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The role of obesity-associated loci identified in genome wide association studies in the determination of pediatric BMI

Jianhua Zhao[1,*], **Jonathan P. Bradfield**[2,*], **Mingyao Li**[3], **Kai Wang**[2], **Haitao Zhang**[2], **Cecilia E. Kim**[2], **Kiran Annaiah**[2], **Joseph T. Glessner**[2], **Kelly Thomas**[2], **Maria Garris**[2], **Edward C. Frackelton**[2], **F. George Otieno**[2], **Julie L. Shaner**[2], **Ryan M. Smith**[2], **Rosetta M. Chiavacci**[2], **Robert I. Berkowitz**[4,5], **Hakon Hakonarson**[1,2,6,**], and **Struan F.A. Grant**[1,2,6,**]

[1]Division of Human Genetics, The Children's Hospital of Philadelphia, Philadelphia, Pennsylvania 19104, USA

[2]Center for Applied Genomics, Abramson Research Center, The Children's Hospital of Philadelphia, Philadelphia, Pennsylvania 19104, USA

[3]Department of Biostatistics and Epidemiology, University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA; [3]Department of Pediatrics, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104, USA

[4]Behavioral Health Center and Department of Child and Adolescent Psychiatry, The Children's Hospital of Philadelphia, Philadelphia PA 19104, USA

[5]Center for Weight and Eating Disorders, Department of Psychiatry, University of Pennsylvania, Philadelphia PA 19104, USA

[6]Department of Pediatrics, University of Pennsylvania, Philadelphia PA 19104, USA

Abstract

The prevalence of obesity in children and adults in the United States has increased dramatically over the past decade. Besides environmental factors, genetic factors are known to play an important role in the pathogenesis of obesity. A number of genetic determinants of adult BMI have already been established through genome wide association studies. In this study, we examined 25 single nucleotide polymorphisms (SNPs) corresponding to thirteen previously reported genomic loci in 6,078 children with measures of BMI. Fifteen of these SNPs yielded at least nominally significant association to BMI, representing nine different loci including *INSIG2*, *FTO*, *MC4R*, *TMEM18*, *GNPDA2*, *NEGR1*, *BDNF*, *KCTD15* and 1q25. Other loci revealed no evidence for association, namely at *MTCH2*, *SH2B1*, 12q13 and 3q27. For the 15 associated variants, the genotype score explained 1.12% of the total variation for BMI z-score. We conclude that among thirteen loci that have been reported to associate with adult BMI, at least nine also contribute to the determination of BMI in childhood as demonstrated by their associations in our pediatric cohort.

In the past three years, thirteen genetic loci have been implicated for BMI from the outcomes of genome wide association studies (GWA) studies primarily in adults. Insulin-induced gene 2 (*INSIG2*) was the first locus to be reported by this method to have a role in obesity[1] but replication attempts have yielded inconsistent outcomes[2–6]. The second reported locus, the fat mass- and obesity-associated gene (*FTO*)[7] has been more robustly

**To whom correspondence should be addressed. grants@chop.edu or hakonarson@chop.edu.
*These authors contributed equally to this work.

observed by others[8–11]. Subsequent larger studies have uncovered eleven additional genes[12–14], firstly melanocortin 4 receptor (*MC4R*) from a multi-center meta-analysis[12], then the GIANT consortium revealed six more genes [transmembrane protein 18 (*TMEM18*), potassium channel tetramerisation domain containing 15 (*KCTD15*), glucosamine-6-phosphate deaminase 2 (*GNPDA2*), SH2B adaptor protein 1 (*SH2B1*), mitochondrial carrier 2 (*MTCH2*) and neuronal growth regulator 1 (*NEGR1*)][14] , five of which were confirmed in the GWA study reported from Iceland (but not *GNPDA2* due to an unavailable proxy SNP), who also uncovered and reported loci on 1q25, 3q27 and 12q13[13] and verified association with the brain-derived neurotrophic factor (*BDNF*) gene[15].

In this study we aimed at examining these finding in a large pediatric cohort with BMI measures and to determine the relative impact of these variants in childhood. For this purpose, we leveraged genotyping data from our ongoing GWA study of BMI variation in children. The twenty five SNPs corresponding to the thirteen previously reported obesity loci were investigated with respect to their association to normalized pediatric BMI (Table 1; also Supplementary Table 1 for analyses by age categories).

In summary, fifteen of these SNPs yielded at least nominally significant association to BMI ($P < 0.05$), representing nine different loci with the same direction of effect as previously reported. Of these nine loci, variants at the *FTO* locus yielded the strongest association with $P<10^{-4}$, namely rs8044769 and rs3751812 ($P = 7.26\times10^{-5}$ and 9.68×10^{-5}, respectively); in addition, this locus also yielded association with rs8050136 and rs7190492 ($P = 1.40\times10^{-4}$ and 0.021, respectively) but not with rs6499640.

