

# Dominance Genetic Variation Contributes Little to the Missing Heritability for Human Complex Traits

Zhihong Zhu,<sup>1</sup> Andrew Bakshi,<sup>1</sup> Anna A.E. Vinkhuyzen,<sup>1</sup> Gibran Hemani,<sup>1,2</sup> Sang Hong Lee,<sup>1</sup> Ilja M. Nolte,<sup>3</sup> Jana V. van Vliet-Ostaptchouk,<sup>3,4</sup> Harold Snieder,<sup>3</sup> The LifeLines Cohort Study,<sup>5</sup> Tonu Esko,<sup>6,7,8,9</sup> Lili Milani,<sup>6</sup> Reedik Mägi,<sup>6</sup> Andres Metspalu,<sup>6,10</sup> William G. Hill,<sup>11</sup> Bruce S. Weir,<sup>12</sup> Michael E. Goddard,<sup>13,14</sup> Peter M. Visscher,<sup>1,15</sup> and Jian Yang<sup>1,15,\*</sup>

For human complex traits, non-additive genetic variation has been invoked to explain “missing heritability,” but its discovery is often neglected in genome-wide association studies. Here we propose a method of using SNP data to partition and estimate the proportion of phenotypic variance attributed to additive and dominance genetic variation at all SNPs ( $h_{SNP}^2$  and  $\delta_{SNP}^2$ ) in unrelated individuals based on an orthogonal model where the estimate of  $h_{SNP}^2$  is independent of that of  $\delta_{SNP}^2$ . With this method, we analyzed 79 quantitative traits in 6,715 unrelated European Americans. The estimate of  $\delta_{SNP}^2$  averaged across all the 79 quantitative traits was 0.03, approximately a fifth of that for additive variation (average  $h_{SNP}^2 = 0.15$ ). There were a few traits that showed substantial estimates of  $\delta_{SNP}^2$ , none of which were replicated in a larger sample of 11,965 individuals. We further performed genome-wide association analyses of the 79 quantitative traits and detected SNPs with genome-wide significant dominance effects only at the *ABO* locus for factor VIII and von Willebrand factor. All these results suggest that dominance variation at common SNPs explains only a small fraction of phenotypic variation for human complex traits and contributes little to the missing narrow-sense heritability problem.

## Introduction

Phenotypic variation of most traits related to human health (e.g., obesity and blood pressure) is due to many genes and their interplay with environmental factors.<sup>1</sup> These traits are called “complex traits” to differentiate them from Mendelian traits. In 1918, Fisher reconciled biometrical and Mendelian modeling of complex traits and partitioned total genetic variance into sources of variation due to additive, dominance (allelic interaction within locus), and epistatic (allelic interaction between loci) effects.<sup>2</sup> Fisher’s subsequent work predicted that for fitness and fitness-related traits, the amount of additive genetic variation in the population should be small because of natural selection.<sup>3</sup> Yet despite nearly a century of theoretical and empirical work since 1918, the quantification and relative importance of non-additive genetic variation remains controversial. In humans, additive and non-additive variance components are usually estimated by comparing resemblance between close relatives, for example in twin studies, and there have been many efforts to estimate non-additive genetic variance in twin studies.<sup>4–8</sup> Such estimates, however, can be biased due to confounding with common environmental effects within families.

In theory, the total genetic variance can be partitioned into the variance components due to additive, dominance, additive-by-additive, additive-by-dominance, and dominance-by-dominance epistatic variation as well as many higher-order terms.<sup>9,10</sup> In practice, however, even with data from large pedigrees, it is difficult to estimate all these genetic variance components, not only because of the partial confounding in coefficients of relatedness for these genetic components but also because the coefficients for the higher-order epistatic variance are small and therefore the sampling errors of their estimates are large.<sup>11</sup> Further, theory shows that rather small proportions of non-additive variance due to dominance and multi-locus epistatic are expected to be found in outbred populations.<sup>11,12</sup>

On the other hand, genome-wide association studies (GWASs) facilitated by high-throughput genotyping technologies have been enormously successful in identifying SNPs that are associated with complex traits.<sup>13</sup> For most complex traits, however, a large portion of trait narrow-sense heritability ( $h^2$ ) remains unexplained, the so-called “missing heritability” problem.<sup>14,15</sup> SNP-trait associations are most often identified by fitting additive models so that phenotypic variation explained by the top associated SNPs in GWASs is per definition additive, and per definition  $h^2$  does not include non-additive genetic variance.

<sup>1</sup>Queensland Brain Institute, The University of Queensland, Brisbane, QLD 4072, Australia; <sup>2</sup>MRC Integrative Epidemiology Unit (IEU) at the University of Bristol, School of Social and Community Medicine, Bristol BS8 1TH, UK; <sup>3</sup>Department of Epidemiology, University of Groningen, University Medical Center Groningen, Groningen 9700 RB, the Netherlands; <sup>4</sup>Department of Endocrinology, University of Groningen, University Medical Center Groningen, Groningen 9700 RB, the Netherlands; <sup>5</sup>University of Groningen, University Medical Center Groningen, Groningen 9700 RB, the Netherlands; <sup>6</sup>Estonian Genome Centre, University of Tartu, Tartu 51006, Estonia; <sup>7</sup>Division of Endocrinology, Boston Children’s Hospital, Cambridge, MA 02141, USA; <sup>8</sup>Program in Medical and Populational Genetics, Broad Institute, Cambridge, MA 02242, USA; <sup>9</sup>Department of Genetics, Harvard Medical School, Boston, MA 02115, USA; <sup>10</sup>Institute of Molecular and Cell Biology, University of Tartu, Tartu 51010, Estonia; <sup>11</sup>Institute of Evolutionary Biology, School of Biological Sciences, University of Edinburgh, Edinburgh EH9 3JT, UK; <sup>12</sup>Department of Biostatistics, University of Washington, Seattle, WA 98195, USA; <sup>13</sup>Department of Food and Agricultural Systems, University of Melbourne, Parkville, VIC 3010, Australia; <sup>14</sup>Biosciences Research Division, Department of Primary Industries, Bundaberg, VIC 3083, Australia; <sup>15</sup>The University of Queensland Diamantina Institute, The Translation Research Institute, Brisbane, QLD 4102, Australia

\*Correspondence: [jian.yang@uq.edu.au](mailto:jian.yang@uq.edu.au)

<http://dx.doi.org/10.1016/j.ajhg.2015.01.001>. ©2015 by The American Society of Human Genetics. All rights reserved.

Hence, missing narrow-sense heritability appears not relevant to non-additive variation. However, it has been suggested that estimates of  $h^2$  could be inflated in the presence of non-additive variation such as epistatic variation,<sup>16,17</sup> and highly non-additive models of biology appear consistent with the resemblance of relatives.<sup>17</sup> Therefore, to place the findings from SNP discoveries in context, precise and unbiased partitioning of total genetic variance is required. In this study, we proposed a method of estimating dominance genetic variance by using genome-wide SNP data and applied the method in the analyses of 79 quantitative traits in humans.

## Material and Methods

### Statistical Models

In quantitative genetics theory,<sup>2,9,10</sup> additive (A) variance at a single locus is defined as the genetic variance explained by the regression of genotypic value (expected value of phenotypic mean in each genotypic class) on genotype, and dominance (D) variance is defined as the residual genetic variance that is not explained by the regression. Let  $a = (\mu_{BB} - \mu_{AA}) / 2$  and  $d = \mu_{AB} - (\mu_{AA} + \mu_{BB}) / 2$  with  $\mu_{AA}$ ,  $\mu_{AB}$ , and  $\mu_{BB}$  being the phenotypic means in the three genotypic classes AA, AB, and BB, respectively. Under the assumption of Hardy-Weinberg equilibrium (HWE), additive variance ( $\sigma_a^2$ ) is  $2p(1-p)[a + (1-2p)d]^2$ , dominance variance ( $\sigma_d^2$ ) is  $[2p(1-p)d]^2$ , and genotypic variance ( $\sigma_g^2$ ) is  $\sigma_a^2 + \sigma_d^2$ , with  $p$  being the frequency of allele B. Additive variance is the variance for the average effect of allele substitution,<sup>10</sup> i.e.,  $\beta = a + (1-2p)d$ , which contains a term due to dominance interaction between two alleles. Such difference between interaction and variance resulting from the interaction is a source of great confusion, not least in the discussion of the importance of epistatic interaction and epistatic variance.<sup>12</sup> Dominance variance is the variation in the deviations of the genotypic values from the regression. These definitions are consistent with the question we seek to ask, i.e., how much extra genetic variance can be explained by dominance variation on top of the A-only model. In GWASs, however, the analysis is often performed based on the model<sup>18</sup>

$$y = \mu + x_A b_A + x_D b_D + e, \quad (\text{Equation 1})$$

where  $y$  is the phenotypic value;  $\mu$  is the mean term;  $x_A$  is coded as 0, 1, or 2 and  $x_D$  is coded as 0, 1, or 0 for the three genotypic classes AA, AB, and BB; and  $e$  is the residual,  $e \sim N(0, \sigma_e^2)$ . However, this model is not orthogonal because  $x_A$  and  $x_D$  are correlated, i.e.,  $\text{cov}(x_A, x_D) = 2p(1-p)(1-2p)$  under HWE. We cannot simply partition additive and dominance variance as  $\text{var}(x_A b_A)$  and  $\text{var}(x_D b_D)$  because they do not add up to the total genetic variance, i.e.,  $\text{var}(x_A b_A) + \text{var}(x_D b_D) \neq \text{var}(x_A b_A + x_D b_D)$ . In a multiple regression analysis of the A+D model, the true parameters of the regression coefficients are  $b_A = a$  and  $b_D = d$ , whereas in a simple regression analysis of the A-only model,  $b_A = a + (1-2p)d$ . We therefore re-parameterize Equation 1 as

$$y = \mu + x_A \beta + x'_D d + e \quad (\text{Equation 2})$$

where  $\beta = a + (1-2p)d$ , which is the same as the regression coefficient of  $y$  on  $x_A$  in a GWAS based on the A-only model, and  $x'_D = 0, 2p$ , or  $(4p-2)$  for genotypes AA, AB, or BB. This model is orthogonal because  $\text{cov}(x_A, x'_D) = 0$ , meaning that the estimate

of  $\beta$  is independent of whether  $d$  is fitted in the model or not and vice versa, and the definitions of additive and dominance variances are exactly consistent with those defined in classical quantitative genetics, i.e.,  $\sigma_a^2 = \text{var}(x_A \beta) = 2p(1-p)[a + (1-2p)d]^2$  and  $\sigma_d^2 = \text{var}(x'_D d) = [2p(1-p)d]^2$  with  $\sigma_a^2 + \sigma_d^2 = \sigma_g^2$ .

