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Pathways Involved in *Sasang* Constitution from Genome-Wide Analysis in a Korean Population

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Abstract

Objective: *Sasang* constitution (SC) medicine, a branch of Korean traditional medicine, classifies the individual into one of four constitutional types (*Taeum*, TE; *Soeum*, SE; *Soyang*, SY; and *Taeyang*, TY) based on physiologic characteristics. The authors of the current article recently reported individual genetic elements associated with SC types via genome-wide association (GWA) analysis. However, to understand the biologic mechanisms underlying constitution, a comprehensive approach that combines individual genetic effects was applied.
Design: Genotypes of 1222 subjects of defined constitution types were measured for 341,998 genetic loci across the entire genome. The biologic pathways associated with SC types were identified via GWA analysis using three different algorithms—namely, the Z-static method, a restandardized gene set assay, and a gene set enrichment assay.
Results: Distinct pathways were associated ($p < 0.05$) with each constitution type. The TE type was significantly associated with cytoskeleton-related pathways. The SE type was significantly associated with cardio- and amino-acid metabolism–related pathways. The SY type was associated with enriched melanoma-related pathways. TY subjects were excluded because of the small size of that sample. Among these functionally related pathways, core-node genes regulating multiple pathways were identified. *TJP1, PTK2*, and *SRC* were selected as core-nodes for TE; *RHOA*, and *MAOA/MAOB* for SE; and *GNAO1* for SY ($p < 0.05$), respectively.
Conclusions: The current authors systematically identified the biologic pathways and core-node genes associated with SC types from the GWA study; this information should provide insights regarding the molecular mechanisms inherent in constitutional pathophysiology.

Introduction

Sasang constitution (SC) medicine is a branch of Korean traditional medicine in which an individual is classified into one of four constitutional types (*Taeum*, TE; *Soeum*, SE; *Soyang*, SY; and *Taeyang*, TY) based on the nature of his/her physiologic and physical characteristics.[1,2] Specifically, the balance among the physiologic functions of four representative internal organs—the Lung, Spleen, Liver, and Kidney (which represent the respiratory, digestive, preservative, and excretory functions, respectively)—is the most important factor for determining SC type. The balance among these organs determines the physiologic characteristics of SC types.[3] For example, the TY type is associated with the developed nape of the neck and slender waist, the TE type is associated with large body and waist size, the SY type is associated with developed chest and small hip, and the SE type is associated with the hip and a weak chest.[2,3] Recent genetic studies also found that polymorphisms of some genes were associated with SC types. For example, *FTO* and *MC4R* polymorphisms are associated with control of body mass, according to SC type.[4] Interleukin-1α and -β polymorphisms were also associated with the SC type in obese women.[5,6] These results support the notion of genetic involvement in determining constitution type.[3–6]

Considering that constitution is a concept that encompasses a diverse set of physiologic characteristics, such a single-gene approach is not sufficient to delineate the complex nature of the physiology underlying constitution. Therefore, Yin and colleagues reported previously that diverse genetic loci are associated with SC types in a genome-wide association (GWA) study of 60 subjects.[7] Recently, the current authors greatly expanded the number of subjects to 1222 to identify genetic elements associated with SC.[8] In that report, single-nucleotide polymorphisms (SNPs) associated with each constitution type were identified, and the relevant

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biologic functions were measured on the basis of these selected SNPs, but not on the whole genome level. Therefore, in this study, the current authors identified the biologic pathways involved in SC types, using a GWA study on the whole genome level. This pathway-based approach could provide novel insights into the molecular delineation of SC.

Materials and Methods

Subjects and diagnosis of constitution type

Peripheral blood samples were collected from a total of 1348 subjects who visited Korean Oriental hospitals between 2006 and 2009. All subjects provided informed consent to the use of their blood samples and clinicopathologic data for research purposes. All experiments were approved by the ethics committee of the Korea Institute of Oriental Medicine (KIOM) and conducted in accordance with the Institutional Guidelines for Human Experimentation. All samples and clinical information were deposited in the *Sasang* Constitutional Medicine databank (DB-SCM) in the KIOM.

The SC type of an individual was determined via three procedures. First, prediagnosis of the SC type of each individual was conducted based on the physical body shape, appearance, temperament, and pathologic symptoms of the individual by a licensed medical specialist who had been in clinical practice in SC medicine for at least 5 years. Second, subjects who were constitutionally prediagnosed were treated with constitution-specific herbal formulae, including *Panax ginseng, Ephedra herba, Schisandra chinensis,* etc., according to the individual's constitution.[4,9,10] After taking the medicine for 30 days or more, any reductions in preexisting symptoms or occurrence of adverse effects (AEs) were recorded. It has been reported that prescriptions that were not appropriate for the SC type induced adverse reactions, such as indigestion, stomach pain, and evacuation troubles.[11] Finally, the SC type of individual was determined by a review of at least 3 specialists in SC medicine. Therefore, subjects included only if they had good medication responses, showing clear reductions in their chief complaints and ordinary symptoms without any adverse effects. Among 1348 subjects, 16 subjects diagnosed with an obscure constitution type were excluded from the analysis.

Genotyping and quality control

The genotypes of genomic DNA isolated from the subjects' peripheral blood were determined using an Affymetrix Genome Wide Human SNP array 5.0, as previously described.[8,9] From a total of 440,092 genotypes of SNPs (>95% call rate), the current authors discarded 12,039 markers following a Hardy-Weinberg equilibrium test ($p<0.001$) and 86,324 markers following minor allele frequency (MAF; $p<0.01$), which left 341,998 SNPs for subsequent analysis. Eighty-three (83) subjects with a high degree of similarity among genotypes (>0.8 of identity by state [IBS] index), and 27 subjects with heterogeneous genotypes (>0.05 on a multidimensional scaling [MDS] index) were excluded, leaving a final total of 1222 samples.

Association analysis

Differences in allele frequencies of each SNP among each constitution type (case) and other constitution types (control) were measured via a χ^2 test using PLINK,[12] and the list of SNPs significantly associated with each constitution type was provided in the authors' previous report.[8]

Pathway analysis

Three different algorithms were used—the *Z*-statistic; a restandardized gene set assay (GSA); and a gene set enrichment assay (GSEA). These were incorporated into GSA-SNP software to evaluate the pathways from GWA data.[13] Initially, each SNP was assigned to a gene whose extent encompassed the SNP within a range of ±20 kb in the neighborhood of each gene. In the *Z*-statistic algorithm, each gene set (GS) was assigned to the *Z*-statistic, which was defined as:

$$(\overline{X} - m)/(\sigma/\sqrt{n}),$$

in which $\overline{X}$ is the average of the gene scores, $-\log(k\text{-th best } p\text{-value})$ in a gene set, m and σ are the mean and the standard deviation (SD) of all the gene scores, and n is the number of genes in the gene set. The k-th best p-value means the k-th smallest value of the association p-values from GWA analysis. The present study used a k number of 2. The current authors also considered two sample permutation-based methods, which also incorporated into GSA-SNP. One of these is the recently developed restandardizated GSA and the other is a modified version of the GSEA. Both algorithms use the maxmean statistic (nonnegative mean) rather than the Kolmogorov-Smirnov statistic originally used in GSEA to summarize gene sets. Integrative analysis of pathways was carried out using KEGGgraph—specifically, the R package for KEGG pathway analysis, which uses a graph-theoretical model to dissect graphs.[14] This program parses KEGG XML files into a graph model. The selected subgraph of nodes and the edges were merged into a new graph and visualized. The most important nodes were computed and visualized by measuring the centrality in which the number of outgoing edges reflects the regulatory role, and the number of "ingoing" edges reflects the degree to which the protein is subject to intermolecular regulation.