With a similar magnitude of association to *FTO* was *TMEM18*, with rs2867125 yielding a $P = 9.72\times10^{-5}$, together with almost as strongly associated SNPs, rs7561317 and rs4854344 ($P = 1.02\times10^{-4}$ and 1.52×10^{-4}, respectively). Indeed, it is interesting to note that the two adult cohorts that uncovered *TMEM18* in obesity also showed it to be second only in significance to *FTO*[13, 14]. The third most significantly associated locus was at *GNPDA2*, (rs13130484; $P = 1.32\times10^{-4}$).

Overall, in addition to *FTO*, *TMEM18* and *GNPDA2*, we found evidence for association at the *INSIG2*, *MC4R*, *NEGR1*, 1q25, *BDNF* and *KCTD15* loci. One could argue that we have carried out multiple testing in our BMI cohort for these previously reported SNPs, albeit at a number of magnitudes less than for a full GWA study. If we were to apply the strictest correction, i.e. the Bonferroni correction based on twenty five SNPs, then *FTO*, *TMEM18*, *GNPDA2* and *MC4R* would still be considered significant. This is very much in line with the observations made with the pediatric cohort utilized by Willer *et al*[14]; however the one exception is that we do not observe strong association with *KCTD15*.

It was also observed that SNPs residing at the 12q13, 3q27, *MTCH2* and *SH2B1* loci did not reveal any evidence of association with BMI in our pediatric cohort.

The positive results for *FTO* and *MC4R* come as no surprise as we have previously reported their association with the CDC-defined 95th percentile of BMI, i.e. obesity, in our pediatric cohort, but limited to ages 2–18 years old[8, 16]. In this current study, where we utilized BMI z-score on all children 0–18 years old, it is satisfying that they continue to show association at a similar magnitude.

One of the more notable results is the positive association with *INSIG2*. This association with pediatric BMI, albeit at just the nominal level, will further add to the debate on the relevance of *INSIG2* in BMI determination.

For the loci we did not observe any evidence for association for at all may be due to power issues, but could also indicate that they have a less pronounced role in a pediatric setting. Indeed, many of the newly uncovered genes have been implicated in neurological functions, genes which may be more important in BMI determination in adults rather than in children, where other more direct metabolic genes could play a more important role.

Finally, we investigated the fifteen significant SNPs further by testing for association between BMI Z-score and the genotype score by summing the number of BMI increasing alleles across all these SNPs. The resulting *P*-value for the genotype score was 2.53×10^{-16} (Figure 1). The genotype score explains 1.12% of the total variation for BMI z-score. We also tested pair-wise interactions between the fifteen significant SNPs, but none of the interaction effects were significant (Supplementary Table 2), suggesting that these fifteen SNPs act additively on the pediatric BMI z-score. As such, we did observe a cumulative effect but not as striking as reported by the GIANT consortium in their adult cohorts[14].

From this analysis, it is clear that a number of loci previously reported from GWA analyses of adult BMI and / or obesity also play a role in our phenotype of interest. While these recently discovered loci unveil several new biomolecular pathways not previously associated with obesity, it is important to note that these well established genetic associations with obesity explain very little of the genetic risk for this pediatric phenotype, suggesting the existence of additional loci whose number and effect size remain unknown. Once our GWA study is complete, we will have the opportunity to look for other variants in the genome that are associated with BMI in childhood.

RESEARCH METHODS AND PROCEDURES

Study Subjects

All subjects were consecutively recruited from the Greater Philadelphia area from 2006 to 2008 at the Children's Hospital of Philadelphia. Our study cohort consisted of 6,078 children of European ancestry with BMI information. All subjects were biologically unrelated and were aged between 0 and 18 years old. This study was approved by the Institutional Review Board of the Children's Hospital of Philadelphia. Parental informed consent was given for each study participant for both the blood collection and subsequent genotyping.

Genotyping

We performed high throughput genome-wide SNP genotyping using either the Illumina Infinium™ II HumanHap550 or Human 610 BeadChip technology in the same manner as our center has reported previously[17]. The SNPs analyzed survived the filtering of the genome wide dataset for SNPs with call rates <95%, minor allele frequency <1%, missing rate per person <2% and Hardy-Weinberg equilibrium $P < 10^{-5}$.

