Cross-ancestry analysis identifies genes associated with obesity risk and protection

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ABSTRACT

Gene discoveries in obesity have largely been based on European cohorts, leading to an ancestral bias, that limits their generalizability across populations. We performed a gene-based rare variant association study of 721,941 individuals and identified 116 novel BMI-associated genes with consistent effects across ancestries, including 50 risk-conferring and 66 protective genes against obesity. Protective genes such as *DCUN1D3* and *NEUROD6* had effect sizes comparable to high-risk genes such as *MC4R* and *BSN*, and nearly twice that of known protective genes such as *GPR75*, which, along with five other genes, showed strong European bias. Notably, 82 of the 116 genes showed functional relevance to obesity including adiposity, energy homeostasis, and glucose metabolism. While polygenic risks or an obesogenic lifestyle amplified the effect of 15 genes on BMI, including the combination of low physical activity and *MACROD1*, 23 genes including *VIRMA*, *AQP3*, and *PML* retained protective effects even at high polygenic scores. Our findings provide further insights into the genetic basis of obesity that is conserved across ancestries and their interactions with obesogenic factors.

MAIN

Obesity is a complex, heritable disorder with significant global impact, contributing to numerous comorbidities and public health challenges[1]. It is influenced by a combination of genetic and lifestyle factors[2,3], yet the genetic component underlying the etiology of obesity remains a key area of investigation. While large-scale studies have identified roles for common variants[4], including significant effects of polygenic risk scores (PGS)[5], and rare protein-truncating variants in obesity risk genes such as *MC4R* and *BSN*[6–9], as well as protective genes such as *GPR75*[9], these studies have predominantly focused on populations of European ancestry. Non-European populations are often underrepresented or included only for replication purposes[6–8]. This lack of diversity in genetic studies has resulted in two major issues: a bias in gene discovery towards a specific ancestry, and a limited ability to detect associations that may be significant in non-Europeans but lack statistical power in predominantly European cohorts[10]. *For example*, clinically significant variants in *APOL1* and *PCSK9* associated with kidney disease and low LDL cholesterol, respectively, were both discovered in populations of African ancestry[11,12]. The consequences of this bias extend beyond gene discovery, affecting the generalizability of obesity-related findings to the broader global population and potentially limiting the effectiveness of precision medicine approaches[13].

Here, we conducted a rare variant association analysis of body-mass index (BMI) using genetic and phenotypic data from 721,941 adults of diverse ancestries, leveraging cohorts from the UK Biobank (UKB) and the All of Us (AoU) initiative. Unlike previous rare variant association studies of BMI that prioritized European populations for discovery and non-Europeans for replication, we adopted a discovery approach in non-European populations as well. Combining statistics across populations, we identified 121 genes, 116 of which have not been previously linked to BMI in the context of rare variants, that showed consistent risk-conferring and protective effects across ancestries. Our findings also revealed a strong European bias in previously identified obesity genes, with effect sizes varying significantly in non-European populations. These disparities highlight the critical need for more inclusive genomic studies to ensure equitable obesity interventions.

RESULTS

Cross-ancestry analysis to identify BMI-associated genes

We analyzed genetic and electronic health record data of 721,941 adults from the UKB and AoU cohorts representing six ancestries[14,15]. We divided the cohorts into five populations, each with at least 50,000 individuals (**Table 1**). We used REGENIE v3.3[16] to perform gene-burden association tests by collapsing ultra-rare (minor allele frequency <0.1%) protein truncating variants (PTVs, defined as predicted loss of function or deleterious missense variants) for each gene and measuring their effect on BMI for each independent population. Using Bonferroni multiple testing correction ($P<8.34 \times 10^{-7}$, 20,000 genes, and three variant collapsing models, see **Methods**) and sample size ($N\geq20$) thresholds, we identified a total of 11 BMI-associated genes from the UKB British (*MC4R*, *PCSK1*, *DIDO1*, *BSN*, *UBR2*, *ATP5PO*, *APBA1*, *SLC12A5*, and *ATP13A1*) and AoU European (*MC4R*, *HECTD4* and *YLPM1*) populations, while no significant genes were found in the UKB non-British, AoU African, or AoU mixed populations, potentially due to insufficient statistical power to detect any association (**Supplementary Table 1**). Therefore, to identify genetic associations that are consistent across populations, we performed random-effect inverse variance weighted meta-analysis[17] by aggregating summary statistics from each population into European (UKB British and AoU European populations), non-European (UKB non-British, AoU African, and AoU mixed populations), and combined (all five populations) results. Gene-burden associations that passed multiple testing correction in either the European or non-European meta-analysis, as well as in the combined result, were considered to be significant gene-BMI associations across ancestries. In contrast to previous cross-ancestry studies of BMI where European meta-analysis was typically used for discovery and non-European analysis for replication, we did not define a single discovery population. Instead, we applied the same discovery approach in both populations, such that discoveries in Europeans were replicated in non-Europeans, and vice versa. This strategy allowed us to detect association signals for BMI in both populations, with consistent effect sizes.

Using our cross-ancestry approach, we discovered 116 novel genes (50 associated with increased BMI and 66 with decreased BMI), along with five genes (*APBA1*, *BSN*, *MC4R*, *ROBO1*, and *UBR2*) that were previously identified in rare-variant studies on BMI[6,7,9,18] (**Fig. 1**, **Supplementary Tables 2, 3 and 4**). We found that 28 out of the 33 known BMI-associated genes did not pass the exome-wide significance threshold, including six genes (*GPR75*, *PTPRG*,

SPARC, *RAB21*, *ATP13A1,* and *DIDO1*) that were only significant in Europeans but not in the combined meta-analysis (**Supplementary Table 5**). This result is consistent with a recent study by Zhao and colleagues where the association of *ATP13A1* with BMI did not replicate in a non-European cohort[7]. In contrast, we found that *GIPR* showed a significant effect in non-Europeans (β=-1.12 kg/m^2, 95% CI: -1.54, -0.70, P=1.67x10^{-7}) but not in Europeans (β=-0.44 kg/m^2, 95% CI: -0.89, 0.05, P=0.09). We did not find significance for some genes such as *KSR2*, *ZFHX3,* and *RAPGEF3,* where previous associations were driven by specific protein-altering variants[6], while for other genes such as *ANO4*, *DPP9,* and *TOX4* cohort-specific biases may have driven previous associations. *For instance*, Zhao and colleagues failed to replicate the association of *TOX4* in a non-European cohort[7] (**Supplementary Fig. 1, Supplementary Table 5**).

Table 1: Cohort and population statistics analyzed in this study.

Population	European	Non-European	Combined
UKB British	419,228		419,228
UKB non-British (includes African, East Asian, South Asian ancestries)		68,070	68,070
AoU European	127,644		127,644
AoU African		54,865	54,865
AoU Mixed (includes Latino/admixed American, East Asian, South Asian and Middle Eastern ancestries)		52,134	52,134
Meta population (N)	**546,872**	**175,069**	**721,941**

In our discovered genes, *MYOG* (β=3.87 kg/m^2, 95% CI: 3.14, 4.6, P=0), *ZFC3H1* (β=1.78 kg/m^2, 95% CI: 1.53, 2.02, P=0), and *TMEM229A* (β=1.77 kg/m^2, 95% CI: 1.47, 2.07, P=0) contributed to the highest increase in BMI, with effect sizes comparable to *MC4R*, *BSN*, and *UBR2*. In fact, we identified 40 such high-effect genes, including 24 BMI increasing and 16 BMI decreasing, which contributed to at least 1 kg/m^2 change in BMI (**Fig. 1, Supplementary Table 4**). The effect sizes of several BMI-increasing genes, including *MACROD1*, *ICE1*, *ADNP, NACC2,* and *LPCAT4,* were robust and showed minimal variability across ancestries

(interpopulation variance, V_β<0.15) (**Fig. 2, Supplementary Table 6**). Similarly, BMI-decreasing genes such as *B3GNT2* (V_β=0.06), *SNAP29* (V_β=0.10), *VIRMA* (V_β=0.13), and *CCT7* (V_β=0.13) showed more consistent effect sizes than previously reported protective genes, including *GPR75* (V_β=1.06) and *GPR151* (V_β=0.49) (**Fig. 2, Supplementary Table 6**). Further, the absolute effect sizes of BMI-decreasing genes, *DCUN1D3* (β=-3.84 kg/m^2, 95% CI: -4.6, -3.09, P=0), *NEUROD6* (β=-3.02 kg/m^2, 95% CI: -4.16, -1.89, P=1.8x10^{-7}), and *SH3GL2* (β=-2.34 kg/m^2, 95% CI: -3.05, -1.63, P=1.16x10^{-10}) were comparable to those of *MC4R* and *BSN*. These results highlight the robustness of our cross-ancestry gene discovery approach.

