

Phenome-wide Burden of Copy-Number Variation in the UK Biobank

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Copy-number variations (CNVs) represent a significant proportion of the genetic differences between individuals and many CNVs associate causally with syndromic disease and clinical outcomes. Here, we characterize the landscape of copy-number variation and their phenome-wide effects in a sample of 472,228 array-genotyped individuals from the UK Biobank. In addition to population-level selection effects against genic loci conferring high mortality, we describe genetic burden from potentially pathogenic and previously uncharacterized CNV loci across more than 3,000 quantitative and dichotomous traits, with separate analyses for common and rare classes of variation. Specifically, we highlight the effects of CNVs at two well-known syndromic loci *16p11.2* and *22q11.2*, previously uncharacterized variation at *9p23*, and several genic associations in the context of acute coronary artery disease and high body mass index. Our data constitute a deeply contextualized portrait of population-wide burden of copy-number variation, as well as a series of dosage-mediated genic associations across the medical phenome.

Introduction

Copy-number variants (CNVs) are a class of structural variation typically defined as large deletions or duplications of at least 50 base-pairs of genomic sequence.^{1,2} CNVs exhibit substantial variability in both size and frequency in the population and have been implicated across a variety of common and rare health outcomes.³ Regional deletion and duplication syndromes have also been described at many loci, clustering near areas of segmental duplication which may potentiate non-allelic homologous recombination.^{4–6} For example, CNV-based architectures for neuropsychiatric (e.g., autism spectrum disorder), developmental (e.g., *16p11.2* [MIM: 611913]),^{7,8} and syndromic cardiac disease (e.g., *22q11.2* [MIM: 188400]) (see GeneReviews in Web Resources) phenotypes have been well established.

Despite a growing body of research on CNV-related syndromes and disease etiologies, large-scale studies of CNV effects have been limited by their rarity in the general population. However, burden testing methods that address this rarity by pooling observed variation across gene regions have yielded reproducible associations in the context of congenital heart disease and various neurocognitive outcomes.^{10,11} Moreover, as studies which include either microarray or NGS-based genotype data have grown in size and scope, it has become possible to describe the distribution of CNVs at kilobase-level resolution in the general population.^{12,13} Furthermore, the aggregation of richly annotated phenotype data in biobanks has diversified the set of phenotypes available for well-powered association studies, and allows for more precise characterization of well-established pathogenic CNVs.^{14–17}

We here describe the genome-wide landscape of copy-number variation and their associations with 3,157 pheno-

types in a cohort of 332,584 participants from the UK Biobank.¹⁸ We replicate well-established syndromic effects of common CNVs—namely *22q11.2* deletion (DiGeorge) syndrome and two variants of *16p11.2* deletion syndrome—and highlight associations for body mass index (BMI), acute coronary artery disease (CAD), and related adipose and cardiovascular phenotypes. Summary statistics from traditional genome-wide associations for common CNVs as well as from gene-level aggregate burden tests of rare variants across all phenotypes are available for download on the Global Biobank Engine.¹⁹

Material and Methods

CNVs were called using PennCNV v.1.0.4 on raw signal intensity data from each genotyping array. Phenotype data were derived from data-fields collected for UK Biobank corresponding to various body measurements, biomarkers, disease diagnoses, and medical procedures from medical records, as well as a questionnaire about lifestyle and medical history. Summary-level data from all statistical tests described here, as well as more thorough documentation on phenotyping, are available on the Global Biobank Engine¹⁹ and can be found in related publications.²⁰

CNV Calling in UK Biobank

Methods for genetic data acquisition and quality control as performed by the UK Biobank have been previously described.¹⁸ In brief, two similar arrays were used for targeted genotyping within the study population: the UK BiLEVE Axiom Array (n = 49,950) by Affymetrix and the UK Biobank Axiom Array (n = 438,427), which was custom designed by Applied Biosystems. Samples and array markers were subject to threshold-based filtration and quality control prior to public release. Specifically, markers were tested for discordance across control replicates, departures from Hardy-Weinberg equilibrium, as well as effects due to batch, plate, array, and sex; affected markers were set as missing in affected batches or

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removed. Similarly, samples were tested for missingness (>5%) and heterozygosity across a set of high-quality markers, but samples identified as low quality ($n = 968$) were not excluded. We also chose to include these samples in this analysis, considering that large structural variants may have been responsible for their poor quality with respect to metrics used for filtration.

We used PennCNV v.1.0.4²¹ to call CNVs within each of the 106 genotyping batches from UK Biobank. We first estimate genomic runs of heterozygosity (RoH) for each sample using a previously developed pipeline in PLINK^{22,23} using the `-homozyg` option. We then select $n = 100$ samples with total RoH covering less than 20 Mb to train a hidden markov model (HMM) of copy state on each chromosome. HMM training was initialized with conditions optimized for Affymetrix arrays (`affywg6.hmm`), provided in PennCNV resources. We used the general calling mode, which performs likelihood-based testing for copy-number state ($CN = 0,1,2,3,4$) at each input marker using its log-normalized signal intensity and allele balance in a given sample. We also apply adjustment for GC content across sites using waviness factor correction.²⁴ After CNV calling, we exclude 1,360 samples with more than 30 called CNVs from downstream analysis, resulting in a cohort of 472,734 individuals with 275,180 unique variants.

