UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
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ESTUDOS FARMACOGENÉTICOS DA RESPOSTA AO TRATAMENTO COM ANTIPSICÓTICOS EM ESQUIZOFRÊNICOS

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SUMÁRIO

LISTA DE ABREVIATURAS, SÍMBOLOS E UNIDADES

alfa1 – receptor alfa adrenérgico tipo 1

BDNF – Fator neutrotrófico derivado do cérebro

COMT – Catecol-O-metil-transferase

CYP – Cytocromo P450

CYP1A2 – Cytocromo P450, família 1, sub-família A, polipeptídeo 2

CYP2C9 – Cytocromo P450, família 2, sub-família C, polipeptídeo 9

CYP2C19 – Cytocromo P450, família 2, sub-família C, polipeptídeo 19

CYP2D6 – Cytocromo P450, família 2, sub-família D, polipeptídeo 6

CYP3A4 – Cytocromo P450, família 3, sub-família A, polipeptídeo 4

CYP3A5 – Cytocromo P450, família 3, sub-família A, polipeptídeo 5

DAO – D-amino-ácido oxidase

DAOA – Ativador da D-amino-ácido oxidase

DAT1 ou SLC6A3 – Transportador de dopamina

DISC-1 – Proteína modificada na esquizofrenia

DRD2 – Receptor de dopamina tipo 2

DRD3 – Receptor de dopamina tipo 3

DRD4 – Receptor de dopamina tipo 4

DTNBP1 – Disbindina

GABA – ácido gama-aminobutírico

GNB3 – subunidade Beta3 da proteina G

GPCRs – receptores de catecolaminas acoplados à proteina G

H1/H2 – receptor histamínicos tipo 1/2

M1 – receptore colinérgico muscarínico tipo 1

NMDA-R – receptor N-metil-D-aspartato

NRG1 – neuregulina

ProDH – prolina desidrogenase

RGS4 – regulador da sinalização da proteína G

5-HT – receptores de serotonina

5-HT1-7 – receptores de serotonina tipo 1-7

5-HT2A e 5-HT2C – receptores de serotonina sub-tipo 2A e 2C

5-HTT ou SLC6A4 – Transportador de serotonina

RESUMO

A farmacogenética busca entender a base hereditária da variabilidade da resposta e dos efeitos adversos dos agentes farmacológicos entre os indivíduos. Os medicamentos antipsicóticos são utilizados em tratamentos bastante efetivos para a esquizofrenia, mas seu uso apresenta complicações por diversos fatores. Embora boa parte dos pacientes responda às terapias com antipsicóticos, 20-40% mostram resposta inadequada, e o custo de cada tentativa de medicação não-efetiva para os pacientes pode levar a semanas de permanência da doença, ocorrência de efeitos adversos potenciais e não-aderência ao tratamento. Este estudo tem como objetivo identificar genes que podem potencialmente influenciar a resposta ao tratamento com antipsicóticos em pacientes esquizofrênicos. Essa abordagem envolveu genes que determinam aspectos farmacocinéticos (enzimas metabolizadoras de fármacos: *CYP2D6, CYP3A4* e *CYP3A5*) e farmacodinâmicos (receptores: *DRD2, DRD3, DRD4, HTR2A, HTR2C* e *HTR1B,* transportadores de neurotransmissores: *SLC6A3, SLC6A4, e* outros genes relacionados: *COMT, MAOA, BDNF* e *GNB3*) potencialmente envolvidos na eficácia do tratamento. No presente trabalho foram estudados 208 pacientes esquizofrênicos, sendo 135 refratários aos neurolépticos e 121 em tratamento com clozapina. Os polimorfismos investigados foram estudados por métodos baseados na Reação em Cadeia da Polimerase (PCR), e alguns deles seguidos por análises de alta resolução, através da plataforma Sequenom (Sequenom, Inc.), em colaboração com o laboratório de Medicina Molecular na Espanha. Os principais resultados incluem alguns inéditos, como a associação de polimorfismos nos genes *GNB3* (*825C>T*) e *COMT* (*Val158Met*) com ocorrência de convulsão no tratamento com clozapina; a associação de polimorfismos em *DRD3* (*Ser9Gly*) e *CYP3A5* (*6986A>G*) com resposta aos neurolépticos típicos; e a primeira descrição detalhada de freqüências alélicas e genotípicas no gene *CYP2D6* em brasileiros. Adicionalmente, polimorfismos nos genes *5HTT* (*HTTLPR*) e *GNB3* (*825C>T*) foram associados significativamente com resposta à clozapina. Estes resultados sugerem que variantes genéticas podem desempenhar um papel importante na efetividade do tratamento com antipsicóticos, e podem ser considerados como

sugestivos do efeito de polimorfismos em genes candidatos para estudos farmacogenéticos na esquizofrenia.

ABSTRACT

The focus of pharmacogenetics is the knowledge of hereditary basis of response and adverse drug reactions to pharmacologic agents among individuals. Antipsychotics are quite effective for schizophrenia treatment, but their use remain complicated by several factors. Although a number of patients may respond to antipsychotic therapy, 20-40% of them exhibit inadequate response and the cost of ineffective medication trial for patients may entail weeks of unremitted illness, potential adverse drug reactions and nonadherence to treatment. This study aims to identify genes that potentially influence treatment response with antipsychotic in schizophrenics. This approach involved genes which determine pharmacokinetic (drug-metabolizing enzymes: *CYP2D6, CYP3A4*, and *CYP3A5*) and pharmacodynamic pathways (receptors: *DRD2, DRD3, DRD4, HTR2A, HTR2C*, and *HTR1B*, neurotransmitter-transporters: *SLC6A3* and *SLC6A4,* and other related genes: *COMT, MAOA, BDNF*, and *GNB3*) potentially involved with treatment efficacy. In the present study, we studied 208 schizophrenic patients, 135 neuroleptic resistants and 121 under clozapine treatment. The investigated polymorphisms were analyzed by Polymerase Chain Reaction (PCR), and some of them were followed by high-throughput analyzes of Sequenom (Sequenom, Inc.), in collaboration with the Laboratory of Molecular Medicine, in Spain. The main results include original findings, such as the association of *GNB3* (*825C>T*) and COMT (*Val158Met*) gene polymorphisms and seizure occurrence under clozapine treatment; the association of *DRD3* (*Ser9Gly*) and *CYP3A5* (*6986A>G*) gene polymorphisms and response to typical neuroleptics; and the first detailed description of *CYP2D6* allele and genotype frequencies in Brazilians. Additionally, the *5HTT* (*HTTLPR*) and *GNB3* (*825C>T*) gene polymorphisms were significantly associated to clozapine response. These results suggest that genetic variants may have an important role in antipsychotic treatment effectiveness, and they may be considered as suggestive of the effect of candidate polymorphisms for pharmacogenetic studies in schizophrenia.

CAPÍTULO I

INTRODUÇÃO

I.1. Considerações gerais

A esquizofrenia foi originalmente chamada *dementia praecox* por Emil Kraepelin, o grande psiquiatra do século XIX, que criou o termo para categorizar um grupo de pacientes jovens que rumavam para o desenvolvimento de demência (Meltzer e cols., 1999). A partir dos anos 50, Eugen Bleuler, ao verificar que a maioria dos pacientes com psicose similar e características afetivas não se tornavam severamente dementes, re-nomeou a doença como esquizofrenia, também conceitualizando a doença como uma patologia neurocognitiva (Andreasen, 1999). Para Bleuler, a esquizofrenia não era uma doença unitária: o "grupo de esquizofrenias" incluía múltiplos transtornos que compartilhavam várias características clínicas, mas diferiam em etiologia e patogênese.

A esquizofrenia está entre os transtornos psiquiátricos mais graves e devastadores, e afeta cerca de 1% da população. Geralmente manifesta-se clinicamente no final da adolescência e início da vida adulta e apresenta igual prevalência entre homens e mulheres. O pico da idade de início da doença em homens é entre 15-25 anos, sendo que em mulheres ocorre um atraso de 3-5 anos (Pearlson, 2000). Esta doença, além de comprometer pacientes e familiares, representa um grande custo para toda a sociedade. No Brasil, a esquizofrenia ocupa 30% dos leitos psiquiátricos hospitalares, ou cerca de 100 mil leitos/dia. Ocupa ainda o segundo lugar das primeiras consultas psiquiátricas ambulatoriais (14%) e o 5º lugar na manutenção de auxílio-doença. Nos Estados Unidos, representa um custo anual de 33-40 bilhões de dólares (Pádua e cols., 2005).

Os transtornos esquizofrênicos caracterizam-se por distorções profundas e características no pensamento e na percepção, e por afeto inapropriado ou embotado. Em outras palavras, são caracterizados por uma ampla variedade de sintomas que refletem alterações nos processos cognitivo, psicomotor e emocional, ou seja, pensamentos desorganizados, incluindo delírios e disparidade de pensamento lógico, e distúrbios de percepção, tal como alucinações (Lesch, 2001). Os critérios diagnósticos para a esquizofrenia (Anexo 1) seguem

principalmente aqueles apresentados no Manual Diagnóstico e Estatístico de Transtornos Mentais (DSM-IV) (American Psychiatric Association, 1994).

Os sintomas da esquizofrenia, por serem bastante complexos e diversos, são simplificados através de subdivisões em categorias naturais. A abordagem mais aceita é a divisão em sintomas “positivos” e “negativos”. Esta terminologia deriva de Hughlings-Jackson, que referia os sintomas positivos como reflexos de processos cerebrais que são desinibidos ou liberados excessivamente pelo cérebro lesionado, e os negativos como resultantes diretamente de lesões em áreas cerebrais responsáveis pela produção do comportamento humano, provavelmente uma conseqüência de perda neuronal (Andreasen, 1995).

Atualmente, os sintomas positivos são referidos como alterações de funções normais (presença de algo que deveria estar ausente) e os negativos como perda da função normal (ausência de algo que deveria estar presente) (Lesch, 2001). Os sintomas positivos podem ser adicionalmente subdivididos em um grupo psicótico (ilusões, delírios e alucinações) e um desorganizado (pensamento, fala e comportamento desorganizados). Os sintomas negativos incluem embotamento afetivo, isolamento social, falta de interesse e escassez de pensamentos. Considera-se que a base biológica dos sintomas psicóticos positivos está ligada a uma super-atividade de neurônios dopaminérgicos, especificamente aqueles envolvidos na rota dopaminérgica mesolímbica, que controla o comportamento, e especialmente pode produzir ilusões e alucinações quando super-ativa (Teoria Dopaminérgica da Esquizofrenia). Já os sintomas negativos da esquizofrenia podem envolver outras regiões do cérebro, tal como o córtex dorsolateral-prefrontal e outros sistemas neurotransmissores (Stahl, 1996). De fato, o déficit comportamental conseqüente dos sintomas negativos implica baixa atividade de alguns sistemas neuronais (Andreasen, 1995).

I.2. Etiologia da esquizofrenia

Apesar do grande progresso em pesquisa básica da esquizofrenia, a identificação conclusiva de fatores etiológicos ou processos patogênicos

específicos da doença permanece elusiva. Dados experimentais sugerem diferentes mecanismos biológicos para explicar as manifestações comportamentais deste transtorno. Tais evidências são bastante diversas, incluindo, além de mecanismos dopaminérgicos, também gabaérgicos, colinérgicos, glutamatérgicos, serotoninérgicos, adenosinérgicos e fosfolipídicos (Lara e cols., 1999; Gattaz, 2000).

I.2.1. Hipóteses dos neurotransmissores

A hipótese dopaminérgica da esquizofrenia propõe uma hiper-estimulação subcortical da transmissão dopaminérgica em receptores de dopamina do tipo 2 (D2), responsáveis pelos sintomas positivos da doença (Guillin e cols., 2007). Esta hipótese é apoiada pela estreita correlação entre doses terapêuticas de antipsicóticos convencionais e suas afinidades por receptores D2 e adicionalmente devido a evidências de aumento do número de receptores D2 em cérebros de esquizofrênicos, mas que também pode ser devido ao uso de medicação antipsicótica (Pearlson, 2000; Miyamoto e cols., 2003; Wong e van Tol, 2003). Anormalidades dopaminérgicas pré-sinápticas implicam em disfunção no estoque pré-sináptico, transporte vesicular, liberação, re-captação e mecanismos metabólicos em sistemas dopaminérgicos mesolímbicos. Ao contrário, a atividade dopaminérgica pode estar diminuída no neocórtex, o que pode ser relacionado, pelo menos em parte, aos distúrbios cognitivos e sintomas negativos (Miyamoto e cols., 2003; Guillin e cols., 2007).

Recentemente, a atenção tem sido focada na serotonina (5-HT). A hipótese da serotonina propõe que este neurotransmissor tenha um papel importante na etiologia da doença, já que receptores de serotonina estão envolvidos nas propriedades psicomiméticas e psicotogênicas de alucinógenos e na ação de antipsicóticos. Há sugestões de que a função trópica da serotonina no neurodesenvolvimento possa estar alterada (Miyamoto e cols., 2003). Como os sistemas serotoninérgico e dopaminérgico são interdependentes, ambos podem estar simultaneamente alterados na esquizofrenia (Lieberman e cols., 1998).

Adicionalmente, o sistema glutamatérgico também é apontado como envolvido na etiologia da doença. Uma função diminuída do receptor N-metil-D-aspartato (NMDA-R) poderia ser um fator de predisposição à esquizofrenia (Coyle, 1996). A existência de inter-relações anatômicas e funcionais entre os sistemas glutamatérgico e dopaminérgico no SNC sugerem que a inibição de NMDA-R pode afetar a neurotransmissão dopaminérgica (Deutsch e cols., 1989).

I.2.2. Hipótese de neurodesenvolvimento

A hipótese de neurodesenvolvimento postula que anormalidades no desenvolvimento cerebral inicial aumentem o risco de emergência subseqüente de sintomas clínicos (Miyamoto e cols., 2003). Portanto, a doença seria causada por lesões cerebrais determinadas por uma combinação de fatores genéticos e/ou ambientais iniciais e que eventualmente interagem com o processo de maturação normal do cérebro para facilitar sintomas como a psicose (Cannon e cols., 2002).

Estudos de imagem revelam claras anormalidades estruturais cerebrais (deficiências no lobo temporal, volume cerebral reduzido, aumento ventricular, perda de massa cinzenta do lobo temporal, redução do volume do tálamo) e redução do volume do giro temporal superior (Hirayasu e cols., 2000). Estas características podem fornecer a base anatômica para os mecanismos patofisiológicos que dão origem a deficiências de linguagem e processamento do pensamento que ocorrem na esquizofrenia.

I.2.3. Fatores genéticos

A prevalência da esquizofrenia através das populações traz à tona a questão da natureza genética da doença. Kraepelin observou, em suas primeiras descrições, que um forte componente herdado para o processo da doença estaria presente (DeLisi, 1996). Jablensky e cols. (1992) concluíram que esta doença é ubíqua, aparece com incidência similar em diferentes culturas e apresenta características clínicas que são mais marcantes devido às suas similaridades

através das populações do que por suas diferenças. Por essa generalização, parece que o componente genético para a esquizofrenia seria mais forte do que o ambiental.

De fato, estudos baseados em famílias, irmãos gêmeos e adotivos, dão evidência de uma importante contribuição genética para a doença, sendo a herdabilidade estimada entre 60 e 80% (Tamminga e Holcomb, 2005; Sanders e Gill, 2007). Heterogeneidade etiológica e de lócus são amplamente suspeitas (Miyamoto e cols., 2003). Apesar disso, diversas dificuldades são encontradas na pesquisa de genes de suscetibilidade à doença. As razões para essa dificuldade incluem a complexidade do fenótipo, heterogeneidade, diagnóstico impreciso e falta de marcadores biológicos (Tamminga e Holcomb, 2005). O modo de transmissão também é complexo, pois envolve o efeito combinado de muitos genes, cada um conferindo efeitos moderados ao fenótipo como um todo, além da interação com fatores de risco ambientais (Gottesman, 1991; McGuffin e cols., 1995). Devido a esta grande complexidade, ainda não há certeza a respeito do número de genes envolvidos nem do grau de interação e da contribuição de cada um para a suscetibilidade total desta doença multifatorial (Prasad e cols., 2002).

Diversos estudos de ligação e de associação com esquizofrenia, realizados até o presente, sugerem a influência de uma série de genes associados à suscetibilidade à doença. Evidências de ligação foram obtidas nos cromossomos 8p, 22q, 2, 3, 5q, 6p, 11q, 13q e 20p. Genes nestas regiões têm sido associados com a doença, incluindo o gene da neuregulina (*NRG1*, 8p12), disbindina (*DTNBP1*, 6p22), D-aminoácido oxidase (*DAO*, 12q24), ativador da D-aminoácido oxidase (*DAOA*, 13q34), proteína modificada na esquizofrenia (DISC-1, 1q42), regulador da sinalização da proteína G (*RGS4*, 1q21-22), prolina desidrogenase (*ProDH*, 22q11.21) e catecol-O-methiltransferase (*COMT*, 22q11) (Tamminga e Holcomb, 2005; Maier e cols., 2006; Sanders e Gill, 2007). Cada um destes genes codifica uma proteína que poderia estar associada a algum mecanismo da doença. As regiões genômicas que têm sido identificadas como suspeitas de possuir genes que predispõem para a doença variam de estudo para estudo e de família para família, sugerindo um alto grau de heterogeneidade da doença.

Estudos de ligação demonstraram também forte influência da etnia e do gênero na predisposição genética ao desenvolvimento de esquizofrenia (Vaswani e Kapur, 2001).

Apesar dos grandes esforços na tentativa de identificação de genes de suscetibilidade à esquizofrenia, os resultados ainda são bastante controversos e, portanto, muitas lacunas permanecem abertas no conhecimento da influência genética da doença. E quando os genes mais relevantes forem encontrados, a interpretação de suas ações ainda será difícil, devido ao limitado entendimento de aspectos fundamentais da organização e desenvolvimento funcional do cérebro (Barondes e cols., 1997).

I.2.4. Fatores ambientais

Por ser uma doença altamente complexa, o ambiente, juntamente com outros fatores de risco, tem um papel importante no desenvolvimento da esquizofrenia. Dentre os fatores de risco ambiental podemos salientar: nascimento em zonas urbanas, nascimento no final inverno/início primavera, infecções maternas (efeito neurotóxico no desenvolvimento de neurônios), complicações obstétricas (complicações na gravidez, crescimento e desenvolvimento fetal anormal, complicações no parto), seqüências retrovirais (ativação transcricional de certos elementos retrovirais no cérebro), filhos de mulheres que receberam radiação no primeiro trimestre de gravidez (Miyamoto e cols., 2003), entre outros.

I.3. Tratamento da esquizofrenia

I.3.1. Classes de antipsicóticos

Os medicamentos comumente utilizados para tratar a esquizofrenia pertencem principalmente a dois grandes grupos: antipsicóticos típicos (ou convencionais) e atípicos (ou de nova geração) (Figura 1). Estes se diferenciam

quanto a sua efetividade principalmente de acordo com seus perfis de sítios de ação específicos.

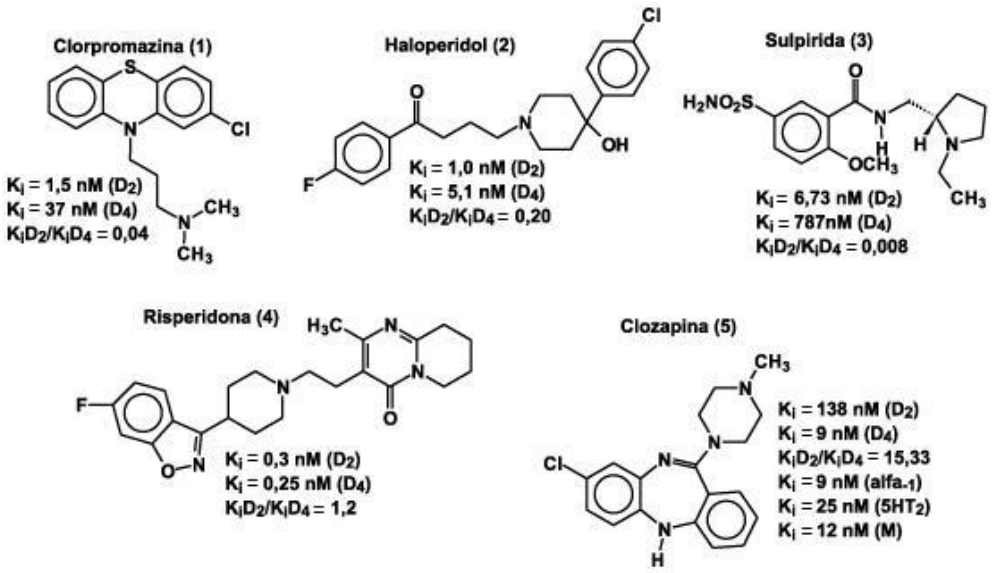

Figura 1: Fármacos antipsicóticos típicos 1-3 e atípicos 4-5 utilizados no tratamento da esquizofrenia. Fonte: Menegatti e cols. (2004)

I.3.2. Mecanismos de ação dos antipsicóticos típicos

Os primeiros fármacos antipsicóticos convencionais foram descobertos por acidente nos anos 50, quando um medicamento considerado um anti-histamínico (clorpromazina) apresentou efeitos antipsicóticos únicos quando testado em pacientes esquizofrênicos, pois além de alguma atividade histamínica, a clorpromazina apresentou uma atividade mais importante em receptores de dopamina (Stahl, 1996). Com a descoberta de outros medicamentos típicos, observou-se que todos esses agentes causavam neurolepsia (falta de movimento) em animais experimentais, de onde derivou a designação neuroléptico (efeitos adversos característicos sobre o sistema motor extrapiramidal, incluindo distonia, parkinsonismo, discinesia e acatisia). Entre os anos 60 e 70 do século XX, reconheceu-se amplamente que todos os neurolépticos clássicos conhecidos compartilhavam a propriedade comum de bloqueio dos receptores de dopamina,

particularmente receptores D2 (Carlson e Lindquist, 1963; Ereshefsky e Pharm, 1999; Prasad e cols., 2002; Meltzer, 2004). Estes fármacos possuem pelo menos mais três sitos de ação: bloqueio de receptores colinérgicos muscarínicos (M1), alfa adrenérgicos (alfa1) e histamínicos (H1). Devido à ação de bloqueio sobre receptores D2 da dopamina (rota mesolímbica), os neurolépticos típicos mostraram-se eficientes na redução, e às vezes na eliminação, dos sintomas positivos da esquizofrenia. Entretanto, o bloqueio de receptores de dopamina na rota dopaminérgica estriatal (rota que controla movimentos) causa os diversos efeitos adversos extrapiramidais. Desta forma, o antagonismo do receptor D2 medeia não somente os efeitos terapêuticos dos agentes antipsicóticos, mas também os efeitos adversos dos mesmos (Stahl, 1996; Kawanishi e cols., 2000).

I.3.3. Mecanismos de ação dos antipsicóticos atípicos

Tentativas posteriores para melhorar o perfil terapêutico dos neurolépticos clássicos basearam-se nas pesquisas que revelaram que receptores de dopamina da rota estriatal mediavam os efeitos adversos extrapiramidais, mas que os receptores de dopamina da rota mesolímbica mediavam as ações terapêuticas antipsicóticas dos neurolépticos. Os esforços centraram-se então na procura de agentes mais seletivos para receptores da rota mesolímbica do que para os da estriatal. Os chamados antipsicóticos atípicos, como tioridazina e sulpiride, por exemplo, mostraram ter menos propensão à produção de efeitos extrapiramidais e ao mesmo tempo ainda apresentando boas propriedades antipsicóticas, quando comparados com os típicos (Stahl, 1996). Portanto, o termo “atípico” derivou-se do conceito: eficácia típica com efeitos adversos atípicos. Com a introdução da Clozapina nos anos 80 do século passado, o conceito “atípico” foi expandido para eficácia aumentada, com efeitos adversos diminuídos, pois os mesmos produzem significantemente menos efeitos extrapiramidais do que os neurolépticos típicos, em doses clinicamente equivalentes, além de mostrarem vantagem no tratamento de sintomas positivos, negativos e de disfunção cognitiva (Jibson e Tandon, 1998; Kawanishi e cols., 2000; Malhotra, 2001), talvez por sua maior afinidade por receptores de dopamina do tipo 4 (D4). Atualmente, a clozapina permanece como

a medicação antipsicótica com eficácia superior às tradicionais para o tratamento de pacientes com resposta pobre ao tratamento ou ainda aqueles resistentes ao mesmo (Malhotra, 2001). O efeito antipsicótico da clozapina tem sido atribuído, em parte, à sua habilidade de intenso bloqueio ao estímulo de receptores de serotonina do tipo 2A (5-HT2A), principalmente quando associado ao fraco bloqueio (rápida dissociação, altos valores de k_{off}, Figura 2) de receptores D2 (Miyamoto e cols., 2005). Como essa característica não explica a maior eficácia da clozapina, seu significado ainda permanece incerto.

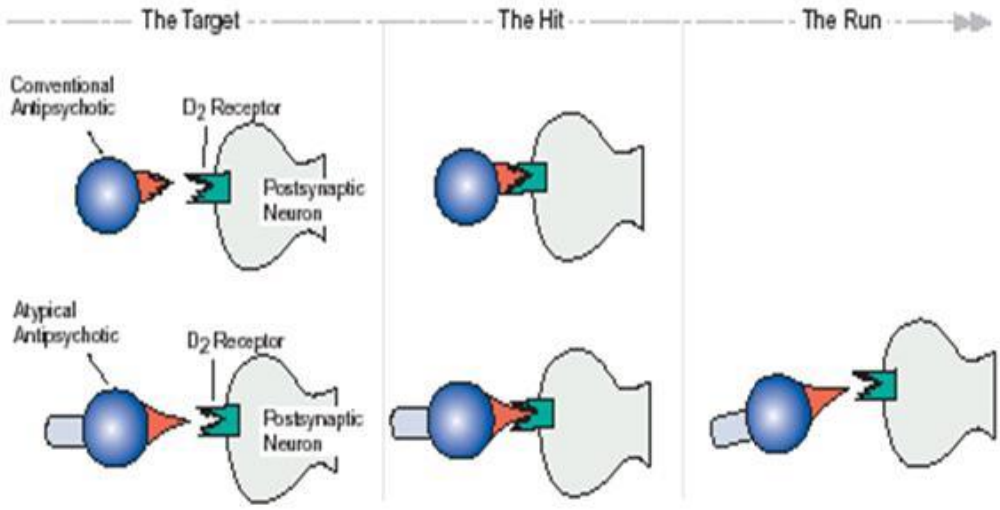

Figura 2: Ação sobre receptores D2: antipsicóticos típicos X atípicos. Fonte: Stahl (1996).

