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Associação de polimorfismos no gene do receptor *DRD2* e nos genes das enzimas *MAOA* e *COMT* com a ingestão alimentar e parâmetros de adiposidade entre crianças de três a quatro anos de idade.

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Patogênese e Fisiopatologia

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Mahatma Gandhi

ABSTRACT

Place of Origin / Performance: Laboratory of Molecular Biology, Centro de Pesquisa e Pós-Graduação Heitor Cirne Lima, UFCSPA.

Background: The increased prevalence of obesity in the world is becoming one of the most important clinical and epidemiological phenomena at present day. Environmental factors such as changes in lifestyle and feeding behavior associated with poorly characterized genetic determinants are thought to play the most important roles in the pathogenesis of this disease. During the last years great advances were obtained in the characterization of the hypothalamic mechanisms involved in food intake control. Such advances are unveiling a complex and integrated system. Despite the contribution of genetic factors to the development of weight gain being widely recognized, the real quantitative contribution of them is a complex question yet to be answered. Evidence obtained from studies with humans and animal models indicate the importance of dopaminergic function in the development of obesity for their role in regulating appetite. Power is associated with the release of dopamine in the dorsal striatum and the degree of pleasure experienced during food intake is directly related to the amount of dopamine released in this region. These evidences are divided about the direction of causal association. One argument is that a Reward Deficiency Syndrome is the risk factor, while others contend that hyper-sensitivity to reward enhances the motivation for pleasurable activities like eating. The genes of the dopamine receptor 2 (*DRD2*), the enzyme monoamine oxidase A (*MAOA*) and catechol-*O*-methyltransferase (*COMT*), encode proteins that control the availability of these neurotransmitters and have polymorphisms (*TaqIA* and-*141C Ins/Del - DRD2; MAOA-u VNTR* and *T941G - MAOA*; *Val158Met - COMT*), which alter their activity and expression. The variants of these genes may be associated with differential intake of food in their

carriers. Whereas the prevalence of obese children has increased in recent decades, and obesity increases the risk for various diseases, the identification of individuals who are genetically more susceptible could be important for the prevention and / or treatment of obesity.

Objectives: To investigate the association of variants *TaqIA* and *-141C Ins/Del* of dopamine receptor 2 gene (*DRD2*); variant *MAOA-u VNTR* and *T941G* of monoamine oxidase A gene (*MAOA*) and *Val158Met* variant of the gene for catechol-O - methyltransferase (*COMT*) in the regulation of body weight and the aspects related to obesity and food consumption.

Material and Methods: We conducted a cross-sectional study aligned to a randomized clinical trial. DNA was extracted from blood samples of 354 children, polymorphisms *DRD2 TaqIA, DRD2 -141C Ins / Del, MAOA T941G* and *COMT Val158Met* were amplified by PCR and further subjected to cleavage with restriction endonuclease, the genotypes were visualized after gel electrophoresis agarose and acrylamide (*COMT Val158Met*); The fragment containing the *MAOA-u VNTR* polymorphism was analyzed by PCR followed by electrophoresis on acrylamide gels.

Results and Conclusions: The distributions of genotype frequencies found are in agreement with the expected under Hardy-Weinberg equilibrium. Regarding the polymorphism *DRD2 -141C Ins / Del* and *MAOA T941G* there were no statistically significant differences regarding the intake of foods with high sugar density (HSD) and high lipid density (HLD), total caloric intake, the BMI Z score, the percentile skinfolds and waist circumference among patients of different genotypes. For *DRD2 TaqIA* polymorphism, the sample was divided into whites and non-whites. Children of the sample of non-whites with genotype *TaqIA C/C* had a higher intake of HLD (159.4 kcal [44.26-272.34]) compared with

children with the *T* allele (82.2 kcal [0.00-195.01], p = 0.009). To analyze the polymorphisms in the *MAOA* gene, the sample was divided into boys and girls. In the sample of boys, the *MAOA-u*long* allele was associated with higher consumption of HLD (134.975kcal [26437-270162]) when compared to *MAOA-u*short* allele (60.1kcal [0.000-192.31], p = 0.009). The consumption of HSD was also higher in boys with the *MAOA-u*long* allele (100.455kcal [54406-163325]) compared to carriers of *MAOA-u*short* allele (80.015kcal [37.45-127.115], p = 0.034). In the sample of girls, the *MAOA-u VNTR* polymorphism was not associated with food intake and anthropometric data. *COMT Val158Met* polymorphism in the carriers *COMT*158Val* allele had a higher consumption of HLD compared with homozygous for the *COMT*158Met* allele (p = 0.008), the medians were 133.79kcal [44.23-265.80] and 83.37Kcal [0.00-252.95] respectively. These findings indicate the association of polymorphisms in *DRD2, MAOA* and *COMT* in the regulation of food intake and as a potential risk factor for obesity.

Keywords: *DRD2, MAOA* and *COMT* polymorphisms; food intake; children obesity

RESUMO

Local de Origem/Realização: Laboratório de Biologia Molecular, Centro de Pesquisa e Pós-Graduação Heitor Cirne Lima, UFCSPA.

Introdução: O aumento da prevalência de obesidade em todo o mundo vem se revelando como um dos mais importantes fenômenos clínico-epidemiológicos da atualidade. Fatores como a mudança do hábito alimentar e o estilo de vida sedentário, aliados a determinantes genéticos ainda pouco conhecidos, desempenham um papel relevante na patogênese desta doença. Nos últimos anos avanços consideráveis foram obtidos na caracterização dos mecanismos hipotalâmicos do controle da ingestão alimentar. Tais avanços têm revelado as particularidades de um sistema complexo e integrado. Apesar da contribuição de fatores genéticos no desenvolvimento do ganho de peso ser amplamente reconhecida, a real contribuição quantitativa dos mesmos em fenótipos relacionados é ainda uma questão complexa que precisa ser elucidada. Evidências obtidas, a partir de estudos com humanos e modelos animais, indicam a importância da função dopaminérgica no desenvolvimento da obesidade por seu papel na regulação do apetite. A alimentação está associada com a liberação de dopamina no estriado dorsal e o grau de prazer sentido durante a ingestão alimentar está diretamente associado à quantidade de dopamina liberada nessa região. Essas evidências estão divididas sobre a direção da causa da associação. Um argumento é que a Síndrome de Deficiência da Recompensa é um fator de risco, enquanto outros afirmam que hiper-sensibilidade à recompensa aumenta a motivação para atividades prazerosas como comer. Os genes do receptor dopaminérgico 2 (*DRD2*), das enzimas monoaminoxidase-A (*MAOA*) e catecol-*O*-metiltransferase *(COMT)*, codificam

proteínas que controlam a disponibilidade destes neurotransmissores e possuem polimorfismos (*TaqIA* e *-141C Ins/Del – DRD2; MAOA-u VNTR* e *T941G – MAOA; Val158Met - COMT*), que alteram as suas atividades e expressão. As variantes destes genes podem estar associadas com ingestão diferencial de alimentos em seus portadores. Considerando que a prevalência de crianças obesas tem aumentado nas últimas décadas e a obesidade aumenta o risco para diversas doenças, a identificação dos indivíduos que são geneticamente mais susceptíveis pode ser importante para a prevenção e/ou no tratamento da obesidade.

Objetivos: Investigar a associação das variantes *TaqIA* e *-141C Ins/Del* do gene do receptor de dopamina 2 *(DRD2)*; variante *MAOA-u VNTR* e *T941G* do gene da monoaminooxidase A *(MAOA)*; e variante *Val158Met* do gene da catecol-*O*-metiltransferase *(COMT)* na regulação do peso corporal e os aspectos relacionados à obesidade e consumo alimentar.

Material e Métodos: Foi realizado um estudo transversal alinhado a um ensaio clínico randomizado. O DNA foi extraído de amostras de sangue de 354 crianças, os polimorfismos *DRD2 TaqIA, DRD2 -141C Ins/Del, MAOA T941G e COMT* Val158Met foram amplificados por PCR e após submetidos a clivagem com endonuclease de restrição, os genótipos foram visualizados posteriormente a eletroforese em gel de agarose e acrilamida (*COMT* Val158Met); O fragmento contendo o polimorfismo *MAOA-u VNTR* foi analisado por PCR seguida de eletroforese em gel de acrilamida.

Resultados e Conclusões: As distribuições das frequências genotípicas encontradas estão de acordo com o esperado sob equilíbrio de Hardy-Weinberg. Com relação ao polimorfismo *DRD2 -141C Ins/Del* e *MAOA T941G* não foram encontradas diferenças estatisticamente significantes quanto à ingestão de alimentos com alta densidade de

açúcar (HSD) e alta densidade de gordura (HLD), ingestão calórica total, ao escore Z do IMC, ao percentil de dobras cutâneas e a circunferência da cintura entre os portadores dos diferentes genótipos. Para o polimorfismo *DRD2 TaqIA*, a amostra foi separada em indivíduos brancos e não-brancos. As crianças da amostra de não-brancos com o genótipo *TaqIA C/C* apresentaram maior ingestão de HLD (159.4 kcal [44.26–272.34]) quando comparadas com crianças portadoras do alelo T (82.2 kcal [0.00–195.01], *p*=0.009). Para analisar os polimorfismos no gene da *MAOA* a amostra foi dividida em meninos e meninas. Na amostra de meninos, o alelo *MAOA-u*longo* foi associado com maior consumo de HLD (134.975kcal [26.437–270.162]) quando comparado ao *MAOA-u*curto* (60.1kcal [0.000–192.31]; *p* = 0.009). O consumo de HSD também foi maior em meninos portadores do alelo *MAOA-u*longo* (100.455kcal [54.406–163.325]) quando comparados aos portadores do alelo *MAOA-u*curto* (80.015kcal [37.45–127.115]; *p* = 0.034). Na amostra de meninas, o polimorfismo *MAOA-u VNTR* não foi associado com a ingestão alimentar e dados antropométricos. No polimorfismo *COMT Val158Met* os portadores do alelo *COMT*158Val* tiveram um consumo maior de HLD em comparação aos homozigotos para o alelo *COMT*158Met* (*p* = 0.008), as medianas foram 133.79kcal [44.23–265.80] e 83.37Kcal [0.00–252.95], respectivamente. Estes achados indicam a associação destes polimorfismos nos genes *DRD2, MAOA* e *COMT* com a regulação da ingestão alimentar e como fator de risco potencial para obesidade.

Palavras-chave: Polimorfismos em *DRD2, MAOA* e *COMT*; consumo alimentar; obesidade infantil.

SUMÁRIO

CAPÍTULO 1

INTRODUÇÃO

1.1 Obesidade

A obesidade é um fator de risco para múltiplos problemas de saúde nos adultos, entre estes problemas destacam-se a doença cardíaca, a aterosclerose, a elevação do colesterol, a pressão sanguínea elevada, determinados tipos de cânceres e diabetes (LEWIS & MAN, 2007). Em um estudo epidemiológico, no qual foi examinada a variação de peso de crianças e adolescentes detectou-se que a partir dos três anos de idade o excesso de peso torna-se definitivamente determinante de obesidade futura; e que se a criança é obesa aos seis anos de idade ela apresenta 50% de chance de tornar-se um adulto obeso (MORAN, 1999). Dados de pesquisas populacionais brasileiras mostram que a prevalência de obesidade em crianças de seis a nove anos triplicou entre 1974 e 1997 (WANG *et al*., 2002a). O excesso de peso como problema de saúde pública tornou-se mais prevalente que a desnutrição no Brasil e no restante do mundo (POPKIN *et al.*, 2001). A epidemia recente de obesidade infantil despertou interesse no seu estudo em função das conseqüências possíveis sobre a saúde clínica e pública (REILLY *et al.*, 2003). O que se pode presumir é que estas terão impacto considerável no futuro sobre custos e serviços de saúde tornando-os mais onerosos (MOZAFFARI & NABAEI, 2007). O desenvolvimento de obesidade resulta de um conjunto de fatores comportamentais e ambientais que conduzem ao balanço energético positivo.

1.1.1 – Comportamento alimentar

O comportamento alimentar envolve o apetite (sensação de fome e saciedade), os estados motivacionais e a necessidade de ingestão energética (processos fisiológicos e metabólicos), coordenados pela atividade dos sistemas nervosos periférico e central (vias neurais e receptores) (NETTO, 1998) É consenso que modificações no comportamento alimentar se impõem para prevenir doenças relacionadas à alimentação e promover a saúde do indivíduo. Uma vez que é na

infância que o hábito alimentar se forma, é necessário o entendimento dos seus fatores determinantes (ANGELIS, 1995; SPLETT, 1991). A literatura sobre nutrição infantil evidencia que o comportamento alimentar do pré-escolar é determinado em primeira instância pela família, da qual ela é dependente e, secundariamente, pelas outras interações psicossociais e culturais da criança (BIRCH, 1998; ROZIN, 1997). O padrão da alimentação do pré-escolar é determinado por suas preferências alimentares. A dificuldade é fazer com que a criança aceite uma alimentação variada, aumentando suas preferências e adquirindo um hábito alimentar mais adequado (KOIVISTO & SJÖDÉN, 1996). A aprendizagem é central no desenvolvimento do padrão alimentar da criança (BIRCH, 1997). Entende-se hábito como sendo um ato, uso e costume, ou um padrão de reação adquirido por freqüente repetição da atividade (aprendizagem). Esse termo também pode ser aplicado, por generalização, a normas de comportamento (FERREIRA, 2004; CABRAL, 1974). Assim, os alimentos ou tipo de alimentação que os indivíduos consomem rotineiramente e repetidamente no seu cotidiano caracterizam o seu hábito ou comportamento alimentar. No entanto, não é simplesmente a repetição do consumo do alimento que desenvolve o comportamento alimentar. Existe um grande número de fatores inter-relacionados, de origem interna e externa ao organismo, que influenciam a aquisição desse comportamento. Cabe ressaltar que o hábito alimentar não necessariamente é sinônimo das preferências alimentares do indivíduo. Porém, no caso específico dos pré-escolares, o hábito alimentar caracteriza-se fundamentalmente pelas suas preferências alimentares. As crianças desta faixa etária acabam consumindo somente alimentos de que gostam, entre os disponíveis no seu ambiente, refutando aqueles de que não gostam (BIRCH, 1998; ROZIN, 1997). Os fatores psicossociais influenciam as experiências alimentares desde o momento do nascimento da criança, proporcionando a aprendizagem inicial para a sensação da fome e da saciedade e para a percepção dos sabores. A adequada introdução dos novos alimentos no primeiro ano de vida, com uma correta socialização

alimentar, a partir deste período, bem como a disponibilização de variados alimentos saudáveis em ambiente alimentar agradável, permite à criança iniciar a aquisição das preferências alimentares responsáveis pela determinação do seu padrão de consumo (BIRCH, 1998). A tendência das preferências alimentares das crianças na idade pré-escolar conduz ao consumo de alimentos com quantidade elevada de carboidrato, açúcar, gordura e sal, e baixo consumo de alimentos como vegetais e frutas, se comparados às quantidades recomendadas (KREBS-SMITH *et al.,* 1996). Esta tendência é originada na socialização alimentar da criança e depende, em grande parte, dos padrões da cultura alimentar do grupo social ao qual ela pertence. A sensibilidade ao sabor doce já aparece na fase pré-natal, sendo, portanto, uma preferência inata. Possivelmente, devido a esta sensibilidade ao doce estimulada pelas substâncias químicas do líquido amniótico durante a fase pré-natal (BEAUCHAMP & MENNELLA, 1994), verifica-se um aumento da aceitação de alimentos desconhecidos, quando estes estão associados ao açúcar ou a alimentos naturalmente adocicados. Neste tipo de aprendizagem, o sabor está associado ao prazer e provavelmente por esta razão se mantém ao longo do tempo, ou seja, é durável e sua modificação só é possível quando outra experiência aprendida substitua ou neutralize a experiência anterior (CAPALDI, 1997). Por este motivo observa-se que a preferência por consumo de alimentos ricos em açúcar e gordura pode levar o indivíduo a obesidade na idade adulta.

Segundo MARTI & MARTINEZ (2006) o risco de obesidade, na prática, depende pelo menos de dois fatores importantes, que interagem mutuamente: 1) as variantes e a expressão genética e 2) a exposição aos fatores de risco ambientais. Quando existe predisposição genética para o desenvolvimento da obesidade, o ambiente e o estilo de vida do indivíduo potencializam o seu desenvolvimento. Portanto, as diferenças genéticas podem esclarecer algumas das discrepâncias encontradas no ganho de peso entre populações, entretanto, a prevalência da obesidade na sociedade deve refletir também mudanças no estilo de vida (hábitos, sedentarismo,

etc.), já que a predisposição genética pode ser influenciada por mudanças na exposição ambiental.

