Published in final edited form as:

Prog Neuropsychopharmacol Biol Psychiatry. 2012 March 30; 36(2): 213–219. doi:10.1016/j.pnpbp.2011.08.001.

Validation of candidate genes associated with cardiovascular risk factors in psychiatric patients

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Abstract

The purpose of this study was to identify genetic variants predictive of cardiovascular risk factors in a psychiatric population treated with second generation antipsychotics (SGA). 924 patients undergoing treatment for severe mental illness at four US hospitals were genotyped at 1.2 million single nucleotide polymorphisms. Patients were assessed for fasting serum lipid (low density lipoprotein cholesterol [LDLc], high density lipoprotein cholesterol [HDLc], and triglycerides) and obesity phenotypes (body mass index, BMI). Thirteen candidate genes from previous studies of the same phenotypes in non-psychiatric populations were tested for association. We confirmed 8 of the 13 candidate genes at the 95% confidence level. An increased genetic effect size was observed for triglycerides in the psychiatric population compared to that in the cardiovascular population.

Keywords

Antipsychotics; Lipid metabolism; Obesity; Personalized medicine; Pharmacogenetics; Triglycerides

1. Introduction

Psychiatric patients face a substantial and potentially growing risk of metabolic syndrome and cardiovascular disease (Franciosi and Kasper, 2005; Newcomer, 2005). Of the 57.7 million patients with diagnosable mental illness in the U.S. (NIMHS, 2009), 30–70%, or 17

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Policy and ethics

The work described in this article was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) (<http://www.wma.net/en/30publications/10policies/b3/index.html>) and the manuscript was prepared according to the Uniform Requirements for manuscripts submitted to Biomedical journals (<http://www.icmje.org>).

to 40 million, are estimated to have metabolic syndrome (Correll et al., 2006; Lamberti et al., 2006; Mackin et al., 2007; McEvoy et al., 2005). The introduction of “second generation” antipsychotics (SGA) has coincided with a disproportionate increase in metabolic syndrome in patients with severe mental illness (Newcomer, 2005). SGAs are approved in the U.S. for treatment of schizophrenia, bipolar disorder, psychotic depression and irritability in children with autistic disorder, but are increasingly being used for other psychoses, dementia, obsessive–compulsive and mood disorders in both adults and children (Barbui, 2004; Olfson et al., 2006). SGAs collectively are now more widely utilized than first generation antipsychotic medications (Snyder and Murphy, 2008). SGAs currently available in the U.S. include clozapine, olanzapine, risperidone, quetiapine, ziprasidone, and aripiprazole. SGAs are associated with unwanted metabolic side effects (*MSE*) including weight gain and glucose and lipid disturbances (Correll et al., 2009; Wampers et al., in press), and patients receiving SGA therapy are 9% more likely to have diabetes mellitus than those treated with “first generation” antipsychotics (Sernyak et al., 2002). Hypertriglyceridemia and hypercholesterolemia can develop in patients in as little as 3 months after initial exposure to these drugs (Meyer et al., 2006).

The development of *MSE* in psychiatric patients at such rapid rates has become a serious problem in clinical psychiatry, mirroring concerns about the development of tardive dyskinesia in patients taking first generation anti-psychotic agents. Indeed, the development of weight gain, hyperlipidemia and diabetes mellitus may present threats to health as significant as the underlying disorders being treated and has already limited the use of SGAs for some patients. It is of clinical interest that *MSE* do not develop in all patients (Newcomer, 2005) and it would be of enormous clinical value to be able to predict vulnerable populations. Adverse drug effects are often related to genetic factors (Blanc et al., 2010; Foster et al., 2010; Gurwitz and McLeod, 2007), and understanding the genetic basis for the variable responses to SGAs is the focus of the Psychotropic Induction and Metabolic Side Effects (PIMS) study ([ClinicalTrials.gov](http://clinicaltrials.gov/ct2/show/NCT00752960?term=metabolic+side+effects+and+psychotropics&rank=4) identifier #NCT00752960, available online at <http://clinicaltrials.gov/ct2/show/NCT00752960?term=metabolic+side+effects+and+psychotropics&rank=4>).