Loci described from GWA studies published to date have been found using either the Affymetrix or Illumina platform. In the event a locus was reported using both the Illumina and Affymetrix arrays, we used the SNPs present on the Illumina array. In the event of a signal only being described on the Affymetrix array, we either already had that SNP on our Illumina array or we identified and used the best surrogate SNP available. As such, as rs7566605 at *INSIG2* and rs10938397 at *GNPDA2* were not available on the BeadChip, we employed perfect surrogates for the association analysis i.e. rs17047697 and rs13130484 respectively ($r^2=1$). With rs7498665 at the *SH2B1* locus, rs8049439 and rs4788102 are already in strong LD with this SNP so no additional surrogate was selected.

Analysis

From our database of heights and weights for our multi-dimensional scaling (MDS) determined Caucasians, we eliminated BMI outliers using 2% cutoff for each age category in order to remove measurement error and syndromic forms of obesity. As BMI values vary widely across pediatric age groups, each BMI value was adjusted for age and sex then expressed as a z-score.

We queried the data for the indicated SNPs in our pediatric samples. All statistical analyses were carried out using the software package *plink*[18]. By treating BMI as a quantitative trait, association analysis for each SNP was carried out using linear regression with the SNP included as an independent variable (coded as 0, 1, and 2).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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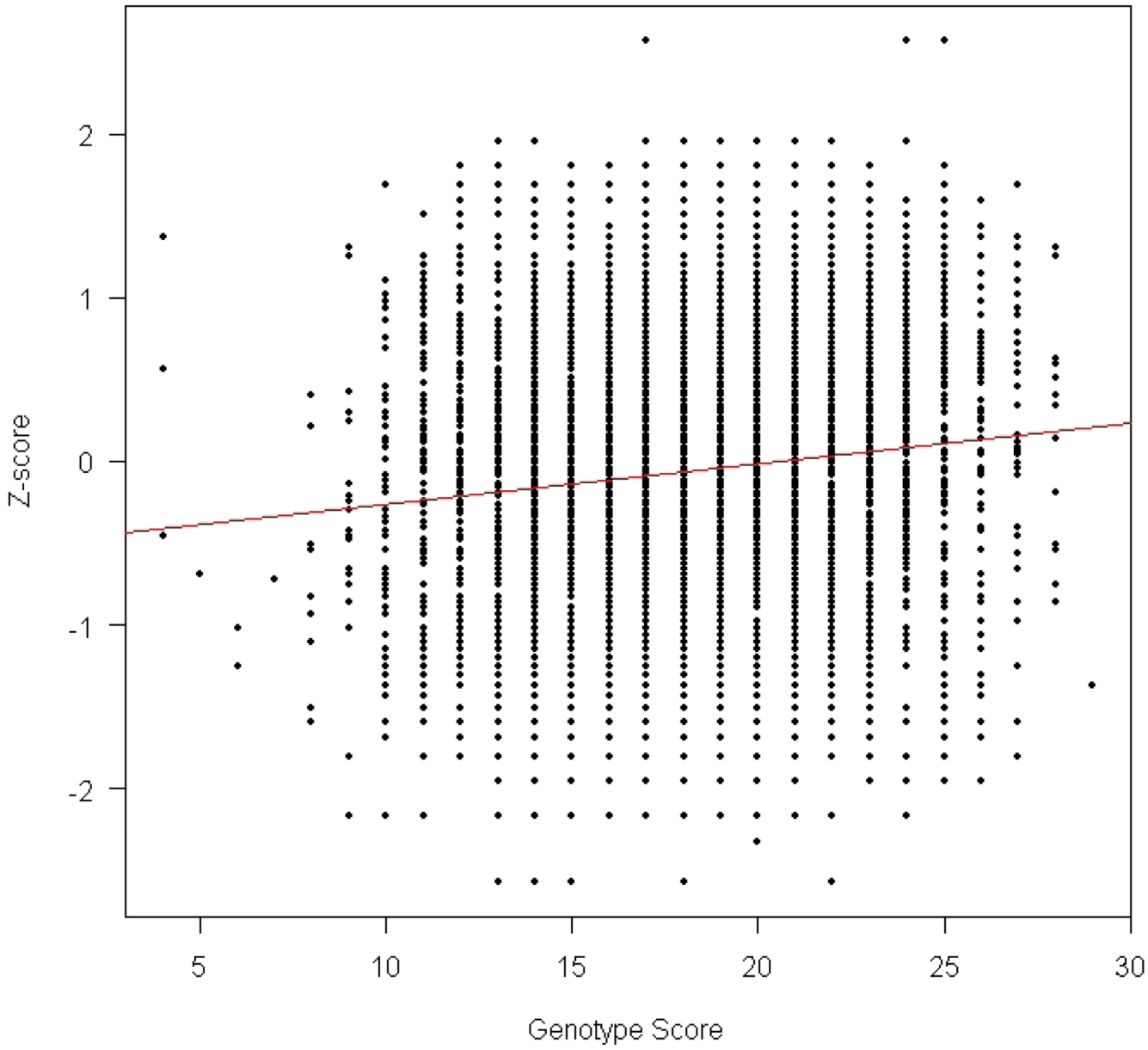

Figure 1.
Scatter plot for association between BMI z-score and the genotype score by summing the number of BMI increasing alleles across all fifteen BMI-associated SNPs.

