5. PSYCHOPHARMACOGENETICS OF SCHIZOPHRENIA AND PSYCHOSIS

Dr. Joachim Scharfetter*

1. INTRODUCTION

Psychotic disorders are severe psychiatric conditions, frequent, disabling, with patients at high risk for suicide, for addiction and social decline. The most prominent of psychotic disorders is schizophrenia with a lifetime prevalence of about 1%. Almost all insights into the pharmacogenetics of psychosis are derived from schizophrenic patients therefore all the following comments will generally refer to this disorder.

Until the development of antipsychotic medication means for therapy have been sparse and the course of the disease frustrating, often leading to chronic illness and social decline. The situation improved profoundly with the advent of antipsychotic medication in the 1950's, later called the typical antipsychotics or neuroleptics. These pharmacological substances are very potent in their antipsychotic efficacy but often inducing severe side effects, for the most part extrapyramidal motor disorders like akathisia, parkinsonism, and early and late onset dyskinesias, beyond else.

The next milestone in pharmacological antipsychotic treatment was the development of the so called atypical antipsychotics (second generation antipsychotics) with Clozapine as their prototype in the 1970's. The term atypical was coined for pharmacological agents (almost) without extrapyramidal side effects that generally have a higher affinity to the 5-HT2A receptor than to the Dopamine D2 receptor. Clozapine was a big success, improving psychotic conditions in patients that were hitherto refractory to treatment. Nevertheless Clozapine was withdrawn from the market due to the severe side effect of agranulozytosis. Owing to its unique and indispensable efficacy in treatment refractoriness the substance was reintroduced in the late 1980's under stringent safety regulations. The 1990's saw the advent of further atypical antipsychotics and today we are equipped with a multitude of pharmacological treatment options.

* Joachim Scharfetter, Universitatsklinik fur Psychiatrie, Abteilung fur Allgemeine Psychiatrie, Wahringer Gurtel 18-20, A-1090 Wien, Austria. joachim.scharfetter@akh-wien.ac.at.

There are various strategies for the clinical decision on what substances to prescribe for which patient. These strategies are partially dependent on pharmacodynamic properties of the substances, on the specific symptomatology of the patients, side effect profiles and treatment history. Substances like Clozapine or Quetiapine are preferred when extrapyramidal motor side effects are to be omitted, Amisulpride is administered in patients with predominantly negative symptomatology, Ziprasidone is renown for causing less weight gain, Clozapine is the substance for treatment resistance, to name a few.

Nevertheless a considerable portion of patients still fail to respond to treatment, despite adequate dosage and duration and moreover patients are often suffering from severe side effects. Consequently psychosis itself and its treatment remains a cost intensive and sometimes frustrating endeavor. Despite considerable efforts there has been no success so far in determining predictive factors for treatment response and relapse. Antipsychotic therapy still remains try and error to a considerable degree.

In this context pharmacogenetics of schizophrenia have gained much attention recently. Targeting treatment more efficiently on the basis of a simple genetic test would have a considerable economic and not the least ethical impact. There have even been proposals for presymptomatic genetic diagnosis and prophylactic treatment [Tsuang et al. 2000, Hurko 2001], after all with considerable ethical caveats of such a proposal to be considered.

Many considerations on methodology and study design in the following have been proposed by Rietschel et al. [Rietschel et al. 1999] in a consensus conference on the application of pharmacogenetics to psychotic disorders, valuable input comes from Masellis et al. [Masellis et al. 2000] in their publication about pharmacogenetics of Clozapine.

2. METHODOLOGY

Dealing with pharmacogenetic studies in psychosis we are almost exclusively confronted with case-control association study designs where it is investigated if the presence (case) or absence (control) of a specific phenotype can be paralleled to a specific genotype. A methodological prerogative for such studies is the fact that the genes whose genetic polymorphisms are investigated have to have an a priori evidence of being involved into the pathogenesis of the phenotype.

It has recently been proposed to preferentially investigate only polymorphisms with functional consequences (that is alteration of the transcribed proteins or changing gene expression by being located in regulatory regions) with the aim to increase the prior probability of detecting valid associations. Silent mutations in the “degenerated” third position of codon triplets, in introns or in the vast areas of non-coding “junk” DNA can nevertheless not be completely disregarded. Seen apart from the possibility that they might be in linkage disequilibrium with functionally relevant sites nearby, they might introduce alternative splicing sites or affect stability or accessibility of DNA. The functional significance of non-coding DNA regions is being intensively discussed just recently. “Junk-DNA”, as these non-coding regions have been termed due to their seemingly uselessness, seem to be highly

conserved throughout evolution and they would not be if really useless [Bejerano et al. 2004]. Many of these non-coding regions are after all transcribed into non-protein-coding RNA with so far unknown purpose [Cawley et al. 2004].

Another methodological issue controversially discussed recently in medical statistics is correction for multiple testing. The latter has often been demanded for reasons of methodological rigor. Authors as for example Dettling et al. [2001b] and Illi et al. [2003] refrained from applying a multiple hypotheses testing adjustment referring to recently published critical comments on this issue [Pernegger 1998].

3. PHENOTYPE

The phenotype definition for pharmacogenetic studies in psychosis is complex since the phenotype is trait- (psychosis) as well as state- (being under pharmacological treatment) dependent. All patients enrolled must be pharmacologically treated with antipsychotics and must have a diagnosis of psychosis that is schizophrenia or schizophrenia and schizoaffective disorder respectively.

Schizophrenia is most likely a complex trait with multiple genetic, developmental and environmental factors contributing to the liability to develop the disease. Consequently there is heterogeneity in symptomatology and pathogenetic pathways probably leading to individual peculiarities in the accessibility to pharmacological treatment. A stringent phenotypic definition of schizophrenia therefore seems to be a prerogative for informative study designs.

Diagnostic subtypes of schizophrenia however could not be related to variability in treatment response and individual psychotic symptoms could not be specifically targeted by different antipsychotic drugs [Nimgaonkar et al. 1988]. Almost all pharmacogenetic studies cited in the following do not take schizophrenia subtypes into account. Schizophrenia and schizoaffective disorder in these studies are primarily diagnosed according to DSM-IV (Diagnostic and Statistical Manual of Mental Disorder, Fourth Edition) [American Psychiatric Association 1994] or ICD-10 (International Classification of Disease, Tenth Edition) [World Health Organization 1992]. Sometimes detailed diagnostic manuals like SCID (Structured Clinical Interview for DSM-IV Diagnoses) [Spitzer et al. 1990], DIGS (Diagnostic Interview for Genetic Studies) [Nurnberger et al. 1994] or SADS (Schedule for Affective Disorders and Schizophrenia) are applied [Endicot and Spitzer 1978].

Another confounding variable in phenotype definition is ethnic heterogeneity in patients that has in fact been found to influence antipsychotic response [Frackiewicz et al. 1997]. This latter observation is imperatively asking for ethnically homogenous samples or better for methods to control for ethnic heterogeneity [Rosenberg et al. 2002].

On the other hand a stringent phenotype definition asks for patients that are uniformly treated with the same antipsychotic, a prerequisite that is not always met by study designs. Since molecular targets and metabolic pathways of different antipsychotic agents are quite divers, genotypes putatively influencing their efficacy are most likely divers too.

Based on this universal set of schizophrenic/schizoaffective/psychotic patients treated with an antipsychotic drug, the phenotype under investigation in the stricter sense is the clinical effect of treatment. In the desired form this effect is defined as amelioration or disappearance of symptoms, in the following called response, and in the undesired variant it is called side effects.

Assessment of response is preferentially done by applying scales, response is then defined either by proposing a cut-off or as a continuous measure. It has been demanded that the scales applied should be able to differentiate between psychopathological dimensions as positive symptoms (hallucinations, delusions) and negative symptoms (avolition, affective flattening). Widely used scales are the PANNS (Positive and Negative Syndrome Scale) [Kay et al. 1987], BPRS (Brief psychiatric Rating Scale) [Overall and Gorham 1962], GAS (Global Assessment Scale) [Endicott et al. 1976], SANS [Andreasen 1989] and CGI [Guy 1976].

The definition of the phenotype response has to take the dimension of time into account since a minimal period of pharmacological treatment is required for symptoms improvement. An evaluation of treatment response after four to six weeks is commonly applied, a continuation up to six month is recommendable.