1.2 Fatores Genéticos

Muitos genes têm sido investigados pelo seu provável papel na determinação da composição corporal (RANKINEN *et al*., 2006). Os genes candidatos para estudo são os que codificam proteínas relacionadas às diferentes vias metabólicas, como a regulação do apetite, sensibilidade à insulina e diferenciação de adipócitos, e as envolvidas na termogênese, no catabolismo e transporte de ácidos graxos (MALCZEWSKA-MALEC *et al*., 2004). Uma das linhas muito pouco investigadas na avaliação do componente genético da obesidade é o estudo de variantes dos genes relacionados ao comportamento de ingestão alimentar de adultos e crianças.

Grandes avanços têm sido alcançados no campo da genética das doenças multifatoriais, tais como obesidade, cardiopatias, diabetes e câncer. A evidência de associações entre genes candidatos e fenótipos relacionados à obesidade passam de 127 genes em que foram encontradas associações significativas (RANKINEN *et al*., 2006). A conclusão do Projeto Genoma Humano (INTERNATIONAL HUMAN GENOME SEQUENCING CONSORTIUM, 2001) é considerada um importante passo no objetivo de revelar as bases genéticas de doenças complexas (HUMPHRIES & ORDOVAS, 2001). Variações em um grande número de genes envolvidos na síntese de proteínas estruturais e enzimas relacionadas no metabolismo de neurotransmissores envolvidos com o consumo alimentar poderiam, a princípio, responder por variações de comportamento alimentar de cada indivíduo. Desta maneira, qualquer gene que seja responsável pela produção de uma proteína envolvida nesta rota metabólica poderia ser um “gene candidato” na investigação de determinantes genéticos da obesidade. Assim, o somatório de

variações com pequeno efeito em cada um destes genes poderia levar à alteração do comportamento alimentar de um indivíduo, predispondo à obesidade.

Como estas variantes genéticas são bastante freqüentes na população em geral (de 1% a 80% dos indivíduos), seu impacto é muito maior na saúde pública quando comparadas com mutações de grande efeito, mas que são muito mais raras. Estas variações quando são freqüentes são chamadas polimorfismos (mais de 1% de freqüência do alelo mais raro). A base genética para esta variação pode ser uma troca de bases no DNA, uma duplicação ou deleção de um ou vários pares de bases. Estimativas atuais sugerem que variações de uma única base entre indivíduos (*single nucleotide polymorphisms*, ou SNPs) ocorrem na freqüência de um SNP a cada 1.300pb, ou seja, existem mais de 1.4 milhão de polimorfismos de substituição de uma única base em nosso genoma (INTERNATIONAL HUMAN GENOME SEQUENCING CONSORTIUM, 2001). O estudo da associação de variações no DNA com suscetibilidade a certas doenças ou características é uma área promissora da genética. Investigações nesta área podem ser realizadas basicamente de duas maneiras: (1) avaliando-se a distribuição da frequência alélica e genotípica entre grupos de indivíduos portadores de uma determinada doença ou característica, como obesidade, por exemplo; (2) analisando-se as médias de parâmetros, relacionados com a característica ou a patologia, entre os grupos de indivíduos portadores dos diferentes genótipos.

1.3 Sistema Dopaminérgico

Existem diversos aspectos a serem considerados na regulação do comportamento alimentar. Dentre os aspectos qualitativos do controle de apetite, podemos citar os mecanismos relacionados à seleção de certos nutrientes específicos ou grupos de nutrientes, os quais são determinados pelo grau de prazer experimentado pelos indivíduos (sistema mesolímbico de

recompensa). Neste contexto, a dopamina, um neurotransmissor endógeno, é especialmente interessante, pois, além de modular uma variedade de funções fisiológicas, como o transporte iônico, o tônus vascular e a pressão sanguínea, possui um papel principal na regulação do apetite (FANG *et a.l,* 2005). Evidências obtidas, a partir de estudos com humanos e modelos animais, indicam a importância da função dopaminérgica no desenvolvimento da obesidade por seu papel na regulação do apetite (SCHACHTER *et al.,* 1968). Em humanos, a obesidade foi associada com uma ação anormal da dopamina no cérebro (NOBLE, 2000a).

Quando baixa o nível de dopamina nos neurônios pós-sinápticos da via mesolímbica quer por diminuição da produção desse neurotransmissor, quer por redução do numero de seus receptores D2, as mesmas condições que antes atuavam como autênticos estímulos naturais já não se mostram suficientes para gerar sensações prazerosas ou de bem estar. O indivíduo passa, então, a buscar, através de alterações comportamentais, o aumento da liberação de dopamina para o sistema límbico. Nesse caso incluem-se o uso e abuso de substâncias químicas, como álcool, cafeína, que parecem induzir o aumento da liberação de dopamina, assim como aumento do consumo alimentar, levando assim a um aumento da obesidade entre esses indivíduos (OLIVEIRA, 1999). FANG *et al*., (2005) demonstraram que drogas agonistas do sistema dopaminérgico são capazes de suprimir o apetite e, subseqüentemente, reduzir o peso. A participação do sistema dopaminérgico na recompensa e no reforço, após a ingestão alimentar, conduziu à hipótese de que há baixa atividade de dopamina no cérebro em pacientes obesos e pacientes pré-dispostos ao consumo excessivo de alimentos (WANG *et al.*, 2002b). BLUM *et al*., (2007), sugeriram que um fator genético, ainda desconhecido, agiria sobre a atividade dopaminérgica no centro da recompensa do cérebro influenciando o comportamento - aumentando o desejo de consumir doces e tendo como conseqüência a obesidade. Este defeito estimula os indivíduos para encontrar nos excessos (alimentares ou entorpecentes) um aumento

da função da dopamina no cérebro, categorizando o que foi denominado de Síndrome da Deficiência da Recompensa (RDS). Atualmente considera-se que o alimento poderia ser o mais importante estimulador natural do sistema de recompensa no cérebro (EPSTEIN & LEDDY, 2006). Portanto, comer em excesso pode representar uma tentativa de compensar a deficiência de recompensa em condições de atividade dopaminérgica reduzida. A deficiência dopaminérgica relativa pode ser causada por diferentes condições, por exemplo, a predisposição genética ou após regulação negativa adaptativa do sistema dopaminérgico, devido à hiperestimulação anterior. Um efeito rebote do comportamento alimentar após hiperestimulação dopaminérgica poderia explicar o ganho de peso, muitas vezes associados a cessação do tabagismo, porque durante o fumo, a nicotina estimula células que contém dopamina, na área ventral tegmental, resultando na liberação desta dopamina nas projeções mesolímbica e mesocortical (KAUER, 2005). Além disso, o aumento de peso é um efeito colateral de muitas drogas comumente usadas. Até a data, os mecanismos subjacentes são ainda mal compreendidos, embora as interações com o sistema de dopamina já tenham sido implicadas (GOUDIE *et al*., 2003). Outro mecanismo de ação proposto sobre a influência do sistema dopaminérgico sobre o comportamento alimentar, caminha na direção contrária e afirma que uma hiper-sensibilidade à recompensa aumentaria a motivação para atividades prazerosas como comer. Em vários estudos, a elevada sensibilidade à recompensa contribui para o aumento do risco de desenvolvimento de comportamentos aditivos para abordagem de atividades potencialmente prazerosa tais como o consumo de drogas e comer, tendo sido inclusive associado à preferência por alimentos ricos em gordura, compulsão alimentar, e ânsia pelo alimento, bem como com o consumo perigoso de álcool (DAVIS *et al*., 2004, 2007; DAVIS & WOODSIDE, 2002; FRANKEN & MURIS, 2005; LOXTON & DAWE, 2001, 2006).

O sistema dopaminérgico no cérebro possui três principais vias de transmissão – nigroestriatais, mesocorticais e mesolímbicas. A via nigroestriatal projeta-se da A9 na Substância Nigra Pars compacta (SNc) do mesencéfalo para o estriado dorsal, em particular, núcleos caudado e putâmem e está envolvida no controle motor. Manifestações da doença de Parkinson são atribuídas à redução de entrada dopaminérgica no estriado devido à degeneração de neurônios dopaminérgicos da SNc (WEINER, 2000). A via mesocortical tem sua origem em corpos celulares da Área Tegmental Ventral (VTA). Seus axônios enviam projeções excitatórias para o córtex pré-frontal afetando funções com a formação de memórias de curto prazo, motivação, atenção e planejamento de estratégia para solução de problemas. Os corpos celulares dos neurônios do sistema mesolímbico estão também localizados na VTA (A8 e A10). Estas células projetam-se para várias partes do sistema límbico, incluindo o núcleo accumbens, a amígdala, o hipocampo, o córtex cingulado e o córtex entorrinal. O núcleo accumbens tem um importante papel nos efeitos reforçadores de certos tipos de estímulos e nos comportamentos orientados a metas (*goal-directed*). Assim, os neurônios dopaminérgicos mesolímbicos estão envolvidos com as propriedades reforçadoras de várias drogas de abuso, incluindo os psicoestimulantes tais como, cocaína e anfetamina (VOLKOW *et al*., 2002) e também com consumo alimentar exagerado, como citado anteriormente.

1.4 Receptores de Dopamina – Gene *DRD2*

Além das diferenças anatômicas, existem também diferenças funcionais entre essas vias, demonstrando a heterogeneidade da população de neurônios cuja dopamina é neurotransmissor. Nessas células, cinco subtipos de receptores dopaminérgicos foram encontrados: DRD1 – DRD5 (SEEMAN *et al*. 1993). Estes receptores são divididos em duas subfamílias, DRD1-like (DRD1 e

DRD5) cuja ação intracelular é ativar a adenil-ciclase, a qual catalisa a formação de AMPc, que por sua vez ativa a proteína quinase A (PKA) regulando canais iônicos e fatores de transcrição; e *DRD2*-like (*DRD2*, DRD3 e DRD4) que inibe a adenil-ciclase e ativa os canais de K^+.

O gene que codifica o receptor de dopamina D2 (*DRD2*) foi mapeado por Grandy em 1989 no cromossomo 11 (q22-q23), sendo a sua seqüência codificadora interrompida por seis introns, originando uma proteína com sete domínios transmembrana (NOBLE, 2000b). WANG *et al*. (2007) em seu estudo demonstraram que a obesidade e o índice de massa corporal estão correlacionados negativamente com a densidade de receptores D2 no estriato (WANG *et al.,* 2001; HALTIA *et al*., 2007), o que pode refletir uma neuroadaptação secundária a superestimulação com alimentos palatáveis (COLANTUONI *et al.,* 2001; BELLO *et al*., 2002). Assim, o aumento da ingestão de alimentos pode ser um comportamento compensatório para a baixa quantidade de receptores dopaminérgicos (DAVIS *et al*., 2004). Está bem estabelecido que a alimentação está associada com a liberação de dopamina no estriado dorsal e que o grau de prazer sentido durante a ingestão alimentar está diretamente associado à quantidade de dopamina liberada nessa região. No entanto, essas evidências estão divididas sobre a direção da causa da associação. Um dos argumentos é que a Síndrome de Deficiência da Recompensa é um fator de risco para o comer compulsivo, enquanto que outros afirmam que hiper-sensibilidade à recompensa aumenta a motivação para atividades prazerosas como comer.

1.4.1 Polimorfismo *TaqI*A

STICE *et al*. (2008) descreveu recentemente menor ativação estriatal em resposta à ingestão de alimentos em indivíduos obesos. Além disso, esta relação foi modulada pela disponibilidade do receptor D2 geneticamente determinada pelo polimorfismo *TaqIA* (STICE *et al.,*

2008). Os dados transversais e prospectivos obtidos a partir de dois estudos de ressonância magnética funcional dão suporte às hipóteses de que a alimentação está associada com a liberação de dopamina no estriato dorsal, e o grau de prazer de comer se correlaciona com a quantidade de dopamina liberada, o que indicaria que os indivíduos podem comer demais para compensar um hipofuncionamento no estriato dorsal, particularmente àqueles com variantes genéticas que atenuam a sinalização de dopamina na região (SMALL *et al*., 2003; SZCZYPKA *et al*., 2001). O gene *DRD2* é altamente polimórfico, mas muita atenção foi focalizada no polimorfismo denominado Taq1A (C32806T ou RS1800497), substituição de C>T situada a 10Kb da posição 3' do gene, uma região não codificadora, o qual parece afetar a disponibilidade do receptor D2 (MUNAFO *et al*., 2005). O alelo C resulta em um sítio de restrição para a enzima *Taq*I; o alelo T que não gera este sítio de restrição era descrito anteriormente como alelo A1. NOBLE *et al*. (1994), encontraram associação entre o alelo T e tempo de latência aumentado para as ondas cerebrais P300 (que estão ligadas à atenção) em filhos de dependentes de álcool, indicando que variações na função dopaminérgica podem ser herdadas. NOBLE (1997), ao analisar sujeitos sem diagnóstico de abuso de álcool ou drogas, detectou que o metabolismo cerebral de glicose está reduzido em portadores do alelo T nas áreas envolvidas no sistema de recompensa cerebral, como *nucleus accumbens*, ou reguladoras de função frontal, como córtex pré-frontal. POHIALAINEN *et al*. (1998), estudando voluntários saudáveis em uma população finlandesa, também encontraram associação entre o alelo T e baixa disponibilidade de receptores D2. Em um estudo sobre hiperprolactinemia induzida pela medicação antipsicótica antagonista do receptor de dopamina D2, observou-se que os pacientes com o alelo T que recebem medicação antipsicótica tiveram níveis mais elevados de prolactina e este alelo foi altamente predominante entre aqueles com hiperprolactinemia (YOUNG *et al*., 2004). LAWFORD *et al*. (2003) em um estudo sobre o tratamento do estresse pós-traumático (PTSD), mostrou que pacientes portadores do alelo T

(genótipo T/C), comparados aos indivíduos não portadores deste alelo (genótipo C/C), apresentaram mais problemas psicopatológicos e maior prevalência de ansiedade/insônia, disfunção social e depressão.

MORTON *et al*. (2006) demonstraram que esta variação genética no gene *DRD2* modifica as características sobre o ato de fumar e também sobre a obesidade recompensa-motivada. Em 2007, EPSTEIN *et al.* observaram em seu estudo que o reforço alimentar foi maior nos obesos do que em indivíduos não obesos, especialmente em indivíduos obesos com o alelo T. O consumo de energia foi maior para indivíduos com alto reforço alimentar e maior ainda naqueles com alto reforço alimentar e com o alelo T (EPSTEIN *et al*., 2007). Em um estudo realizado por NISOLI *et al.* (2009) confirmam que a presença do alelo T não está simplesmente relacionado com o peso corporal, mas que ele pode ser um marcador de uma condição genética em pessoas com alto risco de desenvolver comportamento alimentar patológico. Em outro estudo, que avaliou subgrupos de pessoas obesas, observou-se associação entre ausência do alelo T do polimorfismo *DRD2 TaqIA1* e indivíduos obesos que apresentavam transtorno de compulsão alimentar periódica (TCAP) (DAVIS *et al*., 2009). Estes resultados contraditórios demonstram a necessidade de estudos que demonstrem relação de casualidade entre o alelo T e comportamentos alimentares que predispõem a obesidade. Além de que, o que se observa é que estes estudos na grande maioria foram realizados em brancos e já foi verificado que há diferença significativa entre a freqüência do polimorfismo entre as diversas etnias como demonstrado por DAVIS *et col*. (2009).

1.4.2 Polimorfismo -141C Ins/Del

Outro polimorfismo, no gene de *DRD2*, que vem sendo estudado é o -141C Ins/Del (RS1799732) que corresponde a uma variante genética caracterizada pela presença (**Ins**ertion) ou

ausência (**Del**etion) da base nitrogenada citosina (C) na posição 141 da região promotora do *DRD2* (PARSONS *et al*., 2007). Este polimorfismo já foi associado à diminuição média de 68% da expressão de receptores D2 (ARINAMI *et al*., 1997) e também a redução estriatal destes receptores (JÖNSSON *et al*., 1999). ARINAMI (1997) encontrou associação entre o alelo -141C*Ins e susceptibilidade ao desenvolvimento de esquizofrenia que é caracterizada pelo aumento da disponibilidade de dopamina. Outros estudos que se seguiram também encontraram associação entre o alelo -141C*Ins e esquizofrenia (OHARA *et al*., 1998; SCHINDLER *et al*., 2002). Já um estudo que avaliou a influência do polimorfimo -141-C Ins/Del sobre a eficácia de medicação ansiolítica para pacientes depressivos, constatou uma resposta melhor à medicação nos pacientes que não portavam o alelo -141-C*Del, (SUZUKI *et al*., 2001). Acredita-se que estes resultados opostos se devam à possibilidade da freqüência alélica desse polimorfismo variar de acordo com o grupo étnico. A maioria dos estudos realizados com os polimorfimos no gene que codifica os receptores de dopamina 2 (*DRD2*) estão relacionados a distúrbios psiquiátricos. Até o momento é de nosso conhecimento apenas um estudo que avaliou associação entre o polimorfismo *-141C Ins/Del* e comportamento alimentar, e este estudo realizado por DAVIS *et col*. (2009), não encontrou associação entre portadores do alelo Del com TCAP.

