Exome sequencing identifies high-impact trait-associated alleles enriched in Finns

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ABSTRACT

As yet undiscovered rare variants are hypothesized to substantially influence an individual's risk for common diseases and traits, but sequencing studies aiming to identify such variants have generally been underpowered. In isolated populations that have expanded rapidly after a population bottleneck, deleterious alleles that passed through the bottleneck may be maintained at much higher frequencies than in other populations. In an exome sequencing study of nearly 20,000 cohort participants from northern and eastern Finnish populations that exemplify this phenomenon, most novel trait-associated deleterious variants displayed frequencies 10-173 times higher than in other European populations. These enriched alleles underlie 30 novel associations with 20 disease-related quantitative traits and demonstrate a geographical clustering equivalent to that of Mendelian disease mutations characteristic of the Finnish population. Sequencing studies in populations without this unique history would require hundreds of thousands to millions of participants for comparable power.

INTRODUCTION

Genotyping studies of common genetic variants (defined here as minor allele frequency [MAF]>1%) have identified tens of thousands of genome-wide significant associations with common diseases and disease-related quantitative traits[1]. For most traits, however, these associations account for only a modest fraction of trait heritability, and the mechanisms through which associated variants contribute to biological processes remain mostly unknown. These observations have led to two related expectations. First, since most common variants are well-tagged by the single-nucleotide variants (SNVs) on

genome-wide genotyping arrays, rare variants (defined here as MAF≤1%) are probably responsible for much of the heritability that remains unexplained[2]. Second, since purifying selection acts to remove deleterious alleles from the population, deleterious alleles with a large effect on complex traits should be particularly rare. These expectations suggest that discovering associated alleles not already identified by array-based studies will require exome or genome sequencing of very large numbers of individuals. However, the sample size required to reliably identify rare-variant associations remains uncertain; most sequencing studies to date have identified few novel associations, and theoretical analyses confirm that they have been underpowered to do so[3]. These analyses also suggest that power to detect rare variant associations varies enormously between populations.

In isolated populations that expand rapidly following a bottleneck, alleles that pass through the bottleneck often rise to a much higher frequency than in other populations[4,5]. If the bottleneck was recent, even deleterious alleles under negative selection may remain relatively frequent in these populations, resulting in increased power to detect association with disease-related traits. The Finnish population exemplifies this type of history. It grew from bottlenecks occurring 2,000-4,000 years ago in the founding of the early-settlement regions of southern and western Finland; internal migration in the 15th and 16th centuries to the late-settlement regions of northern and eastern Finland created additional bottlenecks[6]. The subsequent rapid growth of the Finnish population (to ~5.5 million, larger than any other human isolate) generated sizable geographic sub-isolates in late-settlement regions.

Geneticists first took advantage of Finland's demographic history to identify mutations responsible for 36 Mendelian disorders much more common in Finland than in other European countries ("the Finnish Disease Heritage"[7]); most of these disorders concentrate in late-settlement regions. Additional studies demonstrated, in these regions, an overall enrichment of deleterious variants more extreme compared to other isolates or to Finland generally[8-10]. We reasoned that this enrichment would enable exome sequencing studies of late-settlement Finland to be better powered than studies in other populations to systematically investigate the impact of low-frequency variants on disease-related quantitative traits. Based on this expectation, we formed such a sample ("FinMetSeq") from two Finnish population-based cohort studies: FINRISK and METSIM (see Methods).

Using >1.4M variants identified and genotyped by successful exome sequencing of 19,292 FinMetSeq participants, we conducted single-variant association analysis with 64 clinically relevant quantitative traits[11,12]. We identified 43 novel associations with deleterious variants in 25 traits: 19 associations (11 traits) in FinMetSeq and 24 associations (20 traits) in a four-cohort meta-analysis of FinMetSeq with an additional 24,776 Finns from three cohorts for which imputed array-based genome-wide genotype data were available. The MAF of 16 of the 26 variants underlying these 43 novel associations was enriched >10-fold in FinMetSeq compared to non-Finnish Europeans (NFE).

We demonstrate that (1) a well-powered exome sequencing study can identify effects of numerous rare alleles on complex traits that are of a clinically meaningful magnitude for individuals who carry them, and (2) exome sequencing in a population that has expanded after recent population bottlenecks is an extraordinarily efficient strategy to discover such effects. As most of the novel putatively deleterious trait-associated variants that we identified are >10-fold more frequent in FinMetSeq than in non-Finnish Europeans (NFE), similarly powered studies in non-Finnish populations might require hundreds of thousands or even millions of participants. Additionally, the geographical clustering of these enriched alleles (like the Finnish Disease Heritage mutations) demonstrates that the distribution of trait-associated rare alleles may vary significantly between locales within a country.

RESULTS

Genetic variation

We attempted to sequence the protein-coding regions of 23,585 genes covering 39MB of genomic sequence in 20,316 FinMetSeq participants. After extensive quality control, we included in downstream analysis 19,292 individuals sequenced to 47x mean depth (Methods, **Supplementary Table 1**). We identified 1,318,781 SNVs and 92,776 insertion/deletion (indel) variants, with a mean of 20,989 non-reference SNVs and 604 indel alleles per individual. 87.5% of SNVs identified were rare (MAF<1%); 40.5% were singletons (**Table 1**). Each participant carried 15 singleton variants on average, 17 rare (MAF<1%) protein truncating variants (PTVs; annotated as stop gain, essential splice site, start loss, or frameshift) alleles, and 171 common (MAF>1%) PTVs (**Supplementary Table 2**). Frameshift indels accounted for the largest proportion of

PTVs (31% of rare, 42% of common), while stop gain variants were the most frequent type of protein truncating SNVs (29% of rare, 20% of common).

FinMetSeq displays a notable excess of variants with increased MAF compared to NFE from the Exome Aggregation Consortium (ExAC, v0.3.1, **Extended Data Fig. 1**). As in previous smaller-scale comparisons of Finnish and NFE exomes, in FinMetSeq we observe a depletion of alleles with MAF<0.5% and enrichment of alleles with MAF 1-5% compared to NFE for all classes of variants classes. This effect is particularly marked for alleles predicted to be deleterious (PTVs + damaging missense, **Extended Data Fig. 2**).

Single-variant association analyses

We tested for association between genetic variants in FinMetSeq and 64 clinically relevant quantitative traits measured in both cohorts (**Supplementary Table 3)**. We adjusted lipid and blood pressure traits for lipid lowering and antihypertensive medication use, respectively, adjusted all traits for covariates using linear regression (**Supplementary Table 4**), and inverse normalized trait residuals to generate normally distributed traits for genetic association analysis that assumed an additive model (Methods). Based on common GWAS variants, 62 of 64 traits exhibited significant heritability ($P<0.05$, mean $h^2=19.3\%$, **Fig. 1A, Supplementary Table 5**), and there was substantial correlation between traits, phenotypically and genetically (**Fig. 1B).**

We tested the 64 traits for single-variant associations with the 362,996 to 602,080 genetic variants with minor allele count (MAC)≥3 among the 3,558 to 19,291 individuals

measured for each trait (**Supplementary Tables 3, 4**). Association results are available for download and can be explored interactively with PheWeb (http://pheweb.sph.umich.edu/FinMetSeq/) and via the Type 2 Diabetes Knowledge Portal (www.type2diabetesgenetics.org). We identified 1,249 trait-variant associations ($P<5\times10^{-7}$) at 531 variants (**Supplementary Table 6**), with 53 of 64 traits associated with at least one variant (**Fig. 2A**). All 1,249 associations remained significant after multiple testing adjustment across the exome and across the 64 traits with a hierarchical procedure setting average FDR at 5% (Methods). Using the hierarchical FDR procedure, we detected an additional 287 trait-variant associations at these 531 variants (**Supplementary Table 7**). These additional associations reflect the high correlation between a subset of lipid traits, e.g. high-density lipoprotein cholesterol (HDL-C) and apolipoprotein A1 (ApoA1). Given the diversity of traits assessed in these cohorts, these associations may shed additional light on the biology of measures that have been less frequently assayed in large GWAS, such as intermediate density lipoproteins (IDL) and very-low-density lipoprotein (VLDL) particles. Of the 531 associated variants, 59 (11%) were rare (MAF<1%); by annotation, 200 (38%) were coding, 108 (20%) missense, and 11 (2%) protein truncating. Furthermore, minor alleles at >10-fold increased frequency in FinMetSeq compared to NFE are substantially more likely to be associated with a trait compared to variants with similar or lower MAF in FinMetSeq compared to NFE ($OR=6.11$, $P=2.8\times10^{-7}$; **Extended Data Fig. 3**).

We clumped associated variants within 1Mb and with $r^2>0.5$ into a single locus, irrespective of the associated traits (Methods). After clumping, the 531 associated

variants represented 262 distinct loci (597 trait-locus pairs, **Supplementary Table 6**); 158 of the 262 loci (60%) consisted of a single trait-associated variant. As expected, the number of associated loci per trait was positively correlated with trait heritability (r=0.38, $P=8.8\times10^{-4}$). Height was a noticeable outlier, with relatively few associations considering its high estimated heritability (**Fig. 2B**).

61% of variants and loci were associated with a single trait; 4% were associated with ≥10 traits. Overlapping associations (**Fig. 2C**) strongly reflect the relationships exhibited by both trait and genetic correlations (**Fig. 1B**). For example, rs113298164, a missense variant in *LIPC* (p.Thr405Met), is associated with 11 traits, including cholesterols, fatty acids, apolipoproteins, and cholines. Similarly, the estimated genetic correlation of trait pairs is a strong predictor of the probability for a trait pair to share associated loci (**Fig. 2D**).

To determine which of the 1,249 single-variant associations were distinct from known GWAS associations for the same traits, we repeated association analysis for each trait conditional on published associated variants ($P<10^{-7}$) for the corresponding trait in the EBI GWAS Catalog (December 2016 release). 478 of the 1,249 trait-variant associations (213 of 531 variants) remained significant ($P<5\times10^{-7}$) after conditional analysis, representing 126 of the original 262 loci, including at least one conditionally significant locus for each of 48 traits (**Supplementary Table 8**). The conditionally-associated variants were more often rare (24% vs. 11%), more likely to alter or truncate the resulting

protein (31% vs. 22%), and more frequently >10x enriched in FinMetSeq relative to NFE (14% vs. 9%) compared to the full set of associated variants.

Gene-based association analyses

To identify genes associated with the 64 traits, we performed aggregate tests of protein coding variants, grouping variants using three different masks. Mask 1 comprised PTVs of any frequency; Masks 2 and 3 also included missense variants with MAF<0.1% or 0.5% predicted to be deleterious by five algorithms (Methods). We identified 54 gene-based associations at $P<3.88\times10^{-6}$ (adjusting for a maximum of 12,890 genes tested; Methods), 15 of which required ≥2 variants to achieve significance (**Supplementary Table 9**). Extremely rare (MAC≤3) PTVs drove the association of eight traits with *APOB* (**Extended Data Fig. 4**). We found a novel association between two very rare stop gain variants in *SECTM1* and HDL2 cholesterol ($P=7.2\times10^{-7}$, **Extended Data Fig. 5**). *SECTM1* encodes an interferon-induced transmembrane protein that is negatively regulated by bacterial lipopolysaccharide (LPS)[13]. The association could reflect the role of HDL particles in binding and neutralizing LPS in infections and sepsis[14].

