A phenomics-based approach for the detection and interpretation of shared genetic influences on 29 biochemical indices in Chinese south men

CURRENT STATUS: ACCEPTED

yanling hu
Guangxi Medical University

aihua tan
Guangxi Medical University

lei yu
Guangxi Medical University

chenyang hou
Guangxi Medical University

haofa kuang
Guangxi Medical University

qunying wu
Guangxi Medical University

Jinghan sun
Guangxi Medical University

Qingniao zhou
Guangxi Medical University

yuanyuan zhu
Guangxi Medical University

Chenqi Zhang
Guangxi Medical University

wei wei
Guangxi Medical University

Lianfeng li
Guangxi Medical University

weidong li
Guangxi Medical University
yuanjie huang
Guangxi Medical University

hongli huang
Guangxi Medical University

xing xie
Guangxi Medical University

tingxi lu
Guangxi Medical University

haiying zhang
Guangxi Medical University

xiaobo yang
Guangxi Medical University

yong gao
Guangxi Medical University

tianyu li
Guangxi Medical University

yonghua Jiang  jiangyonghua@126.com
Guangxi Medical University
Corresponding Author
ORCiD: 0000-0003-2927-4921

zengnan mo
Guangxi Medical University

DOI:
10.21203/rs.2.12060/v4

SUBJECT AREAS
Epigenetics & Genomics

KEYWORDS
phenomics, FAMHES cohort, biochemical indices, shared genetics, lipid metabolism
Abstract

Background: Phenomics provides a new technologies and platforms as a systematic phenome-genome approach. However, few studies have reported on the system mining of shared genetics among clinical biochemical indices based on Phenomics methods, especially in China. This study aimed to apply phenomics to systematically explore shared genetics among 29 biochemical indices based on the Fangchenggang Area Male Health and Examination Survey cohort.

Result: A total of 1,999 subjects with 29 biochemical indices and 709,211 single nucleotide polymorphisms were subjected to phenomics analysis. Three bioinformatics methods, namely, Pearson test, Jaccard index, and linkage disequilibrium score regression, were used. Results showed that 29 biochemical indices were from a network. IgA, IgG, IgE, IgM, HCY, AFP and B12 were in the central community of 29 biochemical indices. Key genes and loci associated with metabolism traits were further identified, shared-genetics analysis showed that 29 SNPs (P < 10^-4) were associated with three or more traits. After integrating the SNPs related to two or more traits with the GWAS catalog, 31 SNPs were found to be associated with several diseases (P < 10^-8). Taking ALDH2 as an example to preliminarily explore its biological function, we also confirmed that rs671 (ALDH2) polymorphism affected multiple traits of osteogenesis and adipogenesis differentiation in 3T3-L1 preadipocytes.

Conclusion: All these findings indicated a network of shared genetics and 29 biochemical indices, which will helpfully understand the genetics participated in biochemical metabolism.

Background

Complex traits are the product of various biological signals and some intermediate traits
that may be affected either directly or indirectly by these signals [1]. A phenome is the sum of many phenotypic characteristics (phenomics traits) that signifies the expression of the whole genome, proteome and metabolome under a specific environmental influence [2, 3]. The study of phenomes (called phenomics) provides a suite of new technologies and platforms that have enabled a transition from focused phenotype-genotype studies to a systematic phenome-genome approach [4]. Many recent studies have found that, compared to consider only the binary patients vs. healthy controls, mapping intermediate steps in disease processes, such as various disease-related clinical quantitative traits or gene expression, are more informative[5, 6].

Pleiotropy, which is a DNA variant or mutation that can affect multiple traits, is a common phenomenon in genetics [7]. For example, Joseph Pickrell and colleagues [8] performed genome-wide association studies (GWAS) of 42 traits or diseases to compare the genetic variants associated with multiple phenotypes, and identified 341 loci associated with multiple traits. Heid IM et al [9] performed a GWAS of fasting insulin, high-density lipoprotein cholesterol (HDL-C) and triglyceride (TG) levels to identify 53 loci associated with a limited capacity to store fat in a healthy way, and this multi-trait approach could increase the power to gain insights into an otherwise difficult-to-grasp phenotype.

Furthermore, many evidences have found that diseases or clinically quantitative traits can be interconnected. For example, the increasing of circulating fatty acids (Fas) could lead to the development of obesity-associated metabolic complications, such as insulin resistance [10]. Goh et al [11] found that essential human genes tended to encode hub proteins and widely expressed in multiple tissues. Many shared genetics variants are identified in linkage disequilibrium with variants associated with other human traits or diseases, and these pleiotropic connections make the human traits connect together [8, 12]. Therefore, understanding the complex relationships among human traits and diseases
is important for learning about the molecular function of hub genes.

The Fangchenggang Area Male Health and Examination (FAMHES) cohort, which was initiated in 2009 in Fangchenggang City, Guangxi, China. It is a comprehensive demographic and health survey focus on investigating the interaction between environment and genetic factors on man’s health. In previously study, we had reported biochemical indices are closely associated with disease. For example, higher complement 3 (C3) and complement 4 (C4) were associated with an increase in metabolic syndrome (MetS) [13]. Low serum osteocalcin level was a potential marker for MetS [14] and impaired glucose tolerance [15]. Uric acid (UA) was positively correlated with the prevalence of MetS [16]. Additionally, genome-wide assay indicated that gene or loci associated with lipid traits would related with biochemical indices. For example, alcohol consumption and ALDH2 rs671 polymorphism affected serum triglyceride (TG) levels [17].

Although the role of genetic factor and gene polymorphisms lead to affect biochemical indices are reported, the network of biochemical indices themselves, biochemical indices and genetic type are still puzzling. As the rapid advances in bioinformatics techniques, clarifying the biochemical indices network with genetic types becomes feasible.

The aim of this study was to identify the shared genetics responsible for 29 biochemical indices in the FAMHES cohort, using a phenomics approach. Our findings shed light on the relationships between these 29 biochemical indices, including their shared genetic basis and genetic risk loci.

Results

Genetic and trait-based characteristics of 1,999 samples

A total of 1,999 subjects with 29 biochemical indices that passed the QC call rate of 95% were analyzed, and a total of 709,211 SNPs in these subjects were subjected to the
subsequent genetic analysis. The average GWAS inflation factor for all 29 biochemical indices was 1.029 (range: 0.975–1.060), suggesting that the stratification correlation worked well (Table S1). The heatmaps based on the Pearson correlation coefficient showed that 106 correlated pairs were found among these 29 traits (correlation coefficient was over 0.3 or less than −0.3 and the P value was less than 0.01) (Figure 1). In addition, cluster analysis with the hclust package in R package could be classified these 29 biochemical indices into 2 groups, one group including blood urea nitrogen (BUN), Cholesterol, Glucose, testosterone (TE), follicle-stimulating hormone (FSH), Insulin, immunoglobulin G (IgG), homocysteine (HCY), folate (FOL), alpha-fetoprotein (AFP), immunoglobulin A (IgA), low-density lipoprotein cholesterol (LDL-C), immunoglobulin M (IgM), C3, how-density lipoprotein cholesterol (HDL), Triglyce, C-reactive protein (CRP). The other group including vitamin B12 (B12), Ferritin (FRRR), Uricacid, immunoglobulin E (IgE), anti-streptococcus hemolysin “O” (ASO), Creatinine, osteocalcin (OSTEOC), Estradiol, sex hormone binding globulin (SHBG), alanine transaminase (ALT) (Figure S1). All of each group contained common lipid metabolism indices, suggesting that these traits were correlated with lipid metabolism.

