Author's response to reviews

Title: Susceptible Gene of Stasis-Stagnation Constitution from Genome-Wide Association Study Related to Cardiovascular Disturbance and Possible Regulated Traditional Chinese Medicine

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Author's response to reviews: see over
Answers of the comment

Title: Susceptible Gene of Stasis-Stagnation Constitution from Genome-Wide Association Study Related to Cardiovascular Disturbance and Possible Regulated Traditional Chinese Medicine

Reviewer: Kyung-Won Hong

Major comment

1. In the introduction, the authors listed the previous genetic reports such as Chen et al, Wang et al., Wu et al. Please include the results and discussion for the previously reported SNPs, candidate genes and the up and down regulated gene regions from the GWAS result.

Ans:

Yes, we revised our article to describe more detail as follows:

“Chen et al reported that allele frequencies of human leukocyte antigens including DPB1*0501 in the Yin-deficiency group (Frequency 51.6% vs 35.6%, relative risk 1.9), DRB1*09012 in the Phlegmwetness group (Frequency 23.4% vs 12.8%, relative risk 2.1), and DQB1*03032 in the Qi-deficiency (Frequency 22.2% vs 8.1%, relative risk 3.2) and Phlegm-wetness groups (Frequency 19.9% vs 8.1%, relative risk 2.8) differ significantly from those in the normal constitution [2]. Wang et al conducted an expression array and identified 785 upregulated genes and 954 downregulated genes in the Yang-deficiency constitution, compared with those in normal individuals. The most significant enriched Gene Ontology Cluster of upregulated genes is “response to stress” which contained interleukin factors and their receptors. The most significant enriched Gene Ontology Cluster of downregulated genes is “nucleobase, nucleoside, nucleotide and nucleic acid metabolism” which contained thyroid hormone receptor signal pathway [3]. A study of polymorphisms further identified the biased distribution of single nucleotide polymorphisms (SNPs) in PPARD (peroxisome proliferator-activated receptors delta) rs2267669 and rs2076167 and APM1 (adipose most abundant gene transcript 1) rs7627128 and rs1063539 in the Yang-deficiency constitution; PPARD rs2076167 and APM1 rs266729 and rs7627128 in Phlegm-wetness constitution; and in PPARG (peroxisome proliferator-activated receptors gamma) Pro12Ala in the Yin-deficiency constitution [4].”

Reference:

2. Generally, GWAS studies used the highest SNP selection based on the adjusted \( p \)-values after controlling the confounding factors. But this paper selected the SNPs from unadjusted \( p \)-values and then they adjusted for the selected SNP only. Please check the other GWAS reports.

**Ans:**

Two-steps GWAS studies have been adapted to publish several papers. For example:


3. They should display the Manhattan plots and quantile-quantile plots for each genetic model test.

**Ans:**

Yes, we revised our paper to add quantile-quantile plots and Manhattan plots in figure 1 and 2.
4. It is difficult to understand the rationale that they predict the PON2 protein structure and the TCM. Based on their GWAS results, the most significant SNP was rs8093481 located on the PIEZO2 gene region. Also, their finding of the exonic variant (rs7493) is the nonsynonymous SNP 'Ser311Cys' or 'Ser299Cys', but they discuss the His114 residue for the TCM target. They should explain the reason.

Ans:

1. Patients with type 2 diabetes have a higher risk of cardiovascular disease. So we particularly focused on the PON2 gene, a cardiovascular risk factor, and performed the PON2 protein structure analysis to screened potential herbal medicine by molecular docking and dynamic simulation. PIEZO2 protein is a component of the mechanosensitive channel. Dysfunction of PIEZO2 may be only one reason of abnormal sensations of YZ constitution.

2. In the study of Sebastian et al. (1), they indicated that the polymorphism of Ser/Cys$^{311}$ could impaired PON2 activity claimed by Stoltz et al. (2); however, the effect did not observed in the experiment of Sebastian et al., but His$^{114}$ is essential for PON2 activity. Due to the polymorphism of Ser/Cys$^{311}$ of PON2 is controversial; hence, we considered residue His$^{114}$ as key residues for TCM target.


Minor comment

Page 3, Line 6: remove a redundant constitution from 'YZ constitution constitution'.

**Ans:**
Yes, we corrected the sentence as follow:
“Components of the YZ constitution were assessed by a self-reported questionnaire.”

About problems of English, we have sent our manuscript to receive English editing.

