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APOH Interacts with *FTO* to Predispose to Healthy Thinness

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Abstract

We identified 8 candidate thinness predisposition variants from the Illumina HumanExome chip genotyped on members of pedigrees selected for either healthy thinness or severe obesity. For validation, we tested the candidates for association with healthy thinness in additional pedigree members while accounting for effects of obesity-associated genes: *NPFFR2*, *NPY2R*, *FTO*, and *MC4R*. Significance was obtained for the interaction of *FTO* rs9939609 with *APOH* missense variant rs52797880 (minor allele frequency = 0.054). The thinness odds ratio was estimated as 2.15 ($p<0.05$) for the combination of *APOH* heterozygote with the homozygote for the non-obesity *FTO* allele. Significance was not obtained for any other combination of a candidate variant with an obesity gene or for any of the 8 candidates tested independently.

Keywords

Body mass index; Likelihood; Exome chip; Pedigrees

Introduction

For some individuals, thinness is a life-long healthy state rather than the result of malnutrition or disease. Nevertheless, most genetic studies of low body mass index (BMI) have focused not on healthy thinness, but on eating disorders (Helder and Collier 2011) or sarcopenia (Tan et al. 2012). This neglect is unfortunate since healthy thinness may more readily yield its underlying genes than will obesity, for which environmental factors obscure the effects of genes. Furthermore, knowledge of thinness predisposition genes promises to provide insight not only into thinness, but also into obesity. As a model for disease treatments inspired by genetic variants associated with a healthy state, drugs that inhibit *PCSK9* mimic the effect of genetic variants that lower cholesterol levels (Gouni-Berthold and Berthold 2014). Similarly, thinness predisposition variants, redefined as obesity resistance variants, might be the inspiration for drugs to combat obesity. A number of BMI-

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and obesity-associated single nucleotide polymorphisms (SNPs) have been identified by genome-wide association studies; however, even in combination, these SNPs fall short of accounting for the heritability of BMI (Sandholt et al. 2012). The small effect sizes that characterize most associated SNPs are consistent with polygenic inheritance: the accumulation of small effects from many risk variants. Nevertheless, the ability of polygenic inheritance to roughly predict obesity risk (Domingue et al. 2014) does not preclude the existence of variants with large effects on BMI, especially variants that affect the less studied lower end of the range.

Even if unaffected by environmental factors, genetic interactions may obscure the genotype-phenotype relationship for thinness. If a causal variant promotes thinness only when co-inherited with an interacting variant, association between the causal variant and thinness may appear weak because many non-thin subjects carry the causal variant but not the interacting variant. Therefore, accounting for genetic interactions can facilitate the discovery of thinness predisposition variants. Herein, we identified candidate thinness predisposition variants from the Illumina HumanExome chip on members of pedigrees selected either for severe obesity or for healthy thinness, thereby excluding individuals with malnutrition or eating disorders, the focus of most previous genetic studies of the lower end of the BMI range (Helder and Collins 2011). We then validated the candidates in additional pedigree members while accounting as well for the effects of four obesity-associated genes, and their interactions with the candidates.

Methods

Subjects

We ascertained obesity pedigrees using the Health Family Tree, a Utah high school-based family history program designed to teach genetics and disease prevention as part of a mandatory health class (Williams et al. 1988). Students, with parental input, reported disease information and risk factors for the parents' first-degree relatives. We identified 435 severely obese sib pairs from the Trees, chose 107 sibships for expansion, and examined 3,333 relatives of the sibs. Similarly, we identified 40 thin probands who reported multiple thin relatives and examined 400 family members including 265 thin individuals. All subjects were Utah residents with European ancestry. The obesity and thinness projects have been approved by the Institutional Review Board of the University of Utah.

For each subject, height was measured to the nearest centimeter using a Harpenden anthropometer (Holtain, Ltd). Weight was measured in a hospital gown using a Scaletronix scale (model 5100) (Scaletronix Corporation, Wheaton, IL, USA), which has an 800-pound capacity and weighing accuracy of 0.1 kg. BMI was computed as weight divided by height squared.