Table 1

Quantitative association results for the candidate loci in the European American BMI cohort (n=6,078), sorted by chromosomal location.

Chr	SNP	Position (Build 36)	Nearby genes(s)	NMISS	BETA	SE	R2	T	P
1p31	rs3101336	72523773	*NEGR1*	6077	−0.04721	0.01637	0.001367	−2.884	0.0039
1p31	rs2568958	72537704	*NEGR1*	6078	−0.04654	0.01636	0.00133	−2.844	0.0045
1q25	rs10913469	176180142	*SEC16B/RASAL2*	6076	0.04859	0.02037	0.000936	2.385	0.017
2p25	rs2867125	612827	*TMEM18*	6076	−0.07964	0.02042	0.002498	−3.9	9.72×10^{-5}
2p25	rs4854344	628144	*TMEM18*	6046	−0.07734	0.0204	0.002372	−3.791	1.52×10^{-4}
2p25	rs7561317	634953	*TMEM18*	6065	−0.07905	0.02033	0.002488	−3.889	1.02×10^{-4}
2q14	rs17047697*	118544280	*INSIG2*	6072	0.03516	0.01672	0.000728	2.103	0.036
3q27	rs7647305	187316984	*SFRS10/ETV5/DGKG*	6075	0.01377	0.01932	0.00008368	0.7129	0.48
4p13	rs13130484**	44870448	*GNPDA2*	6078	0.06016	0.01572	0.002404	−3.826	1.32×10^{-4}
11p12	rs10838738	47619625	*MTCH2*	6070	−0.01098	0.01659	0.00007224	−0.6621	0.51
11p14	rs4074134	27603861	*BDNF*	6078	−0.03607	0.0191	0.0005866	−1.889	0.059
11p14	rs4923461	27613486	*BDNF*	6076	−0.03339	0.0191	0.0005027	−1.748	0.081
11p14	rs925946	27623778	*BDNF*	6078	0.01886	0.01758	0.0001895	1.073	0.28
11p14	rs10501087	27626684	*BDNF*	6077	−0.02955	0.0191	0.0003937	−1.547	0.12
11p14	rs6265	27636492	*BDNF*	6078	−0.04517	0.01997	0.0008411	−2.262	0.024
12q13	rs7138803	48533735	*BCDIN3D/FAIM2*	6078	0.02931	0.01623	0.0005365	1.806	0.071
16p11	rs8049439	28745016	*SH2B1*	6077	−0.003809	0.01622	0.000009073	−0.2348	0.81
16p11	rs4788102	28780899	*SH2B1*	6075	−0.003331	0.01626	0.00000691	−0.2049	0.84
16q12	rs6499640	52327178	*FTO*	6077	−0.01216	0.01624	0.00009223	−0.7485	0.45
16q12	rs8050136	52373776	*FTO*	6078	0.06086	0.01597	0.002385	3.811	1.40×10^{-4}
16q12	rs3751812	52375961	*FTO*	6071	0.06232	0.01598	0.002501	3.901	9.68×10^{-5}
16q12	rs7190492	52386253	*FTO*	6024	−0.03824	0.01656	0.0008843	−2.309	0.021
16q12	rs8044769	52396636	*FTO*	6077	−0.06233	0.0157	0.002588	−3.97	7.26×10^{-5}
18q21	rs12970134	56035730	*MC4R*	6078	0.05519	0.01806	0.001534	3.056	0.0023
19q13	rs29941	39001372	*KCTD15*	6057	−0.03519	0.01715	0.0006952	−2.052	0.040

NMISS: number of individuals tested; BETA: regression coefficient for the test SNP; SE: standard error of the regression coefficient; R2: r^2 value in linear regression; T: test statistic; P: two-sided trend test *P*-value. The direction of effect is shown for the minor allele in each case. *NEGR1* r^2 between rs3101336 and rs2568958 = 1; *TMEM18:* r^2 between rs2867125 rs4854344 and rs7561317 = 1; *BDNF* r^2

between rs4074134, rs4923461 and rs10501087 =1 and r^2 between rs4074134 and rs925946, rs6265 = 0.14 and 0.85 respectively; SH2B1: r^2 between rs8049439 AND rs4788102 = 0.965; *FTO* r^2 between rs8050136 and rs3751812, rs7190492, rs8044769, rs6499640 = 1, 0.38, 0.61 and 0.18 respectively.

* perfect surrogate for rs7566605

** perfect surrogate for rs10938397