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Genetics & Epigenetics

Effect of *FTO* Gene and Physical Activity Interaction on Trunk Fat Percentage Among the Newfoundland Population

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ABSTRACT

OBJECTIVE: To explore the effect of *FTO* gene and physical activity interaction on trunk fat percentage.

DESIGN AND METHODS: Subjects are 3,004 individuals from Newfoundland and Labrador whose trunk fat percentage and physical activity were recorded, and who were genotyped for 11 single-nucleotide polymorphisms (SNPs) in the *FTO* gene. Subjects were stratified by gender. Multiple tests and multiple regressions were used to analyze the effects of physical activity, variants of *FTO*, age, and their interactions on trunk fat percentage. Dietary information and other environmental factors were not considered.

RESULTS: Higher levels of physical activity tend to reduce trunk fat percentage in all individuals. Furthermore, in males, rs9939609 and rs1421085 were significant ($\alpha = 0.05$) in explaining central body fat, but no SNPs were significant in females. For highly active males, trunk fat percentage varied significantly between variants of rs9939609 and rs1421085, but there is no significant effect among individuals with low activity. The other SNPs examined were not significant in explaining trunk fat percentage.

CONCLUSIONS: Homozygous male carriers of non-obesity risk alleles at rs9939609 and rs1421085 will have significant reduction in central body fat from physical activity in contrast to homozygous males of the obesity-risk alleles. The additive effect of these SNPs is found in males with high physical activity only.

KEYWORDS: *FTO*, rs9939609, rs1421085, physical activity, Newfoundland population

CITATION: Payne et al. Effect of *FTO* Gene and Physical Activity Interaction on Trunk Fat Percentage Among the Newfoundland Population. *Genetics & Epigenetics* 2014:6 21–30 doi:10.4137/GEG.S14957.

RECEIVED: February 18, 2014. **RESUBMITTED:** April 1, 2014. **ACCEPTED FOR PUBLICATION:** April 2, 2014.

ACADEMIC EDITOR: Christian Bronner, Editor in Chief

TYPE: Original Research

FUNDING: Research was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC) and Research and Development Corporation Newfoundland and Labrador (RDC). The CODING study has been funded by Canadian Institutes of Health Research (CIHR) operating grants OOP-77984 and MOP192552 to Dr Guang Sun.

COMPETING INTERESTS: Authors disclose no potential conflicts of interest.

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This paper was subject to independent, expert peer review by a minimum of two blind peer reviewers. All editorial decisions were made by the independent academic editor. All authors have provided signed confirmation of their compliance with ethical and legal obligations including (but not limited to) use of any copyrighted material, compliance with ICMJE authorship and competing interests disclosure guidelines and, where applicable, compliance with legal and ethical guidelines on human and animal research participants.

Introduction

Overweight and obesity have become a major epidemic problem in Newfoundland and Labrador (NL) and throughout Canada. These conditions can lead to high risks of contracting heart diseases, musculoskeletal disorders, and some cancers that can be detrimental to health and even fatal.[1] Data from the 2012 Canadian census show that 61.9% of Canadian adults are overweight or obese, which is of great concern.[2] NL has had the highest percentage of self-reported overweight/obese residents in Canada since 2008 with 63.2% of adults reporting being overweight or obese that year.[3] It is of interest to explore potential causes of these escalating rates of obesity in NL in order to educate individuals and stratify patients into risk classes to improve the treatment of obesity.

Although many recent studies (including genome-wide association studies) confirm significant genetic associations with obesity-related traits such as body mass index (BMI) and waist circumference, the variability of the traits attributable to genetic factors is still small.[4–6] Heritability estimates for obesity-related traits indicate that a considerable percentage of

phenotypic variation still needs to be explained.[7] It is suggested that the interaction between certain genes and environmental factors may contribute to the variability in those traits, since most studies have not taken gene–environment interactions into consideration.

BMI is commonly used when examining obesity due to its ease and low cost of measurement. Trunk fat percentage is a more precise measurement of central body fat not commonly explored in obesity studies. Trunk fat percentage, which for this study has been measured by dual-energy X-ray absorptiometry (DXA), is more closely associated with obesity-related detrimental effects on health.[8–10] DXA is superior to BMI in the measurement of body fat, and BMI has been shown to significantly misclassify obesity compared to DXA measurement,[11] with one study referring specifically to the NL population.[12]

Many studies have shown association between the *FTO* gene (fat mass and obesity associated) and obesity-related traits in populations of different ethnic backgrounds.[13–15] More specifically, two single-nucleotide polymorphisms (SNPs) in the *FTO* gene, namely rs9939609 and rs1421085, have been previously found to have association with obesity.[15–17] These studies have all contributed to the worldwide attribution of the *A* and *C* alleles, respectively, in these SNPs to an increase in BMI. Other SNPs in the *FTO* gene have been explored less often; however, nine other SNPs will be considered in this study.

Many studies show that higher levels of physical activity can reduce the likelihood of being overweight or obese.[18–20] However, none of these studies consider the effect of genes as a modifier. Low physical activity has been thought to accentuate the effect of the *FTO* gene,[21] and it has been shown that the effect of genetic susceptibility to obesity caused by *FTO* variants can actually be attenuated by increased physical activity.[22] One study recently found that rs9939609 was associated with trunk obesity as early as in adolescence.[23] However, the sample size was much smaller than in this study, and the adiposity measurements were made using a lipometer. A lipometer measures subcutaneous fat only and its accuracy may not be as dependable as the DXA measurements of trunk fat percentage used here.[24]

Our goal was to further explore the relationships between physical activity, *FTO* SNPs, and trunk fat percentage in an association study with a large sample of individuals from the NL population. NL has been found to have unique genetic architecture, and based on homogeneity, isolation, and extended linkage disequilibrium, it has been proposed that this population provides advantageous genetic mapping of complex diseases compared to many admixed populations.[25] Due to the unique genetic population in NL and significant findings in this study, we believe that our results pertaining to trunk fat percentage variation add important and widely applicable findings to the literature.