Dataset

The KEGG pathway database (www.genome.jp/kegg/) was used. It is provided as a curated .gmt file format in MSigDB (www.broadinstitute.org/gsea/msigdb/). SNP data were obtained from dbSNP (www.ncbi.nlm.nih.gov/snp).

Statistical analysis

All statistical analyses, including an analysis of variance (ANOVA), were performed using R software (version 2.12.0). p-Values of <0.05 were considered statistically significant.

Results

Subjects

The 1222 subjects were ultimately included in the present study after careful examination of the quality of the genotypes of individual subjects. The clinical distribution and

pathophysiologic association of SC types reported previously[8] are provided in Table 1. Five hundred and twelve (512) subjects were classified into TE, 302 into SE, 389 into SY, and 19 into TY. The TE type showed significant increases in body weight, total cholesterol level, and low-density lipoprotein level (LDL) in both genders. The TY type was excluded for the following analysis because of the small sample size of TY subjects.

Identification of pathways involved in SC types: One-versus-all

Three different algorithms were used—namely the Z-static, a restandardized GSA, and a GSEA method, all incorporated into GSA-SNP software[13]—to evaluate the pathways involved in SC from a GWA study of the 1222 subjects. The detailed list of SNPs associated with constitution types and clinical information regarding constitution types was previously reported.[8]

Initially, the pathways involved with each constitution type were identified by comparing one constitution type with the others, via a one-versus-all approach. Some significant pathways involved in each type of constitution are listed in Table 2 ($p<0.05$ for all algorithms). Pathways involved in different cellular functions were enriched in each constitution type. For example, the biosynthesis of unsaturated fatty acids, melanoma, and cardiac-muscle contraction–pathway were associated with the TE type. While pathways including phenylalanine metabolism, viral myocarditis, and tryptophan metabolism were associated with the SE type, long-term depression, arrhythmogenic right ventricular cardiomyopathy, and regulation of the actin cytoskeleton pathway were associated with the SY type. Table 2 shows that some pathways were commonly enriched across constitution types. A Venn diagram was constructed to illustrate the numbers of commonly significant pathways ($p<0.05$ for all algorithms) among constitution types (Fig. 1A). Table 2 shows that the cardiac-muscle contraction pathway was associated with all three constitution types. Axon guidance, adherens junction, and hypertrophic cardiomyopathy pathways were associated with the TE and SE types. Melanoma and regulation of the actin cytoskeleton pathway were associated with the TE and SY types, whereas tryptophan metabolism was associated with the SE and SY types.

Identification of pathways involved in SC types: One-versus-one

In addition to the one-versus-all approach adopted herein, the current authors also measured the pathways associated with constitution types via a one-versus-one approach. Some significant pathways were enriched differently between two types of constitution—namely, TE versus SE, SE versus SY, and TE versus SY (Table 3). The Venn diagram for significant pathways shown in Figure 1B also confirmed the commonly identified pathways. Axon guidance, adherens junction, regulation of actin cytoskeleton, and the focal adhesion pathway were involved in all combinations of constitution type. Cardiac-muscle contraction, hypertrophic cardiomyopathy, and cell-adhesion molecule pathways were associated with both TE-SE and SE-SY combinations. While long-term depression, tight junction, and Fcε RI signaling

Table 1. Clinical Implications of *Sasang* Constitution Types[a]

	Male					*Female*				
Variable	*TE* (n=239)	*SE* (n=103)	*TY* (n=8)	*SY* (n=130)	p[b]	*TE* (n=273)	*SE* (n=199)	*TY* (n=11)	*SY* (n=259)	p[b]
Age (year)	46.1±15.4	41.5±16.5	51.7±14.5	48.1±16.0	0.0094	50.3±14.7	43.9±14.8	41.7±10.4	45.1±14.5	<0.0001
Height (cm)	169.9±7.1	169.6±7.6	169.5±4.4	168.6±5.8	0.39	157.0±5.6	158.3±6.0	161.7±3.3	157.3±6.0	0.0093
Weight (kg)	73.7±11.0	61.7±8.7	66.6±8.7	65.9±8.2	<0.0001	62.1±9.4	52.7±6.5	56.1±9.6	55.1±6.9	<0.0001
Aspartate aminotransferase (IU/L)	25.2±8.2	24.5±11.9	18.6±3.0	23.5±8.3	0.12	23.7±14.6	20.7±5.5	23.1±10.4	21.7±8.5	0.022
Alanine aminotransferase (IU/L)	29.3±14.7	23.8±23.2	16.4±6.0	25.9±15.7	0.011	22.7±25.8	16.8±7.8	20.8±16.2	19.9±19.0	0.018
Alkaline phosphatase (IU/L)	70.6±43.5	75.4±54.1	67.1±9.4	72.1±41.8	0.83	62.7±27.6	58.4±22.2	59.0±19.5	58.9±23.8	0.21
Total cholesterol (mg/dL)	192.0±37.2	177.9±32.1	182.9±38.9	184.3±31.5	0.0061	194.9±37.87	181.5±33.3	173.5±28.2	188.5±33.5	0.00042
Triglycerides (mg/dL)	162.2±115.5	113.7±65.0	138.8±52.4	166.4±111.9	0.00043	132.7±83.5	91.3±46.0	106.9±62.1	109.3±62.9	<0.0001
High-density lipoprotein (mg/dL)	39.8±9.4	43.8±9.1	36.8±6.5	40.6±10.6	0.0034	44.9±11.3	51.0±12.1	45.5±9.8	48.6±12.0	<0.0001
Low-density lipoprotein (mg/dL)	111.9±31.6	100.3±26.1	109.5±30.4	103.1±27.1	0.0030	112.3±32.4	98.7±28.7	96.0±21.0	105.6±30.5	<0.0001
Bilirubin (mg/dL)	0.82±0.29	0.86±0.42	0.70±0.24	0.82±0.37	0.51	0.70±0.25	0.74±0.27	0.84±0.31	0.78±0.34	0.024
Blood urea nitrogen (mg/dL)	15.4±4.0	14.5±3.7	16.0±4.9	14.9±4.3	0.20	14.2±4.0	13.5±3.8	13.3±4.0	13.6±3.4	0.15

[a]This table was previously reported.[8]
[b]Significance was measured by analysis of variance.
TE, *Taeum*; SE, *Soeum*; SY, *Soyang*; TY, *Taeyang*, IU, international units.