Os agentes antipsicóticos atípicos possuem uma capacidade antagonista em receptores D2 da dopamina diminuída (30-60%) quando comparados à neurolépticos típicos (70-90%) e uma afinidade adicional aos receptores de serotonina (5-HT2) (Miyamoto e cols., 2005). A ação destes agentes não se resume somente a receptores 5-HT2 (em especial 5-HT2A e 5-HT2C) e a um menor grau a receptores D2, mas também a outros receptores de dopamina (D1, D3, D4), histamínicos, adrenérgicos e colinérgicos (Stahl, 1996; Ereshefsky e Pharm, 1999).

I.3.4. Efeitos adversos dos antipsicóticos

Um ponto importante com respeito à eficácia da medicação antipsicótica é o potencial das mesmas em causar efeitos adversos potencialmente sérios. O tratamento com antipsicóticos típicos está associado com efeitos adversos neuromusculares (os chamados efeitos adversos extrapiramidais), tanto agudos quanto crônicos, incluindo discinesia aguda, acatisia, parkinsonismo, síndrome neuroléptica maligna e discinesia tardia (Albers e Ozdemir, 2004; Thomas, 2007). Além disso, estes medicamentos podem causar taquicardia, visão borrada, boca seca, constipação, retenção urinária, hipotensão, disfunção sexual, aumento da função hepática e dos níveis plasmáticos de prolactina, ganho de peso, entre outros (Malhotra, 2001; Thomas, 2007). Estes podem limitar a efetividade dos antipsicóticos típicos, como evidenciado através da falta de adesão ao tratamento devido aos efeitos adversos do mesmo.

Os medicamentos antipsicóticos atípicos, embora menos propensos a causar efeitos adversos neuromusculares, são também associados a alguns destes efeitos adversos, e, em particular, ao ganho de peso (Malhotra, 2001).

A Clozapina é uma dibenzodiazepina que foi utilizada em ensaios clínicos na Europa no final dos anos 60 e nos Estados Unidos no início dos anos 70. Sua utilização foi suspensa em alguns países, após ter causado agranulocitose, que pode ser fatal. Em 1990 foi liberada nos Estados Unidos, após estudo multicêntrico controlado e, no Brasil, a clozapina foi lançada comercialmente em agosto de 1992.

A clozapina foi o primeiro antipsicótico a produzir eficácia no tratamento sem ocorrência de efeitos adversos extrapiramidais (Malhotra, 2001). Entretanto, outras reações estão associadas a este medicamento. Dentre os principais efeitos colaterais da clozapina estão: sonolência, tontura, salivação noturna, cefaléia, constipação, convulsões generalizadas, hipotensão postural e ganho de peso (Haddad e Sharma, 2007). O efeito adverso mais grave é a diminuição dos glóbulos brancos (agranulocitose), que ocorre em cerca de 1% dos indivíduos em tratamento com a clozapina. Com o controle hematológico adequado - exames de

sangue semanais no início e mensais posteriormente - a clozapina pode ser considerada uma medicação segura e eficaz, em boa parte dos casos.

A convulsão é um importante efeito adverso conseqüente do tratamento com clozapina. As convulsões são causadas por um conjunto complexo de alterações e adaptações neurobiológicas (Post, 2004). São distúrbios clínicos ou sub-clínicos da função cortical devido a descargas repentinas, anormais, excessivas e desorganizadas das células do cérebro, que se propagam para todas as regiões do cérebro, levando a uma alteração de toda atividade cerebral. Durante uma crise convulsiva, ocorre um desbalanço entre excitação e inibição, levando a um aumento da atividade excitatória, através de falhas nos mecanismos inibitórios [fechamento de canais de K_+, Cl^-, e receptores de ácido gama-aminobutírico (GABA)] com conseqüente despolarização dos neurônios. Dentre os tipos de convulsão, as convulsões generalizadas do tipo tônico-clônico são caracterizadas por perda de consciência seguida por contrações mantidas (tônicas) e relaxamento (clônicos) e apresentam uma duração média de 1-2 minutos.

A clozapina tem sido associada a um alto risco cumulativo de ocorrência de convulsões (Devinsky e cols., 1991). Este medicamento diminui o limiar de convulsões (o mecanismo ainda não é conhecido), que podem estar presentes em 0,6-2% dos pacientes com doses abaixo de 300 mg/dia, mas podem alcançar até 5-14% em doses que ultrapassem 600 mg/dia (Ereshefsky, 1999). Adicionalmente, um risco cumulativo de 10% é estimado após 3,8 anos de tratamento (Devinsky e Pacia, 1994). A clozapina inibe canais de K^+ (Kobayashi e cols., 1998) e o complexo receptor GABA-canal de Cl^- (Yokota e cols., 2002) e esta modificação poderia estar envolvida no mecanismo convulsivo. O tratamento das convulsões envolve a redução da dose (a interrupção do tratamento raramente é necessária) e utilização concomitante de anticonvulsivantes (ácido valpróico, por exemplo).

Devido ao alto risco de ocorrência de convulsões durante o tratamento com clozapina, surge a necessidade de busca de marcadores biológicos de sucetibilidade a convulsões em indivíduos em tratamento com esta medicação. A

farmacogenética pode ser utilizada como uma importante avaliação da contribuição de fatores genéticos que possam estar envolvidos no processo determinante destas convulsões. Como não existem trabalhos relacionados a este tema, a análise de genes candidatos possivelmente envolvidos na variabilidade da resposta aos antipsicóticos pode ser utilizada como um primeiro passo na investigação farmacogenética de convulsões induzidas pela clozapina.

I.3.5. O tratamento da esquizofrenia no Brasil

A primeira alternativa no tratamento da esquizofrenia do Brasil consiste no uso de um antipsicótico típico. Posteriormente, várias alternativas podem ser utilizadas (Figura 3).

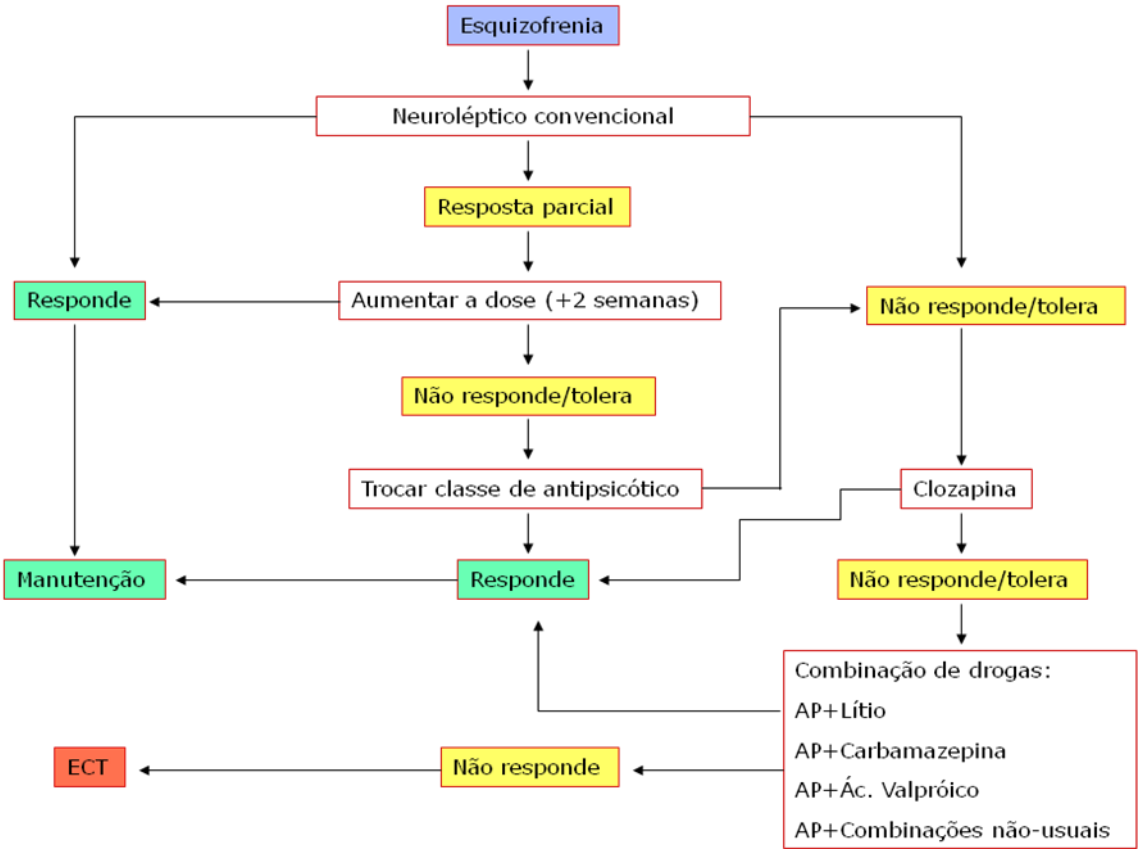

Figura 3: Algoritmo para o tratamento da esquizofrenia. AP= antipsicótico; ECT= eletroconvulsoterapia. Fonte: Ministério da Saúde, 2002.

A clozapina não pode ser utilizada como um fármaco de primeira escolha devido a restrições legais que surgem com o alto risco de indução de agranulocitose (Ministério da Saúde, 2002). Os antipsicóticos comumente utilizados na prática clínica encontram-se na Tabela 1.

Tabela 1: Medicações antipsicóticas comumente utilizadas.

Antipsicótico	Dose recomendada (mg/dia)	Equivalente em clorpromazina (mg/dia)	Meia-vida (horas)
Típicos			
Fenotiazinas			
Clorpromazina	300-1000	100	6
Flupenazina	5-20	2	33
Perfenazina	16-64	10	10
Tioridazina	300-800	100	24
Trifluoperazina	15-50	5	24
Butirofenona			
Haloperidol	5-20	2	21
Atípicos			
Clozapina	150-600	-	12
Risperidona	2-8	-	24
Olanzapina	10-30	-	33

Fonte: American Psychiatric Association (1994).

I.3.6. Resistência ao tratamento

A variabilidade no tratamento de pacientes com esquizofrenia tem um impacto marcante. Cerca de 60-80% dos pacientes com esquizofrenia melhoram com o uso de antipsicóticos convencionais, portanto, 20-40% não respondem a doses elevadas desses antipsicóticos (resistentes ao tratamento), mesmo quando combinados a outras formas de tratamento psicológico e social (Arranz e cols.,

2001). Adicionalmente, estudos clínicos controlados demonstram que a ausência de melhora clínica em pacientes refratários ao tratamento convencional em uso de clozapina ocorre em aproximadamente 40% dos casos (Masellis e cols., 2000).

Outra consideração a ser levantada em relação ao tratamento com medicamentos antipsicóticos é o potencial dos mesmos em causar sérios efeitos adversos, que podem levar à interrupção do tratamento ou até mesmo a morte. Aproximadamente 1% dos pacientes em tratamento com antipsicóticos sofrem de efeitos adversos graves. Antipsicóticos atípicos causam efeitos mais severos que os típicos e, excluindo-se a clozapina, típicos e atípicos parecem ser similares na ocorrência de reações adversas (Bender e cols., 2004).

I.4. Farmacogenética

O efeito do tratamento medicamentoso depende de diversos fatores individuais, que influenciam de diversas maneiras a resposta ao tratamento. A heterogeneidade observada na resposta aos medicamentos resulta parcialmente de diferenças fisiológicas e ambientais, que afetam os indivíduos na população em geral. Dentre estes fatores encontram-se: idade, sexo, etnia, função hepática e renal, dieta, uso concomitante de medicações, severidade e tipo de doença, consumo de álcool e fumo (Goldstein e cols., 2007). Em alguns casos, entretanto, a heterogeneidade da resposta não pode ser somente explicada por estes fatores e por isso fatores genéticos devem ser considerados como uma possível fonte de variabilidade na resposta.

O estudo de variantes genéticas que influenciam a resposta à medicação e a ocorrência de efeitos adversos, e um conseqüente entendimento de como genes interagem para determinar a variabilidade individual nesta resposta faz parte do ramo da farmacogenética. A farmacogenética é definida como o estudo da variabilidade na resposta à medicação devida à hereditariedade. Uma área mais global, a farmacogenômica, examina o papel do genoma como um todo, na tentativa de identificar genes específicos associados com doenças específicas e que podem ser utilizados como alvos para novos fármacos.

O início da farmacogenética deu-se nos anos 50, com a emergência da genética bioquímica humana, através do trabalho de Motulsky (1957) sobre reações adversas, enzimas e genética bioquímica. O termo “farmacogenética” foi determinado por Friedrich Vogel em 1959, na Alemanha. Já o termo “farmacogenômica” foi introduzido nos anos 90, com a emergência do “Projeto Genoma Humano” e o desenvolvimento das ciências do genoma humano. Os estudos de associação são ferramentas extremamente poderosas na identificação de variantes genéticas que influenciam tanto a susceptibilidade às doenças quanto a resposta a um fármaco particular (Risch, 2000).

Atualmente observam-se avanços científicos significantes na conexão gene-resposta e a farmacogenética tornou-se progressivamente popular. Com o avanço de tecnologias de *high-throughput*, novas dimensões na pesquisa de múltiplos genes e suas expressões afetando a resposta a medicação estão sendo geradas. Entretanto, o conhecimento farmacogenético e a prática clínica não têm avançado tão rapidamente quanto a tecnologia, e poucas aplicações foram realizadas até hoje no manejo do tratamento de doenças e no desenvolvimento de novos fármacos (Grossman, 2007).

I.4.1. Farmacogenética e Esquizofrenia

Como mencionado na seção *I.3.6*, observa-se uma ampla variação na resposta clínica individual em pacientes tratados com medicamentos antipsicóticos, permanecendo como um problema crítico no manejo da esquizofrenia. Embora uma minoria dos pacientes possa apresentar completa remissão dos sintomas, uma grande proporção de pacientes (50-70%) continua a sofrer significantemente com os mesmos (Kane, 1999).

A resposta ao tratamento com antipsicóticos pode ser influenciada por diversos fatores, atuando de diferentes formas e em vias distintas. Abordagens farmacogenéticas fornecem uma nova metodologia para desvendar a heterogeneidade de respostas à medicação psicotrópica, já que fatores genéticos podem modificar os efeitos farmacocinéticos e dinâmicos dos fármacos

(absorção, metabolismo, eliminação, transporte e interação fármaco-alvo; discutidos posteriormente) (Poolsup e cols., 2000).

A detecção de diferenças genéticas individuais na resposta à medicação antipsicótica pode levar a novas estratégias importantes para o tratamento de psicoses como a esquizofrenia. A aplicação de dados farmacogenômicos, disponíveis até o presente, vem sendo analisada por estudos mais recentes verificando o impacto que os polimorfismos genéticos têm em relação a resultados positivos e reações adversas no tratamento com antipsicóticos. Além disso, poderiam ser realizados cálculos de ajuste de dose que compensariam as diferenças na concentração sangüínea causadas por variações genéticas em genes relevantes (Kirchheiner e cols., 2005; Arranz e de Leon, 2007).

I.4.2. Farmacogenética de medicamentos antipsicóticos – Variantes genéticas em enzimas de metabolização – Farmacocinética

A farmacocinética consiste na disseminação de um fármaco em um organismo, que depende de processos de absorção, distribuição, metabolismo e excreção, ou seja, refere-se à concentração plasmática do fármaco neste organismo.

O metabolismo de fármacos é geralmente dividido em reações de fase I e II. Reações de fase I, incluindo oxidação, redução e hidrólise, introduzem um grupo polar na molécula, enquanto reações de fase II conjugam uma substância hidrofílica endógena com o grupo polar na molécula, resultando em compostos mais hidrossolúveis. Das reações de fase I, a oxidação é a mais importante no metabolismo de muitos fármacos (Poolsup e cols., 2000).

A maioria dos medicamentos utilizados, atuantes no Sistema Nervoso Central (SNC), são extensivamente metabolizados no fígado por enzimas do Citocromo P450 (CYP), que são membros de uma super-família de enzimas oxidativas e que fazem parte de um sistema maior do metabolismo oxidativo de fase I de substâncias terapêuticas (Figura 4) (Kawanishi e cols., 2000; Poolsup e

cols., 2000; Ozaki, 2004). Em adição, evidências recentes mostram que algumas enzimas CYP são expressas no cérebro e podem influenciar a disponibilidade do fármaco no sítio de ação (Siegle e cols., 2001).

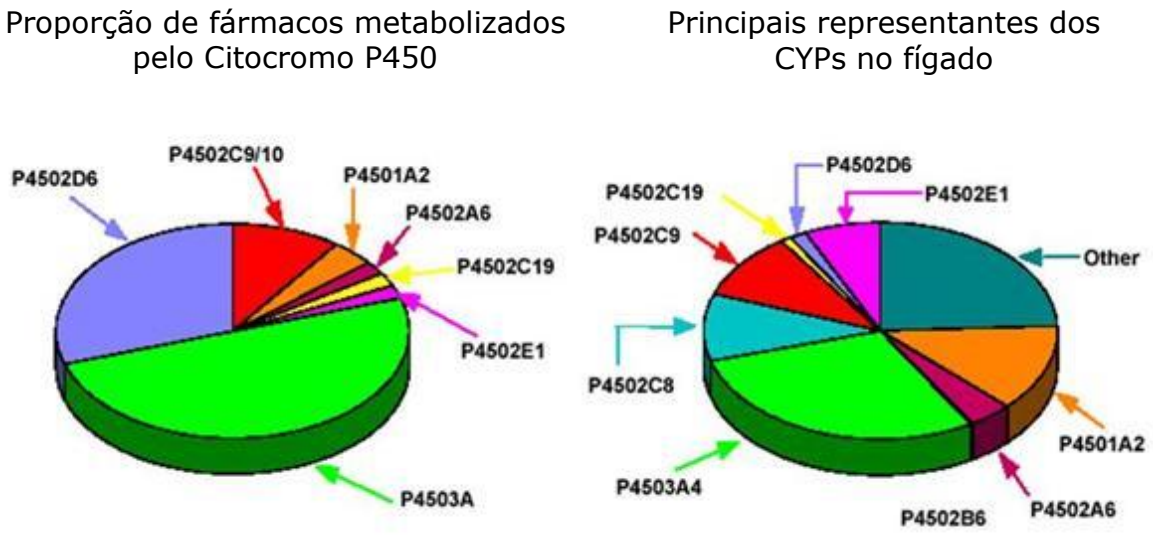

Figura 4: Proporção de fármacos metabolizados pelo Citocromo P450 e seus principais representantes no fígado. Fonte: Shimada e cols. (1994).

Entre estas enzimas, CYP2D6, CYP1A2, CYP3A4, CYP2C9 e CYP2C19 são relacionadas ao metabolismo de medicamentos utilizados em psiquiatria (Tabela 2).

Estudos farmacogenéticos relatam que fenótipos CYP, geneticamente determinados e com grande variação inter-individual, influenciam fortemente a sensibilidade ou resposta à medicação, resultante de taxas de eliminação, concentração e biotransformação (Kawanishi e cols., 2000), pois mutações nestes genes podem resultar em ausência da enzima, enzima deficiente ou com uma atividade aumentada (*Human Cytochrome P450 (CYP) Allele Nomenclature Committee, online*). A maior implicação clínica destes polimorfismos é obviamente relacionada à toxicidade do fármaco e falha terapêutica (Poolsup e cols., 2000).

Tabela 2: Principais enzimas de metabolização e sítios de ação (descritos a seguir) de alguns antipsicóticos típicos e da clozapina.

Antipsicóticos	Enzimas de metabolização[a]	Sítios de ação[b]
Típicos		
Haloperidol	CYP2D6	DRD2↑
	CYP3A4	DRD3↓
	CYP3A5	DRD4↓
	CYP1A2	M1[d]
Tioridazina[c]	CYP2D6	H1[e]
	CYP1A2	α1[f]
	CYP2C19	
Clorpromazina[c]	CYP2D6	
	CYP1A2	
Perfenazina[c]	CYP2D6	
	CYP1A2	
	CYP2C19	
	CYP3A4	
Clozapina	CYP1A2	5-HTR1A↓
	CYP3A4	5-HTR2A ↑
	CYP2D6	5-HTR2C ↑
	CYP2C19	5-HTR3-7↓
		DRD2↓
		DRD3↓
		DRD4↑
		M1[d]
		H1[e]
		α1[f]

[a]Arranz e cols. (2001); Scordo e Spina (2002); Dahl (2002); Kalgutkar e cols., 2003; [b]Stahl (1996); Ereshefsky e Pharm (1999); [c]Enzimas ainda não caracterizadas completamente; [d]Receptores colinérgicos muscarínicos; [e]Receptores histamínicos; [f]Receptores alfa adrenérgicos.

I.4.2.1. CYP2D6

CYP2D6 representa, em média, 2% do conteúdo CYP hepático (Figura 2). O gene que codifica sua síntese localiza-se no braço longo do cromossomo 22 (Scordo e Spina, 2002). Embora expresso em níveis mais baixos quando comparado a outros CYPs humanos, esta isoforma exerce um importante papel no metabolismo de medicamentos psicotrópicos, sendo parcialmente ou inteiramente responsável pela biotransformação oxidativa da maioria dos neurolépticos clássicos e alguns atípicos (Scordo e Spina, 2002; Vandel e cols., 2007).

A população em geral pode ser dividida em quatro grupos (ou fenótipos) principais, de acordo com a habilidade de metabolização de fármacos específicos. Metabolizadores pobres ou lentos (PMs) têm capacidade de metabolização deficiente quando comparados aos indivíduos com atividade metabólica normal, e nestes a toxicidade pode ocorrer devido ao acúmulo do fármaco ou metabólitos ativos, sendo mais suscetíveis aos efeitos adversos. Metabolizadores extensivos (EMs) apresentam atividade enzimática normal e metabolizadores intermediários (IMs) apresentam níveis de metabolização entre EMs e PMs. No grupo de metabolismo ultra-rápido (UMs), os indivíduos catalisam fármacos muito mais rapidamente que o usual, necessitando doses mais altas para produzir uma resposta terapêutica (Poolsup e cols., 2000), e pode ser um dos fatores importantes que induzem refratariedade ao tratamento psiquiátrico (Ozaki, 2004).

O gene *CYP2D6* é extremamente polimórfico, sendo que mais de 90 variantes e sub-variantes alélicas já foram descritas (*Human Cytochrome P450 (CYP) Allele Nomenclature Committee, online*). Estudos envolvendo polimorfismos no gene CYP2D6 mostraram associações significantes entre alguns destes polimorfismos e resposta ao tratamento com o antipsicótico típico haloperidol (Someya e cols., 1999; Roh e cols., 2001), apesar de outros estudos não encontrarem esta associação, ou observarem somente uma tendência (Aitchison e cols., 1999; Brockmöller e cols., 2002; Ohnuma e cols., 2003). Um único estudo com o antipsicótico atípico clozapina (Arranz e cols., 1995a) não foi

capaz de encontrar uma associação significante entre alelos de *CYP2D6* e resposta a esta medicação.

Uma interessante meta-análise (Kirchheiner e cols., 2004) revelou que a dosagem de cerca de 50% dos antipsicóticos comumente utilizados é dependente do genótipo *CYP2D6*. Adicionalmente, uma influência destes genótipos foi também observada na ocorrência de efeitos adversos decorrentes do uso de antipsicóticos típicos (Ingelman-Sundberg, 2005).

Desta forma, a identificação dos genótipos de *CYP2D6*, principalmente PMs que apresentam atividade metabólica deficiente, e UMs que apresentam atividade enzimática aumentada, tem considerável importância clínica tanto na psiquiatria quanto em outras especialidades médicas.

I.4.2.2. CYP3A4

CYP3A4 é expresso no fígado, rim e intestino e é responsável pela metabolização de uma grande variedade de compostos importantes clinicamente, fisiologicamente e toxicologicamente (Sata e cols., 2000; Tabela 2) e é responsável por mais de 30% dos CYPs totais presentes no fígado humano (Dahl, 2002) (Figura 2). O envolvimento desta enzima na metabolização do haloperidol foi descrito por Fang e cols. (1997), e posteriormente descobriu-se que outros antipsicóticos eram também metabolizados por CYP3A4 (Tabela 2). Adicionalmente, casos de pacientes em uso de clozapina que receberam inibidores de CYP3A4 mostraram aumento dos níveis de clozapina sérica, que normalizaram após a interrupção do inibidor (Cohen e Wan, 1996), mostrando a importância desta enzima também na metabolização da clozapina. O primeiro polimorfismo funcional no gene *CYP3A4* (*15713T>C*, alelo **2*) foi descrito por Sata e cols. (2000) e, posteriormente, outras alterações foram descritas neste gene, sendo que alguns destes polimorfismos já foram caracterizados por levarem a alterações na atividade enzimática de CYP3A4. Dentre eles encontram se -*392A>G* (alelo **1B*), *13908G>A* (**8*), *21867C>T* (**11*), *21896C>T* (**12*), *22026C>T*

(**13*), *15603C>G* (**16*), *15615T>C* (**17*), *20070T>C* (**18*), *1461_1462insA* (**20*) (*Human Cytochrome P450 (CYP) Allele Nomenclature Committee, online*).

I.4.2.3. CYP3A5

CYP3A5 representa 50% do total hepático de CYP3A. Esta enzima compartilha alta homologia com CYP3A4 e também muitos substratos. Entretanto, a relevância clínica desta enzima permanece obscura, pois parece haver uma relação entre CYP3A5 e CYP3A4 no fígado e intestino no nível de expressão destas enzimas (Wojnowski e Kadem, 2006). A participação desta enzima no metabolismo do haloperidol já foi observada (Kalgutkar e cols., 2003). Atualmente, existem mais de 26 variantes alélicas no gene *CYP3A5*, sendo que boa parte delas leva a uma atividade reduzida da enzima (*Human Cytochrome P450 (CYP) Allele Nomenclature Committee, online*). Dentre estas variantes encontra-se *6986A>G* (levando a um defeito no *splicing* do RNA mensageiro), que determina o alelo **3* e confere uma atividade severamente reduzida à CYP3A5 (Kuehl e cols., 2001), e que em homozigose pode ser chamado de genótipo de baixa expressão (Wojnowski e Kamdem 2006).

Desta forma, verifica-se que a eficácia da medicação e a presença de efeitos adversos podem ser influenciadas por variações genéticas em enzimas do citocromo P450. Por isso, estudos farmacogenéticos de antipsicóticos têm apostado grandes esforços na associação entre polimorfismos genéticos em genes CYP e o metabolismo de medicamentos antipsicóticos. Atualmente, o conhecimento prévio do *status* de metabolização de determinado fármaco, que pode ser obtido por técnicas de genotipagem simples, torna-se fundamental para o ajuste da dose terapêutica, com o objetivo de minimizar efeitos tóxicos e diminuir a falha terapêutica (Arranz e Kerwin, 2000).

