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Genome-wide analysis of health-related biomarkers in the UK Household Longitudinal Study reveals novel associations

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Serum biomarker levels are associated with the risk of complex diseases. Here, we aimed to gain insights into the genetic architecture of biomarker traits which can reflect health status. We performed genome-wide association analyses for twenty serum biomarkers involved in organ function and reproductive health. 9,961 individuals from the UK Household Longitudinal Study were genotyped using the Illumina HumanCoreExome array and variants imputed to the 1000 Genomes Project and UK10K haplotypes. We establish a polygenic heritability for all biomarkers, confirm associations of fifty-four established loci, and identify five novel, replicating associations at genome-wide significance. A low-frequency variant, rs28929474, (beta = 0.04, P = 2 \times 10^{-10}) was associated with levels of alanine transaminase, an indicator of liver damage. The variant is located in the gene encoding serine protease inhibitor, low levels of which are associated with alpha-1 antitrypsin deficiency which leads to liver disease. We identified novel associations (rs78900934, beta = 0.05, P = 6 \times 10^{-12}; rs2911280, beta = 0.09, P = 6 \times 10^{-10}) for dihydroepiandrosterone sulphate, a precursor to major sex-hormones, and for glycated haemoglobin (rs12819124, beta = −0.03, P = 4 \times 10^{-9}; rs761772, beta = 0.05, P = 5 \times 10^{-9}). rs12819124 is nominally associated with risk of type 2 diabetes. Our study offers insights into the genetic architecture of well-known and less well-studied biomarkers.

Serum biomarker levels are associated with the risk of complex diseases and are therefore increasingly used in clinical practice to assist with diagnosis, status monitoring and disease management. Well-known examples include the measurement of lipid levels in the context of cardiovascular disease or liver enzymes and albumin to assess liver function.

Serum biomarker levels have a polygenic basis. As demonstrated in the case of lipids, identifying genetic associations can provide new insights into disease aetiology which can in turn guide drug discovery and be useful for diagnosis and risk stratification1–4. However, the genetic architecture of most health-related biomarkers has not been studied as extensively as for lipids. Alleles identified to be associated with protein biomarkers to date are predominantly common (minor allele frequency (MAF) >5%). This is primarily driven by genotyping technology and composition of arrays or imputation reference panels used to date4–6. Systematically evaluating the association of low frequency and rare variants can provide new insights regarding the genetic architecture of protein biomarkers.

The importance of studying the joint impact of genetic and non-genetic factors on health has been recognised by the UK Household Longitudinal Study (UKHLS, www.understandingsociety.ac.uk), also known as Understanding Society. Involving a total of 40,000 households representative of the UK population, UKHLS is the largest panel survey in the world to support social research. A wide range of social, economic, environment,
| Variable                  | units   | Female                     | Male                      |
|---------------------------|---------|----------------------------|---------------------------|
| Age                       | years   | 0 5574 52.1                | 25 16 99 4387 52.82      |
| BMI                       |         | 285 5416 28.02 7.4        | 14.5 75.7 4260 28.09 5.6 |
| Albumin                   | G/L     | 137 5501 46.24            | 4 36 57 4323 47.48 4     |
| Alkaline Phosphatase      | lu/L    | 228 5451 70.99 26         | 22 191 4282 71.86 24     |
| Alanine Transaminase      | lu/L    | 230 5458 23.66 10         | 5 152 4273 32.23 16      |
| Aspartate Transaminase    | lu/L    | 498 5321 28.09 8          | 13 84 4142 32.12 9       |
| Fibrinogen                | G/L     | 199 5468 2.87 0.7         | 1.5 5.2 4294 2.76 0.7   |
| Total Cholesterol         | Mmol/L  | 144 5495 5.49 1.5         | 2.2 10 4322 5.29 1.6    |
| Dihydroepiandrosterone Sulphate | Umol/L  | 239 5414 3.76 3.3        | 0.4 19 4308 5.67 4.9    |
| Creatinine                | Umol/L  | 158 5497 68.27 14         | 33 173 4306 85.9 17     |
| Gamma Glutamyl Transferase| lu/L    | 214 5467 27.33 16         | 5 382 4280 39.75 25     |
| Glycated haemoglobin      | Mmol/mol| 525 5288 36.05 6          | 15 57 4148 36.56 6     |
| HDL cholesterol           | Mmol/L  | 165 5482 1.68 0.5         | 0.5 3.4 4314 1.37 0.5   |
| Haemoglobin               | G/L     | 294 5392 130.49 13        | 82 174 4275 145.62 14   |
| C-Reactive Protein (hs assay) | Mg/L    | 420 5350 3.53 3           | 0.2 115.5 4191 3        |
| Insulin-like growth factor 1 | Nmol/L  | 229 5455 17.74 8          | 2 47 4277 18.43 8      |
| Ferritin                  | G/L     | 143 5499 92.93 82         | 3 1292 4319 189.11 143  |
| Testosterone (for males only) | Nmol/L  | 5702 NA NA NA NA NA 4259 15.59 7.3 |
| Triglycerides             | Mmol/L  | 216 5482 1.58 1          | 0.3 6.3 4263 1.99 1.3  |
| Urea                      | Mmol/L  | 143 5498 5.94 2          | 2 16.5 4320 6.53 2     |

Table 1. Descriptive statistics for the sample and the measured biomarkers. *IQR = inter quartile range.

