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Research Paper

Systems Signatures Reveal Unique Remission-path of Type 2 Diabetes Following Roux-en-Y Gastric Bypass Surgery

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ABSTRACT

Roux-en-Y Gastric bypass surgery (RYGB) is emerging as a powerful tool for treatment of obesity and may also cause remission of type 2 diabetes. However, the molecular mechanism of RYGB leading to diabetes remission independent of weight loss remains elusive. In this study, we profiled plasma metabolites and proteins of 10 normal glucose-tolerant obese (NO) and 9 diabetic obese (DO) patients before and 1-week, 3-months, 1-year after RYGB. 146 proteins and 128 metabolites from both NO and DO groups at all four stages were selected for further analysis. By analyzing a set of bi-molecular associations among the corresponding network of the subjects with our newly developed computational method, we defined the represented physiological states (called the edge-states that reflect the interactions among the bio-molecules), and the related molecular networks of NO and DO patients, respectively. The principal component analyses (PCA) revealed that the edge states of the post-RYGB NO subjects were significantly different from those of the post-RYGB DO patients. Particularly, the time-dependent changes of the molecular hub-networks differed between DO and NO groups after RYGB. In conclusion, by developing molecular network-based systems signatures, we for the first time reveal that RYGB generates a unique path for diabetes remission independent of weight loss.

The plasma samples of normal glucose-tolerant obese (NO) and diabetic obese (DO) patients, before and after Roux-en-Y Gastric Bypass (RYGB) surgery, were subjected to proteomic and metabolomic analysis. With our newly developed computational method, we defined the physiological states based on bi-molecular associations among proteins and metabolites (edges) instead of the concentrations of those biomolecules (nodes) for each individual in each stage. Based on integration of these multi-omics molecular networks, we showed that RYGB generates a unique remission-path of type 2 diabetes independent of weight loss.

1. Introduction

Roux-en-Y gastric bypass surgery (RYGB) used for treatment of obesity has turned out to be often leading to apparent remission of diabetes (Mosinski and Kirwan, 2016; Rubino et al., 2010; Vetter et al., 2009). Based on the conceptions of reductionism, researchers have generally tried to identify particular effectors in order to understand the diabetes

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remission. Thus, a number of previous studies have identified individual RYGB-induced effectors that may improve glucose homeostasis, such as certain gut hormones (Jorgensen et al., 2013; Karamanakos et al., 2008), metabolites (Baud et al., 2016; Gerhard et al., 2013; Patti et al., 2009), and proteins (Ryan et al., 2014). On one hand, the regulations of these molecules presumably are direct consequences of RYGB, for example, the postprandial plasma concentrations of glucagon-like peptide-1 (GLP-1) and peptide YY (PYY) of obese patients were elevated already 2 days after RYGB (Saeidi et al., 2013). On the other hand, there is no evidence that they (or some of them) also are the main effectors of diabetes remission independent weight loss. Our previous works have shown that the postprandial GLP-1 secretion was increased more than 10-fold after RYGB in both groups of obese patients with normal glucose tolerance and obese diabetic patients (Jorgensen et al., 2012). Furthermore, other study has demonstrated that RYGB-induced changes of hormone expressions such as ghrelin, secretin, GLP-1, PYY and glucagon in the small-intestinal enteroendocrine cells, were quite similar across both groups of obese patients with normal and abnormal glucose tolerance (Rhee et al., 2015), implying that those RYGB-induced individual molecules might be the only relevant effectors for diabetes remission.

From a systems biology point of view, type 2 diabetes is a complex disease involving complicated interactions between many genes, proteins and metabolites. Thus, it has been reported that the RYGB-induced diabetes remission involves significant alterations of plasma metabolomic and lipidomic profiling (Hansen et al., 2013), implying that global changes of the patient's biomolecules may be required for diabetes remission. In addition, several previous reports showed that the surgery could generate significant tissue changes such as increasing the size and mass of gut wall (Hansen et al., 2013), and this rearranged gut itself improved the glucose homeostasis (Saeidi et al., 2013). It should also be noted that improvements in pancreatic beta-cell secretary capacity and in hepatic and peripheral insulin sensitivity are also major regulators of glucose metabolism and remission of diabetes after RYGB (Jorgensen et al., 2013; Bojsen-Moller et al., 2014).

In the present study, the plasma samples of normal glucose-tolerant obese (NO) and diabetic obese (DO) patients, who were operated with RYGB (Jorgensen et al., 2012), were subjected to proteomic and metabolomic analysis by mass-spectrometry. With our newly developed computational method (Zhang et al., 2015), we defined the represented bi-associations between plasma proteins and metabolites of each subject. Based on these multi-omics molecular networks, we show that RYGB generates a unique remission-path of type 2 diabetes independent of weight loss.

2. Materials and Methods

Briefly, plasma samples were collected from 10 normal glucose-tolerant obese (NO) and 9 diabetic obese (DO) patients before and 1-week, 3-months, 1-year after RYGB. At each stage, the plasma samples were obtained at three time points for each individual, including the fasting state and 30 min and 45 min after a standardized liquid meal (Jorgensen et al., 2012). Signed informed consents from participants were obtained, and the study protocol has been approved by ethical committee. The collection procedures were conformed to standards indicated by the Declaration of Helsinki.

For proteome profiling, the plasma proteins were removed high-abundance proteins, in-solution digested, randomized labeled with six-plex TMT reagents, and analyzed by high resolution mass spectrometry. The metabolome profiling involved non-targeted analysis, targeted amino acid analysis and targeted acyl-carnitine analysis as well. Collectively, 146 proteins and 128 metabolites were measured from both NO and DO groups at all four stages, and used for bioinformatics analysis.

