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Research article

FTO gene SNPs associated with extreme obesity in cases, controls and extremely discordant sister pairs

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Abstract

Background: FTO is a gene located in chromosome region 16q12.2. Recently two studies have found associations of several single nucleotide polymorphisms (SNPs) in FTO with body mass index (BMI) and obesity, particularly rs1421085, rs17817449, and rs9939609.

Methods: We examined these three SNPs in 583 extremely obese women with current BMI greater than 35 kg/m^2 and lifetime BMI greater than 40 kg/m^2, and 544 controls who were currently normal weight (BMI<25 kg/m^2) and had never been overweight during their lifetimes.

Results: We detected highly significant associations of obesity with alleles in all three SNPs (p < 10^{-9}). The strongest association was with rs1421085 (p = 3.04 × 10^{-10}, OR = 1.75, CI = 1.47–2.08). A subset of 99 cases had extremely discordant sisters with BMI<25 kg/m^2. The discordant sisters differed in allele and genotype frequencies in parallel with the overall case and control sample. The strongest association was with rs17817449 (z = 3.57, p = 3.6 × 10^{-4}).

Conclusion: These results suggest common variability in FTO is associated with increased obesity risk or resistance and may in part account for differences between closely related individuals.

Background

Obesity has become the most common human disorder and it poses a serious threat to health and quality of life through its association with diabetes, hypertension, cardiovascular disease, and cancer. Rates have been increasing during the past century, particularly during the past two decades, and show no sign of leveling off. The average body mass index (BMI, weight in kg/height in m^2) of the U.S. population is well into the overweight range (BMI = 28.1 kg/m^2), more than 30% of the people in the United States are obese (BMI ≥ 30 kg/m^2) and more than 64% are overweight (BMI > 25 kg/m^2)[1]. Recent predictions are

for a reduction in life expectancy and quality of life for the next generation [2].

Recently, a genome wide association study revealed an association with a common variant in single nucleotide polymorphisms located in intron 1 of FTO, a gene with previously unknown function located in chromosome region 16q12.2 [3]. A second study found associations with nearby SNPs[4]. The 3 primary SNPs, rs9939609 in the Frayling et al. [3] study and rs1421085 and rs17817449 in the Dina et al study[4], are located within 20 kb and are in strong linkage disequilibrium (LD). The FTO association is notable because of its strength and the

consistency of replication across several samples, both within and between studies. For example, FTO associations with physical activity related body fat accumulation [5] and obesity related traits [6,7] were found in studies having large sample sizes. To date, the obesity association has been found only in Europeans or individuals of European descent. FTO was not associated with obesity in Han Chinese [8] or with six Oceanic populations [9]. Field et al. [10] tested FTO gene variants in type 1 diabetes cases and controls, however, no significant association was found. The association with type II diabetes is mediated entirely by BMI [3].

In this report we examined three SNPs to determine whether FTO variability could distinguish between extremely obese cases and thin controls. In addition, we extended our investigation to examine extremely discordant sister pairs, because the shared genetic background may be useful in future attempts to identify functional variants.

Methods
Cases and controls
Cases were obese women (BMI $\geq$ 35 kg/m^2) with a lifetime BMI >40 kg/m^2. Independent controls were selected who had a current and lifetime BMI $\leq$ 25. All cases and controls were non-Hispanic Caucasians. Sample characteristics are summarized in Table 1. The individuals in the samples were of approximately the same age but differed in average BMI by 29 kg/m^2. As would be expected for

extreme obesity, most cases had an early onset. The median age at obesity onset was 12 and 90% had an onset prior to age 26. This result is consistent with the observation of Frayling et al. [3] of an FTO association in children as young as seven years.

All subjects in the three samples gave informed consent, and the protocol was approved by the Committee on Studies Involving Human Beings at the University of Pennsylvania.

Discordant Sister Pairs
A subset of the case sample had extremely discordant sisters. Characteristics of the sister pairs are presented in Table 1. All thin sisters had BMI < 25 kg/m^2 and had never been obese (BMI $\geq$ 27 kg/m^2), while obese sisters had BMI $\geq$ 35 kg/m^2. The sisters were similar in age but differed markedly in BMI by an average of 26 kg/m^2. This difference is more than double the average BMI of the thin sisters, making the comparison comparable in magnitude to some animal models (Table 1). The percentage body fat of normal weight and obese sisters were 26.41 ± 5.58 and 49.36 ± 5.18, respectively.

