The Precision Medicine to Enhance Depression and Anxiety Outcome (PMEDA) Study

CONTENT

STUDY SUMMARY

This multi-center study aims to investigate the effects of the CYP2D6 genotype and metabolic phenotype on paroxetine exposure and therapeutic outcomes. The primary outcome is defined as the steady-state concentration of paroxetine, while secondary outcomes encompass treatment response and adverse drug reactions. Blood samples are collected and analyzed for plasma concentration using comprehensive LC-MS/MS analysis. The CYP2D6 copy number is ascertained by the qPCR method. The study involves evaluating the treatment efficacy of paroxetine and assessing adverse drug reactions (ADR) with laboratory tests. Regression analyses are conducted to assess the association between the CYP2D6 genotype and metabolic phenotype and the outcomes. Covariates such as age, sex, smoking and drinking habits, clinical diagnosis, and use of adjunctive medicine are taken into account in the analysis, which is followed by a few exploratory analyses.

1. Background and study rationale

1.1 Background and Relevant studies

Despite CYP2D6 accounting for less than 2% of all CYP enzymes in the liver, it is implicated in the hepatic metabolism of approximately 25% of drugs. Noteworthy substrates metabolized by CYP2D6 encompass tricyclic antidepressants (such as clomipramine, nortriptyline, doxepin), selective serotonin reuptake inhibitors (like fluoxetine, fluvoxamine, paroxetine), and antipsychotics (including olanzapine, risperidone, fluphenazine).

CYP2D6 activity exhibits significant inter-individual variation and can be classified into four phenotypes: ultrarapid metabolizers (UM), normal metabolizers (NM), intermediate metabolizers (IM), and poor metabolizers (PM).

The gene encoding CYP2D6, located on chromosome 22q13.1, comprises nine exons encoding a 497 amino acid enzyme. The CYP2D6 gene possesses extensive single nucleotide variants (star alleles) and copy number variants (CNVs), leading to substantial variations in CYP2D6 enzyme activity. The CPIC and the DPWG have recently updated a consensus standard to infer CYP2D6 metabolizer status from the CYP2D6 activity score derived from the CYP2D6 genotype (Caudle et al., 2020; Sawamura et al., 2004). However, it should be noted that this revised classification is arrived at by considering the results of surveys from several medical laboratories and clinical pharmacologists. Consequently, there remains a lack of clinical trial evidence based on evidence-based medicine to support this new classification.

Existing research indicates that oral clearance rates of paroxetine vary significantly depending on the CYP2D6 metabolic phenotype. Under identical dosing conditions, PMs have the highest blood concentrations of paroxetine, rendering them more susceptible to ADRs. Therefore, in clinical practice, when prescribing paroxetine to patients with different CYP2D6 metabolizer statuses, it is advisable to initiate therapy at a different dose. The Clinical Pharmacogenetics Implementation Consortium (CPIC)

and the Dutch Pharmacogenetics Working Group (DPWG) have developed clinical guidelines to assist clinicians in precision dosing based on CYP2D6 metabolizer status.

However, these clinical guidelines, developed from studies focused on Caucasians, may not be applicable to the Chinese population. Approximately 95% of Caucasian PMs carry some combination of two copies of *CYP2D6* *3, *4, *5, and 6, whereas these alleles are infrequently found in the Chinese population, resulting in a low frequency of PMs. 51% of the Chinese population carry *CYP2D6* *10, which encodes an enzyme with low and unstable activity. Although not classified as PMs, individuals with this allele are generally IMs. Therefore, CYP2D6 activity is lower in the Chinese population than in Caucasians. These differences may lead to distinct effects of CYP2D6 metabolizer statuses in the Chinese population.

1.2 Overview of Trial Design

This trial is an 8-week, multi-center, single-drug prospective cohort study evaluating the effects of CYP2D6 metabolic phenotype and genotype on paroxetine outcomes in Chinese Han patients with major depressive disorder (MDD), generalized anxiety disorder (GAD), or panic disorder (PD) from the Precision Medicine to Enhance Depression and Anxiety Outcome (PMEDA) Consortium.