Entretanto, mutações em enzimas de metabolização não podem ser totalmente responsáveis pela heterogeneidade observada na resposta de um indivíduo ao tratamento. Os efeitos farmacológicos totais das medicações não são tipicamente traços monogênicos; mais do que isso, eles são determinados pela

interposição de diversos genes codificando proteínas envolvidas em múltiplas rotas que determinam os efeitos e disposição dos fármacos, em adição ao metabolismo (Kawanishi e cols., 2000). Os tópicos a seguir tratarão deste ponto.

I.4.3. Farmacogenética de medicamentos antipsicóticos – Variantes genéticas em sítios de ação – Farmacodinâmica

A farmacodinâmica refere-se ao impacto da droga no organismo, que depende da interação dos fármacos com seus receptores e das mudanças celulares e fisiológicas subseqüentes a esta interação.

Os medicamentos antipsicóticos têm, em geral, uma ampla variedade de alvos, incluindo diversos receptores de sistemas neurotransmissores. A dopamina e a serotonina são neurotransmissores chaves (Arranz e Kerwin, 2000; Prasad e cols., 2002), e alvos potenciais não estriatais são: D2, D3, D4, 5-HT2A e 5-HT2C (Kerwin e Owen, 1999) (Tabela 2). Variações na seqüência de DNA de receptores-alvo de fármacos podem alterar a estrutura da proteína, propriedades de ligação, função ou níveis de expressão do receptor. Portanto, mutações em receptores podem ter um efeito direto no mecanismo desencadeado pelo fármaco e influenciar sua eficácia terapêutica.

Entretanto, não está totalmente claro quais desses sistemas são responsáveis pela ação terapêutica dos antipsicóticos. A pesquisa farmacogenética pretende não somente desvendar a variabilidade genética relacionada à eficácia do fármaco, mas também determinar quais receptores são de valor terapêutico (Arranz e de Leon, 2007).

Estudos farmacogenéticos de resposta aos medicamentos antipsicóticos vêm sendo realizados com diversos fármacos utilizados em psiquiatria, mas o foco principal destes estudos tem sido na clozapina, tanto em análises prospectivas quanto retrospectivas.

I.4.3.1. Polimorfismos relacionados ao sistema dopaminérgico

A clozapina é um antagonista de receptores de dopamina, portanto estudos iniciais focaram na relação entre o gene do receptor D4 de dopamina (*DRD4*) e resposta à clozapina (devido à maior afinidade por esse receptor). Entretanto, a maioria dos trabalhos foi incapaz de detectar uma associação significante entre o polimorfismo de um VNTR funcional dentro da terceira alça citoplasmática do receptor e resposta à clozapina (Rao e cols., 1994; Kerwin e cols., 1994; Shaikh e cols., 1995; Rietschel e cols., 1996; Kohn e cols., 1997; Kaiser e cols., 2000; Zhao e cols., 2005). Em contraste, associações modestas foram detectadas entre este polimorfismo e resposta aos neurolépticos típicos (Hwu e cols., 1998; Cohen e cols., 1999).

A importância do receptor de dopamina D3 (DRD3) vem do seu sítio predominantemente mesolímbico e do fato de mostrar alta afinidade de ligação a ambos antipsicóticos, típicos e atípicos, que também elevam o nível de RNA mensageiro de D3 (Kerwin e Owen, 1999). A maioria dos estudos aponta associação do gene *DRD3* com resposta à antipsicóticos (Jonsson e cols., 1993, 2003; Mant e cols., 1994; Krebs e cols., 1998; Scharfetter e cols., 1999; Cohen e cols., 1999; Szekeres e cols., 2004; Lane e cols., 2005; Reynolds e cols., 2005), mas três estudos falharam em replicar estes achados (Ohara e cols., 1996; Joober e cols., 2000; Cordeiro e cols., 2006).

Outro candidato óbvio para estudos farmacogenéticos é o gene do receptor tipo D2 (*DRD2*), pelo fato da maioria dos antipsicóticos possuírem uma forte afinidade por esse receptor (Seeman, 1992). Estudos envolvendo polimorfismos no gene *DRD2* têm produzido resultados contraditórios. Alguns estudos demonstraram associação entre este gene e resposta a neurolépticos típicos (Suzuki e cols., 2001; Schäfer e cols., 2001; Wu e cols., 2005), que não são confirmados por outros estudos (Ohara e cols., 1998; Mihara e cols., 2001). Já para os medicamentos atípicos, resultados positivos foram encontrados por Yamanouchi e cols. (2003) e Lane e cols. (2004) para o antipsicótico risperidona, e por Malhotra e cols. (1999) e Hwang e cols. (2005, 2006) para a clozapina,

resultado não encontrado por Arranz e cols. (1998c). Portanto, o papel do gene *DRD2* na resposta ao tratamento ainda permanece incerto.

Estudos envolvendo polimorfismos no gene do transportador de dopamina (*DAT1* ou *SLC6A3*) não encontraram nenhuma associação significante com resposta aos antipsicóticos (Joober e cols., 2000; Szekeres e cols., 2004; Kim e cols., 2005). O gene da enzima catechol-o-metiltransferase (*COMT*) é outro candidato em estudos de associação com resposta à medicação antipsicótica. Associações significantes foram observadas com o polimorfismo *Val158Met* (Weickert e cols., 2004; Anttila e cols., 2004; Woodward e cols., 2007). Outros estudos verificaram a influência do gene do fator neutrotrófico derivado do cérebro (*BDNF*), com resultados positivos (Krebs e cols., 2000) e negativos (Hong e cols., 2003) para dois polimorfismos distintos em cada estudo.

I.4.3.2. Polimorfismos relacionados ao sistema serotoninérgico

Dos sete subtipos de receptores de serotonina (5-HT1-7), os subtipos 5-HT2 são os alvos mais fortes de fármacos antipsicóticos atípicos (Arranz e Kerwin, 2000). Um dos primeiros polimorfismos (*HTR2A T102C*) que atraiu interesse dos pesquisadores foi estudado por Arranz e cols. (1995b), que verificou uma associação significante entre o alelo *102C* e falha em responder a clozapina em pacientes esquizofrênicos tratados com o medicamento. Estes resultados foram replicados posteriormente por alguns investigadores (Arranz e cols., 1996, 1998b, 2000b; Yu e cols., 2001). Entretanto, vários outros estudos não obtiveram o mesmo resultado significante (Nothen e cols., 1995; Masellis e cols., 1995, 1998; Malhotra e cols., 1996a; Nimgaonkar e cols., 1996; Lin e cols., 1999). Outro polimorfismo menos comum dentro do gene *HTR2A*, *His452Tyr*, que parece produzir efeitos funcionais *in vitro*, tem fornecido resultados de associação com resposta à clozapina tanto positivos (Arranz e cols., 1996, 1998a e b, 2000b; Masellis e cols., 1998), quanto negativos (Masellis e cols., 1995; Nothen e cols., 1995; Malhotra e cols., 1996a). Adicionalmente, uma meta-análise mostrou que esses dois polimorfismos desempenham uma função importante na determinação

da resposta à clozapina (Arranz e cols., 1998b). Outro polimorfismo já considerado é o *-1438A>G*, para o qual se observou associação significante em dois estudos (Arranz e cols., 1998a; Hamdani e cols., 2005). Com base nestes resultados, espera-se que estudos com amostras maiores possam auxiliar na elucidação do papel deste gene na resposta aos medicamentos antipsicóticos.

O polimorfismo *Cys23Ser* do gene *HTR2C* foi associado com resposta à clozapina por Sodhi e cols. (1995) e posteriormente por Arranz e cols. (2000b), mas outros estudos não replicaram estes resultados (Malhotra e cols., 1996b; Rietschel e cols., 1997; Masellis e cols., 1998).

Outros genes relacionados à serotonina analisados em estudos farmacogenéticos da clozapina incluem o gene do transportador de serotonina (*5HTT* ou *SLC6A4*). Arranz e cols. (2000a) verificaram uma tendência entre resposta à clozapina e o polimorfismo de *HTTLPR* (480pb/520pb) na região do promotor do gene, onde indivíduos homozigotos para o alelo curto tenderam a uma resposta pobre ao tratamento com clozapina em pacientes esquizofrênicos. Outros estudos não observaram uma relação significante entre o mesmo polimorfismo e resposta à clozapina (Tsai e cols., 2000; Kaiser e cols., 2001), sugerindo que esta variante não teria influência na resposta aos antipsicóticos.

Arranz e cols. (2000b), em análises com diferentes polimorfismos, verificaram uma forte associação de seis polimorfismos combinados com resposta à clozapina (*HTR2A T102C e His452Tyr, HTR2C -330-GT/-244-CT e Cys23Ser, 5HTTLPR, H2 –1018-G/A*), sendo que estes achados constituem um grande potencial em estudos farmacogenéticos, como uma chave para futura melhora e individualização do tratamento clínico de pacientes que sofrem de esquizofrenia.

I.4.3.3. Variantes genéticas em outros genes candidatos

Já que receptores de catecolaminas são acoplados à proteína G (GPCRs) e os antipsicóticos exercem seus efeitos por antagonismo competitivo de GPCRs pós-sinápticos, esta proteína pode ter uma importante influência na função dos

sistemas dopaminérgico e serotoninérgico. Müller e cols. (2005) encontraram o polimorfismo *825C>T*, no gene que codifica a subunidade Beta3 da proteína G (*GNB3*), associado com resposta à clozapina em pacientes com esquizofrenia. Já Anttila e cols. (2007) observaram uma tendência à associação deste polimorfismo com resposta à antipsicóticos típicos, em pacientes homens.

CAPÍTULO II

JUSTIFICATIVA E OBJETIVOS

A esquizofrenia, por ser um transtorno psiquiátrico grave, que afeta cerca de 1% da população, associado à incapacitação, desemprego, fardo de cuidado, suicídio e custos elevados na promoção de saúde e para a sociedade, tem sido alvo de diversos estudos que visam uma melhora da qualidade de vida dos indivíduos afetados pela doença.

A primeira barreira do tratamento da esquizofrenia tanto com fármacos antipsicóticos convencionais como os de nova geração, é a eficácia relativamente baixa. Apesar do fato de que os efeitos adversos induzidos pela droga possam ocorrer rapidamente após a administração da mesma, a eficácia da droga não pode ser verificada antes de quatro a doze semanas após o início do tratamento (Malhotra, 2001). Durante esse tempo, pacientes tratados podem experimentar diminuição no rendimento, disfunção social, morbidade médica, abuso de substâncias e, em uma significante proporção dos casos, o suicídio, além do sofrimento causado por sintomas psicóticos perturbadores. Claramente, a obtenção de informações a respeito da probabilidade de um paciente individual responder ou não a uma medicação antipsicótica seria de grande valor na redução da morbidade e mortalidade associada à esquizofrenia.

Outra consideração a ser levantada em relação ao tratamento com antipsicóticos é o potencial dos mesmos em causar sérios efeitos adversos (acatisia, distonia, parkinsonismo, hiperprolactinemia, efeitos extrapiramidais, ganho de peso, sedação, convulsão, etc) que podem levar à interrupção do tratamento ou até mesmo a morte.

Portanto, a ausência ou pobreza na resposta ao tratamento é pouco entendida e geralmente a medicação é selecionada com base na tentativa e erro. Uma média de 30-40% dos pacientes tratados não mostra nenhuma melhora

após o tratamento com antipsicóticos e 60-70% daqueles que respondem apresentam sérios efeitos adversos (Arranz e cols., 2001).

Infelizmente, apesar de diversas décadas de investigação, nenhum preditor clínico ou biológico de resposta à medicação antipsicótica ou de desenvolvimento de efeitos adversos foi identificada. Por esta razão, a esperança está na introdução de abordagens farmacogenômicas na pesquisa psiquiátrica, que podem fornecer dados informativos para auxiliar na escolha do tratamento individual adequado desta doença tão devastadora, sempre levando em consideração que uma combinação de genes contribuindo para o efeito do fármaco é uma explicação mais provável para a variabilidade entre indivíduos na resposta ao tratamento (Arranz e Kerwin, 2000).

Considerando-se, portanto, a importância da obtenção de informações a respeito da contribuição da variabilidade genética na resposta ao tratamento com medicação antipsicótica, em pacientes sofrendo de esquizofrenia, o presente estudo tem como objetivo específico: verificar a influência de 56 polimorfismos (Tabela 3), em genes de enzimas de metabolização e em genes envolvidos, direta ou indiretamente, no mecanismo de ação de antipsicóticos na resposta ao tratamento com estas medicações, em uma amostra de pacientes tratados com antipsicóticos típicos e com o atípico clozapina, além da avaliação do efeito destes mesmos genes na incidência de um efeito adverso específico da clozapina (convulsão).

Tabela 3: Relação dos polimorfismos analisados na presente tese.

Gene	Símbolo	Localização cromossômica	Polimorfismos	Classificação
Receptor de dopamina D2	*DRD2*	11q23	*-141C Ins/Del*	5’ regulatória
Receptor de dopamina D3	*DRD3*	3q13.3	*rs6280*	Não-sinônima
			rs963468	Intron
			rs2134655	Intron
			rs1486012	3’ não traduzida
			rs7631540	3’ não traduzida
Receptor de dopamina D4	*DRD4*	11p15.5	*48pb VNTR exon III*	VNTR[1]
Transportador de dopamina	*SLC6A3*	5p15.3	*-844C>T*	5’ regulatória
Catechol-O-Metiltransferase	*COMT*	22q11.21	*Val158Met*	Não-sinônima
Receptor de serotonina 2A	*HTR2A*	13q14-q21	*102T>C*	Sinônima
			-1438A>G	5’ regulatória
			His452Tyr	Não-sinônima
Receptor de serotonina 2C	*HTR2C*	Xq24	*Cys23Ser*	Não-sinônima
Receptor de serotonina 1B	*HTR1B*	6q13	*861G>C*	Sinônima
Transportador de serotonina	*SLC6A4*	17q11.1-q12	*HTTLPR*	5’ regulatória
			Stin2 VNTR	VNTR - Intron
Monoamina oxidase A	*MAOA*	Xp11.23-11.4	*-uVNTR*	VNTR

Proteína G Subunidade Beta-3	*GNB3*	12p13	*825C>T*	Variante de *splicing*
			814G>A	Não-sinônima
Fator neutrotrófico derivado do cérebro	*BDNF*	11p13	*Val66Met*	Não-sinônima
Citocromo P450 2D6	*CYP2D6*	22q13.1	*-1584C>G*	5’ regulatória
			31G>A	Não-sinônima
			100C>T	Não-sinônima
			124G>A	Não-sinônima
			138insT	Não-sinônima
			883G>C	Variante de *splicing*
			1023C>T	Não-sinônima
			1039C>T	Sinônima
			1659G>A	Não-sinônima
			1661G>C	Sinônima
			1707T>Del	Não-sinônima
			1758G>A	Sem sentido
			1846G>A	Variante de *splicing*
			1863-1864Ins TTTCGCCCC	Não-sinônima
			1973-1974insG	Não-sinônima
			2539-2542delAACT	Não-sinônima
			2549A>del	Não-sinônima

Citocromo P450 2D6			*2613-2615 delAGA*	Deleção de um aa[2]
			2850C>T	Não-sinônima
			2935A>C	Não-sinônima
			3183G>A	Não-sinônima
			3198C>G	Não-sinônima
			4042G>A	Não-sinônima
			4180G>C	Não-sinônima
			Deleção gênica	-
			Multiplicação gênica	-
Citocromo P450 3A4	*CYP3A4*	7q21.1	*-392A>G*	5’ regulatória
			13908G>A	Não-sinônima
			15603C>G	Não-sinônima
			15615T>C	Não-sinônima
			21867C>T	Não-sinônima
			21896C>T	Não-sinônima
			20070T>C	Não-sinônima
			22026C>T	Não-sinônima
			1461_1462insA	Não-sinônima
Citocromo P450 3A5	*CYP3A5*	7q21.1	*6986A>G*	Variante de splicing

[1] VNTR: número variável de repetições em tandem;
[2] aa: aminoácido.

CAPÍTULO III

TREATMENT-RESISTANCE TO TYPICAL NEUROLEPTICS IN SCHIZOPHRENICS IS INFLUENCED BY POLYMORPHISMS AT DRD3, CYP3A4 AND CYP3A5 GENES.

PHARMACOGENETICS AND GENOMICS (submetido)

Treatment-resistance to typical neuroleptics in schizophrenics is influenced by polymorphisms at *DRD3, CYP3A4 and CYP3A5* genes.

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ABSTRACT

Objectives: The current study aimed to explore the influence of variation in *DRD3, CYP3A4* and *CYP3A5* genes on treatment-resistance to typical neuroleptics in a Brazilian sample of patients with schizophrenia.

Methods: Five polymorphisms at *DRD3* gene, nine at *CYP3A4* gene, and one at *CYP3A5* gene were genotyped in a sample of 186 patients with schizophrenia.

Results: Significant associations were observed between the *CYP3A4 -392A>G* and efficacy of neuroleptic treatment. Homozygous individuals for the *-392A* variant (*P* = 0.014, OR = 3.32) were more frequent in the treatment-resistant group, compared to carriers of one copy of the *-392G* variant. The *CYP3A5* low expressor genotype (*CYP3A5*3*/*CYP3A5*3*) was found to be associated with refractoriness to neuroleptic treatment (*P* = 0.003, OR = 3.16). Among the haplotypes observed in *DRD3* gene, the *T/A/G/A/C* haplotype showed an association with refractoriness to neuroleptics (χ^2 = 5.342, *P* = 0.021, OR = 1.75). This association showed a dose effect, carriers of one copy of this haplotype presented intermediate values between non-carriers and homozygous individuals for the haplotype. Multiple logistic regression analyses revealed that the number of copies of *DRD3 T/A/G/A/C* haplotype and *CYP3A5* low expressor genotype were predictors of refractoriness to neuroleptic after controlling for selected risk factors. *CYP3A5*3 + T/A/G/A/C* homozygotes showed an eleven-fold higher risk to be refractory to neuroleptics than *CYP3A5*3* homozygotes + *non-T/A/G/A/C* carriers (χ^2 = 9.395, *P* = 0.004, OR = 11.07).

Conclusions: Our results suggest a role for *CYP3A5* and *DRD3* gene variants on refractoriness to neuroleptic treatment in Brazilians with schizophrenia.

Key words: pharmacogenetics; typical neuroleptics; *DRD3; CYP3A*; treatment resistance.

INTRODUCTION

Schizophrenia is a highly debilitating illness and one of the most important public health problems confronted by society. It is a complex psychiatric disorder characterized by perceptual abnormalities including hallucinations and delusions, conceptual disorganization, cognitive impairment, and, frequently, loss of usual behaviors, described as negative symptoms such as alogia, affective flattening, anhedonia and avolition [1].

Antipsychotic drugs are the mainstay of treatment of schizophrenia. When they were developed in the 1950s, typical neuroleptic drugs revolutionized the treatment of psychosis. These medications control the excitation, aggression, and restlessness as well as conceptual disorganization and thought disorders of psychosis in a considerable proportion of cases. However, they are limited in their use by side effects especially those from extrapyramidal origin, due to their relatively narrow therapeutic range [2]. Furthermore, they are less effective towards negative symptoms [3]. Besides discovery of newer and more efficient antipsychotic drugs, the first choice for treatment of schizophrenia in Brazil, for economic reasons, is based on typical neuroleptic drugs.

Regarding efficacy, 40-80% of patients fail to respond or demonstrate only a partial response to typical antipsychotic agents [4]. These large interindividual differences in clinical response during neuroleptic drug treatments might be caused by a variety of factors, and among them genetic components presumably play an important role [5]. Adequate prediction of genetic factors associated to treatment response will have major economic, medical and safety implications. It can be expected that the development of reliable assays for pre-prescribing

genotyping with adequate predictive value would reduce the burden and suffering of a large amount of affected subjects that otherwise would have to try several different drugs until they reach adequate balance among clinical control and tolerability-safety.

Different factors can regulate response to drugs, at pharmacodynamic and pharmacokinetic level. At the first level, genetic variation in drug-targeted neurotransmitter receptors can influence binding and functional capabilities whereas at the pharmacokinetic level, metabolic enzymes can modify drug disposition, affecting treatment efficacy. Usually studies concentrate in one of these levels, failing to look at the joint effect of kinetics and dynamics. Therefore, studies focusing on simultaneous occurrence of polymorphisms at these two groups of genes could provide insights about individual variability in neuroleptic drug response in schizophrenic subjects.

Neuropsychopharmacological evidence supports the assumption that all clinically useful neuroleptics exert most of their effect trough reduction of dopaminergic transmission [6], although other targets, like serotonergic, cholinergic, histaminergic, glutamatergic and alpha-adrenergic transmission, would influence drug effects. Independent of that, dopaminergic regulation still stands as a major issue. Therefore, genetic polymorphisms at different dopamine receptors can be considered good candidates for treatment response. Among all dopamine receptors, the dopamine type 3 receptor (DRD3) is of particular interest because DRD3 mRNA is mainly expressed in brain limbic areas that are associated with cognitive, emotional, and endocrine functions [7] showing a potential involvement in the genesis of schizophrenia. Since all typical neuroleptics have a reasonable

high affinity for this receptor [8] its importance on the treatment of schizophrenia, although less investigated, might also be useful. The *Ser9Gly* polymorphism in *DRD3* gene has been intensively studied and several associations were found with tardive dyskinesia [9, meta-analysis in 10], but little information can be found in these studies that can adequately explain the typical neuroleptic response, and inconclusive results were found [11-18]. Albeit the 100 timer lower abundance in the brain than D2 receptors, D3 receptors can contribute for the understanding of differential actions of neuroleptics due to its high expression at limbic areas, considered of relevance for psychotic symptoms, and because of the large variability of binding constant K1 for the different neuroleptics, with DRD3 K1 ranging from 3.2 nmol/l for Haloperidol, to 200 nmol/l for clozapine, whereas DRD2 K1 varied from 1.3 nmol/l for haloperidol and 83 for clozapine. Despite the current uncertainty about the biological relevance of these differences in binding affinity, it is noteworthy the similarity of ratio on binding affinity of Haloperidol and Clozapine for D3/D2 receptors (Ratios of 2.4) [19].

Another issue about the variability of effect of antipsychotic drugs can be pursued on regulators of kinetics of drugs. Among these, genetic variants in Phase I enzymes are of interest, because they are known to reduce or increase antipsychotic activity and alter drug metabolic ratios. The CYP3A subfamily constitutes the most abundant CYP proteins in human liver (up to 30%) and small intestine [20] and plays an important role in the metabolism of neuroleptics [2,21,22]. Interindividual variability in the expression of CYP3A is very high (20 to 40-fold) [23], making members of this subfamily of genes major candidates for pharmacogenetic investigations. Two enzymes with similar substrate specificities –

CYP3A4 and, to a lesser extent, CYP3A5 – are the main members of this subfamily [24].

The current study aimed to explore the influence of five Single Nucleotide Polymorphisms (SNPs) in *DRD3* gene and ten SNPs in *CYP3A* subfamily genes (*CYP3A4* and *CYP3A5*) on typical neuroleptic response in a Brazilian sample of patients of European ancestry with DSM-IV Diagnosis of schizophrenia.

METHODS

Subjects and study design

One hundred and eighty six Brazilian schizophrenic patients were recruited from the program of Schizophrenia and Demency at Hospital de Clínicas de Porto Alegre, Brazil, for a naturalistic pharmacogenetic study of typical neuroleptic response. All individuals included in the study were of European ancestry.

All patients received a comprehensive assessment by Board-Certified Psychiatrists with at least 4 interviews with the presence of an informative relative, and met clinical criteria for schizophrenia according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) [25], with an additional confirmation by a standardized computerized interview algorithm using the Operational Criteria Checklist for Psychotic Disorders – OPCRIT [26]. Ethics Committee of the Hospital de Clínicas de Porto Alegre (Porto Alegre, Brazil) approval was obtained for the study and all subjects and relatives provided written informed consent to participate in this study.

The most commonly prescribed typical neuroleptics were haloperidol and chlorpromazine. The patients were separated in two groups according to their

response to typical neuroleptics. The non-refractory group (n=65, 35%) consisted of patients who had experienced adequate and long-lasting response to treatment with typical neuroleptics. The treatment-resistant group (n=121, 65%) was comprised of patients that fulfilled the following criteria: (1) Failure to respond to haloperidol (20-40mg/day oral doses) or to chlorpromazine (1g/day oral doses) for at least twelve weeks, or to thioridazine (400-800mg/day oral doses), for at least twenty four weeks; (2) did not show appropriated behavior control in the last two years; (3) presence of continuous symptomatology in the last two years [27]. The criteria for treatment-resistant include treatment refractoriness (with inadequate clinical response) and treatment-intolerance (with adverse response), and were approved by the Clinical Protocol and Therapeutic Proceedings of the Brazilian Health Ministry [28]. The response status was defined after a thorough evaluation of psychiatrists and personal hospital records. Co-medication was recorded by clinical interview and medical chart review.

SNP selection

Five SNPs in *DRD3* gene (*rs6280*: exon 2, *rs963468*: intron 4, *rs2134655*: intron 5, *rs1486012* and *rs7631540*: 3' near gene region) were selected according to previous results from association studies of *DRD3* haplotypes and schizophrenia [29]. Nine polymorphisms determining nine alleles of the *CYP3A4* gene (**1B -392A>G*, **8 13908G>A*, **11 21867C>T*, **12 21896C>T*, **13 22026C>T*, **16 15603C>G*, **17 15615T>C*, **18 20070T>C*, **20 1461_1462insA*), and one SNP determining one allele of the *CYP3A5* gene (**3 6986A>G*) were selected according to their effect in enzyme activity (http://www.cypalleles.ki.se/).

Genotyping

Genomic DNA was isolated from peripheral blood leukocytes by standard procedures [30]. Genotyping was performed by the MassARRAY SNP genotyping system (Sequenom, Inc., San Diego, CA, USA), following the manufacturer's instructions. The method involves multiplex PCR and minisequencing assays, designed with the SpectroDESIGNER software (Sequenom, Inc., San Diego, CA) followed by mass spectrometry analysis with the Bruker Bi-Flex MALDI-TOF mass spectrometer (Bruker Daltonics, Billerica, MA, USA). Spectral output was analyzed using SpectroTYPER–RT software (Sequenom, Inc., San Diego, CA) and by manual review. The principles of this method are detailed in Buetow *et al.* [31]. This genotyping platform is at the Spanish National Genotyping Center's facilities at Santiago de Compostela University. It is subjected to regular quality control procedures (such as re-genotyping of random samples) and has average accuracy of >99.9%.