To illuminate the stage-wise data involved the molecular network, the data structure were firstly rebuilt from node-state to edge-state. After fine-tuned neighborhood selection, the group similarity were calculated and evaluated for group-temporal smoothed network generation. Following model comparisons, regularization, and optimization, artificial data with known network structures were evaluated in silico, and were further used to analyze the experimental data. Finally, the network and state were inspected for the influence by RYGB, and distance and PCA analysis were performed to visualize the trajectories or routes of the state transitions from the RYGB for NO and DO subjects, respectively.

Details regarding methods, including experimental methods for proteomics and metabolomics analyses, as well as computational methods, are available in the Supplementary information. The mass spectrometry proteomics data have been deposited to the ProteomeX change Consortium via the PRIDE (Vizcaino et al., 2016) partner repository with the dataset identifier PXD008071.

3. Results

3.1. Computational Analysis of Edge-states Based on Bi-molecular Associations Among Plasma Proteins and Metabolites

Plasma samples from our previously described clinical cohort (Jorgensen et al., 2012), 10 NO and 9 DO subjects obtained pre-RYGB, 1 week (1w), 3 months (3 m), and 1 year (1y) after RYGB (Jorgensen et al., 2012), were included in this analysis. Plasma samples were obtained at three time points for each individual, including the fasting state and30 min and 45 min after a standardized liquid meal (Fig. 1a). Significant decrease of fasting glucose was observed in the DO group already 1w after RYGB (Fig. 1b), but significant declines of BMI were not seen until 3 m and 1 y after RYGB in both groups. This indicates that the early remission of diabetes observed in the DO group is independent of weight loss.

We applied proteomic and metabolomic analyses on those plasma samples (Supplementary information). We quantified 146 plasma proteins (Supplementary Table S1) and 128 metabolites (Supplementary Table S2) that could be identified in all subjects at three time points (fasting, postprandial 30 min and 45 min) of four stages (pre-RYGB, post-RYGB at 1w, 3 m and 1y) (Fig. 1c). All 274 molecules were recognized as nodes for composing the molecular networks.

We first defined pathological states of the subjects before and after RYGB based on the concentrations of these molecular nodes and called them node-states (Fig. 1d, left panel). Then we analyzed the relationships among the node-states of the patients under various treatments in all stages based on the plasma molecular concentrations of all protein- and metabolite-nodes. By using Kruskal-Wallis test (Gregory and Corder, 2009), we selected the “differential” nodes based on their differential molecular concentrations at the four stages. Both hierarchical clustering analysis (HCA) and principal components analysis (PCA) showed that the states of the patients were not clearly classified into the corresponding phenotypes, and the stage-related changes of patient-states were not presented (Fig. 2a and b). Interestingly, both NO and DO individuals at 1w after RYGB (Fig. 2a, model I) were separated from the pre-RYGB group and the groups at 3 m and 1y after RYGB (Fig. 2a, model II), implying that the node-states derived from differential node-concentration could demonstrate the changes of the surgical stress rather than the changes of metabolic homeostasis.

In previous papers, we reported that the edge-state derived from all edges of the network was insensitive to the perturbations (Zeng et al., 2016; Zhang et al., 2015). Therefore we developed a computational method Group-Temporal Lasso (GTLasso) to identify each subject's molecular network as well as all edges (Supplementary information), which were in turn employed to characterize the subject's state, i.e., the edge-state. Specifically, GTLasso transforms the node-state to the corresponding edge-state for individual subject at each particular stage by integrating omics data of time-series (three time-points in this case: fasting, postprandial 30 min and 45 min) (Fig. 1a, and Supplementary information). Note that an edge-state could be represented by all edges or selected edge-biomarkers (network biomarkers) that are