Heritability and genetic overlap analyses. For all biomarkers except overall and LDL-cholesterol, alanine transaminase and ferritin there was significant \( (p < 3.6 \times 10^{-5}) \) evidence for a heritable polygenic component (Table 2). Alkaline phosphatase and testosterone had the highest array heritability estimates with \( h^2 = 27.7\% \) (standard error (SE): 0.040) and \( h^2 = 27.1\% \) (SE: 0.084), respectively. Creatinine, GGT, HbA1c, HDL, IGF1, and triglycerides all had estimates larger than 0.20 while the lowest estimate was observed for ferritin \( (h^2 = 61.9\%, SE: 0.037) \). We found statistically significant \( (p < 5.5 \times 10^{-4}) \) evidence of genome-wide pleiotropy between different biomarkers (Fig. 1). There was genetic correlation between lipid biomarkers: triglyceride and HDL-cholesterol levels (genetic correlation \( r_g = -0.67, p = 9.9 \times 10^{-18} \)). Triglyceride levels were also inversely genetically linked with DHEAS \( (r_g = -0.53, p = 4.0 \times 10^{-4}) \). The genetic correlation between two markers of inflammation, C-reactive protein and fibrinogen, was also significant \( (p = 0.60, p = 3.2 \times 10^{-8}) \). Finally, the genetic factors for creatinine and urea were positively correlated \( (r_g = 0.56, p = 1.2 \times 10^{-8}) \).

Genome-wide association analyses. The genome-wide significance threshold of \( P < 3.56 \times 10^{-8} \) for this study was derived by taking the conventional genome-wide significance threshold \( (P < 5 \times 10^{-8}) \) divided by the effective number of independent traits analysed \( (N = 14.05, details in Methods) \). Across fifteen biomarkers, we observed associations of 54 previously reported loci at this threshold (Fig. 2). This includes a low frequency variant, rs148685782 at 4q31 in the fibrinogen gamma chain gene (weighted effect allele frequency [WEAF] = 0.4%, beta[SE] = -0.18[0.02], \( P = 4.0 \times 10^{-21} \)), associated with levels of fibrinogen, a glycoprotein that assists in the
blood clot formation. This variant is a missense mutation and has been previously reported to be associated with fibrinogen levels as well as with hypofibrinogenemia and haemorrhage.

From the discovery phase we carried forward 573 independent (pairwise $r^2 < 0.01$) variants that were associated with biomarker levels at $P < 1 \times 10^{-5}$ and were located more than 500 kb away from any known index variant for the respective biomarker. Using data from up to 25,897 samples from 4 independent studies (Supplementary Table S1), five loci provided evidence of replication and reached $P < 3.6 \times 10^{-9}$ for the combined analysis of discovery and replication data (Table 3).

rs28929474 at 14q32 (WEAF = 2%, beta[SE] = 0.04[0.01], $P = 1.7 \times 10^{-10}$), a low-frequency variant associated with alanine transaminase (ALT), resides in the serpin family A member 1 (SERPINA1) gene (Figs 3A and 4).

| biomarker name             | $h^2$  | standard error | $p$-value |
|----------------------------|--------|----------------|-----------|
| Albumin                    | 0.15   | 0.04           | $8.9 \times 10^{-6}$ |
| Alkaline Phosphatase       | 0.28   | 0.04           | $2.2 \times 10^{-13}$ |
| Alanine Transaminase       | 0.09   | 0.04           | $6.8 \times 10^{-7}$ |
| Aspartate Transaminase     | 0.09   | 0.04           | $2.9 \times 10^{-3}$ |
| Fibrinogen                 | 0.17   | 0.04           | $6.5 \times 10^{-6}$ |
| Total Cholesterol          | 0.07   | 0.04           | 0.023     |
| Dihydroepiandrosterone Sulphate | 0.17 | 0.04           | $4.7 \times 10^{-6}$ |
| in men                     | 0.14   | 0.08           | 0.045     |
| in women                   | 0.20   | 0.07           | $1.6 \times 10^{-3}$ |
| Creatinine                 | 0.21   | 0.04           | $5.2 \times 10^{-9}$ |
| eGFR                       | 0.12   | 0.04           | $9.0 \times 10^{-4}$ |
| Gamma Glutamyl Transferase | 0.22   | 0.04           | $2.4 \times 10^{-9}$ |
| Glycated haemoglobin       | 0.22   | 0.04           | $2.8 \times 10^{-9}$ |
| HDL cholesterol            | 0.23   | 0.04           | $5.9 \times 10^{-10}$ |
| LDL cholesterol            | 0.08   | 0.04           | 0.013     |
| Haemoglobin                | 0.17   | 0.04           | $5.5 \times 10^{-7}$ |
| C-Reactive Protein (hs assay) | 0.16 | 0.04           | $1.1 \times 10^{-5}$ |
| Insulin-like growth factor 1 | 0.20  | 0.04           | $4.6 \times 10^{-9}$ |
| Ferritin                   | 0.06   | 0.04           | 0.043     |
| Testosterone (for males only) | 0.27 | 0.08           | $4.8 \times 10^{-4}$ |
| Triglycerides              | 0.23   | 0.04           | $3.6 \times 10^{-10}$ |
| Urea                       | 0.14   | 0.04           | $2.1 \times 10^{-4}$ |

