# Rigorous Evaluation of Genetic and Epigenetic effects on Clinical Laboratory Measurements using Japanese Monozygotic Twins

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ABSTRACT

The investigation of environmental effects on clinical measurements using individual samples is challenging because their genetic and environmental factors are different. However, using monozygotic twins (MZ) makes it possible to investigate the influence of environmental factors as they have the same genetic factors within pairs because the difference in the clinical traits within the MZ mostly reflect the influence of environmental factors. We hypothesized that the within-pair differences in the traits that are strongly affected by genetic factors become larger after genetic risk score (GRS) correction. Using 278 Japanese MZ pairs, we compared the change in within-pair differences in each of the 45 normalized clinical measurements before and after GRS correction, and we also attempted to correct for the effects of genetic factors to identify Cytosine-phosphodiester-Guanine (CpG) sites in DNA sequences with epigenetic effects that are regulated by genetic factors. Five traits were classified into the high heritability group, which was strongly affected by genetic factors. CpG sites could be classified into three groups: regulated only by environmental factors, regulated by environmental factors masked by genetic factors, and regulated only by genetic factors. Our method has the potential to identify trait-related methylation sites that have not yet been discovered.

73 Keywords: Monozygotic twin, genetic factor, epigenetic factor, environmental factor,

74 laboratory test

## Introduction

Clinical laboratory measurements are important intermediate phenotypes of complex diseases. Clarification of the association between phenotypes, genetic factors, and environmental factors and clinical laboratory traits can reveal the cause of diseases and develop methods for the prediction and prevention of diseases. Recent genome-wide association studies (GWASs) have shown various associations between traits including metabolic traits (1) and genetic factors, and have enabled the prediction of the genetic effects on each trait by calculating the genetic risk score (GRS), which expresses the power of genetic effect as the sum of the effect size of risk alleles related to each trait (2). Previous studies, in which a GWAS of 58 quantitative traits in 162,255 Japanese individuals identified 1,407 trait-associated loci, proposed a prediction model of clinical traits (3, 4). However, the environmental factors were not considered in the prediction model. Therefore, the effects of environmental factors on the GRS calculated in these studies are not yet clear.

It is difficult to investigate environmental effects on phenotypes using individual samples because their genetic and environmental factors differ. However, using monozygotic twins (MZ) makes it possible to investigate the influence of environmental factors because they have the same genetic factors within pairs. The advantage of our twin study is that we examined the differences in clinical traits, which corrected evidential genetic factors, within the MZ pair and clarified the influence of environmental factors more strictly. In this study,

94 as shown in Figure 1A, we hypothesized that the traits that are strongly affected by genetic
95 factors indicate similar values before GRS correction, and their within-pair differences
96 become larger after GRS correction. However, when the within-pair differences in traits are
97 weakly affected by genetic factors, there are only small differences between before and after
98 GRS correction. This hypothesis is more practical than classic twin methods such as ACE
99 model because it takes in correction evidential genetic factors. Based on this hypothesis,
100 we attempted to verify the validity of the genetic prediction model by applying it to our
101 genetic data of MZ pairs.

102 Two approaches were used to verify the prediction models. First, we compared
103 within-pair intraclass correlation (ICC) between before and after GRS correction. When the
104 clinical trait is strongly affected by genetic factors, each clinical laboratory measurement in a
105 pair shows similar values, and the within-pair ICC is closer to 1.0. However, the ICC is be
106 closer to zero when the trait is strongly affected by environmental factors. Second, we
107 compared the changes in within-pair differences in each normalized clinical measurement
108 before and after GRS correction. We defined a smaller change between before and after GRS
109 correction as indicating a stronger influence of genetic factors.

110 Studies examining the effects of non-genetic environmental factors often focus on
111 DNA methylation. However, as some instances of DNA methylation are regulated by genetic
112 factors (5), it is necessary to consider the influence of genetic factors to clarify the

relationship between DNA methylation and environmental factors. In our additional study, we attempted to correct for the effects of genetic factors using GRS to identify Cytosine-phosphodiester-Guanine (CpG) sites that are regulated by genetic factors.

## Subjects and Methods

### Subjects

A total of 278 Japanese MZ pairs (72 men and 206 women pairs) were recruited from a registry established by the Center for Twin Research, Osaka University (Supplementary Table 1 and Supplementary Table2) (6). Blood samples were collected at 9:00 am from each subject after a 12-h fast. Clinical examination was performed as shown in Supplementary Table 3, and the twins completed health-related questionnaires. Excluding criteria in each analysis were presented in Supplemental Table 4. Twins were examined on the same day. Genomic DNA was isolated from peripheral blood mononuclear cells using a commercial kit (QIAamp DNA Mini Kit; QIAGEN, Hilden, Germany). The zygosity of the twin pair was confirmed by perfectly matching 15 short tandem repeat (STR) loci using the PowerPlex 16 System (Promega, Madison, WI, USA).

131

132 **Phenotypes**

133 We targeted 45 traits that were measured by Beckman Coulter, Inc., according to the
134 method used in the International Federation of Clinical Chemistry and Laboratory Medicine
135 (IFCC) project (7). The measured values of each quantitative trait were adjusted for age, sex,
136 top ten principal components of genetic ancestry, and any necessary trait-specific covariates
137 in a linear regression model, as performed in previous studies (3, 4). If traits had a log-normal
138 distribution, a common log-transformation was conducted before the regression. We then
139 normalized the residuals by applying an appropriate trait-specific transformation (Z-score or
140 rank-based inverse normal transformation). In some traits, Smoking status is added in
141 covariates, because smoking is known as affecting the atherosclerosis from endothelial
142 dysfunction to acute clinical events. (Supplementary Table 4)

143

144

145 **Genotyping and imputation**

146 Single nucleotide polymorphism (SNP) genotyping was performed using Illumina
147 Infinium HumanOmni5-Quad v1-0 Bead Chips (Illumina, San Diego, CA) and Illumina
148 Infinium HumanOmni v1-1 BeadChips (Illumina). Sample exclusion was performed under
149 the following conditions: (i) sample call rate < 0.98, (ii) closely related individuals identified

by identity-by-descent analysis, and (iii) non-East Asian outliers identified by principal component analysis of the studied samples and three major reference populations (African, European, and East Asian) in the International Haplotype Map (HapMap) Project. Variants were then applied to standard quality-control criteria and were excluded through the following criteria: (i) SNP call rate < 0.98, (ii) minor allele frequency < 1%, and (iii) Hardy-Weinberg equilibrium P value < $1.0 \times 10^{-7}$. Genotype prephasing was performed using SHAPEIT2 software, and imputation was performed using minimac3 and the 1000 Genomes Project Phase1 (version 3) East Asian reference haplotypes. For the X chromosome, prephasing and imputation were performed separately for the women and men. In total, 268 samples and 27,428,601 variants were retained after editing.