Gene-Level Constraint Estimation

Regional selective constraint to CNV was estimated for all autosomal protein-coding genes, with genic CNV defined as any variant overlapping within 10 kb of the HGNC gene region. We estimate a null model of structural mutation empirically as in a previous study,¹² and model burden of genic CNV as a linear function of gene size, fraction of genic sequence covered by regions of segmental duplication as extracted from the UCSC Genome Browser.^{25,26} We also account for biased observations due to array genotyping (as compared to exome sequence) by including the number of genic markers as a covariate. The formula for this null model can be written as:

$$n_{cnv} = \beta_1 \cdot len(gene) + \beta_2 \cdot frac(segdup) + \beta_3 \cdot n_{markers} + \epsilon$$

From this model, we compute constraint z-scores for each gene using its negated standardized residual for each gene, winsorizing the negative tail at the lowest 5% of values. We also compute the probability of intolerance to CNV (akin to probability of loss of function intolerance/pLI) as the non-normalized residual over the number of expected CNV, with negative values rounded to zero.

Genetic Associations

Variant-level associations in UK Biobank were estimated with PLINK v2.00a (2 April 2019). We used the `-glm firch-fallback` option for computation. This option is a hybrid algorithm for logistic regression which defaults to a standard regression solver for computation, falling back to Firth's regression in cases where one of the cells of the 2×2 contingency table is zero, or where the traditional method fails to converge in a pre-specified number of iterations. These analyses were performed in a subset of 332,584 unrelated individuals of self-reported white British ancestry with CNV genotype calls and were controlled for age, sex, and four marker-based genomic principal components from the UK Biobank PCA calculation. To ensure adequate power for estimating genetic effects, we perform these tests on 7,038 CNVs observed at a frequency of $\sim 0.005\%$ (1 in $\sim 20,000$, or 15 individuals) in the whole sample of individuals with called CNVs.

Gene-level burden tests were conducted across all gene:phenotype pairs using the same methods and cohort as the variant-level GWAS. Genic burden was encoded as a binary variable which indicates whether an individual has a CNV which contains any overlap within 10,000 base pairs of the HGNC gene region. CNVs which overlapped several gene regions were used for analysis in each gene. We treat deletions and duplications identically, with the assumption that any CNV which overlaps a gene in this fashion will disrupt its normal function. We included the following as covariates in both models: age, sex, four marker-based genomic principal components from UK Biobank's PCA calculation, and the number and combined length of CNVs in each individual.

Targeted variant-level GWAS was performed for both BMI and CAD in the same population, methods, and covariates as in the CNV GWAS. We display summary statistics for variants imputed from the Haplotype Reference Consortium^{18,27} (HRC) which overlap regions of interest as identified in each of these analyses. Lead variants for the BMI GWAS were identified by LD-clumping these variants with PLINK's `-clump` option using a p value threshold of 10^{-10} and r-squared cutoff of 0.2 between lead variants. The lead variant at *9p23* was selected by inspection. Correlation between lead variants and all nearby variation was computed with PLINK's `-r2` option.

Two-sample mendelian randomization was performed via the MR Base web app using GWAS summary statistics for *LDLRAD3* expression QTLs from a CARDIoGRAMplusC4D meta-analysis.²⁸ We report Wald summary statistics from inverse-variance weighted Egger regression; these are the default analysis options for the web interface.

Results

Landscape of Common and Rare CNVs in a Large Volunteer Cohort

To call copy-number variants in UK Biobank, we apply PennCNV²¹ separately within each genotyping batch, resulting in 275,180 unique CNVs among 472,724 individuals after sample quality control. We also observe heavy-tailed distributions in size and allele count of CNVs, with average CNV length ~ 226 kb and the majority of called variants singleton in the cohort (Figures 1A and 1B). This translates to notable burden of variation for nearly all individuals, with 439,464 (93.1%) of the individuals possessing at least one CNV detectable at kilobase resolution (Figures 1C and 1D). Among individuals with at least one CNV, we estimate an average burden of 5.5 variants covering >200 kb of genomic sequence (median 3 variants affecting ~ 100 kb; Figures 1C and 1D). While in line with previous reports,¹² these estimates of individual-level burden are likely conservative, as regions where array markers are sparse or missing limit the accuracy of variant calling. Furthermore, we are unable to call smaller (<1 kb) variants due to inconsistent marker density across all chromosomal regions on the Axiom and BiLEVE UK Biobank genotyping arrays. This limitation is visible in the histogram of called CNV lengths (Figure 1A); we call substantially fewer variants on the order of hundreds of base-pairs than on the order of thousands.

