Universidade Federal do Rio Grande do Sul
Faculdade de Medicina
Programa de Pós-Graduação em Ciências Médicas: Endocrinologia

GENE LIGADO A OBESIDADE E MASSA GORDA (FAT MASS AND OBESITY ASSOCIATED; FTO), MENOPAUSA E FATORES DE RISCO CARDIOVASCULAR EM MULHERES NA PÓS-MENOPAUSA

Ramon Bossardi Ramos

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Dissertação apresentada ao Programa de Pós-Graduação em Ciências Médicas: Endocrinologia, como requisito parcial para obtenção do título de Mestre

Orientadora: Profª. Drª. Poli Mara Spritzer

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Esta Tese de Mestrado segue o formato proposto pelo Programa de Pós-Graduação em Ciências Médicas: Endocrinologia, Faculdade de Medicina, Universidade Federal do Rio Grande do Sul, sendo apresentada na forma de 1 revisão geral e 1 manuscrito sobre o tema da Tese:

- Revisão: Polimorfismos no gene do FTO e associação com obesidade/IMC e variáveis metabólicas e de risco cardiovascular: Revisão da literatura.

- Artigo original 1: Variations in the fat mass and obesity-associated (FTO) gene are related to hypertension and higher lipid accumulation product in postmenopausal women from South Brazil.

SUMÁRIO

Resumo

Obesidade é uma doença crônica multifatorial que no Brasil atinge cerca de 12,4% dos homens e 16,9% das mulheres com mais de 20 anos.

No período pós menopáusico, as mulheres apresentam uma série de alterações fisiológicas, entre elas alteração na distribuição de gordura corporal, sobretudo um aumento na região abdominal.

A obesidade apresenta uma etiologia poligênica e diversos genes vêm sendo estudados, entre eles o gene ligado a obesidade e massa gorda (FTO) que já se mostrou fortemente associado com a obesidade/IMC. Mas em relação a parâmetros metabólicos e marcadores de risco cardiovascular, os estudos ainda são bastante controversos dependendo da população em estudo. Com esses dados nosso trabalho buscou a associação entre os polimorfismos rs9939609 e rs8050136 do gene FTO com variáveis metabólicas e de risco cardiovascular em mulheres na pós menopausa.

Nosso estudo mostrou que no SNP rs9939609, o genótipo homozigoto para o alelo A está associado com aumento da relação cintura quadril, índice de acumulação lipídica (LAP), que é um marcador de risco cardiovascular, que é um marcador de risco cardiovascular e hipertensão. Enquanto que no SNP rs8050136, o genótipo em homozigose para o alelo A foi associado com pressão arterial diastólica e LAP. Tanto o genótipo polimórfico do SNP rs9939609 e selvagem do rs8050136 foram associados com alteração nos níveis de glicose plasmática.

Deste modo, podemos concluir que o polimorfismo rs9939609 no gene do FTO pode ser um preditor de maior risco cardiovascular em mulheres na menopausa.

Polimorfismos no gene do FTO e associação com obesidade/IMC e variáveis metabólicas e de risco cardiovascular: Revisão da literatura.

Introdução

A obesidade é um sério problema que afeta os países desenvolvidos e também parte dos países em desenvolvimento (Zimmet, Alberti et al. 2001). A prevalência de obesidade nos EUA constitui cerca de 25%, enquanto que no Brasil a obesidade atinge 12,4% dos homens e 16,9% das mulheres com mais de 20 anos (IBGE, 2009). As últimas estimativas da organização mundial da saúde (OMS) indicam que em 2005 havia em todo o mundo cerca de 1,6 bilhões de adultos com sobrepeso e pelo menos 400 milhões eram obesos. Além disso, a OMS estima que até 2015, aproximadamente 2,3 bilhões de adultos terão excesso de peso e mais de 700 milhões serão obesos. A obesidade está associada a um maior risco de desenvolver diabetes melitus do tipo 2 (DM2), hipertensão, doenças cardiovasculares e certas formas de câncer (Katzmarzyk, Janssen et al. 2003; Janssen and Mark 2007).

A obesidade apresenta uma etiologia poligênica, a partir disso vários estudos vêm buscando encontrar genes candidatos para maior risco de desenvolver obesidade. Em 2007, um estudo de associação realizado através da técnica de *genome-wide scan* identificou múltiplos polimorfismos no primeiro intron do gene ligado a obesidade e massa gorda (FTO), e que foram associados com diabetes melitus do tipo 2 (DM2). Contudo após ajuste para o índice de massa corporal (IMC) esta associação desapareceu e mostrou que possivelmente estes polimorfismos estavam associados com IMC, aumento de peso e também como um fator de risco para o desenvolvimento de DM2 (Frayling, Timpson et al. 2007).

A expressão do gene FTO já foi observada em diversos tecidos, entre eles o sistema nervoso central (SNC), mais especificamente na região do núcleo arqueado do hipotálamo, que é responsável pelo controle energético e também nas glândulas hipófise e adrenais, sugerindo um potencial papel no eixo hipotálamo-hipófise-adrenal (Dina, Meyre et al. 2007; Frayling, Timpson et al. 2007; Gerken, Girard et al. 2007; Stratigopoulos, Padilla et al. 2008). Além disso, estudos mostraram a presença da proteína do FTO em menor quantidade no tecido adiposo, pâncreas, fígado, musculatura esquelética estriada, cardíaca e rins (Gerken, Girard et al. 2007; Stratigopoulos, Padilla et al. 2008). Essa expressão em diversos tecidos indica que o FTO pode ter uma função importante, possivelmente na regulação do peso corporal através da homeostase de

energia a qual controlaria o gasto energético, (Frayling, Timpson et al. 2007; Sanchez-Pulido and Andrade-Navarro 2007; Fischer, Koch et al. 2009) mas seu real papel e mecanismos de ação ainda permanecem desconhecidos.