Statistical analysis

Allele frequencies were estimated by gene counting. χ^2 test assessed deviations from Hardy-Weinberg equilibrium. Differences in genotype and allele frequencies between refractory and non-refractory patients with schizophrenia were assessed by Pearson's χ^2 test, or if any cell count was less then 5 by Fisher's exact test. Residual analyses were calculated using the PEPI version 4.0 software [32]. A significant *P value* was set as < 0.05. The POWR program from

PEPI Software Version 4.0 was used to calculate the statistical power of the samples.

Allele determination of *CYP3A4* and *CYP3A5* was achieved from the Human Cytochrome P450 (*CYP*) Allele Nomenclature Committee (http://www.cypalleles.ki.se/), according to the SNPs analyzed in this work. All non-detectable alleles determined by any other SNPs not considered here were set by **1*.

Haplotype frequencies for five polymorphisms in *DRD3* gene were computed from phase unknown multiple locus genotypes using the Multiple Locus Haplotype Analysis program (MLOCUS) [33]. D_{max} (D theoretical maximum) and D' (the relative magnitude of D as compared to its theoretical maximum, calculated as $D/D_{max.}$) values were calculated as described by Lewontin [34].

A multivariate logistic regression was performed in order to assess the odds ratio (OR) and 95% confidence interval (95% CI) for genetic risk factors as well as to control for confounding factors. Risk factors included in the model as independent variables were: illness duration ≥ 16 years, age at medication starting ≥ 21 years, and presence of co-medication. The variables were dichotomized because the assumption of a linear relation between these variables and refractoriness to neuroleptic drugs was not met.

RESULTS

Sample characteristics

Demographic and clinical characteristics of the sample of patients are shown in Table 1. Patients were aged between 16 and 65 years (34.69 ± 9.04

years) and 83.3% were men. The most commonly prescribed typical neuroleptic for non-refractory patients was haloperidol (73.8%). A total of 18.5% of these non-refractory patients were treated with only one typical neuroleptic and 50.9% with one typical neuroleptic associated to other drug (anticholinergics, lithium or antidepressants). Among refractory patients, different drug combinations were identified. A total of 108 from 121 (89%) patients treatment-resistant to typical neuroleptics switched to clozapine, and 13 (11%) changed to another atypical antipsychotic.

Genotype and allele activity frequency for CYP 3A4 and 3A5 genes and association with refractoriness to typical neuroleptics

From the nine studied *CYP3A4* SNPs, only the *-392A>G* was polymorphic, and genotype distributions did not reveal statistically significant differences compared to those expected under Hardy-Weinberg equilibrium. Significant associations were observed between the *CYP3A4 -392A>G* polymorphism and the inefficacy of neuroleptic treatment (Table 2). Carriers of two copies of the *-392A* variant (*CYP3A4*1A*, *wild-type; P* = 0.014, OR = 3.32) were more frequent in the treatment-resistant group, compared to carriers of one copy of the *-392G* variant (*CYP3A4*1B*). Since haloperidol metabolism is known to be mediated by CYP3A4, the data were reanalyzed including patients in this medication and we still found a significant association (*P* = 0.04, data not shown).

The observed frequency of the variant defining the *CYP3A5*3* allele (*6986G*, low expression) from the *6986A>G* polymorphism was 90.3%. Genotype frequencies observed were in accordance with Hardy-Weinberg equilibrium.

Because *CYP3A5*3* homozygous individuals express significant lower amounts of CYP3A5 protein than *CYP3A5*1/CYP3A5*3* heterozygotes [35], we compared the low expressor genotype against other genotypes. Refractoriness to neuroleptic treatment was more frequent in low expressors (*CYP3A5*3*/*CYP3A5*3*), compared to carriers of at least one allele with high expression (P = 0.003, OR = 3.16; Table 2). The frequency of the functional allele *CYP3A5*1* (10%) was in agreement with reports from other Caucasian populations (10-15%) [36].

Since CYP3A expression is known to be affected by many co-administered drugs that are CYP3A substrates, inhibitors or inducers [24,37,38], data for association of *-392A>G* and *6986A>G* polymorphisms were reanalyzed excluding patients taking CYP3A substrates, inhibitors or inducers, and the previous associations were maintained (P = 0.024 for *CYP3A4*, and P = 0.021 for *CYP3A5*).

Allele and haplotype frequencies for DRD3 gene and association with refractoriness to typical neuroleptic

Allele frequencies for all the SNPs in *DRD3* gene and the associations with neuroleptic refractoriness are shown in Table 3. The *T*-variant of *rs6280*, *A*-variant of *rs963468*, and *C*-variant of *rs7631540* were more frequent in refractory than non-refractory patients (P = 0.017, P = 0.021, P = 0.026, respectively). Dose effects for the risk-allele were observed for *rs6280* (*T/C* genotype, OR = 1.62; *T/T* genotype, OR = 3.00; P = 0.015) and *rs963468* (*G/A* genotype, OR = 1.60; *A/A* genotype, OR = 3.35; P = 0.023) polymorphisms, whereas a dominant effect for *rs7631540* was observed (*C/T* genotype, OR = 1.83; *C/C* genotype, OR = 2.84; P = 0.021).

Haplotype frequencies for all five *DRD3* gene polymorphisms are shown in Table 4. All SNPs were in strong linkage disequilibrium (D' > 0.91, *P* < 0.001), except *rs6280*, which showed weaker linkage disequilibrium with *rs1486012* and *rs7631540* (D' < 0.60, P < 0.002). Three haplotypes (*T/G/A/T/T*, *C/G/G/T/T* and *T/A/G/A/C*) accounted for about 79% of the genetic variability related to the five *DRD3* polymorphisms investigated. These three most frequent haplotypes were compared between refractory and non-refractory patients and a significant association was observed (χ^2 = 8.171, *P* = 0.017). Residual analyses demonstrated that refractory patients showed a higher *T/A/G/A/C* haplotype frequency (46.8%) than those observed in the non-refractory group (30.5%, *P* = 0.006), and this last group showed a higher *C/G/G/T/T* haplotype frequency (38%) than the refractory group (25%, *P* = 0.029).

Based on that, the *T/A/G/A/C* haplotype – the most frequent and the only one including the three variants individually associated with refractoriness – was compared with all other ten haplotypes. An association was found between this haplotype and refractoriness to neuroleptics (χ^2 = 5.342, *P* = 0.021, OR = 1.75, Table 4). A dose effect was observed among *T/A/G/A/C* haplotype carriers (*T/A/G/A/C* heterozygotes, OR = 1.50; *T/A/G/A/C* homozygotes, OR = 4.08; *P* = 0.022), showing that this haplotype has an important influence on refractoriness of neuroleptic treatment in schizophrenics. In an opposite way, as observed in the residual analysis, the *C/G/G/T/T* haplotype showed a protective effect when analyzed against all other haplotypes (χ^2 = 5.325, *P* = 0.021, OR = 0.56, Table 4).

CYP3A5 and DRD3 genes together associated with refractoriness to typical neuroleptic

Multiple logistic regression analyses checking for independent predictors of refractoriness risk are described in Table 5. The analysis revealed that *DRD3 T/A/G/A/C* haplotype (with a dose effect, OR = 1.97, *P* =0.016) and *CYP3A5* low expressor homozygous genotype (OR = 2.64, *P* = 0.034) were predictors of refractoriness to neuroleptics after controlling for risk factors, and no effect was observed for *CYP3A4 -392A>G*. It was also revealed that increased illness duration (>16 years) was an independent and important risk factor to treatment-resistance to neuroleptics.

To evaluate a possible interaction between those two polymorphisms, we tested treatment refractoriness among *CYP3A5*3* homozygous with two, one or no copies of the *DRD3 T/A/G/A/C* haplotype (Fig. 1). We found that *CYP3A5*3/CYP3A5*3* individuals carrying two copies of the *T/A/G/A/C* haplotype were at eleven-fold increased risk to treatment refractoriness than *CYP3A5*3/CYP3A5*3* individuals with no copy of the *T/A/G/A/C* haplotype (χ^2 = 9.395, *P* = 0.004, OR = 11.07).

DISCUSSION

In this study, we examined the possible influence of several polymorphisms in *DRD3, CYP3A4* and *CYP3A5* genes and the efficacy of typical neuroleptic treatment in schizophrenic patients. We found associations of *CYP3A5* genotypes and *DRD3* gene variants and haplotypes with ineffectiveness of neuroleptic treatment. The role of *CYP3A4* polymorphisms appears to be unclear.

The physiology and pharmacology of the D3 receptor and the pathophysiological changes in illnesses are only beginning to be understood. Gurevich *et al.* [39] postulated a D3 overexpression in the etiology of schizophrenia and suggested that antipsychotic drugs alleviate the symptomatology by blocking the D3 receptor and/or decreasing its expression. The only SNP located in the coding region of the *DRD3* gene is *Ser9Gly* (*rs6280*) and the functional significance of this polymorphism is not fully clear. Since the *Ser9Gly* mutation is situated at the extracellular N-terminus of the receptor, it should not affect agonist or antagonist binding or signal generation. Lundstrom and Turpin [40] observed that the D2-like antagonists haloperidol and chlorpromazine showed no significant differences in binding affinity for the homo- and heterozygotic mutant receptors compared to the wild-type, but a higher binding affinity for D3 and dopamine was observed in the homozygote mutant receptor (*C/C* or *Gly9/Gly9*) when expressed in recombinant cell lines. Although this polymorphism has been intensively studied, the results still remain inconclusive. Most previous association studies of antipsychotic drug response have focused on clozapine (an atypical agent), while very few addressed genetic correlates of refractoriness to typical neuroleptics. Although the majority of the studies with typical neuroleptics have pointed to a higher frequency of homozygous genotypes in good-responders [12,14,17,18], two studies found a higher frequency of genotype *Gly9/Gly9* in non-responders compared to controls [13,16] with other two failing to replicate these findings [11,15]. The heterogeneity and various criteria used to define treatment efficacy in these previous reports could at least partly explain the discrepancies of these studies. Scharfetter [41]

detected a trend for association between the *Gly9* allele and better response to antipsychotic treatment in general, in agreement with our results. The other two SNPs (*rs963468* and *rs7631540*) individually associated with refractoriness and also present in the risk haplotype have no evidence of functional significance. It is noteworthy that the effect of D3 receptor in treatment response is still largely conjectural, still requiring additional efforts to integrate this evidence with the different symptoms and neural correlates involved in this disease and in treatment response to build a comprehensive model for treatment response.

CYP3A4 catalyses the biotransformation of a large number of drugs, and is the most important CYP isoenzyme involved in the biotransformation of the antipsychotic agent haloperidol [22,42-44], the most frequently medication prescribed in this study. Very limited data are available on the enzymes catalysing the metabolism of chlorpromazine, and only one *in vitro* study indicated that 7-hydroxylation of chlorpromazine is catalyzed mainly by CYP2D6 and partially by CYP1A2 [45]. The most common variant *CYP3A4*1B* (-392A>G) results from an A to G transition in the 5' flanking region [46]. This variant occurs in about 4% of Caucasians [47], similar to the frequency described herein (4.8%). It was associated with neuroleptic response in the present study, but the effect of *CYP3A4*1B* allele on CYP3A4 activity remains unclear. Some in vitro studies indicate an association between the presence of *CYP3A4*1B* and higher expression or decreased enzyme activity. Rebbeck *et al.* [46] suggested that the *-392G* variant might alter disposition of androgenic substrates of CYP3A4 as a result of decreased enzymatic activity. However, Amirimani *et al.* [48] suggested that the phenotypic effects of the variant *CYP3A4*1B* may be associated with

enhanced CYP3A4 expression due to reduced binding of a transcriptional repressor (which presents consistently higher binding affinities to the wild-type sequence).

The CYP3A5 enzyme shares high homology with CYP3A4 and appears to share many substrates. This isozyme represents 50% of the total hepatic CYP3A content in individuals polymorphically expressing it [36]. However, the clinical relevance of the CYP3A5 absence remains unclear, because the expression of the *CYP3A5* polymorphism in relation to drug metabolism strongly depends on the concomitant expression of CYP3A4 in the liver and intestine [35]. Huang *et al.* [49] suggested that, under conditions where CYP3A5 content represents a significant fraction of the total hepatic CYP3A pool, the contribution of CYP3A5 to the clearance of some drugs might be an important source of interindividual variability. Kalgutkar *et al.* [42] reported that CYPs 3A4 and 3A5 catalyze haloperidol and reduced haloperidol oxidation. The *CYP3A5*3* allele consists of a *6986A>G* transition within intron 3 and creates an alternative splice site in the pre-mRNA and protein truncation resulting in the absence of CYP3A5 [36]. We identified this variant associated with refractoriness to neuroleptics in our sample. This is the first report of an association between this genetic variant and the efficacy of typical neuroleptic treatment in patients with schizophrenia.

When the multiple logistic regression results are taken into account, at least three points should be considered about the influence of *CYP3A* genes in neuroleptic refractoriness: (1) the individual association between no refractoriness to neuroleptics and *CYP3A4*1*B may reflect the effect of *CYP3A5* (located within 200 kb of CYP3A4), since *CYP3A4*1B* is in linkage disequilibrium with *CYP3A5*1*

[36] (in this study D' = 0.876, $P < 0.001$); (2) the functional effect of *CYP3A4*1B* variant is still not clear; and (3) at present, heritability seems to account for only a fraction of the interindividual variability in CYP3A activity observed in vivo [34]. Even considering the third assumption, *CYP3A5* low expressor genotype appears to be an important risk factor for neuroleptic refractoriness.

Predicting interactions between polymorphisms in drug-metabolizing enzymes and drug receptor/transporter polymorphisms might increase the clinician's ability to develop individualized antipsychotic treatment and reduce the time to drug response. In this investigation it was evidenced that the risk for treatment-resistance to typical neuroleptics was increased when both *DRD3* and *CYP3A5* polymorphisms were considered. The combination of two specific genotypes involved in drug action and disposition (DRD3 *T/A/G/A/C* homozygote + *CYP3A5*3/CYP3A5*3*) showed an additive effect in the response to neuroleptics. Further confirmation of these results in different samples and increased number of subjects can provide substantial cues for development of specific patterns of drug prescription. This approach may be very useful in a pharmacogenomic view, since the response status is multifactorial and results from interactions among many genetic variants scattered over several specific genes influencing treatment response.

A successful treatment should evolve over time. During the initial phase of illness, treatments target the control of acute symptoms such as psychotic behavior/agitation, with adequate and acceptable safety profile that minimizes side effects. As long as psychotic behavior and agitation get under control, other therapeutic targets gain emphasis, such as negative symptoms, cognitive deficits,

co-morbid diagnoses, and adverse effects [50]. The aim of long-term management is effectiveness rather than efficacy (the control of both positive and negative symptoms, minimization of side effects and enabling of independent living). The benefits of alleviation of negative symptoms and cognitive deficits usually are not observed very often with typical agents, and this decreases the likelihood of successful rehabilitation [51]. This point is in agreement with our finding that increased time of illness was associated to neuroleptic drug refractoriness, independently from other covariates.

This report illustrates that differences in target receptors like D3, and CYP3A allelic differences between subjects may give rise to significant variation in the action and biotransformation of neuroleptics and consequently to significant interindividual variation in treatment refractoriness. Nevertheless, some limitations to our study should be pointed: (1) we could not address all potential variables of interest, such as cognitive function, antipsychotic side effect profile, and patients' attitudes to medication that can all affect treatment outcome in the real-life clinical practice; (2) considering CYP activity, physiologic and environmental differences that affect individuals, such as hepatic and renal function, and diet can also results in heterogeneous response; (3) some metabolites of antipsychotic drugs are pharmacologically active and may themselves inhibit CYP enzymes with potencies that are similar to or greater than the parent drugs; (4) we had no access to the measurements of plasma levels and dosage of the drugs. Nevertheless, it is unlikely that the presence of significant number of patients under polidrug use would be a confounding factor, since the primary mechanism of action of all typical neuroleptics is similar.

It is reasonable to be aware that there are several processes that influence and may contribute to the complexity of CYP function. To our knowledge, this is the first study describing an association between these genetic variants and the safety of typical neuroleptic treatment. Despite of the relatively small sample size, our investigation displayed sufficient statistical power to detect an influence of differential neuroleptic refractoriness as a function of *CYP3A4* (67%) and *CYP3A5* genotype (83%), and *DRD3* haplotype (65%) emphasizing the pharmacogenetic relevance of these SNPs. This study may be considered as exploratory and suggestive of the effect of candidate SNPs for pharmacogenetic studies in schizophrenia. Additional studies will be needed for better assessment of the role of *DRD3, CYP3A4* and *CYP3A5* polymorphisms in the treatment refractoriness to typical neuroleptics.

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Table 1: Sample characteristics of neuroleptic-treated patients with DSM-IV diagnosis of schizophrenia.

Characteristics	Typical neuroleptic status		Total
	Non-refractory	Refractory	
Male	52/65 (80.0%)	103/121 (85.1%)	155/186 (83.3%)
Age[a]	36.49± 10.34	33.71 ± 8.14	34.69 ± 9.04
Age of illness onset[b]	20.78 ± 6.88	18.99 ± 5.26	19.62 ± 5.92
Illness duration[c]	15.62 ± 9.39	14.82 ± 7.87	15.10 ± 8.42
Age of first neuroleptic use[d]	23.57 ± 8.40	20.91 ± 5.99	21.85 ± 7.03
Co-medication[e]	81.5%	100%	93.5%

[a]Student's t-test: t = -2.016, *P* = 0.045; mean ± SE (standard error), [b]Mann-Whitney Test: z = -1.504, *P* = 0.133; mean ± SE (standard error), [c]Student's t-test: t = -0.615, *P* = 0.539; mean ± SE (standard error), [d]Mann-Whitney Test: z = -1.839, *P* = 0.066; mean ± SE (standard error), [e]Typical neuroleptics associated with any of these medications: other typical neuroleptics, anticholinergics, lithium or antidepressants.

Table 2: Genotype and allele frequencies of the *CYP3A* variants in refractory and non-refractory groups.

SNP	Genotypes			Alleles	
CYP3A4 –392A>G[a]	*A/A*	*A /G*	*G/G*	*A*	*G*
Non-refractory	54 (83%)	11 (17%)	0 (0%)	119 (92%)	11 (8%)
Refractory	114 (94%)	7 (6%)	0 (0%)	235 (97%)	7 (3%)
P		0.014		0.017	
CYP3A5 6986A>G[b]	*A/A*	*A/G*	*G/G*	*A*	*G*
Non-refractory	1 (1%)	18 (28%)	46 (71%)	20 (15%)	110 (85%)
Refractory	2 (2%)	12 (10%)	107 (88%)	16 (7%)	226 (93%)
P		0.006[c]		0.006	

[a]*-392A* variant =*CYP3A4*1A*; *-392G* variant = *CYP3A4*1B;* [b]*6986A = CYP3A5*1* (High expression); *6986G = CYP3A5*3* (Low expression); [c]*CYP3A5 Low/Low expressor* genotype vs other genotypes, *P* = 0.003, OR = 3.16 (95%CI 1.36-7.40).

Table 3: Allele frequencies and single marker analysis of htSNPs at *DRD3* gene in 186 patients with DSM-IV diagnosis of schizophrenia.

SNP	Location[a]	Type[b]	MFA[c]	Non-refractory	Refractory	*P*
rs6280	115211716	nsSNP	T	0.53	0.66	0.017
rs963468	115183788	Intronic	G	0.74	0.62	0.021
rs2134655	115179102	Intronic	G	0.75	0.78	0.505
rs1486012	115160323	3'downstream	T	0.67	0.57	0.074
rs7631540	115151420	3'downstream	T	0.59	0.47	0.026

[a]Position on NCBI B34 assembly; [b]nsSNP: non-synonymous SNP; [c]Most frequent allele in the non-refractory sample.

Table 4: Distribution of *DRD3* haplotypes between neuroleptic refractory and non-refractory groups.

DRD3 haplotype[a]	Non-refractory		Refractory	
	n	%	n	%
T/G/G/T/T	4	3.1	12	5.0
T/G/A/T/T	34	26.2	53	22.0
C/G/G/T/T	39	30.0[b]	47	19.4
T/G/G/T/C	0	0	2	0.8
T/G/G/A/C	0	0	1	0.4
T/G/A/A/C	0	0	1	0.4
C/G/G/T/C	10	7.7	23	9.5
C/G/G/A/C	9	6.9	11	4.5
T/A/G/T/T	0	0	2	0.8
T/A/G/A/C	32	24.6	88	36.4[c]
C/A/G/A/C	2	1.5	2	0.8

[a]*rs6280/rs963468/rs2134655/rs1486012/rs7631540;* [b]*C/G/G/T/T* haplotype vs all other haplotypes, *P* = 0.021, OR = 0.56 (95% CI 0.33-0.95); [c]*T/A/G/A/C* haplotype vs all other haplotypes, *P* = 0.021, OR = 1.75 (95% CI 1.06-2.92). CI=confidence interval.

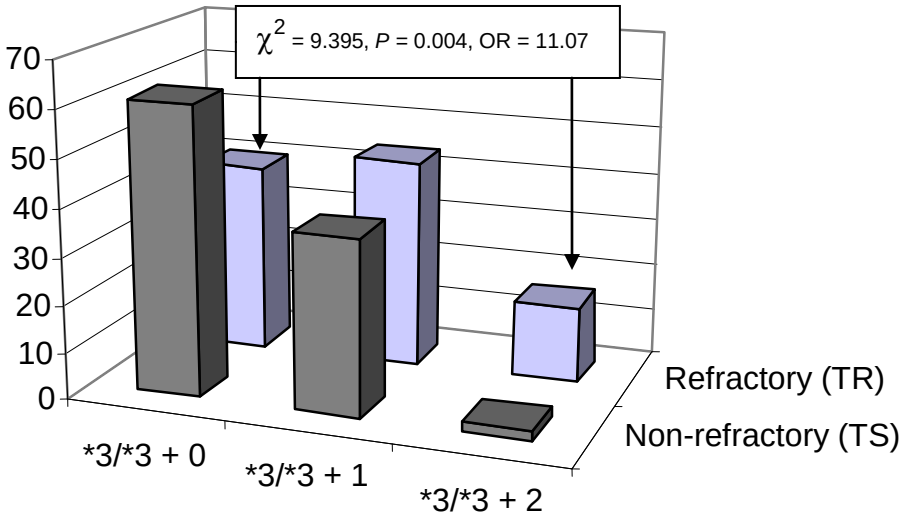
Table 5: Logistic regression analysis between neuroleptic refractory and non-refractory groups.

Variables in the model	β	SE	Wald	*df*	*P*	OR (95% CI)
Age at medication starting (≥21y)	0.008	0.346	0.001	1	0.981	1.01 (0.51-1.99)
Illness duration (≥16y)	0.869	0.350	6.160	1	0.013	2.38 (1.20-4.73)
Co-medication (present)	-0.062	0.045	1.903	1	0.168	0.94 (0.86-1.03)
T/A/G/A/C haplotype[a]	0.678	0.282	5.801	1	0.016	1.97 (1.13-3.42)
CYP3A5[b]	0.970	0.458	4.483	1	0.034	2.64 (1.07-6.48)
CYP3A4[c]	0.934	0.593	2.478	1	0.115	2.54 (0.79-8.13)

β=estimated coefficient; SE=standard error; *df*=degree of freedom; OR=odds ratio; CI=confidence interval. χ^2 model = 38.53, $P < 0.001$; Percent correct classification 72.6%.

[a]*rs6280/rs963468/rs2134655/rs1486012/rs7631540*; covariate counts the number of haplotypes each patient contains; [b]*Low /Low expressor* genotype; [c]*-392A/-392A* genotype.

Figure 1:


χ^2 = 9.395, *P* = 0.004, OR = 11.07
70
60
50
40
30
20
10
0
*3/*3 + 0
*3/*3 + 1
*3/*3 + 2
Refractory (TR)
Non-refractory (TS)

Legend for Figure 1:

Figure 1: Comparison of frequencies among *CYP3A5*3/CYP3A5*3* individuals carrying two, one or no copies of the *DRD3 T/A/G/A/C* haplotype. *3*3 + 0 = *CYP3A5*3/CYP3A5*3* + *T/A/G/A/C* non-carriers*;* *3*3 + 1 = *CYP3A5*3/CYP3A5*3* + carriers of one *T/A/G/A/C* copy*;* *3*3 + 2 = *CYP3A5*3/CYP3A5*3* + carriers of two *T/A/G/A/C* copies.

CAPÍTULO IV

LACK OF ASSOCIATION BETWEEN POLYMORPHISMS OF THE DOPAMINE AND SEROTONIN SYSTEMS AND RESPONSE TO NEUROLEPTIC TREATMENT

AMERICAN JOURNAL OF MEDICAL GENETICS PART B: NEUROPSYCHIATRIC GENETICS (em preparação)

Lack of association between polymorphisms of the dopamine and serotonin systems and response to neuroleptic treatment

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Short title: Pharmacogenetics of neuroleptic response.

Abstract

Pharmacogenetic studies of typical neuroleptic response in schizophrenia are very relevant considering the importance of the treatment with this class of neuroleptics, its low cost and global distributive justice issues in access to medicines. Interindividual variability suggests that polymorphisms at genes encoding neuroleptic targets might be related to neuroleptic response. Pharmacogenetic data on the response to typical antipsychotics are limited and have focused only on dopamine receptors. Studies focusing response-related variants directly or indirectly associated to the disease or its treatment may have a significant value to pharmacogenetic research, mainly through combinations of polymorphisms in multiple genes. We adopted a multiple candidate gene approach to examine whether neuroleptic refractoriness was related to polymorphisms in eleven different pathway genes. The sample included 208 Brazilian schizophrenic patients of European ancestry separated in two groups according to their response to typical neuroleptics. Fifteen polymorphisms were amplified by PCR follow restriction mapping or direct genotyping. Individual and multi-locus analyses did not show any significant association of all polymorphisms with refractoriness to typical neuroleptics. In conclusion, the serotonergic, dopaminergic and other related-systems polymorphisms investigated seem to have a small impact on typical antipsychotic drug response. Additional studies employing more polymorphisms are needed to address the real role of these neurotransmitter-related systems.

Key words: schizophrenia, pharmacogenetics, refractoriness, drug targets, polymorphisms.