Page 3, Line 7: 'Whole genome Genotyping ..’ should be changed to
'Genome-wide SNP genotypes were obtained using the Illumina HumanHap550 platform'

**Ans:**
Yes, we corrected the sentence as reviewer’s suggestion, thank’s.

Page 4, Line 9: Please include the reference for the Huang Di Nei Jing textbook.

**Ans:**
Yes,
The reason for “According to *Huang Di Nei Jing*, a textbook of TCM internal medicine written approximately 2,000 years ago, a certain constitution is partially developed from congenital factors” we referred to the following book:
Their theory was from the chapter 54 of the Lingshu, *Huang Di Nei Jing* textbook:
“Huang Di: “I would like you to inform me about fertilization. What is the energy that constitutes the foundation (base) and what is the energy that serves as shield (protection)?” Qi Bo: “Mother is the foundation, and father, the shield.””
We have added the theory source in our Reference.
Reference:

Page 4, Line 14: Please specify the SNP ID and HLA gene name, and describe the difference the allele frequencies.

**Ans:**
Yes, we revised our article to describe more detail as follows:
“Chen et al reported that allele frequencies of human leukocyte antigens including DPB1*0501 in the Yin-deficiency group (Frequency 51.6% vs 35.6%, relative risk 1.9), DRB1*09012 in the Phlegm-wetness group (Frequency 23.4% vs 12.8%, relative risk 2.1), and DQB1*03032 in the Qi-deficiency (Frequency 22.2% vs 8.1%, relative risk 3.2) and Phlegm-wetness groups.
(Frequency 19.9% vs 8.1%, relative risk 2.8) differ significantly from those in the normal constitution.”

Page 4, Line 17 - : Please include the explanation of the relationship between Yang-deficiency and Yu-Zhi.

**Ans:**
In that report, Wang et al conducted an expression array and identified 785 upregulated genes and 954 downregulated genes in the Yang-deficiency constitution, compared with those in normal individuals. We are sorry that the authors did not explain the relationship between Yang-deficiency and Yu-Zhi (or stagnant blood). We refer to Wang’s report to explain the previous genetic studies in the investigation of congenital factors of other constitution but not Yu-Zhi constitution.

Page 8, Line 17: Please describe the median score.

**Ans:**
Yes, we corrected the sentence to add the median score as follow:
“Participants were divided into high and low YZ score groups according to the median score (YZ score = 10).”

In the Method, Statistical Analysis section: include the statistics software used in this study for GWAS.

**Ans:**
Yes, we revise the Statistical Analysis section of Method as follow to include the statistics software used in this study for GWAS:
“Association analysis was carried out to compare allele frequency and genotype distribution between the high and low YZ score groups using 6 single point methods for each SNP: genotype, allele, trend (Cochran-Armitage test), additive, dominant, and recessive models using PLINK (PLINK 1.07, http://pngu.mgh.harvard.edu/~purcell/plink/contact.shtml#cite) and SAS (SAS Institute Inc., 100 SAS Campus Drive, Cary, NC 27513-2414, USA).”

In Results, please include the individual numbers for each YZ group.

**Ans:**
Yes, we revise the sentence in Result as follow to add the individual numbers for each YZ group: 
“This study enrolled 1,021 patients with type 2 diabetes who were 20 years old or older. Participants were divided into a high YZ score group and low YZ score group according to the median (numbers of high YZ score: low YZ score = 583: 438).”
“Genotyping data were obtained from 947 (numbers of high YZ score: low YZ score = 539: 408) of the 1,021 participants using Illumina HumanHap550duov3 chips.”
In table 2, please list the risk allele frequency, effect sizes and the most significant genetic model for each SNP.

