Published in final edited form as:

Pharmacogenomics. 2009 December ; 10(12): 1929–1939. doi:10.2217/pgs.09.122.

Obesity-related dyslipidemia associated with *FAAH*, independent of insulin response, in multigenerational families of Northern European descent

Yi Zhang^{*}, Gabriele E Sonnenberg^{*}, Tesfaye Mersha Baye^{*}, Jack Ann DeLaForest, Erin MacKinney, Cecilia J Hillard, Ahmed H Kissebah, Michael Olivier, and Russell A Wilke[†]

Abstract

A more thorough understanding of the genetic architecture underlying obesity-related lipid disorders could someday facilitate cardiometabolic risk reduction through early clinical intervention based upon improved characterization of individual risk. In recent years, there has been tremendous interest in understanding the endocannabinoid system as a novel therapeutic target for the treatment of obesity-related dyslipidemia.

Aims—*N*-arachidonyl ethanolamine activates G-protein-coupled receptors within the endocannabinoid system. Fatty acid amide hydrolase (FAAH) is a primary catabolic regulator of *N*-acyl ethanolamines, including arachidonyl ethanolamine. Genetic variants in *FAAH* have inconsistently been associated with obesity. It is conceivable that genetic variability in *FAAH* directly influences lipid homeostasis. The current study characterizes the relationship between *FAAH* and obesity-related dyslipidemia, in one of the most rigorously-phenotyped obesity study cohorts in the USA.

Materials & methods—Members of 261 extended families (pedigrees ranging from 4 to 14 individuals) were genotyped using haplotype tagging SNPs obtained for the *FAAH* locus, including 5 kb upstream and 5 kb downstream. Each SNP was tested for basic obesity-related phenotypes (BMI, waist and hip circumference, waist:hip ratio, fasting glucose, fasting insulin and fasting lipid levels) in 1644 individuals within these 261 families. Each SNP was also tested for association with insulin responsiveness using data obtained from a frequently sampled intravenous glucose tolerance test in 399 individuals (32 extended families).

Results—A well characterized coding SNP in *FAAH* (rs324420) was associated with increased BMI, increased triglycerides, and reduced levels of high-density lipoprotein cholesterol. Mean (standard deviation) high-density lipoprotein cholesterol level was 40.5 (14.7) mg/dl for major allele homozygotes, 39.1 (10.4) mg/dl for heterozygotes, and 34.8 (8.1) mg/dl for minor allele

[†]Author for correspondence: Medical College of Wisconsin, Milwaukee, Wisconsin, WI 53226, USA Tel.: +1 414 456 4532 Fax: +1 414 456 6516 rwilke@mcw.edu.

^{*}These authors contributed to this work equally

For reprint orders, please contact: reprints@futuremedicine.com

Financial & competing interests disclosure

This work was supported through grants 1R01DK080007 (Wilke), 5R01DK65998 (Kissebah), 1R01DK54026 (Sonnenberg), 1R01HL74168 (Olivier) and funds made available through Advancing a Healthier Wisconsin. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

homozygotes ($p < 0.01$, Family-Based Association Test). This SNP was not associated with insulin sensitivity, acute insulin response to intravenous glucose, glucose effectiveness or glucose disposition index.

Conclusion—Genetic variability in *FAAH* is associated with dyslipidemia, independent of insulin response.

Keywords

candidate gene; cholesterol; endocannabinoid; insulin resistance; metabolic syndrome

The current obesity epidemic represents a major international health crisis [1]. Although prevalence differs across races, the increased cardiovascular mortality associated with obesity appears to be common and related primarily to the dysmetabolic complications of weight gain [2]. For example, obese individuals tend to have lower circulating levels of high-density lipoprotein (HDL) cholesterol [3], and each 1 mg/dl reduction in circulating HDL level is known to be associated with a 6% increase in the risk for cardiovascular disease [4]. Diet and weight-related changes in lipid homeostasis therefore have an enormous impact on public health.

The endocannabinoid system (eCS) represents a novel therapeutic target for the treatment of obesity-related dyslipidemia [5]. It has been known for thousands of years that cannabis causes an increase in appetite, particularly for highly palatable foods, resulting in the development of metabolic disturbances and change in body composition [6]. Signaling within the eCS involves complex lipid-derived ligands, and two cannabinoid receptors, CB₁ and CB₂ [7]; and emerging data support the hypothesis that the onset of obesity-related dyslipidemia is specifically influenced by over-active signaling at the level of CB₁ [5]. This claim is supported by multiple recent reports [8–11]. For example, genetic variation in CB₁ has been associated with dyslipidemia in one of the most rigorously phenotyped family-based obesity study cohorts in the USA. [12].

The endogenous CB₁ receptor ligand *N*-arachidonyl ethanolamine (AEA) is metabolized by fatty acid amide hydrolase (FAAH) [13,14]. This enzyme catalyzes the hydrolysis of AEA to arachidonic acid and ethanolamine [15]. The human *FAAH* gene spans 19,582 nucleotides on chromosome 1, and a nonsynonymous SNP in this gene (C385A) was originally found to be associated with BMI in subjects of European ancestry [16]. However, this observation was not replicated in two population-based samples of similar ancestry [17,18]. Furthermore, a subsequent case-control study of French subjects with class III obesity (BMI $> 40 \text{ kg/m}^2$) versus lean controls from the same community (BMI $< 25 \text{ kg/m}^2$) reported data suggesting that the minor allele may in fact be protective against extreme weight gain [19].

