Clinical relevance of discoveries in psychopharmacogenetics[1]

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Abstract Individual genetic variation accounts for some of the variability in response to drugs used routinely in clinical psychiatry. Psychopharmacogenetics focuses on how polymorphisms in genes affecting the mechanism of action of a drug's effect and/or metabolism (both peripheral and central) can influence an individual's clinical response to the drug, in terms of both therapeutic efficacy and adverse effects. Pharmacogenetics promises to be of substantial help in the field of psychiatric pharmacotherapy, but before research findings can be applied to clinical practice, ethical and methodological problems have to be addressed and overcome. This review summarises the most robust findings in the field and outlines how psychopharmacogenetic studies could lead to treatment individualisation.

Clinical differences in drug response in terms of beneficial and adverse effects, or no effect, are well established throughout medicine. In interpreting how this variability in outcome of drug therapy might affect an individual, many factors must be considered: the patient's health profile, prognosis and compliance with therapy; the severity of the disease; the quality of drug prescribing and dispensing; and the genetic profile of the individual.

Pharmacogenetics focuses on the study of gene–drug interactions and examines the extent to which variability in the human genetic make-up is responsible for the differences between patients in therapeutic efficacy, effectiveness and adverse reactions. The term pharmacogenomics has more recently been introduced and it encompasses pharmacogenetics. It refers to a genome-wide search for genes and their products relevant to the use of drugs in humans. The search includes genes determining disease susceptibility and those causing individual variations in drug response, and it is based on the knowledge derived from the Human Genome Project (Aitchison & Gill, 2002).

Pharmacogenetics and pharmacogenomics have a shared goal, in that they both seek to guide pharmacotherapy and improve outcome by providing individualised treatment decisions. A long-term aim of pharmacogenomics is to develop novel diagnostic procedures and therapeutic products for effective and safe individualised drug prescription. Both fields, therefore, hold great potential, particularly in psychiatry, where biologically based treatment guidelines are lacking.

Individual variability in drug response can often be understood as a combination of factors affecting the pharmacokinetic and pharmacodynamic effects of drugs (Fig. 1). Candidate genes for pharmacogenetic studies include polymorphic drug-metabolising enzymes, drug transporters and polymorphic drug targets that affect disease-related pathways.

Pharmacogenetics of antipsychotic treatment
Genetic variants of drug-metabolising enzymes

Antipsychotics are used to treat schizophrenia. There is increasing evidence to suggest that the *CYP2D6* genotype might partially affect response to typical antipsychotics, and also drug side-effects. For example, on average, Asians develop higher plasma levels than Europeans and thus seem to have an increased sensitivity to antipsychotics, including haloperidol (Aitchison *et al*, 2000*b*).

1. A glossary of genetic terms used in this article appears in Box 1. The National Human Genome Research Institute website (http://www.genome.gov/) offers information at various levels of complexity on genes and genetics, including a very simple diagram of a gene and its parts (http://www.accessexcellence.org/RC/VL/GG/gene2.html).

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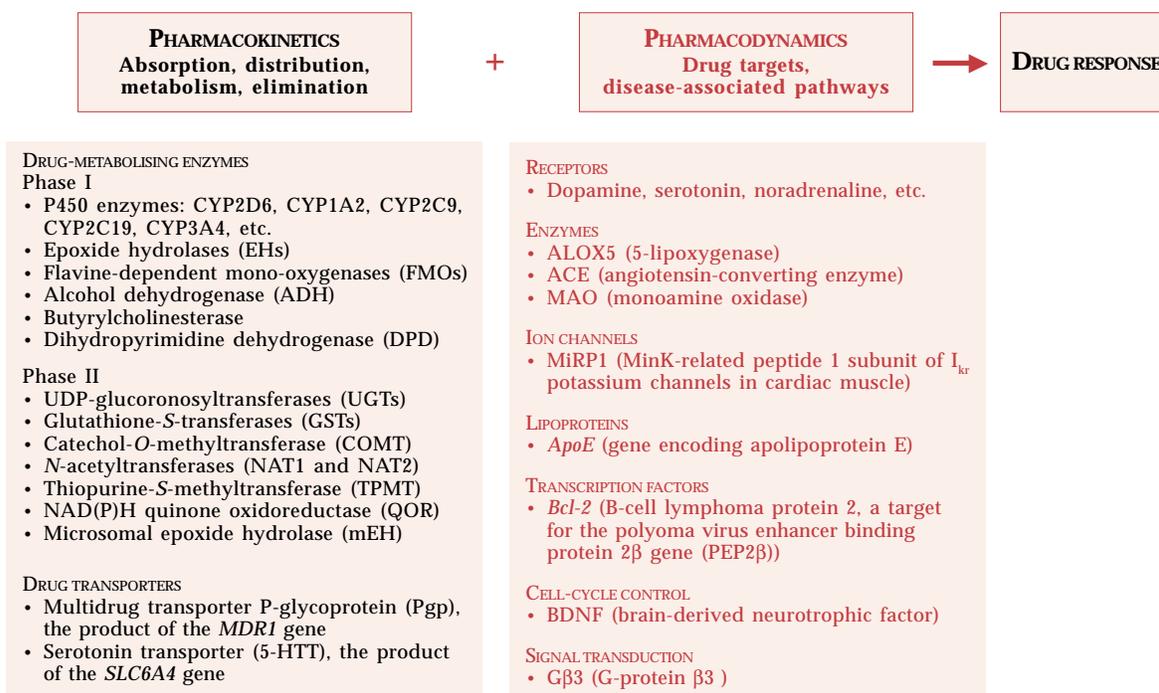
| **PHARMACOKINETICS** Absorption, distribution, metabolism, elimination | + | **PHARMACODYNAMICS** Drug targets, disease-associated pathways | → | **DRUG RESPONSE** |

