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ΤΜΗΜΑ ΙΑΤΡΙΚΗΣ
ΠΜΣ «Μεθοδολογία Βιοϊατρικής Έρευνας, Βιοστατιστική και Κλινική Βιοπληροφορική»

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The role of CYP450 polymorphisms in treatment resistant depression: Study protocol for a pharmacogenetic trial.

Ο ρόλος πολυμορφισμών του κυτοχρώματος P450 στην ανθεκτική στην θεραπεία κατάθλιψη: Πρωτόκολλο για μια φαρμακογενετική μελέτη.

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Περίληψη

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ΕΙΣΑΓΩΓΗ: Εάν και η ανθεκτική στην θεραπεία κατάθλιψη είναι μια από τις ασθένειες με την μεγαλύτερη επίδραση στην λειτουργικότητα των ασθενών παγκοσμίως, ένας ικανοποιητικός ορισμός της παραμένει δύσκολος. Το CYP450 είναι ένα σύστημα ενζύμων που παρουσιάζει πολλούς πολυμορφισμούς και παίζει βασικό ρόλο στον μεταβολισμό των φαρμάκων. Τα αντικαταθλιπτικά που χρησιμοποιούνται σήμερα μεταβολίζονται όλα από το CYP450. Ενώ η βιβλιογραφία καταλήγει με βεβαιότητα στην ύπαρξη συσχέτισης μεταξύ των γενοτύπων του CYP450 και συγκεντρώσεων των αντικαταθλιπτικών στο πλάσμα, η σχέση τους με το κλινικό αποτέλεσμα είναι διφορούμενη.

ΣΚΟΠΟΙ:

Το να εξερευνηθεί ο αντίκτυπος πολυμερισμών των CYPC19 και CYP2D6 στην αντίσταση στην θεραπεία με αντικαταθλιπτικά σε πληθυσμό διαγνωσμένων με μείζονα κατάθλιψη στην Ελλάδα.

ΜΕΘΟΔΟΙ:

Σε αυτή την μελέτη παρατήρησης ενήλικοι εξωτερικοί ασθενείς διαγνωσμένοι με μείζονα κατάθλιψη θα συμμετέχουν στην μελέτη με κριτήρια αποκλεισμού: την διπολική διαταραχή, την σχιζοφρένεια, την κατάχρηση ουσιών την ύπαρξη ασθένειας που μπορεί να επηρεάσει την κατάθλιψη. Οι ασθενείς αξιολογούνται με την κλίμακα HDRS στην αρχή καθώς και τις εβδομάδες 2,4,8,12, οι αξιολογητές θα είναι τυφλοί απέναντι στην ανάλυση των γονοτύπων. Η μεταβολή HDRS% είναι η κύρια μέτρηση της μελέτης και η σχέση της με τα διάφορα αλληλόμορφα των CYPC19 και CYP2D6 θα διερευνηθεί με Χ τετράγωνο τεστ και το μέγεθος της σχέσης θα υπολογιστεί με βάση γενετικά μοντέλα, επίσης θα πραγματοποιηθεί ανάλυση μεταβλητότητας .

ΑΠΟΤΕΛΕΣΜΑΤΑ:

Τα αποτελέσματα της μελέτης αναμένεται να συμφωνήσουν με άλλες έρευνες και να αποδείξουν μια στατιστικά σημαντική σχέση μεταξύ πολυμορφισμών του CYP450 και κλινικού αποτελέσματος.

ΣΥΜΠΕΡΑΣΜΑΤΑ:

Στόχος μας είναι να συνεισφέρουμε στην καλύτερη κατανόηση και θεραπεία της ανθεκτικής κατάθλιψης καθώς και την αξιολόγηση της χρησιμότητας της γενετικής ταυτοποίησης των γονιδίων του P450 για εξατομικευμένη θεραπεία της κατάθλιψης.