**Methylation levels of CpG islands**

Analysis of methylation levels was performed using the Infinium HumanMethylation450 Bead Kit (Illumina) and Infinium MethylationEPIC Kit (Illumina), according to the manufacture's standard protocol, which considered 485,577 highly informative CpG sites at a single-nucleotide resolution for each sample. The experiment was performed with 0.5μg of high-quality genomic DNA. There were two bead types for each CpG site per locus on the chip. The raw data were analyzed using Genome Studio software (Illumina), and the fluorescence intensity ratios between the two bead types were calculated.

169 The ratio of 0 was regarded as total methylation. These raw data were corrected to normalize
170 the differences in detection ranges between the two probes of the Infinium assay using a
171 peak-based correction method. Normalized data were filtered to exclude invalid probes such
172 as null probes and those with low reliability.
173
174 **GRS**
175 GRS indicates the magnitude of the influence of genetic factors on traits. The GRS
176 was added as a covariate when normalized standardization was performed to correct the
177 influence of genetic factors. The SNPs used to calculate GRS were those that were significant
178 in previous GWAS results (7). GRS was calculated using the following equation:
179 $$\mathrm{GRS} = \sum_{k=1}^{n} ( N_{snpk} \beta_{snpk} )$$
180 where n is the number of trait-associated SNPs; $N_{snpk}$ is the number of *k*th SNP risk alleles
181 (0, 1, or 2); and $\beta_{snpk}$ is the weight of *k*th SNP, which was calculated by linear regression
182 analysis of GWAS.
183
184 **ICC**
185 We used ICC to evaluate the correlation of clinical laboratory measurements within
186 pairs (8). With twin data, it is difficult to use the standard Pearson's correlation coefficient

because the classification of either member of the pair as X or Y is arbitrary. ICC was calculated using the following equation:

$$\mathrm{ICC} = \frac{MS_{between} - MS_{within}}{MS_{between} + MS_{within}}$$

where $MS_{between}$ is the mean-square estimate of the between-pair variance, and $MS_{within}$ is the mean-square estimate of within-pair variance. The maximum ICC value was 1.0, and the ICC was closer to 1.0 when the correlation was stronger.

**The effect size for clinical measurement (ESC) and the effect size for environmental factors (ESE)**

To identify CpG sites controlled by genetic factors, we defined and compared ESC and ESE (Supplementary Figure 1). The ESC indicates the relationship between the methylation levels of each CpG site and normalized clinical measurements as the effect size of the multiple regression analysis. The ESE indicates the effect size of multiple regression analysis between the methylation levels at CpG sites and the differences between the GRS and normalized clinical measurements as a purely environmental factor. By comparing these two scores, we classified CpG sites into three groups: regulated only by environmental factors, regulated by environmental factors masked by genetic factors, and regulated only by genetic factors.

We observed ten traits with extremely low mean P values. We considered most of them to be false positives, which is difficult to study in detail. We excluded traits with mean P-values $<1.0 \times 10^{-8}$ from subsequent studies of the results.

**Statistical analysis**

The association analysis between methylation at CpG sites and phenotype was performed using the 'CpGassoc' package and R statistic software (https://cran.r-project.org/web/packages/CpGassoc/index.html). Bonferroni correction was used to reduce the number of false-positive results (type I error). We set the threshold of the P-value for determining significance to $<1.0 \times 10^{-7}$ and the threshold for determining suggestive to $<1.0 \times 10^{-5}$.

**Results**

**Changes in ICC before and after GRS correction**

We calculated the within-pair ICC of each normalized clinical measurement and the differences between ICCs before and after GRS correction for each clinical trait (Table 1 and

Figure 1B). Based on the difference in within-pair ICC before and after GRS correction (Figure 1B), clinical traits could be classified into three groups: high, moderate, and low heritability, as shown in Figure 1B. Five blood biochemical test values (alkaline phosphatase [ALP], uric acid [UA], high-density lipoprotein cholesterol [HDL], high-density lipoprotein cholesterol [LDL], and total cholesterol [T. chol]) were classified into the high-heritability group, which was strongly affected by genetic factors. In addition, we calculated the averages of 100 differences between within-pair ICC before and after random correction that replaced the GRS used when calculating the Z-score of traits with a normally distributed random number for each clinical trait (Figure 1C). Differences in ICC after random correction were gathered at 0, suggesting that the correction of GRS is effective in correcting the influence of genetic factors.

**Changes in within-pair difference before and after GRS correction**

The average changes in within-pair differences in each normalized clinical measurement before and after the GRS correction are shown in Table 2 and Figure 1D. According to the within-pair differences before and after GRS correction, we classified the clinical traits into three groups: high-, moderate-, and low-heritability groups, as shown in Figure 1D. The five blood biochemical test values (ALP, UA, HDL, LDL, and T. chol) were classified into the high-heritability group using the ICC method.

**Identification of CpG sites regulated by genetic factors**

Table 3 presents the CpG sites showing suggestive effects for both ESC and ESE, indicating that these CpG sites were highly associated with traits in both ESC and ESE. This means that these CpG sites are regulated only by environmental factors and may reflect the effects of purely environmental factors because they are still suggestive when corrected for the effects of genetic factors, and some of these CpG sites (indicated by asterisks) have a common effect on two or three traits. T. chol and LDL were commonly associated with CpG sites in RG9MTD1 (cg22315683, cg01537928, cg20574900, and cg24445570), and Hb and Ht were commonly associated with CpG on ADCY7 (cg23580000). Systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean arterial pressure (MAP) were commonly associated with CpG sites on SDHAF1 (cg26643967).

Table 4 presents the CpG sites showing suggestive effects for ESC, but not for ESE, indicating that these CpG sites were associated with the trait in ESC analysis only. These CpG sites are regulated by genetic factors and not environmental factors. The ratio of P-value in ESC/ESE was large for some CpG sites, such as CpG on MEGF6 (cg20277126), which has been shown to be associated with Hb, and for CpG on SLC29A4 (cg02632809), which is a common gene in mean corpuscular hemoglobin (MCH) and mean corpuscular volume (MCV).