Genotypes

We selected a discovery sample comprised of 504 members of the thin and obese pedigrees, chosen for distant relatedness within pedigrees in order to increase statistical power at reduced cost, and a validation sample comprised of 3,569 additional pedigree members. We

used the HumanExome chip to genotype the discovery sample and 351 members of the validation sample. We used the LightScanner (BioFire Diagnostics, SLC, UT) to genotype the remaining members of the validation sample for 8 candidate thinness-associated variants selected based on analysis of the discovery sample. Genotypes of 13 obesity-associated SNPs were available on 3,661 members of the pedigrees (Hunt et al. 2011), henceforth designated the OBgene sample. The Illumina HumanExome chip contains >240,000 variants selected from exome sequencing of >10,000 individuals, including subjects in studies of obesity-related conditions (e.g., NHLBI Exome Sequencing Project, T2D diabetes project, Lipid Extremes, BMI Extremes). Any variant observed ≥2 times in these samples and passing quality control was included on the chip (see: genome.sph.umich.edu/wiki/Exome_Chip_Design for additional information about this chip platform). Duplicate blind repeated controls within the sample showed over 99.8% genotype consistency. Standard quality control was applied.

We merged all sources of genotypes for a combined sample of 5,517 subjects each with genotypes for one or more of the 21 variants (8 thinness-candidate and 13 obesity-associated) using PLINK (Purcell et al. 2007). For each variant, we computed each individual's genotype probability in jPAP allowing an error rate of 2% for genotypes uncorrected for Mendelian errors. Similarly, we haplotyped SNP pairs within each obesity-associated gene and computed each individual's diplotype probabilities. Each probability accounted for allele or haplotype frequencies and genotypes of relatives. A dosage effect of a variant or haplotype for a subject was computed as 0/1/2, corresponding to the number of copies of the allele or haplotype, weighted by the corresponding genotype/diplotype probability to account for genotyping errors and to impute missing genotypes. For interaction effects, dosages of two variants or haplotypes were multiplied, reversing the dosage for any that decreased thinness predisposition.

Discovery Statistical Analysis

To identify thinness candidates among the autosomal exome chip variants, we applied 3 statistical tests to the complete discovery sample. First, the transmission disequilibrium test (TDT) in jPAP (Hasstedt 2005; Hasstedt and Thomas 2011) detected over-transmission of the minor allele from a heterozygous parent to a thin (BMI ≤23 kg/m^2) offspring. Second, a test designated ASSOC in jPAP tested each autosomal exome chip variant for an additive (genotypes coded as 0/1/2) effect on inverse-transformed BMI while accounting for gender, age, and heritability. Finally, famSKAT (Chen et al. 2013), the family-based implementation of the sequence kernel association test SKAT (Wu et al. 2011) evaluated sets of variants within a gene for association with BMI, accounting for gender, age, and heritability. Correction for multiple testing was unnecessary in the discovery phase of the study.

The TDT statistic equaled twice the natural logarithm of the ratio of the likelihood maximized over the transmission probability (τ) to the likelihood with $\tau = 0.5$; the ASSOC test statistic equaled twice the natural logarithm of the ratio of the likelihood maximized over a negative effect on BMI to the likelihood with effect size of zero. Since the null hypothesis constrained τ to the boundary for TDT and constrained the effect size to the boundary for ASSOC, we obtained P-values assuming the statistic approximated a 50:50

mixture of chi square distributions with 0 and 1 degree of freedom (Self and Liang 1987). P-values for famSKAT results were computed using Kuonen's saddlepoint method (Kuonen 1999).

For the TDT we restricted the test to rarer (minor allele frequency (MAF) ≤5%) variants to increase the likelihood that the allele was present in ungenotyped parents only when present in an offspring. jPAP probabilistically inferred missing parental genotypes using information from measured genotypes and the allele frequency. Because genotype inference was not independent of τ, we simulated all missing genotypes fixing $\tau = 0.5$ and allele frequency = 0.001, estimated τ, computed the TDT statistic for the simulated genotypes, then averaged τ and the TDT statistic over 1000 replicates.

