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Genetics of novel therapeutic targets in schizophrenia

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For many years, following the introduction of chlorpromazine in the 1950s, little progress was made in the discovery of new drugs for schizophrenia (Reynolds, 1992). Dopamine D_2 receptor blockade was recognised as the only therapeutic target for antipsychotics (Creese *et al*, 1976) and the inevitable consequences of striatal blockade remained problematic. However, the strategies and stimuli for discovery of new drugs changed with the introduction of new, atypical antipsychotics in the 1990s. These include clozapine, remoxipride (now withdrawn), olanzapine, risperidone and sertindole (Kerwin & Taylor, 1996). The goal of antipsychotic drug development has always been to widen the therapeutic ratio between efficacy and adverse effects. These new drugs have in the main achieved this. However, which therapeutic targets these drugs employ remains a mystery, and this information is clearly important for future research into more selectively targeted agents.

When the atypical drugs clozapine and olanzapine were introduced it became clear that these drugs do not act at D_2 receptors, so that all their other receptor targets represent candidates for sites of action (Pilowsky *et al*, 1992, 1996). Risperidone and sertindole also have a rich pharmacology. It is postulated that the balance between D_2 and 5-HT_2 receptor blockade is responsible for actions against negative symptoms and protection against extrapyramidal side-effects (EPS) (see, for instance, Reyntjens *et al*, 1986). However, this remains speculative. The latest antipsychotics include quetiapine and ziprasidone, both of which have broad-spectrum pharmacology. It is clearly important to identify what may be novel sites from the pharmacology of these drugs.

The main sites of interest are the novel dopamine receptors, especially the D_2 isoforms, D_3 and D_4, and some 5-HT receptors, especially 5-HT_{2a}, 5-HT_{2c}, 5-HT_6 and 5-HT_7. However, these sites are not amenable to conventional studies such as functional receptor imaging and post-mortem studies. Fortunately, a molecular genetic approach has proved very fruitful in assessing whether these receptors are likely to be of interest. This has been achieved in two ways: first, using a case–control association approach, with these sites as candidates for predisposition to schizophrenia, and hence automatically as candidates for sites of drug action; and, second, using a pharmacogenetic association approach, attempting to see if any functional variation in polymorphisms for these receptor sites is associated with altered clinical response.

It can be seen from Table 1 that potential non-striatal targets are D_2, D_3, D_4, 5-HT_{2a} and 5-HT_{2c}. The next section will review gene association studies and pharmacological genetic studies of candidate sites which have attempted to confirm these sites as useful targets for antipsychotic drug effects.

APPROACHES TO PHARMACOGENETICS

Candidate gene/case–control association studies

A 'candidate gene' is one which a knowledge of pathophysiology or of the mode of action of effective treatments suggests may have a role in the disorder. Once a candidate gene has been identified, it can be studied by a variety of genetic methods.

The most effective strategy is to identify polymorphisms or mutations within the gene that are likely to influence protein structure, function or expression, and to compare the frequency of these between matched groups of patients and controls. Needless to say, genes identified in this way will be of considerable interest as potential pharmacological targets, though of course lack of association does not rule out the gene products as therapeutic targets.

The D_3 receptor

The 'candidacy' for the D_3 receptor stems from its predominantly limbic site and from the fact that it shows high-affinity binding to both typical and atypical antipsychotics which also elevate D_3 mRNA (Buckland *et al*, 1993). A serine-to-glycine polymorphism at position 9, close to the N-terminal of the protein, has been widely studied. This produces a digestion site for the restriction endonuclease Bal I. Two groups initially reported an excess homozygosity in patients suffering from schizophrenia, both in Wales (Crocq *et al*, 1992). This finding has subsequently been confirmed in several studies (Nimgaonker *et al*, 1993; Mant *et al*, 1994; Williams *et al*, 1997), though a number of non-replications have also been reported. A meta-analysis of all data published at that time showed significant evidence for an association between schizophrenia and the 1-1 (Ser-Ser) genotype (Shaikh *et al*, 1996). A more recent and complete meta-analysis of published and unpublished data from 24 studies showed significant evidence for homozygosity, with an odds ratio of 1.21 as well as significant excess of the 1-1 genotype alone (Williams *et al*, 1997). Although this is a weak susceptibility gene which has little effect, this does not diminish its importance as a possible major target. It does not necessarily imply that any pharmacologically mediated effect would be small, or would not apply in a wider group of patients.