Table 5 presents the CpG sites showing a suggestive effect for ESE but not for ESC, indicating that these CpG sites were associated with the trait in the ESE analysis only. These CpG sites were associated with environmental factors hidden by genetic factors, because their statistical significance became strong when corrected for the effects of genetic factors. Large increases in P-values were observed for several CpG sites on A2BP (cg00514665) and A2BP1 (cg03986562) in MCV, GTF2H4; VARS2 (cg16914989) in UA; and TRPM8 (cg08343899) in ALP.

## Discussion

In this study, we identified five clinical traits (ALP, UA, HDL, LDL, and T. chol) that were strongly affected by genetic factors.

### ALP

ALP was the most inherited trait in the present study. Consistent with this, ALP also showed high heritability in a previous GWAS using a non-twin Japanese population (4). Strong associations of serum ALP and SNPs located in ABO and FUT1 genes were observed as genetic factors in previous GWAS targeting Japanese, Chinese, and European populations (9). In addition, the association between serum ALP levels and the ABO blood type has been reported in a biological study (10, 11). Therefore, we suggest that serum ALP levels are strongly regulated by genetic factors, which is consistent with our results.

### UA

UA exhibited high heritability in our twin study and a previous GWAS using non-twin Japanese individuals (4). The heritability of UA ranged 39–63% in previous family studies (12, 13) and 46–80% in classical twin studies (14, 15). These results indicate that high heritability was observed in serum UA levels, and this high heritability was confirmed.

**Metabolic traits**

Most metabolic traits were affected by genetic factors compared to other traits in our twin study. HDL, LDL, and T. chol were categorized as high-heritability traits, and triglyceride (TG) and blood sugar (BS) were categorized as moderate-heritability traits in our two methods. However, in a Japanese non-twin GWAS (4), the heritability of metabolic traits, except HDL, was not higher than that in our twin study. Their heritability has been reported in several studies: 16–74% for HDL, 47–100% for LDL, 35–72% for T.chol, and 19–81% for TG in twin and family studies (16), and 40–69% for HDL, 40–66% for LDL, 43% for T.chol, and 19–58% for TG in non-twin studies (17-19). These results indicate that heritability varies widely between studies; however, overall, the heritability of HDL, LDL, T. chol, and TG is considered high. In a study comparing the ICC between two types of MZ, some having grown up separately and others having grown up together, no difference was found in the ICC of HDL between the two MZ groups. In the case of T. chol and TG, the ICC of MZ who grew up together was higher than that of MZ who grew up separately (16). These results indicate that the serum levels of T. chol and TG may be affected by the growth environment and that MZ who grew up together present similar T. chol and TG levels. Therefore, T. chol and TG may show higher heritability in MZ who grew up together than in general individuals. The similarity of the environment of the MZ in their growing up is

considered to be the reason for the discrepancy between our twin study and a previous non-twin study (4).

**Other traits**

All other traits were categorized as moderate- or low-heritability traits in our twin study, and most results were consistent with those of previous reports. For example, in the category of blood pressure, we targeted SBP, DBP, and MAP, and the genetic effects of SBP and MAP were categorized into the moderate-heritability class in our twin study. The heritability of SBP, DBP, and MAP has been calculated in numerous studies: 18–45% for SBP, 24–43% for DBP, and 33–34% for MAP (20-22), suggesting that these traits have considerable associations with genetic factors, and our results in the twin study are similar. However, some traits in our study were not consistent with those reported in previous studies. Although we classified RBC and MCH as low heritability traits and MCV as moderate-heritability traits, their heritability is at the top of all traits in a Japanese non-twin study (4). Strong effects of genetic factors on RBC, MCV, and MCH have been reported in several GWAS (23, 24). In addition, high heritability of RBC, MCV, and MCH was also observed in other twin studies using the traditional ACE method (25). Although the reasons for the discrepancy between our study and others are still unclear, the differences in the number of samples may be the cause.

**Association between GRS and CpG sites**

The CpG sites observed in G9MTD1, ADCY7, and SDHAF1 exhibited no significant change in the relationship between methylation levels and traits before and after GRS correction, suggesting that methylation levels are regulated by environmental factors. RG9MTD1, which has been observed to be associated with T. chol and LDL, encodes the protein TRMT10C and is involved in the 5' processing of mitochondrial tRNA. It is essential for transcriptional processing, RNA modification, translation, and mitochondrial respiration and has been reported to be associated with mitochondrial metabolic diseases. No direct association between RG9MTD1 and T. chol or LDL levels has been reported. However, previous studies have suggested that mitochondrial dysfunction affects blood cholesterol (26) and that the regulation of TRMT10C expression may be related to blood cholesterol. ADCY7, which has been observed to be associated with Hb and Ht, encodes a membrane-bound adenylate cyclase that catalyzes the formation of cAMP from adenosine triphosphate (ATP). Although no association between this gene and Hb or Ht has been reported, numerous studies have shown an association between adenylate cyclase cAMP system activation and erythrocyte deformability (27, 28). SDHAF1, which has been observed to be associated with blood pressure, is related to the synthesis of succinate dehydrogenase (SDH) in the mitochondria, and no direct association with blood pressure has been reported. Mitochondrial

345 dysfunction has been reported to occur in several cardiovascular diseases, including
346 atherosclerosis (29), may affect blood pressure. None of the genes have been reported to be
347 directly associated with any trait, but previous studies have suggested that each gene may be
348 associated with a trait, albeit not directly. MEGF6, which has been observed to be associated
349 with Hb, is also known as EGFL3, and the protein EGFL3 encoded by this gene belongs to
350 the epidermal growth factor EGF group. It induces a wide range of biological functions, such
351 as proliferation, differentiation, apoptosis, adhesion, and migration, and has been reported to
352 be associated with colorectal cancer, osteoporosis, and angiogenesis (30-32), but not with Hb.
353 We observed two regions, MEGF6 and SLC29A4, in which genetic factors may
354 have masked the association between environmental factors and traits. ENT4, encoded by
355 SLC29A4, is a member of the ENT family and plays an important role in the transport of
356 nucleosides and their analogs. Although no association with blood pressure has been reported
357 in humans, an association with blood pressure has been reported in mice (33). ENT4 is also
358 highly expressed in the brain and is known to transport monoamine neurotransmitters (34).
359 ENT4 transports adenosine and that adenosine lowers blood pressure (35, 36), which is
360 consistent with the results observed in this study. Although we could not confirm the
361 association between MEGF6 and Hb, an association between SLC29A4 and blood pressure
362 has been reported and is consistent with the results observed in the present study. We

