NRXN3 Is a Novel Locus for Waist Circumference: A Genome-Wide Association Study from the CHARGE Consortium

The Harvard community has made this article openly available. Please share how this access benefits you. Your story matters.

Citation
Heard-Costa, Nancy L., M. Carola Zillikens, Keri L. Monda, Asa Johansson, Tamara B. Harris, Mao Fu, Talin Haritunians, et. al. 2009. NRXN3 Is a Novel Locus for Waist Circumference: A Genome-Wide Association Study from the CHARGE Consortium. PLoS Genetics 5(6): e1000539.

Published Version
doi://10.1371/journal.pgen.1000539

Citable link
http://nrs.harvard.edu/urn-3:HUL.InstRepos:4739277

Terms of Use
This article was downloaded from Harvard University’s DASH repository, and is made available under the terms and conditions applicable to Other Posted Material, as set forth at http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA
NRXN3 Is a Novel Locus for Waist Circumference: A Genome-Wide Association Study from the CHARGE Consortium

Nancy L. Heard-Costa1,4, M. Carola Zillikens2,5, Keri L. Monda3,5, Åsa Johansson4,6, Tamara B. Harris5,7, Mao Fe6,9, Talin Haritunians7,9, Mary F. Feitosa8,9, Thor Aspelund9,10, Gudny Eiriksdottir9, Melissa Garcia5, Lenore J. Launer5, Albert V. Smith9, Braxton D. Mitchell6, Patrick F. McArdle6, Alan R. Shuldiner6, Suzette J. Bielski11,12, Fred Boerwinkle12, Fred Brancati13, Ellen W. Demerath14, James S. Pankow14, Alice M. Arnold15, Yii-Der Ida Chen7, Nicole L. Glazer16, Barbara McKnight15, Bruce M. Psaty17, Jerome I. Rotter7, Najaf Amin18, Harry Campbell19, Ulf Gyllensten4, Cristian Pattaro20, Peter P. Pramstaller20,21,22, Igor Rudan19,23,24, Maksim Struchalin18, Veronique Vitart25, Xiaoyi Gao6, Aldi Kraja8, Michael A. Province8, Quynyan Zhang8, Larry D. Atwood1, Josée Dupuis26, Joel N. Hirschhorn27, Cashell E. Jaquish28, Christopher J. O’Donnell29, Ramachandran S. Vasan30,31, Charles C. White26, Yuri S. Aulchenko18, Karol Estrada7, Albert Hofman18, Fernando Rivadeneira2,18, André G. Uitterlinden2,18, Jacqueline C. M. Witteman18, Ben A. Oostra32, Robert C. Kaplan33, Vilmundur Gudnason9,10*, Jeffrey R. O’Connell5*, Ingrid B. Borecki8, Cornelia M. van Duijn18*, L. Adrienne Cupples26*, Caroline S. Fox29,34*, Kari E. North35*

1 Department of Neurology, Boston University School of Medicine, Boston, Massachusetts, United States of America, 2 Department of Internal Medicine, Erasmus Medical Center, Rotterdam, The Netherlands, 3 Department of Epidemiology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, United States of America, 4 Department of Genetics and Pathology, Uppsala University, Uppsala, Sweden, 5 Laboratory of Epidemiology, Demography, and Biometry, Intramural Research Program, National Institute on Aging, Bethesda, Maryland, United States of America, 6 Division of Endocrinology, Diabetes, and Nutrition, University of Maryland School of Medicine, Baltimore, Maryland, United States of America, 7 Medical Genetics Institute, Cedars-Sinai Medical Center, Los Angeles, California, United States of America, 8 Department of Genetics, Washington University School of Medicine, St. Louis, Missouri, United States of America, 9 Heart Preventive Clinic and Research Institute, Icelandic Heart Association, Kopavogur, Iceland, 10 University of Iceland, Reykjavik, Iceland, 11 Division of Epidemiology, Mayo Clinic, Rochester, Minnesota, United States of America, 12 Human Genetics Center and Institute of Molecular Medicine, University of Texas Health Science Center, Houston, Texas, United States of America, 13 Department of Medicine and Epidemiology, Johns Hopkins University, Baltimore, Maryland, United States of America, 14 Division of Epidemiology and Community Health, University of Minnesota, Minneapolis, MN, United States of America, 15 Department of Biostatistics, University of Washington, Seattle, Washington, United States of America, 16 Department of Internal Medicine, University of Washington, Seattle, Washington, United States of America, 17 Department of Epidemiology, Medicine, & Health Services, University of Washington, Seattle, Washington, United States of America, 18 Department of Epidemiology and Biostatistics, Erasmus University Medical Center, Rotterdam, The Netherlands, 19 Department of Public Health Sciences, University of Edinburgh Medical School, Edinburgh, Scotland, United Kingdom, 20 Institute of Genetic Medicine, European Academy Bozen/Bolzano, Bolzano, Italy, 21 Department of Neurology, University of Lübeck, Lübeck, Germany, 22 Department of Neurology, Central Regional Hospital, Bolzano, Italy, 23 Croatian Centre for Global Health, University of Split Medical School, Split, Croatia, 24 Institute for Clinical Medical Research, University Hospital “Sestre Milosrdnice,” Zagreb, Croatia, 25 Human Genetics Unit, Institute of Genetics and Molecular Medicine, Edinburgh, Scotland, United Kingdom, 26 Department of Biostatistics, Boston University School of Public Health, Boston, Massachusetts, United States of America, 27 Program in Genomics and Divisions of Endocrinology and Genetics, Harvard Medical School, Boston, Massachusetts, United States of America, 28 Division of Prevention and Population Sciences, National Heart, Lung, and Blood Institute, Bethesda, Maryland, United States of America, 29 Division of Intramural Research, National Heart, Lung and Blood Institute, Framingham Heart Study, Framingham, Massachusetts, United States of America, 30 Boston University School of Medicine, Boston, Massachusetts, United States of America, 31 The Framingham Heart Study, Framingham, Massachusetts, United States of America, 32 Department of Clinical Genetics, Erasmus University Medical Center, Rotterdam, The Netherlands, 33 Department of Epidemiology and Population Health, Albert Einstein College of Medicine, Bronx, New York, United States of America, 34 Division of Endocrinology, Metabolism, and Diabetes, Department of Medicine, Harvard Medical School, Boston, Massachusetts, United States of America

