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RAPID COMMUNICATION

Investigation of prodromal features in metabolic syndrome based on transcriptome analysis

Metabolic syndrome (MetS) is a complex condition that significantly increases the risk of several diseases, including heart disease and diabetes. Patients with MetS show both pro-inflammatory and pro-thrombotic states; however, the state that takes precedence in MetS progression is unclear. The molecular mechanisms underlying the prodromal features in MetS have still not been well-elucidated. In this cohort-based study, we analyzed the characteristics of pre-MetS by dividing 100 participants into three groups: normal, pre-MetS, and MetS. We performed a systematic transcriptome analysis using RNA-seq data obtained from the peripheral blood of individuals. Gene expression and pathway enrichment analyses revealed that immune-related dysregulation was prominent in the pre-MetS group. In contrast, the MetS group showed significant coagulation-related dysregulation. These results suggest that the pro-inflammatory state may be a prodromal feature of pre-MetS, followed by a pro-thrombotic state during progression to MetS. Our findings may contribute to the prevention of MetS progression by encouraging the development of diagnostic and therapeutic strategies that target the identified pre-MetS-associated genes and pathways.

Pro-inflammatory and pro-thrombotic states are the two main characteristics common to patients with MetS.[1] To investigate the molecular signature of prodromal features of MetS, we recruited 100 participants from the Korean Medicine Daejeon Citizen Cohort (KDCC) study[2] (see Supplementary Methods). Clinical information for the participants was obtained, including the five risk factors of MetS: (1) systolic and diastolic blood pressure; (2) glucose level; (3) HDL cholesterol level; (4) triglyceride level; and (5) waist circumference. Based on gender-adjusted Korean-specific diagnostic criteria, MetS was defined as having three or more of these risk factors above the cut-off point, and normal subjects as having all risk factors below the cut-off point. The remaining patients were assigned to the intermittent group, which was defined as pre-MetS with 1–2 risk factors exceeding the cut-off.[3,4] Using the above definitions, the 100 participants were classified as normal (33), pre-MetS (42), and MetS (25) (Fig. 1A; Fig. S1, and Table S1).

To identify the molecular signatures of pre-MetS, we analyzed RNA-seq data from the peripheral blood of the 100 participants. We identified differentially expressed genes (DEGs) in the pre-MetS and MetS groups compared to the normal group, termed PvsN and MvsN, respectively (Fig. S2, left or middle; Data S1). We also identified DEGs between the MetS and pre-MetS groups, termed MvsP (Fig. S2, right; Data S1). DEGs ($P < 0.05$) in the three comparisons were further categorized as those with either downregulated or upregulated expression (Data S2). We observed that PvsN and MvsN DEGs with upregulated or downregulated expression were relatively distinct with little overlap (left panels of Figure. 1B; Fig. S3). MvsP DEGs overlapped with MvsN DEGs but not with PvsN DEGs. These results suggest that MetS progression is not a linear process, and none of the genes exhibited a significant successive change in expression from normal to pre-MetS and from pre-MetS to MetS. Five and nine common DEGs were upregulated or downregulated in both pre-MetS and MetS, respectively (right panels of Fig. 1B; Fig. S3). The expression of these common genes was dysregulated in pre-MetS, and their altered expression was maintained after the disease progressed to MetS.

For DEGs with upregulated and downregulated expression in all three comparisons, we performed pathway enrichment analysis by EnrichR using curated gene sets (Data S3). The Venn diagram for the significantly enriched (adjusted $P < 0.05$) pathways of DEGs with upregulated expression (Fig. 1C, left) showed a similar pattern to the Venn diagram for DEGs. Compared to those of the normal group, the upregulated pathways of pre-MetS and MetS were rather specific. The two common pathways were