Fischer *et al.* mostraram que ratos que não possuem o gene do FTO apresentam uma menor adiposidade devido ao maior gasto energético, quando comparados com ratos que expressavam o mesmo gene (Fischer, Koch et al. 2009). Em camundongos *knockout* para o gene do FTO houve uma maior taxa de morte pós-natal e retardo no crescimento, enquanto que camundongos com uma mutação neste gene apresentaram uma perda parcial nestas mesmas funções (Church, Lee et al. 2009). Gerken e colaboradores mostraram que o FTO pertence à família do Fe^{2+} 2-oxoglutarato, uma classe de proteínas envolvidas em vários processos celulares, incluindo reparo de DNA, metabolismo de ácidos graxos e modificações pós-translacionais (Sanchez-Pulido and Andrade-Navarro 2007). Uma das hipóteses sugere que portadores de polimorfismos no gene FTO possuiriam uma capacidade aumentada para a metilação do DNA (Gerken, Girard et al. 2007). Metilação do DNA é uma das modificações epigenéticas mais importantes, que consiste na adição de um grupo metil na posição 5' da citosina dentro de um dinucleotídeo Cpg, alterando assim a função dos genes (Robertson and Wolffe 2000).

A proteína FTO é composta por 2 domínios: um domínio N-terminal que carrega um núcleo catalítico e um domínio C-terminal com uma estrutura desconhecida (Han, Luo et al. 2010).

O gene do FTO está localizado na região cromossômica 16q12.2, que é composto por 9 éxons e 8 introns, onde já foram identificados mais de 2.348 SNPs (National Center for Biotechnology Information/ NCBI. United States. 2009). Destes SNPs, 92 têm importância científica conhecida, dos quais 26 estão relacionados com IMC (Rampersaud, Mitchell et al. 2008). Entre todos estes polimorfismos podemos destacar os SNPs rs9939609 (troca de uma timina (T) pela adenina (A)) e rs8050136 (troca de uma adenina (A) por uma citosina (C)), ambos localizados no intron 1 do gene. Estes SNPs estão separados por 4.251 pares de base (pb) e foram descritos com um forte desequilíbrio de ligação (DL) (Figura 1) (Hotta, Nakata et al. 2008; Rampersaud, Mitchell et al. 2008; Song, You et al. 2008). Os estudos mostram também uma diferença na freqüência alélica entre as diferentes populações. Assim, mulheres pós-menopáusicas da população espanhola apresentam uma freqüência em torno de

45% enquanto que nas da população asiática a freqüência é em torno de 17% (Song, You et al. 2008) .

Figura 1 Organização genômica do gene do FTO localizado no cromossomo 16 (16q12.2). Composto por 9 exons e 8 introns. SNP rs9939609 em desequilíbrio de ligação com o SNP rs8050136 estão localizados em um bloco de 47- Kb. Figura não corresponde à escala. Retângulos pretos indicam exons.

Desta maneira esta revisão tem como objetivo mostrar os principais estudos realizados que buscaram associação dos polimorfismos rs9939609 e rs8050136 do gene do FTO com variáveis clinicas, metabólicas e marcadores de risco cardiovascular.

Polimorfismo rs9939609 T/A

Frayling et al. foram os primeiros a estudar o polimorfismo rs9939609 em uma amostra com 38.759 pacientes de origem européia. Neste estudo foi observado que pacientes com o genótipo AA tinham aproximadamente 3kg a mais e um aumento de risco para desenvolver obesidade em 1,67 vezes, enquanto portadores do genótipo heterozigoto apresentaram um ganho intermediário de 1,5 kg (Frayling, Timpson et al. 2007).

Posteriormente, diversos outros estudos replicaram e confirmaram a associação do polimorfismo rs9939609 com obesidade/IMC tanto em adultos como em crianças de diversas etnias (Cecil, Tavendale et al. 2008; Chang, Liu et al. 2008; Marvelle, Lange et al. 2008; Doney, Dannfald et al. 2009; Gonzalez-Sanchez, Zabena et al. 2009; Tanofsky-Kraff, Han et al. 2009; Lee, Kim et al. 2010; Liu, Zhu et al. 2010; Ruiz, Labayen et al. 2010; Fang, Li et al. 2011; Mangge, Renner et al. 2011; Rutters, Nieuwenhuizen et al. 2011; Webster, Warrington et al. 2011; Xi, Shen et al. 2011). Bermejo *et a.*, estudando 234 bebês com duas semanas de idade, verificaram que o polimorfismo não estava associado com o peso ao nascer, mas mostrava associação com

o ganho de peso nas duas primeiras semanas de vida bem como com percentual de gordura total, de tronco e abdominal (Lopez-Bermejo, Petry et al. 2008) e em crianças do reino unido o polimorfismo foi associado com uma maior adiposidade (Wardle, Carnell et al. 2008).

Em adultos foi também associado com obesidade mórbida (Villalobos-Comparan, Teresa Flores-Dorantes et al. 2008; Rodriguez-Lopez, Gonzalez-Carpio et al. 2010).