Table 2. Pathways Involved in Each Type of *Sasang* Constitution: One-Versus-All Approach

Constitution	KEGG ID	Pathway	Gene count[a]	Pathway size[b]	Z-score		Restandization		GSEA	
					p-Value	FDR	p-Value	FDR	p-Value	FDR
TE	*hsa01040*	Biosynthesis of unsaturated fatty acids	20	28	1.83×10^{-2}	1.83×10^{-1}	1.46×10^{-3}	1.48×10^{-3}	1.30×10^{-2}	4.97×10^{-1}
	hsa05218	Melanoma	65	77	1.94×10^{-2}	1.83×10^{-1}	3.77×10^{-3}	4.35×10^{-3}	5.08×10^{-3}	5.13×10^{-1}
	hsa04260	Cardiac muscle contraction	64	86	7.50×10^{-3}	9.00×10^{-2}	3.09×10^{-3}	3.41×10^{-3}	5.13×10^{-3}	4.29×10^{-1}
	hsa04810	Regulation of actin cytoskeleton	189	222	1.12×10^{-3}	1.84×10^{-2}	4.37×10^{-3}	5.42×10^{-3}	1.29×10^{-3}	0.00
	hsa04530	Tight junction	119	140	4.99×10^{-4}	9.98×10^{-3}	3.11×10^{-3}	3.45×10^{-3}	6.72×10^{-3}	4.40×10^{-1}
	hsa04360	Axon guidance	118	135	1.37×10^{-8}	2.48×10^{-6}	1.25×10^{-3}	1.26×10^{-3}	3.24×10^{-3}	4.56×10^{-1}
	hsa04510	Focal adhesion	181	207	7.30×10^{-7}	4.38×10^{-5}	2.51×10^{-3}	2.69×10^{-3}	3.13×10^{-3}	5.86×10^{-1}
	hsa04520	Adherens junction	71	81	3.34×10^{-5}	1.20×10^{-3}	1.46×10^{-3}	1.49×10^{-3}	2.06×10^{-2}	5.88×10^{-1}
	hsa05410	Hypertrophic cardiomyopathy	79	91	1.15×10^{-5}	5.20×10^{-4}	1.44×10^{-3}	1.6×10^{-3}	2.24×10^{-2}	5.40×10^{-1}
	hsa04960	Aldosterone-regulated sodium reabsorption	36	78	2.75×10^{-2}	2.36×10^{-1}	2.90×10^{-3}	3.17×10^{-3}	4.51×10^{-2}	7.40×10^{-1}
SE	*hsa00360*	Phenylalanine metabolism	17	24	8.63×10^{-4}	1.55×10^{-2}	1.05×10^{-3}	1.05×10^{-3}	7.10×10^{-6}	0.00
	hsa05416	Viral myocarditis	67	79	4.78×10^{-4}	9.56×10^{-3}	3.20×10^{-3}	3.33×10^{-3}	6.80×10^{-4}	2.30×10^{-1}
	hsa04260	Cardiac muscle contraction	64	86	1.36×10^{-3}	2.05×10^{-2}	3.48×10^{-3}	3.70×10^{-3}	2.33×10^{-4}	2.53×10^{-1}
	hsa00380	Tryptophan metabolism	36	46	1.13×10^{-2}	1.01×10^{-1}	3.46×10^{-3}	3.66×10^{-3}	1.97×10^{-3}	2.53×10^{-1}
	hsa04514	Cell-adhesion molecules	125	140	8.69×10^{-5}	3.11×10^{-3}	4.09×10^{-3}	4.49×10^{-3}	1.18×10^{-2}	3.46×10^{-1}
	hsa04360	Axon guidance	118	135	7.62×10^{-6}	6.86×10^{-4}	3.24×10^{-3}	3.39×10^{-3}	3.79×10^{-2}	4.43×10^{-1}
	hsa05410	Hypertrophic cardiomyopathy	79	91	4.82×10^{-5}	2.89×10^{-3}	2.83×10^{-3}	2.91×10^{-3}	4.10×10^{-2}	4.17×10^{-1}
	hsa04520	Adherens junction	71	81	5.18×10^{-5}	2.33×10^{-3}	2.61×10^{-3}	2.67×10^{-3}	2.29×10^{-2}	3.91×10^{-1}
	hsa04720	Long-term potentiation	64	76	9.45×10^{-5}	2.83×10^{-3}	2.48×10^{-3}	2.52×10^{-3}	1.11×10^{-2}	3.52×10^{-1}
	hsa04270	Vascular smooth-muscle contraction	107	121	1.82×10^{-4}	4.70×10^{-3}	3.94×10^{-3}	4.27×10^{-3}	2.00×10^{-2}	4.08×10^{-1}
	hsa04930	Type II diabetes mellitus	41	53	9.86×10^{-3}	9.86×10^{-2}	3.61×10^{-3}	3.86×10^{-3}	4.17×10^{-2}	4.11×10^{-1}
	hsa05140	Leishmaniasis	57	78	2.14×10^{-2}	1.60×10^{-1}	5.51×10^{-3}	6.49×10^{-3}	1.16×10^{-3}	2.52×10^{-1}
	hsa05332	Graft-versus-host disease	35	48	2.32×10^{-2}	1.67×10^{-1}	4.09×10^{-3}	4.54×10^{-3}	4.67×10^{-3}	2.76×10^{-1}
	hsa05216	Thyroid cancer	27	35	2.33×10^{-2}	1.61×10^{-1}	3.44×10^{-3}	3.62×10^{-3}	7.65×10^{-3}	3.09×10^{-1}
	hsa04144	Endocytosis	159	189	3.28×10^{-2}	2.11×10^{-1}	9.50×10^{-3}	1.30×10^{-2}	1.91×10^{-4}	4.46×10^{-1}
	hsa00340	Histidine metabolism	28	35	3.97×10^{-2}	2.38×10^{-1}	4.14×10^{-3}	4.63×10^{-3}	5.03×10^{-3}	2.62×10^{-1}
	hsa05330	Allograft rejection	34	44	4.56×10^{-2}	2.56×10^{-1}	5.12×10^{-3}	5.91×10^{-3}	3.34×10^{-2}	4.33×10^{-1}
SY	*hsa04730*	Long-term depression	63	76	1.44×10^{-6}	1.30×10^{-4}	1.33×10^{-3}	1.34×10^{-3}	6.73×10^{-3}	1.26×10^{-1}
	hsa05218	Melanoma	65	77	7.82×10^{-3}	6.71×10^{-2}	2.92×10^{-3}	3.33×10^{-3}	8.16×10^{-3}	1.27×10^{-1}
	hsa05412	Arrhythmogenic right-ventricular cardiomyopathy	72	82	5.22×10^{-11}	9.41×10^{-9}	1.03×10^{-3}	1.03×10^{-3}	1.10×10^{-2}	1.47×10^{-1}
	hsa04810	Regulation of actin cytoskeleton	189	222	4.82×10^{-4}	7.88×10^{-3}	3.61×10^{-3}	4.48×10^{-3}	1.29×10^{-2}	1.51×10^{-1}
	hsa04916	Melanogenesis	93	108	7.61×10^{-3}	6.85×10^{-2}	3.51×10^{-3}	4.33×10^{-3}	3.02×10^{-2}	2.19×10^{-1}
	hsa00380	Tryptophan metabolism	36	46	4.14×10^{-3}	4.66×10^{-2}	2.09×10^{-3}	2.18×10^{-3}	3.99×10^{-3}	1.07×10^{-1}
	hsa05215	Prostate cancer	81	95	4.26×10^{-3}	4.51×10^{-2}	3.01×10^{-3}	3.48×10^{-3}	4.15×10^{-3}	1.07×10^{-1}
	hsa04742	Taste transduction	44	58	1.20×10^{-2}	8.36×10^{-2}	2.59×10^{-3}	2.84×10^{-3}	3.04×10^{-2}	2.19×10^{-1}
	hsa04260	Cardiac-muscle contraction	64	86	1.08×10^{-2}	7.81×10^{-2}	3.09×10^{-3}	3.59×10^{-3}	2.67×10^{-2}	2.19×10^{-1}
	hsa04722	Neurotrophin-signaling pathway	118	132	2.87×10^{-2}	1.66×10^{-1}	4.59×10^{-3}	6.12×10^{-3}	2.62×10^{-2}	2.19×10^{-1}

