is a free e-learning platform designed to support early and mid-career researchers throughout their research journey. The "Learn" environment at Researcher Academy offers several interactive modules, webinars, downloadable guides and resources to guide you through the process of writing for research and going through peer review. Feel free to use these free resources to improve your submission and navigate the publication process with ease.

Language (usage and editing services)
Please write your text in good English (American or British usage is accepted, but not a mixture of these). Authors who feel their English language manuscript may require editing to eliminate possible grammatical or spelling errors and to conform to correct scientific English may wish to use the English Language Editing service available from Elsevier's Author Services.

Sex and gender reporting

Appetite encourages researchers to take sex and gender into account when establishing objectives, developing methods of study, obtaining and evaluating data, and reporting the results of their work. We also acknowledge that sex and gender refer to a wide range of physiological, psychological and cultural variables that do not have clear scientific boundaries. Therefore, we recommend that authors describe participants in the terms that they endorsed when choosing to enter the study, e.g., male, female, man, woman, non-binary etc. In addition, authors may wish to refer to sex/gender effects rather than assuming a sharp categorization.

Submission

Our online submission system guides you stepwise through the process of entering your article details and uploading your files. The system converts your article files to a single PDF file used in the peer-review process. Editable files (e.g., Word, LaTeX) are required to typeset your article for final publication. All correspondence, including notification of the Editor's decision and requests for revision, is sent by e-mail.

Submit your article

Please submit your article via https://www.journals.elsevier.com/appetite

Referees

Please submit, with the manuscript, the names, addresses and e-mail addresses of three potential referees along with your reasons for suggesting them. Note that the editor retains the sole right to decide whether or not the suggested reviewers are used.

PREPARATION

Queries

For questions about the editorial process (including the status of manuscripts under review) or for technical support on submissions, please visit our Support Center.

Submission to this journal proceeds totally online and you will be guided stepwise through the creation and uploading of your files. The system automatically converts your files to a single PDF file, which is used in the peer-review process.

Appetite has published an editorial with guidelines on design and statistics, which authors are encouraged to consult.

Cover letter

Cover letters should be addressed to the Editor in Chief.
The letter should: 1) identify the author who has been designated by co-authors as the corresponding author and include the corresponding author's contact details, including email address and full postal address; 2) verify that all authors accept full responsibility for all aspects of to the work described; 3) verify that the manuscript is not under review elsewhere; 4) verify that the corresponding author can provide all original data for review.

In addition, if the study is part of a large study that is or will be published in pieces, an explanation for this choice should be included as well as a verification that the current manuscript includes discussion of how it relates to the other pieces and that full references to the other pieces are included. Multiple manuscripts in which it seems that the dependent variables are just different measures of the same tendencies will not be considered.

If the authors wish to suggest that a particular Executive Editor would be especially appropriate to handle the submission, this may be included in the cover letter.

Ethics

Research involving human participants, human material, or human data must have been performed in accordance with the Declaration of Helsinki and must have been approved by an appropriate independent ethics committee. Similarly, research involving non-human animals or material derived from them must have been approved by an appropriate independent ethics committee. A statement detailing the approval, including the name of the ethics committee and the reference number where appropriate, must appear in the method all manuscripts reporting such research. If an appropriate committee granted an exemption from requiring ethics approval, this should also be detailed in the manuscript, including the name of the ethics committee that granted the exemption. Further information and documentation to support this should be made available to the Editor on request. If an appropriate committee granted an exemption from requiring ethics approval, this should also be detailed in the manuscript, including the name of the ethics committee that granted the exemption. Further information and documentation to support this should be made available to the Editor on request.

Manuscript Preparation

Submission to this journal proceeds totally online, and you will be guided stepwise through the creation and uploading of your files. The system automatically converts your files to a single PDF file, which is used in the peer-review process. Appetite has published an editorial with guidelines on design and statistics, which authors are encouraged to consult.

Peer review

This journal operates a single anonymized review process. All contributions will be initially assessed by the editor for suitability for the journal. Papers deemed suitable are then typically sent to a minimum of two independent expert reviewers to assess the scientific quality of the paper. The Editor is responsible for the final decision regarding acceptance or rejection of articles. The Editor's decision is final. Editors are not involved in decisions about papers which they have written themselves or have been written by family members or colleagues or which relate to products or services in which the editor has an interest. Any such submission is subject to all of the journal's usual procedures, with peer review handled independently of the relevant editor and their research groups. More information on types of peer review.

