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Original Paper

Genome-Wide DNA Methylation Profiles of Phlegm-Dampness Constitution

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Abstract

Background/Aims: Metabolic diseases are leading health concerns in today's global society. In traditional Chinese medicine (TCM), one body type studied is the phlegm-dampness constitution (PC), which predisposes individuals to complex metabolic disorders. Genomic studies have revealed the potential metabolic disorders and the molecular features of PC. The role of epigenetics in the regulation of PC, however, is unknown. ***Methods:*** We analyzed a genome-wide DNA methylation in 12 volunteers using Illumina Infinium Human Methylation450 BeadChip on peripheral blood mononuclear cells (PBMCs). Eight volunteers had PC and 4 had balanced constitutions. ***Results:*** Methylation data indicated a genome-scale hyper-methylation pattern in PC. We located 288 differentially methylated probes (DMPs). A total of 256 genes were mapped, and some of these were metabolic-related. SQSTM1, DLGAP2 and DAB1 indicated diabetes mellitus; HOXC4 and SMPD3, obesity; and GRWD1 and ATP10A, insulin resistance. According to Ingenuity Pathway Analysis (IPA), differentially methylated genes were abundant in multiple metabolic pathways. ***Conclusion:*** Our results suggest the potential risk for metabolic disorders in individuals with PC. We also explain the clinical characteristics of PC with DNA methylation features.

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Introduction

Due to widespread over-nutrition and sedentary lifestyles, metabolic diseases and the subsequent morbidity and mortality are major public health issues in our current

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Yao et al.: DNA Methylation Profiles of PC

society [1]. The steadily increasing prevalence of metabolic disease may be associated with cardiovascular disease and diabetes [2]. It has been estimated that 25% of the world's adult population is suffering from these diseases, and these have notoriously emerged as being the world's major medical and financial burdens [3]. In addition to conventional therapy, early prevention and prediction are beneficial in the promotion of health optimization according to "P4 medicine" [4].

Increasing evidence has revealed that genetic, environmental and dietary factors contribute to the development of metabolic diseases. Epigenetics is thought to be a potential link between postnatal environmental factors and diseases, which refers to a reversible and heritable change that regulates gene expression without a change in the DNA sequence. DNA methylation, as a major epigenetic mechanism that typically occurs at CpG sites in adult cells, is frequently studied [5-9]. Due to its stability in blood samples and transmissibility during cell division, DNA methylation can be used to characterize early disease progression, and it provides an efficient way to prevent and predict diseases in clinical practice. To date, changes in DNA methylation have been widely reported to be involved in the onset of a variety of metabolic diseases, including diabetes mellitus, fatty liver, and metabolic syndrome [10-15].

Phlegm-dampness constitution (PC), one of the nine different body constitution types in Traditional Chinese medicine (TCM), is thought to be the preclinical stage of multiple metabolic disorders [16, 17]. Our previous studies have mainly focused on genomics and single nucleotide polymorphisms (SNPs) of PC, and we discovered correlations between the PC and multiple metabolic disorders [16, 18]. However, there have been no epigenetic studies on PC formation, particularly the effects of DNA methylation. As an ongoing study, we aim to investigate the DNA methylation profile of PC on a genome-wide level using the balanced constitution (BC) as a control group. We also hope to evaluate the difference between the two constitution types (BC and PC) to discover the potential molecular biomarkers of PC. This study could reveal the molecular mechanism behind the preclinical stage of metabolic diseases and shed new light on early prevention and prediction.

Materials and Methods

Study subjects
Eight PC volunteers and four BC volunteers were included according to the standard of *Classification and diagnosis of TCM Constitution* [19]. The participants ranged in age from 30 to 60 years old. They had no diagnosed diseases, and all of them provided written informed consent. This study was approved by the Beijing University of Chinese Medicine ethics committee and performed in accordance with the Declaration of Helsinki.

PBMC isolation
Venous blood samples were extracted with an EDTA anticoagulant tube. Peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll-Hypaque (Histopaque-1077, Sigma-Aldrich, St. Louis, USA) density gradient centrifugation.