**Ans:**
We have re-edit and add the above information in table 2
<table>
<thead>
<tr>
<th>dbSNP ID</th>
<th>Chr.</th>
<th>Position (Mb)</th>
<th>Gene</th>
<th>RA* (NRA)</th>
<th>RA (Best model)</th>
<th>p-value‡</th>
<th>-log (p-value)</th>
<th>best model</th>
<th>Effect size (95% CI)</th>
<th>FDR</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs12036718</td>
<td>1p</td>
<td>48.3</td>
<td>Unknow</td>
<td>T(C)</td>
<td>0.174</td>
<td>0.103</td>
<td>8.10 × 10⁻⁶</td>
<td>Trend</td>
<td>1.68 (0.95 -2.99 )</td>
<td>1.00</td>
</tr>
<tr>
<td>rs1932064</td>
<td>1p</td>
<td>70.8</td>
<td>Unknow</td>
<td>T(C)</td>
<td>0.788</td>
<td>0.700</td>
<td>6.18 × 10⁻⁶</td>
<td>Dominant</td>
<td>1.62 (1.05 -2.51 )</td>
<td>0.97</td>
</tr>
<tr>
<td>rs8179355</td>
<td>1p</td>
<td>70.9</td>
<td>Unknow</td>
<td>C(A)</td>
<td>0.782</td>
<td>0.699</td>
<td>7.53 × 10⁻⁶</td>
<td>Recessive</td>
<td>1.54 (1.25 -1.89 )</td>
<td>0.86</td>
</tr>
<tr>
<td>rs9633289</td>
<td>1p</td>
<td>70.9</td>
<td>Unknow</td>
<td>T(A)</td>
<td>0.217</td>
<td>0.298</td>
<td>6.18 × 10⁻⁶</td>
<td>Recessive</td>
<td>1.55 (1.11 -2.15 )</td>
<td>0.95</td>
</tr>
<tr>
<td>rs7565310</td>
<td>2q</td>
<td>128.9</td>
<td>Unknow</td>
<td>A(G)</td>
<td>0.484</td>
<td>0.380</td>
<td>8.63 × 10⁻⁶</td>
<td>Trend</td>
<td>1.53 (1.27 -1.84 )</td>
<td>0.83</td>
</tr>
<tr>
<td>rs7694118</td>
<td>4q</td>
<td>134.3</td>
<td>PCDH10</td>
<td>C(T)</td>
<td>0.698</td>
<td>0.663</td>
<td>6.31 × 10⁻⁶</td>
<td>Genotype</td>
<td>1.30 (0.85 -1.99 )</td>
<td>1.00</td>
</tr>
<tr>
<td>rs164368</td>
<td>5q</td>
<td>160.6</td>
<td>Unknow</td>
<td>T(C)</td>
<td>0.803</td>
<td>0.785</td>
<td>6.14 × 10⁻⁶</td>
<td>Genotype</td>
<td>1.12 (0.89 -1.40 )</td>
<td>1.00</td>
</tr>
<tr>
<td>rs7493</td>
<td>7q</td>
<td>94.9</td>
<td>PON2</td>
<td>C(G)</td>
<td>0.180</td>
<td>0.177</td>
<td>5.33 × 10⁻⁶</td>
<td>Genotype</td>
<td>1.06 (0.74 -1.51 )</td>
<td>1.00</td>
</tr>
<tr>
<td>rs2299263</td>
<td>7q</td>
<td>94.9</td>
<td>PON2</td>
<td>A(G)</td>
<td>0.180</td>
<td>0.177</td>
<td>5.33 × 10⁻⁶</td>
<td>Genotype</td>
<td>1.06 (0.74 -1.51 )</td>
<td>1.00</td>
</tr>
<tr>
<td>rs17166875</td>
<td>7q</td>
<td>94.9</td>
<td>PON2</td>
<td>T(C)</td>
<td>0.180</td>
<td>0.177</td>
<td>5.33 × 10⁻⁶</td>
<td>Genotype</td>
<td>1.02 (0.81 -1.29 )</td>
<td>1.00</td>
</tr>
<tr>
<td>rs12865228</td>
<td>13q</td>
<td>38.2</td>
<td>FREM2</td>