Use of word processing software

It is important that the file be saved in the native format of the word processor used. The text should be in single-column format. Keep the layout of the text as simple as possible. Most formatting codes will be removed and replaced on processing the article. In particular, do not use the word processor's options to justify text or to hyphenate words. However, do use bold face, italics, subscripts, superscripts etc. When preparing tables, if you are using a table grid, use only one grid for each individual table and not a grid for each row. If no grid is used, use tabs, not spaces, to align columns. The electronic text should be prepared in a way very similar to that of conventional manuscripts (see also the Guide to Publishing with Elsevier). Note that source files of figures, tables and text graphics will be required whether or not you embed your figures in the text. See also the section on Electronic artwork.

To avoid unnecessary errors you are strongly advised to use the 'spell-check' and 'grammar-check' functions of your word processor.

It is important that the file be saved in the native format of the word processor used. The text should be in single-column format. Keep the layout of the text as simple as possible. Most formatting codes will be removed and replaced on processing the article. In particular, do not use the word processor's options to justify text or to hyphenate words. However, do use bold face, italics, subscripts, superscripts etc. When preparing tables, if you are using a table grid, use only one grid for each individual table and not a grid for each row. If no grid is used, use tabs, not spaces, to align columns. The electronic text should be prepared in a way very similar to that of conventional manuscripts (see also the Guide to Publishing with Elsevier). Note that source files of figures, tables and text graphics will be required whether or not you embed your figures in the text. See also the section on Electronic artwork. To avoid unnecessary errors you are strongly advised to use the 'spell-check' and 'grammar-check' functions of your word processor. Please ensure your paper has consecutive line numbering, this is an essential peer review requirement.

Article Structure

Articles should include page and line numbering and the line spacing should be 1.5 or 2 and the font should be no less than 11 point (and ideally 12 point).

Subdivision - numbered sections

Divide your article into clearly defined and numbered sections. Subsections should be numbered 1.1 (then 1.1.1, 1.1.2, ...), 1.2, etc. (the abstract is not included in section numbering). Use this numbering also for internal cross-referencing: do not just refer to 'the text'. Any subsection may be given a brief heading. Each heading should appear on its own separate line.

Introduction

State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

Methods

Provide sufficient details to allow the work to be reproduced by an independent researcher. Methods that are already published should be summarized, and indicated by a reference. If quoting directly from a previously published method, use quotation marks and also cite the source. Any modifications to existing methods should also be described. Include an ethics-approval statement as described above. Include a section describing the statistical approach. This should include: 1) A statement that the hypotheses were specified before the data were collected; 2) A statement that the analytic plan was pre-specified and any data-driven analyses are clearly identified and discussed appropriately

Results

Results should be clear and concise. Authors are encouraged to read and comply with our Guideline on Statistics. When possible, quantify findings and present them with appropriate indicators of measurement error or uncertainty.

Discussion
This should explore the significance of the results of the work, not repeat them. A combined Results and Discussion section is often appropriate. Avoid extensive citations and discussion of published literature.

Appendices
Appendices are not encouraged. Critical details of Method should be described in that section of the manuscript.

Essential title page information

• ***Title.*** Concise and informative. Titles are often used in information-retrieval systems. Avoid abbreviations and formulae where possible.
• ***Author names and affiliations.*** Please clearly indicate the given name(s) and family name(s) of each author and check that all names are accurately spelled. You can add your name between parentheses in your own script behind the English transliteration. Present the authors' affiliation addresses (where the actual work was done) below the names. Indicate all affiliations with a lower-case superscript letter immediately after the author's name and in front of the appropriate address. Provide the full postal address of each affiliation, including the country name and, if available, the e-mail address of each author.
• ***Corresponding author.*** Clearly indicate who will handle correspondence at all stages of refereeing and publication, also post-publication. This responsibility includes answering any future queries about Methodology and Materials. **Ensure that the e-mail address is given and that contact details are kept up to date by the corresponding author.**
• ***Present/permanent address.*** If an author has moved since the work described in the article was done, or was visiting at the time, a 'Present address' (or 'Permanent address') may be indicated as a footnote to that author's name. The address at which the author actually did the work must be retained as the main, affiliation address. Superscript Arabic numerals are used for such footnotes.

