UNIVERSIDADE FEDERAL DO PAMPA

CAMPUS URUGUAIANA

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS FARMACÊUTICAS

RENATA MONTAGNER

POLIMORFISMO ADIPOQ +45T>G DO GENE DA ADIPONECTINA E FATORES DE RISCO CARDIOMETABÓLICO EM PEQUENOS PRODUTORES RURAIS DE UMA COMUNIDADE DO INTERIOR DO RS

Dissertação de Mestrado

Uruguaiana
2019

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Dissertação apresentada ao Programa de Pós-Graduação *Stricto Sensu* em Ciências Farmacêuticas da Universidade Federal do Pampa como requisito parcial para obtenção do Título de Mestre em Ciências Farmacêuticas

Orientador(a): Prof.ª Dr.ª Jacqueline da Costa Escobar Piccoli

Uruguaiana

2019

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M758p Montagner, Renata
Polimorfismo ADIPOQ +45T>G do gene da adiponectina e fatores de risco cardiometabólico em pequenos produtores rurais de uma comunidade do interior do RS / Renata Montagner.
81 p.

Dissertação(Mestrado)-- Universidade Federal do Pampa, MESTRADO EM CIÊNCIAS FARMACÊUTICAS, 2019.
"Orientação: Jacqueline da Costa Escobar Piccoli".

1. Polimorfismo adiponectina. 2. Excesso de peso. 3. Agricultores. I. Título.

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Área de concentração: Farmácia

Dissertação defendida e aprovada em: 29 de março de 2019.

Banca examinadora:

Profª Drª Jacqueline da Costa Escobar Piccoli
Orientadora
UNIPAMPA

Prof. Dr. Matias Nunes Frizzo
UNIJUI

Profª Drª Vanessa Bley Ribeiro
UNIPAMPA

Dedico esta dissertação ao meu anjo, minha metade e eternamente melhor amiga, minha sempre amada irmã Rosália.

AGRADECIMENTOS

A Deus, obrigada pela vida, pela força nos momentos difíceis e pela capacidade de sempre poder aprender mais.

À UNIPAMPA, pela oportunidade de continuar estudando e pelo incentivo à qualificação de seus servidores.

Aos professores do PPGCF, por todo conhecimento transmitido.

À minha querida orientadora, Profª Jacqueline da Costa Escobar Piccoli, que me recebeu de portas abertas, acreditou em mim e sempre me apoiou mesmo nos momentos mais conturbados desse período. Obrigada por toda paciência, dedicação, ensinamentos e amizade. Espero que possamos continuar caminhando juntas.

À Márcia Denise Pavanelo Cattelan, por ter iniciado as pesquisas com os agricultores, permitindo que eu continuasse as investigações nessa área. A todos voluntários que concordaram em participar deste trabalho.

Aos colegas do LabGen pelos bons momentos de convivência e por toda colaboração na pesquisa. Agradeço em especial à Patrícia Maurer e à Débora Rúbio, que foram parceiras de laboratório incansáveis nessa trajetória.

Aos professores Vanessa Bley Ribeiro e Matias Nunes Frizzo, por aceitarem o convite para avaliar e contribuir com este trabalho.

Aos colegas de trabalho na UNIPAMPA, colegas de mestrado e amigos, muito obrigada por cada palavra de conforto, apoio e amizade em todos os momentos.

Ao meu amor Giovani, por toda compreensão, por sempre me dar força e auxiliar no que for preciso. Às nossas cadelinhas Gwen Stacy e Diana Prince, pelos momentos de leveza todos os dias. Nossa família é o meu porto seguro, amo muito vocês!

Aos meus pais que, mesmo à distância, sempre me incentivaram e acreditaram em mim. À minha irmã Rachel, meu orgulho e maior inspiração. À minha irmã Rosália, que tenho certeza que está sempre ao meu lado e guia meu caminho. Obrigada por todo amor. Vocês são o meu motivo para sempre querer ser melhor!

A todos aqueles que contribuíram de alguma forma para essa conquista, meus sinceros agradecimentos.

APRESENTAÇÃO

A presente dissertação foi dividida em três partes principais. Na **parte I** encontram-se a **INTRODUÇÃO**, a **REVISÃO BIBLIOGRÁFICA** e os **OBJETIVOS**. Os resultados que fazem parte desta dissertação, assim como as seções material e métodos, discussão e referências, estão apresentados sob a forma de manuscrito, no item **MANUSCRITO** na **parte II**. Por fim, na **parte III** encontram-se os itens **DISCUSSÃO GERAL, CONCLUSÃO e PERSPECTIVAS**, que apresentam interpretações e comentários gerais sobre os resultados apresentados no manuscrito, bem como o item **REFERÊNCIAS**, que se refere somente às citações que aparecem nos itens introdução, revisão bibliográfica e discussão geral desta dissertação.

RESUMO

POLIMORFISMO ADIPOQ +45T>G DO GENE DA ADIPONECTINA E FATORES DE RISCO CARDIOMETABÓLICO EM PEQUENOS PRODUTORES RURAIS DE UMA COMUNIDADE DO INTERIOR DO RS

Diferentes fatores de natureza física, química, biológica e ergonômica podem afetar a saúde do trabalhador rural e impactar suas atividades laborais, contribuindo para o desenvolvimento de doenças crônicas. Associado a isso, a prevalência de obesidade é cada vez maior na população em geral, inclusive na zona rural. A expansão da gordura abdominal pode alterar a secreção de adipocinas pelo tecido adiposo, aumentando o risco de comorbidades metabólicas e eventos cardiovasculares. Fatores genéticos, como os que afetam os níveis de adiponectina, também podem influenciar tais condições. O presente estudo visou avaliar a associação entre variantes genotípicas do polimorfismo +45T>G do gene da adiponectina e marcadores de risco cardiovascular em pequenos produtores rurais. Os participantes responderam questionário sobre estado de saúde e práticas laborais. Foram avaliados parâmetros antropométricos, hematológicos, bioquímicos e níveis de interleucina-6. O polimorfismo ADIPOQ +45T>G foi determinado pela técnica PCR-RFLP. No total, 82 agricultores participaram do estudo, 20,74% com IMC indicando obesidade, 37,80% com sobrepeso e 41,46% eutróficos. As frequências alélicas e genotípicas encontradas estavam em Equilíbrio de Hardy-Weinberg. Os resultados desse estudo não mostraram relação significativa entre o polimorfismo e excesso de peso, tampouco havia relação com doenças crônicas e medidas laboratoriais, exceto albumina e proteínas totais ($p<0,05$). Os níveis de IL-6 também não se alteraram de acordo com o genótipo, medidas antropométricas e práticas laborais. Dessa forma, é possível afirmar que não houve associação entre o polimorfismo ADIPOQ + 45T> G, obesidade e marcadores de risco cardiovascular nesta população de agricultores. Os níveis de interleucina-6 também não foram alterados em situações de potencial inflamação. Avaliações adicionais são necessárias para melhor compreensão efeitos desse polimorfismo na saúde humana.

Palavras-chaves: obesidade, polimorfismo, ADIPOQ, agricultores, cardiometabólico

ABSTRACT

ADIPOQ +45T>G POLYMORPHISM OF THE ADIPONECTIN GENEAND CARDIOMETABOLIC RISK FACTORS IN SMALL FARMERS OF A COMMUNITY OF THE INTERIOR OF RS

Different factors of a physical, chemical, biological and ergonomic nature can affect the health of rural workers and impact their work activities, contributing to the development of chronic diseases. Associated with this, the prevalence of obesity is increasing in the general population, including in rural areas. Expansion of abdominal fat may alter the secretion of adipokines by adipose tissue, increasing the risk of metabolic comorbidities and cardiovascular events. Genetic factors, such as those affecting adiponectin levels, may also influence such conditions. The present study aimed to evaluate the association between genotypic variants of the + 45T> G polymorphism of the adiponectin gene and cardiovascular risk markers in small farmers. Participants answered questionnaire about health status and work practices. Anthropometric, hematological, biochemical and interleukin-6 levels were evaluated. The ADIPOQ + 45T> G polymorphism was determined by the PCR-RFLP technique. A total of 82 farmers participated in the study, 20.74% with BMI consistent with obesity, 37.80% were overweight and 41.46% were eutrophic. The allelic and genotypic frequencies found were in Hardy-Weinberg equilibrium. The results of this study did not show a significant relationship between polymorphism and overweight, nor was it related to chronic diseases and laboratory measures, except albumin and total proteins ($p < 0.05$). IL-6 levels also did not change according to genotype, anthropometric measures and labor practices. Thus, it is possible to affirm that there was no association between ADIPOQ + 45T> G polymorphism, obesity and cardiovascular risk markers in this population of farmers. Interleukin-6 levels were also not altered in situations of potential inflammation. Additional assessments are needed to better understand the effects of this polymorphism on human health.