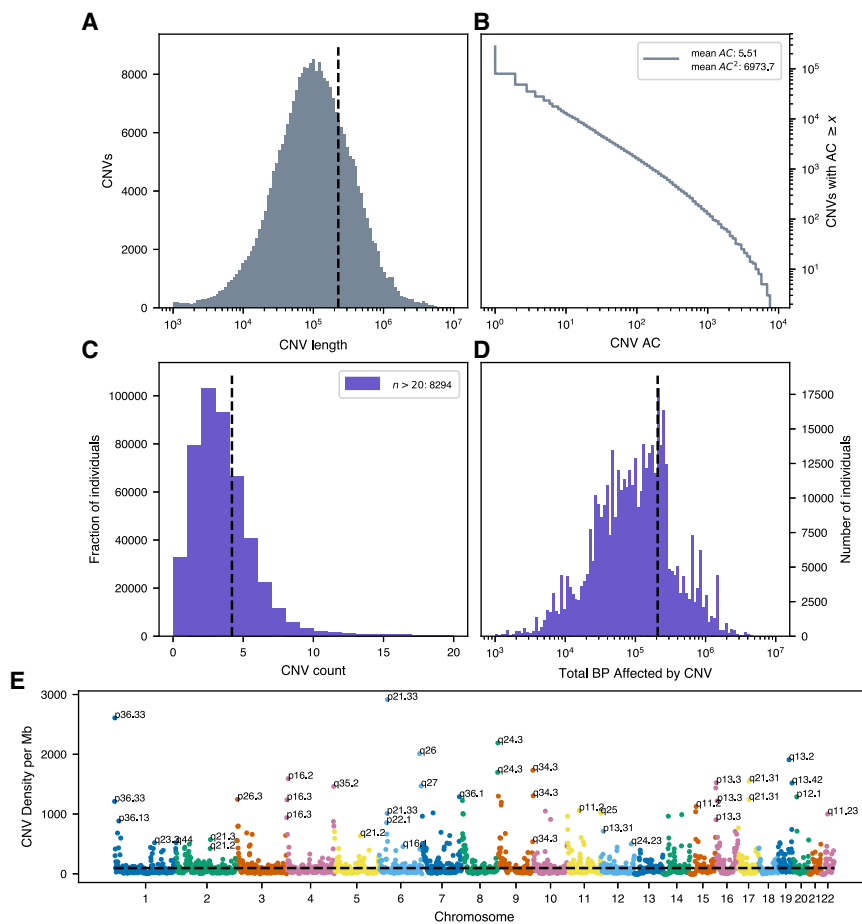


Figure 1. Burden and Distribution of Copy-Number Variation in UK Biobank

(A) Log-scale histogram of CNV lengths. Mean length (dashed line) is 226.5 kb. (B) Cumulative density of CNV allele count (AC), displayed in log-log axes. Average AC is 5.5, but average frequency as experienced by the population (weighted by count, hence AC^2) is $\sim 1.6\%$. (C and D) Histogram of CNV counts (C) and log-scale base-pairs affected by CNV per individual (D). Sample-level burden is heavy-tailed, with the average individual carrying 4.2 variants (dashed line), affecting mean ~ 207.6 kb of genomic sequence. (E) Genome-wide density of CNV, defined as the number of unique CNVs overlapping 10 megabase (Mb) windows tiling each chromosome. Hotspots of structural variation are labeled by cytogenetic band.

“healthy-cohort” enrollment bias³³ and were enrolled between the ages of 40 to 69, which informs our findings. Within the tail of positive constraint z-scores, which indicate the strongest intolerance to structural variation, we observe enrichment for genes which cause early-onset diseases, particularly cancer. Among the top 15 constrained genes (Table 1) are *BRCA1* (MIM: 113705) and

BRCA2 (MIM: 117305), which are associated with early-onset breast cancer;^{34,35} *MLH1* (MIM: 120486), *MSH2* (MIM: 609309), *MSH6* (MIM: 600678), which cause early-onset colorectal cancer (Lynch syndrome [MIM: 120435]);^{36–38} and *ATM* (MIM: 607585) and *APC* (MIM: 611731), which are involved with mismatch repair cancers.^{39,40} In all we find 8,709 genes (47.9% of 18,183 protein coding autosomal genes in the analysis) to be intolerant to CNV, with probability of intolerance (see [Material and Methods](#)) above 0.9; this is a greater than 2.5-fold increase in the set of genes intolerant to loss of function variation identified in ExAC.⁴¹

Selections from the most highly constrained pathways from Gene Ontology Consortium⁴² resources (Table 2) also suggest strong intolerance to CNV among genes involved with core biological processes like protein binding, cellular structural integrity (keratinization), development (growth hormone receptor binding), and immune regulation (natural killer cell activation). Similar results at the gene and pathway level are observed for deletion-specific constraint (Tables S1 and S2), whereas duplication-specific analysis suggests autoimmune-related genes and pathways are most strongly intolerant to dosage effects (Tables S1 and S2). Analysis of medical terms from the Human Phenotype Ontology project⁴³ further validates the observation that genes with carcinogenic variation are

We also observe a highly non-uniform burden of variation across genomic position, with breakpoints most common near the ends of chromosomes, and at known regions of segmental duplication (Figure 1E). Among them are *1p36*, *8q24.3*, *9q34.3*, and *19q13*, all of which have associated microdeletion syndromes causing developmental delay with uniquely characteristic growth patterns.^{29–32} Other CNV hotspots like *6p21.33*, which contains the major histocompatibility complex protein gene family, may be influenced by high marker density (in this case for HLA allelotyping) in addition to these biological features which underlie structural mutagenesis. However, these loci do not categorically correspond to areas where structural variation is commonly observed in the population (Figure S1). For example, *1p36* and *19q13* are also the respective sites of common CNVs overlapping *RHD* (MIM: 111680) and *FUT2* (MIM: 182100) (Rhesus and Lewis blood groups), but there are no such common variants within the telomeric *16p13* cytoband.