Replication and follow-up of single variant associations in three additional Finnish cohorts: Identification of novel coding, deleterious variant associations

We attempted to replicate the 478 single variant associations from FinMetSeq (unconditional and conditional $P<5\times10^{-7}$) and to follow-up the 2,120 suggestive but sub-threshold associations from FinMetSeq (unconditional $5\times10^{-7}<P\leq5\times10^{-5}$, conditional $P\leq5\times10^{-5}$) in 24,776 participants from three Finnish cohort studies for which varying

subsets of the FinMetSeq traits were available: FINRISK[15,16] participants not sequenced in FinMetSeq (n=18,215), the Northern Finland Birth Cohort 1966[17] (n=5,139), and the Helsinki Birth Cohort[18] (n=1,412). For each of the three cohorts, we carried out genotype imputation using the Finnish-specific SISu v2 reference panel (http://www.sisuproject.fi), which is comprised of 5,380 haplotypes from whole-genome based sequencing and 10,184 haplotypes from whole-exome based sequencing in coding regions, and then used the same single variant association analysis strategy employed in FinMetSeq. We then carried out three-study meta-analysis of imputation-based studies to test for replication of FinMetSeq associated variants and four-study meta-analysis (with FinMetSeq) to follow-up suggestive associations (Methods).

We obtained data for 448 of the 478 significant variant-trait associations (191 of the 213 requested variants). 439 of the 448 (98.0%) had the same direction of effect as in FinMetSeq; 392 of the 448 replicated at $P<0.05$ (87.5%; **Supplementary Table 10**). We also obtained data to follow up 1,417 of the 2,120 sub-threshold associations (corresponding to 1,014 of the 1,554 requested variants); >60% of the variants that we could not follow up were very rare in FinMetSeq and were not present in the SISu reference panel. 431 of the 1,417 sub-threshold trait-variant associations reached $P<5\times10^{-7}$ in the four-study meta-analysis (**Supplementary Table 11**).

Of the significant results from FinMetSeq or four-study meta-analysis, 43 associations were with 26 predicted deleterious variants that conditional analysis and literature review suggest are novel (**Table 2**). Nineteen associations, at 15 deleterious coding variants,

were significant in FinMetSeq (**Table 2A; Supplementary Table 10**). Twelve of these associations replicated (P<0.05) in the three-cohort meta-analysis and remained significant in the four-study meta-analysis, while for the other seven associations we either did not have replication data (six associations) or did not replicate but had very low power (<5%) in the replication cohorts (one association). Four of the 15 variants were PTVs; 11 were missense variants predicted to be deleterious by at least one of five prediction algorithms. Another 24 associations, with 16 variants (two PTVs and 14 missense variants predicted to be deleterious), only reached significance in the four-cohort meta-analysis (**Table 2B**; **Supplementary Table 11**). Five variants in this class also showed exome-wide significant associations with different traits in FinMetSeq alone (**Table 2A**).

Of these 43 associations, 30 were with 16 variants enriched by >10-fold in FinMetSeq compared to NFE (10 of 15 variants in FinMetSeq and 10 of 16 variants in four-study meta-analysis with four variants overlapping). Identifying associations for these 16 variants would have required much larger samples in NFE populations than in FinMetSeq (**Fig. 3A & B**). We provide brief summaries relating each of these highly enriched associations to known biology and prior genetic evidence relating to the respective genes in **Supplementary Information.** We highlight a few of the most striking findings, below.

Anthropometric traits. As a group these are among the most extensively investigated quantitative traits, with thousands of common variant associations reported, most of very

small effect[19-23]. *THBS4* encodes thrombospondin 4, a matricellular protein found in blood vessel walls and highly expressed in heart and adipose tissue[24]. It is involved in local signaling in the developing and adult nervous system, and may function in regulating vascular inflammation[25]. We identified a predicted damaging missense variant (rs200373343, p.Arg94Cys) in *THBS4* 13X more frequent in FinMetSeq than in NFE and associated in the four-study meta-analysis with a mean decrease in body weight of 5.9 kg (**Table 2B**). Coding variants in *THBS4* and other thrombospondin genes have been implicated in increased risk for heart disease[26-28].

DLK1 encodes Delta-Like Notch Ligand 1, an epidermal growth factor that interacts with fibronectin and inhibits adipocyte differentiation. We identified a predicted damaging missense variant (rs2273607, p.Val104Met) in *DLK1* that is 13X more frequent in FinMetSeq than in NFE and is associated in the four-study meta-analysis with a mean decrease in height of 1.3 cm ($P_{meta}=1.2\times10^{-10}$, $\hat{\beta}=-0.16$, **Table 2B**). Uniparental disomy of *DLK1* causes Temple Syndrome and Kagami-Ogata Syndrome, characterized by pre- and postnatal growth restriction, hypotonia, joint laxity, motor delay, and early onset of puberty[29-31]. Paternally-inherited common variants near *DLK1* have been associated with child and adolescent obesity, type 1 diabetes, age at menarche, and central precocious puberty in girls[32-34]. Homozygous null mutations in the mouse ortholog Dlk-1 lead to embryos with reduced size, skeletal length, and lean mass[35], while in Darwin's finches, SNVs at this locus have a strong effect on beak size[36].

HDL-C. We identified two novel variants with large effects on HDL-C that were present in FinMetSeq and absent in NFE. The predicted deleterious missense variant rs750623950 (p.Arg112Trp) in *CD300LG* is associated in FinMetSeq with a mean increase in HDL-C of 0.95 mmol/l, and also associated with HDL2-C and ApoA1 (**Table 2A**). *CD300LG* encodes a type I cell surface glycoprotein. A missense variant in *ABCA1* (rs765246726, p.Cys2107Arg) is associated in FinMetSeq with a mean reduction in HDL-C of 0.64 mmol/l (**Table 2A**). Fifteen more variants (including ten with alleles >10X more frequent in FinMetSeq than in NFE) contributed to a strong *ABCA1* gene-based association signal ($P=2.2\times10^{-13}$; **Supplementary Table 9, Extended Data Fig. 6**). *ABCA1* encodes the cholesterol efflux regulatory protein, which regulates cholesterol and phospholipid metabolism. Individuals who are homozygotes or compound heterozygotes for any of several *ABCA1* mutations produce very little HDL-C and experience the manifestations of severe hypercholesterolemia.

Amino Acids. ALDH1L1 encodes 10-formyltetrahydrofolate dehydrogenase, which competes with the enzyme serine hydroxymethyltransferase to alter the ratio of serine to glycine in the cytosol. A stop gain variant (rs780671030, p.Arg722X) in *ALDH1L1* is 173X more frequent in FinMetSeq than NFE, and is associated with a mean reduction in serum glycine levels of 0.03 mmol/l (**Table 2A**), an effect which may increase risk for several cardiometabolic disorders[37]. Although rs780671030 was the strongest associated variant, gene-based association tests suggest that additional PTVs and missense variants in *ALDH1L1* also alter levels ($P=1.4\times10^{-20}$, **Extended Data Fig. 7, Supplementary Table 9**).

Ketone bodies. ACSS1 encodes an acyl-coenzyme A synthetase and plays a role in the conversion of acetate to acetyl-CoA. A predicted damaging missense variant (rs201013770, p.Phe517Ser) in *ACSS1* is 86X more frequent in FinMetSeq than NFE, and is associated in the four-cohort meta-analysis with mean increased serum acetate level of 0.005 mmol/l (**Table 2B**). In rodents, increased acetate levels lead to obesity, insulin resistance, and metabolic syndrome, mediated by activation of the parasympathetic nervous system[38].

Geographical clustering of associated variants

Given the concentration within sub-regions of northern and eastern Finland of most Finnish Disease Heritage mutations[39], we hypothesized that the distribution of rare trait-associated variants discovered through FinMetSeq might also display geographical clustering. In support of this hypothesis, principal component analysis revealed broad-scale population structure within the late-settlement region among 14,874 unrelated FinMetSeq participants whose parental birthplaces are known (**Extended Data Fig. 8**). Consistent with our hypothesis, parental birthplaces were significantly more geographically clustered for carriers of PTVs and missense alleles than for carriers of synonymous alleles, even after adjusting for MAF (**Supplementary Tables 12A, 12B**).

To enable finer scale analysis of the distribution of variants within late-settlement Finland, we delineated geographically distinct population clusters using patterns of haplotype sharing among 2,644 unrelated individuals with both parents known to be born

in the same municipality (Methods, **Extended Data Fig. 9**). Taking the cluster that is most genetically similar to early-settlement Finland as a reference, we compared variant counts for different functional classes and frequencies between this reference cluster and each of the other 12 clusters that contained ≥100 individuals (**Fig. 4, Supplementary Table 13**). In the two clusters that represent the most heavily bottlenecked late-settlement regions (Lapland and Northern Ostrobothnia), we observed a marked deficit of singletons and significant enrichment of variants at intermediate frequency compared to other clusters. This pattern is strongest for PTVs, although its significance is greatest for missense variants, which are present in much larger numbers (**Fig. 4**).

We observed particularly strong geographical clustering among variants >10X enriched in FinMetSeq compared to NFE (**Fig. 5A, Extended Data Fig. 10, Supplementary Table 14**). We further characterized geographical clustering for FinMetSeq-enriched trait-associated variants, by comparing the mean distances between birthplaces for parents of minor allele carriers to those of non-carriers (**Supplementary Table 15**). Most such variants were highly localized. For example, for variant rs780671030 in *ALDH1L1*, which is 173X more frequent in FinMetSeq than in NFE, the mean distance between parental birthplaces is 135 km for carriers and 250 km for non-carriers ($P<1.0\times10^{-7}$, **Fig. 5B**). In contrast, few of the variants that displayed sub-threshold association in FinMetSeq but that showed significant associations in the four-study meta-analysis were significantly geographically clustered within Finland (**Supplementary Table 15**).

Finally, we compared the clustering of FinMetSeq-enriched trait-associated variants to that of 38 Finnish Disease Heritage mutations carried by ≥3 FinMetSeq individuals with known parental birthplaces. FinMetSeq carriers of monogenic Finnish Disease Heritage mutations and FinMetSeq carriers of trait-associated variants identified in FinMetSeq displayed a comparable degree of genetic clustering. This clustering was dramatically greater than that observed for the non-carriers of both sets of variants (**Fig. 5C**), suggesting that rare variants associated with complex traits may be much more unevenly distributed geographically than has been appreciated to date.

DISCUSSION

We have demonstrated that a well-powered exome sequencing study of deeply phenotyped individuals can identify numerous rare variants associated with a wide range of traits. The variants that we identified may provide a useful starting point for studies aimed at uncovering biological mechanisms or fostering clinical translation. For example, further investigation of the p.Arg722X variant in *ALDH1L1* associated with reduced serum glycine could help elucidate the role of this gene in astrocyte function, a topic of growing interest in neurobiology. Glycine is a key inhibitory neurotransmitter localized to astrocytes[40], while the mouse ortholog, *Aldh1l1*, is the primary marker for astrocytes in experimental research, since it is strongly expressed in astrocytes, but not in neurons[41].

The substantial power of this study for discovering rare variant associations derives from the occurrence, in the recently expanded and heavily bottlenecked populations of northern and eastern Finland, of a large pool of deleterious variants at frequencies one to

two orders of magnitude greater than in other populations. This observation motivates a strategy for scaling up the discovery of rare variant associations by prioritizing the sequencing of populations beyond Finland that have expanded in isolation from recent bottlenecks. Examples of other such populations include those of Ashkenazim[42], Iceland[43], Quebec[44], highland regions of Latin America[45], and geographically isolated regions of larger European countries such as the Italian island of Sardinia[46]. In each of these populations, genetic drift has almost certainly caused a different set of alleles to pass through the corresponding bottlenecks, i.e. within any single population, some variants are enriched while others are depleted. The numerous rare-variant associations that could be identified by sequencing already available phenotyped samples across multiple population isolates could rapidly increase our understanding of the genetic architecture of complex traits.