The rising prevalence of metabolic disturbance in the psychiatric population, and the possibility that SGA therapy may be causative, led us to hypothesize the existence of markers for metabolic disturbances specific to the psychiatric population, potentially including pharmacogenetic markers associated with an SGA effect. In various non-psychiatric populations, previous genome wide association studies (GWAS) have focused on obesity (Frayling et al., 2007; Li et al., 2010; Loos et al., 2008), dyslipidemia (Lusis and Pajukanta, 2008; Willer et al., 2008, 2009), glycemia (Paterson et al., 2010; Sparso et al., 2009), hypertension (Newton-Cheh et al., 2009), and type 2 diabetes mellitus (Scott et al., 2007; Sladek et al., 2007; Zeggini et al., 2007). In psychiatric populations, GWAS have focused in the psychiatric diseases (Athanasu et al., 2010). More recently, GWAS studies of drug response have begun to appear in the literature (Adkins et al., 2010; Mick et al., 2011; Uher et al., 2010).

In a previous study, we used a DNA microarray incorporating 384 single nucleotide polymorphisms (SNPs) from 222 cardiometabolic and neuroendocrine genes to search in

subcohorts of the PIMS study for candidate genes that would explain the direct effects of some antipsychotics (olanzapine, quetiapine, risperidone) on body weight and lipids (de Leon et al., 2008; Diaz et al., 2009; Ruaño et al., 2007). We found that weight profiles in patients treated with olanzapine were significantly associated with SNPs in the genes for apolipoprotein E (*APOE*) and apolipoprotein A4 (*APOA4*). Weight profiles in patients treated with risperidone were significantly associated with SNPs in the genes for leptin receptor (*LEPR*) and neuropeptide Y receptor Y5 (*NPY5R*) and paraoxonase (*PONI*). These results are consistent with contrasting mechanisms for the weight profile of patients treated with these drugs. Acetyl coenzyme A carboxylase α (*ACACA*) SNP (rs2229416) was significantly associated with hypertriglyceridemia in 165 patients who were taking the above antipsychotics. Two other SNPs, one in the neuropeptide Y (*NPY*) gene (rs1468271) and the other in the acetyl coenzyme A carboxylase β (*ACACB*) gene (rs2241220), were significantly associated with severe hypercholesterolemia in the same patients. An association between the *ACACA* gene and hypertriglyceridemia is consistent with the *ACACA* enzyme's role in fatty acid synthesis and with the potential use of this enzyme's inhibitors in metabolic syndrome treatments (Harwood, 2005).

In the present study our approach considered candidate genes from prior studies of non-psychiatric populations. This approach allows us to surmount the problem of multiple comparisons through the use of prior information. As a source of candidate genes, we have selected meta-analyses by Willer et al. (2008, 2009) on genetic factors associated with cardiometabolic phenotypes low density lipoprotein cholesterol (LDLc), high density lipoprotein cholesterol (HDLc), triglycerides, and overweight and obesity as indicated by body mass index (BMI).

2. Methods

2.1. Study design and sample

The Psychotropic Induction and Metabolic Side effects (PIMS) study is a non-interventional, cross-sectional study of cardiometabolic risk factors in 924 patients with severe mental illnesses (including schizophrenia, schizoaffective disorder, bipolar disorder, and major depressive disorder) commonly treated with SGAs. Patients were recruited at 4 distinct sites: Institute of Living, Hartford, CT (CT, N=134), Kentucky Eastern State Hospital, Lexington (KYE, N=373), Kentucky Central State Hospital, Louisville (KYC, N=34), and Kentucky Western State Hospital, Hopkinsville (KYW, N=383). The PIMS project was approved by the IRBs of University of Kentucky and Hartford Hospital, and each patient signed a statement of informed consent that included permission to use the sample in genomic studies.

2.2. Definition of phenotype and covariates

For assessment of MSE, we employed a methodology used previously in a 560-patient study (Susce et al., 2005). Body mass index (BMI) and serum metabolic profile including total cholesterol, LDLc, HDLc, and triglycerides were assessed (Cholestech LDX) (Panz et al., 2005). Blood was sampled and DNA was extracted as previously described (de Leon et al., 2005).

2.3. Genotyping

The extracted DNA was genotyped using the Human Hap 1M Duo Genotyping BeadChip of Illumina (San Diego, CA), based on the Infinium Whole-Genome Genotyping platform. An iScan scanner was used to read the fluorescence signals from the chip and the raw data was processed using the GenomeStudio software. Because of the high sensitivity of genome-scale association on genotyping quality, careful quality control was performed, assuring that all loci were called in at least 99% of individuals. SNPs with allele frequency of less than 3% were excluded. We also determined the genetic gender by the presence or absence of a) heterozygote signals of markers on the X chromosome and b) any signal of markers on the Y chromosome. Comparison with clinically reported gender identified 15 suspect reports, which were excluded.