Abstract

A concise and factual abstract is required. The abstract should state briefly the purpose of the research, the principal results and major conclusions. An abstract is often presented separately from the article, so it must be able to stand alone. For this reason, References should be avoided, but if essential, then cite the author(s) and year(s). Also, non-standard or uncommon abbreviations should be avoided, but if essential they must be defined at their first mention in the abstract itself. As per the journal style, the abstract text should not be more than 280 words (1500 characters including spaces). The abstract should be formatted as a single paragraph.

Graphical abstract
Although a graphical abstract is optional, its use is encouraged as it draws more attention to the online article. The graphical abstract should summarize the contents of the article in a concise, pictorial form designed to capture the attention of a wide readership. Graphical abstracts should be submitted as a separate file in the online submission system. Image size: Please provide an image with a minimum of 531 × 1328 pixels (h × w) or proportionally more. The image should be readable at a size of 5 × 13 cm using a regular screen resolution of 96 dpi. Preferred file types: TIFF, EPS, PDF or MS Office files. You can view Example Graphical Abstracts on our information site.
Authors can make use of Elsevier's Illustration Services to ensure the best presentation of their images and in accordance with all technical requirements.

Keywords

Immediately after the abstract, provide a maximum of 6 keywords, using American spelling and avoiding general and plural terms and multiple concepts (avoid, for example, 'and', 'of'). Be sparing with abbreviations: only abbreviations firmly established in the field may be eligible. These keywords will be used for indexing purposes.

Abbreviations

Define abbreviations that are not standard in this field in a footnote to be placed on the first page of the article. Such abbreviations that are unavoidable in the abstract must be defined at their first mention there, as well as in the footnote. Ensure consistency of abbreviations throughout the article.

Acknowledgements

Collate acknowledgements in a separate section at the end of the article before the references and do not, therefore, include them on the title page, as a footnote to the title or otherwise. List here those individuals who provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.).

Author Contributions

Each author should to declare his or her individual contribution to the article. The statement that all authors have approved the final article should be true and included in the disclosure. Such a statement should be included immediately following the Acknowledgements section of the article.

Formatting of funding sources

List funding sources in this standard way to facilitate compliance to funder's requirements:

Funding: This work was supported by the National Institutes of Health [grant numbers xxxx, yyyy]; the Bill & Melinda Gates Foundation, Seattle, WA [grant number zzzz]; and the United States Institutes of Peace [grant number aaaa].

It is not necessary to include detailed descriptions on the program or type of grants and awards. When funding is from a block grant or other resources available to a university, college, or other research institution, submit the name of the institute or organization that provided the funding.

If no funding has been provided for the research, it is recommended to include the following sentence:

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Units

Follow internationally accepted rules and conventions: use the international system of units (SI). If other units are mentioned, please give their equivalent in SI. Ensure that the data are reported with reasonable precision. Age, BMI and similar demographic data rounded to 0.1 at the most, 0-100 VAS to 1, Likert-scale data to 1, etc.

Math formulae

Please submit math equations as editable text and not as images. Present simple formulae in line with normal text where possible and use the solidus (/) instead of a horizontal line for small fractional terms, e.g., X/Y. In principle, variables are to be presented in italics. Powers of e are often more conveniently denoted by exp. Number consecutively any equations that have to be displayed separately from the text (if referred to explicitly in the text).

Footnotes

Footnotes should be used sparingly. Number them consecutively throughout the article. Many word processors can build footnotes into the text, and this feature may be used. Otherwise, please indicate the position of footnotes in the text and list the footnotes themselves separately at the end of the article. Do not include footnotes in the Reference list.

Artwork

Electronic Artwork

General points Make sure you use uniform lettering and sizing of your original artwork Embed the used fonts if the application provides that option Aim to use the following fonts in your illustrations: Arial, Courier, Times New Roman, Symbol, or use fonts that look similar Number the illustrations according to their sequence in the text Use a logical naming convention for your artwork files Provide captions to illustrations separately Size the illustrations close to the desired dimensions of the published version Submit each illustration as a separate file

A detailed guide on electronic artwork is available. **You are urged to visit this site; some excerpts from the detailed information are given here.**

Formats

If your electronic artwork is created in a Microsoft Office application (Word, PowerPoint, Excel) then please supply 'as is' in the native document format. Regardless of the application used other than Microsoft Office, when your electronic artwork is finalized, please 'Save as' or convert the images to one of the following formats (note the resolution requirements for line drawings, halftones, and line/ halftone combinations given below): EPS (or PDF): Vector drawings, embed all used fonts TIFF (or JPEG): Color or grayscale photographs (halftones), keep to a minimum of 300 dpi TIFF (or JPEG): Bitmapped (pure black and white pixels) line drawings, keep to a minimum of 1000 dpi TIFF (or JPEG): Combinations bitmapped line/half-tone (color or grayscale), keep to a minimum of 500 dpi