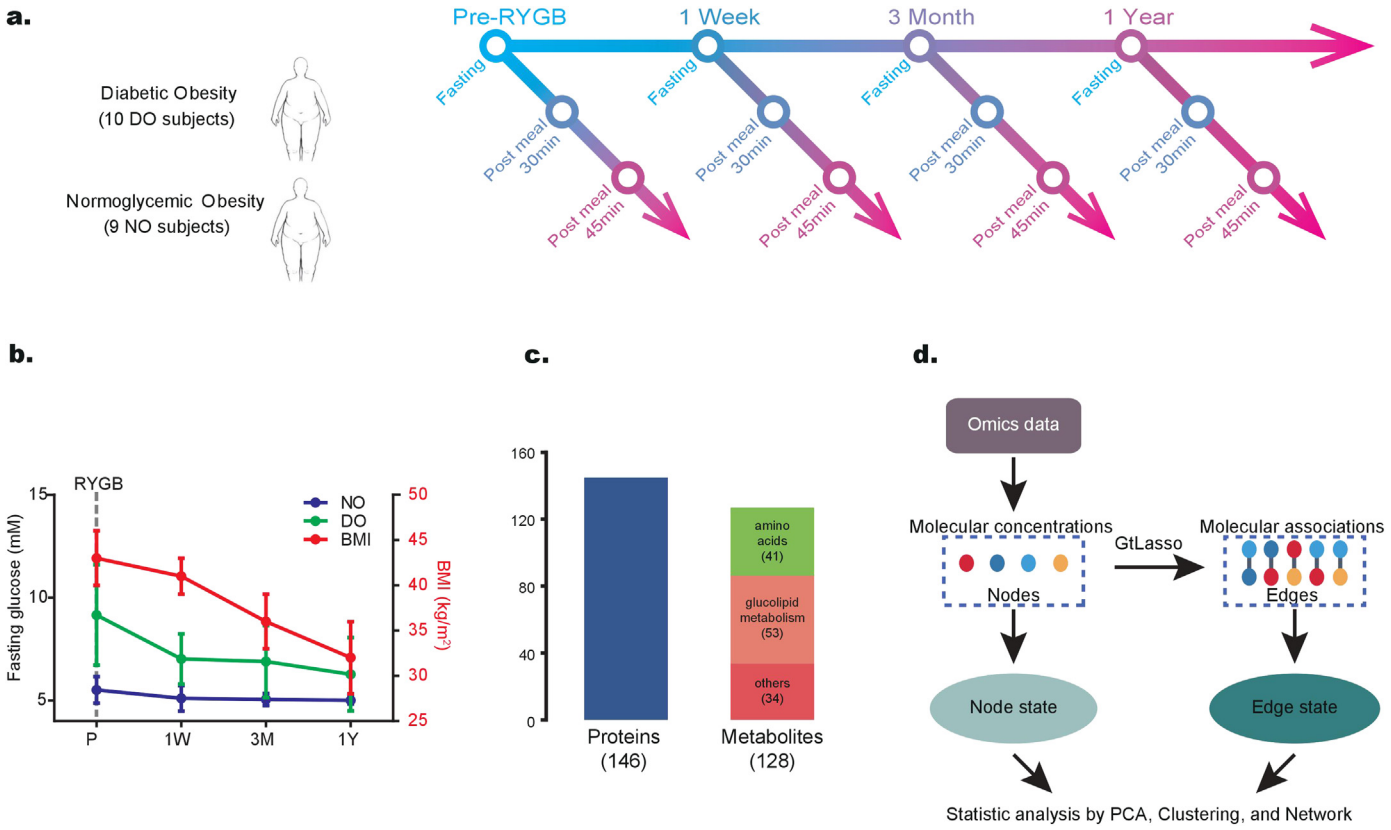


Fig. 1. Workflow of systematic analysis on RYGB-treated subjects. (a) 10 Non-diabetic Obese (NO) and 9 Diabetic Obese (DO) were subjected to RYGB. The plasma samples of each subject on four stages (pre-RYGB, 1w, 3 m, and 1y after RYGB) were collected at three time points, including the fasting, postprandial 30 min and 45 min. (b) BMI and fasting glucose before and after RYGB showed the significant loss of weight in both group and decrease of fasting glucose in DO group. (c) Quantitative proteomic and metabolic data were detected at all four stages of each subject. (d) Sketch shows the computational methods to calculate molecular states either by nodes or by edges.

similar to the node-state characterized by traditional molecular biomarkers (Zhang et al., 2015; Yu et al., 2017).

3.2. RYGB Generates a Unique Remission-path of Type 2 Diabetes Independent of Weight Loss

The HCA result based on calculation of edge-states showed that the DO and NO were grouped together before the operation (Module I, Fig. 2c), clearly separated from all stages after RYGB (Module II, Fig. 2c), indicating that RYGB generated significant changes of the physiological states in both DO and NO subjects.

Furthermore, the PCA result based on the edge-states clearly demonstrated that the DO and NO subjects presented different remission paths after RYGB (Fig. 2d). Firstly, all the NO subjects were separated from the DO subjects by PC1 (Fig. 2d), indicating that the NO edge-states, composed of bi-associations among plasma proteins and metabolites, are significantly different from the DO edge-states at both pre- and after-RYGB stages. Secondly, in agreement with the findings that there was no major weight loss at 1w after RYGB, but significant and continuous weight loss at 3 m and 1y after RYGB (Fig. 1b), both DO and NO subjects at the stages of pre-RYGB and 1w after RYGB were clearly separated from those at the stages of 3 m and 1y after RYGB by PC2 (Fig. 2d), indicating that the sole weight loss is a major factor to separate the subjects with higher BMI from the subjects with lower BMI. Thirdly, consistent with our previous data that the NO subjects showed dramatic improvement of hepatic insulin sensitivity at 1w after RYGB, but more moderate changes at 3 m and 1y (Tables 1 and 2 of Jorgensen et al., 2012), the NO subjects at 1w after surgery were separated from the pre-RYGB NO subjects (Fig. 2d), whereas the NO subjects at 3 m and 1y after RYGB were located in the same PC2-area (Fig. 2d). This suggests that the post-RYGB NO subjects at 3 m and 1y have similar edge-states due to the similar glucose homeostasis of these two groups.

Importantly, consistent with the previous results about continuous improvement of glucose homeostasis of the DO subjects from 1w to 1y after RYGB (e.g. continuously decreasing fasting plasma-glucose concentration; Jorgensen et al., 2012), the PCA result showed that the edge-states of DO subjects from pre-RYGB continuously moved away along PC2-axis at the three stages of 1w, 3 m and 1y after RYGB (Fig. 2d). Taken together, PCA analysis reveals that RYGB generates a unique remission-path of type 2 diabetes independent of weight loss, which involves extensive changes of molecular associations among proteins and metabolites.

3.3. Molecular Hub-networks of DO and NO Subjects Were Differentially Rewired After RYGB

We thereafter tried to answer the related question what is difference between the post-RYGB DO and NO molecular networks. Based on calculating the molecular bi-associations among the detected plasma proteins and metabolites by GTLasso approach, the molecular network representing the time-dependent edges-state after RYGB could be constructed at every particular stage (Supplementary information). Since the molecular hubs, which are connected with multiple molecules, are considered the major players in a particular molecular network, we further analyzed the hub-networks of both DO and NO subjects at all four stages of the RYGB based on these constructed networks consisting of plasma proteins and metabolites.