363 confirmed the association between the gene and the trait, which was not detectable before
364 correction using GRS.
365 The regions that may have been false-positives due to genetic factors were A2BP1,
366 GTF2H4, VARS2, and TRPM8. A2BP1, which has been associated with MCV, is a gene
367 encoding RBFOX1. Mutations in RBFOX1 are associated with decreased SBP, and RBFOX1
368 has been reported to be expressed in organs and tissues, including brain tissue, atrial
369 appendages, left ventricle of the heart, and skeletal muscle tissue, which may be related to
370 blood pressure (37). Neither GTF2H4 nor VARS2, which are associated with UA, were
371 found to be associated with UA. VARS2 has been reported to be in linkage disequilibrium
372 with HIST1H2BF-HIST1H4E, which has been reported to be a susceptibility gene for gout
373 through GWAS (38), and may behave like a trait related to gout-related traits such as UA. In
374 RPM8, no association with ALP has been reported. However, as TRPM8 has been reported to
375 be highly expressed in odontoblasts (39) and ALP is used as an expression marker for
376 odontoblasts (40, 41), an association between TRPM8 and ALP is likely. We found previous
377 studies that have suggested trait associations in these genes. Most of these studies have
378 reported associations with genomic variants in the corresponding gene regions, and we were
379 unable to confirm the association with methylation. Therefore, we were unable to confirm
380 whether the association between methylation and traits was a false positive due to genetic
381 factors, as was hypothesized in this study.

## Conclusion

As a new application of twin studies, we first verified a prediction model of laboratory measurements established using a Japanese non-twin GWAS. The prediction model was verified, specifically for ALP, UA, and HDL, which were strongly affected by genetic factors. Additionally, we observed several methylation sites that may be related to these traits. It is likely that susceptible genes interact with each other to affect methylation levels. There is a limitation in our study. Missing heritability in GWAS is well known and this limitation in GWAS may underestimate genetic factors estimated by GRS. However, even considering this limitation, our method has the potential to identify trait-related methylation sites that have not yet been observed.

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Table 1. ICC and its difference before and after correction

| Category | Trait | Sample N | Excluded N | ICC | ICC-GRS | Difference(normal - GRS) | Difference(normal - random) | Heritability |
|---|---|---|---|---|---|---|---|---|
| Metabolic | TC | 528 | 0 | 0.608 | 0.588 | 0.021 | 0.001 | high |
| | HDL-C | 528 | 0 | 0.715 | 0.697 | 0.018 | 0.001 | high |
| | LDL-C | 528 | 0 | 0.582 | 0.562 | 0.020 | 0.001 | high |
| | TG | 518 | 4 | 0.586 | 0.571 | 0.014 | 0.001 | moderate |
| | BS(GLU) | 480 | 11 | 0.583 | 0.572 | 0.012 | 0.001 | moderate |
| | HbA1c | 500 | 9 | 0.715 | 0.709 | 0.006 | 0.002 | moderate |
| Protein | TP | 520 | 4 | 0.570 | 0.571 | -0.001 | 0.001 | low |
| | Alb | 522 | 3 | 0.500 | 0.498 | 0.002 | 0.002 | low |
| | NAP | 516 | 6 | 0.735 | 0.734 | 0.000 | 0.003 | low |
| | A/G | 522 | 3 | 0.724 | 0.725 | -0.001 | 0.001 | low |
| Kidney-related | BUN(UN) | 528 | 0 | 0.474 | 0.472 | 0.002 | 0.001 | low |
| | sCr(CRE) | 528 | 0 | 0.666 | 0.663 | 0.003 | 0.001 | low |
| | eGFR | 528 | 0 | 0.510 | 0.510 | 0.000 | 0.000 | low |
| | UA | 528 | 0 | 0.599 | 0.569 | 0.029 | 0.001 | high |
| Electrolyte | Na | 522 | 3 | 0.439 | 0.437 | 0.002 | 0.002 | low |
| | K | 526 | 1 | 0.318 | 0.308 | 0.010 | 0.000 | moderate |
| | Cl | 526 | 1 | 0.506 | 0.506 | 0.000 | 0.002 | low |
| | Ca | 526 | 0 | 0.484 | 0.481 | 0.004 | 0.001 | low |
| | P | 528 | 0 | 0.500 | 0.496 | 0.005 | 0.002 | low |
| Liver-related | Tbil | 506 | 5 | 0.579 | 0.577 | 0.003 | 0.004 | low |
| | AST | 502 | 11 | 0.483 | 0.482 | 0.000 | 0.001 | low |
| | ALT | 500 | 12 | 0.438 | 0.434 | 0.004 | 0.001 | low |
| | ALP | 508 | 8 | 0.712 | 0.682 | 0.030 | 0.002 | high |
| | GGT | 474 | 18 | 0.641 | 0.641 | 0.001 | 0.002 | low |
| Other-biochemical | CK | 500 | 11 | 0.535 | 0.535 | 0.000 | 0.001 | low |
| | LDH(LD) | 514 | 5 | 0.716 | 0.712 | 0.004 | 0.002 | low |
| | CRP | 434 | 42 | 0.440 | 0.437 | 0.003 | 0.003 | low |
| Hematological | WBC | 520 | 2 | 0.564 | 0.560 | 0.003 | 0.001 | low |
| | Neutro | 514 | 5 | 0.467 | 0.461 | 0.007 | 0.001 | moderate |
| | Eosin | 506 | 8 | 0.496 | 0.496 | 0.000 | 0.003 | low |
| | Baso | 506 | 3 | 0.618 | 0.616 | 0.002 | 0.003 | low |
| | Mono | 522 | 1 | 0.615 | 0.618 | -0.003 | 0.001 | low |
| | Lym | 518 | 2 | 0.595 | 0.590 | 0.005 | 0.002 | low |
| | RBC | 522 | 1 | 0.712 | 0.711 | 0.000 | 0.001 | low |
| | Hb | 522 | 1 | 0.582 | 0.581 | 0.000 | 0.001 | low |
| | Ht | 522 | 1 | 0.607 | 0.607 | 0.000 | 0.002 | low |
| | MCV | 522 | 1 | 0.676 | 0.669 | 0.006 | 0.002 | moderate |
| | MCH | 514 | 4 | 0.637 | 0.633 | 0.004 | 0.001 | low |
| | MCHC | 520 | 2 | 0.665 | 0.661 | 0.004 | 0.000 | low |
| | Plt | 520 | 2 | 0.701 | 0.701 | 0.000 | 0.002 | low |
| Blood-pressure | SBP | 482 | 1 | 0.447 | 0.440 | 0.007 | 0.002 | moderate |
| | DBP | 482 | 1 | 0.579 | 0.577 | 0.003 | 0.002 | low |
| | MAP | 482 | 1 | 0.542 | 0.536 | 0.006 | 0.001 | moderate |
| | PP | 480 | 2 | 0.327 | 0.327 | 0.000 | 0.001 | low |

