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Integrative Medicine Research

journal homepage: www.imr-journal.com

Original Article

Energy metabolism and whole-exome sequencing-based analysis of Sasang constitution: a pilot study

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ARTICLE INFO

Article history:
Received 11 January 2017
Accepted 2 March 2017
Available online 26 May 2017

Keywords:
energy metabolism
mitochondria
Sasang constitutional medicine
whole-exome sequencing

ABSTRACT

Background: Traditional Korean Sasang constitutional (SC) medicine categorizes individuals into four constitutional types [Tae-eum (TE), So-eum (SE), Tae-yang (TY), or So-yang (SY)] based on biological and physiological characteristics. As these characteristics are closely related to the bioenergetics of the human body, we assessed the correlation between SC type and energy metabolism features.

Methods: Forty healthy, young (22.3 ± 1.4 years) males volunteered to participate in this study. Participants answered an SC questionnaire, and their face shape, voice tone, and body shape were assessed using an SC analysis tool. Thirty-one participants (10 TE, 10 SE, 3 TY, and 8 SY) were selected for further analysis. Collected blood samples were subjected to blood composition analysis, mitochondrial function analysis, and whole-exome sequencing.

Results: The SY type showed significantly lower total cholesterol and high-density lipoprotein cholesterol levels than the SE type. Cellular and mitochondrial Adenosine triphosphate (ATP) levels were similar across types. All types showed similar basal mitochondrial oxygen consumption rates, whereas the TE type showed a significantly lower ATP-linked oxygen consumption rate than the other types. Whole-exome sequencing identified several genes variants that were exclusively detected in particular SC types, including 19 for SE, seven for SY, 11 for TE, and six for TY.

Conclusion: SC type-specific differences in mitochondrial function and gene mutations were detected in a small group of healthy, young Korean males. These results are expected to greatly improve the accurate screening and utilization of SC medicine.

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http://dx.doi.org/10.1016/j.imr.2017.03.002

1. Introduction

Sasang constitutional medicine (SCM) is a Korean medical tradition that classifies physiological and pathological traits into four constitutional types: Tae-yang (TY), So-yang (SY), Tae-eum (TE), and So-eum (SE). The concept that constitution can be "typed" is the most basic underlying paradigm described in the *Donguisusebowon*.[1] Unlike Westernized diagnostic tools based on molecular biological evidence, SCM emphasizes integrative and holistic characteristics of the individual.[1] SCM is not only used for the clinical diagnosis of individual constitution but is also widely used in the SC type-specific treatment of disease.[2] Thus, the SCM tradition can be considered as a complement to the current "personalized medicine" approach.[3,4]

SCM considers balances between food intake and waste discharge, energy consumption and storage, and catabolism and anabolism.[2] TY, SY, TE, and SE types display different hyper- and hypoactive organs. For example, the TE type shows a tendency toward a hyperactive liver and hypoactive lung, whereas the TY type shows the opposite tendency. In the same manner, the SY and SE types also show opposite tendencies toward hyper- and hypoactive organs; the SY type has a hyperactive spleen and hypoactive kidney, whereas the SE type has a hypoactive spleen and hyperactive kidney.[2] Beyond single organ-specific characteristics, SCM is deeply concerned with various biological processes such as food intake, digestion, waste excretion, and energy storage in multiple organs as well as catabolism and anabolism balance at the cellular level. The detailed biological characteristics and bodily features of different SC types have previously been described.[2] According to the SCM theory, the imbalance of energy metabolism under pathological conditions affects the sensitive hypoactive organ of each type, which can cause disease.[2] Because SC types are reflected by integrative systemic features and are closely associated with metabolic status, SCM can facilitate the diagnosis and treatment of metabolic syndromes or diseases including obesity, hyperlipidemia, diabetes, and hypertension.[5–10] These previous studies provide strong evidence of correlations between SC types and particular diseases, suggesting the value of SC types as holistic biomarkers for a wide range of diseases.

SC types are determined by professional oriental medical doctors based on traditional diagnostic methods including seeing, listening, questioning, and touching. However, these traditional diagnostic methods are based on subjective observations and are catechetic methods, making them difficult to objectify and quantify.[1] To overcome difficulties in traditional diagnosis and to establish an evidence-based diagnostic tool, we developed the Sasang constitution analysis tool (SCAT),[11] a system designed to provide objective information for determining the SC type. In addition to the SCAT, several genomic approaches have been used to identify the genetic loci or pathways responsible for different SC types.[12,13] Won et al[12] found a significant link between the constitution and chromosomes 8q11.22–23 and 11q22.1–3 based on a genome-wide scan of a Korean family. Kim et al[13] not only performed a genome-wide association analysis but also analyzed the pathways involved in SC. These studies, therefore, suggest the possibility of genomic differences among SC types.

Next-generation sequencing is a novel and powerful genomics tool that can be used to rapidly sequence whole or specific regions of an individual human genome and provide integrative genomic information about the individual.[14] Whole-exome sequencing (WES) is the most widely used targeted sequencing method, as the exome contains the majority of known disease-causing variants. WES enables the selective capture and sequencing of the protein-coding portion of the genome to understand relationships between gene variants and their associated phenotypes.[15–17] Based on this advantage, we aimed to use WES to identify gene variants of SC types and to link this genomic information to the morphological and physiological characteristics of each SC type.[18]

In the bodies of mammals, intracellular organelles, called the mitochondria, play an essential role in the production, storage, and transformation of biological energy molecules, known as Adenosine triphosphate (ATP), and the regulation of catabolic and anabolic pathways.[19–22] Impaired mitochondrial function results in an imbalance of energy metabolism and an increase in oxidative stress in major organs, including the heart, liver, kidney, and brain, which cause a wide range of diseases including inflammatory diseases, neurodegenerative diseases, metabolic syndromes, cancers, and cardiovascular diseases.[23–28] Recent studies by Shim et al[29,30] show that the TE type has reduced mitochondrial metabolism and an obesity-prone tendency. Therefore, we hypothesized that variations in mitochondrial energy metabolism are associated with different SC types and phenotypic characteristics. The aim of this study was to identify SC type-specific energy metabolic and/or genetic variants that can be used for the precise diagnosis of SC type. We measured the energy status-related metabolites in blood samples, as well as mitochondrial oxidative phosphorylation, and identified SC type-specific gene variants in 31 healthy individuals.

2. Methods

2.1. *Participants*

This study was approved by the Institutional Review Board and Ethics Committee of Inje University Paik Hospital, Busan, Korea (15-0287). The study was performed in accordance with previously established experimental protocols and guidelines. Informed consent was obtained from all participants. Forty healthy males were recruited through posters placed on community boards in the College of Medicine, Inje University. The study included 31 participants after excluding nine individuals for the following reasons: (1) diagnosis of diabetes, hypertension, hyperlipidemia, or other chronic disease, (2) drug supplementation within 3 months of the study, (3) results of initial screening interviews, or (4) SC type diagnosis based on SCAT score and the opinion of a medical doctor with expertise in SCM.

2.2. *SCAT*

The SCAT was used to diagnose the SC type of 40 males at the College of Medicine, Inje University. The diagnosis of constitution was based on face pictures, voice recordings, body

