Individual difference in renal protective effect of dapagliflozin based on *GSK3B* gene polymorphisms and its underlying mechanisms

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Abstract Objective This study aimed to evaluate the variability in the effectiveness of dapagliflozin in reducing urinary protein in Chinese Han patients with diabetic kidney disease (DKD). The study conducted a prospective clinical trial to assess the predictive effect of genetic polymorphisms of key factors on individual differences in the renal protective effect of dapagliflozin. The findings of this study provide a theoretical basis and laboratory evidence for the personalized clinical administration of dapagliflozin in patients with type 2 diabetes mellitus (T2DM).

Methods 1. A retrospective clinical study was conducted to collect clinical indicators of patients with diabetic kidney disease (DKD) who were treated with dapagliflozin, a drug used to lower urinary protein levels. The study defined the response group as those who experienced a decrease of more than 30% in the urinary albumin-creatinine ratio (UACR) after 12 weeks of treatment or a negative urinary protein ratio, while the non-response group was defined as those who experienced a UACR decrease of 30% or less and no negative urinary protein ratio.

2. Then we conducted laboratory studies: drug affinity responsive target stability (DARTS) combined with quantitative proteomics techniques for drug target screening, combined with molecular docking, molecular dynamics simulations, DARTS experiments on recombinant proteins, and molecular level pharmacodynamic screening to validate the method. To explore the key factors of nephroprotective effect of

dapagliflozin (GSK3β was initially identified). The expression and activity of GSK3β were observed in three types of renal parenchymal cells: glomerular podocytes, mesangial cells and tubular epithelial cells under high glucose environment, and the protein expression level of pTyr216-GSK3β was detected by Western blot and the kinase activity of GSK3β was quantified by kinase activity spectrometry.

3. At the animal level, *db/db* mice treated with voglibose were used as the control group and *db/db* mice treated with dapagliflozin were given as the experimental group, and blood glucose was controlled at consistent levels; renal tissues were subjected to PAS staining, Masson staining, Sirius red staining, PASM staining and transmission electron microscopy to observe the glomerular ultrastructure and evaluate the morphological changes in the mice; Western blot detected the expression levels of active pTyr216-GSK3β protein and inhibitory pSer9-GSK3β protein in kidney tissues and quantified the kinase activity of GSK3β by kinase activity spectrometry; Tunel assay detected apoptotic cells in kidney tissues and Western blot detected apoptosis-associated proteins bax, cleaved-caspase3, bcl2 protein expression in kidney tissues to evaluate apoptosis in *db/db* mice; immunofluorescence and Real time PCR to detect the protein expression, distribution and mRNA levels of the podocyte marker proteins Nephrin and Synaptopodin to evaluate renal podocyte function. At the cellular level, mouse podocytes were subjected to high glucose stimulation and given different doses of dapagliflozin for intervention, and the protein expression and distribution of Nephrin, Synaptopodin, pTyr216-GSK3β and pSer9-GSK3β were measured by immunofluorescence, and Western blot for Nephrin, Synaptopodin, bax, cleaved-caspase3, bcl2, pTyr216-GSK3β and pSer9-GSK3β; Real time PCR to detect mRNA levels of Nerphrin and Synaptopodin; flow cytometry to detect apoptosis in high glucose cultured mouse foot cells; using the Transwell model was used as a vehicle to evaluate the barrier function of podocytes by performing albumin influx assay at the level of half organs in vitro. GSK3β was overexpressed in foot cells cultured at normal sugar concentration, and GSK3β was knocked down on high sugar cultured foot cells to observe the effect of modulating GSK3β expression level on the therapeutic effect of dapagliflozin.

4. Back to the clinic, in a prospective clinical study, clinical indices and peripheral blood of DKD patients treated with dapagliflozin for 12 consecutive weeks were collected, and *GSK3B* genotyping was performed by SNaPshot method based on Genotype-Tissue Expression database and thousand genomes database to screen tag SNPs loci. Correlation analysis of *GSK3B* gene polymorphisms with the efficacy of dapagliflozin was performed; genetic risk score and multiple linear regression methods were used to analyze the factors influencing the efficacy of dapagliflozin in lowering urinary protein.