Methods and Procedures

Subjects. All data used were taken from the CODING (*Co*mplex *Di*seases in the *N*ewfoundland Population: Environment and *G*enetics) study. Eligibility of participants for the CODING study was based on the following inclusion criteria: 19 years of age or older; at least a third generation Newfoundlander; and healthy, without any serious metabolic, cardiovascular, or endocrine diseases. The primary method of subject recruitment for the CODING study was the use of posters and handouts. This literature was distributed throughout public facilities (offices and hospitals) in the city of St. John's, NL. Each individual completed a number of questionnaires to obtain information regarding lifestyle and physical activity. Anthropometric, body composition, and biochemical measurements were performed following a 12-hour fasting period. The basic characteristics of the individuals included in this study are presented in Table 1.

Anthropometric and body composition measurements. Height (nearest 0.1 cm) and weight (nearest 0.1 kg)

Table 1. Basic characteristics of participants from the Complex Diseases in the Newfoundland Population: Environment and Diseases (CODING) study by gender.

VARIABLE	CATEGORY	MALE ($n = 808$)		FEMALE ($n = 2196$)	
		MEAN (SD)	%	MEAN (SD)	%
Age (years)	–	39.8 (14.1)	–	43.5 (12.3)	–
Body mass index (BMI)* (kg/m^2)	Overall	27.4 (4.5)	–	26.4 (5.3)	–
	Normal/Underweight	23.0 (1.6)	32.5	22.3 (1.8)	47.4
	Overweight/Obese	29.5 (3.8)	67.5	30.1 (4.7)	52.6
Percent trunk fat	–	29.7 (9.7)	–	38.6 (9.0)	–
Physical activity score**	Low	–	22.8	–	24.2
	Moderate	–	61.4	–	66.4
	High	–	15.8	–	9.4

Notes: *BMI categories from WHO's cut-offs (http://apps.who.int/bmi/index.jsp?introPage=intro_3.html).
**Physical activity scores from the ARIC Questionnaire (*Am J Clin Nutr.* 1982;36(5):936–42).
A table showing the basic characteristics of the individuals considered in this study.

measurements were collected and BMI was calculated. BMI was defined as weight divided by height squared (kg/m^2). Obesity status has been grouped as normal weight (BMI 18.50–24.99), overweight (BMI 25.00–29.99), and obese (≥30) as recommended by the World Health Organization (WHO).[26] Percent trunk fat was measured utilizing DXA (Lunar Prodigy, GE Medical Systems, Madison, WI, USA). DXA produces an accurate measurement of adipose tissue in the body with a low margin of error. The enCORE software package (version 12.2, GE Medical Systems, Madison, WI, USA) was used for DXA data acquisition.

Physical activity. Levels of physical activity were measured using the Ability of the Atherosclerosis Risk in Communities (ARIC) Questionnaire,[27] which consists of a Work Index, Sports Index, and Leisure Time Activity Index. A variable exclusive of workplace activity was used in the data analysis. This variable is continuous, however, three categories were constructed to organize individuals into low, moderate, and high physical activity, in order to generalize results and make them more accessible. Indices of 5 or less were classified as low, greater than 5 but less than 8 were classified as moderate, and indices of 8 and higher were classified as high activity.

Genotyping. Genotyping was completed using a drawn blood sample from each individual. Genotyping of SNPs rs9939609, rs1421085, rs1121980, rs7193144, rs8050136, rs9939973, rs16945088, rs17817449, rs3751812, rs9935401, and rs9941349 was performed using the high-throughput MassARRAY® platform (Sequenom Inc, San Diego, CA, USA). Amplification, Shrimp Alkaline Phosphatase (SAP) digestion, and extension were performed according to the manufacturer's instructions. Unincorporated deoxyribonucleotides were SAP digested prior to MassARRAY® iPLEX Gold allele specific extension with mass-modified dideoxynucleotides using a MassARRAY iPLEX® Gold reagent kit. Extension products were desalted and dispensed onto a SpectroChip (Sequenom Inc, San Diego, CA, USA) using a MassARRAY® Nanodispenser prior to matrix-assisted laser desorption/ionization time-of-flight mass spectrometer analysis with a MassARRAY® Compact Analyzer mass spectrometer. Genotypes were assessed using MassARRAY® Typer Analyzer version 4.0. SNP genotyping success rate was over 99%. The genetic information of the individuals studied is presented in Table 2a.

Statistical analysis. All analyses were conducted using R version 3.0.1 and were repeated using both the complete sample of individuals, as well as a subsample of 1,843 independent, nonfamilial individuals, in order to verify that results were not biased due to genetic resemblance of related individuals. For all SNPs in this study aside from rs16945088, Chi-Squared tests were performed to determine whether or not the population followed Hardy–Weinberg proportions. For rs16945088, due to insufficient observations of individuals with *GG* genotype to warrant a Chi-Squared test, *P* values were calculated from binomial testing. The binomial tests compared the observed cases of these individuals with the expected probabilities under the assumption of Hardy–Weinberg equilibrium. Linkage disequilibrium was examined for each pair of SNPs based on R^2 values. Hardy-Weinberg equilibrium tests and linkage disequilibrium analyses were completed using the *genetics* package (version 1.3.8.1) in R.

A *t*-test comparing the trunk fat percentage for males and females was performed, resulting in stratification by gender. Analysis of association between trunk fat percentage and each SNP was performed using a general linear model, assuming an additive or dominant model for each SNP, depending on the results of Cochran–Armitage Trend Tests. For each SNP, three Cochran–Armitage Trend Tests (one assuming dominant to the first allele, one assuming dominant to the second allele, and one assuming an additive model) were performed, and the model associated with the lowest *P* value for each SNP was considered for further analysis. To compare the effect of genetic variants in each SNP on trunk fat percentage, *t*-tests were also performed to evaluate the difference in mean trunk fat percentage between each pair of allelic variants within each SNP. The tests performed were based on the genetic model resulting from the Cochran–Armitage Trend Test results. Multiple regression models were used to examine the association between physical activity and trunk fat percentage, and were adjusted for age and variants in each SNP. The interaction between the SNP variants and physical activity on trunk fat percentage was examined using analysis of variance tests and non-parametric Kruskall–Wallis tests. *P* values less than 0.05 were considered to be significant for all statistical analysis.