Keywords: obesity, polymorphism, ADIPOQ, farmers, cardiometabolic

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MANUSCRITO

LISTA DE ABREVIATURAS E SIGLAS

Acrp 30 – adipocyte complement-related protein of 30 kDa
ADIPOQ – adiponectina
apM1 – adipose most abundant gene transcript
DCV – doença cardiovascular
DM2 – diabetes mellitus tipo 2
EPI – equipamento de proteção individual
FTO - gene fat mass and obesity associated
GBP28 – gelatin binding protein of 28kDa
GWA – genome wide-association
IDF – International Diabetes Federation
IL-6 – interleucina - 6
IMC – índice de massa corporal
LEP – gene da leptina
LEPR – gene do receptor da leptina
NF-κB – fator nuclear kappa B
OMS – Organização Mundial da Saúde
PCR – proteína C reativa
RCE – relação cintura/estatura
RCQ – relação cintura/quadril
SNP – single nucleotide polymorphism
TAB – tecido adiposo branco
TAM – tecido adiposo marrom
TNF-α – fator de necrose tumoral alfa

LISTA DE ABREVIATURAS E SIGLAS - MANUSCRITO

ADIPOQ – adiponectin

ALKP – alkaline phosphatase

ALT– alanine aminotransferase

AST – aspartate aminotransferase

BMI – body mass index

DBP – diastolic blood pressure

ELISA – enzyme-linked immunosorbent assay

GGT – gamma glutamyl transferase

HDL – high density lipoprotein

IL-6 – interleukin-6

LDL – low density lipoprotein

MS – metabolic syndrome

PCR-RFLP – polymerase chain reaction-restriction fragment length polymorphism

PPE – personal protective equipment

SBP – systolic blood pressure

SNP– single nucleotide polymorphism

T2DM – type 2 diabetes mellitus

SUMÁRIO

PARTE I

1 INTRODUÇÃO

A saúde dos trabalhadores rurais pode ser afetada por diferentes fatores ligados ao dia a dia de trabalho no campo (DIAS, 2006). A exposição a ruídos, clima, uso de produtos químicos, associados à intensa jornada de trabalho podem contribuir para surgimento de doenças musculoesqueléticas e respiratórias, cardiometabólicas, depressão e câncer (ABREU e ALONZO, 2014; MOREIRA et al, 2015). Além desses fatores, a população rural está acompanhando a tendência mundial de aumento da prevalência de sobrepeso e obesidade, causada por uma transição nutricional (TAVARES et al, 2018). O excesso de peso já é considerado um problema de saúde pública, com impactos econômicos e sobre a qualidade de vida dos indivíduos (POHL et al, 2018). Dados da Organização Mundial da Saúde mostram que a obesidade quase triplicou desde 1975 (WHO, 2019). No Brasil, levantamentos apontam que a taxa de adultos com excesso de peso é superior a 50% no conjunto da população adulta das 27 capitais (VIGITEL, 2016).

Além das funções de reserva energética, regulação térmica e proteção mecânica, o tecido adiposo também produz e secreta uma grande variedade de adipocinas (RONTI et al, 2006). Estas moléculas estão envolvidas na regulação do apetite e saciedade, homeostase cardiovascular, metabolismo de glicose e lipídios, além de atuarem em funções inflamatória e resposta imune (RODRIGUEZ et al, 2015; BLÜHER, 2012). A expansão da adiposidade visceral provoca uma disfunção do tecido adiposo (PELLEGRINELLI et al, 2016) e altera a secreção de adipocinas, contribuindo para o surgimento de comorbidades, como Diabetes Mellitus tipo 2, dislipidemias e hipertensão, e potencializa o risco de eventos cardiovasculares (BASTIEN et al, 2014; YANG e CHUANG, 2006).

Um estado de inflamação crônica de baixo grau é observado na obesidade (GIUDICE e GANGESTAD, 2018), acompanhado de elevação dos níveis de citocinas inflamatórias. Uma delas é a interleucina-6 (IL-6), a qual tem sua produção aumetada conforme o índice de massa corporal (HOENE e WEIGERT, 2008). A IL-6 tem grande participação na resposta imune, mas também possui efeitos sobre o metabolismo glicídico e lipídico (ESPINOLA-KLEIN et al, 2011). Níveis elevados

dessa citocina podem levar à resistência à insulina (ESPINOLA-KLEIN et al, 2011) e agravar o risco cardiovascular (BASTARD et al, 2006).

A adiponectina é um hormônio protéico, secretado exclusivamente pelo tecido adiposo, que modula processos metabólicos como regulação da glicemia e catabolismo de ácidos graxos, estando relacionado à sensibilidade à insulina (WANG e SCHERER, 2016). Diferentemente de outras adipocinas, a expansão da massa adiposa reduz a liberação de adiponectina (TURER e SCHERER, 2012). O polimorfismo de único nucleotídeo (SNP) +45T>G no gene da adiponectina, no qual há uma troca silenciosa de uma timina por guanina, leva à diminuição dos níveis séricos dessa adipocina e está frequentemente associado a obesidade, resistência insulínica e síndrome metabólica (LI et al, 2012; RIZK et al, 2013; ZHOU et al, 2016, TU et al, 2014).

Como o sobrepeso é cada vez mais frequente em toda população, inclusive na zona rural, e o fenótipo da obesidade é influenciado de 40 a 70% por fatores genéticos (WARDLE et al, 2008) é interessante investigar a associação de variantes genotípicas do polimorfismo do gene ADIPOQ +45T> G e marcadores de risco cardiovascular em uma população de pequenos agricultores do interior do Rio Grande do Sul.

2 OBJETIVOS

2.1 Objetivo geral:

Investigar a associação entre variantes genotípicas do polimorfismo +45T>G do gene da adiponectina e marcadores de risco cardiovascular em pequenos produtores rurais do município de Santiago/RS.

2.2 Objetivos específicos:

- Descrever as frequências alélicas e genotípicas do polimorfismo +45T>G do gene da adiponectina;
- Verificar condições de saúde e práticas laborais;
- Avaliar os parâmetros laboratoriais hematológicos e bioquímicos e concentração de interleucina-6 sérica.
- Avaliar parâmetros antropométricos;
- Verificar a frequência de Síndrome Metabólica, segundo NCEP-ATPIII;
- Relacionar os resultados encontrados com doenças cardiometabólicas;
- Buscar associações entre os valores médios dos marcadores investigados e genótipos e haplótipos do polimorfismo +45T>G do gene da adiponectina.

3 REFERENCIAL TEÓRICO

3.1 Agricultura e saúde

3.1.1 Produção agrícola e agricultura familiar

O setor agrícola brasileiro apresentou grande crescimento nas últimas três décadas, ocupando um papel importante no desempenho econômico do país. Investimentos, principalmente em produtividade, proporcionaram duplicação do volume de produção agrícola e o triplo na pecuária (OCDE/FAO, 2015).

A agricultura brasileira é considerada como de natureza dualista, na qual coexiste a produção em grande escala e que envolve intenso capital e propriedades agrícolas tradicionais que utilizam recursos reduzidos (OCDE/FAO, 2015). A Lei nº 11.326, de 24 de julho de 2006, considera agricultor e/ou empreendedor familiar aquele indivíduo que pratica atividades no meio rural em área de até quatro módulos fiscais (que variam de acordo com a região), emprega mão de obra da própria família, possui renda vinculada ao próprio estabelecimento/empreendimento, cujo gerenciamento é feito pela própria família. Silvicultores, aquicultores, extrativistas, pescadores, indígenas, quilombolas e assentados da reforma agrária também são considerados agricultores familiares.

Embora o Censo Agropecuário de 2017 só tenha divulgado alguns dados preliminares, informações da edição de 2006 mostram que naquele período as propriedades familiares representavam cerca de 84,4% das unidades de produção e ocupavam 24,3% da área total dos estabelecimentos agropecuários do país. Aproximadamente 4,4 milhões de estabelecimentos e 12,3 milhões de pessoas eram responsáveis pela produção de mais de 70% dos alimentos que vão para a mesa do brasileiro (MDA, 2019; IBGE, 2006).

O Estado do Rio Grande do Sul posiciona-se entre os cinco maiores produtores agrícolas do país, ocupando posição estratégica na oferta nacional de arroz, trigo e aveia e destacando-se como um dos principais exportadores de fumo, soja e arroz. Em relação aos estabelecimentos agropecuários, a maioria pode ser incluída nos critérios da agricultura familiar. Estes estabelecimentos, embora ocupem apenas 30% da área, são responsáveis por uma parcela significativa do pessoal ocupado e do valor da produção agropecuária no RS (FEE, 2019). Em relação à

população, a colonização do RS engloba descendentes indígenas, negros e europeus, o que confere ao estado uma grande diversidade étnica e cultural (ATLAS, 2019).

Na região central do estado encontra-se o município de Santiago, que possui mais de 50.000 habitantes. A população rural corresponde a quase 9% dos habitantes e o número de estabelecimentos rurais ultrapassa 1700. A maior parte das propriedades rurais é de pequeno porte, apresenta cultivo diversificado e pode ser caracterizada como agricultura familiar. A população rural é, em sua maioria, descendente de italianos e alemães (EMATER-RS/ASCAR, 2015; SILVA, 2016).

3.1.2 Saúde dos produtores rurais

Nos dias atuais, são muitos os fatores que influenciam a saúde do agricultor na realização de seu trabalho. Eles vão desde fatores sociais, econômicos, tecnológicos e organizacionais relacionados ao perfil de produção e consumo até fatores de risco de natureza física, química, biológica, mecânica e ergonômica presentes nos processos laborais (DIAS, 2006). A árdua jornada de trabalho, condições climáticas adversas, ruídos, animais peçonhentos, preocupação com a colheita e mau uso de equipamentos de proteção individual (EPI) são exemplos que podem afetar a saúde do trabalhador e causar adoecimento (MENEGAT e FONTANA, 2010; ABREU e ALONZO, 2014). As consequências da exposição a tais riscos é bastante diversificada e decorre das diferentes possibilidades de atuação dentro da produção agrícola (MOREIRA et al, 2015). Estudos já mostraram maior prevalência de doenças musculoesqueléticas, cardiovasculares, respiratórias, diabetes e câncer em diferentes populações de agricultores (MOREIRA et al, 2015; PERKIO-MAKELA, 2010).