DNA extraction
Genomic DNA was extracted from the isolated cell pellets using DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. DNA purity and quantity was assessed using a NanoDrop Spectrophotometer (Thermo Scientific, DE, USA), and the samples were diluted to standard concentrations of 50 ng/μL.

Infinium 450K BeadChip analysis
Bisulfite conversion was conducted on 1μg of DNA sample from each participant using the EZ DNA Methylation kit (Zymo Research Corp, Orange, CA, USA). A genome-wide DNA methylation examination was performed using the Infinium Human Methylation450 BeadChip (Illumina, San Diego, CA, USA), which interrogates 485, 512 CpG sites [20, 21]. BeadChips were scanned with an Illumina iScan; the scanned data and image output files were managed with Genomestudio software (version 1.9.0; Illumina).

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Yao et al.: DNA Methylation Profiles of PC

Bioinformatics and Statistical Analysis

Illumina 450K probe preprocessing. No outlier samples were identified after comparing the methylation beta value box plot and density plot of 12 subjects. All samples were included in the subsequent analysis. At first, we excluded 144, 854 CpG probes that ambiguously mapped to the human genome (hg19), and a total of 340, 658 probes passed the analysis using Bowtie [22]. We also filtered 12, 871 CpG probes that contained common single-nucleotide polymorphisms (SNPs) so that the relative methylation level in each sample was not affected by potential genetic bias. Next, we removed 3965 probes that had a bead count <3 in 5% of samples or >1% of samples with a detection p-value of >0.01. The residual signal intensity values were normalized using the subset-quantile within an arrays normalization (SWAN) method for reducing technical variation, as implemented in the minfi R-package [23]. Although one chip is not irrespective of the batch effect in our data, we excluded 7466 CpG sites on the X- and Y-chromosomes to reduce the effects of differential methylation between males and females on the sex chromosomes. WBC proportion estimation for each sample was necessary for controlling for the effects of the relative proportions of different WBC types on methylation level. In the end, a total of 316, 356 autosomal CpG sites were tested in all samples (Fig. 1A).

To identify differentially methylated CpG sites. The beta value indicated the DNA methylation level of each CpG probes, and beta was defined as: Beta=Meth/ (Meth+Unmeth+100). However, since the beta value was in a proportion range from 0 to 1, we transformed it to M value, which was defined as: M=log (beta/(1-beta)) [24]. Differentially methylated probes (DMPs) were detected by comparing the M values between the two groups. Statistical significance was tested with an unpaired Student's t-test and Wilcoxon signed rank test. Due to the small sample size of this study, we analyzed the data using the two statistical methods separately and identified the probes that overlapped as the DMPs. Finally, the analytic approach we adopted to rank DMPs was to take both statistical significance (p-value) and the magnitude of absolute mean beta value difference (i.e., absolute Δβ).

IPA analysis. The Ingenuity Pathway Analysis (IPA) system was used to analyze potentially altered network relationships, candidate biomarkers, and associated diseases from the list of differentially methylated genes between PC and BC individuals. Using a nonparametric test, IPA measured the likelihood due to chance that genes from the list participated in each network and category of Molecular Function and diseases and calculated the corresponding p-value.

Results

Subjects' characteristics

The characteristics of the study subjects in the PC and BC group are shown in Table 1. No significant differences were noted between these two groups in sex, age, body mass index (BMI), waist circumference, fasting blood glucose, fasting insulin, blood pressure, total cholesterol, triglyceride, low-density lipoprotein, or high-density lipoprotein. All subjects from both groups were healthy individuals with no diagnosable diseases.