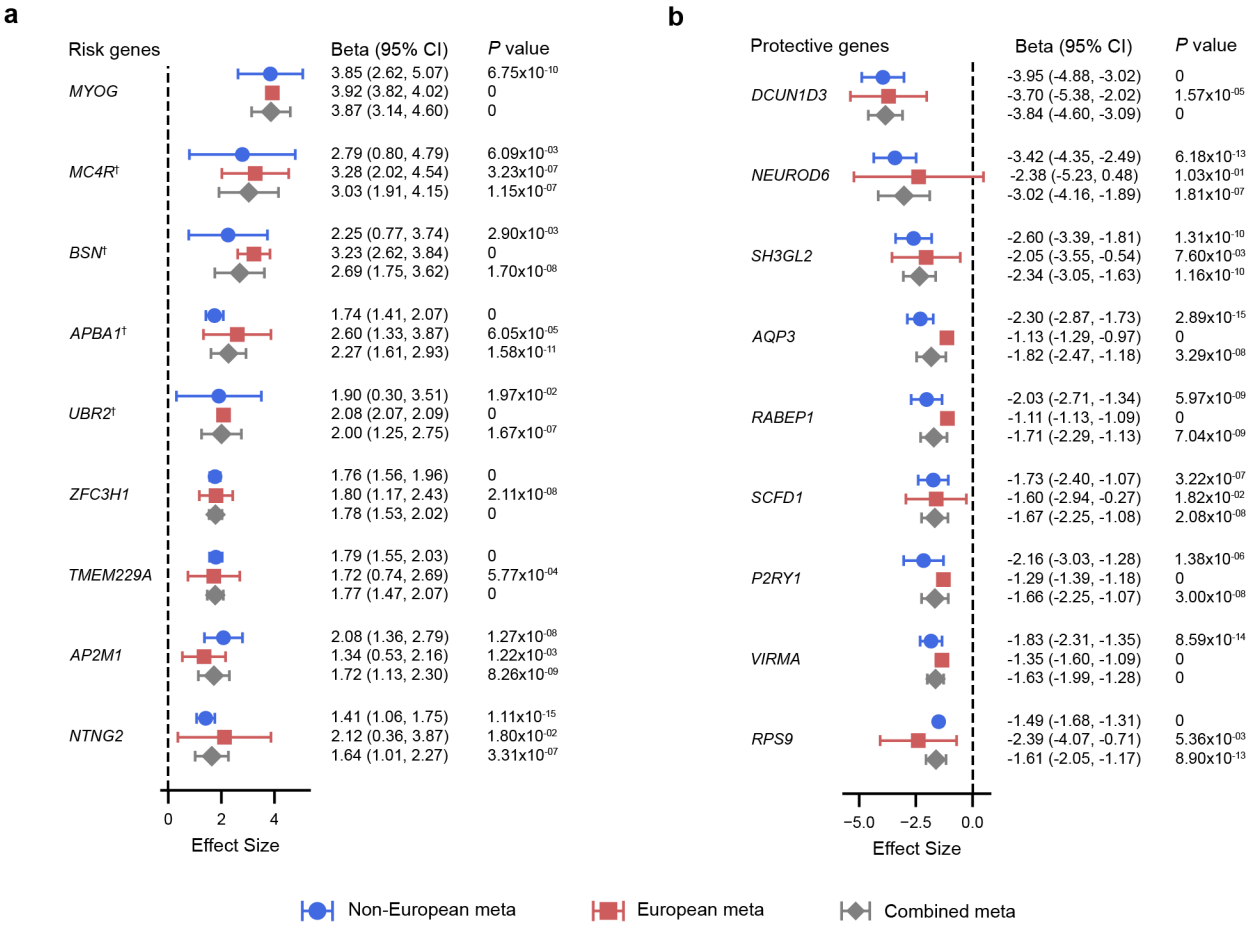
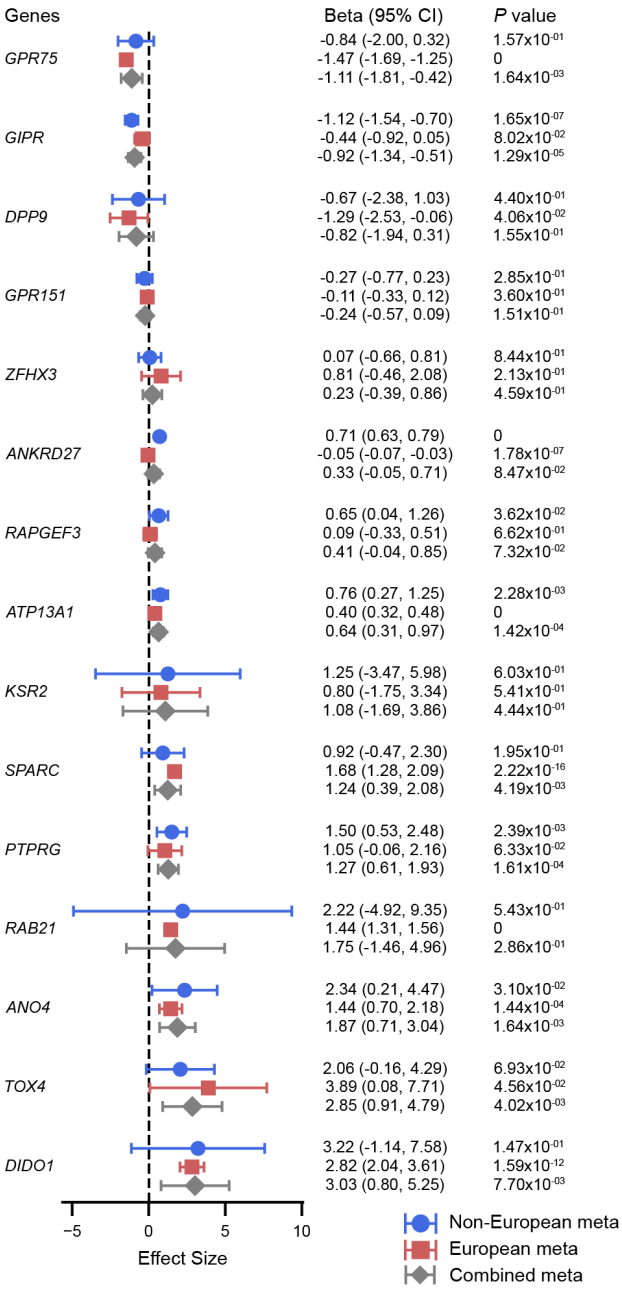


Fig. 1: **Monogenic genes contributing to at least 1.5 kg/m^2 difference in BMI across ancestries**. Effect sizes along with 95% confidence intervals (CI) and significance values (*P* value) in European, non-European, and combined meta-analysis for high-effect (>1.5 kg/m^2) genes associated with (**a**) increased and (**b**) decreased BMI are shown. Three variant collapsing models were tested for each gene and the one with the lowest *P* value in the combined meta-analysis is shown here. † indicates known BMI genes. Data for all genes contributing to change in BMI (including those contributing to >1 kg/m^2) are provided in **Supplementary Table 4**.



Supplementary Fig. 1: **Effect of previously identified BMI-associated genes across ancestries**. Effect sizes along with 95% confidence intervals (CI) and significance values (*P* value) in European, non-European, and combined meta-analysis for genes previously associated with BMI are shown. Three variant collapsing models were tested for each gene and the one with the lowest *P* value in the combined meta-analysis is shown here. Extended data are available in **Supplementary Table 5**.

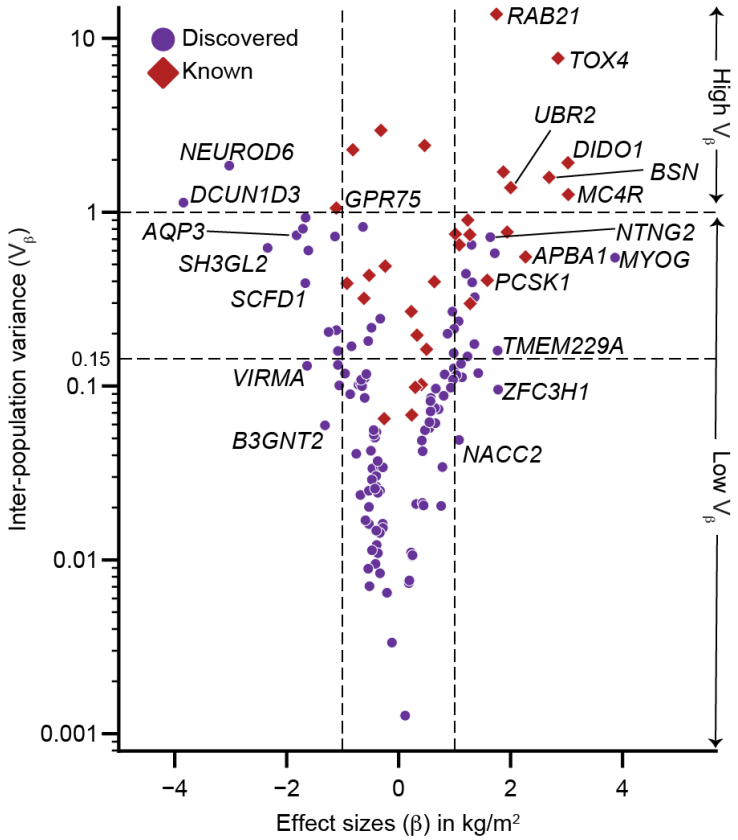


Fig. 2: **Interpopulation variance of known and discovered BMI genes**. Interpopulation variance plot with effect sizes along x-axis and variance of effect sizes across populations along y-axis of previously identified (in red diamond) and discovered (in purple dots) BMI genes are shown. Three variant collapsing models were tested for each gene and the one with the lowest *P* value in the combined meta-analysis is shown here. Extended data are available in **Supplementary Table 6**.

