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project name: Study on Polymorphism of DPP-4 and GLP-1R Genes in Chinese Population and Its Empirical Study on Treatment of Diabetes

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Study protocol

1.1 Introduction

Single Nucleotide Polymorphism (SNP) plays an important role in the differences of clinical manifestations and drug responses of diseases. The vast majority of SNP sites are located in the non-coding region of the gene (about 95%), which is called SNP(non-coding SNP (ncSNP), while the other part of SNP is located in the coding region of the gene, which is called coding SNP (cSNP). Furthermore, cSNP can be divided into two categories: SNP that does not change the encoded amino acid sequence is called synonymous SNP(synonymous SNP, SSNP); SNP that changes amino acid sequence is called SNP(non-synonymous SNP (NSNP)[130] . Although not involved in coding amino acid, some ncSNPs may also affect the regulation of protein expression. Therefore, it is of great significance to study the effects of NC SNP and cSNP on the occurrence and development of diseases and drugs.

DPP-4 enzyme inhibitor is combined with DPP-4 enzyme in human body to reduce hydrolysis of active GLP-1, thus increasing the level of endogenous active GLP-1. Active GLP-1 combines with its receptor GLP-1R to promote insulin release and inhibit glucagon release in hyperglycemia state, and produces opposite effect in hypoglycemia state.

Based on the above principles, we speculate that SNP of genes that may affect the hypoglycemic effect of DPP-4 enzyme inhibitor are:

(1) SNP of DPP-4 enzyme gene. SNP of DPP-4 enzyme gene may affect the enzyme activity and/or protein expression level of DPP-4. Assuming that the effect of DPP-4 enzyme inhibitor is sufficient, patients with higher DPP-4 enzyme activity are more sensitive to DPP-4 enzyme inhibitor drugs; However, for patients with low DPP-4 enzyme activity, DPP-4 enzyme inhibitor drugs cannot play a stronger role in lowering blood sugar.

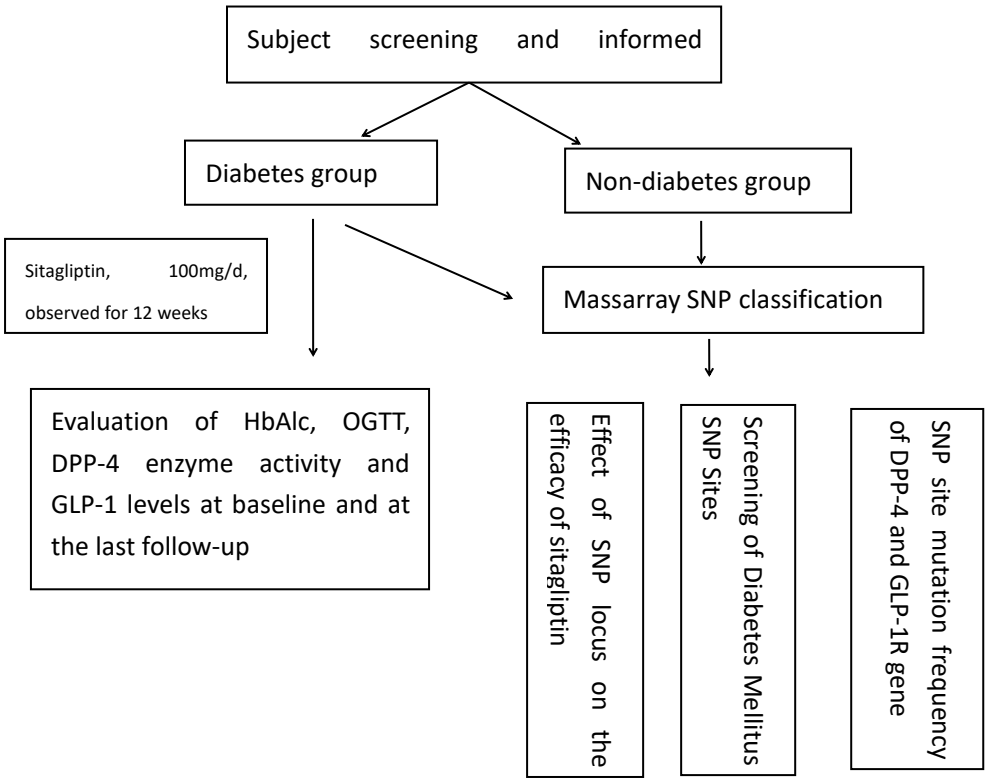
(2) SNP of GLP-1 gene. SNP of GLP-1 gene may affect activity or expression level of GLP-1. Patients with high GLP-1 level are more sensitive to DPP-4 enzyme inhibitor drugs.