Survivorship Bias due to Genetic Selection against Early-Onset Diseases

We estimate gene-level intolerance to structural variation by adapting a method for estimating regional selective constraint.¹² Relative to the general population, the volunteers within the UK Biobank are described to have a

Table 1. 15 Genes Most Intolerant to Copy-Number Variation

Gene	Constraint z	Probability of CNV Intolerance
<i>BRCA2</i>	3.402	0.9911
<i>BRCA1</i>	2.570	0.9840
<i>APC</i>	2.086	0.9456
<i>ATM</i>	1.242	0.9892
<i>MSH2</i>	1.241	0.9883
<i>MLH1</i>	1.224	0.9962
<i>MSH6</i>	0.957	0.9933
<i>RB1</i>	0.905	0.9577
<i>SBDS</i>	0.861	0.9741
<i>SPATA31D1</i>	0.853	0.9979
<i>CYP3A4</i>	0.846	0.9979
<i>PABPC3</i>	0.831	0.9923
<i>OTOP1</i>	0.830	0.9930
<i>KRT16</i>	0.828	0.9979
<i>ZNF302</i>	0.827	0.9979

Columns are gene label, constraint z-score, and probability of CNV intolerance (see [Material and Methods](#) for definitions).

enriched among those most intolerant to CNV ([Table 3](#)). The most constrained HPO terms include carcinomas, neoplasms, and other conditions like chronic fatigue. Deletion-specific analysis of HPO terms ([Table S3](#)) also follows this trend, while duplication-specific constraint suggests strong intolerance to variation altering normal developmental pathways. HPO terms most strongly intolerant to putatively dosage-altering variation include an array of nervous and musculoskeletal abnormalities. These results indicate strong selective effects occurring prior to enrollment in the UK Biobank during childhood and early adulthood against loss of function variation in core developmental, metabolic, and tumor-suppressing genes, and against dosage-altering variation in immune-related genes.

Association Testing Identifies CNVs at Several Genomic Loci

We compute genome-wide associations across 3,157 phenotypes for 7,038 common CNVs observed at 0.005% allele frequency (1 in 20,000) in our GWAS cohort, using regression as implemented in the analysis software PLINK.⁴⁴ We also perform aggregate rare-variant burden tests, pooled by gene. For these tests, we measure the net effect of rare CNVs (AF < 0.1%) overlapping within 10 kb of the gene region as defined by HGNC⁴⁵ for 16,250 autosomal protein coding genes with at least five individuals with overlapping CNV. In sum, we find 14,182 CNV-level associations (about 4 per phenotype) and 102,606 gene-level associations (about 32 per phenotype) with Bonferroni-corrected $p < 0.05/7,038$ (7.1×10^{-6} , for GWAS) or $0.05/16,250$ (3.1×10^{-6} , burden test). It is noteworthy

Table 2. 15 Pathways from Gene Ontology Consortium Most Enriched for Constrained Genes

GO ID	CNV-Intolerant Pathway Name	Delta z	p
GO:0000137	Golgi cis cisterna	0.4086	7.14E-30
GO:0045095	keratin filament	0.2594	8.20E-30
GO:0031436	BRCA1-BARD1 complex	1.4562	2.18E-28
GO:0005515	protein binding	0.0707	5.52E-23
GO:0000800	lateral element	0.4642	1.48E-21
GO:0031424	keratinization	0.1816	1.50E-20
GO:0032301	MutSalpha complex	1.0987	7.98E-20
GO:0008194	UDP-glycosyltransferase activity	0.3525	1.06E-18
GO:0052697	xenobiotic glucuronidation	0.4715	4.53E-18
GO:0070200	establishment of protein localization to telomere	0.9767	1.58E-17
GO:0032300	mismatch repair complex	0.5164	4.29E-17
GO:0008274	gamma-tubulin ring complex	0.3902	2.72E-16
GO:0008202	steroid metabolic process	0.2776	6.76E-16
GO:0042954	lipoprotein transporter activity	0.4233	9.53E-15
GO:0015020	glucuronosyltransferase activity	0.3365	1.24E-14

Columns are GO pathway ID/name, change in z-score between set and non-set members, indicating mean strength of selective effect in the pathway, and p value (t test, gene set members versus all others).

that many of our phenotype observations are correlated (e.g., right/left hand grip strength), and aggregate gene-level tests are also correlated (e.g., cases where a single rare variant overlaps several genes, as in DiGeorge syndrome). A complete list of phenotypes analyzed is available on the Global Biobank Engine ([Web Resources](#)). Here, we describe representative results for one common disease and one quantitative measure with established genetic risk factors and large sample sizes in UK Biobank: acute coronary artery disease (CAD) and body mass index (BMI).

For acute CAD, we identify two statistically significant ($p < 9 \times 10^{-6}$) associations after Bonferroni correction for the common CNV GWAS: an intergenic deletion at chromosome *9p23* and a putative gene fusion event on chromosome 4 ([Figure 2A](#)). The association of the duplicated *FGFR3-TACC3* fusion (MIM: 134934) is unclear; only two individuals with this variant appear in gnomAD SV resource and no previous experimental or genetic data link this locus to cardiovascular disease. However, intergenic variants at the *9p21* locus have been implicated in previous association studies of blood-based biomarkers relevant to cardiac outcomes, specifically, decreases in hematocrit and hemoglobin concentration,⁴⁶ as well as carotid plaque burden.⁴⁷ A recent meta-analysis⁴⁸ using data from UK Biobank and CARDIoGRAMplusC4D identified a lead variant in the vicinity of this locus (rs2891168)

Table 3. 15 Pathways from Human Phenotype Ontology Consortium Most Enriched for Constrained Genes