Table 2. Array heritability ($h^2$) estimates and standard errors for 20 biomarkers.
which is involved in a number of biological processes including signal transduction\(^{13}\), inflammation\(^{14}\) and apoptosis\(^{15}\), as well as whole blood in the GTEx database\(^{17}\).

The index variant at 12q13, rs12819124 (WEAF\(^{7}\)) lies in an intron of the gene encoding c-Maf inducing protein\(^{19}\) (CMIP\(^{7}\)), the product of which is involved in a number of biological processes including signal transduction\(^{19}\), inflammation\(^{14}\) and apoptosis\(^{15}\). At the second novel locus associated with DHEAS the index variant, rs2911280 at 16q13 (WEAF\(^{7}\)) lies within a non-coding exon in the transmembrane channel-like 6 (TM6C) gene and has been shown to affect the expression of TM6C, as well as TNRG6C antisense RNA 1 (TNRG6C-AS1) and transmembrane channel like 8 (TM8C), in cardiac, thyroid, and vascular tissue, as well as whole blood in the GTEx database\(^{17}\).

\(\text{SERPIN1A1}\) encodes alpha-1-antitrypsin (AAT), which is a serine protease inhibitor produced in the liver\(^{11}\). Low levels of this protein are the hallmark of a genetic disorder called alpha-1 antitrypsin deficiency (A1AD), which leads to liver disease\(^{12}\).

We identified two novel replicating associations for DHEAS (Fig. 3B,C). DHEAS is the sulphated form of DHEA, a precursor to major sex-hormones such as testosterone and oestrogen, and is synthesized in the adrenal glands. It is an important marker of adrenal gland function. rs78900934 at chromosome 1p21 (WEAF\(^{30,9}\), \(\beta[SE] = 0.05[0.01], P = 5.9 \times 10^{-12}\)) is located 1 kb upstream of a pseudogene, peptidylprolyl isomerase A pseudogene 7 (PPIAP7). This gene shows a high degree of similarity to cyclophilin A (PPLA), the product of which is involved in a number of biological processes including signal transduction\(^{15}\), inflammation\(^{14}\) and apoptosis\(^{15}\). The second novel locus associated with DHEAS the index variant, rs2911280 at 16q13 (WEAF\(^{7}\)) lies in an intron of the gene encoding c-Maf inducing protein (CMIP\(^{7}\)), thought to play a role in the T-cell signalling pathway\(^{19}\).

Two novel replicating associations with HbA1c levels were identified (Fig. 3D,E). HbA1c represents the three-month average plasma glucose concentration and is used to diagnose as well as manage type 2 diabetes. The index variant at 12q13, rs12819124 (WEAF\(^{46,7}\%), \beta[SE] = -0.03[0.01], P = 4.2 \times 10^{-7}\) lies in an intron of RP1-228F16.4, a long non-coding RNA. The index variant of the second novel locus, rs761772 at 17q25 (WEAF\(^{12,4}\%), \beta[SE] = 0.05[0.01], P = 4.9 \times 10^{-9}\), lies within a non-coding exon in the transmembrane channel-like 6 (TM6C) gene and has been shown to affect the expression of TM6C, as well as TNRG6C antisense RNA 1 (TNRG6C-AS1) and transmembrane channel like 8 (TM8C), in cardiac, thyroid, and vascular tissue, as well as whole blood in the GTEx database\(^{17}\).