In a recent dietary treatment trial, results reported by Aberle and colleagues have raised the intriguing possibility that prior conflicting associations between body composition and genetic variability in *FAAH* may have been attributable to a more direct influence of C385A on lipid homeostasis [20]. In 451 obese European study participants undergoing a 6 week trial of low-fat diet, subjects with a variant *FAAH* genotype experienced greater reductions in circulating lipid levels (i.e., both fasting triglycerides [TGs] and total cholesterol levels) [20]. The subsequent observation, by Durand *et al.*, that this variant may be associated with HDL-cholesterol levels in lean individuals [19], suggests that the relationship between *FAAH* and lipids is worth characterizing further.

The current study therefore evaluates the association between *FAAH* genotype and dyslipidemia in 261 extended families [3]. This study cohort, based within the Midwestern USA and enrolled through a weight loss organization called Take Off Pounds Sensibly, Inc.

(TOPS), has previously contributed to the identification of several major quantitative trait loci influencing visceral adiposity, insulin resistance and dyslipidemia [3,21–26]. The extended family structure of this cohort (i.e., each pedigree ranges from 4 to 14 individuals), accompanied by the availability of refined phenotypic traits (e.g., frequently sampled intravenous glucose tolerance test), makes the TOPS population ideal for the large-scale retrospective characterization of genetic factors influencing the metabolic traits that influence weight gain. In the current study, the C385A variant in *FAAH* was associated with obesity-related dyslipidemia, independent of insulin sensitivity (S_I), acute insulin response to intravenous glucose (AIR_G), glucose effectiveness (S_G) or glucose disposition (D_I).

Materials & methods

Study population

This study was approved by the Institutional Review Board of the Medical College of Wisconsin. The study cohort is family based, and it consists of 1644 individuals in 261 extended families of Northern European Ancestry (TABLE 1). All participating individuals provided informed consent for genotype–phenotype association studies characterizing obesity-related metabolic disorders. Proband was recruited through the TOPS weight loss program based in the Midwestern USA. Participating families ranged in size from 4 to 14 members. The minimum required family structure consisted of an obese proband ($BMI > 30 \text{ kg/m}^2$), an obese sibling, a never-obese sibling ($BMI < 27 \text{ kg/m}^2$) and at least one (usually both) parent(s).

Basic phenotypes

Details regarding the original construction of this cohort have been published [3]. The extended families in the current study have contributed to the identification of several major quantitative trait loci (QTLs) linked with visceral obesity and obesity-related metabolic disorders [3,21–26]. Phenotypes available for the individuals within these families include measurements of fasting glucose, insulin, TGs, cholesterol, and HDL- and LDL-cholesterol after a 10 h overnight fast. Measurement of weight, height, waist and hip circumferences have also been obtained. Anthropometric measurements were performed according to criteria established by the WHO [3]. Height was measured without shoes and weight was measured using an electronic scale. Waist and hip circumferences were measured while each subject was standing upright, using a plastic metric tape. Waist circumference was measured at the minimal circumference of the abdomen, and hip circumference was recorded at the maximal gluteal protuberance of the buttocks. All anthropometric measures were performed by an experienced team of certified research coordinators, and the recorded data reflect the mean value for three separate measurements.

Plasma glucose concentrations were measured on a Glucose Analyzer II (Beckman Instruments, CA, USA) by the glucose oxidase method. Plasma TGs and HDL- and LDL-cholesterol were determined spectrophotometrically. TG kits were obtained from Stanbio Laboratory, Inc. (TX, USA) and cholesterol kits from Roche-Boehringer (Basel, Switzerland). LDL cholesterol was measured directly using the enzymatic selective protection method (EZ LDL) of Sigma Diagnostics (MO, USA). Total HDL cholesterol was determined after phosphotungstic acid/ $MgCl_2$ precipitation. Double-antibody, equilibrium radioimmunoassays (Linco Research, Inc., MA, USA) were used for quantification of plasma insulin and adiponectin concentration.

Refined phenotypes

Based on prior linkage studies conducted in this cohort [3], 32 highly informative families (i.e., families contributing the most to obesity-related QTLs) were selected and more fully

characterized for additional rigorous metabolic phenotypes. In addition to waist circumference and waist:hip ratio (i.e., basic measures of abdominal obesity), visceral and subcutaneous fat mass have been quantified at the mid- abdominal level using computed tomography (CT) scanning (i.e., refined measures of abdominal obesity). Resting Energy Expenditure (REE) was also determined by indirect calorimetry. Each subject was studied using a metabolic cart for analyses of expired gases, to derive the volume of air passing through the lungs, the amount of oxygen extracted from it, and the amount of expired carbon dioxide. All variables were normalized to 1 min intervals, and REE was determined using the abbreviated Weir Equation: $REE = [3.9(VO_2) + 1.1 (VCO_2)]1.44$.