An elegant means to circumvent problems of response definition could be the use of endophenotypes. Endophenotypes are assessed by measuring physiological parameters of pharmacological effects as for example neuro-endocrine responses, receptor occupancy or changes in cerebral blood flow.

Basically the same issues hold true for the definition of side effects. The most prominent of antipsychotics treatments side effects are the extrapyramidal motor side effects, these are dealt with in a chapter of their own. Weight gain as another prominent side effect is commonly defined by a cut off of >7%, sometimes treated as a continuous variable. The Neuroleptic Malignant Syndrome has been investigated using the clinical diagnosis criteria by Pope et al. [Pope et al. 1986]. A treatment regimen with Clozapine apparently is a prerequisite for developing Clozapine induced agranulozytosis (CA). The latter is consistently defined as less than 500 neutrophil counts per mm^3 of whole blood.

Besides these more prominent side effects there are only a few studies on secondary adverse events like urinary incontinence and hypotension. No studies to my knowledge are existing on the pharmacogenetics of neuroleptic induced QT syndrome and diabetes.

4. GENOTYPES

In the following polymorphisms of genes will be discussed that have been targeted in pharmacogenetic studies. These will be presented with special emphasis on their functional importance.

4.1. Overview with respect to specific phenotypes

Since the methodological basis for pharmacogenetic studies in psychosis is a candidate gene association approach, all genetic polymorphisms investigated have to have an a priori evidence for their putative impact on the phenotype under investigation. In other words, the choice of genes whose polymorphisms should be investigated has to be hypothesis driven.

4.1.1 Response (see table 2-6)

One of the first and most prominent hypotheses about the efficacy of antipsychotic medication has been the dopamine hypothesis (renewed and modified by the "fast-off D2 hypothesis" by Kapur [2000] for the quality of neuroleptics to be atypical). Dopamine receptor genes are consequently a prime target for studies investigating the pharmacogenetics of antipsychotic response. Nevertheless the serotonin receptor genes have been the first to be intensively investigated. This is apparently due to the fact that most samples investigated in the beginning of pharmacogenetic studies in psychosis have been in patients treated with the "atypical" drug Clozapine. One of the first hypotheses for the quality of being atypical has been the preferential efficacy of such antipsychotic drugs in the serotonin neurotransmitter system.

Variation in antipsychotics treatment efficacy has repeatedly been assumed to be connected to plasma levels of the prescribed substances. Even though a simple concentration-effect relationship for antipsychotic drugs has never been substantiated [see Bengtsson 2004] the most prominent drug metabolizing enzyme system, the Cytochrome P450, has been focused on in early pharmacogenetic response studies.

Furthermore a variety of neurotransmitter receptor genes have been investigated, namely adrenergic, histaminergic, cholinergic and glutamatergic receptors, due to their putative involvement into the pathophysiology of psychosis and the fact that they are part of the target spectrum of antipsychotic drugs.

The rationale for investigating the HLA system is to be found in the fact that the HLA genes are located on chromosome 6 which has been a positive finding in genetic linkage studies of schizophrenia [see Lahdelma et al. 1998]. Apolipoprotein E ε4 has been associated with less severity of negative symptoms [Hong et al. 2000]. Neurotensin is a neuroregulatory peptide involved into the regulation of neurotransmitter circuits. Catechol-O-methyltransferase (COMT) and monoamine-oxidase (MAO) are enzymes participating in the inactivation of biogenic amines as the neurotransmitters dopamine, serotonin and norepinephrine. The brain derived neurotrophic factor (BDNF) is an important member of the nerve growth factor family involved into neurodevelopment. Brain development abnormalities are one of several paradigms for schizophreniform disorders. Namely dopaminergic systems in the brain and dopamine receptor expression are affected by BDNF [Krebs et al. 2000]. The methylenetetrahydrofolate reductase (THFR) is an enzyme contributing to the folate metabolism and thereby involved into homocysteine regulation. High plasma levels of homocysteine have been paralleled to developmental abnormalities. Schizophreniform symptoms have been described in several homocysteinuric patients and case reports about positive outcome of folate treatment of psychotic patients have been published [Joober et al. 2000b].

Table 1. Antipsychotic Response Cytochrome P450 - CYP2D6

referenc	polymorphism	notes	sample	treatment	phenotype	result
Arranz 1995b	PM(CYP2D6-A and -B) and EM	PCR	123 schizophrenics	Clozapine	GAS 20-point improvement	n.s.
Lane 1997	PM, EM	dextrometho rphan phenotyping	18 chinese schizophrenics	Haloperidol	BPRS improvement >35%	n.s.
Aitchiso n 1999	PM (CYP2D6*3, -*4, -*5), EM, UM (duplications)	PCR	235 treatment resistant schizophrenics/sc hizoaffectives vs. 73 schizophrenic controls	miscellaneous typical	treatment resistance defined as by Kane et al. 1988	n.s., trend for ultrafast metabolizers in response group
Hamelin 1999	EM (genotypes containing of at least 1 CYP2D6*1), PM (all other enotypes with CYP2D6*3, -4, -5, -6, -7)	PCR	39 schizophrenics	miscellaneous NL	BPRS continous	n.s.
Brockm öller 2002	PM, IM, EM, UM (for specific genotypes see publication)	PCR	172 acute psychotics	Haloperidol	PANSS continous	n.s., trend for fast metabolizers in non-response

n.s. not significant

Table 2. Antipsychotic response and combined genotypes?

reference	polymorphism	notes	sample	treatment	phenotype	result
Arranz 2000b	5HT2A: His452Tyr, Thr25Asp, -1438G/A, 102T/C, 516C/T 5HT2C: -330GT/-244CT, Cys23Ser 5HT3A: 178C/T, 1596G/A 5HT5A: -12A/T, -19G/C 5HTT: 5-HTTLPR, VNTR DRD3: Ser9Gly ADRA1A: Arg492Cys ADRA2A: -1291G/C, -261G/A H1: Leu449Ser H2: -1018G/A	no correction for multiple testing treatment response retrospective ly assessed	200 schizophrenic patients	Clozapine	response: GAS	combination of 6 polymorphisms predict clozapin response: 5HT2A: 102T/C His452Tyr 5HT2C: -330GT/-244CT Cys23Ser 5HTT: 5-HTTLPR H2: -1018G/A
Schumacher 2000	Same combination of 6 response predicting genotypes as Arranz 2000b	an attemt to replicate Arranz 2000b	163 schizophrenic patients	Clozapine	4 response groups, group 3 and 4 corresponding to 20 point GAS improvement	n.s. Only H2: -1018 G/A associated with response on the allelic level

n.s. not significant

4.1.2 Agranulozytosis

Table 3. Side Effect: Clozapine-induced Agranulozytosis (CA)
HLA-System

reference	polymorphism	notes	sample	treatment	phenotype	result
Lieberman 1990	HLA-A, -B, -C, -DR, -DQ (HLA-class I and II antigens)	serotyped	schizophrenic and schizoaffective Ashkenazi patients 5 patients with Clozapine induced Agranulozytosis (CA), 26 controls	Clozapine	agranulozytosis: less than 0.5x109/L polymorphonuclear leucocytes	agranulozytosis associated with a haplotype consisting of HLA-B38, -DR4, -Dqw3
Claas 1992	HLA-class I and II antigens	serotyped	103 patients with CA, 95 matched controls	Clozapine	granulozytopenia: less than 1500 granulozytes/ml	n.s. after correction for multiple testing
Yunis 1992	HLA-class I and II antigens	serotyped	11 CA patients, 31 controls, most of jewish ancestry	Clozapine	agranulozytosis: less than 0.5x109/L polymorphonuclear leucocytes	agranulozytosis associated with HLA-B38, -DR4 and –DQw3 haplotype in jewish patients; with HLA-DR2 and –DQw1 in non-jewish
Abt 1992	HLA-class I and II antigens 48 HLA antigens	serotyped diagosis=?	72 patients with granulozytopenia/CA, 74 controls	Clozapine	definition of CA=?	no significant model of HLA subsets
Yunis 1995	HLA-class I and II antigens	serotyped	Ashkenazi and non-Ashkenazi schizophrenics and schizoaffectives, 31 CA, 52 controls extended Yunis 1992 sample	Clozapine	agranulozytosis: less than 500 neutrophils per mm3	Markers for jewish CA patients: B38, DRB1*0402, DRB4*0101, DQB1*0302, DQA1*0301 “protecting alleles”: DR11, DQB1*0301 Markers for non-jewish CA patients: DRB*1601, DRB5*02, DQB1*0502, DQA1*0102, DR2, DQw1
Theodoropoulou 1997	HLA-class I and II antigens	serotyped	43 schizophrenics, 3 of them developing agranulocytosis	Clozapine	agranulozytosis: less than 500 neutrophils per mm3	n.s.