Results

Hardy–Weinberg proportions and linkage disequilibrium. Using Fisher's exact test, and a binomial test for rs16945088, the null hypothesis that the population is in Hardy–Weinberg equilibrium was tested. Since the analysis of related individuals can lead to bias in Hardy–Weinberg proportion testing,[28] each test was also performed on the subsample of unrelated individuals. The *P* values for all SNPs were outside of the rejection region, thus validating the assumption of Hardy–Weinberg equilibrium in the population studied. The *P* values for all SNPs can be seen in Supplementary Tables 1 and 2. The minor allele frequencies observed for rs9939609 were 40.8% and 37.6%, respectively, for males and females. Similarly, the minor allele frequencies for rs1421085 were 43.1% and 39.0% for males and females, respectively. The difference in minor allele frequencies between males and females was significant for both SNPs mentioned. The minor allele frequencies of all other SNPs studied may be seen in Table 2b. Linkage disequilibrium between SNPs was examined using R^2 values. All R^2 values for the full sample may be viewed in Supplementary Table 3. Since all observed values are above 0.8 (aside from those associated with rs16945088)

Table 2a. Basic genotyping information from the Complex Diseases in the Newfoundland Population: Environment and Diseases (CODING) study by gender.

SNP	FREQUENCY*	GENOTYPE	MALE		FEMALE	
			FREQUENCY	%	FREQUENCY	%
rs9939973	893	*GG*	68	29.7	226	34.0
		AG	118	51.5	340	51.2
		AA	43	18.8	98	14.8
rs1421085	2435	*CC*	109	17.7	274	15.1
		CT	312	50.7	872	47.9
		TT	194	31.6	674	37.0
rs1121980	892	*CC*	67	29.6	228	34.2
		TC	116	51.3	339	50.9
		TT	43	19.1	99	14.9
rs7193144	894	*CC*	40	17.5	91	13.7
		TC	113	49.3	322	48.4
		TT	76	33.2	252	37.9
rs16945088	638	*GG*	0	0	4	0.8
		AG	26	17.6	53	10.7
		AA	122	82.4	437	88.5
rs17817449	639	*GG*	25	16.9	80	16.3
		GT	77	52.0	226	46.0
		TT	46	31.1	185	37.7
rs8050136	894	*CC*	76	33.5	249	37.3
		CA	113	49.8	326	48.9
		AA	38	16.7	92	13.8
rs9935401	634	*GG*	48	33.1	184	37.6
		AG	74	51.0	224	45.8
		AA	23	15.9	81	16.6
rs3751812	640	*GG*	46	31.1	183	37.2
		GT	77	52.0	227	46.1
		TT	25	16.9	82	16.7
rs9939609	2428	*AA*	100	16.3	260	14.3
		TA	300	48.9	845	46.6
		TT	213	34.8	710	39.1
rs9941349	631	*CC*	42	29.4	177	36.3
		CT	76	53.1	230	47.1
		TT	25	17.5	81	16.6

Notes: *Frequency refers to the total number of participants for which genetic information was available for each SNP.
A table showing the basic genetic information of the individuals considered in this study.

and have high power to detect associated genetic effects at reasonable levels of heritability (as seen from Supplementary Fig. 1), it is assumed that no putative variant associated with the analyzed SNPs has been missed.

Candidate SNPs association study. Based on a *t*-test comparing the trunk fat percentage for both the genders ($P = 0$) and differences in minor allele frequencies, all models were assessed separately by gender. For each SNP, an additive or dominant model was considered based on the smallest *P* value from Cochran–Armitage Trend Tests. The results of these tests can be seen in Table 3. Multiple *t*-tests were performed for each gender comparing the mean trunk fat percentages of different variants in each SNP. The Bartlett test of homogeneity of variance confirmed the assumption of constant variance within variants of each SNP, thus pooled sample variance was used for each test. The results of the multiple *t*-tests and the *P* value for each test can be seen in Table 4. Each *P* value was compared to an adjusted *P* value using the Benjamini–Hochberg procedure at level 0.05 to determine whether the result was significant, and these outcomes are also

Table 2b. Minor allele frequencies of individuals from the Complex Diseases in the Newfoundland Population: Environment and Diseases (CODING) study.

	FULL SAMPLE			INDEPENDENT SAMPLE		
	OVERALL	MALE	FEMALE	OVERALL	MALE	FEMALE
rs9939973	41.3%	44.5%	40.4%	41.7%	45.6%	40.3%
rs1421085	40.0%	43.1%	39.0%	40.0%	43.4%	39.0%
rs1121980	41.3%	44.7%	40.3%	41.9%	45.5%	40.6%
rs7193144	38.9%	42.1%	37.9%	40.3%	44.4%	38.8%
rs16945088	6.7%	8.8%	6.2%	7.6%	12.1%	6.5%
rs17817449	40.0%	42.9%	39.3%	47.5%	48.2%	46.5%
rs8050136	39.0%	41.6%	38.2%	40.1%	43.7%	38.9%
rs9935401	39.7%	41.4%	39.5%	47.9%	50.0%	47.4%
rs3751812	40.3%	42.9%	39.7%	48.6%	51.7%	47.8%
rs9939609	38.4%	40.8%	37.6%	38.6%	41.1%	37.8%
rs9941349	40.9%	44.1%	40.2%	49.3%	53.8%	48.3%

A table showing the minor allele frequencies for each single-nucleotide polymorphism (SNP) considered in this study. For SNPs with a different minor allele in males and females, the overall minor allele is represented for both genders.