To accurately quantify insulin responsiveness in these individuals, a frequently sampled intravenous glucose tolerance test was employed, using the minimal model (MinMod) of Bergman [27,28]. This model compensates for subject-to-subject variability in insulin kinetics and glucose kinetics. The MinMod has previously been used to identify positional candidate genes associated with insulin resistance [26] in the 32 additional TOPS families that the current study used to characterize *FAAH* as a biological candidate gene. This approach has been validated against euglycemic clamping as a standard both in subjects with normal glucose tolerance and in subjects with impaired glucose tolerance [27,29,30]. Briefly, subjects were characterized after a 10 h fast. While they remained in a supine position, three baseline blood samples were drawn (at -15, -5 and -1 min) and assayed for insulin and glucose levels. Intravenous glucose (0.3 mg/kg up to a maximum of 35 g, using 50% dextrose solution) was administered over 2 min beginning at time zero. At 20 min, an intravenous bolus of insulin (0.03 U/kg of regular insulin) was administered. Blood samples (5 ml) were drawn over a 3 h period. Four discrete parameters were determined from the plasma glucose concentrations using the MinMod program: S_I , AIR_G , S_G and D_I . Each parameter was then tested individually for association with genetic variability in *FAAH*, as defined below.

Genotype

As noted earlier, a nonsynonymous coding SNP in *FAAH* (C385A) has previously been associated with differences in BMI in subjects of European ancestry [16]. This was the primary SNP of interest. To determine whether additional genetic variability in *FAAH* contributes to obesity-related traits in these extended families, haplotype tagging SNPs were also selected for *FAAH* from the Centre d'Etude du Polymorphisme Humain (CEPH) population within the Human HapMap database [31,32], and genotyped within this cohort as well. Chromosomal position for *FAAH* was obtained from the University of California Santa Cruz (UCSC) genome browser [101] for the entire gene, plus 5 kb both upstream and 5 kb downstream (NCBI build 35). Patterns of linkage disequilibrium (LD) were over 98% identical between the current study population and the CEPH population in the HapMap database ($r^2 \geq 0.8$) [33]. The extent of LD was quantified [34], and tagging SNPs were identified using Tagger [35]. A single block of LD was observed across the *FAAH* region of interest. Using this approach, five tagSNPs adequately covered the linkage contained across the entire gene (rs324418, rs324420, rs1984490, rs2145408 and rs4141964).

All tagSNPs were genotyped using an approach that combines thermocycling with fluorescent detection of allele specific oligonucleotides. Primers were designed to amplify products containing each tagSNP: rs324418, forward CAGCAAACAGCAGCCTTTCTAAC, reverse A AGTCTGGCAGGA ACAGGCTACAT; rs324420, forward GAAGTGAACAAAG-GGACCAACTG, reverse TTGTAGGTGAA-GCACTCCTTGAG; rs1984490, forward C TC TG A AGGTCC T T TGC AC AC T T, reverse ACCTCATATGGCTTAGGGAGGAT; rs2145408, forward GTGCTGTGCCTTGA-TGTGTATGT, reverse AAACCACCTTAT-GGCTGGAGGT; rs4141964, forward TGGCC AC AGGT T TAG ATGT TACC, reverse

GGCAGCTGAGACATTTTCTCCTA. Genomic DNA was heated at 94°C for 3 min, then subjected to 40 cycles using the following thermoprofile: 94°C for 30 s, 60°C for 30 s, and 72°C for 30 s. A final extension occurred at 72°C for 10 min then held at 4°C. Custom Invader assays were designed, using software provided by the manufacturer (Third wave Technology, WI, USA). Genotyping was performed in 384-well plates in a total volume of 6 µl, containing 0.5 µl PCR product, 0.02 µl each primary probe, 0.002 µl Invader probe, 1.12 µl 2.6 M betaine (Sigma), 2.75 µl TE buffer, 0.35 µl Cleavase (Third wave Technology) and 1.24 µl fluorescence resonance energy transfer (FRET) mix (Third wave Technology). Reactions were denatured at 95°C for 5 min and then incubated at 65°C for 15 min. Plates were scanned twice on a plate reader (LJL Biosystems [CA, USA] Model Analyst AD 96/384), using separate scans for both the major and minor alleles.

Genotypes were assigned, plate-by-plate, using a proprietary clustering algorithm [33,36,37] implemented in the sequence management pipeline at the Medical College of Wisconsin (WI, USA). Each data point was assigned to one of four clusters, representing either negative controls, homozygotes (AA or BB) or heterozygotes (AB). Final clusters were described by ellipses whose axes were the standard deviations in the x and y directions of the component points. Calculated allele frequencies were then compared with Mendelian laws of segregation. Thus, the analysis software served as a final quality check for the data. Due to observed inconsistencies, two families were removed during the statistical analyses of our primary SNP of interest, rs324420.

Statistical analysis

Pedigree structure was modeled within these 261 extended families using Pedstats. Genotype–phenotype association was then quantified using single-locus Family-Based Association Test. This approach considers simultaneous transmission from both parents, and separately tests for reception of each genotype by the children compared with what would be expected in the absence of association. A general model was applied, and we accepted $p < 0.05$ as significant. More restrictive models include the additive model (in which the statistic used is the number of alleles of interest received by each affected child) and the dominant model (in which the statistic used is the presence or absence of the allele of interest in each affected child). The expected value in all situations is conditional on all available genotype data in the nuclear family.

