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FTO genotype and adiposity in children: physical activity levels influence the effect of the risk genotype in adolescent males

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ABSTRACT

Studies of the fat mass and obesity associated (*FTO*) gene provide compelling evidence of genetic variation in the general population that influences fat levels and obesity risk. Studies of the interaction between genetic and environmental factors such as physical activity will promote understanding of how lifestyle can modulate genetic contributions to obesity. In the current study, we investigated the effect of *FTO* genotype, and interactions with physical activity or energy intake, in young children and adolescents. 1-5 year old children from the GENESIS study ($N = 1980$) and 11-18 year old Greek adolescents ($N = 949$) were measured for adiposity-related phenotypes and genotyped at the *FTO* SNP marker, rs17817449. Adolescents were classified as physically active or inactive based on self-reported levels of physical activity (PA). In adolescents, *FTO* genotype influenced weight ($P = 0.001$), and BMI ($P = 0.007$). There was also a significant SNP*PA*gender interaction ($P = 0.028$) on BMI, which reflected the association between *FTO* genotype and BMI in males ($P = 0.016$) but not females ($P = 0.15$) and significant SNP*PA interaction in males ($P = 0.007$) but not females ($P = 0.74$). The *FTO* genotype effect was more pronounced in inactive males than active. Inactive males homozygous for the G allele had a mean BMI of 3 kg/m^2 higher than T-carriers ($P = 0.008$). In the GENESIS study, no significant association between *FTO* genotype and adiposity was found. The present findings highlight physical activity as a key factor modifying the effect of *FTO* genotype.

INTRODUCTION

Childhood obesity is a leading risk factor for the metabolic syndrome and type 2 diabetes, as well as potentially for subsequent cardiovascular disease in adulthood¹⁻³. Obesity has a complex aetiology but is clearly influenced both by genetic variation and by environmental factors such as diet and lifestyle⁴. Physical activity, for example, is recognised as a prevention tool for obesity, is known to increase energy expenditure and also has the potential to regulate appetite⁵, although the relationship between physical activity and obesity is not clearly defined⁶.

Studies of the recently discovered fat mass and obesity associated (*FTO*) gene have provided the most compelling evidence to date of genetic variation in the general population that influences fat levels and risk of obesity^{7:8}. A set of single nucleotide polymorphisms (SNPs) in the first intron of *FTO* are strongly associated with adiposity/obesity and related phenotypes⁷⁻¹⁷. It has been shown that adults homozygous for the risk allele at rs9939609 weigh on average ~3kg more than those homozygous for the other allele⁷. *FTO* genotype has also been reported to be associated with adiposity in children⁷⁻⁹, and the strength of its influence to increase during childhood¹⁰.

The function of *FTO* is currently unknown but is becoming clearer. It is widely expressed throughout the body and highly so in the arcuate nucleus, a region of the hypothalamus involved in regulating energy balance¹⁸. Fischer et al.¹⁹ reported recently that knocking out *Fto* in mice results in reduced adiposity via a mechanism apparently involving increased energy expenditure, sympathetic tone and levels of

catecholamine hormones, which contrasts with studies to date in humans that implicate hyperphagia without altered energy expenditure^{9;20-23;23}.

Despite having the largest influence on obesity-related phenotypes of any gene reported thus far, *FTO* genotype alone only accounts for a small proportion of the variance in these phenotypes. The full influence of *FTO* will only become clear through more thorough investigation of its interactions with environmental factors such as physical activity levels. Three studies have reported interactions between physical activity and *FTO* genotype in adults, with low activity levels being associated with enhanced genotype effects²⁴⁻²⁶, while other studies have reported no interaction with physical activity^{17;27}. However, to the best of our knowledge, no such studies have been conducted in pre-adults. We report here the influence of rs17817449 on adiposity in two cohorts of children, with ages ranging from one to eighteen years. In adolescent children we also investigated how physical activity levels modulate the *FTO* effects on adiposity-related phenotypes.

METHODS

SUBJECTS

Study participants were those of two previously published cohorts^{28;29}. One cohort comprised subjects from the Growth, Exercise and Nutrition Epidemiological Study In preSchoolers (GENESIS)²⁸, which included 1980 healthy children aged 1–5 years from throughout Greece collected between April 2003 and July 2004. 52% were male. The other cohort comprised 949 children aged 11-18 years, 53% of whom were male,

collected from the Trikala region in central Greece between February 2000 and April 2001²⁹. Both studies were approved by the ethics committee of the University of Glasgow and by local authorities in Greece. Written informed consent was obtained from the parents of all participants prior to the study.

DATA COLLECTION

Data were collected as described below after a period of protocol standardization in both cohorts. In the GENESIS cohort, all measurements were made as previously described in detail²⁸. This included basic body composition measurements (weight, height, and waist, hip and mid-upper arm circumferences), as well as skinfolds at four sites (biceps, triceps, subscapular and suprailiac). Birthweight data for this cohort were also available from the child health booklet. Energy intake data were collected from all children for three days using a combination of weighed food records and 24 hr recall or food diaries, as previously described in detail²⁸ and in the supplementary material. Physical activity (PA) data were not available in the GENESIS cohort. In the adolescent cohort, subjects were measured for height, weight, and triceps and subscapular skinfolds as previously described²⁹ and completed a questionnaire documenting their participation in physical activity both inside and outside of school. Individuals reporting no additional physical activity outside of the compulsory weekly physical education class at school were classified as inactive ($N = 289$; 98 males and 191 females), while those reporting participation in further physical activity were classified as active ($N = 660$)²⁹. Inactive individuals displayed higher subscapular skinfolds, after adjustment for age and gender, than those classified as active ($P = 0.02$) and displayed a trend towards higher BMI ($P = 0.075$) and triceps skinfold ($P =$

0.077) but not weight ($P = 0.39$). Dietary intake data were not available in the adolescent cohort.

