Supplementary Information

Sequencing Panel Analysis

Genomic DNA was extracted from the patient specimen and resuspended in Tris-HCl using AMPure XP (Beckman Coulter). Eluted DNA was quantified via Qubit dsDNA assay (ThermoFisher) using a Biotek Synergy HTX fluorometer, and eluate volume was measured using a BioMicroLab VolumeCheck 100. A combination of Next Generation Sequencing (NGS) and Sanger sequencing technologies to cover the full coding regions of the listed genes plus ~10 bases of non-coding DNA flanking each exon. For NGS, patient DNA corresponding to these regions was captured using an optimized set of DNA hybridization probes. Captured DNA was sequenced using Illumina's Reversible Dye Terminator (RDT) platform (Illumina,SanDiego,CA,USA). For Sanger sequencing, Polymerase Chain Reaction (PCR) was used to amplify targeted regions. After purification of the PCR products, cycle sequencing was carried out using the ABI Big Dye Terminator v.3.1 kit. PCR products were resolved by electrophoresis on an ABI 3730xl capillary sequencer. In nearly all cases, cycle sequencing was performed separately in both the forward and reverse directions.

NGS libraries were prepared using 200 ng DNA and KAPA HyperPlus (Roche) reagents and unique dual indexes (IDT) per the manufacturer's instructions. Libraries were hybridized with custom-designed XGen probes (IDT), pooled, and gene sequenced using 2x100 bp v4 chemistry on an Illumina HiSeq 2500 following the manufacturer's instructions to a mean coverage of 200x. Passing filter reads meeting run and library-level QC criteria were processed via a GATK-based pipeline on DNAnexus (Mountain View, CA, USA). Briefly, BAM files were generated using GRCh37/hg19 reference. Upon additional read filtering and quality recalibration of the aligned BAM files, variants were then called using HaplotypeCaller. Variants with depth >20, quality>100, and variant allele frequency between 0.25 and 0.6 (heterozygous) or greater than 0.925 (homozygous) were included in the analyses. Finally, variants were filtered for rarity (in October 2018) with a <1% maximum continental frequency in the Genome Aggregation Database (gnomAD) and further annotated according to American College of Medical Genetics (ACMG). Human Genome Variation Society (HGVS) recommendations are used to describe sequence variants.[1]

Supplemental Table 1. Genes associated with the Leptin-Melanocortin pathway

Genetic Variants – Upstream Pathway	*AFF4, ALMS1,BBS1-20,CPE, GNAS, HTR2C, INPP5E, IRS2, KSR2, LEP, LEPR, MAGEL2, MC3R, PMCH, MRAP2, NHLH2, PCSK1, POMC, PROK2, RAB23, RAI1, SH2B1, SRC1*
Genetic Variants – Downstream Pathway	*BDNF, MC4R, NTRK2, RPS6KA3, SIM1, THRB*

Supplemental Table 2. Clinical diagnostic codes using ICD-9 and ICD-10

	ICD-9	**ICD-10**
Hypertension[2]	401.xx-405.xx	I10.xx-I15.xx
Diabetes[3]	250.xx	E10.xx–E14.xx
Dyslipidemia[4]	272.xx	E78
Smoking status [5]	305.1	V15.82
CAD[6]	410.xx -414.xx, 429, 996.03	I20.xx–I25.xx, T82.21, T82.85
PAD[4]	437, 440, 441, 443, 444, 445, 447, 556,	I70, I71, I73-I75, I77, I79,
CVA[7]	430-438	G45-46, I63, I65, I66, I67, I69
Abbreviations: CAD, Coronary Artery Disease; CVA, cerebrovascular accident; CVD, cardiovascular disease; ICD, International Classification of Diseases; PAD, Peripheral Arterial Disease		

Supplemental Table 3. Genetic Variants – Upstream and Downstream Pathway. Demographics