(3) SNP of GLP-1R gene. SNP of GLP-1R gene may affect activity or expression level of GLP-1R. Patients with high GLP-1R level are also more susceptible to DPP-4 enzyme inhibitor drugs.

However, studies on the hypoglycemic effect of DPP-4, GLP-1 and their receptors on DPP-4 enzyme inhibitors in the treatment of T2DM are rare[131], which is

not conducive to the evaluation of individualized treatment of such drugs. Therefore, this study intends to select SNP sites with high mutation frequencies of DPP-4, GLP-1 and GLP-1R genes to study the mutation frequencies of these SNPs in diabetic patients and non-diabetic patients and their effects on DPP-4 enzyme inhibitor sitagliptin's hypoglycemic effect on T2DM patients (Fig. 1-1).

Figure 1-1 Study design



1.2 **Research Design and Selection of Subjects**

SNP Site Selection

The SNP site selection in this study is based on the following principles: (1) The SNP site is DPP-4, GLP-1 or its receptor GLP-1R; (2) The mutation frequency of the SNP site in Chinese Han population is greater than or equal to 30% (the query of mutation frequency is based on NCBI database); (3) Studies have reported that the SNP site may be related to the occurrence, development and treatment of T2DM.

Study on the Distribution Difference of SNP Sites between Diabetic Patients and Non-diabetic Patients

In order to study the distribution frequency of different genotypes of selected SNP loci in diabetic patients and non-diabetic patients, inclusion exclusion criteria for two types of subjects are specially set.

Diagnostic criteria for T2DM:

According to the diagnostic criteria for diabetes in the guidelines for the prevention and treatment of T2DM in China[1](2013 edition) published by the Diabetes Credit Association of the Chinese Medical Association in 2014: diabetic symptoms (symptoms of acute metabolic disorders such as polydipsia, polyphagia, polyuria, weight loss, skin pruritus, blurred vision, etc. caused by hyperglycemia), patients diagnosed as T2DM with random blood glucose ≥11.1mmol/L, or fasting blood glucose (FPG) ≥ 7.0 mmol/L, or 2h glucose ≥ 11.1mmol/L after glucose loading.

The included non-diabetic patients have no major diseases such as tumor, no chronic diseases such as dyslipidemia, hypertension, and non-prediabetes whose blood sugar and glycosylated hemoglobin values cannot meet the criteria for diagnosing T2DM, and are over 50 years old.

SNP of DPP-4, GLP-1 and Their Receptors on the Effect of Sitagliptin on T2DM

Inclusion criteria for patients

①According to the Chinese Medical Association, the diagnostic criteria for diabetes in the *Guidelines for the Prevention and Treatment of T2DM in China* published by the Diabetes Credit Association in 2014 are diagnosed as T2DM patients, and glycosylated hemoglobin (calculated by HbA1c) is between 7% and 9%;

② age between 18 and 70 years old;

③ BMI index ≥ 18.5;

④ not receiving hypoglycemic treatment within 4 weeks, or receiving hypoglycemic treatment within 4 weeks, but not changing the treatment plan within 3 months;

⑤ not participating in any clinical trial within 3 months;

⑥ no serious heart, brain, liver, kidney and other diseases;

⑦ signing the informed consent form.