2. Study Objectives

2.1 Primary Objective

The primary objective is to investigate the impact of the CYP2D6 metabolic phenotype on the steady-state concentration of paroxetine.

2.2 Secondary Objective

- To examine the impact of the CYP2D6 metabolic phenotype on the treatment efficacy of paroxetine.

- To investigate the influence of the CYP2D6 metabolic phenotype on adverse drug reactions (ADR) associated with paroxetine.

- To explore the impact of the CYP2D6 genotype on the steady-state concentration, treatment efficacy, and ADR of paroxetine.

- To determine the therapeutic reference range for paroxetine.

3. Investigational Plan

3.1 General Design

Patients are recruited from seventeen hospitals in China, including the Peking University Sixth Hospital, the Fifth Hospital of Tangshan, Renmin Hospital of Wuhan University, Hebei Provincial Mental Health Center, the First Affiliated Hospital of Chongqing Medical University, Tianjin Anding Hospital, Weihai Mental Health Center, the Fourth People's Hospital of Ordos, Hefei Fourth People's Hospital, Fuzhou Neuropsychiatric Hospital, Hebei General Hospital, the First Affiliated Hospital of

Jinan University, the First Affiliated Hospital of Anhui Medical University, Hangzhou Seventh People's Hospital, the First Affiliated Hospital of Air Force Medical University, the Affiliated Mental Health Center of Jiangnan University, and Tongde Hospital of Zhejiang Province. The period of recruitment is between March 2021 and April 2023.

Given that mental disorders are primarily diagnosed based on subjective symptoms, we will conduct training for psychiatrists in each of the seventeen hospitals to ensure coherence. The training content includes research protocols, diagnostic criteria and instruments, scales for assessing symptoms and side effects, blood sample collection procedures, and evaluation of inter-rater reliability. Following this, we will perform several baseline assessments at the start of the study. Patients who meet the criteria will be followed for up to eight weeks or until treatment is discontinued for any reason.

Recruitment of subjects will be conducted through an oral explanation of the study. Interested subjects will provide verbal consent during the interview, facilitated by the clinical doctor. A series of questions will be asked during the interview. Written consent will be obtained prior to conducting the screening labs.

3.2 Study Endpoints

3.2.1 Primary Endpoint

The primary endpoint is defined as the steady-state concentration (Css) of paroxetine in plasma. Blood samples are collected from patients at the 4-week treatment endpoint, ensuring a stable daily dosing regimen for at least ten days. Samples are collected immediately prior to the ingestion of the morning dose, 20-24 hours following the last medication. The Css of paroxetine is detected using liquid chromatography-tandem mass spectrometry (LC-MS/MS, AB5500, AB Sciex).

3.2.2 Secondary Endpoints

The secondary endpoints encompass the treatment efficacy and adverse drug reaction (ADR) of paroxetine.

3.2.2.1 Treatment Efficacy

Treatment efficacy is quantified as the percentage improvement in symptom severity from baseline to the 4-week and 8-week follow-up endpoints. Symptom severity is measured using different scales: the Hamilton Depression Rating Scale-17 (HAMD-17) for patients with MDD, the Hamilton Anxiety Rating Scale (HAMA) for those with GAD, and the Panic Disorder Severity Scale (PDSS) for those with PD. Treatment response is defined as a binary variable, categorized as responder and non-responder. Patients with MDD who show a 50% or greater improvement in the HAMD-17 from baseline at follow-up to the end of the follow-up period are defined as responders, while others are defined as non-responders. Similarly, patients with GAD who show a 50% or greater improvement in the HAMA are considered responders, as are PD patients who show a 50% or greater improvement in the PDSS.

3.2.2.2 Adverse Drug Reaction

ADR is evaluated using the Treatment Emergent Symptom Scale (TESS) in conjunction with laboratory tests. Patients experiencing ADR have at least one item on the TESS with a score exceeding two points, indicating that the ADR can be directly observed in these patients, with some degree of functional impact. These patients may experience discomfort due to the ADR, but it does not seriously impact their lives (three points) or severely affect their daily activities (four points).