Identification of global methylation patterns in PC and BC subjects

A total of 316, 356 sites on the beadchip were included after probe filtering and normalization (Fig. 1A). A genome-wide DNA methylation analysis was performed to explore the global methylation pattern of PC and BC subjects. Methylation levels of CpG sites in the two groups were strongly correlated as shown in Fig. 1B (Pearson's $R^2=0.9978715$, p-value < 2.2e-

Table 1. Characteristics of the study subjects. * P-values were calculated respectively using Student's t-test and Fisher's exact test for continuous and categorical variables

Variables	Phlegm-dampness Constitution (N=8)	Balanced Constitution (N=4)	P-Value*
Male, n (%)	2(25)	1(25)	1
Age (years),Mean (SD)	44.63 ± 3.640	35.25 ± 2.658	0.1231
Body mass index (kg/m2),mean(SD)	23.44 ± 0.6068	23.55 ± 0.5008	0.9072
Waist circumference (cm),mean(SD)	80.50 ± 2.928	79.50 ± 3.304	0.8387
Fasting blood glucose (mmol/L), mean (SD)	5.249 ± 0.1281	5.310 ± 0.1240	0.7694
Fasting insulin (pmol/ml),mean(SD)	9.664 ± 1.731	6.883 ± 1.176	0.3151
Systolic blood pressure (mmHg),mean(SD)	122.8 ± 10.18	107.5 ± 3.227	0.3304
Diastolic blood pressure (mmHg),mean(SD)	73.75 ± 3.063	66.75 ± 3.119	0.1842
Total cholesterol (mmol/L),mean(SD)	4.983 ± 0.3528	4.820 ± 0.5057	0.7965
Triglyceride (mmol/L),mean(SD)	1.281 ± 0.09644	0.9400 ± 0.1802	0.0945
Low density lipoprotein (mmol/L),mean(SD)	2.414 ± 0.1042	2.425 ± 0.2925	0.9646
High density lipoprotein (mmol/L),mean(SD)	1.449 ± 0.09990	1.593 ± 0.1441	0.4282

Fig. 1. Comparison of Global DNA methylation in phlegm-dampness constitution (PC) with balanced constitution (BC) groups. (A) Filter workflow of Illumina 450k Infinium Methylation BeadChip. (B) The overall correlation plot between the beta values of all CpG sites in PC and BC. DNA methylation was measured as the beta value ranging from 0 (unmethylated) to 1 (completely methylated). All available probes (316356 CpG sites) are shown. (C) Volcano plot of all CpG methylation β-values. The data for all available probes (316356 CpG sites) are plotted as β-values versus the −log10 of the P-values. Thresholds are shown as dashed lines. Genes selected as significantly different are highlighted as red dots.
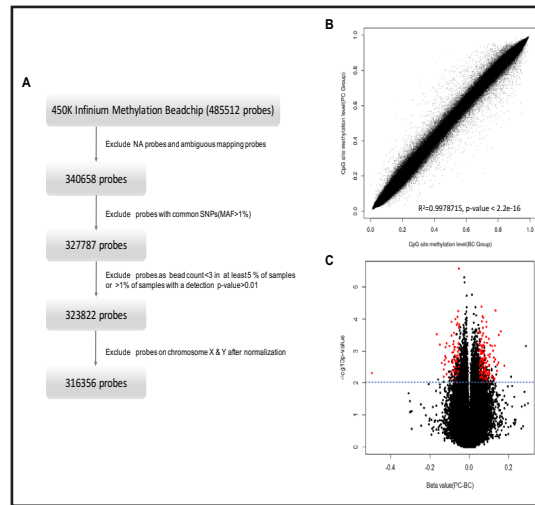
16). The fractions of low methylated CpG sites (<25%) in the PC and BC groups were 30.27% and 30.32%, respectively. The PC group showed 49.16% highly methylated CpG sites fraction (>75%) compared with 47.89% in BC group. As shown in Fig. 2A, the average global levels of DNA methylation were similar between PC and BC subjects.

Using Student's t-test to compare each of the CpG sites individually, 2252 differentially methylated probes were detected in the 316, 356 included probes, while 1658 differential probes were located by the Wilcoxon signed rank test. The overlapping 1011 probes were identified to be significantly (p < 0.01) differentially methylated between PC and BC individuals (Fig. 1C, Fig. 2B). The Δβ was also considered as a criterion of DMP identification, 288 of these 1011 probes met the threshold of |Δβ| ≥ 0.05, including 187 hypermethylated and 101 hypomethylated ones (Fig. 2B), and the top 25 DMPs, sorted by the Δβ, were listed in Table 2.