Validation Statistical Analysis

For the validation analyses, BMI and age restrictions were applied to the discovery, OBgene, and validation samples; “complete” designates samples without these restrictions. Thinness was defined as below the 15th percentile of gender- and age-specific BMI from US reference data for 1999-2002 (McDowell et al. 2005), the approximate years of data collection. Controls were restricted to BMI between the 15th and 85th percentiles, thereby excluding severely obese subjects to detect variants specific for thinness rather than more generally for non-obesity. Age was restricted to 20 to 79 years to eliminate thinness attributable to youth or advanced age.

To test for simultaneous effects of alleles and haplotypes on thinness, we applied logistic regression (LR) in jPAP (Hasstedt and Thomas 2011). We estimated prevalence, heritability, and odds ratios for genotypic dosage effects. Significance was determined from 95% 1-tailed or 2-tailed confidence intervals (CI), depending on presence or absence of a prior knowledge of directionality of the effect. Because all effects were tested simultaneously, no multiple testing correction was required.

Results

The discovery and validation samples comprised non-overlapping subsets of subjects genotyped for 8 candidate thinness variants either separately or from the exome chip; the OBgene sample comprised individuals genotyped for 13 obesity-associated SNPs; the overlap sample comprised members of the validation sample who were also either in the OBgene sample or were genotyped using the exome chip (Figure 1). Every sample subset included more controls than thin subjects and more women than men; controls were on average older than thin subjects (Table 1).

To investigate the role of obesity-associated genes on thinness, we tested for simultaneous effects of the *FTO* SNP and of all common (frequency > 1%) 2-SNP haplotypes of 13 SNPs within *NPFFR2*, *NPY2R*, and *MC4R* (Table 2) in the OBgene sample (1669 members of 909 pedigrees). Starting with all effects, we successively eliminated the least significant until only significant effects remained. One *NPFFR2* haplotype increased thinness predisposition; *FTO* allele T, 2 haplotypes of *NPY2R* and 1 haplotype of *MC4R* decreased thinness predisposition (Table 3).

To identify candidates for thinness predisposition from the exome chip variants, we required high significance on one or more of 3 statistical tests applied to the complete discovery sample (Figure 1) comprising 504 members of 46 pedigrees (Table 4). Two of the selected candidates were common; for the remaining 6 candidates, MAF ≤ 6%. TDT and ASSOC analyses evaluated variants individually; famSKAT analysis evaluated sets of variants within a gene from which we selected for follow-up the variant with the largest minor allele frequency. ASSOC and famSKAT treated BMI as a continuous variable; TDT included only sibships with thin (BMI ≤ 23 kg/m2) members. ASSOC and famSKAT tested all autosomal exome chip variants; TDT tested only variants with MAF ≤ 5%. TDT and ASSOC tested for thinness predisposition of minor alleles; famSKAT did not require assumptions of or imply directionality of the effect. We tested whether the 8 candidate variants, each identified individually, maintained significance when tested simultaneously, accounting for the significant alleles/haplotypes of *NPFFR2*, *NPY2R*, *FTO*, and *MC4R* (Table 3), and following restriction of BMI and age in the discovery sample (156 members of 41 pedigrees ranging from 1 to 13 genotyped members, Figure 1, Table 1). Three of the 8 variants (in *FSIP2*, *HCN1*, and *APOH*) attained significance and A2M occurred only in thin subjects (Table 5). Failed significance for the *SNTB1*, *SPATS2*, and *SCN4A* variants may have been due to the reduced numbers of heterozygotes, 8, 15, and 3 respectively, following age and BMI restrictions. Failed significance for the *GATM* variant may have reflected a non-ideal choice from among the variants showing gene-based significance selected as the variant with the largest minor allele frequency.