Results

Within these 261 families, pedigree size ranged from 4 to 14 individuals per family. Pedigree structure has been summarized for these families using Pedstats: 1941 sib-pairs; 25 half-sib pairs; 2518 parent–child pairs; 620 grandparent–grandchild pairs; 1172 avuncular pairs (Niece–Uncle/Aunt or Nephew–Uncle/Aunt) and 469 pairs of cousins. Figures 1–3 illustrate the relationship of family structure to our primary traits of interest (BMI, TGs and HDL). As anticipated, the strength of correlation was higher for HDL, than for BMI or TG. The heritability of these traits in the current study cohort was H^2 0.66 for HDL-cholesterol level; 0.46 for BMI; and 0.55 for TG level (all $p < 0.001$). These observations are consistent with the established literature [38,39].

Basic obesity-related traits were first tested for association with rs324420 (the nonsynonymous coding SNP in *FAAH*) in the exact same group of individuals (261 families) wherein other eCS signaling genes have been associated with obesity traits [12]. Based upon a single-locus Family-Based Association Test in these 261 families, rs324420 was associated with BMI ($p = 0.042$), fasting TG level ($p = 0.022$) and HDL-cholesterol level ($p = 0.007$). These findings are summarized in Table 1. Fasting glucose level ($p = 0.1$),

homeostasis model assessment of insulin resistance (HOMA-IR) and adiponectin level were not associated with rs324420.

To determine whether additional genetic variability in *FAAH* (i.e., genetic factors beyond rs324420) may contribute to obesity-related phenotypic traits within this family-based cohort, haplotype tagging SNPs were then selected for *FAAH* according to the methods described above. As noted, a single block of LD was observed across the primary region of interest, and five tagging SNPs adequately covered the linkage contained in this region. Beyond the primary SNP of interest (rs324420), none of the other tagSNPs (rs1984490, rs2145408, rs4141964, rs324418) were associated with obesity-related traits in this cohort (i.e., within the 261 extended families shown in Figures 1–3). While *FAAH*-dependent variation in these traits appears to be primarily attributable to rs324420, we cannot exclude the possibility that additional SNPs outside the region of interest could contribute. This seems unlikely, since Lieb *et al.* failed to see an association between BMI and *FAAH* in the Framingham Offspring, using tag-SNPs from a region extending 20 kb upstream and 10 kb downstream [18].

It is plausible that the biological link between rs324420 and obesity-related dyslipidemia in the current cohort may have been driven by changes in insulin responsiveness in these families. As noted earlier, a subset of these 261 families known to contribute to several obesity-related QTLs have been further characterized for additional, more rigorous, metabolic traits. A frequently sampled intravenous glucose tolerance test was conducted on 399 unique individuals in the 32 most highly informative families. As shown in Table 2, rs324420 was tested for association with insulin responsiveness in these 399 individuals. Whereas fasting glucose and fasting insulin levels serve as a general measure of insulin sensitivity and insulin secretion, respectively, the MinMod of Bergman provides more accurate parameters [27,28]: S_I , AIR_G , S_G and D_I . These refined phenotypic traits are all highly heritable within this study cohort: H^2 : 0.43 for S_I ($p < 0.001$), H^2 : 0.54 for AIR_G ($p < 0.001$), H^2 : 0.42 for S_G ($p < 0.001$), H^2 : 0.50 for D_I ($p < 0.001$). Using these traits, rs324420 was further tested for association with insulin responsiveness. None of the rigorous phenotypic parameters derived from the MinMod were associated with rs324420 (AIR_G $p > 0.1$; S_G : $p > 0.1$; S_I : $p > 0.1$; D_I : $p > 0.1$). Conversely, the association between this coding SNP and obesity-related dyslipidemia persisted in this smaller subset of families ($p = 0.0185$ for TGs; $p = 0.0051$ for HDL).

Each effect was then quantified by a copy of the minor allele, for the relationship between rs324420 and obesity-related dyslipidemia within these 32 families. The results have been stratified by genotype in Table 3. Mean BMI was 31.5 kg/m² in subjects homozygous for the major allele (CC), 33.8 kg/m² in heterozygous subjects (CA) and 35.1 kg/m² in subjects homozygous for the minor allele (AA). This finding is consistent with published observations in cohorts of similar ancestry [16]. More importantly, mean HDL-cholesterol level was 40.5 mg/dl in subjects homozygous for the major allele, 39.1 mg/dl in heterozygous subjects and 34.8 in subjects homozygous for the minor allele. Since a 1 mg/dl reduction in circulating HDL level is associated with a 6% increase in the risk for developing coronary artery disease [4], this observation has clinical significance.

Discussion

Genetic variation in *FAAH* has previously been associated with obesity in subjects of European ancestry [16]. This C to A transversion at nucleotide 385 is known to switch proline to threonine at amino acid 129, rendering the enzyme susceptible to increased proteolytic degradation [40]. The current study confirms the observations of Sipe *et al.* in one of the most rigorously phenotyped family-based obesity cohorts in the USA [16], and

extends these findings to report that the C385A coding SNP in *FAAH* is associated with obesity-related dyslipidemia independent of four insulin-responsiveness parameters: S_I , AIR_G , S_G and D_I . Our finding that genetic variability in *FAAH* was not associated with glucose homeostasis is consistent the observations of Durand *et al.*, who excluded a role for genetic variability in *FAAH* in the development of Type 2 diabetes mellitus, but presented suggestive evidence that *FAAH* may directly impact lipid homeostasis [19].

