SUPPLEMENTAL MATERIAL

Detailed Methods

Sample Preparation. Liver (125), adrenal (17), kidney (ten) and small bowel (ten) tissue samples were provided by the Cooperative Human Tissue Network, funded by the National Cancer Institute. We acknowledge use of human adrenal tissues (18) provided by the National Disease Research Interchange (NDRI). Lung biopsy specimens (30) were provided by the Ohio State University Tissue Procurement Pathology Core. Finally, 20 LC samples were provided via the Neuropathology Core of the Emory Neuroscience NINDS Core Facilities. Human autopsy/biopsy tissue samples were either flash frozen in liquid nitrogen, or provided fresh in RNAlater (Qiagen). All samples were obtained under protocols approved by the local Institutional Review Boards. RNA [1] and DNA [2] were isolated from tissues as previously described, and RNA quality was assessed using the Bioanalyzer (Agilent Technologies). cDNA was synthesized via reverse transcription with SuperScript III (Invitrogen), oligo-dT, and gene-specific primers.

Genotyping. The human tissue samples used for the AEI assays were genotyped for the SNPs listed in Online Tables I and II at The Ohio State University as follows. Fluorescent restriction fragment length polymorphism was used if the SNP of interest was included in a restriction enzyme cut site. If not, SNPs were genotyped with allele-specific melting curve analysis. [3] The SNaPshot primer extension assay described below was also used in genotype determination. Primer sequences and assay type used are listed in Online Table IV. Genome-wide genotyping in the clinical cohorts was performed at outside institutions as described in the PheWAS and Genetic Association Study sections below.

Allelic mRNA Expression Analysis. The SNaPshot (Life Technologies) primer extension assay was used to measure allelic mRNA ratios in heterozygous carriers of a marker SNP in the transcribed region. [1] A target region containing the SNP is PCR-amplified. A single base extension reaction adds a fluorescently labeled dideoxynucleotide complementary to the SNP. The signal is quantitated on a 3730 DNA Analyzer capillary electrophoresis instrument (Life Technologies). The allelic mRNA ratios are calculated from the abundance of major over minor allele of the SNP and normalized to the mean of the genomic DNA ratios. The inverse was taken if the ratio was below one for rs77905.

As the two marker SNPs were located near the borders of exons, separate primers were designed for use with DNA and cDNA. In order to ensure there were no differences in amplification, samples homozygous for either the major or minor allele were selected, and a larger piece of DBH, ~550bp, was amplified with a different set of primers. This product was purified and quantitated. The products were combined in differing ratios, 1:4, 2:3, 1:1, and 3:2, diluted, used as template for the PCR reaction, and carried through the SNaPshot procedure. Duplicates were run on the 3730 DNA analyzer, and peak area ratios were compared to template ratios. The reactions were equally efficient, validating the use of different primers sets for the DNA and cDNA (Online Figure I).

Quantitative Real-Time reverse transcriptase PCR (qRT-PCR). *DBH* mRNA expression was measured by qRT-PCR with a 7500 Fast Real-Time PCR System (Life Technologies). Reactions were prepared in 10 ul volumes with Fast SYBR Green Master Mix (Applied Biosystems) and cDNA synthesized from 25 ng of total RNA. Primers spanning the exon 2-3 junction, including rs1108580 were used for DBH detection. Threshold values were set to 0.2 using the 7500 Software v2.0.5 (Applied Biosystems) and threshold cycle numbers (C_T) were computed for each well. [4] The mean *PGK1* amplification C_T value was subtracted from the mean *DBH* C_T value for each sample to calculate the ΔC_T, and obtain standardized values. A lower value is indicative of higher mRNA expression. To assure specific detection of mRNA, assays were replicated for a subset of samples using a Taqman probe spanning the exon 11-12 junction (Life Technologies) with GAPDH as a housekeeping gene.

DNA Sequencing Using Ion Torrent. Regions of the gene (~2-3kb) were PCR amplified using NEB (New England Biolabs) or JumpStart Mastermix (Sigma). Amplicons were combined based on length and concentration and treated with exonuclease followed by phenol-chloroform extraction. The sample was fragmented using Covaris shearing, and barcoded libraries were prepared using NEBNext Fast DNA Library Prep Set for Ion Torrent, the Ion Xpress Barcode Adapters Kit and the Ion One Touch Template Kit. The library was sequenced on the Ion Torrent, using the Ion Torrent Ion PGM Sequencing Kit (Life Technologies).