GENOTYPING AND DATA ANALYSIS

SNP variant rs17817449 was genotyped in both cohorts by a PCR-RFLP method, digesting the PCR product with *AlwNI* enzyme. The forward primer was 5'-CGGTGAAGAGGAGGAGATTG-3' and the reverse primer 5'-CATCTCTGCCCCAGTTTCTC-3'. The G allele generated an undigested 223bp product while the T allele yielded 123bp and 100bp fragments after digestion. Genotyping was successful in 94% of subjects in the GENESIS cohort and in 96% of subjects in the adolescent cohort. G allele frequency at rs17817449 was 0.42 in the adolescent cohort and 0.43 in GENESIS. rs17817449 genotypes were in Hardy-Weinberg equilibrium (HWE) in both the adolescent ($P = 0.17$) and GENESIS cohorts ($P = 0.14$). rs17817449 is in complete linkage disequilibrium (in European populations) with the more commonly measured SNP rs9939609 ($r^2 = 1$).

STATISTICAL ANALYSIS

Groups were tested for normality using the Ryan-Joiner test, and each phenotype transformed (see Supplementary Material) so as to achieve as close to a normal distribution as possible across each cohort. After transformation, most phenotypes were normal, or approaching normality. Data were analyzed using R. We initially investigated the effect of FTO genotype on adiposity phenotypes by ANCOVA. Hypothesizing that genotype effects might also differ in age or gender subgroups, we subsequently tested for interactions using a backward stepwise ANCOVA approach. The starting model contained a four-way (SNP* PA*gender*age) interaction term and

all hierarchically necessary sub-terms in adolescents and SNP*energy intake (Kcal/day)*gender*age in the GENESIS children. Age was classified into single year age categories. Interaction terms were left in the model if $P < 0.1$. The final model for each cohort consisted of all significant interaction terms and additional main effects not contained within those terms. Post-hoc comparisons of phenotypic differences between genotype groups in the adolescent male subgroup were calculated by age-adjusted GLM. To allow comparison of effects across the age range, age and sex specific z -scores were calculated for boys and girls separately in single year age strata in the GENESIS cohort, and in the same manner in adolescents, although 12 and 13 year olds were collapsed into one group, as were 17-18 year olds.

No explicit multiple testing correction was carried out as we view *FTO* as having high prior likelihood of influencing all phenotypes investigated. In the adolescent cohort, point biserial correlations were calculated between physical activity status as a dichotomous variable (Active or Inactive) and adiposity phenotypes. For point biserial correlations, generally r lies between -0.8 and 0.8³⁰. It was used here to investigate the relationship between physical activity and adiposity in all subjects, then in risk and protective genotype groups.

RESULTS

ADOLESCENT COHORT

In a model-free ANCOVA analysis, *FTO* genotype was demonstrated to influence weight ($P = 0.006$) and BMI ($P = 0.02$) in the adolescent cohort (Table 1). A genetic model with dominant effects of the T-allele (T-dominant model) explained most of the genetic variance and all subsequent results were derived using this model (i.e. T-carriers are compared to those homozygous for the G allele). Under the T-dominant model, *FTO* genotype influenced BMI ($P = 0.007$), weight ($P = 0.001$), triceps ($P = 0.034$) and subscapular skinfolds ($P = 0.021$), with GG individuals having higher adiposity than T-carriers for all phenotypes (Table 1). As can be seen in Table 1, those homozygous for the G-allele had measures of adiposity 0.18 – 0.27 standard deviations higher than T-allele carriers. Interactions between *FTO* genotype, age, gender and PA on BMI were then investigated. Non-significant terms were removed from the model until the final model included the simplest significant three way interaction term (SNP*gender*PA: $P = 0.028$) and necessary sub-terms (Supplementary Table 1).

As can be seen in Figure 1, adolescent boys showed differential effects of genotype on BMI in the different physical activity groups, while, while girls did not. Given the significant SNP*gender*PA interactions, we performed gender-stratified analyses that showed that *FTO* genotype influenced BMI in males ($P = 0.016$ – in age-adjusted model not including SNP*PA interaction term) and that there was a significant SNP*PA interaction in males ($P = 0.007$) but there was no main effect of *FTO* genotype on BMI in females ($P = 0.15$), nor any SNP*PA interaction ($P = 0.74$).

Subsequent analyses in males, stratified by physical activity and adjusted for age, showed that inactive males homozygous for the G allele had higher mean BMI than inactive T-carriers ($P = 0.0008$), with *FTO* genotype explaining 10.1% of the variance in BMI amongst inactive males. These inactive male G homozygotes also had higher weight ($P = 0.0009$) and subscapular skinfolds ($P = 0.03$), while effects on triceps skinfolds tended in the same direction ($P = 0.07$). Inactive male G homozygotes had a mean BMI more than 3 units (3.25 kg/m^2 (95% C.I. 1.44 – 5.06); $P = 0.001$, or 0.83 standard deviations) higher than inactive male T-carriers. However, when only active males were considered, *FTO* genotype had no effect on BMI ($P = 0.52$), weight ($P = 0.32$), triceps ($P = 0.79$) or subscapular skinfolds ($P = 0.85$). Age specific effects of *FTO* genotype are shown in Supplementary Table 2, where it can be seen that the direction of effect is consistent throughout the adolescent age range.

Looking at the question the other way round, we asked whether genotype influenced the relationship between physical activity level and the adiposity phenotypes. Analysis of point biserial correlations between physical activity status and adiposity phenotypes (Supplementary Table 3), showed weak but significant correlations in the whole cohort, whose magnitude was strengthened in GG homozygotes and weakened in T-carriers. For example, the correlation between physical activity level and BMI was -0.08 in the entire cohort, -0.06 in T-carriers, but increased in strength to -0.15 (with $P = 0.042$) in G allele homozygotes.

