Original Article
Novel abdominal adiposity genes and the risk of type 2 diabetes: findings from two prospective cohorts

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Abstract: Three loci were recently identified for central adiposity from a genome wide association study (*MSRA* [rs545854; G/C], *LYPLAL1* [rs2605100; G/A], *TFAP2B* [rs987237; A/G]). Central obesity is a strong risk factor for type 2 diabetes (T2D). Therefore, we hypothesized that these single nucleotide polymorphisms (SNPs) would be associated with increased risk of T2D and may influence circulating adipokine concentrations. Participants from two large case control studies nested in the Nurses' Health Study (3394 women, 1245 cases) and the Health Professionals Follow-up Study (2154 men, 862 cases) were genotyped for these SNPs. The association of these SNPs with plasma adipokine concentrations was determined among a subgroup of women without diabetes (n=987). After adjustment for age and other risk factors of diabetes, the *MSRA* variant was associated with an increased T2D risk in men only, with an adjusted OR of 1.30 (95% CI: 1.09-1.56) associated with each copy of the variant allele. In pooled analyses of men and women, each additional copy of the *LYPLAL1* allele (G) was associated with 9% increased T2D risk (adjusted OR 1.09; 95%CI: 0.99-1.19). No significant associations were seen with the *TFAP2B* SNP with a pooled adjusted OR of 1.05 (95% CI: 0.95-1.17) per allele copy. In addition, carriers of the *MSRA* risk variant had lower percent high molecular weight adiponectin (-2.1%, p=0.04). Carriers of the *TFAP2B* risk variant, however, had lower leptin levels (-2.7 ng/ml, p=0.005). These findings suggest potential associations of novel central obesity genes with T2D risk and adipokine regulation.

Keywords: Abdominal adiposity, genes, type 2 diabetes, TFAP2B, MSRA, LYPLAL1

Introduction

Central obesity is a strong risk factor for type 2 diabetes (T2D) [1]. A recent genome-wide association study (GWAS) identified three novel loci (*MSRA* [rs545854; G/C], *LYPLAL1* [rs2605100; G/A], and (*TFAP2B* [rs987237; G/A]) for central adiposity as measured by waist circumference and waist-to-hip ratio (WHR) [2]. Sexual dimorphism was observed, with the association of LYPLAL1 and WHR found to be stronger in women than in men [3,2].

Available data suggest that proteins encoded by the three genes may be implicated in the pathogenesis of T2D. For instance, methionine sulfoxide reductase A (MSRA) plays a role in decreasing oxidative stress by reversing oxidized me-

thionine [4,5], and increased oxidative stress has been implicated in the development of T2D [6] through its destruction of pancreatic beta cells [7]. Lysophospholipase-like protein 1 (*LYPLAL1*), as part of the family of triglyceride lipases, affects fatty acid metabolism including its storage and mobilization [8]. Increased circulating free fatty acids, in turn, have been shown to lead to increased risk of T2D.[9] Transcription factor activating enhancer binding protein 2 beta (TFAP2B) has been associated with increased insulin resistance among adolescents [10] and T2D risk in adults [11]. Its expression is associated with decreased adiponectin levels in subcutaneous tissue [12].

Despite these potential biological links, studies on the association of genetic variants of these

three genes with T2D risk are sparse. Based on data from two independent prospective cohorts, we investigated the association of the three abdominal adiposity SNPs with T2D and with circulating adipokine levels, along with whether these SNPs might interact with major dietary factors (i.e. carbohydrates, fats, and fiber) in relation to T2D risk [2].

Materials and methods

Study population

The Nurses' Health Study (NHS) began with the recruitment of 121,700 female registered nurses aged 30-55 years in 1976. Medical history, lifestyle and dietary information was collected every 2 or 4 years beginning in 1980 [13]. Blood was collected from 32,826 women in NHS between 1989 and 1990. The Health Professionals Follow-up Study (HPFS) is a prospective cohort study which recruited 51,529 U.S. male health professionals aged 40-75 years in 1986 and have conducted ongoing follow-up through biennial mailed questionnaire [14]. Between 1993 and 1999, 18,159 men in HPFS mailed back blood samples.