displayed in Table 4. There is a strong relationship between both rs9939609 and rs1421085, and trunk fat percentage for males, but a similar relationship is not significant for the other SNPs explored, or for females. Male carriers of rs9939609 risk genotype *AA* had mean trunk fat percentage that was 4.2 units higher than homozygotes of the common genotype *TT* (means were different with a *P* value of 0.0001). This difference was not significant for females. For rs1421085, homozygote males of the risk *CC* genotype had mean trunk fat percentage that was 3.8 units higher than homozygote *TT* males (*P* value of 0.0004), while the difference for females was again not significant. When environmental factors are ignored, genetic variants in these two SNPs have a greater effect on trunk fat percentage for males than for females (see Fig. 1). One study states that the rs17817449 SNP in the *FTO* gene was related to an increase in BMI,[29] but as in this study, the relationship between the *FTO* gene and BMI existed only in males and postmenopausal females. This result may relate to our study since 77.3% of the females we considered were below the age of 52 years, the average age of menopause suggested by the Canadian Women's Health Network.[30]

Table 3. Cochran–Armitage trend test results for testing dominance of single-nucleotide polymorphisms (SNPs) explored from the Complex Diseases in the Newfoundland Population: Environment and Diseases (CODING) study.

	MODEL SELECTED	*P* VALUE FOR SELECTED MODEL/S
rs9939973	G dominant	0.055
rs1421085	Additive	0.010
rs1121980	C dominant	0.063
rs7193144	Additive/T dominant	0.050/0.023
rs16945088	Additive/G dominant	0.004/0.004
rs17817449	T dominant	0.069
rs8050136	C dominant	0.029
rs9935401	G dominant	0.112
rs3751812	G dominant	0.069
rs9939609	Additive	0.009
rs9941349	C dominant	0.143

A table showing the results from three Cochran–Armitage trend tests for each SNP explored. These tests were used to determine appropriate dominance models. The model whose p-value was lowest for each SNP was selected.

Physical activity. The three categories of physical activity in this study were introduced as two dummy variables: moderate activity and high activity. Physical activity on its own is a significant explanatory variable for trunk fat percentage, for both males and females. Young females with high levels of physical activity appeared to have lower trunk fat percentage than young inactive females, and this effect seemed to decrease as age increased (although the effect was not statistically significant). The same relationship was not apparent for males (Fig. 2). For males studied, mean trunk fat percentages for individuals with low, moderate, and high activity were 34.6, 29.6, and 22.8, respectively. For females, these values were 42.2, 38.4, and 31.6, respectively. Trunk fat percentages for high and low activity were significantly different for both males and females ($P = 0$). Although increased physical activity results in decreased trunk fat percentage, the following section describes how the magnitude of this effect may change with age and genotype of the SNPs explored in this study.

Interaction of *FTO* variants with age and physical activity. If the magnitude of genetic influence differs as a function of physical activity, it would suggest that gene–environment interaction is present for the outcome of interest, trunk fat percentage. The mean trunk fat percentages