Recent studies have suggested a continuity between the genetic architectures of complex traits and disorders classically considered monogenic[47,48]. Our results offer strong support for this continuity, not only in identifying numerous deleterious variants with large effects on quantitative traits, but in demonstrating that such variants show geographical clustering comparable to that of the mutations responsible for the Finnish Disease Heritage.

The use of a Finland-specific genotype reference panel[49] to impute FinMetSeq variants into array-genotyped samples from three other Finnish cohorts enabled us to identify many additional novel associations. This result suggests that using sequence data from a

subset of individuals in each population to impute variants in array-genotyped samples from the same population is a cost-effective strategy for detecting rare-variant associations. However, the clustering in FinMetSeq of deleterious trait-associated variants within limited geographical regions, and our inability to follow-up >700 sub-threshold associations from FinMetSeq for which the associated variants were not present in the Finnish imputation reference panel, emphasize the importance of extensively representing regional subpopulations when designing such reference panels to account for fine-scale population structure.

To fully realize the value of large-scale sequencing studies in population isolates, it will be necessary to increase the richness of phenotypes available in sequenced cohorts from these populations. For example, in FinMetSeq we identified >23,000 deleterious, highly enriched variants for which we have not yet identified significant association. In Finland, the national health care system and the population's willingness to participate in biomedical research mean that extensive medical records and population registries are available for mining additional phenotype data, and create an opportunity for callback by genotype for further phenotyping and collection of biological samples[50]. The insights gained from such efforts will accelerate the implementation of precision medicine, informing projects in larger, more heterogeneous populations which are still at an early stage[51].

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Author Contributions

AEL, LJS, RKW, AaP, VS, ML, SR, MB, and NBF designed the study. AEL, KMS, HJA, RSF, DCK, DEL, JN, TJN, and JV produced and quality-controlled the sequence data. AEL, AUJ, ArP, HMS, VS, and ML produced and quality-controlled the clinical data. AEL, KMS, CWKC, SKS, ASH, LS, MP, CCC, AUJ, CJK, KK, VR, DR, JV, RW, PY, and XY analyzed data. JGE, MAK, MRJ, and MM provided replication data. HL, SKD, NOS, IMH, CS, SR, MB, and NBF supervised experiments and analyses. AEL, KMS, CWKC, SKS, CS, MB and NBF wrote the paper.

AEL, KMS, CWKC, and SKS contributed equally to this work. NBF and MB jointly supervised this work.

Author Information

Competing interests statements:

VS has participated in a conference trip sponsored by Novo Nordisk and received a honorarium from the same source for participating in an advisory board meeting. He also has ongoing research collaboration with Bayer Ltd.

HL is a member of the Nordic Expert group unconditionally supported by Gedeon Richter Nordics and has received an honorarium from Orion.

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Data Availability: The sequence data can be accessed through dbGaP using the following study numbers: FINRISK: phs000756, METSIM: phs000752. Association results can be accessed at http://pheweb.sph.umich.edu/FinMetSeq/

Table 1. Sequence variants identified using whole-exome sequencing of 19,292 FinMetSeq participants

Variant type	All variants	Variants <1%	Singleton variants
SNV	1,318,781	87.5%	40.5%
Insertion/Deletion	92,776	87.0%	43.1%
Predicted LoF	33,156	96.4%	55.0%
Non-synonymous	353,228	92.2%	46.4%

Variant Annotation	All variants	Variants <1%	Singleton variants
Splice Acceptor	3,180	95.4%	50.8%
Splice Donor	3,795	96.2%	53.3%
Stop Gain	11,382	97.3%	54.3%
Frameshift	12,845	96.6%	58.2%
Stop Loss	621	88.1%	48.1%
Initiator Codon/Start Loss	1,333	93.6%	49.1%
Inframe Insertion	1,673	90.3%	44.5%
Inframe Deletion	4,936	92.9%	46.8%
Missense	353,228	92.3%	46.4%
Splice Region	40,248	87.1%	41.2%
Incomplete Terminal Codon	16	81.3%	50.0%
Stop Retained	217	86.2%	42.4%
Synonymous	180,104	85.7%	40.0%
Coding Sequence	78	88.5%	41.0%
Mature miRNA	239	92.9%	48.5%
5' UTR	35,572	87.8%	38.2%
3' UTR	66,539	86.2%	38.6%
Non-coding Exonic	82,126	85.8%	37.8%
Intronic	601,362	85.1%	37.4%
Upstream	8,820	86.5%	38.3%
Downstream	3,050	84.6%	38.3%
Intergenic	193	85.5%	31.1%

Variant annotation refers to the "most deleterious" annotation for a given variant across all Ensembl (v88) transcripts, following the order defined by VEP (https://useast.ensembl.org/info/genome/variation/prediction/predicted_data.html).

Table 2. Highlighted variants reaching significance in (A) FinMetSeq alone, or (B) in meta-analysis of FinMetSeq with three replication cohorts.

A. Variants reaching significance in FinMetSeq alone

Chr:Pos (GRCh37)	Gene	Anno: Prediction^	FMS MAF	NFE MAF	Fold Enr.	Trait	FMS P	FMS Beta	Repl. P	Repl. Beta	Mean in carriers | non-carriers
1:55076137	*FAM151A*	FS:PTV	.099	.02627*	3.8	Total Chol. in IDL	5.4×10^{-16}	-0.187	2.1×10^{-17}	-0.191	0.84 | 0.87 mmol/l
1:55076137	*FAM151A*	FS:PTV	.099	.02627*	3.8	IDL Particle Conc.	8.9×10^{-14}	-0.172	1.9×10^{-16}	-0.185	0.130 | 0.134 umol/l
3:125831672	*ALDH1L1*	SG:PTV	.0026	.000015	173	Glycine	1.8×10^{-8}	-0.873	4.5×10^{-4}	-0.827	0.24 | 0.27 mmol/l
4:13612630	*BOD1L1*	MS:D;D;D;N;D	.00010	0	∞	WHR adj. BMI	4.7×10^{-7}	-2.501	NA	NA	0.88 | 0.93
5:140181423	*PCDHA3*	FS:PTV	.00010	NA**	NA	WHR adj. BMI	2.7×10^{-7}	2.559	NA	NA	1.14 | 0.93
9:107548661	*ABCA1*	MS:D;D;D;D;D	.00023	0	∞	Serum HDL Chol.	4.8×10^{-10}	-2.046	NA	NA	0.80 | 1.44 mmol/l
11:47282929	*NR1H3*	MS:D;P;P;D;D	.0042	.0001	42	Serum HDL Chol.	1.4×10^{-7}	0.425	6.7×10^{-7}	0.435	1.59 | 1.44 mmol/l
11:117352857	*DSCAML1*	MS:T;B;B;.;D	.016	.0017	9.5	VLDL Chol.	4.1×10^{-8}	0.299	2.0×10^{-3}	0.162	1.01 | .90 mmol/l
16:55862682	*CES1*	MS:D;D;D;D;D	.0018	.0011	1.6	Serum HDL Chol.	1.1×10^{-10}	0.771	3.8×10^{-6}	0.793	1.77 | 1.44 mmol/l
16:56996009	*CETP*	SD:PTV	.0017	.000045	37.3	Serum ApoA1	2.6×10^{-8}	0.834	1.8×10^{-4}	1.034	1.70 | 1.47 g/l
16:56996009	*CETP*	SD:PTV	.0017	.000045	37.3	Serum HDL Chol.	1.1×10^{-14}	0.946	8.8×10^{-21}	1.217	1.84 | 1.44 mmol/l
16:68013570	*DPEP3*	MS:T;B;B;N;D	.0099	.00069	14.3	Serum HDL Chol.	1.6×10^{-7}	-0.295	7.2×10^{-15}	-0.373	1.33 | 1.44 mmol/l
17:6599157	*SLC13A5*	MS:D;D;D;D;D	.00091	.00030	3.0	Citrate	1.3×10^{-9}	1.294	9.5×10^{-12}	1.309	0.14 | 0.11 mmol/l
17:39135270	*KRT40*	MS:D;P;P;N;D	.00013	0***	∞	Serum HDL Chol.	3.2×10^{-8}	2.416	NA	NA	2.51 | 1.44 mmol/l
17:41062979	*G6PC*	MS:T;P;P;D;D	.025	.00052	47	Total MUFA	4.4×10^{-7}	0.275	3.5×10^{-1}	0.067	3.88 | 3.62 mmol/l
17:41926216	*CD300LG*	MS:T;D;P;N;N	.00034	0	∞	Serum HDL Chol.	4.8×10^{-14}	2.061	4.9×10^{-2}	0.801	2.39 | 1.44 mmol/l
17:41926216	*CD300LG*	MS:T;D;P;N;N	.00034	0	∞	HDL2 Chol.	1.3×10^{-7}	2.154	NA	NA	1.88 | .92 mmol/l
17:41926216	*CD300LG*	MS:T;D;P;N;N	.00034	0	∞	Serum ApoA1	8.1×10^{-8}	1.694	NA	NA	2.04 | 1.47 g/l
19:10683762	*AP1M2*	MS:D;D;D;D;D	.015	.0014	10.7	Serum ApoB	5.8×10^{-8}	-0.282	1.5×10^{-3}	-0.199	0.96 | 1.02 g/l

