Published in final edited form as:
Obesity (Silver Spring). 2009 July ; 17(7): 1461–1465. doi:10.1038/oby.2009.53.

Investigation of the locus near *MC4R* with childhood obesity in Americans of European and African ancestry

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Abstract

Recently a modest, but consistently, replicated association was demonstrated between obesity and the single nucleotide polymorphism (SNP), rs17782313, 3' of the *MC4R* locus as a consequence of a meta-analysis of genome wide association (GWA) studies of the disease in Caucasian populations. We investigated the association in the context of the childhood form of the disease utilizing data from our ongoing GWA study in a cohort of 728 European American (EA) obese children (BMI ≥ 95th percentile) and 3,960 EA controls (BMI < 95th percentile), as well as 1,008 African American (AA) obese children and 2,715 AA controls. rs571312, rs10871777 and rs476828 (perfect surrogates for rs17782313) yielded odds ratios in the EA cohort of 1.142 (P = 0.045), 1.137 (P = 0.054) and 1.145 (P = 0.042); however, there was no significant association with these SNPs in the AA cohort. When investigating all thirty SNPs present on the Illumina BeadChip at this locus, again there was no evidence for association in AA cases when correcting for the number of tests employed. As such, variants 3' to the *MC4R* locus present on the genotyping platform utilized confer a similar magnitude of risk of obesity in Caucasian children as to their adult Caucasian counterparts but this observation did not extend to African Americans.

Obesity has become a major health problem in modern societies, with a prevalence of up to 25% in Western societies and an increasing incidence in children[1]. Obesity present in adolescence has been shown to be associated with increased overall mortality in adults[2].

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There is strong evidence for a genetic component to the risk of obesity, including prevalence differences between racial groups[3, 4]. Both the familial occurrence of obesity and higher concordance for fat mass among monozygotic twins has been long noted[5, 6].

The *MC4R* gene, encoding the melanocortin 4 receptor, was the first locus at which mutations were associated with dominantly inherited morbid human obesity and was the commonest genetic cause of human obesity described before the era of genome wide association (GWA) studies.

A common genetic variant located upstream of insulin-induced gene 2 (*INSIG2*) was described in 2006 to be associated with both adult and childhood obesity from the first GWA study published for this phenotype[7]; however, this has proven controversial, with three subsequent technical reports refuting the observation[8–10]. The publication of a second obesity gene, *FTO*[11], almost a year later has been more robust, with independent studies coming out around the same time drawing similar conclusions[12–14]. Indeed, we have reported replication to the *FTO* gene in our pediatric obesity cohort, together with a successful refinement of the signal in African Americans[15].

To identify additional common variants influencing BMI, Loos *et al*[16] analyzed GWA data from ~17,000 individuals of European descent, derived from multiple efforts. After the *FTO* gene, the strongest association signal (rs17782313) mapped 188kb downstream of the *MC4R* gene. They then went on to confirm the BMI association in ~60,000 adults and ~6,000 children, with the latter showing higher odds ratio and higher level of significance of association with variants 3' to *MC4R* in comparison to the other analyses.

We elected to analyze this signal in the context of our ongoing GWA study of childhood obesity. The previously published SNP, rs17782313, was not included on the Illumina BeadChip we are using, but three other SNPs present were perfect surrogates for this SNP i.e. $r^2 = 1$, in the CEU HapMap sample, namely rs571312, rs10871777 and rs476828. Using the allelic chi-squared association test, we observed significant or borderline association between these SNPs and risk for childhood obesity in our current European American (EA) cohort, consisting of 728 obese children (BMI ≥ 95th percentile) and 3,960 controls (BMI < 95th percentile). The minor allele frequencies of rs571312, rs10871777 and rs476828 in the cases were 0.249, 0.249 and 0.257 respectively while they were 0.225, 0.226 and 0.232 in controls respectively, yielding odds ratios of 1.142 (95% CI 1.003 – 1.301; $P = 0.045$), 1.137 (95% CI 0.998 – 1.294; $P = 0.054$) and 1.145 (95% CI 1.005 – 1.305; $P = 0.042$) (Table 1).

As such, from this interim analysis of our ongoing GWA study, we observe replication in the childhood form of the disorder in EA. The three surrogate SNPs conferred risk for the disorder with a comparable magnitude to that previously observed in this ethnicity. However, it should be noted that rs10871777 only gave a *P*-value of 0.054 as a consequence of a lower genotyping yield than the other two SNPs, rs571312 and rs476828, both of which were statistically significant.