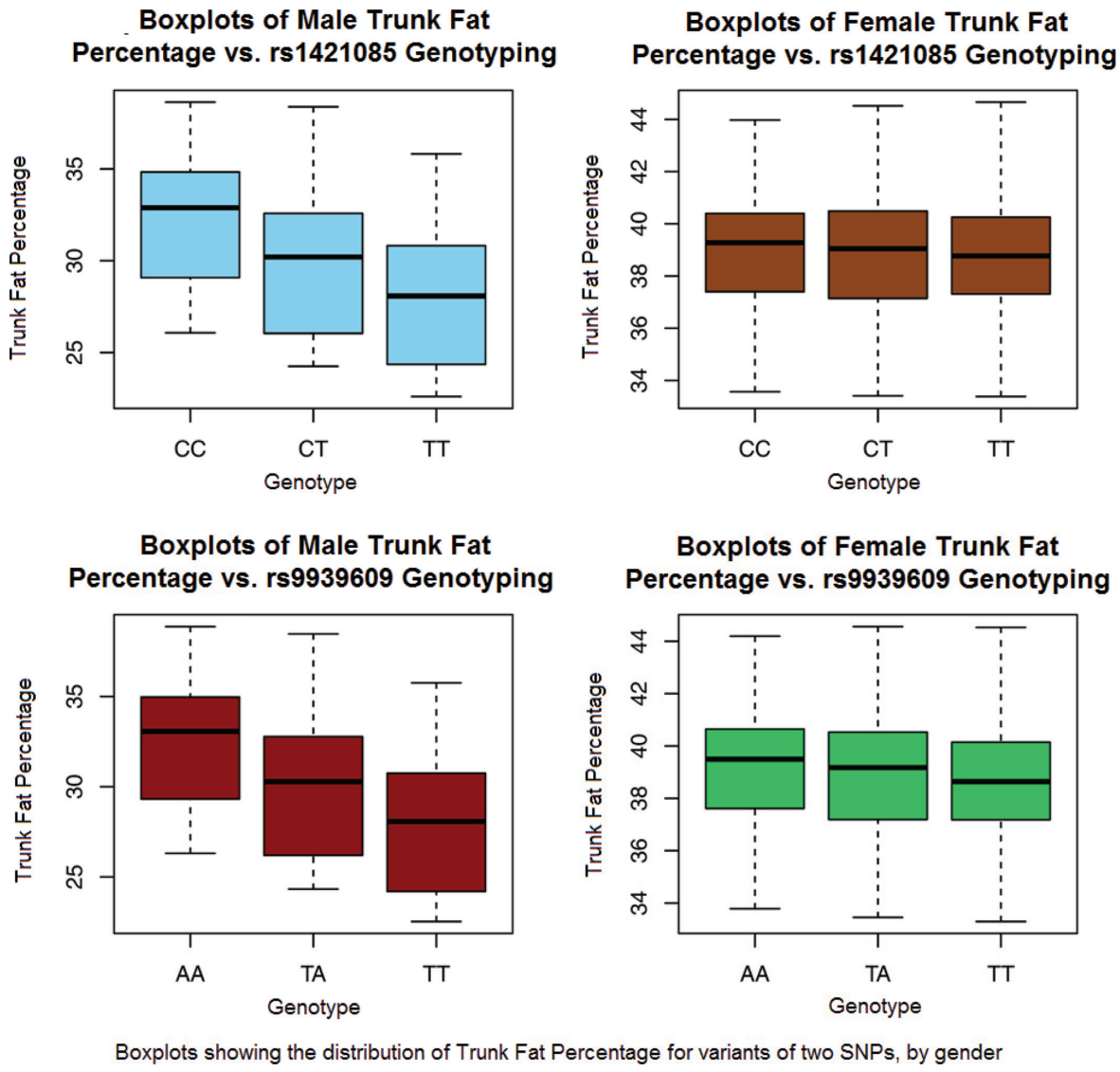


Figure 1. A graph of boxplots showing the distribution of trunk fat percentage for males and females for SNPs rs9939609 and rs1421085. Percent trunk fat is measured in percentage (%).

for highly active males that are homozygotes of the obesity-risk alleles for both rs9939609 and rs1421085 were significantly higher than for highly active homozygote males of the non-obesity-risk alleles. When considering one-way ANOVA, Tukey's test, and the Kruskall–Wallis test, this difference was significant only for rs9939609 and rs1421085, with a maximum *P* value of 0.020 for all tests on these two SNPs. The difference of mean trunk fat percentage between genetic variants was not, however, significant for those males with low or moderate activity.

Figure 3 shows that for those highly inactive individuals, genotype does not tend to affect trunk fat percentage. However, as physical activity increases, those individuals who are less susceptible to obesity tend to have lower trunk fat percentage than homozygotes of the obesity-risk alleles. To view this interaction effect by age, we modeled the interaction of the categorical physical activity variable with the rs9939609 SNP, adding age as a covariate. Figure 4 summarizes the result of this model, again supporting the previous claim. Genetic heritability creates the greatest variation in trunk fat percentage for active males, and has no significant effect for males with lower amounts of activity, or for females.

Discussion

In this study, we applied gene–environment interaction models to test whether or not the magnitude of genetics-associated effects on trunk fat percentage may be modified by physical activity. Our results showed the presence of interactions between genetic susceptibility of obesity caused by two SNPs in the *FTO* gene and physical activity. We found that for males only, physical activity accentuated the effect of rs9939609 and rs1421085 on obesity as indexed by trunk fat

Table 4. *T*-tests for difference in mean trunk fat percentage between variants in single-nucleotide polymorphisms from the Complex Diseases in the Newfoundland Population: Environment and Diseases (CODING) study.

SNP	TEST	MALE		FEMALE	
		P VALUE	ADJUSTED *P* VALUE	*P* VALUE	ADJUSTED *P* VALUE
rs9939973	(GG, AG)–AA	0.177	0.0342	0.234	0.0214
rs1421085	CC–TT	0.000448*	0.00526	0.363	0.0429
	CC–CT	0.00650*	0.00789	0.251	0.0286
	TC–TT	0.106	0.0289	0.331	0.0381
rs1121980	(CC, TC)–TT	0.199	0.0368	0.252	0.0310
rs7193144	(TC, TT)–CC	0.0951	0.0211	0.240	0.0262
	CC–TT	0.0310	0.0132	0.213	0.0167
	CC–TC	0.236	0.0447	0.288	0.0357
	TC–TT	0.0408	0.0184	0.383	0.0476
rs16945088	(GG, AG)–AA	0.0978	0.0237	0.226	0.0190
	GG–AA	–	–	0.381	0.0452
	GG–AG	–	–	0.467	0.0500
	AG–AA	0.0978	0.0263	0.240	0.0238
rs17817449	(GT, TT)–GG	0.201	0.0395	0.0330	0.00714
rs8050136	(CC, CA)–AA	0.129	0.0316	0.210	0.0143
rs9935401	(GG, AG)–AA	0.320	0.0474	0.0269	0.00476
rs3751812	(GG, GT)–TT	0.204	0.0421	0.0333	0.00952
rs9939609	AA–TT	0.000117*	0.00263	0.180	0.0119
	AA–TA	0.00780*	0.0105	0.257	0.0333
	TA–TT	0.0391	0.0158	0.352	0.0405
rs9941349	(CC, CT)–TT	0.320	0.0500	0.0218	0.00238

Notes: *Indicates significant when compared to its adjusted p-value, calculated from using a family confidence level of 0.05 over 19 simultaneous tests for males, and over 21 simultaneous tests for females.
A table showing the *t*-test results for difference in means when comparing mean trunk fat percentage between each pair of possible variants within single-nucleotide polymorphisms. For cases where two possible dominance models were selected from Cochran–Armitage trend tests, mean trunk fat percentages were compared using both plausible models.

percentage. However, a similar result was not significant for the other nine SNPs examined. The novel finding of this study is that physical activity modified the predisposition on trunk fat percentage caused by the *FTO* gene, with a high level of physical activity increasing the additive genetic predisposition of high trunk fat percentage. These models, based on DXA measurements of trunk fat percentage, have not been explored before. We have also shown that high physical activity is significantly associated with a reduction of trunk fat percentage for both males and females, regardless of genetic variants of the SNPs explored. Other studies have yielded consistent results using waist circumference as a measure of obesity instead of trunk fat percentage.[31,32]

Our study also shows that physically active males were significantly affected by the genetic variants of rs9939609 and rs1421085 in contrast to those with low amounts of activity. Although these reductions were similar for females, they were not statistically significant. This inconsistency may result from gender difference. Physically active male homozygotes of the common alleles showed significantly lower trunk fat percentages than physically active homozygotes for the obesity-risk alleles of the above SNPs. These results are similar to some previous studies, which have detected a genetic influence on weight changes for males with high levels of physical activity, but not females.[33,34]

This study benefits from a large sample of the NL population. NL's generally isolated and homogeneous people provide a suitable population for generalized genetic results,[25] and the large sample allowed validating results based on a relatively large subsample of independent (unrelated) individuals. Although there may be possible error or bias in self-reported physical activity level, Baecke's method for assessment of physical activity has been shown to be highly effective.[35] Trunk fat percentage in this study has been measured by DXA, which provides a more accurate measurement of trunk fat percentage compared with other methods of measurement. Gender and age, two major confounders, were considered and controlled as well.