Αντικαταθλιπτικά; CYP2C19, CYP2D6 γονότυπος, φαρμακογενετική

A. Abstract

Abstract

INTRODUCTION:

Though Treatment Resistant Depression maybe the most important cause for disability in the world now, it is still hard to be defined.CYP450 is an enzyme system that is important for drug metabolism and is highly polymorphic. Antidepressants that are in use today are all metabolized by CYP450.Current literature definitely supports a relation between CYP450 genotypes and plasma levels of antidepressants but there is no consensus yet regarding a relation with clinical outcome after treatment.

OBJECTIVE:

To investigate the influence of common CYPC19 and CYP2D6 polymorphisms on antidepressant-treatment resistance to a psychiatric outpatient population diagnosed with Major Depressive disorder in Greece.

METHOD:

In this naturalistic setting study adult psychiatric outpatiens diagnosed with Major Depressive disorder will be enrolled with exclusion criteria being psychotic depression, other major psychiatric diagnosis such as bipolar disorder, schizophrenia or substance abuse and medical condition that can affect symptoms. Subjects will have their condition assessed with HDRS on baseline, 2,4,8,12 weeks, assessment will be blind to genotyping. HDRS% change is the study's prime outcome and its association with CYPC19 and CYP2D6 will be investigated with Xsquared test then the magnitude of association will be examined for genetic models, and analysis of co-variance will be performed.

RESULTS:

The results of the study are expected to be in accordance with current literature and confirm a statistically significant relation between common CYP polymorhisms and clinical response.

CONCLUSIONS:

It is our goal for our results to contribute to the better understanding of treatment of TRD and the potential of P450 genotyping for deression

Antidepressants; CYP2C19; CYP2D6; Genotype; Pharmacogenetics,

B. Introduction

B1. Major Depressive Disorder, Treatment Resistant Depression

Major Depressive Disorder (MDD) is a very common mental disorder, often causing serious morbidity and accompanied by significant deterioration of patients' functioning. According to the World Health Organization (WHO), MDD is currently the leading cause of disability worldwide and a major contributor to the overall global burden of disease. About 10% of the population become depressed annually and a 20% will develop depression at some point during their life time (1-3).

The disability caused by MDD is more prevalent in female population and has an early onset in life. Despite the lack of separate disability assessments concerning treatment-resistant depression (TRD), experts consider patients with TRD to be the most severely disabled (4).TRD is also accountable for 50% of the cost of treatment of MDD, as one third of patients do not respond to initial treatment and about 50% achieve only partial response.

Despite its obvious major role in public health there is no consensus yet, concerning a definition for TRD, amongst researchers (5-6). It has been suggested though that TRD can be defined as Depression that fails to remit after two or more unsuccessful guideline –concordant index antidepressant treatment trials. In addition, five models for staging TRD have been proposed to evaluate the severity of resistance in relation to a variety of treatments and combinations of treatments. Finally evidence support that the outcome of initial treatments is a consistent predictor for long term recovery from MDD as initial remitted state compared to "response with residual depressive symptoms" is connected with higher long term full recovery rates (7).

B2. The Cytochrome P450 enzyme system and antidepressants

The Cytochrome P450 (CYP450) is a superfamily of hemecontaining isozymes that are located in most cells but are primarily found in the liver and GI tract, its main clinical relevancy is catalyzing Phase I reactions in drug metabolism. Due to a variety of genes encodings those enzymes, there are a lot different CYP isoenzymes ,four of those (CYP2D6, CYP2C8/9 and CYP1A2) are the main agents participating in most Phase I reactions, there is relative specificity for different substrates but overlapping is common, as more than one isoforms can catalyze the metabolism of the same drug.(8)

There is considerable genetic variability as polymorphisms have been identified for most P450 isoenzymes. The polymorphic nature of CYP genes affects individual drug response and adverse reactions to a great extent (8). Thus individuals could be classified into 3 major categories: 1) **the ultrarapid metabolizers (UM)**, with more than 2 active genes for a certain P450 isoform.2) **the extensive metabolizers (EM),** with 2 functional genes. 3) **the poor metabolizers (PM),** lacking functional enzyme due to defective or deleted genes. In addition, a more subtle phenotype occur that is commonly called **the intermediate metabolizers (IM)**, usually carrying 1 functional and 1 defective allele or 2 partially defective alleles.(9)