Statistical Analyses. SVS software (Golden Helix) was used to calculate linkage disequilibrium (LD) and R^2 values between SNPs, allele frequencies, Hardy-Weinberg equilibrium, and perform genotype association tests and regression analyses. One-way ANOVA and t-test were performed with GraphPad Prism and $p<0.05$ was considered to be significant when a specific hypothesis was tested. In SPSS v21 (IBM), stepwise linear regression using age, race and gender was used to determine the effect of subject demographics on DBH mRNA expression

5′ RACE (rapid amplification of cDNA ends). 5′UTRs (untranslated regions) were captured and sequenced using the FirstChoice RLM-RACE Kit (Ambion).

Jackson Laboratories Mouse Phenome Database. This resource is a collection of microarray and phenotype data from different laboratories on standard inbred mouse strains and can be accessed at: http://phenome.jax.org. In the analysis 2,483 phenotypes were included although many were related and therefore not entirely independent.

Measurements of cardiac pre-ejection period (PEP) as an index of myocardial contractility and sympathetic control. All participants were recruited from the University of Chicago and surrounding area. The subjects were 47% male with an average age of 21.2+2.5 years. Ethnicity of the population was diverse with 52% Caucasian, 27% Asian, 9% African American, 5% Hispanic and 7% other. Participants reported no chronic mental or physical illness. The research was approved by the University of Chicago IRB. Cardiovascular sympathetic cardiac control was assessed as pre-ejection period (PEP), derived from the electrocardiogram and the impedance cardiogram, representing the period between the electrical invasion of the ventricular myocardium (Q wave of the ECG) and the opening of the aortic valve. The impedance cardiogram was obtained using the standard tetrapolar electrode system as described. [5] The ECG (lead II configuration) and basal thoracic impedance (Z0) were measured using a Bionex system (Mindware) and the dZ/dt waveforms were analyzed to obtain PEP. For each subject, ECG and impedance data were ensemble averaged for each minute of the stress task to produce estimates of the PEP. PEP depends on the time development of intraventricular pressure, widely used as an index of myocardial contractility. Because variations in contractility are largely under sympathetic control, PEP is commonly used as a noninvasive measure of sympathetic cardiac control, [6] with lower PEP values representing higher levels of sympathetic cardiac control. A subset of participants underwent a Trier Social Stress Test (TSST), a widely used laboratory psychosocial stressor known to elicit cardiovascular responses in adults. [7] The modified TSST protocol consisted of a two minute preparation period and a three minute speech. PEP data were averaged across all five minutes to provide a single value for the stress condition. Regression analysis using stress PEP as the quantitative dependent variable included ethnicity as a covariate.