**Discussion**

We identify five new biomarker loci, across common and low frequency variants, associated with DHEAS, HbA1c and ALT. We demonstrate polygenic heritability of the majority of biomarkers included in this study and observe large differences in their polygenic component. To our knowledge this is the first report of SNP-based heritability estimates for DHEAS, insulin-like growth factor 1, testosterone and urea. The large sample set with homogeneous biomarker measurements afforded by UKHLS enables reliable estimation for this population. We also identify genetic correlations between several of the biomarkers. Genetic correlation between two traits is an indicator of shared genetic factors and consequently genome-wide pleiotropy. The patterns of heritability and genetic correlations between several of the biomarkers. Genetic correlation between two traits is an indicator of shared genetic factors and consequently genome-wide pleiotropy. The patterns of heritability and genetic correlations between several of the biomarkers. Genetic correlation between two traits is an indicator of shared genetic factors and consequently genome-wide pleiotropy. The patterns of heritability and genetic correlations between several of the biomarkers. Genetic correlation between two traits is an indicator of shared genetic factors and consequently genome-wide pleiotropy. The patterns of heritability and genetic correlations between several of the biomarkers. Genetic correlation between two traits is an indicator of shared genetic factors and consequently genome-wide pleiotropy. The patterns of heritability and genetic correlations between several of the biomarkers. Genetic correlation between two traits is an indicator of shared genetic factors and consequently genome-wide pleiotropy. The patterns of heritability and genetic correlations between several of the biomarkers. Genetic correlation between two traits is an indicator of shared genetic factors and consequently genome-wide pleiotropy. The patterns of heritability and genetic correlations between several of the biomarkers. Genetic correlation between two traits is an indicator of shared genetic factors and consequently genome-wide pleiotropy. The patterns of heritability and genetic correlations between several of the biomarkers. Genetic correlation between two traits is an indicator of shared genetic factors and consequently genome-wide pleiotropy. The patterns of heritability and genetic correlations between several of the biomarkers. Genetic correlation between two traits is an indicator of shared genetic factors and consequently genome-wide pleiotropy. The patterns of heritability and genetic correlations between several of the biomarkers. Genetic correlation between two traits is an indicator of shared genetic factors and consequently genome-wide pleiotropy. 

Finally, the genetic correlation we observe between creatinine and urea is a previously unreported association.
and highly biologically plausible finding as both markers are increased in blood when glomerular filtration rate declines, reflecting impaired kidney function. Characterising the genetic architecture of health-related biomarkers in this way is informative with respect to their biology as well as the design of future association studies. While each known locus individually explains only a small proportion of the variance in biomarker levels, these analyses demonstrate that the joint effect of many variants can be much larger.

We examined less-well studied health-related biomarkers in addition to routine blood measures used in clinical practice. This made it possible to identify novel associations of common and low frequency variants with DHEAS, HbA1c and ALT. These associations could provide novel biological insights. rs2911280, which we found to be associated with DHEAS, is located in an intron of the gene encoding c-Maf inducing protein (CMIP). CMIP is a highly pleiotropic gene, and is associated with several metabolic traits such as adiponectin and HDL cholesterol levels. Cholesterol is a precursor of DHEA in its synthesis process.

rs28929474 at 14q32 is associated with levels of ALT, which is used in clinical practice to assess liver damage. This variant is located in SERPINA1, encoding the serine protease inhibitor alpha-1-antitrypsin (AAT), which is largely produced in the liver. Mutations of this gene can cause alpha-1-antitrypsin deficiency (A1AD) which can lead to an accumulation of aberrant proteins in hepatocytes causing liver damage. This in turn may elevate levels of ALT, warranting future assessment of the association between this signal and liver-related clinical endpoints.

We identify two novel associations with HbA1c levels. In a lookup using published data from an independent large-scale meta-analysis by the MAGIC consortium, rs12819124 was associated with HbA1c levels with P = 0.04 (0.01), 1.47 × 10^-10. The direction of effect was consistent with our findings. rs12819124 was also nominally associated with risk of type 2 diabetes at P = 0.025 using data from the DIAGRAM study. Moreover, association results from published cohorts suggest a possible pleiotropic association with mental disorders and wellbeing (P = 9.0 × 10^-6 for bipolar disorder and schizophrenia, P = 6.4 × 10^-5 for subjective wellbeing). No HbA1c association results were available for rs761772 in MAGIC. For a proxy SNP, rs429216 (r^2 = 0.92) was associated with HbA1c levels.

### Methods

#### Ethics

Participants gave informed written consent for their blood to be taken and stored for future scientific analysis. The UKHLS has been approved by the University of Essex Ethics Committee and the nurse data collection by the National Research Ethics Service (10/H0604/2).

#### Study population

The United Kingdom Household Longitudinal Study, also known as Understanding Society (https://www.understandingsociety.ac.uk) is a longitudinal panel survey of 40,000 UK households from England, Scotland, Wales and Northern Ireland. Participants are surveyed annually since 2009 and contribute information relating to their socioeconomic circumstances, attitudes, and behaviours via a computer assisted