The D_4 receptor

The candidacy of the D_4 receptor stems from its unique limbic site (Van Tol *et al*, 1992) and its uniquely high affinity for clozapine and other atypical antipsychotics. Particular interest has focused upon a polymorphic 48bp repeat that codes for variation in the third intracytoplasmic loop and which apparently alters sodium-dependent clozapine binding when expressed *in vitro* (Van Tol *et al*, 1992). However, without exception, both linkage and case–control association studies have failed to provide evidence that this variant predisposes to schizophrenia. Linkage studies have been performed by British (Shaikh *et al*, 1994), Swedish (Barr *et al*, 1993), Italian (Macciardi *et al*, 1994) and French (Campion *et al*, 1994) groups. Several case–control studies (Petronis *et al*, 1993; Daniels *et al*, 1994) have failed to find any significant association. One small study

Table 1 Receptor binding profiles of antipsychotics

Drug	Affinity for receptor [Ki (nmol/l)]			α_1	α_2	Histamine	Serotonin	Serotonin
	Dopamine D_1	Dopamine D_2	Dopamine D_4	adrenergic	adrenergic	H_1	5-HT_{2a}	5-HT_{2c}
Existing antipsychotics								
Clozapine	85	126	9	7	8	6	12	8
Haloperidol	25	1	5	46	360	>1000	78	>1000
Remoxipride[1]	>1000	274	>1000	>1000	>1000	>1000	>1000	>1000
Risperidone	75	3	7	3	155	0.6	26	
New antipsychotics								
Olanzapine	31	112	27	19	228	7	4	11
Quetiapine (ICI 204636; Seroquel™)	455	160	NA	7	87	11	220	615
Sertindole[2]	28	41	NA	3.4	350	600	0.39	NA
Ziprasidone	68	8	7.4	7.9	NA	7.3	9	8

1. Drug withdrawn from the market due to adverse effects.
2. Values expressed as IC_{50}.
IC_{50}, concentration of the drug that inhibited binding of a radioligand by 50%; Ki, inhibition constant; NA, no data.

has shown an association between a polymorphism in exon 1 on the D_4 gene and delusional disorder (Catalano *et al*, 1993), but this awaits replication. Although these findings do not rule out the D_4 receptor as a therapeutic target, there is no evidence that variation in this gene predisposes to schizophrenia.

5-HT_{2a} receptors

The 5-HT_2 receptor family has for long been a candidate in this area. The psychotomimetic lysergic acid diethylamide I (LSD) is a 5-HT_2 agonist (Bennet *et al*, 1979). A wide range of atypical antipsychotics, including clozapine, olanzapine, risperidone and sertindole, have high affinities for the 5-HT_{2a} receptor. Again single photon emission tomography (SPET) and positron emission tomography (PET) studies indicate high occupancies *in vivo* of atypical antipsychotics at 5-HT_2 receptors. The first polymorphism to be studied was a thymidine(T)-to-cytosine(C) polymorphism at position 102 of the gene. A preliminary report indicated that higher than expected proportions of allele 2 (C) and genotype 2/2 were found in patients with schizophrenia (Inayama *et al*, 1996). This was confirmed by the European multicentre association study of schizophrenia (Williams *et al*, 1996) and subsequently in a study from a German group (Erdmann *et al*, 1996). Although the odds ratios were small (1.7 for possession of one or more copies of allele 2), the attributable fraction is high (0.38) because allele 2 is relatively common in the population. This association is therefore of considerable potential therapeutic importance. Some studies have failed to replicate these findings (Arranz *et al*, 1996; Jonsson *et al*, 1996; Malhotra *et al*, 1996; Nimgaonkar *et al*, 1996; Sasaki *et al*, 1996). These studies were all small-scale and lacked power. Indeed, the results of a recent meta-analysis which included all published studies support the presence of an association between schizophrenia and allele 2, with no evidence of publication bias (Williams *et al*, 1998). However, the T102C polymorphism is 'silent' and does not alter the structure of the protein. It has therefore been hypothesised that a nearby polymorphism within the gene or its promoter region is the true pathogenic variant and is in linkage disequilibrium with T102C. Two low-frequency structural variants, His452Tyr and Thr25ASN, are not associated with schizophrenia (Erdmann *et al*, 1996). Current interest is focused upon polymorphisms in the promoter as possible functionally important variants. This association with a large attributable risk makes this a highly interesting candidate for a gene predisposing to schizophrenia, and makes it a very strong candidate for a selective therapeutic target.

5-HT_{2c} receptor

The 5-HT_{2c} receptor is a relatively weak candidate. It was originally thought to be restricted to the choroid plexus but has now been found in fronto-temporal regions, which have been associated with the neuropathology of schizophrenia. Although atypical antipsychotics usually have modest affinities at 5-HT_{2c} receptor (Meltzer, 1994), when clozapine passes by iontophoresis onto areas rich in 5-HT_{2c}, it has powerful effects on neuronal firing rates (Ashby & Wang, 1990). There are virtually no case–control association studies of 5-HT_{2c}. The only available published information is from Sodhi *et al* (1995), as part of an allelic association study (see below), which could not find any excess of cystine-to-serine variation at position 23 of the 5-HT_{2c} gene. More work is required in this area.

In conclusion, the finding that variation within receptor genes predisposes to schizophrenia certainly suggests that relevant receptors might be useful therapeutic targets. A complementary strategy for identifying therapeutic targets is to seek genetic variation that influences clinical responses in so-called pharmacological genetic association studies.

