Metabolomics Yield a Novel Biomarker for Predicting Diabetes Mellitus Risk in Humans

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Study Hypothesis
Recent advances in omics technologies have yielded fascinating opportunities for risk prediction of common diseases like hypertension and diabetes mellitus. Identifying biomarkers for predicting risk of diabetes mellitus can help in selecting individuals that would benefit a great deal from the proven preventative strategies for diabetes mellitus. In a landmark initiative, Wang et al. sought to identify metabolite biomarkers of diabetes mellitus risk using high-throughput metabolite profiling from plasma samples of 2 large epidemiological cohorts and followed-up their findings with a series of mechanistic studies in a mice model and human tissues.

How Was the Hypothesis Tested?
The authors performed a nested case–control study using samples from 2 large prospective epidemiological cohorts. The discovery cohort consisted of 188 individuals from the Framingham Offspring study (FHS) who developed new-onset diabetes mellitus (mean age, 56 years and 42% women) during a 12-year follow-up period. These individuals were compared with 188 propensity matched controls. The replication cohort consisted of 163 cases and 163 controls (mean age, 58 years, 55% women) from the Malmö Diet and Cancer study. The authors did metabolite profiling using liquid chromatography-tandem mass spectrometry. Metabolite concentrations were log-transformed and were analyzed using conditional logistic regression analyses. In the pooled analysis, P value threshold of 7×10−4 was used to claim significance. To claim generalizability of their results, the author went on to perform metabolite profiling in additional 1561 randomly selected individuals from the FHS offspring study.

The authors started mechanistic studies by performing feeding experiments using high-fat diet (HFD) and standard chow diet (SCD) to examine the effect of different diets on circulating 2-aminoadipic acid (2-AAA) levels in C57BL/6 mice. Next on both dietary backgrounds, the authors conducted intervention studies with an isotope-labeled reference compound for 2-AAA. Two cohorts (n=6) each of C57BL/6 mice fed on HFD or SCD were included in the study protocol at 6 weeks of age. Half of the mice on each dietary background received 500 mg/kg per day of 2-AAA via the drinking water for next 5 weeks. After 5 weeks of 2-AAA treatment, the authors went on and did intraperitoneal glucose tolerance testing. On completion of the study protocol, the authors harvested tissues (muscle, liver, fat, and pancreas) for metabolite profiling analysis to better understand in which organ 2-AAA is functionally important. Finally, the authors studied the insulin secretion in insulinoma cell line cells and human and murine islet cell systems.

Principal Findings
The authors were able to detect 70 metabolites in the plasma from FHS individuals. Of which, 2-AAA had the strongest association with risk of future diabetes mellitus (P=0.0009). Individuals with plasma 2-AAA concentration in the top quartile had a 4-fold higher odds of developing diabetes mellitus during the 12-year follow-up period compared with those in the lowest quartile (odds ratio, 4.49; 95% confidence interval, 1.86–10.89). Similar results were seen in the replication cohort. The striking association of 2-AAA levels for predicting diabetes mellitus risk was unchanged after adjusting for measures of insulin resistance and β-cell function (hemostatic model assessment, hemoglobin A1C, prediabetes mellitus, oral glucose tolerance test). The 2-AAA levels remained significantly associated with future risk of diabetes mellitus (0=0.0003) in the randomly selected 1561 individuals from the FHS study, which further strengthened the initial observations.

HFD increased circulating 2-AAA levels by 51% (P=0.01) compared with the animals fed with SCD. The treatment with 2-AAA decreased fasting plasma glucose levels and increased plasma insulin levels in animals fed with HFD or SCD (n=12 for each group). After the intraperitoneal glucose tolerance testing, the peak glucose concentration was lower in the 2-AAA–treated mice on both HFD and SCD dietary background. Next, the authors demonstrated that 2-AAA was most abundant in pancreas. Accordingly, the authors sought to

study whether 2-AAA stimulated insulin secretion from the pancreas in a time- and dose-dependent manner. They also found that 2-AAA administration enhanced insulin secretion significantly in the BTC6 cells, primary murine islets, and human islets. However, no effect on peripheral insulin sensitivity in mice by insulin tolerance tests was evident by 2-AAA supplementation. Neither did the 2-AAA administration have any effect on reduced food intake nor obesity status highlighting the complex nature of diabetes mellitus and different molecular pathways playing a role.

**Implications**

In a collaborative translational effort, the study by Wang et al. yield a promising plasma metabolite biomarker, 2-AAA (byproduct of lysine degradation) for predicting future risk of diabetes mellitus in normoglycemic individuals. The mechanistic aspect of the article highlights that 2-AAA is functionally important in the disease pathogenesis of glucose hemostasis and diabetes mellitus. Confirming these results in additional cohorts including obese individuals will be of great interest to the scientific community. Provoking findings in the mice fed with 2-AAA raises an intriguing question on whether administration of 2-AAA as a nutritional supplement can be helpful in the fight against diabetes mellitus in humans.

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**Disclosures**

None.

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**KEY WORDS:** biomarkers ■ diabetes mellitus ■ metabolomics
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