At present, the eCS system represents one of many interesting therapeutic targets for the treatment of lipid disorders accompanying weight gain [5]. Recent data indicate that obesity-related dyslipidemia is influenced by overactive signaling at the level of CB_1 [12]. The endogenous CB_1 receptor agonist AEA is not stored in vesicles [41]; rather, it is synthesized 'on demand' from the acylated phospholipid, *N*-arachidonylphosphatidyl-ethanolamine by phospholipases [42,43]. Since AEA is not stored in vesicles, ligand concentration is determined by its rate of degradation via *FAAH*, a serine amidase. This enzyme catalyzes the hydrolysis of AEA to arachidonic acid and ethanolamine [15]. Immunohistochemical studies reveal that *FAAH* is found in cells postsynaptic to neurons expressing CNR_1 in many brain regions [44–46]. *FAAH* null mice exhibit a 5- to 15-fold increase in brain AEA content and demonstrate no appreciable AEA hydrolysis [13,47], indicating that *FAAH* plays a critical role in the regulation of AEA content in the brain. *FAAH* is also expressed in the small intestine, pancreas and skeletal muscle.

The current observation that genetic variability in *FAAH* is associated with dyslipidemia, independent of insulin responsiveness, suggests that eCS signaling may directly influence lipid homeostasis. Animal data indicate that signaling through CB_1 can modulate energy homeostasis through physiologic mechanisms other than enhanced food intake [48]. CB_1 -dependent signaling occurs in adipose tissue [49], and CB_1 receptor blockade results in enhanced lipolysis through the stimulation of enzymes involved in β -oxidation and the tricarboxylic acid cycle [50]. CB_1 receptor blockade also increases energy expenditure in adipose via futile cycle induction, and it upregulates expression of glucose transporter type 4, resulting in improved glucose utilization [50]. In white fat, the activation of CB_1 inhibits the secretion of adipocytokines [51], and in brown fat CB_1 activation downregulates the thermogenic factor, UCP-1 [51]. The role of thermogenic uncoupling was under-appreciated in humans until recently [52]. While it is conceivable that proteolytic degradation of *FAAH* (due to C385A) might adversely influence lipid homeostasis through increased tissue levels of *N*-arachidonyl-ethanolamine, it should be noted that *FAAH* also catalyzes the hydrolysis of other *N*-acyl-ethanolamines (e.g., palmitoyl-ethanolamine and oleoyl-ethanolamide). Further studies will be needed before the mechanistic link between variation in eCS/ CB_1 signaling and lipoprotein biology is completely understood.

In summary, our data reveal that genetic variability in *FAAH* (rs324420) is associated with obesity-related dyslipidemia in multi-generational families of Northern European descent. While the findings demonstrate that this coding SNP influences fasting TG levels as well as circulating levels of HDL cholesterol, they do not clearly indicate whether this association reflects decreased lipid biosynthesis or increased lipoprotein clearance. Future experiments directed toward characterizing the relationship between eCS/ CB_1 signaling and lipid homeostasis may lead to novel intervention strategies for the management of obesity-related dyslipidemia.

Executive summary

- Obesity-related lipid disorders have tremendous public health significance.
- Markers of cardiometabolic risk may be used to individualize clinical intervention.

Results

- rs324420 is associated with obesity-related dyslipidemia (high triglycerides and reduced high-density lipoprotein cholesterol level) within one of the largest family-based obesity study cohorts in the USA.
- Haplotype tagging SNPs do not reveal any additional association, suggesting that rs324420 is causative.
- rs324420 is not associated with insulin secretion and insulin sensitivity within this family-based cohort.

Conclusion

- Genetic variation in *FAAH* is associated with obesity-related dyslipidemia, independent of insulin response.

Acknowledgments

The authors would like to express their gratitude to Roland James and all participants in the TOPS Metabolic Research Program, based in Milwaukee at the Medical College of Wisconsin (WI, USA).

Bibliography

Papers of special note have been highlighted as:

• of interest

•• of considerable interest

1. Wang Y, Beydoun MA, Liang L, Caballero B, Kumanyika SK. Will all Americans become overweight or obese? Estimating the progression and cost of the US obesity epidemic. *Obesity* (Silver Spring) 2008;16:2323–2330. [PubMed: 18719634]
2. Yusuf S, Hawken S, Ounpuu S, et al. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. *Lancet* 2004;364:937–952. [PubMed: 15364185]
3. Kissebah AH, Sonnenberg GE, Myklebust J, et al. Quantitative trait loci on chromosomes 3 and 17 influence phenotypes of the metabolic syndrome. *Proc Natl Acad Sci USA* 2000;97:14478–14483. [PubMed: 11121050]
4. Ashen MD, Blumenthal RS. Clinical practice. Low HDL cholesterol levels. *N Engl J Med* 2005;353:1252–1260. [PubMed: 16177251]
5. Scheen AJ, Paquot N. Use of cannabinoid CB1 receptor antagonists for the treatment of metabolic disorders. *Best Pract Res Clin Endocrinol Metab* 2009;23:103–116. [PubMed: 19285264]
6. Berry EM, Mechoulam R. Tetrahydrocannabinol and endocannabinoids in feeding and appetite. *Pharmacol Ther* 2002;95:185–190. [PubMed: 12182965]
7. Howlett AC. The cannabinoid receptors. *Prostaglandins Other Lipid Mediat* 2002;68–69:619–631.
8. Benzinou M, Chèvre JC, Ward KJ, et al. Endocannabinoid receptor 1 gene variations increase risk for obesity and modulate body mass index in European populations. *Hum Mol Genet* 2008;17:1916–1921. [PubMed: 18375449]
9. Russo P, Strazzullo P, Cappuccio FP, et al. Genetic variations at the endocannabinoid type 1 receptor gene (*CNR1*) are associated with obesity phenotypes in men. *J Clin Endocrinol Metab* 2007;92:2382–2386. [PubMed: 17405839]
10. Gazzerri P, Caruso MG, Notarnicola M, et al. Association between cannabinoid type-1 receptor polymorphism and body mass index in a southern Italian population. *Int J Obes* 2007;31:908–912.