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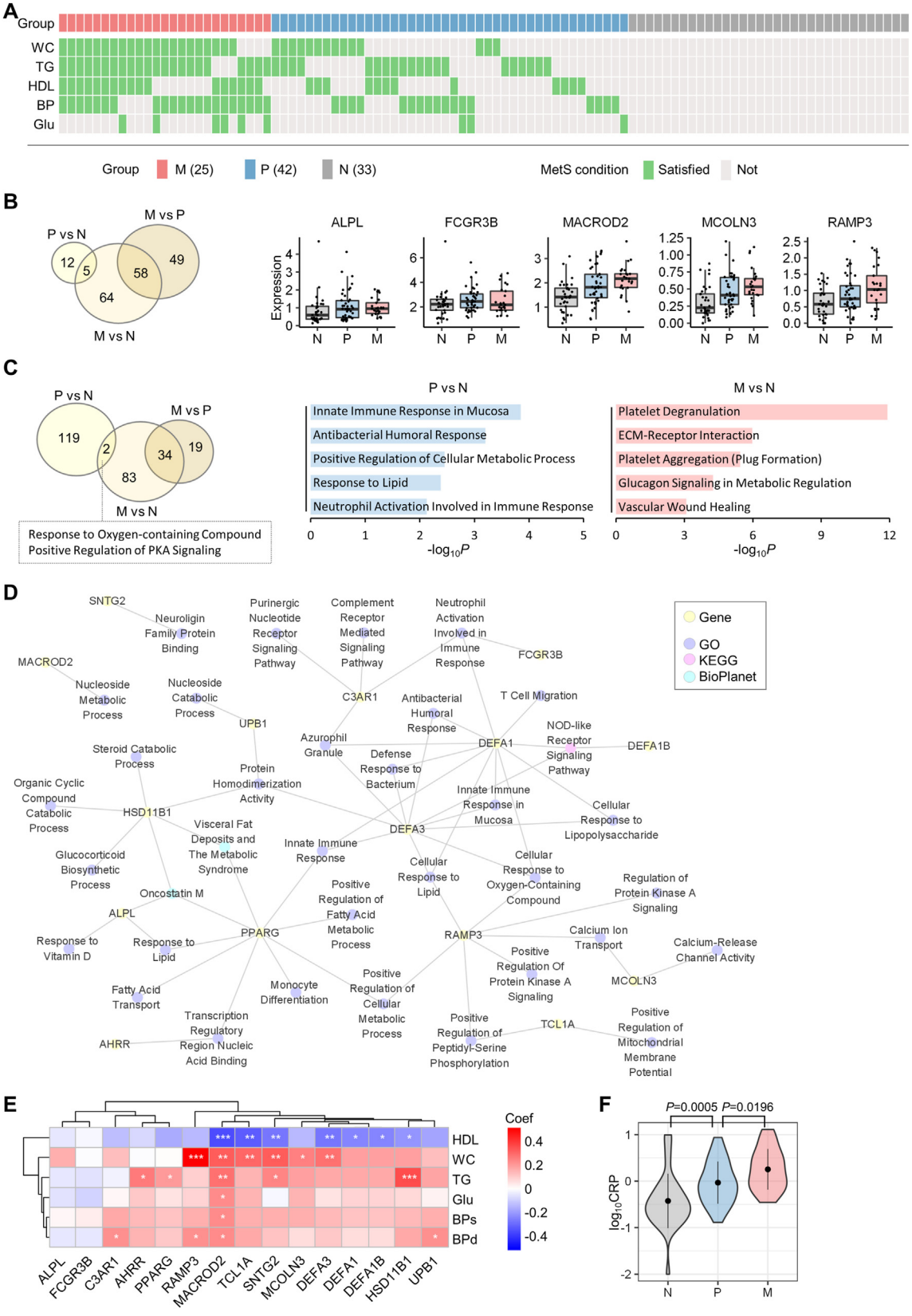

Figure 1 Systematic transcriptome analysis of pre-MetS. **(A)** The five risk factors for metabolic syndrome in the 100 participants. Green or light gray color denotes satisfaction of criteria or not, respectively. The subjects were categorized into three groups depending on the number (*n*) of satisfied conditions: N ($n = 0$), P ($n = 1$ or 2), and M ($n = 3$ or more). **(B)** Venn diagrams showed the number of genes with significantly upregulated expression in the three comparisons: P *vs*. N, M *vs*. N, and M *vs*. P (left). Expression levels of DEGs commonly upregulated between P *vs*. N and M *vs*. N comparisons (right). **(C)** Venn diagrams showed the number of

associated with the response to oxygen-containing compounds and protein kinase A signaling, which regulates glycogen, sugar, and lipid metabolism. The common DEG with upregulated expression, *RAMP3*, was involved in both pathways. A comparison of the upregulated pathways in pre-MetS and MetS revealed different characteristics at different stages (Fig. 1C, right). Pre-MetS was characterized by immune-related pathways, including innate immune responses in mucosa, immune responses against bacteria, and neutrophil activation. In contrast, MetS was characterized by thrombosis-related pathways, including platelet degranulation, plug formation, and vascular wound healing. Additionally, MetS was characterized by glucagon signaling-associated pathways and interactions between the extracellular matrix and membrane receptors.