Table 2. Average of differences within-pair and its amount of change between before and after GRS correction

| Category | Trait | Sample N | Excluded N | Difference | Heritability |
|---|---|---|---|---|---|
| Metabolic | TC | 528 | 0 | 0.017 | high |
| | HDL-C | 528 | 0 | 0.018 | high |
| | LDL-C | 528 | 0 | 0.016 | high |
| | TG | 518 | 4 | 0.012 | moderate |
| | BS(GLU) | 480 | 11 | 0.010 | moderate |
| | HbA1c | 500 | 9 | 0.003 | low |
| Protein | TP | 520 | 4 | 0.001 | low |
| | Alb | 522 | 3 | 0.002 | low |
| | NAP | 516 | 6 | 0.001 | low |
| | A/G | 522 | 3 | -0.001 | low |
| Kidney-related | BUN(UN) | 528 | 0 | 0.001 | low |
| | sCr(CRE) | 528 | 0 | 0.003 | low |
| | eGFR | 528 | 0 | -0.002 | low |
| | UA | 528 | 0 | 0.023 | high |
| Electrolyte | Na | 522 | 3 | 0.001 | low |
| | K | 526 | 1 | 0.006 | moderate |
| | Cl | 526 | 1 | 0.000 | low |
| | Ca | 526 | 0 | 0.002 | low |
| | P | 528 | 0 | 0.002 | low |
| Liver-related | Tbil | 506 | 5 | 0.001 | low |
| | AST | 502 | 11 | 0.000 | low |
| | ALT | 500 | 12 | 0.003 | low |
| | ALP | 508 | 8 | 0.023 | high |
| | GGT | 474 | 18 | 0.001 | low |
| Other-biochemical | CK | 500 | 11 | 0.000 | low |
| | LDH(LD) | 514 | 5 | 0.005 | moderate |
| | CRP | 434 | 42 | 0.000 | low |
| Hematological | WBC | 520 | 2 | 0.003 | low |
| | Neutro | 514 | 5 | 0.008 | moderate |
| | Eosin | 506 | 8 | 0.000 | low |
| | Baso | 506 | 3 | 0.003 | low |
| | Mono | 522 | 1 | -0.004 | low |
| | Lym | 518 | 2 | 0.002 | low |
| | RBC | 522 | 1 | 0.001 | low |
| | Hb | 522 | 1 | 0.000 | low |
| | Ht | 522 | 1 | 0.000 | low |
| | MCV | 522 | 1 | 0.005 | moderate |
| | MCH | 514 | 4 | 0.002 | low |
| | MCHC | 520 | 2 | 0.003 | low |
| | Plt | 520 | 2 | 0.000 | low |
| Blood-pressure | SBP | 482 | 1 | 0.005 | moderate |
| | DBP | 482 | 1 | 0.002 | low |
| | MAP | 482 | 1 | 0.005 | moderate |
| | PP | 480 | 2 | 0.000 | low |
| BMI | BMI | 494 | 1 | 0.001 | low |

Table 3. CpGs with relatively strong associations with the trait and showing suggestive effect both for ESC and ESE

| Category | Trait | CpG_ID | Chr | Position | Gene | P_value |
|---|---|---|---|---|---|---|
| Metabolic | Tchol | cg22315683 | 3 | 101280662 | RG9MTD1* | 3.27E-07 |
| | HDL | cg16187528 | 17 | 79609161 | TSPAN10 | 1.41E-07 |
| | | cg10565662 | 8 | 2670187 | NULL | 1.21E-07 |
| | LDL | cg20574900 | 3 | 101280596 | RG9MTD1* | 8.70E-07 |
| | | cg01537928 | 3 | 101280610 | RG9MTD1* | 8.27E-07 |
| | TG | cg02032984 | 7 | 35490860 | NULL | 6.39E-08 |
| | GLU | cg02895278 | 8 | 26195897 | PPP2R2A | 2.78E-06 |
| | HbA1c | cg11245990 | 11 | 68621969 | NULL | 2.09E-07 |
| Protein | TP | cg22176018 | 2 | 242867611 | NULL | 2.25E-11 |
| | | cg20888499 | 2 | 426252 | NULL | 4.98E-10 |
| Kidney-related | UN | cg01689405 | 1 | 26370407 | SLC30A2 | 9.99E-07 |
| | CRE | cg15714227 | 5 | 2225482 | NULL | 3.06E-09 |
| | | cg22179059 | 6 | 29714945 | LOC285830 | 2.83E-08 |
| | UA | cg18823637 | 14 | 104789524 | NULL | 5.11E-07 |
| | | cg03590420 | 11 | 132662963 | OPCML | 2.31E-06 |
| Electrolyte | Na | cg21755709 | 21 | 45149398 | PDXK | 2.39E-06 |
| | K | cg26528484 | 2 | 103387474 | TMEM182 | 2.80E-08 |
| | Cl | cg01380194 | 11 | 72452482 | ARAP1 | 1.66E-06 |
| | Ca | cg23972915 | 14 | 104809218 | NULL | 6.52E-07 |
| | | cg13046524 | 20 | 48728642 | UBE2V1;TMEM189-UBE2V1 | 1.29E-07 |
| | P | cg04923746 | 1 | 211666304 | RD3;RD3 | 1.29E-07 |
| Liver-related | Tbil | cg20459037 | 17 | 9546550 | WDR16 | 1.43E-06 |
| | AST | cg05229454 | 17 | 80494379 | FOXK2 | 2.00E-09 |
| | | cg24715928 | 15 | 61197517 | RORA | 4.49E-09 |
| | ALT | ch.3.2133154F | 3 | 107908987 | IFT57 | 5.12E-08 |
| | | cg20954484 | 3 | 42727014 | KBTBD5 | 3.85E-08 |
| | ALP | cg13605646 | 20 | 57486019 | GNAS | 8.46E-08 |
| | | cg25210580 | 17 | 744036 | NXN | 1.47E-06 |
| | GGT | cg15347434 | 6 | 37592871 | NULL | 2.72E-08 |
| | | cg03851878 | 15 | 23887710 | NULL | 1.87E-08 |
| Other-biochemical | CK | cg13184225 | 1 | 201956029 | RNPEP | 3.86E-06 |
| | LD | cg21984481 | 17 | 79567631 | NPLOC4 | 3.51E-08 |
| | | cg21275932 | 21 | 46410877 | NULL | 7.94E-08 |
| | CRP | cg19821297 | 19 | 12890029 | NULL | 3.78E-06 |
| Hematological | RBC | cg06657096 | 9 | 140024858 | NULL | 1.60E-09 |
| | Hb | cg23580000 | 16 | 50322156 | ADCY7* | 4.57E-11 |
| | | cg06741653 | 12 | 4351720 | NULL | 7.50E-11 |
| | | cg06897661 | 16 | 50322074 | ADCY7* | 2.50E-10 |
| | Ht | cg23580000 | 16 | 50322156 | ADCY7* | 4.74E-10 |
| | MCV | cg15004787 | 5 | 72802430 | NULL | 8.99E-10 |
| | | cg16285566 | 13 | 33682212 | STARD13 | 9.35E-08 |
| | MCH | cg00791764 | 4 | 53727839 | RASL11B | 2.59E-07 |
| | | cg08505111 | 5 | 98541783 | NULL | 4.53E-07 |
| | MCHC | cg11891431 | 5 | 178209272 | NULL | 1.48E-08 |
| | | cg01375719 | 3 | 184298977 | EPHB3 | 3.87E-08 |
| | Plt | cg17656426 | 19 | 2137788 | AP3D1 | 2.17E-08 |
| | | cg21175585 | 13 | 30095759 | SLC7A1 | 7.13E-09 |
| Blood-pressure | SBP | cg26643967 | 19 | 36485282 | SDHAF1* | 1.62E-07 |
| | | cg19389372 | 19 | 36485356 | SDHAF1* | 6.50E-07 |
| | | cg23895963 | 12 | 117471115 | NULL | 5.05E-07 |
| | DBP | cg06638515 | 6 | 33169581 | RXRB;SLC39A7 | 4.29E-08 |
| | | cg09027985 | 17 | 46973122 | ATP5G1;ATP5G1 | 4.40E-08 |
| | MAP | cg20361540 | 12 | 123380447 | VPS37B | 4.51E-07 |
| | | cg26643967 | 19 | 36485282 | SDHAF1* | 1.83E-07 |
| | PP | cg16190718 | 6 | 31939106 | DOM3Z;STK19 | 2.35E-07 |
| BMI | BMI | cg21075784 | 19 | 54637076 | NULL | 1.42E-06 |