GENESIS COHORT (ages 1-5 years)

In the GENESIS cohort, we also tested the association between *FTO* genotype and energy intake, as well as measures of adiposity: BMI, weight, waist and hip

circumference and bicep, tricep, subscapular and suprailiac skinfold, and birthweight. As can be seen in Supplementary Table 4, none of these phenotypes showed any association with *FTO* genotype after adjustment for age and gender. We further tested for any age, gender or energy intake interactions to establish if any subgroup effects were being obscured. However, no significant interactions between *FTO* genotype and energy intake were observed on BMI ($P > 0.1$) using a backward stepwise approach. Furthermore, as can be seen in Supplementary Table 2, only the 3 year old children showed a significant effect of *FTO* genotype on age and sex-specific BMI z -scores. While this was in a direction consistent with effects in the adolescent cohort, the other age groups showed no significant effects, and directions were inconsistent.

DISCUSSION

We report here that genotype at *FTO* SNP rs17817449 influences variation in obesity-related measures in adolescents, and that T-allele carriers display lower levels of adiposity than G allele homozygotes. We also report an interaction between physical activity status, gender and *FTO* genotype, with the obesogenic influence of *FTO* largely confined to inactive males. In young children, in contrast, there was no association between *FTO* and adiposity phenotypes or energy intake.

In the adolescent cohort, weight and BMI, adjusted for age, were associated with *FTO* genotype ($P < 0.01$), while skinfold thicknesses showed genotypic differences with P

values slightly higher than this (Table 1). This influence of genotype was strongest when analysed under a T-allele-dominant model. In terms of effect size, genotype alone explained 0.4-0.8% of the phenotypic variance for these traits. A significant interaction between *FTO* genotype and activity status was observed in males and the effect of genotype was stronger when analysed in inactive males, with up to 10% of variance explained (Table 1c). The proportion of females classed as inactive in the current study was greater than that of males (42% Vs. 20%). It has been shown previously that adolescent females are less active than boys³¹. However, if the subjective PA measurement method used in the present study is a less specific measure of inactivity in females than males, the inclusion of moderately active females in the inactive group may dilute any interaction, as it appears that the *FTO* effect is strongest in the least active individuals^{17:26}. Nevertheless, the attenuation of the *FTO* effect by physical activity is in agreement with previous findings in adults²⁴⁻²⁶. While some studies have failed to find such an effect^{17:27}, a similar trend is in fact visible in the study of Tan et al.¹⁷. The equivocal findings may be attributable in part to the subjective measures of physical activity used in these studies, and further, large studies, using objective monitoring of PA are required. Inter-population differences in allele frequencies and patterns of linkage disequilibrium may also contribute to heterogeneity of findings. Previous studies have found that the influence of *FTO* on adiposity phenotypes is apparent from as young as 7 years old^{7:32} and that the strength of the association increases into early adolescence¹⁰. In the GENESIS cohort we observed no association between *FTO* genotype and adiposity, which is in line with previous findings reporting no association of *FTO* genotype and childhood BMI in 2-5 year old children³³, nor with energy intake (Supplementary Table 4). However, given the impact of physical activity on *FTO* associations in the adolescent cohort, if

such interaction effects also operate in these younger age groups, the lack of reliable physical activity data in the GENESIS cohort may confound efforts to detect subgroup-specific genotype effects in the younger children.

Obesity phenotypes show a high degree of heritability in familial studies ¹⁰. It has also been shown that having one or two overweight parents increases the risk of being overweight in childhood from a very young age ^{34;35}, further implicating genetic origins for a significant portion of the inter-individual variation in adiposity. However, evidence based on genetic association studies, particularly from genome-wide studies, does not account for a large proportion of this heritability. The *FTO* gene harbours variation that has the largest effect on adiposity phenotypes of any yet reported, accounting for around 1% of the total phenotypic variance in, for example, BMI in adults ⁷. Here, we have shown that the influence of *FTO* may be small overall but it has a significantly greater influence in the physically inactive portion of the population, with *FTO* genotype explaining, after age adjustment, as much as 10% of phenotypic variance in inactive adolescent males (Table 1). Studies that do not consider physical activity levels may well underestimate the potential magnitude of the *FTO* effect on obesity in the most susceptible groups. Our results support the idea that certain genetic effects may be operating most strongly on groups in a particular environmental milieu and extend the finding that *FTO* acts most strongly in physically inactive subgroups to the pre-adult life stages. This supports the idea that some genetic influences may be missed in situations where unknown confounding environmental factors are operating to obscure real genetic effects acting on environmentally distinct subgroups.

As well as influencing risk of obesity, our results also suggest that *FTO* genotype modulates the relationship between adiposity and physical activity, since the genotype/activity interaction implies that, on one hand, genotypic effects are observed only in certain environments but also that, on the other, activity effects are different among genotype groups. Increasing physical activity should play a key role in attaining a negative energy balance by increasing energy expenditure, although the relationship between increasing physical activity and decreasing obesity is equivocal (reviewed by Wareham *et al.*,⁶). In the present study, we find that the weak relationship between physical activity and obesity is strengthened when examined only in those subjects homozygous for the risk allele (Supplementary Table 3). It must be acknowledged, however, that these findings arise from cross-sectional data with subjective physical activity measurement and with relatively modest sample size. Whilst the direction of causality in this relationship is not evident from our cross-sectional data³⁶, the results do support the use of prospective studies with objective activity measurements to investigate the influence of activity on adiposity further. The influence of activity may have been obscured in previous studies of this type due to the inclusion of genetic ‘non-responders’. Although limited evidence is available from lifestyle intervention studies to date^{37;38}, future studies of the relationship between physical activity and obesity could consider a paradigm involving stratification by *FTO* genotype to reveal previously obscured environmental effects.