The validation sample comprised 757 members of 187 pedigrees ranging from 1 to 83 genotyped members (Table 1); 30 pedigrees within the validation sample also contained subjects from the discovery sample. Because of the pedigree overlap, we made an ascertainment correction in the LR analysis by dividing the likelihood of the validation and discovery samples combined by the likelihood of the discovery sample. The combined discovery and validation samples included 913 members of 198 pedigrees ranging from 1 to 95 genotyped members. None of the 8 candidates attained significance (Table 5).

Next we tested for interaction between the thinness candidates and the obesity genes. For each significant obesity allele/haplotype in turn, we repeated the LR analysis, replacing each candidate dosage with its product with the obesity allele/haplotype dosage. To preclude imputation of either candidate or obesity gene variants, we replaced the validation sample with the overlap sample. The combined discovery and overlap sample included 761 members of 175 pedigrees ranging from 1 to 84 members (Table 1), with ascertainment correction again the discovery sample. The *APOH* variant attained significance in interaction with the *FTO* SNP (Table 5). None of the 8 candidates attained significance in interaction with any other obesity alleles/haplotypes (results not shown).

Thinness was more than twice as common for *APOH* rs52797880 heterozygotes paired with *FTO* rs9939609 T/T (71%) compared to any other genotype pair (21-30%) (Table 6). It should be noted that these estimates ignore relatedness in the sample. However, in analysis that accounted for relatedness (Table 5), the per-allele OR estimate of 1.47 corresponds to 2-allele OR estimate of 2.15; dosage effect of 2 was assigned for the heterozygous *APOH*/homozygous *FTO* pairing.

Discussion

This study identified *APOH* (apolipoprotein H (beta-2-glycoprotein I)) rs52797880 as a potential thinness predisposition variant in interaction with *FTO* rs9939609. The chance of thinness was more than double for *APOH* rs52797880 genotype T/C in combination with *FTO* rs9939609 genotype T/T compared to all other genotypes. Small numbers precluded evaluation of the association for homozygous genotype *APOH* C/C.

Located on chromosome 17q24.2, *APOH* participates in a variety of physiologic pathways including lipoprotein metabolism, coagulation, and the production of antiphospholipid autoantibodies (Athanasiadis et al. 2013). More relevant to thinness, *APOH* regulates diet-induced obesity in zebrafish and mammals (Oka et al. 2010) and an *APOH* SNP is associated with BMI in diabetics (Ruaño et al. 2009). In addition, APOH plasma levels are associated with metabolic and cardiovascular risk factors (Castro et al. 2010) and *APOH* transcript levels differed significantly by porcine body composition (Ponsuksili et al. 2005) and by lipids in beef cattle (Romao et al. 2014).

APOH variant rs52797880 is a missense substitution of threonine for isoleucine with no predicted effect on function and no reported disease associations. However, rs1801690, a possibly deleterious variant at 8.5 kb and with LD $r^2 = 0.77$ in our sample, is a missense substitution of serine for triptophan that associates with serum lipid levels (Guo et al. 2015). Although less strongly associated in the complete discovery analysis, rs1801690 interacts more strongly with FTO genotypes in the sample subset genotyped using the exome chip. Among 27 rs1801690 heterozygotes, thinness occurred in 8 of 8 with FTO T/T, 0 of 4 with A/A, and 8 of 7 with T/A. In comparison, among 27 rs52797880 heterozygotes, thinness occurred in 8 of 8 with FTO T/T, 3 of 7 with A/A, and 7 of 12 with T/A.

The nature of the interaction between *APOH* and *FTO* remains to be explained, although both are associated with lipid levels (Asselbergs et al. 2012). The effect of *FTO* on BMI may be mediated through impaired responsiveness to satiety (Benedict et al. 2014) and BMI-increasing alleles of *FTO* SNPs are associated with increased protein intake (Tanaka et al. 2013). However, obesity-associated *FTO* intronic variants and *FTO* activity appear functionally connected not with *FTO* but with two neighboring genes: *IRX3* and *RPGRIP1L*, suggesting that noncoding variants exert a functional impact through the alteration of gene expression (Tung et al. 2014).