[a]Number of genes analyzed in each pathway.
[b]Number of total genes in each pathway.
FDR, false discovery rate; TE, *Taeum*; SE, *Soeum*; SY, *Soyang*.

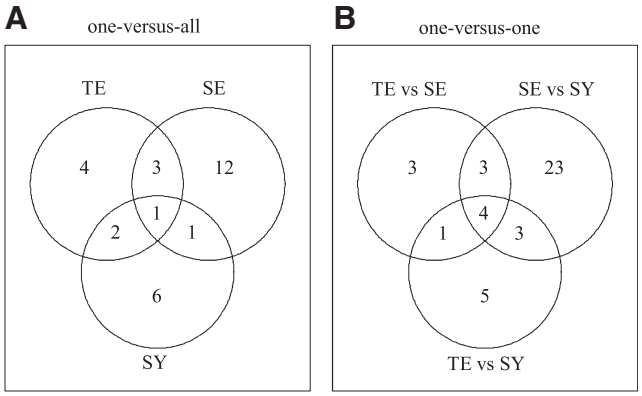

FIG. 1. Venn-diagram illustration of number of common significant pathways among constitution types. **(A)** Pathways were selected as significant ($p < 0.05$ for all algorithms; *Z*-statistic, restandardized gene set assay, and gene set enrichment assay) via comparison of one constitution type with others. **(B)** Pathways were selected as significant ($p < 0.05$ for all algorithms) between any two types of constitution, namely, TE-versus-SE, SE-versus-SY, and TE-versus-SY. TE, *Taeum*; SE, *Soeum*; SY, *Soyang*.

pathways were involved in both SE–SY and TE–SY type combinations, aldosterone-regulated sodium reabsorption pathways were associated with both TE–SE and TE–SY combinations.

Distribution of SNPs in pathways

Tables 2 and 3 show that many pathways were identified as commonly significant using both approaches; namely, one-versus-all and one-versus-one approaches. Moreover, some pathways were commonly selected among three constitutional types, as shown in Fig. 1, thereby indicating that these pathways could be critical for discriminating among constitution types. The distribution of all commonly significant pathways is addressed in Table 4. The current authors measured the distribution of *p*-values of SNPs included in some commonly significant pathways in all or two constitution types (Fig. 2); specifically, the cardiac-muscle contraction, adherens junction, actin cytoskeleton regulation, and tryptophan metabolism pathways. From the distribution of the *p*-values of the most significant 100 SNPs in a pathway, at least one constitution type had a different *p*-value distribution, compared with those of other constitution types. These results indicated that these pathways were more significantly enriched in one type of constitution than in other types.

Identification of core elements in pathways

Interestingly, among the significant pathways, functionally related pathways were enriched in each constitution type, as shown in Tables 2 and 3. For example, cytoskeleton-related pathways—such as the focal adhesion, regulation of actin cytoskeleton, tight junction, and adherens junction pathways—were significantly associated with the TE type. Cardio-related pathways, such as hypertrophic cardiomyopathy, vascular smooth-muscle contraction, and cardiac-muscle contraction pathways, were enriched in the SE type. In addition, amino-acid metabolism pathways—including phenylalanine, tryptophan, and histidine metabolism pathways—were also selected in the SE type, whereas melanoma-related pathways—including the melanoma pathway, melanogenesis pathway, and neurotrophin-signaling pathway—were associated with the SY type. The presence of functionally related pathways in each constitution type indicates a possible implication of common regulators acting on multiple pathways. From the network analysis of functionally related pathways, the current authors identified *CTNNB1, PTK2, ACTB, SRC*, and *TJP1* as the most important nodes in cytoskeleton-related pathways for the TE type (Fig. 3). In the SE type, *GNA12, GNA13*, *RHOA, ROCK1,* and *ROCK2* were identified as central nodes in the cardio-related pathway and *DDC, IDO1, IDO2, MAOA*, and *MAOB* were central nodes in the in the amino-acid metabolism pathways. However, *GNAO1, GSK3B, HRAS, NRAS*, and *NGFR* were selected in melanoma-related pathways for the SY type. Finally, the current authors assessed if these identified core-node genes were associated with each constitution type. Table 5 shows the constitution-associated SNPs ($p < 0.05$) located within the region encompassing ± 10 kb in either direction from the core-node genes. *TJP1* (*rs11073279*), *PTK2* (*rs3639* and *rs11991796*), and SRC (*rs747182*) were associated with the TE type. *RHOA* (*rs6997*) and *MAOA/MAOB* (*rs6609257* and *rs3859959*) were associated with the SE type. *GNAO1* (*rs9927506*) was associated with the SY type. However, the roles of these constitution-associated core-node genes should be investigated in further detail at the molecular level to delineate the mechanism underlying the determination of constitution type.