In contrast to the enriched pathways of DEGs with downregulated expression (Fig. S4), which showed relative overlap with each other, the enriched pathways of PvsN DEGs with upregulated expression were specific to pre-MetS. Thus, to further investigate the differentiating characteristics of pre-MetS, we examined a total of 121 pathways that were significantly enriched for PvsN DEGs with upregulated expression (Data S2). We reconstructed the integrated functional network of pre-MetS by simplifying the equivalent pathway terms and linking them with the associated DEGs (Fig. 1D). Several genes, such as *DEFA1*, *DEFA3*, *PPARG*, *FCGR3B*, and *C3AR1*, were linked to multiple immune-related pathways highlighted in Figure 1C, including antibacterial humoral response and cellular response to lipopolysaccharide. *PPARG*, along with *HSD11B1*, has been linked to pathways associated with visceral fat accumulation, a risk factor for MetS. In addition, *MACROD2*, *RAMP3*, and *UPB1* were linked to cellular response to lipid and regulation of metabolic and catabolic pathways.

To characterize the pre-MetS-associated genes, we further analyzed the association between the expression profile of these genes and risk factors of MetS (Data S4). Among the DEGs with upregulated expression in pre-MetS, we focused on five DEGs shared with MetS (Fig. 1B) and an additional 10 DEGs associated with enriched pathways (Fig. S5). We found that overall, the expression of most genes was positively associated with blood pressure, glucose and triglyceride levels, and waist circumference but negatively associated with HDL levels (Fig. 1E). Particularly, *MACROD2* expression was significantly associated with all risk factors. Conversely, nine DEGs whose expression was significantly downregulated in the pre-MetS group showed the opposite relationship (Fig. S6). Finally, to confirm that immune system dysregulation had already been initiated in pre-MetS, we investigated the levels of CRP, a plasma marker of chronic low-grade inflammation, finding that CRP levels were significantly increased in pre-MetS and MetS compared to those in the normal group (Fig. 1F).

Taken together, our results showed that aberrations in the immune response predominate as prodromal features in MetS progression (see Supplementary Text). The genes and pathways associated with pre-MetS and increased CRP levels suggest that pre-MetS is an inflammatory condition. Pre-MetS-associated genes may act as transcriptional regulators of obesity, adipogenesis, and immunity, which in turn may act as key metabolic regulators of MetS development. Using these genes as diagnostic markers, it will be possible to identify potential patients who are more likely to develop MetS at an earlier stage and to initiate clinical management in advance.

This study has some limitations: it is a cross-sectional study of a small population, and the additional validation group was from the same cohort study. Therefore, it is necessary to confirm the changes from pre-MetS to MetS through individual follow-up investigations. To further clarify the mechanism underlying MetS progression, the identified pre-MetS-associated genes and pathways need to be validated in follow-up cohorts as well as in independent cohorts in future large-scale studies. In addition, future studies may reveal whether nutritional/pharmacological interventions that target pre-MetS-associated genes and their related pathways could help reduce the incidence of MetS.

Conflict of interests

Authors declare no conflict of interests.

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Author contributions

Sang-Min Park: Conceptualization, methodology, formal analysis, visualization, and writing - original draft. **Musun Park, Hyo-Jeong Ban, Su-Jin Baek, and Soo Yeon Kim:** Writing, review & editing. **Siwoo Lee:** Resources. **Hee-Jeong Jin:** Conceptualization, writing - original draft, supervision, and project administration.

significantly upregulated pathways in three comparisons: P *vs*. N, M *vs*. N, and M *vs*. P (left). The selected significantly upregulated pathways for the P (middle) and M (right) groups compared to the N group. **(D)** Integrated functional network for Pre-MetS using DEGs with upregulated expression and significantly enriched pathways (adjusted $P < 0.05$). Nodes represent DEGs or pathways, and the associations between them are represented by links. **(E)** Pearson's correlation coefficients between the criteria parameters and the selected DEGs with upregulated expression in the P group compared to the N group. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$. **(F)** CRP levels in blood from the patients in the three groups. Since CRP levels in the normal group did not follow a Gaussian distribution ($P = 0.013$ for the normal group; $P = 0.48$ for the pre-MetS group; $P = 0.65$ for the MetS group by Shapiro–Wilk test), the Mann–Whitney U test was performed to test statistical significance between groups. N, Normal; P, Pre-MetS; M, MetS; WC, waist circumference; TG, triglyceride level; BPs, systolic blood pressure; BPd, diastolic blood pressure; HDL, HDL cholesterol level; Glu, glucose level.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gendis.2022.07.021.

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