B. Variants reaching significance in 4-study meta-analysis

Chr:Pos (GRCh37)	Gene	Anno: Prediction^	FMS MAF	NFE MAF	Fold Enr.	Trait	FMS P	FMS Beta	Meta P	Meta Beta	Mean in carriers | non-carriers
2:120848049	*EPB41L5*	MS:T;B;B;N;D	.085	.048	1.8	eGFR	1.7×10^{-6}	-0.093	4.8×10^{-12}	-0.107	88.6 | 89.9 SI
2:120848049	*EPB41L5*	MS:T;B;B;N;D	.085	.048	1.8	Creatinine	2.5×10^{-6}	0.091	2.5×10^{-12}	0.098	81.62 | 80.64 umol/l
5:79336091	*THBS4*	MS:D;D;D;D;D	.0045	.00035	12.8	Weight	6.7×10^{-7}	-0.377	3.2×10^{-7}	-0.252	74.6 | 80.5 kg
9:136501728	*DBH*	MS:D;D;P;N;N	.050	.0049	10.1	Diastolic BP	1.5×10^{-6}	-0.115	2.8×10^{-12}	-0.110	83.1 | 84.1 mmHg
11:47282929	*NR1H3*	MS:D;P;P;D;D	.0042	.0001	40.0	HDL2 Chol.	3.2×10^{-6}	0.473	1.3×10^{-8}	0.458	1.07 | .92 mmol/l
11:47282929	*NR1H3*	MS:D;P;P;D;D	.0042	.0001	40.0	VLDL Chol.	4.0×10^{-6}	-0.469	3.1×10^{-7}	-0.412	0.75 |0 .91 mmol/l
11:116692293	*APOA4*	MS:T;D;P;N;N	.0096	.011	0.9	Serum HDL Chol.	2.2×10^{-5}	0.225	1.5×10^{-7}	0.196	1.51 | 1.44 mmol/l
14:101198426	*DLK1*	MS:T;B;B;N;D	.023	.0018	13.1	Height	2.7×10^{-5}	-0.149	1.2×10^{-10}	-0.163	170.7 | 172.0 cm
16:55862682	*CES1*	MS:D;D;D;D;D	.0018	.0011	1.6	Serum ApoA1	1.9×10^{-6}	0.668	4.0×10^{-9}	0.718	1.65 | 1.47 g/l
16:68013570	*DPEP3*	MS:T;B;B;N;D	.0099	.00069	14.3	Serum ApoA1	5.2×10^{-6}	-0.294	4.0×10^{-7}	-0.253	1.40 | 1.47 g/l
16:68732169	*CDH3*	MS:D;D;D;D;D	.0044	.00071	6.2	Pyruvate	3.7×10^{-5}	0.417	6.6×10^{-10}	0.471	0.08 | 0.07 mmol/l
17:7129898	*DVL2*	MS:D;D;D;D;D	.020	.022	0.9	Valine	4.2×10^{-5}	-0.239	5.7×10^{-9}	-0.232	0.210 | 0.217 mmol/l
17:41062979	*G6PC*	MS:T;P;P;D;D	.023	.00052	43.2	Glycerol	5.8×10^{-6}	0.218	4.1×10^{-7}	0.183	0.092 | 0.088 mmol/l
17:41062979	*G6PC*	MS:T;P;P;D;D	.023	.00052	43.2	Plasma CRP	1.6×10^{-5}	0.175	4.0×10^{-9}	0.185	2.47 | 2.17 mg/l
17:41062979	*G6PC*	MS:T;P;P;D;D	.023	.00052	43.2	Triglycerides	1.0×10^{-6}	0.230	1.3×10^{-7}	0.197	1.60 | 1.46 mmol/l
18:47091686	*LIPG*	SA:PTV	.0025	.000015	164	HDL2 Chol.	1.2×10^{-5}	0.579	5.6×10^{-10}	0.624	1.13 | .92 mmol/l
18:47091686	*LIPG*	SA:PTV	.0025	.000015	164	Phosphocholines	3.1×10^{-6}	0.624	1.1×10^{-8}	0.578	2.44 | 2.20 mmol/l
18:47091686	*LIPG*	SA:PTV	.0025	.000015	164	Phosphoglycerides	9.0×10^{-6}	0.594	1.1×10^{-7}	0.538	2.50 | 2.25 mmol/l
19:10683762	*AP1M2*	MS:D;D;D;D;D	.015	.0014	10.7	Total Chol. in IDL	1.1×10^{-6}	-0.289	6.9×10^{-14}	-0.319	0.81 |0 .87 mmol/l
19:10683762	*AP1M2*	MS:D;D;D;D;D	.015	.0014	10.7	IDL Particle Conc.	2.1×10^{-6}	-0.281	8.5×10^{-14}	-0.318	0.125 | 0.133 umol/l
19:10683762	*AP1M2*	MS:D;D;D;D;D	.015	.0014	10.7	Remnant Chol.	8.0×10^{-6}	-0.268	2.7×10^{-12}	-0.301	1.65 | 1.77 mmol/l
19:11350904	*ANGPTL8*	SG:PTV	.0025	.000084	29.8	HDL2 Chol.	3.4×10^{-6}	0.564	1.1×10^{-8}	0.574	1.06 | .92 mmol/l
19:49318380	*HSD17B14*	MS:D;D;D;D;D	.046	.051	0.9	Valine	3.4×10^{-5}	-0.152	2.1×10^{-7}	-0.144	0.212 | 0.217 mmol/l
20:24994201	*ACSS1*	MS:D;D;D;D;D	.0026	.000031	85.8	Acetate	1.3×10^{-5}	0.626	2.1×10^{-12}	0.631	0.046 | 0.041 mmol/l

^Annotations from VEP: FS=Frameshift; SG=Stop Gain; SD=Splice Donor; SA=Splice Acceptor; MS=Missense. All but MS are PTV. Predictions for missense variants are from SIFT (D=Damaging; T=Tolerated); PolyPhen2 - human diversity (D=Probably Damaging; P=Possibly Damaging; B=Benign); PolyPhen2 - hum variation (D=Probaby Damaging; P=Possibly Damaging; B=Benign); Mutation Taster (A=Automatic Disease Causing; D=Disease Causing; N=Polymorphism; P=Automatic Polymorphism); and LRT (D=Deleterious; N=Neutral; U=Unknown).

Fold Enrichment (Fold Enr.) is MAF in FinMetSeq/MAF in ExAC NFE (unless otherwise noted).

*Filtered in ExAC, frequency from gnomAD; **Not found in ExAC or gnomAD; ***Not found in ExAC, frequency from gnomAD

METHODS

METSIM and FINRISK studies: designs, phenotypes, and sequenced participants

METSIM is a single-site study comprised of 10,197 men randomly selected from the population register of Kuopio, Eastern Finland, aged 45 to 73 years at initial examination from 2005 to 2010[12,52]. The goal of METSIM is to investigate genetic and non-genetic factors associated with Type 2 Diabetes (T2D), cardiovascular disease (CVD), insulin resistance, and related traits. The METSIM study protocol includes collection of data on CVD history and risk factors, measurements of height, weight, waist, hip, blood pressure, and collection of a blood sample for laboratory measurements and DNA extraction. Diagnoses of myocardial infarction[53], stroke[54], and peripheral vascular disease were verified based on medical records at baseline. We attempted exome sequencing of all METSIM study participants.

FINRISK is a series of health examination surveys carried out by the National Institute for Health and Welfare (formerly National Public Health Institute) of Finland every five years beginning in 1972[55]. The surveys are based on random population samples from five (six in 2002) geographical regions of Finland. Participants were selected by 10-year age group, sex, and study area. Survey sample sizes have varied from 7,000 to 13,000 individuals and participation rates from 60% to 90%. The age-range was 25 to 64 years until 1992 and 25 to 74 years since 1997. The survey includes a self-administered questionnaire, a standardized clinical examination carried out by specifically trained study nurses, and collection of a blood sample for laboratory measurements and DNA extraction.[56] For exome sequencing, we chose 10,192 participants from the 1992, 1997, 2002, and 2007 FINRISK surveys from northeastern Finland (former provinces of North Karelia, Oulu, and Lapland). This selection was based on the hypothesis that the rapid

growth in isolation of the populations of this region from severe bottlenecks in the 16th-17th centuries might have resulted in deleterious variants rising to a much higher frequency than in other populations.

METSIM participants fasted for more than 10 hours prior to blood draw. FINRISK participants were instructed to fast for four hours before the scheduled examination and to avoid heavy meals earlier in the day; duration of fasting was recorded. Laboratory measurements in METSIM included an oral glucose tolerance test with samples at 0, 30, and 120 minutes (only fasting measurements in known diabetics) for glucose, insulin, proinsulin, and free fatty acids, as well as fasting laboratory measurements including lipids, lipoproteins, apolipoproteins, adiponectin, and hs-CRP. Most of these measurements were carried out in FINRISK non-fasting samples; two-hour oral glucose tolerance tests with glucose and insulin measured at 0 and 120 minutes were carried out in subsets of FINRISK 1992, 2002 and 2007 participants. Both studies include proton NMR metabolomics measurements of lipoprotein subclasses, their lipid concentrations and composition, apolipoprotein A-I and B, multiple cholesterol and triglyceride measures, albumin, various fatty acids, and numerous low-molecular-weight metabolites, including amino acids, glycolysis related measures and ketone bodies[57,58].

METSIM and FINRISK participants are followed up regularly via record linkage using the Finnish health registries, allowing for near complete follow-up of multiple disease diagnoses; participants may also be called back in person for additional studies. Participants in both studies provided informed consent, and all study protocols were approved by the Ethics Committees at the participating institutions (FINRISK cohorts 1992 & 1997: National Public Health Institute of Finland; FINRISK cohorts 2002, 2007 & 2012: Ethical Review Board of the Hospital District of

Helsinki and Uusimaa; METSIM: Research Ethics Committee, Hospital District of Northern Savo IRB#1).

Selection of traits, harmonization, exclusions, covariate adjustment, and transformation

Of the 257 quantitative metabolic and cardiovascular traits measured in both METSIM and FINRISK, we selected 64 traits for association analysis in the current study based on clinical relevance for cardiovascular and metabolic health (**Supplementary Tables 3, 4**).

Exclusions

We excluded 126 individuals, 92 with type 1 diabetes and 34 women who were pregnant at the time of phenotyping, from all analyses, and 3,088 individuals with T2D from analyses of glycemic traits. For traits influenced by food consumption (amino acids, fatty acids, LDL cholesterol, total triglycerides, and glycemic traits), we excluded individuals not fasting for at least 8 hours after their last meal. A complete list of exclusions can be found in **Supplementary Table 4**.

Trait adjustments

For individuals on antihypertensive medication at the time of testing, we added 15 and 10 mmHg to the measured values of systolic and diastolic blood pressures, respectively[59,60]. For individuals on lipid regulating medications, we multiplied the measured total cholesterol by 1.25[61]. For FINRISK participants, we calculated LDL cholesterol (LDL-C) levels using the Friedewald equation (LDL-C = adjusted total cholesterol – HDL-C – (triglycerides/2.2)) and excluded individuals with elevated triglycerides (>2.5mmol/l); LDL-C was measured directly in METSIM

participants and for participants on lipid regulating medication, values were divided by 0.7[62]. All trait adjustments are listed in **Supplementary Table 4**.

Trait transformations and adjustment for covariates

We prepared quantitative traits for association analysis separately for METSIM and FINRISK participants by linear regression on trait-specific covariates; skewed variables were log transformed prior to linear regression analysis. Covariates for these regression analyses always included covariates age and age^2 for METSIM and sex, age, age^2, and cohort year for FINRISK. Trait transformations and trait-specific covariates are listed in **Supplementary Table 4**. We transformed residuals from these initial regression analyses to approximate normality by inverse normal scores.

Exome sequencing

We carried out exome sequencing in two phases.

Phase 1 We quantified the 10,379 Phase 1 DNA samples with the Quant-iT PicoGreen dsDNA reagent on a Varioskan Microplate Reader (ThermoFisher Scientific) and randomly parsed samples with adequate DNA (>250ng) into cohort specific files. We then re-arrayed samples using the BioMicroLab XL20 (USA Scientific) to ensure equal numbers of METSIM and FINRISK samples on each 96-well plate, alternating samples between studies in consecutive positions within and across plates, to reduce opportunities for between-study batch effects.

We constructed dual indexed libraries using 100-250ng of genomic DNA and the KAPA HTP Library Kit (KAPA Biosystems) on the SciClone NGS instrument (Perkin Elmer). The target

insert size was 250bp. We then pooled twelve libraries prior to hybridization with the SeqCap EZ HGSC VCRome (Roche) reagent that targets 45.1Mb (23,585 genes and 189,028 non-overlapping exons) of the human genome. Each library pool contained samples from both cohorts and was comprised of 300-400ng of each individual library for a total library input of 3.6-4.8μg into the initial hybridization. We estimated the concentration of each captured library pool by qPCR (Kapa Biosystems) to produce appropriate cluster counts for the HiSeq2000 platform (Illumina). We then generated 2x100bp paired-end sequence data yielding ~6Gb per sample to achieve a coverage depth of ≥20x for ≥70% of targeted bases for every sample.

Phase 2 We quantified, prepared, pooled, and captured the 9,937 Phase 2 samples just as in Phase 1. Here we generated 2x125bp paired-end sequencing reads on the HiSeq2500 1T to again achieve a coverage depth of ≥20x for ≥70% of targeted bases for every sample.

