Receptor pharmacogenetics: relevance to CNS syndromes

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Pharmacogenetic research dedicated to the investigation of inherited factors that influence drug response has produced exciting results over the past decade. Adding to the knowledge that genetic variation in metabolic enzymes may cause drug-related adverse reactions, recent studies indicate that variation in neurotransmitter receptors can also be the cause of treatment failure. In addition, recent studies have attempted to use genetic information for the prediction of treatment outcome. The aim of this review is to summarize the most significant findings in pharmacogenetic research in relation to CNS drugs and to outline how these studies could lead to the individualization of drug treatment.

Keywords: individualization of treatment, neurotransmitter receptors, pharmacogenomics

Introduction

Over the past decade, genetic studies have evolved from the discovery of genes responsible for simple Mendelian diseases, to the discovery of genes involved in complex neurodevelopmental disorders. A more recent application of genetics has however, assumed a role in offering a superior alternative in the design of pharmaceutically effective products. The future goal is based on the extraction of genetic data such that drugs can ideally be tailored to fit the genetic profile of an individual. In this way, the most likely drug to produce a positive therapeutic effect can be selected, allowing the necessary dose adjustments whilst avoiding potential side-effects. The study of genetic variation in molecular targets of drug behaviour is known as pharmacogenetics or pharmacogenomics. The idea is to investigate variant forms of genes coding for molecular targets of drug metabolism, disposition and effect in order to determine the differential response to medications in individuals.

Inherited differences in drug effects were observed and documented as far back as the 1950s [1]. Much of the ground breaking work in pharmacogenetics has however, only followed the cloning and characterization of the gene coding for the cytochrome P450 (CYP)2D6 drug metabolizing enzyme, also known as debrisoquine hydroxylase [2]. This was subsequently followed by studies of the genetic variation in the metabolism of a range of drugs [3]. Other CYP enzymes (CYP2C19, CYP2A6,

CYP2C9) have also now been implicated as being polymorphically expressed with genetic variation affecting the metabolic rate of a number of drugs [4]. More recently, however, studies have shown a genetic contribution in other components involved in the effector signalling pathway of drugs, such as receptors and transporters. For example, a single nucleotide polymorphism (SNP) in the mu opioid receptor gene affects the activity of the receptor, changing its sensitivity to the endogenous agonist β -endorphin [5], whilst serotonin transporter genes have been associated with drug efficacy [6, 7].

Common central nervous system (CNS) diseases such as schizophrenia, bipolar affective disorder, Alzheimer's disease and anorexia nervosa are known to have a strong genetic component [8]. Taken together with observations that heterogeneity in the way individuals respond to different medications cannot be completely accounted for by clinical factors provides the basis for present day drug development through genetic investigations. Several approaches are currently being pursued: the identification of genes responsible for drug response to pre-existing medications; the identification of genes involved in the aetiology of the disease that will allow entry into a critical protein pathway mechanism leading to the identification of potential new drug targets; and the identification of genes coding for novel receptors that have no known ligands. Studies using the first approach have been reported most commonly and are described below.

CNS disease and neurotransmitter gene studies

Pharmacogenetic publications of CNS diseases to date, have focused mainly on investigations of genes involved in neurotransmitter pathways. Dopaminergic, serotonin-

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Table 1 Neuroreceptor targets associated with altered response to drug metabolism.

Neurotransmitter target gene	Drug	Drug effect associated with gene	Reference
Alpha-7 nicotinic receptor	Acetylcholine(–) nicotine	Increased sensitivity to agonists	[9]
Serotonin 5-HT _{2A} receptor	Clozapine	Treatment of schizophrenia	[16–18]
Serotonin 5-HT _{2C} receptor	Clozapine	Treatment of schizophrenia	[19, 20]
Serotonin transporter 5-HTT	Fluvoxamine	Treatment of delusional depression	[7]
Dopamine D ₂ receptor	Bromocriptine	Late onset hallucinations in Parkinson's	[22]
Dopamine D ₂ receptor	Typical neuroleptics	Tardive dyskinesia	[27]
Dopamine D ₃ receptor	Antipsychotics	Tardive dyskinesia	[29–32]
Dopamine D ₄ receptor	Typical neuroleptics	Treatment of schizophrenia	[38–41]

ergic, muscarinic, adrenergic and histaminergic signalling systems are being extensively investigated for their possible involvement in disease aetiology and treatment. Several receptors for these neurotransmitters have been identified through pharmacogenetic investigations as having a potentially significant role on drug behaviour with regard to drug efficacy and toxicity (see Table 1). The following sections give a general overview of the type of investigations currently being conducted in relation to CNS receptor genetics, but are not an exhaustive account of all published investigations.