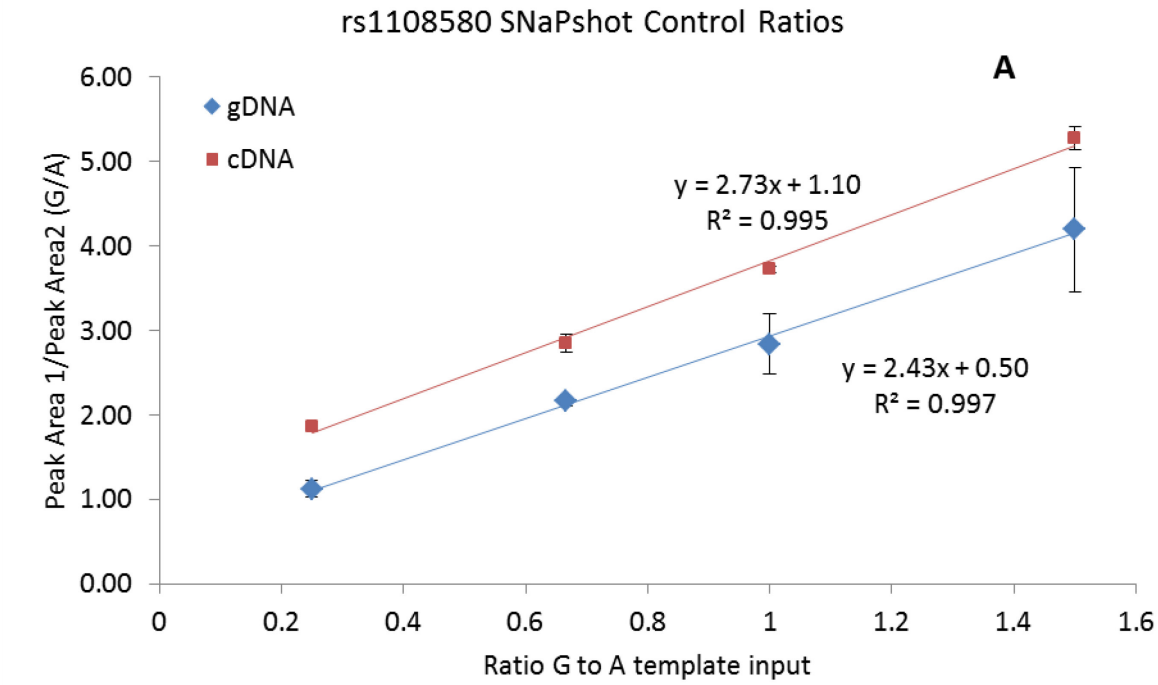
Phenome-Wide Association Study (PheWAS). A PheWAS was performed using data from the Geisinger Clinic *MyCode* biorepository, on 3,035 subjects. [8] Clinical and demographic information was obtained from electronic health records (EHR). We defined case-control status using International Classification of Diseases, Ninth Edition (ICD-9) diagnosis codes from the EHR. We required > 3 of the same ICD-9 code per individual and >10 case subjects, resulting in 482 phenotypes. All phenotypes meeting the

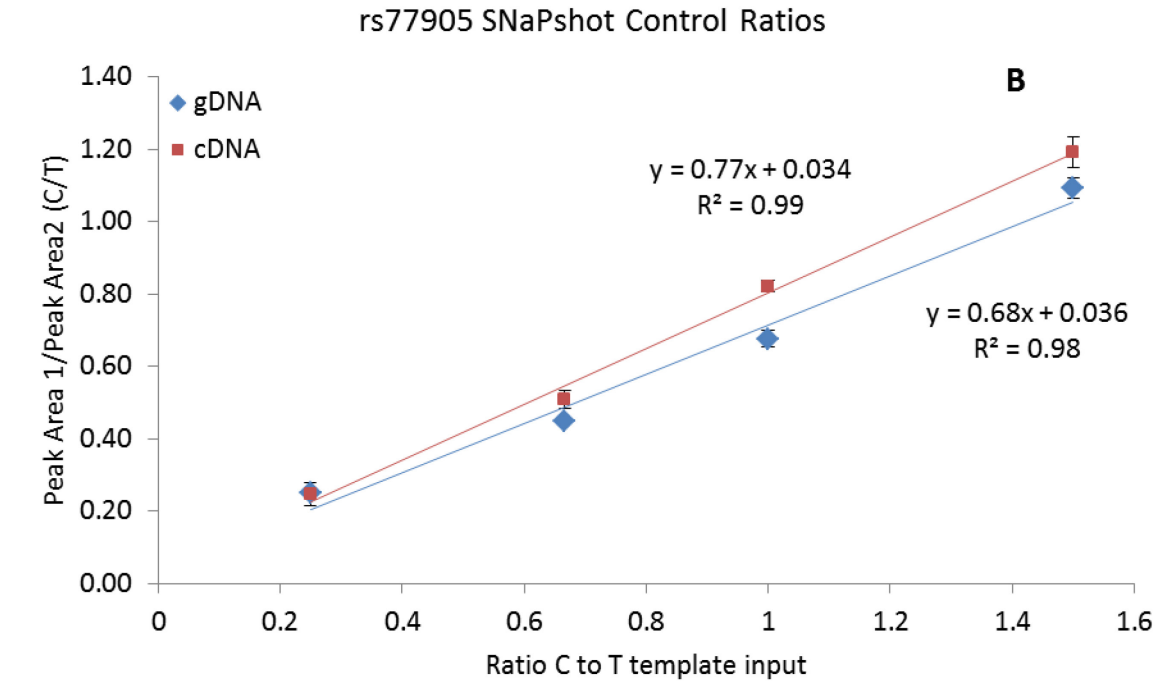
inclusion criteria were analyzed and not filtered to target specific diseases. The nervous system related disorders in the analysis were: convulsions, nondependent abuse of drugs, depression, bipolar, episodic mood disorders, anxiety dissociative and somatoform disorders, special symptoms or syndromes not elsewhere classified, adjustment reaction, organic sleep disorders, other cerebral degenerations, Parkinson's disease, other extrapyramidal disease and abnormal movement disorders, pain not elsewhere classified, multiple sclerosis, migraine, trigeminal nerve disorders, mononeuritis of upper limb and mononeuritis multiplex, hereditary and idiopathic peripheral neuropathy, inflammatory and toxic neuropathy. Using the case/control status and the two SNPs [rs1611115 MAF (T) 0.22; rs1108580 MAF (A) 0.47] we calculated associations using logistic regression via custom script in R, [9] adjusting models for age and sex. Associations were calculated using additive, recessive, and dominant genetic encoding, where the minor allele was the coded allele. We also tested pairwise SNP-SNP interactions. We determined the significance of the interaction via likelihood ratio test (LRT), comparing full versus reduced models, using logistic regression. The full model was: SNP1 + SNP2 + SNP1*SNP2 and the reduced model was: SNP1 + SNP2.