1.5 Monoaminooxidases – Gene *MAOA*

As monoaminooxidases (MAO) são enzimas que catalisam a desaminação oxidativa de monoaminas naturais, entre elas a serotonina, a adrenalina, a noradrenalina e a dopamina (WEYLER *et al*., 1990), o que sugere importante papel na manutenção da homeostase destes neurotransmissores (CHEN, 2004). São flavoenzimas localizadas nas membranas mitocondriais, nos terminais nervosos, no fígado e em outros órgãos. Os subtipos *MAOA* e *MAOB* podem ser

distinguidos de acordo com suas propriedades: peso molecular, afinidade pelo substrato e propriedades imunológicas. Estas enzimas são expressas em todo corpo, mas diferem em expressão e em seu desenvolvimento em células específicas. A enzima *MAOA* é expressa em níveis mais altos em neurônios catecolaminérgicos (FOWLER *et al*., 1987; THORPE *et al*., 1987). *MAOA* preferencialmente oxida as aminas biogênicas como a serotonina, a noradrenalina e a epinefrina. Dopamina, tiramina e triptamina são substratos comuns para ambas as formas (CHIBA *et al.,* 1984). Posteriormente os genes que codificam esses subtipos foram clonados e descobriu-se que as seqüências de aminoácidos de suas estruturas protéicas apresentam 70% de homologia (LIONEL, 1995), além de ter uma organização gênica (número de éxons e introns) idêntica (GRIMSBY *et al*., 1991). EKLUND *et al*. (2005) relatou em seu estudo que a dificuldade de atenção foi associado com baixa atividade de MAO. Os baixos níveis de atividade de MAO foram associados com desatenção e impulsividade em meninos com TDAH (SHEKIM *et al*., 1986). A administração de substâncias que inibem atividade da MAO tem demonstrado significativa melhora do humor e da ansiedade. Estas provas fisiológicas e farmacológicas tornam o gene da monoamina oxidase A um candidato para um estudo de associação com ansiedade e ingestão alimentar.

1.5.1 Polimorfismo *MAOA-u VNTR*

O gene *MAOA* está localizado no cromossomo X (p11.23 - p11.4) e é composto por 15 exons e 14 introns. Este gene possui diversos polimorfismos, entre eles um polimorfismo de número variável de repetições em tandem (*MAOA-u VNTR*) com unidade de repetição de 30pb, localizado na região promotora que influencia a expressão do gene; sendo que os alelos com 3,5 e 4 repetições induzem uma transcrição de 2 até 10 vezes mais eficiente que o alelo com 3

repetições (DENNEY *et al*., 1999). Alguns estudos foram discordantes com relação ao alelo raro de 5 repetições: SABOL *et al*. (1998) e DECKERT *et al*. (1999) relataram baixa e alta atividade transcrição, respectivamente. Os alelos de 3,5 e 4 repetições também foram associados com maiores níveis de degradação de dopamina e serotonina no líquido cerebroespinal em mulheres saudáveis (JÖNSSON *et al*., 2003).

Um estudo observou interação entre *MAOA* e a variante T do polimorfismo Taq1A do gene *DRD2* com ansiedade e dependência de álcool (ANS / DEP ALC), assim variantes no gene de *MAOA* podem modificar a associação entre as variantes de *DRD2* e fenótipo ANS / DEP ALC (HUANG *et al*., 2007). Também foi encontrada associação entre o alelo *MAOA-u**3 e Transtorno de déficit de atenção e hiperatividade (TDAH) em uma população de Taiwan (XU *et al*., 2007). KIM *et al*. (2006) descreveu uma associação entre variantes da *MAOA* e relato de dor máxima no pós-operatório. Um estudo que avaliou genes envolvidos com o funcionamento do sistema serotoninérgico e dopaminérgico encontrou associação entre o polimorfismo *MAOA-u VNTR* e a predição das categorias de IMC observando que o alelo de baixa atividade era associado a um maior IMC em uma amostra de homens obesos (FUEMMELER *et al*., 2008). Outro estudo ao realizar a análise do polimorfismo *MAOAu-VNTR* revelaram uma tendência para um desequilíbrio de transmissão entre o alelo de baixa atividade transcricional (alelo curto) era transmitido preferencialmente aos filhos que apresentavam obesidade (CAMARENA *et al*., 2004). Embora os resultados dos trabalhos acima indiquem uma participação do polimorfismo *MAOAu-VNTR* e obesidade, ainda não é possível determinar um alelo de risco.

1.5.2 Polimorfismo *MAOA T941G*

Outro polimorfismo no gene *MAOA* é encontrado no exon 8 é o T941G, um SNP funcional onde a ocorrência de guanina (G) na posição 941 cria um sítio de restrição para a enzima Fnu4H1 dentro da região codificante do gene. Embora esta substituição T/G ocorra na terceira base do códon, e desta forma não afete a estrutura da proteína, ela foi associada com diferentes níveis de atividade enzimática. Hotamisligil e Breakefield relataram uma associação do alelo 941T com baixa atividade da enzima *MAOA* em 40 linhagens de células de atividade conhecida da *MAOA* (HOTAMISLIGIL *et al*., 1991). Já em uma amostra de irlandeses o alelo 941G, de alta atividade, foi associado com TDAH (DOMSCHKE *et al*., 2005). Xu *et al*., também encontraram associação entre o alelo 941G e risco de desenvolver TDAH (XU *et al*., 2007). O primeiro estudo realizado observando a associação entre o polimorfismos T941G e Transtorno de ansiedade generalizada (TAG), que também tem como fator etiológico aumento nos níveis de serotonina e dopamina no cérebro, foi publicado em 2003 e encontrou associação entre o alelo 941T e TAG (TÁDIC *et al*., 2003). Devido a estas inconsistências da literatura é importante que outros estudos investiguem o papel do polimorfismo *MAOA T941G* no gene *MAOA* e seu papel sobre a ansiedade e comorbidades associadas como o excesso de ingestão alimentar. Estudos que investiguem a associação diretamente do polimorfismo *MAOA T941G* e comportamento alimentar ou obesidade até o momento não foram realizados.

1.6 Catecol-o-metiltransferase – Gene *COMT*

A COMT exerce um papel proeminente na inativação e degradação de catecolaminas e estrógeno (ANNERBRINK *et al.*, 2008). Esta enzima é expressa de duas formas: uma forma solúvel (s-*COMT*) e uma forma ligada a membrana com 50 resíduos adicionais no resíduo N-

terminal (mb-*COMT*) (TENHUNEN *et al*., 1994). A *COMT* catalisa a transferência de um grupo metil da S-adenosilmetionina (SAM) para uma variedade de catecolaminas, incluindo o neurotransmissor dopamina (MANNISTO & KAAKKOLA, 1999). Ela é uma enzima extracelular que *O*-metila a dopamina, sendo a única enzima que é capaz de metabolizar a dopamina neste espaço. Esta reação pode ocorrer antes ou depois da desaminação pela MAO, localizada na mitocôndria intraneuronal (SHIELD *et al*., 2004). Há evidências de que mudanças na atividade da *COMT* trazem efeitos centrais e periféricos por alterar as quantidades de dopamina e noradrenalina na fenda sináptica (KRING *et al*., 2009). Sendo assim, esta enzima está envolvida em vários mecanismos recompensa motivado como o aumento da ingestão alimentar (WANG *et al.,* 2001; HALFORD *et al*., 2004), humor e outros processos mentais (MANNISTO & KAAKKOLA, 1999; MALHOTRA *et al*., 2007). Considerando que catecolaminas e hormônios esteróides exercem influência sobre consumo alimentar (HALFORD et al., 2004) e metabolismo (LOUET *et al*., 2004), pode-se correlacionar que a atividade da enzima *COMT* pode influenciar na determinação do índice de massa corporal (IMC) e/ou na distribuição de gordura.

1.6.1 Polimorfismo *COMT Val158Met*

Vários polimorfismos no gene da *COMT* têm sido descritos, mas o *COMT Val158Met* (24938A/G, rs4680) é o mais amplamente estudado, mostrando as associações com câncer de mama (YIM *et al*., 2001; WEDRÉN et al., 2003), esquizofrenia (STROUS *et al.,* 2003), transtorno obsessivo compulsivo (KARAYIORGOU *et al*., 1997), alcoolismo (KAUHANEN *et al*., 2000), transtornos alimentares (MIKOLAJCZYK et al., 2006) e obesidade (WANG *et al*., 2007). Ele está localizado no quarto éxon do cromossomo 22q11.21 e se caracteriza pela troca de uma valina por uma metionina na posição na posição 158 na forma mb-COMT (*Val158Met*) em conseqüência da

troca da base nitrogenada guanina (alelo G) pela base adenina (alelo A) (LUNDSTRÖM *et al*., 1995). O polimorfismo *Val158Met* afeta a atividade e a expressão da COMT, afetando potencialmente os níveis de dopamina. O alelo Met produz uma proteína termolábil, diminuindo sua atividade e permitindo, assim, aumento na concentração de dopamina extracelular (MANNISTO & KAAKKOLA, 1999; SHIELD *et al*., 2004). Indivíduos portadores do genótipo Met/Met têm redução de 3 a 4 vezes na atividade de degradação enzimática quando comparados com portadores do genótipo Val/Val; heterozigotos possuem uma atividade intermediária (LACHMAN *et al.,* 1996). Os estudos de associação deste polimorfismo com comportamento alimentar e obesidade apresentam resultados controversos, os dois alelos já foram associados com aumento de risco para estas condições. Em um estudo publicado em 2006 por NEED *et cols*., não foi detectada associação entre o polimorfismo *COMTVal158Met* e obesidade.

JUSTIFICATIVA

De acordo com a revisão mais compreensiva na área, o Obesity Gene Map (RANKINEN *et al*., 2006), a influência de diversas variantes genéticas no desenvolvimento de obesidade está sendo investigada por muitos grupos, mas poucos estudos analisaram a influência destas variantes em crianças e, também, seu efeito na variação de peso com a idade. Os poucos estudos realizados em relação ao componente genético da obesidade na população brasileira restringem-se a adultos. A identificação dos indivíduos que são geneticamente mais susceptíveis a responder às mudanças dietéticas particulares pode ser importante para a intervenção bem sucedida no tratamento da obesidade. A amostra analisada neste trabalho possui uma característica inédita, pois numerosos estudos já foram realizados em adultos, mas relativamente poucos em crianças e, mesmo estes, na sua quase totalidade consistiram em estudos que não avaliaram as diferenças étnicas como o aqui proposto.

OBJETIVOS

Investigar a associação das variantes Taq1A e -141C Ins/Del do gene *DRD2*; variante *MAOA-u VNTR* e T941G do gene *MAOA*; e variante Val158Met do gene *COMT* na regulação do peso corporal e os aspectos relacionados à obesidade, dados antropométricos e consumo alimentar de crianças com idade entre três e quatro anos de idade.

Objetivos específicos

- Analisar a associação das variantes Taq1A e -141C Ins/Del do gene *DRD2*; variante *MAOA-u VNTR* e T941G do gene *MAOA*; e variante Val158Met do gene *COMT* e o escore Z do índice de massa corporal.
- Investigar a associação das variantes Taq1A e -141C Ins/Del do gene *DRD2*; variante *MAOA-u VNTR* e T941G do gene *MAOA*; e variante Val158Met do gene *COMT* e o escore Z do percentil de dobras cutâneas.
- Verificar a associação das variantes Taq1A e -141C Ins/Del do gene *DRD2*; variante *MAOA-u VNTR* e T941G do gene *MAOA*; e variante Val158Met do gene *COMT* e circunferência da cintura.
- Verificar a associação das variantes Taq1A e -141C Ins/Del do gene *DRD2*; variante *MAOA-u VNTR* e T941G do gene *MAOA*; e variante Val158Met do gene *COMT* com ingestão alimentar.

CAPÍTULO 2

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CAPÍTULO 3

MANUSCRITO 1
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Association of *DRD2 TaqIA* and -141C *InsDel* polymorphisms with food intake and anthropometric data in Brazilian children

Association of *DRD2 TaqIA* and *-141C InsDel* polymorphisms with food intake and anthropometric data in Brazilian children

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Running title: *DRD2* gene: nutritional and anthropometric data

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Effective interventions to reduce obesity and related health risks are increasingly important because the number of obese adults and children has reached epidemic proportions. There are data supporting a role for allelic variants of the D2 dopamine receptor (DRD2) gene in susceptibility to obesity. We assessed the relationships between food intake, anthropometric data and the *DRD2 Taq1*A and *-141C InsDel* genotypes in children. Our sample consisted of 354 children from three to four years of age whose race or ethnicity was self-defined by skin colour (i.e. white or non-white). Among white children, only 23.6% exhibited the *TaqIA***T* allele as compared to 32% in the non-white sample (p=0.028). A similar pattern was observed for the 141C Del allele (18.4% in white children versus 10.8% in non-white children; p=0.011). In non-white children, the *Taq*IA *C/C* homozygous genotype was associated with higher HLD intake (median 666.8 kJ) when compared with children carrying the *T* allele (343.9 kJ, p=0.009). Further studies evaluating the effects of this polymorphism on the genetic profiles associated with food consumption patterns and the anthropometry of children from different populations will provide a better understanding of polymorphisms relationship to nutritional outcomes.

Paediatric obesity rates have dramatically increased over the past decade [1]. Surveys during the 1990's in Brazil and the USA showed that an additional 0.5% of the child population became overweight during each year of that decade [2, 3]. This tendency is especially alarming because an increase in body weight is a major cause of cardiovascular disease and affects physical and social functioning as well as life quality [4]. Obesity development is influenced by a complex array of genetic, metabolic and neural frameworks along with behaviour, eating habits and physical activity [5]. Obesogenic modern environments may either overshadow the observable effects of genetic differences or boost them, by providing a permissive substrate for the expression of susceptibility [6, 7]. Despite increased knowledge of the neural pathways that control appetite and satiety, obesity prevalence continues to rise. The ingestion of energy-rich diets and palatable foods has been linked to changes in stress and reward pathways in the brain [8].

Dopaminergic (DA) pathways are hypothesized to regulate the behavioural and metabolic responses associated with the development of obesity through feeding and satiety [9]. The central DA reward pathway appears to be involved in the reinforcing effect received by the brain after a pleasurable experience such as the use of certain drugs [10–12]. Drugs that stimulate this pathway have a positive reinforcing action that leads to addiction. Food has also been proposed to be such a reinforcing agent [10, 12]. Feeding is associated with dopamine release in the dorsal striatum, and the degree of pleasure from eating correlates with the amount of dopamine release [13, 14]. Stimulation of this pathway may reduce the effectiveness of satiety factors, thus promoting overeating and leading to an increase in body weight [10]. Since this hypothesis was proposed, several efforts have been made to identify the key molecular components of dopamine neurotransmission that influence obesity. A growing area of research has begun to explore the potential association between specific candidate genes that regulate the brain dopamine system and obesity [15]. In the context of a molecular genetic approach to the problem, several candidate genes among potentially many others related to this hypothesised vulnerability would include 1) any of the five known dopamine receptor genes, 2) genes coding for important enzymes of dopamine metabolism, and 3) the dopamine transporter protein [16]. Pharmacological data have suggested that the dopamine D2 receptor (DRD2) may be involved in excessive body fat accumulation. It was observed that dopamine antagonist administration increases appetite, energy intake and weight gain in rats and in schizophrenic patients [17, 18], whereas dopamine agonists reduce food intake and produce

weight loss [19]. The *DRD2* gene harbours at least two polymorphic functional variants. Civelli et al. [20] cloned the human *DRD2* gene in rats and humans [21] and described the *DRD2 Taq*IA polymorphism (rs1800497), which is a C/T single nucleotide polymorphism (SNP) approximately 10 kb centromeric to the *DRD2* stop codon on 11q23. This polymorphism correlates with the reduced expression of D2 receptors [22]. Another polymorphism described by Ohara et al. [23] (*DRD2 -141C InsDel*; rs1799732) is a single base pair cytosine insertion/deletion at position -141 in the promoter region and is directly correlated with receptor expression *in vitro* [24].