Amar 1998	HLA-class I and II antigens	serotyped	18 schizophrenics, 5 of them with granulozytopenia/CA	Clozapine	granulozytopenia: less than 1000 and agranulozytosis: less than 500 neutrophils per mm3	granulozytopenia/agranulozytosis associated with HLA-DQB1*0201
Valevski 1998	HLA-class I and II antigens	serotyped	61 jewish schizophrenics, 11 of them with CA	Clozapine	agranulozytosis: less than 500 neutrophils per mm3	HLA-B38 associated with agranulozytosis
Meged 1999	HLA-class I	serotyped	88 Jewish schizophrenics, 3 of them with CA	Haloperidol, Clozapine	agranulozytosis: less than 500 neutrophils per mm3	n.s. trend for HLA-B38 to be associated with agranulozytosis
Lahdema 2001	HLA-A, -B	serotyped, partially genotyped	26 schizophrenic patients with Granulozytopenia/Agranulozytosis and 19 schizophrenic controls	Clozapine	granulozytopenia: less than 1,5x109/L agranulozytosis: less than 0.5x109/L	granulozytopenia/agranulozytosis associated with absence of HLA-A1
Dettling 2001a	HLA-class I and II antigens	genotyped no correction for multiple testing	107 caucasian paranoid schizophrenics, 30 of them with CA	Clozapine	agranulozytosis: less than 500 neutrophils per mm3	agranulozytosis associated with HLA-DQB1*0502, -DRB5*02, trend for –DQB1*0201
Dettling 2001b	HLA-class I and II antigens	genotyped; no correction for multiple testing	108 caucasian paranoid schizophrenics, 31 of them with CA sample seems to be identical with Dettling 2001a	Clozapine	agranulozytosis: less than 500 neutrophils per mm3	agranulozytosis associated with HLA-Cw*7, -DQB*0502, -DRB1*0101, -DRB3*0202

Heat Shock Protein

reference	polymorphism	notes	sample	treatment	phenotype	result
Corzo 1995	HSP70-1 HSP70-2	HSP70 is part of the HLA-class III cluster	75 schizophrenic and schizoaffektive patients, 32 of them with CA	Clozapine	agranulozytosis: less than 0.5x109/L polymorphonuclear leucocytes	HSP70-1 A and HSP70-2 9.0kb in linkage disequilibrium with each other and associated to CA in jewish patients

Tumor Necrosis Factor

reference	polymorphism	notes	sample	treatment	phenotype	result
Turbay 1997	TNF microsattelites a-b, d-e		12 jewish, 21 non-jewish schizophrenics vs 33 controls	Clozapine	agranulozytosis: less than 500 neutrophils per ml	CA associated with d3 and b4, inversely associated with b5

NQO2

reference	polymorphism	notes	sample	treatment	phenotype	result
Ostrousky 2003	NQO2: 1536C/T, 1541G/A, 372C/T (Phe/Leu), 202G/A, -367A/G, -394G/C	Sample seems to be partially identical to Valevski 1998	98 schizophrenics 18 of these with CA	Clozapine	agranulozytosis: less than 500 neutrophils per mm3	CA patients predominantly heterozygous for several exon and intron SNP's

n.s. not significant

The basis for the development of Clozapine induced agranulozytosis/granulozytosis/granulozytopenia (CA) is still hypothetically. Theories are ranging from immune-mediated toxicity, induction of apoptosis unto direct toxicity of Clozapine via certain degradation products acting as free radicals [see Ostousky et al. 2003]. All association studies done to date are dealing either with HLA polymorphisms or polymorphisms of genes mapping to the MHC III region like TNF, HSP70 or NQO2. The latter gene seems to be involved into the degradation or detoxification of Clozapine.

4.1.3 Weight gain

Regarding weight gain the serotonin system is a prime target. The 5HT2C receptor seems to be involved in the appetite regulation propensities of leptin, a peptide secreted by adipozytes and acting in the hypothalamus as a catabolic regulator. 5HT2A seems to mediate the effect of neuropeptide Y (NPY) another regulatory peptide in the hypothalamus with anabolic effects. 5HT1A agonists have been shown to induce hyperphagia in rats. Further genotypic targets out of the serotonergic system have been 5HT6 and the serotonin transporter 5HTTLPR.

Histamine H1 receptor antagonism seems to increase food intake in rats making this receptor gene a candidate. Adrenergic receptors as part of the sympathetic nervous system are involved into the body's energy management probably via a mitochondrial pathway. The cytochrome P450 enzyme CYP1A2 has been investigated in the pharmacogenetics of antipsychotics induced weight gain due to its involvement in the degradation of Clozapine, an antipsychotic drug with pronounced weight increasing properties. The tumor necrosis factor TNF-α too has been implicated in the regulation of metabolic regulatory processes. See Basile et al. [2001] for a synopsis.

Table 4. Side Effect: Weight Gain

reference	polymorphism	notes	sample	treatment	phenotype	result
Rietschel 1997	5-HT2C: Cys23Ser		152 schizophrenics	Clozapine	?	n.s.
Hong 2001b	5-HTTLPR, 5-HT2A 102T/C, 5-HT2C 68G/C, 5-HT6 267C/T		93 schizophrenic	Clozapine	weight gain continous	n.s.
Basile 2001	5HT2C: Cys23Ser, 5HT1A: CAn repeat, 5HT 2A: 102T/C and His452Tyr, H1:?, H2: -1018G/A, Cyp1A2: Intron1 C/A, ADRA1A: Arg347Cys, ADRB3: Trp64Arg, TNFa -308G/A		80 schizophrenics	Clozapine	weight gain continous	trends for ADRB3, ADRA1A, TNFa, 5HT2C
Reynolds 2002	5-HT2C: -759C/T		123 chinese schizophrenics	miscellaneous NL	cut off: weight gain >7%	-759C associated with weight gain
Tsai 2002	5-HT2C: -759C/T		80 chinese schizophrenics	Clozapine	weight gain: BMI continous and cut off >7%	n.s.
Basile 2002a	5-HT2C: -759C/T		80 schizophrenics	Clozapine	Cut off: weight gain >7%	n.s.
Reynolds 2003	5-HT2C: -759C/T	subsample of Reynolds 2002	32 chinese schizophrenics	Clozapine	cut off: weight gain >7%	-759T associated with less weight gain
Theisen 2004	5-HT2C: -759C/T		97 german schizophrenic patients	Clozapine	Cut off: weight gain >7%	n.s.

n.s. not significant

4.1.4 NMS

Regarding neuroleptic malignant syndrome (NMS) two candidate genes have been targeted, the cytochrome P450 CYP2D6 and the dopamine receptor D2. The rationale for the first is the fact that many antipsychotic drugs are metabolized by CYP2D6 and a poor metabolizer state with increased plasma levels has been hypothesized as a risk factor for developing NMS. The latter is due to the fact that predominantly D2 blocking agents are increasing the risk for NMS and discontinuation of this medication improved this condition.