HPO ID	CNV-intolerant HPO Term	Delta z	P
HP:0006725	Pancreatic adenocarcinoma	0.5545	2.50E-46
HP:0012432	Chronic fatigue	0.7301	3.00E-39
HP:0025318	Ovarian carcinoma	0.6659	1.94E-38
HP:0003003	Colon cancer	0.4031	2.95E-37
HP:0004389	Intestinal pseudo-obstruction	0.6735	3.16E-36
HP:0100787	Prostate neoplasm	0.5044	2.72E-34
HP:0012125	Prostate cancer	0.5044	2.72E-34
HP:0100273	Neoplasm of the colon	0.3444	9.78E-34
HP:0030406	Primary peritoneal carcinoma	0.5883	8.40E-32
HP:0012334	Extrahepatic cholestasis	0.5551	4.59E-28
HP:0003002	Breast carcinoma	0.3147	3.65E-27
HP:0002885	Medulloblastoma	0.5220	2.38E-26
HP:0002254	Intermittent diarrhea	0.5046	4.04E-26
HP:0100834	Neoplasm of the large intestine	0.2734	7.87E-26
HP:0009592	Astrocytoma	0.5033	2.38E-24

Columns are HPO ID/term, change in z-score between set and non-set members, indicating mean strength of selective effect in the pathway, and p value (t test, gene set members versus all others).

associated with 6% unit increase in risk for similarly defined coronary artery disease. However, the CNV we here identify confers an estimated 12.4-fold increased risk (95%CI: 7.2–21.3, $p = 3.7 \times 10^{-6}$) and is at least 2 Mb distant from the nearest SNPs (rs10961206) at genome-wide significance near the *9p21/9p23* locus in the meta-analysis. This and the absence of linkage between the *9p23* CNVs and rs10961206 ($r^2 = 0.013$) are suggestive of independent effects. However, translocation of flanking regulatory elements has been suggested as a mechanism for CNV-derived phenotypic effect;⁴⁹ given the proximity of this variant to a well-established susceptibility region (*9p21*) for CAD, we cannot rule out the possibility that *trans*-regulatory effect on known regions drives this association.

Gene-level burden testing of rare CNVs in individuals with CAD implicates *LDLRAD3* (MIM: 617986), a member of the low-density lipoprotein (LDL) receptor family that did not meet pre-specified significance criteria in revised analyses but remains strongly associated with disease, and is a clear outlying genome-wide signal (Figure 2B). The CNVs called in this gene are predominantly deletions affecting the coding sequence—in aggregate ($n = 27$), these variants confer an estimated 11-fold increase in risk of acute CAD (95% CI: 6.5–19.0, $p = 6.7 \times 10^{-6}$). Though the role of lipoprotein receptors in cholesterol metabolism is a well-established mechanism of risk for cardiovascular disease, *LDLRAD3* is not known to participate in cholesterol metabolism. It is, however, a receptor widely expressed throughout adult tissues which may participate

in proteolysis in the central nervous system.^{50,51} We therefore sought to replicate these findings using two-sample mendelian randomization⁵² on expression quantitative trait loci (eQTLs) from CAD summary statistics from a CARDIoGRAMplusC4D meta-analysis.²⁸ We identify a nominally significant protective effect between an eQTL increasing expression of *LDLRAD3* and CAD (OR = 0.85 [95%CI: 0.62–0.97], $p = 0.012$), the direction of which is consistent with a dosage model of *LDLRAD3*-mediated risk for CAD.

We find multiple significant associations for BMI; three deletions at chromosome *16p11.2*, a locus implicated in syndromic early-onset obesity and developmental delay (Figure 3A). Each of these CNVs appears to correspond to a distinct form of *16p11.2* deletion syndrome. The smaller $\sim 220\text{kb}$ deletion ($\beta = 0.92$ SD [95%CI: 0.72–1.12], $p = 5.1 \times 10^{-6}$, AC = 24 [MIM: 613444]) has been associated with early-onset obesity and spans nine distinct genes, with *SH2B1* [MIM: 608937] the suspected causal obesity gene.⁷ Obesity is also a phenotypic consequence of a larger $\sim 593\text{kb}$ deletion ($\beta = 1.35$ SD [95%CI: 1.18–1.51], $p = 3.3 \times 10^{-16}$, AC = 37 [MIM: 611913]), which is further associated with neurodevelopmental delay and related conditions.⁸ However, this deletion spans a wholly distinct set of genes which are suspected to play complex dosage-dependent roles in the phenotypic consequences of the syndrome.⁵³ As both subtypes of *16p11.2* deletion syndrome may present in early childhood, it is noteworthy that the effect we measure on BMI is in a cohort comprised entirely of older individuals, indicating burden of adult disease associated with the CNV locus. Moreover, these effects are consistent, though slightly higher, than previous meta-analysis or targeted study of this locus in UK Biobank.^{14,54}

After controlling for multiple comparisons, burden testing for BMI identifies *KLHL22* at chromosome *22q11.2*, recapitulates the *16p11.2* deletions at the gene level (*SH2B1*, *BOLA2* [MIM: 613182]), and associations at five additional loci each with strong evidence for causality (Figure 3B). Mechanisms for these loci are related to risk of diabetes; mouse knockouts of *USP2* (MIM: 604725) reduce insulin resistance,⁵⁵ *NEUROD1* (MIM: 601724) variation is a known cause of diabetes,⁵⁶ and *BHLHE40* (MIM: 604256) may modify diabetes in mice via disturbances in circadian rhythm.⁵⁷ While *SPTAN1* (MIM: 182810) has been previously associated with severe neurological disease,⁵⁸ neither *SPTAN1* nor *RHD* have a previous association with BMI. Variation at the *22q11.2* locus, also known as DiGeorge syndrome, has variable phenotypic consequences including craniofacial dysmorphisms and conotruncal congenital heart disease, along with increased risk for an adverse cardiovascular outcomes and neuropsychiatric disease later in life. Among individuals affected with *22q11.2* deletion syndrome, obesity is a recognized manifestation of disease, and we estimate a 1.5–2.0 point increase in BMI for genic CNVs near *22q11.2* in *KLHL22*. as well as 3.0–3.8 for genic CNV at *16p11.2*—these effects