We went on to analyze 27 additional SNPs on the BeadChip in the region of linkage disequilibrium (LD) harboring the association signal. Table 1 shows that three other SNPs (rs633265, rs2051311 and rs1350341) that are in strong, but not perfect, LD with rs17782313 were also nominally associated with childhood obesity in EA.

Variants found in populations of both African and Caucasian ancestry may represent more universally important genes to the disorder. A cohort of African ancestry can also potentially aid in refining associations made with the GWA approach due to differing LD in this ethnicity, as was the case in our study of the *FTO* gene and its role in childhood obesity[15]

(see Supplementary Figure 1 for a direct comparison of LD patterns (r^2) at this locus, 3' to *MC4R*, between the CEU and YRI HapMap sample sets). As such, we also analyzed rs571312, rs10871777 and rs476828 in our African American (AA) cohort, consisting of 1,008 obese children (BMI ≥ 95th percentile) and 2,715 controls. Of these three SNPs, which are in complete LD with rs17782313 in CEU HapMap sample, only rs10871777 is in strong LD with this marker in the YRI HapMap sample (r^2=0.927) while rs571312 and rs476828 are in weak to moderate LD (r^2 = 0.149 and 0.526 respectively). The resulting genomic inflation factor was only 1.05; however, there was no significant association observed with these SNPs in this cohort (Table 2). With respect to all 30 SNPs present on the BeadChip in this region, although rs9966951 and rs12457166 yielded nominally significant association (and also rs1942880 when re-analyzing this data adjusting for admixture), there was no significant association with any these SNPs in this ethnicity when correcting for the number of tests employed (significance threshold P = 0.0017) (Table 2).

Therefore, we failed to show evidence of association in the AA cohort, despite the fact that the AA case cohort was larger than the EA set. However, we may have missed a *bona fide* association at this locus in AA due to the fact that the SNPs assayed using the BeadChip employed in this study were not selected for optimal haplotype tagging for the YRI HapMap sample; as such, additional SNP genotyping would be required for a more comprehensive appraisal of this locus in this ethnicity. As we did not observe association at this locus in AA, we were unable to refine this signal working with this ethnicity. It should, however, be noted that rs10871777, which was the only SNP in strong LD with rs17782313 in both ethnicities, did yield the same direction of effect in the AA cohort, albeit non-significantly, with a very modest odds ratio.

In conclusion, we have demonstrated that SNPs 3' to the *MC4R* locus confer a similar magnitude of risk for obesity in our pediatric Caucasian cohort as previously reported in both adults and children with the same phenotype. This observation further supports the notion that this pathway is causally linked to the disorder in children, over and above the previously described role of this gene in the rarer syndromic form of obesity, suggesting that interventions at this pathway level may be of value in patients who suffer from the more general form of the disease. The variants that we observe association to may directly dictate expression levels or some other regulatory mechanism but are more likely to be in LD with the causative variant(s). However, unlike with the *FTO* gene[15], we were unable to observe association at this locus in African Americans with the genotyping platform we employed.

RESEARCH METHODS AND PROCEDURES

Study Subjects

All subjects were consecutively recruited from the Greater Philadelphia area from 2006 to 2007 at the Children's Hospital of Philadelphia (CHOP) with self-reported ethnicity. Our study consisted of 728 EA obese children (BMI ≥ 95th percentile), 3,960 EA controls (BMI < 95th percentile), 1008 AA obese children and 2,715 AA controls. All of these participants had their blood drawn in to an 8ml EDTA blood collection tube and were subsequently DNA extracted for genotyping. BMI ≥ 95th percentile was defined using the Center for Disease control (CDC) z-score=1.645 (http://www.cdc.gov/nchs/about/major/nhanes/growthcharts/datafiles.htm). All subjects were biologically unrelated and were aged between 2 and 18 years old. All subjects were between −3 and +3 standard deviations of CDC corrected BMI i.e. outliers were excluded to avoid the consequences of potential measurement error or Mendelian causes of extreme obesity. This study was approved by the Institutional Review Board of CHOP. 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 $P < 10^{-5}$.

Analysis

All statistical analyses were carried out using the software package *plink* (http://pngu.mgh.harvard.edu/~purcell/plink/index.shtml)[18]. The single marker association analysis was carried out using the 1-df allelic chi-squared test. Odds ratios and the corresponding 95% confidence intervals were calculated for each SNP. All thirty SNPs employed were in Hardy-Weinberg equilibrium in both the cases and controls. Adjustment for admixture in the African American cohort was carried out using logistic regression that utilized multi-dimensional scaling values derived through *plink* for our cohort.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We would like to thank Adrienne Alexander, Chioma Onyiah, Elvira Dabaghyan, Kenya Fain, Maria Garris, Wendy Glaberson, Kisha Harden, Andrew Hill, Crystal Johnson-Honesty, Lynn McCleery, Robert Skraban, Kelly Thomas and Alexandria Thomas for their expert assistance with genotyping or data collection and management. We would also like to thank Smari Kristinsson, Larus Arni Hermannsson and Asbjörn Krisbjörnsson of Raförninn ehf for their extensive software design and contribution.