4. Study Population and Duration of Participation

4.1 Inclusion Criteria

Patients are diagnosed by psychiatric clinicians using the Structured Clinical Interview of the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5). Included patients are those who have a diagnosis of MDD, GAD, or PD, are aged between 18 and 65 years, are of Han Chinese ancestry, have not undergone systemic antidepressant treatment or used CYP2D6-inducing or -inhibiting drugs within two weeks prior to enrollment, have no language barriers, and can cooperate with assessment and treatment.

Moreover, patients with MDD score 17 or higher on the 17-item Hamilton Depression Scale (HAMD-17) and 13 or lower on the Hypomania Checklist-32 (HCL-32). Patients with GAD score 14 or higher on the Hamilton Anxiety Scale (HAMA). Patients with PD score 7 or higher on the Panic Disorder Severity Scale (PDSS). Patients who are either experiencing their first episode or have relapsed after discontinuing antidepressant treatment for over two weeks are enrolled.

4.2 Exclusion Criteria

Excluded patients are those who have other mental disorders, are pregnant or lactating, have severe suicidal tendencies or pose harm to others, have severe or unstable physical illnesses, have secondary depressive and anxiety disorders due to endocrine disease, epilepsy, Parkinson’s disease, Huntington’s disease, or traumatic brain injury, are participating in another trial, or are unwilling or unable to complete this trial. Patients who have taken less than 80% of the prescribed medication are considered to have poor adherence and are excluded.

4.3 Subject Recruitment

Subject recruitment is conducted by the members of the PMEDA from 17 research centers. All recruitment materials, which will be viewed by potential participants, require approval by the Institutional Review Board (IRB) of each center.

4.4 Duration of Study Participation

The duration of the study subjects' participation, encompassing the screening, study intervention phase, and any follow-up time period, is approximately 8 weeks.

4.5 Total Number of Subjects and Sites

A total of 1100 subjects will be enrolled across the 17 sites.

4.6 Vulnerable populations

During the execution of the project, we will implement a series of protective measures: 1) We will introduce the research plan and the associated risks and benefits to participants, with the signing of informed consent forms by subjects (and legal guardians) on a voluntary basis. Participants can withdraw from the project unconditionally during the execution; 2) This project will only involve participants aged 18 to 65 and will not include children, elderly individuals, or pregnant women.

5. Study Intervention

5.1 Intervention Regimen

Following baseline assessments and laboratory tests, patients receive paroxetine hydrochloride tablets (Seroxat) at a dosage of 10mg/d as monotherapy in the first week and 20-40mg/d after one week. If necessary, adjunctive medicine that does not undergo CYP2D6 metabolism can be used. Based on DrugBank V5.0, drugs that inhibit or increase the biosynthesis or actions of the CYP2D6 enzyme, referred to as CYP2D6-inhibiting or inducing drugs, are prohibited two weeks before enrollment and during the study. Physical therapy is restricted during the study.

Doctors from the PMEDA consortium follow up on patients at four and eight weeks, which includes clinical scale assessments and laboratory tests.

5.2 Receipt and Storage

The investigational drug will be obtained from the PMEDA hospitals. The investigational drug will store in the pharmacy of each research center at room temperature, keeping in a cool dry room.

5.3 Blinding

Percentage improvement and ADR rater are blinded to the results of CYP2D6 metabolic phenotype and paroxetine concentration. The psychiatrists and patients are not blinded.

6. Study Procedures

TABLE 1: SCHEDULE OF STUDY PROCEDURES

	Baseline visit (Day 0)	Visit 1 (Day 28)	Visit (Day 56)
Informed consent	*		
Subjects screen	*		
General information	*		

Symptoms and medical history	*		
DSM-5	*		
Body and nervous system examination	*	*	*
Vital signs	*	*	*
Weight and waist circumference	*	*	*
HAMD/HAMA/PDSS	*	*	*
TESS	*	*	*
Laboratory examination	*	*	*
Electrocardiograph (ECG)	*	*	*
Blood drawing for genotyping		*	
Plasma Concentration		*	
Concomitant medications monitoring	*	*	*
Adverse events	*	*	*
Form for the ending			*

6.1 Screening

The screening visit will consist of screening tests, patient history, laboratory tests, and physical and psychiatric examinations. Patients who meet all criteria will be enrolled in the study.