A better understanding of the role of inter-individual variation in the DA system with respect to motivation for energy intake will guide the development of prevention strategies and better therapeutic and/or behavioural interventions for obesity. The purpose of our study was to investigate potential associations of the *DRD2 Taq*IA and *-141C InsDel* polymorphisms with food intake and anthropometric measurements in children from three to four years of age.

Materials and Methods

Subjects

The sample used in this study consisted of 354 children from three to four years of age. This study is a cross-sectional examination of children who had participated in a randomised trial that occurred during their first year of life. All eligible mothers were informed by fieldworkers about both the overall aims of the study (advice on feeding of infants and its effects on the child's health) as well as all research procedures, including the use of a questionnaire, anthropometric and blood haemoglobin measurements, dental examinations and differences between the intervention and control groups. Race or ethnicity was self-defined by skin colour (i.e., whites and non-whites) as officially used in demographic censuses in Brazil. More details of the traits studied are described in Vitolo et al. [25]. This study was conducted according to the guidelines laid down in the Declaration of Helsinki. The study protocol was approved by the Ethics Committee of the Universidade Federal de Ciências da Saúde de Porto Alegre (n. 286/06), and all participants provided written informed consent before commencing the study.

Nutritional status assessed at 3-4 years old

The child's nutritional status was assessed by means of anthropometric measures in all visits. Weight was assessed using a portable digital scale and height was assessed using a portable stadiometer. Their nutritional status was estimated using the body mass index (BMI) for age charts from the International Child Growth Standards released by the World Health Organization [26]. The waist circumference was measured at the minimum circumference between the iliac crest and the rib cage using an inflexible measuring tape. Triceps skinfold and subscapular skinfold were measured using a Lange skinfold caliper to the nearest 1.0 mm. Each skinfold was measured two times on the right side of the body and was analysed as z-score according to *Multicentre Growth Study* standard charts [26].

Dietary data assessed at 3-4 years old

Two 24-hour dietary recalls were collected for each child on two randomly selected days. A food portion measurement aid and the common household measures (eg, teaspoons, tablespoons, cups) were used to quantify portion sizes.

Dietary information was entered into the Nutrition Support Program software from the Escola Paulista de Medicina, Federal University of São Paulo, based on United States Department of Agriculture chemical composition tables. The energy intake was calculated using the average of two diet recalls. We considered high sugar density food (HSD) if the percentage of simple carbohydrates in 100 grams was higher than 50% and high lipid density food (HLD) if greater than 30% fat per 100 grams. The calories provided by those foods groups were studied separately, for statistical analysis.

DNA analyses

Genomic DNA was extracted from peripheral blood leukocytes by the Lahiri and Nurnberger procedure [28]. *DRD2 Taq*IA (rs1800497) and *-141C InsDel* (rs1799732) polymorphisms were detected by PCR-RFLP analysis using primer sequences and conditions described by Hamarman at al. and Ohara et al. [29, 23], respectively. Primers sequences (IDT Coralville, IA, USA) were as follows: *Taq*IA, forward primer 5'-CACCTTCCTGAGTGTCATCAA -3' and reverse primer 5'-AGACAACTTGGCCAGCCGTG-3'; *-141C InsDel*, forward primer 5'-ACTGGCGAGCAGACGGTGAGG and reverse primer 5'-TGCGCGCGTGAGGCTGCCGGT. PCR products were digested separately with either *Taq*I (*Taq*IA polymorphism) or *Mva*I (*-141C InsDel* polymorphism*)* enzyme (Fermentas, Glen

Burnie, MD, USA), according to the manufacturer's instructions. Genotypes were determined after electrophoresis in 2% or 3% agarose gels that had been stained with ethidium bromide. For the *DRD2 Taq*IA polymorphism, the *C* allele contains a *Taq*I restriction site and is also designated as the *A2* allele, while the *T* allele is designated as the *A1* allele. For the *-141C InsDel* polymorphism, the *-141C Ins* allele contains a restriction site for *Mva*I while the *-141C Del* allele does not.

Statistical analyses

Allele frequencies were estimated by gene counting. A χ^2 test for goodness-of-fit was used to determine whether the observed genotype frequency distributions agreed with those expected under Hardy-Weinberg equilibrium. Haplotype frequencies and linkage disequilibrium were estimated using the Multiple Locus Haplotype Analysis program, Version 2.0 [30, 31] and Arlequin software, Version 3.1 [32].

Pearson's chi-squared or Fisher's Exact Test was used to compare genotype or allele frequencies, respectively, for polymorphisms between white and non-white children. The association analyses were performed separately in white and non-white samples. Mean or median kilocalories of food cluster intake (HSD and HLD), total energy/day and anthropometric parameters (waist circumference, triceps skin fold Z-score and subscapular skin fold Z-score) were compared among the different genotypes by the Student's t test for independent samples or the Mann-Whitney U test, depending on the variable distributions. All tests and transformations were performed using the Statistical Package for Social Sciences, Version 16.0 (SPSS®, Chicago, IL, USA).

Results

Mean ages for the children in this study were 4.1 ± 0.9 years old (mean ± SD), of which 41.7% were white and 57.2% were boys. Genotype frequency distributions observed for both polymorphisms studied did not reveal statistically significant differences when compared to those expected under Hardy-Weinberg equilibrium, for both the *DRD2 Taq*IA polymorphism in white (χ^2=0.973, 2DF, p=0.614) and non-white children (χ^2=0.000, 2DF, p=1.000) and for the *DRD2 -141C InsDel* polymorphism in non-white (χ^2=0.251, 2DF, p=0.882) and white children (χ^2=0.560, 2DF, p=0.756). Allele and genotype frequencies for the two studied polymorphisms are presented in Table 1. The frequency of the *DRD2 Taq*IA**T* allele was significantly higher in non-white (0.320) than in white children (0.236; *p*=0.028), and genotype frequencies were

also different for non-white versus white children (p=0.048). The frequency of the *DRD2 -141C Del* allele was significantly higher in non-white (0.184) than in white children (0.108; p=0.011), and the *DRD2 -141C InsDel* genotype frequencies were different between non-white and white children (p=0.034). The two gene variants were not in linkage disequilibrium (white children: D'=0.689, χ^2=0.040, DF=1, p= 0.840; non-white children: D'=0.031, χ^2=0.141, DF=1, p=0.707). The *DRD2 -141C InsDel* polymorphism was not associated with food intake and anthropometric data when analyzed in white and non-white children (Tables 2 and 3). In non-white children, the *Taq*IA *C/C* homozygous genotype was associated with higher HLD intake (median 666.8 kJ) when compared with children carrying the *T* allele (343.9 kJ, p=0.009; Table 2).

Discussion

The allele and genotype frequencies of the polymorphisms found in white and non-white samples were similar to those described in other European-American and African-American populations, respectively [33, 34]. In the present study, as also reported by O'Hara et al. [35], the *-141C InsDel* polymorphism in the promoter region of *DRD2* and the *Taq*IA polymorphism were not in linkage disequilibrium (LD). This finding can be explained because these two polymorphisms are distant from each other in the *DRD2* chromosomal region. However, Gelernter et al. [34] found significant LD between the *-141C InsDel* and *Taq*IA polymorphisms in African-American subjects, a finding that we did not replicate when analysing only non-white samples.

There is increasing evidence for a role of the dopaminergic pathway in the development of obesity. More specifically, DA hypoactivity might lead to overcompensatory food intake. Eating and DA signalling are closely related. Food rewards and their associated stimuli both elevate dopamine levels in crucial components of brain reward circuits [36]. In fact, food might be the most important natural stimulus for the reward system in the brain [37]. Small et al. [13] suggest that the amount of released dopamine correlates with the degree of experienced pleasure. Some studies have shown that the presence of the *T* (*A1*) allele (polymorphism *Taq*IA) is associated with a 40% reduction in D2 receptor expression without changing the receptor affinity, and this phenomenon results in DA hypoactivity [38, 39].

The *DRD2 Taq*IA polymorphism is associated with increased BMI [40, 41], obesity [42-45], Reward Deficiency Syndrome [45] and the differential therapeutic effects of chromium picolinate with respect to weight loss and changes in body fat [46]. This variant has been

associated with food intake, such that individuals carrying the *T* (*A1*) allele have higher food reinforcement (are willing to work harder for food) and consume more food than their counterparts without the *T* (*A1*) allele [47, 48]. These findings suggest that *DRD2* variations affect nutritional status through modulating food craving behaviour. It is noteworthy that none of the studies cited above included children in their samples. The few studies that investigated the influences of the *Taq*IA polymorphism in children have focused on Attention Deficit Hyperactivity Disorder [29, 48], and a recent study evaluated food consumption in response to methylphenidate, focusing on polymorphisms related to the availability of dopamine. That study found an association between C/C homozygous children and reduced dietary intake in response to methylphenidate [49].

In the present study, the *Taq*IA *C/C* (*A2/A2*) homozygous genotype was associated with higher intake of HLD when compared with the *Taq*IA**T* (*A1*) allele carriers in non-white children. However, the *T* (*A1*) allele of the *DRD2 Taq*IA polymorphism is generally associated with decreased *DRD2* function or expression [50]. Thus, our results are apparently contradictory to what should be expected regarding the functional effect of this polymorphism. Nevertheless, Miyake et al. [51] investigated the association of idiopathic short stature with the *DRD2 TaqIA* polymorphism in a Japanese population, and their results suggest that the *T* (*A1*) allele may differ in its linkage disequilibrium among different populations. One other studies conducted by Davis *et al.* (2008) also found an inverse relationship to what was expected. In this study they observed in the Individuals with binge eating disorder (BED) and obese participants, significantly higher reward sensitivity was found in the *T (A1)* + groups. One reason could be that the BED and obese (but not the normal-weight) participants possess another genetic variant that interacts with the *T (A1)* allele to produce higher DA activity. The findings of a recent study are cognate to this possibility [52]. Reuter et al. (2006) have provided evidence for a gene interaction model between the catabolic enzyme activity of catechol-O-methyl transferase (COMT) and DRD2 receptor density, whereby disequilibrium is associated with higher DA levels and higher *Behavioural Activation* (BAS) scores. In other words, high enzyme activity (associated with the *Val* allele of COMT) and low D2 receptor density (associated with the *T (A1)* allele) contribute to relatively high DA levels and correspondingly elevated reward sensitivity scores on the BAS scale [53]. Since the *T* (*A1*) allele is not itself thought to be a functional polymorphism in the *DRD2* gene, variations in genetic background (i.e., different patterns of linkage with actual functional genetic

polymorphisms) could explain varying results across populations. There are several studies that have replicated a previously reported disease-marker association, with the effect of the risk allele being opposite from that in the previous report. These associations with contradictory results may indeed be confirmations but multilocus effects and variation in interlocus correlations might contribute to a flip-flop phenomenon [54].

Based on previous research suggesting that BMI associations with dopamine receptor polymorphisms are due to increased food craving [24], we expected that BMI would be related to the dopamine receptor genotype. However, food craving in Western environments of food surpluses might have very different contexts than in populations with different food availability. Adeyemo et al. [55] performed a genome-wide scan in a black population for markers associated with obesity and found an association with chromosome 11, in the exact area where the *DRD2* gene is located. There have been many positive reports suggesting an association between *DRD2* alleles and obesity in people of European descent but not in African-American subjects [15, 18, 40- 44, 46, 48, 56].

Luciferase reporter assays demonstrated that transient enzymatic activity and therefore transcription is significantly silenced in clones containing the deletion variant (*-141C Del*) compared to the insertion variant (*-141C Ins*) [57]. Arinami et al. [24] reported that the *-141C Del* allele decreases the promoter activity of the *DRD2* gene in cell cultures. Other studies produced conflicting results [58, 59]. We found no association between alleles of the polymorphism *DRD2 -141C InsDel* and any anthropometric variables or food consumption. To our knowledge, no other studies to date have been conducted to analyse the association of the polymorphism *DRD2 -141C InsDel* with food intake or with anthropometric data.

An inherent limitation to our study is the moderate sample size, which may not have enough power to detect an association of polymorphisms with small effects on food intake and anthropometric measurements, such as *-141C InsDel*. However, we believe the size of our sample was sufficient to detect relatively large genetic effects, reinforcing the importance of our findings relating *Taq*IA to a higher intake of HLD. The present results must be regarded with caution and should be confirmed in a larger study. At this time, it is important to highlight another detail that is relevant to our results: the observation that a non-association with obesity-related anthropometric phenotypes may be due to the fact that children are small and their time of exposure to an obesogenic environment has not been sufficient to counteract excessive weight gain. We believe that a follow-up to the present sample may provide

interesting findings for the future. Further studies evaluating the effects of these polymorphisms on the genetic profiles of food consumption patterns and the anthropometry of children of different populations would be very relevant for a better understanding of polymorphism influence on nutritional outcomes.

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A. C. S. G. performed DNA and statistical analyses and drafted the manuscript. M.R.V. supervised the sample collection and participated in writing the manuscript. P.D.B.C. carried sample data collection. V.S.M. participated in statistical analysing and writing the manuscript. S.A. supervised the study and participated in statistical analysing and writing the manuscript. All authors read and approved the final manuscript.

The authors declare no conflicts of interest.

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Table 1

Distributions of the *TaqIA* e -141 *InsDel* polymorphisms alleles and genotype frequencies in white and non-white children from three to four years of age.

TaqIA	**White (n = 127)**	**Non-white (n = 175)**	***P***
Alelle/genotype	Frequency (n)	Frequency (n)	
T allele	0.236 (55)	0.320 (94)	0.028[a]
C/C	0.566 (72)	0.462(81)	
T/C	0.393 (50)	0.434 (76)	
T/T	0.039 (5)	0.102 (18)	0.037[b]
-141C InsDel	**White (n = 127)**	**Non-white (n = 172)**	***P***
Alelle/genotype	Frequency (n)	Frequency (n)	
***Del* allele**	0.108 (25)	0.184 (58)	0.011[a]
Ins/Ins	0.806 (104)	0.672 (119)	
Ins/Del	0.170 (22)	0.288 (51)	
Del/Del	0.023 (3)	0.039 (7)	0.034[b]

[a]Fisher's Exact Test

[b]Pearson Chi-Square

n = individuals number carrier allele/genotype

Table 2

Food intake according *TaqIA* and *-141C* I*nsDel* polymorphisms genotypes in white and non-white children from three to four years of age.

	White Children					Non-white Children				
TaqIA	**T Carriers**	**N**	**C/C homozygotes**	**N**	***p***	**T Carriers**	**N**	**C/C homozygotes**	**N**	***p***
HSD (kcal)	84.60[37.45–126.86][a]	55	101.56[53.06–175.25][a]	72	0.063[b]	95.56 [36.90–178.11][a]	94	91.83 [44.19–156.12][a]	81	0.957[b]
HLD (kcal)	108.57[18.66–265.95][a]	55	179.81[43.54–290.95][a]	72	0.139[b]	82.20 [0.00–195.01][a]	94	159.39 [44.26–272.34][a]	81	0.009[b]
Average energy intake daily (kcal)	1510.40±378.15[c]	55	1593.60±449.22[c]	72	0.271[b]	1451.50±347.25[c]	94	1549.30±422.30[c]	81	0.095[d]
-141C InsDel	***Del* Carriers**	**N**	**Ins/Ins homozigotes**	**N**	***p***	***Del* Carriers**	**N**	**Ins/Ins homozigotes**	**N**	***p***
HSD (kcal)	94.12 [48.10–174.97][a]	25	95.05[44.55–143.12][a]	102	0.714[a]	90.30 [45.01–164.00][a]	55	92.52 [37.37 – 159.30][a]	117	0.941[b]
HLD (kcal)	118.00 [38.47–268.42][a]	25	140.73[34.08–291.68][a]	102	0.504[a]	91.74 [18.66–224.29][a]	55	118.00 [17.07–250.66][a]	117	0.735[b]
Average energy intake daily (kcal)	1629.50±399.20[c]	25	1539.90±425.422[c]	102	0.341[b]	1548.20±401.71[c]	55	1469.90±373.26[c]	117	0.212[d]

HSD indicates high sugar density foods; HLD indicates high lipid density foods; [a] Median [Interquartile Range]; [b] Mann-Whitney U Test; [c] Mean ± standard deviation, [d] Test T for independent sample.

n = individuals number carrier genotype

Table 3

Anthropometric data according *TaqIA* and *-141C InsDel* polymorphisms genotypes in white and non-white children from three to four years of age.