Few studies on pharmacogenetics of typical neuroleptics have been conducted. Schizophrenia is a disease of high morbidity that affects 1% of the population worldwide [American Psychiatric Association, 1994]. The treatment with typical antipsychotics has been successful against positive symptoms (delusions, hallucinations, thought disorder and disorganized behavior), but has a lower effect on negative symptoms (poverty of thought, blunted affect and social withdrawal) and is associated with extrapyramidal symptoms (EPS) [Sawa and Snyder, 2002]. Many patients with schizophrenia do not respond to the typical antipsychotic drugs, and this variable clinical efficacy may be due to inter-individual variability in their binding to target neurotransmitter receptors. Possible pharmacological variability suggests that polymorphisms at genes that encode neuroleptic targets might be related to the patophysiological mechanism mediating the neuroleptic treatment response in schizophrenia.

Because of economic considerations, typical antipsychotics are still widely prescribed in Brazil. Additionally, failure to antipsychotic treatment has substantial clinical and economic cost. Results from recent studies such as CATIE [Lieberman *et al*., 2005; Lieberman, 2007] raised important considerations about the place of typical antipsychotics in the treatment of schizophrenia, subject to appropriate risk-benefit considerations. The availability of genetic predictors of therapeutic response to these drugs would further improve the risk-benefit ratio, and should be the focus of intensive research in the field of psychiatric pharmacogenetics.

Most pharmacogenetic studies to antipsychotic drug response in schizophrenia focused on patients treated with clozapine [Foster *et al*., 2007].

Pharmacogenetic data on the response to typical antipsychotics are scarcer and have focused only on dopamine receptors [Miyamoto *et al*., 2005]. Association studies between neuroleptic response and polymorphisms at dopamine receptor D2 gene (*DRD2*) were the most prevalent, but inconclusive results were reported with positive and negative findings [review in Arranz and de Leon, 2007].

If varied pharmacological profiles denote complex pathways and consequently little is known about the molecular mechanisms of antipsychotic activity, studies focusing response-related variants in drug-targeted areas indirectly related to disease via interactions with systems affected in schizophrenia may have a significant value to pharmacogenetic research. The polygenic basis of pharmacogenetic traits is an issue of major importance and for most traits it is unclear how many genes are involved, and genes that have been implicated thus far in well-studied phenotypes are of small effect [Lerer *et al*., 2006].

Promising efforts point to test that individual polymorphisms on their own may be unable to explain variability to treatment, and a combination of polymorphisms in multiple genes may provide a better predictive value of response. Thus, a multiple candidate gene approach has been adopted in this study. We examined whether neuroleptic refractoriness was related to polymorphisms of eleven different pathway genes. Genes coding for neurotransmitter receptors targeted by typical antipsychotics or indirectly involved in their effects are good candidates for pharmacogenetic studies, because therapeutic effects result from the interaction of these drugs and neurotransmitter receptors. The research on neurotransmitter-related genes affecting the availability and function of dopaminergic and serotonergic components, as serotonin

transporter (*SLC6A4*), monoamine oxidase A (*MAO-A*), dopamine transporter (*SLC6A3*), catechol-O-methyltransferase (*COMT*), G-protein (since catecholamine receptors are G-protein-coupled) (*GNB3*), and the brain-derived neurotrophic factor (*BDNF*) may also provide useful information.

The sample included 208 Brazilian schizophrenic patients of European ancestry, attending the program of Schizophrenia and Demency at Hospital de Clínicas de Porto Alegre, Brazil for at least 4 years. All patients met the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) [American Psychiatric Association, 1994] criteria for schizophrenia, with an additional confirmation using the Operational Criteria Checklist for Psychotic Disorders – OPCRIT [McGuffin *et al*. 1991]. Written informed consent was signed by all patients participating in this study, which was approved by the Ethics Committee of the hospital.

The patients were separated in two groups according to their response to typical neuroleptics. The non-refractory group (n=73, 35%) consisted of patients with sufficient and long-lasting response to treatment with typical neuroleptics. The treatment-resistant group (showing inadequate clinical response or intolerance; n=135, 65%) comprised patients that fulfilled the following criteria: (1) failure to respond to haloperidol (20-40mg/day oral doses) or to chlorpromazine (1g/day oral doses) for at least twelve weeks, or to thioridazine (400-800mg/day oral doses), for at least twenty four weeks; (2) did not show appropriated behavior control in the last two years; (3) presence of continuous symptomatology in the last two years [Kane *et al*., 1988]. These criteria were approved by the Clinical Protocol and Therapeutic Proceedings of the Brazilian Health Ministry [2002]. The response

status was verified after a thorough evaluation of psychiatrists and personal hospital records. Co-medication was recorded by clinical interview and review of medical records.

Genomic DNA was isolated from peripheral blood leukocytes by standard procedures [Lahiri and Nurnberger, 1991]. Fifteen polymorphisms were amplified by PCR using oligonucleotide primers and restriction mapping or direct genotyping, following methods described elsewhere (Table I).

Allele frequencies were estimated by gene counting. Deviation from Hardy-Weinberg equilibrium were assessed by χ^2 tests or, when appropriate, by Fisher's exact test. Statistical analyses were carried out using the SPSS software package. The significance level was set at $P < 0.05$.

Data were adjusted for multiple comparisons using the set-association method proposed by Hoh *et al*. [2001]. According to this method, the smallest of the empirical significance levels identifies the best and most parsimonious model predicting neuroleptics refractoriness. Permutation testing accessed the overall significance of the best model adjusting for multiple testing. This set-association approach was performed using the Sumstat software (http://linkage.rockefeller.edu/ott/sumstat.html). As established by the software, only biallelic polymorphisms were added to these analyses.

The characteristics of the sample of patients are shown in Table II. The studied sample included 169 men and 39 women with DSM-IV schizophrenia, and patients were aged between 16 and 65 years (35.00 ± 9.28 years). Among non-refractory patients, the most commonly prescribed typical neuroleptics was haloperidol (72.6%). During the study period, 19.2% of the non-refractory patients

were treated with only one typical neuroleptic and 51.7% with one typical neuroleptic plus an additional drug such as anticholinergics, lithium or antidepressants. Among refractory patients, many combinations of medications were adjusted. From 135 patients refractory to typical neuroleptics, 121 switched to clozapine and 14 changed to another atypical antipsychotic.

The set-association model based on Hoh *et al*.'s method [2001] is shown on Table III. This multi-locus analysis showed that any of the eleven Single Nucleotide Polymorphisms (SNPs) was significantly associated to refractoriness to typical neuroleptics. No significant association was also observed for the VNTRs *SLC6A4 Stin2*, *MAOA-uVNTR*, and *DRD4 48bp* in the univariate analyses (data not shown). The polymorphism *HTR2C Cys23Ser*, not included in the multi-locus analysis because is X-linked, also did not show any association with neuroleptic response (data not shown).

Previous associations were observed between the -141C Ins/Del polymorphisms at *DRD2* gene and typical neuroleptics response [Dahmen *et al*., 2001; Suzuki *et al*., 2001; Himei *et al*., 2002; Wu *et al*., 2005; Lencz *et al*., 2006], but one study could not replicate these findings [Ohara *et al*., 1998;]. A number of studies also addressed the influence of *COMT* [Inada *et al*., 2003; Illi *et al*., 2003a,b, 2007; Weickert *et al*., 2004; Anttila *et al*., 2004; Nolan *et al*., 2006; Molero *et al*., 2007], *BDNF* [Krebs *et al*., 2000; Anttila *et al*., 2005], and *DRD4* [Hwu *et al*., 1998; Cohen *et al*.; 1999; Kaiser *et al.*, 2000] polymorphisms on typical neuroleptics response, showing more negative than positive results. In a recent study with a serotonergic approach, Anttila *et al*. [2007] observed associations between *5HT2A T102*C and *GNB3 C825T*, and response to typical

neuroleptics. Their response criteria were the same used in the present report, nevertheless their findings for these two polymorphisms were not replicated in our larger sample. Serotonin transporter (*SLC6A4*) studies also did not report positive associations [Kaiser *et al*., 2001] and one monoamine-oxidase A (*MAO-A*) study found an association only in combination with a *COMT* polymorphism [Illi *et al*., 2003], which was not confirmed by Nolan *et al*. [2006].

Discrepancies among studies possibly reflect methodological issues such as sample size or study design but other features (ethnicity and comorbidities, for instance) could also contribute to conflicting results. The naturalistic design is also a limitation of the present study since it is difficult to control for all confounders in an observational study but the observational-naturalistic design is valuable to better appreciate the role of genetic factors in routine clinical practice beyond the realm of controlled clinical trials. Since we performed analyses in a moderate sample size we also cannot exclude Type II errors.

In conclusion, genetic variations in serotonergic, dopaminergic and other related-systems seem to have a small impact on typical antipsychotic drug response. More studies are needed to address the real role of polymorphisms at these neurotransmitter-related genes. Studies which employ a great number of genes and polymorphisms have higher potential to screen large portions of the genome for genes that have possible influence on response to antipsychotics [Kane and Malhotra, 2003]. Additionally, negative results are relevant [Colhoun *et al*., 2003] especially in an area with few studies because it may help researchers to select genes and polymorphisms, and to ensure which genes are truly related to antipsychotic response.

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Table I: Polymorphisms investigated in this study.

Gene[a] Polymorphisms	Chromosome	Enzyme	Reference
HTR1B 861G>C	6q13	*Hinc*II	(Hawi *et al*., 2002)
HTR2A 102T>C	13q14-q21	*Msp*I	(Quist *et al*., 2000)
HTR2A -1438A>G	13q14-q21	*Msp*I	(Arranz *et al*., 1998)
HTR2A His452Tyr	13q14-q21	*Bsm*I	(Quist *et al*., 2000)
HTR2C Cys23Ser	Xq24	*Hinf*I	(Lappalainen *et al* 1995)
SLC6A4 5HTTLPR	17q11.1-q12	-	(Heils *et al*., 1996)
SLC6A4 Stin2 VNTR	17q11.1-q12	-	(Ito *et al*., 2002)
MAOA -uVNTR	Xp11.23-11.4	-	(Sabol *et al*., 1998)
DRD2 -141C ins/del	11q23	*Mva*I	(Ohara *et al*., 1998)
DRD4 VNTR 48bp exon3	11p15.5	-	(Kohn *et al*., 1997)
SLC6A3 -844C>T	5p15.3	*Msp*I	(Rubie *et al*., 2001)
COMT Val158Met	22q11.21	*Nla*III	(Kunugi *et al*., 1997)
GNB3 825C>T	12p13	*Bsa*JI	(Rosskopf *et al*., 2000)
GNB3 814G>A	12p13	*Pst*I	(Rosskopf *et al*., 2000)
BDNF Val66Met	11p13	*Eco*721	(Neves-Pereira *et al*., 2002)

[a]*HTR1B*, *HTR2A*, and *HTR2C*: 5-hydroxytryptamine (serotonin) receptor *1B*, *2A* and *2C* genes; *SLC6A4*: solute carrier family 6 (serotonin transporter), member 4; *MAOA*: monoamine oxidase A gene; *DRD2* and *DRD4*: dopamin receptor D2 and D4 genes; *SLC6A3*: solute carrier family 6 (dopamine transporter), member 3; *COMT*: catechol-O-metyltransferase gene; *GNB3*: G-protein Beta-3 subunit gene; *BDNF*: brain-derived neurotrophic factor gene.

Table II: Sample characteristics of 208 neuroleptic-treated patients with schizophrenia.

Characteristics	Typical neuroleptic status		Total
	Non-refractory	Refractory	
Male	56/73 (76.7%)	113/135 (83.7%)	169/208 (81.3%)
Age[a]	36.58 ± 10.08	34.13 ± 8.73	35.00 ± 9.28
Age of schizophrenia onset[b]	20.97 ± 6.87	18.99 ± 5.19	19.68 ± 5.90
Illness duration[c]	15.52 ± 9.05	15.16 ± 8.41	15.29 ± 8.62
Age at medication starting[d]	23.69 ± 8.39	20.89 ± 5.88	21.88 ± 6.99
Co-medication[e]	80.8%	100%	93.3%

[a] Student's t-test: t = -1.818, *P* = 0.070; mean ± SE (standard error)

[b] Mann-Whitney Test: z = -1.823, *P* = 0.068; mean ± SE (standard error)

[c] Student's t-test: t = -0.284, *P* = 0.777; mean ± SE (standard error)

[d] Mann-Whitney Test: z = -2.085, *P* = 0.037; mean ± SE (standard error)

[e] Typical neuroleptics associated with any of the following medications: other typical neuroleptics, anticholinergics, lithium or antidepressants.

Table III: Set association approach results for typical neuroleptic refractoriness in patients with schizophrenia (corrected for multiple comparisons).

Polymorphism[a]	χ^2 statistic	Sum	*P*-value
HTR2A His452Tyr	3.0668	3.0668	0.9085
HTR1B 861G>C	1.2727	4.3396	0.9655
BDNF Val66Met	0.7293	5.0688	0.9844
GNB3 825C>T	0.5821	5.6510	0.9899
SLC6A3 -844C>T	0.5726	6.2236	0.9913
SLC6A4 HTTLPR	0.5140	6.7375	0.9922
HTR2A 102T>C	0.3522	7.0898	0.9931
COMT Val158Met	0.3332	7.4230	0.9931
HTR2A -1438A>G	0.2749	7.6979	0.9922
DRD2 -141C ins/del	0.0996	7.7975	0.9923
GNB3 814G>A	0.0226	7.8202	0.9924

[a]Only bialelic polymorphisms.

CAPÍTULO V

CYP2D6 GENETIC VARIATION IN HEALTHY AND SCHIZOPHRENIC BRAZILIAN SUBJECTS: LACK OF ASSOCIATION WITH ANTIPSYCHOTIC TREATMENT

THE PHARMACOGENOMICS JOURNAL (em preparação)

CYP2D6 genetic variation in healthy and schizophrenic Brazilian subjects: Lack of association with antipsychotic treatment

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Running title: CYP2D6 genetic variation in Brazilian subjects

Abstract

CYP2D6 is a polymorphic drug-metabolizing enzyme which contributes to the metabolism of almost 25% of drugs in common clinical use, including antipsychotics. We investigated twenty-four *CYP2D6* polymorphisms, gene deletion and gene multiplication in 278 individuals of European ancestry, among them 186 were patients with schizophrenia 87 African Brazilians healthy individuals, besides, with the aim of characterize *CYP2D6* allele and genotype frequencies in Brazilians as well as its influence on antipsychotics response. Twenty-three different alleles and seven allele duplications were identified. No significant differences were observed between the healthy group and the schizophrenic patients, but significant differences were observed between African- and European-Brazilians**.** The metabolic phenotype distribution between the two groups showed a significant difference (P = 0.014). The distribution of reduced alleles led to a higher frequency of IM (19.6%) in the African-ancestry sample than in the European-ancestry individuals (7.3%; P = 0.001). Antipsychotic response analyses did not show any significant association with *CYP2D6* allele function/genotype. In conclusion, *CYP2D6* may not be the major determining factor for the variability in efficacy of antipsychotic treatment in Brazilians. However, the recognition of common functionally significant polymorphisms in *CYP2D6* might be important to the outcome of other drug treatments in the Brazilian population.

INTRODUCTION

Cytocrome P450 enzymes (CYPs) are important in the biosynthesis and degradation of several endogenous compounds such as steroids, lipids and vitamins, chemicals present in diet and environment, as well as medications (Nebert and Russel, 2002). Ingelman-Sundberg and Rodriguez-Antona (2005) estimated that 15-20% of all drug treatments are influenced by polymorphisms at the cytochrome P450 genes, and they are many times responsible for the development of adverse drug reactions (Phillips *et al*., 2001). While the aim of pharmacogenetics is to promote individualized medicine, differences between populations can also be considered in order to improve healthcare quality, since polymorphisms show variable allele frequencies among populations.

CYP2D6 represents an average of 2% of hepatic CYP content, it is one of the best-known polymorphic drug-metabolizing enzymes, and it is not induced by environmental factors (Tiwari *et al*., 2005). It also contributes to the metabolism of almost 25% of drugs in common clinical use, such as β-blockers, antiarrythmic agents, opiates and many psychotropic drugs, including antipsychotics (Bradford, 2002) such as haloperidol, thioridazine, perphenazine, and chlorpromazine (Ingelman-Sundberg, 2004). The *CYP2D6* gene spans a 4.2-kb region and forms a CYP2D cluster with two pseudogenes, *CYP2D8P* and *CYP2D7P*, arranged in tandem on chromosome 22q13.1 (Kimura *et al*., 1989). At present, more than 90 different *CYP2D6* allelic variants and sub-variants are known (http://www.imm.ki.se/CYPalleles/cyp2d6.htm), including those produced by major rearrangements (whole-gene deletion or duplication). However there are only four major CYP2D6 phenotypes: ultrarapid (three or more functional copies), extensive

(at least one functional allele), intermediate (one reduced activity allele and one inactive allele or two reduced activity alleles), and poor metabolizers (two inactive alleles) (de Leon, 2006).

Most antipsychotic drugs are highly lipophilic and also undergo extensive metabolism by CYP enzymes. In schizophrenic subjects, 40-80% of patients in treatment with classical antipsychotics does not fully respond to treatment, even after several attempts, and are thus termed treatment-refractory cases (Lane *et al*., 2005). CYP enzymes involved in the metabolism of antipsychotics are therefore good candidates for investigation of refractoriness. An interesting analysis by Kirchheiner *et al*. (2004) revealed that the dosage of about 50% of the commonly prescribed antipsychotics is *CYP2D6* genotype dependent.

Since the delineation of allele distribution and frequency is required to effectively translate pharmacogenetics to the clinic and given the paucity of *CYP2D6* data in the Brazilian population, the purpose of this research was to characterize the *CYP2D6* alleles and genotype frequencies in Brazilians of European and African ancestries as well as its influence on antipsychotics response in this population.

RESULTS

Twenty-three different alleles and seven allele duplications were identified (Table 1), while eight alleles (**2B, *11, *12, *14A, *20, *25, *30, *31*) were tested but not found in Brazilians.

In African Brazilians, the most frequent alleles were **1, *2A, *4A, *17*, and **41*. These alleles accounted for 79% of the chromosomes investigated. Allele

duplications were observed in 3.4% whereas allele **5* (deletion) was seen in 4% of the subjects from this ethnic group (Table 1). Table 2 shows that alleles coding for reduced function (**10B, *17, *29, *41, *41XN*) were observed in 31% of the sample. Non-functional (**3, *4ADJ, *4XN, *5, *15*) alleles accounted for 13.2% of the alleles identified, and 54% (**1, *2A*) were functional alleles. Gene duplications (**2AXN*) resulting in high enzyme activity were observed in 1.7% of the African-Brazilians.

In healthy Brazilians of European ancestry, alleles **1, *2, *4, *35*, and **41* were the most frequent. These five alleles accounted for 73% of the chromosomes investigated in the healthy individuals from this ethnic group. Allele duplications were seen in 5.2% (Table 1). Alleles coding for reduced function (**9, *10, *17, *29, *41, *41XN*) showed a frequency of 14.2%, non-functional alleles (**3, *4, *4XN, *5, *6A/BC, *15, *19*) were detected in 18.5%, and functional (**1, *2A, *35*) in 63.0**%** of the chromosomes investigated (Table 2). High enzyme activity due to gene duplications (**1XN, *2AXN, *35XN*) was observed in 4.1% of European-Brazilians (Table 1 and 2).

Overall little differences were observed between the healthy group and the schizophrenic patients from the same ethnic group. Nineteen different alleles (**1, *2A, *2D, *3, *4ABDJK, *5, *6A/B, *9, *10AB, *15, *17, *35, *40, *41*) and five allele duplications (**1XN, *2AXN, *4AXN, *35XN, *41XN*) were detected in patients with schizophrenia (Table 1). The most frequent allele in this sample was the wild-type allele (**1;* 38%). Seven alleles (**3, *4BK, *10A, *40, *35XN, *41XN*) detected in low frequencies in schizophrenic patients were not observed in the European-Brazilian healthy sample, while other six alleles (**6C, *14B, *19, *29, *4DXN,*

4JXN) were observed, also in low frequencies, only in healthy individuals. Duplication of *CYP2D6* gene (**1XN, *2AXN, *4AXN, *35XN, *41XN*) was observed in seventeen (4.6%) individuals with schizophrenia, and 3.8% was related to high activity alleles (**1XN, *2AXN, *35XN*).

The frequencies of alleles with normal, decreased, none, and high activity (Table 2) were similar between patients with schizophrenia and healthy controls (χ^2 = 0.836, *P* = 0.841). Consequently, the same pattern of phenotypes were observed in relation to the distribution of UM, EM, IM, and PM phenotype status (*P* = 0.413).

The sample of African Brazilian individuals showed a higher frequency of **5* (4.0%), **17* (9.2%), **29* (6.3%), and **41* (10.9%) alleles, whereas the European Brazilian sample showed a higher frequency of **4* (12.2%) and **35* (6.6%) alleles (Table 1). We observed significant differences among the distribution of the alleles with normal, decreased, none, and high activity between African- and European-Brazilians (χ^2 = 26.632, *P* < 0.0001), with an over-representation of reduced activity alleles in those individuals with African ancestry (*P* < 0.0001) and of normal activity alleles in the European-derived subjects (*P* = 0.033) (Table 2).

Phenotype (UM, EM, IM, PM) frequencies are shown in Figure 1. As expected, the most frequent metabolic status was EM in both African and European Brazilians (73.6% and 82.5%), however the metabolic phenotype distribution between the two groups showed a significant difference (*P* = 0.014). The distribution of reduced alleles shown in Table 2 led to a higher frequency of IM (19.6%) in the African-ancestry sample than in the European-ancestry individuals (7.3%; *P* = 0.001).

Antipsychotic response analyses did not show any significant association between *CYP2D6* allele function/genotype and typical neuroleptics/clozapine response (Table 3).

DISCUSSION

The present study describes the range of genetic polymorphisms occurring in the drug metabolizing Cytochrome P450 2D6 and the influence of ethnic origin in variation on allele frequencies in the Brazilian population. Additionally, the relevance of the various polymorphisms to antipsychotic response was also evaluated.

The Brazilian population is a highly heterogeneous population that results from interethnic admixture among people from European, African and Amerindian origins. However this admixture is uneven across the country. This heterogeneity was documented in several genetic studies, using either uniparental or autosomal markers, which demonstrated a typical although non-uniform triethnic pattern for the Brazilian population gene pool. Southern populations presented lower levels of African and higher degrees of European contributions when compared to other Brazilian groups (Salzano and Bortolini, 2002; Callegari-Jacques *et al*., 2003; Zembrzuski *et al*., 2006).

The influence of ethnicity in the distribution of *CYP2D6* alleles and consequently phenotypes (PM, IM, EM, UM) is already known. High heterogeneity in the African continent has been observed. The prevalence of PMs ranged from 0 to 19%. The frequency of PMs in African-Brazilians was 3.4%, which is comparable with that described for Nigerians (4%) and higher than that reported

for African Americans (1.5-1.9%) (Masimirembwa and Hasler, 1997; Cai *et al*., 2006). The West African contribution to the African Brazilian gene pool was already known from beta-globin haplotype investigations (Salzano and Bortolini, 2002). The high frequency of the IM genotype in our sample (19.6%) is due at least in part to the presence of the **17* (9.2%) and **29* (10.9%) alleles. In Africans, the **17* has been detected at a median frequency of 24% (Bradford, 2002), also contributing to elevate the frequency of IMs. Gene duplication frequencies vary in Subsaharan Africa from 0.8 to 3.5% (Sistonen *et al*., 2007). The observed frequency (3.4%) of gene duplication and UMs in our sample is in the upper range described for Africans. In African American studies, gene duplications ranged from 1.4 to 5.4% (Bradford, 2002; Cai *et al*., 2006), generating 4.6% of UMs in this population (Cai *et al*., 2006).

Depending on the precise country of origin, 1 to 10% of Europeans have one extra copy of **1* or **2* alleles, resulting in faster than average metabolism (UM, Lovlie *et al*., 1996). Gene duplication generally is observed at a low frequency of 1 to 2%, with Spaniards having the highest frequency of duplicated alleles (7-10%) (Agúndez *et al*., 1995; Bernal *et al*., 1999). Additionally, investigations of genotype frequencies in different populations of Europe revealed 10% of UMs in Spaniards and Italians (Ingelman-Sundberg, 2005). The colonization of South Brazil is marked by a strong contribution of these two populations therefore the European ancestry of Brazilians could explain the high frequency of UMs observed in our study (9.8%). It is well established that about 5% of Europeans lacks CYP2D6 activity and are known as poor metabolizers (Daly, 2004). The observed lower

frequency of PMs in European-derived Brazilians (3.3%) is concomitant with the low frequency of **4* and all the PMs detected in our study presented this allele.

We found significant differences between African- and European-descendants with respect to the distribution of activity alleles and metabolic genotypes (Table 2 and Figure 1). An over-representation of reduced activity alleles and consequently a higher frequency of IMs were observed in African Brazilians, as expected (Bradford, 2002), reflecting the influence of some characteristic alleles (**17, *29, *41*) in the metabolic status of this population.

Ten alleles **1, *2, *4, *5* (gene deletion)*, 9, *10, *17, *29*, 35 and **41* alleles, besides detection of gene multiplication accounted for 92% of CYP2D6 variability in Brazilians independent of skin color, therefore this subset should be preferentially genotyped in pharmacogenetic studies in Brazil.

There is still a lack of evidence of significant relationship between *CYP2D6* activity or genotype and therapeutic response to antipsychotic treatment, but genetic knowledge of metabolic status may be useful for adjustments of therapeutic doses (Kirchheiner *et al*., 2005; Arranz and de Leon, 2007). Moreover, caution must be considered because of the inter-ethnic differences in the frequency of genotypes. We observed no significant difference between patients with schizophrenia and European-derived healthy individuals, as well as no correlation between *CYP2D6* allele function/genotype and typical neuroleptics/clozapine response was observed. Some studies have tried to find effects of *CYP2D6* genotypes and metabolism on response to neuroleptics. Regarding effects on plasma concentration of haloperidol, Someya *et al*. (1999) observed significant tendency to higher plasma concentrations of haloperidol and

reduced haloperidol in Japanese carriers of **5* allele than in non-carriers. However, Roh *et al*. (2001) suggested the involvement of *CYP2D6* genotypes in the metabolism of haloperidol only at low doses of this drug, and no difference was found with respect to reduced haloperidol concentrations, in a Korean sample of schizophrenic patients. These findings were not confirmed by Ohnuma *et al*. (2003). Brockmöller *et al*. (2002) significantly correlated the number of active alleles and reduced haloperidol levels, and a trend toward lower therapeutic efficacy with increasing number of *CYP2D6* alleles. Results in the same direction were also found by Aitchison *et al*. (1999). In a retrospective follow-up study, Mulder *et al*. (2006) found significant findings between PM genotype and changes in dosage regimen in a sample of hospitalized patients treated with antipsychotics (class of antipsychotic was not specified). Arranz *et al*. (1995) reported an absence of correlation between *CYP2D6* alleles and response to clozapine, suggesting that CYP2D6 is not the major enzyme responsible for the metabolism of clozapine, which concur with the findings reported herein.