Following the GREML approach<sup>19</sup> we developed previously, we can fit dominance effects of all SNPs together as random effects in a mixed linear model, i.e.,

$$y = \mu + \sum_i w_{A(i)} u_{A(i)} + \sum_i w_{D(i)} u_{D(i)} + e. \quad (\text{Equation 3})$$

For a SNP  $i$ ,  $w_{A(i)} = (x_{A(i)} - 2p_i) / \sqrt{2p_i(1-p_i)}$  and  $w_{D(i)} = (x'_{D(i)} - 2p_i^2) / [2p_i(1-p_i)]$ , which are essentially the standardized forms of  $x_A$  and  $x_D$  because  $E(x_A) = 2p$ ,  $E(x'_D) = 2p^2$ ,  $\text{var}(x_A) = 2p(1-p)$ , and  $\text{var}(x'_D) = 4p^2(1-p)^2$ .  $u_A$  and  $u_D$  are additive and dominance effects (random effects) corresponding to the standardized genotype variables  $w_A$  and  $w_D$ , respectively. The SNP-based model can be transformed to an individual-based model as

$$y = \mu + g_A + g_D + e, \quad (\text{Equation 4})$$

where  $g_A = \sum_i w_{A(i)} u_{A(i)}$  and  $g_D = \sum_i w_{D(i)} u_{D(i)}$ , which can be defined as the genome-wide additive and dominance genetic values of an individual, respectively. Then, the phenotypic covariance between individuals  $j$  and  $k$  is  $\text{cov}(y_j, y_k) = \pi_{A(jk)} \sigma_A^2 + \pi_{D(jk)} \sigma_D^2 + \sigma_e^2$ , where  $\sigma_A^2 = \text{var}(g_A)$ ,  $\sigma_D^2 = \text{var}(g_D)$ ,  $\pi_{A(jk)}$  and  $\pi_{D(jk)}$  are the additive and dominance genetic relationships between individuals  $j$  and  $k$ , respectively, and  $\sigma_e^2$  is the residual variance. Using the method of equating the SNP-based model (Equation 3) to the individual-based model (Equation 4),<sup>19</sup> we get

$$\pi_{A(jk)} = \frac{1}{m} \sum_i (w_{A(ij)} w_{A(ik)}) = \frac{1}{m} \sum_i \frac{(x_{A(ij)} - 2p_i)(x_{A(ik)} - 2p_i)}{2p_i(1-p_i)}$$

$$\pi_{D(jk)} = \frac{1}{m} \sum_i (w_{D(ij)} w_{D(ik)}) = \frac{1}{m} \sum_i \frac{(x'_{D(ij)} - 2p_i^2)(x'_{D(ik)} - 2p_i^2)}{4p_i^2(1-p_i)^2},$$

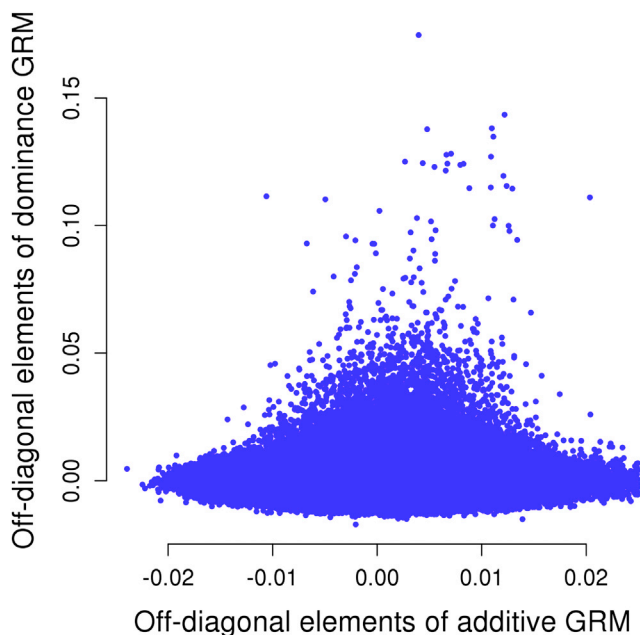
where  $m$  is the number of SNPs. Because  $\text{cov}(x_A, x'_D) = 0$ , the correlation between  $\pi_A$  and  $\pi_D$  is also expected to be zero, and therefore the estimates of  $\sigma_A^2$  and  $\sigma_D^2$  are independent in a sample of unrelated individuals. More generally, if there are fixed covariates such as principal components, we can re-write Equation 4 in matrix form as

$$\mathbf{y} = \mathbf{C}\mathbf{b} + \mathbf{g}_A + \mathbf{g}_D + \mathbf{e}, \quad (\text{Equation 5})$$

where  $\mathbf{y}$  is a  $n \times 1$  vector of phenotypes of all the individuals,  $\mathbf{C}$  is a  $n \times c$  matrix of  $c$  covariates,  $\mathbf{b}$  is a  $c \times 1$  vector of the effects of the covariates,  $\mathbf{g}_A$  and  $\mathbf{g}_D$  are  $n \times 1$  vectors of genome-wide additive and dominance values of all individuals, respectively, and  $\mathbf{e}$  is an  $n \times 1$  vector of residuals. If there are no covariates,  $\mathbf{C}$  will be a  $n \times 1$  vector of ones and  $\mathbf{b} = \mu$ . The (co)variance matrix of phenotypes is

$$\text{var}(\mathbf{y}) = \text{var}(\mathbf{g}_A) + \text{var}(\mathbf{g}_D) + \text{var}(\mathbf{e}) = \mathbf{\Theta}_A \sigma_A^2 + \mathbf{\Theta}_D \sigma_D^2 + \mathbf{I} \sigma_e^2$$

where  $\mathbf{\Theta}_A$  and  $\mathbf{\Theta}_D$  are the additive and dominance genetic relationship matrices (GRM), respectively. This is a typical mixed linear model, and the variance components can be estimated by the REML approach.<sup>20</sup> The variance explained by additive and dominance variation at all SNPs are defined as  $h_{SNP}^2 = \sigma_A^2 / (\sigma_A^2 + \sigma_D^2 + \sigma_e^2)$  and  $\delta_{SNP}^2 = \sigma_D^2 / (\sigma_A^2 + \sigma_D^2 + \sigma_e^2)$ , respectively, where  $h_{SNP}^2$  is interpreted as the narrow-sense heritability ( $h^2$ ) captured by



**Figure 1. Off-Diagonal Elements of the Additive GRM against Those of the Dominance GRM**

The correlation is  $3.40 \times 10^{-4}$ , which is not significantly different from zero ( $p = 0.11$ ).

SNPs and  $H_{SNP}^2 = h_{SNP}^2 + \delta_{SNP}^2$  is the broad-sense heritability ( $H^2$ ) captured by SNPs. We can assess the significance of  $h_{SNP}^2$  or  $\delta_{SNP}^2$  by likelihood ratio test (LRT) and calculate the standard errors (SEs) of the estimates of  $h_{SNP}^2$  or  $\delta_{SNP}^2$  via the delta method.<sup>10</sup>

We named this method GREMLd following the previous nomenclature<sup>21</sup> and have implemented it in the GCTA software tool (see [Web Resources](#)).

### Analysis of GWAS Data

We used SNP genotype data from three published GWASs, i.e., the Atherosclerosis Risk in Communities (ARIC) study ( $n = 8,682$  European Americans),<sup>22</sup> the population-based biobank of the Estonian Genome Center at the University of Tartu (EGCUT) study ( $n = 10,652$ ),<sup>23</sup> and the LifeLines (LL) study ( $n = 13,386$ ).<sup>24</sup> Informed consent was obtained from all subjects. To partition and estimate the proportions of variance explained by additive and dominance variation at all common SNPs ( $h_{SNP}^2$  and  $\delta_{SNP}^2$ ) for quantitative traits, we first performed analyses in the ARIC cohort for a number of quantitative traits and used the EGCUT and LL data as a replication dataset for a few traits that showed a substantial component of dominance variance from the analysis of the ARIC data.

Information on genotyping and quality controls (QC) in the three data sets are summarized in [Table S1](#). To be able to merge multiple datasets, genotype data from different genotyping platforms were imputed separately to 1000 Genomes (1000G) reference panels<sup>25</sup> via IMPUTE v.2.<sup>26</sup> After imputation, we excluded SNPs with MAF  $< 0.01$ , HWE test  $p$  value  $< 10^{-6}$ , or imputation  $R^2 < 0.6$ . We then extracted SNPs on HapMap phase 3 (HM3) for two reasons. First, the HM3 SNP set was optimized to capture common genetic variation in the human genome.<sup>27</sup> Second, there has been a debate on applying the SNP-based heritability estimation approach in dense coverage SNP data (e.g., 1000G imputed data), which has not led to a clear conclusion<sup>28,29</sup> and needs

further investigation. We finally retained 1,174,402, 1,177,501, and 1,158,700 SNPs in the ARIC, EGCUT, and LL cohorts, respectively, for analysis. To remove cryptic relatedness, we used all the HM3 SNPs to estimate the additive genetic relationships between all the individuals in each cohort and removed one of each pair of individuals with estimated genetic relatedness  $> 0.025$ . We retained 6,715, 6,420, and 7,850 unrelated individuals in the ARIC, EGCUT, and LL cohorts, respectively. In the combined dataset of the EGCUT and LL cohorts, there were 1,140,901 HM3 SNPs in common across the two cohorts and 11,965 unrelated individuals (pairwise genetic relatedness  $< 0.025$ ).

There are hundreds of phenotypes (including those measuring the same trait at multiple visits) in the ARIC data, which are related to height, obesity, lipoproteins, diabetes, blood phenotypes, carotid artery, heart function, smoking, etc. We used data at the first visit because the sample size was smaller in the follow-up visits. We did not use the mean phenotype averaged across multiple visits because (strictly speaking) mean phenotype is a different trait. We excluded traits with missing rate  $> 40\%$  and excluded those categorical traits with the number of classes  $< 10$ . There were 79 quantitative traits included in the analysis. A summary description of the phenotypes is presented in [Table S2](#). We replicated the estimates of  $h_{SNP}^2$  and  $\delta_{SNP}^2$  in the EGCUT and LL cohorts for four traits (see [Results](#)), i.e., systolic blood pressure (SBP), BMI, weight (WT), and waist circumference (WC). Each of the phenotypes was corrected for age, standardized to z-score, and inverse normal transformed, in males and females separately, in each cohort. Pairwise correlations between the 79 traits in ARIC are shown in [Figure S1](#). The first 20 principal components (PCs) estimated from the SNP data<sup>30</sup> were included as fixed covariates in the GREMLd analyses.

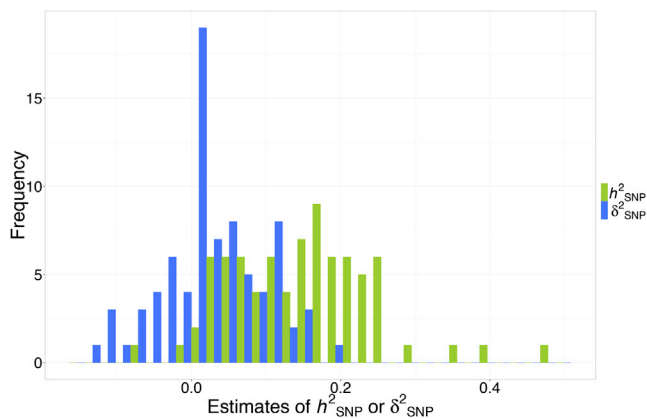
### Genome-wide Association Tests for Dominance Variance at Individual SNPs

We used the method described in [Equation 3](#) to perform genome-wide association tests for dominance variance at individual SNPs for all the 79 traits in the ARIC data, and further for 4 traits that showed a substantial estimate of  $\delta_{SNP}^2$  in the ARIC data, in the combined data of the LL and EGCUT cohorts. The first 20 PCs were also fitted as covariates in the association analyses.

### Results

We estimated  $h_{SNP}^2$  and  $\delta_{SNP}^2$  via the GREMLd method for the 79 traits using  $\sim 1.17$ M SNPs and 6,715 unrelated individuals in the ARIC cohort ([Materials and Methods](#)). The method uses genome-wide SNP data to estimate the additive and dominance GRMs and fits both GRMs in a mixed linear model to estimate  $h_{SNP}^2$  and  $\delta_{SNP}^2$  simultaneously. The additive and dominance genotype variables at single SNPs are parameterized such that genome-wide additive and dominance GRMs are uncorrelated. Therefore, the estimate of  $h_{SNP}^2$  is independent of whether  $\delta_{SNP}^2$  is fitted in the model or not, and vice versa. This is demonstrated empirically by the tiny correlation ( $r = 0.0003$ ) of the off-diagonal elements between the additive and dominance GRMs in the ARIC data ([Figure 1](#)).

The estimates of  $h_{SNP}^2$  and  $\delta_{SNP}^2$  for the 79 traits are shown in [Table S3](#), with their distribution being presented in



**Figure 2. Distribution of the Estimates of  $h^2_{\text{SNP}}$  and  $\delta^2_{\text{SNP}}$  for 79 Traits in the ARIC Cohort**

To get an unbiased estimate of the mean of  $h^2_{\text{SNP}}$  or  $\delta^2_{\text{SNP}}$  across all the traits, the estimates of  $h^2_{\text{SNP}}$  and  $\delta^2_{\text{SNP}}$  for each trait were not constrained to be positive in the REML analysis. The mean estimates of  $h^2_{\text{SNP}}$  and  $\delta^2_{\text{SNP}}$  are 0.15 and 0.03, respectively.