Contamination detection, sequence alignment, sample QC, and variant calling

We aligned sequence reads to human genome reference build 37 using bwa-mem (v0.7.7), marked duplicates with picard MarkDuplicates (v1.113; http://broadinstitute.github.io/picard), and realigned indels with the GATK[63] IndelRealigner (v2.4). We used BamUtil clipOverlap (v1.0.11; http://genome.sph.umich.edu/wiki/BamUtil:_clipOverlap) to mark overlapping bases from paired reads resulting from short insert fragments.

For each sample, we required ≥70% of target bases sequenced at ≥20x depth, and SNV genotype array concordance >90% if SNV array data were available. We used verifyBamID[64] (v1.1.1) to detect and exclude samples with estimated contamination >3%. Where available, we also used existing genotype data with verifyBamID to detect and exclude sample swaps. Of 20,316

samples attempted, 13 failed sequencing, 35 were sample swaps, 760 either had low genotype concordance, sex mismatch, or estimated contamination >3%, and four had discrepancies between reported and genotype-estimated relationships (**Supplementary Table 1**).

We called SNVs and short indels using the recommended best practices for cohort-level calling with GATK[63] (v3.3). For each individual, we called bases and identified variant sites for all targeted exome bases and 500bp of sequence up and downstream of each target region using HaplotypeCaller, resulting in calling substantial numbers of non-exonic variants. We merged these calls in batches of 200 individuals using CombineGVCFs, and recalled genotypes for all individuals at all variable sites with GenotypeGVCFs.

After merging genotypes for the 19,378 samples that passed preliminary QC checks, we filtered SNVs and indels separately using the recommended best practices for Variant Quality Score Recalibration (VQSR). We used the set of true positive variants provided in the GATK resource bundle (v2.5; build37) for training the VQSR model after restricting to sites in targeted exome regions. After assessment with VQSR, we retained variants for which we identified ≥99% of true positive sites used in the training model (i.e. truth sensitivity) for both SNVs and indels.

Following initial variant filtering, we decomposed multi-allelic variants into bi-allelic variants, left-aligned indels, and dropped redundant variants using vt[65] (version 0.5). We filtered variants with >2% missing calls and/or Hardy-Weinberg p-value$<10^{-6}$. We applied an additional filter removing variants with an overall allele balance (AB; alternate AC/sum of total AC) <30% in genotyped samples. We then excluded 86 individuals with >2% missing variant calls yielding a final analysis set of 19,292 individuals.

Array genotypes, genotype imputation, and integrated exome+imputation panel

METSIM participants were previously genotyped with the Illumina OmniExpress array; genotyping and quality control are described elsewhere[66]. FINRISK participants were previously genotyped in several batches on different arrays[16]. 1,488 participants (57 METSIM, 1,431 FINRISK) lacked genotype-array data. From the available genotype array data, we generated three datasets: 1) a merged array-based genotype call set of all variants present in ≥90% of array-genotyped individuals across both cohorts; 2) a merged Haplotype Reference Consortium (HRC) v1.1 imputed data set of the array-based genotypes; 3) an integrated data set containing genotyped and imputed array-based variants and exome sequence variants (HRC+exome). Where there was overlap between the sequence and imputed genotypes, we kept the sequence-based genotypes. We excluded the 1,488 individuals with no array data from the HRC+exome panel.

We prepared array genotypes for imputation using the Imputation Preparation and Checking tool (http://www.well.ox.ac.uk/~wrayner/tools/HRC-1000G-check-bim.v4.2.5.zip) and used the Michigan Imputation Server[67] (www.imputationserver.sph.umich.edu) to impute genotypes using the HRC (v1.1) reference panel[68]. METSIM samples were imputed in a single batch. FINRISK samples were imputed in batches based on the genotyping array and/or center where genotypes were generated.

Annotation

We annotated the final set of variants passing QC using Ensembl's variant effect predictor (VEP v76)[69] and Combined Annotation-Dependent Depletion[70] (CADD v1.2). We employed five *in*

silico algorithms implemented in dbNSFP v2.4 (https://sites.google.com/site/jpopgen/dbNSFP) to predict the functional impact of missense variants: PolyPhen2 HumDiv and HumVar[71], LRT[72], MutationTaster[73], and SIFT[74].

Association testing

Single variants

We carried out single-variant association tests for transformed trait residuals with genotype dosages for variants with MAC≥3 assuming an additive genetic model. We used the EMMAX[75] linear mixed model approach, as implemented in EPACTS (v3.3.0; http://genome.sph.umich.edu/wiki/EPACTS), to account for relatedness between individuals. We used genotypes for sequenced variants with MAF≥1% to construct the genetic relationship matrix (GRM).

Conditioning on associated variants from prior GWAS

To differentiate association signals identified in this study from known association signals, for each trait we performed exome-wide association analysis conditioning on variants previously associated ($P<10^{-7}$) with that trait. We compiled a list of known variants for each trait from the EBI GWAS catalog (https://www.ebi.ac.uk/gwas/downloads; December 4, 2016 version), from recent papers, and from manuscripts in preparation of which we were aware[66,76-78]. The keywords from the GWAS catalog we used to assign known variants to each of our traits are listed in **Supplementary Table 16**. We also manually curated the associations from Inouye et al.[79] and Kettunen et al.[76] to attribute "blood metabolite" associations to the specific associated metabolites.

Using the combined HRC+exome panel (see above), we pruned each trait-specific list of associated variants ("GWAS variants") based on linkage disequilibrium (LD) (r^2>0.95). Twenty-three GWAS variants were not present in the HRC+exome panel. For 17 of these 23 variants, we identified a proxy (r^2>0.80) variant instead; we excluded the remaining six variants from conditional analysis. The list of variants included in conditional analysis for each trait is included in **Supplementary Table 17**. We extracted genotypes for variants used in conditional analysis from the integrated HRC+exome panel and converted dosages to alternate allele counts by rounding to the nearest integer (0, 1, or 2). We imputed missing genotypes for the 1,488 individuals without array data using the mean genotype dosage for purposes of conditional analysis.

For conditional analysis for each trait, we ran association analysis using the same linear mixed model approach as in unconditional analysis but including the complete set of pruned GWAS variants as covariates in the association test. Following conditional association, we further determined novelty based on absence of the variant from OMIM descriptions, ClinVar, and extensive literature searches.

Defining loci

The set of >1.4M variants tested for association includes variants in LD. To identify the number of distinct associations for each trait, we performed LD clumping using Swiss (https://github.com/welchr/swiss). We selected the subset of variants with (1) unconditional $P<5\times10^{-7}$ or (2) both unconditional and conditional $P<5\times10^{-5}$ for at least one trait. For each variant in this subset, we provided Swiss with the minimum unconditional p-value across all traits. The clumping procedure starts with the variant with the smallest p-value (index variant),

and merges into one locus all variants within ±1Mb that have r^2>0.5 with the index variant. The procedure is repeated iteratively until no variants remain. Trait associations with variants in the same locus are considered to represent the same signal and trait associations with variants in different loci to be distinct signals.

Gene-based testing

We carried out gene-based association tests using the mixed model implementation of SKAT-O[80], which tests for the optimal mixture of burden and dispersion-style multi-marker tests while adjusting for relatedness between individuals using the same GRM calculated for the single-variant tests. EMMAX and the mixed model version of SKAT-O (mmskat) are implemented in EPACTS.

We implemented gene-based tests using three different, but nested, sets of variants (variant "masks"):

(1) PTVs at any allele frequency with VEP annotations: frameshift_variant, initiator_codon_variant, splice_acceptor_variant, splice_donor_variant, stop_lost, stop_gained;

(2) PTVs included in (1) plus missense variants with MAF<0.1% scored as "damaging" or "deleterious" by all five functional prediction algorithms;

(3) PTVs included in (1) plus missense variants with MAF<0.5% scored as "damaging" or "deleterious" by all five functional prediction algorithms.

Each mask contained a different number of genes with at least two qualifying variants: up to 7,996, 12,795, and 12,890 for the three masks, respectively, with specific numbers depending on variation in sample size by trait. Using a Bonferroni-corrected exome-wide significance

threshold for 12,890 genes corresponds to a threshold of $P<3.88\times10^{-6}$. Similar to single variant association, we passed genes meeting this association threshold forward for additional consideration with hierarchical FDR correction as described below.

Hierarchical FDR correction for testing multiple traits and variants

In controlling for multiple testing our goal was to make sure that, by looking across 64 traits, we did not increase the proportion of falsely discovered variants. To accomplish this objective, we adopted a FDR controlling procedure described in Peterson et al.[81], which uses a hierarchical strategy to increase power while controlling type I error (**Supplementary Methods**). This procedure has two stages. Stage 1 identifies the set of R variants that are associated with at least one trait, controlling genome-wide FDR across all variants at 0.05. Stage 2 identifies all the traits that are associated with the discovered variants in a manner that guarantees an average FDR<0.05.

In Stage 1 we restricted ourselves to the R=531 variants that have an unconditional association $P<5\times10^{-7}$ with at least one trait. For these, we calculated a p-value for the hypothesis of no association between the variant and any of the 64 traits using Simes' rule[82], a combination rule that is robust to dependence between phenotypes. To account for the fact that we did an initial selection of these R variants from the total number of variants tested (T), we passed the Simes p-values to a Benjamini-Hochberg (BH) procedure that controls FDR at target level $0.05\times R/T$, a modification[83] which guarantees that the FDR in the set of S variants discovered to be associated with at least one trait is less than 0.05.

In stage 2, to determine which traits are associated with the set of the S selected variants we apply the Benjamini and Bogomolov[84] procedure. This procedure applies a multiplicity correction variant by variant, passing the 64 trait association p-values from each of the S selected variants and all 64 traits to a BH procedure that controls FDR at target level 0.05×S/T.

We applied this hierarchical correction to two different sets of results: the set of single-variant unconditional results and the set of gene-based test results. The gene-based tests used a threshold of $P<3.88\times10^{-6}$ to identify the R nominally significant genes in the first stage of the hierarchical procedure.

Genotype validation

We validated exome sequence-based genotype calls using Sanger sequencing for METSIM carriers of 13 trait-associated very rare variants with MAF<0.1% in seven genes. 107 of 108 (99.1%) non-reference genotypes were concordant.

Association replication in additional Finnish cohorts

We performed replication analysis of significant single-variant associations ($P<5\times10^{-7}$) and follow-up analysis of suggestive single-variant associations ($P<5\times10^{-5}$) in up to 24,776 individuals from three GWAS cohort studies: Northern Finland Birth Cohort 1966 (NFBC1966), the Helsinki Birth Cohort Study (HBCS), and FINRISK study participants not included in the exome sequencing portion of FinMetSeq.

A detailed description of the NFBC1966 study has been published previously and additional information is available at: http://www.oulu.fi/nfbc/node/18091[17]. The data used here, including

clinical measurements and blood samples for genetic and NMR metabolite analyses, were collected at the 31-year follow-up in 1997. NFBC1966 samples (n=5,139) were genotyped on the Illumina 370k array.

The HBCS includes participants born in Helsinki from 1934-1944 and has been described elsewhere[18]; a basic description is available at https://thl.fi/fi/web/thlfi-en/research-and-expertwork/projects-and-programmes/helsinki-birth-cohort-study-hbcs-idefix. HBCS samples (n=1,412) were genotyped on the Illumina 610k array.

The FINRISK cohort was described in detail above, and participants (replication n=18,125) were genotyped in several batches on the Illumina 610k, CoreExome, or OmniExpress arrays[15,16].