Cholinergic system

The cholinergic pathway has been found to play an important role in Alzheimer's disease (AD) pathology. The disease is often treated using cognition-enhancing agents such as acetylcholinesterase inhibitors that target acetylcholinesterase (ACh) activity and nicotinic receptor binding. An early investigation reported a differential response to this treatment in association with the apo ϵ 4 gene variant of the apolipoprotein E (apoE), implicating that it influences the integrity of the cholinergic system in the brain [9]. Recent studies however, have been contradictory showing no association between drug response and the ϵ 4 allele [10]. Cholinergic neurotransmitter receptors, such as the nicotinic and muscarinic receptors, are also likely to demonstrate differential drug response phenotypes. Systematic screening of the muscarinic genes has however, failed to clearly identify functional variants [11]. Nevertheless, a receptor binding study has recently demonstrated how single amino acid changes that occur naturally in the human alpha-7 nicotinic receptor can alter receptor pharmacology [12].

Serotonergic system

The serotonergic pathway has been implicated in mood related illnesses such as schizophrenia and bipolar affective disorder. The involvement of the serotonergic system has attracted much interest as a potential target for the

development of antipsychotic medications for the treatment of psychiatric diseases [13]. Serotonergic receptors are targeted by atypical antipsychotics, a novel group of drugs characterized by a low prevalence of extrapyramidal side-effects [14]. It has been hypothesized that serotonergic targeting greatly contributes to their therapeutic success [15]. Genetic studies have strengthened this hypothesis through the finding of an association between polymorphisms in the 5-HT_{2A} and 5-HT_{2C} receptor genes and a therapeutic response to the clozapine, the prototypical antipsychotic drug [16–19]. A combined analysis of genetic variants in the 5-HT₂ receptors, the serotonin transporter (5-HTT) and the histamine receptor showed predictive properties for treatment response to clozapine [20]. A second study attempting to replicate these results however, failed to show similar findings [21]. Allelic variation in the promoter region of the 5HTT gene also appears to play a role in influencing response to the antidepressant fluvoxamine [7].

Dopaminergic system

The dopaminergic system is best known for its involvement in Parkinson's disease and for the long-standing dopamine hypothesis in schizophrenia. In Parkinson's disease, dopamine receptor agonism can control the motor symptoms of the disease by increasing dopaminergic neurotransmission. This has however, also been linked with the occurrence of side-effects such as hallucinations. Interestingly, variation in the D₂ receptor gene has been shown to correlate with the occurrence of late onset hallucinations in patients with Parkinson's disease [22].

Dopamine receptor blockade is believed to be the site of action for most of the antipsychotic medications used in treating the symptoms of schizophrenia. Typical antipsychotics are thought to act by blocking dopamine D₂ receptors, whilst the atypical antipsychotic clozapine demonstrates a higher affinity for D₄ receptors [23] and selective affinity for D₂ receptors [24]. However, antagonism at the dopamine receptors has also been associated

with the occurrence of side-effects [14]. It is therefore not surprising that current genetic investigation of the D₂ receptors has been extended to evaluate the relationship between the gene and side-effects of these drugs.

In schizophrenia, an early study identified a DNA sequence that coded for an important drug-binding site in the D₂ receptor, the Asp (114) residue, which if mutated had severe functional consequences to the binding properties of the receptor [25]. However, no genetic variation has been found at this site in schizophrenic patients with an altered response to typical antipsychotics. More recent studies have demonstrated an association between a *Taq I* polymorphism in this gene and response to nemonapride, a selective dopamine antagonist, in schizophrenic patients [26]. An investigation of side-effects such as tardive dyskinesia after long-term administration of antipsychotic treatment in schizophrenic patients has also implicated the same D₂ receptor polymorphism [27].

Analysis of the D₃ receptor gene in schizophrenic patients has also shown that genetic variation may affect drug response. Marginal evidence for an association between the Ser9Gly polymorphism in the D₃ receptor gene and response to clozapine has been demonstrated [28]. More interestingly, the same polymorphism has been reported to be associated with drug-induced tardive dyskinesia by several authors [29–32].

Pioneering studies of the pharmacogenetics of psychiatric disease were conducted on the dopamine D₄ receptor gene. The response profile of the atypical antipsychotic drug clozapine was investigated in relation to D₄ receptor gene variants [33]. No association was demonstrated, and this has been supported by several other investigators [34–38]. Other studies have, however, reported contradictory findings, showing an association between polymorphisms in the D₄ receptor gene and response to typical antipsychotic medication [39, 40]. The recent finding of a functional polymorphism in the promoter region of the D₄ gene [41] may help to clarify the contribution of this receptor to therapeutic benefit from antipsychotics.