To seek replication for our PheWAS results, we used data on 4,027 individuals from The Marshfield Personalized Medicine Research Project (PMRP) biobank and linked EHR. [10] ICD-9 codes are organized in a hierarchical manner, with main categories and sub-categories. We first identified the main ICD-9 code category for any results within our discovery *MyCode* that had an association $p < 0.01$. Then we identified all ICD-9 codes within those categories - as different medical centers often use different ICD-9 codes by habit of practice, resulting in 92 phenotypes. We then defined case/control status for all of those ICD-9 codes using the same criteria used for the discovery *MyCode* PheWAS and performed the analysis the same way.

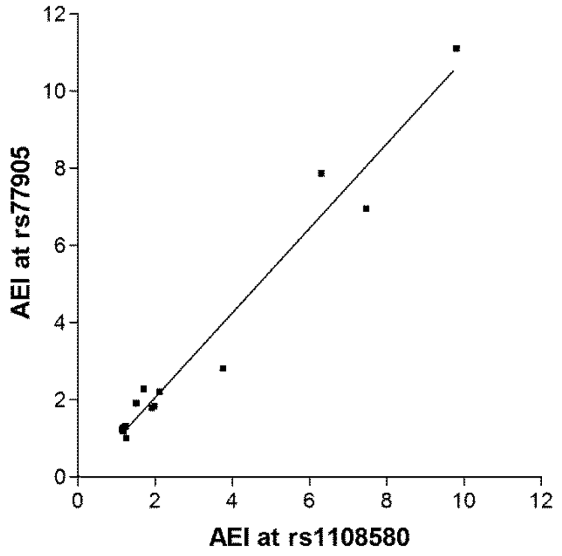
In the Marshfield PMRP dataset, genome-wide genotyping was performed at the Center for Inherited Disease Research (CIDR) using the Illumina 660W-Quad Beadchip. Genotyping calls were made at CIDR using BeadStudio version 3.3.7. For the Geisinger MyCode data set genotyping was performed at the University of Pittsburgh Genomics and Proteomics Core Laboratories using the Illumina Omni Express Beadchip. Genotyping calls were made at the University of Pittsburgh using GenomeStudio. Data for both sets were cleaned using the eMERGE QC pipeline developed by the eMERGE Genomics Working Group. [11] This process includes evaluation of sample and marker call rate, gender mismatch, duplicate and HapMap concordance, batch effects, Hardy-Weinberg equilibrium, sample relatedness, and population stratification. For the current PheWAS, only the two SNPs of interest were extracted from the GWAS dataset.