Exclusion criteria for patients

① BMI is greater than 40;

② GLP-1 or DPP-4 drugs were taken orally in the past month;

③ immunocompromised persons;

④ patients with C peptide < 0.3ng/ml;

⑤ being allergic to GLP-1 and DPP-4 enzyme inhibitor drugs;

⑥ pregnant or lactating patients;

⑦patients taking drugs that may affect DPP-4 enzyme inhibitor metabolism;

⑧ patients with severe cardiac, renal, liver and respiratory insufficiency, patients with past or family history of medullary thyroid carcinoma (MTC), and patients with type 2 multiple and endocrine tumor syndrome (MEN2);

⑨ pancreatitis and drug abusers have occurred in the past year.

Exit criteria:

① Serious adverse reactions occurred during the implementation of clinical research;

② compliance with medication＞120% or＜80%;

③ patients unwilling to continue this clinical study.

Index Detection and Sample Collection

Subjects with T2DM will receive OGTT and HbA1c tests after entering the group. OGTT tests will be carried out routinely in the nuclear medicine department of Sichuan Academy of Medical Sciences and Sichuan People's Hospital, and HbA1c tests will be carried out routinely in the laboratory of Sichuan Academy of Medical Sciences and Sichuan People's Hospital Medical Center. The subjects collected blood samples on an empty stomach in the morning. Professional nursing staff collected 8ml of venous blood from the upper limbs of the patients using disposable venous blood collection needles, and EDTA anticoagulation tube was used for preservation. 5ml of whole blood was reserved for DNA extraction, centrifuged at 3000rpm for 10min, and plasma and blood cells were separated and stored at -80℃.

Sitagliptin Treatment Scheme for T2DM Subjects

The diabetes patient was treated with sitagliptin phosphate 100mg qd for 12 weeks without changing the original treatment scheme. Sitagliptin Phosphate Tablets were purchased by the research group from Mercadon (China) Investment Co., Ltd. according to the scientific research and drug purchasing process of Sichuan Academy of Medical Sciences and the Ministry of Science and Technology of Sichuan Provincial People's Hospital.

Benefits and risks of subjects

For non-diabetic patients, they will receive diabetes knowledge education once free of charge and take 5ml of venous blood from upper limbs. For diabetic patients, they will receive 100 mg qd of sitagliptin phosphate for 12 weeks free of charge on the basis of the original treatment, and will receive OGTT examination and glycosylated hemoglobin examination free of charge at the time of admission and at the end of the study. During OGTT examination, an additional 8ml venous blood was drawn for SNP site, DPP-4 enzyme activity and GLP-1 level detection. Patients will receive free professional consultation from clinicians with senior professional titles in endocrinology when they leave the group, and the treatment plan will be readjusted according to the blood glucose results of patients.

1.3 Statistical analysis

Microsoft EXCEL is used for data sorting and preprocessing. The HbA1c at the end of clinical study minus HbA1c at baseline was used to obtain the HbA1c reduction value, and the same was true for fasting blood glucose and postprandial blood glucose. For each group of data difference (e.g. Δ HbA1c) data of each genotype, the position test is performed, i.e. whether the difference is statistically significant with 0 is tested. When the group of data meets the normal distribution, the student t test is used for analysis, and when the normal distribution is not met, the symbolic rank test (S test) is used for analysis. Continuous variables are described by means of average standard deviation, and qualitative variables are described by means of percentage. The following methods are used to test the difference of continuous variables in patients with different genotypes: when the data of each group conform to the normality and the homogeneity of variance, the hypothesis test is carried out by variance analysis; when one group does not meet the normality or the homogeneity of variance, wilcoxon rank sum test is used for analysis, Kruskal-Wallis Test is used for non-parametric test, and the two comparisons between each group are carried out by T test based on the estimation of the difference within the group of population samples and LSmeans method. The difference test of blood glucose index control rate (HbA1c<7%，FPG< 7mmol/L，PPG<10mmol/L) in patients with different genotypes was conducted by χ^2 test, and Fisher exact test was used for hypothesis test in case of theoretical cell frequency< 5. SAS 9.21 is used to implement the statistical process. Set the significance level at 0.05. If there are less than 3 data in a single group,

statistical analysis will not be conducted because intra-group differences cannot be estimated.