| biomarker                  | rs-id     | function nearest gene cytoband | EA/NEA | discovery EA/NEA beta (SE), p-value | replication EA/NEA beta (SE), p-value | combined EA/NEA beta (SE), p-value |
|----------------------------|-----------|---------------------------------|--------|-------------------------------------|--------------------------------------|-----------------------------------|
| Alanine Transaminase       | rs28929474| missense SERPINA1 1q32 T/C | 0.02   | 0.04 (0.01), 2.61 × 10^-5            | 0.04 (0.01), 1.47 × 10^-10          | 0.04 (0.01), 1.72 × 10^-10         |
| Dihydroepiandrosterone Sulphate | rs28909094| upstream gene PPiap7 1q21 A/C | 0.31   | 0.05 (0.02), 7.95 × 10^-6            | 0.08 (0.02), 4.32 × 10^-4           | 0.05 (0.01), 5.88 × 10^-12         |
| Dihydroepiandrosterone Sulphate | rs2911280| intron CMIP 16q23 A/G | 0.08   | 0.09 (0.02), 2.25 × 10^-6            | 0.08 (0.03), 8.63 × 10^-4           | 0.09 (0.01), 5.97 × 10^-10         |
| Glycated haemoglobin       | rs12819124| intron RP1-228F16.4 12q31 A/C | 0.47   | -0.04 (0.01), 5.94 × 10^-6           | -0.02 (0.01), 1.12 × 10^-3          | -0.03 (0.01), 6.20 × 10^-6         |
| Glycated haemoglobin       | rs761772  | non-coding exonic TMC8 17q25 C/T | 0.13   | 0.06 (0.01), 1.83 × 10^-5            | 0.03 (0.01), 1.83 × 10^-5           | 0.05 (0.01), 4.86 × 10^-4         |

### Table 3. Association results of replicating novel signals. function: variant functional consequence; nearest gene: gene nearest to lead variant with 500Kb from either side; chr: chromosome; EA/NEA: effect allele/non-effect allele; EA: effect allele frequency; beta(SE), p-value: effect size (standard error) and p-value; N: total number of individuals analysed for this variant; r^2: imputation accuracy.
As recruitment was household based, the study contains related individuals. The study includes phenotypical data for a representative sample of participants for a wide range of social and economic indicators as well as a biological sample collection encompassing biometric, physiological, biochemical, and haematological measurements and self-reported medical history and medication use (https://www.understandingsociety.ac.uk/d/100/7251_User_Guide_Health_Assmt_w2_w3.pdf?1392855567). For each participant non-fasting blood samples were collected through venepuncture, were centrifuged to separate plasma and serum, aliquoted and frozen at −80 °C. DNA has been extracted and stored for genetic analyses.

For replication, data were available for 5533 individuals from ELSA32, 9888 from Fenland33 (Supplemental Table 1), 7621 from HRS (http://hrsonline.isr.umich.edu)34, 2859 from NCDS35. These studies have been described in detail elsewhere. Sample collection were carried out consistently and analysed by the same laboratories for UKHLS, ELSA and NCDS.

**Biomarker measurements.** In total, biomarker data were successfully obtained from 13,107 eligible individuals who gave consent to give blood samples to be stored for future analysis (https://www.understandingsociety.ac.uk/d/154/7251-UnderstandingSociety-Biomarker-UserGuide-2014.pdf?1418057881). All biomarkers were measured from serum (non-fasting), using a variety of suitable assays, and the majority analysed on a single Roche P module analyser36. On each machine Internal Quality Controls (IQC) were at regular intervals per day. External Quality Assurance (EQA) systems were in place to monitor all tests.
Phenotype transformations and exclusions. The measurements for biomarkers used in the association analyses were prepared according to protocols from the largest genetic association study published for each specific trait at the time when analyses commenced, details for which are available in Supplementary Table S2.

Genotyping. In total, 10,484 UKHLS samples have been typed using the Illumina Infinium HumanCoreExome BeadChip Kit® (12v1-0). This array contains a set of >250,000 highly informative genome-wide tagging single nucleotide polymorphisms as well as a panel of functional (protein-altering) exonic markers, including a large proportion of low-frequency (MAF 1–5%) and rare (MAF <1%) variants. Genotype calling was performed with the gencall algorithm using GenomeStudio (Illumina Inc.). For quality control (QC) we excluded individuals based on the following criteria: sample call rate <98%, autosomal heterozygosity outliers (>3SD), gender mismatches, duplicates as established by identity by descent (IBD) analysis (PI_HAT >0.9). Individuals with non-European ancestry were also excluded. For this we estimated the genomic kinship between all pairs of individuals along with 1000 Genomes Project data. These were converted to distances and subjected to multidimensional scaling. Prior to variant QC, we first mapped all 538,448 variants to the human reference genome build 37. Variants with Hardy-Weinberg equilibrium p-value <1 × 10−4, call rate below 98% or poor genotype clustering values (<0.4) were excluded, leaving 525,314 variants passing QC. For typed variants in our GWAS analyses that were brought forward for replication we inspected cluster plots manually using Scattershot 0.75 beta (Supplementary Fig. S1). All QC procedures were carried out using PLINK (v1.07) and R.

Imputation. We imputed our genotype data using a combined reference panel consisting of 7,562 haplotypes from the UK10K project and 2,184 haplotypes from 1000 Genomes phase 3. Details regarding the creation of this combined imputation panel are described elsewhere37. Prior to imputation, we first pre-phased using SHAPEIT (v2.r). Data were then imputed using IMPUTE2 (v2.3.0), resulting in an initial set of 38,310,212 variants. Variants with an IMPUTE info score <0.4, and variants with a Hardy-Weinberg p-value <1 × 10−4 were excluded, leaving 26,851,013 variants for analysis.