Discussion

Since the completion of the Human Genome Project, modern medicine has focused on searching for the genetic elements relevant to the differences between individuals.[15] Despite the rapid progress made in recent years, most of this research is still conducted at the laboratory level. An alternative and practical approach to laboratory research would involve subgrouping human populations according to their homogeneous biologic characteristics. The classification of human populations based on individual constitution is a common feature of many traditional medicines, including Traditional Chinese Medicine and Ayurveda, an ancient Indian system of personalized medicine.[16–18] In SC medicine, an individual is classified into one of four types based on physiologic characteristics.[3] As shown in Supplementary Table S1 (online only at: www.liebertpub.com/acm), physical, psychologic, and clinical features differed among SC types.[2,3,8] Although, these physiologic traits of an individual were measured to diagnose constitution type in the present study, as previously described,[8,9] it will be necessary to apply more-objective criteria for determinations of constitution type. Therefore, the current authors have struggled to develop a diagnostic tool, using only objective physical measurements, such as facial features or body mass index to exclude subjective judgment.[19,20]

Considering the complex nature of the constitution, the whole-genome approach rather than the single-gene approach should prove to be an effective method for establishing a genetic basis for constitution.[7,9] For example, genes and molecular functions correlated with the phenotypic class

Table 3. Pathways Involved in Each Type of *Sasang* Constitution: One-Versus-One Approach

Constitution	*KEGG ID*	*Pathway*	*Gene count*[a]	*Pathway size*[b]	*Z-score*		*Restandization*		*GSEA*	
					p-*Value*	*FDR*	p-*Value*	*FDR*	p-*Value*	*FDR*
TE vs. SE	*hsa00360*	Phenylalanine metabolism	17	24	6.34×10^{-3}	7.61×10^{-2}	1.74×10^{-3}	1.74×10^{-3}	1.44×10^{-4}	0.00
	hsa04260	Cardiac muscle contraction	64	86	5.24×10^{-3}	6.74×10^{-2}	4.51×10^{-3}	4.84×10^{-3}	1.30×10^{-3}	1.25×10^{-2}
	hsa05416	Viral myocarditis	67	79	3.79×10^{-3}	5.26×10^{-2}	4.31×10^{-3}	4.59×10^{-3}	5.00×10^{-3}	1.61×10^{-2}
	hsa04930	Type II diabetes mellitus	41	53	7.80×10^{-3}	8.77×10^{-2}	3.48×10^{-3}	3.62×10^{-3}	2.60×10^{-2}	2.95×10^{-2}
	hsa04360	Axon guidance	118	135	1.88×10^{-6}	3.39×10^{-4}	2.98×10^{-3}	3.04×10^{-3}	1.06×10^{-2}	5.36×10^{-1}
	hsa04960	Aldosterone-regulated sodium reabsorption	36	48	3.67×10^{-2}	2.75×10^{-1}	5.05×10^{-3}	5.61×10^{-3}	4.44×10^{-2}	5.20×10^{-1}
	hsa04520	Adherens junction	71	81	8.29×10^{-5}	3.73×10^{-3}	2.75×10^{-3}	2.79×10^{-3}	2.60×10^{-2}	5.03×10^{-1}
	hsa05410	Hypertrophic cardiomyopathy	79	91	4.73×10^{-5}	2.84×10^{-3}	2.87×10^{-3}	2.91×10^{-3}	3.27×10^{-2}	4.83×10^{-1}
	hsa04514	Cell adhesion molecules (CAMs)	125	140	2.79×10^{-4}	8.37×10^{-3}	4.72×10^{-3}	5.12×10^{-3}	2.22×10^{-2}	5.98×10^{-1}
	hsa04810	Regulation of actin cytoskeleton	189	222	2.61×10^{-2}	2.23×10^{-1}	9.83×10^{-3}	1.34×10^{-2}	2.65×10^{-2}	4.65×10^{-1}
	hsa04510	Focal adhesion	181	207	2.04×10^{-4}	7.36×10^{-3}	5.75×10^{-3}	6.68×10^{-3}	4.98×10^{-2}	5.14×10^{-1}
SE vs. SY	*hsa05211*	Renal cell carcinoma	67	76	1.03×10^{-2}	5.99×10^{-2}	2.11×10^{-3}	2.56×10^{-3}	3.54×10–5	3.18×10^{-3}
	hsa04360	Axon guidance	118	135	4.45×10^{-10}	8.01×10^{-8}	1.03×10^{-3}	1.05×10^{-3}	2.07×10–5	3.18×10^{-3}
	hsa04012	ErbB signaling pathway	80	93	1.17×10^{-4}	1.75×10^{-3}	1.35×10^{-3}	1.48×10^{-3}	1.84×10^{-4}	1.10×10^{-2}
	hsa05223	Non-small cell lung cancer	50	60	3.90×10^{-3}	3.34×10^{-2}	1.42×10^{-3}	1.60×10^{-3}	1.37×10^{-3}	2.75×10^{-2}
	hsa00532	Glycosaminoglycan biosynthesis-chondroitin sulfate	19	28	1.28×10^{-3}	1.45×10^{-2}	6.48×10^{-4}	6.51×10^{-4}	1.23×10^{-2}	7.16×10^{-2}
	hsa04662	B cell receptor signaling pathway	67	81	9.98×10^{-3}	6.19×10^{-2}	2.11×10^{-3}	2.55×10^{-3}	2.14×10^{-3}	2.24×10^{-1}
	hsa05214	Glioma	58	71	2.95×10^{-2}	1.47×10^{-1}	2.26×10^{-3}	2.86×10^{-3}	3.88×10^{-3}	2.45×10^{-1}
	hsa04730	Long-term depression	63	76	2.41×10^{-5}	5.42×10^{-4}	1.19×10^{-3}	1.25×10^{-3}	4.76×10^{-3}	2.29×10^{-1}
	hsa04722	Neurotrophin signaling pathway	118	132	3.23×10^{-2}	1.57×10^{-1}	2.81×10^{-3}	4.15×10^{-3}	5.88×10^{-4}	2.21×10^{-1}
	hsa05410	Hypertrophic cardiomyopathy	79	91	2.43×10^{-6}	8.76×10^{-5}	1.17×10^{-3}	1.22×10^{-3}	3.12×10^{-3}	2.53×10^{-1}
	hsa04320	Dorso-ventral axis formation	23	31	6.51×10^{-3}	4.50×10^{-2}	1.15×10^{-3}	1.19×10^{-3}	2.03×10^{-2}	2.73×10^{-1}
	hsa00500	Starch and sucrose metabolism	47	58	2.37×10^{-3}	2.24×10^{-2}	1.35×10^{-3}	1.47×10^{-3}	3.18×10^{-2}	2.85×10^{-1}

(*continued*)

Table 3. (Continued)