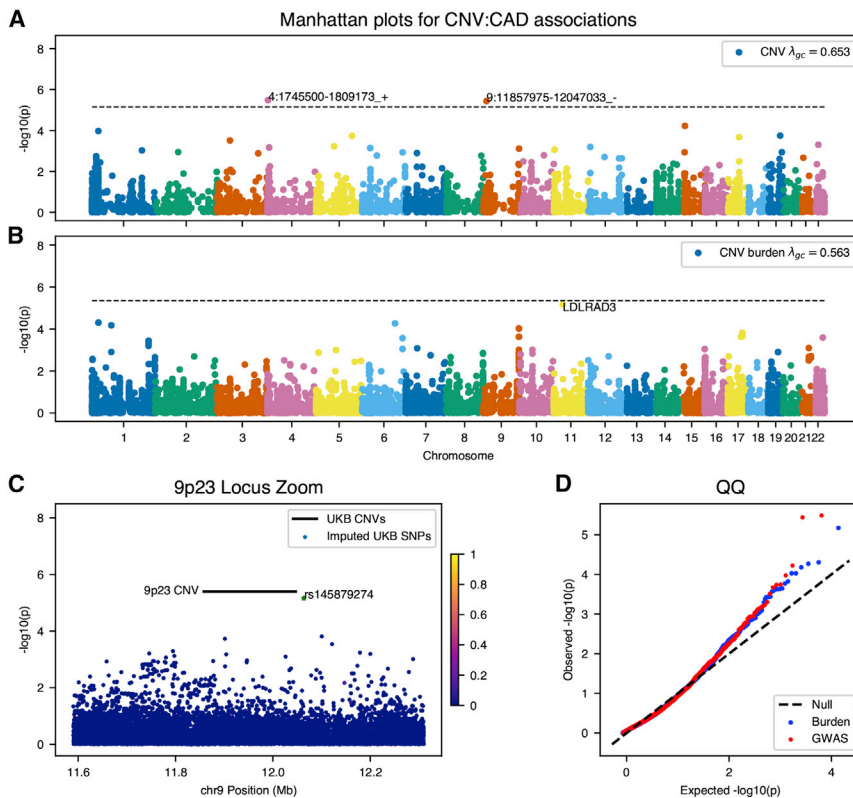


Figure 2. Genome-wide CNV Associations for Acute Coronary Artery Disease (CAD)

(A and B) Manhattan plots for (A) genome-wide association of common copy-number variants and (B) genome-wide burden test of rare variants for genes with at least ten individuals observed with CNVs.

(C) Locus inset of 9p23 CNV and summary statistics from GWAS of coronary artery disease using variants imputed on the same study population used in the CNV analysis. Variants are colored by marker LD with lead regional GWAS SNPs (rs145879274) from the analysis. This marker is highly stratified by continental ancestry and does not show significant correlation with any other variant in the region.

(D) Quantile-quantile plots for genome-wide summary statistics from CNV associations.

Discussion

In calling copy-number variants and performing genetic association studies at scale from a large cohort of array-genotyped individuals with

are attenuated relative to clinically estimated effect of DiGeorge syndrome on obesity,⁵⁹ our own estimates of the effect of *16p11.2* deletion, and previous studies,^{14,54} likely due to the inclusion of non-causal variants which overlap non-candidate genes in these regions. The presence of these associations in a large volunteer cohort offers further evidence that these potentially pathogenic CNV contribute to population-scale risk for common diseases.

Phenome-wide associations for each of the CNVs at *16p11.2* further highlight changes in biomarkers, biometrics, and increased risk of common disease, consistent with high BMI over the course of a lifetime (Figure 4). Genome-wide significant phenotypes for the 220 kb CNV recapitulate the established syndromic effects from early-onset obesity. We observe significant increases, on the order of one standard deviation, in weight, BMI, hip and waist circumference, reticulocyte count, and Cystatin C measures for these individuals. The larger 593 kb CNV associates with similar measures of body size and fat, as well as hypertension, diabetes/HbA1c, and abdominal hernia. These results are also indicative of effects due to developmental delay; namely, decreased measures of memory, higher Townsend deprivation (an index of material deprivation which considers employment, home/auto ownership, and household overcrowding in a person's neighborhood), and lower lung capacity (FEV, FVC), with higher associated risk of respiratory failure. Taken together, these results highlight the variable expressivity of CNV-related disease, as well as its long-term effects across the medical phenome.

richly annotated phenotype data, we provide a portrait of the phenome-wide burden of genomic copy-number variation. Our estimates of the individual-level burden of CNV and population-wide allele frequencies are consistent with previous reports, and the deep phenotypic information available in the UK Biobank permits more finely tuned measures of the genic intolerance to CNV which include estimates of variation absent from our cohort of predominantly healthy, middle-aged individuals.