Table 5. Side Effect: Neuroleptic Malignant Syndrome

reference	polymorphism	notes	sample	treatment	phenotype	result
Ueno 1996	CYP2D6: 1795T del, 1934G/A, Arg296Cys (Hha I)		9 NMS patients	miscellaneous NL ?	?	n.s.
Iwahashi 1997	CYP2D6: HhaI	identical to Iwahashi 1999	56 japanese schizophrenics, 8 of them with NMS	miscellaneous NL ?		n.s.
Kawanishi 2000	Cyp2D6: Pro34Ser		36 patients with NMS, 107 schizophrenic controls	miscellaneous NL ?	NMS diagnosis according to the criteria of Pope et al. 1986	n.s.
Suzuki 2001c	DRD2: TaqI A		153 schizophrenic patients, 15 with NMS	miscellaneous NL ?	NMS criteria by Pope et al. 1986	A1 allele associated with NMS
Kishida 2003	DRD2: TaqI A		49 patients with NMS, 123 schizophrenic controls	miscellaneous NL ?	NMS criteria Pope et al. 1986	n.s.
Kishida 2004	DRD2: TaqI A, -141C Ins/Del, Ser311Cys		164 japanese schizophrenics, 32 with NMS	miscellaneous NL ?	criteria by Pope et al. 1986	-141C Del more frequent in NMS

n.s. not significant

4.2. Specific genotypes

4.2.1. Pharmacokinetic phase

The traditional pharmacogenetic paradigm has been genetic variability in major drug metabolizing enzymes [Massellis et al. 1998] consequently scientists have almost exclusively focussed on drug metabolizing enzymes in the advent of pharmacogenetics. There are several pharmacokinetic enzyme systems involved in drug metabolism recently described as ADME (absorption, distribution, metabolism and excretion) [Ring and Kroetz 2002]. The most prominent of these are the phase I and II reactions as delineated in the following.

4.2.1a. Cytochrome P450

Oxidative reactions as the most prominent of the phase I group of drug metabolizing reactions are mediated by the Cytochrome P450 system (CYP), situated in the liver as a group of heme-containing enzymes. There are over 30 different CYP enzymes in humans, organized in 14 families. Specific pharmacological agents are preferentially detoxified by specific CYP enzymes. Clozapine for example appears to be metabolized by CYP*1A2,* CYP*3A4*, CYP*2C19* and CYP*2D6*, it inhibits CYP*2C9* and CYP*2C19*, induces CYP*1A*,

CYP*3A* and CYP*2B*; Risperidone seems to be metabolized by CYP*2D6*; Olanzapine by CYP*1A2* andCYP*2D6*; Quetiapine by CYP*3A4*; Sertindole by CYP*2D6* (see table x). Further information on CYP enzymes and their antipsychotic drug substrates can be found in Dahl [2002] and in Scordo and Spina [2002].

Table 6.

Isoenzyme	Substrates	Inhibitors	Inducers
CYP-1A2	Chlorpromazine, Clozapine, Haloperidol, Olanzapine, Perphenazine, Thioridazine, Zotepine		
CYP-2C9	Perazine	Clozapine	
CYP-2C19	Clozapine, Perphenazine, Thioridazine	Clozapine	
CYP-2D6	Bromperidol, Chlorpromazine, Clozapine, Fluphenazine, Haloperidol, Olanzapine, Perphenazine, Risperidone, Thioridazine, Zotepine, Zuclopenthixol	Clozapine, Haloperidol, Perphenazine, Thioridazine	
CYP-3A4	Bromperidol, Clozapine, Haloperidol, Olanzapine, Perazine, Perphenazine, Quetiapine, Risperidone, Ziprasidone, Zotepine		Clozapine

adapted from Prior et al. [1999], Scordo and Spina [2002], Dahl [2002]

Traditionally low and high metabolizing phenotypes have been defined by probe reactions like the debrisoquine/sparteine reaction of the cytochrome P450 enzyme CYP2D6, more recently it has become the practice to identify specific alleles by genotyping genetic polymorphisms.

For CYP2D6, the most polymorphic of the P450 isoenzyme, 4 different phenotypes have been described: “poor”, “intermediate”, “extensive” and “ultra rapid metabolizer” [Arranz et al. 2001]. These are defined by varying combinations of different active/inactive alleles, differentiated by point mutations, deletions, duplications and conversions [Sachse et al. 1997]. A unified nomenclature of these alleles was developed by Daly et al. [1996].

Allele CYP2D6*1 is the wild type allele. The most frequent inactivating mutation among caucasians is the CYP2D6*4 allele, a 1934G/A splice-site mutation, former known as type-B mutation. Duplications are described for the 1, 2 and 4 allele, in case of the 2 allele resulting in an ultra rapid metabolizing state [Sachse et al. 1997]. The CYP2D6*2 allele is, beyond else, resulting from a 2938C/T point mutation, detected by a HhaI RFLP, leading to an Arg296Cys amino acid substitution. CYP2D6*6 is another inactivating mutation, alternatively known as type-A mutation, a 1795Tdel mutation [Ueno et al. 1996]. An 188C/T mutation resulting in a Pro24Ser substitution is found in allele CYP2D6*10 and constitutes a further poor metabolizing variant [Kawanishi 2000]. The CYP1A2 genotype investigated by Basile et al. [2001] in neuroleptic induced weight gain is a C/A polymorphism in the first intron of the gene, with the C/C genotype being less inducible by smoking [Sachse et al. 1999].

4.2.1b. NQO2

Dihydronicotinamide riboside (NRH) quinone oxidoreductase 2 (NQO2) has been deemed a candidate gene for Clozapine induced agranulozytosis

(AGR) for several reasons. Starting from MHC association findings for AGR by different groups [Lieberman et al. 1990, Corzo et al. 1997] Ostrousky et al. [2003] found evidence for a gene or genes mapped telomeric to the MHC complex to be associated to AGR in experiments with microsatellite markers spanning the entire MHC region (unpublished data). The gene for NQO2 is mapping to this latter region of the chromosome 6, 6p25, its transcript is an enzyme catalyzing reduction of quinones and quinoid compounds. It has been suggested that NQO2 should play an important role in detoxification of chemicals and in the protection of cells against drug-induced oxidative and electrophilic stress. Clozapine has been hypothesized to be oxidized on the membranes of activated neutrophils to chemical reactive nitrenium ions acting as free radicals leading to apoptosis [Ostrousky et al. 2003].

Of the polymorphisms investigated 1536C/T and 1541G/A are situated in the first intron and the mutation of either is leading to the disruption of a myeloid zinc finger protein (MZF1) binding site. MZF1 is specifically expressed in myeloid cells lineages; it may have a general role in the regulation of hematopoietic gene expression. The -367A/G and -394G/C polymorphisms are situated in the promoter region of the NQO2 gene implying gene expression regulatory functionality, 202G/A is a silent mutation in exon 5 and 372C/T in exon 3 is conferring a Phenylalanin to Leucin substitution in position 47 with yet undetermined functionality [Ostrousky 2003].

4.2.1c. COMT

The catechol O-methyl-transferase (COMT) is a phase II reaction (conjugation) enzyme involved in the degradation and inactivation of dopamine and norepinephrine. Other prominent phase II enzymes are for example N-acetyltransferase and glutathione-S-transferase. There are low, intermediate and high activity variants of the enzyme due to a common polymorphism consisting of a G to A transition at codon 158 of the membrane-bound form of COMT (codon 108 of the soluble form). This transition results in a valine (Val) to methionine (Met) substitution with the Met/Met genotype being 3 to 4 fold lower in enzyme activity than the wild type Val/Val, the Met/Val genotype being in between. [Illi 2003]

4.2.1d. MAOA

The enzyme monoamine oxidase consists of 2 isoenzymes, MAOA and MAOB, involved in the degradation of biological amines with different substrate affinities. The former is involved in the metabolic inactivation of dopamine, norepinephrine and serotonin. A polymorphism in the promoter region 1.5kb upstream of the coding sequence consisting of a 30-bp repeat in 3, 3.5, 4 or 5 copies (30bp VNTR) has been shown to affect transcriptional activity of the MAOA promoter. [Sabol et al. 1998]

4.2.2. Pharmacodynamic phase

With growing knowledge about the action of pharmacological agents at target structures like neurotransmitter receptors, pharmacogenetics of the pharmacodynamic aspect of drug actions have increasingly been focussed on.

4.2.2a. Serotonergic System

5-HT1A: The rationale for 5-HT1A to be investigated in neuroleptic induced weight gain is derived from the fact that agonists of this receptor have been shown to increase food intake in rats. Furthermore 5-HT1A receptors are localized in high density in the brains satiety control centers as shown by autoradiographic studies. A (CA)n dinucleotide repeat polymorphism has been utilized to detect association of 5-HT1A with weight gain. [Basile 2001]

5-HT2A: Of the 5-HT2A polymorphisms the most frequently investigated is the 102T/C substitution. It is a silent mutation and might consequently be considered a weak candidate for association studies. However it is in complete linkage disequilibrium with the -1438G/A polymorphism residing in the promoter region of the 5-HT2A gene and therefore most likely of greater functional importance [Ellingrod et al. 2003].

