SUPPLEMENTAL MATERIALS

Appendix A

The grade of diabetic retinopathy

Professional photographers used a digital non-mydriatic fundus camera to obtain bilateral retinal 45-degree images of the macula (centered on the fovea). Fundus photographs were graded preliminarily for the level of DR and other fundus lesions by two graders depending on the retinal photographs assessment[1]. If one eye was unavailable for the classification, the other one was graded. DR grading and its severity was determined depending on the worse eye, and each eye was assessed as follows: no apparent DR and any DR, which would be further classified into mild, moderate and severe non-proliferative DR (NPDR) as well as proliferative DR (PDR) according to International Clinical Diabetic Retinopathy Disease Severity Scale[1]. To further investigate the consistency of the diagnosis on DR independently finished by two professional ophthalmologists, an investigation on the interrater reliability was applied using kappa coefficient, which was introduced by Cohen (1960) and has been a frequently used index to estimate interrater reliability in practice. The associated kappa coefficient for two graders' agreement on fundus photographs were 0.86, which strongly indicated that the diagnosis and grading of DR had excellent agreement in the two ophthalmologists (In statistics, kappa coefficient over 0.80, from 0.81 to 1.00, would be generally accepted as almost perfect). Few inconsistent samples were graded again by another experienced ophthalmologist of our fundus reading center.

Appendix B

Widely targeted UPLC-MS/MS-based metabolic profiling

Serum samples were taken out of the -86℃ ultra-low temperature refrigerator, thawed to 4°C and determined in the central laboratory according to the following steps. A total of 100 μL serum was weighted and extracted overnight at 4℃ with 300 μL aqueous methanol, then respectively centrifuged twice at 12,000 rpm for 10 min and 3 min. Finally, the extracts were carefully absorbed and filtrated for further assessment. To evaluate the stability and reliability of UPLC-MS/MS platform, quality control (QC) samples were carefully prepared in advance by mixing equal volume of each serum sample, pretreated as described above and inserted into each block of 20 testing samples once when performing serum metabolites determination. The 2-μL extracted supernatant was transferred into the UPLC (Shim-pack UFLC SHIMADZU CBM30A, https://www.shimadzu.com/) - electrospray ionization (ESI) - MS/MS (Applied Biosystems 6500 QTRAP, https://sciex.com/) system for acquiring serum metabolic profiles. The chromatographic separation was performed at 40 °C using HSS C18 (positive ion mode) and HSS T3 (negative ion mode) columns with a flow rate of 0.35 mL/min. The mobile phase was a mixture of (A) ultra-pure water with 0.04% formic acid, and (B) acetonitrile with 0.04% as follows: 0 min with 95% A and 5% B, 11 min with 5% A and 95% B, 12 min with 5% A and 95% B, and 14 min with 95% A and 5% B. The mass spectrometer was operated under ESI in 500 °C, 5.5 kV of voltage and 25 psi of curtain gas as well as high parameter of collision-activated dissociation. In the triple quadrupole, each ion pair was scanned depending on the optimized declustering potential and collision energy. The peak area of metabolites including trehalose and glutamate were acquired with Analyst Software V.1.6.3 (AB Sciex) and extracted in a widely targeted manner using MultiQuant Software (AB Sciex).

1. Wilkinson CP, Ferris FL, Klein RE, Lee PP, Agardh CD, Davis M, Dills D, Kampik A, Pararajasegaram R, Verdaguer JT: Proposed international clinical diabetic retinopathy

and diabetic macular edema disease severity scales. Ophthalmology 2003, 110:1677-1682.