According to the analysis of these 288 DMPs, the subjects in the PC group exhibited higher methylation levels than those in the BC group (Fig. 2C). The proportions of 288 DMPs measured by location relative to CpG island regions and gene regions are presented in Fig. 3A and 3B, respectively.

Fig. 2. Whole methylome analysis of peripheral blood mononuclear cells (PBMCs) from PC and BC subjects. (A) Differences in the global methylation levels of the overall available CpGs between PC and BC groups. (B) Venn diagram showing the methods for identifying differentially methylated probes (DMPs) based on two statistic methods and Δβ threshold. A total of 2252 and 1658 DMPs are detected by Student's t-test and Wilcoxon signed rank test respectively (P<0.01). Among the overlapped probes of the two sets, 288 DMPs met the criterion of |Δβ| ≥ 0.05. (C) Global differences in methylation levels of the 288 DMPs identified by 450K array analysis between PC and BC groups (P<0.001).

Most of the hypermethylated DMPs are located in the Open Sea, followed by the North Shore and the South Shore. For the hypomethylated DMPs, CpG islands were ranked first and were followed by the North Shore and the Open Sea. In terms of gene regions, both hyper and hypomethylated DMPs were predominantly located at the promoters, included TSS1500, TSS200, UTR5 and 1stExon. Supervised hierarchical clustering based on the data of 288 DMPs demonstrated that there were distinctly different DNA methylation patterns between subjects in the PC and BC groups, which are displayed as a heatmap in Fig. 3C.
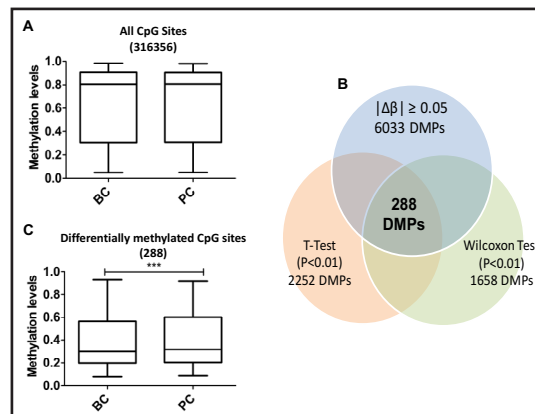
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Yao et al.: DNA Methylation Profiles of PC

Table 2. Top 25 differently methylated CpG sites based on the Δβ (P < 0.01)

IllumID	Gene symbol	Gene feature	Δβ	Associated diseases
cg21498547	DLGAP2	3'UTR	0.495	epithelial cancer, adenocarcinoma, Alzheimer's disease, carcinoma, endometrioid cancer, non-insulin-dependent diabetes mellitus
cg13925773	DUSP19	TSS200	-0.179	adenocarcinoma, epithelial cancer
cg26542412	TGFBR3	5'UTR	0.166	acute myeloid leukemia, ovarian neoplasia, Duchenne muscular dystrophy,etc.
cg26029734	PAX6	Body	-0.161	aniridia, congenital aniridia, irido-corneo-trabecular dysgenesis, aniridia, etc.
cg16155081	TAF1B	TSS1500	-0.149	epithelial cancer, adenocarcinoma, carcinoma
cg04066190	TFAP2A	Body	-0.149	branchiooculofacial syndrome, epithelial cancer, etc.
cg00818680	TMCO7	Body	0.148	melanoma, melanoma cancer,non-insulin-dependent diabetes mellitus
cg15082992	——	——	-0.142	——
cg08627825	——	——	-0.141	——
cg12891252	HNRNPF	5'UTR	0.141	infection by HIV-1, endometrioid cancer, endometrioid carcinoma, epithelial cancer, melanoma, melanoma cancer
cg17796323	N4BP3	Body	-0.134	epithelial cancer, adenocarcinoma, breast cancer, carcinoma
cg04206742	TGDS	TSS1500	-0.132	Catel-Manzke syndrome, productive infection by HIV-1
cg14553705 ——		——	-0.13	
cg15443732	GALR1	TSS1500	0.13	melanoma cancer, melanoma, neoplasia, tumor
cg24768902	PLXDC1	TSS1500	0.129	cancer, osteosarcoma, melanoma cancer, angiomatosis, hemangioma, squamous cell carcinoma
cg23936410 ——		——	-0.129	
cg12486814	C1orf192	TSS200	0.126	melanoma cancer
cg21571166	ZIC1	3'UTR	-0.126	——
cg10547050	PHF12	Body	-0.125	endometrioid cancer, endometrioid carcinoma, epithelial cancer, infection by HIV-1
cg05033239	GPR98	Body	-0.125	type IIC Usher syndrome, melanoma cancer, melanoma, epithelial cancer,etc.
cg07310406	MLC1	TSS1500;TSS200	-0.124	megalencephalic leukoencephalopathy with subcortical cysts 1, adenocarcinoma, etc.
cg22151644	HOXC4	5'UTR	0.123	adenocarcinoma, epithelial cancer, rheumatoid arthritis
cg25373372	FAM46A	Body	-0.123	adenocarcinoma, epithelial cancer, neoplasia, tumor
cg26345888	DAB1	TSS200	-0.121	melanoma cancer, melanoma, adenocarcinoma, epithelial cancer, schizophrenia, amyotrophic lateral sclerosis, non-insulin-dependent diabetes mellitus