Como a regulação do peso corporal é, naturalmente um equilíbrio entre a ingestão e o gasto energético, muitos estudos posteriores buscaram avaliar a influência de SNPs no gene do FTO sobre variáveis relacionadas com fome e saciedade, ingestão de alimentos e gasto energético. Pacientes homozigotos para o alelo A no polimorfismo rs9939609 parecem apresentar uma ingestão alimentar maior (Speakman, Rance et al. 2008; Wardle, Carnell et al. 2008), diminuição da resposta à saciedade (Wardle, Carnell et al. 2008; den Hoed, Westerterp-Plantenga et al. 2009; Tanofsky-Kraff, Han et al. 2009), preferência por alimentos mais calóricos, maior massa gorda, tecido adiposo subcutâneo e visfatina (Cecil, Tavendale et al. 2008; Lopez-Bermejo, Petry et al. 2008; Timpson, Emmett et al. 2008; Mangge, Renner et al. 2011) e também menores níveis de adiponectina (Qi, Kang et al. 2008; Fang, Li et al. 2011) do que sujeitos portadores do genótipo homozigoto para o alelo T.

Outros estudos mostraram que SNPs no gene do FTO não estão associados com gasto energético reduzido (Speakman, Rance et al. 2008; Haupt, Thamer et al. 2009), atividade física (Jonsson, Renstrom et al. 2009; Ruiz, Labayen et al. 2010), realização de dieta, maior perda de peso corporal (Reinehr, Hinney et al. 2009) e maior consumo de gordura saturada, trans e calorias (Timpson, Emmett et al. 2008). No entanto, existem algumas evidências que mostram uma associação com o aumento do gasto energético, bem como aumento dos níveis de atividade física (Jonsson and Franks 2009; Lee, Kim et al. 2010).

O polimorfismo rs9939609 como vista, mostra-se associado com obesidade/IMC em grande número de estudos, mas também parece relacionado com DM2 e outras comorbidades como hipertensão, alterações em variáveis metabólicas e fatores de risco cardiovascular (tabela 1). Pacientes com DM portadores do genótipo homozigoto para o alelo A apresentaram maiores níveis de IMC e circunferência da cintura (Legry, Cottel et al. 2009; Gu, Alvarsson et al. 2010) enquanto que dois outros estudos com diabéticos

não encontraram associação com obesidade em japoneses (Hotta, Nakata et al. 2008) e africanos (Hennig, Fulford et al. 2009).

Este polimorfismo foi associado com uma maior predisposição a DM2 em diversas etnias (Legry, Cottel et al. 2009; Yajnik, Janipalli et al. 2009; Li, Song et al. 2010; Rees, Islam et al. 2011), mas não na população chinesa (Chang, Liu et al. 2008) e nem em portadores de nefropatia diabética (Gu, Alvarsson et al. 2010).

Estudos avaliando variáveis metabólicas observaram associação entre o genótipo polimórfico e maiores níveis de glicemia (Grunnet, Brons et al. 2009) e de insulinemia em jejum (Grunnet, Brons et al. 2009; Karasawa, Daimon et al. 2010), colesterol total (Villalobos-Comparan, Teresa Flores-Dorantes et al. 2008; Hakanen, Raitakari et al. 2009), triglicerídeos (Doney, Dannfald et al. 2009; Fang, Li et al. 2011), menores níveis de HDL-c (Hunt, Stone et al. 2008; Doney, Dannfald et al. 2009). níveis de pressão arterial (Hunt, Stone et al. 2008; Hakanen, Raitakari et al. 2009; Karasawa, Daimon et al. 2010) e síndrome metabólica (Al-Attar, Pollex et al. 2008; Sjogren, Lyssenko et al. 2008).

No entanto, após ajuste pelo IMC alguns destes estudos e outros não observaram associação com insulina, HOMA – ß, insulina, glicose, triglicerídeos e HDL-c (Freathy, Timpson et al. 2008; Kring, Holst et al. 2008; Villalobos-Comparan, Teresa Flores-Dorantes et al. 2008; Xi, Shen et al. 2011).

Quanto à associação entre o genótipo polimórfico homozigoto AA e níveis de proteína C reativa, um estudo verificou aumento de 15% entre os homens portadores deste genótipo e 12% entre as mulheres (Fisher, Schulze et al. 2009). Após este estudo outros descreveram essa mesma associação (Sun, Sun et al. 2010; Zimmermann, Skogstrand et al. 2011), enquanto outro não observou esta associação (Mangge, Renner et al. 2011).

Um estudo longitudinal evidenciou nos indivíduos portadores do genótipo AA menores níveis de HDL-c em homens mesmo após o ajuste pelo IMC e pressão sistólica e diastólica mais elevada em mulheres. Além disso, no seguimento de 10 anos o genótipo AA mostrou-se associado com um aumento de eventos cardiovasculares apenas nos homens. Em um subgrupo com DM2, o polimorfismo mostrou-se associado a um aumento do risco de infarto do miocárdio (Lappalainen, Kolehmainen et al. 2010).

Este dado já havia sido sugerido em estudo anterior, em que o seguimento de 4897 indivíduos com DM2 mostrou que o alelo A está associado com o aumento do risco de infarto do miocárdio fatal ou não fatal mesmo após ajuste para sexo, fumo, uso de estatinas e insulina (Doney, Dannfald et al. 2009).