In conclusion, *CYP2D6* genotype may not be the major determining factor for the variability in efficacy of antipsychotic treatment in Brazilian patients with schizophrenia. However, the recognition of common functionally significant polymorphisms in *CYP2D6* might be important to the outcome of other drug treatments, such as β-blockers, antidepressants, and opioids (Lynch and Price, 2007), making the establishment of relevant alleles very useful for the future application of pharmacogenetics in the Brazilian population.

MATERIAL AND METHODS

Subjects

Porto Alegre is the capital of Brazil's southernmost state; the city was founded in 1752 by 60 couples from the Azores islands. At present the European-derived subjects from Porto Alegre are still mainly of Portuguese descent, but Italians, Spaniards and Germans have also contributed to its gene pool. African Brazilians constitute approximately 14% of the population. They are descendants of slaves who were brought to Brazil between the 15th and18th centuries, from Africa's West Coast but also from Angola and Mozambique.

The European derived sample consisted of 92 healthy individuals who came to the Genetics Department of the Federal University of Rio Grande do Sul for paternity testing, their mean age being 31.0±11.5 years. Eighty-seven (mean age: 35.0±15.2) African Brazilians were ascertained at the Central Laboratory of a General Public Hospital to which they went for routine blood determinations. A total of 186 nonrelated patients with schizophrenia were also recruited at Hospital de Clínicas de Porto Alegre, the University Hospital. All subjects with schizophrenia were of European ancestry. All patients met clinical criteria for schizophrenia according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) (American Psychiatric Association, 1994). Furthermore an Operational Criteria Checklist for Psychotic Disorders (OPCRIT) (McGuffin *et al*., 1991) was completed for each patient on the basis of a standardized semi-structured diagnostic interview. Informed consent was signed by all subjects. The project was approved by the Hospital de Clínicas de Porto Alegre Ethics Committee.

Patients were grouped according to their response to neuroleptics. The group of responders (n=65, 35%) consisted of patients who had experienced sufficient and long-lasting response to treatment with typical neuroleptics. The treatment-resistant group (n=121, 65%) fulfilled the criteria as follow: (1) failure to respond to haloperidol (20-40mg/day oral doses) or to chlorpromazine (1g/day oral doses) for at least twelve weeks, or to thioridazine (400-800mg/day oral doses), for at least twenty four weeks; (2) did not show appropriated behavior in the last two years; (3) presence of continuous symptomatology in the last two years (Kane *et al*., 1988). The term treatment-resistant includes those who are treatment refractory (show inadequate clinical response) and those who are treatment-intolerant (exhibit adverse responses). A total of 108 from 121 patients treatment-resistant to typical neuroleptics switched to clozapine, and 13 changed to another atypical antipsychotic.

Clozapine response was evaluated by the treating physician using the Brief Psychiatric Rating Scale (BPRS) (Overall and Gorham, 1962), and a 20% reduction of the scores was considered as the threshold for appropriate response. Sixty-two patients were classified as responders and 46 as non-responders to clozapine treatment. Co-medication was recorded by clinical interview and review of medical records. Any additional information about the subjects was reviewed from the patient records. Both criteria of response were approved by the Clinical Protocol and Therapeutic Proceedings of the Brazilian Health Ministry (2002).

SNP selection

Twenty-four polymorphisms determining thirty-one alleles, gene deletion and gene duplication in *CYP2D6* gene (Table 4) were selected according to their effect in CYP2D6 enzyme activity (http://www.cypalleles.ki.se/).

Genotyping

High-molecular-weight genomic DNA was isolated from venous blood using standard procedures (Lahiri and Nurnberguer, 1991). Before genotyping, the entire *CYP2D6* gene was amplified in long-PCR reaction using primers CYP2D6-F (5'-GCTGCCATACAATCCACCTG-3') and CYP2D6-R (5'-GCCTCAACGTACCCCTGTCTC-3') to separate the gene from the flanking highly homologous *CYP2D7* and *CYP2D8P* pseudogenes, producing a ~6.2-kb fragment. The reaction mixture contained 2U Expand Long Template Enzyme Mix (Roche), Expand Long Template Buffer 1 1x, 0.25mM each deoxynucleotide triphosphate and ~100ng of genomic DNA, and it was carried out on a 9700 Thermal Cycler (Applied Biosystems).

CYP2D6 genotyping was performed by using the MassARRAY SNP genotyping system (Sequenom, Inc., San Diego, CA, USA), following the manufacturer's instructions. The method involves multiplex PCR and minisequencing assays, designed with SpectroDESIGNER software (Sequenom, Inc., San Diego, CA), and followed by mass spectrometry analysis with the Bruker Bi-Flex MALDI-TOF mass spectrometer (Bruker Daltonics, Billerica, MA, USA). Spectral output was analyzed using SpectroTYPER–RT software (Sequenom, Inc., San Diego, CA) and by manual review. The principles of this method are

detailed in Buetow *et al*. (2001). This genotyping platform is at the Spanish National Genotyping Center's facilities at Santiago de Compostela University. It is subjected to regular quality control procedures (such as re-genotyping of random samples) and has average accuracy of >99.9%.

Gene duplications and deletion were detected by long-range PCR in separated reactions containing a set of three primers. With the primer combination CYP-207-F (5'-CCCTCAGCCTCGTCACCTCAC-3') and CYP-32-R (5'-CACGTGCAGGGCACCTAGAT-3') we amplified a duplication-specific 3.2kb fragment. In addition, a 3.8kb fragment was amplified as an internal control fragment using primer CYP-13-F (5'-ACCGGGCACCTGTACTCCTCA-3'), to rule out false-negative results. To detect the gene deletion we combined CYP-207-F and CYP-13-F plus a deletion-specific primer CYP-24-R (5'-GCATGAGCTAAGGCACCCAGAC-3'), yielding a 3.5kb fragment and a 3.0kb control fragment. Long-PCR was carried out on a 9600 Thermal Cycler (Applied Biosystems) and the reaction mixture contained 2U Platinum® Taq DNA Polymerase High Fidelity (Invitrogen), Buffer $MgCl_2$ free 1x, 1mM $MgCl_2$, 0.2mM each deoxynucleotide triphosphate and ~100ng of genomic DNA. The primer concentrations were as follow: 0.6μM CYP-207-F, 0.4μM CYP-32-R or CYP-24R, and 0.2μM CYP-13F. The temperature cycling profile was the same as described in Sistonen *et al*. (2005). The resulting long-PCR products were separated and detected in ethidium bromide-containing 1% agarose gels.

To determine which allele was duplicated we used an approach of dose analysis, comparing peak highs in the spectral output from Sequenom platform (Sequenom, Inc., San Diego, CA).

Statistical analysis

Allele determination of *CYP2D6* was based on the Human Cytochrome P450 (*CYP*) Allele Nomenclature Committee recommendation (http://www.cypalleles.ki.se/), according to the SNPs analyzed in this work (Table 4). Alleles were classified on the basis of haplotype combinations. The **1* allele was set when any change was observed in all SNPs studied here. Metabolic status was defined as: ultra-rapid metabolizer (UM): at least three active duplications; extensive metabolizer (EM): one or two active alleles; intermediate metabolizer (IM): one or two low activity alleles; poor metabolizer (PM): two inactive alleles (de Leon, 2006).

Allele frequencies were estimated by gene counting. Differences in *CYP2D6* genotype and allele frequencies between samples as well as between schizophrenic responders and non-responders to antipsychotics were assessed by Pearson's χ^2 test, or if any cell count was less than 5 by Fisher's exact test. The data analysis was carried out using SPSS v10.0. Residual analyses were made using PEPI Software, Version 4.0 (Abramson and Gahlinger, 2001). A significant *P value* was set as < 0.05.

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Table 1: *CYP2D6* allele frequencies observed in African-derived, European-derived control and schizophrenia samples of South-Brazilian population.

Alleles	African-derived (%)	European-derived (%)	Schizophrenics (%)
**1*	72 (41.4)	79 (42.9)	143 (38.4)
**2A*	22 (12.6)	19 (10.3)	65 (17.5)
**2D*	0 (0)	4 (2.2)	3 (0.8)
**3*	2 (1.1)	0 (0)	4 (1.1)
**4A*	8 (4.6)	10 (5.4)	35 (9.4)
**4B*	0 (0)	0 (0)	1 (0.3)
**4D*	2 (1.1)	5 (2.7)	3 (0.8)
**4J*	1 (0.6)	4 (2.2)	7 (1.9)
**4K*	0 (0)	0 (0)	3 (0.8)
**5*	7 (4.0)	2 (1.1)	8 (2.2)
**6A/B*	0 (0)	3 (1.6)	2 (0.5)
**6C*	0 (0)	1 (0.5)	0 (0)
**9*	0 (0)	4 (2.2)	6 (1.6)
**10A*	0 (0)	0 (0)	2 (0.5)
**10B*	7 (4.0)	5 (2.7)	6 (1.6)
**14B*	0 (0)	1 (0.5)	0 (0)
**15*	1 (0.6)	1 (0.5)	6 (1.6)
**17*	16 (9.2)	4 (2.2)	5 (1.3)
**19*	0 (0)	2 (1.1)	0 (0)
**29*	11 (6.3)	1 (0.5)	0 (0)
**35*	0 (0)	14 (7.6)	23 (6.2)

**40*	0 (0)	0 (0)	2 (0.5)
**41*	19 (10.9)	13 (7.1)	31 (8.3)
**1XN*	0 (0)	1 (0.5)	2 (0.5)
**2AXN*	3 (1.7)	8 (4.3)	11 (3.0)
**4AXN*	0 (0)	1 (0.5)	2 (0.5)
**4DXN*	2 (1.1)	1 (0.5)	0 (0)
**4JXN*	0 (0)	1 (0.5)	0 (0)
**35XN*	0 (0)	0 (0)	1 (0.3)
**41XN*	1 (0.6)	0 (0)	1 (0.3)
Total	174	184	372

Table 2: *CYP2D6* allele activity frequencies observed in African- and European-derived samples of South-Brazilian population.

		European-derived (%)		
Allele activity	African-derived (%)	Controls	Schizophrenics	Total
None	23 (13.2)	31 (16.8)	72 (19.4)	103 (18.5)
Reduced	54 (31.0)	27 (14.7)	52 (14.0)	79 (14.2)
Normal	94 (54.0)	116 (63.0)	234 (62.9)	350 (63.0)
High	3 (1.7)	9 (4.9)	14 (3.8)	23 (4.1)
Unknown	0 (0)	1 (0.5)	0 (0)	1 (0.2)
Total	174	184	372	556

χ^2 test: Schizophrenics and healthy controls (European-derived, excluding the individual with unknown activity N) = 0.836, *P* = 0.841; African-derived and European-derived (excluding the individual with unknown activity N) = 26.632, *P* < 0.0001.

Table 3: Antipsychotic response and *CYP2D6* genotype and allele function.

	Neuroleptics (%)		Clozapine (%)	
Activity	Non-refractory	Refractory	Responders	Non-responders
Allele Activity				
None	24 (18.5)	48 (19.8)	25 (20.2)	19 (20.7)
Reduced	19 (14.6)	33 (13.7)	13 (10.5)	17 (18.5)
Normal	82 (63.1)	152 (62.8)	82 (66.1)	51 (55.4)
High	5 (3.8)	9 (3.7)	4 (3.2)	5 (5.4)
Total	130	242	124	92
	P = 0.986		*P* = 0.264	
Genotype				
PM	2 (3.1)	5 (4.2)	3 (5.0)	2 (4.6)
IM	5 (7.7)	10 (8.5)	4 (6.5)	6 (13.6)
EM	54 (83.1)	98 (83.1)	50 (82.0)	35 (79.5)
UM	4 (6.1)	5 (4.2)	4 (6.5)	1 (2.3)
Total[1]	65	118	61	44
	P = 0.932		*P* = 0.506	

[1]Three schizophrenia individuals with no determination of duplication were excluded.

Table 4: Polymorphisms in *CYP2D6* gene and correspondent alleles.

Polymorphisms	Alleles[1]
-1584C>G	**2A, *2B, *2D, *14B, *35*
31G>A	**35*
100C>T	**4A, *4B, *4D, *4J, *4K, *10A, *10B, *14A*
124G>A	**12*
138insT	**15*
883G>C	**11*
1023C>T	**17, *40*
1039C>T	**2B, *4D, *10B*
1659G>A	**29*
1661G>C	**2A, *2B, *4A, *4D, *4J, *4K, *10A, *10B, *11, *14B, *17, *19, *20, *29, *30, *31, *35, *40, *41*
1707T>del	**6A/B, *6C*
1758G>A	**14A, *14B*
1846G>A	**4A, *4B, *4D, *4J, *4K*
1863RepeatIns	**30, *40*
1973-1974insG	**20*
2539-2542delAACT	**19*
2549A>del	**3*
2613-2615delAGA	**9*
2850C>T	**2A, *2B, *2D, *4K, *11, *12, *14A, *14B, *17, *19, *20, *29, *30, *31, *35, *40, *41*
2935A>C	**7*
3183G>A	**29*
3198C>G	**25*
4042G>A	**31*
4180G>C	**2A, *2B, *2D, *4A, *4B, *4D, *4K, *6C, *10A, *10B, *11, *12, *14A, *14B, *17, *19, *20, *29, *30, *31, *35, *40, *41*
Gene deletion	**5*
Gene duplication	**1XN, *2XN, *4XN, *10XN, *17XN, *35XN, *41XN*

[1]Alleles were classified on the basis of haplotype combinations. Wild-type allele (**1*) was set when any change was observed in all SNPs studied here.

Figure 1

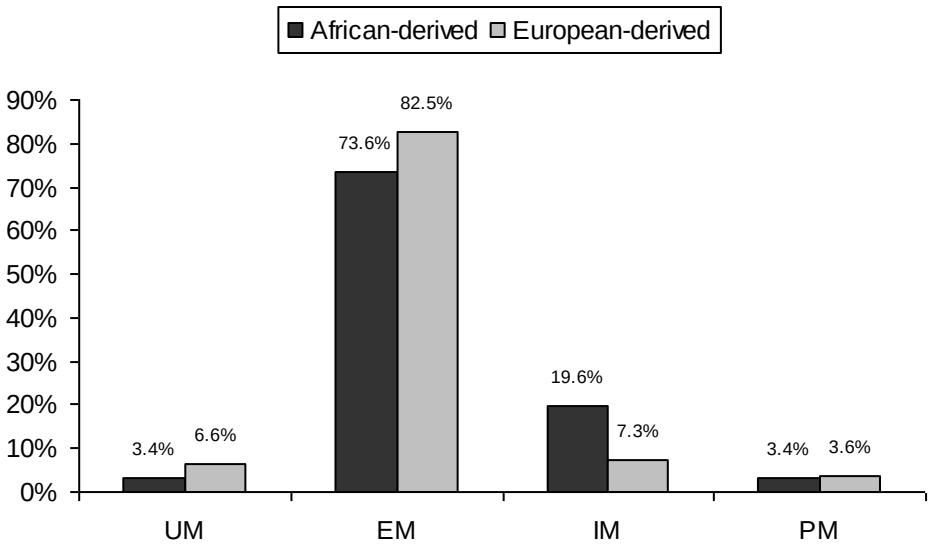
African-derived
European-derived
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
3.4%
6.6%
73.6%
82.5%
19.6%
7.3%
3.4%
3.6%
UM
EM
IM
PM

Figure 1: Frequency of *CYP2D6* phenotype classes deduced by genotype in African-derived and European-derived (controls and schizophrenics) samples of South-Brazilian population.

Legend for Figure 1: Phenotypes were predicted from genotypes. UM: Ultrarapid Metabolizer; EM: Extensive Metabolizer; IM: Intermediate Metabolizer; PM: Poor Metabolizer. Fisher's exact tests: African-derived and European-derived: P = 0.014; European-derived: schizophrenics and healthy controls: P = 0.413.

CAPÍTULO VI

POLYMORPHISMS AT G-PROTEIN GENE AND SEROTONIN TRANSPORTER GENE ARE ASSOCIATED WITH RESPONSE TO CLOZAPINE.

PHARMACOGENOMICS (em preparação)

Polymorphisms at G-Protein gene and Serotonin Transporter gene are associated with response to clozapine.

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Running title: Pharmacogenetics of clozapine response.

Abstract

Schizophrenia is a severe mental illness that can greatly limit the patient's ability to function normally and thus impairs the quality of life. Its treatment with clozapine is effective in resistant cases of schizophrenia and this medication is associated to a superior clinical profile than other antipsychotics. However, only 30-60% of individuals resistant to typical antipsychotics respond to clozapine. Since genetic factors are believed to play a significant role in the variation of response to antipsychotics, the aim of the present study was to verify the effect of serotonergic, dopaminergic, and related system genes on clozapine response, in Brazilian patients with schizophrenia, using multivariate approaches. One hundred twenty one schizophrenic patients in treatment with clozapine were genotyped for 16 polymorphisms in 12 genes by the polymerase chain reaction. At the univariate level of analysis, significant associations were observed with *SLC6A4 HTTLPR* and *GNB3 C825T* polymorphisms (χ^2 = 6.927, p = 0.031 and χ^2 = 7.708, p = 0.021, respectively). These results were confirmed by a multilocus test and by logistic regression analyses in which these findings were controlled for confounders. In conclusion, our data provide additional evidence of a role for genetic factors influencing inter-individual variation in drug response in persons with schizophrenia treated with clozapine.

Keywords: pharmacogenetics; clozapine; polymorphisms; *GNB3*; *SLC6A4*.

INTRODUCTION

Schizophrenia is a severe mental illness that can greatly limit the patient's ability to function normally and thus impairs the quality of life. Antipsychotic drugs are the best means available to symptomatically treat individuals suffering from schizophrenia. The atypical antipsychotic clozapine, available clinically in the 1970's, is effective in treatment-resistant cases of schizophrenia and significantly reduces negative symptoms (Kane *et al.,* 1988). Although it is associated to a superior clinical profile than other antipsychotics (Davis *et al.,* 2003), only 30-60% of individuals resistant to typical antipsychotics respond to clozapine (Arranz *et al.,* 2001; Basile *et al.,* 2002). This substantial unexplained interindividual variation in clinical response to antipsychotic drug treatment remains a critical problem in the management of schizophrenia (Malhotra *et al.,* 2004).

Clozapine's action is thought to result from interactions between dopaminergic and serotonergic neurotransmitter systems. Clozapine is a prototype 'broad-spectrum' antagonist. Its binding profile is quite different from other antipsychotics. It has relatively low affinity for D2 receptors in the striatum, while its in vitro affinity for the D4 receptors is approximately 10 times greater than that for D2 receptors and it has also been shown to bind to the D1, D3 and D5 receptors. Clozapine has been recognized to show significant activity at a broad range of receptors outside the dopaminergic system. It has high affinity for serotonin (5-HT) receptors including 5-HT2, 5-HT3, 5-HT6 and 5-HT7 subtypes. It also has affinity for $\alpha_{1\text{-}2}$- and $\beta_{1\text{-}3}$-adrenergic, muscarinic cholinergic $M_{1\text{-}5}$, and histaminergic H_1 and H_3 receptors (Wilffert *et al.,* 2005).

Since genetic factors are believed to play a major role in the variation of response to antipsychotics (Perlis *et al*., 2005), possible pharmacodynamic effects may result from polymorphisms in genes associated with dopamine and serotonin synthesis, availability, transport, and receptor subunits targeted by clozapine, because they can influence therapeutic results changing the interaction of these drugs with neurotransmitter receptors. This is the case of serotonin transporter (*SLC6A4*), monoamine oxidase A (*MAO-A*), dopamine transporter (*SLC6A3*), and catechol-O-methyltransferase (*COMT*). The G-protein gene might also have important influence on the function of these systems, since catecholamine receptors are G-protein-coupled (GPCRs) and antipsychotics exert their therapeutic effects by competitive antagonism of postsynaptic GPCRs, reducing their activity (Teitler *et al.,*, 2002). Since antipsychotic drugs operate by blocking the dopamine transmission at the dopamine D2-like receptors, and the brain-derived neurotrophic factor gene (*BDNF*) controls the expression of one of these D2-like receptors (the dopamine D3 receptor) (Guillin *et al.*, 2007), the hypothesis of a link between BDNF and the dopamine neurotransmission pathway could be raised in schizophrenia and its treatment, making the *BDNF* gene a good candidate for pharmacogenetic studies.

Previous efforts point out that single polymorphisms may be unable to explain variability to treatment, and a combination of polymorphisms in multiple genes may provide a better predictive value of response. Thus, a multiple candidate gene approach needs to be adopted in the pharmacogenomics of antipsychotics. Therefore, in the present study we used multivariate approaches to consider the effect of serotonergic, dopaminergic, and related system genes on

the response to clozapine, in a Brazilian sample of patients of European ancestry with DSM-IV diagnosis of schizophrenia. Many of these polymorphisms have a functional significance and have been investigated in association studies of schizophrenia susceptibility, clozapine response and/or adverse drug reactions incidence (Arranz and de Leon, 2007, Foster *et al.,* 2007).

MATERIAL AND METHODS

Sample characteristics

The sample consisted of 121 schizophrenic patients of European ancestry in treatment with clozapine, attended at the HCPA (Hospital de Clínicas de Porto Alegre) Schizophrenia Program. All patients have been comprehensively assessed by Board-Certified Psychiatrists with at least 4 interviews in the presence of a relative, and must meet clinical criteria for schizophrenia according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) (American Psychiatric Association, 1994), with additional assessment by standardized interviews using the Operational Criteria Checklist for Psychotic Disorders - OPCRIT (McGuffin *et al.*, 1991). All patients have signed a written informed consent to participate in the study, which was approved by the Ethics Committee of the Federal University of Rio Grande do Sul (Porto Alegre, Brazil).

All patients using clozapine included in our study have met the criteria for treatment refractoriness or intolerance to typical antipsychotic therapy, defined as lack of satisfactory clinical response to at least two or more standard antipsychotics, administered at doses equivalent to at least 1000 mg chlorpromazine, for at least six weeks and a poor level of functioning over the past

5 years (Kane *et al.,* 1988). Clozapine response was evaluated by the treating physician using the Brief Psychiatric Rating Scale (BPRS) (Overall and Gorham, 1962), and a 20% reduction of the scores was considered as the threshold for appropriate response. This criterion for clozapine response was approved by the Clinical Protocol and Therapeutic Proceedings of the Brazilian Health Ministry (2002). Sixty-six patients were classified as responders and 55 as non-responders to clozapine treatment. Any additional information about the subjects was reviewed from the patient records. Almost all non-responders received co-medications, because the protocol of treatment (Brazilian Health Ministry, 2002) suggests that individuals showing unsatisfactory response to clozapine should try combinations of other medications with clozapine. These co-medications prescribed for non-responders included typical antipsychotics, anticholinergics, lithium and/or antidepressants.

The patients have been treated with a stable dose of clozapine for at least 3 months, since 2001. The characteristics of the individuals investigated are shown in Table 1. The mean daily dose of clozapine was 540.91mg/day, but it varied from 100 to 900mg/day.

Laboratory procedures

Genomic DNA was isolated from peripheral blood leukocytes by a salting-out procedure (Lahiri and Nurnberguer, 1991). Sixteen polymorphisms in 12 genes were genotyped by the polymerase chain reaction with primers and protocols previously described (Table 2).

Statistical analysis

Allele frequencies were estimated by gene counting. The agreement of genotype frequencies with Hardy-Weinberg equilibrium was assessed by χ^2 test. Significance of allelic and genotype frequencies differences between responders and non-responders was assessed by Pearson's χ^2 test, or if any cell count was less then 5 by Fisher's exact test. Residual analysis and Odds Ratio (OR) calculations were performed using PEPI Software, Version 4.0 (Abramson and Gahlinger, 2001).

The data were adjusted to multiple comparisons using the set association method proposed by Hoh *et al.* (2001) According to this method, the smallest of the empirical significance levels identifies the best and most parsimonious model predicting response status. Permutation testing accessed the overall significance of the best model adjusting for multiple testing. This set association approach was performed using the Sumstat software, and as established by the software, only biallelic polymorphisms were added to these analyses. (http://linkage.rockefeller.edu/ott/sumstat.html).

Logistic regression was performed to estimate the effect of different polymorphisms on response status after adjusting for possible confounders. The regression model was adjusted for two independent risk variables: illness duration (≥ 16 years) and illness duration before starting clozapine treatment. The covariates were dichotomized before analysis because the assumption of a linear relation between refractoriness and the log odds of these variables was not met. Co-medication was not included in the model because it is highly correlated with non-response to clozapine (Pearson Correlation = 0.901, $P < 0.0001$). Statistical

analyses were performed using the SPSS software package version 10.0. The significance level was set at $P < 0.05$. The PEPI Software, Version 4.0 POWR program was used to calculate sample statistical power.

RESULTS

Table 1 shows demographic and clinical characteristics of the patient's sample. Patients were aged between 16 and 64 years (34.02 $\pm$ 8.79 years) and 83.5% were men. Among patients with good response, only one individual (1.5%) received co-medication (paroxetine), and all others were in monotherapy with clozapine.

The genotype frequencies observed for all studied polymorphisms did not show statistically significant differences compared to those expected under Hardy-Weinberg equilibrium. Among the sixteen polymorphisms, we found good response to clozapine significantly associated with *SLC6A4 HTTLPR* and *GNB3 C825T* polymorphisms.

There were significant differences observed between responders and non-responders to clozapine for the 5' regulatory region of the human *SLC6A4 HTTLPR* polymorphism (see Table 3). Patients who failed to respond had a *S*-allele frequency of 0.54, as compared to 0.37 in those who did respond (χ^2 = 6.600, p = 0.010, OR = 1.97 [95%CI 1.13-3.43]). Examining genotypes, individuals who did not respond to clozapine were more likely to be *S/S* homozygous or *S/L* heterozygous than those who did respond (χ^2 = 6.927, p = 0.031 for genotype frequency relative to clozapine response and χ^2 = 5.869, p = 0.015 if the *S*-allele was considered dominant, OR = 2.79 [95%CI 1.12-7.30]).

An association between the *GNB3 825C>T* polymorphism and clinical response to clozapine was observed (Table 3). Homozygosis for the *T825* allele was more frequent among non-responders (27.3%) than in responders (10.6%) and oppositely homozygosis for *C825* allele was observed in higher frequency among responders (47.0% versus 27.3% in non-responders) (χ^2 = 7.708, p = 0.021). When the *T825* allele was considered dominant, we also observed significant differences between responders and non-responders (χ^2 = 4.939, p = 0,026, OR = 2.36 [95%CI 1.03-5.50]). Analyses of allele frequencies also showed a significant association between allele *T825* and unsatisfactory response to clozapine (χ^2 = 8.259, p = 0.004, OR = 2.14 [95%CI 1.23-3.74]).