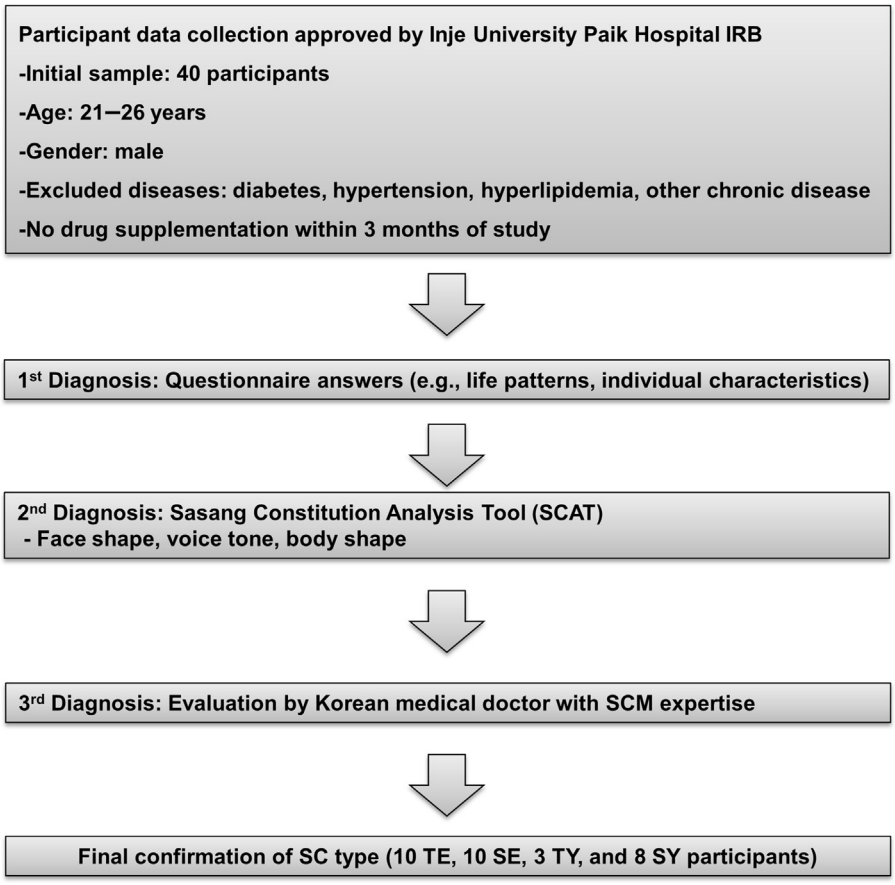

Fig. 1 – Flow diagram of SC type diagnosis.
IRB, institutional review board; SCM, Sasang constitutional medicine; SE, So-eum; SY, So-yang; TE, Tae-eum; TY, Tae-yang.

measurements, and responses to survey questions.[11] First, front- and side-view pictures of the face were taken. Second, an audio recording of the participant, making a specific verbal statement, was made. Third, circumference measurements of the eight parts of the body were obtained. Finally, individual responses to an SC structure classification survey were recorded. SCAT scoring results were confirmed by a medical doctor with expertise in SCM. We only included individuals with a difference ratio of at least 5% between the top- and second-ranked SC types. We included 10 SE, eight SY, 10 TE, and three TY participants (Fig. 1).

2.3. *Blood collection and composition analysis*

Blood samples were collected into serum-separating tubes or EDTA-containing anticoagulation tubes under fasting conditions for composition analysis or WES analysis, respectively. Blood samples were allowed to clot at room temperature for 30 minutes and centrifuged at 3000 *g* for 15 minutes. The separated serum was stored at −80 °C until further analysis. Serum glucose, insulin, total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) were determined using enzymatic techniques based on a colorimetric assay as previously described.[31] Blood samples in EDTA tubes were kept at −4 °C until WES analysis.

2.4. *Mitochondrial function analysis*

2.4.1. *Measurement of cellular and mitochondrial ATP levels*

Mitochondrial ATP levels were measured by Mitochondrial ToxGlo assay (Promega, Madison, WI, USA) according to the manufacturer's protocol. Briefly, isolated human platelets were plated at 1×10^4 cells/well in a white and clear-bottomed 96-well culture plate. Plates were centrifuged at 200 *g* for 10 minutes to remove the medium. The medium was replaced with 50 μL fresh medium lacking glucose and supplemented with 10 mM glucose (cellular ATP) or 10 mM galactose (mitochondrial ATP). The plate was incubated at 37 °C in a humidified and CO_2-supplemented incubator for 90 minutes. The assay solution (100 μL) was added to the plate, which was incubated at room temperature for 30 minutes. Luminescence was measured using a luminometer (Molecular Device, Sunnyvale, CA, USA).

2.4.2. *Oxygen consumption analysis*

Oxygen consumption rate (OCR) was measured using an XF24 analyzer (Seahorse Bioscience, Billerica, MA, USA) as previ-

ously described.[32,33] Briefly, isolated human platelets were plated at 2×10^4 cells/well in a Corning Cell-TAK cell and tissue adhesive (Corning Inc., Corning, NY, USA)-treated XF24 cell culture plate (Seahorse Bioscience, Billerica, MA, USA). Plates were centrifuged at 200 *g* for 10 minutes to remove the medium. The medium was replaced with 500 μL XF Assay medium-modified Dulbecco's modified Eagle's medium (Seahorse Bioscience, Billerica, MA, USA) and incubated at 37 °C without CO_2 for 1 hour. OCR was measured using an XF24 analyzer and software. We measured basal OCR, ATP-linked OCR, maximal OCR, and spare respiration capacity using specific mitochondrial inhibitors including oligomycin (ATPase inhibitor for ATP-linked OCR) and carbonyl cyanide *p*-[trifluoromethoxy]-phenyl-hydrazone (FCCP; uncouples the mitochondrial inner membrane and allows maximum electron flux through the electron transport chain). Basal OCR was measured without any mitochondrial inhibitor. ATP-linked OCR was calculated as the difference in OCR prior to and after treatment with oligomycin. Spare respiration capacity was calculated as the difference between maximal and basal OCR.[32]

2.5. *WES and variant analyses*

WES and variant analyses were performed using previously described methods.[15] An Illumina Hiseq2500 machine and Sureselected Exome V5 kit were used as a sequencing platform. On average, 6.25 gigabases of raw sequences were used for the analyses. Quality control was performed using FastQC,[34] and raw sequence reads were aligned to a reference genome (NCBI b37) using the Burrows–Wheeler Aligner-MEM algorithm.[35] The initial alignment was refined by local realignment and base quality recalibration using GATK tools.[36] Variants were called by the GATK HaplotypeCaller and filtered by the GATK Variant Recalibrator walker.[16] These variants were annotated by SnpEff and filtered by SnpSift.[37]

2.6. *Gene set enrichment analyses*

We obtained gene-to-function annotation from a gene ontology (GO) database[38] (*Homo sapiens*, "go.obo", June 2016). Cate-

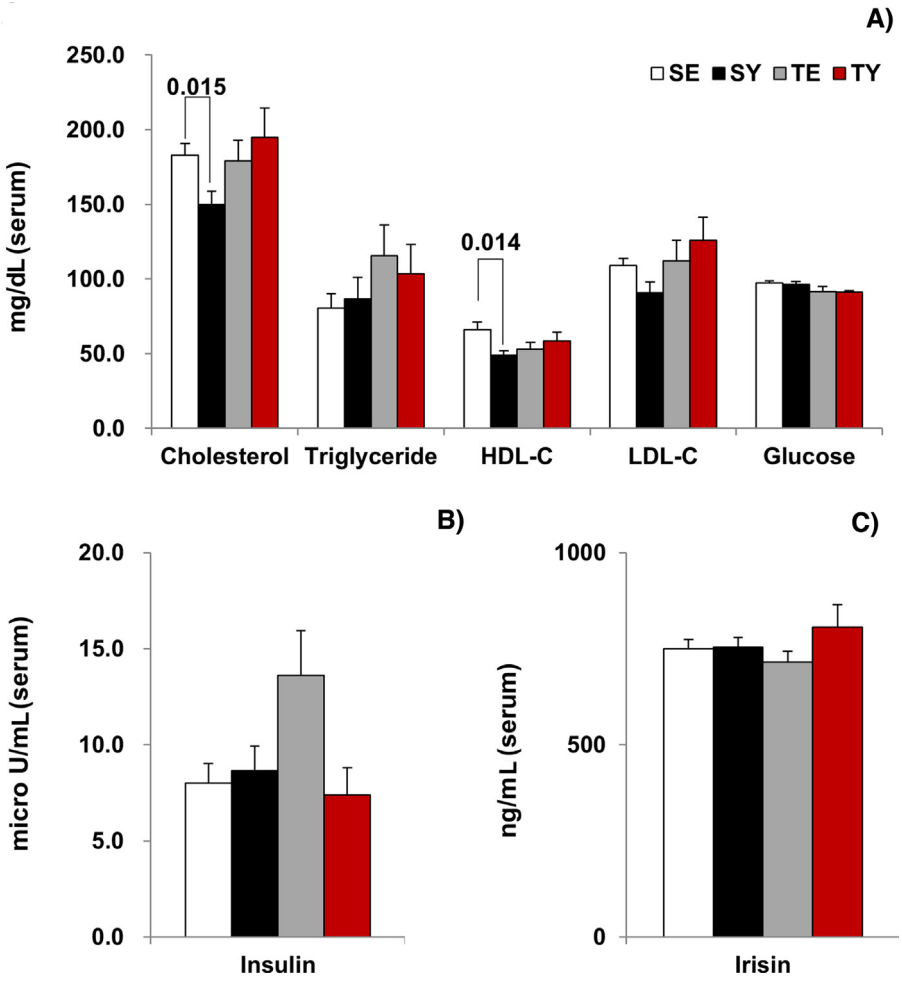

Fig. 2 – Blood composition analysis. (A) Comparison of serum cholesterol, triglyceride, HDL-C, LDL-C, and glucose levels between SC types. (B) Comparison of serum insulin levels between SC types. (C) Comparison of serum irisin levels between SC types.

HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SC, Sasang constitutional; SE, So-eum; SY, So-yang; TE, Tae-eum; TY, Tae-yang.

Table 1 – Participant characteristics.

	SE ($n=10$)	SY ($n=8$)	TE ($n=10$)	TY ($n=3$)	F
Age (y)	22.50 ± 0.45	21.62 ± 0.26	22.80 ± 0.64	21.66 ± 0.33	1.345
Height (cm)	172.10 ± 1.50	176.37 ± 1.13	174.70 ± 0.86	176.00 ± 2.64	3.076
Body weight (kg)	60.20 ± 1.91[a]	70.50 ± 1.08[b]	82.40 ± 3.23[c]	68.33 ± 2.02	23.021
BMI (kg/m^2)	20.20 ± 0.55[a]	22.62 ± 0.32[a]	26.90 ± 1.19[b]	22.00 ± 0.00[a]	17.655

Values are expressed as mean ± SEM. Values with different letter superscripts (a, b, and c) are significantly different.
SE, So-eum; SEM, standard error of the mean; SY, So-yang; TE, Ta-eum; TY, Tae-yang.

Table 2 – Average values of face shape variables.

Type	Jaw width (mm)	Angle inside eyes (degree)	Ratio of face width to nose length	Nose aspect ratio
SE	116.11	32.75	0.36	1.94
SY	122.48	34.23	0.34	1.82
TE	130.79	31.08	0.33	1.83
TY	123.18	33.75	0.35	1.81
Overall	123.13	32.78	0.34	1.86

SE, So-eum; SY, So-yang; TE, Ta-eum; TY, Tae-yang.

Table 3 – Average values of voice tone variables.

Type	MFCC1	MFCC3	MFCC8	sF50	sF10
SE	−2.67	1.54	0.82	125.06	1047.29
SY	−2.62	0.86	−5.12	128.59	1094.00
TE	−2.93	−0.40	−3.42	127.77	1082.56
TY	−2.19	2.91	0.42	121.48	321.27
Overall	−2.69	0.87	−2.30	126.62	3545.13

Mel-frequency cepstral coefficients (MFCC), 50th and 10th percentile of average fundamental frequency distribution (sF50 and sF10, respectively).
SE, So-eum; SY, So-yang; TE, Ta-eum; TY, Tae-yang.

gories used for analyses were "biological process," "molecular function," and "cellular component." Biological pathway information was obtained from the GSEA (Gene Set Enrichment Analysis) database (http://software.broadinstitute.org/gsea), which contains the BIOCRATA, KEGG (Kyoto Encyclopedia of Genes and Genomes), and REACTOME pathways.[39] Disease phenotypes were obtained from the Menche et al[40] dataset containing OMIM (Online Mendelian Inheritance in Man) disease and GWAS (genome-wide association study) information. We estimated the enrichment p values using hypergeometric tests to determine which biological modules were enriched in SC type-specific variants. Hypergeometric p values were obtained by comparing the fraction of genes included in the specific modules within the whole genome and those in the corresponding modules within SC type-specific variants (enriched p values: <0.01).

2.7. *Statistical analysis*

Data are presented as mean ± standard error of the mean. Statistical analyses were performed using IBM SPSS Statistics for Windows, version 23.0 (IBM Corp., Armonk, NY, USA). One-way analysis of variance and Duncan *post hoc* test were used to determine differences between the groups. Statistical significance was set at $p < 0.05$. Because the sample size of the TY type was very small ($n = 3$), we eliminated TY values from the mean comparison tests.

3. Results

3.1. *Participant characteristics*

Participant characteristics, including age, height, body weight, and body mass index (BMI), are shown in Table 1. Age and height were not significantly different among the CS types. Body weight and BMI for the TE type were significantly higher than those for the other types, which was consistent with previous studies.[41,42]

3.2. *SCAT results*

The specific SC type of participants was determined by the SCAT and expert opinion of medical doctors in the Korea Institute of Oriental Medicine.[11] The average values for face shape, voice tone, and body shape are shown in Tables 2–4, respec-

Table 4 – Average values of body shape variables.

Type	Rib circumference (cm)	Hip–waist circumference
SE	71.18	1.27
SY	81.65	1.19
TE	88.40	1.15
TY	79.33	1.17
Overall	80.31	1.20

SE, So-eum; SY, So-yang; TE, Ta-eum; TY, Tae-yang.

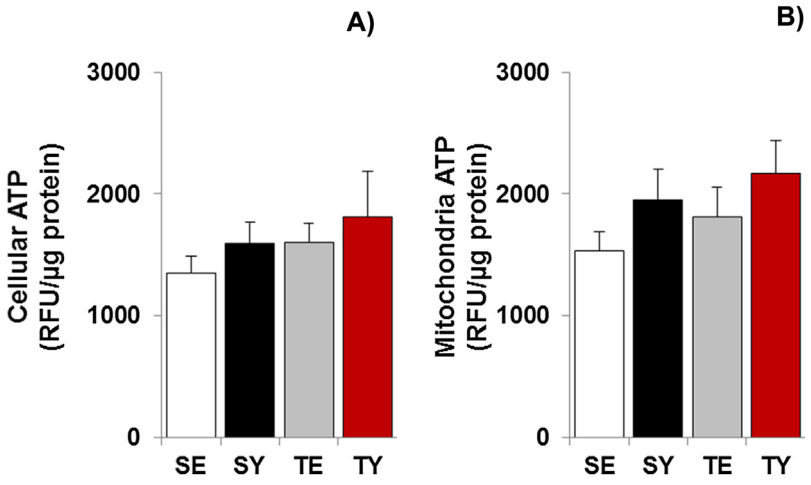

Fig. 3 – Cellular and mitochondrial ATP analysis. (A) Cellular ATP levels were measured in platelet cells in the presence of 10 mM glucose. (B) Mitochondrial ATP levels were measured in platelet cells in the presence of 10 mM galactose. ATP, ; SE, So-eum; SY, So-yang; TE, Tae-eum; TY, Tae-yang.

tively. Based on the results of this analysis, we confirmed the SC type of 31 participants (10 TE, 10 SE, 3 TY, and 8 SY) and collected their blood samples for further testing.

3.3. *Blood composition results*

Blood composition analysis revealed significant differences in blood TC and HDL-C levels between the SE and SY types ($p < 0.05$); however, all other components were comparable among the groups ($p > 0.05$; Table 5 and Fig. 2). The SY type showed the lowest blood TC and HDL-C levels, which were significantly different from the levels for the SE type (Fig. 2A). However, the ratio of TC/HDL-C was similar across types. Importantly, all measured values were within normal physiological ranges.

3.4. *Mitochondrial function analysis results*

We investigated differences in the mitochondrial function among the four constitution types. To validate the mitochondrial oxidative phosphorylation capacity, we measured the cellular ATP and mitochondrial ATP levels in isolated platelets from each participant (Fig. 3). Both ATP levels were similar across the four SC types. We further analyzed OCR using an XF24 analyzer (Fig. 4). Basal OCR levels were comparable across the SC types (Fig. 4A). ATP-linked OCR was significantly different between the TE and SY types ($p < 0.05$), with lower ATP-linked OCR in the TE type than in the SY type (Fig. 4B). The TE type also showed a significantly higher maximal OCR than the SE and SY types ($p < 0.05$; Fig. 4C). Spare respiration capacity was also significantly higher for the TE type than for the SE type ($p < 0.05$; Fig. 4D).

3.5. *WES and variant analysis results*

We investigated which gene variants were SC type-specific by WES analysis. We used GO terms, pathway, and disease information to identify which biological roles were associated with each SC type. Gene variants were defined as genes containing at least one altered amino acid. We investigated the number of gene variants and their associated amino acid residue alterations. We found 3344, 3070, 3291, and 1986 gene variants for the SE, SY, TE, and TY types, respectively. Of these gene variants, 886, 702, 828, and 247 genes were specific to SE, SY, TE, and TY types, respectively (Fig. 5A). We also counted the number of amino acid residue variations for each SC type to measure the amino acid level alterations that might affect protein function. The numbers of specific amino acid variants were 2983, 2348, 2754, and 751 for the SE, SY, TE, and TY types, respectively (Fig. 5B). A total of 1369 gene variants were commonly found across all SC types (Fig. 5A), and these genes contained an average of 1.85 altered residue variants.