<td>G(T)</td>
<td>0.429</td>
<td>0.414</td>
<td>3.38 × 10⁻⁶</td>
<td>Genotype</td>
<td>1.06 (0.88 -1.28 )</td>
<td>1.00</td>
</tr>
<tr>
<td>rs4526895</td>
<td>13q</td>
<td>38.2</td>
<td>FREM2</td>
<td>C(T)</td>
<td>0.428</td>
<td>0.414</td>
<td>1.79 × 10⁻⁶</td>
<td>Genotype</td>
<td>1.38 (0.93 -2.04 )</td>
<td>1.00</td>
</tr>
<tr>
<td>rs17118382</td>
<td>14q</td>
<td>82.8</td>
<td>Unknow</td>
<td>A(G)</td>
<td>0.815</td>
<td>0.730</td>
<td>9.75 × 10⁻⁶</td>
<td>Allele</td>
<td>1.45 (1.03 -2.04 )</td>
<td>0.98</td>
</tr>
<tr>
<td>rs194045</td>
<td>16p</td>
<td>29.2</td>
<td>Unknow</td>
<td>G(A)</td>
<td>0.943</td>
<td>0.888</td>
<td>6.31 × 10⁻⁶</td>
<td>Trend</td>
<td>2.13 (1.51 -3.02 )</td>
<td>0.83</td>
</tr>
<tr>
<td>rs8093481</td>
<td>18p</td>
<td>10.7</td>
<td>PIEZO2</td>
<td>A(G)</td>
<td>0.757</td>
<td>0.656</td>
<td>9.64 × 10⁻⁷</td>
<td>Trend</td>
<td>1.79 (1.16 -2.77 )</td>
<td>0.93</td>
</tr>
<tr>
<td>rs11660953</td>
<td>18p</td>
<td>10.7</td>
<td>PIEZO2</td>
<td>T(C)</td>
<td>0.756</td>
<td>0.656</td>
<td>1.60 × 10⁻⁶</td>
<td>Trend</td>
<td>1.79 (1.16 -2.77 )</td>
<td>0.93</td>
</tr>
<tr>
<td>rs1133146</td>
<td>19q</td>
<td>58.4</td>
<td>ZNF665</td>
<td>A(G)</td>
<td>0.990</td>
<td>0.956</td>
<td>4.77 × 10⁻⁶</td>
<td>Allele</td>
<td>1.52 (1.01 -2.29 )</td>
<td>0.99</td>
</tr>
<tr>
<td>rs12971799</td>
<td>19q</td>
<td>58.4</td>
<td>ZNF665</td>
<td>C(T)</td>
<td>0.700</td>
<td>0.599</td>
<td>4.77 × 10⁻⁶</td>
<td>Allele</td>
<td>1.49 (0.99 -2.25 )</td>
<td>1.00</td>
</tr>
<tr>
<td>rs4801958</td>
<td>19q</td>
<td>58.4</td>
<td>ZNF665</td>
<td>T(C)</td>
<td>0.700</td>
<td>0.599</td>
<td>4.77 × 10⁻⁶</td>
<td>Allele</td>
<td>1.56 (1.29 -1.89 )</td>
<td>0.83</td>
</tr>
<tr>
<td>rs12460170</td>
<td>19q</td>
<td>58.4</td>
<td>ZNF665</td>
<td>G(A)</td>
<td>0.699</td>
<td>0.600</td>
<td>7.56 × 10⁻⁶</td>
<td>Allele</td>
<td>1.56 (1.29 -1.89 )</td>
<td>0.83</td>
</tr>
<tr>
<td>rs4803055</td>
<td>19q</td>
<td>58.4</td>
<td>ZNF665</td>
<td>C(T)</td>
<td>0.737</td>
<td>0.639</td>
<td>4.87 × 10⁻⁶</td>
<td>Allele</td>
<td>1.56 (1.02 -2.38 )</td>
<td>0.98</td>
</tr>
<tr>
<td>rs871913</td>
<td>20p</td>
<td>16.1</td>
<td>Unknow</td>
<td>A(G)</td>
<td>0.099</td>
<td>0.045</td>
<td>8.24 × 10⁻⁶</td>
<td>Trend</td>
<td>1.86 (0.78 -4.44 )</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Case number: High YZ score = 538, Low YZ score = 409