The most commonly prescribed antidepressants in clinical practice are all metabolized in the liver by the cytochrome P450 (CYP) enzyme system. (10) The isoforms that metabolize specific antidepressant agents are shown in table 1. (11)

CYP1A2 plays a major role to the metabolism of agomelatine and a lesser one for Selective Serotonin Re-uptake Inhibitors (SSRI's), mirtazapine and duloxetine.(11)

CYP2C19 extensively metabolizes Tricyclic antidepressants (TCA) and there is replicated evidence that support the relevancy of CYP2C19 polymorphisms to the metabolism of these drugs. As 2-defected gene carriers in several trials scored consistently higher plasma concentrations than those carrying one or none mutated gene. As for SSRI's, evidence show a significant effect of CYP2C19 polymorphisms to plasma concentration level for patients administered Escitalopram, Citalopram or Sertaline but the effect is unclear when clinical response is measured as an outcome. Nevertheless some researchers have suggested guidelines for dosage regulation based on genotype metabolizer status i.e. UM, EM, PM (11).

CYP2D6 may be the most well documented isoenzyme in current literature in regards to the significant effect of its polymorphisms to the outcome of treatment with antidepressant medication. For TCAs ultra rapid metabolizers have been proven to need higher doses to achieve clinical improvement and poor metabolizers are suggested by evidence to be more susceptible to adverse effects in regular doses. Whereas this effect is much less well documented for SSRI's such as escitalopram and fluoxetine and SNRI's like venlafaxine some researchers do have suggested genotype based guidelines for these drugs too (11).

Table 1

CYP isoform	Antidepressant Substrate
CYP1A2	tricyclics,fluvoxamine,trazodone, duloxetine,mirtazapine,agomelatine
CYP2B6	bupropion
CYP2C9	fluoxetine
CYP2C19	tricyclics,sertaline,citalopram,escitalopram
CYP2D6	Trycyclics,fluoxetine,fluvoxamine, paroxetine,escitalopram,venlafaxine, Mirtazapine,duloxetine,vortioxetine
CYP3A4	tricyclics, sertraline, citalopram, escitalopram,venlafaxine, mirtazapine, trazodone

B3. Further evidence supporting significant effect of CYP450 polymorphisms to treatment resistance

Two independent, peer reviewed 2004 pilot studies found non-responders to TCA and/or SSRI treatment to have a significantly higher CYP2D6 UM phenotype incidence -5 to 10 times higher than expected-(12-13) .

In addition recent peer reviewed studies conclude that the efficacy of treatment with escitalopram is significantly correlated to CYP2D6 and/or CYP2C19 genotype (14-15).

Furthermore, numerous studies show significant correlation between CYP2D6 and/or CYP2C19 genotypes and plasma concentrations for escitalopram, citalopram, venlafaxine, mirtazapine, sertaline and other antidepressants (11, 14-18).

C. Objective

Compared to evidence confirming a correlation between CYP genotypes and antidepressant plasma levels, there are relatively scarce data on the effect of CYP genotype to the clinical outcome of antidepressant treatment (11).Taking into account what is currently considered a pharmacologic dogma for psychotropic drug experts i.e. *that plasma concentration levels for psychotropic drugs, cannot predict clinical response*, the need for further studies becomes more apparent (11, 19).

The main objective of this study is to investigate the influence of common CYPC19 and CYP2D6 polymorphisms on antidepressant-treatment resistance to a psychiatric outpatient population diagnosed with Major Depressive disorder in Greece. Also to compare that influence to other possible factors contributing to resistance.

A secondary aim is collecting relevant pharmacogenetic data for Greek population, as there is a documented scarcity of pharmacogenetic studies for Greek psychiatric patients (20).

D. Methods

D1. Study population

The subjects of this study will be men and women of 18 year of age or older that receive psychiatric treatment at various outpatient clinics in Greece and have been diagnosed with Major Depressive Disorder according to DSM-5-Diagnostic and Statistical Manual for Mental Disorders Fifth Edition criteria (21) and with a Hamilton Depression Rating Scale score of at least 18 at baseline .