We showed that the NO and DO hub-networks at all four stages are quite different (compare Fig. 3a–d, h–g). This is consistent with the PCA result that all the edge-states of the NO subjects were separated from that of the DO subjects by PC1 (Fig. 2d). Also, the hub-network of the pre-RYGB NO subjects (Fig. 3a) was significantly different from that of

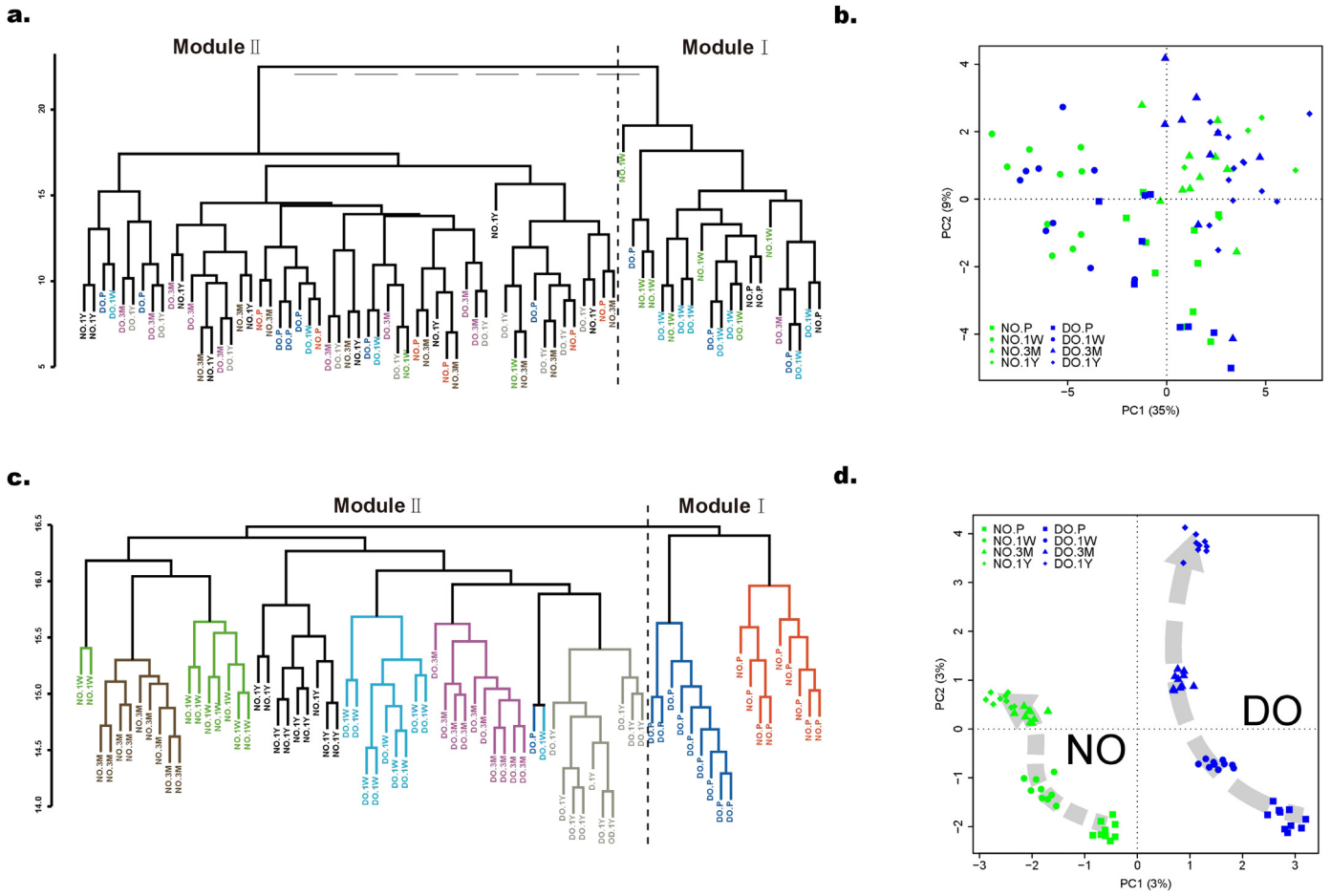


Fig. 2. Individual state classification using both proteomic and metabolic data. (a, b) HCA and PCA based on differential nodes; (c, d) HCA and PCA based on all edges. Obviously, the edge-states of the patients were clearly classified into the corresponding phenotypes.

the pre-RYGB DO subjects (Fig. 3e): the former was mainly composed of metabolites, which could be divided into one amino-acid sub-network and one glycol-lipid sub-network (Fig. 3a), whereas the latter mainly consisted of proteins (Fig. 3e). These results imply that the dysfunctional metabolic networks of the DO patients do not present in the NO subjects. Since the hub-networks of both NO and DO subjects were broken into small fragments at 1w after RYGB (Fig. 3), RYGB had generated similar consequences either for the metabolite-based hub-network of the NO subjects or for the protein-based hub-network of the DO subjects.