Fig. 3. Analysis of 288 DMPs between PC and BC groups. (A) Proportions of 288 DMPs measured by location relative to CpG isle regions. The six categories of CpG island, North Shelf, North Shore, Open Sea, South Shelf and South Shore are identified according to Illumina 450K annotation. (B) Proportions of 288 DMPs measured by functional location relative to gene regions. The regions of 200 bp and 200 to 1500 bp upstream of transcription starting site are categorized as TSS 200 and TSS 1500, respectively. The other five groups are 5' UTR, first exon, gene body, 3' UTR, and intergenic regions. (C)

Heatmap of the 288 DMPs between PC and BC groups. Columns represent the samples (8 PC subjects and 4 BC subjects) and each row represents a CpG site. Higher methylation levels are shaded in red and lower levels are in green. The dendrogram shows the results of unsupervised hierarchical clustering of the 288 CpG sites, which separates PC subjects from BC subjects distinctly.
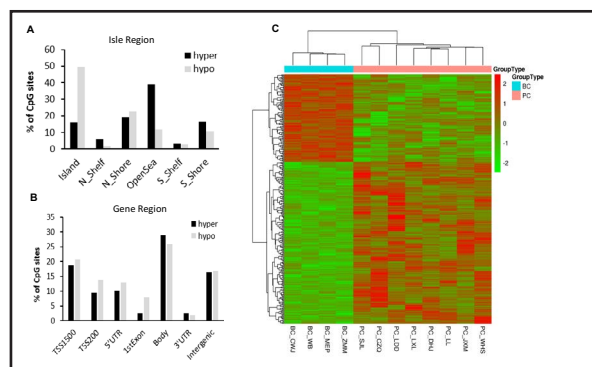
Fig. 4. Manhattan plot and Quantile-quantile (QQ) plot. (A) Manhattan plot showing global distribution of DMPs according to the chromosomes sequence. The x-axis represents chromosomes; the y-axis represents -log10 (p-values). DMPs above the blue line represent a genome-wide significance (p<1e-04). (B) QQ plot of p-values from all 316356 probes. The x-axis shows the expected –log10(p-value). The y-axis displays

the observed –log10(p-value). The red line indicates the expected distributions under the hypothesis. The gray shaded region represents the 95% confidence interval.
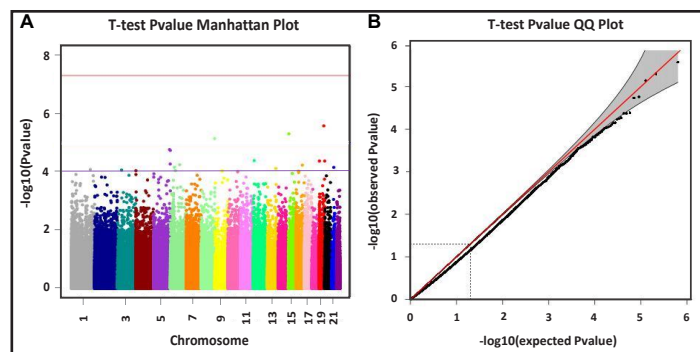
DMPs between PC and BC groups significantly associated with metabolic diseases