As a further quality control measure, we investigated the genetic population structure using high resolution genome-wide allelic dissimilarity analysis. Allelic dissimilarity was calculated as:

$$d_{ij} = \sqrt{\sum_k^{N_{loc}} (g_{ik} - g_{jk})^2}$$

between any pair of individuals, involving the genotypes g_{ik} for subject i at locus k , for all 866,914 loci. Genotypes g_{ik} were 0 for reference homozygotes, 1 for heterozygotes, and 2 for variant homozygotes. Analysis of the resulting distance matrix permitted identification of sample duplications, and unreported family relationships among patients. We compared the population structure as determined genetically using hierarchical clustering (neighbor joining) of the distance metric d_{ij} , and found it to be perfectly correlated with the heritage as reported clinically. This provides evidence that population structure is adequately accounted for by adjusting the risk factors for reported heritage, which was borne out by the results of our p-value distribution analysis. The final number of samples used after quality control was 924, the final number of SNPs, 866,914.

2.4. Statistical analysis

We performed genome wide association screens to find markers associated with any of the four phenotypes listed above. All response variables were adjusted for covariates as described above, and then analyzed quantitatively using linear regression vs. marker allele count (0, 1, or 2, indicating the number of alternative alleles). The regression p-values were converted to log-scores according to the formula $s = -\log_{10} p$. In order to guard against false positive associations caused by non-normal phenotype distribution and other reasons, we also performed a non-parametric permutation analysis, which requires more computation but results in a different set of p-values free of bias caused by divergence from distribution assumptions. The two sets of p-values were compared to identify any statistical anomalies.

2.5. Nearby associated SNPs

For confirmation of existing hypothesized associations we considered the actual reported SNP, if available, and also any SNP 200 kb upstream or downstream of the reported position.

To account for multiple comparisons, we applied a sliding Bonferroni correction by dividing the p-value of association by the number of SNPs located closer to the index SNP than the SNP in question. All SNPs in the 200 kb window were annotated with these corrected p-values and the highest scoring SNP was selected as the nearby associated SNP.

3. Results

3.1. Clinical characteristics of the cohort

There were 232, 105, 228, 66, and 68 patients, respectively, on risperidone, olanzapine, quetiapine, ziprasidone and aripiprazole. 152 patients did not take an antipsychotic, and the remaining 73 patients were taking multiple SGAs or other antipsychotics. Patients ranged in age from 18 to 75 years (mean $\pm$ sd=37 $\pm$ 11 years) and were mostly Caucasian (Table 1). Diagnoses included schizophrenia (21%, N=194), major depression (19%, N=177), schizoaffective disorder (17%, N=156), bipolar disorder (14%, N=126), depressive disorder NOS (5%, N=45), psychotic disorder NOS (3%, N=30), and mood disorder NOS (2%, N=19). The remaining patients were diagnosed with a variety of less common disorders (19%, N=174).

Clinical data including demographics, physical measurements, blood chemistry, diagnoses, pharmacotherapy (SGAs and concomitant medications), and psychiatric care history, were gathered and curated for all patients in the study. The percentages of clinical diagnoses by treating physicians were 7% for diabetes mellitus, 19% for hypertension and 10% for hyperlipidemia. Metabolic phenotypes prepared for analysis were BMI, and fasting serum levels of high density lipoprotein cholesterol (HDLc), low density lipoprotein cholesterol (LDLc), and triglycerides (TG). Table 1 shows the distributions of the phenotypes among patients. We did not attempt to include a metabolic syndrome diagnosis, because glucose levels and blood pressure were not available. In our database, 16.3% was taking antihypertensive treatment; 6.7%, antidiabetic treatment; 6.2%, anticholesterol treatment; and 0.9%, treatment for triglyceridemia.

Before association testing, the metabolic phenotypes were adjusted for age, gender, heritage, drug, and study site. Heritage was defined as combination of race and ethnicity with Hispanic ethnicity treated as an additional category to the usual racial groups (Table 1). We did not include diagnosis as a covariate in the association test, because of its heterogeneity and the potential for introducing noise. However, we did perform a covariate regression analysis of the phenotypes with diagnosis added, and found no association. All lipid levels were adjusted according to BMI, to control for confounding effects of obesity. Generally, 3–5% of variation was explained by these covariates, the strongest being the effect of BMI on triglycerides ($R^2=5.5\%$). Variation explained (partial R^2) for each significant covariate on all phenotypes is listed in Table 2.