Please do not: Supply files that are optimized for screen use (e.g., GIF, BMP, PICT, WPG); these typically have a low number of pixels and limited set of colors; Supply files that are too low in resolution; Submit graphics that are disproportionately large for the content

Color artwork
Please make sure that artwork files are in an acceptable format (TIFF (or JPEG), EPS (or PDF), or MS Office files) and with the correct resolution. If, together with your accepted article, you submit usable color figures then Elsevier will ensure, at no additional charge, that these figures will appear in color online (e.g., ScienceDirect and other sites) regardless of whether or not these illustrations are reproduced in color in the printed version. **For color reproduction in print, you will receive information regarding the costs from Elsevier after receipt of your accepted article**. Please indicate your preference for color: in print or online only. Further information on the preparation of electronic artwork.

Illustration services
Elsevier's Author Services offers Illustration Services to authors preparing to submit a manuscript but concerned about the quality of the images accompanying their article. Elsevier's expert illustrators can produce scientific, technical and medical-style images, as well as a full range of charts, tables and graphs. Image 'polishing' is also available, where our illustrators take your image(s) and improve them to a professional standard. Please visit the website to find out more.

Figure captions
Ensure that each illustration has a caption. A caption should comprise a brief title (**not** on the figure itself) and a description of the illustration. Keep text in the illustrations themselves to a minimum but explain all symbols and abbreviations used.

Tables

Please submit tables as editable text and not as images. Tables can be placed either next to the relevant text in the article, or on separate page(s) at the end. Number tables consecutively in accordance with their appearance in the text and place any table notes below the table body. Be sparing in the use of tables and ensure that the data presented in them do not duplicate results described elsewhere in the article. Please avoid using vertical rules and shading in table cells.

References

Citation in text
Please ensure that every reference cited in the text is also present in the reference list (and vice versa). Any references cited in the abstract must be given in full. Unpublished results and personal communications are not recommended in the reference list, but may be mentioned in the text. If these references are included in the reference list they should follow the standard reference style of the journal and should include a substitution of the publication date with either 'Unpublished results' or 'Personal communication'. Citation of a reference as 'in press' implies that the item has been accepted for publication.

Web references
As a minimum, the full URL should be given and the date when the reference was last accessed. Any further information, if known (DOI, author names, dates, reference to a source publication, etc.), should also be given. Web references can be listed separately (e.g., after the reference list) under a different heading if desired, or can be included in the reference list.

Data references
This journal encourages you to cite underlying or relevant datasets in your manuscript by citing them in your text and including a data reference in your Reference List. Data references should include the following elements: author name(s), dataset title, data repository, version (where available), year, and global persistent identifier. Add [dataset] immediately before the reference so we can properly identify it as a data reference. The [dataset] identifier will not appear in your published article.

Preprint references
Where a preprint has subsequently become available as a peer-reviewed publication, the formal publication should be used as the reference. If there are preprints that are central to your work or that cover crucial developments in the topic, but are not yet formally published, these may be referenced. Preprints should be clearly marked as such, for example by including the word preprint, or the name of the preprint server, as part of the reference. The preprint DOI should also be provided.

References in a special issue
Please ensure that the words 'this issue' are added to any references in the list (and any citations in the text) to other articles in the same Special Issue.

Reference management software
Most Elsevier journals have their reference template available in many of the most popular reference management software products. These include all products that support Citation Style Language styles, such as Mendeley. Using citation plug-ins from these products, authors only need to select the appropriate journal template when preparing their article, after which citations and bibliographies will be automatically formatted in the journal's style. If no template is yet available for this journal, please follow the format of the sample references and citations as shown in this Guide. If you use reference management software, please ensure that you remove all field codes before submitting the electronic manuscript. More information on how to remove field codes from different reference management software.