	White Children					Non-white Children				
TaqIA	**T Carriers**	**N**	**C/C homozygotes**	**N**	***p***	**T Carriers**	**N**	**C/C homozygotes**	**N**	***p***
Z-score of BMI	0.334±0.980[a]	57	0.151±0.8787[a]	71	0.269[b]	0.360±0.978[a]	98	0.205±1.516[a]	82	0.412[b]
Waist circumference in cm	51.254±3.611[a]	57	50.628±2.815[a]	70	0.275[b]	51.007±3.226[a]	98	50.615±4.631[a]	82	0.511[b]
Z-score of triceps skinfolds	-0.383±1.124[a]	57	-0.341±1.045[a]	70	0.829[b]	-0.463±1.226[a]	98	-0.425±1.356[a]	82	0.846[b]
Z-score of subescapular skinfolds	-0.032±1.207[a]	57	-0.312±1.230[a]	70	0.200[b]	-0.497±1.423[a]	98	-0.433±1.640[a]	82	0.777[b]
-141C InsDel	***Del* Carriers**	**N**	**Ins/Ins homozigotes**	**N**	***p***	***Del* Carriers**	**N**	**Ins/Ins homozigotes**	**N**	***p***
Z-score of BMI	0.316±1.114[a]	25	0.2127±0.8798[a]	103	0.619[b]	0.132±1.450[a]	58	0.389±1.146[a]	119	0.204[b]
Waist circumference in cm	51.820±3.599[a]	25	50.6863±3.0716[a]	102	0.113[b]	50.686±4.624[a]	58	50.941±3.576[a]	119	0.680[b]
Z-score of triceps skinfolds	-0.458±1.075[a]	25	-0.3357±1.0819[a]	102	0.611[b]	-0.628±1.275[a]	58	-0.335±1.290[a]	119	0.162[b]
Z-score of subescapular skinfolds	-0.118±1.188[a]	25	-0.2034±1.2367[a]	102	0.758[b]	-0.574±1.640[a]	58	-0.376±1.459[a]	119	0.411[b]

BMI indicates Body Mass Index; [a] Mean ± standard deviation; [b] Test T for independent sample

n = individuals number carrier genotype

CAPÍTULO 3

MANUSCRITO 2
(em preparação para ser submetido a revista Diabetes, Obesity and Metabolism)

Association evaluate of *MAOA-u VNTR*, *MAOA T941G* polymorphisms and *COMT Val158Met* with food intake and anthropometric data in Brazilian children

Association of MAOA *MAOAu-VNTR* and *T941G* and COMT *Val158Met* polymorphisms with food intake and anthropometric data in Brazilian children

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Abstract

Several studies have implicated dopamine in appetite regulation. Dopamine availability is controlled by the enzymes COMT and MAOA, and each gene has a well-characterized functional variant. In this study we have examined three functional polymorphisms these genes - T941G and 30-bp repeat polymorphism in the MAOA and the Val158Met in the COMT, to investigate how heritable variation in dopamine levels influences the anthropometrics data and food intake in a cross-sectional examination of 354 children. The MAOA T941G polymorphism showed no association with anthropometrics data or food intake. We found, however, that both MAOA-u VNTR and COMT Val158Met polymorphisms, the children with the high-activity genotypes was associated with increased high lipid density (HLD) food intake (MAOA-u*long - 134.97kcal [26.43–270.16]; *COMT*158Val* - 133.79kcal [44.23–265.80]) when compared with children who are low-activity genotypes (MAOA-u*short carriers - 60.1kcal [0.00–192.31]; *COMT*158Met* - 83.37Kcal [0.00–252.95]), p=0.009 and p=0.008 respectively. The MAOA-u*long was still associated with an increased high sugar density (HLS) food intake when compared with MAOA-u*short carriers (p=0.034), the medians were 100.45kcal [54.40–163.32] and 80.01kcal [37.45–127.11]. This study provides the first indication that dopamine availability in implicating both the MAOA and COMT variants is involved in human obesity.

1. Introdution

Obesogenic modern environments have caused marked increases in mean of the weight in populations over the past few decades [1], but there is still remarkable variability in weight within populations. There is growing evidence from family, twin, and adoption studies that there is a heritability component to obesity [2]. In several studies, obese adults were shown to have less effective down-regulation of appetite after food consumption [3], and lower sensitivity to gastric motility [4]. Obese adults also exhibited stronger up-regulation of intake in response to palatability than did normal-weight controls [5]. Similar findings were reported in children, obese children show poorer caloric compensation after a preload [6, 7], increase their food intake more than normal-weight controls after exposure to food cues [6], have higher levels of snack consumption in the absence of hunger [8], and score higher on psychometrically assessed “external eating” [9]. Other strong body of research pays particular attention to the fact that food, as like nicotine and other psychoactive drugs, induces pleasurable sensations. In 1985, Hoebel [10] suggested that the mesocorticolimbic dopaminergic reward pathways of the brain had a central role in the neuromodulation of appetite. Further evidence for this theory came from the observation that dopaminergic agonists suppress appetite whereas antagonists tend to enhance appetite [11, 12]. Since formulation this hypothesis, several efforts have been made to identify the key molecular components of dopamine transmission in obesity. A growing area of research has begun to explore the potential association between specific candidate genes regulating brain dopamine system with obesity [13]. In the context of a molecular genetic approach to the problem, candidate genes related to this hypothesized

vulnerability would include any of the five known dopamine receptor genes, genes coding for important enzymes of the dopamine metabolism, and the dopamine transporter protein, among potentially many others [14].

Monoamine oxidase (MAO) is a mitochondrial enzyme involved in the degradation of biological amines including serotonin, dopamine, and norepinephrine. In humans, there are two isozymes: monoamine oxidase A (MAOA) and monoamine oxidase B (MAOB) [15]. The *MAOA* gene is located on the short arm of chromosome X [16]. The promoter region of this gene has a variable number tandem repeat (VNTR) polymorphism, *MAOAu-VNTR,* which has been shown to influence gene transcription, alleles with 3.5 and 4 repeats were found to transcribe the protein more efficiently than the 3 repeat allele. The studies were discordant with regard to a rare 5 repeat allele [17; 18]. Another polymorphism in *MAOA* gene is the *MAOA T941G* polymorphism (rs6323), a missense mutation caused by G/T transversion located at position 941 in exon 8, and was reported to be associated with high (G allele) and low (T allele) MAOA activity in 40 cell lines in which the activity of MAOA was know [16].

Another enzyme that acts in the degradation of dopamine is the catechol-O-methyltransferase (COMT). This enzyme plays a prominent role in the inactivation and degradation of catecholamines and estrogen [19]. There is evidence that changes in activity of COMT bring central and peripheral effects by altering the amounts of dopamine and norepinephrine in the synaptic cleft [20]. This enzyme has been involved in several mechanisms of reward-motivated behavior, such as obesity [21], mood and other mental processes [22]. The chromosome 22q11.21 region coding for the human COMT gene [23]. Several polymorphisms in the

COMT gene have been described, but the *Val158Met* (24938A/G, rs4680) polymorphism is the most widely studied [24]. This polymorphism in the fourth exon of this gene is characterized by a G to A transition, results in a valine to methionine substitution at codon 158 of the COMT protein, respectively [23].

On the basis of the hypothesis of dopaminergic reward pathways on the brain has a central role in the neuromodulation of appetite, we propose that the altered activities or levels of dopamine-regulating enzymes might be relate to the pathophysiology of obesity. We therefore, investigated if *MAOAu-VNTR*, *MAOA T941G* and *COMT Val158Met* polymorphisms contribute to the risk of increase food intake and consequent obesity in Brazilian children.

2. Materials and Methods

Subjects

This study is a cross-sectional examination of children who had participated in a randomised trial that occurred during their first year of life. The sample consisted of 354 children from three to four years of age. The 24-hour diet recall, carried out by Nutrition students, recorded the child's food intake on the day before the last home visit. The blood samples were collected in São Leopoldo and analysed in the Clinical Analysis Laboratory of the Cardiology Institute of Porto Alegre. Race or ethnicity was self-defined by skin colour (i.e., whites and non-whites) as officially used in demographic censuses in Brazil. More details of the traits studied are described in Vitolo et al., 2008 [25]. All eligible mothers were informed by fieldworkers about both the overall aims of the study (advice on feeding of infants and its effects on the child's health) as well as all research procedures, including the use of a questionnaire, anthropometric and blood haemoglobin measurements, dental examinations and differences between the intervention and control groups. The study protocol was approved by the Ethics Committee of the Universidade Federal de Ciências da Saúde de Porto Alegre, and all participants provided written informed consent before commencing the study.

Nutritional status assessed at 3-4 years old

The child's nutritional status was assessed by means of anthropometric measures in all visits. Weight was assessed using a portable digital scale and height was assessed using a portable stadiometer. Their nutritional status was

estimated using the body mass index (BMI) for age charts from the International Child Growth Standards released by the World Health Organization [26]. The waist circumference was measured at the minimum circumference between the iliac crest and the rib cage using an inflexible measuring tape. Triceps skinfold and subscapular skinfold were measured using a Lange skinfold caliper to the nearest 1.0 mm. Each skinfold was measured two times on the right side of the body and was analysed as z-score according to *Multicentre Growth Study* standard charts [26].

Dietary data assessed at 3-4 years old

Two 24-hour dietary recalls were collected for each child on two randomly selected days. A food portion measurement aid and the common household measures (eg, teaspoons, tablespoons, cups) were used to quantify portion sizes.

Dietary information was entered into the Nutrition Support Program software from the Escola Paulista de Medicina, Federal University of São Paulo, based on United States Department of Agriculture (USDA) chemical composition tables. The energy intake was calculated using the average of two diet recalls. We considered high sugar density food (HSD) if the percentage of simple carbohydrates in 100 grams was higher than 50% and high lipid density food (HLD) if greater than 30% fat per 100 grams. The calories provided by those foods groups were studied separately, for statistical analysis.

DNA analyses

Genomic DNA was extracted from peripheral blood leukocytes by the Lahiri and Nurnberger procedure [27]. MAOA *MAOAu-VNTR* polymorphism was genotyped using polymerase chain reaction (PCR). The primer sequences were as follows: forward 5'- ACAGCCTGACCGTGGAGAAG - 3 and reverse 5'- GAACGGACGCTCCATTCGGA -3'. The PCR products containing the tandem repeat polymorphism were resolved by electrophoresis on 6% poliacrylamide gel with ethidium bromide. The primers used yielded 291, 321, 336, 351 and 381 base pair (bp) fragments corresponding to the 2-, 3-, 3.5-, 4-and 5-repeat alleles, respectively. They were then visualized under U.V light to determine the fragment sizes by comparison with a 100 bp DNA ladder. The MAOA *T941G* (rs6323) polymorphism was detected by PCR-RFLP analysis, using same primers sequence and conditions previously described by Tádic et al., 2003 [28]. Sequences of primers were: forward 5'- GACCTTGACTGCCAAGAT -3' and the reverse 5'- CTTCTTCTTCCAGAAGGCC -3'. The PCR products were digested with *Fnu*IV restriction enzyme according to the manufacturer's instructions. Genotypes were determined after electrophoresis on agarose gel 1.5%, stained with ethidium bromide. COMT Val108/158Met (rs4680) polymorphism was detected by PCR-RFLP analysis. Sequences of primers were: forward 5' – TCGTGGACGCCGTGATTCAGG - 3' and the reverse 5' – AGGTCTGACAACGGGTCAGGC - 3'. The PCR products were digested with *Nla*III restriction enzyme according to the manufacturer's instructions. Genotypes were determined after electrophoresis on poliacrylamide gel 7%, stained with ethidium bromide.

Statistical analyses

Allele frequencies were estimated by gene counting. A χ^2 test for goodness of fit was used to determine whether observed genotype frequencies distribution agreed with those expected under Hardy-Weinberg equilibrium. For MAOA polymorphisms the association analyses were performed separately in boys and girls, because de MAOA gene is located in X chromosome. Kilocalories mean or median of food clusters intake (HSD and HLD) and total/day energy and anthropometric parameters (Waist circumference, Z-score of skinfolds of triceps and Z-score of skinfolds of subescapular were compared among carriers of the different genotypes by one-way analyses of variance (ANOVA) or Kruskal Wallis, depending on variables distribution. All tests and transformations were performed using the Statistical Package for Social Sciences Version 16.0 (SPSS®, Chicago, IL, USA).

3. Results

The mean age of the children in this study was 4.075 ± 0.948 years age (mean ± SD), 41.7% of children were white and the percentage of boys in the sample was 57.2%. The genotype frequencies distributions observed for polymorphisms studied did not reveal statistically significant differences compared to those expected under Hardy–Weinberg equilibrium, for *MAOAu-VNTR* polymorphism in girls (χ^2 = 0.546, 2DF, p = 0.761); for *MAOA T941G* polymorphism in the girls (χ^2 = 0.405, 2DF, p = 0.817); for *COMT Val158Met* polymorphism (χ^2 =0.027, 2DF, p = 0.9866). The allele and genotype frequencies for three polymorphisms studied are presented in Table 1. Were not detected statistically significant differences in genotype frequency distributions of polymorphisms analyzed between white and non-white samples (data not show). In the boy's sample, the *MAOAu-long* allele presence was associated with higher intake of HLD (median: 134.975kcal [interquartile range: 26.437–270.162]) when compared with *MAOAu-short* allele (60.1kcal [0.000–192.31]; *p* = 0.009; Table 2); the HSD intake was also higher in boys carriers of *MAOAu-long* allele (100.455kcal [54.406–163.325]) when compared with *MAOAu-short* allele carriers (80.015kcal [37.45–127.115]; *p* = 0.034; Table 2). In the girl's sample, *MAOAu-VNTR* polymorphism was not associated with food intake and anthropometric data (Table 3). The *MAOA T941G* polymorphism was not associated with food intake and anthropometric data when analyzed in boys and girls (Table 2 and 3). The *COMT 158Val* allele was associated with higher intake HLD when compared with *COMT 158Met* homozygous (*p* = 0.008), the medians were 133.79kcal [44.23–265.80] and 83.37kcal [0.00–252.95], respectively (Table 4).

4. Discussion

There is increasing evidence for a role of the dopaminergic pathway in the development of obesity. Food rewards and their associated stimuli both elevate dopamine levels in crucial components of brain reward circuits [29]. In fact, food might be the most important natural stimulus for the reward system in the brain [30]. Small et al. [31] suggest that the amount of released dopamine (DA) correlates with the degree of experienced pleasure. Individual differences in reward sensitivity have been implicated in food intake. In this work, we evaluate polymorphisms in genes of enzymes that affect dopamine availability; together MAOA and COMT determine a part of DA available at the synapse. MAOA and COMT are affecting both the degradation of norepinephrine and dopamine, a decrease in activity of the gene product will increase the after-effects of these neurotransmitter receptors. In this context, it is interesting that re-uptake inhibition on these two systems, that will tend to have the same effect, is an effective way to produce a weight loss in humans [32, 22].

Some studies have shown that the presence of the long allele of MAOA-u VNTR polymorphism is associated with high MAOA activity and this phenomenon might results in lower levels of DA in pre synaptic neuron [33, 34]. No studies have quantified the effect of low or high activity isoforms on the availability of DA, however, has been shown that women with one copy of high activity MAOA allele (long allele) have higher levels of homovanillic acid (HVA), the main metabolite of DA [35]. This finding implies that those who have a high activity MAOA allele have increased metabolism DA and, consequently, might lead to overcompensatory

palatable food intake. In this study, our results demonstrated that MAOA u-VNTR polymorphism was associated with amount of palatable food intake in boys sample, boys with high activity allele (long allele) intake more HSD and HLD foods than them with low activity allele (short allele). This polymorphism was not associated with anthropometrical parameters in our study, probably because the children are little and their time of exposure to obesogenic environment was not enough, however, this food intake behaviour might confer increased risk of developing obesity in future. Evidences obtained from studies with humans and animal models indicating the importance of dopaminergic function in the development of obesity are divided about the direction of causal association. One argument is that a Reward Deficiency Syndrome (RDS) is the risk factor, while others contend that hyper-sensitivity to reward enhances the motivation for pleasurable activities like eating. Most studies that evaluated the polymorphisms in the MAOA goes toward that hyper-sensitivity to reward, like in a transmission disequilibrium test, the low activity allele of this same polymorphism was shown to be preferentially transmitted to obese offspring of parents [34]. In addition, Visentin et al. 2004 [33] compared the activity MAOA fat in obese and nonobese patients, and found that the levels were halved in the obese. However, several studies will prove that RDS is implicated in the genesis of obesity support our results, such as the study by Wang et al. 2001 which found that the availability of dopamine D2 receptor was decreased in obese individuals in proportion to their BMI. Dopamine modulates motivation and reward circuits and hence dopamine deficiency in obese individuals may perpetuate pathological eating as a means to compensate for decreased activation of these circuits.