Next, we tested the hypothesis that SC type-specific gene mutations, existing in the exome region, could be used as genetic markers to identify the SC type of an individual. We identified variant genes that were more frequently found in certain SC types and examined their biological roles (Data S1). We detected 19, seven, 11, and six SC type-specific genes for the SE, SY, TE, and TY types, respectively (Fig. 6 and Table 6). We positively selected the genes observed at least two human samples in the group.

For SE type-specific gene variants, those associated with musculoskeletal disease (*SCN4A* and *DNM2*) were found in six of 10 participants, and those associated with nervous system diseases (*CC2D1A*, *SKOR2*, *SCN4A*, *SPOCK2*, and *DNM2*) were found in nine of 10 participants (Fig. 6A). SY type-specific gene variants were confirmed to have biological roles in immune response, ion transport, and cell–cell adhesion (Fig. 6B). TE type-specific variants, associated with GTP-mediated signaling, were found in seven of 10 participants, including *ARL5C*, which directly binds to GTP, and *OR4M2* and *OR5D13*, which are involved in the G-protein-coupled signaling pathway (Fig. 6C). As the TY type included a small number of participants, only six frequent TY-specific variants were found, which were associated with protein homoligomerization, axoneme formation, and cell differentiation (Fig. 6D).

Table 5 – Blood composition.

	SE ($n=10$)	SY ($n=8$)	TE ($n=10$)	TY ($n=3$)
TC (mg/dL)	182.70 ± 8.04^{a}	150.00 ± 8.93^{b}	179.10 ± 13.54^{ab}	195.00 ± 19.34
TG (mg/dL)	80.30 ± 9.70	86.62 ± 14.53	115.40 ± 20.79	103.33 ± 19.70
HDL-C (mg/dL)	66.00 ± 5.24^{a}	48.75 ± 3.33^{b}	53.00 ± 4.43^{ab}	58.33 ± 6.00
LDL-C (mg/dL)	108.80 ± 5.10	90.75 ± 7.36	112.20 ± 13.46	125.66 ± 15.45
TC/HDL-C	3.08 ± 0.16	2.84 ± 0.17	3.60 ± 0.41	3.36 ± 0.27
LDL/HDL	1.88 ± 0.14	1.70 ± 0.14	2.30 ± 0.36	2.17 ± 0.26
HOMA-IR	2.18 ± 0.31	1.73 ± 0.17	3.06 ± 0.54	1.65 ± 0.29
Glucose (mg/dL)	97.40 ± 1.14	96.25 ± 2.19	91.50 ± 3.22	91.00 ± 1.15
Insulin (nU/mL)	8.00 ± 1.01	8.65 ± 1.29	13.60 ± 2.31	7.40 ± 1.40
Irisin (ng/mL)	750.63 ± 23.56	753.66 ± 25.60	715.99 ± 27.04	806.07 ± 58.78

Values are expressed as mean ± SEM. Values with different letter superscripts (a, ab, and b) are significantly different.
HOMA-IR, homeostatic model assessment insulin resistance; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SEM, standard error of the mean; TC, total cholesterol; TG, triglyceride.

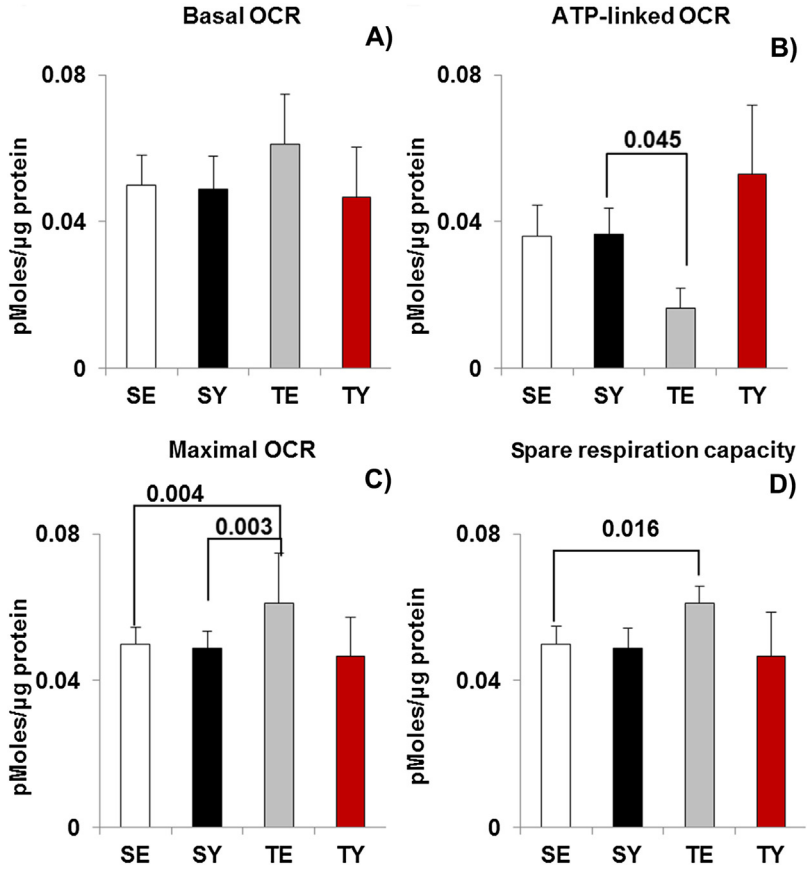

Fig. 4 – Mitochondrial OCR analysis. (A) Basal OCR was measured in platelet cells by an XF24 analyzer without a mitochondrial inhibitor. (B) ATP-linked OCR was calculated as the difference in OCR prior to and after treatment with oligomycin, a mitochondrial ATPase inhibitor. (C) Maximal OCR was measured in the presence of FCCP, a mitochondrial uncoupler. (D) Spare respiration capacity was calculated as the difference between maximal and basal OCR.
ATP, ; FCCP, *p*-[trifluoromethoxy]-phenyl-hydrazone; OCR, oxygen consumption rate; SE, So-eum; SY, So-yang; TE, Tae-eum; TY, Tae-yang.

To assess correlations between specific gene variants and their biological roles, we isolated SC type-specific gene variants and performed module enrichment tests to determine which biological terms were primarily associated with SC type-specific variants (Data S2). The module analysis utilized GO terms, REACTOME, BIOCRATA, and KEGG pathways, and disease phenotypes. Enrichment tests were conducted for module sizes >4 and <100. The SE, SY, TE, and TY type-specific variants were enriched for 299, 286, 219, and 114 modules, respectively. The top five terms based on enrichment significance for SE type-specific variants were “detection of visible light” (GO: 0009584), “cytochrome-b5 reductase activity, acting on NAD(P)H” (GO: 0004128), “negative regulation of actin filament polymerization” (GO: 0030837), “G-protein coupled

Table 6 – SC type-specific gene variants.