For each replication cohort, prior to phasing we performed genotype quality control batch-wise using standard quality thresholds for both sample-wise and variant-wise filtering: call rate>95%, HWE>10^{-6}, MAF>5%. We pre-phased array genotypes with Eagle[85] (v2.3) and imputed genotypes genome-wide with IMPUTE[86] (v2.3.1) using the SISu v2 reference panel consisting of 2,690 sequenced Finnish genomes and 5,092 sequenced Finnish exomes. Following imputation, we assessed imputation quality by confirming sex, comparing sample allele frequencies with reference population estimates, and examining imputation quality (INFO score) distributions. We excluded any variant with INFO<0.7 within a given batch from all replication/follow-up analyses.

For each of the three cohorts, we matched, harmonized, covariate adjusted, and transformed available phenotypes as described above for FinMetSeq. We used the same covariates as for

FINRISK. For each cohort, we ran single-variant association using the EMMAX linear mixed model implemented in EPACTS after generating kinship matrices from LD-pruned (command: plink --indep-pairwise 50 5 0.2) directly genotyped variants with MAF>5%.

Population genetic analyses

Identifying unrelated individuals

To identify a set of nearly independent common autosomal SNVs, we removed SNVs with MAF<5% and pruned the remaining SNVs in windows of 50 SNVs, in steps of 5 SNVs, such that no pair of SNVs had r^2>0.2. We used the resulting 26,036 SNVs to estimate pairwise relationships among the 19,292 exome-sequenced individuals using KING[87]. We then removed one individual from each of the 4,418 pairs inferred by KING to have a relationship of 3rd degree or closer, resulting in a set of 14,874 (nearly) unrelated individuals for population genetic analyses.

Identifying sub-population clusters in FinMetSeq

We first combined exome sequenced variants and a genome-wide set of 220,798 SNVs from GWAS arrays to provide a genome-wide backbone to aid in phasing and computing haplotype sharing. After removing variants with MAC<3, variants in known regions of long range LD[88] and variants with HWE<10^{-4}, we phased the remaining 764,696 variants using SHAPEIT[89] (version 2, r837). To assess the substructure in our dataset while minimizing the effect of mixing due to recent population mobility, we focused on the 2,644 unrelated individuals born by 1955 whose parents were both born in the same municipality (irrespective of the birth location of the individual).

We identified sub-populations of the 2,644 individuals using ChromoPainter (version 2) and fineSTRUCTURE[90] (version 2.0.8). We first used ChromoPainter to generate a pairwise co-ancestry matrix, which represents each individual's DNA as a count of haplotype blocks copied from every other individual in the dataset. Following previous practices[91], for computational efficiency, we estimated and fixed the switch and global emission rates as input for ChromoPainter on a subset of the data; cluster inference is known to be robust to up to 10-fold deviations of the estimated switch and emission rates[92]. For further computational speedup, we generated an initial clustering by applying a normal mixture model clustering[93] (mclust package in R, version 5.1) to the top ten principal components of the coancestry matrix and used this initial cluster solution as seed to the fineSTRUCTURE analysis. We conducted 1 million Markov chain Monte Carlo (MCMC) iterations retaining one sample for every 1,000 iterations after discarding 3 million iterations as burn-in. After MCMC, we used fineSTRUCTURE to perform *post-hoc* refinement of cluster membership; we started with the MCMC sample with the highest posterior probability and reassigned membership, taking into account the cluster membership at each of the recorded MCMC samples[92].

In total, we ran five MCMC chains using fineSTRUCTURE, retaining the configuration with highest posterior probability for further analysis. We confirmed convergence of the fineSTRUCTURE MCMC runs by calculating Geweke's convergence diagnostic using the coda package (version 0.18) in R to compare the number of inferred clusters in the first 10% and last 50% of the MCMC chain, and visual inspections of the general consistency of cluster memberships between independent MCMC chains. In total, we inferred 245 sub-population clusters among the 2,644 individuals.

We inspected the initial clustering solution from fineSTRUCTURE by examining for each individual the estimated proportion of their haplotype length derived from each of the inferred clusters using non-negative least squares[92,94]. This approach showed many individuals derived a substantial proportion of their haplotype length not from the cluster initially assigned by fineSTRUCTURE, but instead from a different but related sub-cluster on the fineSTRUCTURE hierarchical clustering tree, suggesting redundancy in fineSTRUCTURE-inferred clusters. We therefore combined related clusters by successively merging pairs of clusters that resulted in the smallest decrease in the posterior probability of the fineSTRUCTURE hierarchical clustering tree. At each merge, we reorganized individuals into merged cluster memberships and re-estimated the haplotype-sharing profile for each individual. We iteratively merged the hierarchical tree until ≥90% of individuals were assigned to the cluster where they also derive the highest proportion of haplotype sharing, resulting in 16 clusters for the 2,644 reference individuals, each named based on the most common parental birthplaces of its members (**Supplementary Table 13**).

Enrichment of predicted functionally deleterious alleles in Finland

We assessed enrichment of predicted functionally deleterious alleles in Finland by comparing the 14,874 nearly unrelated FinMetSeq individuals to the 33,370 NFE in ExAC. We analyzed sites with base quality score >10, mapping quality score >20, and coverage equal to or greater than that found in ≥80% of variable sites (25.55X in FinMetSeq, 29.95X in ExAC NFE), resulting in ~33.8Mbp for comparisons. We considered only the two most common alleles at each site. We contrasted the proportional site frequency spectra for FinMetSeq and NFE for three functional variant categories (PTVs, missense, synonymous) after accounting for sample size differences between datasets by down-sampling both datasets to 28,000 chromosomes.

We also assessed the enrichment of functional alleles within subpopulations of the FinMetSeq dataset. Of the 16 sub-population clusters identified by fineSTRUCTURE, we used as the reference population a cluster for which the highest proportion of the parents of its members were born in the southwestern, "early-settlement" part of Finland (NSv3, **Supplementary Table 13**). Twelve of the remaining 15 clusters also have >100 members, and were used in subsequent analyses (**Supplementary Table 13**). We then compared the ratio of the site frequency spectra to the reference for the same three functional variant categories, again down-sampling both datasets to 200 haploid chromosomes to account for sample size differences. For a given comparison, we computed statistical evidence for enrichment or depletion at a given allele count bin by exact binomial test against a null of equal number of variants found in both the test and reference cluster.

Geographical clustering of predicted functionally deleterious alleles

We first generated a distance matrix tabulating the pairwise geographical distance in kilometers between the birthplaces of all available parents of unrelated sequenced individuals. For each variant of interest, we computed for the minor allele carriers in FinMetSeq the mean distance among all parent pairs. For example, for a variant with three carriers with information for five (of the possible six) parents, we computed the mean for all (5-choose-2 = 10) distances. We evaluated statistical significance of geographical clustering by comparing the mean distance to the means for up to 10,000,000 sets of randomly drawn non-carrier individuals matched by cohort status and number of parents with birthplace information available.

To assess whether PTVs or missense variants may be more geographically clustered than synonymous variants, we first identified a set of near-independent variants (r^2>0.02) with MAC≥3 and MAF≤5% among the 14,874 unrelated individuals. This set included 4,312 PTVs, 91,851 missense variants, and 49,842 synonymous variants. For each variant, we computed the mean pairwise geographical distance in kilometers between the birthplaces across all pairs of the available parents of carriers of the minor allele and regressed this mean distance on variant class (PTVs, missense, or synonymous) and MAC, MAC^2, and MAC^3 (**Supplementary Table 12**).

We also assessed whether variants showing stronger enrichment (compared to NFE) are more likely to be geographically clustered. Starting with the three functional classes of variants identified above, we further restricted analysis to those variants found in ExAC so we could calculate the enrichment in frequency over ExAC NFE. We included 2,539 PTVs, 67,860 missense, and 39,605 synonymous variants in this analysis after pruning variants for LD with PLINK. As above, we computed the mean pairwise distances among parents of carriers of the minor allele and regressed mean distance on the logarithm of enrichment and MAC, MAC^2, and MAC^3 (**Supplementary Table 14**). In both analyses, we first assessed a model with the interaction terms but reported only the model without interactions if the interactions were not significant.

Heritability estimates and genetic correlations

We used genome-wide array genotype data on the 13,326 unrelated individuals for whom both exome sequence and array data were available to estimate heritability and genetic correlations for the 64 traits. We constructed a GRM with PLINK[95] (v.1.90b, https://www.cog-genomics.org/plink2) by applying additional filters for MAF>1% and genotype missingness rate

<2% to the set of previously-used genotyped SNVs, leaving 205,149 SNVs for GRM calculation. We used the exact mixed model approach of biMM[96] (v.1.0.0, http://www.helsinki.fi/~mjxpirin/download.html) to estimate the heritability of our 64 traits and the genetic correlation of the 2,016 trait pairs.

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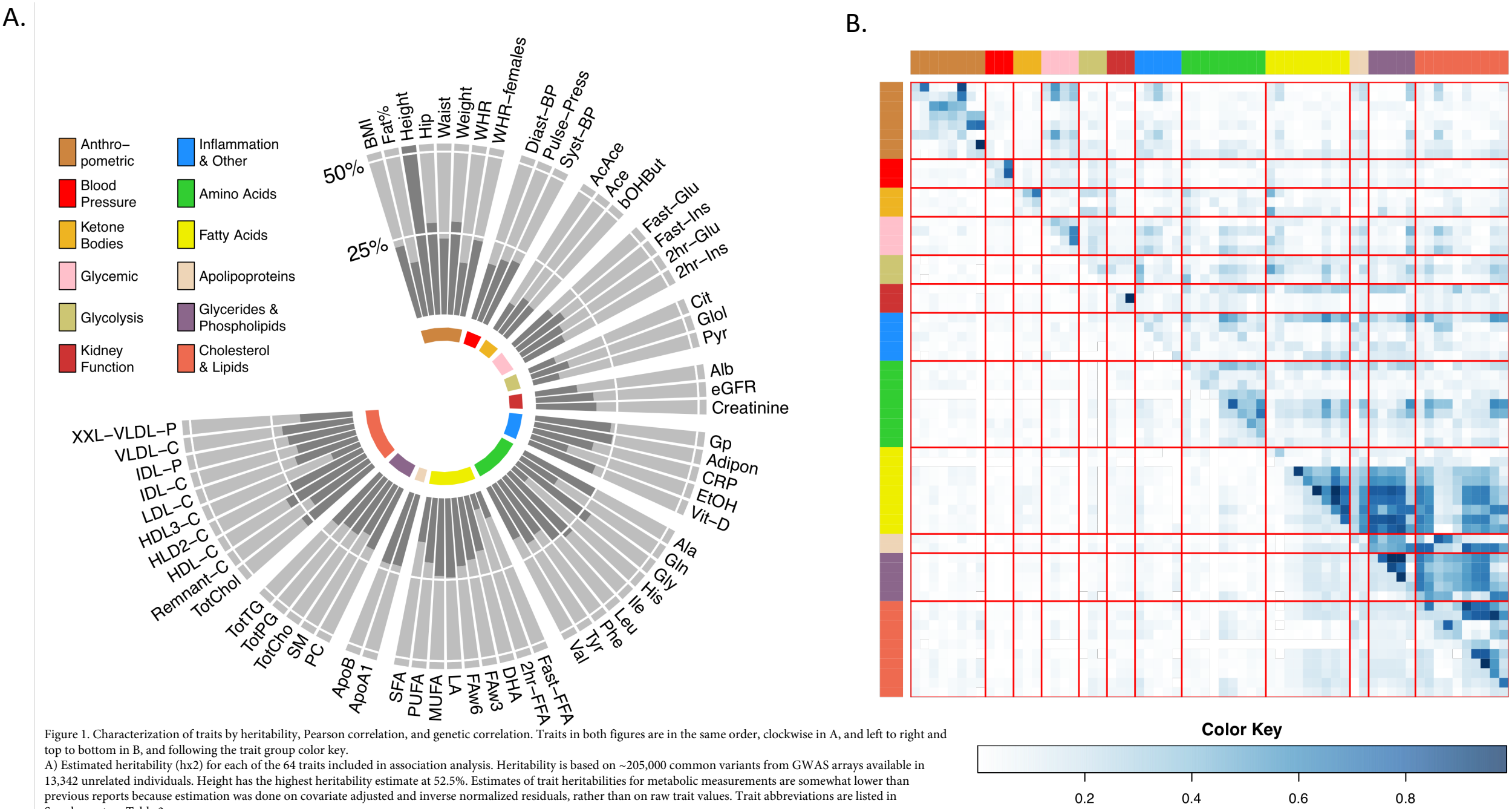


Figure 1. Characterization of traits by heritability, Pearson correlation, and genetic correlation. Traits in both figures are in the same order, clockwise in A, and left to right and top to bottom in B, and following the trait group color key.