Reference formatting
There are no strict requirements on reference formatting at submission. References can be in any style or format as long as the style is consistent. Where applicable, author(s) name(s), journal title/ book title, chapter title/article title, year of publication, volume number/book chapter and the article number or pagination must be present. Use of DOI is highly encouraged. The reference style used by the journal will be applied to the accepted article by Elsevier at the proof stage. Note that missing data will be highlighted at proof stage for the author to correct. If you do wish to format the references yourself they should be arranged according to the following examples:

Reference Style
Text: Citations in the text should follow the referencing style used by the American Psychological Association. You are referred to the Publication Manual of the American Psychological Association, Sixth Edition, ISBN 978-1-4338-0561-5, copies of which may be ordered online or APA Order Dept., P.O.B. 2710, Hyattsville, MD 20784, USA or APA, 3 Henrietta Street, London, WC3E 8LU, UK.
List: references should be arranged first alphabetically and then further sorted chronologically if necessary. More than one reference from the same author(s) in the same year must be identified by the letters 'a', 'b', 'c', etc., placed after the year of publication.
Examples:
Reference to a journal publication:
Van der Geer, J., Hanraads, J. A. J., & Lupton, R. A. (2010). The art of writing a scientific article. *Journal of Scientific Communications, 163*, 51–59. https://doi.org/10.1016/j.Sc.2010.00372.
Reference to a journal publication with an article number:
Van der Geer, J., Hanraads, J. A. J., & Lupton, R. A. (2018). The art of writing a scientific article. *Heliyon*, *19*, e00205. https://doi.org/10.1016/j.heliyon.2018.e00205.
Reference to a book:
Strunk, W., Jr., & White, E. B. (2000). *The elements of style.* (4th ed.). New York: Longman, (Chapter 4).
Reference to a chapter in an edited book:

Mettam, G. R., & Adams, L. B. (2009). How to prepare an electronic version of your article. In B. S. Jones, & R. Z. Smith (Eds.), *Introduction to the electronic age* (pp. 281–304). New York: E-Publishing Inc.
Reference to a website:
Cancer Research UK. Cancer statistics reports for the UK. (2003). http://www.cancerresearchuk.org/aboutcancer/statistics/cancerstatsreport/ Accessed 13 March 2003.
Reference to a dataset:
[dataset] Oguro, M., Imahiro, S., Saito, S., Nakashizuka, T. (2015). *Mortality data for Japanese oak wilt disease and surrounding forest compositions*. Mendeley Data, v1. https://doi.org/10.17632/xwj98nb39r.1.
Reference to a conference paper or poster presentation:
Engle, E.K., Cash, T.F., & Jarry, J.L. (2009, November). The Body Image Behaviours Inventory-3: Development and validation of the Body Image Compulsive Actions and Body Image Avoidance Scales. Poster session presentation at the meeting of the Association for Behavioural and Cognitive Therapies, New York, NY.

Journal abbreviations source
Journal names should be abbreviated according to the List of Title Word Abbreviations.

Video
Elsevier accepts video material and animation sequences to support and enhance your scientific research. Authors who have video or animation files that they wish to submit with their article are strongly encouraged to include links to these within the body of the article. This can be done in the same way as a figure or table by referring to the video or animation content and noting in the body text where it should be placed. All submitted files should be properly labeled so that they directly relate to the video file's content. In order to ensure that your video or animation material is directly usable, please provide the file in one of our recommended file formats with a preferred maximum size of 150 MB per file, 1 GB in total. Video and animation files supplied will be published online in the electronic version of your article in Elsevier Web products, including ScienceDirect. Please supply 'stills' with your files: you can choose any frame from the video or animation or make a separate image. These will be used instead of standard icons and will personalize the link to your video data. For more detailed instructions please visit our video instruction pages. Note: since video and animation cannot be embedded in the print version of the journal, please provide text for both the electronic and the print version for the portions of the article that refer to this content.

Data visualization
Include interactive data visualizations in your publication and let your readers interact and engage more closely with your research. Follow the instructions here to find out about available data visualization options and how to include them with your article.

Supplementary material
Supplementary material such as applications, images and sound clips, can be published with your article to enhance it. Submitted supplementary items are published exactly as they are received (Excel or PowerPoint files will appear as such online). Please submit your material together with the article and supply a concise, descriptive caption for each supplementary file. If you wish to make changes to supplementary material during any stage of the process, please make sure to provide an updated file. Do not annotate any corrections on a previous version. Please switch off the 'Track Changes' option in Microsoft Office files as these will appear in the published version.