Constitution	KEGG ID	Pathway	Gene count[a]	Pathway size[b]	Z-score		Restandization		GSEA	
					p-Value	FDR	p-Value	FDR	p-Value	FDR
	hsa04020	Calcium signaling pathway	167	184	8.29×10^{-8}	7.46×10^{-6}	1.39×10^{-3}	1.53×10^{-3}	4.45×10^{-4}	2.87×10^{-1}
	hsa00983	Drug metabolism - other enzymes	44	57	2.87×10^{-3}	2.58×10^{-2}	1.33×10^{-3}	1.45×10^{-3}	3.82×10^{-2}	2.91×10^{-1}
	hsa04512	ECM-receptor interaction	80	90	6.06×10^{-7}	2.73×10^{-5}	1.09×10^{-3}	1.12×10^{-3}	3.81×10^{-3}	2.64×10^{-1}
	hsa04520	Adherens junction	71	81	1.82×10^{-5}	4.68×10^{-4}	1.20×10^{-3}	1.27×10^{-3}	4.63×10^{-3}	2.41×10^{-1}
	hsa00860	Porphyrin and chlorophyll metabolism	37	47	1.30×10^{-2}	7.32×10^{-2}	1.54×10^{-3}	1.75×10^{-3}	4.94×10^{-2}	2.93×10^{-1}
	hsa00140	Steroid hormone biosynthesis	50	61	4.32×10^{-3}	3.38×10^{-2}	1.48×10^{-3}	1.68×10^{-3}	4.18×10^{-2}	2.88×10^{-1}
	hsa04062	Chemokine signaling pathway	171	196	7.25×10^{-3}	4.83×10^{-2}	2.70×10^{-3}	3.08×10^{-3}	1.09×10^{-3}	2.61×10^{-1}
	hsa04666	Fc gamma R-mediated phagocytosis	81	103	1.00×10^{-2}	6.00×10^{-2}	2.20×10^{-3}	2.77×10^{-3}	5.59×10^{-3}	2.29×10^{-1}
	hsa00640	Propanoate metabolism	32	39	3.46×10^{-2}	1.64×10^{-1}	1.89×10^{-3}	2.19×10^{-3}	2.93×10^{-2}	2.79×10^{-1}
	hsa04540	Gap junction	77	96	2.05×10^{-4}	2.84×10^{-3}	1.41×10^{-3}	1.57×10^{-3}	7.05×10^{-3}	2.16×10^{-1}
	hsa04940	Type I diabetes mellitus	39	50	2.41×10^{-2}	1.31×10^{-1}	1.93×10^{-3}	2.25×10^{-3}	3.81×10^{-2}	2.95×10^{-1}
	hsa04260	Cardiac muscle contraction	64	86	3.94×10^{-2}	1.77×10^{-1}	2.44×10^{-3}	3.24×10^{-3}	1.13×10^{-2}	2.39×10^{-1}
	hsa04530	Tight junction	119	140	1.80×10^{-3}	1.90×10^{-2}	2.19×10^{-3}	2.69×10^{-3}	5.61×10^{-3}	2.17×10^{-1}
SY vs. TE	hsa03450	Nonhomologous end-joining	13	20	1.52×10^{-2}	1.31×10^{-1}	9.07×10^{-4}	9.12×10^{-4}	6.52×10^{-3}	2.34×10^{-1}
	hsa05218	Melanoma	65	77	9.80×10^{-3}	1.10×10^{-1}	2.33×10^{-3}	2.67×10^{-3}	5.48×10^{-3}	2.34×10^{-1}
	hsa04810	Regulation of actin cytoskeleton	189	222	4.48×10^{-4}	7.33×10^{-3}	2.92×10^{-3}	3.44×10^{-3}	3.04×10^{-3}	2.34×10^{-1}
	hsa04730	Long-term depression	63	76	2.62×10^{-6}	1.18×10^{-4}	9.25×10^{-4}	9.36×10^{-4}	8.02×10^{-3}	2.40×10^{-1}
	hsa04530	Tight junction	119	140	2.41×10^{-4}	4.35×10^{-3}	2.05×10^{-3}	2.26×10^{-3}	1.23×10^{-2}	3.03×10^{-1}
	hsa05215	Prostate cancer	81	95	1.51×10^{-2}	1.35×10^{-1}	2.93×10^{-3}	3.46×10^{-3}	1.35×10^{-2}	6.48×10^{-1}
	hsa04960	Aldosterone-regulated sodium reabsorption	36	48	1.10×10^{-2}	1.16×10^{-1}	1.57×10^{-3}	1.69×10^{-3}	3.05×10^{-2}	7.07×10^{-1}
	hsa04520	Adherens junction	71	81	9.81×10^{-6}	3.53×10^{-4}	1.02×10^{-3}	1.04×10^{-3}	2.19×10^{-2}	8.28×10^{-1}
	hsa05412	Arrhythmogenic right ventricular cardiomyopathy	72	82	2.13×10^{-9}	3.84×10^{-7}	6.85×10^{-4}	6.85×10^{-4}	2.61×10^{-2}	8.31×10^{-1}
	hsa00380	Tryptophan metabolism	35	46	3.06×10^{-2}	1.97×10^{-1}	2.11×10^{-3}	2.35×10^{-3}	4.81×10^{-2}	8.24×10^{-1}
	hsa04664	Fc ε RI signaling pathway	74	85	1.12×10^{-2}	1.12×10^{-1}	2.56×10^{-3}	2.97×10^{-3}	3.99×10^{-2}	8.23×10^{-1}
	hsa04510	Focal adhesion	181	207	1.83×10^{-6}	1.10×10^{-4}	1.87×10^{-3}	2.04×10^{-3}	2.67×10^{-2}	6.92×10^{-1}
	hsa04360	Axon guidance	118	135	1.26×10^{-7}	1.13×10^{-5}	1.13×10^{-3}	1.16×10^{-3}	4.33×10^{-2}	8.19×10^{-1}

[a]Number of genes analyzed in each pathway.
[b]Number of total genes in each pathway.
FDR, false discovery rate; TE, *Taeum*; SE, *Soeum*; SY, *Soyang*.