6.2 Study Intervention Phase

After baseline assessments and laboratory tests, patients receive paroxetine hydrochloride tablets (Seroxat) at 10mg/d monotherapy in the first week and 20-40mg/d after one week.

6.2.1 Baseline Visit

·Informed consent

·Subjects screen

·General information

·Symptoms and medical history

·DSM-5

·Body and nervous system examination

·Vital Signs

·Weight and waist circumference

·HAMD/HAMA/PDSS

·TESS

·Laboratory Tests

·Electrocardiograph (ECG)

·Adverse reaction assessment

·Concomitant medications monitoring

6.2.2 Visit 2

·Body and nervous system examination

·Vital Signs

·Weight and waist circumference

·HAMD/HAMA/PDSS

·TESS

·Laboratory Tests

·Electrocardiograph (ECG)

·Blood drawing for genotyping

·Plasma concentration

·Adverse reaction assessment

·Concomitant medications monitoring

6.2.3 Visit 3

·Body and nervous system examination

·Vital Signs

·Weight and waist circumference

·HAMD/HAMA/PDSS

·TESS

·Laboratory Tests

·Electrocardiograph (ECG)

·Adverse reaction assessment

·Concomitant medications monitoring

·Form for the ending

6.3 Subject Withdrawal

Patients who meet any exclusion criteria, have intolerable ADRs, or request to withdraw will withdraw from the study. Doctors from the PMEDA consortium provide a final assessment and develop alternative treatment plans.

7. Study Evaluations and Measurements

7.1 Physical Examinations

The baseline evaluations include the medical history, physical examination (weight, height, and blood pressure) demographic characteristics and other information that will be collected.

7.2 Laboratory Evaluations

Fasting blood glucose level, hemoglobin, lipid profile (total cholesterol, high-density lipoprotein [HDL] cholesterol, triglycerides), complete blood count, ALT, AST, BUN, and serum prolactin level.

Plasma concentration detection of paroxetine is performed using LC-MS/MS (AB5500, AB Sciex). Blood samples are collected using EDTA anticoagulant tubes and centrifuge at 1700 g for 10 minutes at 4℃. The supernatant is used to prepare three dried blood spots, with each spot containing 25 μL of the sample. Investigators air-dry these dried blood spots at room temperature for 4-6 hours before being transported to a centralized testing laboratory for analysis.

Genomic DNA is extracted from dried blood spots using the Mag-MK Blood Spot DNA Extraction Kit (QIAGEN, Hilden, Germany). *CYP2D6* *3, *4, *5, *6, *7, *8, *10, *11, *12, *14A, *14B, *15, *17, and *41 alleles are genotyped using a nucleotide Matrix-Assisted Laser Desorption/Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) assay (Shanghai Conlight Medical Laboratory Co., Ltd.). The copy number of *CYP2D6* is identified using the ΔΔCt relative quantitative method by CopyCaller V2.3.1 software (Thermo Fisher, Waltham, USA). The activity score is derived from the genotyping result.

8. Statistical Plan

8.1 Sample Size and Power Determination

The calculation of the sample size is based on an expected small effect size (f^2= 0.015) for the increase in explained variance of three additional predictors (dummy variables for CYP2D6 metabolizer status) in the multiple regression model, which includes five pre-defined demographic predictors (covariates). A target sample size of 822 is projected to achieve an 85% power at a significance (alpha) level of 0.05. Adjusting for

an anticipated dropout rate of approximately 20-30% results in a target sample size of about 1100 participants.

8.2 Statistical Methods

Predictors encompass CYP2D6 metabolic phenotype (categorical variable: metabolizer status, continuous variable: activity score), and *CYP2D6* CNV. Potential covariates include demographic factors such as gender, age, smoking, and drinking status, and clinical factors such as diagnosis, course, whether adjunctive medicine is received or not, and the daily dose of paroxetine.