**Figure 2.** The estimate of  $h^2_{\text{SNP}}$  averaged across all the 79 traits was 0.15 (ranging from  $-0.07$  to  $0.48$ ), consistent with that from a previous study in Asians.<sup>31</sup> The estimate of  $\delta^2_{\text{SNP}}$  averaged across traits was 0.03 (ranging from  $-0.13$  to  $0.19$ ). These results suggest that on average dominance variance is approximately a fifth of additive variance, consistent with  $\sigma^2_{\text{D}}$  being much smaller than  $\sigma^2_{\text{A}}$  as predicted from classical quantitative genetics theories<sup>11</sup> and observed in pedigree-based analyses of thousands of gene expression traits.<sup>32</sup> We plotted the estimate of  $\delta^2_{\text{SNP}}$  against that of  $h^2_{\text{SNP}}$  for each of these traits and did not observe a significant correlation between the estimates of  $h^2_{\text{SNP}}$  and  $\delta^2_{\text{SNP}}$  (Figure S2), suggesting that traits that have a large component of  $h^2_{\text{SNP}}$  do not necessarily have a substantial component of  $\delta^2_{\text{SNP}}$ . We further performed analyses with the genotyped data (593,521 SNPs genotyped on Affymetrix 6.0 array after QC, Table S1), and the results were similar to those using the imputed data (Figure S3).

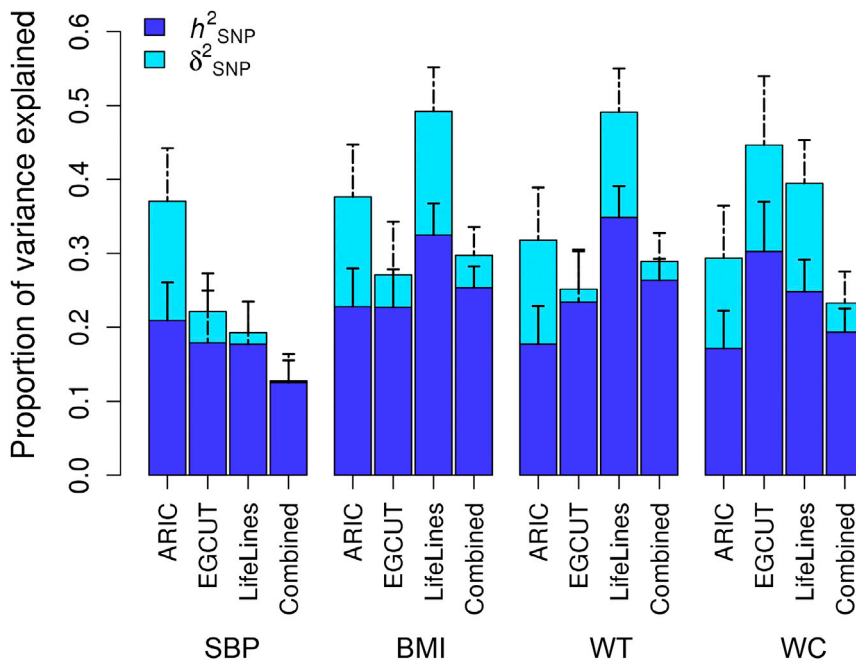
There were eight traits, related to obesity, blood pressure, and heart rate, which had nominally significant estimates of  $\delta^2_{\text{SNP}}$  ( $p < 0.05$ ) (Table S3), e.g., systolic blood pressure (SBP,  $\delta^2_{\text{SNP}} = 0.16$ , SE = 0.07) and BMI ( $\delta^2_{\text{SNP}} = 0.15$ , SE = 0.07). We then replicated the results for four of the eight traits (SBP, BMI, WT, and WC) having data available in the EGCUT ( $n = 6,420$ ) and LL ( $n = 7,850$ ) studies (Materials and Methods) and performed analyses in a combined set of EGCUT and LL samples ( $n =$  up to 11,965) (Figure 3). To avoid bias due to winner's curse (the estimates of  $\delta^2_{\text{SNP}}$  for these four traits were selected by  $p$  values in the ARIC data), we did not include the ARIC cohort in the analysis of combined data. All the four traits appeared to have a strong component of additive variance, consistently across all the datasets. For dominance variance, however, none of estimates were replicated in the combined dataset of EGCUT and LL.

Having not found any evidence of dominance variance for all the traits using all genome-wide SNPs, we asked

whether there are any specific SNPs that have strong dominance effects. Using the orthogonal model as described above (Equation 2), we then performed genome-wide association analyses to test for dominance effect of each SNP for the 79 traits in the ARIC data using  $\sim 1.17\text{M}$  HM3 SNPs (Materials and Methods). We identified the *ABO* blood group gene locus on chromosome 9 that had a genome-wide significant ( $p < 5 \times 10^{-8}$ ) dominance effect on two traits: factor VIII (FVIII,  $p$  value for dominance effect  $P_{\text{D}} = 5.0 \times 10^{-27}$ ) and von Willebrand factor (vWF,  $P_{\text{D}} = 1.1 \times 10^{-25}$ ) (Figure 4). These are two correlated traits with a phenotypic correlation of 0.72. The top associated SNPs at the *ABO* gene locus are rs505922 for FVIII (MAF = 0.35) and rs612169 for vWF (MAF = 0.35), which are in high linkage disequilibrium (LD) with  $r = 0.96$ . The additive variation at this locus is known to explain more than 10% of the phenotypic variance for vWF.<sup>33</sup> In our study, the additive variation at the top associated SNP explained 11.4% of variance for FVIII (13.6% for vWF), and the dominance variation at the SNP explained 1.4% of variance for FVIII (1.3% for vWF), also consistent with additive genetic variance being several-fold larger than dominance genetic variance, even at a single SNP level. The estimates of  $a$  and  $d$  were 0.44 (SE = 0.02) and 0.26 (SE = 0.02) at rs505922 for FVIII, 0.47 (SE = 0.02) and 0.25 (SE = 0.02) at rs612169 for vWF, respectively, suggesting a partial dominance model of gene action. Even under a full dominance model, e.g., assuming  $a = d = 0.44$  at rs505922 for FVIII, the additive variance (0.147) is still  $\sim 3.8$  times larger than dominance variance (0.039). In addition, we did not find SNPs that were associated with any other traits at genome-wide significance level ( $P_{\text{D}} < 5 \times 10^{-8}$ ). We further performed GWAS analyses for the four traits (SBP, BMI, WT, and WC) in the combined EGCUT and LL sample of up to 11,965 unrelated individuals and did not find any SNP with dominance effect at genome-wide significance level (Figure S4).

## Discussion

Results from GREMLd analyses show that on average across all the 79 quantitative traits, dominance genetic variance is about a fifth of additive genetic variance and that none of the traits show significant estimates of dominance variance. There are two possible explanations for these results: either dominance variance at causal variants is small or dominance variance at the underlying causal variants is not small but the observed dominance variance at the SNPs is small due to imperfect LD between SNPs and causal variants. In theory<sup>34</sup> and simulations (Figure S5), the proportion of genetic variance at a causal variant captured by a SNP is  $r^2$  for additive variance, with  $r$  being the LD correlation between the SNP and the causal variant, and  $r^4$  for dominance variance, suggesting that if LD between SNPs and causal variants are weak to moderate, the observed dominance variance at SNPs will tend to be



**Figure 3. Estimates of  $h^2_{SNP}$  and  $\delta^2_{SNP}$  in Three Independent Cohorts of ARIC, EGCUT, and LL and in the Combined Dataset of EGCUT and LL for Four Traits**  
Error bar represents the standard error.

smaller than the observed additive variance even if the actual additive and dominance variance components at causal variants are equal. However, in a variance estimation analysis using genome-wide SNPs, an unobserved causal variant can be tagged by multiple SNPs. Therefore, variance explained by SNPs should be proportional to the multi-correlation between the causal variants and the SNPs in LD with the causal variants.

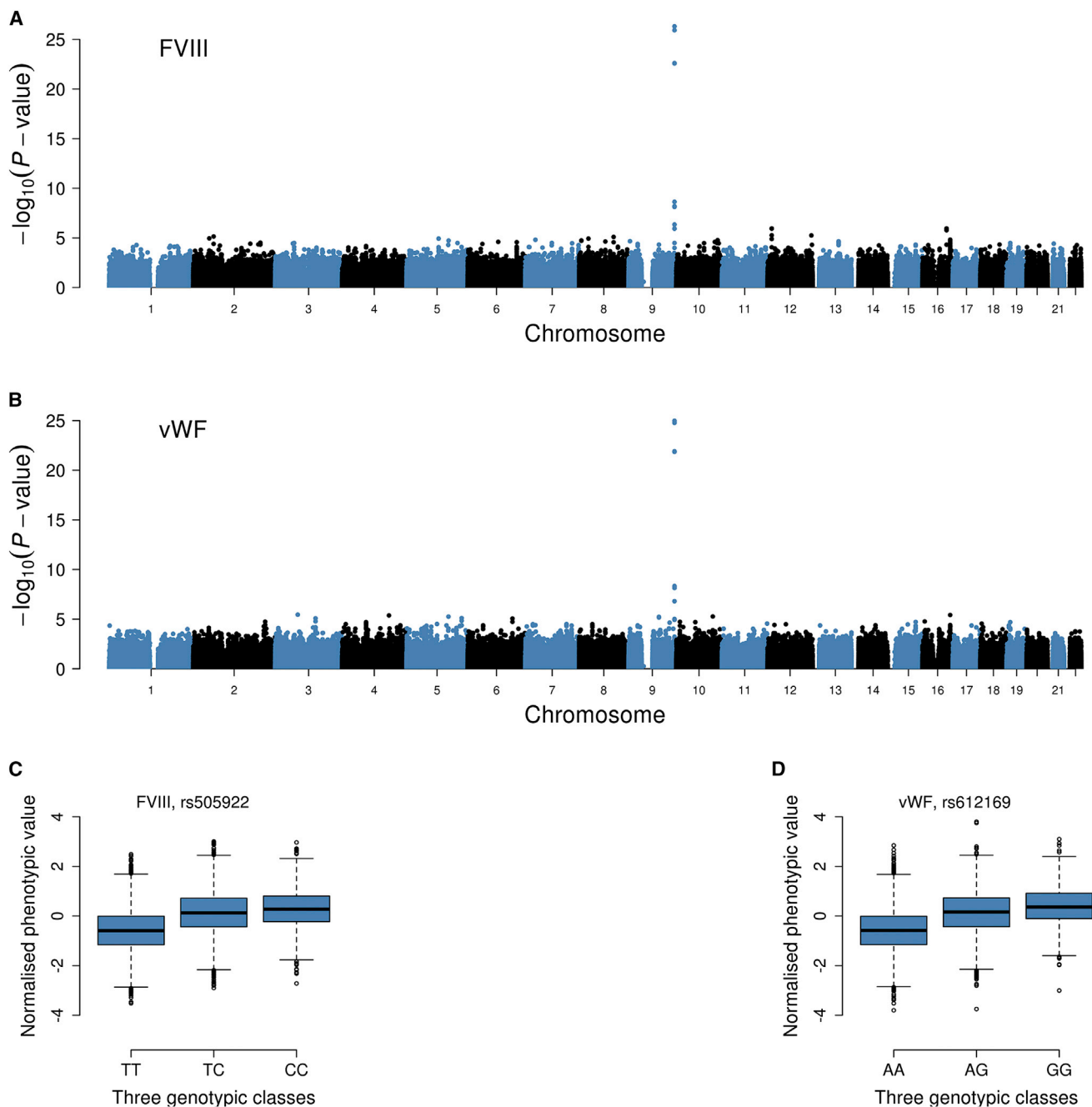
To calibrate the extent to which dominance variance decreases due to the imperfect LD between SNPs and causal variants, we performed two additional analyses. We calculated the multi-correlation  $R^2$  between a SNP and all other SNPs within 1 Mb distance. Multi-correlation  $R^2$  for additive variance (multi- $R^2_{x(A)}$ ) is defined as the multiple regression  $R^2$  of  $x_A$  of the target SNP on  $x_A$  of other SNPs in the region, and that for dominance variance (multi- $R^2_{x(D)}$ ) is defined as the multiple regression  $R^2$  of  $x'_D$  of the target SNP on  $x'_D$  of other SNPs. Both multi- $R^2_{x(A)}$  and multi- $R^2_{x(D)}$  were adjusted for chance correlation due to the use of multiple variables, analogous to the adjusted  $R^2$  in a multiple regression analysis. In the ARIC dataset, the average multi- $R^2_{x(A)}$  and multi- $R^2_{x(D)}$  over all SNPs were 0.96 and 0.84, respectively, suggesting that if any one of the SNPs is missing, on average 96% of its additive variance and 84% of its dominance variance can be captured by the other SNPs, and that even if all causal variants are not present in the HapMap3 SNP panel, only 16% of dominance variance at common causal variants is missing, which is small given the mean  $\delta^2_{SNP}$  of 3.0%.