Table 4. Distribution of Common Significant Pathways (*$p < 0.05$ for all Algorithms)

KEGG	Pathway	One-vs.-all			One-vs.-one		
		TE	SE	SY	TE vs. SE	SE vs. SY	TE vs. SY
hsa04520	Adherens junction	*	*		*	*	*
hsa04360	Axon guidance	*	*		*	*	*
hsa04260	Cardiac-muscle contraction	*	*	*	*	*	
hsa04810	Regulation of actin cytoskeleton	*		*	*	*	*
hsa04510	Focal adhesion	*			*	*	*
hsa05410	Hypertrophic cardiomyopathy	*	*		*	*	
hsa04960	Aldosterone-regulated sodium reabsorption	*			*		*
hsa04514	Cell-adhesion molecules		*		*	*	
hsa04730	Long-term depression			*		*	*
hsa05218	Melanoma	*		*			*
hsa04530	Tight junction	*				*	*
hsa00380	Tryptophan metabolism		*	*			*
hsa05412	Arrhythmogenic right-ventricular cardiomyopathy			*			*
hsa04664	Fcε RI signaling pathway					*	*
hsa04720	Long-term potentiation		*			*	
hsa04722	Neurotrophin-signaling pathway			*		*	
hsa00360	Phenylalanine metabolism		*		*		
hsa05215	Prostate cancer			*			*
hsa04930	Type II diabetes mellitus		*		*		
hsa04270	Vascular smooth-muscle contraction		*			*	
hsa05416	Viral myocarditis		*		*		
hsa05330	Allograft rejection		*				
hsa04662	B-cell receptor–signaling pathway					*	
hsa01040	Biosynthesis of unsaturated fatty acids	*					
hsa04020	Calcium signaling pathway					*	
hsa04062	Chemokine signaling pathway					*	
hsa00472	D-Arginine and D-ornithine metabolism					*	
hsa04320	Dorso-ventral axis formation					*	
hsa00983	Drug metabolism—other enzymes					*	
hsa04512	ECM-receptor interaction					*	
hsa04144	Endocytosis		*				
hsa04012	*ErbB* signaling pathway					*	
hsa04666	Fc γ R-mediated phagocytosis					*	
hsa04540	Gap junction					*	
hsa05214	Glioma					*	
hsa00532	Glycosaminoglycan biosynthesis-chondroitin sulfate					*	
hsa05332	Graft-versus-host disease		*				
hsa00340	Histidine metabolism		*				
hsa05140	Leishmaniasis		*				
hsa04916	Melanogenesis			*			
hsa03450	Nonhomologous end-joining						*
hsa05223	Non–small cell lung cancer					*	
hsa00860	Porphyrin and chlorophyll metabolism					*	
hsa00640	Propanoate metabolism					*	
hsa05211	Renal-cell carcinoma					*	
hsa05222	Small-cell lung cancer					*	
hsa00500	Starch and sucrose metabolism					*	
hsa00140	Steroid hormone biosynthesis					*	
hsa04742	Taste transduction			*			
hsa05216	Thyroid cancer		*				
hsa04940	Type I diabetes mellitus					*	

TE, *Taeum*; SE, *Soeum*; SY, *Soyang*.

of constitution were also identified using the whole-genomic approach in Ayurveda.[21] The current authors recently reported SNPs associated with SC types and biologic functions of these associated SNPs by using the same dataset used in the present study.[8] However, only a small number of genes or SNPs via that method were obtained, and, thus, it was difficult to determine the biologic significance of selected elements in the context of the whole-genome level.[22] Therefore, in this study, the current authors used information from whole genome-wide results and pathway information in an effort to elucidate the biologic functions associated with each constitutional type. As shown in Tables 2 and 3, diverse pathways were associated ($p < 0.05$) with each constitutional type. The *p*-value distribution of SNPs included in these

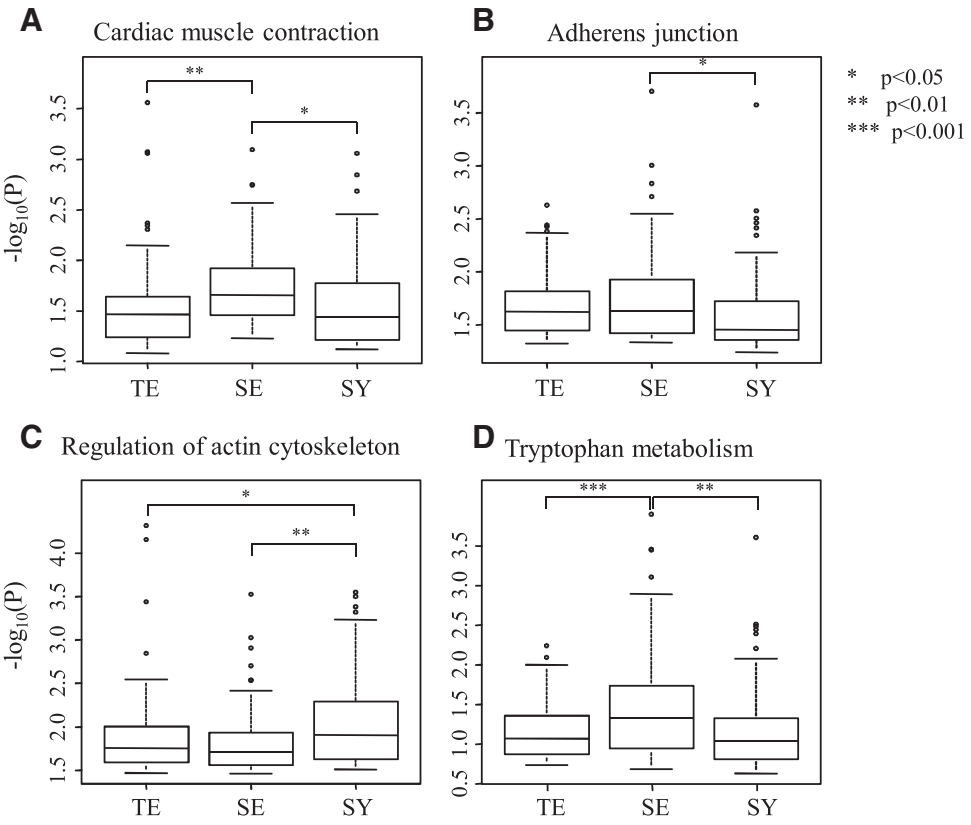

FIG. 2. Distribution of single-nucleotide polymorphisms (SNPs) in common significant pathways among constitution types. In the cardiac muscle contraction pathway **(A)**, adherens junction pathway **(B)**, regulation of actin cytoskeleton pathway **(C)**, and tryptophan metabolism pathway **(D)**, the top 100 significant SNPs were selected from each constitution type. The differences in SNP distributions were measured using Tukey's test after an analysis of variance. TE, *Taeum*; SE, *Soeum*; SY, *Soyang*.