CONCLUSION

It has been suggested that moderate and controlled dietary restriction may prevent *FTO*-induced obesity⁹ and previous studies on the genetic influence on obesity have highlighted the importance of genes regulating energy intake³⁹. However, the finding

that physical activity levels in adolescent males influence the obesogenic effects of *FTO* risk genotypes highlights that both sides of the energy balance equation: intake and expenditure, should be considered in combating obesity. In the present study, it was found that *FTO* genotype had no strong influence in physically active adolescents but did so in inactive males, with those homozygous for the risk, G allele having a mean BMI as much as 3 kg/m² higher than those who are physically active or T-carriers. This is a promising advert for the benefit of a physically active lifestyle, particularly in those carrying *FTO* risk variants.

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Figure Legends

Figure 1. Effect of *FTO* genotype on BMI in adolescents and its interaction with Physical Activity and gender. Males showed a significant SNP*PA interaction ($P_{\text{interaction}} = 0.01$ in model-free ANCOVA, as above, and $P_{\text{interaction}} = 0.0007$ in T-dominant model). Females showed no significant SNP*PA interaction ($P = 0.54$ as above and $P = 0.74$ in T-dominant model). Data are presented as mean \pm SEM (adjusted for age). N for each group as follows: Active males - GG: 145, GT: 189, TT: 67; Inactive males - GG: 29, GT: 43; TT: 26 Active females - GG: 96, GT: 118, TT: 44; Inactive females - GG: 61, GT: 91, TT: 39.

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Running title: **FTO Gene interaction with physical activity**

ABSTRACT

Studies of the fat mass and obesity associated (*FTO*) gene provide compelling evidence of genetic variation in the general population that influences fat levels and obesity risk. Studies of the interaction between genetic and environmental factors such as physical activity will promote understanding of how lifestyle can modulate genetic contributions to obesity. In the current study, we investigated the effect of *FTO* genotype, and interactions with physical activity or energy intake, in young children and adolescents. 1-5 year old children from the GENESIS study ($N = 1980$) and 11-18 year old Greek adolescents ($N = 949$) were measured for adiposity-related phenotypes and genotyped at the *FTO* SNP marker, rs17817449. Adolescents were classified as physically active or inactive based on self-reported levels of physical activity (PA). In adolescents, *FTO* genotype influenced weight ($P = 0.001$), and BMI ($P = 0.007$). There was also a significant SNP*PA*gender interaction ($P = 0.028$) on BMI, which reflected the association between *FTO* genotype and BMI in males ($P = 0.016$) but not females ($P = 0.15$) and significant SNP*PA interaction in males ($P = 0.007$) but not females ($P = 0.74$). The *FTO* genotype effect was more pronounced in inactive males than active. Inactive males homozygous for the G allele had a mean BMI of 3 kg/m^2 higher than T-carriers ($P = 0.008$). In the GENESIS study, no significant association between *FTO* genotype and adiposity was found. The present findings highlight physical activity as a key factor modifying the effect of *FTO* genotype.

INTRODUCTION

Childhood obesity is a leading risk factor for the metabolic syndrome and type 2 diabetes, as well as potentially for subsequent cardiovascular disease in adulthood¹⁻³. Obesity has a complex aetiology but is clearly influenced both by genetic variation and by environmental factors such as diet and lifestyle⁴. Physical activity, for example, is recognised as a prevention tool for obesity, is known to increase energy expenditure and also has the potential to regulate appetite⁵, although the relationship between physical activity and obesity is not clearly defined⁶.

Studies of the recently discovered fat mass and obesity associated (*FTO*) gene have provided the most compelling evidence to date of genetic variation in the general population that influences fat levels and risk of obesity^{7,8}. A set of single nucleotide polymorphisms (SNPs) in the first intron of *FTO* are strongly associated with adiposity/obesity and related phenotypes⁷⁻¹⁷. It has been shown that adults homozygous for the risk allele at rs9939609 weigh on average ~3kg more than those homozygous for the other allele⁷. *FTO* genotype has also been reported to be associated with adiposity in children⁷⁻⁹, and the strength of its influence to increase during childhood¹⁰.

The function of *FTO* is currently unknown but is becoming clearer. It is widely expressed throughout the body and highly so in the arcuate nucleus, a region of the hypothalamus involved in regulating energy balance¹⁸. Fischer et al.¹⁹ reported recently that knocking out *Fto* in mice results in reduced adiposity via a mechanism apparently involving increased energy expenditure, sympathetic tone and levels of

catecholamine hormones, which contrasts with studies to date in humans that implicate hyperphagia without altered energy expenditure^{9;20-23;23}.

Despite having the largest influence on obesity-related phenotypes of any gene reported thus far, *FTO* genotype alone only accounts for a small proportion of the variance in these phenotypes. The full influence of *FTO* will only become clear through more thorough investigation of its interactions with environmental factors such as physical activity levels. Three studies have reported interactions between physical activity and *FTO* genotype in adults, with low activity levels being associated with enhanced genotype effects²⁴⁻²⁶, while other studies have reported no interaction with physical activity^{17;27}. However, to the best of our knowledge, no such studies have been conducted in pre-adults. We report here the influence of rs17817449 on adiposity in two cohorts of children, with ages ranging from one to eighteen years. In adolescent children we also investigated how physical activity levels modulate the *FTO* effects on adiposity-related phenotypes.

METHODS

SUBJECTS

Study participants were those of two previously published cohorts^{28;29}. One cohort comprised subjects from the Growth, Exercise and Nutrition Epidemiological Study In preSchoolers (GENESIS)²⁸, which included 1980 healthy children aged 1–5 years from throughout Greece collected between April 2003 and July 2004. 52% were male. The other cohort comprised 949 children aged 11-18 years, 53% of whom were male,

collected from the Trikala region in central Greece between February 2000 and April 2001²⁹. Both studies were approved by the ethics committee of the University of Glasgow and by local authorities in Greece. Written informed consent was obtained from the parents of all participants prior to the study.