Cardiometabolic profile of carriers of PTVs in BMI-associated genes

We next examined whether PTV carriers in the BMI-associated genes were enriched across different obesity categories, namely, underweight/normal (BMI<25), overweight (25≤BMI<30), obese (30≤BMI<40), and severely obese (BMI≥40). Of the BMI-increasing genes, PTV carriers in 35 genes, including *NCOR1* (OR=1.28, 95% CI: 1.04, 1.58, *P*=0.02), *SEC24B* (OR=1.46, 95% CI: 1.04, 2.04, *P*=0.02), *ADNP* (OR=1.77, 95% CI: 1.15, 2.7, *P*=0.007), and *ANGPT2* (OR=1.62, 95% CI: 1.08, 2.42, *P*=0.01), showed higher odds for obesity, while PTV carriers in 22 genes, including *MACROD1* (OR=2.11, 95% CI: 1.31, 3.25, *P*=0.002), *ICE1* (OR=1.74, 95% CI: 1.06, 2.73, *P*=0.01), and *MYOG* (OR=7.47, 95% CI: 1.73, 25.21, *P*=0.004), showed higher odds for severe obesity (**Fig. 3a, Supplementary Fig. 2, Supplementary Table 7**). Of the BMI-

decreasing genes, PTV carriers in 15 genes, including *NEUROD6* (OR=2.78, 95% CI: 1.05, 7.61, *P*=0.03), *DCUN1D3* (OR=2.49, 95% CI: 1.00, 6.22, *P*=0.04), *SH3GL2* (OR=2.4, 95% CI: 1.49, 3.87, *P*=0.0001), and *VIRMA* (OR=1.47, 95% CI: 1.11, 1.94, *P*=0.006), were more likely to be underweight or normal than individuals without PTVs in those genes (**Fig. 3a, Supplementary Fig. 2, Supplementary Table 7**). Further, individuals with PTVs in *VIRMA* (OR=0.51, 95% CI: 0.35, 0.73, $P=7.18\text{x}10^{-5}$), *RABEP1* (OR=0.53, 95% CI: 0.32, 0.85, *P*=0.005), *CDK7* (OR=0.67, 95% CI: 0.46, 0.96, *P*=0.03), and *P2RY1* (OR=0.44, 95% CI: 0.18, 0.96, *P*=0.03) were less likely to be obese, while individuals with PTVs in *AQP3* (OR=0.13, 95% CI: 0.003, 0.76, *P*=0.01) and *TSPAN4* (OR=0.46, 95% CI: 0.22, 0.86, *P*=0.01) were also less likely to be severely obese. This analysis confirmed obesity risk and protective effects of the BMI-associated genes discovered in our study.

We also found significantly altered risks for 15 obesity-related comorbidities in carriers of PTVs in both obesity-risk conferring and protective genes compared to non-carriers (**Fig. 3b, Supplementary Table 8**). *For instance*, individuals with PTVs in *LPGAT1* showed higher risks for 5 out of 15 comorbidities, including aortic valve stenosis (OR=4.05, 95% CI: 1.46, 9.07, *P*=0.005), heart failure (OR=2.33, 95% CI: 1.17, 4.21, *P*=0.01), and knee osteoarthritis (OR=2.27, 95% CI: 1.40, 3.55, *P*=0.0006). Notably, *TMEM229A*, despite conferring obesity risk, showed depletion for heart failure phenotype in carriers (OR=0, 95% CI: 0, 0.93, *P*=0.03). Among the obesity protective genes, PTV carriers in *VIRMA* and *SCFD1* showed reduced risk for most of the tested cardiometabolic phenotypes, including significant depletion for venous thromboembolism (OR=0, 95% CI: 0, 0.65, *P*=0.008) and hypertension (OR=0.49, 95% CI: 0.28, 0.85, *P*=0.007), respectively. Further, PTV carriers in the BMI-decreasing *PANK1* and *P2RY1* exhibited an increased risk for type 2 diabetes (OR=1.74, 95% CI: 1.16, 2.54, *P*=0.005) and heart failure (OR=2.82, 95% CI: 0.87, 7.11, *P*=0.04), respectively. While obesity risk-conferring genes typically associated with worse cardiometabolic profiles and protective genes with better profiles, notable exceptions were observed where obesity protective genes conferred risk and risk genes were protective for certain cardiometabolic phenotypes.

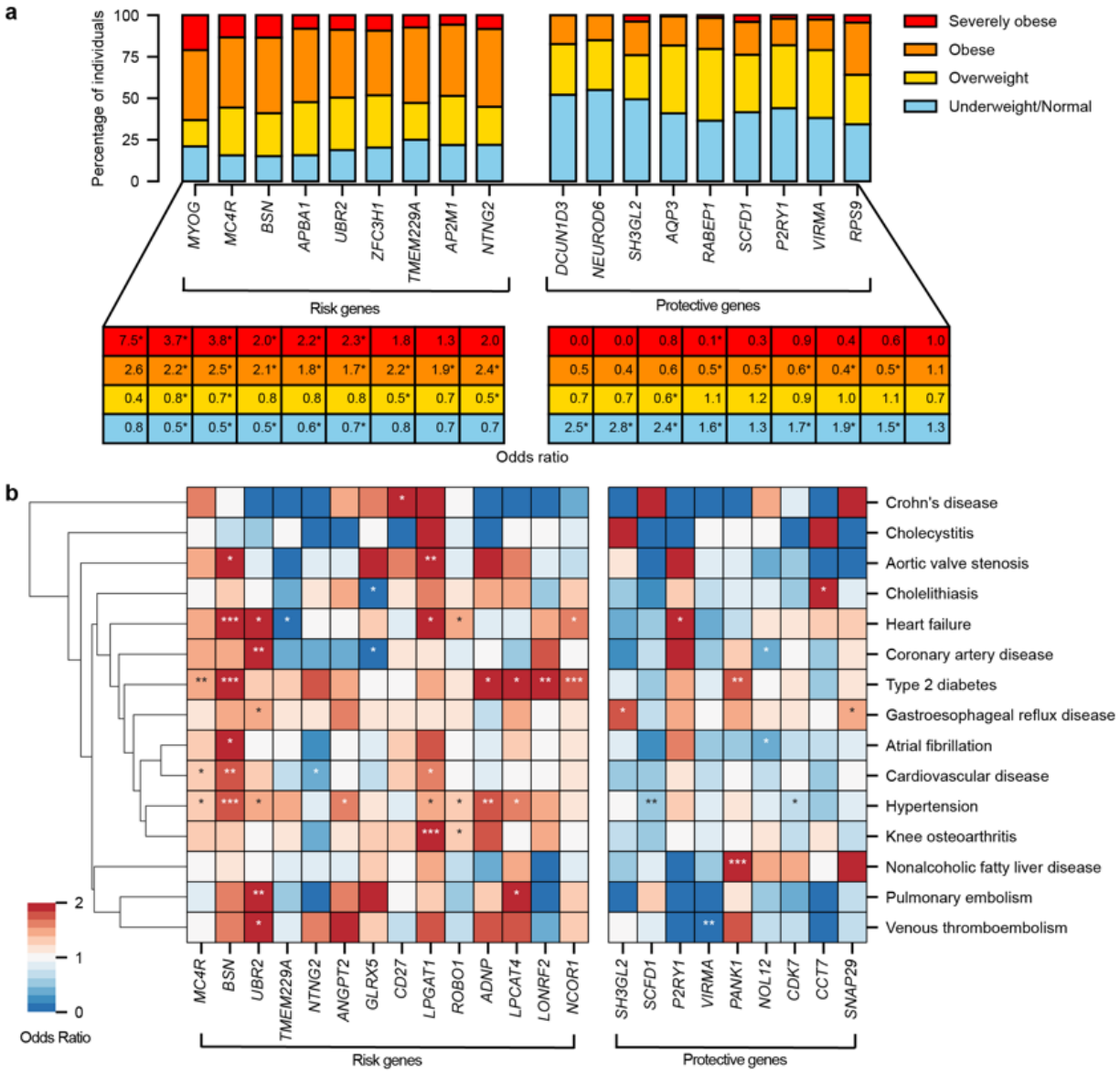
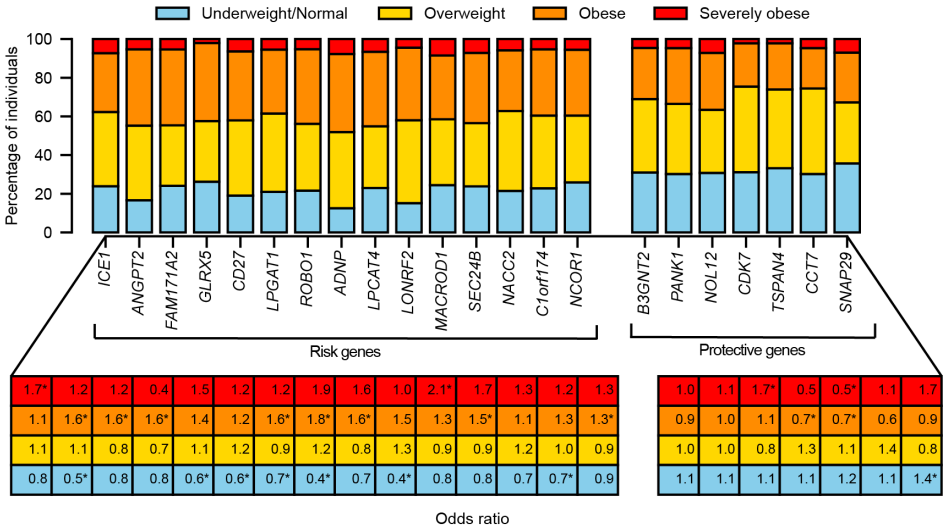