Our study has significant limitations that inform our analysis. While arrays are an inexpensive way to genotype large cohorts, permitting straightforward algorithms to infer the presence of structural variation, the resulting CNV calls are limited by the density and placement of markers across chromosomes. For UK Biobank genotyping arrays in particular, there are large portions of genomic sequence with low marker density (in particular near centromeric regions) which bias our resulting genotype calls away from such regions. Array-derived CNV likewise cannot differentiate structural events like inversions or translocations, or determine breakpoint position at base-pair resolution.⁶⁰ Complicating these barriers is the fact that our sample was genotyped on two distinct arrays, which may cause identical CNVs to present with different breakpoints across individuals in the call set—as evident in the two calls of the 593 kb form of *16p11.2* deletion syndrome (Figure 3A). Our choice to present gene-level burden tests which include the vast majority of variants included in our CNV GWAS was informed by this realization. Given

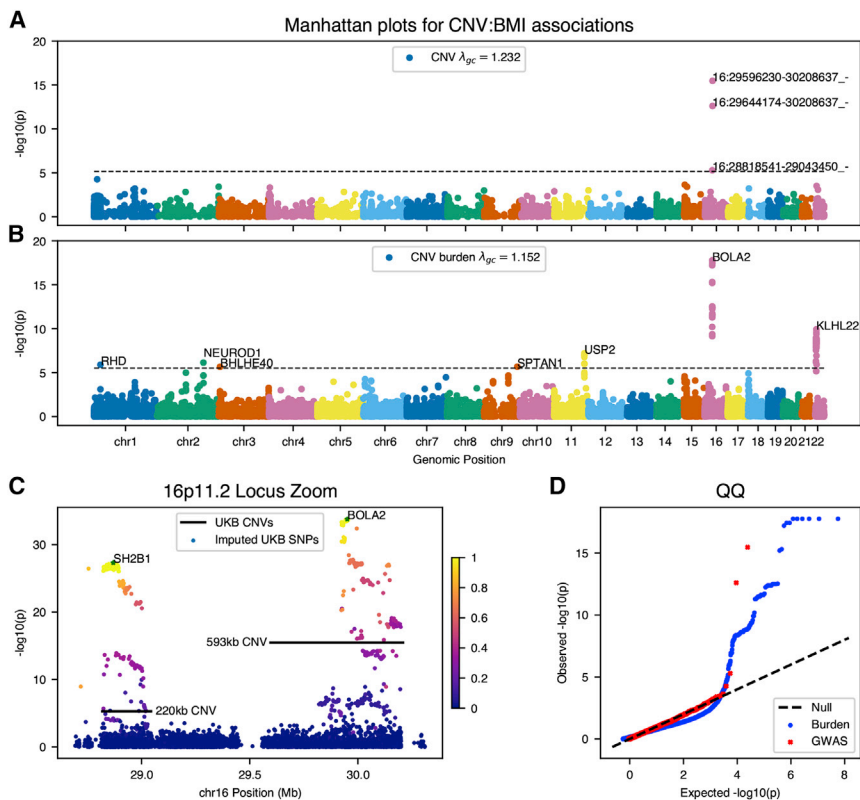


Figure 3. Genome-wide CNV Associations for Body Mass Index (BMI) (A and B) Manhattan plots for (A) genome-wide association of common copy-number variants and (B) genome-wide burden test of rare variants for genes with at least five individuals observed with CNVs. (C) Locus inset of 16p11.2 CNVs and summary statistics from GWAS of BMI using variants imputed on the same study population used in the CNV analysis. Variants are colored by marker LD with lead regional GWAS SNPs overlapping each CNV (rs62037365 in *SH2B1*; rs12716975 in non-coding *BOLA2*). (D) Quantile-quantile plots for genome-wide summary statistics from CNV associations.

the release of exome-sequence data for 50,000 UK Biobank participants,⁶¹ it is worth noting that NGS-based analysis of structural variants is a natural extension of this work which would complement the limitations of our genotyping.

Our associations are also heavily impacted by a known “healthy-cohort” bias, which may influence null results for phenotypes with known genetic contributions; notably, there are no genome-wide significant hits in our burden test for breast cancer. With this in mind, our constraint scores constitute a sobering observation of genetic survivorship bias. We take this opportunity to honor these non-participating individuals and their implicit contribution to our understanding of genetic disease. Though consideration of genic intolerance to variation may complement association studies, we find no novel candidate genes for early-onset disease among our results. However, the observation of selection bias in UK Biobank suggests that findings from biobank studies around the world will be influenced by implicit and explicit barriers to participation.

Despite selection against high-penetrance alleles causing early-onset disease, we detect a strong association for coronary artery disease at *LDLRAD3*. While this locus has prior putative association with bone mineral density,⁶² existing large-scale GWASs do not detect a strong association with coronary artery disease or established cardiometabolic risk-factors. In our study, CNVs at this locus are associated with some established cardiometabolic risk factors, such as diabetes onset, smoking status, and arterial stiffness, but

not obesity or other fat-related phenotypes (Figure S6). Consistent with our findings that a decrease in *LDLRAD3* dosage increases the risk of disease, a strong eQTL increasing *LDLRAD3* expression decreases the risk of disease when used as an instrument in a two-sample mendelian randomization in a large-scale study of coronary artery disease. These results highlight

the utility of analyzing genic CNV which, when directly impacting mRNA dosage, offer an interpretable mechanism distinct from alterations of protein structure or small changes in transcriptional regulation.