Abstract

Central abdominal fat is a strong risk factor for diabetes and cardiovascular disease. To identify common variants influencing central abdominal fat, we conducted a two-stage genome-wide association analysis for waist circumference (WC). In total, three loci reached genome-wide significance. In stage 1, 31,373 individuals of Caucasian descent from eight cohort studies confirmed the role of FTO and MC4R and identified one novel locus associated with WC in the neurexin 3 gene [NRXN3 (rs10146997, p = 6.4 × 10−7)]. The association with NRXN3 was confirmed in stage 2 by combining stage 1 results with those from 38,641 participants in the GIANT consortium (p = 0.009 in GIANT only, p = 5.3 × 10−8 for combined analysis, n = 70,014). Mean WC increase per copy of the G allele was 0.0498 z-score units (0.65 cm). This SNP was also associated with body mass index (BMI) (p = 7.4 × 10−6, 0.024 z-score units (0.10 kg/m²) per copy of the G allele) and the risk of obesity (odds ratio 1.13, 95% CI 1.07–1.19; p = 3.2 × 10−5 per copy of the G allele). The NRXN3 gene has been previously implicated in addiction and reward behavior, lending further evidence that common forms of obesity may be a central nervous system-mediated disorder. Our findings establish that common variants in NRXN3 are associated with WC, BMI, and obesity.
Body mass index (BMI) is a commonly used measure of overall adiposity. However, specific fat depots may confer differential metabolic risk. In particular, central abdominal fat, as measured by waist circumference (WC), may be more strongly associated with metabolic risk factors and cardiovascular disease, have been difficult to identify and replicate.

Early genome-wide association studies (GWAS) identified both FTO and MC4R as genes related to BMI and WC [7–10]. Many new loci have been identified in recent obesity related GWAS studies [11–13]. However, collectively these variants explain only a small proportion of the variation in adiposity [7–13]. In addition, no GWAS exist exclusively to identify genes for central fat. Thus, identifying new variants, we carried out a large-scale meta-analysis of GWAS from eight studies to detect variants associated with central body fat distribution.

Methods

Study Samples

Participants for the current analysis were drawn from 8 cohort studies, including the Age, Gene/Environment Susceptibility-Reykjavik Study (AGES- Reykjavik Study), the Atherosclerosis Risk in Communities Study (ARIC), the Cardiovascular Health Study (CHS), the European Special Population Network consortium (EUROSPAN), the Family Heart Study, the Framingham Heart Study, Old Order Amish (OOA), and the Rotterdam Study (RS). These groups comprise the CHARGE (Cohorts for Heart and Aging Research in Genomic Epidemiology) Consortium. All
participants provided informed consent. Local ethical committees at each institution approved the individual study protocols. Text S1 contains details regarding all participating cohorts.

Imputation and Statistical Analysis

Common to all analyses were use of the raw WC measures and the assumption of an additive model; study specific details follow. Each study reported an effect allele which was meta-analyzed consistently across all studies. Results are currently presented relative to the minor G allele for the ARXV3 SNP. In all studies except CHS, MACH (version 1.0.15 in Family Heart, Framingham, EUROSPAN and RS; version 1.0.16 in ARIC, AGES, and OOA) was used to impute all autosomal SNPs on the HapMap, using the publicly available phased haplotypes (release 22, build 36, CEU population) as a reference panel. In CHS, the program BIMBAM was used [14]. Details are provided in Table S1 regarding covariates and trait creation.

In ARIC, Framingham, and RS, sex- and either cohort-specific or study center-specific residuals were created after adjustment for age, age-squared, and smoking status. In CHS and Family Heart, linear regression models were used to adjust for age, age-squared, sex, smoking, and study center. In AGES, linear regression models using PLINK v1.04 [15] were used to adjust for age, age-squared, sex, and smoking. In the OOA the measured genotype mixed effects model was used adjusting for age, age-squared, sex and family structure based on the complete 14-generation pedigree as implemented in ITSBNB [16]. Framingham employed the linear mixed effect model for continuous traits and the generalized estimating equations for dichotomous traits in R [17] to account for family relatedness. In RS, linear regression models were run using MACHEQTL [18]. In ARIC and EUROSPAN, all regression models were run using the ProhABEL package from the ABEL set of programs [19] and in EUROSPAN genomic control [20] was used to correct standard errors of the effect estimates for relatedness among individuals. The Family Heart Study determined the effect of each SNP using linear mixed effects models to account for the siblings present in the data using SAS.

Principal components calculated using EIGENSTRAT [21] were adjusted for in the individual studies when significant in order to account for population substructure.

Meta-Analysis

A weighted z-score approach was used to conduct meta-analyses with METAL (www.sph.umich.edu/csg/abecasis/metal/). Genomic control correction was applied to each study prior to the full meta-analysis. P-values less than $4.4 \times 10^{-7}$ were considered genome-wide significant [22].

In Silico Exchange with the GIANT Consortium

In stage 2 of our study, we conducted an in silico exchange of the results of 48 SNPs with the GIANT consortium. To create our list of SNPs to exchange, we first selected the top 34 SNPs from independent loci (defined as SNPs with $R^2 < 0.2$) from our meta-analysis of WC, excluding SNPs in known loci for adiposity. An additional 14 SNPs of independent loci with a p-value $< 1.0 \times 10^{-5}$ from a secondary list that focused on SNPs for WC with corresponding BMI p-values $> 0.01$ were also included in an attempt to isolate genes that might be specifically associated with central fat deposition. Our a priori threshold for replication was a p-value $< 0.001 (0.05/48$ SNPs) and/or reaching genome-wide significance in a combined meta-analysis. CHARGE and GIANT results were then meta-analyzed using METAL.