Fig. 3: **Enrichment of obesity and cardiometabolic comorbidities in PTV carriers of the discovered BMI associated genes**. (**a**) *Top*: Proportion of PTV carriers within the obesity clinical categories (underweight or normal, overweight, obese, and severely obese) that led to at least 1.5 kg/m^2 increase (risk) or decrease (protective) in BMI. *Bottom*: Odds ratio of obesity clinical categories in carriers compared to non-carriers. **P*<0.05. Extended data with the odds ratios, 95% confidence intervals and exact *P* values are available in **Supplementary Table 7.** (**b**) Odds ratio for obesity-related comorbidities in PTV carriers of risk or protective genes compared to non-carriers. **P*<0.05, ***P*<0.01, ****P*<0.001. Extended data with the odds ratios, 95% confidence intervals and exact *P* values are available in **Supplementary Table 8**.



Supplementary Fig. 2: **Enrichment in obesity clinical categories for BMI-associated genes**. Proportion of PTV carriers in obesity clinical categories that led to between 1 to 1.5 kg/m^2 increase (risk) or decrease (protective) in BMI. The odds ratio of being underweight or normal, overweight, obese, and severely obese among carriers compared to non-carriers is also shown in the table below (*P<0.05). All data with the odds ratio, 95% confidence intervals, and exact P values are available in **Supplementary Table 7**.

Functional analysis of obesity-associated genes

We evaluated the impact of natural variation in plasma protein levels of the discovered genes on BMI of individuals from the UK Biobank, irrespective of their PTV carrier status. Among the discovered BMI-associated genes, six had plasma protein measurements from approximately 50,000 individuals. We constructed linear models using protein expression as the independent variable and BMI, corrected for covariates, as the dependent variable. All six proteins had significant model coefficients, with DNER having the highest absolute effect on BMI (**Fig. 4a, Supplementary Table 9**), consistent with the association of *DNER* PTVs with severe obesity. Protein levels of FGF2 (β=0.21, P=3.87 x 10^{-14}) and GLOD4 (β=0.79, P=7.02 x 10^{-53}) associated with increased BMI, supporting the observation that disruption of these genes leads to a decrease in BMI (**Supplementary Fig. 3**). Interestingly, increased CD27 and ROBO1 levels associated with increased BMI, contrary to the expected effect from PTVs in these genes, which resulted in increased BMI. This suggests a complex relationship between protein expression of these genes and their effect on BMI. Our results show that natural variation in protein expression levels, potentially due to other common or rare non-coding variants, of the genes identified in our study are associated with BMI.

We next assessed whether the 116 novel genes identified in our study had been previously linked to obesity or related pathways using genetic and functional approaches beyond rare variant association studies. Nineteen genes, including *MACROD1*, *VIRMA*, and *RABEP1*, had common variant signals associated with BMI recorded in the GWAS catalog[19] (**Supplementary Table 10 and 11**). We also queried the Common Metabolic Diseases Knowledge Portal[20], which aggregates and scores genotype-phenotype relationships (Human Genetic Evidence, or HuGE scores[21]) based on evidence from both rare and common variant association studies. This analysis identified 16 more genes, including *LAMB2*, *FARP2*, *CELSR3*, and *ANGPT2*, that showed strong associations (HuGE score≥10) with BMI or related phenotypes such as waist-hip ratio adjusted BMI or fasting insulin-adjusted BMI (see **Supplementary Table 10 and 11**).

We further investigated the functional effects of knocking out obesity-associated genes using data from the International Mouse Phenotyping Consortium[22] and previous studies in mouse models. We found that 15 of our novel genes (five associated with risk and ten protective against obesity) exhibited directionally consistent obesity-related phenotypes in mice, such as changes in total body fat, lean mass, and glucose tolerance (**Table 2**). Among the obesity risk-conferring genes, we found impaired glucose tolerance, and increased weight gain in mouse models of *Lonrf2*[22], *Ncor1*[23], and *Gabra5*[24], respectively. Mouse studies further highlighted the involvement of these genes in obesity-related processes such as thermogenesis (*Fgf2*[25] and *Gabra5*[24]), adipogenesis (*Ncor1*[23]), glucose metabolism (*Abca1*[26]), fatty acid metabolism (*Pml*[27]), and skeletal muscle metabolism (*Mipep*[28]). We also found direct evidence of protective effects from homozygous knockouts of *Aqp3*, *Kctd7*, and *Fgf2* in mice, resulting in increased lean mass, reduced total body fat, and resistance to obesity with a high-fat diet, respectively (**Table 2**). In addition, *Dcun1d3* homozygous knockout mice showed a significant reduction in total body fat, increased lean mass, and improved glucose tolerance. Finally, we performed a systematic literature review and found evidence for 71 genes with functional roles in obesity, including *MMP3*, *SLC25A1*, *CYP3A5*, *APMAP*, and *UCP3* (**Fig. 4b, Supplementary Table 12**). Overall, we found evidence linking 82 out of the 116 discovered genes to obesity or related phenotypes, based on previous association studies, plasma proteomics, and mouse models (**Fig. 4b, Supplementary Table 11**).

Table 2: List of mouse models of BMI-associated genes showing obesity-related phenotypes.

Risk gene	**Evidence from mouse models**
ABCA1	Increased weight gain in adipocyte specific *Abca1*$^{-ad/-ad}$ KO mouse when fed a high fat diet[26]
GABRA5	Increased weight gain in chemogenetically inhibited (by clozapine N-oxide) mouse when fed a high fat diet[24]
NCOR1	Increased weight gain in adipocyte specific *NCoR*$^{fl/fl}$*aP2-Cre*$^{+/-}$ KO mouse when fed a high fat diet[23]
LONRF2	Impaired glucose tolerance observed in *Lonrf2*$^{em2(IMPC)H}$ homozygous KO mouse model
ANGPT2	Protection from high fat diet induced obesity in adipose tissue specific overexpressed (by doxycycline) mouse[29]
Protective gene	**Evidence from mouse models**
DCUN1D3	Decreased total body fat mass, increased lean body mass and improved glucose tolerance observed in *Dcun1d3*$^{em1(IMPC)Wtsi}$ homozygous KO mouse
AQP3	Increased lean body mass observed in *Aqp3*$^{tm2b(EUCOMM)Wtsi}$ homozygous KO mouse
SNAP29	Increased lean body mass observed in *Snap29*$^{tm1a(EUCOMM)Wtsi}$ heterozygous KO mouse
KCTD7	Decreased total body fat amount observed in *Kctd7*$^{em2(IMPC)Bay}$ homozygous KO mouse model
FGF2	Protection from high fat diet induced obesity in *Fgf2*$^{-/-}$ homozygous KO mouse[25]
MTFP1	Protection from high fat diet induced obesity in liver specific *Alb-Cre*$^{tg/+}$*Mtfp1*$^{LoxP/LoxP}$ KO mouse[30]
SETX	Improved glucose tolerance observed in *Setx*$^{tm1b(EUCOMM)Wtsi}$ homozygous KO mouse
MIPEP	Protection from high fat diet induced obesity in adipocyte specific *miPEP*$^{flox/flox}$*Adiponectin-Cre*$^{+/-}$ KO mouse[28]
AHNAK	Improved glucose tolerance observed in *Ahnak*$^{tm1b(KOMP)Mbp}$ heterozygous KO mouse
PML	Protection from western diet induced obesity in *Pml*$^{-/-}$ mouse[27]