SC type	Gene	Accession	Frequency	HGVS.p
SE	*HSPBAP1*	rs71270423	6	p.Pro456_Gln457insSer
SE	*KNTC1*	—	3	p.Phe684Tyr
SE	*KNTC1*	—	3	p.Phe721Tyr
SE	*KNTC1*	rs186936079	3	p.Leu1511Phe
SE	*KNTC1*	—	2	p.Lys106Asn
SE	*KNTC1*	—	2	p.Lys1110Asn
SE	*KNTC1*	—	2	p.Lys2185Asn
SE	*KNTC1*	rs75696429	2	p.Val71Gly
SE	*KNTC1*	rs186936079	3	p.Leu70Phe
SE	*KNTC1*	rs75696429	2	p.Val1512Gly
SE	*VIPR1-AS1*	rs413042	5	intron_variant
SE	*COBLL1*	rs3841875	4	p.Ser1210fs
SE	*COBLL1*	rs3841875	4	p.Ser1181fs
SE	*COBLL1*	rs3841875	4	p.Ser1143fs
SE	*COBLL1*	rs3841875	4	p.Ser1105fs
SE	*C6orf163*	rs9353479	5	p.Ala72Val
SE	*C14orf166B*	rs201329545	2	p.Lys449Asn
SE	*C14orf166B*	rs139864900	3	p.Ile132Thr
SE	*C14orf166B*	—	2	p.Glu139Lys
SE	*C14orf166B*	—	2	p.Glu122Lys
SE	*ACOT8*	rs201025211	4	p.Met1fs
SE	*NT5C1B-RDH14*	—	2	p.Ser176Trp
SE	*NT5C1B-RDH14*	rs145698850	3	p.Gly353Arg
SE	*NT5C1B-RDH14*	—	2	p.Ser118Trp
SE	*NT5C1B-RDH14*	rs145698850	3	p.Gly411Arg
SE	*CC2D1A*	rs76113658	3	p.Ala338Thr
SE	*CC2D1A*	—	2	p.Glu363Lys
SE	*CC2D1A*	—	2	p.Glu909Lys
SE	*CC2D1A*	—	2	p.Glu910Lys
SE	*SKOR2*	rs77291182	2	p.Pro927Leu
SE	*SKOR2*	—	2	p.Ser899Cys
SE	*SKOR2*	—	2	p.Pro291del
SE	*RNF166*	rs117837843	4	p.Arg134Met
SE	*DIAPH3*	rs36084898	4	p.Asn293Ser
SE	*DIAPH3*	rs36084898	4	p.Asn352Ser
SE	*DIAPH3*	rs36084898	4	p.Asn363Ser
SE	*DIAPH3*	rs36084898	4	p.Asn317Ser
SE	*TMEM176A*	rs35972858	4	p.Gly63Asp
SE	*TMEM176A*	rs35972858	4	p.Gly52Asp
SE	*TMEM176A*	rs35972858	4	p.Gly111Asp
SE	*SCN4A*	—	2	p.Val1589Ala
SE	*SCN4A*	rs7218917	3	synonymous codon
SE	*FAM132B*	rs111241405	3	p.Ala260Ser
SE	*FAM132B*	rs117194634	3	p.Val185Met
SE	*FAM132B*	rs117199696	2	p.Ala81Ser
SE	*PPIG*	rs76754479	4	p.Thr76Met
SE	*SPOCK2*	rs2306322	4	p.Gly353Ser
SE	*DNM2*	rs3745674	4	p.Pro263Leu
SE	*DNM2*	rs3745674	4	p.Pro15Leu
SY	*METTL1*	rs118007790	5	p.Ser46Phe
SY	*SULT1A2*	rs138147609	3	p.Glu217*
SY	*SULT1A2*	—	2	p.Val176Gly
SY	*SULT1A2*	rs138147609	3	p.Glu184*
SY	*TTYH1*	rs3745433	2	p.Gly86Arg
SY	*TTYH1*	rs3745433	2	p.Gly21Arg
SY	*TTYH1*	rs3745433	2	p.Gly82Arg
SY	*TTYH1*	rs144026046	3	p.Gln51*
SY	*TTYH1*	rs3745433	2	p.Gly135Arg
SY	*VWA2*	rs200171942	2	p.Arg591Trp
SY	*VWA2*	rs201721125	2	p.Ser378Cys
SY	*PSMA6*	rs17103147	4	p.Arg5Trp
SY	*PACSIN2*	rs139155473	4	p.Cys27Phe
SY	*LPCAT2*	—	2	p.Ser51fs
SY	*LPCAT2*	—	2	p.Ser194fs
SY	*LPCAT2*	rs144432562	3	p.Arg167*

Table 6 (*Continued*)

SC type	Gene	Accession	Frequency	HGVS.p
TE	*CHD1*	rs138635992	5	p.Pro1684del
TE	*SP4*	rs139491266	4	p.Leu241Val
TE	*MICAL3*	—	2	p.Thr122Ile
TE	*MICAL3*	rs148674116	2	p.Gly309Ser
TE	*MICAL3*	—	2	p.Thr1829Ile
TE	*MICAL3*	rs61739477	2	p.Val319Ile
TE	*MICAL3*	—	2	p.Thr84Ile
TE	*FLNC*	rs180834558	3	p.Leu2538Phe
TE	*FLNC*	—	2	p.Ser589Leu
TE	*FLNC*	rs180834558	3	p.Leu2505Phe
TE	*ARL5C*	rs151045610	4	p.Trp171Arg
TE	*KLC3*	rs186054339	2	p.Ala219Ser
TE	*KLC3*	rs182912549	2	p.Glu333Lys
TE	*KLC3*	—	2	p.Arg440_Gly441insGluSerIleArgArg
TE	*KLC3*	—	2	p.Arg455_Gly456insGluSerIleArgArg
TE	*KLC3*	—	2	p.Arg441_Gly442insGluSerIleArgArg
TE	*KLC3*	rs186054339	2	p.Ala189Ser
TE	*KLC3*	rs182912549	2	p.Glu319Lys
TE	*KLC3*	rs182912549	2	p.Glu318Lys
TE	*KLC3*	rs186054339	2	p.Ala205Ser
TE	*KLC3*	rs186054339	2	p.Ala204Ser
TE	*PRG3*	rs115707133	4	p.Cys215*
TE	*OR4M2*	rs140079625	4	p.Ile200Met
TE	*TNK2*	rs148791867	2	p.Pro402Leu
TE	*TNK2*	—	2	p.Arg6Leu
TE	*TNK2*	rs148791867	2	p.Pro434Leu
TE	*TNK2*	rs200619114	2	p.Leu198Gln
TE	*TNK2*	rs200619114	2	p.Leu589Gln
TE	*TNK2*	rs200619114	2	p.Leu667Gln
TE	*TNK2*	rs148791867	2	p.Pro465Leu
TE	*TNK2*	rs200619114	2	p.Leu156Gln
TE	*TNK2*	rs200619114	2	p.Leu621Gln
TE	*OR5D13*	rs74548274	4	p.Gln198*
TE	*MNT*	—	2	p.Arg232fs
TE	*MNT*	rs185455119	3	p.Gly32Arg
TY	*RP11-274B21.1*	rs77554651	3	splice donor variant
TY	*SHKBP1*	—	2	p.Gln111Glu
TY	*SHKBP1*	rs114918214	2	p.Ala207Thr
TY	*SHKBP1*	rs114918214	2	p.Ala130Thr
TY	*SHKBP1*	rs114918214	2	p.Ala201Thr
TY	*PCDP1*	rs182093842	2	p.Met105Ile
TY	*PCDP1*	rs200947466	2	p.Gly611Arg
TY	*PCDP1*	rs200947466	2	p.Gly325Arg
TY	*PCDP1*	rs187802437	2	p.Pro106Thr
TY	*PCDP1*	rs200947466	2	p.Gly38Arg
TY	*PCDP1*	rs200947466	2	p.Gly169Arg
TY	*FAM211A*	rs140374173	3	p.Arg210*
TY	*MYCBPAP*	rs78242165	3	p.Ala535Val
TY	*MYCBPAP*	rs78242165	3	p.Ala546Val
TY	*MYCBPAP*	rs78242165	3	p.Ala572Val
TY	*MYCBPAP*	rs78242165	3	p.Ala5Val
TY	*C1orf222*	—	2	p.Thr111Met
TY	*C1orf222*	—	2	p.Pro45Leu
TY	*C1orf222*	—	2	p.Pro167Leu

SE, So-eum; SY, So-yang; TE, Ta-eum; TY, Tae-yang.

photoreceptor activity" (GO: 0008020), and "exit from mitosis" (GO: 0010458). SY type-specific variants were involved in "the regulation of protein localization to plasma membrane" (GO: 1903076), "polynucleotide adenylyl transferase activity" (GO: 0004652), "hepatocyte differentiation" (GO: 0070365), "vitamin D metabolic process" (GO: 0042359), and "citrate metabolic process" (GO: 0006101). TE type-specific variants were identified as "RNA transport" (GO: 0050658), "dorsal/ventral neural tube patterning" (GO: 0021904), "S-adenosylhomocysteine metabolic process" (GO: 0046498), and "regulation of fibroblast growth factor receptor signaling pathway" (GO: 0040036). Finally, TY type-specific variants were identified as "mitochondrial fragmentation involved in apoptotic process" (GO: 0043653), "cellular response to arsenic-containing substance"

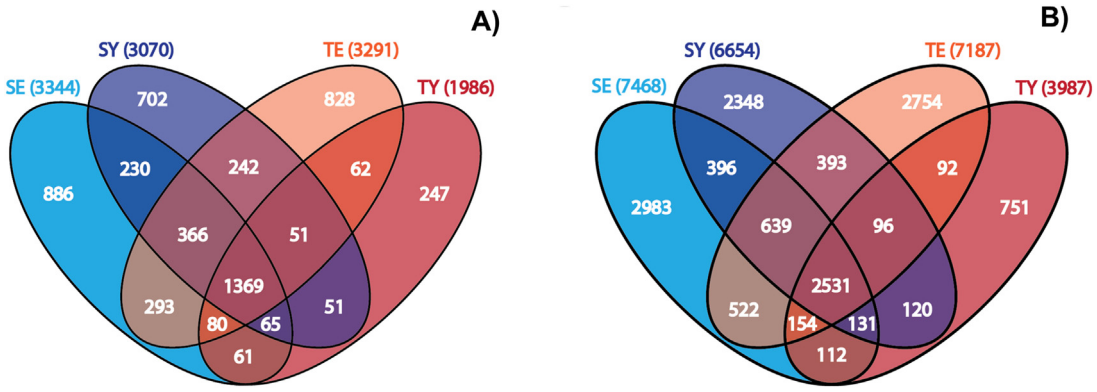

Fig. 5 – Venn diagram of gene variants for each SC type. (A) Number of gene variants for each SC type. (B) Number of amino acid residue alterations for each SC type.
SC, Sasang constitutional; SE, So-eum; SY, So-yang; TE, Tae-eum; TY, Tae-yang.