The MAOA T941G polymorphism showed no association with anthropometrics data or food intake of children among three to four years. This SNP is located in the third base of a codon and does not affect the amino acid sequence. However, Hotamisligil and Breakefield, 1991 [16] reported an association of the 941T allele with lower MAOA enzyme activity in 40 cell lines of known MAOA activity. When the sample was divided into two groups on the basis of lower versus higher MAOA activity, the less common 941G, present in 25% of the cell lines, was over-represented in the higher activity group. Functional differences can be caused by other polymorphisms that are in disequilibrium with the T941G variant. Gade et al., 1998 [36] recently reported MAOA gene VNTR allele groups being associated with behavioral phenotypes in drug abusers and patients suffering from Tourette syndrome. In this study, the longest alleles were associated with the greatest phenotypic effect. The authors also examined the T941G polymorphism in the patient group and found a linkage disequilibrium between the VNTR polymorphism and the T941G polymorphism. Gade et al. postulated that the gene variants with the higher number of repeats would be less active than the shorter variants, which would, in respect to the function of the T941G allele variant, be in accordance with the report of Hotamisligil and Breakfield [16] mentioned above. Few studies have evaluated this polymorphism and most of the time were studies that analyzed this variant only making it difficult to evaluate a possible linkage disequilibrium with other variants.

COMT is an extracellular enzyme that O-methylates DA, and is the only enzyme that can act on the extracellular DA. This O-methylation may occur before

or after deamination by MAO. The Met allele of COMT Val158Met polymorphism produces a labile protein, with activity significantly lower [22, 25], thus conferring slow detoxification of neurotransmitters [30], such as the degradation and inactivation of dopamine [22, 30] resulting in higher levels of dopamine. Individuals with genotype Met/Met have a reduction of 3 to 4 times the activity of enzymatic degradation in comparison with homozigotes Val/Val; heterozygotes have an intermediate activity [37]. We found an association between the Val allele and more HSD and HLD food (palatable food) intake, conferring increased risk of developing obesity which is consistent with the suggested role of COMT Val158Met polymorphisms in obesity [20]. Further study corroborate a our results a showing the exercise-induced weight loss in women to be slightly smaller in met carriers [38]; in contrast, Need et col., 2006 assessing the possible association between the COMT Val158Met polymorphism and BMI did not find any such relationship [39]. In a study by Happonen et al., 2006 [40], no marked effects of the COMT val158-met polymorphism on waist-hip ratio (WHR) were found. While Annerbrink et. col., 2008 found that subjects homozygous for the low-activity allele (Met) displayed higher WHR and abdominal sagittal diameter as compared with heterozygous and homozygous for the high-activity allele (Val). Our results also implied that genotypes of MAOAu-VNTR and COMT Val158Met migth have an impact on the food intake and possibly about development of obesity. The present and potential discrepancies between studies underline the continuing challenges of studies with different populations with different characteristics and pathophysiological and life-style related characteristics that may modify the effects of the examined gene variants.

Our results indicate that is evidence that dopamine levels are associated with feeding behavior respecting that same significant associations, in the same direction were observed for both low activity variants of the MAOA and COMT genes. These results therefore strongly suggest a role of heritable variation in DA metabolism in risk with higher intake of palatable food, resulting in increased risk of developing obesity and underscore the need for additional research in order to replicate the results and to identify the complex interplay between the examined psychophysiological genes that may further characterize their functionality in feeding behavior.

An inherent limitation to our study is the moderate sample size, which may not have enough power to detect an association of polymorphisms with small effects on food intake and anthropometric measurements, such as MAOA T941G. However, we believe the size of our sample was sufficient to detect relatively large genetic effects, reinforcing the importance of our findings relating MAOA-u VNTR polymorphism with higher intake of HLD and HSD foods and COMT Val158Met polymorphism with higher intake of HLD foods. The results of this should be considered with caution and should be confirmed in a larger study. It is now important to point out another detail that is relevant to our results: the observation that a non-association with phenotypes related to obesity anthropometric may be due to the fact that children are small and their time of exposure to an obesogenic environment was not enough to offset the excessive weight gain. We believe that a follow-up to this sample may provide interesting results for the future.

Conflict of interest statement

The authors have declared no conflict of interest.

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Table 1

Distributions of the *MAOA-u VNTR, MAOA T941G and COMT Val158Met* polymorphisms alleles and genotype frequencies in children from three to four years of age.

MAOA-u VNTR	**Boys (186)**	**Girls (139)**
Alelle/genotype	Frequency (n)	Frequency (n)
Long allele	0.655 (122)	0.641 (119)
Long/Long	-	0.428 (59)
Long/Short	-	0.428 (60)
Short/Short	-	0.144 (20)
MAOA T941G	**Boys (186)**	**Girls (139)**
Alelle/genotype	Frequency (n)	Frequency (n)
***G* allele**	0.207 (39)	0.213 (55)
G/G	-	0.036 (5)
G/T	-	0.359 (50)
T/T	-	0.605 (84)
COMT Val158Met	**n=326**	
Alelle/genotype	Frequency (n)	
Met allele	0.615 (278)	
Met/Met	0.377 (123)	
Val/Met	0.475 (155)	
Val/Val	0.148 (48)	

n = individuals number carrier allele/genotype

Table 2

Food intake and anthropometric data according *MAOA-u VNTR* and *MAOA T941G* polymorphisms genotypes in boys from three to four years of age.

Food intake

MAOA-u VNTR	**Long allele**	**N**	**Short allele**	**N**	***p***
HSD (kcal)	100.45 [54.40–163.32][a]	120	80.01 [37.45–127.11][a]	63	0.034[b]
HLD (kcal)	134.97 [26.43–270.16][a]	120	60.10 [0.00–192.31][a]	63	0.009[b]
Average energy intake daily (kcal)	1544.49±389.73[c]	120	1512.37±423.94[c]	63	0.608[d]
T941G	**G allele**	**N**	**T allele**	**N**	***p***
HSD (kcal)	82.307kcal [40.75– 128.10][a]	38	97.15kcal [49.43–159.47][a]	145	0.105[b]
HLD (kcal)	53.23kcal [0.00– 228.19][a]	38	127.71kcal [20.58–256.50][a]	145	0.154[b]
Average energy intake daily (kcal)	1545.58±430.21[c]	38	1530.25±394.45[c]	145	0.834[d]

Anthropometric data

MAOA-u VNTR	Long allele	N	Short allele	N	*p*
BMI Z-score	0.16±1.19[c]	122	0.36±1.12[c]	63	0.271[d]
Waist circumference in cm	50.65± 3.53[c]	122	51.55± 3.42[c]	63	0.099[d]
Z-score of skinfolds of triceps	7.37±2.46[c]	122	7.85± 2.46[c]	63	0.207[d]
Z-score of skinfolds of subescapular	5.36±2.45[c]	122	5.79±2.38[c]	63	0.261[d]
T941G	**G allele**	**N**	**T allele**	**N**	***p***
BMI Z-score	0.52±1.16[c]	38	0.16±1.16[c]	147	0.086[d]
Waist circumference in cm	51.77±3.67[c]	38	50.75±3.45[c]	147	0.110[d]
Z-score of skinfolds of triceps	8.03±2.66[c]	38	7.40±2.40[c]	147	0.160[d]
Z-score of skinfolds of subescapular	5.81±2.78[c]	38	5.43±2.33[c]	147	0.391[d]

HSD indicates high sugar density foods; HLD indicates high lipid density foods; BMI indicates Body Mass Index; [a] Median [Interquartile Range]; [b] Mann-Whitney U; [c] Mean ± standard deviation, [d] Test T for independent sample.

n = individuals number carrier genotype

Table 3

Food intake and anthropometric data according *MAOA-u VNTR* and *MAOA T941G* polymorphisms genotypes in girls from three to four years of age.

Food intake

MAOA-u VNTR	**Long/Long**	**N**	**Long/Short**	**N**	**Short/Short**	**N**	***p***
HSD (kcal)	97.62 [44.70–144.74][a]	57	100.66 [28.06–169.45][a]	58	151.68 [41.69–199.26[a]]	20	0.318[b]
HLD (kcal)	106.84 [47.73–282.79][a]	57	146.31 [37.25–271.34][a]	58	98.67 [0.00–254.53][a]	20	0.671[b]
Average energy intake daily(kcal)	1472.04±406.36[c]	57	1543.14±434.21[c]	58	1406.79±235.16[c]	20	0.370[d]
T941G	**G/G**	**N**	**G/T**	**N**	**T/T**	**N**	***p***
HSD (kcal)	139.37 [56.81–203.52][a]	5	108.80 [39.14–173.46][a]	49	94.75 [36.95–154.27][a]	81	0.586[b]
HLD (kcal)	253.60 [15.67–303.82][a]	5	122.11 [46.88– 247.55][a]	49	106.84 [20.77–282.79][a]	81	0.265[b]
Average energy intake daily (kcal)	1308.26±214.69[c]	5	1477.26±402.75[c]	49	1513.79±405.83[c]	81	0.509[d]

Anthropometric data

MAOA-u VNTR	**Long/Long**	**N**	**Long/Short**	**N**	**Short/Short**	**N**	***p***
BMI Z-score	0.24±0.97[c]	58	0.39±1.12[c]	59	0.12±0.98[c]	20	0.537[d]
Waist circumference(cm)	50.40± 3.04[c]	58	51.23±4.37[c]	59	50.32±3.24[c]	20	0.412[d]
Z-score of triceps skin folds	7.84±2.12[c]	58	8.01±2.34[c]	59	7.67±2.32[c]	20	0.822[d]
Z-score of subescapular skin folds	6.29±1.84[c]	58	6.50±2.82[c]	59	5.75±2.14[c]	20	0.537[d]
T941G	**G/G**	**N**	**G/T**	**N**	**T/T**	**N**	***p***
BMI Z-score	-0.03 ±0.82[c]	5	0.27±1.15[c]	49	0.31±0.98[c]	83	0.269[d]
Waist circumference(cm)	50.60±2.07[c]	5	51.07±4.21[c]	49	50.57±3.46[c]	83	0.282[d]
Z-score of triceps skin folds	6.90±1.24[c]	5	7.73±2.32[c]	49	8.04±2.22[c]	83	0.813[d]
Z-score of subescapular skin folds	4.90±1.02[c]	5	6.34±2.98[c]	49	6.36±1.94[c]	83	0.922[d]

HSD indicates high sugar density foods; HLD indicates high lipid density foods; BMI indicates Body Mass Index; [a] Median [Interquartile Range]; [b] Kruskal Wallis Test; [c] Mean ± standard deviation, [d] One-Way ANOVA.

n = individuals number carrier genotype

Table 4

Food intake and anthropometric data according *COMT Val158Met* polymorphism genotype in children from three to four years of age.

	Food Intake				
	Non carriers	**n**	**Carries Val**	**n**	***p***
HSD (kcal)	88.25 [43.65–138.14[a]]	120	101.64 [42.35–168.61][a]	198	0.154[b]
HLD(kcal)	83.37 [0.00–252.95][a]	120	133.79 [44.23–265.80][a]	198	0.008[b]
Average energy intake daily (kcal)	1501.93±424.77[c]	120	1524.90±385.27[c]	198	0.620[d]
	Anthropometric data				
IMC Z-score	0.31±1.16[c]	121	0.22±1.08[c]	202	0.518[d]
Waist circumference(cm)	51.76±7.23[c]	122	50.85±3.59[c]	203	0.132[d]
Z-score of triceps skin folds	7.75±2.68[c]	120	7.68±2.21[c]	203	0.814[d]
Z-score of subescapular skin folds	5.88±2.69[c]	120	5.85±2.27[c]	203	0.905[d]

HSD indicates high sugar density foods; HLD indicates high lipid density foods; BMI indicates Body Mass Index; [a] Median [Interquartile Range]; [b] Mann-Whitney U; **[c]** Mean ± standard deviation, [d] Test T for independent sample.

n = individuals number carrier genotype

CAPÍTULO 5

ANEXOS

ANEXO A - PARECER DO COMITÊ DE ÉTICA EM PESQUISA DA UFCSPA

Título do Projeto: Incidência de obesidade e anemia em uma coorte de nascimento acompanhada até 4 anos de idade: avaliação do componente genético

Pesquisador Responsável : Márcia Regina Vitolo **Parecer** 719/08

Data da Versão	Cadastro 143/06	Data do Parecer 13/11/2008

Grupo e Área Temática Classificação utilizada pela CONEP

Objetivos do Projeto

Geral: Investigar a associação de variantes de genes envolvidos nas diferentes vias de controle e regulação do peso corporal e parâmetros antropométricos e de ingesta alimentar em uma coorte de crianças entre 3 e 4 anos de idade. .

Sumário do Projeto

Itens Metodológicos e Éticos	Situação
Título	Adequado
Autores	Adequados
Local de Origem na Instituição	Adequado
Projeto elaborado por patrocinador	Não
Aprovação no país de origem	Não necessita
Local de Realização	Outro (citar no comentário)
Outras instituições envolvidas	Sim
Condições para realização	Adequadas

Comentários sobre os itens de Identificação

Instituto de Cardiologia do RS; Laboratório de Biologia Molecular da UFCSPA.

Introdução	Adequada

Comentários sobre a Introdução

Objetivos	Adequados

Comentários sobre os Objetivos

Não apresenta o objetivo no protocolo de pesquisa.

Pacientes e Métodos	
Delineamento	Adequado
Tamanho de amostra	Total 354 Local
Cálculo do tamanho da amostra	Adequado
Participantes pertencentes a grupos especiais	Menores de 18 anos
Seleção eqüitativa dos indivíduos participantes	Adequada
Critérios de inclusão e exclusão	Adequados
Relação risco- benefício	Adequada
Uso de placebo	Não utiliza
Período de suspensão de uso de drogas (wash out)	Nâo utiliza
Monitoramento da segurança e dados	Adequado
Avaliação dos dados	Adequada - quantitativa
Privacidade e confidencialidade	Adequada
Termo de Consentimento	Adequado
Adequação às Normas e Diretrizes	Sim

Comentários sobre os itens de Pacientes e Métodos

Cronograma	Adequado
Data de início prevista	2007
Data de término prevista	2010
Orçamento	Adequado
Fonte de financiamento externa	Agência de fomento

Comentários sobre o Cronograma e o Orçamento

Referências Bibliográficas	Adequadas

Comentários sobre as Referências Bibliográficas

Recomendação

Aprovar

Comentários Gerais sobre o Projeto

O projeto acima descrito foi analisado para inclusão de dois autores e alteração no cronograma. Em relação aos itens (inclusão e alteração de cronograma) está de acordo com as Resoluções para pesquisa envolvendo seres humanos vigentes.

ANEXO B – TERMO DE CONSENTIMENTO INFORMADO

TERMO DE CONSENTIMENTO INFORMADO

O presente estudo (**Investigação dos Fatores de Risco para Obesidade Precoce e Anemia em uma Coorte de Crianças Submetidas a um Programa de Intervenção Nutricional no Primeiro Ano de Vida**) pretende dar continuidade ao trabalho realizado no 1º ano de vida de seu filho, visando acompanhar as condições de crescimento e desenvolvimento por meio das medidas de peso, altura, quantidade de gordura corporal, as quais não conferem riscos nem dor para seu filho. Utilizaremos um questionário para fazer-lhe perguntas sobre sua família, o qual conterá: condições de vida (sociais e econômicas), moradia, práticas alimentares de seu filho, atividades diárias e presença de doenças. Em data marcada com o pesquisador, será verificada a pressão arterial e será realizada coleta de sangue por profissional treinado com agulhas descartáveis, sem risco de contaminação, para análise dos níveis de colesterol, LDL, triglicerídeos, proteína-C reativa e glicemia, além disso, será avaliado alterações genéticas que podem estar associadas à obesidade e anemia. A criança sentirá um pequeno desconforto o momento da picada, porém não haverá riscos a sua saúde. Entretanto, não há outra forma de verificação que possa fornecer resultados mais precisos. Essas informações serão transformadas em números e a identidade da sua família não será divulgada em nenhum momento. Este estudo é importante para se conhecer os fatores que são responsáveis pela obesidade e anemia na infância e dessa forma intervir de forma mais ampla na população. A senhora receberá todos os resultados das avaliações e orientações ou encaminhamentos se necessário para o melhor bem estar seu e de seu filho. A senhora também terá toda a liberdade de interromper a entrevista em qualquer momento ou de pedir maiores esclarecimentos caso tenha alguma dúvida. Assinará 2 cópias desse consentimento, ficando 1 em seu poder e outra com a responsável do programa.

São Leopoldo, ____ de _____ de 200___.