T2D cases were self-reported and confirmed by validated questionnaires in both cohorts [15]. In a validation study, 99% of cases were confirmed by medical record review (61 out of 62 women) [15]. The National Diabetes Data Group criterion was used for diagnosis among cases reported prior to 1998, after which the American Diabetes Association criterion was used. Participants of this study consisted of those who provided blood samples and were free of cancer or cardiovascular disease at baseline [15]. Healthy control subjects were matched on age, month and year of blood draw, and fasting status with diabetic patients. Adipokines (i.e. high molecular weight (HMW) and total adiponectin, leptin, soluble leptin receptor and resistin) were measured among a subset of women without diabetes (n=987) [16].

Participants who were not Caucasians of European ancestry or missing genotype data for all three SNPs were excluded. Prevalent T2D cases reported prior to the collection of dietary data in 1980 for women and 1986 for men were excluded (380 female cases, 377 male cases) so that the risk of incident type 2 diabetes could be determined. In total, the sample consisted of

3394 women (1245 cases) and 2154 men (862 cases). All participants provided written informed consent, and the Human Research Committee at the Brigham and Women's Hospital, Boston, approved the study.

Genotyping

The three abdominal adiposity genetic variants (*MSRA* (rs545854; G/C), *LYPLAL1* (rs2605100; G/A), *TFAP2B* (rs987237; G/A)) previously identified by GWAS[2] were genotyped. DNA was extracted from buffy coat using a QIAmp blood kit (Qiagen, Chatsworth, CA). SNPs were genotyped using TaqMan SNP allelic discrimination by means of an ABI 7900HT (Applied Biosystems, Foster City, CA). Blinded samples typed in duplicate assured the internal quality of the genotyping, and resulted in concordance of >99%. The call rate was >98%, respectively. No significant departures from Hardy-Weinberg equilibrium were detected among controls by Chi-square test.

Covariate assessment

Information on family history of diabetes, smoking status, alcohol intake, and physical activity were collected at baseline for each study [17]. Information on weight at baseline (1976 for NHS and 1986 for HPFS), and current body weight updated during follow-up were self-reported. Body mass index (BMI) was calculated based on weight divided by height squared (kg/m²). Dietary intake was obtained by semi-quantitative food frequency questionnaire [18]. Participants reported on frequency of intake on food items during the previous year through responses to 9 possible choices ranging from "never or 1/mo" to "6/d." Nutrient intakes were derived by multiplying the frequency of intake by the average nutrient content of the specified portion size obtained from the Harvard University food composition database, which used U.S. Department of Agriculture data[19] and was supplemented with information from the manufacturer.

Statistical analysis

Odds ratios and 95% confidence intervals (CI) on the risk of T2D by genotypes were estimated using logistic regression, adjusting for age, physical activity, smoking status, alcohol consumption, coffee, ratio of polyunsaturated to

saturated fat, trans fat, cereal fiber and meno-pausal status combined with hormone replace-ment therapy use among women. Associations of the SNPs with anthropometric measures were conducted using a linear regression model. The sample size was reduced in those analyses involving waist and hip measurements to include only controls in each cohort with available data (1415 women, 1283 men). Analyses of the associations between the SNPs and adipokines were adjusted for age alone. Nominal significance was determined at p<0.05. All analyses were conducted using SAS statistical package (v.9.1 for UNIX; SAS Insti-tute, Cary, NC).

Results

The frequencies of the alleles previously associ-ated with increased abdominal adiposity [2] were 18% for the rs545854 allele G (*MSRA*), 71% for the rs2605100 allele G (*LYPLAL1*), and 18% for rs987237 allele G (*TFAP2B*) among women, and 15%, 73%, 17%, respectively, among men. These allele frequencies were simi-lar to those previously reported in Caucasians (18%, 69%, 16%, respectively) [2]. Only the *MSRA* SNP rs545854 was nominally related to increase WHR among 1415 female controls with data available. Women with the C allele had a mean WHR of 0.78 compared with 0.81 among those with the GG genotype (p=0.01). No significant associations of these SNPs with anthropometry measures (including waist and hip circumference and WHR) were found among men.

The *MSRA* G/C variant was related to an in-creased risk of incident T2D in men alone, with an OR of 1.30 (95% CI: 1.09-1.56) associated with each copy of the G allele after adjusting for risk factors including age, smoking, physical activity, menopausal status for women, and intakes of coffee, alcohol, ratio of polyunsatu-rated to saturated fat, trans fat, and cereal fi-ber. (**Table 1**) Results adjusting for age alone did not yield different inferences. The pooled OR after including women was not statistically sig-nificant (1.05, 95%CI: 0.91-1.20). The test for heterogeneity of the association among women and men was borderline significant (p=0.06). In pooled analyses of men and women, each effec-tive allele (G) of *LYPLAL1* was borderline associ-ated with a 9% increased risk of incident T2D (OR 1.09, 95%CI: 0.99-1.19). Additional adjust-ment for BMI strengthened the association slightly (OR 1.13: 1.03-1.19) although it may be over-adjusted. No association was found for the *TFAP2B* variant and incident T2D in either co-hort (pooled OR = 1.05, 95% CI: 0.95-1.17).

