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Multi-layered network structure of amino acid (AA) metabolism characterized by each essential AA-deficient condition

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Short Abstract: We inferred the network structure of plasma amino acids from plasma samples of rats fed diet deficient in one of nine essential amino acids, with using multi-level digraph analysis method. This revealed several interesting interrelations between plasma amino acids, which could not be drawn from the metabolic pathway map.

Long Abstract:
Background
The plasma amino acid concentrations have distinctive features for various physiological conditions. Specific abnormalities in plasma amino acid profiles are reported in some diseases, and in some cases, it is used for diagnostic markers. One of such examples is the Fisher's ratio, which is a ratio of branched-chain amino acids to aromatic amino acids, used for the marker of liver fibrosis. These evidences show that plasma amino acid profile can be a useful tool for monitoring the physiological state of an organism. However, the control mechanism behind the change in the amino acid profile is so complicated that the further investigations to uncover the trigger reasons for these changes have not been succeeded. The reasons for this complexity come from the interrelatedness of the amino acids and the numerous factors affecting their concentrations. Amino acids are related to each other within a large metabolic pathway, and their concentrations in the plasma are equivalent to the whole sum of the metabolic flow in each organs and tissues. The metabolic flow in organs and tissues are affected by various factors, and so does the plasma amino acid profile. Thus, the change in the concentration of plasma amino acids can not be simply explained by the topological network structure of metabolic pathway map.
Objectives
In this study, we inferred the interrelated network structure of plasma amino acids without any prior topological information of metabolic pathways based on our experimentally observed results of plasma amino acid profiles of rats in a single amino acid-deficient (minus-one) diet.
Results
Constructing binary interaction matrix for plasma amino acids.
The sample plasma was obtained from the rats fed amino acid minus-one diet, which is completely depleted of single essential amino acid. The plasma concentration of the deficient amino acid of these rats is less than half of that of the control diet fed rats. Deficiency in one

essential amino acid triggers the change in concentrations of all the other amino acids in plasma. This change is converted to the directional binary relation from the deficient amino acids to all the other amino acids using threshold-test analysis method. If the degree of change in amino acid a passes the arbitrarily set threshold, the relation from the deficient amino acid to the amino acid a is set to 1, and otherwise it is set to 0. Thus a binary interaction matrix is constructed. Two different values were adopted to set the threshold. One is the fold-change value (θ-value). The other value is the p-value (level of significance) for the average difference. Each experimental groups consist of 6 samples, and for all the amino acids measured, the p-value for the average difference between the experimental group and the control group was calculated using Dunnet's multiple comparison method.

Network structure estimated by multi-level digraph method.

From the binary interaction matrix, multi-scale digraph was drawn. In the analysis using θ-value as the threshold, the number of amino acids (nodes) composing the network decreases as the filtering threshold becomes severer. This decrease is not observed in the analysis using the p-value. In either analysis, some amino acids formed an equivalence group, in which the interactions (links) form a loop and the direction of the effect on one another cannot be decided. When the filtering condition becomes severer, the equivalence group disperses into the smaller groups or individual amino acids. In the case using θ-value, the equivalence group disperses at the threshold of 3.0, and 3-level digraph with 9 amino acids is drawn. In the case using p-value, the equivalence group does not completely disappear and at the threshold p=0.001, and 4-level digraph with 11 amino acids is drawn. Since we should like to infer network model as many nodes and links as possible, we decided to use p-value for the threshold for the further analysis.

Effect of each amino acid on the network structure.

The same analysis was carried out using the partial dataset. By excluding one amino acid minus-one dataset, we can infer the network structure without considering the effect of the excluded amino acid. At the threshold p=0.05, the removal of threonine minus-one dataset changed the network structure drastically. The equivalence group disappears and the interactions between the amino acids which belonged to the equivalence group are alternatively revealed. This indicates that the relation responsible for holding the equivalence group together was the effect of threonine on the other amino acids, leucine, methionine, tryptophan and valine, in the same group. At the threshold p=0.001, the only amino acids forming the equivalence group are threonine and methionine. By excluding threonine minus-one dataset, the effects of methionine on other amino acids are revealed, and by excluding methionine minus-one dataset, the effects of threonine are revealed. Integrating the information obtained from the analysis of partial dataset, the estimated network structure is further refined.

In the inferred network, we could draw several interesting interrelations between plasma amino acids as follows: 1) Lysine is located at the top control level. The change in the concentration of lysine affects almost all of the other plasma amino acids, however, lysine itself is not affected by the deficiency of any other amino acids. 2) Threonine plays a role in a hub in the network, which has direct links to the most number of other amino acids. 3) Threonine and methionine are interrelated each other, (loop structure), which leads to the oscillatory behavior of these two amino acids and might act as a biological and metabolic switch in the network.

Conclusion

The network analysis of the plasma amino acid revealed the new interrelation between the amino acids which could not be drawn from the topological metabolic pathway. This data

driven analysis should lead us to the further understanding of the dynamics of the plasma amino acid profiles and gives us some clues for the factors affecting the interrelated network.