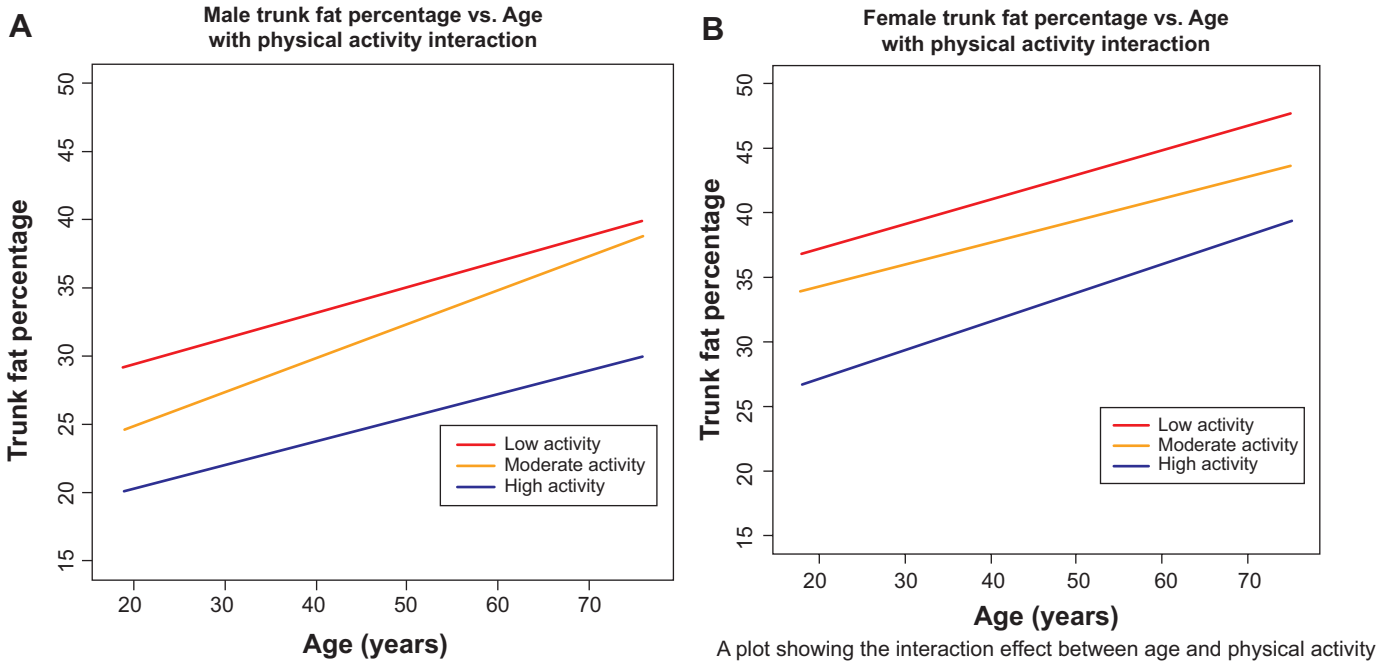
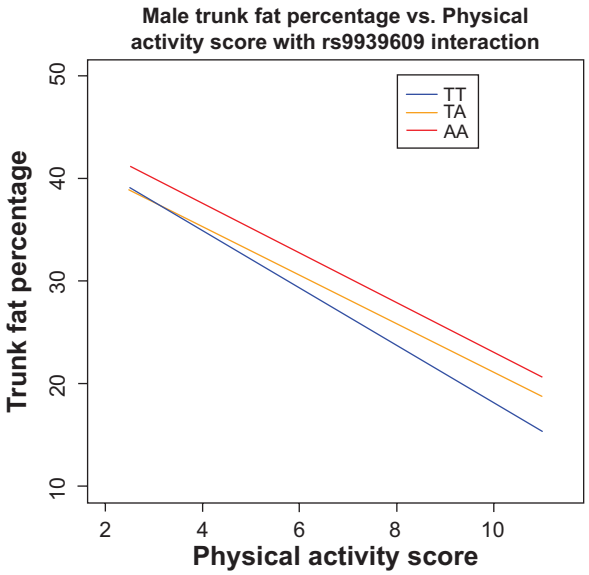


Figure 2. Graphs showing the effects of different levels of physical activity on trunk fat percentage for males and females, by age. Percent trunk fat is measured in percentage (%), and age is measured in years.

In our study, age was found to be a significant moderator, as expected. In physical activity–age interaction models, our results (although not significant) suggest that the effect of high physical activity in males on the heritability of trunk fat percentage strengthens with increasing age. However, further studies are required to address the contribution of age groups.

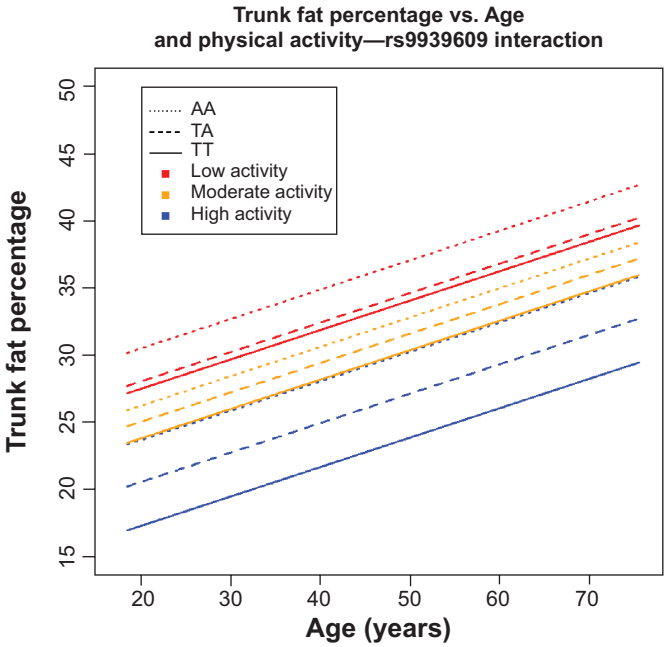


A plot showing how rs9939609 modifies the effect of physical activity

Figure 3. The effect of increased physical activity for each variant of rs9939609. Percent trunk fat is measured in percentage (%), and physical activity is measured by the score obtained using the ARIC Questionnaire (*Am J Clin Nutr.* 1982;36(5):936–42).