A) Estimated heritability (hx2) for each of the 64 traits included in association analysis. Heritability is based on ~205,000 common variants from GWAS arrays available in 13,342 unrelated individuals. Height has the highest heritability estimate at 52.5%. Estimates of trait heritabilities for metabolic measurements are somewhat lower than previous reports because estimation was done on covariate adjusted and inverse normalized residuals, rather than on raw trait values. Trait abbreviations are listed in Supplementary Table 3.

B) Heatmap of: 1) absolute Pearson correlations of standardized trait values in upper triangle, 2) estimated heritability (hx2) on the diagonal, and 3) absolute values of rhoG(x,y)(hx2,hy2) in lower triangle, where rhoG(x,y) is the estimated genetic correlation of traits x and y.

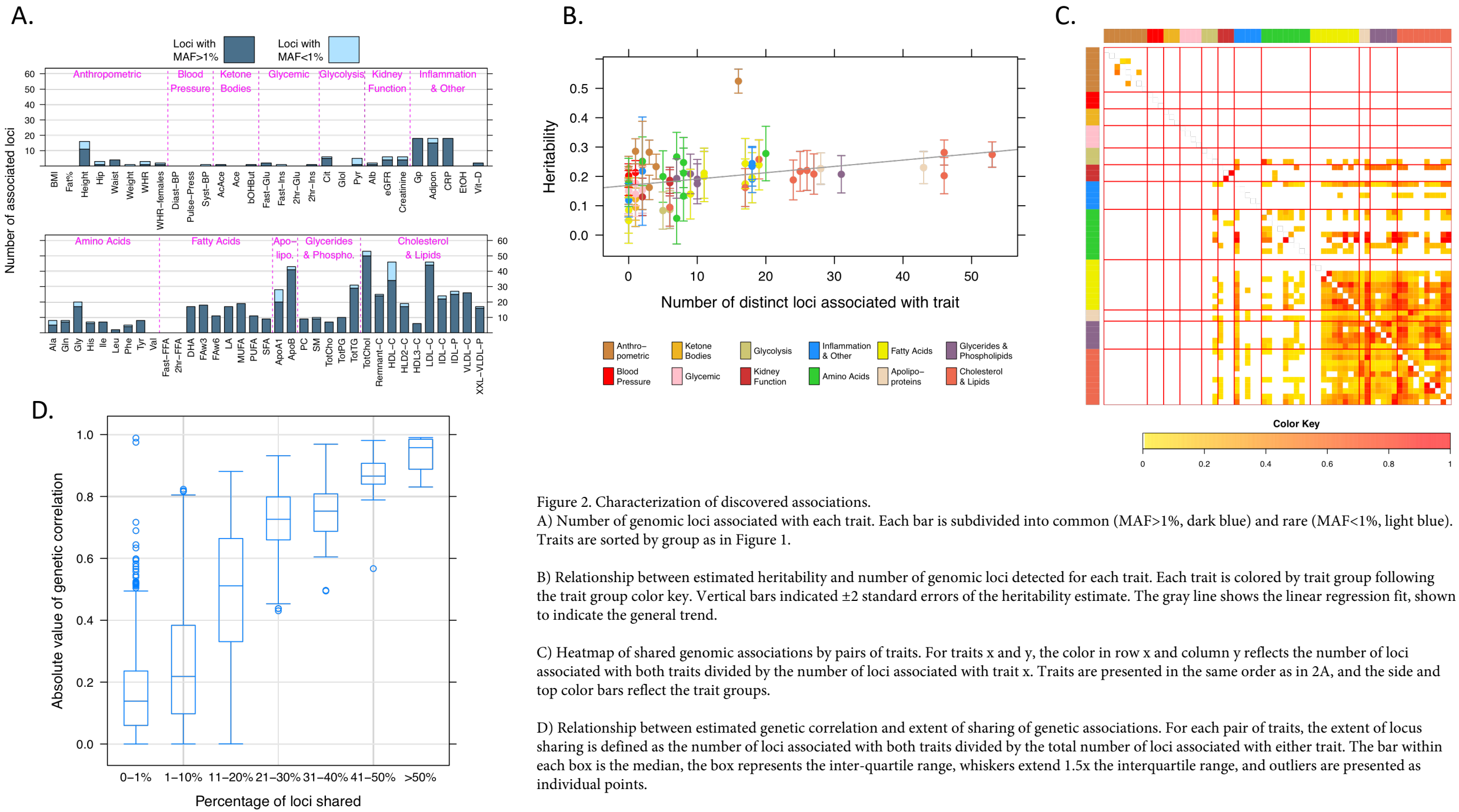


Figure 2. Characterization of discovered associations.

A) Number of genomic loci associated with each trait. Each bar is subdivided into common (MAF>1%, dark blue) and rare (MAF<1%, light blue). Traits are sorted by group as in Figure 1.

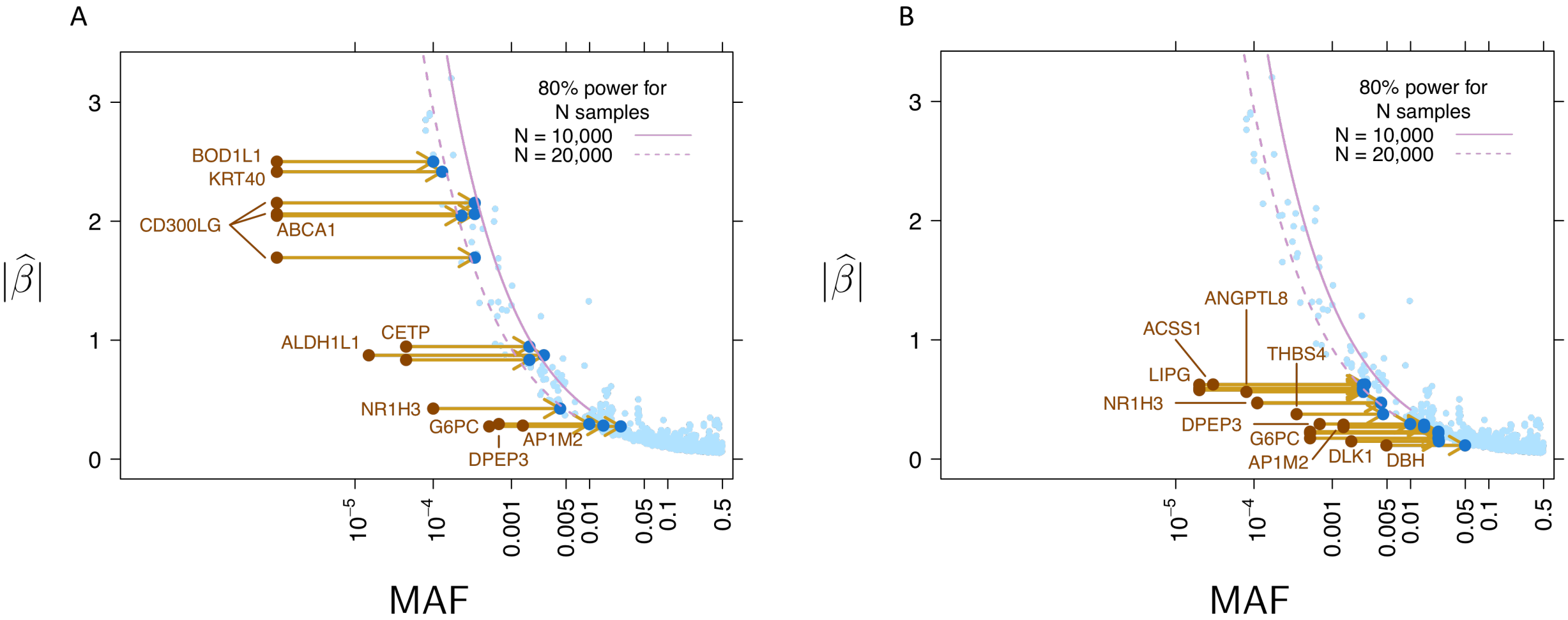
B) Relationship between estimated heritability and number of genomic loci detected for each trait. Each trait is colored by trait group following the trait group color key. Vertical bars indicated ±2 standard errors of the heritability estimate. The gray line shows the linear regression fit, shown to indicate the general trend.

C) Heatmap of shared genomic associations by pairs of traits. For traits x and y, the color in row x and column y reflects the number of loci associated with both traits divided by the number of loci associated with trait x. Traits are presented in the same order as in 2A, and the side and top color bars reflect the trait groups.

D) Relationship between estimated genetic correlation and extent of sharing of genetic associations. For each pair of traits, the extent of locus sharing is defined as the number of loci associated with both traits divided by the total number of loci associated with either trait. The bar within each box is the median, the box represents the inter-quartile range, whiskers extend 1.5x the interquartile range, and outliers are presented as individual points.

Figure 3. Allelic enrichment in the Finnish population and its effect on genetic discovery.
A) Relationship between MAF and estimated effect size for associations discovered in FinMetSeq exomes alone. Each variant reaching significance in FinMetSeq is plotted. Those associations highlighted in Table 2A are represented with a dark blue point (FinMetSeq MAF) and a corresponding brown point reflecting the NFE MAF (ExAC or gnomAD). The purple lines indicate the 80% power curves for significance at 5x10-7 for sample sizes of 10,000 and 20,000. Plots show the dramatic increase in power due to higher relative frequency in Finland.

B) Relationship between MAF and estimated effect size for associations discovered in 4-cohort meta-analysis. Same plot as in A, highlighting the variants in Table 2B only reaching significance in the 4-study meta-analysis.