DATA COLLECTION

Data were collected as described below after a period of protocol standardization in both cohorts. In the GENESIS cohort, all measurements were made as previously described in detail²⁸. This included basic body composition measurements (weight, height, and waist, hip and mid-upper arm circumferences), as well as skinfolds at four sites (biceps, triceps, subscapular and suprailiac). Birthweight data for this cohort were also available from the child health booklet. Energy intake data were collected from all children for three days using a combination of weighed food records and 24 hr recall or food diaries, as previously described in detail²⁸ and in the supplementary material. Physical activity (PA) data were not available in the GENESIS cohort. In the adolescent cohort, subjects were measured for height, weight, and triceps and subscapular skinfolds as previously described²⁹ and completed a questionnaire documenting their participation in physical activity both inside and outside of school. Individuals reporting no additional physical activity outside of the compulsory weekly physical education class at school were classified as inactive ($N = 289$; 98 males and 191 females), while those reporting participation in further physical activity were classified as active ($N = 660$)²⁹. Inactive individuals displayed higher subscapular skinfolds, after adjustment for age and gender, than those classified as active ($P = 0.02$) and displayed a trend towards higher BMI ($P = 0.075$) and triceps skinfold ($P =$

0.077) but not weight ($P = 0.39$). Dietary intake data were not available in the adolescent cohort.

GENOTYPING AND DATA ANALYSIS

SNP variant rs17817449 was genotyped in both cohorts by a PCR-RFLP method, digesting the PCR product with *A*/wNI enzyme. The forward primer was 5'-CGGTGAAGAGGAGGAGATTG-3' and the reverse primer 5'-CATCTCTGCCCCAGTTTCTC-3'. The G allele generated an undigested 223bp product while the T allele yielded 123bp and 100bp fragments after digestion. Genotyping was successful in 94% of subjects in the GENESIS cohort and in 96% of subjects in the adolescent cohort. G allele frequency at rs17817449 was 0.42 in the adolescent cohort and 0.43 in GENESIS. rs17817449 genotypes were in Hardy-Weinberg equilibrium (HWE) in both the adolescent ($P = 0.17$) and GENESIS cohorts ($P = 0.14$). rs17817449 is in complete linkage disequilibrium (in European populations) with the more commonly measured SNP rs9939609 ($r^2 = 1$).

STATISTICAL ANALYSIS

Groups were tested for normality using the Ryan-Joiner test, and each phenotype transformed (see Supplementary Material) so as to achieve as close to a normal distribution as possible across each cohort. After transformation, most phenotypes were normal, or approaching normality. Data were analyzed using R. We initially investigated the effect of FTO genotype on adiposity phenotypes by ANCOVA. Hypothesizing that genotype effects might also differ in age or gender subgroups, we subsequently tested for interactions using a backward stepwise ANCOVA approach. The starting model contained a four-way (SNP* PA*gender*age) interaction term and

all hierarchically necessary sub-terms in adolescents and SNP*energy intake (Kcal/day)*gender*age in the GENESIS children. Age was classified into single year age categories. Interaction terms were left in the model if $P < 0.1$. The final model for each cohort consisted of all significant interaction terms and additional main effects not contained within those terms. Post-hoc comparisons of phenotypic differences between genotype groups in the adolescent male subgroup were calculated by age-adjusted GLM. To allow comparison of effects across the age range, age and sex specific z-scores were calculated for boys and girls separately in single year age strata in the GENESIS cohort, and in the same manner in adolescents, although 12 and 13 year olds were collapsed into one group, as were 17-18 year olds.

No explicit multiple testing correction was carried out as we view *FTO* as having high prior likelihood of influencing all phenotypes investigated. In the adolescent cohort, point biserial correlations were calculated between physical activity status as a dichotomous variable (Active or Inactive) and adiposity phenotypes. For point biserial correlations, generally r lies between -0.8 and 0.8³⁰. It was used here to investigate the relationship between physical activity and adiposity in all subjects, then in risk and protective genotype groups.

RESULTS

ADOLESCENT COHORT

In a model-free ANCOVA analysis, *FTO* genotype was demonstrated to influence weight ($P = 0.006$) and BMI ($P = 0.02$) in the adolescent cohort (Table 1). A genetic model with dominant effects of the T-allele (T-dominant model) explained most of the genetic variance and all subsequent results were derived using this model (i.e. T-carriers are compared to those homozygous for the G allele). Under the T-dominant model, *FTO* genotype influenced BMI ($P = 0.007$), weight ($P = 0.001$), triceps ($P = 0.034$) and subscapular skinfolds ($P = 0.021$), with GG individuals having higher adiposity than T-carriers for all phenotypes (Table 1). As can be seen in Table 1, those homozygous for the G-allele had measures of adiposity 0.18 – 0.27 standard deviations higher than T-allele carriers. Interactions between *FTO* genotype, age, gender and PA on BMI were then investigated. Non-significant terms were removed from the model until the final model included the simplest significant three way interaction term (SNP*gender*PA: $P = 0.028$) and necessary sub-terms (Supplementary Table 1).

As can be seen in Figure 1, adolescent boys showed differential effects of genotype on BMI in the different physical activity groups, while, while girls did not. Given the significant SNP*gender*PA interactions, we performed gender-stratified analyses that showed that *FTO* genotype influenced BMI in males ($P = 0.016$ – in age-adjusted model not including SNP*PA interaction term) and that there was a significant SNP*PA interaction in males ($P = 0.007$) but there was no main effect of *FTO* genotype on BMI in females ($P = 0.15$), nor any SNP*PA interaction ($P = 0.74$).