(GO: 0071243), "morphogen activity" (GO: 0016015), and "actin-dependent ATPase activity" (GO: 0030898). A summary of enriched terms for each SC type is shown in Fig. 7.

4. Discussion

During the past decade, more than 100 studies have contributed to improving the evidence-based analysis of SCM. A wide range of approaches has successfully been used to accumulate unbiased evidence for SCM. To our knowledge, this is the first study to investigate the mitochondrial function characteristics and WES-based SC type-specific gene variants in healthy participants. Our results reveal the novel finding that aspects of mitochondrial oxygen consumption, including ATP-linked OCR, maximal OCR, and spare respiration capacity, differ between the TE type and other SC types.

Our study included student participants from a single university who were healthy (i.e., nondiseased), a single sex (i.e., male), and within a narrow age range (22.3 ± 1.4 years). A major hurdle in genomic studies is the wide variation among individuals in age, sex, environmental factors, and pathological history. Therefore, the similarity of participants in the present study could serve to minimize such biases among individuals. As DNA mutations accumulate during aging and in pathological conditions, the inclusion of relatively young participants in the current study could serve to minimize acquired DNA mutations and preserve inborn gene variants, thereby strengthening links between WES results and SC types.

Examination of basic physical characteristics showed that the TE type had a significantly greater body weight and BMI and a tendency toward higher insulin levels than the other SC types, which is consistent with previous studies.[41,42] Various studies suggest that the TE type is prone to metabolic disorders and cardiovascular diseases, including obesity, diabetes, and ischemic stroke.[10,18,29,30,43–45] Although we found no significant pathological abnormalities in the serum indices of any tested sample, the higher BMI and body weight of the TE type could be risk factors for these diseases.

We also found significantly lower serum TC and HDL-C levels for the SY type than for the SE type, with the SY type showing the lowest HDL-C level among the four SC types. A lower HDL-C level is a well-known risk factor for cardiovascular disease.[46] Importantly, a recent study suggests that the SY type has a higher cardiovascular disease risk ratio than other SC types.[45] Thus, our finding that the SY type has the lowest HDL-C level could be one reason for their higher cardiovascular disease risk ratio. Irisin is a recently discovered muscle-derived hormone that is implicated in metabolic homeostasis and various metabolic diseases.[47–49] However, we found no differences in serum irisin levels among SC types. Because irisin levels are only altered by high-intensity exercise,[50] examining exercise response and alterations in blood irisin levels after long-term exercise would be valuable for further investigations in the differences in energy metabolism among the SC types. We note that the agreement between the results of the present study and previous SCM studies reflects the high reliability of our sample group despite its small size.

Mitochondria consume oxygen for oxidative phosphorylation, which converts various substrates (i.e., glucose, free fatty acids, and amino acids) into the high-energy biomolecule ATP. Thus, measurements of ATP level and OCR are essential for assessing the mitochondrial function. Although ATP contents and basal OCR were comparable among the SC types, some specific components, including ATP-linked OCR, maximal OCR, and spare respiration capacity, differed between the TE type and the SE or SY types. ATP-linked OCR is the amount of oxygen required to produce ATP. A higher ATP-linked OCR indicates a greater efficiency of ATP generation, whereas a lower ATP-linked OCR indicates a lower efficiency of substrate and oxygen utilization. Generally, a lower efficiency is found in metabolic disease such as type 2 diabetes.[51,52] Maximal OCR and spare respiration capacity are measured in the presence of FCCP, a hydrogen ion (or proton, H^+) uncoupler. The proton gradient is a fundamental driving force of ATP production via ATPase of the mitochondrial inner membrane. Under physiological conditions, mitochondria are not fully activated and have spare respiration capacity; thus, maximal OCR can be measured only in *ex vivo* conditions with FCCP.[32] The higher maximal OCR and spare respiration capacity of the TE type might indicate that this constitutional type maintains

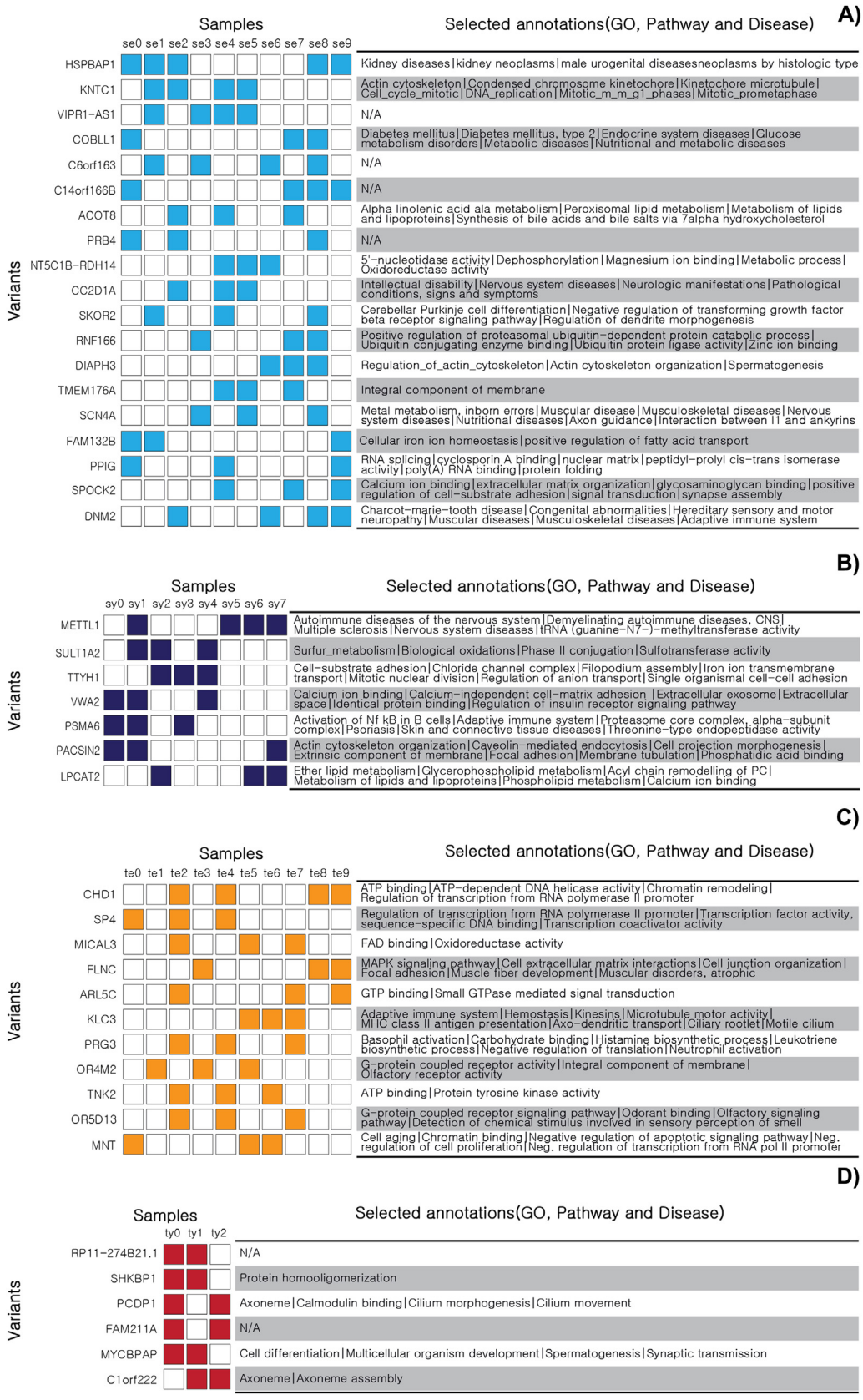

Fig. 6 – Selected gene–biological module associations. Gene variants and biological module associations from GO terms, pathways, and diseases. (A) SE type-specific variants. (B) SY type-specific variants. (C) TE type-specific variants. (D) TY type-specific variants.