SNP Markers
The three SNPs, rs1421085, rs17817449, and rs9939609, are located within 19.6 kb in intron 1 of the FTO gene and are in strong LD (pairwise r^2>0.97) based on our samples. There are two primary haplotypes, C-G-A (42.0 %) and T-T-T (55.5 %). The T-C-T haplotype was observed at 1.7 %

Table 1: Clinical characteristics of cases/controls and discordant sister pairs

	N	Minimum	Maximum	Mean	Std. Deviation
Controls					
Age	544	16	65	42.63	8.75
BMI	544	15.99	24.93	20.77	1.79
% Fat	534	7.50	40.00	23.65	5.61
Cases					
Age	583	18	64	41.05	9.42
BMI	583	35.08	96.95	49.41	8.81
% Fat	529	30.70	70.70	50.01	5.92
Onset Age	462	0.1	55	13.90	9.01
Thin Sisters					
Age	99	14	56	37.61	9.10
BMI	99	17.01	24.90	21.80	1.88
% Fat	81	15.10	38.40	26.41	5.58
Obese Sisters					
Age	99	17	65	39.55	9.27
BMI	99	35.08	71.35	47.46	8.10
% Fat	89	37.00	58.90	49.36	5.18
Onset Age	78	1	42	14.04	8.10
Differences (Obese-Thin)					
Age	99	-19.00	18.00	1.94	6.39
BMI	99	13.14	50.26	25.65	8.27
% Fat	79	6.30	40.20	23.33	7.41

and 5 others had very low frequencies. Pairwise linkage disequilibrium measures used Proc Allele and haplotype analyses the Proc Haplotype EM algorithm [11] in SAS 9.1.

Genotyping

DNA was extracted from whole blood or lymphoblastoid cell lines using a high salt method. PCR reactions and genotyping were completed using an Applied Biosystems (ABI, Foster City, CA) TaqMan 7300 system. SNP primers and probes were provided by ABI Assays-on-Demand. Detailed conditions are available on request.

Statistical analyses

Descriptive statistics, allele and genotype frequencies were calculated using SPSS 15.0. Tests of Hardy-Weinberg (HW) equilibrium were based on a Chi Square goodness-of-fit test programmed in Excel. The program Structure was used to test for possible population structure. For this purpose, more than 300 SNP markers were available for cases, while a subset of 37 were available for both cases and controls. Association analyses were completed using the Case Control procedure in SAS 9.1, with the overall association with genotype based on the Armitage trend test and odds ratios based on allele counts, reflecting additive effects.

For discordant sister pairs, mean values for BMI and percent fat were compared using one-way ANOVA and analysis of covariance used General Linear Models in SPSS 15.0. Association analyses for discordant sisters used the sibling transmission disequilibrium test (S-TDT) [12]

Power

For the case/control comparisons, power was estimated using parameters from the original Frayling et al. [3] study, since that study had the largest sample size. Values reported by Dina et al. [4] were similar. Assuming a risk allele frequency of .45, obesity prevalence = 0.2 (BMI > 35 kg/m^2), linkage disequilibrium (D') = 0.80, relative risk (RR) = 1.67, p < .05, and additive, dominant or recessive expression, we have greater than 80% power with 520 unpaired cases and controls, the minimum number of individuals with genotypes. This estimate considers only the risk for the upper extreme phenotype, and conservatively assumes a population prevalence based on current BMI>35 rather than lifetime BMI>40. Power is higher still if risk and protection are considered in the respective tails of the distribution (analyses not shown).

Results

Genotyping

Genotype frequencies for controls, cases and the combined sample were all in Hardy-Weinberg equilibrium, and there was no evidence of structure (analyses not shown). Genotype and allele frequencies for cases and controls are given in the Table 2. Obese cases have consistently higher minor allele frequencies that are near or above 0.5, while those for controls were about 0.13 lower. The genotype frequencies were similar for controls and thin sisters and minor allele frequencies were 9–12% lower than their obese sibling. Significance for case-control and thin-obese sister allele frequency comparisons are given in the right column. All reach statistical significance except for rs1421085 in discordant sisters.

Table 2: Genotype and allele frequencies of three FTO SNPs in cases/controls and discordant sisters. Significance for case-control and thin-obese sister allele frequency comparisons are given in the right column. Genotype comparisons are presented in Table 3.

	N	Genotypes			Alleles		Allele Freq. Difference
rs1421085		TT	TC	CC	T	C	
Controls	521	.370	.518	.111	.630	.370	
Cases	523	.256	.474	.270	.493	.507	p = 3.5 × 10^{-10}
Thin Sisters	92	.337	.554	.109	.614	.386	
Obese Sisters	93	.247	.548	.204	.522	.478	p = 0.07
rs17817449		TT	TG	GG	T	G	
Controls	522	.393	.496	.111	.641	.359	
Cases	527	.275	.469	.256	.509	.491	p = 1.2 × 10^{-9}
Thin Sisters	98	.347	.571	.082	.633	.367	
Obese Sisters	93	.258	.516	.226	.516	.484	p = 0.02
rs9939609		TT	TA	AA	T	A	
Controls	520	.398	.494	.108	.645	.355	
Cases	527	.288	.459	.252	.518	.482	p = 3.7 × 10^{-9}
Thin Sisters	98	.367	.541	.092	.638	.362	
Obese Sisters	92	.272	.489	.239	.516	.484	p = 0.02