Another frequently investigated single nucleotide polymorphism (SNP) is the one encoding a His452Tyr substitution. The 452Tyr variant has been shown to have a reduced ability to activate phospholipase C and D through the G-protein signaling pathway [Hazelwood and Sanders-Bush 2004].

Regarding the functional relevance of Thr25Asn and 516T/C a literature search yielded no specific information.

5-HT2C: The most frequently investigated 5-HT2C polymorphism in response, a 68G/C substitution resulting in a Cys23Ser amino acid substitution, seems to have no specific functional importance of its own and might only be relevant as being in linkage disequilibrium with a relevant site [Fentress et al. 2005].

The -759C/T SNP in the promoter region of the 5-HT2C gene seems to be functionally relevant by reducing transcriptional activity through its -759T allele [Buckland et al. 2005].

No information could be found about the -330GT/-244CT repeat as reported by Arranz et al. [2000b]

5-HT3A/B: The potent antagonism of clozapine on the 5HT3 receptors has been hypothesized to contribute to the antipsychotic properties of atypical antipsychotics. Of the polymorphisms investigated by Gutierrez et al. [2002] 5HT3A 178C/T and 1596A/G and a CA-repeat of 5HT3B no information about functional importance could be found.

5-HT5A: The rationale for an investigation of 5-HT2A receptors in psychosis is not as stringent as for the afore mentioned 5-HT receptors. The polymorphisms investigated are a -19G/C substitution in the promoter region and a silent 12A/T substitution without further information on functional impact to be found [Birkett et al. 2000].

5-HT6: The 267T/C polymorphism of 5-HT6 is a silent mutation situated in exon 1. There is no further information on its functional importance.

5-HTT: Two polymorphisms of the serotonin transporter (SERT) gene have been pharmacogenetically investigated. The 5-HTTLPR is a variable number tandem repeat (VNTR) polymorphism with 14 to 16 copies of a 22 bp repeat sequence. The most common alleles are the 16 repeat or long allele and the 14 repeat or short allele. Consequently the polymorphism is alternatively termed 44 bp Ins/Del. The short allele predicts lower levels of 5-HTT mRNA and HTT activity in vitro [Heils et al. 1996, Lesch et al. 1996]. A second VNTR polymorphism of 17bp is situated in intron 2 of the 5-HTT gene and seems to influence gene transcription in an allele dependant manner [Hranilovic et al. 2004, De Luca et al. 2005].

4.2.2b. Dopaminergic System

DRD1: The D1/D2 receptor balance is a major hypothesis for the mode of antipsychotic action of clozapine. The DdeI polymorphism investigated in DRD1 is a restriction fragment length polymorphism (RFLP) situated in the 5' untranslated region of the DRD1 gene [Basile 2002b, Potkin 2003].

DRD2: There is convincing evidence for the -141C Ins/Del polymorphism in the 5' flanking region of the DRD2 gene to be functionally relevant. It seems to affect DRD2 expression [Arinami et al. 1997] and in vivo PET studies [Jönsson et al. 1999] showed an increased striatal DRD2 density in subjects with the Del allele, a result however that is not supported by a second PET study [Pohjalainen et al. 1999].

A further clue to functionality might be derived from a remarkable study on synonymous (or silent) polymorphisms in the DRD2 gene influencing mRNA folding, stability and consequently DRD2 expression in a complex manner. One of the discussed polymorphisms was even found to be in linkage disequilibrium with -141C Ins/Del and Taq1 A [Duan et al. 2003].

In post mortem studies on the functional consequences of the Taq1 A polymorphism the DRD2 receptor density in the striatum has been demonstrated to be lower in subjects with A1 alleles [Tompson et al. 1997] and in vivo PET studies revealed a decreased binding potential in individuals with the A1 allele [Pohjalainen et al. 1998]. The latter result however has not been replicated by [Laruelle et al. 1998].

For the Ser311Cys polymorphism in the 3rd cytoplasmatic loop it has been shown that the 311Cys allele was markedly less effective in inhibiting cAMP synthesis than the 311Ser allele, probably due to the reduced ability of those variant receptors to activate the receptor coupled G protein [Cravchik et al. 1996].

DRD3: Ser9Gly is a SNP causing a serine to glycine amino acid substitution in the N-terminal extracellular part of DRD3. Mutations in this region could disturb membrane insertion as has been demonstrated for similar mutations in other receptors [Lundstrom and Turpin 1996]. Furthermore a higher binding affinity for D3 selective ligands and dopamine, but not for D2 selective ligands, such as Haloperidol, has been demonstrated for the Gly-9 homozygote [Lundstrom and Turpin 1996].

It has been demonstrated that DRD3 receptors form oligodimers and moreover even heterodimers with DRD2 [Scarselli et al. 2001] giving ample opportunity to speculate about functional implications. Since typical and atypical antipsychotics differ in their potency to bind to D2 and D3 receptors the formation of heterodimers might be responsible for the above discussed genetic association findings pattern.

The 5'-leader SNP's investigated by Sivagnanasundaram et al. [2000] and the -205A/G polymorphism were found to be in linkage disequilibrium with the Ser9Gly polymorphism.

DRD4: There is sparse evidence that long alleles should be functionally different from short alleles of the 48bp VNTR [Asghari et al. 1995]. A twofold reduction of dopamin potency to reduce forscolin stimulated cAMP formation was observed for the allele 7 variant. However in his study only 3 out of 10 alleles have been tested and consequently results can not easily be generalized to short versus long.

The 12bp repeat polymorphism is located in exon 1, it is occurring 1 to 3 fold and coding for 4 amino acids at the N-terminal part of the receptor protein. The 13bp deletion in the first exon causes a frameshift mutation and is probably a complete loss of function mutation. [Rietschel et al. 1996]

4.2.2c. Adrenergic, Cholinergic and Histaminergic System

Different polymorphisms of the adrenergic system (ADRA1A: Arg492Cys, Arg347Cys, RsaI; ADRA2A: -1291C/G, -261G/A; ADRB3: Trp64Arg), the cholinergic system (CHRM1: 267C/A, 1044G/A, 1221C/T, 1353C/T; CHRM3: 193G/A; CHRM4: 1338C/T) the histaminergic system (H1: -17C/T, -974C/A, -1023A/G, -1536C/G; H2: -294A/G, -592A/G, -1018G/A, -1077G/A, Leu449Ser) and the glutamatergic system (NMDA-GRIN2B: 1664C/T) have been investigated but there is only sparse evidence about functional importance of these polymorphisms.

4.2.2d. HLA System

HLA alleles and haplotypes have found to be associated with various immune- and non-immune mediated diseases. An overview on their functional importance would considerably exceed the scope of this chapter and I would therefore recommend the consultation of a review like Wright et al. [2001].

4.2.2e. Neurotensin

The VNTR polymorphism of the neurotensin receptor 1 (NTSR1) and a 3020T/C polymorphism have both been found to resides in the untranslated regions of the neurotensin gene and seems therefore to be without functional impact [Huezo-Diaz 2004, Austin et al. 2000].

4.2.2f. TNF

The TNFα polymorphism 308G/A does not seem to be functionally relevant [Baylay et al. 2004]

4.2.2g. BDNF

The Val66Met polymorphism in the 5'region of the BDNF gene has been shown to affect intracellular storage and secretion of BDNF [Egan et al. 2003].

4.2.2h. THFR

The 677C/T point mutation of the THFR gene results in a valine substitution for an alanine. Heterozygotes (CT) and homozygotes (TT) have 71% and 33%, respectively, of the activity of the wild-type THFR (CC). [Chiuve et al. 2005]

5. FINDINGS

5.1. Response

5.1.1. Cytochrome P450 (see table 2)

The pharmacogenetic studies of genes of importance for the pharmacokinetic phase of drug action have almost exclusively focused on

Cytochrome P450 and its CYP2D6 variant. All but one study [Lane et al. 1997] utilized PCR techniques for identification of allele carrier status, the latter utilized a probe drug approach with dextromethorphan.