Na análise da expressão do mRNA, maiores níveis de mRNA foram encontrados no tecido adiposo subcutâneo dos obesos mórbidos quando comparado com os controles. Uma correlação negativa foi verificada entre a expressão do gene FTO no tecido subcutâneo e triglicerídeos, e uma correlação positiva com leptina e visfatina (Zabena, Gonzalez-Sanchez et al. 2009), na população mexicana. Além disso, a expressão do mRNA de FTO também em tecido adiposo subcutâneo foi maior em obesos grau III (Villalobos-Comparan, Teresa Flores-Dorantes et al. 2008).

Polimorfismo rs8050136 A/C

Em termos gerais, o polimorfismo rs8050136 também vem sendo associado com obesidade/IMC (tabela 2). Neste polimorfismo os estudos indicam que o genótipo de risco é o selvagem AA tanto em adultos como em crianças de diversas etnias (Haupt, Thamer et al. 2008; Ng, Park et al. 2008; Pecioska, Zillikens et al. 2010; Mitchell, Church et al. 2010; Ramya, Radha et al. 2011). Alguns estudos parecem também evidenciar uma associação do genótipo selvagem com maior predisposição à DM2 e a um pior perfil metabólico. Horikoshi et al. mostraram que o genótipo selvagem está associado com DM2 em populações asiáticas (Horikoshi, Hara et al. 2007; Han, Luo et al. 2010), mas este resultado não foi confirmado em outro estudo com a mesma etnia, após ajuste para IMC, sexo e idade (Ng, Park et al. 2008) ou em população indiana (Ramya, Radha et al. 2011).

O genótipo selvagem AA vem sendo associado a níveis mais elevados de insulina basal (Han, Luo et al. 2010; Haupt, Thamer et al. 2008) e no tempo 120 (Haupt, Thamer et al. 2008), de glicemia basal e no tempo 120 (Wen, Ronn et al. 2010), peptídeo C (Wen, Ronn et al. 2010) e HOMA-IR (Han, Luo et al. 2010). No entanto, outros estudos não observaram associação do genótipo de risco AA com variáveis metabólicas (glicemia, insulina e HOMA-IR) (Horikoshi, Hara et al. 2007; Li, Wu et al. 2008; Ng, Park et al. 2008; Mitchell, Church et al. 2010), maior prevalência de hipertensão (Ahmad, Chasman et al. 2010; Ahmad, Lee et al. 2011), síndrome

metabólica, menores níveis de HDL-c (Ahmad, Chasman et al. 2010) e redução no peso após atividade física (Haupt, Thamer et al. 2008; Mitchell, Church et al. 2010) quando comparado com o genótipo CC. Estes resultados discrepantes podem ter ocorrido, pelo menos em parte, devido à variabilidade das populações estudadas.

Finalmente, um estudo observou uma associação entre o genótipo AA e o risco de desenvolver doença cardiovascular, sendo a taxa de risco por alelo de 1,14 no modelo ajustado para consumo total de calorias, gorduras, fibras e também para história familiar de infarto do miocárdio. Já no modelo ajustado para o IMC não foi encontrada uma associação entre o genótipo e o risco de doença cardiovascular (Ahmad, Chasman et al. 2010).

Com base nos dados atuais podemos observar que o gene do FTO foi um dos primeiros genes associado fortemente com aumento de IMC, mas suas funções biológicas, locais e mecanismos de ação ainda não estão totalmente esclarecidos.

O conjunto dos resultados descritos na literatura indica que homozigotos para o alelo A tanto para o SNP rs9939609 como rs8050136 apresentam maior susceptibilidade a desenvolver obesidade. No que se refere à possibilidade de associação entre estes polimorfismos e doenças crônicas não transmissíveis associadas ao excesso de gordura corporal, os dados disponíveis são incompletos ou discrepantes, sugerindo que mais estudos são necessários. Além disso, apenas um estudo em mulheres no período pós menopáusico foi publicado até o momento.

Com base no que foi descrito nesta revisão, julgou-se oportuno investigar se, entre mulheres na pós-menopausa recente, e aparentemente saudáveis, ocorre associação entre os genótipos dos polimorfismos rs9939609 e rs8050136 e o fenótipo clínico, como variáveis metabólicas e fatores de risco cardiovascular.

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Variations in the fat mass and obesity-associated (FTO) gene are related to hypertension and higher lipid accumulation product in postmenopausal women from South Brazil

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Capsule

In postmenopausal women from South Brazil, the AA genotype of the rs9939609 polymorphism in the FTO gene was related to increased waist-to-hip ratio, blood pressure and lipid accumulation product index.

ABSTRACT

Study objective: To test the association between polymorphisms rs9939609 T>A and rs8050136 A>C of the fat mass and obesity-associated **(**FTO) gene and metabolic and cardiovascular variables in postmenopause.

Design: Cross-sectional study.

Setting: University Hospital.

Patients: 135 postmenopausal women (mean age 52±4 years).

Interventions: Anthropometric measurements and collection of blood samples.

Main outcome measure(s): Blood pressure, metabolic variables, and FTO genotype.