dbSNP ID, SNP database identification; Chr, chromosome; RA, risk allele; NRA, nonrisk allele; YZ, Yu-Zhi; FDR, false discovery rate; PCDH10, protocadherin 10; PON2, paraoxonase 2; FREM2, FRAS1 related extracellular matrix protein 2; BANP, BTG3 associated nuclear protein; PIEZO2, piezo-type mechanosensitive ion channel component 2; ZNF665, zinc finger protein 665

*Risk allele is the allele with higher frequency in subjects with high Yu-Zhi score compared with low Yu-Zhi score

‡p-value (best model) is the p-value of the most significant statistic obtained from 6 models: genotype, allele, trend, additive, dominant, and recessive
In the supplementary materials, please include the questionnaire item.

**Ans:**
Yes, Questionnaire items for measuring Yu-Zhi constitution was added to the supplementary materials.

Questionnaire items for measuring Yu-Zhi constitution

1. I feel numbness in the limbs.
2. I feel chest tightness or my chest seems to be oppressed by something.
3. There is tingling pain in my body which makes me uncomfortable.
4. I have a dull sensation or pain over the lateral side (costal region) of my body.
5. Bruises appear on my skin without an apparent cause.
6. My skin gets dry, cracked, scaly, or tough.
7. My face feels dull and lustreless.
8. My lips or tongue have a dull purple color, or I find petechiae on them.
**Reviewer:** Bu-Yeo Kim

**Major Compulsory Revisions**

1. Authors should focus more on YZ constitution. What does the YZ constitution mean in terms of biological and physiological aspects? Any population can be scored on YZ constitution? Then why authors selected only type 2 diabetes population. Can authors obtain same result with normal population? In table 1, age and sex were associated with YZ constitution. Does this result mean the biased recruitment of samples?

   **Ans:**
   1. We revised this portion in introduction as follow to explained YZ constitution more detail:
      “The Yu-Zhi (YZ) constitution in TCM indicates stasis and stagnation, which expressed dull, lusterless skin color; dry, cracked, scaly or tough skin; dull purple lips or tongue; localized pain or numbness; knotted, intermittent, or uneven pulse. It is one of the body constitutions that tend to express blood stasis syndrome (BSS), a morbid state caused by blood circulation disturbance, included extravasated blood, blood circulating sluggishly, or blood congested in viscera, that may turn into pathogenic factors.”
   2. Any population can be scored on YZ constitution but we selected type 2 diabetes because this population has a higher risk of cardiovascular disease that we have more opportunity to discover a gene that associated cardiovascular risk. Normal population is fewer ratio of YZ constitution and fewer ratios of cardiovascular events. We may need very large population to obtain the same result.
   3. In traditional Chinese medicine theories, constitutions were not constant; it could slowly progress due to acquired factors. Aging is an important factor for patients to express YZ constitution. On the other hand, we put these parameters into multivariate logistic regression model to adjust the bias.

2. The risk of rs7493 polymorphism on coronary artery disease has been known in normal population. Authors should explain the meaning of rs7493 polymorphism associated with YZ constitution in type 2 diabetes population.

   **Ans:**
   We have explained that in Discussion and summary as follow:
   1. The rs7493 located in PON2 on chromosome 7q, belong to one of the paraoxonase (PON) gene families, and has antioxidative and antiatherosclerotic properties.
   2. PON2 has been shown to prevent LDL oxidation, to reverse the oxidation of mildly oxidized LDL, inhibit oxidized LDL-induced monocyte chemotaxis, increases cholesterol efflux and decreases the size of atherosclerotic lesions, protects electron transport chain complexes against oxidative stress.
   3. PON2 plays a role in hepatic insulin signaling. PON2 deficiency is associated with inhibitory insulin-mediated phosphorylation of hepatic insulin receptor substrate-1. PON2 may enhance
the influence of the macrophage-mediated inflammatory response in hepatic insulin sensitivity.

4. Type 2 diabetes patients with a strongly YZ constitution may have PON2 polymorphism with a low protein function, resulting in cardiovascular disturbance and hyperglycemia.

3. If authors want to identify drug candidates against specific protein like PON2, experimental supports should be presented. Although all figures focused on this issue, only in silico result was presented here.

**Ans:**

In fact, we hope to identify a target gene and regulated herbal medicine or nature compound to improve cardiovascular risk through the improvement of pathophysiology of YZ constitution. Further experiment will be conducted in future.

4. Analytical methods for GWAS should be explained in detail. How many SNPs were included in analysis? The p-values does not consider to be statistically significant in Table 2. Multiple adjustment result (FDR etc.) for GWAS was missing. What is the definition of nearest gene?

**Ans:**

1. We had written the following data in Methods, Genotyping section:

   “A total of 560,184 SNPs were genotyped, 38,700 SNPs were excluded due to quality control criteria, 12,723 SNPs were excluded due to Hardy-Weinberg equilibrium principle (P <0.0001) and 508,761 SNPs were used in final analysis.”

2. We rewritten this portion in Result as following:

   “SNPs in autosomal chromosomes with a p-value < 9.8 × 10^-8 were not detected in all the 6 statistical models. Table 2 summarizes the SNPs selected from results showing p-values < 10^-5 under the most significant test statistic obtained from any of the 6 statistical models. However, the measured false discovery rate was high.”