Genetic Association Study. Data from the Jackson Heart Study (JHS), which investigates cardiovascular risk factors were used. [12] Participants are African-American men and women from the three counties surrounding Jackson, MS. For the current analysis, rs1611115 was genotyped directly using the CARe IBC Array [13] in 2,762 participants at the Broad Institute of Harvard and MIT, and was imputed in an additional 262 individuals, using Affymetrix 6.0 genotypes and a 1000 Genomes reference panel as described. [14] Genotypes for rs1108580, were imputed in all 3,024 participants. After excluding participants with coronary heart disease (CHD) at baseline or with missing data for incident myocardial infarction (MI), 2,378 participants were available for analysis. In addition to MI, procedures included in the incident CHD outcome include coronary artery bypass grafts and coronary angioplasty. Genotype association tests were performed with an additive model, counting the number of copies of the *T* allele for rs1611115 and *A* allele for rs1108580. The data were adjusted for baseline age, sex, hypertension, and type II diabetes, as well as 10 principal components to adjust for potential ethnic stratification. All analyses were approved by the Institutional Review Board of the University of Mississippi Medical Center.

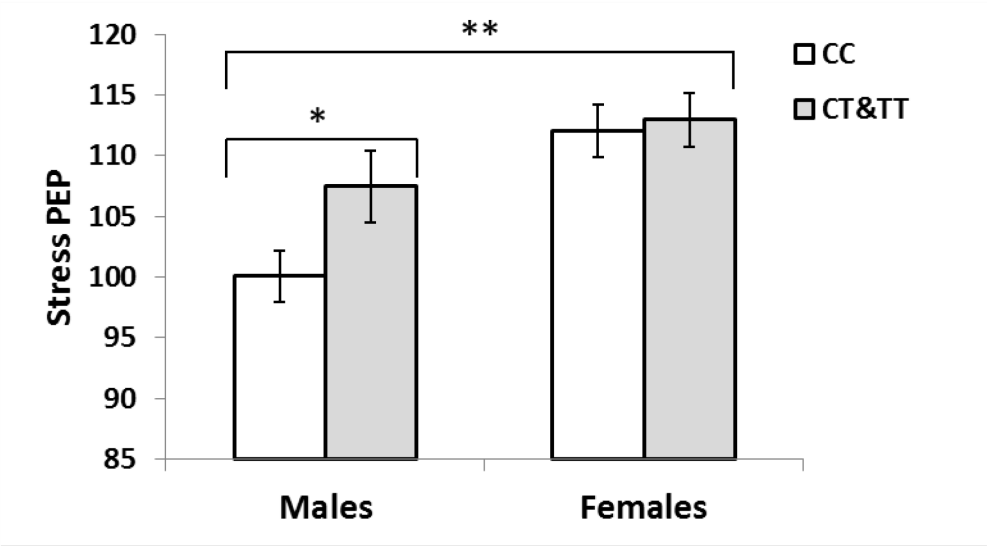




Online Figure I: Efficiency comparison of primers for PCR amplification of DNA and RNA around rs1108580 (A) and rs77905 (B). Separate primer sets were used as both SNPs are near the border of an exon. The products were combined in differing ratios, 1:4, 2:3, 1:1, and 3:2, diluted, used as template for the PCR reaction, and used in the SNaPshot assay. Peak area ratios were compared to input ratios. A diamond indicates gDNA primers, while a square indicates cDNA primers.



Online Figure II: Linear regression of average AEI magnitude measured at two marker SNPs: rs1108580 and rs77905 in any samples heterozygous for both marker SNPs in all tissues. The equation of the line was y = 1.1x - 0.14 with R^2=0.96. In order to compare the magnitude of the values, we used the inverse of ratios below 1.



Online Figure III: Myocardial contractility and sympathetic control measured by cardiac pre-ejection period (PEP) with subjects sorted by gender and rs1611115 genotype. Average stress PEP was calculated for each genotype, pooling heterozygous and homozygous minor alleles together. There was a significant difference between males and females (p=1.9E-05), therefore they were analyzed separately. There was no significant genotype effect in females (p=0.77). In males, a t-test demonstrated a significant difference in stress PEP between subjects homozygous for the major allele and those carrying at least one copy of the minor allele ($p<0.05$, effect size d = 0.54).