An *NPFFR2* haplotype also contributed to thinness predisposition, while haplotypes of *NPY2R* and *MC4R* decreased thinness predisposition. Haplotypes of each of these obesity genes were previously shown to be associated with obesity (Hunt et al. 2011). Therefore, the obesity genes affect BMI across the range, affecting risk at the lower as well as the upper end of the range. Upon restricting controls to BMI<50th percentile, only effects of *FTO* and *MC4R* remained (results not shown), possibly because of smaller sample size.

In summary, we identified *APOH* as an obesity-resistance gene that interacts with *FTO* rs9939609 to more than double the occurrence of thinness.

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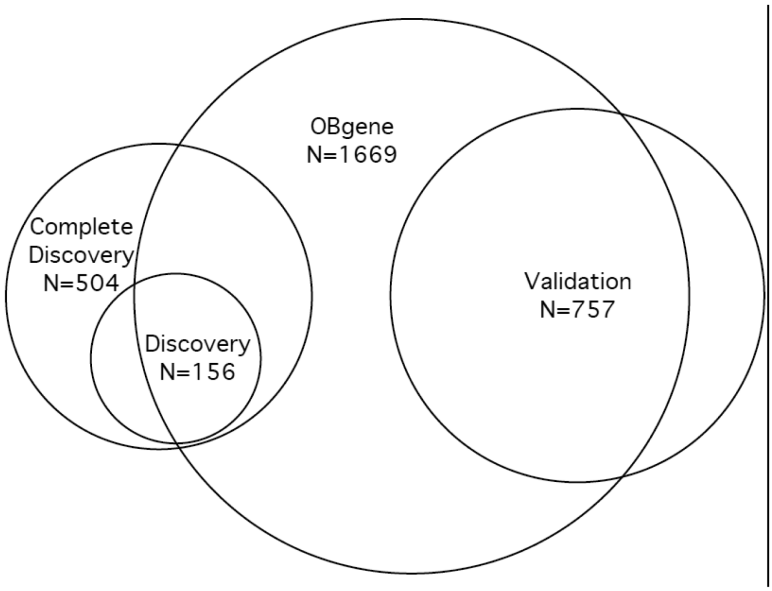

Fig. 1.

Samples used in the tests for association with thinness. Except for the Complete discovery Sample, all samples were restricted to BMI < 85th percentile and 20 ≤ age < 80 years. The Overlap Sample (Table 1) comprised the overlap of the OBgene Sample with the validation Sample with the inclusion of an additional 28 subjects from the validation Sample who were genotyped using the exome chip

Table 1

Counts and mean age (restricted to 20 yrs ≤age < 80 yrs) by gender, phenotype, and sample.

Sample	Discovery				Validation				OBgene				Overlap			
	N		Mean Age		N		Mean Age		N		Mean Age		N		Mean Age	
Phenotype	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Thin[a]	34	54	28.15	29.33	62	87	42.47	43.24	137	208	42.93	42.36	57	79	42.23	42.89
Controls[b]	17	51	40.82	42.63	233	375	49.01	48.14	558	766	50.54	49.30	179	290	48.87	48.78
Total	51	105	32.37	35.79	295	462	47.63	47.21	695	974	49.04	47.82	236	369	47.26	47.52

[a] Gender-, age-specific for BMI < 15th percentile

[b] Gender-, age-specific for BMI 15th to 85th percentile

Table 2

Obesity-associated SNPs included in the analysis.

SNP	Gene	Chromosome	Position (bp)[a]	N	MAF
rs12510838	*NPFFR2*	4	72961538	3661	0.1776
rs4129733	*NPFFR2*	4	72963020	3661	0.3063
rs9291171	*NPFFR2*	4	72981626	3661	0.2882
rs11940196	*NPFFR2*	4	73003569	3661	0.3614
rs12649641	*NPY2R*	4	156125333	3875	0.3880
rs12507396	*NPY2R*	4	156129044	3661	0.1114
rs17376826	*NPY2R*	4	156130948	3910	0.0359
rs10461238	*NPY2R*	4	156132216	3661	0.4447
rs10461239	*NPY2R*	4	156132447	3661	0.0460
rs2880415	*NPY2R*	4	156136027	3661	0.4527
rs9939609	*FTO*	16	53820527	4110	0.4356
rs17782313	*MC4R*	18	57851097	4131	0.2602
rs477181	*MC4R*	18	57896038	3898	0.3570

[a]Build GRCh37

Table 3

Thinness OR and 95% 2-tailed CI for obesity-associated haplotypes on the OBgene sample.