DRUG-METABOLISING ENZYMES

Phase I
- P450 enzymes: CYP2D6, CYP1A2, CYP2C9, CYP2C19, CYP3A4, etc.
- Epoxide hydrolases (EHs)
- Flavine-dependent mono-oxygenases (FMOs)
- Alcohol dehydrogenase (ADH)
- Butyrylcholinesterase
- Dihydropyrimidine dehydrogenase (DPD)

Phase II
- UDP-glucoronosyltransferases (UGTs)
- Glutathione-*S*-transferases (GSTs)
- Catechol-*O*-methyltransferase (COMT)
- *N*-acetyltransferases (NAT1 and NAT2)
- Thiopurine-*S*-methyltransferase (TPMT)
- NAD(P)H quinone oxidoreductase (QOR)
- Microsomal epoxide hydrolase (mEH)

DRUG TRANSPORTERS
- Multidrug transporter P-glycoprotein (Pgp), the product of the *MDR1* gene
- Serotonin transporter (5-HTT), the product of the *SLC6A4* gene

RECEPTORS
- Dopamine, serotonin, noradrenaline, etc.

ENZYMES
- ALOX5 (5-lipoxygenase)
- ACE (angiotensin-converting enzyme)
- MAO (monoamine oxidase)

ION CHANNELS
- MiRP1 (MinK-related peptide 1 subunit of I_{kr} potassium channels in cardiac muscle)

LIPOPROTEINS
- *ApoE* (gene encoding apolipoprotein E)

TRANSCRIPTION FACTORS
- *Bcl-2* (B-cell lymphoma protein 2, a target for the polyoma virus enhancer binding protein 2β gene (PEP2β))

CELL-CYCLE CONTROL
- BDNF (brain-derived neurotrophic factor)

SIGNAL TRANSDUCTION
- Gβ3 (G-protein β3)

Fig. 1 Pharmacokinetic and pharmacodynamic factors that affect individual patients' responses to drugs.

Genetic variants of drug targets

Drug response studies for antipsychotics have focused largely on genes that code for the neuronal targets of these drugs. These include the genes encoding dopaminergic D_2, D_3 and D_4 receptors (*DRD2*, *DRD3*, and *DRD4*); the serotonergic 5-HT$_{2A}$ (*HTR2A*), 5-HT$_{2C}$ (*HTR2C*) and 5-HT$_6$ (*HTR6*) receptors; the histaminergic H_1 and H_2 receptors (*H_1* and *H_2*); the muscarinic cholinergic receptors; neurotransmitter transporters; and other intra-cellular signalling molecules.

Of the antipsychotics, clozapine is thought to be the most efficacious in improving both positive and negative symptoms of schizophrenia. Clozapine is most useful for patients who fail to respond to typical antipsychotics, but even among this group only about two-thirds of patients respond to it. Clozapine exhibits large inter-individual variations in bio-availability, steady-state plasma concentrations and clearance. It is metabolised by several CYP enzymes, including CYP1A2, CYP3A4 and CYP2C19, but not by CYP2D6 (Collier, 2003). Aitchison *et al* (2000*a*) found that clozapine is primarily metabolised by CYP1A2, since CYP1A2 knock-out mice given clozapine demonstrated significantly lower clozapine clearance than did wild-type mice. These results suggested that *CYP1A2* polymorphisms might be associated with clozapine response, but so far this has not been confirmed (Basu *et al*, 2004*a*).

There are only a few reports on pharmacogenetic studies based on the metabolic pathways of other atypical antipsychotics such as risperidone, olanzapine and quetiapine (Staddon *et al*, 2002).

Dopamine receptors

The dopamine D_2 receptor is a major site of action of antipsychotics, but a functional polymorphism (–141 Ins/Del) affecting promoter activity and *DRD2* expression was not shown to be associated with clinical response to clozapine or typical anti-psychotics. This is in contrast to reports of missense variants (Val96Ala, Pro310Ser and Ser311Cys) determining response to various antipsychotics, including clozapine. Furthermore, the *DRD3* Ser9Gly missence variant has been associated with response to typical antipsychotics, but association with response to clozapine remains controversial (for a review see Shaikh & Kerwin, 2002). Several studies on the association of a 16 amino acid repeat polymorphism in exon 3 of *DRD4* have been carried out, and surprisingly, given the relatively high affinity of clozapine for the D_4 receptor, suggest that this polymorphism does not seem to correlate with the degree of response to clozapine, in contrast to the positive association between this polymorphism and a rapid response to acute treatment with typical antipsychotics.