Correlation analysis based on network medicine

For each trait, we used a linear mixed model estimate fixed value, adjusted with PC1 and PC2 of population stratification and age, respectively, to perform GWAS. A total of 86,556 SNPs (p-value $1 \times 10^{-3}$) associated with all 29 biochemical indices were obtained and then annotated using the SNP Function database with default parameters and the south Asian population option[18]. A total of 12,521 genes were obtained, and protein-protein interactions were determined using the BioGRID database [19]. A total of 5,313 genes with known proteins were obtained, and the interactional network was built with Cytoscape
The topological coefficient, clustering coefficient and degree distribution were important indices to evaluate network nodes. Details of these three factors for 5,313 genes are shown in Figure S2 (A, B, C, D).

The Jaccard correlation matrix heatmaps showed that there were 63 correlated pairs among 435 pairwise combinations among these 29 traits indices with MCI were over 0.6 (Figure 2). In these pairs, HCY, IgG, SHBG, B12, IgA and C4 were closely related with more than other six traits. However, in view of the information regarding gene/protein interactions in public databases is limited, interaction information for most of the genes/proteins in this study could not be obtained, and the Jaccard index was computed based on a small number of genes/proteins.

**Correlation analysis based on linkage disequilibrium score regression (LDSC)**

Genetics can help to elucidate cause and effect. However, single variants tend to have minor effects, and reverse causation involves an even smaller list of confounding factors. Therefore, interrogating genetic overlap via GWAS that focuses on genome-wide significant SNPs is predicted to be an effective means of mining the correlation between different phenotypes. The GWAS effect size estimate for a given SNP will capture information about SNPs near the linkage disequilibrium [21]. The correlations based on GWAS of the 29 quantitative clinical traits were estimated using cross-trait LDSC. The genetic correlation estimates for all 435 pairwise combinations among these 29 traits. After removing the outlier values, 68 significant correlated pairs (p<0.05) were found (Figure 3). The details for these 68 selected pairs of traits are shown in Table S2.

Integration and interpretation of important pairs identified by these three methods
To identify the correlation pairs among these three methods, we integrated the correlated traits fitting at least one of the following: Pearson coefficient was greater than 0.3 or less than -0.3 and P value less than 0.01, Jaccard coefficient was greater than 0.6, or P value of LDSC was less than 0.05. Totally 208 correlated pairs among biochemical indices were found, among them 106, 63, 68 correlated pairs were found by Pearson coefficient, Jaccard coefficient, LDSC, respectively. Only 1 correlated pairs was found by all three methods. 10 correlated pairs both by Pearson coefficient and LDSC, 10 correlated pairs were found both by Pearson coefficient and LDSC, 15 by Pearson and Jaccard coefficient, and 5 by Jaccard coefficient and LDSC. (Figure S3, A). The related traits were integrated if they fulfilled the following conditions: the Pearson coefficient was greater than 0.3 and p-value less than 0.01, the Jaccard coefficient was greater than 0.6, or the LDSC p value was less than 0.05. Six traits (IgA, IgG, HCY, AFP, IgE and B12) were the first top factors in the network of these 29, and related to more than 20 traits. Additionally, IgM, CRP, C4, BUN, TG, Creatinine and FSH were the second top factors and connected with more than 15–20 traits, and OSTEOC, Estradiol, Glucose, FOL, TE, SHBG, FERR, BMI, ALT and HDL were the third top traits which correlated with more than 10 traits (Figure S3, B).

Genes and SNPs those are potentially important across multiple traits

We selected SNPs with \( P<10^{-3} \) for each trait, resulting in a total of 60,644 SNPs for all 27 traits. The essential genes have a tendency to express in multiple tissues, and are topologically and functionally central [12]. After integrating all 5,313 genes and removing the free notes in the total network among 29 biochemical indices, 427 genes (with \( P<10^{-3} \) at least one SNP) were correlated with more than 5 traits. After filtering the genes with SNPs (\( P<10^{-4} \)), there were 71 genes correlated with more than or equal to 3 traits, especially aldehyde dehydrogenase 2 family member (ALDH2), BRCA1 associated protein
(BRAP), cadherin 13 (CDH13) and CUB and Sushi multiple domains 1 (CSMD1) which was related with more than 5 traits. In these 71 genes, 38 genes were found to connect more than 5 other genes in the interactional network annotated from BioGRID database [19] (Table S3). This showed that essential genes related with multiple traits were sure to locate in the central gene interactional network.

Among all the genome wide variations SNPs, 481 ($P<1 \times 10^{-3}$) were associated with three or more clinical biochemical quantitative traits, and 13 of these 481 SNPs were related with more than 5 traits. In these SNPs, rs12229654 (near cut like homeobox 2 (CUX2)), rs2188380 (locates in CUX2), rs3809297 (locates in CUX2) and rs3782886 (locates in BRAP) was related with more than 10 traits. Six SNPs in CUX2 were correlated with more than 5 traits, which denote that CUX2 should play an important role on this net. In addition, for all the SNPs with $P<1 \times 10^{-4}$, 29 SNPs were related with three or more biochemical indices (Figure 4). After annotated 29 SNPs with $P<1 \times 10^{-4}$ using the HaploReg database[22], we found that almost all these SNPs were related to enhancer histone binding, promoter DNase binding and transcript binding, which affected protein binding or the presence of eQTL (Table S4).