Subsequent analyses in males, stratified by physical activity and adjusted for age, showed that inactive males homozygous for the G allele had higher mean BMI than inactive T-carriers ($P = 0.0008$), with *FTO* genotype explaining 10.1% of the variance in BMI amongst inactive males. These inactive male G homozygotes also had higher weight ($P = 0.0009$) and subscapular skinfolds ($P = 0.03$), while effects on triceps skinfolds tended in the same direction ($P = 0.07$). Inactive male G homozygotes had a mean BMI more than 3 units (3.25 kg/m^2 (95% C.I. 1.44 – 5.06); $P = 0.001$, or 0.83 standard deviations) higher than inactive male T-carriers. However, when only active males were considered, *FTO* genotype had no effect on BMI ($P = 0.52$), weight ($P = 0.32$), triceps ($P = 0.79$) or subscapular skinfolds ($P = 0.85$). Age specific effects of *FTO* genotype are shown in Supplementary Table 2, where it can be seen that the direction of effect is consistent throughout the adolescent age range.

Looking at the question the other way round, we asked whether genotype influenced the relationship between physical activity level and the adiposity phenotypes. Analysis of point biserial correlations between physical activity status and adiposity phenotypes (Supplementary Table 3), showed weak but significant correlations in the whole cohort, whose magnitude was strengthened in GG homozygotes and weakened in T-carriers. For example, the correlation between physical activity level and BMI was -0.08 in the entire cohort, -0.06 in T-carriers, but increased in strength to -0.15 (with $P = 0.042$) in G allele homozygotes.

GENESIS COHORT (ages 1-5 years)

In the GENESIS cohort, we also tested the association between *FTO* genotype and energy intake, as well as measures of adiposity: BMI, weight, waist and hip

circumference and bicep, tricep, subscapular and suprailiac skinfold, and birthweight. As can be seen in Supplementary Table 4, none of these phenotypes showed any association with *FTO* genotype after adjustment for age and gender. We further tested for any age, gender or energy intake interactions to establish if any subgroup effects were being obscured. However, no significant interactions between *FTO* genotype and energy intake were observed on BMI ($P > 0.1$) using a backward stepwise approach. Furthermore, as can be seen in Supplementary Table 2, only the 3 year old children showed a significant effect of *FTO* genotype on age and sex-specific BMI z-scores. While this was in a direction consistent with effects in the adolescent cohort, the other age groups showed no significant effects, and directions were inconsistent.

DISCUSSION

We report here that genotype at *FTO* SNP rs17817449 influences variation in obesity-related measures in adolescents, and that T-allele carriers display lower levels of adiposity than G allele homozygotes. We also report an interaction between physical activity status, gender and *FTO* genotype, with the obesogenic influence of *FTO* largely confined to inactive males. In young children, in contrast, there was no association between *FTO* and adiposity phenotypes or energy intake.

In the adolescent cohort, weight and BMI, adjusted for age, were associated with *FTO* genotype ($P < 0.01$), while skinfold thicknesses showed genotypic differences with *P*

values slightly higher than this (Table 1). This influence of genotype was strongest when analysed under a T-allele-dominant model. In terms of effect size, genotype alone explained 0.4-0.8% of the phenotypic variance for these traits. A significant interaction between *FTO* genotype and activity status was observed in males and the effect of genotype was stronger when analysed in inactive males, with up to 10% of variance explained (Table 1c). The proportion of females classed as inactive in the current study was greater than that of males (42% Vs. 20%). It has been shown previously that adolescent females are less active than boys³¹. However, if the subjective PA measurement method used in the present study is a less specific measure of inactivity in females than males, the inclusion of moderately active females in the inactive group may dilute any interaction, as it appears that the *FTO* effect is strongest in the least active individuals^{17;26}. Nevertheless, the attenuation of the *FTO* effect by physical activity is in agreement with previous findings in adults²⁴⁻²⁶. While some studies have failed to find such an effect^{17;27}, a similar trend is in fact visible in the study of Tan et al.¹⁷. The equivocal findings may be attributable in part to the subjective measures of physical activity used in these studies, and further, large studies, using objective monitoring of PA are required. Inter-population differences in allele frequencies and patterns of linkage disequilibrium may also contribute to heterogeneity of findings. Previous studies have found that the influence of *FTO* on adiposity phenotypes is apparent from as young as 7 years old^{7;32} and that the strength of the association increases into early adolescence¹⁰. In the GENESIS cohort we observed no association between *FTO* genotype and adiposity, which is in line with previous findings reporting no association of *FTO* genotype and childhood BMI in 2-5 year old children³³, nor with energy intake (Supplementary Table 4). However, given the impact of physical activity on *FTO* associations in the adolescent cohort, if

such interaction effects also operate in these younger age groups, the lack of reliable physical activity data in the GENESIS cohort may confound efforts to detect subgroup-specific genotype effects in the younger children.

Obesity phenotypes show a high degree of heritability in familial studies¹⁰. It has also been shown that having one or two overweight parents increases the risk of being overweight in childhood from a very young age^{34;35}, further implicating genetic origins for a significant portion of the inter-individual variation in adiposity. However, evidence based on genetic association studies, particularly from genome-wide studies, does not account for a large proportion of this heritability. The *FTO* gene harbours variation that has the largest effect on adiposity phenotypes of any yet reported, accounting for around 1% of the total phenotypic variance in, for example, BMI in adults⁷. Here, we have shown that the influence of *FTO* may be small overall but it has a significantly greater influence in the physically inactive portion of the population, with *FTO* genotype explaining, after age adjustment, as much as 10% of phenotypic variance in inactive adolescent males (Table 1). Studies that do not consider physical activity levels may well underestimate the potential magnitude of the *FTO* effect on obesity in the most susceptible groups. Our results support the idea that certain genetic effects may be operating most strongly on groups in a particular environmental milieu and extend the finding that *FTO* acts most strongly in physically inactive subgroups to the pre-adult life stages. This supports the idea that some genetic influences may be missed in situations where unknown confounding environmental factors are operating to obscure real genetic effects acting on environmentally distinct subgroups.