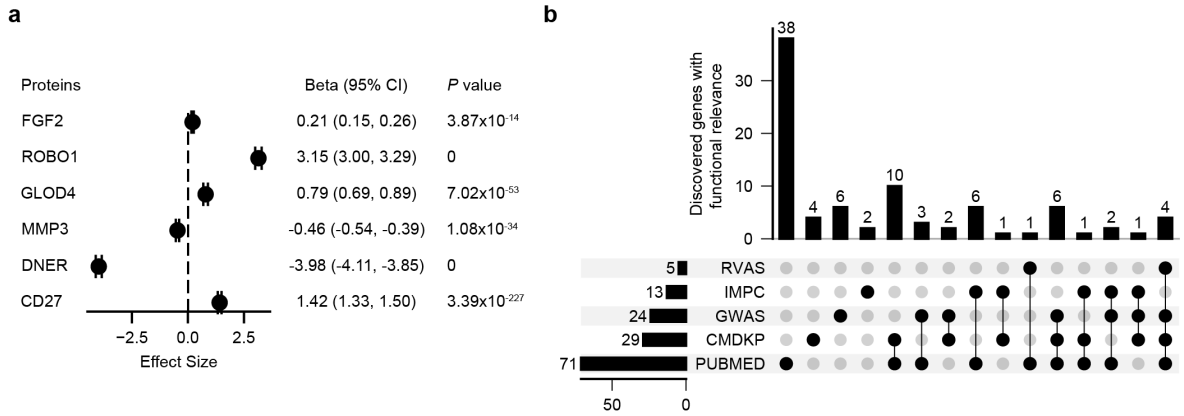
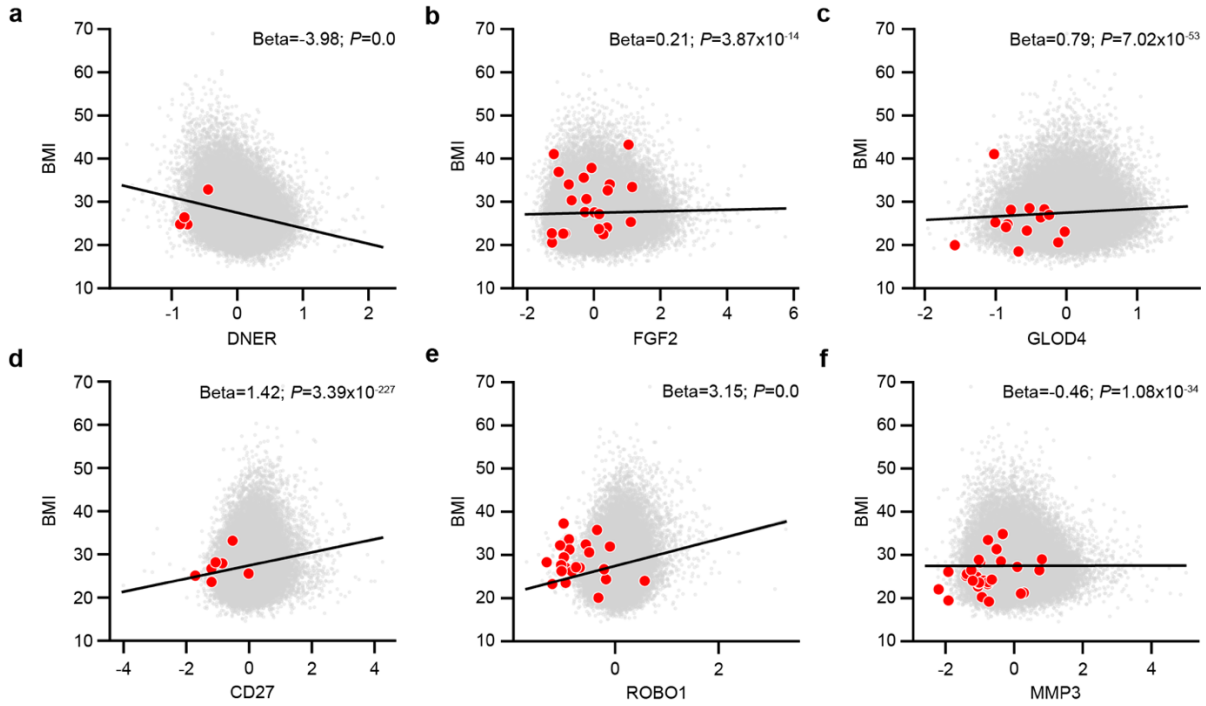


Fig. 4: **Functional validation of BMI associated genes**. (**a**) Model coefficients, with 95 % CI and *P* value, of a linear model constructed using plasma protein expression values of BMI-associated genes as independent variable and BMI as the dependent variable. Data with exact *P* values, model coefficients and 95% confidence interval are available in **Supplementary Table 9. (b**) Upset plot of evidence for a role of the identified BMI-associated genes in obesity-related functions and phenotypes. The bars represent the number of genes with functional relevance found from the respective study, which is represented by the dots. RVAS, Rare variant association study; IMPC, International mouse phenotypic consortium; GWAS: Genome wide association study; CMDKP: Common metabolic diseases knowledge portal.



Supplementary Fig. 3: Relationship between plasma protein levels and BMI. Association of the plasma protein levels with BMI in the population (grey scatters) and the PTV carriers (red scatters) for (**a**) DNER, (**b**) FGF2, (**c**) GLOD4, (**d**) CD27, (**e**) ROBO1, (**f**) MMP3.

Interactions of obesity-associated genes with polygenic risk and lifestyle factors

We assessed how PGS modulated the effect of the identified genes on BMI (**Supplementary Fig. 4a**). We used linear regression to test multiplicative interactions between PGS and each disrupted gene towards changes in BMI. We identified four scenarios where PGS contributed to the variability in BMI among individuals with PTVs in the same gene (**Fig. 5a and b, Supplementary Fig. 4b**). In the *first* scenario, BMI was synergistically increased by both PGS and PTVs in risk genes, leading to more severe obesity. *For example*, individuals with PTVs in *TSFM*, *LPGAT1*, *FAM171A2*, *ICE1*, *TNK2,* and *TPRX1* and eight other genes showed a synergistic increase in BMI in concert with PGS. This pattern was more evident in carriers of *BSN* PTVs where a non-additive increase in BMI was observed across PGS quintiles (**Fig. 5c**). *Second*, the combined effect of PGS and PTVs in five risk genes, including *UBR2* and *PAM*, was much lower than their expected additive effects on BMI (**Fig. 5c**). *Third*, PGS was found to override the effect of six protective genes such as *SH3GL2* and *APMAP*, and individuals with high PGS still showed increased BMI despite carrying PTVs in BMI-decreasing genes (**Fig. 5c**). In the *fourth* scenario, we noted that 23 protective genes override the strong obesity risk-conferring effect of PGS, and individuals remain protected despite carrying high polygenic risk for obesity. *For example*, the effect of PGS in the highest quintile among carriers of PTVs in *VIRMA* was 2.53 kg/m^2 less than in non-carriers (**Fig. 5c**). We did not identify non-additive effects of PGS on other disrupted genes such as *MC4R*, *APBA1* and *ROBO1*, consistent with previous reports[7,9] (**Supplementary Table 13**).

We next investigated the effect of obesity-inducing lifestyle factors on the BMI of PTV carriers after controlling for PGS. We found that the effects of PTVs in *MACROD1* and *VIRMA* was modulated by the degree of physical activity of individuals. In particular, individuals carrying *MACROD1* PTVs with low physical activity (UKB) or bad physical health (AoU) had a higher BMI compared to non-carriers, in both UKB and AoU cohorts (**Fig. 5d, Supplementary Table 14**). On the other hand, the protective effect of *VIRMA* was more pronounced in individuals with low physical activity in the UKB cohort but was diminished in individuals self-reported to be in bad physical health in the AoU cohort (**Supplementary Fig. 4c, Supplementary Table 14**). This observation could be attributed to differences in how lifestyle factors are measured across UKB and AoU, or other cohort-specific factors. We also identified associations for carriers of *MC4R*, *TNK2,* and *GADL1* PTVs with diet, sleep, and sedentary

lifestyle factors, respectively, in UKB (**Supplementary Table 15**), but these associations could not be tested in AoU due to the lack of comparable data. Our results underscore the influence of PGS and obesogenic factors towards the effect conferred by obesity risk and protective genes.