After integrating the SNPs associated with more than 2 traits $P<1 \times 10^{-4}$ with GWAS catalog[23], we found that 31 SNPs in 18 genes were found in GWAS catalog (Table S5). Among those SNPs, five SNPs (rs579459, rs649129, rs507666, rs495828, and rs651007) in ABO were associated with more than 10 quantitative traits and diseases. One SNP (rs671) in ALDH2 was related to 21 traits, six SNPs (rs10519302, rs16964211, rs2305707, rs2414095, rs6493487 and rs727479) in or near CYP19A1 were associated with mainly with hormone measurements. This finding supports the idea that shared genetics on traits can produce correlations among these traits.
The rs671 polymorphism in ALDH2 affects osteogenic and adipogenic differentiation of 3T3-L1 preadipocytes

An interaction between SNP (rs671) in ALDH2 was related to 13 traits were found in this study. The relations between rs671 and lipid metabolism or osteocalcin have been found in some literatures [24, 25], however, their function need to invest. Rs671 is a nonsynonymous (ns) SNP (G504L) in the ALDH2 gene, which is located on chromosome 12. To evaluate the effects of the rs671 polymorphism on osteogenic and adipogenic differentiation of 3T3-L1 preadipocytes, a lentivirus vector was used to overexpress ALDH2-WT or ALDH2-G504L-mut in 3T3-L1 preadipocytes (Figure S4). The cell growth curve of ALDH2-G504L-mut showed no obvious change compared with that of the control, but expression of ALDH2-WT induced a significant increase in cell proliferation (Figure 5A). The cell apoptosis results were consistent with this finding; overexpression of ALDH2-WT resulted in a 3.935-fold decrease in late apoptotic cells in comparison to that of ALDH2-G504L-mut or control cells (Figure 5B, C). We next investigated the impact of the ALDH2 G504L mutation on osteogenic and adipogenic differentiation of 3T3-L1 preadipocytes. At 7 days after osteoblast induction, cells were subjected to Alizarin red-S staining. ALDH2-WT cells showed more mineralized nodules than the control cells or those expressing ALDH2-G504L-mut (Figure 5D, E). In addition, mRNA expression of osteoblast-related genes, such as alkaline phosphatase (AKP), osteocalcin, RUNX family transcription factor 2 (Runx2), and collagen type I (Col1), was significantly higher in ALDH2-WT cells than in ALDH2-G504L-mut or control cells (Figure 5F). After 7 days of adipogenic induction, the ALDH2-WT cells displayed accumulation of lipid vacuoles, as detected by oil red O staining, compared with ALDH2-G504L-mut or control cells (Figure 5G, H). The expression levels of adipogenesis-related proteins, such as adiponectin, C/EBPα (CCAAT/enhancer
binding protein α), C/EBPβ, adipocyte fatty acid-binding protein (Fabp4), and Pparγ (peroxisome proliferator-activated receptor), were much higher in ALDH2-WT cells than in ALDH2-G504L-mut or control cells (Figure 5I). Taken together, these results suggest that ALDH2-G504L-mut affected the osteogenic and adipogenic differentiation of 3T3-L1 preadipocytes.

Discussion

A network of shared genetics and 29 biochemical indices were found in this research. Not only did one intermediate phenotype have multiple associated SNPs, interestingly, one SNP associated with multiple intermediate phenotypes was also common. The phenomenon of some genes or loci have the ability to affect multiple distinct phenotypic traits called pleiotropy. More and more attention has been paid to pleiotropy. In 2011, according to the data of the NIH GWAS website, Sivakumaran found that nearly 5% SNPS and 17% genes or gene regions were related to two or more diseases or traits[26]. In 2018, Chesmore used the same method and database, he found 44% of genes or gene regions were associated with two or more diseases or traits, a nearly two-fold increase of which Sivakumaran S found [27]. It is proved that pleiotropy facilitating accurate diagnosis and treatment of human diseases [28]. Moreover, pleiotropy research is also helpful for understand the association between sequence variation and phenotype in plant or animal. Gene co-expression networks, novel mutation associated with many phenotypic traits were identified in maize [29, 30]. It has been proved that wing shape of drosophila was affected by multiple genetic sites[31].

Immunoglobulin is produced by plasma cells and lymphocytes and characteristic of these types of cells, and plays an essential role in the body’s immune system. In this study, we found that IgG, IgA, IgE and IgM were the central traits in biochemical indices network, and these traits could link to 19 and more traits. HCY, a naturally occurring amino acid
found in blood plasma, plays the central role in biochemical indices by connecting with 23 traits. High levels of HCY have proved to associate with several body dysfunction, such as the vasculature[32] and endothelial injury [33]. Interesting, Vitamin B12 was identified its central role in biochemical indices network through correlating to 21 other traits. Which is similar to previous studies, Vitamin B12 correlates with several quantitative traits, such as bone mineral density, FOL and FERR [34–36].

Pleiotropy refers that some genes or loci have the ability to affect multiple distinct phenotypic traits. After integrating all the related genes among 29 biochemical indices, it is surprised that *ALDH2* and *BRAP* can be related with 9 traits, and connected with 19 and 13 genes, respectively. *ALDH2* belongs to the aldehyde dehydrogenase family of proteins, which is the second enzyme of the major oxidative pathway of alcohol metabolism. *ALDH2* dysfunction will lead to several disease, such as cancer [33, 37], alcoholic fatty liver [38], cardiovascular diseases [39]. *BRAP* is a cytoplasmic protein which can bind to the nuclear localization signal of BRCA1 and other proteins [40]. The polymorphisms in this gene associated with myocardial infarction [41], metabolic syndrome [42]. Additionally, the common *CSMD1* was related to 8 traits. *CSMD1* is a large (∼390 kDa) membrane-bound complement inhibitor [43]. Mutations of this gene participate in complement activation and inflammation in central nervous system, which leads to Parkinson disease [44]. This denotes that these three genes may be the hub genes in biochemical indices networks.

If the SNPs locate sites related to promoter DNase binding, enhancer histone binding and transcript binding, the marginally significant SNPs work as regulatory roles affecting protein binding or the presence of eQTL [45, 46]. In this research, 29 SNPs (P < 10–4) were associated with three or more traits and correlated with each other. These results revealed that the shared regulatory genetics are most likely to drive association signals and play important roles in clinical biological function. This phenomenon may provide
important “scaffolding” to support a framework to explore the basic mechanism of biochemical indices.

Shared genetics are commonly applied to build disease-diseased relationship and mine the common disorder of diseases [47, 48]. An important general insight from this study was that associated genes across traits tend to gather in trait specific network modules, we had found 31 SNPs in 18 genes were associated with several traits and diseases, such as five SNPs (rs579459, rs649129, rs507666, rs495828 and rs651007) of ABO were associated with cholesterol, LDL levels. Six SNPs (rs10519302, rs16964211, rs2305707, rs2414095, rs6493487, rs727479) of CYP19A1 associated with estradiol levels. Rs671 in ALDH2 associated with glucose, OSTEOC, SHBG levels. These founding suggest that shared genetics on traits can produce correlations between different traits of disease. For example, ABO gene located around 9q34.2, encodes glycosyltransferases related to the first discovered ABO blood group system [49]. The abnormal expression or polymorphism of this gene is correlated with several body dysfunctions, such as ischemic stroke [50], large artery atherosclerotic stroke [51] and pancreatic cancer [52]. CYP19A1 gene located on 15q21.2, encodes key enzyme for estrogen biosynthesis. SNP in CYP19A1 might affect the aromatase activity the influence the estradiol levels then impacts on human health. Previously research reported that correlation with SNPs of CYP19 and disease, such as polycystic ovarian syndrome [53], coronary heart disease[54], and its correlation with coronary artery disease (CAD). ALDH2 gene located on 12q24.12, encode aldehyde dehydrogenase, the second enzyme of the major oxidative pathway of alcohol metabolism. Rs671 is nonsynonymous mutation sit on exon 12. rs671 mutation was found to be associated with several traits (BMI, osteocalcin, renal function-related traits[55], response to alcohol consumption[56, 57], triglyceride[17], hematological and biochemical traits[58], intracranial aneurysm [59], mean corpuscular hemoglobin [17]). Taking ALDH2
as an example to preliminarily explore its biological function, the in vitro function testing of rs671 played a role in the proliferation and osteogenic and adipogenic differentiation of 3T3-L1 preadipocytes.