Table 4 presents the multilocus analyses based on the method of Hoh *et al.* (2001). This set association model confirmed the results cited above: G-protein Beta-3 subunit (*825C>T*) and the serotonin transporter (*HTTLPR*) gene polymorphisms were significantly associated with clozapine response. The minimum value of *P* (*P* = 0.0109) was reached in the model that included only these polymorphisms. When more SNPs were added to the model, the *P*-values increased. The final experiment wise *P*-value for the model was 0.028. After controlling for confounders in logistic regression analyses, the same pattern was observed: *HTTLPR* and *825C>T* polymorphisms were associated to clozapine response (Table 5).

DISCUSSION

In this study, we examined the possible influence of several neurotransmitter-related polymorphisms and the efficacy of treatment with

clozapine. Our findings point to associations between *GNB3 825C>T* and *SLC6A4 HTTLPR* polymorphism and clozapine response.

The transcriptional activity of the 5HT transporter gene is modulated by the polymorphic repetitive element (*SLC6A4* gene-linked polymorphic region, *HTTLPR*) located upstream of the transcription start site (Lesch and Gutknecht, 2005). This polymorphism consists of a 44-base pair insertion/deletion resulting in a short (*S*) and a long (*L*) variant, the first resulting in twofold decreased expression and transport activity *in vitro* (Heils *et al.,* 1996) and this genetic difference is manifested from the beginning of development (Murphy *et al.,* 2001). A study of Arranz *et al.* (2000b) with schizophrenic patients demonstrated an importance of this polymorphism to predict clozapine response when combined with other polymorphisms. However, the same authors only found a trend to significance in a previous study (Arranz *et al.,* 2000a). Other studies could not replicate these findings (Tsai *et al.,* 2000; Schumacher *et al.,* 2000; Kaiser *et al.,* 2001). In our study, we found an association between the short allele and poor response to clozapine. Since the *HTTLPR* influences serotonin (5HT) concentrations at all synapses, this allelic variation in 5HT function likely affects the response to clozapine modulating the availability of extracellular serotonin.

Most monoaminergic receptors (e.g., DRD1-5, 5-HT2A, 5-HT2C) are intracellularly coupled to G-proteins (i.e. G-protein-coupled receptors, GPCRs). G-proteins are composed of α, β and γ subunits and each subunit is coded by many different gene products. The *GNB3* gene codes the β-subunit 3 of G-proteins. The *825C>T* polymorphism located in exon 10 of the *GNB3* gene has been shown to be associated with alternative splicing of exon 9. The T-allele results in an in-frame

deletion of 41 amino acids and this shorter protein leads to an increased intracellular signal transduction by stimulation of several GPCR (Siffert *et al.,* 1998). Antipsychotics exert their therapeutic effects by competitive antagonism of postsynaptic receptors (thus reducing subsequent GPCR activity) (Teitler *et al.,* 2002). Since both dopamine and serotonin receptor subtypes activate intracellular pathways through GPCR, the effect of the variability in the *GNB3* gene might affect medication response and be important in the efficacy of clozapine drug treatment. Müller *et al*. (2005) hypothesized that this polymorphism could be associated with response to antipsychotics in a population of chronic schizophrenic patients and found the *C/C* genotype significantly associated with clinical improvement to clozapine, suggesting that genetic susceptibility for decreased signal transduction may enhance antipsychotic efficacy. Anttila *et al.* (2007) found a trend-like positive association among male patients with good response to typical neuroleptics compared with non-responders. The findings of our study also showed an association between the *825C>T* polymorphism of the *GNB3* gene and clozapine efficacy. We found a significant effect of the *T*-allele with an increasing chance of unfavorable clozapine response in about two times, and a positive effect on response of *C/C* genotype. The association of *T/T* and *T/C* genotypes (or a higher signal transduction) with unfavorable response suggests that not only genetic susceptibility for decreased signal transduction may enhance antipsychotic efficacy, but also that increased signal transduction may reduce that efficacy.

The multilocus analyses showed a combination of those two polymorphisms with the strongest association with clozapine response, and the logistic regression

analyses confirmed these results. Possession of both genotypes, *T825/T825* or *T825/C825* in the *GNB3* gene and *S/S or S/L* in the serotonin transporter gene, would be predictive of unsatisfactory response to clozapine.

There were some limitations to our study. The sample size is not large, thus these data require additional confirmation in a larger sample. Despite that, our investigation has statistical power to detect an influence of differential clozapine response as a function of *GNB3* (62%) and *SLC6A4* (66%) genotypes, emphasizing the relevance of these SNPs. Another important limitation is the impossibility to study clozapine plasma levels in our patients, a possible factor contributing to the final phenotype.

In conclusion, the data presented here provide additional evidence of a role for genetic factors influencing inter-individual variation in drug response in persons with schizophrenia treated with clozapine. The adoption of a pharmacogenomics approach represents a unique opportunity for the prediction of response to antipsychotic drugs by investigating genes implicated with drug response. In this study we have moved beyond single-gene associations and considered a good number of polymorphisms in *a priori* biologic candidate genes influencing drug response. In the future, technological and methodological advances will likely provide further candidate genes and refine association analyses of existing candidates, enabling the prescription of medications that are personalized to the individual's genetic make-up.

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Table 1: Characteristics of the 121 investigated patients with schizophrenia under clozapine.

Characteristics	Responders	Non-responders	Total
Gender	66	55	121
Male	56 (84.8%)	45 (81.8%)	101 (83.5%)
Female	10 (15.2%)	10 (18.2%)	20 (16.5%)
Age[1]	33.92 ± 7.92	34.13 ± 9.84	34.02 ± 8.79
Age at illness onset[2]	19.76 ± 6.02	17.71 ± 3.31	18.83 ± 5.06
Age at clozapine starting[3]	30.24 ± 8.44	30.12 ± 10.15	30.18 ± 9.20
Illness duration	14.26 ± 7.59	16.37 ± 9.50	15.21 ± 8.53
Clozapine dose[5]	497.12 ± 177.95	593.45 ± 184.90	540.91 ± 186.7
Co-medication[6]	1 (1.5%)	50 (90.9%)	51 (42.1%)

[1,2,3,4] in years, mean ± SE (standard error); [5] mg/day; mean ± SE (standard error); [6] presence of co-medication

[1]Student's t-test: t = 0.127, *P* = 0.899; [2]Mann-Whitney Test: z = -1.715; 86, *P* = 0.09; [3]Mann-Whitney Test: z = -0.665, *P* = 0.506; [4]Mann-Whitney Test: z = -1.387, *P* = 0.165; [5]Mann-Whitney Test: -2.853, *P* = 0.004.

Table 2: Polymorphisms investigated in this study.

Polymorphisms[a]	Chromosome	Function	Enzyme for RFLP	Reference
HTR1B 861G>C	6q13	Synonymous	*Hinc*II	Hawi *et al.,* 2002
HTR2A 102T>C	13q14-q21	Synonymous	*Msp*I	Quist *et al.,* 1996
HTR2A -1438A>G	13q14-q21	5'-promoter region	*Msp*I	Arranz *et al.,* 1998
HTR2A His452Tyr	13q14-q21	Nonsynonymous	*Bsm*I	Quist *et al.,* 1996
HTR2C Cys23Ser	Xq24	Nonsynonymous	*Hinf*I	Lappalaien *et al.,* 1995
SLC6A4 HTTLPR	17q11.1-q12	5'-promoter region	-	Heils *et al.,* 1996
SLC6A4 STin2 VNTR	17q11.1-q12	-	-	Ito *et al.,* 2002
MAOA -uVNTR	Xp11.23-11.4	-	-	Sabol *et al.,* 1998
DRD2 -141C ins/del	11q23	5'-promoter region	*Mva*I	Ohara *et al.,* 1998
DRD3 Ser9Gly	3q13.3	Nonsynonymous	*Msc*I	Scharfetter *et al.,* 1999
DRD4 VNTR exon III	11p15.5	-	-	Kohn *et al.,* 1997
SLC6A3 -839C>T	5p15.3	5'-promoter region	*Msp*I	Rubie *et al.,* 2001
COMT Val158Met	22q11.21	Nonsynonymous	*Nla*III	Kunugi *et al.,* 1997

GNB3	*825C>T*	12p13	Splice variant	*Bsa*JI	Rosskopf *et al.,* 2000
GNB3	*814G>A*	12p13	Nonsynonymous	*Pst*I	Rosskopf *et al.,* 2000
BDNF	*Val66Met*	11p13	Nonsynonymous	*Eco*721	Neves-Pereira *et al.,* 2002

[a] *HTR1B*, *HTR2A*, and *HTR2C*: 5-hydroxytryptamine (serotonin) receptor *1B*, *2A* and *2C* genes; *SLC6A4*: solute carrier family 6 (serotonin transporter), member 4; *MAOA*: monoamine oxidase A gene; *DRD2*, *DRD3*, and *DRD4*: dopamin receptor D2, D3, and D4 genes; *SLC6A3*: solute carrier family 6 (dopamine transporter), member 3; *COMT*: catechol-O-metyltransferase gene; *GNB3*: G-protein Beta-3 subunit gene; *BDNF*: brain-derived neurotrophic factor gene.

Table 3: Genotype and allele frequencies of *C825T* and *HTTLPR* polymorphisms in responders and non-responders to clozapine.

Genotype or allele		Responders	Non-responders	OR	*P*
SLC6A4 HTTLPR					
Genotype	*L/L*	26 (39.4%)	10 (18.9%)	-	-
	S/L	31 (47.0%)	29 (54.7%)	2.43	0.089
	S/S	9 (13.6%)	14 (26.4%)	4.04	0.023
			P = 0.031		
Allele	*L*	0.63	0.46	-	-
	S	0.37	0.54	1.97	0.010
GNB3 825C>T					
Genotype	*C/C*	31 (47.0%)	15 (27.3%)	-	-
	T/C	28 (42.4%)	25 (45.4%)	1.84	0.279
	T/T	7 (10.6%)	15 (27.3%)	4.43	0.011
			P = 0.021		
Allele	*C*	0.68	0.50	-	-
	T	0.32	0.50	2.14	0.004

Table 4: Set association results for clozapine response in 121 patients with schizophrenia under clozapine treatment (corrected for multiple comparisons).

Polymorphism[a]	Chi square statistic	Sum	*P*-value
GNB3 825C>T	0.3636	0.3636	0.0414
SLC6A4 HTTLPR[b]	0.3330	0.6967	0.0109
BDNF Val66Met	0.1697	0.8664	0.0217
SLC6A3 –839C>T	0.1431	1.0095	0.0297
HTR2A 102T>C	0.0788	1.0883	0.0572
HTR1B 861G>C	0.0485	1.1368	0.0953
GNB3 814G>A	0.0485	1.1852	0.1273
DRD3 Ser9Gly	0.0481	1.2334	0.1466
COMT Val158Met	0.0457	1.2791	0.1548
DRD2 –141c ins/del	0.0286	1.3077	0.1673
HTR2A –1438A>G	0.0209	1.3286	0.1746
HTR2A His452Tyr	0.0152	1.3438	0.1736

[a]Only bialelic polymorphisms; [b]Final experiment *P*-value for the model with the lowest *P*-value = 0.028

Table 5: Logistic regression analyses predicting clozapine response.

Variables in the model	β	SE	Wald	*df*	*P* value	OR (95% CI)
Duration of illness before clozapine starting (≥14years)	0.004	0.008	0.286	1	0.593	1.00 (0.99-1.02)
Illness duration (≥16y)	0.327	0.408	0.642	1	0.423	1.39 (0.62-3.09)
GNB3 C825T[a]	0.879	0.407	4.657	1	0.031	2.41 (1.08-5.35)
SLC6A4 HTTLPR[b]	1.000	0.456	4.801	1	0.028	2.71 (1.11-6.65)

β=estimated coefficient; SE=standard error; *df*=degree of freedom; OR=odds ratio; CI=confidence interval. χ^2 model = 11.999, p = 0.0174; Percent correct classification 67.23%; [a]*T/T* and *T/C* genotypes; [b]*S/S* and *S/L* genotypes.

CAPÍTULO VII

CLOZAPINE-INDUCED GENERALIZED SEIZURES IN SCHIZOPHRENICS ARE INFLUENCED BY GENETIC POLYMORPHISMS: A MULTI-LOCUS APPROACH.

JOURNAL OF NEURAL TRANSMISSION (submetido)

Clozapine-induced generalized seizures in schizophrenics are influenced by genetic polymorphisms: a multi-locus approach.

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Running title: Pharmacogenetics of clozapine-induced generalized seizures.

Summary

Genetic variability may contribute to the occurrence of seizures under clozapine treatment. We have investigated associations between sixteen variants and New-Onset-Generalized Seizures (NOGS) after Clozapine in schizophrenic patients, to screen for a pharmacogenetic component of the pro-convulsive effect of clozapine. Positive associations were detected between NOGS and three polymorphisms: *Val158Met* in catechol-O-metyltransferase gene (*COMT)*, *861G>C* in serotonin receptor subtype 1B (*HTR1B*), and *825C>T* in G-protein Beta-3 subunit gene (*GNB3*). The *COMT Met/Met* genotype has shown a protective effect for NOGS (P = 0.008). *C/C* individuals at the *HTR1B 861G>C* were more frequently observed in the NOGS group (P = 0.014). Differences in genotype and allele frequencies for the *GNB3 825C>T* polymorphism varied between patients with or without NOGS (P = 0.033 and P = 0.007 for genotype distribution and allele frequencies, respectively). Multi-locus analyses have assessed these associations, but after controlling for covariates by logistic regression, significant associations were seen only with *GNB3 825C>T* and *COMT Val158Met* polymorphisms, suggesting an influence of these polymorphisms on specific neurological side effects (NOGS) under clozapine treatment.

Keywords: pharmacogenetics; clozapine; generalized seizures; COMT; GNB3.

Introduction

Schizophrenia is a complex psychiatric disorder characterized by clusters of specific clinical symptoms (positive, negative, and disorganized, in most studies), with extensive variation between individuals (Sawa and Snyder, 2002). Among the repertoire of drugs used to treat the disorder, several meta-analyses have evidenced that the antipsychotic clozapine displays greater effectiveness compared to typical neuroleptic drugs, with effect size of 0.48 relative to typical antipsychotics (Davis et al., 2003). This medication has a unique receptor-binding profile, which includes a number of target affinities, ranging from low to high, with dopamine $D_{1\text{-}5}$; serotonin 5-$HT_{1\text{-}3}$, 5-HT_6, and 5-$HT_{7;}$ $\alpha_{1\text{-}2}$, and $\beta_{1\text{-}3}$-adrenergic, muscarinic cholinergic $M_{1\text{-}5}$; and histaminergic H_1 and H_3 receptors (Wilffert et al., 2005). It displays preferential affinities with serotonin receptors, and consequently, lack of extra-pyramidal side effects compared to classical neuroleptics. Therefore, the dopaminergic and serotonergic receptor systems have been suggested to play major roles in schizophrenia and are the main targets of antipsychotic treatment (Arranz and Kerwin, 2000).

Clozapine is associated to a superior clinical profile, especially in measurements of positive and negative symptoms, and quality of life, besides a significantly lower incidence and intensity of treatment-emergent extra-pyramidal symptoms and tardive dyskinesia compared to conventional agents (Kawanishi et al., 2000). At the same time, it displays greater frequency of other undesirable effects, like weight gain, hypotension, somnolence, constipation and several types of convulsive symptoms, including new-onset-generalized seizures (NOGS). Despite the relatively low frequency, it represents a great burden in schizophrenic

patients' care, since there are no drugs with superior therapeutic effect than clozapine. Adverse drug reactions (ADRs) are frequent, and about 5-15% the patients experience unexpected complications after using the medication (Amacher, 2006). ADRs may have a substantial impact on medication adherence, and many patients may not receive optimal therapy due to dose-dependent side effects. Many of these reactions are thought to have a genetic predisposition (Pirmohamed and Park, 2003).

New-onset-generalized seizures (NOGS) consist of an undesirable dose-related side effect of clozapine treatment, which lowers the threshold for generalized seizures. Seizures can occur at any dosage of clozapine, and higher doses increase risks. During low-dosage treatment (<300 mg/day), the risk is similar to that of other antipsychotics, but it significantly increases at higher doses (>600mg/day) (Hedges et al., 2003). The exact mechanism of threshold reduction is still unknown, but several biochemical mechanisms involving different neurotransmitters have been identified in NOGS under clozapine. There is strong evidence of serotonin neurotransmission defects in this feature, which is of interest because clozapine has different effects on serotonergic neurotransmission (Meltzer, 1989).

The frequency of seizures in schizophrenic patients has been estimated between 1 and 20% (Devinsky and Pacia, 1994; Welch et al., 1994). Investigations looking for predictors of clozapine-induced seizures have shown that age, gender, dosage, plasma levels, and electroencephalogram (EEG) changes have limited value as good predictors of seizures (Devinsky et al., 1991; Wong and Delva, 2007). Once there are no good predictors established for clozapine-induced

seizures and they represent significant treatment complications, we have raised the hypothesis that genetic variation in neurotransmitters or related genes may be used as an indicator of susceptibility to seizures in patients with schizophrenia under clozapine treatment.

Genes coding for neurotransmitter receptors targeted by clozapine are good candidates for pharmacogenetic studies, because both therapeutic and side effects result from the interaction of these drugs and neurotransmitter receptors. The research on neurotransmitter-related genes affecting the availability and function of dopaminergic and serotonergic components may also provide useful information. This is the case of serotonin transporter (*SLC6A4*), monoamine oxidase A (*MAO-A*), dopamine transporter (*SLC6A3*), and catechol-O-methyltransferase (*COMT*). The G-protein gene might also have important influence on the function of these systems, since catecholamine receptors are G-protein-coupled (GPCRs) and antipsychotics exert their therapeutic effects by competitive antagonism of postsynaptic GPCRs, reducing their activity (Teitler et al., 2002). Since antipsychotic drugs operate by blocking the dopamine transmission at the dopamine D2-like receptors, and the brain-derived neurotrophic factor gene (*BDNF*) controls the expression of one of these D2-like receptors (the dopamine D3 receptor) (Guillin et al., 2007), the hypothesis of a link between BDNF and the dopamine neurotransmission pathway could be raised in schizophrenia and its treatment, making the *BDNF* gene a good candidate for pharmacogenetic studies.

The present study aims to identify possible pharmacogenetic components of the pro-convulsive effect of clozapine. For this purpose, we have investigated

several polymorphisms of serotonergic, dopaminergic, and related system genes, focusing on genes already identified in studies on schizophrenia genetic risks. Many of these polymorphisms have a functional significance and have been investigated in some association studies of clozapine response and/or ADRs (Arranz et al., 2000; Tsai et al., 2000; Hong et al., 2003; Matsumoto et al., 2004; Muller et al., 2005; Guillin et al., 2007).

Material and Methods

Subjects

One hundred and twenty one patients with schizophrenia were recruited for a pharmacogenetic study on clozapine side effects, using a naturalistic approach. All patients included in the study were of European ancestry to avoid false positive results due to population stratification (Zembrzuski et al., 2006).

The patients have been treated with a stable dose of clozapine for at least 3 months, since 2001. All patients have been comprehensively assessed by Board-Certified Psychiatrists with at least 4 interviews in the presence of a relative, and must meet clinical criteria for schizophrenia according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) (American Psychiatric Association, 1994), with additional assessment by standardized interviews using the Operational Criteria Checklist for Psychotic Disorders - OPCRIT (McGuffin et al., 1991). Each patient has signed a written informed consent to participate in the study, which was approved by the Ethics Committee of the Federal University of Rio Grande do Sul (Porto Alegre, Brazil).

All patients using clozapine have fulfilled the criteria for treatment refractoriness or intolerance to typical antipsychotic therapy, defined as lack of satisfactory clinical response to at least two or more standard antipsychotics, administered at doses equivalent to at least 1,000-mg chlorpromazine, for a minimal period of six weeks, and poor functioning level over the past 5 years (Kane et al., 1988).

The occurrence of clozapine-induced generalized (new-onset) seizures was assessed by clinical interviews with patients and relatives, reporting clear descriptions of tonic-clonic generalized seizures (from 2001 to 2005) collected by clinical psychiatrists that were not aware of genotypes. For all subjects, new-onset generalized seizures during clozapine treatment had not required drug discontinuation.

The characteristics of the individuals investigated are shown in Table 1. Most of them were male (83%), 34 years old, in average. The daily clozapine dose was 540.91 mg/day, in average, varying from 100 to 900 mg/day. Co-medication was recorded by clinical interviews and the review of medical records. A generalized convulsive episode had been observed by relatives in 27 out of 121 subjects (22.3%), after prolonged exposure to clozapine. Considering NOGS is completely known as an adverse reaction to clozapine, the seizure diagnosis was not assessed using EEG. According to Wong and Delva (2007) it is limited practical value to investigate plasma levels and/or to perform an EEG to diagnose a seizure episode due to clozapine treatment. Therefore, according to standard procedures, those patients suffering convulsions remained using clozapine, also receiving anti-convulsivant medication (sodium valproate) after the episode.

Laboratorial procedures

High-molecular-weight genomic DNA was isolated from venous blood using standard procedures (Lahiri and Nurnberger, 1991). Among 16 SNPs (Single Nucleotide Polymorphisms) investigated, 8 were serotonergic genes (*5HT*), 5 from the dopaminergic system (*DA*), 2 from the G-protein Beta-3 subunit gene (*GNB3*) and one variant of the brain-derived neurotrophic factor gene (*BDNF*). The genotyping methods were those previously described (Table 2).

Statistical analysis

Allele frequencies were estimated by gene counting. Deviations from Hardy-Weinberg equilibrium were assessed by χ^2 test. Significance of allele and genotype frequency differences between presence and absence of a convulsion episode was assessed by the χ^2 test, or Fisher's exact test, if any cell count was lower than 5.

Data were adjusted for multiple comparisons using the set-association method proposed by Hoh et al (Hoh et al., 2001). According to this method, the smallest of the empirical significance levels identifies the best and most parsimonious model predicting seizure occurrence. Permutation testing accessed the overall significance of the best model adjusting for multiple testing. This set-association approach was performed using Sumstat software (http://linkage.rockefeller.edu/ott/sumstat.html). As established by the software, only biallelic polymorphisms were added to these analyses.

Logistic regression was performed to estimate the effect of different polymorphisms on the occurrence of seizures, adjusting for the effect of clozapine dose and presence of co-medication (sodium valproate was not considered a co-medication for statistic analyses purposes, except when another medication was also present). Clozapine doses were dichotomized (cut point: 650 mg/day) before analysis because the assumption of a linear relation between refractoriness and the log odds of dose was not met. Statistical analysis was performed by SPSS package, version 10.0. Residual analyses were made using PEPI Software, Version 4.0 (Abramson and Gahlinger, 2001). A *P*-value < 0.05 was considered significant. The PEPI Software, Version 4.0 POWR program was used to calculate sample statistical power.

Results

Among 16 polymorphisms investigated (Table 2), those associated to NOGS are shown in Table 3. Genotype distribution of *Val158Met* polymorphism in catechol-O-metyltransferase (*COMT*) gene was significantly different between patients with and without generalized seizures (*P* = 0.010). The low activity *COMT* genotype (*COMT*L/*L*) consisting of *Met/Met* homozygous genotype has shown convulsion protective effect when compared to high activity *COMT* genotypes (*High COMT Val/Val* and *Val/Met* genotypes; *P* = 0.008, OR = 10.24). At the serotonin receptor *HTR1B 861G>C* polymorphism, *C* allele homozygous individuals were more frequent in patients suffering convulsion compared to those who did not (18% versus 3% respectively). Additional grouping of *G/G* and *G/C* genotypes has increased the association (*P* = 0.014, OR = 6.89). Genotype

distribution and allele frequencies for *GNB3 825C>T* polymorphism have significantly varied between NOGS positive and negative subjects (*P* = 0.033 for genotype distribution, χ^2 = 7.279, *P* = 0.007, OR = 2.17 for allele frequencies). Subjects with *T* allele had increased risk for NOGS (χ^2 = 5.607, *P* = 0.018, OR = 3.40), when a dominant model was considered.

Additional multi-locus analyses based on Hoh et al.'s method (2001) are shown on table 4. The set-association model confirmed that the three previously identified polymorphisms, *HTR1B 861G>C*, *GNB3 825C>T* and *COMT Val158Met* were significantly associated to NOGS under clozapine. *P* minimum value (*P* = 0.007) was only reached in the model that included these three polymorphisms. Additional SNPs to the model were associated to higher *P*-values. The experiment final *P*-value for the model was 0.016. However, after controlling for dose and co-medication in a logistic regression analysis, only *GNB3 825C>T* and *COMT Val158Met 825C>T* polymorphisms were confirmed to be associated to NOGS (Table 5).

Discussion

The rate of seizure in our sample (22%) is high, when compared to that shown in the literature (Hedges et al., 2003). The rate of people having seizure under clozapine treatment may be explained by the fact that this is a four-year study (a cumulative risk of tonic-clonic seizures is estimated after four years of treatment) (Devinsky and Pacia, 1994), and our sample included mild- to-severe-affected patients who took 540-mg/day clozapine in average, a high dose when seizure episodes are more likely to occur at doses higher than 450 mg/day.

In the current study, we have shown an association between clozapine-induced generalized seizures and *GNB3* and *COMT* gene polymorphisms. These findings may be better understood under the current dopamine and glutamate hypothesis of schizophrenia.