The study is supported in part by a Research Development Award from the Cotswold Foundation (H.H. & S.F.A.G) and NIH grant 1R01HD056465-01A1.

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Table 1

Childhood obesity Caucasian case-control association study results for markers in the downstream region of *MC4R* perfectly correlated with rs17782313 in Caucasians (**bold**) plus all other markers present on the BeadChip in the corresponding HapMap CEU region of LD.

(a) Caucasians

Chr	SNP	r^2 to rs17782313	B35 location	Minor Allele	Aff MAF (n=728)	Ctrl MAF (n=3960)	OR	95% CI	*P*-value
18	rs3897644	0.358	55880049	G	0.462	0.450	1.049	0.938 – 1.174	0.401
18	rs4940927	0.729	55883669	A	0.285	0.262	1.125	0.994 – 1.274	0.063
18	rs8085349	0.275	55884408	G	0.478	0.454	1.100	0.983 – 1.230	0.097
18	rs11152208	0.056	55890778	C	0.129	0.126	1.030	0.872 – 1.218	0.727
18	rs6567155	0.729	55906097	T	0.267	0.248	1.106	0.975 – 1.256	0.118
18	rs9948303	0.033	55911746	G	0.080	0.077	1.044	0.849 – 1.284	0.686
18	rs11660783	0.591	55918615	C	0.194	0.174	1.142	0.991 – 1.317	0.067
18	rs17066582	0.032	55919507	G	0.072	0.070	1.031	0.830 – 1.280	0.783
18	rs9966951	0.558	55926275	A	0.335	0.311	1.112	0.987 – 1.252	0.080
18	rs1893512	0.558	55938539	C	0.343	0.319	1.114	0.990 – 1.254	0.073
18	rs1942880	0.558	55944189	T	0.339	0.315	1.114	0.990 – 1.255	0.073
18	rs17772748	0.039	55948798	G	0.096	0.097	0.993	0.822 – 1.200	0.941
18	rs633265	0.452	55982448	A	0.457	0.421	1.156	1.033 – 1.293	0.011
18	rs2051311	0.445	55987860	G	0.458	0.422	1.157	1.034 – 1.294	0.011
18	**rs571312**	**1**	**55990749**	**T**	**0.249**	**0.225**	**1.142**	**1.003 – 1.301**	**0.045**
18	rs1350341	0.452	55993513	T	0.459	0.421	1.166	1.042 – 1.305	0.007
18	**rs10871777**	**1**	**56002743**	**G**	**0.249**	**0.226**	**1.137**	**0.998 – 1.294**	**0.054**
18	**rs476828**	**1**	**56003567**	**G**	**0.257**	**0.232**	**1.145**	**1.005 – 1.305**	**0.042**
18	rs9954571	0.061	56010857	A	0.125	0.123	1.023	0.864 – 1.212	0.793
18	rs921971	0.812	56012643	C	0.268	0.248	1.110	0.978 – 1.261	0.106
18	rs9947403	0.618	56020730	T	0.347	0.323	1.115	0.991 – 1.254	0.071
18	rs8094523	0.021	56029135	A	0.077	0.073	1.062	0.859 – 1.312	0.579
18	rs646749	0.46	56034105	A	0.399	0.377	1.099	0.980 – 1.232	0.107
18	rs12970134	0.811	56035730	A	0.267	0.247	1.105	0.974 – 1.255	0.121
18	rs477181	0.596	56047018	T	0.350	0.324	1.121	0.997 – 1.261	0.057
18	rs12457166	0.024	56054253	A	0.067	0.066	1.009	0.804 – 1.267	0.937

(a) Caucasians

Chr	SNP	r^2 to rs17782313	B35 location	Minor Allele	Aff MAF (n=728)	Ctrl MAF (n=3960)	OR	95% CI	*P*-value
18	rs12964203	0.78	56054584	C	0.264	0.246	1.099	0.967 – 1.248	0.148
18	rs9956274	0.043	56063683	C	0.054	0.060	0.889	0.695 – 1.137	0.350
18	rs4450508	0.517	56064414	A	0.350	0.328	1.104	0.982 – 1.242	0.099
18	rs752720	0.186	56066949	T	0.507	0.494	1.053	0.941 – 1.177	0.369

Table 2

Childhood obesity African American case-control association study results for markers in the downstream region of *MC4R* perfectly correlated with rs17782313 in Caucasians (**bold**) plus all other markers present on the BeadChip in the corresponding region.