With the emergence of GWAS analysis, a large number of loci and disease-related information were excavated. However, due to its strict restriction on the P value of correlation analysis, a lot of potential information were lost while significant loci obtained. Some loci didn’t achieve P cutoff value but itself, but if these loci site in a short range, or they involve in similar function, these lower p value loci may also affect biologically function [60]. Furthermore, it was challenged at identified common pathways and biological functionality core regulatory network of across loci. For more efficient analysis these lower p value loci functions, more complex models were emerged. Raychaudhuri have designed GRAIL to set a lower threshold in considering relatedness for those genes in narrow regions. They systematically examined 370 SNPs from 179 independent loci with P < 1x10^-3, three gene regions of CD28, PRDM1 and CD2/CD58 were identified to be closely related to rheumatoid arthritis [61]. To assess new asthma risk loci Demenais interrogated the GWAS catalog by set P value thresholds from 5x10^-8 to 10^-3, meta-analysis on genetic variation and blood indexes and environmental exposure histories [62]. Kostem analysis follow-up SNPs association with disease by set lower cut off value, then analysis the particular values of tag SNP statistic, pairwise correlation, and the effect size of the candidate SNP[63].

For there have no mature methods of research on the genetic relationship between traits at the level of genome-wide summary statistics, we set the lower threshold value for get more SNP for analysis, analysis these candidate SNP association net by three different method, Pearson correlation coefficient, LDSC or Jaccard correlation. As we show, even in
three different calculate methods, the most of the top important traits are similar. Of these, IgA, IgG, HCY, AFP, IgE and B12 were the first top factors in the network. Our research is an experimental attempt to assess the network of shared genetics and 29 biochemical indices.

Conclusion

We investigated the correlations among 29 biochemical indices through three biological information methods. Firstly, we found that IgA, IgG, IgE, IgM, HCY, AFP and B12 were in the central community of 29 biochemical indices. Secondly, the shared genetics analysis showed that 29 SNPs (P<10^{-4}) were associated with more than 3 traits. 31 SNPs can be found to associate with several diseases (P<10^{-8}) by integrating the SNPs related with 2 or more traits with the GWAS catalog. Thirdly, taking ALDH2 as an example to preliminarily explore its biological function, we found that the rs671 (ALDH2) polymorphism could affect the osteogenic and adipogenic differentiation of 3T3-L1 preadipocytes. We clarified 29 biochemical indices were from a network, hub variations/genes that played a vital role in biological processes, these findings highlight a network of shared genetics and 29 biochemical indices.

Methods

Study sample

Our study included 2,012 unrelated healthy Chinese men aged 20–69 years from the FAMHES[14, 15], which was conducted among non-institutionalized Chinese men in Guangxi and was designed to investigate the effects of environmental and genetic factors and their interaction with the development of age-related chronic diseases. Men aged ≥18 years were requested to participate in the study upon large-scale physical examination at the Medical Center of Fangchenggang First People’s Hospital from September 2009 to
December 2009. The included participants all self-reported that they were free of hyperthyroidism, diabetes mellitus, stroke, coronary heart disease, rheumatoid arthritis, impaired hepatic or renal function, and tumors. Our study research protocol was approved by the Guangxi medical university Ethics Committee, and each participant provided written informed consent.

Measurements of 29 Biochemical indices

Overnight (≥8 h) fasting venous blood specimens were obtained between 7:00 am and 10:00 am, and serum samples were extracted and stored at -80°C. Triglyceride, cholesterol, HDL-C, LDL-C, glucose, ALT, BUN, uric acid and creatinine were measured enzymatically on a Dimension-RxL Chemistry Analyzer (Dade Behring, Newark, DE) in the Department of Clinical Laboratory Science at the Fangchenggang First People’s Hospital. CRP, C3, C4, IgA, IgE, IgG, IgM, ASO were measured with immunoturbidimetric methods on a HITACHI 7600 biochemistry analyzer (Hitachi Corp, Tokyo, Japan). Ferritin, folate and vitamin B12, TE, estradiol, FSH, SHBG, insulin, AFP and OSTEOC were measured with the same batch of reagents by electrochemiluminescence immunoassay and HCY assayed by enzyme cycle method using a COBAS 6000 system E601 (Elecsys module) immunoassay analyzer (Roche Diagnostics, GmbH, Mannheim, Germany).

SNP genotyping and quality control (QC) analysis

Genome-wide SNP genotyping was performed with an Illumina Omni 1 M chip (Illumina, San Diego, USA). Among 2,012 genotyped subjects, 1,999 passed the QC call rate of 95% and were included in the final data analysis. A total of 709,211 SNPs in these subjects passed the QC criteria as follows: the $P$ value for the Hardy-Weinberg Equilibrium (HWE) test was greater than $1 \times 10^{-3}$, minor allele frequency (MAF) was greater than 0.01, and genotype call rate was greater than 95%. The inferred genotypes of SNPs in the genome
that were not directly genotyped were computed by the IMPUTE program[64] (e.g., SNPs catalogued in HapMap Phase II CHB population release #24). All genotypes with a posterior probability of >90% based on IMPUTE software imputation were retained.

Jaccard coefficient

Phenotypes are linked if they share alterations in genetics. The pathobiology of human diseases might be understood by creating molecular and phenotypic networks [65, 66]. We used SNP function[18] (https://snpinfo.niehs.nih.gov/) tool to identify the genes containing all of the SNPs which the p-value for the GWAS was less 1×10^{-3}. The human interactome was obtained by combining protein-protein interaction (PPI) information from the BioGRID database [19].

We built the correlations among 29 clinical phenomes based on the common genes/proteins between two traits. To minimize the bias in estimating of the correlation between two given traits, we calculated the molecular comorbidity index (MCI) by adapting the formula from Grosdidier S[67] to further consider the different coefficients of distance between the two diseases. The MCI was defined as follows:

$$MCI_{\text{trait1,trait2}} = \frac{(proteins_{\text{trait1}} \cap proteins_{\text{trait2}}) \cup proteins_{\text{trait1} \rightarrow \text{trait2}} \cup proteins_{\text{trait2} \rightarrow \text{trait1}}}{(proteins_{\text{trait1}} \cup proteins_{\text{trait2}})}$$

Where and are the proteins related to clinical traits 1 and 2, respectively. are those proteins related to trait 1 that interact with the proteins associated with trait 2 (and vice versa). The two operators \(\cap\) and \(\cup\) denote the intersection and union between the two sets of elements (and), respectively.