Box 1 Glossary of terms

Allele   One of the variant forms of a gene at a particular locus, or location, on a chromosome. Different alleles produce variation in inherited characteristics such as hair colour or blood type. In an individual, one form of the allele (the dominant one) may be expressed more than another form (the recessive one).

Candidate gene   A gene, located in a chromosome region suspected of being involved in a disease, whose protein product suggests that it could be the disease gene in question.

CYP enzymes   Cytochrome P450 enzymes; a large family of enzymes responsible for the oxidative (Phase I) metabolism of a wide range of compounds, including many medications.

Deletion   A particular kind of mutation: loss of a piece of DNA from a chromosome. Deletion of a gene or part of a gene can lead to a disease or abnormality.

Duplication   A particular kind of mutation: production of one or more copies of any piece of DNA, including a gene or even an entire chromosome.

Exon   The region of a gene that contains the code for producing the gene's protein. Each exon codes for a specific portion of the complete protein. In some species (including humans), a gene's exons are separated by long regions of DNA (called introns or junk DNA) that have no apparent function.

Gene expression   The process by which proteins are made from the instructions encoded in DNA.

Genetic marker   A segment of DNA with an identifiable physical location on a chromosome and whose inheritance can be followed. A marker can be a gene, or it can be a section of DNA with no known function. Because DNA segments that lie near each other on a chromosome tend to be inherited together, markers are often used as indirect ways of tracking the inheritance pattern of a gene that has not yet been identified, but whose approximate location is known.

Genetic screening   Testing a population group to identify a subset of individuals at high risk for having or transmitting a specific genetic disorder.

Genome   All the DNA contained in an organism or a cell, which includes both the chromosomes within the nucleus and the DNA in mitochondria.

Genotype   The genetic identity of an individual that does not necessarily show as outward characteristics.

Insertion   A type of chromosomal abnormality in which a DNA sequence is inserted into a gene, disrupting its normal structure and function.

Intron   A noncoding sequence of DNA that is initially copied into RNA but is cut out of the final RNA transcript.

Linkage   The association of genes and/or markers that lie near each other on a chromosome. Linked genes and markers tend to be inherited together.

Locus   The place on a chromosome where a specific gene is located, a kind of address for the gene. The plural is loci.

Microsatellite   Repetitive stretches of short sequences of DNA used as genetic markers to track inheritance in families.

Missense mutation   A mutation that changes a codon so that it codes for a different amino acid.

Mutation   A permanent structural alteration in DNA. In most cases, DNA changes either have no effect or cause harm, but occasionally a mutation can improve an organism's chance of surviving and passing the beneficial change onto its descendants.

Nonsense mutation   A single DNA base substitution resulting in a stop codon.

Phenotype   The observable traits or characteristics of an organism, for example hair colour, weight or the presence or absence of a disease. Phenotypic traits are not necessarily genetic.

Polymorphism   A common variation in the sequence of DNA among individuals.

Promoter   The part of a gene that contains the information to turn the gene on or off. The process of transcription is initiated at the promoter.

Substitution   Replacement of one nucleotide in a DNA sequence by another nucleotide or replacement of one amino acid in a protein by another amino acid.

Variable number tandem repeat (VNTR)   A microsatellite consists of a specific sequence of DNA bases or nucleotides which contains mono, di, tri, or tetra tandem repeats, the so-called variable number tandem repeats.

Serotonin receptors

In addition to the undoubted pivotal role of dopamine in the mechanism of action of anti-psychotics, mechanisms mediated by serotonin (5-HT) may also be involved (Meltzer, 1995). Several lines of research have implicated the 5-HT_{2A}, 5-HT_{2C}, 5-HT_{5A} and 5-HT_6 receptors in response to treatment of schizophrenia. A strong association reported between the 102T/C polymorphism in *HTR2A* and clozapine response was not replicated by others, but a meta-analysis showed that the 102T/C silent polymorphism played a major role in determining clozapine response (Arranz *et al*, 1998). Similarly, a −1438G/A polymorphism in the promoter region of this gene was strongly associated with clozapine response. These polymorphisms have also been associated with response to typical antipsychotics (Joober *et al*, 1999) and risperidone (Lane *et al*, 2002). With respect to *HTR2A*, positive associations were also reported between clozapine response and a structural His452Tyr variant of potential functional significance. An association has also been reported between a potentially functional Cys23Ser structural change in *HTR2C* and clozapine response but other studies have failed to replicate this finding.

Furthermore, investigation of the 5-HT$_5$ and the 5-HT$_6$ receptor genes has pointed towards a minor contributing role in clozapine treatment response (Yu *et al*, 1999; Birkett *et al*, 2000).