Further, we performed simulation studies based on real SNP data in the ARIC cohort (Appendix A). We randomly sampled 10% of SNPs as a pool of “causal variants.” In each simulation, we randomly sampled 1,000 causal variants from the pool and simulated phenotypes with  $h^2 =$

$\delta^2 = 0.3$ . The analyses of the simulated data were performed in two scenarios: (1) using all the SNPs (including the pool of causal variants) and (2) using only 90% of the SNPs (excluding the pool of causal variants). In scenario 1 where the causal variants were a random subset of all SNPs and were included in the analysis, the estimates of  $h^2$  and  $\delta^2$  were unbiased (Table S4). In scenario 2 where the causal variants were not included in the analysis, the estimate of  $h^2_{SNP}$  was biased downward, more so for  $\delta^2_{SNP}$ . We further performed analyses reducing the number of SNPs used from 90% to 10%

(Figure S6). Because the pool of causal variants (10% of the SNPs) was always left out of the analysis, reducing the number of SNPs used in the analysis (randomly sampled from the remaining 90% SNPs) decreased the LD between SNPs and causal variants. We observed a slightly faster decline of the estimate of  $\delta^2_{SNP}$  due to imperfect LD than that of  $h^2_{SNP}$ , consistent with that predicted from theory. Even in a very extreme scenario, where only 10% SNPs were included in the estimation analysis, the ratio of  $\hat{h}^2_{SNP}$  (0.20) to  $\hat{\delta}^2_{SNP}$  (0.13) was 1.48, not inconsistent with a ratio of average multi- $R^2_{x(A)}$  (0.67) to average multi- $R^2_{x(D)}$  (0.40) of 1.68 calculated in a random subset of 10% SNPs (see above for the method of calculating multi- $R^2$ ), but much smaller than that observed in the analysis of the 79 real phenotypes ( $\hat{h}^2_{SNP}/\hat{\delta}^2_{SNP} = \sim 5$ ). As suggested by Yang et al.,<sup>19</sup> if causal variants tend to be in lower MAF than SNPs, the estimate of  $h^2_{SNP}$  will be biased downward, more so if the causal variants are not included in estimation analysis. We then sought to test whether the observed  $\hat{h}^2_{SNP}/\hat{\delta}^2_{SNP}$  at SNPs would become larger if the unobserved causal variants tend to be in lower MAF than the SNPs by sampling causal variants from SNPs with  $MAF \leq 0.1$  ( $h^2 = 0.3$  and  $\delta^2 = 0.3$ ). We found that both  $h^2$  and  $\delta^2$  were underestimated ( $\hat{h}^2_{SNP} = 0.18$  and  $\hat{\delta}^2_{SNP} = 0.18$ ); however, the biases in  $\hat{h}^2_{SNP}$  and  $\hat{\delta}^2_{SNP}$  were roughly equal so that  $\hat{h}^2_{SNP}/\hat{\delta}^2_{SNP}$  is still approximately equal to 1. All these results suggest that the observed large difference between  $\hat{h}^2_{SNP}$  and  $\hat{\delta}^2_{SNP}$  in the analysis of real phenotypes is unlikely to be driven by imperfect tagging.

Taking all results together, the most plausible reason why we did not find a significant component of dominance variance for all the traits is that  $\delta^2_{SNP}$  is small so



**Figure 4. Genome-wide Association Tests for Dominance Effects for Factor VIII and von Willebrand Factor**

(A and B) Manhattan plots of p values for dominance effects from the model of fitting both additive and dominance effects for factor VIII (FVIII) (A) and von Willebrand factor (vWF) (B). SNPs with genome-wide significant dominance effects are located at the *ABO* gene locus. (C and D) Genotype-phenotype maps at the top SNP rs505922 for FVIII (C) and the top SNP rs612169 for vWF (D). The normalized phenotypic means in the three genotypic classes are  $-0.57$ ,  $0.12$ , and  $0.30$  at the SNP rs505922 for FVIII (C), and  $-0.57$ ,  $0.15$ , and  $0.37$  at the SNP rs612169 for vWF (D). Bars represent 2.5% and 97.5% quartiles of the phenotype distribution at each of the three genotypic classes.

that we do not have sufficient power to detect it with statistical significance given the sample size used in this study. The power to detect  $\delta_{SNP}^2$  is determined by the non-centrality parameter (NCP) of the chi-square statistic, i.e.,  $NCP = \delta_{SNP}^4 / \text{var}(\hat{\delta}_{SNP}^2)$ . For additive genetic variance, we have derived in a previous study<sup>21</sup> that  $\text{var}(\hat{h}_{SNP}^2)$  is approximately equal to  $2 / [N^2 \times \text{var}(\text{GRM}_A)]$ , where  $N$  is the sample size and  $\text{var}(\text{GRM}_A)$  is the variance of the off-diagonal

elements of the additive GRM, which is approximately  $2 \times 10^{-5}$  using all common SNPs. We show by empirical data that  $\text{var}(\text{GRM}_A)$  is approximately twice that of  $\text{var}(\text{GRM}_D)$ , i.e.,  $\text{var}(\text{GRM}_D) = 1 \times 10^{-5}$ , meaning that  $\text{var}(\hat{\delta}_{SNP}^2) \approx 2 / [N^2 \times \text{var}(\text{GRM}_D)] \approx 2 / (1 \times 10^{-5} N^2)$  (Figure S7). Given a sample size of 7,000, we will have only  $\sim 12\%$  and  $\sim 35\%$  of power to detect  $\delta_{SNP}^2$  of 0.05 and 0.1, respectively, at the significance level of 0.05.

Very little dominance variance is attributable to rare causal variants because that at a single variant is proportional to  $[2p(1-p)]^2$ . For a rare variant with MAF  $< 0.01$ , even if the dominance effect is large (e.g., 1 standard deviation), the proportion of variance explained by dominance variation at it is tiny ( $< 0.04\%$ ). If variants are deleterious there is reason to expect that degree of dominance is associated with the size of effect, i.e., those of largest effect are likely to be at lowest frequency, contributing to inbreeding depression but not generating much dominance variance.<sup>35</sup>

We observed a significant estimate of dominance variance at the *ABO* gene locus for von Willebrand factor (1.28% of variance explained) and for factor VIII (1.36% of variance explained), which were also several-fold smaller than those for additive variation ( $> 10\%$  of variance explained). We then used simulations to test whether or not the observed dominance variation at the SNP was caused by the unexplained additive variation at the unobserved causal variant due to imperfect LD between the SNP and the causal variant. As shown in Figure S8, if the genetic effect at an unobserved causal variant is purely additive, there is no inflation in the test statistic for dominance effect at the linked SNP, suggesting that dominance variation at the *ABO* SNP is not driven by additive variation at the underlying causal variant.

We have shown by theory, simulations, and data analyses the use of SNP data to partition and estimate additive and dominance variance in unrelated individuals based on an orthogonal model. We found that, on average, dominance variation at all the common SNPs explain only 3% of variance for the traits analyzed in this study, 5-fold smaller than that for additive variation. Because rare variants contribute little to the dominance variance and a very large proportion (multi- $R^2_{x(D)} = 0.84$ ) of dominance variation at common variants can be captured by common SNPs, the variance explained by dominance variation at all causal variants is also likely to be small ( $3\% / 0.84 < 4\%$ ). Hence, even if the missing heritability problem is partly due to the overestimation of  $h^2$  in family/twin studies, it is highly unlikely to be caused by dominance variation. Therefore, dominance variation contributes little to the missing heritability.

## Appendix A

### Simulations

We performed a series of simulations based on the real genotypes of  $\sim 1.17\text{M}$  HapMap3 SNPs and 6,715 unrelated individuals in the ARIC cohort. To mimic the incomplete LD between the unobserved causal variants and the observed SNPs, we randomly sampled 10% of SNPs ( $\sim 117\text{K}$  SNPs) as a pool of causal variants, and used the other 90% as the observed SNPs. In each simulation replicate, we randomly sampled 1,000 causal variants from this pool

and generated the phenotype of each individual based on Equation 3, where the additive and dominance effects were generated from the standard normal distribution and the residuals were generated from a normal distribution with mean 0 and variance  $\text{var}(g_A + g_D)[1 / (h^2 + \delta^2) - 1]$  (see Equation 4 for the definitions of  $g_A + g_D$ ). We chose  $h^2 = 0.3$  and  $\delta^2 = 0.3$ . We then estimated  $h^2_{\text{SNP}}$  and  $\delta^2_{\text{SNP}}$  based on Equation 5 in two scenarios: (1) all the SNPs (including the pool of causal variants) were included in the GREMLd estimation analysis, and (2) only the observed SNPs (excluding the pool of causal variants) were included in the GREMLd analysis. We repeated the simulation 100 times. In each scenario, we calculated the mean estimates of  $h^2_{\text{SNP}}$  and  $\delta^2_{\text{SNP}}$  and their standard errors across all replicates.

We extended the simulations by reducing the number of observed SNPs included in the GREMLd analysis from 90% to 10% by steps of 10%. With the decreasing number of observed SNPs used in analysis, on average the LD between causal variants used for generating phenotype and the SNP used in analysis decreased. This simulation was to test whether or not the reduction in the estimate due to incomplete LD for  $\delta^2_{\text{SNP}}$  is faster than that for  $h^2_{\text{SNP}}$ .

We further performed simulations to mimic causal variants tending to have lower minor allele frequency (MAF) than SNPs by randomly sampling causal variants from SNPs with MAF  $< 0.1$ . We randomly sampled 10% of SNPs as a pool of causal variants, simulated phenotype with the same parameter setting as above (1,000 causal variants,  $h^2 = 0.3$ ,  $\delta^2 = 0.3$ , and 100 simulation replicates), and estimated  $h^2_{\text{SNP}}$  and  $\delta^2_{\text{SNP}}$  using the other 90% of SNPs (excluding the pool of causal variants).

### Supplemental Data

Supplemental Data include eight figures, four tables, and Supplemental Acknowledgments and can be found with this article online at <http://dx.doi.org/10.1016/j.ajhg.2015.01.001>.

### Consortia

The members of the LifeLines Cohort Study are Behrooz Z. Alizadeh, Rudolf A. de Boer, H. Marika Boezen, Marcel Bruinenberg, Lude Franke, Pim van der Harst, Hans L. Hillege, Melanie M. van der Klauw, Gerjan Navis, Johan Ormel, Dirkje S. Postma, Judith G.M. Rosmalen, Joris P. Slaets, Harold Snieder, Ronald P. Stolk, Bruce H.R. Wolffenbuttel, and Cisca Wijmenga.

### Acknowledgments

This research was supported by the Australian Research Council (130102666), the Australian National Health and Medical Research Council (1052684, 613601, 1048853), the USA NIH (GM057091, GM099568, MH100141), the Sylvia & Charles Viertel Charitable Foundation, and the UQ Foundation. This study makes use of data from the database of Genotypes and Phenotypes (dbGaP) under accession phs000090, the Lifelines study, and the

EGCUT study (see the [Supplemental Acknowledgments](#) for the full set of acknowledgments for these data).