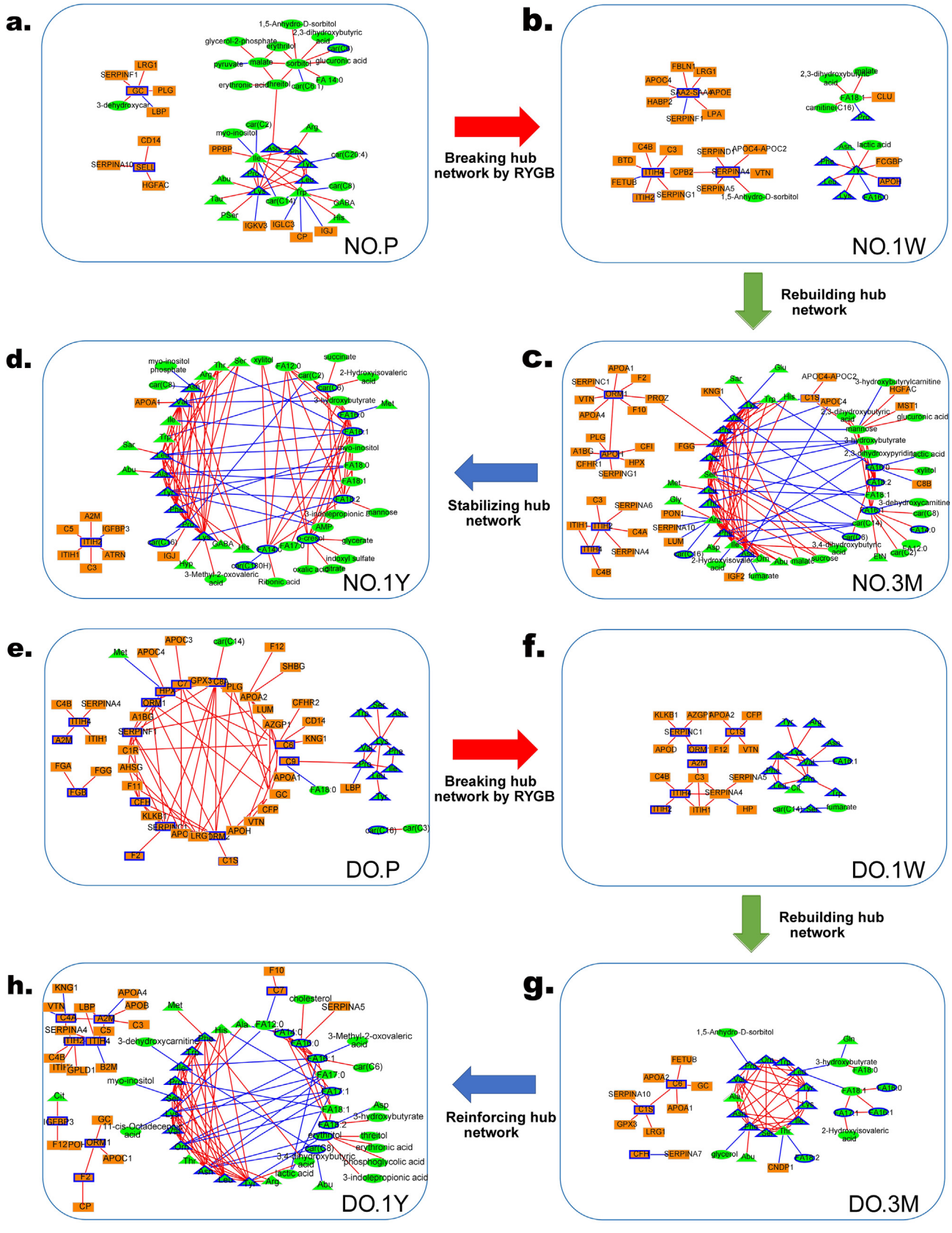
Furthermore, a rebuilt hub-network mainly containing metabolites of the NO subjects at 3 m after RYGB (Fig. 3c) was quite similar to that at 1y after RYGB (Fig. 3d), which throws light on the PCA result that the edge-states of NO subjects at 3 m and 1y after RYGB were grouped in the same PC2-area (Fig. 2d). Intriguingly, the re-built hub-network of the DO subjects at 3 m after RYGB (Fig. 3g) was significantly different from that at 1y after RYGB (Fig. 3h). This is consistent with the observation that the edge-states of post-RYGB DO subjects at 3 m were separated from those at 1y in the PCA map (Fig. 2d). In particular, the amino acids were getting connected at 3 months after RYGB in the DO individuals, which was earlier than the connection of fatty acids after RYGB (Fig. 3g), indicating the crucial role of amino acid metabolism in diabetes remission. This result is consistent with the previous observations of accelerated protein digestion and amino acid absorption after meal intake after RYGB (Bojsen-Moller et al., 2015), while fasting circulating concentrations of total amino acids decreased significantly (Laferrere et al., 2011a). Interestingly, the overall metabolic hub-network structure of the DO subjects at 1y after RYGB (Fig. 3h) looks quite similar to that of the NO subjects at 3 m (Fig. 3c) or 1y after RYGB (Fig. 3d). Taken together, we conclude that the DO subjects at 1y after RYGB have built a novel metabolic network similar to that of the post-RYGB NO subjects, which could generate an improved glucose homeostasis and result in the remission of type 2diabetes.

4. Discussion

Generally, biological networks show characteristic changes during disease progression. Thus, to define a physiological or pathological state is to measure a set of bio-molecules such as the concentrations of particular RNAs, proteins or metabolites, which are called nodes in term of network (Luonan Chen et al., 2010; Zhang et al., 2015). The state of a biological system difined by calculating the concentrations or concentration-changes of the nodes therefore is called the node-state (see left panel of Fig. 1d).