A global view of all included CpG sites is presented in a Manhattan plot (Fig. 4A), indicating that the significant DMPs were mainly located on chromosomes 5, 6, and 19. A list of 256 genes, including 169 hypermethylated and 87 hypomethylated genes, were mapped based on the 288 DMPs. IPA analysis was used to investigate the associated top diseases and bio-

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logical functions of these differentially methylated genes. As shown in Table 3, hypermethylated genes were mainly related to cancer, gastrointestinal disease, cell morphology and lipid metabolism. Hypomethylated genes were mainly related to dermatological diseases, immunological and inflammatory diseases (Table 4). Further gene pathway analysis indicated that the hypomethylated genes were enriched in the pathways related to immune responses (Cytotoxic T lymphocyte-mediated apoptosis of target cells) and metabolism (D-glucuronate

Table 3. IPA summary of 169 hypermethylated genes on top diseases and bio-functions

	Name	P-value	#Molecules
Diseases and Disorders	Dermatological Diseases and Conditions	8.47E-03 - 9.55E-04	5
	Immunological Disease	1.27E-02 - 9.55E-04	9
	Inflammatory Disease	6.64E-03 - 9.55E-04	5
	Inflammatory Response	4.25E-03 - 9.55E-04	2
	Cancer	4.56E-02 - 4.25E-03	62
Molecular and Cellular Functions	Cell-To-Cell Signaling and Interaction	4.17E-02 - 4.25E-03	4
	Cellular Assembly and Organization	3.76E-02 - 4.25E-03	5
	Cellular Growth and Proliferation	3.76E-02 - 4.25E-03	7
	Cellular Movement	4.25E-03 - 4.25E-03	1
	Lipid Metabolism	3.76E-02 - 4.25E-03	3

Table 4. IPA summary of 87 hypomethylated genes on top diseases and bio-functions

	Illumina ID	Chromosome	Gene symbol	Gene features	Δβ	p-value	Associated disease	Reference
Sorted by |Δβ|	cg21498547	chr8	DLGAP2	3'UTR	0.495	0.005014063	diabetes mellitus	[24]
	cg26345888	chr1	DAB1	TSS200	-0.121	0.000985864	diabetes mellitus	[25]
	cg18473521	chr12	HOXC4	Body	-0.104801945	0.001258699	obesity	[26]
	cg24458896	chr3	MECOM	Body	-0.103044391	0.004821182	hypertension	[30]
	cg06714180	chr13	HOXC4; HOXC5; HOXC6	TSS1500	-0.075991233	0.005506578	obesity	[26, 27, 28, 29]
Sorted by p-value	cg07291563	chr19	GRWD1	1stExon	0.056162301	2.66E-06	insulin resistance	[33]
	cg01788205	chr15	ATP10A	Body	0.013843073	5.01E-06	obesity, insulin resistance	[34, 35, 38]
	cg22198907	chr5	SQSTM1	Body	0.011992258	1.85E-05	diabetes mellitus	[31]
	cg04703197	chr16	SMPD3	5'UTR	0.00081136	5.92E-05	obesity	[36]
	cg22664450	chr13	MYO16	TSS1500	-0.042456757	7.70E-05	metabolic syndrome	[39]
	cg23982237	chr6	FBXO9	TSS1500	-0.069287866	9.09E-05	diabetes mellitus	[32]
	cg03521085	chr4	CTBP1	Body	0.004355016	9.17E-05	obesity	[37]