Online Studies

Online studies are becoming increasingly common and they have some advantages over in person studies e.g. easier recruitment of larger and more diverse samples . However, there are potential limitations e.g. low data quality due to malicious programs, or bots, generating invalid data. Therefore authors are requested to outline in their manuscript the steps they have taken to improve data quality and detect bots. Such strategies might include the use of recaptcha at the start of a survey, dummy/implausible questions, attention check questions, collecting timing and page use data, and consistency checks.

Research data

This journal requires and enables you to share data that supports your research publication where appropriate, and enables you to interlink the data with your published articles. Research data refers to the results of observations or experimentation that validate research findings. To facilitate reproducibility and data reuse, this journal also encourages you to share your software, code, models, algorithms, protocols, methods and other useful materials related to the project.

Below are a number of ways in which you can associate data with your article or make a statement about the availability of your data when submitting your manuscript. When sharing data in one of these ways, you are expected to cite the data in your manuscript and reference list. Please refer to the "References" section for more information about data citation. For more information on depositing, sharing and using research data and other relevant research materials, visit the research data page.

Data linking

If you have made your research data available in a data repository, you can link your article directly to the dataset. Elsevier collaborates with a number of repositories to link articles on ScienceDirect with relevant repositories, giving readers access to underlying data that gives them a better understanding of the research described.

There are different ways to link your datasets to your article. When available, you can directly link your dataset to your article by providing the relevant information in the submission system. For more information, visit the database linking page.

For supported data repositories a repository banner will automatically appear next to your published article on ScienceDirect.

In addition, you can link to relevant data or entities through identifiers within the text of your manuscript, using the following format: Database: xxxx (e.g., TAIR: AT1G01020; CCDC: 734053; PDB: 1XFN).

Data in Brief

You have the option of converting any or all parts of your supplementary or additional raw data into a data article published in *Data in Brief*. A data article is a new kind of article that ensures that your data are actively reviewed, curated, formatted, indexed, given a DOI and made publicly available to all upon publication (watch this video describing the benefits of publishing your data in *Data in Brief*). You are encouraged to submit your data article for *Data in Brief* as an additional item directly alongside the revised version of your manuscript. If your research article is accepted, your data article will automatically be transferred over to *Data in Brief* where it will be editorially reviewed, published open access and linked to your research article on ScienceDirect. Please note an open access fee is payable for publication in *Data in Brief*. Full details can be found on the Data in Brief website. Please use this template to write your *Data in Brief* data article.

Data statement

To foster transparency, we require you to state the availability of your data in your submission if your data is unavailable to access or unsuitable to post. This may also be a requirement of your funding body or institution. You will have the opportunity to provide a data statement during the submission process. The statement will appear with your published article on ScienceDirect. For more information, visit the Data Statement page..

AFTER ACCEPTANCE

Online proof correction

To ensure a fast publication process of the article, we kindly ask authors to provide us with their proof corrections within two days. Corresponding authors will receive an e-mail with a link to our online proofing system, allowing annotation and correction of proofs online. The environment is similar to MS Word: in addition to editing text, you can also comment on figures/tables and answer questions from the Copy Editor. Web-based proofing provides a faster and less error-prone process by allowing you to directly type your corrections, eliminating the potential introduction of errors.
If preferred, you can still choose to annotate and upload your edits on the PDF version. All instructions for proofing will be given in the e-mail we send to authors, including alternative methods to the online version and PDF.

We will do everything possible to get your article published quickly and accurately. Please use this proof only for checking the typesetting, editing, completeness and correctness of the text, tables and figures. Significant changes to the article as accepted for publication will only be considered at this stage with permission from the Editor. It is important to ensure that all corrections are sent back to us in one communication. Please check carefully before replying, as inclusion of any subsequent corrections cannot be guaranteed. Proofreading is solely your responsibility.

Offprints

The corresponding author will, at no cost, receive a customized Share Link providing 50 days free access to the final published version of the article on ScienceDirect. The Share Link can be used for sharing the article via any communication channel, including email and social media. For an extra charge, paper offprints can be ordered via the offprint order form which is sent once the article is accepted for publication. Both corresponding and co-authors may order offprints at any time via Elsevier's Author Services. Corresponding authors who have published their article gold open access do not receive a Share Link as their final published version of the article is available open access on ScienceDirect and can be shared through the article DOI link.

AUTHOR INQUIRIES

Visit the Elsevier Support Center to find the answers you need. Here you will find everything from Frequently Asked Questions to ways to get in touch.
You can also check the status of your submitted article or find out when your accepted article will be published.