GO, gene ontology; SE, So-eum; SY, So-yang; TE, Tae-eum; TY, Tae-yang.

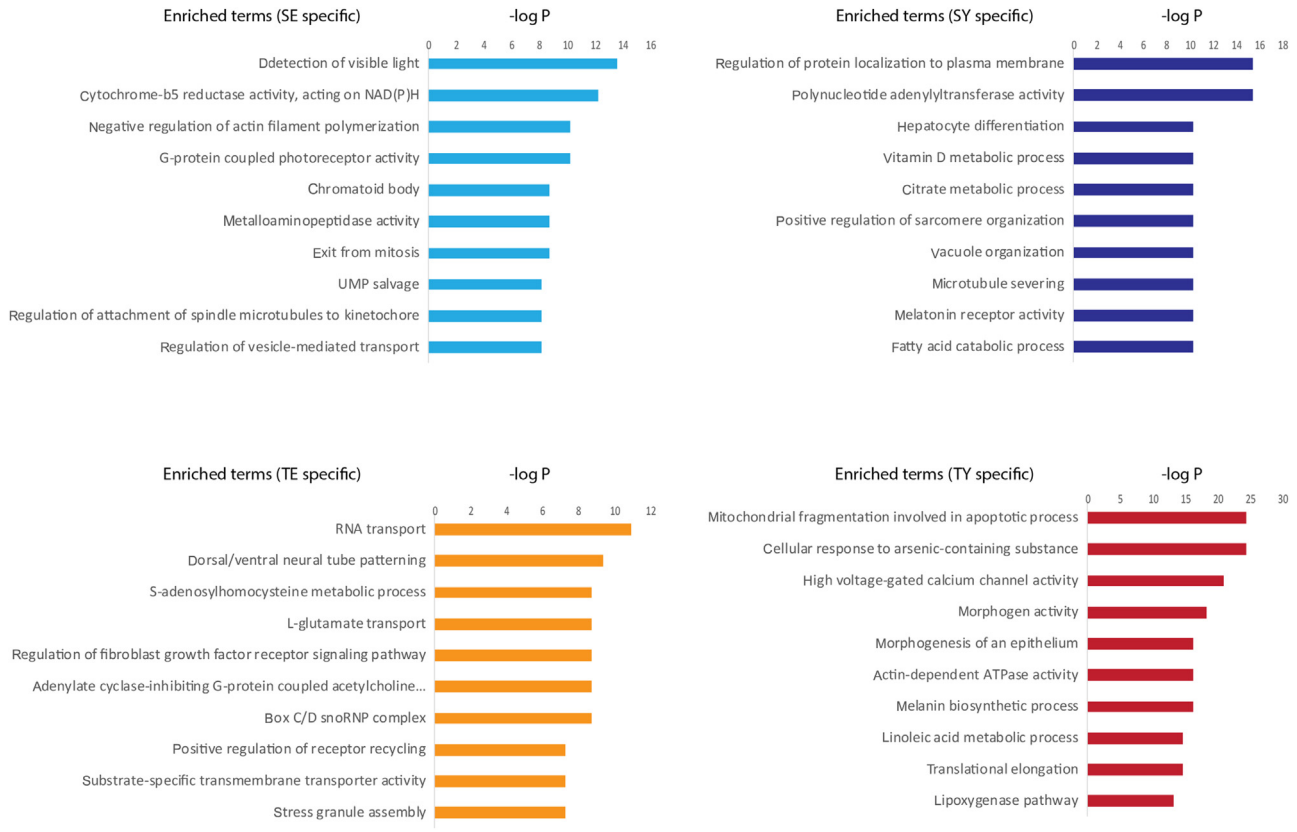

Fig. 7 – Enriched terms for each SC type. Different colors indicate the enriched biological terms for different SC types. Enrichments were calculated with hypergeometric tests. Values on the x-axis denote the significance of enrichment tests. SC, Sasang constitutional; SE, So-eum; SY, So-yang; TE, Tae-eum; TY, Tae-yang.

a low respiration rate for survival under normal conditions but shows full activation in high energy-requiring environments, which might be reflected by a higher obesity rate (i.e., energy-storing condition).

In summary, we found that the specific energy metabolism-related features of the TE type were a higher BMI, lower ATP-linked OCR, and higher maximal OCR without a significant change in the ATP level. These features are most likely characteristic of the TE type under normal conditions. Similar biological features are found in peripheral blood mononuclear cells of type 2 diabetes patients.[53] Coincidently, the TE type is known to have a higher risk of metabolic syndromes, including obesity and diabetes.[29,30] However, in the present study, we found that basal OCR rate and ATP level were not significantly modified in TE-type healthy individuals. These results may explain why TE-type individuals have a higher risk of metabolic syndromes.

An important finding of the present study is the identification of gene variants exclusively detected in different SC types with a high frequency (19, 7, 11, and 6 variants in the SE, SY, TE, and TY types, respectively). Although there is a limit to associating gene variants with constitutional features or specific energy metabolism-enriched pathways, we found that the top 10 enriched biological pathways of each type were completely distinct without overlap. Thus, these independent sets of gene variants could serve as genetic markers for SC type diagnosis.

The present and previous[41,42] findings, showing that the TE type has a significantly higher BMI and a greater tendency toward obesity than other SC types, leads us to question whether the TE type-specific gene variants are primarily associated with obesity or other TE type-specific features. However, the BMI of the TE type fell in the overweight (25.0–29.9), but not the obese (>30), range. In addition, the tested blood component indices (e.g., TC, TG, HDL-C, LDL-C, and insulin) were within normal ranges for all types. Importantly, the TE type-specific gene variants were also found in TE participants with normal BMI (18.5–24.9). Therefore, we believe that the specific genetic variants of the TE type are not merely associated with the overweight tendency of the TE type but rather are integrative features of the TE type.

Although the quality of our collected samples was good and nongenetic variance among participants was minimal, the small number of tested samples is a major limitation of the present study. Therefore, validations of mitochondrial function and SC type-specific gene variations within larger controlled populations are needed for the clinical application of these findings in SC-based Korean medicine.

In conclusion, our study demonstrates SC type-specific differences in mitochondrial function and gene mutations. An understanding of the TE type-specific mitochondrial function differences could be helpful for the diagnosis and treatment of metabolic diseases among TE individuals. Our findings sug-

gest that mitochondria-mediated energy metabolism analysis and WES can be effective methods for SCM research.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgments

This study was supported by grants from the Priority Research Centers Program and Basic Science Research Program through the National Research Foundation of Korea funded by the Ministry of Education, Science and Technology (2010-0020224, 2015R1A2A1A13001900, and 2015R1D1A1A01057937) and the Korea Institute of Oriental Medicine (K15809), Republic of Korea.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.imr.2017.03.002.