3.2. Validation of previously discovered associations

We modeled our genome wide approach after studies of lipid and metabolic phenotypes in non-psychiatric populations (Willer et al., 2008, 2009). Of 58 SNPs associated to cardiometabolic phenotypes reported by Willer et al. (2008, 2009), 30 were genotyped by us

directly. For the other 28 we found markers in the immediate neighborhood of the reported SNP which were strongly linked, as described in Methods. In most cases, we found a much stronger nearby association to another SNP on our array within 200 kb of the index SNP, even when accounting for multiple comparisons within the local area including approximately 200 SNPs. The gene associations reported by Willer et al. (2008, 2009) are shown in detail and combined with our results in Table 3. We show association parameters (log-score and effect) for a) the original report, b) the exact match for the reported SNP on our array (if available), and c) a nearby linked SNP found to be associated in our data. Fig. 1 summarizes the comparison of significance scores grouped by gene. Log-scores for our associations (ordinate) are plotted against those of Willer et al. (2008, 2009) (abscissa). For each gene, scores for the top associated SNP are shown. Given alpha values as shown (p-values of 10^{-7} for screening and 0.05 for validation, vertical and horizontal lines), we validated 8 of 13 gene associations, including 2 of 4, 3 of 4, 2 of 4, and 1 of 1 associations for HDLc, LDLc, triglycerides, and BMI, respectively (filled circles, ●). Note that some of the genes reported by Willer have no SNPs reported that reach genome-wide significance. These are not counted among the 13.

Fig. 2 shows effect sizes of the present study compared to Willer et al. (2008, 2009). Effect sizes in the psychiatric population tend to be stronger compared to the non-psychiatric population for the triglyceride phenotype, roughly equivalent for the HDLc phenotype, and weaker for the LDLc and BMI phenotypes.

4. Discussion

With the cost of genotyping arrays decreasing rapidly, it is becoming economical to perform whole-genome genotyping even when only a small number of candidate genes are to be studied. Samples can be genotyped for the whole genome ahead of candidate selection (i.e. pre-genotyped), and then multiple candidate studies can be performed using that data, without further DNA analysis. This approach is demonstrated by the present study, where we utilize genome-wide genotypes to successfully interrogate candidate genes for associations that are not strong enough to be detected using hypothesis-free design.

We have successfully validated, in a psychiatric patient sample, 8 of 13 candidate genes previously found to be associated with cardiovascular risk factors in non-psychiatric GWAS studies. We have found an increased effect size for the validated associations with triglycerides, similar effect size for HDLc, and decreased effect size for LDLc and BMI. This is interesting, as triglycerides have been implicated in a possible direct effect of SGA (de Leon et al., 2007; Kohen and Manu, 2010; Meyer, 2001; Ruaño et al., 2007), and most of our patients were taking SGA medications.

SGAs have been reported to exert a direct effect on dyslipidemia, particularly hypertriglyceridemia, that is independent of weight gain (de Leon et al., 2007; Kohen and Manu, 2010; Meyer, 2001; Ruaño et al., 2007) and develops within as little as 3 months (Meyer et al., 2006). The present study supports these findings given the increased effect size on triglycerides as compared with non-psychiatric studies.

In approaching the interface of disease susceptibility, drug-induced effects, and cardiometabolic risk, we believe our findings suggest a close alignment in mental illness and psychotropic therapy. Innate susceptibility can be unmasked by drug exposure, which triggers additional sequelae as induction of pharmacological effect takes hold. The effect sizes for the cardiometabolic risk genes validated in this study exceed those reported by Willer et al. (2008, 2009) in non-psychiatric populations. The implications for the psychopharmacologic treatment of severe mental illness are considerable. The indiscriminant prescription of SGAs is challenged by the significant sequelae of metabolic syndrome and the need to stratify degrees of risk for populations and individuals is compelling. Indeed, it is difficult to imagine a future in which SGA prescription remains widespread without guidance by genotype analysis.