(b) African Americans

Chr	SNP	r^2 to rs17782313	B35 location	Allele	Aff MAF (n=1008)	Ctrl MAF (n=2715)	OR	95% CI	*P*-value	Adjusted *P*-value
18	rs3897644	0.002	55880049	A	0.275	0.286	0.948	0.845 – 1.062	0.354	0.394
18	rs4940927	0.003	55883669	A	0.470	0.477	0.973	0.878 – 1.077	0.595	0.537
18	rs8085349	0.046	55884408	A	0.319	0.326	0.970	0.869 – 1.082	0.580	0.291
18	rs11152208	0.027	55890778	C	0.120	0.113	1.069	0.912 – 1.253	0.411	0.619
18	rs6567155	0	55906097	C	0.457	0.468	0.957	0.864 – 1.061	0.406	0.567
18	rs9948303	0.053	55911746	G	0.127	0.118	1.086	0.930 – 1.268	0.296	0.581
18	rs11660783		55918615	C	0.032	0.030	1.095	0.817 – 1.468	0.543	0.119
18	rs17066582	0.002	55919507	G	0.103	0.094	1.104	0.932 – 1.309	0.253	0.344
18	rs9966951	0.082	55926275	A	0.444	0.419	1.109	1.000 – 1.230	0.049	0.014
18	rs1893512	0.154	55938539	T	0.331	0.330	1.007	0.903 – 1.122	0.902	0.329
18	rs1942880	0.227	55944189	C	0.481	0.506	0.904	0.817 – 1.002	0.054	0.008
18	rs17772748		55948798	G	0.024	0.022	1.079	0.769 – 1.514	0.660	0.615
18	rs633265	0.09	55982448	C	0.244	0.250	0.967	0.859 – 1.089	0.581	0.075
18	rs2051311	0.063	55987860	A	0.251	0.258	0.963	0.856 – 1.083	0.527	0.071
18	**rs571312**	**0.149**	**55990749**	**T**	**0.337**	**0.335**	**1.009**	**0.906 – 1.125**	**0.867**	**0.348**
18	rs1350341	0.097	55993513	C	0.246	0.251	0.972	0.863 – 1.094	0.637	0.123
18	**rs10871777**	**0.927**	**56002743**	**G**	**0.294**	**0.283**	**1.057**	**0.944 – 1.183**	**0.336**	**0.161**
18	**rs476828**	**0.526**	**56003567**	**G**	**0.411**	**0.412**	**0.993**	**0.894 – 1.103**	**0.898**	**0.825**
18	rs9954571	0.145	56010857	A	0.202	0.211	0.945	0.832 – 1.072	0.378	0.172
18	rs9947403	0.224	56020730	C	0.409	0.418	0.963	0.868 – 1.068	0.477	0.287
18	rs8094523	0.097	56029135	A	0.138	0.144	0.948	0.818 – 1.100	0.482	0.126
18	rs646749	0.035	56034105	G	0.329	0.332	0.990	0.888 – 1.104	0.862	0.602
18	rs12970134	0.022	56035730	A	0.139	0.139	0.996	0.859 – 1.154	0.954	0.563
18	rs477181	0.116	56047018	G	0.501	0.488	1.051	0.949 – 1.164	0.339	0.252
18	rs653048	0.217	56047203	T	0.131	0.128	1.021	0.877 – 1.189	0.786	0.934
18	rs12457166	0.003	56054253	A	0.213	0.236	0.876	0.771 – 0.994	0.040	0.013

(b) African Americans

Chr	SNP	r^2 to rs17782313	B35 location	Allele	Aff MAF (n=1008)	Ctrl MAF (n=2715)	OR	95% CI	*P*-value	Adjusted *P*-value
18	rs12964203	0.144	56054584	C	0.109	0.101	1.086	0.921 – 1.282	0.326	0.069
18	rs9956274	0.048	56063683	T	0.415	0.411	1.020	0.919 – 1.131	0.715	0.296
18	rs4450508	0.166	56064414	A	0.290	0.283	1.035	0.925 – 1.159	0.548	0.588
18	rs752720	0.02	56066949	T	0.387	0.389	0.993	0.894 – 1.103	0.892	0.893