Results: The frequency of polymorphism rs9939609 was 43.7% for the wild TT genotype, 43.0% for TA, and 13.3% for AA. The frequency of the rs8050136 polymorphism was 12.6% for the wild AA genotype, 39.3% for AC, and 48.1% for CC. The polymorphic AA genotype of the SNP rs9939609 was associated with higher waist-to-hip ratio, blood pressure and lipid accumulation product (LAP) index. Wild AA genotype of the SNP rs8050136 was associated with higher diastolic blood pressure and LAP. SNP rs9939609 AA genotype was associated with hypertension. AA genotypes of both SNP rs9939609 and rs8050136 were associated with more frequent abnormal glucose.

Conclusions: The rs9939609 polymorphism in the FTO gene is a candidate for predicting cardiovascular risk in postmenopause. Further studies are needed in different ethnic backgrounds to confirm the clinical relevance of these associations.

Keywords: FTO gene polymorphism, cardiovascular risk, menopause, LAP, blood pressure.

INTRODUCTION

In the postmenopausal period, estrogen deficiency may adversely affect the metabolic profile, including glucose and lipid metabolism. This leads to an increase in the incidence of cardiovascular morbidity and mortality in postfertile years (1).

Variants of the fat mass and obesity-associated (FTO) gene have been identified as determinants of human obesity in the recent past. The FTO gene is located in chromosome region 16q12.2. Single nucleotide polymorphisms rs9939609 T/A and rs8050136 A/C have been linked to fat mass and body mass index (BMI), risk of obesity, metabolic syndrome and high cardiovascular risk in healthy men and women (2-5). The FTO gene has also been associated with higher plasma levels of C-reactive protein (CRP) (6, 7) and increased degree of insulin resistance (8). Evidence suggests that the FTO genotype probably affects obesity by increasing food intake rather than energy expenditure (9, 10) or physical activity (11).

Only one case-control study from the Women's Health Initiative Observational Study (WHI-OS) was conducted to assess the influence of the FTO gene specifically in postmenopausal women. In that study, the clinical phenotype of participants, stratified in four ethnic groups, was analyzed according to FTO gene variants. Polymorphisms rs9939609 and rs8050136 were found to be associated with BMI and waist circumference in white and Hispanic women, but not in black and Asian/Pacific Islander women (12).

Therefore, the aim of the present study was to assess whether polymorphisms rs9939609 T>A and rs8050136 A>C in intron 1 of the FTO gene are associated with anthropometric and metabolic variables and cardiovascular risk factors in postmenopausal women from South Brazil.

MATERIALS AND METHODS

Patients

The study population included postmenopausal women consulting for climacteric symptoms. The study was performed in the Gynecological Endocrinology Unit at a university hospital (Hospital de Clínicas de Porto Alegre) in Brazil. One hundred and thirty-five postmenopausal women fulfilling the following inclusion criteria were consecutively enrolled: last menstrual period at least 6 months before the beginning of the study plus follicle stimulating hormone (FSH) levels higher than 35 IU/L; being older than 40 years of age; no use of any medication known to interfere with hormonal, glucose, or lipoprotein levels in the past 3 months; no use of steroidal or no steroidal anti-inflammatory drugs in the last 15 days. Patients with previous hysterectomy, endometrial thickness >0.5cm, history of cancer, thromboembolism, or established cardiovascular disease were excluded. Approval for this study was obtained from the Institutional Review Board and the local Ethics Committee, and written informed consent was obtained from every subject.

Study protocol

Anthropometric measurements included body weight, height, BMI (current weight in kg/m^2), waist circumference (WC, waist measured at the midpoint between the lower rib margin and the iliac crest, perpendicularly to the long axis of the body, with the subject standing balanced on both feet, spread approximately 20 cm apart, with both arms hanging freely) (13-15), hip circumference (widest circumference over the buttocks), waist to hip ratio (WHR, waist circumference divided by hip circumference). Blood pressure was measured with the patient in the supine position after a 10-minute rest. The same calibrated mercury manometer attached to a 12.5 x 23 cm inflatable cuff

was used in all patients by the same operator, who adopted the fifth Korotkoff sound to determine diastolic pressure (16). Hypertension was defined as systolic blood pressure (SBP) ≥ 130 mmHg and/or diastolic blood pressure (DBP) ≥ 85 mmHg and abnormal fasting blood glucose ≥ 110 mg/dL, in accordance with the National Cholesterol Education Program/ Adult Treatment Panel III (17).

Laboratory measurements

Blood samples were obtained between 08:00 and 10:00 a.m. After a 12-hour overnight fast, blood samples were drawn from an antecubital vein for determination of plasma glucose and insulin and lipid profile (total cholesterol, HDL cholesterol and triglycerides). Blood samples were also drawn for highly sensitive C-reactive protein (hs-CRP) before and 2 hours after the ingestion of 75g of glucose. LDL cholesterol was estimated indirectly with the Friedewald formula (18). Homeostasis model assessment index to estimate insulin resistance (HOMA-IR) was calculated by multiplying insulin (μIU/mL) by glucose (mmol/L) and dividing this product by 22.5 (19). The lipid accumulation product (LAP) index was calculated using the formula [waist (cm) – 58] x triglyceride concentration (mmol/L) (20).

Biochemical and hormonal assays

Total cholesterol, HDL cholesterol and triglycerides were determined by colorimetric-enzymatic methods with intra and interassay coefficient of variation (CV) < 3%. Glucose was determined by the hexokinase method with intra-assay CV < 3.4% and interassay CV < 2.1%. Serum hs-CRP concentrations were measured using latex enhanced immunoturbidimetry, with intra and interassay CV < 3.5%, as previously

reported (21). Serum insulin was measured with electrochemiluminescent immunoassays with intra-assay CV < 3.5% and interassay CV < 7.5%.