Monoaminergic receptors as DRD1-5, 5-HT2A, 5-HT2C are intra-cellular G-protein-coupled receptors (GPCRs), and changes in the structure of G-proteins (GPs) may affect intracellular signal transduction, hence affecting cell excitability and seizure threshold. GPs are composed of α, β and γ subunits, and each subunit is coded by different genes. The studied *825C>T* polymorphism located at *GNB3* gene exon 10 has been shown to be associated to alternative splicing of exon 9. The T-allele results in an in-frame deletion of 41 amino acids and this shorter protein leads to an increased intracellular signal transduction by stimulation of several GPCRs (Siffert et al., 1998). Antipsychotics exert their therapeutic effects by competitive antagonism of postsynaptic receptors, reducing subsequent GPCR activity (Teitler et al., 2002). Since both dopamine and serotonin receptor subtypes activate intracellular pathways through GPCR, *825C>T* polymorphism might increase central nervous system toxicity of clozapine. Defects in ion channels, which are directly involved in regulation of neuronal excitability, increase susceptibility to seizure (Kitami et al., 2004). G protein-activated inwardly rectifying K^+ (GIRK) channels play an important role in the inhibitory regulation of neural excitability in most brain regions through activation of various GPCRs (Blednov et al., 2003). It has been shown that GIRK channels are involved in seizure susceptibility (Signorini et al., 1997), and dose-related inhibition of this function by antipsychotics can increase seizure susceptibility. Since Clozapine strongly

inhibits GIRK channels (Kobayashi et al., 1998), this effect can explain the relationship between *GNB3* polymorphisms and seizures in patients exposed to this drug. Individuals homozygous for the *C*-allele of the *825C>T* polymorphism are supposed to have a lower signal transduction, and a potentially reduced inhibition of GIRK channels under clozapine exposure; this could reduce the risk of clozapine-induced seizures. Additional effects over seizures can be assigned to the metabolism of gamma-aminobutyric acid (GABA), which is the major inhibitory neurotransmitter. Clozapine-induced seizures had already been correlated with reduced inhibitory effect on the $GABA_A$ receptor-Cl^- channel complex (Yokota et al., 2002). Although this receptor is not a GPCR, polymorphisms in the G-protein gene may influence its action. Alternatively, G-proteins translated from *C/C* genotypes may increase the activation of $GABA_B$ receptors (GPCRs), facilitating K^+ channels opening (reducing the post-synaptic excitability), also reducing the risk of seizure.

Dopamine is catabolized by COMT, which converts dopamine into 3-methoxytyramine (Matsumoto et al., 2004). The *Val158Met* polymorphism is caused by a G to A transition at codon 158 of membrane-bound COMT. This amino acid change affects the stability of the enzyme, and it was found that homozygosity for the methionine-allele leads to a three-to-four fold reduction in enzyme activity compared with homozygosity for the valine-allele (Matsumoto et al., 2004). We have found an association between the high activity genotypes (*Val/Val* and *Val/Met*), and an increased susceptibility to NOGS under clozapine. This finding could be related to a subsequent reduction in dopamine concentrations in high activity genotypes. Vriend et al. (1993) have observed

substantial decreases in concentrations of dopamine in seizure-prone mice, suggesting a reduction of dopamine release in this state. If this information is confirmed, the high activity genotype, and perhaps the dopamine metabolite 3-methoxytyramine, could be involved in the increase of seizure frequency. Czlonkowska et al. (2001) have found a significantly decrease in the concentrations of 3-methoxytyramine after administration of midazolam (a benzodiazepine anticonvulsant). Therefore, dopamine reduction, and 3-methoxytyramine increase might affect the occurrence of generalized seizures.

When confounders such as clozapine dose and co-medication were considered, the *HTR1B* polymorphism was no longer significant. However, due to the importance of this receptor, it should be further discussed. This receptor seems to mediate the deleterious effects of excessive serotonergic (5HT) levels, by presynaptic inhibition of glutamate release reducing excitatory neurotransmission (Salichon et al., 2001). If the *861C* allele was associated with decreased 5-HT1B binding (Huang et al., 1999), the balance between essential neurotransmitters during the development of the nervous system could be contributing to a convulsive episode.

High clozapine doses and co-medication were not significantly associated to NOGS when the genotypes were considered. Therefore, we suggest that high doses and drug-drug interaction may not be the only major risk factors for clozapine-induced generalized seizures, and may not be the only predictors for this side effect.

Our study has limitations that require careful consideration. The sample size is not large, mainly in the clozapine-induced seizures group; thus, these data

require additional assessment with larger samples. Despite the small sample size, our investigation has statistical power to detect an influence of differential occurrence of NOGS under clozapine treatment as a function of *COMT* (84%) and *GNB3* genotype (70%), emphasizing the pharmacogenetic relevance of these SNPs. Another important limitation is the impossibility to study clozapine plasma levels in our patients, a possible factor contributing to the final phenotype. The choice of candidate genes to be examined was somewhat arbitrary, although each is putatively linked to clozapine mechanism of action. As no studies have been conducted focusing this important treatment side effect, the genes investigated, as well as the number of SNPs within these genes were considered as exploratory to start with. Certainly more studies including other genes such as those involved in the epilepsies might also have been a reasonable approach. Clearly, more studies with other genes and more SNPs genotyping are needed to disclose the genetic role on clozapine-induced seizures. We have to keep in mind that it is currently difficult to estimate the impact of genetic variability on side effects because this would require complex data on many factors that are still unknown, as side effects may be a function of variant alleles at independently segregating loci, as well as environmental exposures (Phillips et al., 2001), and co-medications. This could be a reasonable explanation to the effect of dose and co-medication in the role of the *HTR1B* gene in generalized seizure occurrence, and our sample was not able to detect the real association. Despite these methodological limitations, our results should be considered as positive preliminary evidence, since there are no records of other studies addressing the relationship between SNPs and clozapine-associated generalized seizures.

Finally, side effects actually considered as non-preventable may become at least partially preventable, as a first step in optimizing drug therapy with genetic information. This study provides empirical evidence that the use of pharmacogenomics could potentially reduce adverse drug reactions like NOGS, for example, by co-administrating valproate to individuals susceptible to seizures to prevent a problem of major significance in the treatment of severe cases of schizophrenia.

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Table 1: Characteristics of the sample of 121 patients with schizophrenia under clozapine use.

Characteristics	New-Onset Generalized Seizures (NOGS) under clozapine		Total
	Positive	Negative	
Male gender	19/27 (70%)	82/94 (87%)	101/121 (83%)
Age[a]	34.37 ± 9.41	33.91 ± 8.66	34.02 ± 8.79
Age of schizophrenia onset[b]	17.78 ± 3.52	19.13 ± 5.40	18.83 ± 5.06
Illness duration[c]	16.81 ± 8.84	14.74 ± 8.44	15.21 ± 8.53
Age at clozapine starting[d]	29.04 ± 9.03	30.52 ± 9.27	30.18 ± 9.20
Mean clozapine dose[e]	638.52 ± 146.57	512.87 ± 188.18	540.91 ± 186.7
Co-medication[f]	15 (56%)	36 (38%)	51 (42%)

[a] Student's t-test: t = -0.236, *P* = 0.813; mean ± SE (standard error); [b] Mann-Whitney Test: z = -1.140, *P* = 0.254; mean ± SE (standard error); [c] Student's t-test: t = -1.112, *P* = 0.268; mean ± SE (standard error); [d] Mann-Whitney Test: z = -0.804, *P* = 0.421; mean ± SE (standard error); [e] Mann-Whitney Test: z = -3.085, *P* = 0.002; mean ± SE (standard error); [f] medication used to treat convulsion not included

Table 2: Polymorphisms investigated in this study.

Polymorphisms[a]	Chromosome	Function	Enzyme for RFLP	Reference
HTR1B 861G>C	6q13	Synonymous	*Hinc*II	Hawi *et al.,* 2002
HTR2A 102T>C	13q14-q21	Synonymous	*Msp*I	Quist *et al.,* 1996
HTR2A -1438A>G	13q14-q21	5'-promoter region	*Msp*I	Arranz *et al.,* 1998
HTR2A His452Tyr	13q14-q21	Nonsynonymous	*Bsm*I	Quist *et al.,* 1996
HTR2C Cys23Ser	Xq24	Nonsynonymous	*Hinf*I	Lappalaien *et al.,* 1995
SLC6A4 HTTLPR	17q11.1-q12	5'-promoter region	-	Heils *et al.,* 1996
SLC6A4 STin2 VNTR	17q11.1-q12	-	-	Ito *et al.,* 2002
MAOA -uVNTR	Xp11.23-11.4	-	-	Sabol *et al.,* 1998
DRD2 -141C ins/del	11q23	5'-promoter region	*Mva*I	Ohara *et al.,* 1998
DRD3 Ser9Gly	3q13.3	Nonsynonymous	*Msc*I	Scharfetter *et al.,* 1999
DRD4 VNTR exon III	11p15.5	-	-	Kohn *et al.,* 1997
SLC6A3 -839C>T	5p15.3	5'-promoter region	*Msp*I	Rubie *et al.,* 2001
COMT Val158Met	22q11.21	Nonsynonymous	*Nla*III	Kunugi *et al.,* 1997

GNB3	*825C>T*	12p13	Splice variant	*Bsa*JI	Rosskopf *et al.,* 2000
GNB3	*814G>A*	12p13	Nonsynonymous	*Pst*I	Rosskopf *et al.,* 2000
BDNF	*Val66Met*	11p13	Nonsynonymous	*Eco*721	Neves-Pereira *et al.,* 2002

[a] *HTR1B*, *HTR2A*, and *HTR2C*: 5-hydroxytryptamine (serotonin) receptor *1B*, *2A* and *2C* genes; *SLC6A4*: solute carrier family 6 (serotonin transporter), member 4; *MAOA*: monoamine oxidase A gene; *DRD2*, *DRD3*, and *DRD4*: dopamin receptor D2, D3, and D4 genes; *SLC6A3*: solute carrier family 6 (dopamine transporter), member 3; *COMT*: catechol-O-metyltransferase gene; *GNB3*: G-protein Beta-3 subunit gene; *BDNF*: brain-derived neurotrophic factor gene.

Table 3: Significant associations observed between polymorphisms and occurrence of clozapine-induced seizures.

		NOGS			
Genotype or allele		Positive	Negative	OR	*P*-value
COMT Val158Met					
Genotype	*Met/Met*	1 (4%)	26 (28%)	-	-
	Val/Met	17 (63%)	36 (39%)	12.28	0.003
	Val/Val	9 (33%)	30 (33%)	7.80	0.039
		P = 0.010			
Allele	*Met*	0.35	0.48	-	-
	Val	0.65	0.52	1.71	0.085
HTR1B 861G>C					
Genotype	*G/G*	17 (64%)	47 (50%)	-	-
	G/C	5 (18%)	44 (47%)	0.31	0.050
	C/C	5 (18%)	3 (3%)	4.61	0.094
		P = 0.002			
Allele	*G*	0.72	0.73	-	-
	C	0.28	0.27	1.05	0.874
GNB3 825C>T					
Genotype	*C/C*	5 (18%)	41 (44%)	-	-
	T/C	14 (52%)	39 (41%)	2.94	0.091
	T/T	8 (30%)	14 (15%)	4.69	0.030
		P = 0.033			
Allele	*C*	0.45	0.64	-	-
	T	0.55	0.36	2.17	0.007

Table 4: Results of the set association approach for clozapine-induced seizure occurrence in patients with schizophrenia (corrected for multiple comparisons).

Polymorphism [a]	χ^2 statistic	Sum	*P*-value
HTR1B 861G>C	11.4925	11.4925	0.035650
COMT Val158Met	10.2487	21.7411	0.010300
GNB3 825C>T	6.7983	28.5394	0.007450[b]
DRD2 –141C ins/del	1.1919	29.7313	0.016400
SLC6A4 HTTLPR	1.1361	30.8674	0.027900
DRD3 Ser9Gly	0.7888	31.6562	0.041050
SLC6A3 –844C>T	0.4000	32.0562	0.055300
BDNF Val66Met	0.2686	32.3248	0.069700
HTR2A –1438A>G	0.1641	32.4890	0.083350
HTR2A 102T>C	0.0841	32.5731	0.093350
GNB3 814G>A	0.0541	32.6272	0.100450
HTR2A His452Tyr	0.0340	32.6612	0.103400

[a] Only bialelic polymorphisms; [b] Final experiment *P*-value for the model with the lowest *P*-value = 0.016

Table 5: Results from logistic regression analysis predicting NOGS in clozapine treated patients.

Variables	*P*-value	OR	95%CI
GNB3 825C>T	0.027	3.67[a]	1.16-11.63
COMT Val158Met	0.009	19.49[b]	2.10-181.01
HTR1B 861G>C	0.061	6.29[C]	0.91-43.24
Clozapine dose	0.193	2.05[d]	0.70-6.02
Co-medication	0.207	1.93[e]	0.69-5.36

$\chi 2$ model = 27.103, *P* = 0.0001; Percent correct classification 81.51%; [a] *T/T* and *T/C* genotypes; [b] *Val/Val* and *Val/Met* genotypes; [c] *C/C* genotype; [d] clozapine dose > 650mg/day; [e] co-medication present

CAPÍTULO VIII

DISCUSSÃO

Discussões referentes aos resultados específicos obtidos no presente trabalho encontram-se nos capítulos anteriores (III, IV, V, VI e VII). Neste capítulo serão abordados aspectos gerais, referentes à aplicabilidade da farmacogenética, principalmente no Brasil.

O objetivo da farmacogenética é encontrar polimorfismos em genes codificadores de proteínas e enzimas envolvidas no transporte, metabolismo e ação dos fármacos, desta forma possibilitando o conhecimento da aplicabilidade de um fármaco particular e aumentando sua eficácia. O estudo da farmacogenética da esquizofrenia teve seu marco no trabalho de Arranz e cols. (2000) há mais de sete anos atrás. Este trabalho teve um grande impacto na época devido à sua abordagem multi-gênica, observando-se uma forte associação entre um conjunto específico de seis polimorfismos e resposta à clozapina em pacientes esquizofrênicos, com um valor preditivo positivo de 76%. A partir daí, poucos trabalhos foram desenvolvidos com este tipo de abordagem. A maioria envolve estudos de associação individuais que acabam por ter um valor prático mínimo, pois como a maioria dos antipsicóticos tem alvos múltiplos, seria muito improvável que somente um destes alvos seja responsável por toda sua variabilidade terapêutica e, portanto, estes estudos não preveriam de uma maneira abrangente o resultado do tratamento. Da mesma forma, estes estudos enfocam somente um dos dois principais níveis importantes no processo do fármaco dentro do organismo: metabolismo e ação. O objetivo da presente tese foi exatamente este: determinar variantes genéticas que influenciam a variabilidade multifatorial na eficácia e/ou efeitos adversos aos antipsicóticos, analisando-as de maneira combinada e verificando suas inter-relações determinantes do fenótipo final. Para tanto, utilizamos uma abordagem combinada que inclui genes que codificam proteínas envolvidas na farmacodinâmica e na farmacocinética, no intuito de investigar o efeito de um grande número de genes e polimorfismos abrangendo estas duas dimensões do fenótipo, já que poucos estudos visam este objetivo que é de importância indiscutível. Da mesma maneira, a análise de um efeito adverso da clozapina, a convulsão, foi pela

primeira vez analisada através deste trabalho, também de forma multi-gênica. Adicionalmente, este trabalho tem uma importante colaboração no estudo da resposta aos antipsicóticos típicos, menos estudados que a clozapina, já que estes medicamentos são amplamente utilizados no Brasil (Ministério da Saúde, 2002). A expectativa é de que, independente de custos, futuramente os medicamentos possam ser selecionados a partir de abordagens farmacogenômicas em nosso país.

Os dados obtidos neste trabalho são principalmente exploratórios, alguns inéditos e, portanto, a aplicabilidade dos mesmos não ocorrerá em uma via direta. As associações observadas poderão ser utilizadas como um substrato inicial para replicações em estudos com amostras maiores ou ainda em ensaios clínicos randomizados.

Os estudos de associação são o tipo de estratégia mais adequada para pesquisas farmacogenéticas (Risch, 2000). Entretanto, a determinação da relevância prática de variantes farmacogenéticas permanece difícil em parte devido aos problemas com o delineamento e replicação de tais estudos na área. Muitos estudos iniciais com resposta aos antipsicóticos não foram passíveis de replicação em populações clinicamente similares e a implicação dos poucos replicados ainda não foi estabelecida. Além de erros estatísticos do tipo I e II, a dificuldade está principalmente na uniformização das amostras clínicas com relação ao número de indivíduos apropriado e informação clínica detalhada da melhora dos sintomas e desenvolvimento de efeitos adversos. A complexidade dos fatores genéticos nas doenças psiquiátricas e na resposta à medicação também é um fator complicador. O envolvimento de muitos genes na etiologia da esquizofrenia torna difícil a identificação e caracterização de muitos daqueles que seriam relevantes como alvos terapêuticos e como estes componentes interagem ou se combinam no processo. Outras dificuldades incluem o conhecimento incompleto dos mecanismos da esquizofrenia, a complexidade do funcionamento cerebral e a influência de fatores não genéticos (principalmente no metabolismo dos fármacos) incluindo idade, dieta, interações e exposições ambientais, comorbidades, interações entre fármacos uma vez que raros são os pacientes

esquizofrênicos em monoterapia, fatores estes difíceis de serem controlados. A dinâmica de eventos epigenéticos podem também se apresentar como responsáveis pelas variações clínicas observadas na resposta aos antipsicóticos. Alterações epigenéticas em genes dopaminérgicos e serotoninérgicos, relacionados à reposta aos antipsicóticos, já foram sugeridas (Flomen e cols., 2004; Abdolmaleky e cols., 2005).

A estratificação populacional em estudos caso-controle é uma das responsáveis por grande parte das associações falso-positivas (Pritchard e Rosenberg, 1999), pois diferenças genéticas entre grupos étnicos muitas vezes levam a diferenças na resposta ao tratamento. Adicionalmente, a ausência de publicação de achados negativos também gera uma problemática na replicação de resultados (Arranz e cols., 2000c). Para uma maior uniformização dos estudos farmacogenéticos em esquizofrenia, algumas medidas básicas deveriam ser tomadas, dentre elas: (1) utilização de amostras grandes, já que a maioria dos genes influenciando a resposta tem um efeito pequeno no fenótipo, (2) padronização de dados clínicos importantes no desfecho do fenótipo (como dose, duração do tratamento, idade de início do tratamento, entre outros) (Lerer e cols., 2006), (3) tipo de medicação e co-medicações, (4) padronização da determinação da resposta, (5) gravidade da doença, (6) estudos prospectivos, entre outros.

As limitações deste trabalho foram descritas nos capítulos anteriores. A principal delas é nosso pequeno número amostral. Entretanto, outros estudos farmacogenéticos em esquizofrênicos apresentam tamanhos amostrais bastante similares. Além disso, nossa amostra foi capaz de identificar efeitos de polimorfismos que influenciam a resposta e ocorrência de efeitos adversos de fármacos antipsicóticos, tanto de típicos quanto da clozapina. Outras limitações incluem a obtenção dos níveis séricos dos medicamentos. De fato, medidas como esta poderiam ser incluídas em estudos posteriores na tentativa de melhorar o perfil amostral, mas no Brasil não faz parte da rotina clínica este tipo de análise, dificultando assim sua obtenção. Um outro ponto interessante seria a inclusão da fenotipagem da atividade metabólica de CYP2D6 nos indivíduos pertencentes à amostra para comprovação dos genótipos obtidos.

O objetivo principal da farmacogenética é a individualização do tratamento. Discussões sobre a questão da aplicação da identificação de variantes em enzimas metabolizadoras de fármacos são particularmente presentes no momento. Um exemplo interessante é o sistema *microarray AmpliChip® CYP450* (Roche®), que foi aprovado pela *Food and Drug Administration* (FDA) apesar de não existir protocolos validados para seu uso na prática clínica. O estudo *Clinical Antipsychotic Trials of Intervention Effectiveness* (CATIE) (Lieberman e cols., 2005) e um estudo duplo-cego investigando dose de antipsicóticos (Stroup e cols., 2003) controlados por variáveis de confusão específicas da prática clínica, indicam que variações em enzimas de metabolização desempenham um papel pequeno na determinação da resposta clínica aos antipsicóticos. Entretanto, quando combinados simultaneamente aos marcadores farmacodinâmicos, cada um conferindo efeitos moderados, poderão ser mais válidos e apresentar relações custo-efetividade significantes na prática clínica. Os resultados apresentados no capítulo III apontam nessa direção.

A população brasileira apresenta uma origem tri-étnica, com europeus, africanos e ameríndios contribuindo para sua constituição genética (Salzano e Bortolini, 2002). Atualmente, o Brasil já recebeu imigrantes de todas as partes do mundo, tornando nossa população altamente heterogênea, com uma variedade de mistura inter-étnica (Suarez-Kurtz e Pena, 2006). Entretanto, é evidenciado que populações do sul do Brasil apresentam contribuições genéticas provenientes de europeus bem maiores do que de africanos (Callegari-Jacques e cols., 2003). Neste ponto, a aplicabilidade da farmacogenética no Brasil torna-se diferenciada, pois os modelos farmacogenéticos atuais não são aplicáveis às populações com alto grau de miscigenação. Portanto, indivíduos pertencentes à populações como a brasileira devem ser analisados como indivíduos, mais do que como exemplares de uma “raça” (Suarez-Kurtz, 2007). No presente estudo, determinaram-se, pela primeira vez em brasileiros, as freqüências de um considerável número de variantes alélicas do gene que codifica a enzima *CYP2D6*. Adicionalmente, observamos diferenças significantes nas freqüências alélicas e genotípicas entre sul-brasileiros de ancestralidade africana e européia. Com isso, estes dados de *CYP2D6* nunca descritos terão um impacto bastante importante em estudos

farmacogenéticos no Brasil, não somente em relação aos antipsicóticos, mas também a uma ampla gama de medicamentos metabolizados por esta enzima. Como já mencionado na discussão específica, apenas dez alelos respondem por 90% da variabilidade independentemente da cor de pele do indivíduo e poderiam ser utilizados no Brasil como um todo.

Mesmo com o avanço de tecnologias de alta velocidade, de análises de DNA *high-throughput*, de expressão gênica, da bioinformática e da redução dos custos destas tecnologias, o Brasil ainda não conta com recursos suficientes para estudos em grande escala. Portanto, a relação custo-benefício de modelos farmacogenéticos pode fazer com que sua aplicação, já dificultada pelos outros fatores já citados, seja ainda mais lenta.

A futura prescrição farmacogenética de medicamentos focados para os genótipos farmacocinéticos e dinâmicos dos pacientes poderá possibilitar a otimização da seleção de medicações e de suas doses. Entretanto, no tratamento de doenças de origem multifatorial a farmacogenética deve ser apenas uma de uma variedade de abordagens que devem ser analisadas, como as análises de expressão e a proteômica. É importante salientar, como nas palavras de Munir Pirmohamed (Mayor, 2007), “a farmacogenética está chegando, mas não para tudo”. Portanto, se tais predições de eficácia dos medicamentos irão reduzir o sofrimento e melhorar a qualidade de vida dos pacientes, somente o tempo, através de mais investigações, irá dizer.

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ANEXO 1 – Critérios diagnósticos para esquizofrenia segundo o DSM-IV.

A. *Sintomas característicos*

Dois (ou mais) dos seguintes, cada qual presente por um período significativo durante 1 mês (ou menos, se tratados com sucesso):

(1) Delírios

(2) Alucinações

(3) Discurso desorganizado (p.ex., freqüente descarrilhamento ou incoerência)

(4) Comportamento amplamente desorganizado ou catatônico

(5) Sintomas negativos, isto é, embotamento afetivo, alogia ou avolição

Nota: Apenas um sintoma do critério A é necessário se os delírios são bizarros ou se as alucinações consistem de vozes que comentam o comportamento ou os pensamentos da pessoa, ou duas ou mais vozes conversando entre si.

B. *Disfunção social/ocupacional*

Por um período significativo desde o início da perturbação, uma ou mais áreas importantes do funcionamento, tais como trabalho, relações inter-pessoais ou cuidados pessoais, estão acentuadamente abaixo do nível alcançado antes do início (ou, quando o início dá-se na infância ou adolescência, fracasso em atingir o nível esperado de aquisição inter-pessoal, acadêmica ou ocupacional).

C. *Duração*

Sinais contínuos da perturbação persistem por pelo menos seis meses. Este período de seis meses deve incluir pelo menos um mês de sintomas (ou mesmo, se tratados com sucesso) que satisfazem o critério A (isto é, sintomas da fase ativa), podendo incluir períodos de sintomas prodrômicos ou residuais.

Durante esses períodos prodrômicos ou residuais, os sinais da perturbação podem ser manifestados apenas por sintomas negativos ou por dois ou mias sintomas relacionados no critério A presentes de forma atenuada (P. ex., crenças estranhas, experiências perceptuais incomuns).

D. *Exclusão de transtorno esquizoafetivo e transtorno de humor*

O transtorno esquizoafetivo e o transtorno de humor com aspectos psicóticos foram descartados, porque (1) nenhum episódio depressivo maior, maníaco ou misto ocorreu concomitantemente aos sintomas da fase ativa; ou (2) se os episódios de humor ocorreram durante os sintomas da fase ativa, sua duração total foi breve relativamente à duração dos períodos ativo e residual.

E. *Exclusão de substância/condição médica geral*

A perturbação não se deve aos efeitos fisiológicos diretos de uma substância (p. ex., uma droga de abuso, um medicamento) ou a uma condição médica geral.

F. *Relação com um transtorno invasivo do desenvolvimento*

Se existe história de transtorno autista ou outro transtorno invasivo do desenvolvimento, o diagnóstico adicional de esquizofrenia é feito apenas se delírios ou alucinações proeminentes também estão presentes por pelo menos um mês (ou menos, se tratados com sucesso).

Fonte: American Psychiatric Association (1994).

HCPA - HOSPITAL DE CLÍNICAS DE PORTO ALEGRE
Grupo de Pesquisa e Pós-Graduação
COMISSÃO CIENTÍFICA E COMISSÃO DE PESQUISA E ÉTICA EM SAÚDE

RESOLUÇÃO

A Comissão Científica e a Comissão de Pesquisa e Ética em Saúde, que é reconhecida pela Comissão Nacional de Ética em Pesquisa (CONEP)/MS como Comitê de Ética em Pesquisa do HCPA e pelo Office For Human Research Protections (OHRP)/USDHHS, como Institucional Review Board (IRB0000921) analisaram o projeto:

Projeto: 01-130

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Título: ESTUDO DAS BASES MOLECULARES DA ESQUIZOFRENIA NAS POPULAÇÕES BRASILEIRAS E PORTUGUESA, COOPERAÇÃO INTERNACIONAL CAPES-ICCTI - PROJETO DE DESENVOLVIMENTO.

- Este projeto foi Aprovado em seus aspectos éticos e metodológicos, inclusive quanto ao seu Termo de Consentimento Livre e Esclarecido, de acordo com as Diretrizes e Normas Internacionais e Nacionais, especialmente as Resoluções 196/96 e complementares do Conselho Nacional de Saúde. Os membros do CEP/HCPA não participaram do processo de avaliação dos projetos onde constam como pesquisadores. Toda e qualquer alteração do Projeto, assim como os eventos adversos graves, deverão ser comunicados imediatamente ao CEP/HCPA. Somente poderão ser utilizados os Termos de Consentimento onde conste a aprovação do GPPG/HCPA.

- Por pertencer a uma área temática especial este projeto somente poderá ser iniciado após a sua aprovação pela Comissão Nacional de Ética em Pesquisa (CONEP).

Porto Alegre, 17 de agosto de 2001.

Profª Themis Reverbel da Silveira
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