PHARMACOLOGICAL GENETIC ASSOCIATION STUDIES

A drug like clozapine is a useful tool for this type of study. We know that the drug has multiple receptor actions and that it does not seem to act conventionally via D_2 receptors (Pilowsky *et al*, 1992). It follows that pharmacological genetic studies of the response to clozapine can be used to test the involvement of the other receptors upon which it acts. The technique is being increasingly used to define and quantify drug

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effects, not only at pharmacodynamic sites of action, but also to understand the differential types of metabolism for certain drugs. This technique identifies therapeutic targets, explains why individuals are resistant or react idiosyncratically to drugs, and explains why individuals may be particularly resistant or vulnerable to the side-effects of drugs. The technique requires large samples of well-characterised patients on a particular drug.

These studies describe large cohorts of patients on clozapine, comparing polymorphic variation with clinical response. It is hoped that this will ultimately generate selective or oligoselective drugs acting at single therapeutic targets in the brain.

D_3 receptor

The D_3 Ser-gly polymorphism has also been studied in pharmacological genetic studies. There are only three published studies but all are in agreement. Jonsson *et al* (1993) found an excess of 1-1 homozygosity in good responders to antipsychotic medication, and Mant *et al* (1994) demonstrated the same phenomenon, which was also associated with male gender and a positive family history. Shaikh *et al* (1996) found genotype 1-1 to be associated with non-response to clozapine in patients who had not previously responded to typical neuroleptics. This makes the D_3 receptor even more likely to be an important target for selective drugs.

D_4 receptor

Pharmacological genetic association studies have also ruled out a role for the 48bp repeat in the third intracytoplasmic loop of D_4 in determining clozapine response. The initial findings of Van Tol *et al* (1992) generated a particular hypothesis, that poor responders have an excess of longer repeats and good responders an excess of shorter repeats. Several studies have now addressed this issue. Shaikh *et al* (1993) could find no association in variants between 41 responders and 23 non-responders. Kennedy and colleagues, in two studies (1993, 1994), also failed to show any association. In order to test for weak effects, we looked at a larger series of over 200 patients (Shaikh *et al*, 1994) and again failed to find any relationship. A fourth study was later performed (Rao *et al*, 1994), this time using an external comparison with fluphenazine or placebo, but no associations were revealed.

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Thus functional polymorphism does not appear to determine clozapine response. However, there may be other structural or regulatory polymorphisms in D_4 that are involved in drug response.

5-HT_{2a} receptor

In support of the associations found in candidate gene case–control studies, we also reported an association between T102C in the 5-HT_{2s} receptor gene and clinical response to clozapine (Arranz *et al*, 1995). This finding has not been unambiguously replicated (e.g. Masellis *et al*, 1995; Noethen *et al*, 1995). However, a further association has been found with a structural polymorphism His452Tyr and response to clozapine (Arranz *et al*, 1996), and this has been replicated (Badri *et al*, 1996). But, this polymorphism is rare, and the association seen with clozapine response is weaker than the one seen with T102C. A novel G-to-A base change in the promoter region at position -1438 has been detected. Unfortunately, initial functional studies did not obtain evidence that this variant alters promoter function and further work is needed to determine the nature of the 'active' polymorphism in the 5-HT_{2a} receptor gene. However, these results continue to add strength to the notion of the 5-HT_{2a} receptor as an important mediator of antipsychotic action.

The 5-HT_{2c} receptor

Again, in contrast to the 5-HT_{2a} receptor, there has been little work in this area. One study has been published, and this shows quite a strong effect (Sodhi *et al*, 1995). Ninety per cent of subjects who had one or more of the 23 serine alleles (19/21) were clozapine responders, compared with 59% (84/141) without this allele. This result, in its own right, indicates that the 5-HT_{2c} receptor may contain a site for antipsychotic action, but further work on this understudied receptor needs to be performed.

CONCLUSIONS

It is useful to look at the candidate gene and the pharmaco-association studies together, to see what firm conclusions can be drawn, where further work is needed and what future studies should be performed.

Clearly the negative results for D_4 in both sets of studies are not encouraging for its major role as a therapeutic site. Comprehensively positive studies certainly rule-in the 5-HT_{2a} site, and this should be considered for the next phase, looking at functional changes in binding and second messenger systems, using *in vitro* studies.

It should also be possible to look for functional differences in binding and gene expression in genotyped brain tissue. Functional differences may also be seen *in vivo* with SPET. If these studies support the findings from association studies, consideration should be given to the development of ultra-selective 5-HT_{2a} drugs. The D_3 receptor comes out weakly positive in both candidate gene and pharmacogenetic studies, and further replication studies are required here. Work on the 5-HT_{2a} receptor remains trivial but interesting, and a great deal more effort should be focused on this site. Finally, it is vital that this approach is followed through on large samples of other atypical antipsychotics, such as olanzapine, risperidone and sertindole.

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