Arranz et al. [1995b] reported no association of CYP2D6 metabolizer status with response to Clozapine, the same as Lane et al. [1997] in a Haloperidol sample and Hamelin et al. [1999] in a miscellaneous neuroleptics sample.

Aitchison et al. [1999] and Brockmöller et al. [2002] too found no significant association with miscellaneous neuroleptics and Haloperidol respectively. Both however report a trend, the first of ultra fast metabolizing status to be associated with response, the second of fast metabolizer genotypes with non-response. This latter finding after all seems more apt to meet expectations.

5.1.2. Serotonergic System

5.1.2a. 5-HT2A

All the earlier studies with the 5-HT2A polymorphism 102T/C dealt with Clozapine treated patients. Arranz et al. [1995a] reported a significant association between 102C homozygotes and treatment non-response, a finding that was not replicated by Masellis et al. [1995], Nöthen et al. [1995] and Malhotra et al. [1996a].

In a meta-analysis of the above mentioned studies Arranz et al. [1998a] reported an overall significant association between the 102C allele and treatment non-response. Subsequent Clozapine studies by Masellis et al. [1998] and Lin et al. [1999] once again yielded insignificant results, as well as studies by Nimgaonkar et al. [1996a] and Jönsson et al. [1996], the latter treating patients with varying neuroleptics.

Significant results have then been reported by Joober et al. [1999], who, treating patients with typical neuroleptics, found an association of the 102C/102C genotype with male poor responders. Ellingrod et al. [2002] with an Olanzapine design reported an association of the 102T/102T genotype with improvement in negative symptoms. Both studies are in accordance with Arranz et al. [1998a]'s meta-analysis report of the 102C allele being associated with non-response. Lane et al. [2002] however, in a Risperidone sample, reported the 102C/102C genotype to be associated with better outcome. Yamanouchi et al. [2003] reported a trend for a diplotype containing a C allele to be associated with better response in a small Risperidone sample.

102C/T however is a silent mutation and might be considered a weak candidate for association studies. -1438G/A is a polymorphism residing in the promoter region of the 5-HT2A gene and therefore most likely of greater functional importance. This polymorphism is in strong linkage disequilibrium with 102C/T. Arranz et al. [1998b] reported an association of –1438G with non response, however in a sample that is identical to their 1995 sample with the significant 102C association result. In a second sample they could only see a trend in the same direction. Two other studies by Masellis et al. [1998] and Ellingrod et al. [2003] yielded insignificant results.

Another candidate polymorphism with promising association results is His452Tyr. Whilst there is no significant result reported by Nöthen et al. [1995]

and Malhotra et al. [1996a] with Clozapine treated patients, Arranz et al. [1996] reported association of Tyr452 with non-response and Arranz et al. [1998b] a trend in the same direction. A meta analysis of the above cited studies was done by Arranz et al. [1998a] and the association result was upheld. In the meantime the association of Tyr452 with non-response has been replicated by Masellis et al. [1998] with Clozapine treated patients, Ellingrod et al. [2002] were not able to do so in an Olanzapine sample.

Finally there are two studies with the polymorphism Thr25Asn both with insignificant results: Nöthen et al. [1995] and Ellingrod et al. [2002]. The latter was additionally investigating the 516T/C polymorphism with insignificant results too.

To summarize: all studies on 5-HT2A 102C/T genotype report either insignificant findings or association of 102C with non-response, except one that reports association of 102C with response and a second that reports a trend in the same direction, both of the latter with an atypical antipsychotics sample. All studies about His452Tyr that are not reporting insignificant findings, demonstrate association of Tyr452 with non-response. However these results have to be interpreted with caution: positive and negative predictive values based on the meta analyses remain moderate, haplotype analyses do not show stronger association with response [Masellis et al. 1998] and the predicted population frequency of the Tyr452 allele is very low [Veenstra-VanderWeele et al. 2000].

Table 7. Antipsychotic Response and Serotonergic System, the 5HT2A studies

reference	polymorphism	notes	sample	treatment	phenotype	result
Arranz 1995a	102T/C	retrospective	149 schizophrenics resistent to typical NL	Clozapine	response: 20 point improvement in GAS	102C homozygotes more frequent in non-responders
Masellis 1995	102T/C		126 schizophrenics	Clozapine	response: 20% improvement in BPRS	n.s.
Nöthen 1995	102T/C	retrospective	146 schizophrenics	Clozapine	response: clinical rating	n.s.
Malhotra 1996a	102T/C		70 typical neuroleptics responder	Clozapine	response: 20% improvement in BPRS	n.s.
Arranz 1998a	102T/C	meta-analysis	Masellis 1995, Arranz 1995, Nöthen 1995, Nimgaonkar 1996a, Malhotra 1996a	Clozapine	response	102C associated with non-response
Masellis 1998	102T/C		185 schizophrenics	Clozapine	response: 20% improvement in BPRS	n.s.

Lin 1999	102T/C	sample identical with Tsai 2000, 2001 and Yu 1999, Hong 2000	97 chinese schizophrenics	Clozapine	response: BPRS continous	n.s.
Nimgaonkar 1996a	102T/C		174 patients	miscellaneous NL	response: clinical rating	n.s.
Jönsson 1996	102T/C		118 schizophrenics	miscellaneous NL	response: clinical ratings	n.s.
Joober 1999	102T/C		schizophrenics: 39 responders, 63 nonresponders	Typical neuroleptics	response: clinical rating according to the criteria by Brenner 1990	102C homozygotes more frequent in male non-responders
Lane 2002	102T/C		100 Chinese schizophrenics	Risperidone	response: PANS subscales continous	102C homozygotes associated with better outcome
Ellingrod 2002	102T/C		41 schizophrenics	Olanzapine	response: BPRS and SANS continous	Trend for 102T homozygotes to be associated with reduction of negative symtoms
Arranz 1998b	-1438G/A,	"in strong linkage disequilibrium with 102T/C"	Sample I: 160 schizophrenics (same sample as Arranz 1995); Sample II: 114 patients	Clozapine	response: 20 point improvement in GAS	-1438G associated with non-response
Masellis 1998	-1438G/A		185 schizophrenics	Clozapine	response: 20% improvement in BPRS	n.s.
Ellingrod 2003	-1438G/A	sample identical to Ellingrod 2002	41 schizophrenics	Olanzapine	response: BPRS and SANS continous	n.s.
Arranz 1996	His452Tyr		153 schizophrenics, 178 normal controls	Clozapine	response: 20 point improvement in GAS	Tyr452 associated with poor response
Nöthen 1995	His452Tyr		146 schizophrenics	Clozapine	response: clinical rating	n.s.
Malhotra 1996a	His452Tyr		70 typical neuroleptics responder	Clozapine	response: 20% improvement in BPRS	n.s.
Arranz 1998b	His452Tyr		Sample I: 160 schizophrenics (same sample as Arranz 1995); Sample II: 114 patients	Clozapine	response: 20 point improvement in GAS	Tyr452 trend to be assoziated with non-response

Arranz 1998a	His452Tyr	meta-analysis	Nöthen 1995, Malhotra 1996a, Arranz 1998b	Clozapine	response	Tyr452 associated with poor response
Masellis 1998	His452Tyr		185 schizophrenics	Clozapine	response: 20% improvement in BPRS	Tyr452 associated with non-response
Ellingrod 2002	His452Tyr		41 schizophrenics	Olamzapine	response: BPRS and SANS continous	n.s.
Nöthen 1995	Thr25Asn,		146 schizophrenics	Clozapine	response: clinical rating	n.s.
Ellingrod 2002	Thr25Asn		41 schizophrenics	Olamzapine	response: BPRS and SANS continous	n.s.
Ellingrod 2002	516T/C		41 schizophrenics	Olamzapine	response: BPRS and SANS continous	n.s.
Masellis 1998	102T/C, -1438A/G, His452Tyr	haplotype	185 schizophrenics	Clozapine	response: 20% improvement in BPRS	n.s.
Yamanou chi 2003	102T/C, -1438G/A	haplotype	73 japanese schizophrenics	Risperidone	response: PANS subscales continous	n.s. but diplotype A-T/A-T trend to worse outcome than A-T/G-C

5.1.2b. 5-HT2C

In several studies with Clozapine treated patients only Sodhi et al. [1995] were able to report a significant finding for the Cys23Ser polymorphism, they found 23Ser being associated with better response. The publications by Malhotra et al. [1996b] and Rietschel et al. [1997] yielded insignificant results, as well as Masellis et al. [1998], the latter two at least reported a trend for 23Ser and better response, the former a contradictory trend of 23Ser and association with non-response.