Our discovery of potential drug-related effects on triglyceridemias affirms the special role of triglycerides and supports a pharmacogenetic correlation. Clinically both innate disease susceptibility and pharmacogenetic risk can be anticipated to become evident upon treatment. The dual effects of pharmacotherapy pose challenges to psychiatric practice where genotype guidance could play a pivotal role for personalized therapy.

4.1. Limitations

The Psychotropic Induction and Metabolic Side Effects (PIMS) study herein reported is one of the largest examinations of cardiovascular phenotypes in a psychiatric population to date. The cohort in this study can be merged with others to create a meta-analysis of psychiatric cohorts since every patient is already genotyped with a comprehensive Total Genome Array. The cross-sectional study design limits its relevance to drug effects. Another limitation of this study is that data were not available to quantify the exact durations of illness and SGA treatment. Incorporation of these durations in analysis may alter our estimated associations between genetic variants and the cardiometabolic risk factors examined. However, because of the relative homogeneity of the treatment mode, variation in durations is limited. Psychiatric illness for all patients was categorically severe enough to require hospital treatment, suggesting advanced disease states. Nonetheless, we cannot rule out residual confounding associated with variable SGA treatment.

Not all the second generation antipsychotic drugs have the same metabolic side effects, which further limits the drug dependence of the effect and related gene associations. For example, the metabolic side effects of the newer SGAs aripiprazole and ziprasidone may not be so prominent as for the earlier SGAs. Together, these drugs were taken, respectively, by 66 (7.1%) and 68 (7.3%) patients of the cohort. Further, drug-specific gene associations are not supported by the cohort size of each individual drug. For example, at 232 patients, the risperidone cohort was the largest in the study. Larger cohorts in future studies could support drug-specific associations, which are likely given the differences in pharmacokinetics and dynamics among SGAs.

The association between atypical antipsychotics and the hypertensive and hyperglycemic components of metabolic syndrome is complex. One of the unanswered questions is whether the effects of atypical antipsychotics on metabolic syndrome are mediated only by the drug or whether mental illness itself also determines other syndrome components. We have not

attempted genetic correlations but have surveyed the epidemiological data extensively at our own institutions. At the Institute of Living (IOL, Hartford, Connecticut), the patient population's prevalence of hyperglycemia is higher than the general population reflected in National Health and Nutrition Examination Survey (NHANES) data. Comparing individuals ages 18–59 years, IOL patients admitted in 2005–2007 were approximately 38% more likely (14.3% versus 10.4%) to meet the Adult Treatment Panel (Anon, 2002) (ATP-III) criterion for fasting blood sugar than were NHANES (2003–2004) (Centers for Disease Control and Prevention (CDC): National Center for Health statistics (NCHS), 2008) participants (Blank et al., 2010; Goethe et al., 2009b). In another study of the IOL population looking broadly at patients ages 5–95 years, prevalence of diabetes mellitus was 11% and of hypertension 17% (Goethe et al., 2007). In this study regression analyses showed that patients 50 years old were 6 times as likely to have diabetes mellitus and 8 times as likely to have hypertension (both $p < .001$) (Goethe et al., 2007, 2009a). At the 3 Kentucky State Hospitals (Hopkinsville, Lexington, Louisville), our surveys have indicated that the frequencies of hypertension in psychiatric patients tend to be similar whether taking atypical antipsychotics or not (de Leon and Diaz, 2007; de Leon et al., 2008). Susceptible psychiatric patients who develop hypertension on antipsychotics are most likely to have prior history of hypertension or borderline blood pressure baseline readings. We have opined that the pharmacogenetics of the direct effects of antipsychotics on hypertension and hyperglycemia appear to be methodologically intractable and require hundreds or thousands of psychiatric patients in order to find frequent variants with small effects (de Leon and Diaz, 2007; de Leon et al., 2008). In contrast, genetic studies of genes already associated with hypertension and diabetes mellitus would be more tractable in psychiatric populations.

Different studies have different goals. Although the metabolic syndrome has great interest for clinicians, for pharmacologists and physiologists it is a conceptual *tour de force* since antipsychotics have different effects at different levels for each of the metabolic syndrome components (de Leon and Diaz, 2007). Therefore, multiple different mechanisms may explain the effects of antipsychotics in the metabolic syndrome. Each different component of the metabolic syndrome may have specific genetic associations. The validity of comparisons across the literature and of metabolic syndrome as a predictor of cardiovascular disease is uncertain. We examined undisputed outcome measurements that have been the focus of many high quality previous studies of risk of cardiovascular disease among the seriously mentally ill (Kahn et al., 2005; Szarek et al., 2009). Thus, our approach is focused on the genetic associations to individual cardiovascular risk factors. Other groups may have broader scope in their research and explore the genetic associations of the metabolic syndrome on patients taking antipsychotics. It will be useful to compare the results of these approaches in the future.