Entre todos os fatores que afetam a saúde do trabalhador rural, a utilização de agrotóxicos ganha destaque pela grande exposição e potencial de dano (ABREU e ALONZO, 2014). A contaminação por agrotóxicos pode ocorrer em diferentes etapas da produção (preparo, pulverização, descarte, manipulação das plantas), sendo a via dérmica a forma de absorção mais importante na exposição ocupacional (BLANCO et al, 2008). As intoxicações agudas compõem a maioria dos dados notificados no Brasil. Elas surgem em até algumas após a exposição e os sintomas variam de

acordo com o grupo químico do agrotóxico utilizado (OPA/OMS, 1996; FARIA, 2007). Na intoxicação crônica os sintomas surgem tardiamente após pequenas ou médias exposições a um ou múltiplos produtos. Dificilmente é diagnosticada antes do surgimento de danos irreversíveis e/ou doenças graves, como câncer, disfunções na fertilidade, distúrbios endócrinos, neurológicos e tentativas de suicídio em longo prazo (ECOBICHON, 2001; OPAS/OMS, 1996; INCA, 2010; JOBIM et al, 2010).

Além de atuarem como iniciadores e/ou promotores tumorais (KOIFMAN e HATAGIMA, 2003), o metabolismo dos agrotóxicos pode levar a um aumento nos níveis de radicais livres e conduzir o organismo a um estado de estresse oxidativo. Os danos celulares gerados nessas situações aumentam o envelhecimento e o risco de desenvolvimento de doenças crônicas não transmissíveis (AMR et al, 2015; FONG et al, 2007; BANERJEE et al, 2001, GARCIA-GARCIA et al, 2016). Os agrotóxicos também podem interferir no sistema endócrino e ter efeitos sobre a reprodução, distúrbios de comportamento e doenças autoimunes (RIGOTTO e AGUIAR, 2015), além de resistência à insulina e obesidade (LONG e HOLLOWAY, 2017; BURNS et al, 2014).

3.2 Sobrepeso e obesidade

Sobrepeso e obesidade são definidos como acumulo excessivo de gordura corporal, o que traz riscos e efeitos deletérios à saúde. Dados da Organização Mundial da Saúde mostram que a obesidade quase triplicou desde 1975, sendo que em 2016 39% dos adultos estavam acima do peso e um em cada oito eram obesos. Projeções estimam que em 2025 serão cerca de 2,3 bilhões de adultos com sobrepeso e mais de 700 milhões de obesos (WHO, 2019). Da mesma forma que a nível global, a epidemia de aumento de peso também vem ocorrendo na América Latina e Caribe, com impacto maior nas mulheres e tendência de crescimento nas crianças (OPAS/OMS Américas, 2016). No Brasil, levantamentos apontam que no conjunto da população adulta das 27 capitais, a taxa de adultos com excesso de peso é superior a 50% (VIGITEL, 2016). A elevada prevalência tornou a obesidade um problema de saúde pública, trazendo impactos econômicos e na qualidade de vida da população, visto que o excesso de peso pode comprometer o desenvolvimento das atividades do dia a dia (CORREA, 2014; POHL et al, 2018).

O ganho de peso é resultado de um desequilíbrio crônico entre energia ingerida e energia gasta, possui etiologia multifatorial e envolve interações entre aspectos ambientais, genéticos, socioeconômicos, culturais e comportamentais (MARQUES-LOPES et al, 2004; WANDERLEI e FERREIRA, 2010). Nas últimas décadas, mudanças na sociedade provocaram uma transição nutricional no país, onde a desnutrição foi sendo gradativamente substituída pelo excesso de sobrepeso e obesidade na população em geral. Essa transformação foi impulsionada pela urbanização e crescimento socioeconômico, que levaram a mudanças no estilo de vida, principalmente com alteração de hábitos alimentares, aumento da ingestão calórica e sedentarismo, aumentando o risco de desenvolvimento de doenças crônicas não transmissíveis, como as cardiovasculares (CORREA, 2014; POHL et al, 2018; BATISTA-FILHO e RISSIN, 2003). O perfil epidemiológico de transição também está sendo observado no meio rural pela incorporação de hábitos de vida urbanos e avanços tecnológicos no processo de trabalho rural que reduzem o esforço físico, resultando em prevalência elevada de sobrepeso nessa população (TAVARES et al, 2018, SILVA et al, 2008).

3.2.1 Índices antropométricos

Diferentes índices antropométricos podem ser utilizados para avaliação da composição corporal, devido à sua estreita relação com o estado de saúde (NOGUEIRA et al, 2014; SAVKIN e ASLAN, 2017). Como a distribuição de gordura é mais preditiva de saúde, a combinação de massa corporal e distribuição de gordura tem se tornado uma melhor opção de avaliação. Técnicas mais sofisticadas de determinação de composição corporal, como pesagem hidrostática, bioimpedância, absorciometria com raios-X de dupla energia e técnicas de imagem apresentam bons resultados, mas tem custo elevado e uso limitado na prática clínica (ABESO, 2016).

3.2.1.1.Índice de Massa Corporal (IMC)

O IMC é calculado através da divisão do peso em kg pela altura em metros elevada ao quadrado. Por ser simples, prático e sem custo, é a forma de avaliação de adiposidade mais utilizada. Mesmo sendo um bom indicador, o IMC não considera diferenças na composição corporal decorrentes da idade, sexo e condições de saúde, além de não distinguir massa gordurosa de massa magra (ABESO, 2016).

A OMS propôs uma classificação internacional da obesidade segundo o IMC e risco de doença, dividindo-a em graus e classes (tabela 1). IMC de 25 a 29,9 kg/m² são considerados sobrepeso, enquanto na obesidade o IMC é maior ou igual a 30 kg/m². O excesso de peso, que inclui sobrepeso e obesidade é definido para IMC maior ou igual a 25 kg/m².

Tabela 1. Classificação de obesidade segundo a OMS.

IMC (kg/m^2)	**Classificação**	**Obesidade grau/classe**	**Risco de doença**
< 18,5	Magro ou baixo peso	0	Normal ou elevado
18,5 – 24,9	Normal ou eutrófico	0	Normal
25 -29,9	Sobrepeso ou pré-obeso	0	Pouco elevado
30 – 34,9	Obesidade	I	Elevado
35 – 39,9	Obesidade	II	Muito elevado
≥ 40,0	Obesidade grave	III	Muitíssimo elevado

Fonte: adaptado de ABESO, 2016.

3.2.1.2 Distribuição da massa adiposa

Além da determinação de IMC, é recomendado utilizar índices que levam em conta a distribuição regional de gordura (MANCINI, 2001; ASHWELL et al, 2012). A OMS reconhece a gordura abdominal como importante medida de risco para ocorrência de infarto do miocárdio, acidente vascular encefálico e morte prematura, independentemente do IMC (WHO, 2008). Segundo a Federação Internacional de Diabetes, medidas de circunferência abdominal igual ou superior a 94 cm em

homens e 80 cm em mulheres estão associadas a um maior risco de complicações cardiometabólicas (ABESO, 2016).

A determinação da relação cintura/quadril (RCQ) é uma medida antropométrica amplamente utilizada para estabelecer se a obesidade é central ou periférica. RCQ maior que 0,9 em homens e 0,85 em mulheres indicam acúmulo de gordura abdominal (WHO, 2008; DOBASHI et al, 2017). Outras medidas muito utilizadas como preditoras de risco cardiometabólico são circunferência cervical e razão cintura/estatura (RCE) (ABESO, 2016; SARDINHA et al, 2016; FANTIN et al, 2017).

3.2.2 Disfunções cardiometabólicas

Doença cardiovascular (DCV) é um termo que designa um grande grupo de doenças e alterações do coração e dos vasos sanguíneos. As DCV são a principal causa de morte no mundo, correspondendo a cerca de 31% do total (OPAS/OMS Brasil). A Sociedade Brasileira de Cardiologia estima que em 2017 ocorreram mais de 380 mil mortes por DCV no país. A redução da mortalidade por essas doenças envolve prevenção e estratégias sobre fatores comportamentais de risco, como dietas não saudáveis, excesso de peso, tabagismo, uso de álcool e sedentarismo (FREITAS et al, 2018).

Já é amplamente reconhecido o envolvimento da adiposidade abdominal no desenvolvimento de doenças crônicas não transmissíveis como Diabetes Mellitus tipo 2 (DM2), dislipidemias e hipertensão, os quais são fatores de risco para DCV. O termo cardiometabólico passou a ser empregado a partir da constatação de que muitas vezes há associação dessas doenças (CURTI, 2012; BASTIEN et al, 2014; YANG e CHUANG, 2006). A Síndrome Metabólica (SM) é caracterizada como um conjunto de doenças relacionadas à deposição central de gordura visceral, cuja base é a resistência insulínica (REAVEN, 2011). Segundo as Sociedades Brasileiras de Cardiologia e de Endocrinologia e Metabologia, a presença de SM está relacionada a uma mortalidade geral 1,5 vezes maior, chegando a 2,5 vezes mais mortalidade por motivos cardiovasculares. Não existe uma única forma de diagnosticar a SM, sendo assim, o diagnóstico segue critérios que reconhecem a importância da adiposidade abdominal, hiperglicemia, hipertensão e dislipidemia.

No Brasil, as Diretrizes para Diagnóstico e Tratamento da SM citam os critérios da Organização Mundial da Saúde (OMS) e do National Cholesterol Education Program's Adult Treatment Panel III (NCEP-ATP III) como os mais utilizados na prática clínica. Para a OMS, a SM está presente quando identificada resistência à insulina e outros dois fatores de risco. Por outro lado, a NCEP–ATP III estabelece que a presença de 3 dos 5 fatores de risco são suficientes para diagnóstico da SM. Outro critério muito empregado é o da International Diabetes Federation (IDF). (ALBERTI et al, 2006)

Tabela 2. Critérios diagnósticos de Síndrome Metabólica, segundo OMS, IDF e NCEP.