Table4. Top5 CpGs showing suggetive or significant effect for ESC but not for ESE, with the largest amount of P-values change between two values.

| Category | Trait | CpG_ID | Chr | Position | Gene | P(Measured) | P(Measured-GRS) | P(Measured-GRS)/P(Measured) |
|---|---|---|---|---|---|---|---|---|
| Metabolic | Tchol | cg11026954 | 2 | 132250549 | FAM128A;LOC150776 | 3.32E-05 | 7.87E-06 | 2.37E-01 |
| | | cg08122652 | 3 | 122281939 | PARP9 | 1.21E-05 | 4.00E-06 | 3.30E-01 |
| | | cg07791427 | 12 | 54402704 | HOXC8 | 1.18E-05 | 4.32E-06 | 3.68E-01 |
| | HDL | cg11341011 | 17 | 2632132 | | 2.57E-04 | 5.77E-06 | 2.24E-02 |
| | | cg23470729 | 5 | 140725964 | PCDHGA2;PCDHGA3;PCDHGA1 | 3.83E-05 | 1.49E-06 | 3.89E-02 |
| | | cg08045301 | 16 | 71887487 | ATXN1L | 1.86E-04 | 7.95E-06 | 4.28E-02 |
| | | cg08850438 | 16 | 89609646 | SPG7 | 1.61E-04 | 7.79E-06 | 4.84E-02 |
| | | cg17547577 | 3 | 21265890 | | 1.31E-04 | 8.25E-06 | 6.28E-02 |
| | LDL | cg16283010 | 3 | 101280485 | RG9MTD1 | 1.53E-05 | 5.66E-06 | 3.70E-01 |
| | GLU | cg08247887 | 10 | 44394566 | | 1.06E-05 | 2.02E-06 | 1.90E-01 |
| Protein | TP | cg25809722* | 12 | 133121162 | FBRSL1 | 2.91E-07 | 8.90E-08 | 3.06E-01 |
| | | cg01616956* | 2 | 232393196 | NMUR1 | 1.61E-07 | 6.07E-08 | 3.76E-01 |
| Kidney-related | UN | cg09277709 | 19 | 46224285 | FBXO46 | 1.74E-05 | 4.93E-06 | 2.82E-01 |
| | | cg21868798 | 1 | 199481399 | | 2.57E-05 | 7.63E-06 | 2.96E-01 |
| | | cg15409616 | 14 | 106936446 | | 2.48E-05 | 8.17E-06 | 3.29E-01 |
| | | cg11134777 | 7 | 1522211 | INTS1 | 1.27E-05 | 4.26E-06 | 3.35E-01 |
| | | cg17438457 | 1 | 53094893 | | 1.28E-05 | 4.50E-06 | 3.51E-01 |
| | CRE | cg10724867 | 7 | 27218867 | HOXA10 | 2.09E-05 | 1.20E-06 | 5.77E-02 |
| | | cg03534008 | 9 | 45763353 | | 7.16E-05 | 6.00E-06 | 8.39E-02 |
| | | cg24360241 | 2 | 233370823 | | 2.58E-05 | 2.57E-06 | 9.93E-02 |
| | | cg21287054 | 19 | 54928012 | TTYH1 | 1.38E-05 | 1.42E-06 | 1.03E-01 |
| | | cg17453840 | 15 | 83317526 | CPEB1 | 4.08E-05 | 4.64E-06 | 1.14E-01 |
| | UA | cg22636722 | 17 | 78865263 | RPTOR | 3.04E-05 | 1.90E-06 | 6.26E-02 |
| | | cg06001976 | X | 3790470 | | 2.69E-05 | 3.04E-06 | 1.13E-01 |
| | | cg02401352 | 15 | 89354338 | ACAN | 2.36E-05 | 7.00E-06 | 2.97E-01 |
| | | cg09874643 | 2 | 239362030 | | 1.55E-05 | 6.05E-06 | 3.89E-01 |
| Liver-related | Tbil | cg05409752 | 6 | 28599165 | | 7.90E-05 | 1.98E-06 | 2.51.E-02 |
| | | cg12501004 | 12 | 117287232 | RNFT2 | 4.14E-05 | 3.72E-06 | 8.99.E-02 |
| | | cg01909160 | 1 | 3162066 | PRDM16 | 4.97E-05 | 4.82E-06 | 9.70.E-02 |
| | | cg10209474 | 16 | 9181903 | | 3.37E-05 | 6.88E-06 | 2.05.E-01 |
| | | cg09380555 | 13 | 53277872 | LECT1 | 2.14E-05 | 5.46E-06 | 2.55.E-01 |
| | ALT | cg12252008 | 15 | 72448319 | | 4.75E-05 | 7.51E-06 | 1.58.E-01 |
| | | cg15811719 | 21 | 39047824 | KCNJ6 | 1.21E-05 | 3.95E-06 | 3.25.E-01 |
| | ALP | cg05798111 | 16 | 68560845 | | 7.77E-05 | 7.02E-06 | 9.03.E-02 |
| | | cg06657028 | 6 | 30080495 | TRIM31 | 2.36E-05 | 2.25E-06 | 9.52.E-02 |
| | | cg16262034 | 5 | 628091 | CEP72 | 3.27E-05 | 3.20E-06 | 9.77.E-02 |
| | | cg25601713 | 10 | 92720690 | | 3.46E-05 | 8.32E-06 | 2.41.E-01 |
| | | cg22691639 | 1 | 81794767 | | 2.70E-05 | 8.79E-06 | 3.25.E-01 |
| | GGT | cg02844892 | 6 | 31370412 | MICA | 2.95.E-04 | 8.63E-06 | 2.93.E-02 |
| | | cg27137258 | 16 | 2892782 | TMPRSS8 | 2.70.E-05 | 8.49E-07 | 3.14.E-02 |
| | | cg04753836 | 14 | 65308247 | | 4.98.E-05 | 1.97E-06 | 3.97.E-02 |