Received: October 20, 2014

Accepted: January 2, 2015

Published: February 12, 2015

## Web Resources

The URLs for data presented herein are as follows:

1000 Genomes, <http://browser.1000genomes.org>

dbGaP, <http://www.ncbi.nlm.nih.gov/gap>

GCTA-GREMLd, <http://ctgg.qbi.uq.edu.au/software/gcta/GREMLd.html>

International HapMap Project, <http://hapmap.ncbi.nlm.nih.gov/>

## References

1. Mackay, T.F. (2001). The genetic architecture of quantitative traits. *Annu. Rev. Genet.* 35, 303–339.
2. Fisher, R.A. (1918). The correlation between relatives on the supposition of Mendelian inheritance. *Trans. R. Soc. Edinb.* 52, 399–433.
3. Fisher, R.A. (1930). *The Genetical Theory of Natural Selection* (Oxford: Clarendon press).
4. Jinks, J.L., and Fulker, D.W. (1970). Comparison of the biometrical, MAVA, and classical approaches to the analysis of human behavior. *Psychol. Bull.* 73, 311–349.
5. Treloar, S.A., and Martin, N.G. (1990). Age at menarche as a fitness trait: nonadditive genetic variance detected in a large twin sample. *Am. J. Hum. Genet.* 47, 137–148.
6. Herskind, A.M., McGue, M., Holm, N.V., Sørensen, T.I., Harvald, B., and Vaupel, J.W. (1996). The heritability of human longevity: a population-based study of 2872 Danish twin pairs born 1870–1900. *Hum. Genet.* 97, 319–323.
7. Abney, M., McPeck, M.S., and Ober, C. (2001). Broad and narrow heritabilities of quantitative traits in a founder population. *Am. J. Hum. Genet.* 68, 1302–1307.
8. Ober, C., Abney, M., and McPeck, M.S. (2001). The genetic dissection of complex traits in a founder population. *Am. J. Hum. Genet.* 69, 1068–1079.
9. Falconer, D.S., and Mackay, T.F.C. (1996). *Introduction to Quantitative Genetics* (England: Longman).
10. Lynch, M., and Walsh, B. (1998). *Genetics and Analysis of Quantitative Traits* (Sunderland: Sinauer Associates).
11. Hill, W.G., Goddard, M.E., and Visscher, P.M. (2008). Data and theory point to mainly additive genetic variance for complex traits. *PLoS Genet.* 4, e1000008.
12. Mäki-Tanila, A., and Hill, W.G. (2014). Influence of gene interaction on complex trait variation with multilocus models. *Genetics* 198, 355–367.
13. Welter, D., MacArthur, J., Morales, J., Burdett, T., Hall, P., Junkins, H., Klemm, A., Flicek, P., Manolio, T., Hindorf, L., and Parkinson, H. (2014). The NHGRI GWAS Catalog, a curated resource of SNP-trait associations. *Nucleic Acids Res.* 42, D1001–D1006.
14. Maher, B. (2008). Personal genomes: The case of the missing heritability. *Nature* 456, 18–21.
15. Manolio, T.A., Collins, F.S., Cox, N.J., Goldstein, D.B., Hindorf, L.A., Hunter, D.J., McCarthy, M.L., Ramos, E.M., Cardon, L.R., Chakravarti, A., et al. (2009). Finding the missing heritability of complex diseases. *Nature* 461, 747–753.
16. Eichler, E.E., Flint, J., Gibson, G., Kong, A., Leal, S.M., Moore, J.H., and Nadeau, J.H. (2010). Missing heritability and strategies for finding the underlying causes of complex disease. *Nat. Rev. Genet.* 11, 446–450.
17. Zuk, O., Hechter, E., Sunyaev, S.R., and Lander, E.S. (2012). The mystery of missing heritability: Genetic interactions create phantom heritability. *Proc. Natl. Acad. Sci. USA* 109, 1193–1198.
18. Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A., Bender, D., Maller, J., Sklar, P., de Bakker, P.I., Daly, M.J., and Sham, P.C. (2007). PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am. J. Hum. Genet.* 81, 559–575.
19. Yang, J., Benyamin, B., McEvoy, B.P., Gordon, S., Henders, A.K., Nyholt, D.R., Madden, P.A., Heath, A.C., Martin, N.G., Montgomery, G.W., et al. (2010). Common SNPs explain a large proportion of the heritability for human height. *Nat. Genet.* 42, 565–569.
20. Yang, J., Lee, S.H., Goddard, M.E., and Visscher, P.M. (2011). GCTA: a tool for genome-wide complex trait analysis. *Am. J. Hum. Genet.* 88, 76–82.
21. Visscher, P.M., Hemani, G., Vinkhuyzen, A.A., Chen, G.B., Lee, S.H., Wray, N.R., Goddard, M.E., and Yang, J. (2014). Statistical power to detect genetic (co)variance of complex traits using SNP data in unrelated samples. *PLoS Genet.* 10, e1004269.
22. Heard-Costa, N.L., Zillikens, M.C., Monda, K.L., Johansson, A., Harris, T.B., Fu, M., Haritunians, T., Feitosa, M.F., Aspelund, T., Eiriksdottir, G., et al. (2009). NRXN3 is a novel locus for waist circumference: a genome-wide association study from the CHARGE Consortium. *PLoS Genet.* 5, e1000539.
23. Leitsalu, L., Haller, T., Esko, T., Tammesoo, M.L., Alavere, H., Snieder, H., Perola, M., Ng, P.C., Mägi, R., Milani, L., et al. (2014). Cohort Profile: Estonian Biobank of the Estonian Genome Center, University of Tartu. *Int. J. Epidemiol.*, in press. Published online February 11, 2014. <http://dx.doi.org/10.1093/ije/dyt268>.
24. Stolk, R.P., Rosmalen, J.G., Postma, D.S., de Boer, R.A., Navis, G., Slaets, J.P., Ormel, J., and Wolffenbuttel, B.H. (2008). Universal risk factors for multifactorial diseases: LifeLines: a three-generation population-based study. *Eur. J. Epidemiol.* 23, 67–74.
25. Abecasis, G.R., Altshuler, D., Auton, A., Brooks, L.D., Durbin, R.M., Gibbs, R.A., Hurles, M.E., and McVean, G.A.; 1000 Genomes Project Consortium (2010). A map of human genome variation from population-scale sequencing. *Nature* 467, 1061–1073.
26. Howie, B.N., Donnelly, P., and Marchini, J. (2009). A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. *PLoS Genet.* 5, e1000529.
27. Altshuler, D.M., Gibbs, R.A., Peltonen, L., Altshuler, D.M., Gibbs, R.A., Peltonen, L., Dermitzakis, E., Schaffner, S.F., Yu, F., Peltonen, L., et al.; International HapMap 3 Consortium (2010). Integrating common and rare genetic variation in diverse human populations. *Nature* 467, 52–58.
28. Lee, S.H., Yang, J., Chen, G.B., Ripke, S., Stahl, E.A., Hultman, C.M., Sklar, P., Visscher, P.M., Sullivan, P.F., Goddard, M.E., and Wray, N.R. (2013). Estimation of SNP heritability from dense genotype data. *Am. J. Hum. Genet.* 93, 1151–1155.



29. Speed, D., Hemani, G., Johnson, M.R., and Balding, D.J. (2013). Response to Lee et al.: SNP-based heritability analysis with dense data. *Am. J. Hum. Genet.* *93*, 1155–1157.
30. Price, A.L., Patterson, N.J., Plenge, R.M., Weinblatt, M.E., Shadick, N.A., and Reich, D. (2006). Principal components analysis corrects for stratification in genome-wide association studies. *Nat. Genet.* *38*, 904–909.
31. Yang, J., Lee, T., Kim, J., Cho, M.C., Han, B.G., Lee, J.Y., Lee, H.J., Cho, S., and Kim, H. (2013). Ubiquitous polygenicity of human complex traits: genome-wide analysis of 49 traits in Koreans. *PLoS Genet.* *9*, e1003355.
32. Powell, J.E., Henders, A.K., McRae, A.F., Kim, J., Hemani, G., Martin, N.G., Dermitzakis, E.T., Gibson, G., Montgomery, G.W., and Visscher, P.M. (2013). Congruence of additive and non-additive effects on gene expression estimated from pedigree and SNP data. *PLoS Genet.* *9*, e1003502.
33. Smith, N.L., Chen, M.H., Dehghan, A., Strachan, D.P., Basu, S., Soranzo, N., Hayward, C., Rudan, I., Sabater-Lleal, M., Bis, J.C., et al.; Wellcome Trust Case Control Consortium (2010). Novel associations of multiple genetic loci with plasma levels of factor VII, factor VIII, and von Willebrand factor: The CHARGE (Cohorts for Heart and Aging Research in Genome Epidemiology) Consortium. *Circulation* *121*, 1382–1392.
34. Weir, B.S. (2008). Linkage disequilibrium and association mapping. *Annu. Rev. Genomics Hum. Genet.* *9*, 129–142.
35. Kacser, H., and Burns, J.A. (1981). The molecular basis of dominance. *Genetics* *97*, 639–666.

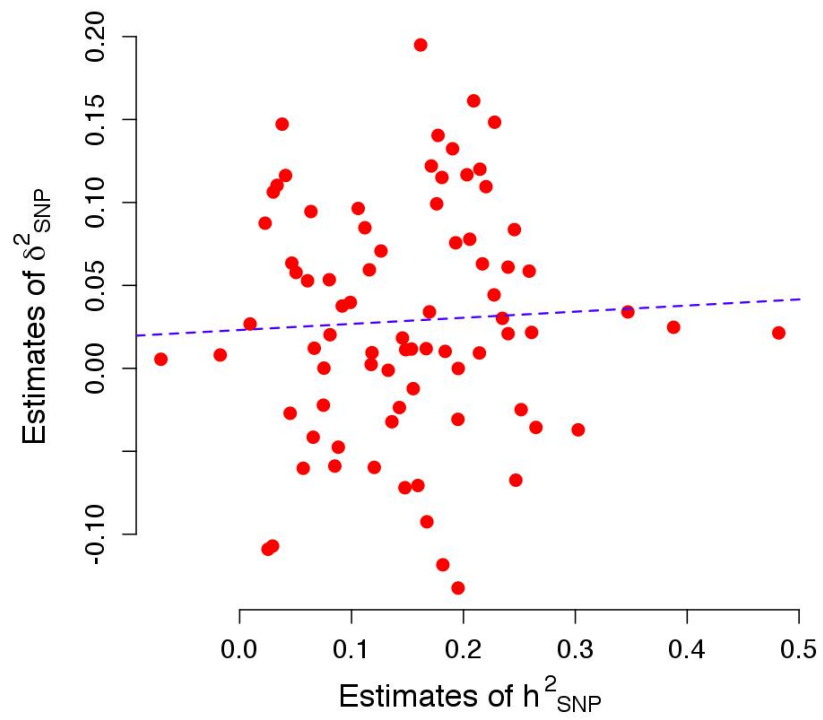
The American Journal of Human Genetics

Supplemental Data

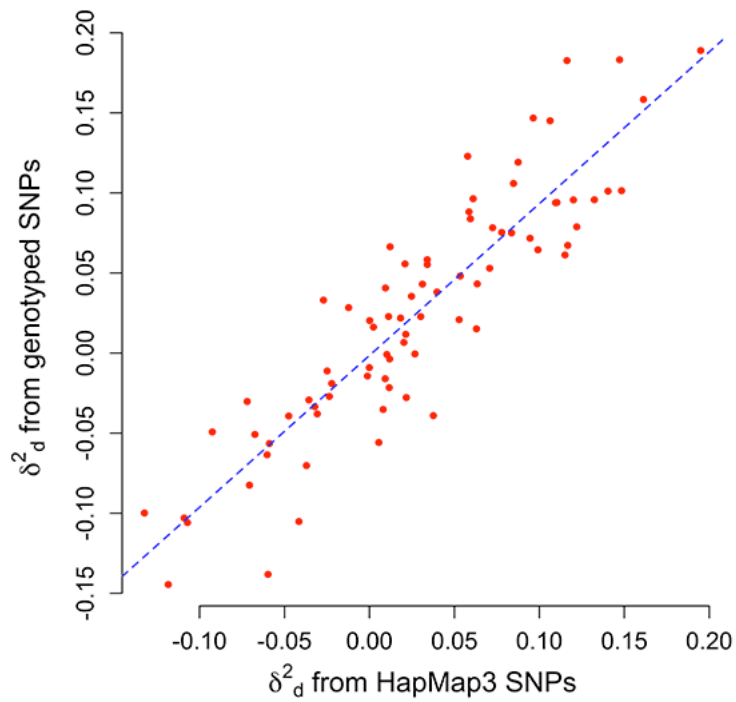
## **Dominance Genetic Variation Contributes Little to the Missing Heritability for Human Complex Traits**

Zhihong Zhu, Andrew Bakshi, Anna A.E. Vinkhuyzen, Gibran Hemani, Sang Hong Lee, Ilja M. Nolte, Jana V. van Vliet-Ostaptchouk, Harold Snieder, The LifeLines Cohort Study, Tonu Esko, Lili Milani, Reedik Mägi, Andres Metspalu, William G. Hill, Bruce S. Weir, Michael E. Goddard, Peter M. Visscher, and Jian Yang