Característica	OMS	IDF	NCEP-ATPIII
Obesidade	IMC > 30 kg/m2 e/ou RCQ>0,9 (H) e>0,85 (M)	Circunferência abdominal > 94 cm (H) e > 80 cm (M)	Circunferência abdominal > 102 cm (H) e > 88 cm (M)
Triglicerídeos	>150 mg/dL	>150 mg/dL	>150 mg/dL
HDL	< 35 mg/dL (H) < 39 mg/dL (M)	< 40 mg/dL (H) < 50 mg/dL (M)	< 40 mg/dL (H) < 50 mg/dL (M)
Glicemia	DM2 ou glicose alterada	Jejum > 100 mg/dL	Jejum > 110 mg/dL
Pressão arterial	PA> 140x90 mmHg ou em tratamento	PA> 130x85 mmHg ou em tratamento	PA> 130x85 mmHg ou em tratamento
Outros	Microalbuminúria > 20ug/min		
Condições para diagnóstico	Resistência à insulina + 2	Obesidade abdominal + 2	Presença de 3 fatores

H = homens; M = mulheres

3.3 Tecido adiposo e inflamação

Nos organismos, o tecido adiposo é dividido em dois tipos: tecido adiposo marrom (TAM), também chamado multilocular, e tecido adiposo branco (TAB), unilocular. O TAB constitui a maior parte do tecido adiposo corporal e encontra-se na região subcutânea e em depósitos viscerais (LEITE et al, 2009). Tradicionalmente, o tecido adiposo era considerado um órgão com funções de armazenamento de energia, regulação térmica e proteção mecânica, porém uma revolução no

entendimento de sua função biológica nas últimas décadas tornou esse tecido um grande foco de pesquisas (PRADO, 2009; TRAYHURN, 2007).

Atualmente, o tecido adiposo é considerado um dos maiores órgãos endócrinos do corpo, visto que o TAB é responsável pela produção e liberação de uma grande variedade de moléculas bioativas, denominadas adipocinas, que influenciam não apenas a função adipocitária autócrina e parácrina, mas também podem atuar a nível sistêmico (RONTI et al, 2006; UNAMUNO, 2018). As adipocinas estão envolvidas na regulação do apetite e saciedade, homeostase cardiovascular, metabolismo glicídico e lipídico, além de terem atuação nas respostas inflamatória e imune (RODRIGUEZ et al, 2015; BLÜHER, 2012). Dentre as mais de 50 adipocinas descritas, se destacam a interleucina-6 (IL-6), o fator de necrose tumoral (TNF-α), a leptina e a adiponectina (PRADO, 2009).

Em indivíduos obesos, o tecido adiposo é remodelado a nível celular e estrutural a fim de se adaptar ao excesso de ingestão calórica. Como consequência, a expansão da gordura visceral desencadeia uma disfuncionalidade do tecido adiposo (PELLEGRINELLI et al, 2016), alterando a secreção de adipocinas para um padrão pró-inflamatório, diabetogênico e aterogênico (OUCHI et al, 2011; YOO e CHOI, 2014). Essas alterações causam estresse celular e contribuem para o surgimento de comorbidades relacionadas ao excesso de peso (JENSEN, 2008).

Nas últimas décadas, a obesidade vem sendo caracterizada como um estado pró-inflamatório crônico, no qual a inflamação do tecido adiposo visceral pode favorecer o desenvolvimento da maioria das doenças cardiometabólicas (SALTIEL e OLEFSKY, 2017). Existem diferentes possibilidades para a origem dos marcadores inflamatórios na obesidade: (1) produção e liberação por outros órgãos, como o fígado, (2) secreção de fatores pelo TAB que estimulam a produção de marcadores inflamatórios por outros órgãos e (3) adipócitos atuando como fonte direta desses marcadores (TRAYHURN e WOOD, 2004; DAS, 2011).

3.3.1 Interleucina-6

A IL-6 é uma citocina secretada por diferentes tipos celulares, como macrófagos, linfócitos, células endoteliais, beta pancreáticas, hepatócitos, musculares esqueléticas e lisas, astrócitos e adipócitos, entre outros (AKIRA et al,

1993). Sua liberação na corrente circulatória ocorre por fatores fisiológicos e patológicos, entre eles hormônios, citocinas, dieta, atividade física, estresse e hipóxia (HELEGDA, 2014).

A IL-6 possui atuação multifuncional, local e sistêmica, participando da resposta imune, inflamação e hematopoese (OLIBONI et al, 2016). Uma das suas funções mais conhecidas é a mediação da resposta inflamatória de fase aguda, com a indução da produção hepática de proteína C-reativa (PCR) e outras proteínas de fase aguda, além de atuar no desenvolvimento de febre, diferenciação de linfócitos e proliferação de células de defesa (GIUDICE e GANGESTAD, 2018).

Paralelamente às ações de defesa, a IL-6 possui efeitos metabólicos, inibindo a ação da insulina nos músculos, fígado e adipócitos (ESPINOLA-KLEIN et al, 2011). Por ser amplamente secretada pelo tecido adiposo visceral, há uma correlação direta entre o aumento da massa adiposa e os níveis de IL-6 na circulação, o que reflete uma inflamação de baixo grau e sugere a participação dessa citocina na patogenia da obesidade. A maior produção de IL-6 em obesos está ligada à maior infiltração de macrófagos no tecido adiposo desses indivíduos. (HOENE e WEIGERT, 2008; GIUDICE e GANGESTAD, 2018).

Valores elevados de IL-6 podem levar a uma resistência à insulina, sobretudo em indivíduos obesos (ESPINOLA-KLEIN et al, 2011) e aumentar o risco de eventos cardiovasculares (BASTARD et al, 2006). Por outro lado, a produção de IL-6 possui alta regulação transcricional e pós-transcricional e, assim que há redução da massa adiposa, a sua produção é diminuída (ANDERSON, 2008). Porém, a síntese contínua e exacerbada de IL-6 já foi relacionada com câncer e outras doenças (TANAKA e KISHIMOTO, 2014; HADDICK et al, 2017; BROOKS et al, 2016).

A exposição a agrotóxicos também pode causar reação inflamatória e uma substancial expressão de citocinas inflamatórias, sobretudo quando não são utilizados EPIs. Tais alterações já foram relacionadas ao desenvolvimento de tumores, asma e distúrbios metabólicos (LIU e DING, 2015; GARCIA-GARCIA, 2016; GANGEMI et al, 2016).

3.4 Aspectos genéticos ligados à obesidade

Como dito anteriormente, o ganho de peso é multifatorial e inclui aspectos genéticos. O fenótipo da obesidade pode ser influenciado de 40 a 70% por fatores hereditários (WARDLE et al, 2008). A conclusão do sequenciamento do genoma humano, associada a avanços tecnológicos e na metodologia, levou à identificação de uma abundância de genes que modulam características antropométricas (PIGEYRE et al, 2016).

Como fenótipo de base genética, a obesidade pode ser dividida em sindrômica, monogênica ou poligênica. Pelo menos 25 síndromes podem levar a obesidade grave, independentemente de estímulos ambientais, acompanhada de atrasos no desenvolvimento (CLEMENT e FERRE, 2003). Na forma monogênica, alterações em um único gene também podem resultar em obesidade grave. Mutações ou polimorfismos raros em genes codificadores das proteínas reguladoras da ingestão de alimentos, como na via leptina/melanocortina, levam a obesidade mórbida no início da infância (CHOQUET e MEYRE, 2011). A forma mais comum de obesidade é a poligênica, na qual a participação de múltiplos genes resulta em uma predisposição genética para ganho de peso, que pode progredir para obesidade dependendo da interação com fatores ambientais e estilo de vida (MARQUES-LOPES et al, 2004; VAN DER KLAAUW e FAROOGI, 2015).

A busca por variantes genéticas associadas a doenças complexas, como a obesidade, foi impulsionada por estudos de Genome-wide association (GWA) que buscam identificar a associação de polimorfismos de nucleotídeo único (SNP) e traços variáveis entre os indivíduos (FAWCETT e BARROSO, 2010). Os genes candidatos à participação na epidemia de obesidade estão relacionados com a regulação da fome e do gasto energético, distribuição da gordura corporal, metabolismo de lipídeos e glicose e na sinalização autócrina e parácrina dos adipócitos (LOOS, 2009). Alguns exemplos são os genes da leptina (LEP) e de seu receptor (LEPR), da adiponectina, gene fat mass and obesity associated (FTO), genes de receptores de melanocortinas, entre outros (MARQUES-LOPES et al, 2004; FERNANDES et al, 2011).

3.4.1 Adiponectina

A adiponectina, hormônio protéico produzido exclusivamente pelo tecido adiposo, foi identificada por 4 grupos independentes há pouco mais de duas décadas. Por essa razão possui diferentes nomenclaturas: Acrp30 (*adipocyte complemente-related protein of 30kDa),* ADIPOQ, apM1 (*adipose most abundant gene transcript*) e GBP28 (*gelatin binding protein of 28kDa*) (WANG e SCHERER, 2016). Essa proteína apresenta peso molecular de 28kDa, é composta por 244 aminoácidos e possui uma sequência sinalizadora na região N-terminal, seguida por uma região variável, um domínio semelhante ao colágeno e um domínio globular na região C-terminal (SCHERER et al, 1995).

A ação da adiponectina é mediada por seus receptores específicos AdipoR1 e AdipoR2. O AdipoR1 é expresso em vários tecidos, principalmente no músculo esquelético, enquanto o AdipoR2 é expresso predominantemente no fígado (YAMAGUCHI et al, 2003; KADOWAKI et al, 2006). Dessa forma, a adiponectina age em diferentes tecidos, através da regulação do metabolismo glicídico e lipídico, aumentando a sensibilidade à insulina. Ela aumenta a oxidação de ácidos graxos livres e a captação de glicose no músculo esquelético, além de suprimir a produção de glicose hepática (WANG e SCHERER, 2016; KADOWAKI e YAMAGUCHI, 2005).

Além dos efeitos metabólicos, a adiponectina apresenta propriedades anti-inflamatorias, aumentando a síntese de citocinas anti-inflamatórias como IL-10 e reduzindo a produção de IL-6, TNF-α e ativação do fator nuclear kappa B (NF-kB) (KUKLA et al, 2011). Adicionalmente, possui propriedades antiaterogências e antitrombóticas.(COSTA et al, 2011).