Results

Table 1 presents the descriptive statistics across the 8 cohorts providing data for the meta-analysis. We had a total sample size of 31,373 individuals of Caucasian descent. Participants were mostly middle-aged with ages ranging from a mean of 45 to 76 years of age.

Figure S1 shows the genome-wide association results for WC in the stage 1 CHARGE-only analysis. The top SNPs for WC were in the $FTO$ and $MC4R$ genes (Table S3). Figure S2 shows the QQ plot for our results excluding SNPs in $FTO$ and $MC4R$. For $FTO$, the top SNP was rs1558902 ($p = 4.6 \times 10^{-15}$). For $MC4R$, the top SNP was rs489693 ($p = 3.5 \times 10^{-7}$). The top results excluding SNPs in $FTO$ and $MC4R$ from our stage 1 meta-analysis are shown in Table 2 along with the stage 2 in silico replication results from the GIANT consortium; additional meta-analysis results from CHARGE are presented in Table S3. The lowest p-value on our list, for SNP rs10146997 in the ARXV3 gene, had a stage 1 meta-analysis p-value of $6.4 \times 10^{-7}$ and was confirmed in 38,641 participants from the GIANT consortium with a p-value of 0.009 and a combined p-value of $5.3 \times 10^{-8}$. The ARXV3 SNP was derived from the list of SNPs associated with WC irrespective of association with BMI. None of the other SNPs that were exchanged were confirmed in GIANT. We do note that while rs10857809 (proxy for rs10857810) in the FAM104A gene had a p-value of 0.003 in GIANT, the results were not direction-consistent with CHARGE and therefore did not replicate in the combined analysis.

Figure 1 presents the genomic region for SNP rs10146997 (intronic) in ARXV3. Table 3 shows detailed results of rs10146997 in the ARXV3 gene by contributing CHARGE study and corresponding results appear in the forest plot in Figure S3; there was no evidence for heterogeneity across the stage 1 studies ($p = 0.64$). The minor allele (G) frequency (MAF) for rs10146997 in our sample ranged from 0.14 in the OOA to 0.24 in the Croatians; the frequency of the ARXV3 SNP G allele is 0.275, 1.0, 1.0, and 0.35, in Hapmap CEU, Han Chinese, Japanese, and Yoruba populations, respectively. This SNP was genotyped in AGES, CHS, Family Heart Study, Rotterdam and all EUROSPAN studies, and imputation scores for the other studies indicated very high quality. Overall, per copy of the G allele, mean WC was increased 0.0498 z-score units (0.65 cm). Beta coefficients (in z-score units) were consistently positive in all samples except the ERF study ($\beta = -0.0098$; $p = 0.86$), which is most likely due to chance. Due to overlap in participants from the Framingham Heart Study and ARIC with those from the Family Heart Study, the CHARGE meta-analysis was re-run for the ARXV3 SNP without the Family Heart Study; results were essentially unchanged ($p = 6.6 \times 10^{-7}$). Individual study-specific results for rs10146997 from the studies comprising the GIANT consortium can be found in Table S2.

Within CHARGE, we also observed an association of rs10146997 with BMI ($p = 7.4 \times 10^{-6}$). Overall, mean BMI was increased 0.024 z-score units per G allele (0.10 kg/m$^2$). When WC was additionally adjusted for BMI, the signal was completely attenuated (0.0065 z-score units per G allele; $p = 0.32$). The association of rs10146997 with WC was similar in women and men and in older and younger individuals (Table 4). After excluding smoking from the covariate adjustment list, results were essentially similar. Per copy of the G allele, the odds ratio of having high WC (≥88 cm in women; ≥102 cm in men) was 1.07 (95% CI 1.02–1.11; Table 4). Similarly, the odds ratio of obesity was 1.13 (95% CI 1.07–1.19).
Table 1. Descriptive statistics across the eight cohorts.

| Cohort               | N    | Age (years) | % Women | Current smokers (%) | Waist Circ (cm) | BMI (kg/m²) |
|----------------------|------|-------------|---------|---------------------|-----------------|-------------|
| AGES                 | 3172 | 76.4 (5.4)  | 58.0 (1840) | 12.7 (402)        | 100.7 (12.1)*   | 27.1 (4.4)  |
| ARIC                 | 8097 | 54.3 (5.7)  | 52.8 (4276) | 25.2 (2036)       | 96.2 (13.4)     | 27.0 (4.9)  |
| CHS                  | 3213 | 72.3 (5.4)  | 60.0 (1942) | 11.0 (354)        | 93.6 (12.6)     | 26.4 (4.3)  |
| Family Heart Study   | 855  | 55.6 (11.0) | 51.5 (440)  | 11.9 (101)        | 98.6 (13.6)     | 27.8 (5.1)  |
| Framingham Heart Study| 7115 | 45.2 (10.9) | 52.7 (3750)| 18.8 (1338)       | 91.4 (15.0)     | 26.0 (5.1)  |
| Old Order Amish      | 1134 | 49.6 (16.8) | 48.4 (549)  | 9.4 (106)         | 88.5 (11.4)     | 27.0 (4.7)  |
| Rotterdam Study      | 5471 | 69.0 (8.8)  | 58.6 (3205) | 23.0 (1258)       | 90.6 (11.2)     | 26.3 (3.7)  |
| EUROSPLAN Consortium |      |             |          |                    |                 |             |
| ERF (Dutch)          | 1239 | 48.3 (14.7) | 60.1 (744)  | 43.6 (540)        | 87.0 (13.7)     | 26.7 (4.7)  |
| CROATIAN             | 784  | 56.5 (15.3) | 58.6 (459)  | 27.7 (217)        | 95.9 (11.8)     | 27.3 (4.3)  |
| MICROS (South Tyrolean)| 293  | 46.3 (15.6) | 59.7 (175)  | 45.3 (125)        | 88.5 (13.3)     | 25.4 (5.4)  |

Data provided as mean (standard deviation) for continuous and % (n) for dichotomous data.