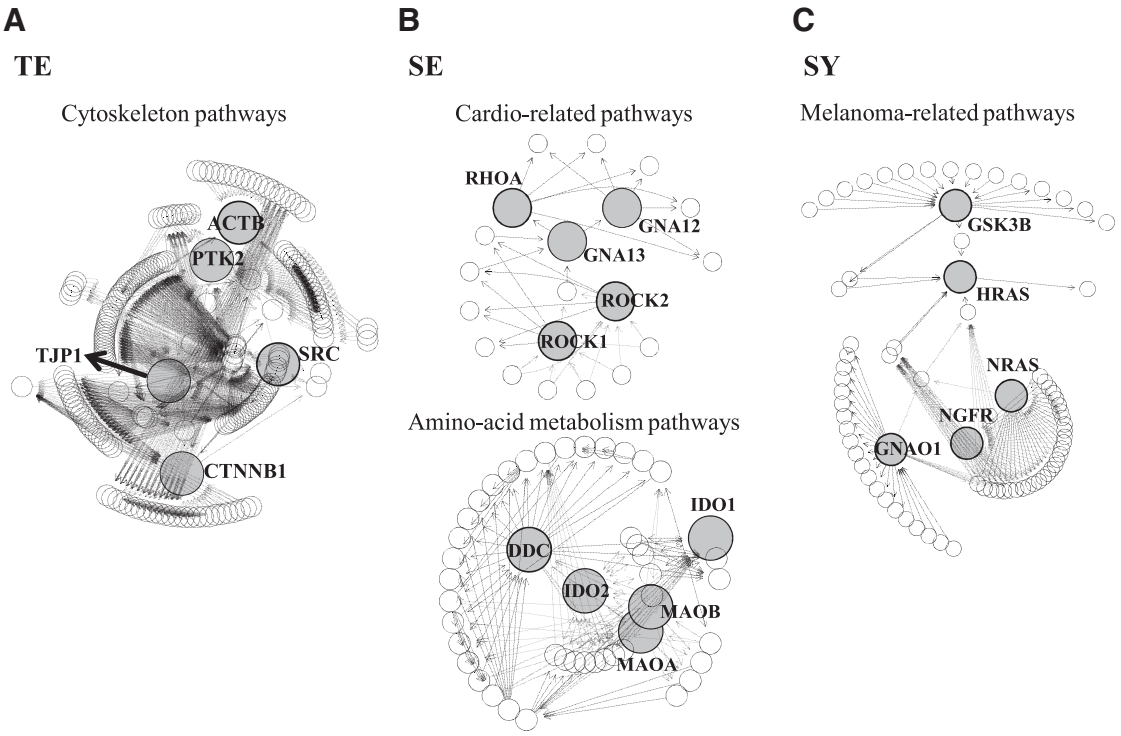

FIG. 3. Network analysis of pathways. Core-node genes were identified from functionally related multipathways. **(A)** For the TE type, cytoskeleton-related pathways, including focal adhesion, regulation of actin cytoskeleton, tight junction, and adherens junction pathways were analyzed. **(B)** For the SE type, cardio-related pathways, including hypertrophic cardiomyopathy, vascular smooth-muscle contraction, and cardiac-muscle contraction pathways were analyzed. In addition, amino-acid metabolism pathways including phenylalanine, tryptophan, and histidine metabolism pathways were also used. **(C)** For the SY type, melanoma-related pathways including melanoma, melanogenesis, and neurotrophin-signaling pathways, were analyzed. From the multipathways, core-node genes (big gray circles) were identified with a KEGGgraph. TE, *Taeum*; SE, *Soeum*; SY, *Soyang*.

Table 5. Core-Node Genes and Related SNPs Identified with KEGGgraph

Constitution	Symbol	SNP	Chromosome	Position	Association p-value[a]	Allele		Odds ratio[b]
						Major	Minor	
TE	*TJP1*	*rs11073279*	15	27893050	4.31×10^{-3}	A	G	0.63 (0.46–0.86)
	PTK2	*rs3639*	8	141753352	1.09×10^{-2}	T	C	0.64 (0.45–0.90)
	SRC	*rs747182*	20	35416303	4.15×10^{-2}	T	C	1.25 (1.00–1.55)
SE	*RHOA*	*rs6997*	3	49428838	3.65×10^{-2}	G	A	1.34 (1.01–1.77)
	MAOA/MAOB	*rs6609257*	X	43497652	1.24×10^{-4}	G	A	1.48 (1.21–1.82)
SY	*GNAO1*	*rs9927506*	16	54896727	4.04×10^{-2}	A	G	0.56 (0.32–0.98)

[a]χ^2 analysis was performed for differences in allele frequencies between each constitution type and other constitution types.
[b]Numbers in parenthesis represent a 95% confidence interval.
SNPs, single-nucleotide polymorphisms; TE, *Taeum*; SE, *Soeum*; SY, *Soyang*.

pathways confirmed that at least one constitutional type was highly enriched (Fig. 2). Among the various pathways identified, some functionally related pathways could be segregated in each constitutional type. Cytoskeleton-related pathways, cardio-related pathways, and melanoma-related pathways were associated with TE, SE, and SY types, respectively (Table 2).

Although the direct association of this pathway information with SC types is difficult to identify, because a pathway is not a clinically or quantitatively measurable variable, some pathways were indirectly related to the clinical characteristics of SC type. For example, cytoskeletal pathways regulating muscle contraction, which was associated with the TE type, has been shown to be a major cause of diseases such as hypertension and vasospasm of the coronary and cerebral arteries via Rho kinase.[23] Actually, a recent study showed that the prevalence of hypertension was highest in the TE type.[24] Moreover, the pathophysiology of diabetes, which is also a highly risky disease in the TE type, has been associated with the loss of tight junction pathways, which regulate permeability in the intestinal membrane or blood–brain barrier.[25,26] Considering that membrane-maintenance pathways, including the tight junction, focal adhesion, and adherens junction, were associated with the TE type, it can be speculated that the TE type would be more vulnerable to diseases such as hypertension and diabetes.[3] In contrast with the TE type, the SE type is the least vulnerable to these diseases.[27,28] This was also partially observed in the samples, in which the SE type had the lowest body weight, total cholesterol level, and LDL level (Table 1). Therefore, the cardio-related pathways identified in SE type may reflect the low susceptibility of the SE type to these diseases, although the current authors did not measure the direction of pathways, such as activation or suppression, in the present analysis. Taken together, the involvement of specific pathways with constitution types could provide some insights into the pathophysiologic differences among constitution types.

In addition, it is very important to isolate core-node genes that can control functionally related multipathways. In this study, core genes were identified with a high number of inward or outward connections with other genes in functionally related multiple pathways (Fig. 3). Furthermore, among core genes, *TJP1* (rs11073279), *PTK2* (rs3639, and *rs11991796*) and SRC (*rs747182*) were associated with the TE type, whereas *RHOA* (*rs6997*) and *MAOA/MAOB* (*rs6609257* and r*s3859959*) were associated with the SE type, and *GNAO1* (*rs9927506*) was associated with the SY type ($p<0.05$; Table 5). Because SNPs associated with core genes were located in the untranslated or intron regions of genes, the current authors are now measuring the *cis*-effects of these SNPs on the expression of core genes. Moreover, the activities of pathways based on gene expression levels also should be elucidated in detail. As core-node genes can regulate functionally related multiple pathways, these genes may play a crucial role in delineating the complex molecular physiology of constitution.

Conclusions

The current authors identified the biologic pathways and core-node genes associated with SC types from the GWA study; however, the biologic functions of the pathways identified herein should be evaluated further in association with each constitutional type.

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Disclosure Statement

No competing financial interests exist.

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