Correlation analysis by LDSC

The genetic correlations derived from the summary statistics are evaluated by the GWAS
effect size for a given SNP and integrated the effects of all SNPs which are in linkage disequilibrium (LD) with that SNP. The LDSC (which targets genetic correlation) uses variants across the whole genome and is a symmetrical (i.e., nondirectional) analysis for the risk factor and the outcomes [21]. In short, LDSC assumes that, for polygenic traits, SNPs will also capture information about SNPs near the LD. This relationship between the LD and the associated signal can also be used to test the relationship between the two traits for all SNPs in the genome. To further elucidate the correlations of these 29 biochemical indices in FAMHES from the genetic architecture, we applied LDSC to estimate the correlation of these 29 traits.

Osteogenic and adipogenic differentiation of 3T3-L1 preadipocytes

Full-length ALDH2-WT and ALDH2-G504L-mut cDNA were cloned into the pTSBOE-CMV-MSC-3flag-EF1-tRFP-F2A-Puro lentivirus vector (Quanyang, Shanghai). The 3T3-L1 preadipocytes were cultured in Dulbecco’s modified Eagle’s medium (DMEM) with 10% fetal bovine serum (FBS) at 37°C in a humidified atmosphere with 5% CO₂. The osteoblast-inducing medium used was α-MEM (α-minimum Eagle’s medium) containing 10% FBS fetal bovine serum, 100 nM dexamethasone, 5 mM β-phosphoglyceride and 5 μg/mL vitamin C. The adipogenesis-inducing medium included A and B medium. A medium was DMEM containing 10% FBS, 100 nM dexamethasone, 0.5 mM 3-isobutyl-1-methylxanthine and 5 μg/mL insulin. B medium was DMEM containing 10% FBS and 5 μg/mL insulin. For adipocyte induction, cells were cultured for two cycles of A medium for 2 days, then B medium for 1 day. Cell proliferation was measured by a CCK-8 assay according to the manufacturer’s instructions (DOJINDO, Japan). Cell apoptosis was examined by Annexin V-APC/7-AAD staining followed by flow cytometry detection. For Oil Red O or Alizarin Red S staining, cells were fixed with 4% paraformaldehyde for 30 min and stained with 4% Oil
Red O solution or 0.4% Alizarin Red S. Lipid droplets and calcium nodules were quantified using Image J software. Cellular RNA was extracted using an RNA extraction kit (Promega, China). Reverse transcription was performed with a Transcriptor Reverse Transcriptase kit (Kangwei, China). Quantitative reverse transcriptase-PCR was performed using a Roche Light Cycler 480 and KANGWEI qPCR Kit (KANGWEI, China). Per primer sequences were listed in Table S6.

Statistical analysis

The correlations among the 29 biochemical indices were computed by the CORR procedure using SAS 9.0 and defined as the Pearson correlation coefficient between the rank variables. With the exception of BUN, HCY, B12, FERR, OSTEOC, creatinine, Uricacid, cholesterol, HDL, LDL, TE and C3, 17 traits without normal distribution were logarithmically transformed to normalize the distribution. The association of the SNPs with 29 clinical quantitative traits was evaluated using a linear regression adjusted for population stratification factors (PC1 and PC2) and age, respectively. Population stratification was evaluated by a principal component approach with EIGENSTRAT software[68].

List Of Abbreviations

AFP: alpha-fetoprotein
ALDH2: aldehyde dehydrogenase 2 family member
ALT: alanine transaminase
ASO: anti-streptococcus hemolysin “O”
BUN: blood urea nitrogen
CRP: C-reactive protein
C3: complement 3
C4: complement 4

FAMHES: The Fangchenggang Area Male Health and Examination cohort

Fas: fatty acids

FOL: folate

FRRR: Ferritin

FSH: follicle-stimulating hormone

GWAS: genome-wide association studies

HCY: homocysteine

HDL-C: high-density lipoprotein cholesterol

IgA: immunoglobulin A

IgE: immunoglobulin E

IgG: immunoglobulin G

IgM: immunoglobulin M

LDL-C: low-density lipoprotein cholesterol

LDSC: linkage disequilibrium score regression

MetS: metabolic syndrome

OSTEOC: osteocalcin

SNPs: single nucleotide polymorphism

TE: testosterone

TG: triglyceride

UA: Uric acid

Declarations

Ethics approval and consent to participate

The study was approved by the Ethical Committee of Guangxi Medical University.
Consent for publication

All the authors are aware of and approve the manuscript as submitted to BMC genomics.

Availability of data and materials

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by the National Natural Science Foundation of China (No. 81770759, 81472414, 81560608 and 81460388), Guangxi Natural Science Foundation (No. 2015GXNSFBB139008), Guangxi Medical University Training Program for Distinguished Young Scholars, Youth Science Foundation of Guangxi Medical University (No. GXMUYSF201603) and the Guangxi Colleges and Universities Key Laboratory of Biological Molecular Medicine Research Foundation (No. GXBMR201603).

Author’s contributions

Yanling Hu, Yonghua Jiang, Zengnan Mo conceived and designed the experiments. Aihua Tan, Haiying Zhang, Xiaobo Yang, Yong Gao, Tianyu Li, Zengnan Mo performed epidemiologic study, Yonghua Jiang performed Cell biology experiment. Lei Yu, Chenyang Hou, Haofa Kuang, Qunying Wu, Jinghan Su, Qingniao Zhou, Yuanyuan Zhu, Chenqi Zhang, Wei Wei, Lianfeng Li, Weidong Li, Yuanjie Huang, Hongli Huang, Xing Xie, Tingxi Lu performed bioinformatic analysis experiments. Yanling Hu, Yonghua Jiang wrote the manuscript.

Acknowledgements

Not applicable
References

1. Falconer D. S. MTF: Introduction to quantitative genetics (4th edn). Trends Genet 1996, 2.

2. Oti M, Huynen MA, Brunner HG: Phenome connections. Trends Genet 2008, 24(3):103–106.

3. Houle D, Govindaraju DR, Omholt S: Phenomics: the next challenge. Nat Rev Genet 2010, 11(12):855–866.

4. Cai T, Zhang Y, Ho YL, Link N, Sun J, Huang J, Cai TA, Damrauer S, Ahuja Y, Honerlaw J et al: Association of Interleukin 6 Receptor Variant With Cardiovascular Disease Effects of Interleukin 6 Receptor Blocking Therapy: A Phenome-Wide Association Study. JAMA Cardiol 2018, 3(9):849–857.

5. Emilsson V, Thorleifsson G, Zhang B, Leonardson AS, Zink F, Zhu J, Carlson S, Helgason A, Walters GB, Gunnarsdottir S et al: Genetics of gene expression and its effect on disease. Nature 2008, 452(7186):423–428.