However, several reports indicate that the calculated node-states may be misleading due to both high fluctuations of the nodes' concentrations and individual variations (Chen et al., 2012; Liu et al., 2014). In addition, many molecules/nodes may show time-dependent variations and are sensitive to small perturbations of various factors that are irrelevant to the observed phenotypes, and thus are not reliable markers to characterize the states of the network (Liu et al., 2016; Zeng et al., 2016).

Based on our previous studies (Zeng et al., 2016; Zhang et al., 2015), we developed an alternative way to characterize biological systems by a


a.
NO.P
Breaking hub network by RYGB
b.
NO.1W
Rebuilding hub network
c.
NO.3M
Stabilizing hub network
d.
NO.1Y
e.
DO.P
Breaking hub network by RYGB
f.
DO.1W
Rebuilding hub network
g.
DO.3M
Reinforcing hub network
h.
DO.1Y
Proteins
Amino acids
Other metabolites
Protein hubs
Amino acid hubs
Other metabolite hubs
positive correlation
negative correlation

set of bi-molecular associations (i.e., edges) within a given network, which is called the edge-state (see right panel of Fig. 1d). The edges directly represent the associations or correlation between the molecules within the bio-networks and are much more robust in contrast to the fluctuations of node concentrations (Yu et al., 2014; Zeng et al., 2016). Therefore, an edge-state is composed of all edges of the network, which is considered to be insensitive to various small perturbations irrelevant to the phenotypes. Hence, the edge-state is a reliable marker to characterize the bio-network or physiological states of organisms. In the present study, we showed that, based on the edge-states, the remission-paths of both DO and NO subjects after RYGB was clearly differentiated (Fig. 2c and d), whereas the node-states could not define the remission-path of the DO subjects from that of the NO subjects after RYGB (Fig. 2a and b).

The analysis of edge-states seems to be another advantage that even small fraction of proteins and metabolites (e.g. 146 plasma proteins and 128 metabolites in the present study) could provide clearly and defined information to characterize the physiological or pathological states since we calculate the molecular associations rather than the concentrations of bio-molecules. In addition, all the proteins and metabolites analyzed in the present study are identified from patient plasma, which must reflect the states of whole body rather than the states of particular cells or tissues.

Recently, Boyle and colleagues have proposed an "omnigenic" model for disease-related complex traits, "we propose that gene regulatory networks are sufficiently interconnected such that all genes expressed in disease-relevant cells are liable to affect the functions of core disease-related genes and that most heritability can be explained by effects on genes outside core pathways" (Boyle et al., 2017). This model fully supports our finding: "core disease-related genes" are corresponding to "molecular nodes", and "extensive interconnected gene regulatory networks" are corresponding to our "edge-states". We believe that the edges/associations among small number of bio-molecules derived from plasma can be used for definition of global biological states of the body due to the extensive interconnection of bio-molecules in a biological system. And, just because of this reason, we first endeavored to establish the entire workflow by integrating the proteomics and metabolomics data to illuminating the value of edge-states for this type of complex network analysis behind gastric bypass surgery.

Based on integration of phenotype data derived from our previous study (Bojsen-Moller et al., 2014), and the hub-network analysis (Fig. 3) in the present study, we revealed that RYGB broke the problematic molecular networks of diabetic patients and resulted in re-building a well-connected metabolic molecular network for glucose homeostasis, which was reflected as the unique remission-path of diabetes patients independent of weight loss (Fig. 2d).

By analyzing the re-wired hub networks in detail, we observed that the interconnection of amino acids was significantly increased in the hub-networks of the post-RYGB DO subjects (Fig. 3g), whereas the highly interconnected protein hub-networks of pre-RYGB DO subjects (Fig. 3e) was completely disrupted after the surgery (Fig. 3f–h). This implies that the RYGB-induced rebuilding the hub-network of amino acids plays important role to establish normal glucose homeostasis of type 2 diabetes patients. Previous reports showed that serum levels of branched-chain and aromatic amino acids (BCAAs and AAAs), including leucine, isoleucine, valine, phenylalanine and tyrosine, were positively correlated to insulin resistance and obesity (Laferrere et al., 2011b; Wurtz et al., 2013). In addition, the BCAA levels were associated with the improvement in insulin resistance independent of weight loss after gastric bypass surgery (Shah et al., 2012). A reported 35% decrease in fasting plasma BCAA concentrations after gastric bypass surgery was associated with an increase in two key BCAA catabolic enzymes, and these changes in expression of BCAA catabolic enzymes actually enhanced metabolic flux and increased catabolism of BCAAs after gastric bypass surgery (She et al., 2007). Taken together, these results imply that the most important effect of RYGB for remission of type 2 diabetes might be to break the dysfunctional network of amino acids and then result in reconstruction of a new metabolic network that is similar to that of normal glucose homeostasis.

We consider the present study based on the molecular edge-states derived from plasma proteins and metabolites as a pilot study. Certainly, further validation with larger clinical cohorts is needed. In addition, the recovering-path of patients after RYGB is a dynamical process, and thus detecting its tipping point before transiting to a stable state after RYGB by dynamic network biomarker (DNB) (Chen et al., 2012; Liu et al., 2014) is also an interesting problem for further dissolving. In addition, the more studies with animal-models on the mechanisms of metabolic improvement, particular rebuilding the networks of amino acids by RYGB, are worth doing in the near future.

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Conflicts of Interest

T.R.C. works for Novo Nordisk A/S and own shares in Novo Nordisk A/S and Zealand Pharma A/S.