Table 5. Metabolic-related genes mapped based on 288 DMPs

Variables	Phlegm-dampness Constitution (N=8)	Balanced Constitution (N=4)	P-Value*
Male, n (%)	2(25)	1(25)	1
Age (years),Mean (SD)	44.63 ± 3.640	35.25 ± 2.658	0.1231
Body mass index (kg/m2),mean(SD)	23.44 ± 0.6068	23.55 ± 0.5008	0.9072
Waist circumference (cm),mean(SD)	80.50 ± 2.928	79.50 ± 3.304	0.8387
Fasting blood glucose (mmol/L), mean (SD)	5.249 ± 0.1281	5.310 ± 0.1240	0.7694
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Systolic blood pressure (mmHg),mean(SD)	122.8 ± 10.18	107.5 ± 3.227	0.3304
Diastolic blood pressure (mmHg),mean(SD)	73.75 ± 3.063	66.75 ± 3.119	0.1842
Total cholesterol (mmol/L),mean(SD)	4.983 ± 0.3528	4.820 ± 0.5057	0.7965
Triglyceride (mmol/L),mean(SD)	1.281 ± 0.09644	0.9400 ± 0.1802	0.0945
Low density lipoprotein (mmol/L),mean(SD)	2.414 ± 0.1042	2.425 ± 0.2925	0.9646
High density lipoprotein (mmol/L),mean(SD)	1.449 ± 0.09990	1.593 ± 0.1441	0.4282

degradation Ⅰ, Glutamate degradation III). The hypermethylated genes were enriched in pathways, including acyl carrier protein metabolism, super pathway of cholesterol biosynthesis Ⅱ, tetrahydrobiopterin biosynthesis, VDR/RXR activation, and lysine degradation, that were all involved in various metabolic processes (Fig. 5A).

Some genes, which were mapped by top DMPs with the most distinct Δβ, were related to the metabolism according to the gene function annotation. In Table 5, we showed that DLGAP2 and DAB1 were involved with diabetes mellitus [25, 26]; HOXC4, HOXC5, and HOXC6 were related to obesity and fat accumulation in differentiated brown adipocytes [27-30]; MECOM was associated with hypertension [31]. It is interesting to note that, of these 288 DMPs, two probes (cg22151644 and cg18473521) were both annotated to gene HOXC4. This implied that this gene might be linked to PC identification.

By the analysis of Manhattan plot in Fig. 4, some specific sites (p<1e-04) were also screened out. The genes mapped by these sites were reported to be closely associated with multiple metabolic diseases (Table 5). For instance, SQSTM1 and FBXO9 were related with diabetes mellitus [32, 33]. GRWD1 and ATP10A were involved with insulin resistance [34-36]. SMPD3, ATP10A and CTBP1 were associated with obesity [37-39]. MYO16 was involved with metabolic syndrome [40]. Network visualization and Gene Oncology (GO) analysis of these metabolic-related genes is displayed in Fig. 5B.

Discussion

This study is the first comprehensive analysis to explore the genome-wide DNA methylation profiles in human PBMCs of PC and BC subjects and to detect the differences between these two constitution types using an Infinium 450K BeadChip assay. The contrasting

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DNA methylation profiles of PC and BC groups provided solid support for the constitution classification system in TCM. Furthermore, this study can verify the reliability of the TCM theory that the healthy individuals within an ethnic population can be divided into distinct groups with corresponding disease risks. Our previous study examined the gene expression patterns of PBMCs isolated from PC and BC volunteers, and 355 differentially expressed genes were identified between these two groups [18]. As an ongoing study, this work goes further based on previous studies and confirms the efficacy of TCM constitution classification at the epigenetic level for the first time.