Online Table I: SNPs genotyped and genotype association test using the F statistic

Marker	Location	Minor Allele	MAF	Association of SNP with rs77905 AEI values (F-test p)	Association of SNP with rs1108580 AEI values (F-test p)
rs1079783	upstream	G	0.25	0.16	0.92
rs3025343	upstream	A	0.10	0.48	0.51
rs141116007 and CA repeat	upstream	B	0.41	0.047	0.098
rs1076150	upstream	T	0.49	0.28	0.60
rs1989787	upstream	A	0.34	0.54	0.26
rs1611115	upstream	T	0.21	0.0016	2.0E-07
rs2519143*	intron 1	A	0.20	0.0016	2.9E-07
rs1108580	exon 2	A	0.46	0.20	x†
rs77905	exon 9	T	0.46	x†	0.57
rs6271	exon 11	T	0.06	0.67	0.24
rs129882	exon 12	T	0.25	0.43	0.46

*rs2519143 scored with similar p values compared to rs1611115 but was excluded as the causative SNP because it was homozygous in a tissue showing strong AEI.
†When used as the marker SNP, rs1108580 and rs77905 could not be tested as all samples were heterozygous.

Online Table II: LD values (D′ and R^2) for all SNPs genotyped in liver

	D'	R^2	D'	R^2	D'	R^2	D'	R^2	D'	R^2	D'	R^2	D'	R^2	D'	R^2	D'	R^2	D'	R^2	D'	R^2
Marker	rs1611115		rs129882		rs1108580		rs77905		rs2519143		rs3025343		rs1989787		rs1079783		rs1076150		rs141116007 & CA rep		rs6271	
rs1611115	1.00	1.00	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
rs129882	0.29	0.00	1.00	1.00	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
rs1108580	0.73	0.15	0.40	0.04	1.00	1.00	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
rs77905	0.11	0.00	0.06	0.00	0.04	0.00	1.00	1.00	.	.	.	.	.	.	.	.	.	.	.	.	.	.
rs2519143	0.91	0.79	0.02	0.00	0.77	0.16	0.44	0.02	1.00	1.00	.	.	.	.	.	.	.	.	.	.	.	.
rs3025343	0.99	0.03	0.98	0.04	0.07	0.00	1.00	0.18	0.99	0.03	1.00	1.00	.	.	.	.	.	.	.	.	.	.
rs1989787	1.00	0.14	0.63	0.13	1.00	0.40	0.26	0.06	1.00	0.12	0.22	0.01	1.00	1.00	.	.	.	.	.	.	.	.
rs1079783	0.50	0.01	0.27	0.06	0.55	0.06	0.58	0.08	0.69	0.02	1.00	0.04	0.70	0.19	1.00	1.00	.	.	.	.	.	.
rs1076150	1.00	0.26	0.27	0.02	0.94	0.80	0.06	0.00	0.87	0.19	0.16	0.00	1.00	0.46	0.39	0.03	1.00	1.00	.	.	.	.
rs141116007 & CA rep	0.82	0.27	0.60	0.05	0.38	0.10	0.35	0.04	0.86	0.27	0.75	0.04	0.83	0.21	0.56	0.04	0.40	0.10	1.00	1.00	.	.
rs6271	1.00	0.02	0.99	0.02	0.49	0.01	1.00	0.09	1.00	0.02	0.32	0.05	0.53	0.03	0.97	0.02	0.54	0.02	1.00	0.04	1.00	1.00

Online Table III: Liver gDNA genotypes obtained from Ion Torrent Sequencing
See Excel sheet.
*"Het" indicates a heterozygous sample, whereas "hom" indicates homozygous for the variant allele. Samples that were homozygous for the reference allele were not called by the software and therefore not listed in the table.

Online Table IV: Primer sequences and reaction conditions.
See Excel sheet.
*All PCR reactions begin with a 5 minute denaturation step at 95°C, 30 cycles of PCR (unless otherwise noted), followed by a 10 minute extension step at 72°C. Cycling conditions are listed with denaturation, annealing extension times in seconds (s) or minutes (m) with corresponding temperatures in parentheses.