6. Korte A, Vilhjalmsson BJ, Segura V, Platt A, Long Q, Nordborg M: A mixed-model approach for genome-wide association studies of correlated traits in structured populations. Nat Genet 2012, 44(9):1066–1071.

7. Visscher PM, Yang J: A plethora of pleiotropy across complex traits. Nat Genet 2016, 48(7):707–708.

8. Pickrell JK, Berisa T, Liu JZ, Segurel L, Tung JY, Hinds DA: Detection and interpretation of shared genetic influences on 42 human traits. Nat Genet 2016, 48(7):709–717.

9. Heid IM, Winkler TW: A multitrait GWAS sheds light on insulin resistance. Nat Genet 2016, 49(1):7–8.

10. Schweiger M, Romauch M, Schreiber R, Grabner GF, Hutter S, Kotzbeck P, Benedikt P, Eichmann TO, Yamada S, Knittelfelder O et al: Pharmacological inhibition of adipose
triglyceride lipase corrects high-fat diet-induced insulin resistance and hepatosteatosis in mice. Nat Commun 2017, 8:14859.

11. Goh KI, Cusick ME, Valle D, Childs B, Vidal M, Barabasi AL: The human disease network. Proc Natl Acad Sci U S A 2007, 104(21):8685-8690.

12. Lauc G, Huffman JE, Pucic M, Zgaga L, Adamczyk B, Muzinic A, Novokmet M, Polasek O, Gornik O, Kristic J et al: Loci associated with N-glycosylation of human immunoglobulin G show pleiotropy with autoimmune diseases and haematological cancers. PLoS Genet 2013, 9(1):e1003225.

13. Liu Z, Tang Q, Wen J, Tang Y, Huang D, Huang Y, Xie J, Luo Y, Liang M, Wu C et al: Elevated serum complement factors 3 and 4 are strong inflammatory markers of the metabolic syndrome development: a longitudinal cohort study. Sci Rep 2016, 6:18713.

14. Tan A, Gao Y, Yang X, Zhang H, Qin X, Mo L, Peng T, Xia N, Mo Z: Low serum osteocalcin level is a potential marker for metabolic syndrome: results from a Chinese male population survey. Metabolism 2011, 60(8):1186-1192.

15. Liang Y, Tan A, Liang D, Yang X, Liao M, Gao Y, Jiang Y, Yao Z, Lin X, Lu Z et al: Low osteocalcin level is a risk factor for impaired glucose metabolism in a Chinese male population. J Diabetes Investig 2016, 7(4):522-528.

16. Chen D, Zhang H, Gao Y, Lu Z, Yao Z, Jiang Y, Lin X, Wu C, Yang X, Tan A et al: Cross-sectional and longitudinal associations between serum uric acid and metabolic syndrome: Results from Fangchenggang Area Male Health and Examination Survey in China. Clin Chim Acta 2015, 446:226-230.

17. Tan A, Sun J, Xia N, Qin X, Hu Y, Zhang S, Tao S, Gao Y, Yang X, Zhang H et al: A genome-wide association and gene-environment interaction study for serum triglycerides levels in a healthy Chinese male population. Hum Mol Genet 2012, 21(7):1658-1664.

18. Xu Z, Taylor JA: SNPinfo: integrating GWAS and candidate gene information into
functional SNP selection for genetic association studies. Nucleic Acids Res 2009, 37(Web Server issue):W600–605.

19. Chatr-Aryamontri A, Breitkreutz BJ, Oughtred R, Boucher L, Heinicke S, Chen D, Stark C, Breitkreutz A, Kolas N, O’Donnell L et al: The BioGRID interaction database: 2015 update. Nucleic Acids Res 2015, 43(Database issue):D470–478.

20. Lotia S, Montojo J, Dong Y, Bader GD, Pico AR: Cytoscape app store. Bioinformatics 2013, 29(10):1350–1351.

21. Bulik-Sullivan B, Finucane HK, Anttila V, Gusev A, Day FR, Loh PR, ReproGen C, Psychiatric Genomics C, Genetic Consortium for Anorexia Nervosa of the Wellcome Trust Case Control C, Duncan L et al: An atlas of genetic correlations across human diseases and traits. Nat Genet 2015, 47(11):1236–1241.

22. Ward LD, Kellis M: HaploReg: a resource for exploring chromatin states, conservation, and regulatory motif alterations within sets of genetically linked variants. Nucleic Acids Res 2012, 40(Database issue):D930–934.

23. Welter D, MacArthur J, Morales J, Burdett T, Hall P, Junkins H, Klemm A, Flicek P, Manolio T, Hindorff L et al: The NHGRI GWAS Catalog, a curated resource of SNP-trait associations. Nucleic Acids Res 2014, 42(Database issue):D1001–1006.

24. Imatoh T, Yengo L, Rocheleau G, Kamimura S, Maeda S, Miyazaki M, Froguel P: ALDH2 Polymorphism rs671, but Not ADH1B Polymorphism rs1229984, Increases Risk for Hypo-HDL-Cholesterolemia in a/a Carriers Compared to the G/G Carriers. Lipids 2018, 53(8):797–807.

25. Hoshi H, Hao W, Fujita Y, Funayama A, Miyauchi Y, Hashimoto K, Miyamoto K, Iwasaki R, Sato Y, Kobayashi T et al: Aldehyde-stress resulting from Aldh2 mutation promotes osteoporosis due to impaired osteoblastogenesis. J Bone Miner Res 2012, 27(9):2015-2023.
26. Sivakumaran S, Agakov F, Theodoratou E, Prendergast JG, Zgaga L, Manolio T, Rudan I, McKeigue P, Wilson JF, Campbell H: Abundant pleiotropy in human complex diseases and traits. Am J Hum Genet 2011, 89(5):607–618.

27. Chesmore K, Bartlett J, Williams SM: The ubiquity of pleiotropy in human disease. Hum Genet 2018, 137(1):39-44.

28. Solovieff N, Cotsapas C, Lee PH, Purcell SM, Smoller JW: Pleiotropy in complex traits: challenges and strategies. Nat Rev Genet 2013, 14(7):483–495.

29. L Z, Q Y, S JC: Functionally, structurally, and evolutionarily distinct set of genes linked to phenome wide variation in maize. bioRxiv preprint first posted online 2019.

30. Schaefer RJ, Michno JM, Jeffers J, Hoekenga O, Dilkes B, Baxter I, Myers CL: Integrating Coexpression Networks with GWAS to Prioritize Causal Genes in Maize. Plant Cell 2018, 30(12):2922–2942.

31. Pitchers W, Nye J, Marquez EJ, Kowalski A, Dworkin I, Houle D: A Multivariate Genome-Wide Association Study of Wing Shape in Drosophila melanogaster. Genetics 2019, 211(4):1429–1447.