* N = 3167 for WC by tape measure; mean (SD) of WC measured by computed tomography is 125.9(14.0) cm.

We calculated a risk score of FTO (rs9939609), MC4R (rs17782313), and NRXN3 with possible scores ranging from 0–6 risk alleles (Figure 2). Across this range, mean WC increased from 92.4 cm among those with 0 risk alleles, to 95.7 cm among those with 4 or more risk alleles. To put our findings in perspective, per copy of the effect allele, the NRXN3 SNP resulted in a WC difference of 0.65 cm; FTO 0.73 cm, and MC4R 0.37 cm.

CHARGE consortium meta-analysis results for BMI can be found in Table S4; Manhattan and QQ plots for BMI can be found in Figure S4 and Figure S5, respectively.

Discussion

In a discovery sample of more than 30,000 individuals from several cohort studies, we identified a novel locus in the NRXN3 gene associated with WC. In combination with data from the GIANT consortium, the p-value for this finding exceeded our pre-defined threshold for genome-wide statistical significance. This SNP was also significantly associated with BMI and obesity. This gene has previously been associated with addiction and reward behavior, and is a compelling biologic candidate for obesity.

We calculated a risk score of FTO (rs9939609), MC4R (rs17782313), and NRXN3 with possible scores ranging from 0–6 risk alleles (Figure 2). Across this range, mean WC increased from 92.4 cm among those with 0 risk alleles, to 95.7 cm among those with 4 or more risk alleles. To put our findings in perspective, per copy of the effect allele, the NRXN3 SNP resulted in a WC difference of 0.65 cm; FTO 0.73 cm, and MC4R 0.37 cm.

CHARGE consortium meta-analysis results for BMI can be found in Table S4; Manhattan and QQ plots for BMI can be found in Figure S4 and Figure S5, respectively.

Table 1. Descriptive statistics across the eight cohorts.

| Cohort               | N    | Age (years) | % Women | Current smokers (%) | Waist Circ (cm) | BMI (kg/m²) |
|----------------------|------|-------------|---------|---------------------|-----------------|-------------|
| AGES                 | 3172 | 76.4 (5.4)  | 58.0 (1840) | 12.7 (402)        | 100.7 (12.1)*   | 27.1 (4.4)  |
| ARIC                 | 8097 | 54.3 (5.7)  | 52.8 (4276) | 25.2 (2036)       | 96.2 (13.4)     | 27.0 (4.9)  |
| CHS                  | 3213 | 72.3 (5.4)  | 60.0 (1942) | 11.0 (354)        | 93.6 (12.6)     | 26.4 (4.3)  |
| Family Heart Study   | 855  | 55.6 (11.0) | 51.5 (440)  | 11.9 (101)        | 98.6 (13.6)     | 27.8 (5.1)  |
| Framingham Heart Study| 7115 | 45.2 (10.9) | 52.7 (3750)| 18.8 (1338)       | 91.4 (15.0)     | 26.0 (5.1)  |
| Old Order Amish      | 1134 | 49.6 (16.8) | 48.4 (549)  | 9.4 (106)         | 88.5 (11.4)     | 27.0 (4.7)  |
| Rotterdam Study      | 5471 | 69.0 (8.8)  | 58.6 (3205) | 23.0 (1258)       | 90.6 (11.2)     | 26.3 (3.7)  |
| EUROSPLAN Consortium |      |             |          |                    |                 |             |
| ERF (Dutch)          | 1239 | 48.3 (14.7) | 60.1 (744)  | 43.6 (540)        | 87.0 (13.7)     | 26.7 (4.7)  |
| CROATIAN             | 784  | 56.5 (15.3) | 58.6 (459)  | 27.7 (217)        | 95.9 (11.8)     | 27.3 (4.3)  |
| MICROS (South Tyrolean)| 293  | 46.3 (15.6) | 59.7 (175)  | 45.3 (125)        | 88.5 (13.3)     | 25.4 (5.4)  |

Data provided as mean (standard deviation) for continuous and % (n) for dichotomous data.

* N = 3167 for WC by tape measure; mean (SD) of WC measured by computed tomography is 125.9(14.0) cm.

doi:10.1371/journal.pgen.1000539.t001

The small magnitude of the effect size of the NRXN3 variant on WC is consistent with what has previously been reported for FTO and MC4R. These findings highlight the need for large sample sizes in order to facilitate continued gene discovery for obesity-related traits. In particular, genes that emerge for waist circumference will most likely be genes for overall adiposity because of the strong correlation between the two measurements [22]. More specific measures of visceral abdominal fat depots may make it possible to isolate genes involved in regional body composition.

NRXN3 is part of a family of central nervous adhesion molecules and is highly expressed in the central nervous system. Prior studies of NRXN3 point towards an important role in alcohol dependence, cocaine addiction, and illegal substance abuse [23–26]. In addition, opioid dependence has been linked to the chromosome 14q region [23]. In mice, NRXN3 beta expression was observed in the globus pallidus when exposed to cocaine [24]. Many of the neuronal pathways in these sub-cortical regions of the brain in which NRXN3 is expressed are involved with learning and reward training [25].

Obesity and addiction may share common neurologic underpinnings [26]. Other well-replicated obesity loci, including MC4R, have also been shown to be associated with centrally-mediated phenomena including binge eating behavior [11,12,27]. Studies in mice indicate that FTO expression is particularly pronounced in regions of the brain known to regulate energy balance [28], and recent data suggest that variants in the FTO gene may regulate food intake and selection [29].

Additional research is needed to understand the association of rs10146997 with the NRXN3 gene and to identify a causal variant. Since there are no other genes within a distance of more than several hundred kilobases of this SNP, it is unlikely that a different gene accounts for this finding. A search of publically available databases [30–32] did not identify an association between SNPs in NRXN3 and gene expression.

A relationship between WC and causal variants in the NRXN3 gene may have clinical implications. Obesity is a multifactorial trait that results from a complex interaction between genes and environment. The identification of an association between obesity and variants in a gene that has been associated with substance abuse suggests that further exploration of the role of this gene in vulnerability to addiction to food substances should be undertaken.