11. Peeters A, Beckers S, Mertens I, van Hul W, van Gaal L. The G1422A variant of the cannabinoid receptor gene (*CNR1*) is associated with abdominal adiposity in obese men. *Endocrine* 2007;31:138–141. [PubMed: 17873324]
- 12•. Baye TM, Zhang Y, Smith E, et al. Genetic variation in cannabinoid receptor 1 (*CNR1*) is associated with derangements in lipid homeostasis, independent of body mass index. *Pharmacogenomics* 2008;9:1647–1656. Prior work showing that genetic variation in endocannabinoid signaling directly affects lipids. [PubMed: 19018721]
13. Cravatt BF, Demarest K, Patricelli MP, et al. Supersensitivity to anandamide and enhanced endogenous cannabinoid signaling in mice lacking fatty acid amide hydrolase. *Proc Natl Acad Sci USA* 2001;98:9371–9376. [PubMed: 11470906]
14. Hillard CJ, Shi L, Tuniki VR, Falck JR, Campbell WB. Studies of anandamide accumulation inhibitors in cerebellar granule neurons: comparison to inhibition of fatty acid amide hydrolase. *J Mol Neurosci* 2007;33:18–24. [PubMed: 17901541]
15. Ueda N. Endocannabinoid hydrolases. *Prostaglandins Other Lipid Mediat* 2002;68–69:521–534.
16. Sipe JC, Waalen J, Gerber A, Beutler E. Overweight and obesity associated with a missense polymorphism in fatty acid amide hydrolase (FAAH). *Int J Obes (Lond)* 2005;29:755–759. [PubMed: 15809662]
17. Jensen DP, Andreasen CH, Andersen MK, et al. The functional Pro129Thr variant of the *FAAH* gene is not associated with various fat accumulation phenotypes in a population-based cohort of 5,801 whites. *J Mol Med* 2007;85:445–449. [PubMed: 17216208]
- 18•. Lieb W, Manning AK, Florez JC, et al. Variants in the *CNR1* and the *FAAH* genes and adiposity traits in the community. *Obesity* 2009;17:755–760. Recent work showing that genetic variation in endocannabinoid signaling does not influence BMI longitudinally. [PubMed: 19165169]
19. Durand E, Lecoecur C, Delplanque J, et al. Evaluating the association of *FAAH* common gene variation with childhood, adult severe obesity, and type 2 diabetes in the French population. *Obesity Facts* 2008;1:305–309. [PubMed: 20054193]
20. Aberle J, Fedderwitz I, Klages N, George E, Beil FU. Genetic variation in two proteins of the endocannabinoid system and their influence on body mass index and metabolism under low fat diet. *Horm Metab Res* 2007;39:395–397. [PubMed: 17533584]
21. Comuzzie AG, Funahashi T, Sonnenberg G, et al. The genetic basis of plasma variation in adiponectin, a global endophenotype for obesity and the metabolic syndrome. *J Clin Endocrinol Metab* 2001;86:4321–4325. [PubMed: 11549668]
22. Curran JE, Jowett JB, Elliott KS, et al. Genetic variation in selenoprotein S influences inflammatory response. *Nat Genet* 2005;37:1234–1241. [PubMed: 16227999]
23. Martin LJ, Comuzzie AG, Sonnenberg GE, et al. Major quantitative trait locus for resting heart rate maps to a region on chromosome 4. *Hypertension* 2004;43:1146–1151. [PubMed: 14993199]
- 24•. Smith EM, Wang X, Littrell J, et al. Comparison of linkage disequilibrium patterns between the HapMap CEPH samples and a family-based cohort of Northern European descent. *Genomics* 2006;88:407–414. Prior work showing that the linkage structure in our study population is similar to the Centre d'Etude du Polymorphisme Humain (CEPH) population. [PubMed: 16713172]
25. Sonnenberg GE, Krakower GR, Kissebah AH. A novel pathway to the manifestations of metabolic syndrome. *Obes Res* 2004;12:180–186. [PubMed: 14981209]
26. Walder K, Kerr-Bayles L, Civitarese A, et al. The mitochondrial rhomboid protease PSARL is a new candidate gene for Type 2 diabetes. *Diabetologia* 2005;48:459–468. [PubMed: 15729572]
27. Bergman RN, Ider YZ, Bowden CR, Cobelli C. Quantitative estimation of insulin sensitivity. *Am J Physiol* 1979;236:E667–E677. [PubMed: 443421]
28. Pacini G, Bergman RN. MINMOD: a computer program to calculate insulin sensitivity and pancreatic responsivity from the frequently sampled intravenous glucose tolerance test. *Comput Methods Programs Biomed* 1986;23:113–122. [PubMed: 3640682]
29. Saad MF, Anderson RL, Laws A, et al. A comparison between the minimal model and the glucose clamp in the assessment of insulin sensitivity across the spectrum of glucose tolerance. *Insulin Resistance Atherosclerosis Study. Diabetes* 1994;43:1114–1121. [PubMed: 8070611]