Pharmacogenetic prediction of antipsychotic response

Arranz *et al* (2000) performed association studies of multiple candidate genes in an attempt to find the combination of polymorphisms that gave the best predictive value of response to clozapine in patients with schizophrenia. On the basis of clozapine-binding profiles, they studied 19 genetic polymorphisms in eight receptor subtype genes, including the α$_{2A}$-adrenoceptor (*ADRA2A*), *DRD3*, *HTR2A*, *HTR2C*, *HTR3A*, *HTR5A*, *H$_1$*, *H$_2$* and the serotonin transporter (*5-HTT*) genes. A combination of six polymorphisms showing the strongest association with response (*HTR2A* 102T/C and His452Tyr; *HTR2C* −330GT/−244CT and Cys23Ser; *5-HTTLPR*; *H$_2$* −1018G/A) gave a level of prediction of 76.86% (χ^2 = 35.8, *P* = 0.0001) and a sensitivity of 95.89 (s.d. = 0.04) for the identification of patients who will show a satisfactory improvement with treatment. This study was the first to report on the use of combinations of pharmacodynamic factor gene polymorphisms to predict the response to antipsychotic medication. Attempts to replicate this finding have, however, been inconsistent.

Preliminary results from the same group (Clark *et al*, 2002), showed that, in 92 Spanish patients, a combination of polymorphisms in the genes *HTR2C*, *HTR2A*, *DRD3*, *5-HTT* (the variable number tandem repeat (VNTR) and the serotonin-transporter-linked polymorphic region (*5-HTTLPR*) gene) may be used for the prediction of treatment response to olanzapine (positive predicted value = 76%, negative predicted value = 79%, sensitivity = 82%, specificity = 72%, *P* = 0.07). Similar studies have been performed for the response to risperidone, but in smaller groups. The hope is that if these studies are replicated, such methodology could form the basis for pharmacogenetic prediction tests for response to various antipsychotics, in a new era of treatment individualisation.

Adverse effects of antipsychotic treatment

Pharmacogenetic candidate gene studies concerning the side-effects of antipsychotic drugs have investigated the association with the relevant drug-metabolising enzymes or neurotransmitter receptors. Extrapyramidal symptoms and early-stage side-effects of antipsychotic therapy such as postural hypotension and excess sedation have been reported to be associated with overrepresentation of poor metabolisers of *CYP2D6* (for a review see Scordo & Spina, 2002).

Extrapyramidal side-effects

Tardive dyskinesia is an involuntary movement disorder manifested typically in the orofacial area, but frequently extending to the limbs and the trunk. Susceptibility to the development of tardive dyskinesia is currently thought to have a genetic basis, and a positive association between tardive dyskinesia and a functional polymorphism (a cytosine-to-adenine substitution, C/A) in the first intron of *CYP1A2* has been reported (Basile *et al*, 2000). A meta-analysis (Lerer *et al*, 2002) has correlated the *DRD3* Ser9Gly polymorphism with tardive dyskinesia in a large sample (*n*=780) of patients treated with typical antipsychotics. Since both *DRD3* and *CYP1A2* seem to contribute to the development of tardive dyskinesia, a gene–gene interaction analysis was undertaken (Basile *et al*, 2002) using scores on the Abnormal Involuntary Movements Scale (AIMS) to measure symptom severity. This showed that patients who exhibited the homozygous risk genotype at both *DRD3* (Gly/Gly) and *CYP1A2* (C/C) had the most severe tardive dyskinesia (highest mean AIMS scores), whereas those who had none of the risk genotypes at either locus had the lowest mean scores. Following the same rationale, Zhang *et al* (2003) recently reported a possible synergistic effect of *DRD3* Ser9Gly and manganese superoxide dismutase gene (*MnSOD*). Manganese superoxide dismutase is an enzyme that catalyses the dismutation reaction of the toxic superoxide radical to molecular oxygen and hydrogen peroxide and thus forms a crucial part of the cellular antioxidant defence mechanism. This reported interaction may affect susceptibility to tardive dyskinesia by influencing mitochondrial free radical scavenging.

Acute akathisia has also been reported to be associated with polymorphisms in *DRD3* and *DRD2* (Basu *et al*, 2004*b*). *MnSOD* alone has also been shown to be weakly associated with tardive dyskinesia. Furthermore, the contribution of ten polymorphic sites in six candidate dopaminergic and serotonergic genes to the development of tardive dyskinesia was recently examined in a small Jewish sample, with only the dopamine transporter gene (*DAT*) 3'-VNTR polymorphism, the serotonin transporter-linked polymorphic region (*5-HTTLP*) and the tryptophan hydroxylase (*TPH*) intron-7 polymorphism yielding trends towards a positive association (Segman *et al*, 2003).

Hyperprolactinaemia

Antipsychotic-induced hyperprolactinaemia has been shown to be associated with the *DRD2* Taq1A polymorphism (Mihara *et al*, 2000) and, more recently, a significant association between *DRD2* –141C and hyperprolactinaemia consistent with *in vitro* work was demonstrated (further details available from the authors on request). The latter association was strengthened by controlling for *CYP2D6* genotypic category ($P = 0.023$), and a trend for an association with a specific *DRD2* haplotype was also shown.