Data availability. The UKHLS EGA accession number is EGAD00010000918. ELSA EGA accession number is EGAC00001000270. NCDS accession number is EGAC00000000001. HRS is available through dbGAP, Study Accession number phs000428.v1.p1. Genotype-phenotype data access for UKHLS, ELSA and NCDS is available by application to Metadac (www.metadac.ac.uk).

Statistical analyses. Heritability analyses and genetic correlations. The proportion of trait variance explained by the genotyped and imputed variants was estimated using the GREML method as implemented in the GCTA software48, 49 (v1.26). We included all variants with minor allele frequency (MAF) >0.01. We excluded variants with imputation accuracy less than 0.4. We computed the genetic relationship matrix (GRM) for each autosome and then used GCTA to combine them into one matrix. We excluded relatives from the estimation by filtering based on the GRM using a threshold of 0.1 after inspecting the distribution. This led to the exclusion of 672 individuals for this analysis. We also performed bivariate REML analysis in order to estimate genetic correlations between different biomarkers48. We applied a Bonferroni adjusted significance threshold using the effective number of traits for the heritability analyses and using the number of pairs based on the effective number of traits for the genetic correlation analyses.

Association analyses. The association analyses were carried out using a multivariate linear mixed model to account for relatedness as implemented in GEMMA (v0.95). QQ plots and genomic inflation factors, as well as Manhattan plots for traits where we identified novel associations are displayed in Supplementary Fig. S2. Replication analyses were carried out in R and PLINK, following the same trait preparation protocols as used in the discovery stage. The association summary statistics from the replication analyses, as well as the combined discovery and replication stage were meta-analysed using a fixed-effects inverse variance weighted approach.
implemented in METAL (v2011-03-25). We calculated an adjusted genome-wide significance threshold, for the effective number of traits, as several of our biomarkers have correlated levels. The effective number of traits was derived by computing the eigenvalues for the correlation matrix of the 20 biomarkers (effective number: 14.05). The routinely used GWAS threshold of \( p < 5 \times 10^{-8} \) was then adjusted for this using the Bonferroni approach: \( 5 \times 10^{-8}/14.05 = 3.56 \times 10^{-9} \).

**Power calculations.** We carried out power calculations using Quanto (v1.2.4), for discrete per-variant frequency and (standardised) effect sizes combinations, representative of variants identified in the discovery. Per-trait and per selected variant power analyses showed that we would minimally need 5,000 to 15,000 samples to replicate our variants with \( P < 3.56 \times 10^{-6} \), two-sided, for testosterone levels, whereas the largest replication sample of 25,000 to 60,000 would be needed for eGFR (Fig. 4).

**Selection of replication SNPs, and criteria for novel loci.** For replication we selected independent SNPs (LD \( r^2 < 0.1 \)), with MAF > 0.01 and a discovery p-value of \( P < 1 \times 10^{-8} \) and at least \( > 500 \) Kb away from the nearest known reported index SNP for a given trait. We also took forward independent rare variants with a MAF ≤ 0.01 that were typed and reached \( P < 1 \times 10^{-5} \), regardless whether they represented known associations for a given trait. Known index SNPs for all biomarkers analysed in this study were obtained through the GWAS catalog\(^{43} \) (accessed August 4, 2016) >, supplemented by manual searches in PubMed.

**Annotation.** For annotation of our lead variants we used an in-house annotation script that automatically retrieves variant annotations from ENSEMBL\(^{32} \), including variant function, the nearest gene IDs within 500Kb of a given variant, transcript and protein IDs for these genes, as well as conservation scores. It also calculates GWAVA\(^{45} \) scores for non-genic variants amongst other annotations.

All methods were performed in accordance with the relevant guidelines and regulations.

**References**

1. Teslovich, T. M. et al. Biological, clinical and population relevance of 95 loci for blood lipids. *Nature* **466**, 707–713 (2010).

2. Cohen, J. C., Boerwinkle, E., Mosley, T. H. & Hobbs, H. H. Sequence variations in PCSK9, low LDL, and protection against coronary heart disease. *N. Engl. J. Med.* **354**, 1264–1272 (2006).

3. Timpson, N. J. et al. A rare variant in APOC3 is associated with plasma triglyceride and VLDL levels in Europeans. *Nat. Commun.* **5**, 4871 (2014).

4. Manolio, T. A. Bringing genome-wide association findings into clinical use. *Nat. Rev. Genet.* **14**, 549–558 (2013).

5. Voight, B. F. et al. The metabochip, a custom genotyping array for genetic studies of metabolic, cardiovascular, and anthropometric traits. *PLoS Genet.* **8**, e1002793 (2012).