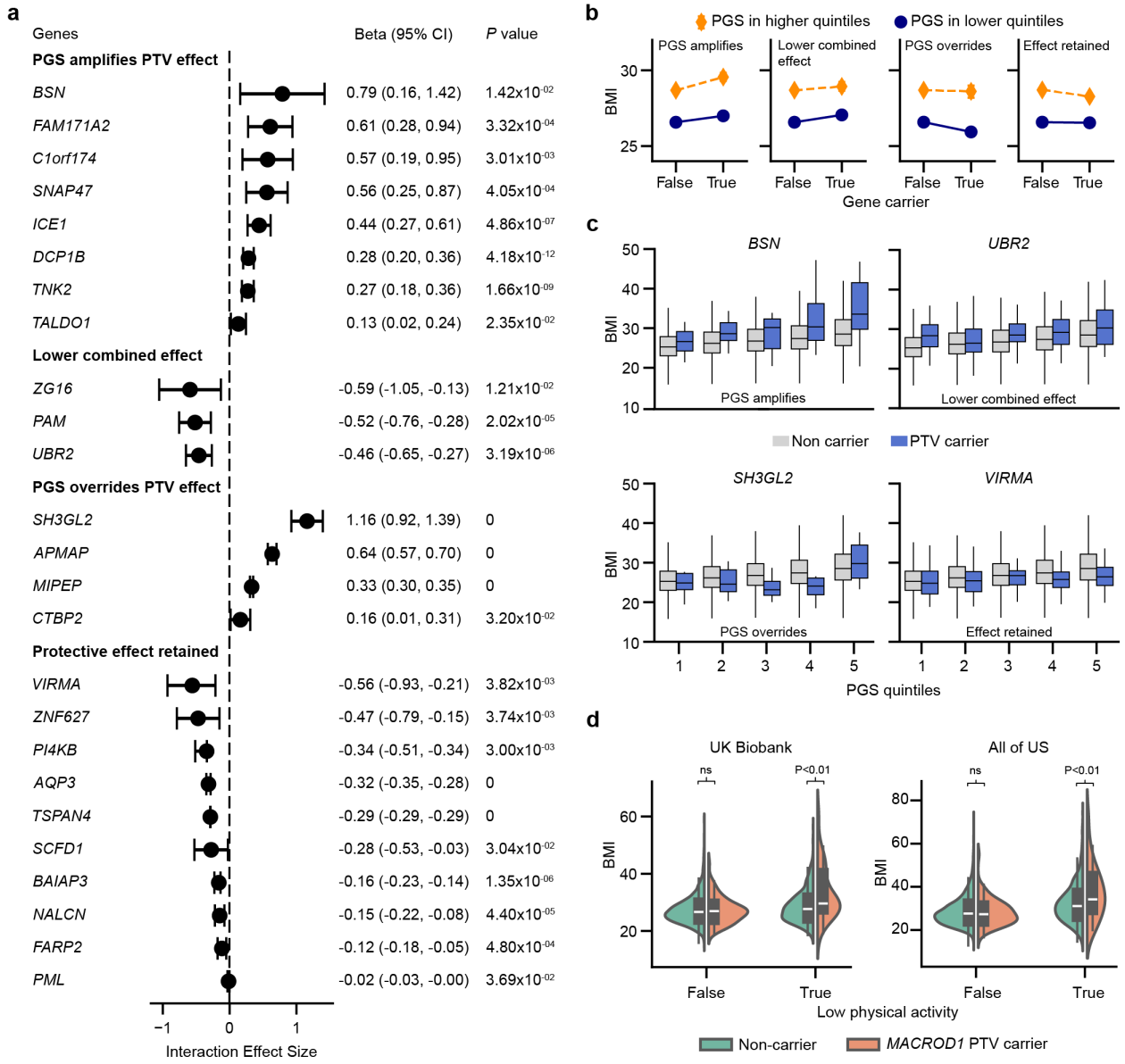
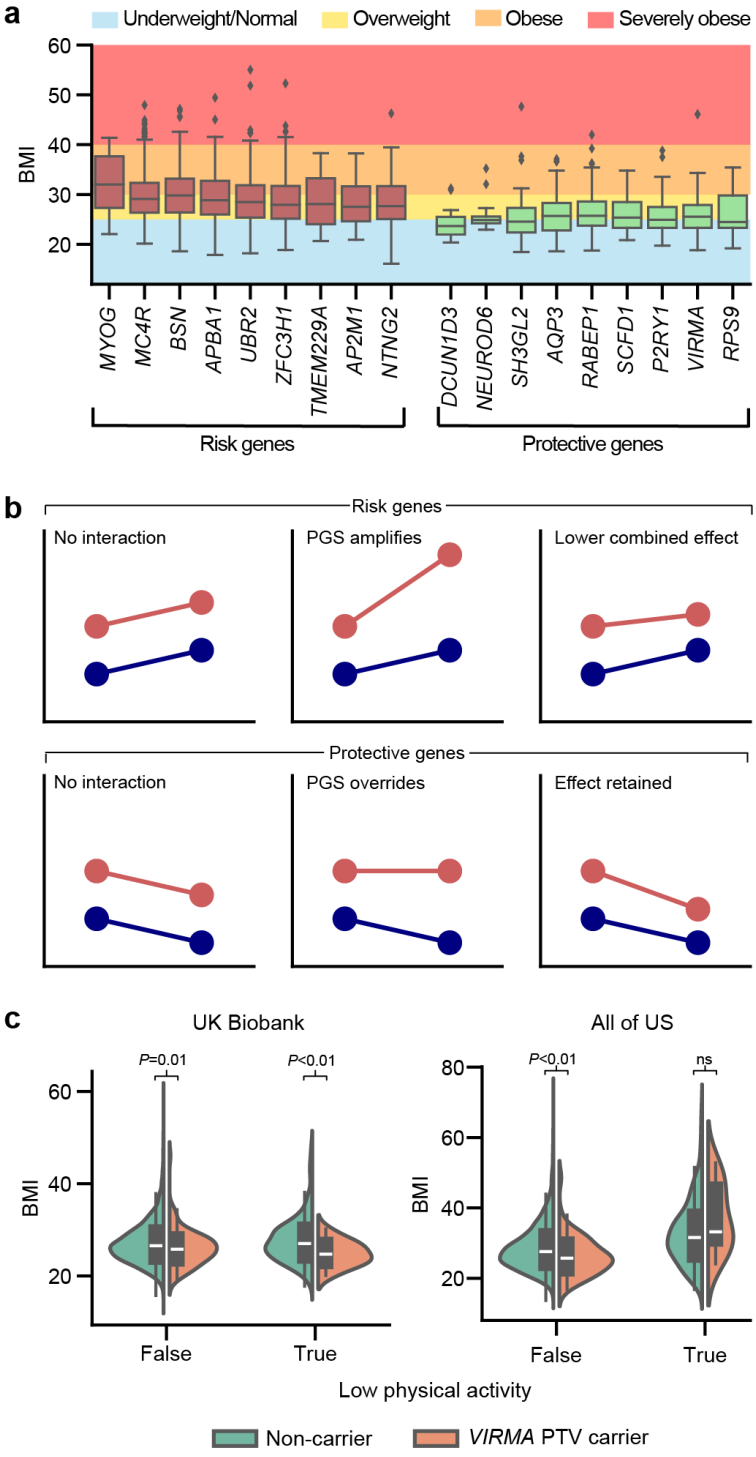


Fig. 5: **Effect of obesity-associated genes modulated by polygenic and obesogenic risk factors**. (**a**) Interaction model coefficient plot of combined risk of PTVs in the discovered genes and PGS on BMI, categorized by the four different scenarios. Data with exact *P* values and other associated statistics are available in **Supplementary Table 13. (b**) Interaction plots showing the mean BMI of PTV carriers and non-carriers in the higher (fourth and fifth) and lower (first, second, and third) PGS quintiles are shown. The PTV carriers of genes were aggregated based on their interaction category with PGS. (**c**) BMI distribution across PGS quintiles of PTV carriers (blue) compared to non-carriers (grey) of the representative examples for each of the four

interaction scenarios. (**d**) BMI distribution of *MACROD1* PTV carriers compared to non-carriers, stratified by their physical activity levels in UKB (left) and AoU (right) cohorts.



Supplementary Fig. 4: **Effect of obesity-associated genes modulated by polygenic and obesogenic risk factors**. (**a**) BMI distribution of PTV carriers in high-effect obesity genes. (**b**) Interaction plots depicting the different scenarios through which PGS modulates the BMI of

individuals with PTVs in the discovered genes. (**c**) BMI distribution of carriers of PTVs in *VIRMA* compared to non-carriers stratified by their physical activity levels in UKB (left) and AoU (right) cohorts.

DISCUSSION

Here, we discovered 116 genes with consistent effects on BMI across ancestries and provide accurate estimates of effect sizes for known obesity genes. By using genetic data from both European and non-European populations, we uncovered gene-discovery biases present in previous studies particularly in genes such as *DIDO1*, *SPARC*, and *RAB21* that showed significant associations only in Europeans. Typically, protective obesity genes have been more challenging to identify than risk genes, but three notable ones, *GPR75*, *GPR151*, and *GIPR*, have emerged more recently through rare variant association studies[6,9,31]. However, our findings suggest that these genes may also be influenced by ancestral biases, with variable effect sizes across populations, which could impact their potential as therapeutic targets for obesity. In contrast to previous studies, we identified genes with consistent effects across ancestries and functional evidence, such as mouse knockout models for these genes, confirm their role in obesity-related pathways. These findings emphasize the value of a cross-ancestry analysis, which enabled the discovery of several novel genes with effect sizes comparable to canonical obesity genes such as *MC4R* and *BSN*.