A list of genes obtained from the annotation of 288 DMPs was screened out between PC and BC individuals. IPA results revealed that the molecular and cellular functions of hypermethylated genes, such as B4GALNT1, ST6GAL-NAC5, DHCR24, and PRKCZ, were significantly associated with lipid metabolism as reported [41-

Fig. 5. Pathway analysis and function annotation of genes mapped by DMPs. (A) summary of significant Canonical pathway analysis derived from ingenuity pathway analysis (IPA) for 288 DMPs. The upper gray part and the lower black part indicates the significant pathways for genes with hypomethylation and hypermethylation respectively. The y-axis displays the functional categories that are identified in the analyses. The x-axis demonstrates the significance which is the value of -log (P-value). (B) Network visualization of metabolic related genes and Gene Ontology (GO) terms. The genes were mapped by the top DMPs that were selected by a Manhattan plot analysis.

43]. The hypomethylated genes (BCL11B, HLA-C, FOXP1, FOXP1) were mainly involved with immunological and inflammatory diseases [44]. It was noteworthy that these molecular features are consistent with previous publications and clinical observations in TCM [16, 44, 45]. In this experiment, the findings were limited by the relatively small numbers of samples. We will try to enlarge the sample scales of PC and BC individuals for further validation. More optimal tissues for obtaining DNA, not only from PBMCs of blood, will also be used for further investigation.

Conclusion

our present study explored the DNA methylation characteristics of PC for the first time. We identified the DMPs and the corresponding genes associated with obesity, diabetes
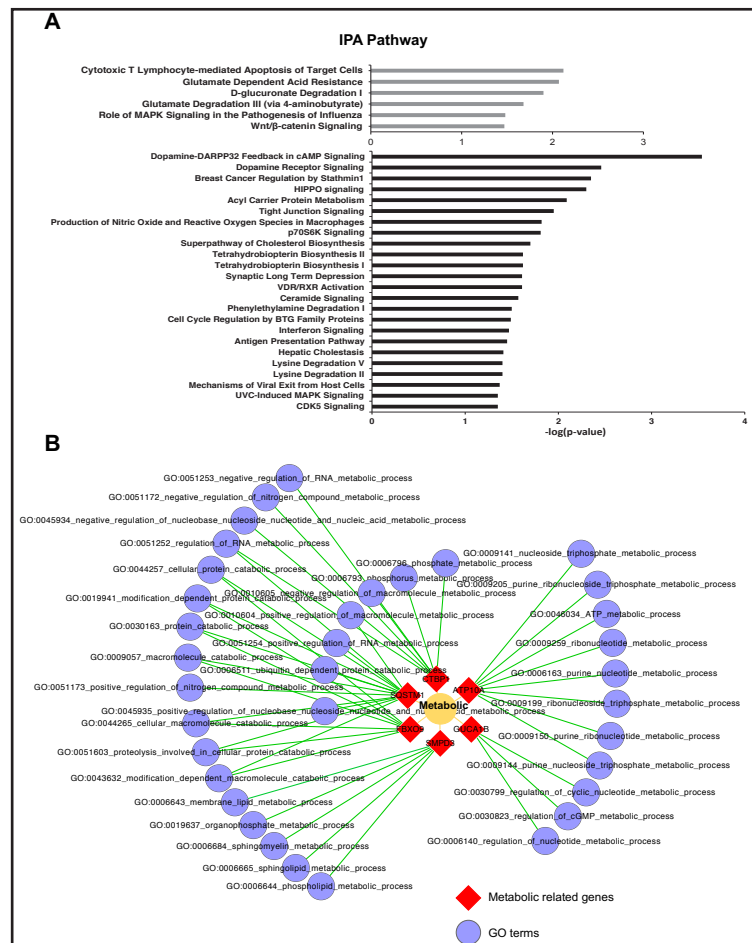
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Yao et al.: DNA Methylation Profiles of PC

mellitus, and other metabolic diseases. These results further support the existing research on molecular biological characteristics of PC. We also provide a possible approach for identifying metabolic disease-susceptible individuals in a healthy population, indicating the objective existence of the subtypes of the healthy population defined by TCM constitution. Apart from the influence of epigenetic mechanisms in the pathogenesis of multiple metabolic disorders [46-49], data from this study suggested that DNA methylation might also play a potential role in the pathological mechanism of PC, which is the incubating stage of multiple metabolic diseases.

Acknowledgements

Disclosure Statement

The authors declare no conflicts of interest.

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