B.D. works for Novo Nordisk A/S and own shares in Novo Nordisk A/S.

J.S.P. works for Novo Nordisk A/S and owns shares in Novo Nordisk A/S.

Fig. 3. Rewiring of molecular hub networks for NO and DO subjects with time-dependent states. (a–d) NO hub-network was broken at 1 week of post-RYGB (b), then re-constructed at 3 months (c), and stabilized at 1 year (d); (e-h) DO hub-network was broken at 1 week of post-RYGB (f), gradually re-constructed from 3 months (g) to 1 year (h). Particularly, the hub-networks heavily composed by the metabolites of pre-RYGB NO and by the proteins of pre-RYGB DO could be observed, respectively. During the process of the hub-network rebuilding from 1w to 1y-state, for both post-RYGB NO and DO subjects, the metabolites-dominated networks were reconstructed.

Author Contributions

S.H.J, N.B.J, C.D, K.N.BM, and S.M obtained plasma samples from study participants and supervised the clinical study. Q.R.L., and D.D.W. performed most of the proteomics and metabolomics-related experiments, supported by Z.D.S. and R.X.L. for proteomic analysis, X.F.G., Q.Q.W., H.P.Z., and L.Z. for metabolomic analysis. Z.M.W. developed computational method. Z.M.W., Q.R.L., N.J.W.A., and D.D.W. interpreted data. J.R.W., R.Z., L.N.C.,J.S.P., S.M., T.R.C., B.D., N.J.W.A. and J.J.H. conceived and supervised the project. J.R.W., R.Z., J.J.H., and L.N.C. wrote the paper. All authors read and approved the manuscript.

Appendix A. Supplementary Data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ebiom.2018.01.018.

References

Baud, G., Daoudi, M., Hubert, T., Raverdy, V., Pigeyre, M., Hervieux, E., Devienne, M., Ghunaim, M., Bonner, C., Quenon, A., et al., 2016. Bile diversion in roux-en-Y gastric bypass modulates sodium-dependent glucose intestinal uptake. Cell Metab. 23, 547–553.

Bojsen-Moller, K.N., Dirksen, C., Jorgensen, N.B., Jacobsen, S.H., Serup, A.K., Albers, P.H., Hansen, D.L., Worm, D., Naver, L., Kristiansen, V.B., et al., 2014. Early enhancements of hepatic and later of peripheral insulin sensitivity combined with increased postprandial insulin secretion contribute to improved glycemic control after roux-en-Y gastric bypass. Diabetes 63, 1725–1737.

Bojsen-Moller, K.N., Jacobsen, S.H., Dirksen, C., Jorgensen, N.B., Reitelseder, S., Jensen, J.E.B., Kristiansen, V.B., Holst, J.J., van Hall, G., Madsbad, S., 2015. Accelerated protein digestion and amino acid absorption after roux-en-Y gastric bypass. Am. J. Clin. Nutr. 102, 600–607.

Boyle, E.A., Li, Y.I., Pritchard, J.K., 2017. An expanded view of complex traits: from polygenic to Omnigenic. Cell 169, 1177–1186.

Chen, L.N., Liu, R., Liu, Z.P., Li, M.Y., Aihara, K., 2012. Detecting early-warning signals for sudden deterioration of complex diseases by dynamical network biomarkers. Sci. Rep. 2.

Gerhard, G.S., Styer, A.M., Wood, G.C., Roesch, S.L., Petrick, A.T., Gabrielsen, J., Strodel, W.E., Still, C.D., Argyropoulos, G., 2013. A role for fibroblast growth factor 19 and bile acids in diabetes remission after roux-en-Y gastric bypass. Diabetes Care 36, 1859–1864.

Gregory, W., Corder, D.I.F., 2009. Nonparametric Statistics for Non-Statisticians: A Step-by-Step Approach. Wiley, New Jersey.

Hansen, C.F., Bueter, M., Theis, N., Lutz, T., Paulsen, S., Dalboge, L.S., Vrang, N., Jelsing, J., 2013. Hypertrophy dependent doubling of L-cells in roux-en-Y gastric bypass operated rats. PLoS One 8.

Jorgensen, N.B., Jacobsen, S.H., Dirksen, C., Bojsen-Moller, K.N., Naver, L., Hvolris, L., Clausen, T.R., Wulff, B.S., Worm, D., Hansen, D.L., et al., 2012. Acute and long-term effects of roux-en-Y gastric bypass on glucose metabolism in subjects with Type 2 diabetes and normal glucose tolerance. Am. J. Physiol. Endocrinol. Metab. 303, E122–E131.

Jorgensen, N.B., Dirksen, C., Bojsen-Moller, K.N., Jacobsen, S.H., Worm, D., Hansen, D.L., Kristiansen, V.B., Naver, L., Madsbad, S., Holst, J.J., 2013. Exaggerated glucagon-like peptide 1 response is important for improved beta-cell function and glucose tolerance after roux-en-Y gastric bypass in patients with type 2 diabetes. Diabetes 62, 3044–3052.

Karamanakos, S.N., Vagenas, K., Kafarentzos, F., Alexandrides, T.K., 2008. Weight loss, appetite suppression, and changes in fasting and postprandial ghrelin and peptide-YY levels after Roux-en-Y gastric bypass and sleeve gastrectomy - A prospective, double blind study. Ann. Surg. 247, 401–407.

Laferrere, B., Reilly, D., Arias, S., Swerdlow, N., Gorroochurn, P., Bawa, B., Bose, M., Teixeira, J., Stevens, R.D., Wenner, B.R., et al., 2011a. Differential metabolic impact of gastric bypass surgery versus dietary intervention in obese diabetic subjects despite identical weight loss. Sci. Transl. Med. 3.