6. Cortes, A. & Brown, M. A. Promise and pitfalls of the Immunochip. *Arthritis Res. Ther.* **13**, 101 (2011).

7. Huffman, J. E. et al. Rare and low-frequency variants and their association with plasma levels of fibrinogen, FVII, FVIII, and vWF. *Blood* **126**, e19–29 (2015).

8. Brennan, S. O., Fellowes, A. P., Faed, J. M. & George, P. M. Hypofibrinogenemia in an individual with 2 coding (gamma82 A–> G and Bbeta235 P–> L) and 2 noncoding mutations. *Blood* **95**, 1709–1713 (2000).

9. Wyatt, J., Brennan, S. O., May, S. & George, P. M. Hypofibrinogenemia with compound heterozygosity for two gamma chain mutations - gamma 82 Ala–> Gly and an intron two GT–> AT splice site mutation. *Thromb. Haemost.* **84**, 449–452 (2000).

10. Ivaskevicius, V. et al. gamma-alfa2A-gly represents a common fibrinogen gamma-chain variant in Caucasians. *Blood Coagul. Fibrolysis Int. J. Haemost. Thromb.* **16**, 205–208 (2005).

11. Stoller, J. K. & Aboussouan, L. S. A review of alpha 1-antitrypsin deficiency increases respiratory capacity and height. *J. Med. Genet.* **35**, 605–607 (1992).

12. Walsh, C. T., Zydowsky, L. D. & McKeon, F. D. Cyclosporin A, the cyclophilin class of peptidylprolyl isomerases, and blockade of T cell signal transduction. *J. Biol. Chem.* **267**, 13115–13118 (1992).

13. Jin, Z.-G. et al. Cyclophilin A is a proinflammatory cytokine that activates endothelial cells. *Arterioscler. Thromb. Vasc. Biol.* **24**, 1186–1191 (2004).

14. Yazdanabakhsh, K., Choi, J. W., Li, Y., Lau, L. F. & Choi, Y. Cyclophilin A blocks apoptosis by inhibiting the DNA binding activity of the transcription factor Nur77. *Proc. Natl. Acad. Sci. USA* **92**, 437–441 (1995).

15. Grimbelt, P. et al. The Fllamin-A is a partner of Tc-mip, a new adaptor protein involved in c-maf-dependent Th2 signaling pathway. *Mol. Immunol.* **40**, 1257–1261 (2004).

16. Carithers, L. J. et al. A Novel Approach to High-Quality Postmortem Tissue Procurement: The GTEx Project. *Biopreservation Biobanking* **13**, 311–319 (2015).

17. The UK10K Consortium. The UK10K project identifies rare variants in health and disease. *Nature* **526**, 82–90 (2015).

18. Bulk-Sullivan, B. et al. An atlas of genetic correlations across human diseases and traits. *Nat. Genet.* **47**, 1236–1241 (2015).

19. Pownall, H. J. et al. Correlation of serum triglyceride and its reduction by omega-3 fatty acids with lipid transfer activity and the neutral lipids and compositions of high-density and low-density lipoproteins. *Atherosclerosis* **143**, 285–297 (1999).

20. Tall, A. R. Plasma cholesterol ester transfer protein. *J. Lipid Res.* **34**, 1255–1274 (1993).

21. Miller, W. L. Androgen biosynthesis from cholesterol to DHEA. *Mol. Cell. Endocrinol.* **198**, 7–14 (2002).

22. Bolton, J. L. et al. Genome Wide Association Identifies Common Variants at the SERPINA6/SERPINA1 Locus Influencing Plasma neutral lipid compositions of high-density and low-density lipoproteins. *Arterioscler. Thromb. Vasc. Biol.* **25**, 1253–1255 P<0.01 and at least \( > 500 \) Kb away from the nearest known reported index SNP for a given trait.

23. Carithers, L. J. et al. A Novel Approach to High-Quality Postmortem Tissue Procurement: The GTEx Project. *Biopreservation Biobanking* **13**, 311–319 (2015).

24. The UK10K Consortium. The UK10K project identifies rare variants in health and disease. *Nature* **526**, 82–90 (2015).

25. Bulk-Sullivan, B. et al. An atlas of genetic correlations across human diseases and traits. *Nat. Genet.* **47**, 1236–1241 (2015).

26. Pownall, H. J. et al. Correlation of serum triglyceride and its reduction by omega-3 fatty acids with lipid transfer activity and the neutral lipids and compositions of high-density and low-density lipoproteins. *Atherosclerosis* **143**, 285–297 (1999).

27. Tall, A. R. Plasma cholesterol ester transfer protein. *J. Lipid Res.* **34**, 1255–1274 (1993).

28. Miller, W. L. Androgen biosynthesis from cholesterol to DHEA. *Mol. Cell. Endocrinol.* **198**, 7–14 (2002).

29. Bolton, J. L. et al. Genome Wide Association Identifies Common Variants at the SERPINA6/SERPINA1 Locus Influencing Plasma neutral lipid compositions of high-density and low-density lipoproteins. *Arterioscler. Thromb. Vasc. Biol.* **25**, 1253–1255 P<0.01 and at least \( > 500 \) Kb away from the nearest known reported index SNP for a given trait.