The strengths of this work include the large discovery sample size. The effect size was small, and achieving conventional levels of genome-wide significance required combining data from more than 70,000 participants in two large consortia. Although the confirmation with the GIANT consortium is promising, the joint p-value based on more than 70,000 participants achieved only borderline genome-wide significance. Our findings warrant the need for further replication in other ethnic groups.

We identified a SNP at a novel locus in the NRXN3 gene associated with WC. This gene has previously been implicated in...
Table 2. Top 48 SNPs exchanged with the GIANT Consortium, GIANT p-values, and the combined results.

| Marker | Chromosome | Position | CHARGE pvalue | GIANT pvalue* | COMBINED pvalue | Nearest Gene** |
|--------|------------|----------|---------------|---------------|-----------------|---------------|
| rs10146997 | 14 | 79014915 | 6.4E-07 | 0.009 | 5.3E-08 | NRXN3 |
| rs981113 | 5 | 75556884 | 9.8E-07 | 0.55 | 3.4E-03 | SV2C |
| rs7338657 | 13 | 62299289 | 1.1E-06 | 0.75 | 4.4E-04 | PCDH20 |
| rs6174750 | 2 | 136499639 | 1.9E-06 | 0.48 | 2.9E-03 | DARS |
| rs1555967 | 6 | 51267954 | 1.9E-06 | 0.07 | 3.3E-06 | PHK1D1 |
| rs4701252 | 5 | 21814911 | 2.5E-06 | 0.45 | 2.3E-06 | CDH12 |
| rs4420638 | 19 | 50114786 | 3.6E-06 | 0.80 | 3.8E-04 | APOC1 |
| rs2365642 | 4 | 199501709 | 4.1E-06 | 0.79 | 3.4E-03 | PKP1 |
| rs17008958 | 3 | 71838178 | 6.5E-06 | 0.18 | 5.7E-05 | FE84E3 |
| rs7932813 | 11 | 7664875 | 9.6E-06 | 0.09 | 5.0E-06 | OVC2 |
| rs569406 | 9 | 77219165 | 4.7E-06 | 0.54 | 3.7E-04 | OSTF1 |
| rs6837818 | 4 | 1681112 | 5.2E-06 | 0.81 | 1.1E-03 | ZNF718 |
| rs17537900 | 13 | 42593449 | 7.3E-06 | 0.07 | 2.9E-03 | DNAJC15 |
| rs17476669 | 2 | 50579975 | 9.9E-06 | 0.27 | 1.1E-04 | NRXN1 |
| rs11857639 | 15 | 71424825 | 8.0E-06 | 0.94 | 3.8E-04 | HCN4 |
| rs3758063 | 8 | 87754664 | 1.2E-05 | 0.76 | 5.4E-03 | CNG83 |
| rs804569 | 20 | 22099652 | 1.4E-05 | 0.29 | 1.7E-04 | FOX2A |
| rs13003246 | 2 | 133761936 | 1.6E-05 | 0.78 | 1.9E-03 | KAP5 |
| rs7138803 | 12 | 48533735 | 1.6E-05 | 0.01 | 8.0E-07 | BCDIN3D |
| rs17201502 | 12 | 48571829 | 1.7E-05 | 0.02 | 4.2E-06 | FAIM2 |
| rs154168 | 5 | 107078981 | 1.7E-05 | 0.86 | 2.0E-03 | Efn5A5 |
| rs1324618 | 9 | 121107783 | 1.8E-05 | 0.62 | 0.01 | DBC1 |
| rs1553754 | 17 | 43918706 | 2.0E-05 | 0.05 | 1.2E-05 | HoxB1 |
| rs12971184 | 18 | 32134683 | 2.1E-05 | 0.43 | 0.03 | FHOD3 |
| rs253414 | 5 | 74992273 | 2.3E-05 | 0.47 | 8.0E-04 | Csur57 |
| rs309193 | 19 | 52317155 | 2.4E-05 | 0.20 | 1.8E-04 | C1orf2 |
| rs12457723 | 18 | 27981438 | 2.4E-05 | 0.14 | 0.08 | RFN138 |
| rs806194 | 14 | 88980606 | 2.5E-05 | 0.61 | 0.01 | FOXN3 |
| rs10172766 | 2 | 205587746 | 3.0E-05 | 0.30 | 0.01 | PAR3D6 |
| rs11096633 | 2 | 20067535 | 3.1E-05 | 0.47 | 5.2E-04 | MATN2 |
| rs8049894 | 16 | 75371885 | 3.1E-05 | 0.67 | 1.9E-03 | CNTNAP4 |
| rs12148445 | 15 | 34703950 | 3.1E-05 | 0.60 | 0.01 | C15orf41 |
| rs9829637 | 3 | 135638752 | 3.5E-05 | 0.10 | 4.9E-05 | ANAPC13 |
| rs7666149 | 4 | 41017949 | 3.7E-05 | 0.06 | 2.1E-05 | LIMCH1 |
| rs13421140 | 2 | 1753016 | 4.2E-05 | 0.97 | 6.1E-03 | MYT1L |
| rs4238692 | 16 | 82149934 | 5.8E-05 | 0.14 | 1.4E-04 | CDH13 |
| rs17833967 | 12 | 13846345 | 6.0E-05 | 0.46 | 1.2E-03 | GRIN2B |
| rs1532206 | 3 | 99153367 | 6.2E-05 | 0.89 | 9.2E-03 | MIA |
| rs6723108 | 2 | 135196450 | 6.2E-05 | 0.27 | 4.4E-04 | TME1M63 |
| rs12704232 | 7 | 85640166 | 7.4E-05 | 0.61 | 0.05 | GRM3 |
| rs12377679 | 9 | 128437576 | 8.0E-05 | 0.12 | 1.1E-04 | LMX1B |
| rs1017643 | 6 | 156835825 | 9.5E-05 | 0.04 | 2.6E-05 | ARID1B |
| rs6485438 | 11 | 43643194 | 1.3E-04 | 0.09 | 9.7E-05 | HSD17B12 |
| rs7116632 | 11 | 129452949 | 1.9E-04 | 0.74 | 0.04 | APLP2 |
| rs422988 | 1 | 4718977 | 2.4E-04 | 0.62 | 3.5E-03 | AIA101 |
| rs5771623 | 22 | 47415000 | 2.9E-04 | 0.07 | 0.28 | FAM159A5 |
| rs6728666 | 2 | 216894986 | 5.3E-04 | 0.76 | 0.02 | MARCH4 |
| rs10857810*** | 1 | 110403320 | 1.8E-04 | 0.03 | 0.97 | FAM40A |