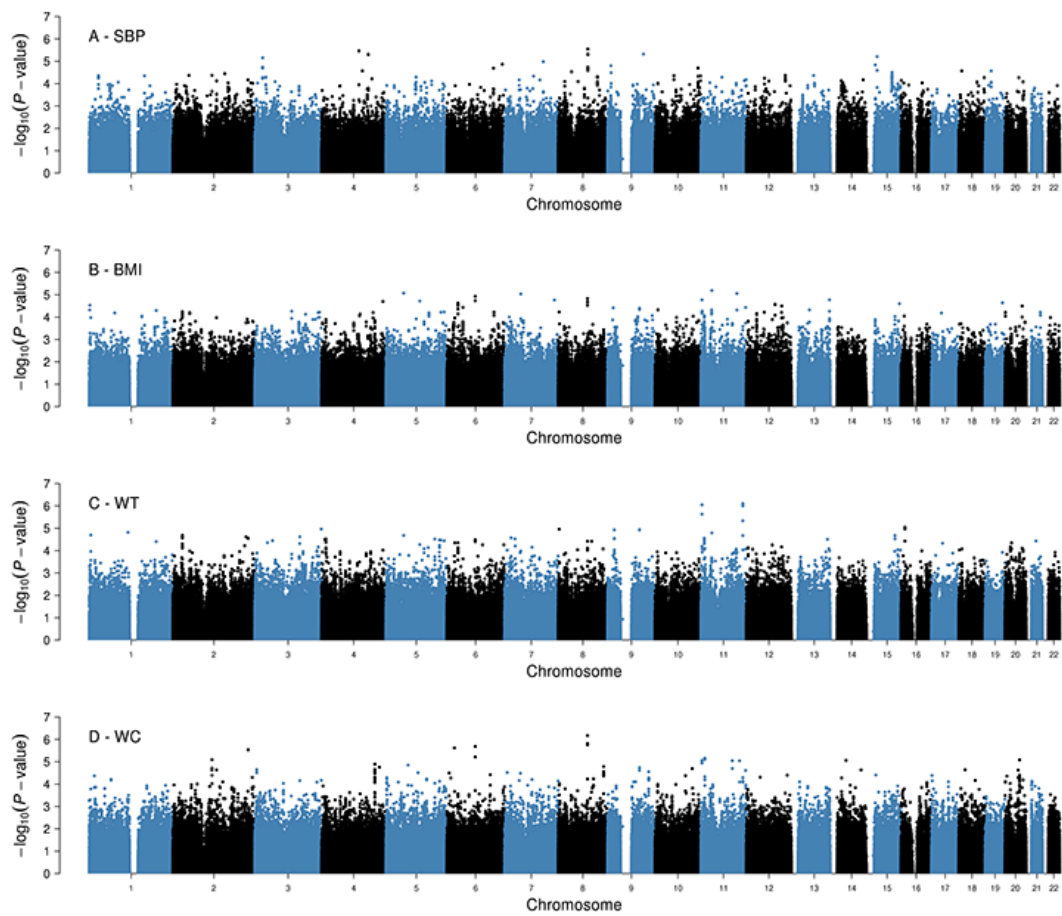




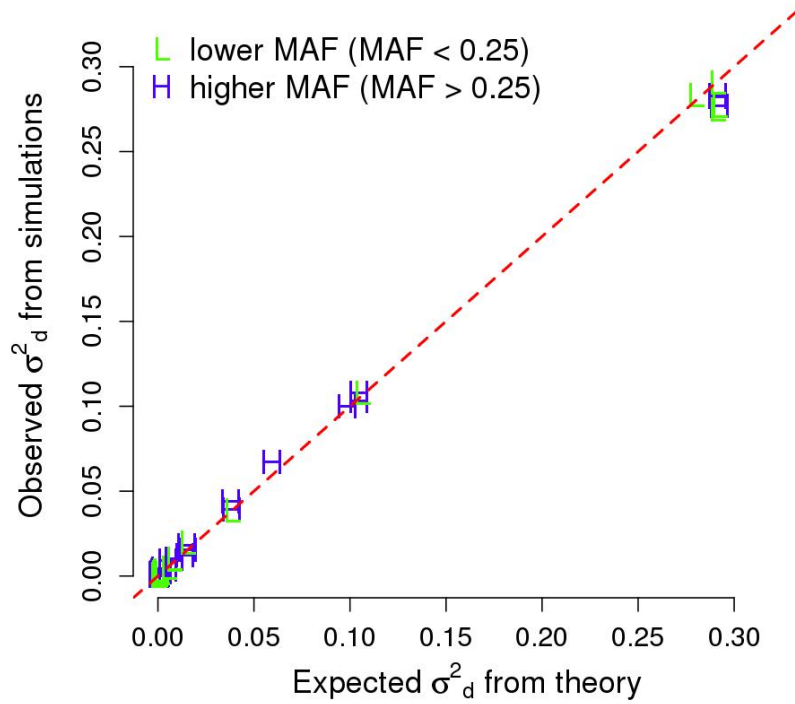
**Figure S2. Estimate of  $\delta^2_{SNP}$  against that of  $h^2_{SNP}$  in the ARIC cohort.** The regression slope is 0.04, which is not significantly different from zero ( $P = 0.67$ ).



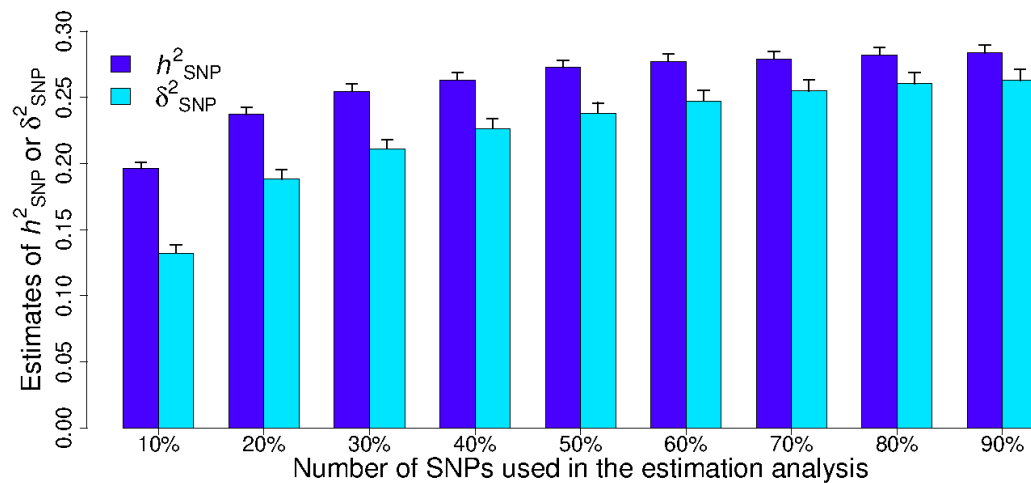
**Figure S3. Plot of the estimates of  $\delta_{SNP}^2$  using genotyped data against those using imputed data for the 79 traits in the ARIC cohort.** The mean estimate of  $\delta_{SNP}^2$  across all the 79 traits is 3% using genotype data, which is exactly the same as that using imputed data (HapMap phase 3 SNPs extracted from 1000G imputed data). The regression slope is 0.95 (SE=0.05), which is not significantly different from 1.



**Figure S4. Genome-wide association tests for dominance effects for 4 quantitative traits in the combined sample of EGCUT and LL cohorts.** These traits are a) SBP (systolic blood pressure), b) BMI (body mass index), c) WT (weight), and d) WC (waist circumference). Plotted are  $-\log_{10}$  of  $p$ -values for dominance effects from the analysis of fitting both additive and dominance effects.

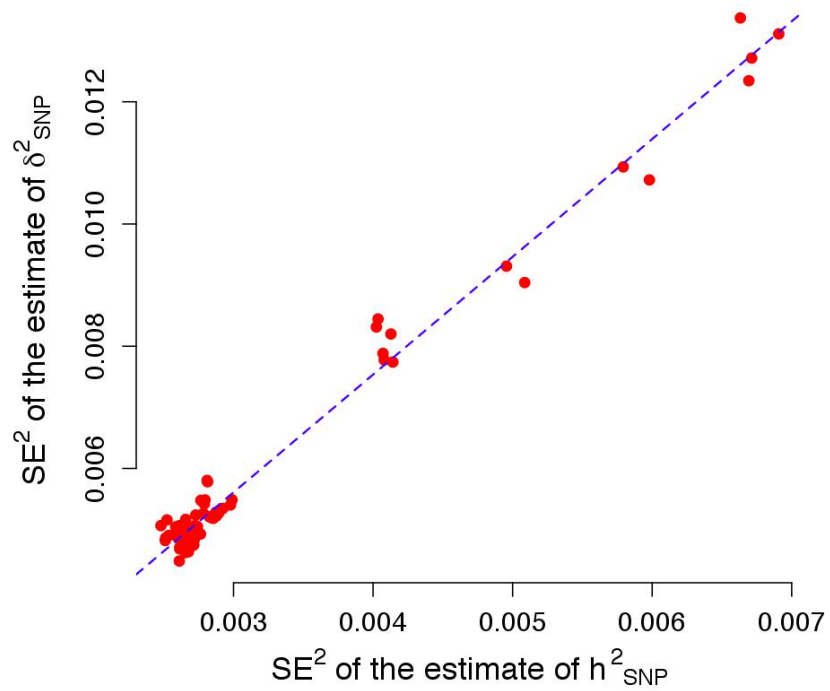


**Figure S5. Plot of the observed  $\sigma_d^2$  from simulations against the expected  $\sigma_d^2$  from theory.** In theory<sup>1</sup>, dominance variance at a SNP equals dominance variance at a causal variant times  $r^4$  with  $r$  being the LD correlation between the SNP and the causal variant, i.e.  $\sigma_d^2 = r^4 \sigma_{d(\text{Causal})}^2$ . To confirm this, we conducted simulations by extracting 1,000 proximal SNPs on chromosome 1 as a pool of causal variants (including 577 SNPs with MAF < 0.25 and 423 SNPs with MAF > 0.25) and one additional SNP (MAF = 0.18) as the observed SNP from the ARIC data set. For each of the causal variants, we generated a quantitative phenotype using the same simulation approach as in **Appendix** with  $\sigma_{a(\text{Causal})}^2 / \sigma_p^2 = 0.3$  and  $\sigma_{d(\text{Causal})}^2 / \sigma_p^2 = 0.3$  with  $\sigma_p^2$  being the phenotypic variance, and estimated  $\sigma_d^2$  from an association analysis of the observed SNP and simulated phenotype using Equation 5. The LD between the observed SNP and the causal variants is a function of MAF difference between the SNP and causal variants, which causes variation in  $\sigma_d^2$ .



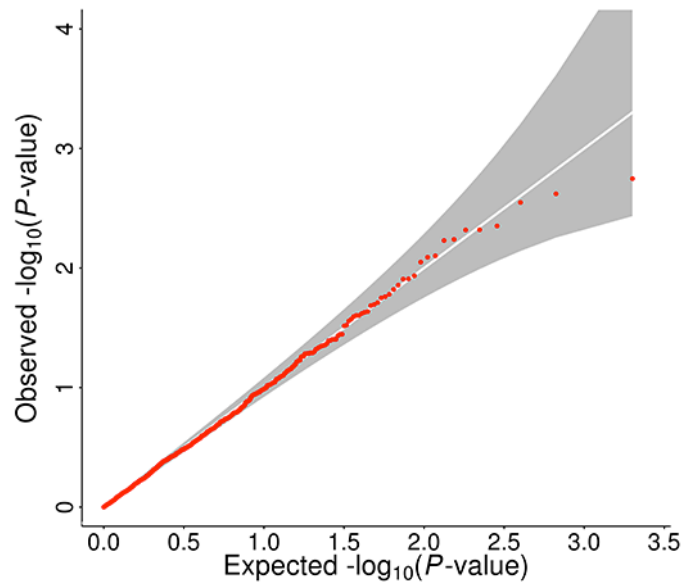
**Figure S6. Estimates of  $h^2_{SNP}$  and  $\delta^2_{SNP}$  based on a range of numbers of SNPs from simulations.** Simulation strategy can be found in the **Appendix**. In brief, we randomly sampled 10% of SNPs as a pool of “unobserved causal variants”, and used the remaining 90% as the “observed SNPs”. We gradually reduced the LD between the observed SNPs and the unobserved causal variants by including a smaller number of “observed SNPs” in the GREMLd estimation analysis. Shown are the mean estimates over 100 simulations. Error bars are the standard errors of mean estimates.