The observation of variation at the *16p11.2* and *22q11.2* loci sheds further light on the penetrance of potentially pathogenic CNVs in the general population. The *16p11.2* recurrent microdeletion syndrome has been previously described in individuals with autism and neuropsychiatric disease and may include seizures, and brain and other anatomic abnormalities. People carrying variation at the *22q11.2* locus within the general population are known to be at increased risk of neuropsychiatric diseases⁶³ for which variable phenotypic penetrance is well recognized.⁶⁴ To wit, individuals with genetic variation at both loci were by and large sufficiently healthy and capable of volunteering to participate in UK Biobank. Our findings support a growing recognition that the penetrance and effect sizes of syndromic alleles may require revision in the context of broad population-based surveys of rare genetic variation.^{65,66}

These described associations suggest a role of structural variation in population-wide burden of common disease and suggest loci where CNV-derived syndromic disease may exist. As such, these resources may be of immediate use by genetic clinicians in classification of CNV detected in clinical testing and for follow-up functional study. Of particular interest would be an analysis of “human knockout” individuals with both gene copies altered by CNV or other loss-of-function variation, as

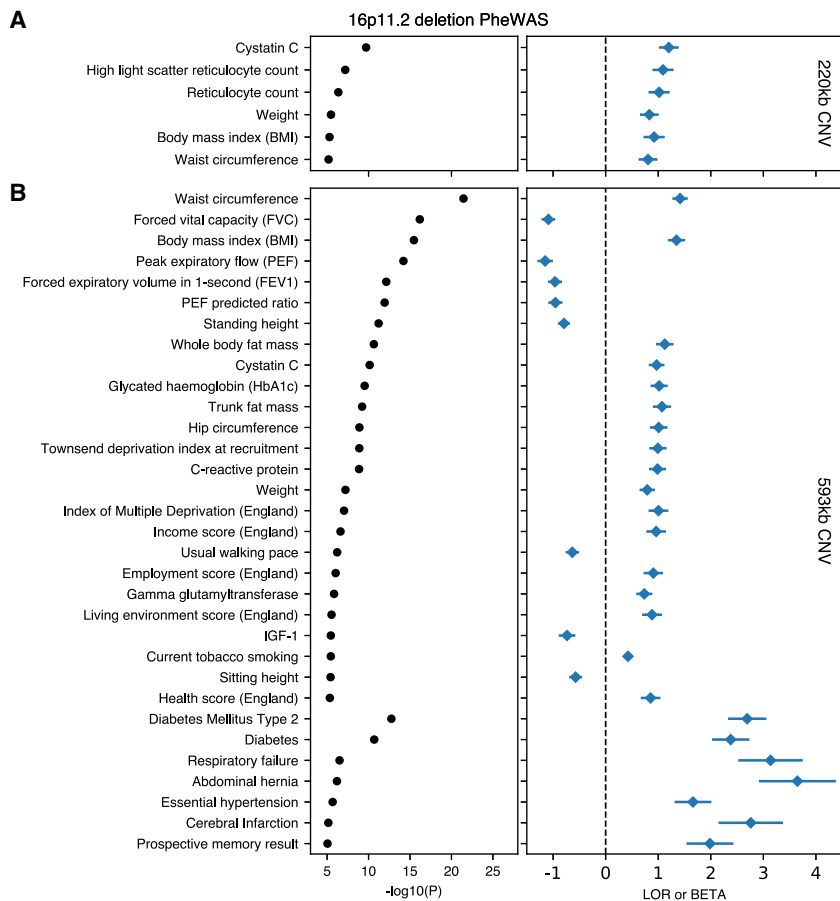


Figure 4. PheWAS of 16p11.2 CNVs
 Selected genome-wide significant ($p < 9 \times 10^{-6}$) associations for 220 kb (top) and 593 kb (bottom) *16p11.2* CNVs, with $n > 500$ binary cases or 15,000 quantitative values. Traits are grouped by type (binary/quantitative) then sorted by p value (left). Log-odds ratio and standardized betas (right) align with trait names on the y axis, with the horizontal dashed line separating positive and negative direction of association.

determined by SNP genotype data from UK Biobank. As with single-nucleotide variation, the functional consequence of and pathogenicity of genic structural variation is difficult to classify. One consequence is a dosage effect upon mRNA transcription; alternatively, dosage compensation to modulate mRNA levels⁶⁷ or fusion of flanking regions may drive phenotypic alteration.^{68,69} Summary statistics from association studies described here, as well as for all phenotypes present on the Global Biobank Engine, are freely available for download on the site. We hope that these data will be leveraged to empower future analyses of the phenome-wide effects of structural variation and gene-level dosage effects.

Supplemental Data

Supplemental Data can be found online at <https://doi.org/10.1016/j.ajhg.2019.07.001>.

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genomic and hospital linkage data,” and the results are displayed in the Global Biobank Engine.

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Declaration of Interests

The authors declare no competing interests.

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Web Resources

GeneReviews, McDonald-McGinn, D.M., Emanuel, B.S., and Zackai, E.H. (1999). 22q11.2 deletion syndrome, <https://www.ncbi.nlm.nih.gov/books/NBK1523/>
 Global Biobank Engine (summary statistics), <https://biobankengine.stanford.edu/downloads>

OMIM, <https://www.omim.org/>
UK Biobank Application 24983, <http://www.ukbiobank.ac.uk/wp-content/uploads/2017/06/24983-Dr-Manuel-Rivas.pdf>
UK Biobank CNV Analysis, <https://github.com/priestlab/cnv-ukb>

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