Genotyping

In addition to serum samples, whole blood samples were collected from all women. Genomic DNA was extracted from peripheral leukocytes following the technique described by Miller and associates (22). The DNA samples were diluted to 2 ng/mL and genotyped for SNP rs9939609 T>A and rs8050136 A>C of the FTO gene by real time PCR (7500 Fast Applied Biosystems, Foster City, CA, USA), using the allelic discrimination assay with TaqMan MGB Primers and Probes (Applied Biosystems, Foster City, CA, USA). The primers and labeled oligonucleotide probes were designed and offered by Applied Biosystems. For a final volume of 4 mL of mix per sample, TaqMAn assay (0.250mL) and H_20 (1.250mL) were added to TaqMan Master mix (2.5 mL). For a total reaction volume of 5 mL, 1mL of DNA was added. The reaction conditions for the rs9939609 SNP were: 95 ºC (10 minutes) after 50 cycles of denaturation at 95 ºC (15 seconds) and annealing at 61 ºC (1 minute). For the rs8050136 SNP, the conditions were: 95 ºC (10 minutes) after 40 cycles of denaturation at 95 ºC (15 seconds) and annealing at 60 ºC (1 minute). Endpoint fluorescent readings were performed by 7500 Fast System SDS software version 1.4.

Statistical analysis

Results are expressed as mean ± SD or median and interquartile range (25% to 75%). Non-normally distributed parameters were log transformed to approximate a normal distribution curve before statistical analysis. The Student t-test was used to test comparisons between groups, and the χ^2-test to compare categorical variables. Previous

studies have shown that the rs8050136 “A” allele is in strong linkage disequilibrium (LD) with the rs9939609 “A” allele in Caucasians (23, 24). Therefore, for each SNP, the two risk alleles – the recessive model for rs9939609 T>A, and the dominant model for rs8050136 A>C – were tested separately.

$P \leq 0.05$ was considered to be statistically significant. All analyses were performed using the Statistical Package for the Social Sciences 18 (SPSS, Chicago, IL, USA).

RESULTS

The mean age of participants was 52 ± 4 years. The mean time since menopause was 18 ± 11 months, age at menopause was 49 ± 3 years and BMI was 26.7 ± 3.6. One hundred and seventeen (87%) of the 135 participants were Caucasians. The remaining participants were of mixed (African and European) descent.

All 135 participants were genotyped. The frequency of polymorphism rs9939609 was 43.7% for the wild TT genotype, 43.0% for the heterozygous polymorphic TA genotype and 13.3% for the homozygous polymorphic AA genotype. The frequency of the rs8050136 polymorphism was 12.6% for the wild AA genotype, 39.3% for the heterozygous polymorphic AC genotype and 48.1% for the homozygous polymorphic CC genotype (table 1). Genotype frequencies were in agreement with Hardy-Weinberg equilibrium for both polymorphisms. The overall minor allele frequency (MAF) observed in this study was 34% for SNP rs9939609 and 32% for SNP rs8050136.

Table 2 shows clinical, metabolic and pro-inflammatory variables according to SNP rs9939609 genotypes. BMI, waist circumference, as well as insulin, total cholesterol, HDL-c, LDL-c, triglycerides and usCRP levels and HOMA-IR were similar

between genotypes. Individuals with the polymorphic AA genotype had significantly higher WHR, SBP, DBP, and LAP in comparison with individuals presenting the wild TT genotype and the TA genotype.

Table 3 shows clinical, metabolic and pro-inflammatory variables according to SNP rs8050136 genotypes. Wild AA genotype was associated with significantly higher DBP and LAP as compared to heterozygous AC and homozygous CC genotypes. BMI, waist circumference, WHR, SBP, insulin, cholesterol, HDL-c, LDL-c, triglycerides, usCRP and HOMA-IR were similar between genotypes.

When participants were stratified according to the presence or not of hypertension, significant differences were found in AA versus AT + TT genotype of the SNP rs9939609. The AA genotype was significantly more frequent in individuals with hypertension than in those without hypertension (61% vs. 39%, $p = 0.036$) (Figure 1A). No significant difference was observed in the prevalence of hypertension for the SNP rs8050136 genotypes ($p = 0.173$) (Figure 1B). Abnormal glucose was also more frequent in the AA genotypes of both SNP rs9939609 (16.7% vs. 3.5%, $p = 0.021$) and SNP rs8050136 (17.6% vs. 3.5%, $p = 0.016$) (Figure 1C and D).

DISCUSSION

In the present study, FTO gene polymorphisms were found to be associated with cardiovascular risk factors in postmenopausal women. The AA genotype of the SNP rs9939609 of the FTO gene was associated with hypertension, hyperglycemia and higher LAP; and the AA genotype of the SNP rs8050136 was related to higher LAP and diastolic blood pressure. To the best of our knowledge, this is the first description of an association between FTO gene variants and the cardiovascular risk marker LAP in postmenopausal women.

The overall MAF for SNP rs9939609 described in the present study was similar to those previously reported in European populations (23, 25-27). Concerning the SNP rs8050136, MAF was 42% in the German population (28), and 45% to 48% in the HapMap HCB population (29).