**Figure S7. Plot of  $SE^2(\hat{h}_{SNP}^2)$  against  $SE^2(\hat{\delta}_{SNP}^2)$  for 79 traits in the ARIC cohort.**

The blue dash line represents the fitted regression with a slope of 1.93.  $SE^2(\hat{h}_{SNP}^2)$  and  $SE^2(\hat{\delta}_{SNP}^2)$  are the estimates of  $\text{var}(\hat{h}_{SNP}^2)$  and  $\text{var}(\hat{\delta}_{SNP}^2)$ , respectively.



**Figure S8. QQ-plot of  $p$ -values for dominance variation at an observed SNP from 1000 simulations where the underlying causal variant has a strong genetic effect that is purely additive.** Genotypes of the causal variant and the SNP are generated from a bivariate binomial distribution, given sample size = 5,000, MAF = 0.4 for the causal variant, MAF = 0.2 for the SNP, and LD  $r^2 = 0.25$ . The phenotype is generated as such that additive variance at the causal variance explained 10% of the phenotypic variance, and the dominance variance is 0. Association test for dominance variance at the SNP is performed based on the method described in Equation (3). The grey area shown on the figure is the 95% confidence interval.

**Table S1.** Genotyping and quality controls in the ARIC, EGCUT and Lifelines cohorts.

Cohort	Platform	Inclusion criteria				SNPs that met QC
		MAF	SNP call rate	Sample call rate	<i>p</i> for HWE	
ARIC	Affymetrix Genome-Wide Human SNP Array 6.0	≥ 1%	≥ 98%	≥ 98%	> 10 <sup>-3</sup>	593,521
EGCUT	Illumina Human370CNV	≥ 1%	≥ 95%	≥ 95%	> 10 <sup>-6</sup>	326,898
	Illumina OMNI-Express	≥ 1%	≥ 95%	≥ 95%	> 10 <sup>-6</sup>	633,182
Lifelines	Illumina Cyto SNP12 v2	≥ 1%	≥ 95%	≥ 95%	> 10 <sup>-6</sup>	245,943

*p* for HWE: *p*-value from Hardy-Weinberg Equilibrium test.

**Table S2.** Descriptive summary of the 79 quantitative traits in the ARIC cohort.

Group	Trait	Description (unit)	Mean (SD) in males	Mean (SD) in females
Height	Height	Height (cm)	176.2 (6.48)	162 (5.9)
	SitHT	Sitting height (cm)	92.8 (3.47)	86.4 (3.34)
Obesity	WT	Weight (lb)	187.5 (30.02)	153.7 (32.64)
	WT@25	Weight at the age of 25 (lb)	165.8 (26.05)	126.4 (19.87)
	BMI	Weight (kg) divided by squared height (m)	27.4 (3.98)	26.6 (5.49)
	WC	Waist circumference (cm)	99.6 (10.37)	93.1 (14.83)
	HC	Hip circumference (cm)	102.7 (7.54)	104.2 (10.86)
	WHR	Waist-to-hip ratio, the ratio of waist to hip	1 (0.05)	0.9 (0.08)
	WHRadjBMI	BMI adjusted WHR	0.8 (0.04)	0.7 (0.07)
Lipoprotein	Wrist	Wrist breadth (mm)	57.8 (3.57)	49.8 (3.32)
	Calf	Calf circumference (cm)	38.2 (3.07)	36.7 (3.85)
	TRI	Triceps skinfold (mm)	19.6 (7.67)	28.9 (7.95)
	SUB	Subscapular skinfold (mm)	21.2 (8.16)	22.4 (10.22)
	Apo	Apolipoprotein (mmol/L)	1.7 (1.13)	1.5 (0.94)
	ApoA	Apolipoprotein (mg/L)	1187.9 (248.74)	1417.7 (314.18)
	ApoB	Apolipoprotein B (mg/L)	958.6 (268.52)	919.6 (289.79)
Diabetes	CHL	Cholesterol (mmol/L)	5.5 (0.99)	5.7 (1.1)
	HDL	High-density lipoprotein (mmol/L)	1.1 (0.32)	1.5 (0.44)
	HDL2	Subfraction of HDL cholesterol (mmol/L)	0.3 (0.15)	0.4 (0.24)
	HDL3	Subfraction of HDL cholesterol (mmol/L)	0.8 (0.25)	1 (0.27)
	LDL	LDL cholesterol (mmol/L)	3.6 (0.92)	3.5 (1.03)
	Glucose	Glucose (mmol/L)	6 (1.64)	5.7 (1.7)
	Insulin	Insulin (pmol/L)	91.1 (120.1)	78.2 (122.21)
Blood pressure	SBP	Systolic blood pressure (mmHg)	120.1 (16.09)	116.9 (17.54)
	BBP	Brachial systolic blood pressure (mmHg)	130.4 (18.81)	123.4 (20.41)
	ABP	Ankle systolic blood pressure (mmHg)	151.1 (22.37)	135.8 (22.93)
	ABPI	Ankle-brachial index, the ratio of ABP to BBP	1.2 (0.14)	1.1 (0.14)
	DBP	Diastolic blood pressure (mmHg)	73.6 (10.01)	69.8 (9.73)
Blood phenotypes	Ht	Hematocrit, the volume percentage (%) of red blood cells in blood	44.7 (2.94)	40 (2.86)
	Hb	Hemoglobin	15 (1.01)	13.3 (1)
	WBC	White blood cells count $\times 1000/\text{mm}^3$	6.5 (2.08)	6.2 (1.88)
	PRO	Blood proteins (gm/dl)	7.2 (0.39)	7.2 (0.41)
	Albumin	Albumin (gm/dl)	3.9 (0.24)	3.9 (0.25)
	FI	Factor I	294 (61.37)	299.4 (60.52)
	FVII	Factor VII	111.5 (24.54)	125.2 (30.97)
	FVIII	Factor VIII	122.8 (33.56)	127.4 (34.41)
	vWF	von Willebrand factor	113.5 (43.23)	111.4 (42.46)
	Na	Sodium (mmol/L)	140.9 (2.32)	140.9 (2.41)
	K	Potassium (mmol/L)	4.6 (0.44)	4.5 (0.48)
	Ca	Calcium (mg/dl)	1.2 (0.16)	1 (0.14)
	Mg	Magnesium (meq/L)	9.7 (0.40)	9.8 (0.42)
P	Phosphorus (meq/L)	1.7 (0.15)	1.6 (0.15)	

	Creatine	Serum creatine (mg/dl)	3.2 (0.44)	3.6 (0.46)
	UA	Uric acid (mg/dl)	6.7 (1.33)	5.3 (1.34)
Carotid artery	Lopamx	Maximum far wall width (L common carotid: opt ang)	0.8 (0.25)	0.7 (0.21)
	Lanamx	Maximum far wall width (L common carotid: anterior angle)	0.8 (0.24)	0.7 (0.19)
	Lpoamx	Maximum far wall width (L common carotid: pst ang)	0.8 (0.27)	0.7 (0.20)
	Ropamx	Maximum far wall width (R common carotid: opt ang)	0.8 (0.25)	0.7 (0.21)
	Ranamx	Maximum far wall width (R common carotid: an ang)	0.8 (0.22)	0.7 (0.19)
	Rpoamx	Maximum far wall width (R common carotid: pst ang)	0.8 (0.25)	0.7 (0.19)
	Rbiamx	Maximum far wall width (R bifurcation)	1.2 (0.69)	1.0 (0.52)
	Lopaav	Average far wall width (L common carotid: opt ang)	0.7 (0.19)	0.6 (0.16)
	Lanaav	Average far wall width (L common carotid: anterior angle)	0.7 (0.18)	0.6 (0.15)
	Lpoaav	Average far wall width (L common carotid: pst ang)	0.7 (0.19)	0.6 (0.16)
	Ropaav	Average far wall width (R common carotid: opt ang)	0.7 (0.19)	0.6 (0.15)
	Ranaav	Average far wall width (R common carotid: ant ang)	0.7 (0.18)	0.6 (0.15)
	Rpoaav	Average far wall width (R common carotid: pst ang)	0.7 (0.19)	0.6 (0.14)
	Rbiaav	Average far wall width (R bifurcation)	0.9 (0.45)	0.8 (0.34)
	Lopada	Average far wall thickness (L opt)	0.7 (0.17)	0.6 (0.15)
	Ropada	Average far wall thickness (R opt)	0.7 (0.17)	0.6 (0.14)
	Lbiada	Derived average far wall thickness (L bifurcation)	0.9 (0.33)	0.8 (0.26)
	Rbiada	Derived average far wall thickness (R bifurcation)	0.9 (0.37)	0.8 (0.29)
Linada	Derived average far wall thickness (L internal carotid)	0.7 (0.28)	0.7 (0.26)	
Rinada	Derived average far wall thickness (R internal carotid)	0.8 (0.35)	0.7 (0.28)	
MeanDA	Mean of the derived average far wall thickness variables	0.8 (0.20)	0.7 (0.17)	
Lopart	Derived average far wall thickness (L opt)	0.7 (0.17)	0.6 (0.15)	
Ropart	Derived average far wall thickness (R opt)	0.6 (0.17)	0.6 (0.13)	
Lbiart	Derived average far wall thickness (L bifurcation)	0.9 (0.33)	0.8 (0.26)	
Rbiart	Derived average far wall thickness (R bifurcation)	0.9 (0.37)	0.8 (0.28)	
Linart	Derived average far wall thickness (L internal carotid)	0.7 (0.28)	0.6 (0.25)	
Rinart	Derived average far wall thickness (R internal carotid)	0.8 (0.34)	0.7 (0.28)	
MeanRT	Mean of the derived average far	0.8 (0.20)	0.7 (0.16)	

wall thickness variables				
Heart function	HR	Heart rate	66.1 (9.95)	69 (9.43)
	HR(ECG)	HR measured by electrocardiography (ECG)	64.9 (10.03)	68 (9.65)
	QT	Q-T interval	408.5 (28.75)	411.7 (27.82)
	PR	P-R interval	167.3 (24.98)	160.5 (24.53)
	QRS	QRS complex, the combination of three of the graphical deflection, Q, R, and S waves.	101.6 (12.79)	94.5 (11.16)
	Cor	Cornell voltage	13.1 (5.10)	10 (4.24)
Smoking	CIG	Cigarette smoking, average number of cigarettes per day times the number of years smoked	470.8 (492.07)	230.2 (350.77)

**Table S3.** Estimates of variance explained by additive and dominance variation at all SNPs for the 79 traits in ARIC cohort.