3. We have to re-edit and add relevant information in table 2

4. We are sorry that our detected SNPs are really located in the gene. So we corrected the “nearest gene” to “gene” in table 2 and table 3
## Table 2 Summary of the SNPs associated with high Yu-Zhi score in type 2 diabetes

<table>
<thead>
<tr>
<th>dbSNP ID</th>
<th>Chr.</th>
<th>Position (Mb)</th>
<th>Gene</th>
<th>RA* (NRA)</th>
<th>RA frequency</th>
<th>-log (p-value)</th>
<th>p-value‡ (Best model)</th>
<th>FDR</th>
<th>Effect size (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs12036718</td>
<td>1p</td>
<td>48.3</td>
<td>Unknow</td>
<td>T(C)</td>
<td>0.174</td>
<td>5.09</td>
<td>Trend</td>
<td></td>
<td>1.68 (0.95 -2.99)</td>
</tr>
<tr>
<td>rs1932064</td>
<td>1p</td>
<td>70.8</td>
<td>Unknow</td>
<td>T(C)</td>
<td>0.788</td>
<td>5.21</td>
<td>Dominant</td>
<td>0.97</td>
<td>1.62 (1.05 -2.51)</td>
</tr>
<tr>
<td>rs8179355</td>
<td>1p</td>
<td>70.9</td>
<td>Unknow</td>
<td>C(A)</td>
<td>0.782</td>
<td>5.12</td>
<td>Recessive</td>
<td>0.86</td>
<td>1.54 (1.25 -1.89)</td>
</tr>
<tr>
<td>rs9633289</td>
<td>1p</td>
<td>70.9</td>
<td>Unknow</td>
<td>T(A)</td>
<td>0.217</td>
<td>5.21</td>
<td>Recessive</td>
<td>0.95</td>
<td>1.55 (1.11 -2.15)</td>
</tr>
<tr>
<td>rs7565310</td>
<td>2q</td>
<td>128.9</td>
<td>Unknow</td>
<td>A(G)</td>
<td>0.484</td>
<td>5.06</td>
<td>Trend</td>
<td>0.83</td>
<td>1.53 (1.27 -1.84)</td>
</tr>
<tr>
<td>rs7694118</td>
<td>4q</td>
<td>134.3</td>
<td>PCDH10</td>
<td>C(T)</td>
<td>0.698</td>
<td>5.20</td>
<td>Genotype</td>
<td></td>
<td>1.30 (0.85 -1.99)</td>
</tr>
<tr>
<td>rs164368</td>
<td>5q</td>
<td>160.6</td>
<td>Unknow</td>
<td>T(C)</td>
<td>0.803</td>
<td>5.21</td>
<td>Genotype</td>
<td></td>
<td>1.12 (0.89 -1.40)</td>
</tr>
<tr>
<td>rs7493</td>
<td>7q</td>
<td>94.9</td>
<td>PON2</td>
<td>A(G)</td>
<td>0.180</td>
<td>5.27</td>
<td>Genotype</td>
<td></td>
<td>1.06 (0.74 -1.51)</td>
</tr>
<tr>
<td>rs2299263</td>
<td>7q</td>
<td>94.9</td>
<td>PON2</td>
<td>A(G)</td>
<td>0.180</td>
<td>5.27</td>
<td>Genotype</td>
<td></td>
<td>1.02 (0.81 -1.29)</td>
</tr>
<tr>
<td>rs17166875</td>
<td>7q</td>
<td>94.9</td>
<td>PON2</td>
<td>T(C)</td>
<td>0.180</td>
<td>5.27</td>
<td>Genotype</td>
<td></td>
<td>1.06 (0.74 -1.51)</td>