5. Conclusion

In conclusion, we have successfully validated, in a psychiatric patient sample, 8 of 13 candidate genes previously found to be associated with cardiovascular risk factors in non-psychiatric GWAS studies. We have observed an increased effect size for triglycerides in our psychiatric population vs. cardiovascular populations, which may indicate a particular importance of that aspect in psychiatric patients.

Acknowledgments

Supported by the NIH Small Business Innovation Research Grant 2 R44 MH073291-02 “DNA Diagnostics for Minimizing Metabolic Side-Effects of Antipsychotics.” The [ClinicalTrials.gov](https://clinicaltrials.gov) identifier for the PIMS study is NCT00752960. Dr. Ruano is Principal Investigator and Dr. Goethe and Dr. de Leon are co-investigators for this NIH grant.

Abbreviations

BMI	body mass index
GWAS	Genome-Wide Association Studies
MSE	metabolic side effects
PIMS	Psychotropic Induction and Metabolic Side effects
SGAs	Second Generation Antipsychotics

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Fig. 1. Comparison of association results with previously published studies in non-psychiatric patient populations. On the abscissa are p-values as reported by Willer et al. (2008, 2009). The ordinate corresponds to our association p-values.

Fig. 2. Effect sizes measured in all validated SNP associations, relative to the maximum effect size per phenotype. Effect size in the original study (ordinate) is plotted against effect size in our psychiatric population (abscissa).

Distribution of phenotypes across patients by various categories. Patient count, mean and standard deviation are given for each phenotype, broken down by covariates.

Table 1

Category	Group	N	HDLc [mg/dl]	LDLc [mg/dl]	TG [mg/dl]	BMI
All		924	50.2±15.3	98.2±36.9	186.6±118.8	28.6±7.4
Gender	Male	570	48.4±14.3	96.6±35.6	194.0±125.0	27.9±6.6
	Female	354	53.1±16.3	100.8±38.7	174.8±107.3	29.8±8.3
Heritage	Caucasian	748	49.5±15.2	98.0±37.4	195.4±121.5	28.5±7.5
	African American	128	55.4±15.5	97.3±34.1	144.5±93.2	29.4±6.7
	Hispanic Latino	37	46.1±12.1	104.1±32.8	165.4±119.5	30.1±7.1
	Other	11	48.7±18.4	108.2±47.4	146.0±80.9	25.2±4.1
Site	CT	134	46.9±12.6	109.1±36.4	183.4±130.6	29.8±6.9
	KYE	373	50.5±14.9	95.4±37.2	177.4±108.6	27.9±7.6
	KYC	34	60.5±20.2	81.0±35.5	173.4±69.4	28.8±5.9
	KYW	383	50.1±15.6	99.2±36.0	197.8±126.9	28.9±7.4
DSM-IV diagnosis	Schizophrenia	194	50.6±15.1	93.2±37.0	191.7±124.4	28.8±8.0
	Schizoaffective	156	49.7±15.5	96.6±35.0	183.5±106.0	28.4±7.1
	Bipolar	126	48.9±15.0	102.4±36.5	186.7±130.4	29.0±7.8
	Depression	177	50.4±15.4	101.5±37.6	183.5±101.6	28.5±6.9
	Psychotic nos	30	47.5±17.7	103.0±35.8	225.8±185.4	29.4±6.9
	Depressive nos	45	49.6±13.0	101.1±37.8	200.0±131.8	28.9±7.0
	Mood nos	19	51.8±16.8	88.8±36.6	152.5±82.2	27.6±8.1
	Other	174	51.4±15.6	98.1±37.8	178.0±111.7	28.5±7.2
	Antipsychotic treatment	152	52.5±17.0	103.9±38.5	181.7±115.8	28.0±7.8
	Risperidone	232	51.1±15.7	95.0±33.1	161.0±94.9	27.8±6.5
	Quetiapine	228	48.0±14.3	99.8±37.5	214.4±129.5	30.0±7.8
	Olanzapine	105	48.6±13.0	105.4±39.1	199.4±145.5	26.6±5.0
	Aripiprazole	66	52.5±17.9	95.1±41.4	177.5±97.0	29.5±9.5
	Ziprasidone	68	50.4±13.4	92.3±33.4	147.6±88.3	30.3±8.1
	Clozapine	11	44.4±12.8	102.9±25.0	256.2±155.9	29.0±2.8
	Other	20	50.3±13.2	83.8±31.4	234.4±141.2	26.2±3.9