A concentração plasmática desse hormônio varia de 3-30ug/ml, correspondendo a até 0,05% das proteínas plasmáticas totais (SCHERER et al, 1995), sendo menor no sexo feminino (NISHIZAWA, 2002). Diferentemente de outras proteínas secretadas por adipócitos, a adiponectina tem sua produção diminuída conforme a expansão do tecido adiposo, apresentando-se em menores níveis em obesos se comparada com indivíduos eutróficos (TURER e SCHERER, 2012). Apesar de parecer controverso devido à maior massa adiposa em obesos e a produção de adiponectina ocorrer exclusivamente em adipócitos, em condições de obesidade a produção de adiponectina é inibida pela presença de citocinas pró-inflamatórias (LI et al, 2009). O emagrecimento é capaz de elevar os níveis

plasmáticos de adiponectina, evidenciando a correlação inversa entre IMC e níveis circulantes desse hormônio (YANG et al, 2001; GELONEZE et al, 2009).

3.4.2 Polimorfismo +45T>G do gene ADIPOQ

O gene humano que codifica a adiponectina é composto por três éxons e dois introns e está localizado no cromossomo 3q27, um locus de suscetibilidade à obesidade (LING et al, 2009). Alterações que levem a hipoadiponectinemia, como SNPs no gene ADIPOQ, já foram relacionadas ao aumento do IMC, doenças metabólicas e cardiovasculares em diferentes populações étnicas (BISWAS et al, 2011; SONE et al, 2010; SIITONEN et al, 2012; OLIVEIRA et al, 2012; LI et al, 2015; MOHAMMADZADEH et al, 2016).

O polimorfismo +45T>G (rs 2241766) é caracterizado pela troca de um nucleotídeo timina (T) por uma guanina (G) na posição 45 do éxon 2 (Figura 1). Essa alteração é silenciosa pois não muda o aminoácido codificado (Gly), porém impacta nos níveis séricos de adiponectina. Estudos mostram que esse SNP está fortemente associado à obesidade, resistência insulínica e SM (LI et al, 2012; RIZK et al, 2013; ZHOU et al, 2016; TU et al, 2014; YAO et al, 2016).

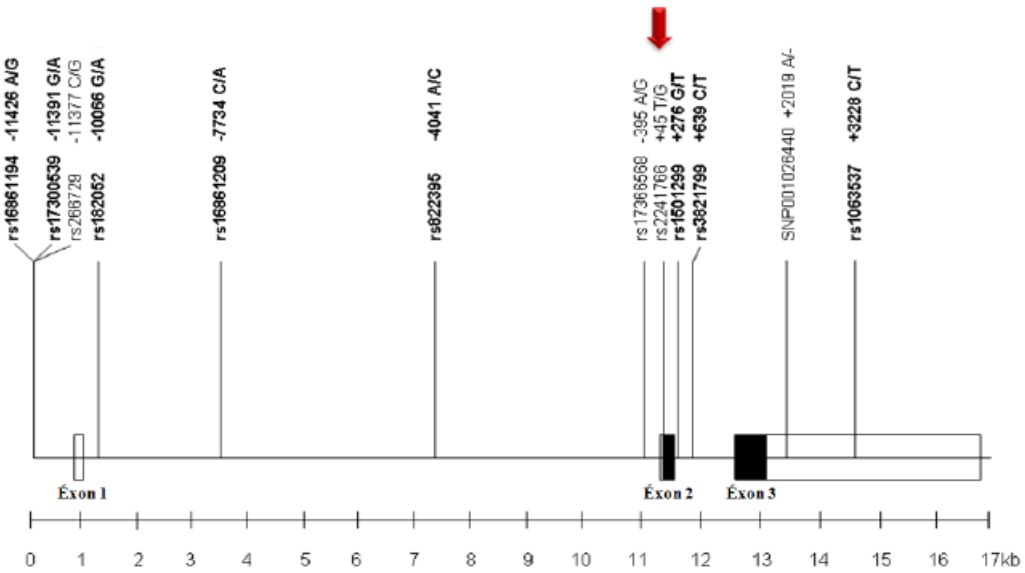


Figura 1. Mapa genômico do gene ADIPOQ. Localização de diferentes polimorfismos, com destaque para o +45T>G. Os boxes representam éxons, sendo que a parte preta corresponde à região traduzida do gene.
Fonte: KYRIAKOU et al, 2008

Como o sobrepeso é cada vez mais frequente na população, inclusive no meio rural, e o fenótipo da obesidade é fortemente influenciado por fatores genéticos, é interessante investigar a associação de variantes genotípicas do polimorfismo do gene ADIPOQ +45T> G e marcadores de risco cardiovascular em uma população de pequenos agricultores do interior do Rio Grande do Sul.

Além disso, esse polimorfismo apresenta resultados inconsistentes de acordo com a população estudada e, até onde foi possível investigar, não existem relatos de estudos específicos desse polimorfismo em populações rurais.

PARTE II

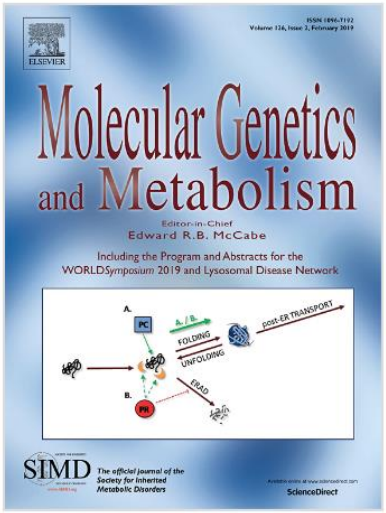
MANUSCRITO

POLIMORFISMO ADIPOQ +45T>G DO GENE DA ADIPONECTINA NÃO TEM INFLUÊNCIA SOBRE FATORES DE RISCO CARDIOMETABÓLICOS EM PEQUENOS PRODUTORES RURAIS DO SUL DO BRASIL

Renata Montagner, Marcia Denise Pavanelo Cattelan, Débora Alejandra Vasques Rubio, Patrícia Maurer, Jacqueline da Costa Escobar Piccoli

Em fase de preparação para submissão para a revista Molecular Genetics and Metabolism

ISSN: 1096-7192. Fator de impacto: 3.774



ELSEVIER

Title page

ADIPOQ + 45T> G POLYMORPHISM OF THE ADIPONECTIN GENE HAS NO INFLUENCE ON CARDIOMETABOLIC RISK FACTORS IN SMALL FARMERS IN SOUTHERN BRAZIL

Renata Montagner[1,2], Marcia Denise Pavanelo Cattelan[2], Débora Alejandra Vasques Rubio[2], Patrícia Maurer[2,3], Vanusa Manfredini[3], Jacqueline da Costa Escobar Piccoli[1,2,3]*

Affiliation

[1] Postgraduate Program in Pharmaceutical Sciences, Federal University of Pampa, Uruguaiana, Rio Grande do Sul, Brazil.
[2] LabGen – Genomic Laboratory, Federal University of Pampa, Uruguaiana, Rio Grande do Sul, Brazil.
[3] Postgraduate Program in Biochemistry, Federal University of Pampa, Uruguaiana, Rio Grande do Sul, Brazil.

* Correspondence should be addressed to Jacqueline C. E. Piccoli, Federal University of Pampa – Campus Uruguaiana, BR 472, Km 585, Uruguaiana, RS, Brazil, CEP: 97500-970. Tel.: +55 55 3911.02.00. E-mail address: jcepiccoli@yahoo.com.br

CONFLICT OF INTERESTS

No conflicts of interest were reported.

ACKNOWLEDGMENT

The research was supported by the National Institute for Health Research (NIHR) Collaboration for Leader-ship in Applied Health Research and Care.

Abstract

Changing lifestyles made obesity more and more prevalent in the general population, including in rural areas. The expansion of visceral fat alters the secretion of adipokines and increases the risk of metabolic and cardiovascular comorbidities. Polymorphisms affecting adiponectin levels may also contribute to the development of obesity and metabolic diseases. This study evaluated the association of ADIPOQ +45T>G polymorphism (rs 2241766) and cardiovascular risk markers in small farmers in southern Brazil. Participants answered questionnaire about health conditions and work practices and had blood collected for hematological, biochemical and IL-6 dosing. Anthropometric measurements were also performed. The ADIPOQ +45T>G polymorphism was determined by the PCR-RFLP technique. Of the 82 farmers who participated in the study, 20.74% were obese, 37.80% were overweight and 41.46% were eutrophic. The allelic and genotypic frequencies found were in Hardy-Weinberg equilibrium. There was no significant relationship between polymorphism and overweight, nor with chronic diseases and laboratory measures, except albumin and total proteins ($p < 0.05$). IL-6 levels remained similar according to genotype, anthropometric measurements and use of pesticides. Thus, it is possible to affirm that there was no association between ADIPOQ +45T>G polymorphism, obesity and cardiovascular risk markers in this population of farmers. Interleukin-6 levels were also not altered in situations of potential inflammation.

Keywords: farmers, obesity, Adiponectin, Interleukin-6, Gene polymorphism

1 Introdution

In recent decades, changes in the population's lifestyle have led to a nutritional transition in Brazil, where malnutrition has gradually been replaced by overweight and obesity [1,2]. This transition is also observed in rural areas, influenced by the incorporation of urban life habits, especially in food, and technological advances in the rural work process that reduce physical effort [3,4]. In Brazil, the rate of adults with excess of weight in all 27 capitals is over 50% [5], being considered a public health problem, with economic and quality of life impacts [1]. Abdominal adiposity is involved

in the development of chronic diseases such as type 2 Diabetes *Mellitus* (T2DM), dyslipidemias and hypertension, potentializing the risk of cardiovascular events [6,7].