To further investigate the potential mechanisms underlying the associations with diabetes, we examined the association of the three SNPs with plasma adipokine levels among women (n=945) in whom these markers were meas-ured. Carriers of the *MSRA* G allele had signifi-cantly lower percent high molecular weight (% HMW) adiponectin than non-carriers (age-adjusted mean of 38.8% vs. 40.9%, p=0.04). (**Figure 1A**) Women with one or two of the *TFAP2B* G alleles, however, had significantly lower leptin (age-adjusted mean of 17.5 vs. 20.1 ng/ml p=0.007). (**Figure 1B**) The *LYPLAL1* SNP was not related to adipokine levels.

Discussion

The present study, based on data from two in-dependent cohorts, found that the *MSRA* SNP [rs545854; G/C] was significantly associated with increased risk of incident T2D in men alone. In addition, *MSRA* was associated with greater WHR and lower %HMW adiponectin among female controls. The *LYPLAL1* SNP [rs2605100; G/A] was borderline significantly associated with an increased risk of T2D. The *TFAP2B* SNP [rs987237; G/A] was not associ-ated with incident T2D risk in either cohort but was found to be associated with lower leptin levels.

The SNP rs545854 is located ~50,000 bp up-stream of the *MSRA* gene. The protein methion-ine sulfoxide reductase A that it encodes pro-tects against oxidative stress by repairing me-thionine [4]. The *MSRA* SNP has recently been found to be associated with early-onset obesity in children and adolescents [20]. However, it was not associated with T2D risk in a meta-analysis of the DIAGRAM consortium data (OR 1.06; 95% CI: 0.92-1.22) [2]. Nevertheless, our results show that the elevated risk associated with the *MSRA* SNP may be gender-specific, with increased risk only found among men. When we pooled the results by meta-analysis, our findings were similar to those of the DIA-GRAM consortium[2] and the test for heteroge-neity of the association among men and women was borderline significant. These findings

Table 1. Risk of type 2 diabetes by genotypes of abdominal adiposity SNPs among women and men

	Women			Men			All	
***MSRA* (8p23.1)**								
[rs545854, G/C]	cases*/controls	Adjusted OR**	p-value	cases*/controls	Adjusted OR**	p-value	Pooled OR**	p-value
CC	786/1393	1.00 (Ref)	n/a	530/926	1.00 (Ref)	n/a	1.00 (Ref)	n/a
CG	347/605	1.03 (0.87-1.21)	0.73	238/330	1.30 (1.06-1.60)	0.01	1.13 (0.99-1.28)	0.07
GG	39/58	1.18 (0.77-1.81)	0.45	23/27	1.70 (0.94-3.09)	0.08	1.34 (0.94-1.89)	0.10
Per G allele	1172/2056	1.05 (0.91-1.20)	0.50	791/1283	1.30 (1.09-1.56)	0.003	1.14 (1.02-1.27)	0.03
***LYPLAL1* (1q41)**								
[rs2605100; G/A]	cases*/controls	Adjusted OR**	p-value	cases*/controls	Adjusted OR**	p-value	Pooled OR**	p-value
AA	89/179	1.00 (Ref)	n/a	55/110	1.00 (Ref)	n/a	1.00 (Ref)	n/a
AG	477/855	1.08 (0.81-1.44)	0.60	321/485	1.32 (0.92-1.91)	0.14	1.17 (0.93-1.46)	0.18
GG	659/1049	1.22 (0.93-1.62)	0.16	421/707	1.25 (0.87-1.79)	0.23	1.23 (0.99-1.54)	0.06
Per G allele	1225/2083	1.12 (1.00-1.25)	0.06	797/1302	1.04 (0.90-1.20)	0.63	1.09 (0.99-1.19)	0.07
***TFAP2B* (6p12)**								
[rs987237, A/G]	cases*/controls	Adjusted OR**	p-value	cases*/controls	Adjusted OR**	p-value	Pooled OR**	p-value
AA	809/1384	1.00 (Ref)	n/a	542/898	1.00 (Ref)	n/a	1.00 (Ref)	n/a
AG	382/651	1.01 (0.87-1.19)	0.88	229/354	1.11 (0.90-1.36)	0.32	1.05 (0.92-1.19)	0.47
GG	42/61	1.23 (0.81-1.87)	0.32	24/44	0.94 (0.56-1.59)	0.82	1.11 (0.80-1.54)	0.52
Per G allele	1233/2096	1.05 (0.92-1.19)	0.52	795/1296	1.06 (0.89-1.25)	0.53	1.05 (0.95-1.17)	0.37