30. Haffner SM, Howard G, Mayer E, et al. Insulin sensitivity and acute insulin response in African-Americans, non-Hispanic whites, and Hispanics with NIDDM: the Insulin Resistance Atherosclerosis Study. *Diabetes* 1997;46:63–69. [PubMed: 8971083]
31. International Consortium: A haplotype map of the human genome. *Nature* 2005;437:1299–1320. [PubMed: 16255080]
32. International Consortium: A second generation human haplotype map of over 3.1 million SNPs. *Nature* 2007;449:851–861. [PubMed: 17943122]
33. Smith EM, Littrell J, Olivier M. Automated SNP genotype clustering algorithm to improve data completeness in high-throughput SNP genotyping datasets from custom arrays. *Genomics Proteomics Bioinformatics* 2007;5:256–259. [PubMed: 18267308]
34. Gabriel SB, Salomon R, Pelet A, et al. Segregation at three loci explains familial and population risk in Hirschsprung disease. *Nat Genet* 2002;31:89–93. [PubMed: 11953745]
35. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 2005;21:263–265. [PubMed: 15297300]
36. Olivier M, Chuang LM, Chang MS, et al. High-throughput genotyping of single nucleotide polymorphisms using new biplex invader technology. *Nucleic Acids Res* 2002;30(12):E53. [PubMed: 12060691]
37. Olivier M. The Invader assay for SNP genotyping. *Mutat Res* 2005;573(1–2):103–110. [PubMed: 15829241]
38. Maes HM, Neale MC, Eaves LJ. Genetic and environmental factors in relative body weight and human adiposity. *Behav Genet* 1997;27:325–351. [PubMed: 9519560]
39. Schousboe K, Willemsen G, Kyvik KO, et al. Sex differences in heritability of BMI: a comparative study of results from twin studies in eight countries. *Twin Res* 2003;6:409–421. [PubMed: 14624725]
40. Sipe JC, Chiang K, Gerber AL, Beutler E, Cravatt BF. A missense mutation in human fatty acid amide hydrolase associated with problem drug use. *Proc Natl Acad Sci USA* 2002;99:8394–8399. [PubMed: 12060782]
41. Devane WA, Hanus L, Breuer A, et al. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* 1992;258:1946–1949. [PubMed: 1470919]
42. Okamoto Y, Morishita J, Tsuboi K, Tonai T, Ueda N. Molecular characterization of a phospholipase D generating anandamide and its congeners. *J Biol Chem* 2004;279:5298–5305. [PubMed: 14634025]
43. Liu J, Wang L, Harvey-White J, et al. A biosynthetic pathway for anandamide. *Proc Natl Acad Sci USA* 2006;103:13345–13350. [PubMed: 16938887]
44. Egertova M, Cravatt BF, Elphick MR. Comparative analysis of fatty acid amide hydrolase and CB(1) cannabinoid receptor expression in the mouse brain: evidence of a widespread role for fatty acid amide hydrolase in regulation of endocannabinoid signaling. *Neuroscience* 2003;119:481–496. [PubMed: 12770562]
45. Gulyas AI, Cravatt BF, Bracey MH, et al. Segregation of two endocannabinoid-hydrolyzing enzymes into pre- and postsynaptic compartments in the rat hippocampus, cerebellum and amygdala. *Eur J Neurosci* 2004;20:441–458. [PubMed: 15233753]
46. Tsou K, Nogueron MI, Muthian S, et al. Fatty acid amide hydrolase is located preferentially in large neurons in the rat central nervous system as revealed by immunohistochemistry. *Neurosci Lett* 1998;254:137–140. [PubMed: 10214976]
47. Clement AB, Hawkins EG, Lichtman AH, Cravatt BF. Increased seizure susceptibility and proconvulsant activity of anandamide in mice lacking fatty acid amide hydrolase. *J Neurosci* 2003;23:3916–3923. [PubMed: 12736361]
48. Colombo G, Agabio R, Diaz G, Lobina C, Reali R, Gessa GL. Appetite suppression and weight loss after the cannabinoid antagonist SR 141716. *Life Sci* 1998;63:PL113–PL117. [PubMed: 9718088]
49. Murdolo G, Kempf K, Hammarstedt A, Herder C, Smith U, Jansson PA. Insulin differentially modulates the peripheral endocannabinoid system in human subcutaneous abdominal adipose tissue from lean and obese individuals. *J Endocrinol Invest* 2007;30:RC17–RC21. [PubMed: 17923791]

50. Jbilo O, Ravinet-Trillou C, Arnone M, et al. The CB1 receptor antagonist rimonabant reverses the diet-induced obesity phenotype through the regulation of lipolysis and energy balance. *FASEB J* 2005;19:1567–1569. [PubMed: 16009704]
51. Perwitz N, Fasshauer M, Klein J. Cannabinoid receptor signaling directly inhibits thermogenesis and alters expression of adiponectin and visfatin. *Horm Metab Res* 2006;38:356–358. [PubMed: 16718635]
52. Jia JJ, Tian YB, Cao ZH, et al. The polymorphisms of *UCP1* genes associated with fat metabolism, obesity and diabetes. *Mol Biol Rep*. 2009 (Epub ahead of print).