There are some limitations in this study. A study with a larger sample size would provide higher power in detecting effects. Increased statistical power would be especially important in the gene–environment interaction analyses. In addition, our study has some limitations regarding the ability to generalize since it consists of volunteers rather than random individuals. Several dietary intake variables were considered in preliminary analysis for this study. However, their interactions with the *FTO* SNPs studied did not have any significant effects on trunk fat percentage for males or females. It is possible, however, that other dietary or environmental information not considered in this study may potentially affect the genetic heritability of high levels of trunk fat percentage. Furthermore, our study was based on cross-sectional models, and we were not able to determine whether high physical activity is effective in explaining trunk fat percentage over time. Aside from rs9939609 and rs1421085 which were selected due to previously studied associations, all other SNPs for this study were selected due to availability of data. The position of each SNP can be seen in Supplementary Table 4. There were far more individuals in our database with information recorded for rs9939609 and rs1421085 compared with the other SNPs. It is possible that other genes and SNPs not studied may further, or even better, explain genetic predisposition to obesity. For example, a recent study suggests that the *IRX3* gene expression may be effective in explaining body mass, and that obesity-associated regions of *FTO* may actually be regulated by *IRX3* expression.[36]

Our study suggests that higher physical activity is beneficial in reducing central obesity. These results are consistent



A plot showing the effect of rs9939609 at different levels of physical activity

Figure 4. The effect of rs9939609 and physical activity interaction, by age. Percent trunk fat is measured in percentage (%), and age is measured in years.

with the basic concept that even moderate physical activity helps to relieve obesity at the population level. However, for people with specific genetic predisposition, particularly male *FTO* risk carriers, a higher level of physical activity is required to achieve a significant beneficial effect in reducing trunk fat percentage.

Acknowledgments

The authors are grateful to Ms. Hongwei Zhang and Ms. Peyvand Amini for their contributions in providing data for analysis, and are also thankful to Dr Laurent Briollais for his generous time in reviewing this manuscript and for providing insight toward its improvement.

Author Contributions

AP produced the original draft of this manuscript, consolidated future drafts, performed the majority of statistical analysis, and produced tables and figures. FC collected subject data, genotyped subjects, assembled the dataset, produced an updated manuscript draft, and was a primary consultant on the subjects and methodology involving data collection. GS acted as the primary genetic consultant throughout the project, produced an updated draft of the manuscript, and is the primary supervisor of the research laboratory from which all data were collected. JCL-O provided consultation and interpretation of linkage disequilibrium analysis, and provided an updated edition of the manuscript. TA supervised research, analysis and manuscript composition, provided statistical interpretation, composed a draft of the discussion, and produced an updated draft of the manuscript. All authors reviewed and approved of the final manuscript.

Supplementary Data

Supplementary table 1. *P*-values obtained from testing whether each SNP is in Hardy–Weinberg proportions under the null hypothesis that the SNP is in Hardy–Weinberg proportions for different subsamples of the full sample.

Supplementary table 2. *P*-values obtained from testing whether each SNP is in Hardy–Weinberg proportions under the null hypothesis that the SNP is in Hardy–Weinberg proportions for different subsamples of the sample of unrelated individuals.

Supplementary table 3. R^2 values for linkage disequilibrium analysis between each pair of SNPs analyzed.

Supplementary table 4. A table showing the position of each SNP studied.

Supplementary figure 1. A graph showing powers corresponding to certain levels of heritability and R^2 values obtained from linkage disequilibrium analysis between SNPs.

REFERENCES

1. World Health Organization [Internet]. Obesity and overweight; 2013 March [cited 2013 May 17]. Available at http://www.who.int/mediacentre/factsheets/fs311/en/
2. Statistics Canada [Internet]. Measured adult body mass index (BMI), by age group and sex, household population aged 18 and over excluding pregnant females, Canada (excluding territories); 2009 June 24 [cited 2013 May 16]. Available at http://www5.statcan.gc.ca/cansim/a26?lang=eng\&retrLang=eng\&id=1050507\&paSer=\&pattern=\&stByVal=1\&p1=1\&p2=-1\&tabMode=dataTable\&csid=
3. Statistics Canada [Internet]. Body mass index, overweight or obese, self-reported, adult, by sex, provinces and territories; 2013 May 2 [cited 2013 May 13]. Available at http://www.statcan.gc.ca/tables-tableaux/sum-som/l01/cst01/health82b-eng.htm
4. Frayling TM, Timpson NJ, Weedon MN, et al. A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. *Science*. 2007;316(5826):889–894.
5. Willer CJ, Speliotes EK, Loos RJ, et al. Six new loci associated with body mass index highlight a neuronal influence on body weight regulation. *Nat Genet*. 2008;41(1):25–34.
6. Thorleifsson G, Walters GB, Gudbjartsson DF, et al. Genome-wide association yields new sequence variants at seven loci that associate with measures of obesity. *Nat Genet*. 2009;41(1):18–24.
7. O'Rahilly S, Farooqi IS. Human obesity: a heritable neurobehavioral disorder that is highly sensitive to environmental conditions. *Diabetes*. 2008;57(11):2905–2910.
8. Björntorp P. Body fat distribution, insulin resistance, and metabolic diseases. *Nutrition*. 1997;13(9):795–803.
9. Larsson B, Svärdsudd K, Welin L, Wilhelmsen L, Björntorp P, Tibblin G. Abdominal adipose tissue distribution, obesity, and risk of cardiovascular disease and death: 13 year follow up of participants in the study of men born in 1913. *Br Med J (Clin Res Ed)*. 1984;288(6428):1401–1404.
10. Donahue RP, Abbott RD, Bloom E, Reed DM, Yano K. Central obesity and coronary heart disease in men. *Lancet*. 1987;1(8537):821–824.
11. Shah NR, Braverman ER. Measuring adiposity in patients: the utility of Body Mass Index (BMI), percent body fat, and leptin. *PLoS One*. 2012;7(4):e33308.
12. Kennedy AP, Shea JL, Sun G. Comparison of the classification of obesity by BMI vs. dual-energy X-ray absorptiometry in the Newfoundland population. *Obesity (Silver Spring)*. 2009;17(11):2094–2099.
13. Hunt SC, Stone S, Xin Y, et al. Association of the FTO gene with BMI. *Obesity (Silver Spring)*. 2008;16(4):902–904.
14. Xi B, Wang C, Wang R, Huang Y. FTO gene polymorphisms are associated with obesity and type 2 diabetes in east Asian populations: an update. *Obesity (Silver Spring)*. 2011;19(2):236–237.
15. Villalobos-Comparán M, Teresa Flores-Dorantes M, Teresa Villarreal-Molina M, et al. The FTO gene is associated with adulthood obesity in the Mexican population. *Obesity (Silver Spring)*. 2008;16(10):2296–2301.