REFERENCES

1. Shim EB, Lee S, Kim JY, Earm YE. Physiome and Sasang constitutional medicine. *J Physiol Sci* 2008;58:433–40.
2. Kim JY, Pham DD. Sasang constitutional medicine as a holistic tailored medicine. *Evid Based Complement Alternat Med* 2009;6:11–9.
3. Cooper EL. Contributions of Sasang constitutional medicine. *Evid Based Complement Alternat Med* 2009;6(Suppl):1–3.
4. Chae H, Lyoo IK, Lee SJ, Cho S, Bae H, Hong M, et al. An alternative way to individualized medicine: psychological and physical traits of Sasang typology. *J Altern Complement Med* 2003;9:519–28.
5. Lee SK, Yoon DW, Lee SW, Kim JY, Kim JK, Cho NH, et al. Association of Sasang constitutional types with incident hypertension: a 12-year follow-up study. *J Altern Complement Med* 2016;22:706–12.
6. Lee HY, Lee WJ, Kim HW, Jang ES, Ahn YC, Ku BC, et al. A systematic review on Sasang constitutional type-associated susceptibility to disorders in Korea. *J Altern Complement Med* 2016;12:950–6.
7. Kim Y, Park K, Yoo J, Jang E. Sasang constitution may play a key role in increasing the number of sub-elements of metabolic syndrome. *J Altern Complement Med* 2016;22:204–11.
8. Lee SK, Yoon DW, Lee SW, Kim JY, Kim JK, Shin C. Non-alcoholic fatty liver disease among Sasang constitutional types: a population-based study in Korea. *BMC Complement Altern Med* 2015;15:399.
9. Jang E, Baek Y, Kim Y, Park K, Lee S. Sasang constitution may act as a risk factor for prehypertension. *BMC Complement Altern Med* 2015;15:231.
10. Cho NH, Kim JY, Kim SS, Lee SK, Shin C. Predicting type 2 diabetes using Sasang constitutional medicine. *J Diabetes Investig* 2014;5:525–32.
11. Do JH, Jang E, Ku B, Jang JS, Kim H, Kim JY. Development of an integrated Sasang constitution diagnosis method using face, body shape, voice, and questionnaire information. *BMC Complement Altern Med* 2012;12:85.
12. Won HH, Lee S, Jang E, Kim KK, Park YK, Kim YJ, et al. A genome-wide scan for the Sasang constitution in a Korean family suggests significant linkage at chromosomes 8q11.22-23 and 11q22.1-3. *J Altern Complement Med* 2009;15:765–9.
13. Kim BY, Jin HJ, Kim JY. Genome-wide association analysis of Sasang constitution in the Korean population. *J Altern Complement Med* 2012;18:262–9.
14. Reis-Filho JS. Next-generation sequencing. *Breast Cancer Res* 2009;11:S12.
15. Lee S, Kim JY, Hwang J, Kim S, Lee JH, Han DH. Investigation of pathogenic genes in peri-implantitis from implant clustering failure patients: a whole-exome sequencing pilot study. *PLoS ONE* 2014;9:e99360.
16. DePristo MA, Banks E, Poplin R, Garimella KV, Maguire JR, Hartl C, et al. A framework for variation discovery and genotyping using next-generation DNA sequencing data. *Nat Genet* 2011;43:491–8.
17. Vissers LE, Veltman JA. Standardized phenotyping enhances Mendelian disease gene identification. *Nat Genet* 2015;47:1222–4.
18. Lee BC, Doo HK, Ahn SY, Byun SH, Kim SI, Park HK, et al. Peroxisome proliferator-activated receptor-gamma Pro12Ala polymorphism is associated with the susceptibility to ischemic stroke in Taeeumin classified by Sasang medicine. *Neurol Res* 2007;29:S32–7.
19. Johannsen DL, Ravussin E. The role of mitochondria in health and disease. *Curr Opin Pharmacol* 2009;9:780–6.
20. Zeviar DD, Gonzalez MJ, Massari JRM, Duconge J, Mikirova N. The role of mitochondria in cancer and other chronic diseases. *JOM* 2014;29:157.
21. Mailloux RJ. Application of mitochondria-targeted pharmaceuticals for the treatment of heart disease. *Curr Pharm Des* 2016;22:4763–79.
22. Murphy MP. Mitochondrial diseases: shortcuts to therapies and therapeutic shortcuts. *Mol Cell* 2016;64:5–6.
23. Mittal M, Siddiqui MR, Tran K, Reddy SP, Malik AB. Reactive oxygen species in inflammation and tissue injury. *Antioxid Redox Signal* 2014;20:1126–67.
24. Kim GH, Kim JE, Rhie SJ, Yoon S. The role of oxidative stress in neurodegenerative diseases. *Exp Neurobiol* 2015;24:325–40.
25. Ando K, Fujita T. Metabolic syndrome and oxidative stress. *Free Radic Biol Med* 2009;47:213–8.
26. Sosa V, Moline T, Somoza R, Paciucci R, Kondoh H, LLeonart ME. Oxidative stress and cancer: an overview. *Ageing Res Rev* 2013;12:376–90.
27. Kim HK, Nilius B, Kim N, Ko KS, Rhee BD, Han J. Cardiac response to oxidative stress induced by mitochondrial dysfunction. *Rev Physiol Biochem Pharmacol* 2016;170:101–27.
28. Pham-Huy LA, He H, Pham-Huy C. Free radicals, antioxidants in disease and health. *Int J Biomed Sci* 2008;4:89–96.
29. Shim EB, Lee SW, Kim JY, Leem CH, Earm YE. Taeeum-type people in Sasang constitutional medicine have a reduced mitochondrial metabolism. *Integr Med Res* 2012;1:41–5.
30. Shim EB, Lee S, Kim SJ, Leem CH, Kwon YK, Baik Y, et al. [Mitochondria Hypothesis on the obesity-prone tendency in Tae-Eum people]. *Korean J Orient Physiol Pathol* 2009;23:1241–6.
31. Seo DY, Lee S, Figueroa A, Kim HK, Baek YH, Kwak YS, et al. Yoga training improves metabolic parameters in obese boys. *Korean J Physiol Pharmacol* 2012;16:175–80.
32. Dranka BP, Benavides GA, Diers AR, Giordano S, Zelickson BR, Reily C, et al. Assessing bioenergetic function in response to oxidative stress by metabolic profiling. *Free Radic Biol Med* 2011;51:1621–35.
33. Jeong S, Song I, Kim H, Lee SR, Song S, Suh H, et al. An analogue of resveratrol HS-1793 exhibits anticancer activity

against MCF-7 cells via inhibition of mitochondrial biogenesis gene expression. *Mol Cells* 2012;34:357–65.
34. Andrews S. FastQC A quality control tool for high throughput sequence data. http://www.bioinformatics.babraham.ac.uk/projects/fastqc. Accessed 2 May 2016.
35. Li H, Durbin R. Fast and accurate short read alignment with Burrows–Wheeler transform. *Bioinformatics* 2009;25:1754–60.
36. McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, et al. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res* 2010;20:1297–303.
37. Cingolani P, Platts A, Wang LL, Coon M, Nguyen T, Wang L, et al. A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of *Drosophila melanogaster* strain w1118; iso-2; iso-3. *Fly (Austin)* 2012;6:80–92.
38. Blake J, Christie KR, Dolan M, Drabkin HJ, Hill DP, Ni L, et al. Gene Ontology Consortium: going forward. *Nucleic Acids Res* 2015;43:D1049–56.
39. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci U S A* 2005;102:15545–50.
40. Menche J, Sharma A, Kitsak M, Ghiassian SD, Vidal M, Loscalzo J, et al. Disease networks. Uncovering disease–disease relationships through the incomplete interactome. *Science* 2015;347:1257601.
41. Lee TG, Koh B, Lee S. Sasang constitution as a risk factor for diabetes mellitus: a cross-sectional study. *Evid Based Complement Alternat Med* 2009;6:99–103.
42. Pham DD, Do JH, Ku B, Lee HJ, Kim H, Kim JY. Body mass index and facial cues in sasang typology for young and elderly persons. *Evid Based Complement Alternat Med* 2011;2011:749209.
43. Baek Y, Park K, Lee S, Jang E. The prevalence of general and abdominal obesity according to sasang constitution in Korea. *BMC Complement Altern Med* 2014;14:298.
44. Choi K, Lee J, Yoo J, Lee E, Koh B. Sasang constitutional types can act as a risk factor for insulin resistance. *Diabetes Res Clin Pract* 2011;91:e57–60.
45. Cho NH, Kim JY, Kim SS, Shin C. The relationship of metabolic syndrome and constitutional medicine for the prediction of cardiovascular disease. *Diabetes Metab Syndr* 2013;7:226–32.
46. Toth PP. High-density lipoprotein and cardiovascular risk. *Circulation* 2004;109:1809–12.
47. Bostrom P, Wu J, Jedrychowski MP, Korde A, Ye L, Lo JC, et al. A PGC1-alpha-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. *Nature* 2012;481:463–8.
48. Liu J, Hu Y, Zhang H, Xu Y, Wang G. Exenatide treatment increases serum irisin levels in patients with obesity and newly diagnosed type 2 diabetes. *J Diabetes Complications* 2016;30:1555–9.
49. Du XL, Jiang WX, Lv ZT. Lower circulating irisin level in patients with diabetes mellitus: a systematic review and meta-analysis. *Horm Metab Res* 2016;48:644–52.
50. Tsuchiya Y, Ando D, Goto K, Kiuchi M, Yamakita M, Koyama K. High-intensity exercise causes greater irisin response compared with low-intensity exercise under similar energy consumption. *Tohoku J Exp Med* 2014;233:135–40.
51. Dabkowski ER, Williamson CL, Bukowski VC, et al. Diabetic cardiomyopathy-associated dysfunction in spatially distinct mitochondrial subpopulations. *Am J Physiol Heart Circ Physiol* 2009;296:H359–69.
52. Bugger H, Abel ED. Mitochondria in the diabetic heart. *Cardiovasc Res* 2010;88:229–40.
53. Hartman ML, Shirihai OS, Holbrook M, Xu G, Kocherla M, Shah A, et al. Relation of mitochondrial oxygen consumption in peripheral blood mononuclear cells to vascular function in type 2 diabetes mellitus. *Vasc Med* 2014;19:67–74.