To handle the missing data in the multivariable analysis, complete-case analysis (CCA) approach wil be employed. All statistical analyses are performed using R software version 4.2.3, with the significance level set at a two-sided *P*-value of < 0.05.

8.2.1 Descriptive Analysis

A descriptive analysis will be performed on the baseline characteristics of the sample. All quantitative variables will be analyzed using the Kolmogorov-Smirnov test with Lilliefors corrections to determine whether they follow a normal distribution. Depending on the results of the normality analysis, either parametric or non-parametric statistics will be used for within and between-group analyses. Normality transformation will be performed for un-normally distributed variables. A one-way ANOVA will be used to test the significance of normally distributed variables among the different CYP2D6 metabolizer statuses.

8.2.2 Main Analysis

Given that previous study have suggested the effect of ex, age, smoking and drinking habits, and BMI on paroxetine steady-state concentration (Feng et al., 2006; Gazzaz et al., 2018; Kim et al., 2015; Nishimura et al., 2016; Oliveira et al., 2017), multiple regression analyses will be conducted to explore the independent effect of the CYP2D6 metabolic phenotype (independent variable) on the steady-state concentration of paroxetine (dependent variable), adjusting for sex, age, smoking and drinking habits, and BMI (covariates).

Similar analyses will also be performed for the effect of the CYP2D6 metabolic phenotype (independent variable) on the percentage improvement and ADR status (dependent variables) with adjusting for sex and age (covariates), as sex and age was associated with both dependent variables and independent variable (Demyttenaere et al., 2005; Gex-Fabry et al., 2008; Serretti et al., 2011)

8.2.3 Sensitivity Analysis

Several sensitivity analyses will be performed, including multiple regression with demographic and clinical covariates, stratified analysis comparing female versus male, and subgroup analysis.

8.2.5 Exploratory Analysis

In the exploratory analysis, we will flexibly model and visualize the associations of the CYP2D6 activity score (independent variable) with paroxetine Css and percentage improvement (dependent variables) using restricted cubic spline (RCS) linear regression, and the association between the CYP2D6 activity score (independent variable) and ADR status (dependent variable) using RCS logistic regression, implemented with the R package "rms". To balance the best fit and overfitting of splines, we will select the number of knots (between three and seven) based on the lowest Akaike information criterion, and assess nonlinearity using the Wald test.

The therapeutic reference range (TRR) is defined as the range of paroxetine's steady-state concentration that is associated with a therapeutic outcome. This range has a lower limit, below which a response is unlikely, and an upper limit, above which tolerability decreases. To estimate the TRR, we will conduct Receiver Operating Characteristic (ROC) analyses to identify the cutoff value that separates responders from non-responders or patients with or without ADR. We will use the Youden index to determine the best cutoff value.

9. Serious Adverse Events and Handling

9.1 Serious Adverse Event

A serious adverse event is defined as any adverse event that is:

- Fatal

- Life-threatening

- Requires or prolongs hospital stay

- Results in persistent or significant disability or incapacity

- A congenital anomaly or birth defect

- An important medical event. Important medical events are those that may not be immediately life-threatening, but are clearly of major clinical significance. They may jeopardize the subject and may require intervention to prevent one of the other serious outcomes noted above. For example, a drug overdose or abuse, a seizure that did not result in in-patient hospitalization, or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

All adverse events that do not meet any of the criteria for serious should be regarded as non-serious adverse events.

9.2 Adverse Event Process and Handling

At each contact with the subject, the investigator will seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events will be recorded immediately in the source document, and also in the appropriate adverse event module of the case report form (CRF).

All adverse events occurring during the study period will be recorded. Serious adverse events that are still ongoing at the end of the study period will be followed up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study intervention or study participation will be recorded and reported immediately.

Doctors from each site will provide symptomatic treatment for adverse reactions experienced by patients. If patients cannot tolerate the adverse reactions, they may withdraw from this study, and doctors will consider switching them to alternative therapeutic medications.

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