Weight gain

Among antipsychotics, clozapine appears to have the greatest weight-gain liability. It is currently thought that weight gain induced by clozapine and other antipsychotics (typical and atypical) results from multiple neurotransmitter receptor interactions, leading to changes in appetite and eating behaviour. Basile *et al* (2001) invesitaged ten genetic polymorphisms across nine candidate genes involved in both central hypothalamic weight regulation and peripheral thermogenic pathways. The nine candidate genes were the *HTR2C, HTR2A, HTR1A*, the H_1 and H_2, the *CYP1A2*, the β_3-adrenergic receptor genes (*ADRB3*) and *ADRA1A* and the tumour necrosis factor a gene (*TNFα*). Only four of these (*HTR2C, ADRB3, ADRA1A* and *TNFα*) demonstrated a modest, non-significant trend towards a positive association with clozapine-induced weight gain. A positive association between a promoter polymorphism (–759C/T), thought to alter *HTR2C* gene expression, has also been reported by Reynolds *et al* (2002), but both studies await replication.

Agranulocytosis

Clozapine-induced agranulocytosis has been associated with a dominant gene within the major histocompatibility complex region marked by heat shock protein 70–1 and 70–2 variants. This finding was, however, reported in two studies with small power of Jewish samples (Valevski *et al*, 1998; Meged *et al*, 1999). In a more recent study, of a non-Jewish Caucasian sample, clozapine-induced agranulocytosis was significantly associated with some human leukocyte antigen (HLA) polymorphisms, and age seemed to be a further major risk factor for clozapine-induced agranulocytosis (Dettling *et al*, 2001). Thus, HLA loci may serve as genetic markers to identify individuals of different ethnic subgroups prone to this severe idiosyncratic drug reaction.

Pharmacogenetics of antidepressant treatment

Pharmacotherapy in depression results in effective treatment for the majority of patients, but it can take up to 6 weeks before response is seen and up to 40% do not respond sufficiently to the initial treatment. It would therefore be highly desirable to identify those who will not respond before therapy is started, thus avoiding a relatively long period of trial and error. The impact on health care costs of a test identifying these patients could be substantial, owing to the large burden of disease exerted by depression.

Polymorphisms of drug-metabolising enzymes

Most interest has focused on the *CYP2D6* gene, which encodes debrisoquine hydroxylase, the enzyme that metabolises many antidepressants, including the tricyclics (TCAs), selective serotonin reuptake inhibitors (SSRIs) and venlafaxine. Studies have identified genetic polymorphisms with over 70 allelic variants; these result in clinically important functional metabolic changes, notwithstanding ethnic differences in the prevalence of both 'poor metabolisers' and 'ultrarapid metabolisers'. About 7–10% of Caucasians are poor metabolisers of *CYP2D6*. These individuals have no functional CYP2D6 because they have no functional copy of a *CYP2D6* gene (e.g. they are homozygous for null alleles, a mutation that leads to a prematurely truncated protein and hence lack of debrisoquine hydroxylase activity). Such people are likely to have increased concentrations of metabolised drugs at conventional doses (Brøsen, 1996; Sachse *et al*, 1997). At the other extreme of the metabolic spectrum, in ultrarapid metabolisers (with *CYP2D6* gene duplications, which result in excess metabolic activity of debrisoquine hydroxylase), who comprise 3–8% of Caucasians and up to 30% of some other ethnic groups, drugs at standard doses often do not reach therapeutic concentrations and an increased dose may be required to achieve therapeutic response. For example, nortriptyline *CYP2D6* poor metabolisers require only 50% of the average effective antidepressant dose, but ultrarapid metabolisers may require up to 230% of this dose (Johansson *et al*, 1993; Kirchheiner *et al*, 2001).

Pharmacodynamic variability

There is considerable evidence supporting the hypothesis that alterations in serotonergic neuronal function are involved in the pathophysiology of

depression (Owens & Nemeroff, 1994). The serotonin transporter (5-HTT) protein acts as the primary mechanism for removing 5-HT from the synaptic cleft. Two polymorphisms have been identified within the human *5-HTT*, a 44 bp insertion/deletion polymorphism in the promoter region (5-HTTLPR), giving rise to a short (*s*) and a long (*l*) variant, and a VNTR polymorphism in intron 2. Since the serotonin transporter is the target for serotonin reuptake inhibitors, including SSRIs, the effect of *5-HTT* variants on clinical response to these drugs has been intensively studied.

The effects of *5-HTTLPR* on clinical response to fluvoxamine and paroxetine in two Italian samples revealed that the presence of at least one *l* allele was significantly associated with greater improvement in scores on the Hamilton Rating Scale for Depression. This finding was replicated by a study of Spanish Caucasians treated with citalopram, where the *s/s* genotype was shown to be more frequent in the group that did not respond to SSRI treatment. Moreover, homozygosity of the *l* allele was associated with a faster response to paroxetine in elderly American patients with depression, and Taiwanese patients with depression were found to respond better to fluoxetine in the presence of the *l/l* genotype.

On the other hand, several studies have suggested an association in the opposite direction, not only in Oriental but also in American patients treated with SSRIs. A significant association between specific SSRI response and the intron 2 VNTR 12/12 genotype has also been reported (Serretti *et al*, 2002). We have found a positive trend towards an association between the *5-HTTLPR l/l* genotype and response to treatment with TCAs in a group of Caucasian patients with unipolar or bipolar affective disorder (Tsapakis *et al*, 2003).