Online Table V: Demographics for liver samples used in qRT-PCR

Demographic	Group (n)	Mean ±SD	DBH Expression (ΔC_T)*	Pearson Correlation (p value) †
Sex	Male (25)		6.6 ± 1.0	0.1
	Female (32)		6.2 ± 1.0	
Race	Caucasian (49)		6.4 ± 1.0	0.5
	Other (8)		6.4 ± 0.9	
Age		62.3 ±13.2		0.3

*The mean PGK1 amplification C_T was subtracted from the mean DBH C_T.
†As p values for the Pearson correlation were all >0.05, no variables were included as covariates in the analysis.

Online Table VI: Correlation between phenotype and *Dbh* liver mRNA expression from Jackson Laboratory database

Phenotype	p value*	Pearson R	Chr location
Grip Strength	0.000030	–0.92	2:27173449
Percent Lean	0.00011	0.79	2:27174217
Percent Fat	0.00011	–0.79	2:27174217
Body Weight day 120 (60d post-exposure)	0.00017	–0.840	2:27174217
Food Intake	0.00019	0.92	2:27173449
Body Weight day 0	0.00020	–0.84	2:27174217
Triglycerides	0.00021	0.87	2:27173449
Total Tissue Mass	0.00028	–0.87	2:27173449
Water Intake	0.00040	0.90	2:27173449
R-wave Amplitude	0.00047	0.81	2:27174217
Body Length	0.00048	–0.81	2:27173449
protein phosphatase 1 regulatory (inhibitor) subunit 1B (PPP1R1B), relative fluorescence intensity in cerebral cortex	0.00053	0.89	2:27173449
Weight of Fat Tissue	0.00056	–0.73	2:27174217
Percent Fat	0.00059	–0.71	2:27174217
Grip Strength	0.00064	–0.84	2:27173449
Elevated plus maze, time spent in center 5 x 5 cm hub	0.00070	0.76	2:27174217
CCAAT/enhancer binding protein (C/EBP), alpha (Cebpa), relative mRNA abundance	0.00071	0.86	2:27173449
Total Tissue Mass	0.00081	–0.79	2:27172582
Fucosyltransferase 9 (FUT9), relative fluorescence intensity in cerebral cortex	0.00083	0.79	2:27174217
Total Tissue Mass	0.00087	–0.77	2:27172582
Body Weight	0.00090	–0.77	2:27174217
Total Body Area	0.00091	–0.78	2:27173449
Total Tissue Mass	0.0010	–0.76	2:27173449
Fear conditioning chamber, percent time spent near chamber walls, day 2	0.0010	0.85	2:27172582

*All results with p≤0.001

Online Table VII: PheWAS analysis results of each individual SNP, using an additive model

SNP	Coded Allele (minor)	Beta	SE	Odds Ratio	95% CI	pval	ICD-9 Code	ICD-9 Description	Cases	Ctrls
rs1108580	A	-0.78	0.19	0.46	0.32-0.66	3.1E-05	722.52	Intervertebral disc disorders: degeneration lumbar or lumbosacral intervertebral disc	71	2964
rs1611115	T	-0.84	0.22	0.43	0.28-0.67	0.0002	413.9	Angina pectoris: unspecified angina pectoris	103	2932
rs1611115	T	0.66	0.21	1.9	1.3-2.9	0.002	250.6	Diabetes mellitus: diabetes with neurological complications	48	2987
rs1108580	A	-0.69	0.24	0.50	0.32-0.80	0.004	780.39	Convulsions	43	2992
rs1108580	A	-0.36	0.13	0.70	0.54-0.90	0.007	784	General symptoms: symptoms involving head and neck	131	2904
rs1108580	A	-0.40	0.15	0.67	0.50-0.89	0.007	413.9	Angina pectoris: unspecified Angina pectoris	103	2932
rs1611115	T	0.60	0.23	1.8	1.2-2.9	0.008	789.01	Other symptoms involving abdomen and pelvis: abdominal pain, right upper quadrant	44	2991

Data is sorted by p value, filtered at a cutoff of 0.01. The following abbreviations are used: SE, standard error; CI, confidence interval; and Ctrls, controls.

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