In the present study, the polymorphic AA genotype for rs9939609 was associated with higher WHR. Previous studies have shown that the AA genotype was associated with greater WHR in children (30) and adults (6), and also with other measures of obesity, such as BMI and waist circumference (31-33). In turn, others did not observe this association with WHR (34, 35).

The mechanism by which FTO polymorphisms affect obesity and WHR in humans is still unclear. Studies have shown that the FTO gene is widely expressed in many tissues, including the arcuate nucleus of the hypothalamus (23, 36, 37). FTO expression seems to be regulated by fasting and feeding (37, 38), indicating a functional involvement of the gene in the central control of energy homeostasis (39). Recently, studies have shown an association of FTO gene polymorphisms with increased energy intake (39-41).

The precise mechanism underlying the effect of FTO protein on metabolism also remains largely unknown. FTO seems to play a role in nucleic acid demethylation (37). The relatively high expression of FTO in the hypothalamus could point to a critical role of this gene in neuroregulation of energy metabolism via the hypothalamic-pituitary-adrenal axis (23, 37, 42).

Associations of FTO variants with several metabolic parameters have been sought by several authors, with inconsistent results. In a large sample of white individuals, Freathy *et al*. found an association of the rs9939609 A allele with increased insulin, glucose, and triglyceride levels, and with lower HDL-cholesterol levels.

However, no evidence of these associations was observed after adjustment for BMI (3). In postmenopausal women, no ethnic-specific associations were observed between FTO and obesity-related metabolic traits such as SBP, DBP, plasma fasting insulin, glucose and high-sensitivity C-reactive protein (12). In the present study, the FTO rs9939609 A allele was not associated with lipid profile, confirming other previous studies in European populations (34) and in men with abnormal glucose metabolism (34, 43).

We observed that the rs9939609 polymorphic AA genotype was associated with SBP and DBP and also with the presence of hypertension. A recent paper by Karasawa *et al.* also reports that polymorphic FTO gene variants are implicated in increased SBP and DBP in a Japanese sample (5). Another study with healthy women and men showed that the AA genotype was associated with high levels of SBP even after adjustment for age, gender and BMI (44). However, studies with European (34) and Chinese participants did not find an association with blood pressure after adjustment for BMI (30, 45). It has been speculated that since FTO is highly expressed in the hypothalamus, the association between FTO variants and increased risk for hypertension may be related, at least in part, to a disturbed regulation of sympathetic modulation of vasomotor tone (46)**.** Moreover, FTO-deficient mice may have their leanness associated with a state of increased energy expenditure and systemic sympathetic activation (47).

Except for higher DBP and LAP, we found no consistent differences between menopausal women with wild AA genotype of rs8050136 *versus* the combined homozygous and heterozygous polymorphic genotypes. The relevance of this gene variant is still unclear, but some studies report an association of the FTO gene with increased risk for adverse metabolic traits, including low HDL-c, hypertension and metabolic syndrome (4), fasting and fed glucose (48), and HOMA-IR (49).

Interestingly, both the homozygous polymorphic AA genotype for rs9939609 and wild AA rs8050136 were associated with higher LAP, a marker of cardiovascular risk (50). Results from the Ludwigshafen risk and cardiovascular health study (LURIC), which included postmenopausal women at high cardiovascular risk, indicate that high LAP levels are associated with heart failure mortality in normal weight women (51). Another study showed an independent association of LAP levels with increased risk of incident cardiovascular disease among women but not men (52). Our group has also recently reported that LAP can be used to screen for insulin resistance and cardiovascular risk in hyperandrogenic women with polycystic ovary syndrome (53). Thus, the present results suggest that women with the AA genotype may have increased cardiovascular risk. Other studies have associated FTO gene polymorphisms with increased levels of CRP and higher cardiovascular risk (4, 6, 43, 54).

It should be noted that SNPs rs9939609 and rs8050136, which are located in intron 1, are in linkage disequilibrium (LD). This extensive LD region (> 30 kb) across the FTO gene intron 1 region makes it difficult to discern the best single SNP surrogate to fully capture genetic variability for this region. In addition, it is plausible that intron 1 of the FTO gene may contain an element that is important in regulating both mRNA splicing and expression (12, 42).

In conclusion, results from the present study indicate an association of AA genotype from both SNP rs9939609 and rs8050136 in FTO gene with LAP in postmenopausal women, whereas increased frequency of hypertension and hyperglycemia is only associated with the AA genotype of SNP rs9939609. Our results thus suggest that the rs9939609 polymorphism in the FTO gene is a candidate for predicting cardiovascular risk in postmenopause. Further studies in populations of

different ethnic backgrounds, as well as and longitudinal studies, are needed to confirm the clinical relevance of these associations.