32. Ganguly P, Alam SF: Role of homocysteine in the development of cardiovascular disease. Nutr J 2015, 14:6.

33. Gao YH, Wu ZX, Xie LQ, Li CX, Mao YQ, Duan YT, Han B, Han SF, Yu Y, Lu HJ et al: VHL deficiency augments anthracycline sensitivity of clear cell renal cell carcinomas by down-regulating ALDH2. Nat Commun 2017, 8:15337.

34. Berenson AB, Rahman M: Effect of hormonal contraceptives on vitamin B12 level and the association of the latter with bone mineral density. Contraception 2012, 86(5):481–487.

35. Bala KA, Dogan M, Kaba S, Mutluer T, Aslan O, Dogan SZ: Hormone disorder and vitamin deficiency in attention deficit hyperactivity disorder (ADHD) and autism spectrum
disorders (ASDs). J Pediatr Endocrinol Metab 2016, 29(9):1077-1082.

36. Mohan IK, Khan SA, Jacob R, Sushma Chander N, Hussain T, Arokayan SA, Radha Rama Devi A, Naushad SM: Application of adaptive neuro-fuzzy inference systems (ANFIS) to delineate estradiol, glutathione and homocysteine interactions. Clin Nutr ESPEN 2017, 20:41-46.

37. Wu C, Kraft P, Zhai K, Chang J, Wang Z, Li Y, Hu Z, He Z, Jia W, Abnet CC et al: Genome-wide association analyses of esophageal squamous cell carcinoma in Chinese identify multiple susceptibility loci and gene-environment interactions. Nat Genet 2012, 44(10):1090-1097.

38. Zhong W, Zhang W, Li Q, Xie G, Sun Q, Sun X, Tan X, Sun X, Jia W, Zhou Z: Pharmacological activation of aldehyde dehydrogenase 2 by Alda-1 reverses alcohol-induced hepatic steatosis and cell death in mice. J Hepatol 2015, 62(6):1375-1381.

39. Millwood IY, Walters RG, Mei XW, Guo Y, Yang L, Bian Z, Bennett DA, Chen Y, Dong C, Hu R et al: Conventional and genetic evidence on alcohol and vascular disease aetiology: a prospective study of 500 000 men and women in China. The Lancet 2019, 393(10183):1831-1842.

40. Asada M, Ohmi K, Delia D, Enosawa S, Suzuki S, Yuo A, Suzuki H, Mizutani S: Brap2 functions as a cytoplasmic retention protein for p21 during monocyte differentiation. Mol Cell Biol 2004, 24(18):8236-8243.

41. Ozaki K, Sato H, Inoue K, Tsunoda T, Sakata Y, Mizuno H, Lin TH, Miyamoto Y, Aoki A, Onouchi Y et al: SNPs in BRAP associated with risk of myocardial infarction in Asian populations. Nat Genet 2009, 41(3):329-333.

42. Avery CL, He Q, North KE, Ambite JL, Boerwinkle E, Fornage M, Hindorff LA, Kooperberg C, Meigs JB, Pankow JS et al: A phenomics-based strategy identifies loci on APOC1, BRAP, and PLCG1 associated with metabolic syndrome phenotype domains. PLoS Genet 2011,
7(10):e1002322.

43. Escudero-Esparza A, Kalchishkova N, Kurbasic E, Jiang WG, Blom AM: The novel complement inhibitor human CUB and Sushi multiple domains 1 (CSMD1) protein promotes factor I-mediated degradation of C4b and C3b and inhibits the membrane attack complex assembly. FASEB J 2013, 27(12):5083–5093.

44. Patel M: Parkinson disease: CSMD1 gene mutations can lead to familial Parkinson disease. Nat Rev Neurol 2017, 13(11):641.

45. Nicolae DL, Gamazon E, Zhang W, Duan S, Dolan ME, Cox NJ: Trait-associated SNPs are more likely to be eQTLs: annotation to enhance discovery from GWAS. PLoS Genet 2010, 6(4):e1000888.

46. Mifsud B, Tavares-Cadete F, Young AN, Sugar R, Schoenfelder S, Ferreira L, Wingett SW, Andrews S, Grey W, Ewels PA et al: Mapping long-range promoter contacts in human cells with high-resolution capture Hi-C. Nat Genet 2015, 47(6):598–606.

47. Brainstorm C, Anttila V, Bulik-Sullivan B, Finucane HK, Walters RK, Bras J, Duncan L, Escott-Price V, Falcone GJ, Gormley P et al: Analysis of shared heritability in common disorders of the brain. Science 2018, 360(6395).

48. Zhao H, Yang Y, Lu Y, Mort M, Cooper DN, Zuo Z, Zhou Y: Quantitative mapping of genetic similarity in human heritable diseases by shared mutations. Hum Mutat 2018, 39(2):292–301.

49. Yamamoto F, Clausen H, White T, Marken J, Hakomori S: Molecular genetic basis of the histo-blood group ABO system. Nature 1990, 345(6272):229–233.

50. Ling X, Zheng Y, Tao J, Zheng Z, Chen L: Association study of polymorphisms in the ABO gene with ischemic stroke in the Chinese population. BMC Neurol 2016, 16(1):146.

51. Zhang H, Zhang Z, Zhang J, Xu L, Ye Z, Hao Y, Cai B, Zhou S, Liu K, Sun L et al: Fine-Mapping of ABO Gene Identifies Two Novel SNPs Associated with Large Artery
Atherosclerotic Stroke in a Chinese Han Population. Mol Neurobiol 2017, 54(3):2107-2113.

52. Nakao M, Matsuo K, Hosono S, Ogata S, Ito H, Watanabe M, Mizuno N, Iida S, Sato S, Yatabe Y et al: ABO blood group alleles and the risk of pancreatic cancer in a Japanese population. Cancer Sci 2011, 102(5):1076-1080.

53. Wang H, Li Q, Wang T, Yang G, Wang Y, Zhang X, Sang Q, Wang H, Zhao X, Xing Q et al: A common polymorphism in the human aromatase gene alters the risk for polycystic ovary syndrome and modifies aromatase activity in vitro. Mol Hum Reprod 2011, 17(6):386-391.

54. Wang B, Fu ZY, Ma YT, Huang D, Liu F, Dong CL, Wang T, Meng YJ: Identification of a CYP19 Gene Single-Nucleotide Polymorphism Associated with a Reduced Risk of Coronary Heart Disease. Genet Test Mol Biomarkers 2016, 20(1):2-10.

55. Okada Y, Sim X, Go MJ, Wu JY, Gu D, Takeuchi F, Takahashi A, Maeda S, Tsunoda T, Chen P et al: Meta-analysis identifies multiple loci associated with kidney function-related traits in east Asian populations. Nat Genet 2012, 44(8):904-909.

56. Quillen EE, Chen XD, Almasy L, Yang F, He H, Li X, Wang XY, Liu TQ, Hao W, Deng HW et al: ALDH2 is associated to alcohol dependence and is the major genetic determinant of “daily maximum drinks” in a GWAS study of an isolated rural Chinese sample. Am J Med Genet B Neuropsychiatr Genet 2014, 165B(2):103-110.