The tryptophan hydroxylase gene (*TPH*) and the brain-expressed *TPH* (*TPH2*) are further candidate genes implicated in the clinical response to SSRI treatment. *TPH* encodes the rate-limiting enzyme in the synthesis of 5-HT from tryptophan. Various non-functional *TPH* polymorphisms have been detected and, in a Finnish sample, the intron-7 779A/C polymorphism has been associated with suicidality and alcoholism. A further polymorphism (218A/C), located in a potential transcription factor binding site, may influence gene expression and, consequently, response to antidepressants. In fact, associations between *TPH* variants and response to both fluvoxamine and paroxetine have been reported (Serretti *et al*, 2001*a,b*).

The postsynaptic 5-HT$_{2A}$ receptor may also influence the efficacy of serotonergic antidepressants. Indeed, the C-containing variants of the 102T/C 5-HT$_{2A}$ polymorphism were associated with response to treatment with SSRIs, tricyclics and electroconvulsive therapy (ECT) (Minov *et al*, 2001). Furthermore, mutations in guanine nucleotide binding proteins (G-proteins), which represent the essential regulatory components in the transmembrane system of many receptors, might affect antidepressant efficacy. Zill *et al* (2000) have described an association between TT homozygosity in *Gβ3* with response to SSRI, TCAs and ECT combinations.

The serotonergic and dopaminergic systems are interconnected in the brain, and serotonergic projections inhibit dopamine function in the midbrain. SSRIs enhance dopamine function in the nucleus accumbens through increased expression of postsynaptic dopamine D$_2$ receptors, and an association between changes in the dopaminergic system and treatment response in major depression has been suggested. However, a study testing a *DRD2* and a *DRD4* polymorphism failed to show evidence of an association with efficacy of SSRIs (Serretti *et al*, 2001*c*). Clinically significant variations within the noradrenaline (norepinephrine) transporter (*NET* or *hNAT*) genes and the *ADRA2A* also seem to determine the side-effect profiles of antidepressant medications. Among others, the rare Ala457Pro variant of *hNAT* has been associated with orthostatic intolerance (a syndrome characterised by light-headedness, fatigue, altered mentation and syncope, and associated with postural tachycardia and plasma noradrenaline concentrations that are disproportionately high in relation to sympathetic outflow), and the transporter coded for by this variant has been shown to have reduced affinity for noradrenaline (Shannon *et al*, 2000). Furthermore, Aitchison *et al* (2002) have identified a complex microsatellite-like repeat region that appeared to be polymorphic, and the most common sequence variant in this region has been reported to be associated with anorexia nervosa (Urwin *et al*, 2002). The α$_2$-adrenergic receptor has been implicated in the aetiology of sexual dysfunction induced by SSRIs and other antidepressants, and the α$_2$ antagonist yohimbine was shown to improve this extremely common adverse effect in a small Swedish open trial (Jacobsen, 1992). Two high-frequency single-nucleotide polymorphisms (787C/G and 1817G/A) have been identified in Sweden (Liljedahl *et al*, 2003).

Pharmacogenetics of treatment with lithium

Lithium response has long been thought to have a genetic component, and changes in the serotonergic system have been implicated in the mechanism of

action of lithium. In a review of the literature, Ikeda & Kato (2003) note that a positive association between the presence of the *l* allele of *5-HTTLPR* and good response to lithium has been reported in a North Italian sample, but an earlier study from the South of Italy reported the *l/l* genotype to be associated with the non-responder phenotype. Furthermore, no association between response to lithium prophylaxis and the 102T/C or the 141C/T *5-HT*$_{2A}$ polymorphisms was found in another Italian sample. A significant association between *TPH* and prophylactic efficacy of lithium has also been reported. At therapeutic doses, lithium inhibits the activity of enzymes involved in the phosphatidylinositol and phospholipase C intracellular pathways, and a higher frequency of a phospholipase C γ-1 gene (*PLCG1*) repeat has been shown to be positively associated with lithium response in the treatment of bipolar disorder. In addition, a *PLCG1*-8 repeat was reported to be more frequent among lithium responders than normal controls, and it was also suggested that the 973C/A polymorphism in the inositol polyphosphate 1-phosphatase gene was an indication of positive lithium response. Washizuka *et al* (2003) have reported on the association of the mitochondrial DNA (mtDNA) 10398 polymorphism and maintenance lithium treatment response in a small Japanese sample of patients with bipolar affective disorder.

Alzheimer's disease

Although the aetiology of Alzheimer's disease remains largely unknown, three genes have been identified whose mutations cause the early-onset familial Alzheimer's disease and show nearly 100% penetrance with autosomal dominant inheritance. These genes encode β-amyloid precursor protein (*APP*), presenilin-1 (*PSEN1*) and presenilin-2 (*PSEN2*). Moreover, the risk of occurrence of Alzheimer's disease has been associated with apolipoprotein E (ApoE) and its three common isoforms Apo E2, E3 and E4. It has further been established that the *ε4* allele confers a significant risk for late-onset, sporadic Alzheimer's disease, the most common form of the illness. As a result, *E4* has served as the primary target for all pharmacogenetic studies related to Alzheimer's disease conducted to date, and it has been demonstrated that the therapeutic response to drugs in Alzheimer's disease is *ApoE* genotype-specific. Some studies have suggested that individuals carrying *ε4* respond poorly to the acetylcholinesterase inhibitor tacrine, compared with people with other genotypes, but other studies have produced conflicting results.