Adipose tissue is currently recognized as one of the largest endocrine organs in the body, responsible for the production and release of bioactive molecules, called adipokines [8,9]. These molecules are involved in the regulation of appetite and satiety, cardiovascular homeostasis, glucose and lipid metabolism, as well as acting in inflammatory and immune response functions [10,11]. The expansion of visceral fat causes a dysfunction of adipose tissue [12], altering the secretion of adipokines to a pro-inflammatory, diabetogenic and atherogenic pattern [13,14].

Interleukin-6 (IL-6) has multifunctional activity, local and systemic, participating in the immune response, inflammation and hematopoiesis [15]. In parallel, it has metabolic effects, inhibiting the action of insulin in muscles, liver and adipocytes [16]. As it is widely secreted by visceral adipose tissue, there is a direct correlation between increased body mass index (BMI) and IL-6 levels, suggesting its participation in the pathogenesis of obesity [17,18]. Elevated IL-6 levels can lead to insulin resistance [16] and aggravate cardiovascular risk [19].

Adiponectin is a protein hormone produced exclusively by adipose tissue [20]. It acts on different tissues by regulating lipid and glucose metabolism, increasing insulin sensitivity [21]. In addition to its metabolic effects, it has anti-inflammatory [22], antiatherogens and antithrombotics properties [23]. Unlike other adipokines, adiponectin production decreases as adipose tissue expands [24]. Changes that lead to hypoadiponectinemia, such as single nucleotide polymorphisms (SNP) in the ADIPOQ gene, have already been related to increased BMI, metabolic and cardiovascular diseases in different ethnic populations [25,26,27]. One of the most studied polymorphisms is SNP +45T>G in exon 2 (rs 2241766), which is frequently associated with obesity susceptibility, insulin resistance and metabolic syndrome (MS) [28,29, 30].

The aim of the present study was to investigate the association between genotypic variants of the ADIPOQ +45T>G gene polymorphism and cardiovascular risk markers in a population of small farmers in the interior of Rio Grande do Sul.

2 Material and methods

2.1 Subjects

The study was carried out with a voluntary population of small farmers in the rural area of Santiago-RS. The ethical aspects of research involving human beings have been respected, according to the Helsinki Declaration. The study was approved by the Ethics Committee in Research of UNIPAMPA with the protocol number 1,216,322 and participants signed an informed consent form and had their rights preserved. Then, all farmers answered a questionnaire where they reported general information, work practices and health conditions, and collected blood by venipuncture, after 12 h of fasting, for analysis.

2.2 Physiological and anthropometric measures

Blood pressure was measured according to the recommendations of the cardiology societies, Brazilian Society of Cardiology, Brazilian Society of Hypertension, and Brazilian Society of Nephrology [31]. The anthropometric data collected were: weight (kg), height (cm), abdominal waist circumference (cm) and neck circumference (cm).

2.3 Determination of obesity and metabolic syndrome

The anthropometric measures were used as criteria for the classification of the participants regarding obesity, according to criteria of the Brazilian Guidelines on Obesity. BMI of 25 to 29.9 kg/m² are considered overweight, while in obesity the BMI is greater than or equal to 30 kg/m² [32]. The metabolic syndrome was diagnosed according to the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATPIII) [33], in the presence of three or more of the following criteria: waist circumference > 88 cm for women and > 102 cm for men, serum HDL cholesterol < 50 mg/dL for women and < 40 mg/dL for men, serum triglycerides ≥ 150 mg/dL, blood pressure ≥ 130/85 mm Hg, and fasting plasma glucose ≥ 110 mg/dL.

2.4 Hematological evaluation

The whole blood, with K_3EDTA anticoagulant, was used to perform hemogram and platelet count. Both were made in automated Sysmex KX 21 equipment using standard commercial kits.

2.5 Biochemical evaluations

Using serum, were measure glucose, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, uric acid, urea, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase,gamma-glutamyl transferase (GGT), total proteins and albumin. The analysis were performed in equipment semi-automated ChemWell T (Labtest®, Lagoa Santa, MG, Brazil), using commercial kits Labtest (Labtest®, Lagoa Santa, MG, Brazil) and/or Bioclin (Bioclin®, Belo Horizonte, MG, Brazil).

2.6 Genetic-molecular analysis

Genomic DNA was isolated from whole blood using the QIAamp DNA Mini Kit® (Qiagen, Hilden, Germany). ADIPOQ +45T>G gene polymorphism was determined by PCR-RFLP technique (polymerase chain reaction-restriction fragment length polymorphism) using the primers F:5' GAA GTA GAC TCT GCT GAG ATG G 3' and R:5' TAT CAG TGT AGG AGG TCT GTG ATG 3', according to conditions described by Curti et al [34]. The restriction enzyme used was SmaI (Invitrogen, California, USA) and the alleles obtained were separated on 2.5% agarose gels. The TT genotype was expected to show single band with 372 pb, the GG to show two bands at the positions of 219 and 153 pb, and the heterozygote TG should to have three bands (372 bp, 219 bp and 153 bp).

2.7 Analysis of interleukin-6

IL-6 levels were measured by enzyme-linked immunosorbent assay (ELISA) using standard kits (Hu IL-6 ELISA Kit, Invitrogen, California, USA) under conditions described by the manufacturer.

2.8 Statistical analysis

Allele and genotype frequencies were analyzed using the Hardy-Weinberg principle. Data were analyzed using statistical program SPSS version 20.0. The descriptive variables were expressed by frequency (%) or means and standard deviation (±). Quantitative variables were analyzed using the Independent T-Test or analysis of variance ANOVA oneway, followed by the Bonferroni post hoc test. Categorical variables were analyzed using the chi-square test. Values of $p<0.05$ were considered significant.

3 Results

A total of 82 small farmers participed in the study, being 43 men and 39 women. The mean age was 52.2 ± 14.2 years. Of the 82 participants, 34 (41.46%) presented BMI consistent with normal weight, 31 (37.80%) were overweight and 17 (20.74%) were considered obese. Other population characteristics and laboratory dosages can be seen in Tables 1 and 2.

Insert here Tables 1 and 2.

ADIPOQ +45T>G (rs2241766) analysis showed that the most frequent genotype was the homozygote TT and that the polymorphic G allele (TG+GG) was present in a small part of the participants, as described in the Table 3. The distribution of genotypes in the studied population is in Hardy-Weinberg equilibrium (p = 0.147 and X^2=2.1). In addition, among individuals with G allele, 63.2% were female.

Insert here Table 3.

No was found relationship between ADIPOQ +45T>G genotypes and chronic diseases, depression and smoking reported in a questionnaire. Table 4 shows that there was also no significant difference between excess weight and other changes in the parameters related to MS in individuals bearing the G allele (TG + GG genotypes).

Insert here Table 4.

In Figure 1 it is possible to see the laboratory measurements, that showed that the mean serum levels of different biochemical markers were similar among genotype groups, except for total proteins ($p < 0.05$) and albumin ($p < 0.005$). It is also possible to observe that there was no significant change in IL-6 dosage and anthropometric measures.

Insert here Figure 1.

Regarding the analysis of IL-6 (Table 5), anthropometric measures, MS, and work practices were not related to significant changes in serum levels.

Insert here Table 5.

4 Discussion

In recent years, several researchers have shown the participation of genetic factors in the development of cardiometabolic pathologies, especially in overweight individuals. In this study, we investigated the SNP ADIPOQ +45T>G and its clinical relevance in a farmers' population. The allelic and genotypic frequency found (Table 3), where the T allele was more frequent (0.87) as well as the TT genotype (76.83%), is in agreement with the database of the National Biotechnology Information Center). [35]. In a study with elderly Brazilians from Retamoso et al (2018) [36], 76.6% of the

participants had TT genotype, 20.7% TG and 2.7% GG. Another study, by Ghazouani et al (2018) [37], showed that among healthy individuals in North Africa, the frequency of the G allele was 0.19 and the T 0.81 allele. Both ADIPOQ +45T>G SNP studies were performed using the PCR-RFLP genotyping technique and corroborate the results found here, confirming the low frequency of the G allele in the general population.

Although this polymorphism is often associated with obesity in studies with different populations, the results are still inconsistent and contradictory [38]. In this study, the frequency of the G allele (TG + GG haplotype) was not related to excess weight (Table 4) in the farmers' population. A similar result has been reported by Oliveira et al (2015) [39], who also did not find a direct relation of the SNP ADIPOQ +45T>G with BMI and waist circumference, nor with risk of obesity and overweight in a study with 249 individuals attended at the university hospital of University of São Paulo.

The presence of the G allele is extensively related to changes in metabolic markers, presence of diabetes and metabolic syndrome [40,41]. A meta-analysis conducted by Zhou in 2016 [30] showed that SNP ADIPOQ +45T>G is associated with increased risk of MS in allelic, dominant, and homozygous genetic models. However, in this study there was no significant association between polymorphism and conditions that increase the risk of cardiometabolic diseases (Table 4). Investigating 400 patients with T2DM, 150 with MS and 300 healthy controls from Kashmir, Farooq et al (2018) [42] did not observe a significant difference in the frequency of genotypes of ADIPOQ +45T>G genotypes between groups. Other studies have also not shown this association in different ethnic groups, such as Tunisian [43], French [44], Swedish [45] and Brazil [36].

Arterial hypertension is another parameter that was not significantly altered in individuals with 1 or more G alleles of the studied polymorphism (Table 4). These findings are in agreement with reports that other SNPs in the ADIPOQ gene, mainly -11377C> G (rs 266729) and + 276GT (rs 1501299), are involved in hypertension and coronary artery disease [37,46,47]. In a study with 401 pregnant women from the University Hospital of the Faculty of Medicine of Ribeirao Preto, Brazil, Machado et al (2014) [48] found no difference in the distributions of genotypes or alleles for the +45T>G polymorphism regarding gestational hypertension, however, there was association of -11377C>G with occurrence of pre-eclampia. Likewise,

Mohammadzadeh et al (2016) [49] reported in 2016 that the TT genotype of +276G>T increased the risk of coronary artery disease when compared to the GG genotype in a group of 200 Iranians with DM2. However, they did not observe any significant difference in relation to the SNP +45T>G.