Category	Group	N	HDLc [mg/dl]	LDLc [mg/dl]	TG [lmg/dl]	BMI
	Multiple	42	50.5±16.1	90.6±40.3	202.7±121.9	30.4±7.6

Abbreviations: CT — Institute of Living, Hartford, Connecticut; KYE — Kentucky Eastern State Hospital, Lexington; KYC — Kentucky Central State Hospital, Louisville; KYW — Kentucky Western State Hospital, Hopkinsville; DSM-IV — Diagnostic and Statistical Manual of Mental Disorders, fourth edition, 1994.

Table 2

Covariate dependencies. R^2 values based on multiple regression ANOVA are shown. Partial R^2 values are derived from the regression sums of squares of the ANOVA table. Examination of cross-correlations confirmed them to be small, allowing the partial R^2 values to be interpreted as fraction of variation explained by each variable.

Covariate	HDLc	LDLc	TG	BMI
Age	0.007	0	0	0.005
Gender	0.028	0	0.011	0.014
Heritage	0.018	0	0.024	0
Drug	0	0.017	0.045	0.031
Site	0.021	0.023	0	0.01
BMI	0.034	0	0.049	–

Table 3

Confirmation results for SNP associations reported by Willer et al. We show the prior score and effect as reported by Willer, and score and effect in our data set for the same SNP (where available) and the nearby associated SNP as defined in Methods. SNPs validated with a score of 2 or better ($p < 0.01$) are indicated in bold.

Gene	SNP	chr	Position	Prior		Same SNP		Nearby SNP		
				Score	Effect	Score	Effect	Rs-Id	Score	Effect
<i>HDL cholesterol</i>										
CETP (NUP93)	rs3764261	16	55550825	18.55	3.47	6.15	3.51	rs1800775	8.99	4.00
	rs1864163	16	55554734	16.52	4.12	6.75	3.87	rs1561139	5.69	3.14
	rs9989419	16	55542640	15.10	1.72	3.19	2.21	rs735144	5.49	3.07
	rs12596776	16	55476849	4.43	1.26	–	–			
LIPC	rs1566439	16	55582163	4.70	0.96	0.03	0.06			
	rs10468017	15	56465804	10.07	1.76	0.96	1.22	rs10468017	0.96	1.22
	rs4775041	15	56461987	8.55	1.38	0.55	0.82	rs4775041	0.55	0.82
	rs261332	15	56514617	8.77	1.41	0.11	0.23	rs4774292	2.65	2.04
LPL	rs12678919	8	19888502	10.89	2.44	0.73	1.47	rs343	3.05	3.92
	rs10503669	8	19891970	9.49	2.09	–	–			
	rs2197089	8	19870653	7.47	1.38	1.02	1.11			
	rs6586891	8	19958878	4.46	1.00	–	–			
GALNT2	rs4846914	1	228362314	6.54	1.15	–	–	rs2281719	0.70	0.81
	rs2144300	1	228361539	6.18	1.11	–	–			
LIPG	rs4939883	18	45421212	6.85	1.87	0.27	0.49	rs7242671	2.03	1.83
	rs2156552	18	45435666	6.08	1.20	0.43	0.83	rs11875600	2.64	3.16
ABCA1	rs4149268	9	106687041	6.48	0.82	0.16	0.25	rs4149268	0.16	0.25
MVK/MMAB (KCTD10)	rs2338104	12	108379551	5.72	0.48	–	–	rs11609019	0.09	0.22
LCAT (DPEP2)	rs255052	16	66582496	5.82	0.74	0.23	0.47	rs255052	0.23	0.47
GRIN3A	rs1323432	9	103402758	7.60	0.03	–	–	rs6479056	1.01	1.31
<i>LDL cholesterol</i>										
APOE/C1/C4	rs4420638	19	50114786	20.49	6.61	0.42	2.17	rs445925	2.48	7.73
	rs10402271	19	50021054	5.01	2.62	0.27	1.24			
CELSR2/PSRC1/SORT1	rs602633	1	109623034	13.32	6.09	0.71	2.36	rs602633	0.71	2.36