16. Tanofsky-Kraff M, Han JC, Anandalingam K, et al. The FTO gene rs9939609 obesity-risk allele and loss of control over eating. *Am J Clin Nutr*. 2009;90(6): 1483–1488.
17. Cha SW, Choi SM, Kim KS, et al. Replication of genetic effects of FTO polymorphisms on BMI in a Korean population. *Obesity (Silver Spring)*. 2008;16(9): 2187–2189.
18. King GA, Fitzhugh EC, Bassett DR Jr, et al. Relationship of leisure-time physical activity and occupational activity to the prevalence of obesity. *Int J Obes Relat Metab Disord*. 2001;25(5):606–612.
19. Bernstein MS, Costanza MC, Morabia A. Association of physical activity intensity levels with overweight and obesity in a population-based sample of adults. *Prev Med*. 2004;38(1):94–104.
20. Yoshioka M, Ayabe M, Yahiro T, et al. Long-period accelerometer monitoring shows the role of physical activity in overweight and obesity. *Int J Obes*. 2005; 29(5):502–508.
21. Andreasen CH, Stender-Petersen KL, Mogensen MS, et al. Low physical activity accentuates the effect of the FTO rs9939609 polymorphism on body fat accumulation. *Diabetes*. 2008;57(1):95–101.
22. Vimaleswaran KS, Li S, Zhao JH, et al. Physical activity attenuates the body mass index-increasing influence of genetic variation in the FTO gene. *Am J Clin Nutr*. 2009;90(2):425–428.
23. Mangge H, Renner W, Almer G, Weghuber D, Möller R, Horejsi R. Rs9939609 variant of the fat mass and obesity-associated gene and trunk. Obesity in adolescents. *J Obes*. 2011;2011:186368.
24. Mcfarlin B, Jackson A, Ellis K, et al. Cross-validation of lipometer estimates of body composition: the effect of gender and skin color. *Obes Res*. 2006;14:A142.
25. Rahman P, Jones A, Curtis J, et al. The Newfoundland population: a unique resource for genetic investigation of complex diseases. *Hum Mol Genet*. 2003; 12(suppl 2):R167–R172.
26. World Health Organization [Internet]. BMI classification; 2013 January [cited 2013 January 13]. Available at http://apps.who.int/bmi/index.jsp?introPage=intro_3.html
27. Baecke JA, Burema J, Frijters JE. A short questionnaire for the measurement of habitual physical activity in epidemiological studies. *Am J Clin Nutr*. 1982;36(5): 936–942.
28. Terwilliger JD. Inflated false-positive rates in Hardy–Weinberg and linkage-equilibrium tests are due to sampling on the basis of rare familial phenotypes in finite populations. *Am J Hum Genet*. 2000;67(1):258–259.
29. Hubacek JA, Pitha J, Adamkova V, Lanska V, Poledne R. A common variant in the FTO gene is associated with body mass index in males and postmenopausal females but not in premenopausal females. Czech post-MONICA and 3PMFs studies. *Clin Chem Lab Med*. 2009;47(4):387–390.
30. Canadian Women's Health Network [Internet]. Menopause; 2006 June [cited 2013 May 16]. Available at http://www.cwhn.ca/en/faq/menopause
31. Mustelin L, Silventoinen K, Pietilainen K, Rissanen A, Kaprio J. Physical activity reduces the influence of genetic effects on BMI and waist circumference: a study in young adult twins. *Int J Obes*. 2009;33:29–36.
32. Waller K, Kaprio J, Kujala UM. Associations between long-term physical activity, waist circumference and weight gain: a 30-year longitudinal twin study. *Int J Obes*. 2008;32(2):353–361.
33. Heitmann BL, Kaprio J, Harris JR, Rissanen A, Korkeila M, Koskenvuo M. Are genetic determinants of weight gain modified by leisure-time physical activity? A prospective study of Finnish twins. *Am J Clin Nutr*. 1997;66(3):672–678.
34. McCaffery JM, Papandonatos GD, Bond DS, Lyons MJ, Wing RR. Gene X environment interaction of vigorous exercise and body mass index among male Vietnam-era twins. *Am J Clin Nutr*. 2009;89(4):1011–1018.
35. Philippaerts RM, Lefevre J. Reliability and validity of three physical activity questionnaires in Flemish males. *Am J Epidemiol*. 1998;147:982–990.
36. Smemo S, Tena JJ, Kim K-H, et al. Obesity-associated variants within *FTO* form long-range functional connections with *IRX3*. *Nature*. 2014;507:371–375.