57. Takeuchi F, Isono M, Nabika T, Katsuya T, Sugiyama T, Yamaguchi S, Kobayashi S, Oghara T, Yamori Y, Fujioka A et al: Confirmation of ALDH2 as a Major Locus of Drinking Behavior and of Its Variants Regulating Multiple Metabolic Phenotypes in a Japanese Population. Circulation Journal 2011, 75(4):911-918.

58. Kamatani Y, Matsuda K, Okada Y, Kubo M, Hosono N, Daigo Y, Nakamura Y, Kamatani N: Genome-wide association study of hematological and biochemical traits in a Japanese population. Nat Genet 2010, 42(3):210-215.

59. Low SK, Takahashi A, Cha PC, Zembutsu H, Kamatani N, Kubo M, Nakamura Y: Genome-
wide association study for intracranial aneurysm in the Japanese population identifies three candidate susceptible loci and a functional genetic variant at EDNRA. *Hum Mol Genet* 2012, 21(9):2102–2110.

60. Raychaudhuri S, Plenge RM, Rossin EJ, Ng AC, International Schizophrenia C, Purcell SM, Sklar P, Scolnick EM, Xavier RJ, Altshuler D et al: Identifying relationships among genomic disease regions: predicting genes at pathogenic SNP associations and rare deletions. *PLoS Genet* 2009, 5(6):e1000534.

61. Raychaudhuri S, Thomson BP, Remmers EF, Eyre S, Hinks A, Guiducci C, Catanese JJ, Xie G, Stahl EA, Chen R et al: Genetic variants at CD28, PRDM1 and CD2/CD58 are associated with rheumatoid arthritis risk. *Nat Genet* 2009, 41(12):1313–1318.

62. Demenais F, Margaritte-Jeannin P, Barnes KC, Cookson WOC, Altmuller J, Ang W, Barr RG, Beaty TH, Becker AB, Beilby J et al: Multiancestry association study identifies new asthma risk loci that colocalize with immune-cell enhancer marks. *Nat Genet* 2018, 50(1):42–53.

63. Kostem E, Lozano JA, Eskin E: Increasing power of genome-wide association studies by collecting additional single-nucleotide polymorphisms. *Genetics* 2011, 188(2):449–460.

64. Marchini J, Howie B, Myers S, McVean G, Donnelly P: A new multipoint method for genome-wide association studies by imputation of genotypes. *Nat Genet* 2007, 39(7):906–913.

65. Barabasi AL, Gulbahce N, Loscalzo J: Network medicine: a network-based approach to human disease. *Nat Rev Genet* 2011, 12(1):56–68.

66. Faner R, Agusti A: Network analysis: a way forward for understanding COPD multimorbidity. *Eur Respir J* 2015, 46(3):591–592.

67. Grosdidier S, Ferrer A, Faner R, Pinero J, Roca J, Cosio B, Agusti A, Gea J, Sanz F, Furlong LI: Network medicine analysis of COPD multimorphidities. *Respir Res* 2014, 15:111.
Figure 1

The heatmaps based on the Pearson correlation for 29 Biochemical indices in the FAMHES cohort. The coefficient in each cell ranges from -1 to 1. A negative value denotes a negative correlation, and a positive value denotes a positive correlation; 1 indicates a complete correlation, and 0 indicates no correlation.
The correlations between clinical quantitative traits shown in this matrix are shown in blue and red. Blue represents a positive correlation, and the darker the color, the stronger the positive correlation. Red indicates a negative correlation, and the darker the color, the stronger the negative correlation. If the correlation coefficients were greater than 0.3 or less than -0.3 and p-value < 0.01, we considered the pairs to be correlated.

Figure 2

Molecular comorbidity index (MCI) for 29 Biochemical indices in the FAMHES cohort. The MCI value is between 0 and 1. The darker of blue the stronger correlation between two clinical biochemical indicators. If the MCI was over 0.6, we considered the pairs to be correlated.
Correlation analysis based on linkage disequilibrium score regression (LDSC) for 29 Biochemical indices in the FAMHES cohort. The genetic correlation estimate \((R_g)\) ranges between \(-1\) and \(1\). A negative value denotes a negative correlation, and a positive value denotes a positive correlation; \(1\) indicates a complete correlation, and \(0\) indicates no correlation. The correlations between clinical biochemical indicators shown in this matrix are represented by blue and red. Blue represents a positive correlation, and the darker the color, the stronger the positive correlation. Red indicates a negative correlation, and the darker the color, the stronger the negative correlation.
Figure 4

Circos plot of shared SNPs related to more than 3 Biochemical indices based on analysis of individuals in the FAMHES cohort. Each plot presents one trait with a specific color. ASO and IgE have no common SNPs in these 481 SNPs, so they are not in this Circos. The black dash denotes the shared SNP, and the upper line denotes the significant value with log (p value). The chromosome number is marked on the outside of the Circos plot. The chromosome positions of 29 common sites (p-value<10-4) associated with more than four Biochemical indices are marked on the outside of the Circos plot.
Figure 5

The impact of ALDH2 rs671 on osteogenic and adipogenic differentiation of 3T3-L1 preadipocytes. (A) The cell growth curve measured as 450 nm absorbance by using cell counting kit-8 Annexin V-FITC/PI–labeled cells were detected by flow cytometry to measure osteoblast apoptosis. Shown are representative dot plots (B) and quantified data as the percentage of total cells (C) At 7 days after osteoblast induction, cells were stained with Alizarin Red S solution to measure calcium content. Shown are the representative photographs (D) and quantified Alizarin red S staining in cells (E) Expression of osteocalcin-related genes (AKP, osteocalcin, Runx2, Col1) in ALDH2 WT- or Glu504Lys-overexpressing 3T3-L1 preadipocytes after 7 days of induction refer to 3T3-L1 RFP. (F) At 7 days after adipocyte induction, cells were stained with Oil-Red O to measure triglyceride (TG) content. Shown are the representative photographs (G) and quantified Oil-Red O staining in cells (H) qPCR analysis of adipogenic (adiponectin, C/EBPα C/EBPβ, Fabp4, Pparγ) expression in ALDH2 WT- or Glu504Lys-overexpressing 3T3-L1 preadipocytes after 7 days of induction refer to 3T3-L1 RFP. (I) Data are shown
as the mean ± SE from 3 independent experiments. * P < 0.05, **P < 0.01; ***P < 0.001.

Supplementary Files

This is a list of supplementary files associated with the primary manuscript. Click to download.

Tabel S2 1102.docx
Figure S4.png
Tabel S4 1102.docx
Formula.jpg
Table S3.pdf
Figure S3.png
Table S1.pdf
Table S6.pdf
Table S5.pdf
Figure S2.png
Figure S1.jpg