20. North, T.-L. et al. A study of common Mendelian disease carriers across ageing British cohorts: meta-analyses reveal heterozygosity for alpha-1 antitrypsin deficiency increases respiratory capacity and height. *J. Med. Genet.* **53**, 280–288 (2016).

21. Lomas, D. A., Evans, D. L., Finch, J. T. & Carrell, R. W. The mechanism of Z alpha-1 antitrypsin accumulation in the liver. *Nature* **357**, 605–607 (1992).

22. Soranzo, N. et al. Common variants at 10 genomic loci influence hemoglobin A(C) levels via glyemic and nonglyemic pathways. *Diabetes* **59**, 3229–3239 (2010).

23. Fuchsberger, C. et al. The genetic architecture of type 2 diabetes. *Nature* **536**, 41–47 (2016).

24. Wang, K.-S., Liu, X.-F. & Aragam, N. A genome-wide meta-analysis identifies novel loci associated with schizophrenia and bipolar disorder. *Schizophr. Res.* **124**, 192–199 (2010).

25. Ökbay, A. et al. Genetic variants associated with subjective well-being, depressive symptoms, and neuroticism identified through genome-wide analyses. *Nat. Genet.* **48**, 624–633 (2016).
30. Kim, Y. J. et al. Large-scale genome-wide association studies in east Asians identify new genetic loci influencing metabolic traits. Nat. Genet. 43, 990–995 (2011).
31. Sluis, S., van der, Verhage, M., Posthuma, D. & Dolan, C. V. Phenotypic Complexity, Measurement Bias, and Poor Phenotypic Resolution Contribute to the Missing Heritability Problem in Genetic Association Studies. PLOS ONE 5, e13929 (2010).
32. Steptoe, A., Breeze, E., Banks, J. & Nazroo, J. Cohort profile: the English longitudinal study of ageing. Int. J. Epidemiol. 42, 1640–1648 (2013).
33. Lotta, L. A. et al. Integrative genomic analysis implicates limited peripheral adipose storage capacity in the pathogenesis of human insulin resistance. Nat. Genet. doi:10.1038/ng.3714 (2016).
34. Sonnega, A. et al. Cohort Profile: the Health and Retirement Study (HRS). Int. J. Epidemiol. 43, 576–585 (2014).
35. Power, C. & Elliott, J. Cohort profile: 1958 British birth cohort (National Child Development Study). Int. J. Epidemiol. 35, 34–41 (2006).
36. M Benzeval, A Davillas, M Kumari, P Lynn Understanding Society: the UK Household Longitudinal Study Biomarker User Guide and Glossary, ISER, University of Essex - Google Search. Available at: https://www.google.co.uk/search?q=M+Benzeval,+A+Davillas,+M+Kumari,+P+Lynn+Understanding+Society:+the+UK+Household+Longitudinal+Study+Biomarker+User+Guide+and+Glossary,+ISER,+University+of+Essex&ie=utf-8&oe=utf-8&gws_rd=cr&ei=XIR5WOjVGdseqAa3djKoDg. (Accessed: 19th December 2016) (2014).
37. Huang, J. et al. Improved imputation of low-frequency and rare variants using the UK10K haplotype reference panel. Nat. Commun. 6, 8111 (2015).
38. Yang, J. et al. Common SNPs explain a large proportion of the heritability for human height. Nat. Genet. 42, 565–569 (2010).
39. Yang, J., Lee, S. H., Goddard, M. E. & Visscher, P. M. GCTA: a tool for genome-wide complex trait analysis. Am. J. Hum. Genet. 88, 76–82 (2011).
40. Lee, S. H., Yang, J., Goddard, M. E., Visscher, P. M. & Wray, N. R. Estimation of pleiotropy between complex diseases using single-nucleotide polymorphism-derived genomic relationships and restricted maximum likelihood. Bioinforma. Oxf. Engl. 28, 2540–2542 (2012).
41. Welte, D. et al. The NHGRI GWAS Catalog, a curated resource of SNP-trait associations. Nucleic Acids Res. 42, D1001–1006 (2014).
42. Yates, A. et al. Ensemble 2016. Nucleic Acids Res. 44, D710–716 (2016).
43. Ritchie, G. R. S., Dunham, I., Zeggini, E. & Flicek, P. Functional annotation of noncoding sequence variants. Nat. Methods 11, 294–296 (2014).

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Author Contributions
B.P.P., K.B.K. and E.Z. drafted the initial manuscript. B.P.P. and K.B.K. performed the statistical analyses. E.Z. designed and supervised the study. Y.B., M.S., D.Z., G.F., J.L. carried out the replication analyses. N.J.W., R.A.S., B.P.P., K.B.K. and E.Z. drafted the initial manuscript. B.P.P. and K.B.K. performed the statistical analyses. E.Z.

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