Gene	Haplotype[a]	Frequency	OR[b]	CI	
				Lower	Upper
NPFFR2	GTXX	0.1774	NS		
NPFFR2	AXGX	0.1116	NS		
NPFFR2	XGXG	0.3140	NS		
NPFFR2	XGXA	0.0130	NS		
NPFFR2	XTXG	0.0414	1.90	1.50	2.42
NPFFR2	XXAA	0.6308	NS		
NPY2R	AXXXXT	0.1792	0.79	0.65	0.96
NPY2R	AXXXXA	0.2168	NS		
NPY2R	CXXXXT	0.2859	0.88	0.79	0.99
NPY2R	CXXXXA	0.3181	NS		
FTO	A	0.4356	0.78	0.73	0.85
MC4R	TG	0.2098	NS		
MC4R	TT	0.0594	0.20	0.07	0.58
MC4R	GG	0.1612	NS		
MC4R	GT	0.5696	NS		

[a] Haplotype designated by base, with order corresponding to Table 2. X indicates the SNP was not included.

[b] NS indicates restriction of the OR to 1 due to non-significance at an earlier analysis stage.

Table 4

Candidate variants with P-values from 3 statistical tests performed on the complete discovery sample.

Variant	Gene	Chr	Position(bp)[a]	N	MAF[b]	TDT	ASSOC	famSKAT
rs79762465	FSIP2	2	186654867	502	0.041	1.5×10^{-8}	1.2×10^{-5}	2.0×10^{-5}
rs981782	HCN1	5	45285718	403	0.462	NA	0.21	2.7×10^{-10}
rs61762674	SNTB1	8	121561028	503	0.002	1.5×10^{-3}	0.04	0.01
rs55761427	A2M	12	9243017	501	0.011	3.1×10^{-3}	0.13	0.01
rs142230440	SPATS2	12	49884473	456	0.004	1.2×10^{-4}	1.8×10^{-5}	1.3×10^{-4}
rs1288775	GATM	15	45661678	501	0.264	NA	0.02	1.8×10^{-7}
rs41280102	SCN4A	17	62028920	456	0.009	1.3×10^{-3}	0.42	0.01
rs52797880	APOH	17	64216854	503	0.054	NA	4.7×10^{-5}	4.0×10^{-6}

[a] Build GRCh37

[b] Estimated using N>3000 with genotypes

Table 5

Thinness OR (per allele) and 95% 1-tailed lower CI in the discovery and validation samples and for interaction with *FTO* in the overlap sample

Sample	**Discovery**		**Validation**		**Overlap**	
Interaction	**None**		**None**		***FTO***	
Gene	**OR**	**CI**	**OR**	**CI**	**OR**	**CI**
FSIP2	2.87	1.39	1.00	NA	1.00	NA
HCN1 [a]	1.52	1.28	1.02	0.93	1.01	0.92
SNTB1	5.27	0.56	1.00	NA	1.00	NA
A2M	∞	NA	1.53	0.46	1.19	0.55
SPATS2	3.43	0.88	1.00	NA	1.00	NA
GATM	1.24	0.91	1.00	NA	1.00	NA
SCN4A	1.00	NA	1.00	NA	1.00	NA
APOH	2.36	1.08	1.21	0.73	1.47	1.01

[a] Major allele.

Table 6

Percentage of the overlap sample that was thin, by APOH and FTO genotypes.

FTO	APOH					
	T/T		T/C		C/C	
	N	Thin (%)	N	Thin (%)	N	Thin (%)
T/T	232	30	31	71	0	
T/A	330	25	51	29	2	100
A/A	98	28	19	21	0	