Gene	SNP	chr	Position	Prior		Same SNP		Nearby SNP		
				Score	Effect	Score	Effect	Rs-Id	Score	Effect
LDLR (SMARCA4)	rs599839	1	109623689	12.92	5.48	0.77	2.46	rs14000	1.14	5.22
	rs6511720	19	11063306	9.17	9.17	0.57	2.94	rs2072382	4.38	18.96
	rs515135	2	21139562	13.51	6.08	-	-	rs1713222	1.54	5.44
	rs562338	2	21141826	10.92	4.89	-	-	rs541041	1.20	3.82
APOB	rs754523	2	21165196	6.15	2.78	0.45	1.82	rs473770	2.18	5.21
	rs693	2	21085700	6.92	2.44	0.04	0.22			
PCSK9	rs11206510	1	55268627	5.12	3.04	0.99	3.91	rs11206510	0.99	3.91
NCAN	rs2228603	19	19190924	6.74	6.46	0.50	3.50	rs2228603	0.50	3.50
B3GALT4 (COL11A2)	rs2254287	6	33251926	5.54	1.91	-	-	rs1799908	0.40	1.56
B4GALT4	rs12695382	3	120430861	5.31	2.23	-	-	rs4234656	0.92	5.17
Triglycerides										
GCKR	rs1260326	2	27584444	14.82	10.25	0.78	7.29	rs1260326	0.78	7.29
	rs780094	2	27594741	13.77	8.59	0.82	7.59	rs780094	0.82	7.59
RBKS/GCKR	rs11127129	2	27930212	3.70	3.77	-	-	rs7426295	0.08	1.22
APOA5	rs964184	11	116154127	15.82	18.12	-	-	rs11823543	1.80	20.50
	rs12286037	11	116157417	6.96	25.82	-	-	rs651821	2.41	29.02
	rs662799	11	116168917	7.37	16.88	-	-			
	rs2000571	11	116090743	4.33	6.93	1.04	10.29	rs1145195	2.78	15.99
	rs486394	11	116031532	3.77	1.50	-	-	rs496958	2.30	19.45
LPL	rs6993414	8	19947198	12.85	14.20	-	-	rs4244457	3.32	18.71
	rs6586891	8	19958878	3.62	4.60	-	-			
	rs10503669	8	19891970	8.85	11.57	-	-	rs2165558	3.59	20.45
	rs2197089	8	19870653	10.51	3.38	0.82	7.43	rs3916027	3.74	21.03
TRIB1	rs2954029	8	126560154	7.55	6.42	-	-	rs4567057	0.69	8.62
	rs17321515	8	126555591	7.17	6.42	-	-	rs4567059	0.73	9.01
MLXIPL (TBL2)	rs17145738	7	72620810	5.39	8.21	1.52	17.96	rs17145738	1.52	17.96
ANGPTL3 (DOCK7)	rs1748195	1	62822181	3.64	7.12	-	-	rs1748201	1.75	12.18
NCAN/SF4	rs10401969	19	19268718	6.64	12.28	2.17	23.23	rs10401969	2.17	23.23
LIPC	rs4775041	15	56461987	4.14	3.62	0.73	7.88	rs4775041	0.73	7.88
	rs2144300	1	228361539	3.31	4.25	-	-	rs1124110	1.48	19.18

Gene	SNP	chr	Position	Prior		Same SNP		Nearby SNP		
				Score	Effect	Score	Effect	Rs-Id	Score	Effect
Body Mass Index										
FTO	rs9939609	16	52378028	16.20	0.33	-	-	rs7202116	2.33	0.97
TMEM18	rs6548238	2	624905	5.90	0.26	-	-	rs6720683	1.48	0.72
MC4R	rs17782313	18	56002077	6.40	0.20	-	-	rs10871777	0.58	0.45
SH2B1	rs7498665	16	28790742	5.30	0.15	-	-	rs8055982	0.09	0.08
MTCH2	rs10838738	11	47619625	5.10	0.07	0.05	0.05	rs1474056	2.07	1.16
KCTD15	rs11084753	19	39013977	6.60	0.06	-	-	rs10853932	1.53	0.74
NEGR1	rs2815752	1	72585028	5.00	0.10	0.01	0.01	rs2815752	0.01	0.01