As well as influencing risk of obesity, our results also suggest that *FTO* genotype modulates the relationship between adiposity and physical activity, since the genotype/activity interaction implies that, on one hand, genotypic effects are observed only in certain environments but also that, on the other, activity effects are different among genotype groups. Increasing physical activity should play a key role in attaining a negative energy balance by increasing energy expenditure, although the relationship between increasing physical activity and decreasing obesity is equivocal (reviewed by Wareham *et al.*,⁶). In the present study, we find that the weak relationship between physical activity and obesity is strengthened when examined only in those subjects homozygous for the risk allele (Supplementary Table 3). It must be acknowledged, however, that these findings arise from cross-sectional data with subjective physical activity measurement and with relatively modest sample size. Whilst the direction of causality in this relationship is not evident from our cross-sectional data³⁶, the results do support the use of prospective studies with objective activity measurements to investigate the influence of activity on adiposity further. The influence of activity may have been obscured in previous studies of this type due to the inclusion of genetic ‘non-responders’. Although limited evidence is available from lifestyle intervention studies to date^{37:38}, future studies of the relationship between physical activity and obesity could consider a paradigm involving stratification by *FTO* genotype to reveal previously obscured environmental effects.

CONCLUSION

It has been suggested that moderate and controlled dietary restriction may prevent *FTO*-induced obesity⁹ and previous studies on the genetic influence on obesity have highlighted the importance of genes regulating energy intake³⁹. However, the finding

that physical activity levels in adolescent males influence the obesogenic effects of *FTO* risk genotypes highlights that both sides of the energy balance equation: intake and expenditure, should be considered in combating obesity. In the present study, it was found that *FTO* genotype had no strong influence in physically active adolescents but did so in inactive males, with those homozygous for the risk, G allele having a mean BMI as much as 3 kg/m² higher than those who are physically active or T-carriers. This is a promising advert for the benefit of a physically active lifestyle, particularly in those carrying *FTO* risk variants.

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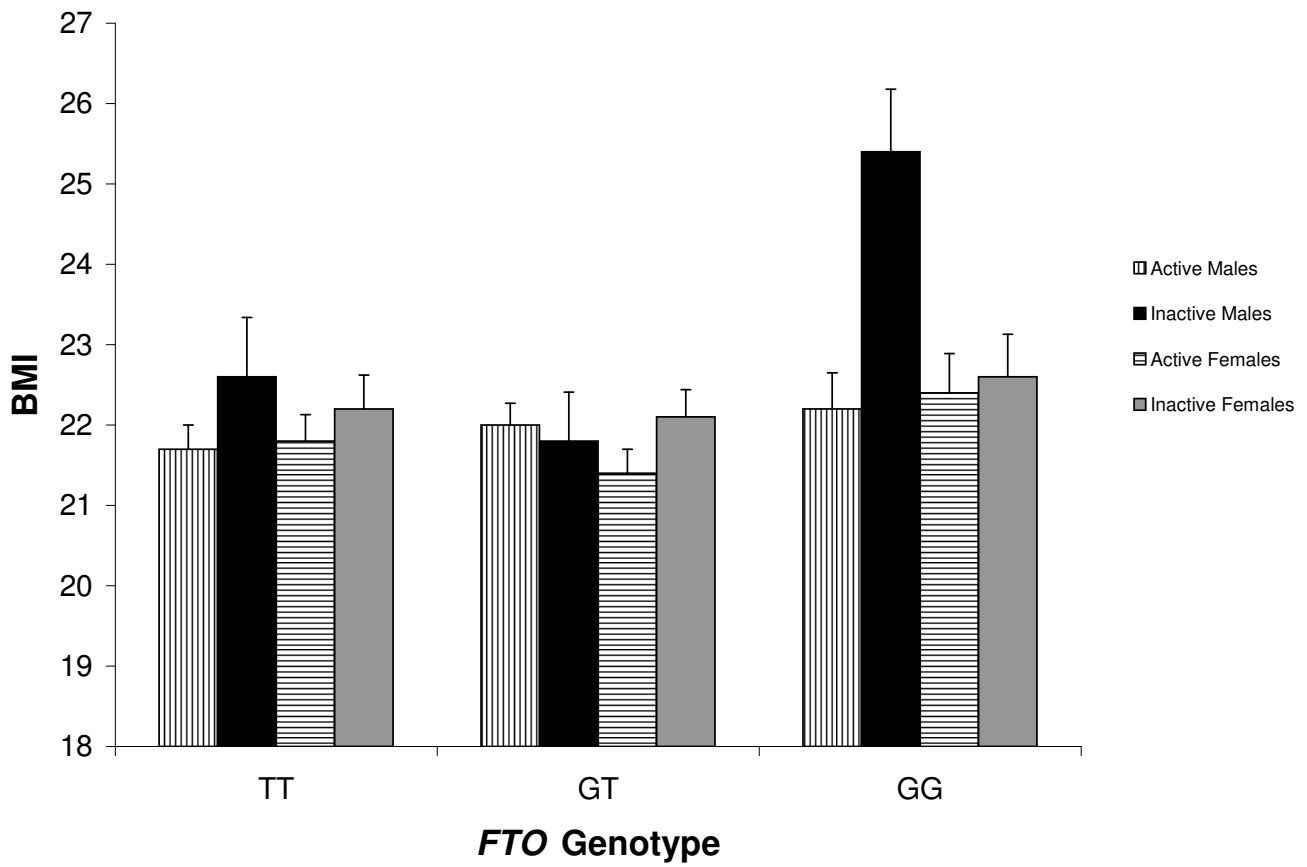
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Figure Legends

Figure 1. Effect of *FTO* genotype on BMI in adolescents and its interaction with Physical Activity and gender. Males showed a significant SNP*PA interaction ($P_{\text{interaction}} = 0.01$ in model-free ANCOVA, as above, and $P_{\text{interaction}} = 0.0007$ in T-dominant model). Females showed no significant SNP*PA interaction ($P = 0.54$ as above and $P = 0.74$ in T-dominant model). Data are presented as mean \pm SEM (adjusted for age). N for each group as follows: Active males - GG: 145, GT: 189, TT: 67; Inactive males - GG: 29, GT: 43; TT: 26 Active females - GG: 96, GT: 118, TT: 44; Inactive females - GG: 61, GT: 91, TT: 39.