*GIANT sample size is 38,641.
**Nearest reference is bolded if SNP is within the reference gene.
***GIANT SNP is proxy rs10857809 (r² = 0.92).

doi:10.1371/journal.pgen.1000539.t002

NRXN3 and Waist Circumference
Figure 1. Regional Association Plot for rs10146997 on chromosome 14 in the stage 1 CHARGE-only analysis. The color scheme is red for strong linkage disequilibrium (LD; $r^2 \geq 0.8$), orange for moderate LD ($r^2 = 0.5$ and $< 0.8$), yellow for weak LD ($r^2 = 0.2$ and $< 0.5$) and white for limited or no LD ($r^2 < 0.2$).

doi:10.1371/journal.pgen.1000539.g001

Table 3. Results per copy of the G allele for rs10146997 by contributing study; beta coefficients expressed as z-scores.

| Cohort                      | N   | MAF (G) | Imputation Quality Score | Beta Coefficient | SE   | p-value |
|-----------------------------|-----|---------|--------------------------|------------------|------|---------|
| AGES                        | 3170| 0.21    | Genotyped               | 0.058            | 0.031| 0.06    |
| ARIC                        | 8097| 0.22    | 0.98                     | 0.032            | 0.019| 0.12    |
| CHS                         | 3213| 0.21    | Genotyped               | 0.103            | 0.030| 0.00048 |
| Family Heart Study          | 855 | 0.21    | Genotyped               | 0.003            | 0.055| 0.65    |
| Framingham Heart Study      | 7115| 0.20    | 1.00                     | 0.068            | 0.022| 0.0019  |
| Old Order Amish             | 1097*| 0.14   | 0.87                     | 0.049            | 0.073| 0.33    |
| Rotterdam Study             | 5471| 0.21    | Genotyped               | 0.042            | 0.024| 0.08    |
| EUROSPAN Consortium         |     |        |                          |                  |      |         |
| ERF (Dutch)                 | 1241| 0.20    | Genotyped               | −0.010           | 0.052| 0.86    |
| Croatia                     | 784 | 0.24    | Genotyped               | 0.039            | 0.059| 0.52    |
| MICROS (South Tyrolean)     | 293 | 0.17    | Genotyped               | 0.057            | 0.101| 0.60    |
| Meta-analysis results       | 31373| 0.21   | N/A                      | 0.0498           | 0.010| 6.4 × 10$^{-7}$ |

SE = standard error; MAF = minor allele frequency.

Sample size reduced from 1134 because smokers excluded due to the low smoking prevalence.

doi:10.1371/journal.pgen.1000539.t003
Table 4. CHARGE consortium secondary analysis results per copy of the G allele for rs10146997 in 31373 individuals; beta coefficients expressed as z-scores.

|                      | Beta Coefficient | SE  | p-value    |
|----------------------|------------------|-----|------------|
| Overall              | 0.0498           | 0.010 | 6.4 × 10⁻⁷ |
| Overall without adjusting for smoking | 0.0460           | 0.010 | 5.6 × 10⁻⁶ |
| Sex stratification   |                  |     |            |
| Women                | 0.0500           | 0.014 | 4.7 × 10⁻⁴ |
| Men                  | 0.0427           | 0.013 | 0.001      |
| Age stratification   |                  |     |            |
| < 55 years           | 0.0520           | 0.017 | 0.002      |
| ≥ 55 years           | 0.0560           | 0.013 | 7.4 × 10⁻⁶ |

Odds Ratio | 95% CI | p-value |
|------------|--------|---------|
| WC category* |       |         |
| High WC (women ≥ 88 cm, men ≥ 102 cm) | 1.07 | 1.02–1.11 | 0.003 |
| BMI categories** |       |         |
| Overweight (BMI 25 to < 30) | 1.03 | 0.98–1.07 | 0.250 |
| Obese (BMI ≥ 30) | 1.13 | 1.07–1.19 | 3.2 × 10⁻⁵ |

*Referent = normal WC category (women < 88 cm; men < 102 cm).
**Referent = normal weight category (BMI 18.5–25 kg/m²).

doi:10.1371/journal.pgen.1000539.t004

Figure 2. Mean waist circumference by number of risk alleles for FTO, MC4R, and NRXN3. Bars represent standard errors. The panel on the left represents the distribution of risk alleles in the overall sample.
doi:10.1371/journal.pgen.1000539.g002
addiction and reward behavior, lending further support to the concept that obesity, in part, is a centrally-mediated disorder.