Regarding the biochemical dosages, the comparison of the values between the 3 genotypes only showed a significant alteration in the serum concentration of albumin and total proteins (Figure 1), although albumin levels are still within the reference values. Albumin is the most abundant protein in the blood and its levels are decreased in malnutrition, malabsorption and various pathological conditions. Increased values, such as those seen in this study, in the absence of other changes are generally not clinically relevant because they are related to dehydration states [50,51,52]. Although SNP +45T>G is frequently associated with a risk of non-alcoholic fatty liver disease [53,54], no change was observed in markers of hepatic damage according to genotype in this farmer population. Similarly, serum triglycerides, total cholesterol, HDL and LDL fractions showed similar values between the groups. These results are in accordance with those found in a study in Bahrain in 2016, where, in the comparison between individuals with T2DM and non-diabetic controls, there was no association between the SNP +45T>G and metabolic parameters of the lipid profile [55]. However, other studies have documented the association between this polymorphism and plasma lipids in children [56] and adults [57].

In addition to investigating the genetic influence on obesity and its cardiometabolic complications, this study evaluated the inflammatory cytokine IL-6 (Table 5). The chronic inflammatory state present in obesity is reflected in elevated levels of this cytokine in excess weight individuals [18] and favors the development of cardiometabolic diseases [14,58]. Here, no difference was found in the serum IL-6 dosage according to genotype, although the mean of all groups is above values already reported as significantly increased in obesity studies [39,15]. This result differs from the cross-sectional population-based study with the participation of 262 adults, conducted by Maintinguer Norde et al (2016) [59], where they showed that the plasma fatty acid profile may interact with ADIPOQ SNP variants, including the +45T>G, to modulate the risk of systemic inflammation, as determined by the concentration of different inflammatory markers, including IL-6. They also found a relationship between waist circumference and inflammatory status.

Elevated levels of IL-6 may also be associated with other sources of inflammation. As this study involves farmers, the relationship of IL-6 with the occupational use of pesticides was investigated. Experimental and epidemiological studies show that exposure to pesticides can lead to chronic diseases [60,61], which can occur due to disturbances in cytokine balance [62,63,64]. Contrary to literature reports, in this study, no significant difference was found between IL-6 levels and use of pesticides. The use of personal protective equipment during farm work, which can significantly reduce the incidence of health problems related to exposure to agrochemicals [61], also did not influence the measurements of this cytokine.

This study may not have demonstrated significant associations between the SNP ADIPOQ + 45T>G and the parameters analyzed, due to the inability to control factors that may cause physiological and biochemical changes, such as heterogeneity of the participant group, environmental factors, drug use and level of physical activity. Absence of serum adiponectin dosage was another limiting factor. In addition, testing only one SNP also prevents the combined analysis of genetic risk factors for the development of chronic diseases.

5 Conclusion

In this study there was no association between SNP ADIPOQ +45T>G, obesity and cardiovascular risk markers in the farmers' population. Interleukin-6 levels were also not altered in situations of potential inflammation. Research with additional participants and investigation of additional parameters are necessary for a better understanding of the effects of SNP ADIPOQ +45T>G on human health.

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Figures

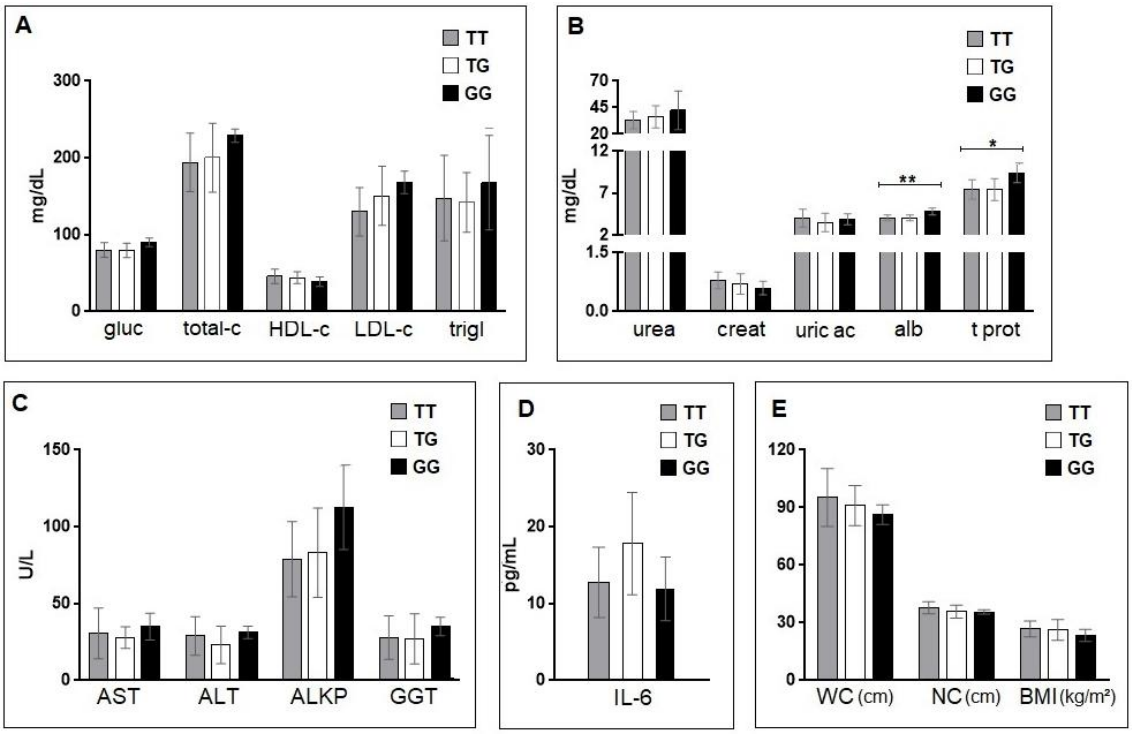

Figure 1. Laboratory and anthropometric measurements according to genotype ADIPOQ +45T> G. A) glycemic status and lipid profile. B) renal profile. C) hepatic profile. D) Interleukin-6. E) anthropometric measures. Data expressed as mean and standard deviation. * p <0.05 between TT-GG and TG-GG, ** p <0.005 between TT-GG and TG-GG (ANOVA oneway).

Gluc = glucose, total-c = total cholesterol, HDL-c = high-density lipoprotein cholesterol, LDL-c = low-density lipoproteins cholesterol, trigl = triglycerides, crat = creatinine, uric ac = uric acid, alb = albumin, t preo = total protein, AST = aspartate aminotransferase, ALT= alanine aminotransferase, ALKP = a*lkaline* phosphatase, GGT = gamma-glutamyl transferase, IL-6 = interleukin-6, WC = waist circumference, NC = neck circumference, BMI= body mass index.

Tables

Table 1. Frequency of metabolic syndrome [33], diseases reported in the questionnaire and use of pesticides.

Variable		Frequency (n=82)	Percent (%)
Metabolic Syndrome	Yes	24	29.3
	No	58	70.7
Hypertension	Yes	19	23.2
	No	63	76,8
Diabetes	Yes	6	7.3
	No	76	92.7
Dyslipidemia	Yes	17	20.7
	No	65	79.3
Depression	Yes	11	13.4
	No	71	86.6
Use of pesticides	Yes	44	53.7
	No	38	46.3

Table 2. Mean values of anthropometric measures, blood pressure, biochemical and hematological evaluation of the small farmers.

Variable	**Min-Max**	**Mean**	**SD (±)**
BMI (kg/m²)	17.6 – 42,2	26.59	4.33
Waist circumference (cm)	71 – 188	94.09	14.18
Neck circumference (cm)	31 – 45	37.34	3.21
SBP (mmHg)	88 – 188	115.45	51.86
DBP (mmHg)	50 – 113	81.07	20.11
Glucose (mg/dL)	56 – 332	83.43	29.33
Total cholesterol (mg/dL)	89 – 328	196.89	42.83
HDL cholesterol (mg/dL)	25 – 67	45.29	8.95
LDL cholesterol (mg/dL)	36 – 237	126.31	39.87
Triglycerides (mg/dL)	47 – 764	135.11	91.25
Urea (mg/dL)	17 – 57	34.05	9.16
Creatinine (mg/dL)	0.4 – 1.4	0.77	0.22
Uric acid (mg/dL)	2 – 8	3.96	1.06
Albumin (mg/dL)	3.2 – 5.3	4.13	0.37
Total protein (mg/dL)	4.4 – 10.8	7.55	1.23
AST (U/L)	12 – 125	30.22	16.46
ALT (U/L)	7 – 158	29.67	21.21
GGT (U/ L)	7 – 152	30.73	22.28
Alkaline phosphatase (U/L)	18 – 147	81.02	25.98
Red blood cells (10^6/mm³)	3.8 – 6.2	4.88	0.47
Hemoglobin (g/dL)	11.5 – 18.6	14.77	1.36
Leukocytes (10^3/mm³)	3.2 – 10.1	5.91	1.32
Platelets (10^3/µL)	129 – 421	216.00	58.42
Interleukin-6 (pg/mL)	0.26 – 80.79	14.31	9.31

Min-Max = minimum and maximum values measured, SD = standard deviation, BMI = body mass index, SBP = systolic blood pressure, DBP = diastolic blod pressure, HDL = high-density lipoprotein , LDL = low-density lipoprotein, AST = aspartate aminotransferase, ALT = alanine aminotransferase, GGT = gamma-glutamyl transferase.

Table 3. Allelic and genotypic frequencies of ADIPOQ +45T>G in the studied population.