FTO genotype and adiposity in children: physical activity levels influence the effect of the risk genotype in adolescent males

Tables

Table 1. Association of *FTO* Genotype with adiposity phenotypes in the adolescent cohort. ANCOVA analysis in a) all participants. b) All participants under T-dominant model. c) Inactive males under T-dominant model. Effect sizes are expressed as age and sex specific z-scores. i.e. an effect size of 1 is equal to a 1 standard deviation increase in BMI.

	Subjects	Model	Weight	BMI	Triceps skinfold	Subscapular skinfold	
a)	All participants *	Model-free	GG (N = 176)	62.3 (60.6-64.1)	22.8 (22.3-23.3)	16.3 (15.3-17.3)	14.2 (13.1-15.3)
			GT (N = 441)	59.2 (58.1-60.3)	21.8 (21.5-22.1)	15.0 (14.4-15.7)	12.8 (12.1-13.5)
			TT (N = 331)	59.2 (57.9-60.4)	21.9 (21.5-22.3)	15.1 (14.4-15.9)	12.7 (11.9-13.5)
			P-Value	P = 0.006	P = 0.02	P = 0.11	P = 0.066
			% Genetic variance†	99%	95%	93%	97%
			% Phenotypic variance‡	0.8%	0.8%	0.4%	0.5%
b)	All participants *	T-dominant	GG (N = 176)	62.3 (60.6-64.1)	22.8 (22.3-23.3)	16.3 (15.3-17.3)	14.2 (13.1-15.3)
			T Carriers (N = 772)	59.2 (58.4-60.0)	21.9 (21.6-22.1)	15.1 (14.6-15.6)	12.8 (12.2-13.3)
			P-Value	P = 0.001	P = 0.007	P = 0.034	P = 0.021
			% Phenotypic variance‡	0.8%	0.7%	0.4%	0.5%
			Effect size (SD (95% C.I))	0.27 (0.1-0.43)	0.26 (0.1-0.43)	0.2 (0.03-0.35)	0.18 (0.02-0.35)
			c)	Inactive males **	T-dominant	GG (N = 26)	75.6 (70.0-81.1)
T Carriers (N = 72)	64.1 (60.8-67.4)	22.1 (21.2-23.0)				12.9 (11.2-14.6)	12.2 (10.1-14.3)
P-Value	P = 0.0009	P = 0.0008				P = 0.07	P = 0.034
% Phenotypic variance‡	8.1%	10.1%				3.3%	4.4%
Effect size (SD (95% C.I))	0.88 (0.4-1.4)	0.83 (0.36-1.3)				0.5 (0.01 – 1)	0.45 (-0.03-0.94)

Mean and 95% C.I. are shown adjusted for age ** or age and sex* Weight data are in kg; Skinfold data are in mm.

Associations with $P < 0.05$ are shown in **bold**.

† % Genetic variance explained by T-dominant model.

‡ % of phenotypic variance explained by *FTO* genotype under model being analysed

FTO genotype and adiposity in children: physical activity levels influence the effect of the risk genotype in adolescent males

Tables

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a)	All participants *	Model-free	GG (N = 176)	62.3 (60.6-64.1)	22.8 (22.3-23.3)	16.3 (15.3-17.3)	14.2 (13.1-15.3)
			GT (N = 441)	59.2 (58.1-60.3)	21.8 (21.5-22.1)	15.0 (14.4-15.7)	12.8 (12.1-13.5)
			TT (N = 331)	59.2 (57.9-60.4)	21.9 (21.5-22.3)	15.1 (14.4-15.9)	12.7 (11.9-13.5)
			<i>P</i> -Value	<i>P</i> = 0.006	<i>P</i> = 0.02	<i>P</i> = 0.11	<i>P</i> = 0.066
			% Genetic variance†	99%	95%	93%	97%
			% Phenotypic variance‡	0.8%	0.8%	0.4%	0.5%
b)	All participants *	T-dominant	GG (N = 176)	62.3 (60.6-64.1)	22.8 (22.3-23.3)	16.3 (15.3-17.3)	14.2 (13.1-15.3)
			T Carriers (N = 772)	59.2 (58.4-60.0)	21.9 (21.6-22.1)	15.1 (14.6-15.6)	12.8 (12.2-13.3)
			<i>P</i> -Value	<i>P</i> = 0.001	<i>P</i> = 0.007	<i>P</i> = 0.034	<i>P</i> = 0.021
			% Phenotypic variance‡	0.8%	0.7%	0.4%	0.5%
			Effect size (SD (95% C.I))	0.27 (0.1-0.43)	0.26 (0.1-0.43)	0.2 (0.03-0.35)	0.18 (0.02-0.35)
			c)	Inactive males **	T-dominant	GG (N = 26)	75.6 (70.0-81.1)
T Carriers (N = 72)	64.1 (60.8-67.4)	22.1 (21.2-23.0)				12.9 (11.2-14.6)	12.2 (10.1-14.3)
<i>P</i> -Value	<i>P</i> = 0.0009	<i>P</i> = 0.0008				<i>P</i> = 0.07	<i>P</i> = 0.034
% Phenotypic variance‡	8.1%	10.1%				3.3%	4.4%
Effect size (SD (95% C.I))	0.88 (0.4-1.4)	0.83 (0.36-1.3)				0.5 (0.01 – 1)	0.45 (-0.03-0.94)

Mean and 95% C.I. are shown adjusted for age ** or age and sex* Weight data are in kg; Skinfold data are in mm.

Associations with $P < 0.05$ are shown in **bold**.

† % Genetic variance explained by T-dominant model.

‡ % of phenotypic variance explained by *FTO* genotype under model being analysed