A significant finding from our study was the identification of 66 protective genes, some of which exhibit effect sizes more than twice that of known protective genes. Notably, *DCUN1D3* showed the highest effect size, with PTVs in these genes leading to approximately 3.8 kg/m^2 decrease in BMI. This gene is highly expressed in adipocytes, and *Dcun1d3* knockout mice showed reduced fat body mass, increased lean body mass and improved glucose tolerance[22]. *DCUN1D3* encodes a protein involved in neddylation, a post translation modification that attaches a small ubiquitin-like molecule, NEDD8, to substrate proteins, altering their properties[32,33]. Disruption of neddylation has been linked to impaired energy metabolism and age-related metabolic disorders[34,35]. While the mechanisms by which *DCUN1D3* might influence these pathways or diseases are unclear, our findings suggest that its inhibition could be a therapeutic target for obesity. Similarly, *VIRMA* consistently led to decreased BMI, is supported by evidence from nearby common variant signals[19], and individuals with PTVs in this gene displayed improved cardiometabolic health. Two other protective genes, *AQP3* and *FGF2*,

not only showed evidence from mouse models for obesity protection but also have well-established functional roles in glycerol metabolism[36] and thermogenesis[25]. Currently, pharmacological inhibitors or negative modulators, such as Bisacodyl and Pentosan polysulfate, exist for *AQP3* and *FGF2*; however, they are used as a laxative and for bladder pain, respectively[37]. We also found that Setmelanotide, the *MC4R* agonist used for weight loss, is also an agonist of *ANGPT2*, an obesity risk gene discovered in our study, with evidence from mouse models demonstrating its role in body weight regulation[29,38]. Thus, our discoveries provide avenues for repurposing existing drugs or devising new ones to alleviate obesity risk.

While polygenic risks have been found to additively influence rare variant risk for obesity[9], a recent study showed *BSN* and PGS interact non-additively. In addition to recapitulating the effect between *BSN* and PGS, we found synergistic effects of PGS on other genes, such as *TSFM*, *LPGAT1*, and *FAM171A2*, as well. Further, synergistic interactions between obesity causing risk factors can also exacerbate the risk of severe obesity and its associated morbidities. *For instance*, carriers of PTVs in *MACROD1*, an obesity risk gene, showed significantly higher BMI when they were physically inactive compared to carriers who were active. While previous studies have shown the adverse effect of lifestyle factors on common variants associated with BMI[2], we provide evidence that rare variant risk can be modulated by obesogenic factors. Notably, carriers of PTVs in protective genes such as *VIRMA* and *PI4KB*, remained protected despite having high PGS, highlighting the complex patterns of interactions between specific obesity genes and PGS. Understanding these genetic and gene-environment interactions could be key to developing more effective interventions, where lifestyle changes may mitigate genetic risk in high-risk individuals, and protective variants might offer resilience against obesogenic factors. Overall, using a multi-ancestry approach, we provide a more comprehensive view of obesity genetics, identifying novel genes, refining the effects of known genes, and demonstrating the importance of including diverse populations in genetic studies.

METHODS

Variant quality control, filtering, and annotation

We analyzed whole exome sequencing data of 469,835 individuals from the UK Biobank (UKB) cohort, available as multi-sample project variant call format (pVCF) files in the UKB Research Analysis Platform (RAP). The sequencing method and preparation of the pVCF files have been previously described[39]. Using the Hail[40] platform on DNANexus, we first split multi-allelic records, and then filtered to retain rare variants with intracohort frequency <0.001. These variants were annotated using variant effect predictor[41] (VEP v109) and dbNSFP v4[42] to identify the predicted variant effects on gene transcripts. All variants within protein coding genes in the autosomes with a call rate of 50% were used for subsequent analysis. We then annotated each variant based on their most deleterious functional impact on the transcript into three categories, from the most harmful to the least harmful, as follows: (1) Loss of function or "lof", which included frameshift, stop gained, splice acceptor, and splice donor VEP annotations, (2) "missense strict", which included missense variants predicted to be deleterious by nine deleteriousness prediction tools (SIFT[43], LRT[44], FATHMM[45], PROVEAN[46], MetaSVM[47], MetaLR[47], PrimateAI[48], DEOGEN2[49], and MutationAssessor[50]) available through dbNSFP database, and (3) "missense lenient", which included missense variants predicted to be deleterious by at least seven out of the nine deleteriousness prediction tools. All UKB analyses were performed in the DNANexus UKB RAP.

We also analyzed whole genome sequencing data of 245,388 individuals in the All of Us (AoU) cohort. Variant call files for regions overlapping with the exomes were available as a Hail matrix table in the AoU portal. After filtering the variants for intracohort frequency (<0.001), we annotated the variants using Nirvana[51], available in the AoU research platform as a Hail table, and determined the functional impact of each variant using the same criteria as defined for the UKB data. All AoU analyses were performed in the Researcher Workbench of the AoU portal.

Phenotype, obesogenic lifestyle, polygenic risk analyses

For the UKB cohort, BMI, genetic sex (Data Field 22001), age (Data Field 21003), ethnic background (Data Field 21000), PGS (Data Field 26216), genetic kinship (Data Field 22021), and the top 40 genetic principal components (PCs; Data Field 22009) on 502,368 individuals in UKB were accessed and preprocessed through the UKB RAP. Numerical fields such as BMI and

age, measured across multiple visits, were averaged. Categorical fields such as sex and ethnic background were searched for inconsistent readings, and samples with conflicting values across multiple assessments were dropped. We further filtered samples with 10 or more third-degree relatives from our analysis based on previously published kinship estimates[14]. We finally divided the cohort into two populations, based on their ethnic background namely, "British" consisting of 419,228 individuals self-reported to be "White British" based on the UKB data-field 21000, and the other 68,070 individuals who we categorized as "non-British" population. We note that the UKB non-British population contains individuals of European ancestry, *for example*, individuals with self-reported "Irish" ethnic background. ***Obesogenic lifestyle factors*** such as Metabolic Equivalent Task (MET) scores, sleep duration, alcohol consumption, or smoking habits were also accessed and preprocessed through the UKB RAP. Numerical fields were averaged and samples with categorical fields having conflicting values across multiple assessments were dropped. All fields were then binarized based on their extreme values and their potential effect on BMI as follows: Numerical fields with the potential to have a directly proportional effect on BMI, such as "time spent watching television", were assigned as 1 for an individual, if their corresponding value was greater than 95% quantile. On the other hand, fields with inversely proportional effect on BMI, such as "consumption of cooked vegetable", were assigned as 1 if the value was less than 5% quantile. Individuals with extreme values from ordinal fields that potentially lead to increased BMI were assigned as 1, and individuals with all other values were assigned as 0. *For example*, individuals who consumed alcohol daily or almost daily, the extreme category for this field, were encoded as 1 for that lifestyle factor. A full description of the lifestyle factors used in this study and the thresholds or categories used to binarize them is available in **Supplementary Table 16**. After binarizing, the fields were combined to define one of the six lifestyle factors, namely, (i) physical activity, (ii) alcohol, (iii) smoke, (iv) diet, (v) sleep, and (vi) sedentary lifestyle, used in this study. Binarized MET scores, alcohol consumption frequency, and sleep patterns were directly used as indicators of physical activity, alcohol, and sleep metrics, respectively. Individuals whose current or past smoking tendencies were high were considered to have the "smoke" lifestyle. Similarly, those spending more time on TV or computer were indicative of "sedentary lifestyle". Diet was denoted as 1 if either the sum of high processed meat-, beef-, mutton-, and pork-intake binarized columns was greater than 0, or the sum of low cooked vegetable, salad, fresh fruit, dried fruit, oily fish, or non-oily fish-

intake binarized columns was greater than 1. Thus, the diet field used in the study is analogous to high-meat and/or low vegetable consumption. International Classification of Disease (ICD-10) 10th revision summary diagnosis codes corresponding to each individual were also extracted from the Hospital Episode Statistics (HES) data available in the UKB RAP.

For the AoU cohort, we obtained BMI, age, genetic sex, previously calculated genetic ancestry[15], 16 genetic PCs, and sample IDs of related individuals from the AoU Workbench. We calculated the age based on the date of birth and the BMI measurement data concepts ("concept" defined here as a collection of similar data stored together) available in AoU. We used the "sex at birth" concept, available in AoU, to denote the genetic sex of an individual. Only individuals with BMI values between 12 and 75 kg/m^2 and "sex at birth" field listed as either "Male" or "Female" were included to ensure consistency between UKB and AoU. Based on the predicted genetic ancestry, we divided the AoU data into three populations namely, (1) European (N=127,644), which included individuals predicted to be of European ancestry, (2) African (N=54,865), which included individuals predicted to be of African ancestry, and (3) Mixed (N=52,134), which included individuals predicted to be of Admixed American ancestry and other ancestries. ***We calculated PGS for BMI in individuals from AoU*** by using the GWAS summary statistics from a previous study[4]. Duplicate and ambiguous single nucleotide polymorphism (SNPs) were filtered from the summary statistics. Genotype data of the AoU cohort filtered for common variants (MAF>0.01) was obtained as a Hail matrix table. We further removed variants with Hardy-Weinberg Equilibrium p-value $<1x10^{-6}$ and retained variants that overlapped between summary statistics data and the AoU genotyped data with a call rate greater than 0.9. We then used the predetermined effect sizes of the SNPs as Bayesian priors, weighed them based on the alternate allele frequency observed in individuals, and summed the weighted effect sizes to obtain individual-specific polygenic risk for BMI. All calculations were conducted using Hail, available in the AoU Researcher Workbench. ***Obesogenic lifestyle factors*** such as physical activity, alcohol consumption, and smoking tendency in the AoU cohort were obtained from survey questionnaires available through the AoU research portal. Individuals falling in the extreme categories for each of the three factors, (1) Physical activity: "General Physical Health: Fair" or "General Physical Health: Poor", (2) Alcohol consumption, "Drink Frequency Past Year: 4 or More Per Week", and (3) Smoking tendency: "Smoke Frequency: Every Day", were annotated as 1, denoting those carrying the respective obesogenic factor, while others not falling

in these extreme categories were annotated as 0. We note that physical activity measurements in AoU were not equivalent to those in UKB, where a more rigorous assessment of an individual's physical health was measured using International Physical Activity Questionnaire guidelines. Additionally, diet and sedentary lifestyle information was not available in AoU, while sleep data were available on less than 10% of the cohort and these lifestyle factors could not be tested in AoU. ICD-10 diagnosis codes corresponding to each individual in the AoU cohort was extracted using the researcher workbench.