| | | cg21185936 | 6 | 29716247 | LOC285830 | 7.86.E-05 | 5.59E-06 | 7.11.E-02 |
| | | cg11693285 | 10 | 131927345 | | 5.58.E-05 | 4.78E-06 | 8.56.E-02 |
| Other-biochemical | CK | cg07777347 | 3 | 194361833 | LSG1 | 8.12.E-05 | 5.73E-06 | 7.05.E-02 |
| | | cg08479073 | 1 | 207038584 | IL20 | 6.51.E-05 | 7.28E-06 | 1.12.E-01 |
| | LD | cg23797887 | 11 | 18477753 | LDHAL6A | 4.41.E-05 | 7.19E-06 | 1.63.E-01 |
| | | cg11277090 | 17 | 72848048 | GRIN2C | 1.23.E-05 | 2.72E-06 | 2.22.E-01 |
| | | cg24553547 | 17 | 19247920 | MIR1180;B9D1 | 1.38.E-05 | 4.78E-06 | 3.46.E-01 |
| | CRP | cg09115646 | 10 | 2978687 | | 1.50.E-05 | 4.25E-06 | 2.84.E-01 |
| Hematological | RBC | cg06734816* | 6 | 116422381 | NT5DC1 | 4.55.E-07 | 2.07E-08 | 4.56.E-02 |
| | | cg03356760* | 6 | 37665051 | MDGA1 | 1.10.E-06 | 5.79E-08 | 5.25.E-02 |
| | | cg08318587* | 2 | 216484453 | | 1.29.E-06 | 7.41E-08 | 5.75.E-02 |
| | | cg24528447* | 5 | 92918517 | NR2F1 | 1.52.E-06 | 8.77E-08 | 5.77.E-02 |
| | | cg15988010* | 4 | 166033722 | TMEM192 | 8.04.E-07 | 6.46E-08 | 8.03.E-02 |
| | Hb | cg20277126* | 1 | 3507151 | MEGF6 | 1.32.E-07 | 3.43E-08 | 2.60.E-01 |
| | Ht | cg18500431* | 7 | 1709489 | | 4.56.E-07 | 5.07E-08 | 1.11.E-01 |
| | | cg06665941* | 21 | 34602869 | IFNAR2 | 4.66.E-07 | 5.44E-08 | 1.17.E-01 |
| | | cg17157516* | 1 | 35332203 | DLGAP3 | 4.25.E-07 | 5.73E-08 | 1.35.E-01 |
| | | cg02538199* | 9 | 37034277 | PAX5 | 3.90.E-07 | 5.35E-08 | 1.37.E-01 |
| | | cg27660165* | 1 | 156784036 | SH2D2A | 3.91.E-07 | 5.61E-08 | 1.43.E-01 |
| | MCV | cg21028156 | X | 2743660 | | 2.30.E-04 | 4.17E-06 | 1.81.E-02 |
| | | cg01820213 | 14 | 104645063 | KIF26A | 1.28.E-04 | 2.93E-06 | 2.29.E-02 |
| | | cg24923509 | 10 | 102498399 | | 3.73.E-05 | 1.53E-06 | 4.11.E-02 |
| | | cg02632809 | 7 | 5336811 | SLC29A4 | 8.72.E-05 | 7.12E-06 | 8.16.E-02 |
| | | cg14345882 | 6 | 26364793 | BTN3A2 | 2.16.E-05 | 1.85E-06 | 8.56.E-02 |
| | MCH | cg14548802 | 9 | 137675380 | COL5A1 | 3.54.E-05 | 7.61E-07 | 2.15.E-02 |
| | | cg07361759 | 1 | 32688314 | C1orf91;EIF3I | 3.91.E-04 | 9.31E-06 | 2.38.E-02 |
| | | cg13791713 | 21 | 40720916 | HMGN1 | 6.48.E-05 | 1.70E-06 | 2.63.E-02 |
| | | cg02632809 | 7 | 5336811 | SLC29A4 | 3.90.E-05 | 1.06E-06 | 2.72.E-02 |
| | | cg10263003 | 2 | 235766844 | | 4.41.E-05 | 1.56E-06 | 3.54.E-02 |
| | MCHC | cg00731395 | 7 | 5265623 | WIPI2 | 2.83.E-05 | 2.37E-06 | 8.37.E-02 |
| | | cg21124940 | 19 | 4090224 | | 8.32.E-05 | 9.54E-06 | 1.15.E-01 |
| | | cg15427520 | 11 | 35252384 | CD44 | 1.90.E-05 | 2.65E-06 | 1.40.E-01 |
| | | cg21685427 | 20 | 42187356 | SGK2 | 6.02.E-05 | 8.54E-06 | 1.42.E-01 |
| | | cg18565023 | 17 | 32503 | DOC2B | 6.45.E-05 | 9.69E-06 | 1.50.E-01 |
| | Plt | cg21180703 | 9 | 90273181 | DAPK1 | 4.61.E-05 | 1.61E-06 | 3.50.E-02 |
| | | cg04121415 | 3 | 38325566 | | 4.00.E-05 | 1.48E-06 | 3.71.E-02 |
| | | cg16734017 | 1 | 3385914 | ARHGEF16 | 1.22.E-04 | 6.26E-06 | 5.14.E-02 |
| | | cg07909498 | 4 | 79627477 | | 3.76E-05 | 2.16E-06 | 5.74.E-02 |
| | | cg22125220 | 1 | 3748957 | KIAA0562 | 7.53E-05 | 4.56E-06 | 6.06.E-02 |
| Blood-pressure | MAP | cg09027985* | 17 | 46973122 | ATP5G1 | 2.44E-07 | 5.90E-08 | 2.42.E-01 |
| | | cg10223982 | 19 | 4302995 | TMIGD2 | 1.97E-05 | 5.62E-06 | 2.86.E-01 |
| | | cg23780110 | 2 | 26045244 | ASXL2 | 1.94E-05 | 7.67E-06 | 3.95.E-01 |
| BMI | BMI | cg23059452 | 9 | 86983432 | SLC28A3 | 3.87E-05 | 4.77E-06 | 1.23.E-01 |
| | | cg26175729 | 7 | 150726136 | ABCB8 | 2.82E-05 | 7.21E-06 | 2.55.E-01 |