* Among incident cases of T2D after first administered food frequency questionnaire (i.e. 1980 for NHS, 1986 for HPFS)
** Odds ratios adjusted for age, smoking, coffee, alcohol, physical activity, ratio of polyunsaturated and saturated fat, trans fat, cereal fiber (& menopausal status for NHS)

A

B
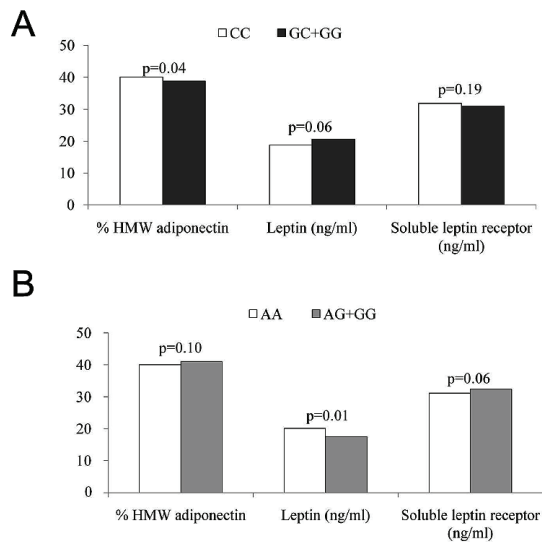
Figure 1. Age-adjusted geometric means of adipokine levels between carriers and non-carriers of the risk alleles of the *MSRA* (**A**) and *TFAP2B* (**B**) genes among 945 women without diabetes in NHS.

suggest that future studies of this gene and T2D risk may consider testing for interaction by gender.

The *LYPLAL1* SNP (rs2605100) is newly identified from a recent GWAS for abdominal adiposity [2] and replicated recently in another large GWAS [3]. Study on its association with T2D has just recently emerged [2]. The SNP is located in a gene desert ~250,000 bp upstream of the *LYPLAL1* gene. Limited information is known about the protein, lysophospholipase-like protein 1, that it encodes after its initial discovery as a novel gene in a proteomics study of mouse adipose tissue [21]. Triglycerides are stored in adipose tissue and are mobilized and released into circulation as free fatty acids through the functions of lipolytic enzymes [21]. A GWAS meta-analysis of over 14,000 individuals showed evidence of the potential role of the *LYPLAL1* SNP in triglyceride mobilization with a positive association found with fasting triglyceride levels (beta=0.05, p=4x10^{-4}) [2]. The mechanism for the association of the *LYPLAL1* variant with an increased T2D risk could be through increasing circulating triglyceride levels. [9] Previously, among DIAGRAM participants the OR (95% CI) for the G allele associated with T2D risk was 1.04 (0.97-1.11) [2]. Differences in the compositions (e.g. sex and age) of the

study populations and risk factor profiles of T2D may partly explain different findings between studies.

We did not find a significant association between the *TFAP2B* SNP and T2D risk in either cohort despite previous studies [10,11]. Our study and another study among adolescents [10] were also unable to confirm the findings that TFAP2B expression is associated with adiponectin expression [12]. Rather, we found that the *TFAP2B* SNP was associated with slightly decreased leptin levels.

This study had several strengths including the availability of data from two large independent cohorts. We had 90% power to detect ORs of 1.11-1.14 (depending on the allele frequency of the SNPs) when both cohorts were pooled together (n=~5000). Thus, the SNPs that were not found significantly associated with T2D may have very weak effects. There were some limitations to the study. We did not have adipokine data among men. In addition, these results may not be generalizable to non-Caucasian populations. Recent genome wide association studies of abdominal adiposity have identified additional polymorphisms that may be of interest for future investigations [3, 22].

In conclusion, the abdominal adiposity *MSRA* SNP was associated with incident T2D risk among men and decreased percent HMW adiponectin in women. More studies are needed to further replicate these findings. In addition, mechanistic studies will be necessary to characterize the role of MSRA in the development of T2D.

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