Group	Trait	<i>n</i>	$h^2_{\text{SNP}}$ (SE)	<i>P</i>	$\delta^2_{\text{SNP}}$ (SE)	<i>P</i>	
Height	Height	6710	0.48 (0.05)	< 1E-16	0.02 (0.07)	3.78E-01	
	SitHT	6704	0.39 (0.05)	8.55E-15	0.02 (0.07)	3.63E-01	
Obesity	<b>*WT</b>	<b>6709</b>	<b>0.18 (0.05)</b>	<b>2.14E-04</b>	<b>0.14 (0.07)</b>	<b>2.28E-02</b>	
	WT@25	6698	0.22 (0.05)	6.23E-06	0.11 (0.07)	5.81E-02	
	<b>*BMI</b>	<b>6709</b>	<b>0.23 (0.05)</b>	<b>3.50E-06</b>	<b>0.15 (0.07)</b>	<b>1.67E-02</b>	
	<b>*WC</b>	<b>6707</b>	<b>0.17 (0.05)</b>	<b>3.06E-04</b>	<b>0.12 (0.07)</b>	<b>4.13E-02</b>	
	HC	6706	0.17 (0.05)	4.55E-04	0.01 (0.07)	4.32E-01	
	WHR	6704	0.18 (0.05)	1.36E-04	0.12 (0.07)	5.10E-02	
	WHRadjBMI	6701	0.13 (0.05)	3.00E-03	0.00 (0.07)	4.93E-01	
	Wrist	6709	0.26 (0.05)	9.81E-08	0.06 (0.07)	2.08E-01	
	<b>*Calf</b>	<b>6708</b>	<b>0.21 (0.05)</b>	<b>1.09E-05</b>	<b>0.12 (0.07)</b>	<b>3.67E-02</b>	
	TRI	6708	0.19 (0.05)	1.25E-04	0.08 (0.07)	1.38E-01	
Lipoprotein	SUB	6689	0.23 (0.05)	2.81E-06	0.04 (0.07)	2.60E-01	
	Apo	6691	0.30 (0.05)	1.39E-09	-0.04 (0.07)	2.98E-01	
	ApoA	6702	0.14 (0.05)	2.26E-03	-0.02 (0.07)	3.65E-01	
	ApoB	6703	0.15 (0.05)	1.06E-03	0.01 (0.07)	4.34E-01	
	CHL	6699	0.26 (0.05)	1.29E-07	0.02 (0.07)	3.79E-01	
	HDL	6705	0.25 (0.05)	5.53E-07	-0.07 (0.07)	1.63E-01	
	HDL2	6702	0.11 (0.05)	1.64E-02	0.08 (0.07)	1.12E-01	
	HDL3	6704	0.20 (0.05)	4.47E-05	-0.13 (0.07)	2.59E-02	
Diabetes	LDL	6595	0.21 (0.05)	1.42E-05	0.01 (0.07)	4.48E-01	
	Glucose	6679	0.17 (0.05)	4.82E-04	-0.09 (0.07)	8.98E-02	
Blood pressure	Insulin	6699	0.06 (0.05)	1.10E-01	0.05 (0.07)	2.34E-01	
	<b>*SBP</b>	<b>6711</b>	<b>0.21 (0.05)</b>	<b>1.71E-05</b>	<b>0.16 (0.07)</b>	<b>1.18E-02</b>	
	BBP	6481	0.18 (0.05)	4.06E-04	0.10 (0.07)	7.81E-02	
	<b>*ABP</b>	<b>6481</b>	<b>0.16 (0.05)</b>	<b>7.78E-04</b>	<b>0.19 (0.07)</b>	<b>3.59E-03</b>	
	ABPI	6472	0.06 (0.05)	1.09E-01	0.09 (0.07)	8.94E-02	
Blood phenotypes	DBP	6710	0.21 (0.05)	3.66E-05	0.08 (0.07)	1.38E-01	
	Ht	6692	0.16 (0.05)	9.22E-04	-0.07 (0.07)	1.55E-01	
	Hb	6691	0.20 (0.05)	7.60E-05	-0.03 (0.07)	3.28E-01	
	WBC	6695	0.26 (0.05)	6.61E-08	-0.04 (0.07)	3.01E-01	
	PRO	6708	0.20 (0.05)	3.30E-05	0.00 (0.07)	5.00E-01	
	Albumin	6709	0.11 (0.05)	1.73E-02	0.10 (0.07)	8.52E-02	
	FI	6685	0.24 (0.05)	1.47E-06	0.02 (0.07)	3.84E-01	
	FVII	6550	0.18 (0.05)	1.77E-04	-0.12 (0.07)	4.89E-02	
	FVIII	6682	0.25 (0.05)	2.96E-07	-0.02 (0.07)	3.57E-01	
	vWF	6684	0.35 (0.05)	3.69E-12	0.03 (0.07)	3.10E-01	
	Na	6708	0.05 (0.05)	1.51E-01	0.06 (0.07)	2.09E-01	
	K	6710	0.15 (0.05)	1.68E-03	-0.07 (0.07)	1.66E-01	
	Ca	6706	0.12 (0.05)	1.02E-02	0.06 (0.07)	1.94E-01	
	Mg	6710	0.07 (0.05)	8.78E-02	0.01 (0.07)	4.30E-01	
	<b>*P</b>	<b>6708</b>	<b>0.19 (0.05)</b>	<b>6.76E-05</b>	<b>0.13 (0.07)</b>	<b>2.68E-02</b>	
	Carotid artery	Creatine	6709	0.24 (0.05)	1.13E-06	0.06 (0.07)	1.97E-01
		UA	6709	0.25 (0.05)	1.42E-07	0.08 (0.07)	1.13E-01
Lopamx		5278	0.04 (0.06)	2.57E-01	0.12 (0.09)	1.02E-01	
Lanamx		4432	0.08 (0.08)	1.42E-01	0.02 (0.10)	4.22E-01	
Lpoamx		4804	0.12 (0.07)	4.37E-02	0.01 (0.10)	4.61E-01	
Ropamx		5216	0.03 (0.06)	3.00E-01	0.11 (0.09)	1.09E-01	

	Ranamx	4143	0.09 (0.08)	1.31E-01	0.04 (0.11)	3.71E-01
	Rpoamx	5199	0.05 (0.06)	2.36E-01	-0.03 (0.09)	3.81E-01
	Rbiamx	4048	-0.07 (0.08)	1.95E-01	0.01 (0.12)	4.81E-01
	Lopaav	5279	0.04 (0.06)	2.73E-01	0.15 (0.09)	5.57E-02
	Lanaav	4435	0.15 (0.08)	2.95E-02	0.02 (0.10)	4.29E-01
	Lpoaav	4805	0.16 (0.07)	1.41E-02	-0.01 (0.10)	4.48E-01
	Ropaav	5224	0.08 (0.06)	9.93E-02	0.05 (0.09)	2.68E-01
	Ranaav	4143	0.12 (0.08)	6.92E-02	-0.06 (0.11)	3.00E-01
	Rpoaav	5200	0.08 (0.06)	1.17E-01	0.00 (0.09)	4.99E-01
	Rbiaav	4050	0.07 (0.08)	2.13E-01	-0.04 (0.11)	3.60E-01
	Lopada	6350	0.02 (0.05)	3.34E-01	0.09 (0.08)	1.27E-01
	Ropada	6352	0.10 (0.05)	3.09E-02	0.04 (0.07)	2.86E-01
	Lbiada	6341	0.15 (0.05)	3.10E-03	0.01 (0.07)	4.39E-01
	Rbiada	6345	0.03 (0.05)	3.17E-01	-0.11 (0.07)	6.96E-02
	Linada	6341	-0.02 (0.05)	3.72E-01	0.01 (0.07)	4.56E-01
	Rinada	6338	0.06 (0.05)	1.43E-01	-0.06 (0.07)	2.02E-01
	MeanDA	6351	0.09 (0.05)	4.91E-02	-0.05 (0.07)	2.57E-01
	Lopart	6350	0.03 (0.05)	2.85E-01	0.11 (0.08)	8.24E-02
	Ropart	6353	0.13 (0.05)	8.50E-03	0.07 (0.07)	1.58E-01
	Lbiart	6341	0.17 (0.05)	8.75E-04	0.03 (0.07)	3.23E-01
	Rbiart	6345	0.03 (0.05)	2.93E-01	-0.11 (0.07)	7.35E-02
	Linart	6343	0.01 (0.05)	4.30E-01	0.03 (0.07)	3.59E-01
	Rinart	6339	0.07 (0.05)	7.97E-02	-0.02 (0.07)	3.80E-01
	MeanRT	6351	0.12 (0.05)	1.41E-02	0.00 (0.07)	4.87E-01
Heart function	HR	6691	0.22 (0.05)	9.10E-06	0.06 (0.07)	1.83E-01
	<b>*HR(ECG)</b>	<b>6611</b>	<b>0.20 (0.05)</b>	<b>3.69E-05</b>	<b>0.12 (0.07)</b>	<b>4.42E-02</b>
	QT	6616	0.23 (0.05)	3.23E-06	0.03 (0.07)	3.43E-01
	PR	6619	0.18 (0.05)	1.41E-04	0.01 (0.07)	4.39E-01
	QRS	6625	0.05 (0.05)	1.83E-01	0.06 (0.07)	1.78E-01
	Cor	6483	0.09 (0.05)	4.72E-02	-0.06 (0.07)	1.91E-01
Smoking	CIG	6636	0.14 (0.05)	4.26E-03	-0.03 (0.07)	3.23E-01

$n$  = sample size;  $h_{SNP}^2$  = variance explained by additive variation at all SNPs;  $\delta_{SNP}^2$  = variance explained by dominance variation at all SNPs; SE = standard error;  $P$  =  $p$ -value from a LRT test; \* traits (in bold) with nominally significant estimates of  $\delta_{SNP}^2$  ( $P < 0.05$ ).



**Table S4.** Mean estimates of  $h_{SNP}^2$  and  $\delta_{SNP}^2$  averaged over 100 simulations

	$h^2$	Est. $h_{SNP}^2$ (SEM)	$\delta^2$	Est. $\delta_{SNP}^2$ (SEM)
Scenario I	0.30	0.30 (0.01)	0.30	0.30 (0.01)
Scenario II		0.28 (0.01)		0.26 (0.01)

Scenario I: all the SNPs including the causal variants were included in the estimation analysis; Scenario II: causal variants were sampled from 10% of the SNPs and the other 90% of SNPs were used in the estimation analysis;  $h^2$ : variance explained by additive variation at the causal variants;  $\delta^2$ : variance explained by dominance variation at the causal variants; Est.: mean estimate over 100 simulations; Est.  $h_{SNP}^2$ : variance explained by additive variation at all SNPs in the estimation analysis (all SNPs in Scenario I, all SNPs excluding causal variants in Scenario II); Est.  $\delta_{SNP}^2$ : variance explained by dominance variation at all SNPs in the estimation analysis; SEM: standard error of the mean estimate.

## **Reference**

1. Weir, B.S. (2008). Linkage disequilibrium and association mapping. *Annu Rev Genomics Hum Genet* 9, 129-142

## **Acknowledgements**

The Atherosclerosis Risk in Communities Study is carried out as a collaborative study supported by National Heart, Lung, and Blood Institute contracts (HHSN268201100005C, HHSN268201100006C, HHSN268201100007C, HHSN268201100008C, HHSN268201100009C, HHSN268201100010C, HHSN268201100011C, and HHSN268201100012C), R01HL087641, R01HL59367 and R01HL086694; National Human Genome Research Institute contract U01HG004402; and National Institutes of Health contract HHSN268200625226C.

The authors thank the staff and participants of the ARIC study for their important contributions. Infrastructure was partly supported by Grant Number UL1RR025005, a component of the National Institutes of Health and NIH Roadmap for Medical Research.

EGCUT received funding through targeted financing from Estonian Government SF0180142s08, Estonian Research Roadmap through Estonian Ministry of Education and Research, and from Center of Excellence in Genomics (EXCEGEN), University of Tartu (SP1GVARENG), Estonian Research Council IUT2-2 grant and European Regional Development Fund.

The LifeLines Cohort Study, and generation and management of GWAS genotype data for the LifeLines Cohort Study is supported by the Netherlands Organization of Scientific Research NWO (grant 175.010.2007.006), the Economic Structure Enhancing Fund (FES) of the Dutch government, the Ministry of Economic Affairs, the Ministry of Education, Culture and Science, the Ministry for Health, Welfare and Sports, the Northern Netherlands Collaboration of Provinces (SNN), the Province of Groningen, University Medical Center Groningen, the University of Groningen, Dutch Kidney Foundation and Dutch Diabetes Research Foundation. The

authors are grateful to the study participants, the staff from the LifeLines Cohort Study and the contributing research centers delivering data to LifeLines and the participating general practitioners and pharmacists. LifeLines Cohort Study: Behrooz Z Alizadeh (1), Rudolf A de Boer (2), H Marike Boezen (1), Marcel Bruinenberg (3), Lude Franke (4), Pim van der Harst (2), Hans L Hillege (1,2), Melanie M van der Klauw (5), Gerjan Navis (6), Johan Ormel (7), Dirkje S Postma (8), Judith GM Rosmalen (7), Joris P Slaets (9), Harold Snieder (1), Ronald P Stolk (1), Bruce HR Wolffenbuttel (5), Cisca Wijmenga (4)

- (1) Department of Epidemiology, University of Groningen, University Medical Center Groningen, The Netherlands
- (2) Department of Cardiology, University of Groningen, University Medical Center Groningen, The Netherlands
- (3) LifeLines Cohort Study, University of Groningen, University Medical Center Groningen, The Netherlands
- (4) Department of Genetics, University of Groningen, University Medical Center Groningen, The Netherlands
- (5) Department of Endocrinology, University of Groningen, University Medical Center Groningen, The Netherlands
- (6) Department of Internal Medicine, Division of Nephrology, University of Groningen, University Medical Center Groningen, The Netherlands
- (7) Interdisciplinary Center of Psychopathology of Emotion Regulation (ICPE), Department of Psychiatry, University of Groningen, University Medical Center Groningen, The Netherlands
- (8) Department of Pulmonology, University of Groningen, University Medical Center Groningen, The Netherlands
- (9) University Center for Geriatric Medicine, University of Groningen, University Medical Center Groningen, The Netherlands