Results 1. A retrospective study included 382 patients with DKD on dapagliflozin as the primary urinary protein-lowering regimen, of whom 125 met the data inclusion criteria and were divided into a responder group (71) and a non-responder group (54), with up to 43.20% of patients in the non-responder group, indicating significant individual differences in the urinary protein-lowering effect of dapagliflozin in Chinese Han Chinese patients with DKD. The results of dichotomous logistic regression analysis showed that the factors associated with the urinary protein-lowering efficacy of dapagliflozin were the duration of diabetes and baseline eGFR, but the predictive effect was limited.

2. DARTS combined with quantitative proteomics studies suggested that dapagliflozin can bind GSK3β, which is the primary target for the independent nephroprotective effect of dapagliflozin. The results of DARTS assay, molecular docking, molecular dynamics simulation and molecular level pharmacodynamic screening assay of recombinant GSK3β protein showed that dapagliflozin could bind GSK3β and inhibit its kinase activity. The levels of active pTyr216-GSK3β protein and the kinase activity of GSK3β in three types of renal parenchymal cells, namely, podocytes, mesangial cells and renal tubular epithelial cells, were increased to different degrees under high glucose induction, with the most significant changes in podocytes.

3. The results of animal experiments showed that dapagliflozin had nephroprotective effects in the DKD model of *db/db* mice independent of glycemic improvement, inhibited GSK3β activity, reduced podocyte apoptosis, protected podocytes, and improved renal morphology and renal function. Further cellular experiments showed that GSK3β activity of podocytes was elevated, apoptosis level of podocytes was

increased, expression of podocyte marker proteins was reduced, and barrier function was impaired in high glucose status, while dapagliflozin had a significant ameliorative effect, and knockdown of GSK3β could further enhance the ameliorative effect of dapagliflozin. Overexpression of GSK3β in normal cultured cells increased podocyte apoptosis, decreased podocyte marker protein expression, and impaired barrier function, while dapagliflozin was able to reverse the damaging effects of GSK3β overexpression on podocytes to a certain extent.

4. In the prospective clinical study, a total of 152 patients with DKD who met the inclusion and exclusion criteria were enrolled. The more DKD patients carrying rs1807075 allele C, the less significant the improvement in TC and UACR; the more DKD patients carrying rs1807075 allele C, the less significant the improvement in TC and UACR; the results of grouping DKD patients based on risk allele score showed that the more DKD patients carrying *GSK3B* risk allele, the worse the efficacy of dapagliflozin. The results of multiple linear regression analysis suggested that baseline UACR, rs60393216, was closely related to UACR improvement after 12 weeks of dapagliflozin treatment; the higher the baseline UACR, the more significant the decrease in UACR after dapagliflozin treatment; for each T allele mutation, the percent decrease in UACR after dapagliflozin treatment in DKD patients was 42.50%.

Conclusions There are significant individual differences in the urinary protein-lowering effect of dapagliflozin in Chinese Han DKD patients; dapagliflozin can directly bind GSK3β and inhibit its activity, and the activity level of GSK3β has an important effect on the podocyte-protective effect of dapagliflozin; baseline UACR, *GSK3B* rs60393216 and improvement of UACR after 12 weeks of dapagliflozin treatment was closely related to the improvement of UACR after 12 weeks of dapagliflozin treatment and was an important predictor of the efficacy of dapagliflozin in lowering urinary protein.

Key words type 2 diabetes mellitus; diabetic kidney disease; dapagliflozin; individual differences; *GSK3B*; gene polymorphisms; podocytes