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FIGURES

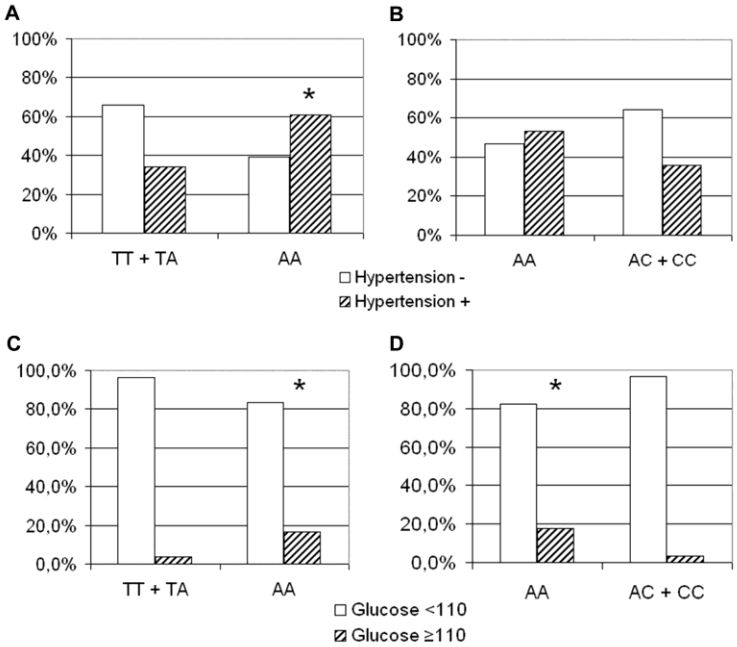

Figure 1. Association of polymorphisms rs9939609 (**A**) and rs8050136 (**B**) with hypertension. Association of polymorphisms rs9939609 (**C**) and rs8050136 (**D**) with abnormal glucose. Data denoted in % (χ^2 test). * $p < 0.05$.

TABLES

Table 1. Genotypic distribution of FTO gene polymorphisms[a]

SNP	A/a	Position on chromosome (pb)	Genotype distribution
			AA/Aa/aa
rs9939609	T/**A**	53820527	43.7/43.0/13.3
rs8050136	**A**/C	53816275	12.6/39.3/48.1

[a] Risk allele denoted in bold

Table 2. Clinical and metabolic characteristics of postmenopausal women according to FTO gene SNP rs9939609 genotypes

	TT TA (117)	AA (18)	p
Waist (cm)	84.0 ± 8.1	87.6 ± 7.4	0.13
BMI (kg/m²)	26.5 ± 3.5	27.8 ± 41	0.15
WHR	0.8 ± 0.0	0.8 ± 0.0	0.02
SBP (mmHg)	120.5 ± 14.3	128.1 ± 16.4	0.04
DBP (mmHg)	76.7 ± 8.6	82.5 ± 8.0	0.01
Fasting glucose (mg/dL)	91.3 ± 9.5	96.7 ± 15.0	0.16
Glucose at 120 min (mg/dL)	100.3 ± 24.0	112.9 ± 31.1	0.05
Fasting insulin (µUl/mL)	7.3 (4.7 – 10.3)	6.9 (4.9 – 9.9)	0.99
Serum cholesterol (mg/dL)	196.0 ± 51.0	211.7 ± 44.5	0.22
HDL-c (mg/dL)	61.6 ± 15.0	60.0 ± 10.3	0.65
LDL-c (mg/dL)	113.2 ± 40.7	120.8 ± 46.1	0.46
Serum triglycerides (mg/dL)	116.7 ± 51.4	127.2 ± 52.4	0.42
C-reactive protein (mg/L)	1.3 (0.4 – 3.0)	2.0 (0.9 – 3.1)	0.09
LAP	31.7 (18.5 – 48.0)	42.6 (29.9 – 51.7)	0.01
HOMA-IR	1.6 (1.1 – 2.4)	1.6 (1.0 – 2.1)	0.82

Data are mean ± SD values or median and interquartile range (25% a 75%) (Student t-test); WHR= waist to hip ratio, BMI= body mass index, HDL-c= high-density lipoprotein, LDL-c= low-density lipoprotein; HOMA-IR = homeostatic model assessment , LAP= lipid accumulation product

Table 3. Clinical and metabolic characteristics of postmenopausal women according to FTO gene SNP rs8050136 genotypes

	AA (17)	AC CC (118)	p
Waist (cm)	87.4 ± 7.1	83.9 ± 8.1	0.13
BMI (kg/m²)	27.3 ± 3.1	26.6 ± 3.7	0.45
WHR	0.85 ± 0.0	0.82 ± 0.0	0.06
SBP (mmHg)	123.8 ± 10.9	121.2 ± 15.3	0.49
DBP (mmHg)	80.2 ± 5.1	76.8 ± 9.2	0.03
Fasting glucose (mg/dL)	96.7 ± 15.1	91.2 ± 9.7	0.16
Glucose at 120 min (mg/dL)	111.3 ± 34.7	100.3 ± 23.6	0.22
Fasting insulin (µUl/mL)	7.0 (4.9 – 10.0)	6.8 (4.6 – 10.3)	0.19
Serum cholesterol (mg/dL)	219.2 ± 40.7	193.6 ± 52.0	0.05
HDL-c (mg/dL)	60.1 ± 11.8	61.7 ± 14.8	0.68
LDL-c (mg/dL)	128.9 ± 43.2	111.2 ± 40.9	0.10
Serum triglycerides (mg/dL)	123.4 ± 53.9	116.4 ± 51.5	0.60
C-reactive protein (mg/L)	2.0 (0.9 – 3.4)	1.3 (0.4 – 2.9)	0.19
LAP	41.8 (30.4 – 50.8)	31.7 (18.5 – 48.0)	0.01
HOMA-IR	1.7 (1.0 – 2.2)	1.6 (1.1 – 2.4)	0.99

Data are mean ± SD values or median and interquartile range (25% a 75%) (Student t-test); WHR= waist to hip ratio, BMI= body mass índex, HDL-c= high-density lipoprotein, LDL-c= low-density lipoprotein; HOMA-IR = homeostatic model assessment , LAP= lipid accumulation product

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