Supporting Information

**Figure S1** CHARGE consortium Manhattan plot for waist circumference. Found at: doi:10.1371/journal.pgen.1000539.s001 (0.61 MB TIF)

**Figure S2** CHARGE consortium QQ plot for waist circumference. Found at: doi:10.1371/journal.pgen.1000539.s002 (0.44 MB TIF)

**Figure S3** Forest plot for rs10146997. Found at: doi:10.1371/journal.pgen.1000539.s003 (0.37 MB TIF)

**Figure S4** CHARGE consortium Manhattan plot for Body Mass Index. Found at: doi:10.1371/journal.pgen.1000539.s004 (0.58 MB TIF)

**Figure S5** CHARGE consortium QQ plot for Body Mass Index. Found at: doi:10.1371/journal.pgen.1000539.s005 (0.34 MB TIF)

**Table S1** Summary of imputation and statistical analysis methods across the cohorts. Found at: doi:10.1371/journal.pgen.1000539.s006 (0.08 MB DOC)

**Table S2** GIANT Study-specific results for rs10146997. Found at: doi:10.1371/journal.pgen.1000539.s007 (0.06 MB DOC)

**Table S3** Comprehensive results from the CHARGE consortium for Waist Circumference with P<9.9×10⁻⁶. Found at: doi:10.1371/journal.pgen.1000539.s008 (0.04 MB XLS)

**Table S4** Comprehensive results from the CHARGE consortium for body mass index with P<9.9×10⁻⁶. Found at: doi:10.1371/journal.pgen.1000539.s009 (0.04 MB XLS)

References

1. Cassano PA, Rosner B, Vokonas PS, Weiss ST (1992) Obesity and body fat distribution in relation to the incidence of non-insulin-dependent diabetes mellitus. A prospective cohort study of men in the normative aging study. Am J Epidemiol 136: 1474–1486.

2. Fulton AR, Kushi LH, Anderson KE, Mink PJ, Olson JE, et al. (2000) Associations of general and abdominal obesity with multiple health outcomes in older women: the Iowa Women’s Health Study. Arch Intern Med 160: 2117–2128.

3. Ohlson LO, Larsson B, Svardsudd K, Welin L, Eriksson H, et al. (1985) The distribution in relation to the incidence of non-insulin-dependent diabetes mellitus. A prospective cohort study of men in the normative aging study. Am J Epidemiol 136: 1474–1486.

4. Wei M, Gaskell SP, Halfner SM, Stern MP (1997) Waist circumference as the best predictor of non-insulin dependent diabetes mellitus (NIDDM) compared to body mass index, waist/hip ratio and other anthropometric measurements in Mexican Americans-a 7-year prospective study. Obes Res 3: 16–23.

5. Rankinen T, Zuberi A, Chagnon YC, Weijsnagel SJ, Argyroudis G, et al. (2006) The human obesity gene map: the 2005 update. Obesity (Silver Spring) 14: 529-644.

6. Lyon HN, Hirschhorn JN (2005) Genetics of common forms of obesity: a brief overview. Am J Clin Nutr 82: 215S–217S.

7. Chambers JC, Elliott P, Zabaneh D, Zhang W, Li Y, et al. (2008) Common genetic variation near MCHR1 is associated with waist circumference and insulin resistance. Nat Genet 40: 717–718.

8. Fryling TM, Timpson NJ, Weedon MN, Zeggini E, Frayth RM, et al. (2007) A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. Science 316: 889–894.

9. Loos RJ, Lindgren CM, Li S, Wheeler E, Zhao JH, et al. (2009) Common variants near MCHR1 are associated with fat mass, weight and risk of obesity. Nat Genet 40: 767–775.

10. Kring SI, Hodt C, Zimmermann E, Jøss T, Berentzen T, et al. (2008) FTO gene associated famines in relation to body fat distribution and metabolic train throughout a broad range of famines. PLoS ONE 3: e9258. doi:10.1371/journal.pone.0009258.

Acknowledgments

The authors acknowledge the essential role of the CHARGE (Cohorts for Heart and Aging Research in Genome Epidemiology) Consortium in development and support of this manuscript. CHARGE members include the Netherland’s Rotterdam Study (RS), the NHLBI’s Framingham Heart Study (FHS), Cardiovascular Health Study (CHS), the NHLBI’s Atherosclerosis Risk in Communities (ARIC) Study, and the Icelandic Heart Association’s and NIA’s Iceland Age, Gene/Environment Susceptibility (AGES) Reykjavik Study, and the European Special Population Network (EUROSPAN).

We are indebted to the staff and participants of the AGES Reykjavik Study, the ARIC Study, the CHS Study, the FHS Study, the Rotterdam Study, and EUROSPAN for their important contributions. A full list of principal CHS investigators and institutions can be found at http://www.chs-nhlbi.org/pi.htm. We acknowledge the National Heart, Lung, and Blood Institute, who has made the SHARE (SNP Health Association Resource) project possible. We thank Pascal Arp, Mda Jhamaiz, Dr. Michael Moorhouse, Marijn Verkerk, and Sander Bervoets for their help in creating the Rotterdam database and Maxim Struchalin for his contributions to the imputations of the Rotterdam data.

Author Contributions

Conceived and designed the experiments: NLHC MCZ TBH MF GE MG LJI BDM ARS EB FB JSP BMP JHR HC UG PP IR MAP LDA JNH CEJ CJO AH FR AGU JCMW BAO RCK VG JRO IBB CMvD LAC KEN. Wrote the paper: NLHC MCZ KLM AJM MF TH TA GE LJL AVS BDM ARS SJB EB CSF KEN. Analyzed the data: NLHC KLM AJM MF TBH TH TA GE LJL AVS BDM ARS JHB JAO FB CSF KEN. Contributed reagents/materials/analysis tools: TBH TH TA GE LJL AVS BDM ARS SJB EB CSF KEN. Conceived and designed the experiments: NLHC MCZ TBH MF GE MG LJI BDM ARS EB FB JSP BMP JHR HC UG PP IR MAP LDA JNH CEJ CJO AH FR AGU JCMW BAO RCK VG JRO IBB CMvD LAC KEN. We are indebted to the staff and participants of the AGES Reykjavik Study, the ARIC Study, the CHS Study, the FHS Study, the Rotterdam Study, and EUROSPAN for their important contributions. A full list of principal CHS investigators and institutions can be found at http://www.chs-nhlbi.org/pi.htm. We acknowledge the National Heart, Lung, and Blood Institute, who has made the SHARE (SNP Health Association Resource) project possible. We thank Pascal Arp, Mda Jhamaiz, Dr. Michael Moorhouse, Marijn Verkerk, and Sander Bervoets for their help in creating the Rotterdam database and Maxim Struchalin for his contributions to the imputations of the Rotterdam data.