Table 3: Association studies of three FTO SNPs in cases and controls

SNP	N Controls	N Cases	Armitage χ^2	p	Additive OR (CI)
Cases and controls					
rs1421085	521	523	39.65	3.04×10^{-10}	1.75 (1.47–2.08)
rs17817449	522	527	36.55	1.51×10^{-9}	1.72 (1.44–2.05)
rs9939609	520	527	34.06	5.34×10^{-9}	1.69 (1.42–2.02)

Association study in cases and controls

The results from the association analyses for cases and controls are presented in Table 3. Results were highly significant ($p < 10^{-9}$) for all three SNPs. The strongest association was with rs1421985 ($p = 3.04 \times 10^{-10}$, OR = 1.75, CI = 1.47–2.08) (Table 3). Considering all 8 observed haplotypes the overall χ^2 for association with case status was 50.69 (df = 7, $p = 1.07 \times 10^{-8}$). For the two common haplotypes C-G-A and T-T-T the values were 36.47 (df = 7, $p = 1.55 \times 10^{-9}$) and 36.50 (df = 7, $p = 1.53 \times 10^{-9}$), respectively.

Association studies in extremely discordant sister pairs

Results from association analyses for discordant sister pairs are presented in Table 4. All associations are significant and parallel those for allele frequencies (Table 2) and associations in cases and controls (Table 3).

Discussion

The results of this study strongly replicate and extend those from previous reports of an association between FTO and human obesity. The effect size was large, with odds ratios to 1.69–1.75 in cases and controls and 1.46–1.65 in discordant sisters. These values are similar to those reported previously [3,4]. The consistency and strength of the associations along with the high frequencies of the risk alleles/haplotype among extremely obese individuals suggest the effects of this gene, while still unknown, are common and pervasive. Given the history of non-replications for other gene associations, the consistency and

strength of the replication is particularly notable. Moreover, because of the very large sample sizes in the original studies [3,4] one might reasonably have expected it would be difficult to find the association with moderate sample sizes like those of the current study. However, the effects are quite strong and detectible even in a relatively small sample of discordant sisters.

Extreme sampling from both tails of the phenotypic distribution is uncommon, because the screening process in sample selection requires much more effort. However, the increase in power to detect associations is substantial [13]. The design should select for both risk and protective variation in cases and controls, respectively The case-control sample of the current study is a powerful one and likely played a role in the strong replication, even though it is only of moderate size.

The use of extremely discordant sibling pairs is particularly uncommon because of the difficulty and expense in accruing such samples. However, pairs that differ in phenotype can increase power to identify associations, particularly when they also differ in genotype [14]. The results from this study indicate that FTO can have a substantial effect on obesity risk between individuals close in age, having similar rearing environments, and sharing half their genes identical by descent. While the sample sizes for discordant siblings may be too small for definitive conclusions, they are highly significant, consistent with other results, and can be particularly useful in isolating causa-

Table 4: Association analyses of extremely discordant sibling pairs.

Obese		Thin		Z	P-value
rs1421085	TT	TC	CC		
TT	15	6	0	2.14	p = 0.032
TC	14	32	2		
CC	2	7	8		
rs17817449	TT	TG	GG		
TT	18	6	0	3.57	$p = 3.6 \times 10^{-4}$
TG	13	34	1		
GG	2	11	8		
rs9939609	TT	TA	AA		
TT	18	7	0	3.09	p = 0.002
TA	14	30	1		
AA	2	12	8		

tive sequence variability once an overall association has been identified.

Although the role of FTO or nearby genes in susceptibility to obesity remains unknown, several recent studies have begun to shed light on its function. For example, FTO is highly expressed in hypothalamic nuclei that control eating behavior [15]. An intriguing observation is that it catalyzes Fe(II)- and 2OG- dependent DNA demethylation [15], although the role of FTO related DNA methylation in obesity is unknown. Further studies are needed to clarify the functional relationship of FTO with obesity susceptibility.

Conclusion
These results suggest common variability in FTO is associated with increased obesity risk or resistance and may in part account for differences between closely related individuals.

Abbreviations
BMI Body mass index

SNP Single nucleotide polymorphism

FTO Homolog of mouse *fatso* (fused toes) gene

LD Linkage disequilibrium

Competing interests
The author(s) declare that they have no competing interests.

Authors' contributions
RAP is PI of the projects that accrued the samples and performed the phenotyping. For this specific study, he conducted statistical analyses and drafted the manuscript. WDL participated in data collection, performed data analyses, conducted genotyping, and made contributions to the manuscript. HZ analyzed data, advised on statistical analyses, and contributed to the manuscript. All authors have read and approved the final manuscript.

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