Gene burden association test using REGENIE

We used REGENIE v3.3[16] to conduct gene burden association tests. Similar to most whole genome regression tools, REGENIE operates in two steps. *First*, it uses SNP data, preferably a genotyped array of SNPs, from across the genome to fit a null model that estimates a polygenic score for the trait to be tested (i.e., BMI in our study). This step accounts for population structure and relatedness between samples. For both UKB and AoU cohorts, we used the genotyped SNP array files available in DNANexus or Researcher workbench for this step. We lifted over the SNP array files in UKB from version hg19 to hg38 using Picard's LiftoverVCF tool[52]. In the *second* step, REGENIE then calculates the association between the genetic variants of interest and the trait after considering the null model calculated in step 1 and other user defined covariates. In our study, we used age, genetic sex, and first ten genetic PCs obtained from both UKB and AoU cohorts as additional covariates. REGENIE is capable of collapsing variants to a gene-level with user defined masks and annotation files apart from the usual variant data as input before running association tests. We supplied our already annotated variant file based on their impact on gene transcripts and defined three variant masks to collapse variants on a gene level, namely, (a) "lof" variants only, (b) "lof" and "missense strict" variants, and (c) "lof", "missense strict" and "missense lenient" variants. We performed gene-based association tests for all the five defined populations across both biobanks and generated statistics for all individual gene-mask pairs.

Meta analysis and variance calculation

We first created three meta populations from the previously defined five populations (UKB British, UKB non-British, AoU European, AoU African, and AoU Mixed), the first composed of

individuals from European ancestry (UKB British and AoU European) termed "European meta", the second from non-European ancestry (UKB non-British, AoU African and AoU Mixed) termed "non-European meta", and the third from all five populations termed "combined meta". We note that a minority of individuals (approximately 15%) in the non-European population were from ethnic groups such as 'Irish' or 'any other white background', and are therefore likely to be of European ancestry.

Results from the individual populations were pooled into the three meta populations using an inverse variance weighted random effects model. The meta-analysis calculation was implemented using the python package statsmodels v0.14.2. Each gene-variant mask was associated with meta statistics for three meta populations. Any gene-variant mask that passed the Bonferroni multiple testing correction threshold of 8.34×10^{-7}, accounting for 20,000 genes and three variant collapsing models, *either* in the European meta *or* the non-European meta *and* in the combined meta population were considered to be significantly associated with BMI across ancestries.

We calculated the interpopulation variance in effect sizes across populations for the discovered genes passing our multiple testing criterion, as well as previously associated BMI genes, using the "var" method available in the python package, pandas v2.1.1.

Odds-ratio calculation for obesity clinical categories and obesity-related disorders

To calculate risk across the obesity clinical categories among carriers of PTVs in the discovered genes compared to non-carriers, we first categorized each individual into their respective clinical category based on their BMI values: underweight or normal (<25), overweight (25 to 30), obese (30 to 40) and severely obese (>40). We then created 2x2 contingency tables using their carrier status as the first variable and whether they belong to the respective obesity category as opposed to any other category as the second variable. Finally, we calculated the conditional odds ratio and the significance value using a two-sided Fisher's exact test.

To calculate risk for obesity related disorders, we obtained the specific ICD codes for comorbidities frequently associated with obesity based on previous BMI related studies and calculated the odds of PTV carriers diagnosed with the comorbidity compared to non-carriers using Fisher's exact test. A list of the comorbidities and the ICD codes used to categorize an

individual as carrying the comorbidity is provided in **Supplementary Table 17**. All odds ratio calculations were conducted using the scipy v1.11.3 package available in python.

Functional analysis of the discovered genes

We overlapped the list of discovered genes with a BMI gene list obtained from the GWAS catalogue[19], where the SNPs identified in previous BMI GWAS studies are mapped to their most likely gene targets. We further overlapped the discovered gene list with gene phenotype associations from Common Metabolic Diseases Knowledge Portal (CMDKP) database[20]. CMDKP aggregates information and scores genotype-phenotype relationships (Human Genetic Evidence or HuGE scores[21]) based on the evidence acquired from previous rare and common variant association studies for all common metabolic disorders, including obesity and related phenotypes. Any gene that showed strong associations (HuGE score≥10, indicating "strong" evidence of gene-phenotype link) with obesity, BMI, or related phenotypes, such as fasting insulin adjusted BMI, or waist hip ratio adjusted BMI, were considered an overlapping BMI related gene. Associations of the discovered genes with previous *in vivo* studies of knockout (KO) mouse models were first identified using data from the International Mouse Phenotyping Consortium (IMPC)[22], which provides comprehensive phenotypic characterization of mouse KOs, including assessments of body weight and obesity-related traits. IMPC represents an unbiased resource for investigating the *in vivo* effects of novel genes that have not been studied in the context of obesity. Further, we conducted a PubMed search of each gene by searching for the gene name in the abstract or title and "obesity" in the text. This provided additional functional evidence for the novel genes, which have been phenotypically characterized using mouse models, or other complementary approaches such as human genetic studies. The BMI-associated GWAS gene list was directly obtained from NHGRI-EBI catalog, while associations from CMDKP, IMPC, and PubMed were accessed programmatically through their REST API using python's requests v2.31.0 module.

Proteomics data analysis of oligogenic combinations

Normalized plasma protein expression data of 1,463 proteins on 50,956 individuals were accessed through the DNANexus portal of UKB RAP. Multiple replicates of protein readouts were averaged, and protein levels of corresponding genes were identified. Using linear

regression models, the effect of protein expression levels for the discovered genes with available protein data on BMI was measured, while accounting for covariates such as age, genetic sex, ten genetic PCs, PGS, and the interaction term between the protein and the PGS. The model was trained using "ols" function from statsmodels v0.14.2 package available in python.

Interaction models for obesity risk factors

To assess interactive effects between each gene and PGS, we used a linear regression model to predict BMI using individual as well as interactive terms between PGS and PTV carrier status for the discovered genes while accounting for age, genetic sex, and ten genetic PCs. We obtained separate model coefficients in UKB and AoU cohorts and then used random effect meta-analysis to combine the statistics from the two cohorts. Interaction terms with meta-analysis *P* value less than 0.05 were considered significant gene-PGS interactions. The models were trained using "ols" function from statsmodels v0.14.2 package, available in python.

To compare interactive effects between lifestyle factors and gene PTV carrier status, we conducted factorial analysis of covariance using the individual gene and lifestyle terms as well as the gene-lifestyle interaction term, while accounting for age, genetic sex, first ten genetic PCs and PGS, separately in UKB and AoU cohorts. Interaction terms that crossed the *P* value threshold (0.05) in both UKB and AoU were considered significant gene-lifestyle interactions. Model training and calculation of *P* value significance and confidence intervals were performed using "ols" and "anova_lm" function from statsmodels v0.14.2 package available in python.

AUTHOR CONTRIBUTIONS

S.G and D.B conceived the study, acquired, analyzed and interpreted the data, and wrote the manuscript.

ACKNOWLEDGEMENTS

We thank Corrine Smolen and Jiawan Sun for critical reading of the manuscript. We thank the participants and investigators in the UK Biobank and All of US research studies who made this work possible. This research has been conducted using the UK Biobank Resource under Application Number 45023. We also thank the National Institutes of Health's All of Us Research Program for making available the participant data examined in this study. This work was supported by NIH R01-GM121907, resources from the Huck Institutes of the Life Sciences and the Pennsylvania State University to S.G. The authors declare no competing interests.

Data and code availability

The UK Biobank and All of Us genetic and phenotypic data analyzed in this study are publicly available to registered researchers through their respective analysis portals. Additional information about registration for access to the data is available at https://www.ukbiobank.ac.uk/ and https://www.researchallofus.org/ for UK Biobank and All of Us, respectively. The code for pre-processing, filtering, annotating genetic data, association tests and statistical analysis is available on GitHub (https://github.com/deeprob/BMI_monogenic).

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