Author Contributions

Conceived and designed the experiments: NLHC MCZ TBH MF GE MG LJI BDM ARS EB FB JSP BMP JHR HC UG PP IR MAP LDA JNH CEJ CJO AH FR AGU JCMW BAO RCK VG JRO IBB CMvD LAC KEN. Wrote the paper: NLHC MCZ KLM AJM MF TH TA GE LJL AVS BDM ARS JHB JAO FB CSF KEN. Contributed reagents/materials/analysis tools: TBH TH TA GE LJL AVS BDM ARS SJB EB CSF KEN. Conceived and designed the experiments: NLHC MCZ TBH MF GE MG LJI BDM ARS EB FB JSP BMP JHR HC UG PP IR MAP LDA JNH CEJ CJO AH FR AGU JCMW BAO RCK VG JRO IBB CMvD LAC KEN.

Text S1 Details of participating cohorts. Found at: doi:10.1371/journal.pgen.1000539.s010 (0.07 MB DOC)

Acknowledgments

The authors acknowledge the essential role of the CHARGE (Cohorts for Heart and Aging Research in Genome Epidemiology) Consortium in development and support of this manuscript. CHARGE members include the Netherland’s Rotterdam Study (RS), the NHLBI’s Framingham Heart Study (FHS), Cardiovascular Health Study (CHS), the NHLBI’s Atherosclerosis Risk in Communities (ARIC) Study, and the Icelandic Heart Association’s and NIA’s Iceland Age, Gene/Environment Susceptibility (AGES) Reykjavik Study, and the European Special Population Network (EUROSPAN).

We are indebted to the staff and participants of the AGES Reykjavik Study, the ARIC Study, the CHS Study, the FHS Study, the Rotterdam Study, and EUROSPAN for their important contributions. A full list of principal CHS investigators and institutions can be found at http://www.chs-nhlbi.org/pi.htm. We acknowledge the National Heart, Lung, and Blood Institute, who has made the SHARE (SNP Health Association Resource) project possible. We thank Pascal Arp, Mda Jhamaiz, Dr. Michael Moorhouse, Marijn Verkerk, and Sander Bervoets for their help in creating the Rotterdam database and Maxim Struchalin for his contributions to the imputations of the Rotterdam data.

**Author Contributions**

Conceived and designed the experiments: NLHC MCZ TBH MF GE MG LJI BDM ARS EB FB JSP BMP JHR HC UG PP IR MAP LDA JNH CEJ CJO AH FR AGU JCMW BAO RCK VG JRO IBB CMvD LAC KEN. Wrote the paper: NLHC MCZ KLM AJM MF TH TA GE LJL AVS BDM ARS JHB JAO FB CSF KEN. Contributed reagents/materials/analysis tools: TBH TH TA GE LJL AVS BDM ARS SJB EB CSF KEN. Conceived and designed the experiments: NLHC MCZ TBH MF GE MG LJI BDM ARS EB FB JSP BMP JHR HC UG PP IR MAP LDA JNH CEJ CJO AH FR AGU JCMW BAO RCK VG JRO IBB CMvD LAC KEN.

**Author Contributions**

Conceived and designed the experiments: NLHC MCZ TBH MF GE MG LJI BDM ARS EB FB JSP BMP JHR HC UG PP IR MAP LDA JNH CEJ CJO AH FR AGU JCMW BAO RCK VG JRO IBB CMvD LAC KEN. Wrote the paper: NLHC MCZ KLM AJM MF TH TA GE LJL AVS BDM ARS JHB JAO FB CSF KEN. Contributed reagents/materials/analysis tools: TBH TH TA GE LJL AVS BDM ARS SJB EB CSF KEN. Conceived and designed the experiments: NLHC MCZ TBH MF GE MG LJI BDM ARS EB FB JSP BMP JHR HC UG PP IR MAP LDA JNH CEJ CJO AH FR AGU JCMW BAO RCK VG JRO IBB CMvD LAC KEN.
22. Gordon AGG, Qui X, Yakovlev A (2007) Control of the mean number of false discoveries, bonferroni and stability of multiple testing. Annals of Applied Statistics 1: 179–190.
23. Lachman HM, Fann CS, Barzilai N, Esgrafio OV, Rosenthal RN, et al. (2007) Genomewide suggestive linkage of opioid dependence to chromosome 14q. Hum Mol Genet 16: 1327–1334.
24. Kelai S, Maussion G, Noble F, Boni C, Ramoz N, et al. (2008) Nrxin3 upregulation in the globus pallidus of mice developing cocaine addiction. Neureport 19: 751–755.
25. Clay SW, Allen J, Parran T (2008) A review of addiction. Postgrad Med 120: E01–07.
26. Rapaka R, Schnur P, Shurtleff D (2008) Obesity and addiction: common neurological mechanisms and drug development. Physiol Behav 95: 2–9.
27. Branson R, Potoczna N, Kral JG, Lentes KU, Hohe MR, et al. (2003) Binge eating as a major phenotype of melanocortin 4 receptor gene mutations. N Engl J Med 348: 1096–1103.
28. Gerken T, Girard CA, Tung YC, Webby CJ, Saudek V, et al. (2007) The obesity-associated FTO gene encodes a 2-oxoglutarate-dependent nucleic acid demethylase. Science 318: 1469–1472.
29. Cecil JE, Tavendale R, Watt P, Hetherington MM, Palmer CN (2008) An obesity-associated FTO gene variant and increased energy intake in children. N Engl J Med 359: 2558–2566.
30. Myers AJ, Gibbs JR, Webster JA, Rohrer K, Zhao A, et al. (2007) A survey of genetic human cortical gene expression. Nat Genet 39: 1494–1499.
31. Dixon AL, Liang L, Moffatt MF, Chen W, Heath S, et al. (2007) A genome-wide association study of global gene expression. Nat Genet 39: 1202–1207.
32. Schadt EE, Molony C, Chudin E, Hao K, Yang X, et al. (2008) Mapping the genetic architecture of gene expression in human liver. PLoS Biol 6: e107. doi:10.1371/journal.pbio.0060107.