</tr>
<tr>
<td>rs12865228</td>
<td>13q</td>
<td>38.2</td>
<td>FREM2</td>
<td>G(T)</td>
<td>0.429</td>
<td>5.47</td>
<td>Genotype</td>
<td></td>
<td>1.06 (0.88 -1.28)</td>
</tr>
<tr>
<td>rs4526895</td>
<td>13q</td>
<td>38.2</td>
<td>FREM2</td>
<td>C(T)</td>
<td>0.428</td>
<td>5.75</td>
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<td>1.38 (0.93 -2.04)</td>
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<tr>
<td>rs17118382</td>
<td>14q</td>
<td>82.8</td>
<td>Unknow</td>
<td>A(G)</td>
<td>0.815</td>
<td>5.01</td>
<td>Allele</td>
<td>0.98</td>
<td>1.45 (1.03 -2.04)</td>
</tr>
<tr>
<td>rs194045</td>
<td>16p</td>
<td>29.2</td>
<td>Unknow</td>
<td>G(A)</td>
<td>0.943</td>
<td>5.20</td>
<td>Trend</td>
<td>0.83</td>
<td>2.13 (1.51 -3.02)</td>
</tr>
<tr>
<td>rs8093481</td>
<td>18p</td>
<td>10.7</td>
<td>PIEZO2</td>
<td>A(G)</td>
<td>0.757</td>
<td>6.02</td>
<td>Trend</td>
<td></td>
<td>1.79 (1.16 -2.77)</td>
</tr>
<tr>
<td>rs11660953</td>
<td>18p</td>
<td>10.7</td>
<td>PIEZO2</td>
<td>T(C)</td>
<td>0.756</td>
<td>5.79</td>
<td>Trend</td>
<td>0.93</td>
<td>1.79 (1.16 -2.77)</td>
</tr>
<tr>
<td>rs1133146</td>
<td>19q</td>
<td>58.4</td>
<td>ZNF665</td>
<td>A(G)</td>
<td>0.990</td>
<td>5.32</td>
<td>Allele</td>
<td>0.99</td>
<td>1.52 (1.01 -2.29)</td>
</tr>
<tr>
<td>rs12971799</td>
<td>19q</td>
<td>58.4</td>
<td>ZNF665</td>
<td>C(T)</td>
<td>0.700</td>
<td>5.32</td>
<td>Allele</td>
<td></td>
<td>1.49 (0.99 -2.25)</td>
</tr>
<tr>
<td>rs4801958</td>
<td>19q</td>
<td>58.4</td>
<td>ZNF665</td>
<td>T(C)</td>
<td>0.700</td>
<td>5.32</td>
<td>Allele</td>
<td></td>
<td>1.56 (1.29 -1.89)</td>
</tr>
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<td>ZNF665</td>
<td>G(A)</td>
<td>0.699</td>
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<td>Allele</td>
<td></td>
<td>1.56 (1.29 -1.89)</td>
</tr>
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<td>58.4</td>
<td>ZNF665</td>
<td>G(C)</td>
<td>0.737</td>
<td>5.31</td>
<td>Allele</td>
<td></td>
<td>1.56 (1.02 -2.38)</td>
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<tr>
<td>rs871913</td>
<td>20p</td>
<td>16.1</td>
<td>Unknow</td>
<td>A(G)</td>
<td>0.099</td>
<td>5.08</td>
<td>Trend</td>
<td>1.00</td>
<td>1.86 (0.78 -4.44)</td>
</tr>
</tbody>
</table>