Table5. Top5 CpGs showing suggetive or significant effect for ESE but not for ESC, with the largest amount of P-values change between two values.

| Category | Trait | CpG_ID | Chr | Position | Gene | P(Measured) | P(Measured-GRS) | P(Measured-GRS)/P(Measured) |
|---|---|---|---|---|---|---|---|---|
| Metabolic | HDL | cg11651932 | 8 | 1327546 | | 1.45E-06 | 2.29E-04 | 158 |
| | | cg11115976 | 17 | 80997086 | B3GNTL1 | 1.83E-07 | 1.70E-05 | 92 |
| | | cg00443543 | 17 | 1645410 | SERPINF2 | 7.35E-06 | 7.90E-05 | 11 |
| | LDL | cg25019526 | 6 | 236559 | | 1.90E-06 | 3.08E-04 | 162 |
| | | cg01672042 | 16 | 49623820 | ZNF423 | 8.86E-07 | 2.21E-05 | 25 |
| | TG | cg12657416 | 9 | 139607421 | FAM69B | 5.19E-06 | 6.31E-05 | 12 |
| | | cg20486551 | 13 | 29329226 | | 1.66E-06 | 1.84E-05 | 11 |
| | HbA1c | cg22325292 | 17 | 80708367 | FN3K | 1.43E-06 | 1.93E-05 | 14 |
| | | cg00809820 | 17 | 80708513 | TBCD;FN3K | 1.40E-06 | 1.64E-05 | 12 |
| Kidney-related | UA | cg16914989 | 6 | 30881764 | GTF2H4;VARS2 | 9.76E-06 | 8.33.E-04 | 85 |
| | | cg15393936 | 10 | 15354631 | FAM171A1 | 1.17E-06 | 5.48E-05 | 47 |
| Liver-related | Tbil | cg01579172 | 8 | 122068905 | | 6.17E-06 | 2.70.E-04 | 44 |
| | ALP | cg08343899 | 2 | 234847554 | TRPM8 | 1.98E-06 | 1.18.E-04 | 59 |
| | | cg06241101 | 17 | 77895684 | | 1.45E-06 | 5.64.E-05 | 39 |
| | | cg12848457 | 10 | 52566320 | | 8.05E-06 | 2.51.E-04 | 31 |
| | | cg00853940 | 2 | 234847683 | TRPM8 | 9.79E-06 | 3.00.E-04 | 31 |
| | | cg13224161 | 6 | 33141279 | COL11A2 | 1.40E-06 | 3.05E-05 | 22 |
| | GGT | cg10189962 | 2 | 175192878 | | 8.78E-06 | 9.67E-05 | 11 |
| | | cg11458473 | 7 | 1424047 | | 8.45E-06 | 9.05E-05 | 11 |
| Other-biochemical | LD | cg07387044 | 8 | 145170347 | KIAA1875 | 7.55E-07 | 1.08E-05 | 14 |
| | | cg17965690* | 19 | 10736049 | SLC44A2;SLC44A2 | 1.59E-08 | 2.11E-07 | 13 |
| Hematological | MCV | cg00514665* | 16 | 7703812 | A2BP | 1.01E-08 | 1.78E-06 | 177 |
| | | cg03986562 | 16 | 7703893 | A2BP1 | 6.05E-07 | 6.41E-05 | 106 |
| | | cg08368788 | 7 | 94537033 | PPP1R9A | 8.19E-06 | 1.97.E-04 | 24 |
| | | cg10395519 | 6 | 151412304 | MTHFD1L | 1.26E-06 | 3.01.E-05 | 24 |
| | MCH | cg02717117 | 2 | 55458694 | C2orf63;RPS27A | 1.62E-06 | 3.30E-05 | 20 |
| | Plt | cg07686394 | 11 | 69448444 | | 4.70E-06 | 3.43.E-04 | 73 |
| | | cg19084794 | 8 | 96086565 | | 2.40E-06 | 9.45.E-05 | 39 |
| | | cg23036452 | 1 | 203644735 | ATP2B4 | 8.73E-06 | 2.81.E-04 | 32 |
| | | cg05693864 | 5 | 844184 | ZDHHC11 | 7.88E-07 | 2.01E-05 | 25 |
| | | cg20944315 | 1 | 200839460 | | 2.98E-06 | 6.95E-05 | 23 |

Figure Legends

Figure 1

GRS correction was used to evaluate the genetic and environmental effects on each trait. (A) Schematic representation of the procedure for evaluating the effects of genetic and environmental factors using within-pair differences before and after GRS correction. (B) Distribution of ICC differences for each trait between before and after GRS correction. (C) Distribution of GRS-corrected and random-corrected superimposed in each trait. (D) Distribution of average within-pair differences in each trait between before and after GRS correction.