An exploratory meta analysis of the above cited studies was undertaken by Veenstra-VanderWeele et al. [2000] with a preliminary affirmation of the 23Ser/response association result. A more recent study by Ellingrod et al. [2002] with Olanzapine treated patients did not yield a significant result.

Table 8. Antipsychotic Response and Serotonergic System, the 5HT2C studies

reference	polymorphism	notes	sample	treatment	phenotype	result
Sodhi 1995	Cys23Ser		162 (168?) treatment resistent Schizophrenics	Clozapine	response: 20 point improvement in GAS	23Ser associated with response
Malhotra 1996b	Cys23Ser		66 schizophrenic and schizoaffective	Clozapine	response: 20% improvement in BPRS	n.s., trend for 23Ser to be associated with non-response

Rietschel 1997	Cys23Ser		152 schizophrenics	Clozapine	?	n.s., trend for 23Ser to be associated with response
Masellis 1998	Cys23Ser		185 schizophrenics	Clozapine	response: 20% improvement in BPRS	n.s. trend for 23Ser to be associated with response
Veenstra-VanderWeele 2000	Cys23Ser	meta-analysis	Sodhi 1995, Malhotra 1996b, Rietschel 1997, Masellis 1998	Clozapine	response	23Ser associated with response
Ellingrod 2002	Cys23Ser		41 schizophrenics	Olanzapine	response: BPRS and SANS	n.s.

5.1.2c. 5-HT3A/B

There is only one study by Gutierrez et al. [2002] with a Clozapine design investigating the 178C/T and the 1596A/G polymorphism of 5-HT3A and a CA repeat polymorphism of 5-HT3B with insignificant results.

Table 9. Antipsychotic Response and Serotonergic System, the 5HT3A/B studies

reference	polymorphism	notes	sample	treatment	phenotype	result
Gutierrez 2002	5HT3A: 178C/T, 1596A/G; 5HT3B: CA-repeat		266 british caucasian schizophrenics	Clozapine	response: 20 point improvement in GAS-scale 3 month after initiation of treatment	n.s.

5.1.2d. 5-HT5A

Birkett et al. [2000] investigated the –19G/C and the 12A/T polymorphism in Clozapine treated patients without significant findings.

Table 10. Antipsychotic Response and Serotonergic System, the 5HT5A studies

reference	polymorphism	notes	sample	treatment	phenotype	result
Birkett 2000	-19G/C, 12A/T		269 schizophrenic	Clozapine	GAS, response definition = ?	n.s.

5.1.2e. 5-HT6

Masellis et al. [2001] could not replicate the finding by Yu et al. [1999], who saw homozygous T/T 267 genotypes of the 267T/C polymorphism being associated with response. Masellis et al. speculated on methodological differences in statistics as possible cause for this divergence but consequently dismissed this possibility again by recalculating their data with Yu et al.'s statistical approach, again yielding insignificant results. Ethnic heterogeneity of their sample and genetic heterogeneity of the phenotype "Clozapine response" were alternative explanations given.

Table 11. Antipsychotic Response and Serotonergic System, the 5HT6 studies

reference	polymorphism	notes	sample	treatment	phenotype	result
Yu 1999	267T/C	sample identical with Tsai 2000, 2001, Lin 1999, Hong 2000	99 chinese schizophrenics	Clozapine	response: BPRS continous	Better response for 267T homozygotes
Masellis 2001	267T/C		185 schizophrenics	Clozapine	response: 20% improvement in BPRS	n.s.

5.1.2f. 5-HTT

Arranz et al. [2000a] found a VNTR polymorphism in the 2nd intron and a VNTR in the promoter region not associated with Clozapine response, Tsai et al. [2000] reported insignificance for the VNTR in the promoter region.

Table 12. Antipsychotic Response and Serotonergic System, the 5HTT studies

reference	polymorphism	notes	sample	treatment	phenotype	result
Arranz 2000a	VNTR in Intron2, 5HTTLPR-VNTR in promoter region		268 treatment resistant schizophrenics	Clozapine	rsponse: 20 point improvement in GAS	n.s.
Tsai 2000	5HTTLPR - VNTR in the promoter region	sample identical with Tsai 2001, Lin 1999 and Yu 1999, Hong 2000	90 chinese schizophrenics	Clozapine	response: BPRS continous	n.s.

n.s. not significant

5.1.3 Dopaminergic System

5.1.3a. DRD1

The 2/2 genotype of DdeI, an upstream DRD1 polymorphism, has been found to be associated with better response to Clozapine and decrease in frontal and cortical metabolism as assessed by FDG-PET [Potkin et al. 2003]. The same polymorphism has been reported to be significantly associated with change in scores on the Wisconsin card sort test assessed before and after treatment with Clozapine in a pilot study by Basile et al. [2002b].

Table 13. Antipsychotic Response and Dopaminergic System, the DRD1 studies

reference	polymorphism	notes	sample	treatment	phenotype	result
Potkin 2003	DdeI		15 schizophrenics	Clozapine	response: BPRS, SANS, CGI: continous; PET scan: metabolism continous	Homozygotes for allele2 better improvement in BPRS and decrease in PET frontal and temporal cortical metabolism.
Basile 2002b	DdeI	pilot study	35 schizophrenic patients	Clozapine	response: changes in score of Wisconsin card sort test, continous	Association with changes in score of Wisconsin card sort test

5.1.3b. DRD2

The –141C Ins/Del polymorphism was primarily investigated by Arranz et al. [1998c] in a mixed and partially Clozapine treated sample yielding insignificant results. Ohara et al. [1998], giving no information about treatment regimes, reported insignificance too. Malhotra et al. [1999] however, in a Clozapine treated sample, found the –141C Ins allele being associated with better treatment response. This latter result was supported by Suzuki et al. [2001a] who investigated schizophrenic patients treated with Bromperidol, a close structural analogue to Haloperidol, and Nemonapride and found –141C Ins allele carriers more responsive to treatment as determined by the anxiety/depression subscale of the BPRS (Brief Psychiatric Rating Scale).

There are two studies combining the –141C Ins/Del polymorphism with the TaqI A polymorphism into a diplotype analysis. Kondo et al. [2003] reported genotypes carrying the –141C Del allele to be associated with worse outcome in the anxiety/depression subscales of the BPRS, a fact that is not surprising, given that their sample seems to be identical with Suzuki et al. [2001a]. Yamanouchi et al. [2003] however reported better response for diplotypes with the –141C Del allele.

The TaqI A polymorphism has been investigated in several samples, all with different regimes of neuroleptics prescribed. Only the study by Suzuki et al. [2001b] reported an insignificant finding. All other studies found either the allele 1 associated with better response (Suzuki et al. [2000], Dahmen et al. [2001] in a diplotype analysis with DRD3 Ser9Gly and Yamanouchi et al. [2003] in a diplotype analysis with –141C Ins/Del) or the allele 2 associated with non-response (Schäfer et al. [2001] and Kondo et al. [2003] in a diplotype analysis with –141C Ins/Del).

Two studies investigating the Ser311Cys polymorphisms reported no significant results (Shaikh et al. [1994] and Ohara et al. [1996]).

To summarize: two studies reported no association of the –141C Ins/Del polymorphism with treatment response, two (three if we include Kondo et al. [2003]) studies report the –141C Ins allele to be associated with better treatment response and one with worse. For TaqI A the situation seems to be clearly in favor of the allele 1 to be associated with better response. The samples of Suzuki et al. [2000, 2001b] and Kondo et al. [2003] however seem to be at least partially identical thus somehow blurring the picture.