		Frequency
Allele	T	142 (0.87)
	G	22 (0.13)
Genotype	GG	3 (3.66%)
	TG	16 (19.51%)
	TT	63 (76.83%)

Table 4. Frequency of change in parameters of cardiometabolic evaluation according to the genotypes for the ADIPOQ +45T>G.

Parameter		**TT (n=63)**	**TG+GG (n=19)**	***p***
Obesity	Excess weigth [(a)]	39 (61.9%)	9 (47.4%)	0,260
	Normal weigth [(a)]	24 (38.1%)	10 (52.6%)	
Waist circumference [(b)] >102cm (M) >88 cm (F)	Yes	25 (39.7%)	7 (36.8%)	0,824
	No	38 (60.3%)	12 (63.2%)	
Glycemia [(b)] ≥ 110 mg/dL	Yes	0 (0.0%)	1 (5.3%)	0,232
	No	63 (100.0%)	18 (94.7%)	
Triglycerides [(b)] ≥ 150 mg/dL	Yes	18 (28.6%)	7 (36.8%)	0,492
	No	45 (71.4%)	12 (63.2%)	
HDL-cholesterol [(b)] < 40 mg/dL (M) <50mg/dL (F)	Yes	30 (47.6%)	11 (57.9%)	0,432
	No	33 (52.4%)	8 (42.1%)	
Arterial hypertension [(b)] ≥ 130 mmHm or ≥ 85 mmHm	Yes	35 (55.6%)	12 (63.2%)	0,557
	No	28 (44.4%)	7 (36.8%)	
Metabolic syndrome [(b)]	Yes	18 (28.6%)	6 (31.6%)	0,801
	No	45 (71.4%)	13 (68.4%)	

[(a)] Weight / obesity classification according to WHO [32]

[(b)] Diagnostic criteria of metabolic syndrome according to NCEP-ATPIII [33]

Normal weigth = BMI (body mass index) ≤ 24.9 kg/m², excess weigth = BMI >25 kg/m², HDL = high-density lipoprotein, M = male, F = female.

Table 5. Dosage of interleukin-6 according to obesity, abdominal circumference, metabolic syndrome, use of pesticides and personal protective equipment by farmers.

Parameter		**IL-6 (pg/mL)**	***p***
Obesity	Obese [a]	15.28 ± 7.03	0.056
	Overweigth [a]	11.43 ± 5.89	
	Normal weigth [a]	16.82 ± 13.21	
Waist circumference [b] >102cm (M) >88 cm (F)	Yes	13.8 ± 6.67	0.679
	No	14.69 ± 11.47	
Metabolic syndrome [b]	Yes	16.83 ± 14.48	0,117
	No	13.32 ± 7.08	
Use of pesticides	Yes	13.91 ± 5.22	0,673
	No	14.78 ± 12.87	
Use of PPE	Yes	14.46 ± 2.32	0.635
	No	15.21 ± 6.23	

[a] Weight / obesity classification according to WHO [32]

[b] Diagnostic criteria of metabolic syndrome according to NCEP-ATPIII [33]

Normal weigth = BMI (body mass index) ≤ 24.9 kg/m², overweigth = BMI 25-29.9 kg/m², obese = BMI > 30 kg/m², HDL = high-density lipoprotein, M = male, F = female, PPE = personal protective equipment.

PARTE III

4 DISCUSSÃO GERAL

A expansão da gordura visceral causa uma disfuncionalidade no tecido adiposo e altera a secreção de citocinas para um padrão pró-inflamatório (PELLEGRINELLI et al, 2016). Como o Brasil está enfrentando uma transição nutricional que pode afetar a qualidade de vida e prejudicar as atividades de trabalho (POHL et al, 2018) e várias pesquisas mostram envolvimento de polimorfismos genéticos na obesidade, este estudo investigou o SNP ADIPOQ +45T>G e sua relevância clínica em uma população de agricultores.

As condições gerais de saúde, avaliadas pelo questionário e pelas dosagens laboratoriais, mostraram bom estado de saúde para a população considerada como um todo. Os valores hematológicos médios estavam dentro dos valores de referência e eram semelhantes aos encontrados em estudo com outra população rural também no interior do RS (ALVES et al, 2016). Porém a análise dos mínimos e máximos evidenciou que alguns parâmetros tinham dosagens abaixo, mas também acima dos valores de referência. Tais achados elevados podem ser resultado de casos pontuais de desidratação, visto que outras dosagens bioquímicas, como albumina e proteínas totais, também sugeriram essa condição.

Os parâmetros bioquímicos mostraram valores médios para glicemia e perfil lipídico dentro dos valores referenciais desejáveis ou levemente elevados, segundo o preconizado pela Sociedade Brasileira de Cardiologia. Entretanto, também foi observada grande variabilidade individual, a qual pode ter influenciado nos resultados das associações pesquisadas neste trabalho. A mesma amplitude nos resultados também foi verificada nas enzimas hepáticas e, principalmente, na dosagem de IL-6.

As altas taxas de sobrepeso e obesidade visualizadas mostram que este é um fenômeno que realmente está afetando toda a população, e que pode trazer consigo uma gama de dificuldades no dia a dia e complicações cardiometabólicas (BASTIEN et al, 2014; TAVARES et al, 2018; POHL et l, 2018). É de suma importância a identificação dessas situações, visto que nas áreas rurais brasileiras geralmente há

menor escolaridade e renda, associada à dificuldade de acesso a serviços sociais e de saúde e políticas governamentais (DIAS, 2006; SANTANA et al, 2016).

Apesar do SNP ADIPOQ +45T>G ser amplamente pesquisado, são escassos os estudos com trabalhadores rurais. As frequências alélica e genotípica desse SNP na população estudada foram semelhantes às encontradas em pesquisas que utilizaram a mesma técnica de genotipagem (GHAZOUANI et al, 2018; RETAMOSO et al, 2018). Este polimorfismo causa uma mutação silenciosa Gly→Gly que, embora não cause alteração de aminoácido, pode alterar a estabilidade do RNA e a expressão da adiponectina, reduzindo seus valores séricos (YANG e CHUANG, 2006) e, consequentemente, predispondo a doenças metabólicas (ZHOU et al, 2016). Relatos da literatura mostram de que ainda há muita discordância e inconsistência em pesquisas desse polimorfismo com diferentes populações étnicas (RIESTRA et al, 2015; TU et al, 2014, WU et al, 2014).

As análises de frequência de parâmetros antropométricos e bioquímicos alterados juntamente com as dosagens laboratoriais mostraram que nessa população de pequenos produtores rurais o polimorfismo estudado não tem relação com risco cardiometabólico. De fato, enquanto diferentes autores citam relação entre o SNP ADIPOQ +45T>G e obesidade, resistência insulínica e síndrome metabólica, tantos outros relatam a falta de influência desse polimorfismo nas mesmas situações (SAHLI et al, 2017; GU et al, 2004, AL HANNAN et al, 2016).

Outro marcador estudado foi a IL-6, devido ao estado pró-inflamatório da obesidade. Pesquisas já mostraram que a inflamação crônica de baixo grau está envolvida na patogênese de doenças metabólicas ligadas ao excesso de peso, como SM, DM2, DCV, hipertensão e doença hepática gordurosa não alcoólica (LUMENG e SALTIEL,2014; OLIVEIRA et al, 2015). No presente estudo não foi encontrada relação entre essa citocina inflamatória e o SNP ADIPOQ +45T>G, diferentemente de relatos de que esse polimorfismo pode modular o estado inflamatório sistêmico, se refletindo em maiores dosagens de moléculas pró-inflamatórias (MAINTINGUER NORDE et al, 2016). Tampouco havia relação com excesso de peso e práticas laborais que poderiam ser outras fontes de inflamação.

Este estudo apresentou algumas limitações as quais podem ter influenciado nos resultados obtidos, principalmente em relação à falta de material biológico para análises adicionais. A dosagem de adiponectina sérica, por exemplo, poderia contribuir fortemente para uma melhor análise dos resultados. Testar apenas um

SNP também impede a análise combinada de fatores genéticos de risco para desenvolvimento de doenças crônicas. Outras limitações que podem ter influenciado nas dosagens são a ampla faixa etária dos participantes e a impossibilidade de controlar fatores ambientais, medicação, alimentação e níveis de atividade física, os quais podem originar alterações fisiológicas e bioquímicas.

5 CONCLUSÃO

Neste estudo não foi verificada associação entre as variantes genotípicas do polimorfismo +45T>G do gene da adiponectina e excesso de peso na população de agricultores. Tampouco foi observada alteração nas dosagens de marcadores bioquímicos, parâmetros de risco cardiovascular e interleucina-6. Pesquisas com mais participantes e investigação de parâmetros adicionais são necessárias para uma melhor compreensão dos efeitos desse SNP na saúde humana.

6 PERSPECTIVAS

Os resultados obtidos nesta pesquisa trazem a necessidade de estudos adicionais. A pretensão futura é contatar novamente os participantes para coleta de material bioquímico que permita a dosagem de outros marcadores relacionados à obesidade, como adiponectina e leptina e marcadores de resposta inflamatória, além da genotipagem de diferentes polimorfismos em genes candidatos à participação na obesidade.

Dessa forma, será possível examinar de forma mais ampla a influência genética no excesso de peso e suas complicações, inclusive com a análise de combinações de haplótipos de risco. Análises adicionais também permitirão investigar de forma mais pontual o fato de que o uso de agrotóxicos pode atuar como um disruptor de glândulas que atuam na regulação do metabolismo energético.

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ANEXO I - Author Guidelines

MOLECULAR GENETICS AND METABOLISM
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R. Dorfman, A. Sandford et al., Complex two-gene modulation of lung disease severity in children with cystic fibrosis, J. Clin. Invest. 118 (2008) 1040-1049, http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2248329.

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