Patients with Alzheimer's disease may benefit from S12024, a drug that increases vasopressinergic activity. A multifactorial therapy combining three different drugs yielded positive results during 6–12 months' treatment in about 60% of patients. With this therapeutic strategy, *APOE-4/4* carriers were the worst responders, and patients with the *APOE-3/4* genotype were the best responders. It was also possible to differentiate the influential effect of *PSEN1* and *PSEN2* polymorphic variants on mental performance in response to multifactorial therapy (Cacabelos, 2003).

Genetic profiling of drug response in Alzheimer's disease was recently extended for tacrine and S12024, and Variagenics, a pharmacogenomics biotechnology company have patented an association with a polymorphism in the butyrylcholinesterase gene (Amouyel, 2002).

Pharmacogenetics of attention-deficit hyperactivity disorder

Family, twin and adoption studies in attention-deficit hyperactivity disorder (ADHD) have shown both an increased risk in the relatives of probands and a moderate to high heritability; transmission is believed to be polygenic, each gene having a small effect on phenotype. As regards the pharmacogenetics of ADHD, homozygosity of the 10-repeat allele at *DAT1* was associated with poor response to methylphenidate, and this finding has been replicated in a Brazilian study. A positive association between the 10-repeat allele at *DAT1* and response to methylphenidate has, however, been reported, and homozygosity of the 9-repeat allele at *DAT1* was associated with a poor response to methylphenidate. Further, it was shown that individuals with the 7-repeat allele at *DRD4* achieved less normalisation of symptoms and required 1.5 times more methylphenidate to achieve improvement than did those without the 7-repeat allele. Individuals who both presented the 7-repeat allele at *DRD4* and were homozygous for the *l* allele of *5-HTTLPR* showed a reduced improvement in general functioning during methylphenidate treatment (Rohde *et al*, 2003).

Newer developments

Besides drug-metabolising enzymes and neurotransmitter receptors and transporters, other important candidates that warrant further investigation have now emerged, mostly focusing on elucidating mechanisms responsible for the slow onset of therapeutic effect of psychotropics

(particularly antidepressants). Such mechanisms may include the evoking of adaptive changes in intracellular signal transduction and synaptic connectivity. There has been an extensive search for genes to explain this phenomenon, and current candidates include the following: brain-derived neurotrophic factor (BDNF), G-proteins, the association between mood stabilisers (e.g. lithium and sodium valproate) and human B-cell lymphoma protein-2 (Bcl-2), and other related genes such as *Bcl-2* associated athanogene-1 protein (Bag-1).

Ethical considerations

The emergence of pharmacogenetics is about to mark a new era in clinical psychiatry, in which genotype or other biomarkers may influence choice of therapy, increasing the safety and efficacy of commonly used medications. The ethical issues that arise from pharmacogenetic research and its clinical applications (Box 2) should, however, be addressed before the benefits of treatment individualisation can be realised (Buchanan *et al*, 2002).

At present, it is unclear whether, or to what extent, pharmacogenetic tests will be included in standard packages of care. Much will depend on whether pharmacogenetic testing appears to be cost-effective in the long-run, when the costs of integrating the methodology into the health care delivery system are taken into account. However, if a test's positive predictive value and utility in determining whether to prescribe a drug, in what dosage and in what combinations with other drugs are well established, then physicians would probably have a duty to offer it if the benefits outweigh the risks and costs of doing so. Consequently, physicians are likely to have to learn the role that pharmacogenomic tests may play in choosing therapy and to respond accordingly. The pace at which these tests will be integrated into psychiatric practice will, no doubt, differ across diseases and between specialists and generalists. Without major changes in the teaching of genetics psychiatric problem include random errors, confounding and bias, and effect modifications and interactions (Box 3). The rigorous application of basic epidemiological principles to avoid confounding, misclassification and bias may not be sufficient. Matching according to ethnicity and/or the ethnic homogeneity of the study population become important, although these are often difficult to achieve in practice. Hence, testing for hidden population stratification is mandatory and can be performed at tolerable expense by use of a limited number of multi-allelic unlinked marker loci (Pritchard & Rosenberg, 1999). This strategy should allow for the detection of moderate or even minor differences in ethnic composition, and therefore the question of statistical power is crucial. For research into disease susceptibility genes, most investigators

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Box 2 Ethical issues in pharmacogenetics

Pharmacogenetic tests would need to be approved and overseen by major national regulatory bodies such as the UK Medicines Control Agency (MCA)

- Over 500 pharmaceutical company and government representatives from around the world recently discussed the Food and Drug Administration's draft guidance for industry on pharmacogenomics data submission (Abbott, 2003)

Appropriate protection for privacy and confidentiality is crucial

- Pharmacogenetic tests can carry several types of potentially psychosocially harmful secondary information
- Genotype-based information about drug response may inform prognosis

Information on poor drug response could easily affect an individual in terms of:
- employment opportunities
- insurance status
- their relatives, who might be genetically predisposed to the same mental illness and therefore might also take the drug.