Nome_____

Assinatura_____

Tel Prof. Márcia Regina Vitolo – tel 81629929 – 32248822 (ramal 153)

Termo de Consentimento Informado ao Paciente

Eu, Profa. Márcia Regina Vitolo, nutricionista, estou realizando a pesquisa: "INCIDÊNCIA DE OBESIDADE E ANEMIA EM UMA COORTE DE NASCIMENTO ACOMPANHADA ATÉ 4 ANOS DE IDADE: AVALIAÇÃO DO COMPONENTE GENÉTICO". Esta pesquisa visa esclarecer como variações genéticas normais podem influenciar no desenvolvimento da obesidade infantil, assim como, no desenvolvimento de anemia. Para que tal pesquisa possa ser realizada peço sua colaboração, autorizando que seja realizado o estudo das variantes genéticas nas amostras de sangue que já foram coletadas de seu filho.

Quais os riscos em participar? Como não se fará nenhuma picada a mais do que aquelas necessárias para os exames que já foram realizados não há risco para a paciente em participar deste projeto.

O que o paciente ganha com este estudo?

Com a análise, poderemos saber quais crianças podem ter maior predisposição ao desenvolvimento de obesidade e anemia. No entanto, os benefícios deste estudo poderão ser obtidos apenas em longo prazo.

Quais são os seus direitos?

Seus registros médicos serão sempre tratados confidencialmente. Os resultados deste estudo poderão ser usados para fins científicos, mas você não será identificado por nome. Sua participação no estudo é voluntária, de forma que, caso você decida não participar, isto não afetará no tratamento normal que você tem direito.

Assinará 2 cópias desse consentimento, ficando 1 em seu poder e outra com a responsável do programa.

Nome : _____

Assinatura:_____

Núm de identificação:_____

Assinantura do responsável:_____

Data: ___/___/_____

Em caso de qualquer dúvida quanto à pesquisa ou sobre os seus direitos, você poderá contatar com

Prof. Márcia Regina Vitolo – tel 81629929 – 32248822 (ramal 153)

ANEXO C – QUESTIONÁRIO

Projeto: Investigação dos Fatores de Risco para Obesidade Precoce e Anemia em uma Coorte de Crianças que Foram Submetidas a um Programa de Intervenção Nutricional no Primeiro Ano de Vida

FICHA DA CRIANÇA

Entrevistador_____

1. Data _____/_____/_____	Data4: ___/___/__

Identificação (criança):

2.Telefones para contato_____

3.Numero de identificação _____ 4.Nome da criança_____ 5.Nome da mãe_____ 6.Endereço: _____ _____ _____ _____ 7.Data de Nascimento: _____/_____/_____	Ident4: _____

Dados Maternos e Socioeconômicos:

8.Qual a sua idade? _____anos	IdMae4:_____
09.Data de nascimento da mãe ____/____/_____	DNm4_____
10.Qual o seu estado civil?	
Casada/ou mora junto (1) Viúva (2) Solteira (3) Separada (4)	EstCivil4 _____
11.Você teve outros filhos?	
(1) Sim (2) Não (pule para a questão 14)	Filhos4 _____
12. Se sim:	
Quantos:_____	Quant4:_____
DN____/____/_____	DNf1:___/___/___
DN____/____/____-	DNf2___/___/___
DN____/____/____	DNf3___/___/___
13.Quantas pessoas moram na sua casa? _____	Famí4:_____
14.Qual o grau de parentesco?	Adul4:_____
(1) Família nuclear	
(2) Família não nuclear	Parente4 _____
15.Qual a sua ocupação?	OcupaMae4:____
(1) Desempregada (2) Empregada c/ carteira assinada (3) Empregada s/ carteira assinada (4) Do lar (5) Estudante	
16.Qual a ocupação do pai do seu (sua) filho (a)? (1) Desempregado (2) Empregado c/ carteira assinada (3) Empregado s/ carteira assinada (4) Aposentado (5) Estudante	OcupaPai4:_____
17.Qual a renda total da família? R$ _____	RendaT4:_____

18.Qual o gasto familiar mensal com alimentação? R$_____ 19.Qual o gasto familiar mensal com transporte? R$_____	GFA:_____ GFT: _____

20.Você é fumante? (1) Sim (2) Não (pule para a 22) (3) Parou de fumar (pule para a 22)	Vcfum4:_____

21.Quantos cigarros você fuma por dia? _____	Ncd4:_____

22.Alguém que mora na sua casa é fumante? Sim (1) Não (2) (Pule para a pergunta 24)	Ncd4:_____

Se sim:

23.Quem é fumante na sua casa? Pai (1) Outros moradores da casa (2) Anotar quantos (3)Pai e outros	QuemFuma: _____

24.Você fumou durante a gestação do seu filho que participou do projeto? (1) Sim (2) Não (pule para a 26)	Fgest4:_____

<u>Se sim:</u>

25.Quantos cigarros você fumava por dia?_____	Ncfum4_____

26. Alguém na família tem ou teve? (**referente a criança**) **Para a pergunta quem: coloque 1 quando sim e 2 quando não** 26.a Obesidade: (1) Sim (2) Não ou (3) Não Sabe (9) IGN	Obesi: ____ Obpai: ___ Obmãe: ___

Se sim:Quem? ( ) Pai ( ) Mãe ( ) Avós ( ) Tios ( )Irmãos (88) NSA (99) IGN	Obavós: ___ Obatio: ___ Obairm: ___
26.b Colesterol Alto: (1) Sim (2) Não ou (3) Não Sabe (9) IGN ***Se sim:***Quem?( ) Pai ( ) Mãe ( ) Avós ( ) Tios ( )Irmãos (88)NSA (99) IGN	ColAlto: ____ Colpai: _____ Colmãe: ____ Colavós: ____ Colatio: _____ Colairm: _____
26.c Doença cardiovascular: (1) Sim (2) Não (3) Não Sabe (9) IGN ***Se sim***:Quem?( ) Pai ( ) Mãe ( ) Avós ( ) Tios ( )Irmãos (88)NSA (99) IGN	DCV: ____ DCVpai: ___ DCVmãe: ___ DCVavós: ___ DCVtio: ___ DCVirm: ___
26.d Diabetes Melitus: (1) Sim (2) Não ou (3) Não Sabe) (9) IGN ***Se sim:***Quem?( ) Pai ( ) Mãe ( ) Avós ( ) Tios ( )Irmãos (88)NSA (99) IGN	DM: ____ DMpai:___ DMmãe:___ DMavós:___ DMtio:___ DMirm:___

26.e Hipertensão (Pressão Alta):(1) Sim (2) Não (3) Não Sabe (9) IGN ***Se sim:***Quem?( ) Pai ( ) Mãe ( ) Avós ( ) Tios ( )Irmãos (88)NSA (99) IGN	PA: _____ PApai: ___ PAmãe:____ PAavós:____ PAtio:____ PAirm:____

27. A criança realizou algum exame de sangue após o realizado quando o seu filho estava com 1 ano de idade, através do nosso projeto? Sim (1) Não (2)	Exam4_____

Se sim anotar:

Data:___/___/___ Data:___/___/___

Hb:_____g/dl Hb:_____g/dl

Ht:_____g/dl Ht:_____g/dl

VCM:_____fl VCM:_____fl

HCM:_____pg HCM:_____pg

28. Atualmente o seu filho esta recebendo algum suplemento de ferro? (1)Sim (2) Não Se sim:	Suple4_____
29.Qual o nome do suplemento?_____	Qual4_____
30.Qual a quantidade? _____gotas ou _____drágeas	Qgotas___
31.O seu filho realmente recebe o suplemento? (1) Sim (2) nao	Qdrag____
32.Que idade a crianca tinha quando iniciou com o uso desse suplemento?_____meses	Receb4____ Idade:____
33. Tempo de uso:_____semanas	Tempu4____

CONDIÇÕES DE SAÚDE NOS ÚLTIMOS 6 MESES

34. Seu (sua) filho (a) foi internado no últimos 6 meses? Sim (1) Não (2) Não sabe (3)	Intern4_____
35.Seu (sua) filho (a) teve episódios de diarréia no últimos 6 meses? Sim (1) Não (2) Não sabe (3)	Diarré4_____
36. Seu (sua) filho (a) apresentou febre importante no últimos 6 meses? Sim (1) Não (2) Não sabe (3)	Febre4_ _____
37. Seu (sua) filho (a) teve infecção no últimos 6 meses? Sim (1) Não (2) Não sabe (3)	Infecç4_____
38. Seu (sua) filho (a) teve infecção urinária nos últimos 6 meses? Sim (1) Não (2) Não sabe (3)	InfUri4_ _____
39.O seu (sua) filho (a) apresentou algum problema respiratório? Sim (1) Não (2) (pule para a 67)	Resp4_____

Leia as alternativas para o entrevistado

40. Qual ou quais problema (s) que seu (sua) filho (a) apresenta?	Tosse4____
Tosse ( ) Coriza ( )	Coriza4____
Obstrução Nasal ( ) Respiração rápida ou difícil ( )	Obstru4_____
Para o quadro ao lado preencher 1 para sim e 2 para não	Respd4_____

Preencher se a criança recebe leite de vaca: (se não recebe pule para a 72)

41. Qual o volume da preparação?_____ml	Vol4_____
42. Qual a freqüência que seu filho toma leite no dia? _____ vezes	freqleit_____
43. Volume total de leite ingerido no dia: _____ml	Volleite_____
(descontar se sobra)	

44. A criança vai a creche? Sim (1) Não (2) 45. Período: meio turno (1) dia inteiro (2) 46 Desde que idade (em meses):_____ 47.Se **não**, no lugar onde ela fica, tem outras crianças junto? Sim (1) Não (2)	Creche4_____ Períod4_____ temp4_____ idcre4_____ ondfica4_____

48. O (a) seu (sua) filho (a) bebe água? Sim (1) Não (2) (Pule para a pergunta 51)	Água4 _____

Se sim:

49. Quanto bebe? _____ 50. Qual o "tipo" da água? Filtrada / Fervida / Torneira tratada e fervida / Mineral (próprias para o consumo) (1) Torneira não tratada (impróprias para o consumo) (2) 51. Seu (sua) filho (a) comeu/come terra ou objetos não alimentares? Sim (1) Não (2) (pule para a 80) Não sabe (3) (pule para a 53)	Quant4_____ Tagua4 _____ Objet4 _____

Se sim:

52. Quais os objetos não alimentares que seu (sua) filho (a) comeu? (1) Terra (2) Sabão/Sabonete (3) Terra + sabão (5) casca da mandioca (4) Outras substancias _____(qual?)	Quais4_____

Estado Nutricional: 53. Peso_____gramas 54. Comprimento_____cm	 Peso4_____ Compri4_____

Atividades diárias(ontem) :	
55. Que horas foi dormir ontem___ Que horas acordou hoje_____/	Hsonoit____
horas de sono:_____	
56. O que fez ontem pela manha:	
( ) Creche / Tempo_____	MCrecheT___
( ) Assistiu TV / Tempo:_____	MTvT_____
( ) Brincou fora de casa / Tempo:_____	MBrifT_____
( ) Brincou dentro de casa / Tempo:_____	MBridT____
( ) Dormiu / Tempo:____	MDormT_____
57. O que fez ontem de tarde:	
( ) Creche / Tempo_____	TCrecheT___
( ) Assistiu TV / Tempo:_____	TTvT_____
( ) Brincou fora de casa / Tempo:_____	TBrifT_____
( ) Brincou dentro de casa / Tempo:_____	TBridT____
( ) Dormiu / Tempo:____	TDormT_____
58. O que fez ontem de noite:	
( ) Assistiu TV / Tempo:_____	
( ) Brincou dentro de casa / Tempo:_____	NTvT_____
( ) Dormiu / Tempo:____	NbrinT_____
OBS:Colocar sempre o tempo de HORAS	NdormT_____
59. Tem alguma atividade física regular na semana:	
( ) Sim ( )Não	
Se sim qual:_____	Ativ_____
Freqüência na semana:_____	Qual_____

60. Você considera seu filho: 1. muito calmo 2. calmo 3. ativo 4. muito ativo 5. agitado	FreqS____ ConFi_____

ANEXO D – INSTRUÇÕES BRITISH JOURNAL OF NUTRITION

Instruções para autores da Revista Britsh Journal of Nutrition a qual foi submetido o artigo intitulado: **Association of *DRD2 TaqIA* and *-141C InsDel* polymorphisms with food intake and anthropometric data in Brazilian children.**

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British Journal of Nutrition

(Revised July 2009)

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20. Department of Health (1997) Committee on Toxicity of Chemicals in Food Consumer Products and the Environment. Statement on vitamin B_6 (pyridoxine) toxicity. http://www.open.gov.uk/doh/hef/B6.htm
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Comparisons between means can be made by using either confidence intervals (CI) or significance tests. The most appropriate of such measures is usually the standard error of a difference between means (SED), or the standard errors of the means (SE or SEM) when these vary between means. The standard deviation (SD) is more useful only when there is specific interest in the variability of individual values. The degrees of freedom (df) associated with SED, SEM or SD should also be stated. The number of decimal places quoted should be sufficient but not excessive. Note that pH is an exponential number, as are the log(10) values often quoted for microbial numbers. Statistics should be carried out on the scalar rather than the exponential values.

If comparisons between means are made using CI, the format for presentation is, e.g. 'difference between means 0·73 (95 % CI 0·314, 1·36) g'. If significance tests are used, a statement that the difference between the means for two groups of values is (or is not) statistically significant should include the level of significance attained, preferably as an explicit *P* value (e.g. $P=0{\cdot}016$ or $P=0{\cdot}32$) rather than as a range (e.g. $P<0{\cdot}05$ or $P>0{\cdot}05$}. It should be stated whether the significance levels quoted are one-sided or two-sided. Where a multiple comparison procedure is used, a description or explicit reference should be given. Where appropriate, a superscript notation may be used in tables to denote levels of significance; similar superscripts should denote lack of a significant difference. Where the method of analysis is unusual, or if the experimental design is at all complex, further details (e.g. experimental plan, raw data, confirmation of assumptions, analysis of variance tables, etc.) should be included.

Figures. In curves presenting experimental results the determined points should be clearly shown, the symbols used being, in order of preference, ○, ●, Δ, ▲, □, ■, ×, +□. Curves and symbols should not extend beyond the experimental points. Scale-marks on the axes

should be on the inner side of each axis and should extend beyond the last experimental point. Ensure that lines and symbols used in graphs and shading used in histograms are large enough to be easily identified when the figure is reduced to fit the printed page. Figures and diagrams can be prepared using most applications but please do not use the following: cdx, chm, jnb or PDF. All figures should be numbered and legends should be provided. Each figure, with its legend, should be comprehensible without reference to the text and should include definitions of abbreviations. Latin names for unusual species should be included unless they have already been specified in the text. Each figure will be positioned near the point in the text at which it is first introduced unless instructed otherwise.

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Refer to a recent copy of the journal for examples of figures.

Plates. The *British Journal of Nutrition* will now also consider the inclusion of illustrations and photomicrographs. The size of photomicrographs may have to be altered in printing; in order to avoid mistakes the magnification should be shown by scale on the photograph itself. The scale with the appropriate unit together with any lettering should be drawn by the author, preferably using appropriate software.

Tables. Tables should carry headings describing their content and should be comprehensible without reference to the text. Tables should not be subdivided by ruled lines. The dimensions of the values, e.g. mg/kg, should be given at the top of each column. Separate columns should be used for measures of variance (SD, SE etc.), the ± sign should not be used. The number of decimal places used should be standardized; for whole numbers 1·0, 2·0 etc. should be used. Shortened forms of the words weight (wt) height (ht) and experiment (Expt) may be used to save space in tables, but only Expt (when referring to a specified experiment, e.g. Expt 1) is acceptable in the heading.

Footnotes are given in the following order: (1) abbreviations, (2) superscript letters, (3) symbols. Abbreviations are given in the format: RS, resistant starch. Abbreviations appear in the footnote in the order that they appear in the table (reading from left to right across the table, then down each column). Abbreviations in tables must be defined in footnotes. Symbols for footnotes should be used in the sequence: *†‡§||¶, then ** etc. (omit * or †, or both, from the sequence if they are used to indicate levels of significance).

For indicating statistical significance, superscript letters or symbols may be used. Superscript letters are useful where comparisons are within a row or column and the level of significance is uniform, e.g. '^{a,b,c}Mean values within a column with unlike superscript letters were significantly different ($P<0{\cdot}05$)'. Symbols are useful for indicating significant differences between rows or columns, especially where different levels of significance are found, e.g. 'Mean values were significantly different from those of the control group: $*P<0{\cdot}05$, $**P<0{\cdot}01$, $***P<0{\cdot}001$'. The symbols used for *P* values in the tables must be consistent.

Tables should be placed at the end of the text. Each table will be positioned near the point in the text at which it is first introduced unless instructed otherwise.