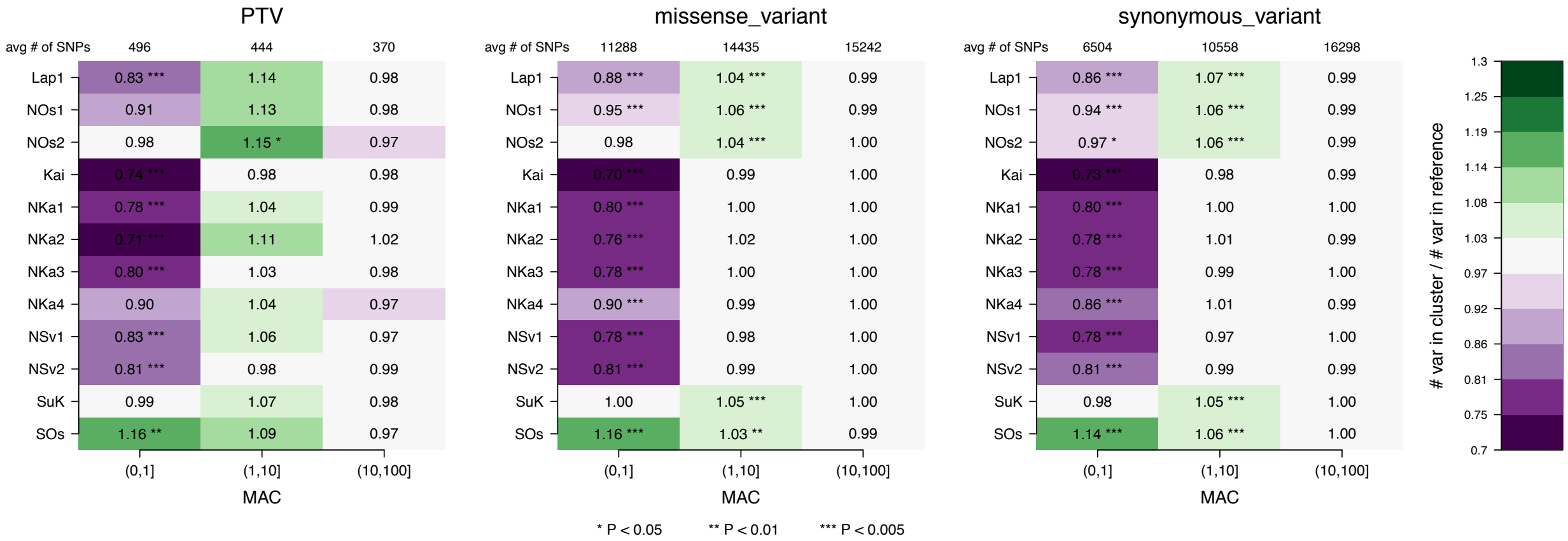
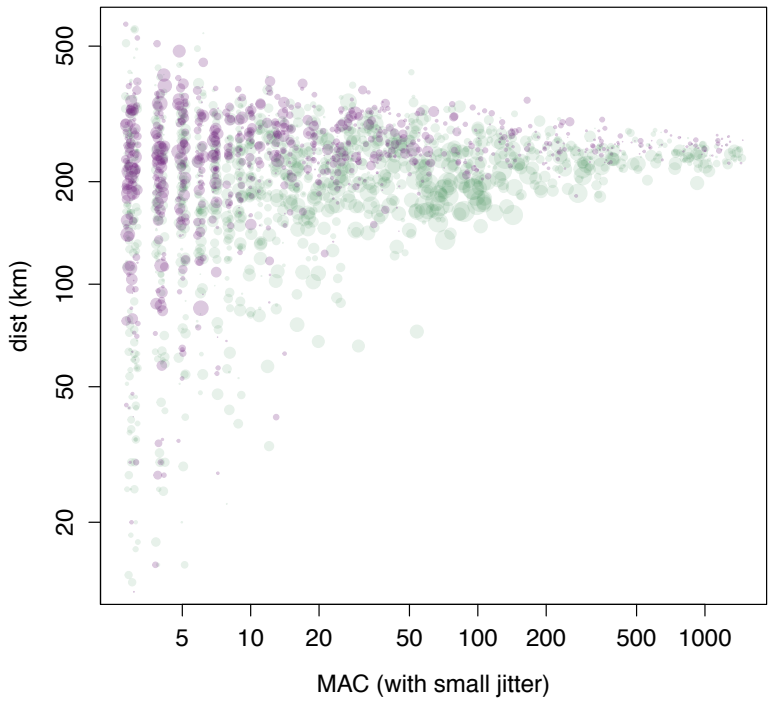


Figure 4. Regional variation in allele frequencies by functional annotation. Enrichment of functional allelic class in sub-populations (regions) of Northern and Eastern Finland. For each minor allele count bin, we computed the ratio of number of variants found in each subpopulation to an internal reference subpopulation (NSv3). Green cells represent an enrichment (ratio >1), purple cells represent a depletion (ratio <1). The 12 sub-populations with sample size >100 are shown. The frequency spectra were down-sampled to 200 chromosomes for comparisons. The results are consistent with multiple independent bottlenecks followed by subsequent drift in Northern and Eastern Finland, particularly for populations in Lapland and Northern Ostrobothnia. Abbreviations for regions: Kainuu (Kai), Lapland (Lap1, Lap2), Northern Karelia (NKa1, NKa2, NKa3, NKa4), Northern Ostrobothnia (NOs1, NOs2, NOs3, NOs4), Northern Savonia (NSv1, NSv2, NSv3), Southern Ostrobothnia (SOs), and Surrendered Karelia (SuK). For more detailed information on region definitions see Supplementary Table S13.

A.



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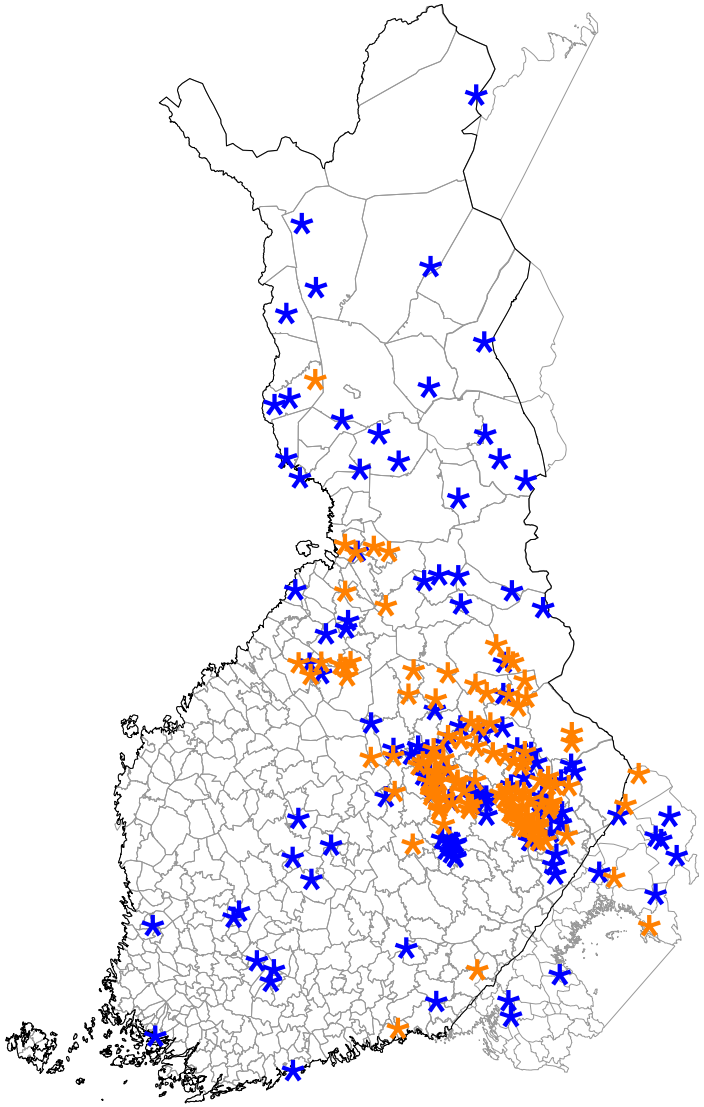

C.

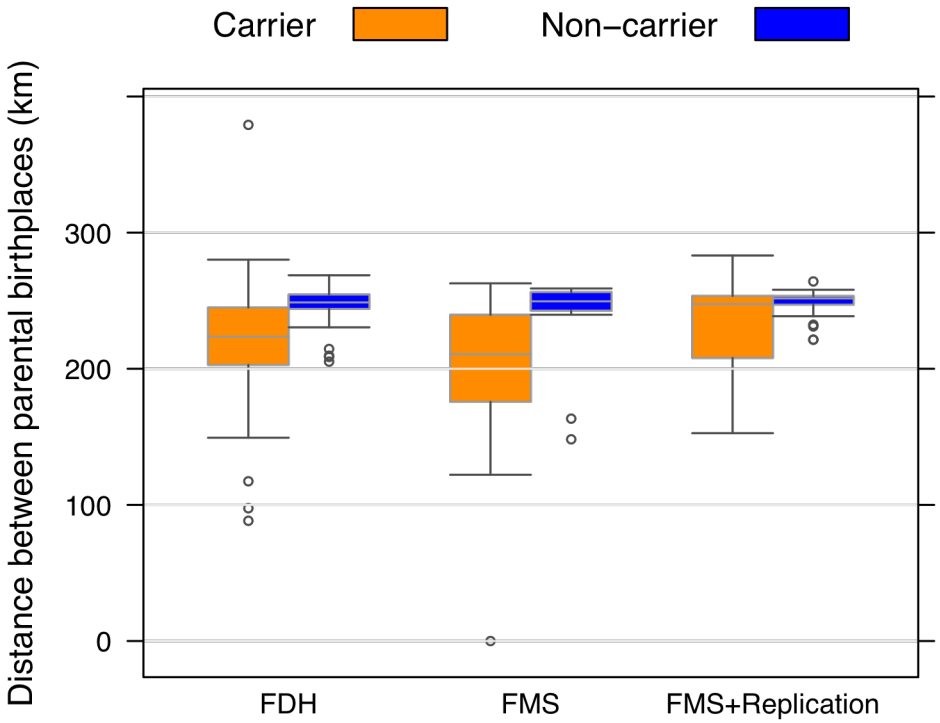

Figure 5. Geographical clustering of associated variants.
A) Geographical clustering of PTV as a function of MAC and fold frequency enrichment over ExAC NFE. For each PTV (r2≤0.02, MAC≥3, MAF≤0.05) we computed the mean distance between birth places of available parents of all carriers of the minor allele. We compared the frequency of the minor allele in FinMetSeq to ExAC NFE. Purple and green colors denote the frequency is lower or higher in FinMetSeq than in ExAC NFE, respectively. The size of the point is proportional to the logarithm of the fold frequency difference. In general, we observe that variants enriched in FinMetSeq are more geographically clustered.

B) Example of geographical clustering for a trait associated variant. The birth locations of all parents of carriers (orange) and a matching number of parents of non-carriers (blue) of the minor allele for variant chr3:125831672 (rs780671030, p.Arg722X) in ALDH1L1 are displayed on a map of Finland. This variant is associated with serum glycine levels in FinMetSeq and shows a 173x enrichment compared to NFE in ExAC. The parents of carriers are born on average 135 km apart, the parents of non-carriers on average 250 km apart (P<10-7 by permutation).

C) Comparison of geographical clustering between Finnish Disease Heritage (FDH) mutations and trait-associated variants that are >10x more frequent in FinMetSeq than in NFE. The degree of geographical clustering (based on parental birthplace) is comparable between carriers of those variants that showed significant associations in FinMetSeq alone (FMS) and carriers of FDH mutations, and greater than that seen in carriers of variants that showed significant association only in the 4-cohort meta-analysis (FMS+Replication). For all variants, carriers of the minor allele displayed greater clustering than non-carriers. The bar within each box is the median, the box represents the inter-quartile range, whiskers extend 1.5x the interquartile range, and outliers are presented as individual points.