Participation in pharmacogenetic research requires informed consent, and participants should be fully informed of:
- the need to obtain DNA for the research
- the protections of confidentiality and privacy (including how the sample and test results will be stored and their privacy and confidentiality maintained)
- whether further research may be done using their DNA in the future
- the risks and benefits of providing DNA for participation in the trial
- whether research results will be disclosed to the participants

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nowadays consider studies with family controls (e.g. so-called trios, where the genotype of an individual is studied with that of two of his or her siblings) as the best option. This approach, however, is not usually feasible for the study of genes that modify drug response or the risk of adverse drug effects. Such studies require families in which several members have the same disease and are exposed to the same drug; this is only rarely seen (even retrospectively). Thus, genetic modifiers of drug response and adverse drug reactions usually have to be studied in unrelated controls, and population stratification may be excluded in these instances. Multiple testing is another major concern in genetic association studies. Sample sizes should be large enough to allow all predefined hypotheses to be tested at the desired confidence level, and all other tests should be identified as explorative.

Future directions

In view of the large numbers of conflicting studies and the need for careful data collection and statistical, epidemiological and genetic analysis during data generation, future pharmacogenomic analysis should be integrated much more widely into clinical psychiatric research. If study participants were stratified or even selected according to their pharmacogenetic traits, variabilities in pharmacokinetics and drug response could be reduced considerably. Sample sizes could then be smaller and the studies less expensive, although selection biases would have to be carefully avoided as they could materially compromise study results. According to accepted criteria of evidence-based medicine, the value of pharmacogenetic testing for routine clinical practice should be verified prospectively in clinical trials. Proven benefits and pharmaco-economic considerations (e.g. the number of tests necessary to avoid one case of toxicity), including the costs of pharmacogenomics test kits, are among the factors that will determine whether or not such tests will become part of routine clinical practice.

Box 3 Causes of discrepant results among different association studies on the same problem (adapted from Meisel *et al*, 2003)

Random errors
- multiple testing
- inappropriate sample size
- inappropriate selection of genetic variants
- errors in genotyping

Confounding and bias
- genetic heterogeneity (the phenotype is affected by variants in numerous genes)
- aetiological heterogeneity (several causes lead to the same phenotype)
- differences in ethnic background within the population studied
- population stratification (multiple sub-groups, each with different allele frequencies)
- inappropriate or missing consideration of haplotypes
- intermixing of incident and prevalent cases of the same phenotype
- inappropriate control groups

Effect modifications and interactions
- undefined gene–gene interactions
- undefined gene–environment interactions

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MCQs

1 With regard to clozapine:

a response to treatment with clozapine is subject to interindividual variations

b it is primarily metabolised by CYP1A2

c it is unlikely to be metabolised by CYP2D6

d dopamine D_4 receptor polymorphisms have been shown to be strongly associated with clozapine response

e combinations of pharmacodynamic gene polymorphisms have been shown to predict response to clozapine.

2 Regarding tardive dyskinesia:

a susceptibility to the development of tardive dyskinesia is thought to have a genetic basis

b the AIMS is a commonly used scale for rating the presence of tardive dyskinesia

c the *DRD3* Ser9Gly polymorphism has been significantly correlated with the development of tardive dyskinesia

d the development of tardive dyskinesia has not been linked with *CYP1A2*

e no association has been shown between the development of tardive dyskinesia and superoxide dismutase genes.

3 The serotonin transporter-linked polymorphic region (*5-HTTLPR*):

a is an insertion/deletion polymorphism

b has been identified in intron 2 of the serotonin transporter gene

c has been associated with clinical response to SSRIs

d has been associated with clinical response to TCAs

e has not been associated with response to lithium treatment.

4 The following are genes thought to be associated with the corresponding phenotypes:

a the *5-HT_{2A}* gene and response to treatment with electroconvulsive therapy

b the HLA gene family and clozapine-induced agranulocytosis

c the phospholipase C γ-1 gene and lithium response in the treatment of bipolar disorder

d the apolipoprotein $\varepsilon 4$ gene and poor response to tacrine in the treatment of Alzheimer's disease

e the dopamine transporter gene and response to methylphenidate in the treatment of ADHD.

5 Rate the following statements as true or false:

a haloperidol is metabolised via CYP2D6

b *CYP2D6* polymorphisms do not affect the metabolism of nortriptyline

c poor CYP2D6 metabolisers have multiple functional copies of a *CYP2D6* gene

d up to 10% of Caucasians may be CYP2D6 ultrarapid metabolisers

e the P450 enzymes are Phase II drug-metabolising enzymes.

MCQ answers

1		2		3		4		5	
a	T	a	T	a	T	a	T	a	T
b	T	b	T	b	F	b	T	b	F
c	T	c	T	c	T	c	T	c	F
d	F	d	F	d	T	d	T	d	F
e	T	e	F	e	F	e	T	e	F