	N of carriers	Sex, females	Age, years	Weight, kg	BMI, kg/m^2
Genetic Variants – Upstream Pathway					
Pro-opiomelanocortin gene (POMC)	39	27 (69.2%)	65.8 (13.1)	122.3 (23.9)	44.2 (8.1)
Steroid receptor coactivator 1 gene (SRC 1)	28	24 (85.8%)	64.1 (15.6)	118.4 (19.3)	41.1 (6.1)
Proprotein convertase subtilisin/kexin type 1 gene (PCSK1)	25	19 (76%)	62.8 (13.8)	119.6 (22.1)	43.8 (7.8)
SH2B adaptor protein 1 gene (SH2B1)	22	17 (85%)	62.7 (13.2)	122.3 (17.8)	44.8 (6.7)
Leptin receptor gene (LEPR)	23	17 (73.9%)	67.1 (15.1)	115.7 (20.5)	41.6 (7.1)
Retinoic Acid Induced 1 gene (RAI 1)	4	2 (50%)	47.3 (5.9)	132.8 (25.9)	43.2 (7.1)
Prokineticin 2 gene (PROK2)	2	1 (50%)	48.5 (10.6)	147.1 (1.5)	46.6 (0.7)
Ras-related protein Rab-23 gene (RAB23)	2	0	62 (15.6)	148.4 (13)	47 (6)
MAGE Family Member L2 (MAGEL2)	1	1 (100%)	33	95.8	37.9
Genetic Variants – Downstream Pathway					
Melanocortin-4-receptor gene (MC4R)	16	8 (50%)	69.3 (19.2)	122.5 (31.3)	43.3 (8.7)
Single-minded homolog 1 gene (SIM1)	6	5 (83.3%)	59.2 (17.4)	124.3 (18.9)	40.5 (4.8)
Abbreviations: BMI, Body Mass Index.					

STROBE Checklist

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls	4
		(*b*) For matched studies, give matching criteria and the number of controls per case	4
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4-5
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	4-5, supplement
Bias	9	Describe any efforts to address potential sources of bias	5
Study size	10	Explain how the study size was arrived at	NA
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	4-5
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	5
		(*b*) Describe any methods used to examine subgroups and interactions	5
		(*c*) Explain how missing data were addressed	NA
		(*d*) If applicable, explain how matching of cases and controls was addressed	NA
		(*e*) Describe any sensitivity analyses	NA

Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	6, Fig 1
		(b) Give reasons for non-participation at each stage	6, Fig 1
		(c) Consider use of a flow diagram	Fig 1
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	6, Table 1
		(b) Indicate number of participants with missing data for each variable of interest	6
Outcome data	15*	Report numbers in each exposure category, or summary measures of exposure	6, Table 2
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	6, Table 3
		(*b*) Report category boundaries when continuous variables were categorized	6, tables
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	NA
Discussion			
Key results	18	Summarise key results with reference to study objectives	7
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	8
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	9
Generalisability	21	Discuss the generalisability (external validity) of the study results	9
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	1

References

1. den Dunnen JT, Dalgleish R, Maglott DR, et al. HGVS recommendations for the description of sequence variants: 2016 update. *Human mutation* 2016; **37**(6): 564-9.
2. Beckman KD. How to document and code for hypertensive diseases in ICD-10. *Family practice management* 2014; **21**(2): 5-9.
3. Khokhar B, Jette N, Metcalfe A, et al. Systematic review of validated case definitions for diabetes in ICD-9-coded and ICD-10-coded data in adult populations. *BMJ open* 2016; **6**(8): e009952.
4. Quan H, Sundararajan V, Halfon P, et al. Coding algorithms for defining comorbidities in ICD-9-CM and ICD-10 administrative data. *Medical care* 2005: 1130-9.
5. Wiley LK, Shah A, Xu H, Bush WS. ICD-9 tobacco use codes are effective identifiers of smoking status. *Journal of the American Medical Informatics Association* 2013; **20**(4): 652-8.
6. Marrie RA, Bo NY, Leung S, et al. Prevalence and incidence of ischemic heart disease in multiple sclerosis: a population-based validation study. *Multiple sclerosis and related disorders* 2013; **2**(4): 355-61.
7. Kokotailo RA, Hill MD. Coding of stroke and stroke risk factors using international classification of diseases, revisions 9 and 10. *Stroke* 2005; **36**(8): 1776-81.