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Chemical formulas. These should be written as far as possible on a single horizontal line. With inorganic substances, formulas may be used from first mention. With salts, it must be stated whether or not the anhydrous material is used, e.g. anhydrous $CuSO_4$, or which of the different crystalline forms is meant, e.g. $CuSO_4.5H_2O$, $CuSO_4.H_2O$.

Descriptions of solutions, compositions and concentrations. Solutions of common acids, bases and salts should be defined in terms of molarity (M), e.g. 0·1 M-NaH_2PO_4. Compositions expressed as mass per unit mass (w/w) should have values expressed as ng, µg, mg or g per kg; similarly for concentrations expressed as mass per unit volume (w/v), the denominator being the litre. If concentrations or compositions are expressed as a percentage, the basis for the composition should be specified (e.g. % (w/w) or % (w/v) etc.). The common measurements used in nutritional studies, e.g. digestibility, biological value and net protein utilization, should be expressed as decimals rather than as percentages, so that amounts of available nutrients can be obtained from analytical results by direct multiplication. See *Metric Units, Conversion Factors and Nomenclature in Nutritional and Food Sciences*. London: The Royal Society, 1972 (para. 8).

Cell lines. The Journal expects authors to deposit cell lines (including microbial strains) used in any study to be published in publicly accessible culture collections, for example, the European Collection of Cell Cultures (ECACC) or the American Type Culture Collection (ATCC) and to refer to the collection and line or strain numbers in the text (e.g. ATCC 53103). Since the authenticity of subcultures of culture collection specimens that are distributed by individuals cannot be ensured, authors should indicate laboratory

line or strain designations and donor sources as well as original culture collection identification numbers.

Nomenclature of vitamins. Most of the names for vitamins and related compounds that are accepted by the Editors are those recommended by the IUNS Committee on Nomenclature. See *Nutrition Abstracts and Reviews* (1978) **48**A, 831–835.

Acceptable name	*Other names**
Vitamin A	
Retinol	Vitamin A_1
Retinaldehyde, retinal	Retinene
Retinoic acid (all-*trans* or 13-*cis*)	Vitamin A_1 acid
3-Dehydroretinol	Vitamin A_2
Vitamin D	
Ergocalciferol, ercalciol	Vitamin D_2 calciferol
Cholecalciferol, calciol	Vitamin D_3
Vitamin E	
α-, β- and γ-tocopherols plus tocotrienols	
Vitamin K	
Phylloquinone	Vitamin K_1
Menaquinone-n (MK-n)†	Vitamin K_2
Menadione	Vitamin K_3, menaquinone, menaphthone
Vitamin B_1	
Thiamin	Aneurin(e), thiamine
Vitamin B_2	
Riboflavin	Vitamin G, riboflavine, lactoflavin
Niacin	
Nicotinamide	Vitamin PP
Nicotinic acid	
Folic Acid	
Pteroyl(mono)glutamic acid	Folacin, vitamin B_c or M
Vitamin B_6	
Pyridoxine	Pyridoxol
Pyridoxal	
Pyridoxamine	
Vitamin B_{12}	
Cyanocobalamin	
Hydroxocobalamin	Vitamin B_{12a} or B_{12b}
Aquocobalamin	
Methylcobalamin	
Adenosylcobalamin	
Inositol	
Myo-inositol	*Meso*-inositol
Choline	
Pantothenic acid	
Biotin	Vitamin H
Vitamin C	
Ascorbic acid	
Dehydroascorbic acid	

*Including some names that are still in use elsewhere, but are not used by the *British Journal of Nutrition*.

†Details of the nomenclature for these and other naturally-occurring quinones should follow the Tentative Rules of the IUPACIUB Commission on Biochemical Nomenclature (see *European Journal of Biochemistry* (1975) **53**, 15–18).

Generic descriptors. The terms **vitamin A**, **vitamin C** and **vitamin D** may still be used where appropriate, for example in phrases such as 'vitamin A deficiency', 'vitamin D activity'.

Vitamin E. The term **vitamin E** should be used as the descriptor for all tocol and tocotrienol derivatives exhibiting qualitatively the biological activity of α-tocopherol. The term **tocopherols** should be used as the generic descriptor for all methyl tocols. Thus, the term **tocopherol** is not synonymous with the term **vitamin E**.

Vitamin K. The term **vitamin K** should be used as the generic descriptor for 2-methyl-1,4-naphthoquinone (menaphthone) and all derivatives exhibiting qualitatively the biological activity of phylloquinone (phytylmenaquinone).

Niacin. The term **niacin** should be used as the generic descriptor for pyridine 3-carboxylic acid and derivatives exhibiting qualitatively the biological activity of nicotinamide.

Vitamin B_6. The term **vitamin B_6** should be used as the generic descriptor for all 2-methylpyridine derivatives exhibiting qualitatively the biological activity of pyridoxine.

Folate. Due to the wide range of C-substituted, unsubstituted, oxidized, reduced and mono- or polyglutamyl side-chain derivatives of pteroylmonoglutamic acid that exist in nature, it is not possible to provide a complete list. Authors are encouraged to use either the generic name or the correct scientific name(s) of the derivative(s), as appropriate for each circumstance.

Vitamin B_{12}. The term **vitamin B_{12}** should be used as the generic descriptor for all corrinoids exhibiting qualitatively the biological activity of cyanocobalamin. The term **corrinoids** should be used as the generic descriptor for all compounds containing the corrin nucleus and thus chemically related to cyanocobalamin. The term **corrinoid** is not synonymous with the term **vitamin B_{12}**.
Vitamin C. The terms **ascorbic acid** and **dehydroascorbic acid** will normally be taken as referring to the naturally-occurring L-forms. If the subject matter includes other optical isomers, authors are encouraged to include the L- or D- prefixes, as appropriate. The same is true for all those vitamins which can exist in both natural and alternative isomeric forms.
Amounts of vitamins and summation. Weight units are acceptable for the amounts of vitamins in foods and diets. For concentrations in biological tissues, SI units should be used; however, the authors may, if they wish, also include other units, such as weights or international units, in parentheses.
See *Metric Units, Conversion Factors and Nomenclature in Nutritional and Food Sciences* (1972) paras 8 and 14–20. London: The Royal Society.
Nomenclature of fatty acids and lipids. In the description of results obtained for the analysis of fatty acids by conventional GLC, the shorthand designation proposed by Farquhar JW, Insull W, Rosen P, Stoffel W & Ahrens EH (*Nutrition Reviews* (1959), **17**, Suppl.) for individual fatty acids should be used in the text, tables and figures. Thus, 18 : 1 should be used to represent a fatty acid with eighteen carbon atoms and one double bond; if the position and configuration of the double bond is unknown. The shorthand designation should also be used in the abstract. If the positions and configurations of the double bonds are known, and these are important to the discussion, then a fatty acid such as linoleic acid may be referred to as *cis*-9,*cis*-12-18 : 2 (positions of double bonds related to the carboxyl carbon atom 1). However, to illustrate the metabolic relationship between different unsaturated fatty acid families, it is sometimes more helpful to number the double bonds in relation to the terminal methyl carbon atom, *n*. The preferred nomenclature is then: 18 : 3*n*-3 and 18 : 3*n*-6 for α-linolenic and γ-linolenic acids respectively; 18 : 2*n*-6 and 20 : 4*n*-6 for linoleic and arachidonic acids respectively and 18 : 1*n*-9 for oleic acid. Positional isomers such as α- and γ-linolenic acid should always be clearly distinguished. It is assumed that the double bonds are methylene-interrupted and are of the *cis*-configuration (see Holman RT in *Progress in the Chemistry of Fats and Other Lipids* (1966) vol. 9, part 1, p. 3. Oxford: Pergamon Press). Groups of fatty acids that have a common chain length but vary in their double bond content or double bond position should be referred to, for example, as C_{20} fatty acids or C_{20} PUFA. The modern nomenclature for glycerol esters should be used, i.e. triacylglycerol, diacylglycerol, monoacylglycerol *not* triglyceride, diglyceride, monoglyceride. The form of fatty acids used in diets should be clearly stated, i.e. whether ethyl esters, natural or refined fats or oils. The composition of the fatty acids in the dietary fat and tissue fats should be stated clearly, expressed as mol/100 mol or g/100 g total fatty acids.
Nomenclature of micro-organisms. The correct name of the organism, conforming with international rules of nomenclature, should be used: if desired, synonyms may be added in parentheses when the name is first mentioned. Names of bacteria should conform to the current Bacteriological Code and the opinions issued by the International Committee on Systematic Bacteriology. Names of algae and fungi must conform to the current International Code of Botanical Nomenclature. Names of protozoa should conform to the current International Code of Zoological Nomenclature.
Nomenclature of plants. For plant species where a common name is used that may not be universally intelligible, the Latin name in italics should follow the first mention of the common name. The cultivar should be given where appropriate.
Other nomenclature, symbols and abbreviations. Authors should consult recent issues of the *British Journal of Nutrition* for guidance. The IUPAC rules on chemical nomenclature should be followed, and the Recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature (see *Biochemical Journal* (1978) **169**, 11–14). The symbols and abbreviations, other than units, are essentially those listed in *British Standard* 5775 (1979–1982), *Specifications for Quantities, Units and Symbols*, parts 0–13. Day should be abbreviated to d, for example 7 d, except for 'each day', '7th day' and 'day 1'.
Elements and simple chemicals (e.g. Fe and CO_2) can be referred to by their chemical symbol (with the exception of arsenic and iodine, which should be written in full) or formula from the first mention in the text; the title, text and table headings, and figure legends can be taken as exceptions,. Well-known abbreviations for chemical substances may be used without explanation, thus: RNA for ribonucleic acid and DNA for deoxyribonucleic acid. Other substances that are mentioned frequently (five or more times) may also be abbreviated, the abbreviation being placed in parentheses at the first mention, thus: lipoprotein lipase (LPL), after that, LPL, and an alphabetical list of abbreviations used should be included. Only accepted abbreviations may be used in the title and text headings. If an author's initials are mentioned in the text, they should be distinguished from other abbreviations by the use of stops, e.g. 'one of us (P. J. H.)…'. For UK counties the official names given in the *Concise Oxford Dictionary* (1995) should be used and for states of the USA two-letter abbreviations should be used, e.g. MA (not Mass.) and IL (not Ill.). Terms such as 'bioavailability' or 'available' may be used providing that the use of the term is adequately defined.
Spectrophotometric terms and symbols are those proposed in *IUPAC Manual of Symbols and Terminology for Physicochemical Quantities and Units* (1979) London: Butterworths. The attention of authors is particularly drawn to the following symbols: m (milli, 10^{-3}), μ (micro, 10^{-6}), n (nano, 10^{-9}) and p (pico, 10^{-12}). Note also that ml (millilitre) should be used instead of cc, μm (micrometre) instead of μ (micron) and μg (microgram) instead of γ.
Numbers. Numerals should be used with units, for example, 10 g, 7 d, 4 years (except when beginning a sentence, thus: 'Four years ago...'); otherwise, words (except when 100 or more), thus: one man, ten ewes, ninety-nine flasks, three times (but with decimal, 2·5 times), 100 patients, 120 cows, 136 samples.
Abbreviations. The following abbreviations are accepted without definition by the *British Journal of Nutrition:*
ADP (GDP) adenosine (guanosine) 5'-disphosphate
AIDS acquired immune deficiency syndrome
AMP (GMP) adenosine (guanosine) 5'-monophosphate
ANOVA analysis of variance
apo apolipoprotein

ATP (GTP) adenosine (guanosine) 5'-triphosphate

BMI body mass index
BMR basal metabolic rate
bp base pair
BSE bovine spongiform encephalopathy
CHD coronary heart disease
CI confidence interval
CJD Creutzfeldt-Jacob disease
CoA and acyl-CoA co-enzyme A and its acyl derivatives
CV coefficient of variation
CVD cardiovascular disease
Df degrees of freedom
DHA docosahexaenoic acid
DM dry matter
DNA deoxyribonucleic acid
dpm disintegrations per minute
EDTA ethylenediaminetetra-acetic acid
ELISA enzyme-linked immunosorbent assay
EPA eicosapentaenoic acid
Expt experiment (for specified experiment, e.g. Expt 1)
FAD flavin-adenine dinucleotide
FAO Food and Agriculture Organization (except when used as an author)
FFQ food-frequency questionnaire
FMN flavin mononucleotide
GC gas chromatography
GLC gas–liquid chromatography
GLUT glucose transporter
GM genetically modified
Hb haemoglobin
HDL high-density lipoprotein
HEPES 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid
HIV human immunodeficiency virus
HPLC high-performance liquid chromatography
Ig immunoglobulin
IHD ischaemic heart disease
IL interleukin
IR infra red
kb kilobases
K_m Michaelis constant
LDL low-density lipoprotein
MHC major histocompatibility complex
MRI magnetic resonance imaging
MS mass spectrometry
MUFA monounsaturated fatty acids
NAD+, NADH oxidized and reduced nicotinamide-adenine dinucleotide
NADP+, NADPH oxidized and reduced nicotinamide-adenine dinucleotide phosphate
NEFA non-esterified fatty acids
NF-κB nuclear factor kappa B
NMR nuclear magnetic resonance
NS not significant
NSP non-starch polysaccharide
OR odds ratio
PAGE polyacrylamide gel electrophoresis
PBS phosphate-buffered saline
PCR polymerase chain reaction
PG prostaglandin
PPAR peroxisome proliferator-activated receptor
PUFA polyunsaturated fatty acids
RDA recommended dietary allowance
RER respiratory exchange ratio
RIA radioimmunoassay
RMR resting metabolic rate
RNA, mRNA etc. ribonucleic acid, messenger RNA etc.
rpm revolutions per minute
RT reverse transcriptase
SCFA short-chain fatty acids
SDS sodium dodecyl sulphate

SED standard error of the difference between means
SFA saturated fatty acids
TAG triacylglycerol
TCA trichloroacetic acid

TLC thin-layer chromatography
TNF tumour necrosis factor
UN United Nations (except when used as an author)
UNICEF United Nations International Children's Emergency Fund
UV ultra violet
VLDL very-low-density lipoprotein
V_{O_2} O_2 consumption
$V_{O_{2max}}$ maximum O_2 consumption
WHO World Health Organization (except when used as an author)
Use of three-letter versions of amino acids in tables: Leu, His, etc.
CTP, UTP, GTP, ITP, as we already use ATP, AMP etc.
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deuterium or tritium (use ^2H and ^3H)
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ANEXO E – COMPROVANTE DE SUBMISSÃO DE ARTIGO

Cópia do comprovante de submissão do artigo intitulado: **Association of *DRD2 TaqIA* and *-141C InsDel* polymorphisms with food intake and anthropometric data in Brazilian children** à Revista British Journal of Nutrition.

Manuscript #	BJN-2010-015087
Current Revision #	0
Submission Date	17th Mar 10
Current Stage	Under Review
Title	Association of DRD2 TaqIA and -141C InsDel polymorphisms with food intake and anthropometric data in Brazilian children
Running Title	DRD2: nutritional and anthropometric data
Manuscript Type	Research Article
Special Section	N/A
Category	Behaviour, Appetite, and Obesity
Corresponding Author	Silvana Almeida (silvana.almeida@pq.cnpq.br) (Universidade Federal de Ciências da Saúde de Porto Alegre)
Contributing Authors	Ananda Galvão , Márcia Vitolo , Paula Campagnolo , Vanessa Mattevi
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Abstract	Effective interventions to reduce obesity and related health risks are increasingly important because the number of obese adults and children has reached epidemic proportions. There are data supporting a role for allelic variants of the D2 dopamine receptor (DRD2) gene in susceptibility to obesity. We assessed the relationships between food intake, anthropometric data and the DRD2 Taq1A and -141C InsDel genotypes in children. Our sample consisted of 354 children from three to four years of age whose race or ethnicity was self-defined by skin colour (i.e. white or non-white). Among white children, only 23.6% exhibited the TaqIA*T allele as compared to 32% in the non-white sample (p=0.028). A similar pattern was observed for the 141C Del allele (18.4% in white children versus 10.8% in non-white children; p=0.011). In non-white children, the TaqIA C/C homozygous genotype was associated with higher high lipid density (HLD) food intake

	(median 666.8 kJ) when compared with children carrying the T allele (343.9 kJ, p=0.009). Further studies evaluating the effects of this polymorphism on the genetic profiles associated with food consumption patterns and the anthropometry of children from different populations will provide a better understanding of polymorphisms relationship to nutritional outcomes.
Key Words	child obesity, DRD2 polymorphisms, food intake
Conflict of Interest	**No**, there is no conflict of interest that I should disclose, having read the above statement.
Word Count	3,157

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