Website

101. Human Genome Browser Gateway. <http://genome.cse.ucsc.edu/cgi-bin/hgGateway>

Figure 1. Pair-wise relationships (e.g., parent–child) are shown for BMI in 261 families

The pedigree structure of our entire study cohort ($n = 1644$ individuals) has been modeled using Pedstats. The following relationships have been illustrated: sibling–sibling (**A**), half-sibling–half-sibling (**B**), grandparent–grandchild (**C**) and parent–child (**D**). Our primary phenotype (BMI) was plotted by relationship for each pair of individuals. While higher generation relationships (e.g., great grandparents, avuncular and cousins) were frequently observed within our cohort, these relationships have not been plotted.

Figure 2. Pair-wise relationships (e.g., parent–child) are shown for TG in 261 families

The pedigree structure of our entire study cohort ($n = 1644$ individuals) has been modeled using Pedstats. The following relationships have been illustrated: sibling–sibling (**A**), half-sibling–half-sibling (**B**), grandparent–grandchild (**C**) and parent–child (**D**). Our primary phenotype (TGs) was plotted by relationship for each pair of individuals. While higher generation relationships (e.g., great grandparents, avuncular and cousins) were frequently observed within our cohort, these relationships have not been plotted. TG: Triglyceride.

Figure 3. Pair-wise relationships (e.g., parent–child) are shown for HDL in 261 families

The pedigree structure of our entire study cohort ($n = 1644$ individuals) has been modeled using Pedstats. The following relationships have been illustrated: sibling–sibling (**A**), half-sibling–half-sibling (**B**), grandparent–grandchild (**C**) and parent–child (**D**). Our primary phenotype (HDL) was plotted by relationship for each pair of individuals. While higher generation relationships (e.g., great grandparents, avuncular and cousins) were frequently observed within our cohort, these relationships have not been plotted. HDL: High-density lipoprotein.

Table 1

Subject characteristics, tested for association with rs324420 (n = 261 families)^{*}.

Phenotype	Units	Mean	SD	Sib correlation	p-value [‡]
Age	Years	46	16	0.677	ns
BMI	kg/m ²	31.9	8.2	0.109	0.042
Waist	cm	102	19	0.117	0.090
Hips	cm	116	17	0.125	ns
WHR	Ratio	0.88	0.11	0.121	ns
T Chol	mg/dl	197	44	0.280	ns
LDL	mg/dl	122	40	0.203	ns
HDL	mg/dl	39	12	0.161	0.007
TGs	mg/dl	127	81	0.098	0.022
Glucose	mmol	91	32	0.071	0.100
HOMA-IR	Derived	4.07	5.25	0.103	ns
Adiponectin	µg/ml	8.16	4.25	0.245	ns

^{*} This cohort represents the same 261 families used previously to describe an association between obesity-related dyslipidemia and *CNR1* haplotype [12].

[‡] p-value, determined by an uncorrected single-locus, family-based association test, for the nonsynonymous coding SNP in *FAAH* (rs324420).

HDL: High-density lipoprotein; HOMA-IR: Homeostasis model assessment of insulin resistance; LDL: Low-density lipoprotein; ns: Not significant; SD: Standard deviation; T Chol: Total cholesterol; TG: Triglyceride; WHR: Waist:hip ratio.

Table 2

Parameters of insulin responsiveness* by genotype (n = 32 families).

rs324420		AIR _g	S _I	S _g	D _I	REE
CC (n = 215)	Mean	511.2	3.211	0.018	1343.6	3336.7
	SD	411.8	2.748	0.010	1102.7	1638.6
AC (n = 76)	Mean	563.0	3.480	0.016	1096.3	3756.0
	SD	709.0	6.715	0.009	1221.2	1816.3
AA (n = 10)	Mean	485.5	3.657	0.019	1471.7	3687.0
	SD	365.9	2.896	0.008	1118.8	1731.1

* All parameters of the minimal model were tested for association with rs324420 by Family-Based Association Test; none were significant (p > 0.10).
AIR_g: Acute insulin response to i.v. glucose; D_I: Disposition index; REE: Resting energy expenditure; S_g: Glucose effectiveness; S_I: Insulin sensitivity.

Table 3

Genotype by effect, for obesity-related dyslipidemia (n = 32 families).

rs324420		BMI	HDL [*]	TG [‡]
CC (n = 276)	Mean	31.5	40.5	105.1
	SD	8.0	14.7	58.8
AC (n = 97)	Mean	33.8	39.1	122.9
	SD	8.7	10.4	71.4
AA (n = 15)	Mean	35.1	34.8	87.0
	SD	8.6	8.1	47.8

* Associated with rs324420 (p < 0.01).

‡ Associated with rs324420 (p < 0.05).

HDL: High-density lipoprotein; SD: Standard deviation; TG: Triglyceride.