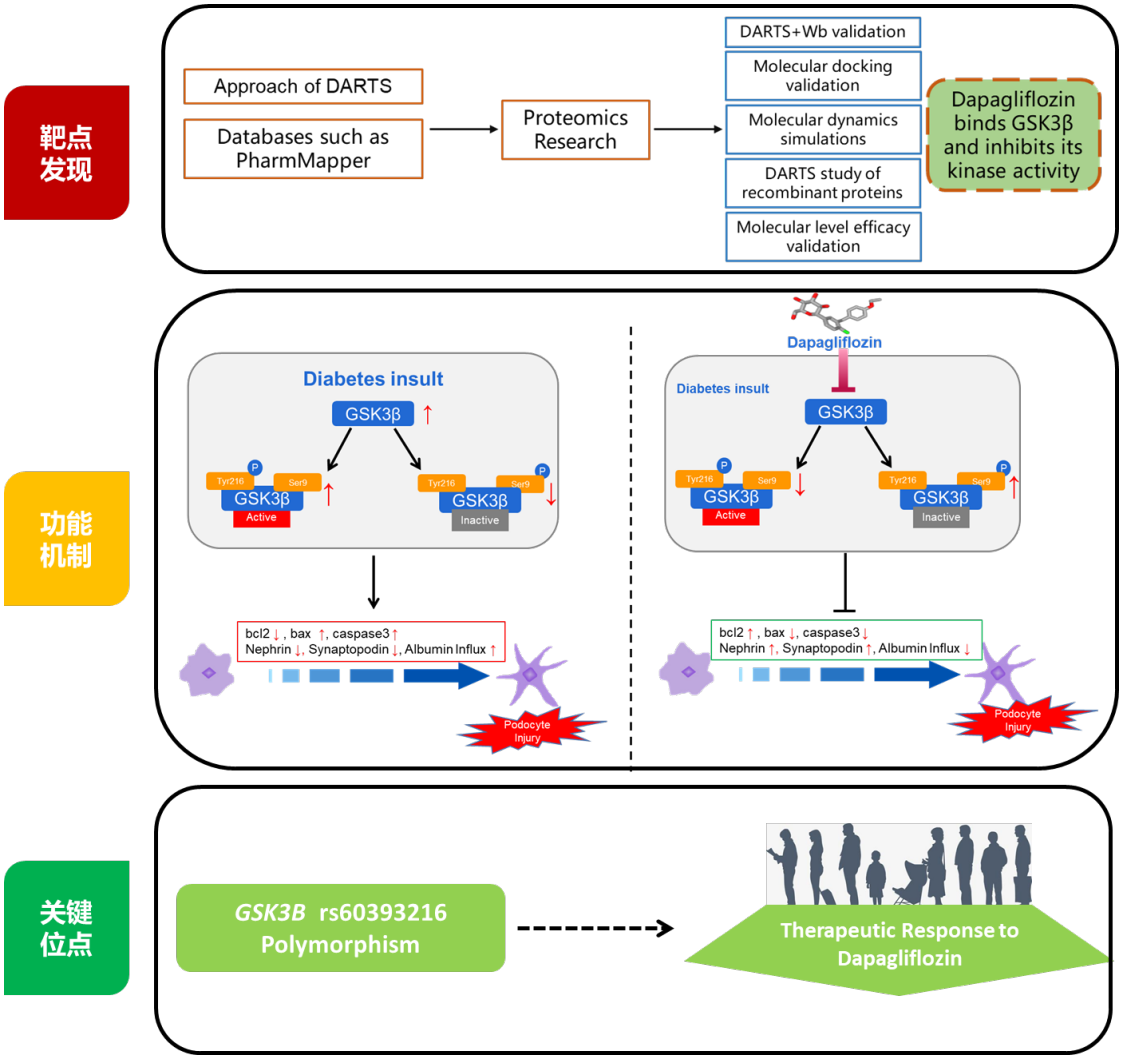

Figure Individual difference in renal protective effect of dapagliflozin based on *GSK3B* gene polymorphisms and its underlying mechanisms. Clinical practice has revealed that there is a phenomenon of insignificant reduction of urinary protein in patients after dapagliflozin treatment. Then, a retrospective clinical study was conducted to confirm that some DKD patients did not respond to the urinary protein-lowering effect of dapagliflozin, and a new binding target of dapagliflozin, GSK3β, was screened by DARTS combined with quantitative proteomics study, which confirmed that GSK3β is a key factor in the cytoprotective effect of dapagliflozin. The prospective clinical study identified *GSK3B* gene polymorphism as a key factor affecting individual differences in urinary protein reduction by dapagliflozin, providing a reference for individualized clinical application of dapagliflozin.