Exclusion criteria for enrollment are co-morbidity or history of other major psychiatric diagnosis i.e. *schizophrenia, schizoaffective disorder, bipolar disorder, neuro-cognitive disorder or substance related and addictive disorder (including alcohol)* as they are described in DSM-5. Also patients diagnosed with MDD with psychotic features as specified in DSM-5 will be considered ineligible for enrollment regardless if those symptoms are mood-congruent or not (21). A medical condition that can imitate or worsen depressive symptoms (e.g. anemia, thyroid disorders, multiple sclerosis etc) and pregnancy are also considered ineligibility criteria.

Three independent psychiatrists will evaluate the subjects' history and diagnosis in order for eligibility to be assessed with the maximum accuracy.

D2. Study Design

The duration of the study will be 12 weeks and will be conducted in 4 outpatient psychiatric clinics in Northern Greece. As this is a study conducted in a naturalistic setting, drugs and clinical and laboratory examinations will be administrated as per patient needs, in accordance to current clinical practice and up to date with current guidelines for MDD treatment. The basic outcome measured in the study is reduction on Hamilton Depression Rating Scale (HDRS) score.

On baseline after eligibility evaluation, each subject will be assessed with HDRS and a blood sample for conducting genotyping will be received after written informed consent of each subject. Also demographic and other relevant information such as smoking status and psychotherapy treatment will be obtained with a proper questionnaire.(..)

Follow-up assessment with HDRS will be repeated on weeks 2, 4, 8 and 12 and it will be blinded to genotype. On weeks 2 to 12 subjects will also be assessed for side effects using the UKU *side effects rating scale for the registration of unwanted effects of psychotropics*. Drug discontinuation, change of drugs, dosage regulation, augmentation with other drug(s) and any other change of treatment will be documented from baseline to week12 in proper forms. Doses during analysis will be converted to fluoxetine equivalents according to current evidence (23).

Genotyping will be performed for CYP2C19 alleles: CYP2C19*2/*3/*4/*17 and CYP2D6 alleles : CYP2D6*3/*4/*5/*10 using standard long-range PCR after DNA extraction from venous blood samples. The patients will be then categorized into CYP2C19 and CYP2D6 genotype-defined metabolizer subgroups according to the number of functioning gene carrying.

D3.Statistical analysis

The number of different CYP2C19 and CYP2D6 polymorphisms that will be found in the study sample will be assessed for deviation from Hardy-Weinberg equilibrium (24) using test.

If response is treated as a categorical variable then a binary outcome can be produced (response/non response) by choosing a suitable cut point on % HDRS change. Then the association between response to treatment and a polymorphism can be tested by test. If the association between genotype distribution and the response outcome is significant, the magnitude for association can be examined for various genetic models. Additive, co-dominant, recessive, dominant .The generalized odds ratio will be computed.

The association of the polymorphisms with response, if response is considered a continuous variable expressed as % HDRS change, can be tested with analysis of co-variance (general linear model with type III sum of square statistics) using age, gender, smoking status, years since first major episode and psychotherapy treatment as co-variants.

SPSS and ORGASMA software will be used for the analysis.

D4. Ethical aspects

All subjects will be thoroughly informed before asked to give written consent for participation; confidentiality of DATA will be secured in accordance to the Declaration of Helsinki. The study will be filed for approval to an independent bioethics committee.

E. Results

The results of the study are expected to be in accordance with current literature and confirm a statistically significant relation between common CYP polymorhisms and clinical response to antidepressant treatment (11-19).Those results are expected to quantify the effect of certain CYP alleles to clinical outcomes of our study sample.

F. Conclusion

It is our goal for our results to contribute to the pursuit of further understanding Treatment Resistant Depression a clinical entity that has been dubbed “resistant to definition” (5) and thus contribute to the evaluation of strategies for its pharmacological and non pharmacological treatment. We also hope that our results will be useful for further evaluating the use of CYP450 genotyping to the guidance of antidepressant treatment of patients with major depressive disorder.

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