In summary, a review of the literature shows that pharmacogenetic investigations based on a case–control association model are often contradictory. There are several reasons for this:

- There may be differences between the populations investigated resulting in heterogeneity between populations;
- the use of different clinical criteria for inclusion in studies; and
- the presence of ethnically unmatched individuals resulting in population stratification giving false negative or positive findings.

Replication of the results within homogeneous populations is ultimately necessary to validate findings.

Receptor genes in the human genome

It is not surprising that receptors are quite often the primary ‘targets’ of interest in pharmacogenetic investigations given their obvious drug binding properties. It is also well known that even single base pair changes within the genomic sequence of a receptor can cause a significant change in receptor binding properties to certain drugs [5]. The recent publications of the sequencing of the human genome estimate the presence of between 25 000 and 40 000 human genes [42, 43]. Of these, genes coding for G-protein coupled receptors (GPCRs) are likely to run into the thousands, and mining of the genome for novel GPCR that lack known ligands is already underway [44].

Direct screening for genes involved in drug behaviour can be conducted rapidly with the identification of critical polymorphisms within the genes (SNPs or VNTRs). Single nucleotide polymorphisms (SNPs) are single base pair substitutions that account for many well-characterized human phenotypes, and are the most abundant type of naturally occurring variant present in the human genome. The human genome screening effort has reported the identification of more than 3 million SNPs [42, 43]. Alternatively, VNTRs are repetitions of a short DNA sequence that can exist in different lengths and have been associated with altered expression or functionality of the neurotransmitter system genes [45]. Due to their abundant nature, SNP discovery has become an integral part of current large-scale pharmacogenetic studies. The identification of all human genes, the discovery of critical polymorphisms and their subsequent effective analysis should ultimately facilitate the identification of new targets for drug behaviour.

A model for therapeutic targets

Current drug discovery for CNS medication has followed a repetitive path. The atypical antipsychotics clozapine (a dibenzodiazepine derivative) and olanzapine (a thienobenzodiazepine) act on dopamine and serotonin receptors. New compounds with similar receptor binding properties are being developed for therapeutic use in schizophrenia. Furthermore, selective serotonin reuptake inhibitors are mechanistically similar to traditional tricyclic antidepressants [12, 44]. By contrast, many drugs are nonselective in their actions and are used in treating several diseases; for example, valproate is used for epilepsy, bipolar disorders and migraine [44].

It seems evident that a single gene is unlikely to account for drug behaviour. A typical drug response

model is therefore likely to involve several genes encoding proteins in multiple pathways of drug metabolism, disposition and effect [46]. For example, variation within one or more genes could govern the way a drug is metabolized, and another set of genes could determine how well a drug binds to a receptor to exert a therapeutic effect. In addition, it also appears likely that in certain cases more than one member of the same or different family of receptor genes may be involved in determining drug effect. For example, a combination of six polymorphisms located on four different receptor genes resulted in approximately 77% success in the predicting the response to clozapine [20].

The challenge for pharmacogenomics is to determine whether it is genetic variation in the receptor that is causing a drug response effect or whether it is the effect of a gene further down-stream of the receptor-ligand binding process. A direct correlation between response and receptor genes outlines an ideal scenario; however, in reality a combination of several genes will be involved.

Future prospects of pharmacogenetics

The financial benefits of developing safe and effective drugs based on genetic information has long been realized by the biotechnology industry, and has made pharmacogenetics one of the fastest growing areas in this field. There are several reasons for the growing demand and investment in this field. The first is in the potential to develop new therapeutic products that are specifically designed to complement an individual's genetic profile ranging from an individual's ability to metabolize a specific drug to the exclusion of potential toxic side-effects. The benefits would thus include the ability to quantify the specific dose of drug required for a therapeutic effect based on individual ability to metabolize the drug. A more immediate benefit would be the ability to predict the effectiveness of pre-existing medications.

The viability of producing pharmaceutical products based on genetic information can only be proven once all genetic components contributing to drug behaviour have been identified. If several genes are involved, this could prove to be a challenging task. Technological advances such as the application of DNA chips will facilitate the process. Ideally, a single chip would contain SNPs from specific genes of importance to identify drug behaviour for a particular disease. An individual requiring treatment would have their genetic profile examined with respect to a specific disease by donating a small drop of blood to allow automated high throughput screening. Based on this information, the most effective drug and the optimum quantity of medication may be prescribed. With future goals such as these, pharmacogenomics still appears to be in its infancy; however, the materialization

of such visions could lead to tremendous benefits in individualization of medicine and patient health care.

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