Case number: High YZ score = 538, Low YZ score = 409

dbSNP ID, SNP database identification; Chr, chromosome; RA, risk allele; NRA, nonrisk allele; YZ, Yu-Zhi; FDR, false discovery rate; PCDH10, protocadherin 10; PON2, paraoxonase 2; FREM2, FRAS1 related extracellular matrix protein 2; BANP, BTG3 associated nuclear protein; PIEZO2, piezo-type mechanosensitive ion channel component 2; ZNF665, zinc finger protein 665

*Risk allele is the allele with higher frequency in subjects with high Yu-Zhi score compared with low Yu-Zhi score

‡p-value (best model) is the p-value of the most significant statistic obtained from 6 models: genotype, allele, trend, additive, dominant, and recessive
5. Many figures should be moved to supplementary figures.

Ans:
After discussion of our team, we considered that each figure has its own importance. Since manuscript does not restrict the length, we hope to reserve all figures in the manuscript for that we could explain more detail.

6. Results and Discussion is too long. It should be concise.

Ans:
We have removed the less important portion of Result and Discussion to make them more concise. Since manuscript does not restrict the length, we hope to reserve the remaining part to explain as clear as possible.

The revise area including:

Result
“Genotyping data were obtained from 947 (numbers of high YZ score: low YZ score = 539: 408) of the 1,021 participants using Illumina HumanHap550duov3 chips. Quantile–quantile plots for each model were shown that the distribution of observed p-values deviated from expected p-values in Figure 1. Manhattan plots of p-values across all chromosomes for each model were shown in Figure 2. SNPs in autosomal chromosomes with a p-value < 9.8 × 10-8 were not detected in all the 6 statistical models. Table 2 summarizes the SNPs selected from results showing p-values < 10-5 under the most significant test statistic obtained from any of the 6 statistical models. However, the false discovery rate was high. The SNP rs7694118 is located on chromosome 4 in the 5’ untranslated region (UTR) of PCDH10 (protocadherin 10). The SNP rs7493 is located on chromosome 7 in an exon region of PON2 (paraoxonase 2) and is in complete linkage disequilibrium with rs2299263 and rs17166875 (D’=1.0, r2=1.0) in intron regions. The SNP rs4526895 is in tight linkage disequilibrium with rs12865228 (D’ = 1; r2 = 0.97). Two of the SNPs are located in an intron of FREM2 (FRAS1 [Fraser syndrome 1] related extracellular matrix protein 2) on chromosome 13. The SNP rs8093481 was strongly associated with the YZ constitution (p = 9.64 × 10-7) and in complete linkage disequilibrium with rs11660953 (D’ = 1; r2 = 1). Two of the SNPs are located in an intron region of the PIEZO2 (piezo-type mechanosensitive ion channel component 2) gene on chromosome 18. The SNP rs4801958 is located on chromosome 19 in an exon region of the ZNF665 (zinc finger protein 665) and is completely linked with rs12460170 (D’=1.0, r2=1.0), which is also in an exon region. It is also tightly linked with rs12971799 (D’=1.0, r2=0.989) and rs1133146 (D’=1.0, r2=0.989) in the 3’ UTR, and with rs4803055 (D’=0.987, r2=0.824) in an intron region of the ZNF665.”

Discussion
“The rs7493, rs2299263, and rs17166875 polymorphisms, located in PON2 on chromosome 7q, belong to one of the paraoxonase (PON) gene families, which encode enzymes participating in the hydrolysis of organophosphates. The PON gene cluster contains 3 adjacent gene members,
PON1, PON2, and PON3. All 3 PON genes share high sequential homology and a similar β propeller protein structure [50] and are thought to have antiatherosclerotic properties. Thus the PON gene cluster has been considered a target in the treatment of atherosclerosis [51, 52]. PON2 has been shown to prevent LDL oxidation, to reverse the oxidation of mildly oxidized LDL, and to inhibit oxidized LDL-induced monocyte chemotaxis [53]. It also increases cholesterol efflux [54] and decreases the size of atherosclerotic lesions [55].

PON2 is a ubiquitously expressed intracellular protein that is expressed in a wide range of tissues [56, 53]. PON2 exhibits antioxidant functions at the cellular level, in addition to a host of intracellular antioxidative enzymes that act against oxidative stress. PON2 is localized in the inner mitochondrial membrane, associated with respiratory complex III, and binds with high affinity to coenzyme Q10. Decreased activity of mitochondrial electron transport chain (ETC) complexes is implicated in the development of many inflammatory diseases, including atherosclerosis. PON2 protects ETC complexes against oxidative stress by lowering reactive oxygen species. The intracellular antioxidative effect plays a role in antiatherosclerosis by avoiding endothelial dysfunction caused by mitochondria dysfunction [57, 58]. A common polymorphism rs7493, also known as Ser311Cys, a missense SNP in PON2, has also been associated with the risk of CAD [59]. In addition, PON2 plays a role in hepatic insulin signalling. PON2-deficient mice display elevated hepatic oxidative stress, coupled with an exacerbated inflammatory response, because of PON2-deficient macrophages. PON2 deficiency is associated with inhibitory insulin-mediated phosphorylation of hepatic insulin receptor substrate-1. PON2 may enhance the influence of the macrophage-mediated inflammatory response in hepatic insulin sensitivity [60]. The PON2 G148 variant has been associated with elevated fasting plasma glucose in patients with type 2 diabetes [61]. The role of PON2 provides the genetic basis underlying the YZ constitution. Patients with a strongly YZ constitution may have PON2 polymorphism with a low protein function which tends to decrease its antioxidative efficacy, resulting in cardiovascular disturbance and hyperglycemia. Thus, PON2 may be a candidate gene for the YZ constitution. Treatment using herbal medicines or natural compounds that could potentially regulate PON2 might be useful in protecting type 2 diabetes patients with a YZ constitution from cardiovascular complications."

Minor Essential Revisions

1. This manuscript is not the first report applying GWAS in TCM with large sample size. Refer to the following paper published in J Altern Complement Med. 2012;18(3):262-9.

   **Ans:**
   Yes, we have revised the sentence and added this reference in our manuscript.
   “This technique had been adapted to explore the genetic base of Korean Sasang constitutional medicine [49].”

   **Reference:**

2. English should be corrected in many sentences.

   **Ans:**
   Yes, we have sent our manuscript to receive English editing. All the English errors has been corrected.