Laferrere, B., Reilly, D., Arias, S., Swerdlow, N., Gorroochurn, P., Bawa, B., Bose, M., Teixeira, J., Stevens, R.D., Wenner, B.R., et al., 2011b. Differential metabolic impact of gastric bypass surgery versus dietary intervention in obese diabetic subjects despite identical weight loss. Sci. Transl. Med. 3, 80re82.

Liu, R., Wang, X.D., Aihara, K., Chen, L.N., 2014. Early diagnosis of complex diseases by molecular biomarkers, network biomarkers, and dynamical network biomarkers. Med. Res. Rev. 34, 455–478.

Liu, X.P., Wang, Y.T., Ji, H.B., Aihara, K., Chen, L.N., 2016. Personalized characterization of diseases using sample-specific networks. Nucleic Acids Res. 44.

Luonan Chen, C.L., Wang, Ruiqi, Aihara, Kazuyuki, 2010. Modelling Biomolecular Networks in Cells: Structures and Dynamics. Springer-Verlag, London.

Mosinski, J.D., Kirwan, J.P., 2016. Longer-term physiological and metabolic effects of gastric bypass surgery. Curr. Diabetes Rep. 16.

Patti, M.E., Houten, S.M., Bianco, A.C., Bernier, R., Larsen, P.R., Holst, J.J., Badman, M.K., Maratos-Flier, E., Mun, E.C., Pihlajamaki, J., et al., 2009. Serum bile acids are higher in humans with prior gastric bypass: potential contribution to improved glucose and lipid metabolism. Obesity 17, 1671–1677.

Rhee, N.A., Wahlgren, C.D., Pedersen, J., Mortensen, B., Langholz, E., Wandall, E.P., Friis, S.U., Vilmann, P., Paulsen, S.J., Kristiansen, V.B., et al., 2015. Effect of Roux-en-Y gastric bypass on the distribution and hormone expression of small-intestinal enteroendocrine cells in obese patients with type 2 diabetes. Diabetologia 58, 2254–2258.

Rubino, F., Schauer, P.R., Kaplan, L.M., Cummings, D.E., 2010. Metabolic surgery to treat type 2 diabetes: clinical outcomes and mechanisms of action. Annu. Rev. Med. 61, 393–411.

Ryan, K.K., Tremaroli, V., Clemmensen, C., Kovatcheva-Datchary, P., Myronovych, A., Karns, R., Wilson-Perez, H.E., Sandoval, D.A., Kohli, R., Backhed, F., et al., 2014. FXR is a molecular target for the effects of vertical sleeve gastrectomy. Nature 509, 183.

Saeidi, N., Meoli, L., Nestoridi, E., Gupta, N.K., Kvas, S., Kucharczyk, J., Bonab, A.A., Fischman, A.J., Yarmush, M.L., Stylopoulos, N., 2013. Reprogramming of intestinal glucose metabolism and glycemic control in rats after gastric bypass. Science 341, 406–410.

Shah, S.H., Crosslin, D.R., Haynes, C.S., Nelson, S., Turer, C.B., Stevens, R.D., Muehlbauer, M.J., Wenner, B.R., Bain, J.R., Laferrere, B., et al., 2012. Branched-chain amino acid levels are associated with improvement in insulin resistance with weight loss. Diabetologia 55, 321–330.

She, P., Van Horn, C., Reid, T., Hutson, S.M., Cooney, R.N., Lynch, C.J., 2007. Obesity-related elevations in plasma leucine are associated with alterations in enzymes involved in branched-chain amino acid metabolism. Am. J. Phys. Endocrinol. Metab. 293, E1552–1563.

Vetter, M.L., Cardillo, S., Rickels, M.R., Iqbal, N., 2009. Narrative review: effect of bariatric surgery on type 2 diabetes mellitus. Ann. Intern. Med. 150 (94-U54).

Vizcaino, J.A., Csordas, A., del-Toro, N., Dianes, J.A., Griss, J., Lavidas, I., Mayer, G., Perez-Riverol, Y., Reisinger, F., Ternent, T., et al., 2016. 2016 update of the PRIDE database and its related tools. Nucleic Acids Res. 44, D447–456.

Wurtz, P., Soininen, P., Kangas, A.J., Ronnemaa, T., Lehtimaki, T., Kahonen, M., Viikari, J.S., Raitakari, O.T., Ala-Korpela, M., 2013. Branched-chain and aromatic amino acids are predictors of insulin resistance in young adults. Diabetes Care 36, 648–655.

Yu, X.T., Li, G.J., Chen, L.N., 2014. Prediction and early diagnosis of complex diseases by edge-network. Bioinformatics 30, 852–859.

Yu, X., Zhang, J., Sun, S., Zhou, X., Zeng, T., Chen, L., 2017. Individual-specific edge-network analysis for disease prediction. Nucleic Acids Res. 45, e170 PMID: 28981699.

Zeng, T., Zhang, W.W., Yu, X.T., Liu, X.P., Li, M.Y., Chen, L.N., 2016. Big-data-based edge biomarkers: study on dynamical drug sensitivity and resistance in individuals. Brief. Bioinform. 17, 576–592.

Zhang, W.W., Zeng, T., Liu, X.P., Chen, L.N., 2015. Diagnosing phenotypes of single-sample individuals by edge biomarkers. J. Mol. Cell Biol. 7, 231–241.