Table 14. Antipsychotic Response and Dopaminergic System, the DRD2 studies

reference	polymorphism	notes	sample	treatment	phenotype	result
Arranz 1998c	-141C Ins/Del		Sample 1: "white british caucasians " 94 responders vs. 57 non-responders Sample 2: "chinese'" 85 responders vs. 65 non-responders	Sample 1: Clozapine Sample 2: ?	response: Sample 1: GAS: 20 point improvement Sample 2: "personal interview"	n.s.
Ohara 1998	-141C Ins/Del		170 schizophrenics	?	Response: PANSS	n.s.

Malhotra 1999	-141C Ins/Del		72 patients	Clozapine	response: BPRS: 20% improvement	-141C Ins carriers greater reduction in psychotic symptoms
Suzuki 2001a	-141C Ins/Del		49 acutely exacerbated schizophrenics	Bromperidol, Nemonapride	response: BPRS (continous) in subgrouped symptoms	-141C Ins carriers better improvement in anxiety/depression
Suzuki 2000	TaqI A		25 schizophrenics	Nemonapride	response: BPRS (continuous)	A1 allele associated with better response
Dahmen 2001	TaqI A	diplotype analysis with DRD3: Ser9Gly	18 patients	miscellaneous NL	response: BPRS (continous)	DRD3-Ser9 homozygozity and at least one allele of DRD2-A1 better improvement
Schäfer 2001	TaqI A		57 patients with “acute psychosis”	Haloperidol	response: PANSS (continuous)	allele 2 homozygotes display less improvement in positive symptoms
Suzuki 2001b	TaqI A		“japanese”: 30 acutely exacerbated schizophrenics	Bromperidol	response: BPRS (continous)	n.s.
Kondo 2003	TaqI A, -141C Ins/Del	sample seems to be identical with Suzuki et al. 2000, 2001a; polymorphisms 250kb apart; diplotype analysis	49 schizophrenics	Bromperidol, Nemonapride	response: BPRS continous	A2/Del poorer improvement in anxiety/depression
Yamanouchi 2003	-141C Ins/Del, Taq1 A;	diplotype analysis	73 japanese schizophrenics	Risperidone	response: PANS subscales continous	Ins-A2/Del-A1 diplotype better response than Ins-A2/Ins-A2
Shaikh 1994	Ser311Cys		“Caucasians”: 87 “treatment-resistant” vs. 100 controls	Clozapine	response: ?	n.s.
Ohara 1996	Ser311Cys		“japanese?”: 45 treatment resistant schizophrenics vs. ?	?	response: PANSS	n.s.

5.1.3c. DRD3

For DRD3 exclusively the Ser9Gly genotype has been investigated, with the exception of Sivagnanasundaram et al [2000] who tried to associate several 5' leader SNP's with Clozapine response and reported no significant results.

The situation for Ser9Gly firstly remains complex. There are several studies with no significant results and even more studies with contradictory ones. If the studies are grouped according to the type of dispensed neuroleptics however there is a remarkable pattern to be observed.

With only the studies with a Clozapine treatment regime taken into account, we find three studies with insignificant findings [Gaitonde et al. 1996, Malhotra et al. 1998, Shaikh et al. 1996], the latter after all with a trend for the genotype Ser9/Ser9 to be associated with non-response. One study reported association of the Gly9 allele and of genotypes including the Gly9 allele with response [Scharfetter et al. 1999]. A meta-analysis incorporating the results of Malhotra et al. [1998], Shaikh et al. [1996] and Scharfetter et al. [1999] substantiated this latter result [Scharfetter et al. 1999].

There are further studies with atypical antipsychotics treatment regimes that reported corresponding findings. Szekeres et al. [2004] with "atypical antipsychotics" reported association of treatment non-response with Ser9 alleles and Ser9/Ser9 genotypes and Staddon et al. [2002] in an analysis combining Ser9Gly with -205A/G and an Olanzapine sample reported the Gly9/-205G diplotype to be associated with better positive symptoms improvement.

All the other studies about Ser9Gly are either administering typical neuroleptics [Kennedy et al. 1995], "mixed" neuroleptics [Dahmen et al. 2001] or give no clue at all about which neuroleptics were administered [Nimgaonkar et al. 1993, Jönsson et al. 1993, Yang et al. 1993, Mant et al. 1994, Ohara et al. 1996, Nimgaonkar et al. 1996B, Durany et al. 1996, Ebstein et al. 1997, Krebs et al. 1998, Joober et al. 2000a, Jönnson et al. 2003]. In most of the latter studies the pharmacogenetic part is just an addition to a classical schizophrenia association study design and it's justifiable to assume that most patients have been treated with typical neuroleptics.

While a majority of these latter studies reported no significant results, there are two studies that reported an association of homozygotes with response [Jönsson et al. 1993, Mant et al. 1994] and one study by Krebs et al. [1998] that reports an association of homozygotes with response and of Gly9/Gly9 genotypes with response, the latter result in accordance with the "atypical neuroleptics studies" by Scharfetter et al. [1999], Szekeres et al. [2004] and Staddon et al. [2002]. Three other studies however associate response with the Ser9 allele or non-response with Gly9 respectively [Jönsson et al. 2003, Dahmen et al. 2001 (in a diplotype analysis with DRD2 TaqI A) and Ebstein et al. 1997].

The above characterized trend was first delineated by Jönssen et al. [2003], incorporating genotypic data from studies where such were available into a meta analysis: Mant et al. 1994, Durany et al. 1996, Krebs et al. 1998, Joober et al. 2000a, Jönnson et al. 2003, Malhotra et al. 1998, Shaikh et al. 1996 and

Scharfetter et al. 1999. Jönsson et al. were parting the studies with typical antipsychotics from the studies with Clozapine and ascertained an association of Gly9 with response in Clozapine treated patients and an association of Ser9 with response in patients treated with traditional antipsychotics. Discussing this finding however Jönnson et al. speculated that this should be due to the fact that patients treated with Clozapine have been recruited mainly from former nonresponders to typical antipsychotics.

Table 15. Antipsychotic Response and Dopaminergic System, the DRD3 studies

reference	polymorphism	notes	sample	treatment	phenotype	result
Shaikh 1996	Ser9Gly		"white european caucasian " : 79 responder vs. 54 non responder	Clozapine	response: GAS: 20 point improvement	trend for Ser9 homozygotes to be associated with non-response
Gaitonde 1996	Ser9Gly		84 "caucasians, except 3 ": ? vs. ?	Clozapine	response: clinical rating	n.s.
Malhotra 1998	Ser9Gly		68 schizophrenics: 19 responders vs. 49 non-responders	Clozapine	response: BPRS: discrete – 20% reduction; continuous	n.s.
Scharfetter 1999	Ser9Gly		"pakistani patients ": 21 responders vs. 11 non-responders	Clozapine	response: BPRS: 50% improvement after 6 month of treatment	Gly9 allele and Gly9 homozygotes as well as Ser9/Gly9 genotypes more frequent in responders
Nimgaonkar 1993	Ser9Gly		53 "caucasian " schizophrenics: ? vs. ?	?	response: clinical rating from hospital records	n.s.
Jönsson 1993	Ser9Gly		76 "caucasian" schizophrenics: ? response present vs. ? response absent	?	response: ?, hospital records	n.s., association between homozygosity and response before corrected for multiple testing
Yang 1993	Ser9Gly		"Han-chinese": 45 responders vs. 98 controls	?	response: clinical rating	n.s.
Mant 1994	Ser9Gly		"western european Caucasians": 68 good response vs. 63 no response	?	response: clinical rating	better response in homozygotes
Kennedy 1995	Ser9Gly		"north American": 38 treatment resistant vs. 38 controls	traditional neuroleptics	response: clinical rating	n.s.
Ohara 1996	Ser9Gly		"japanese?": 45 treatment resistant schizophrenics vs. ?	?	response: PANSS	n.s.

Nimgaonkar 1996b	Ser9Gly		"caucasians" and "afro-americans": ? vs. ?	?	response: clinical rating	n.s.
Durany 1996	Ser9Gly		61 patients with good vs. 43 with bad response	?	response: ?	n.s.
Ebstein 1997	Ser9Gly		"israeli sample": "41 Ashkenazi": 10 good vs. 31 poor responders; "46 non-Ashkenazi": 9 good vs. 37 poor responders "italian sample": 39 good vs. 21 poor responders	?	response: clinical rating	association of homozygosity with poor response in the non-Ashkenazi and the combined sample
Krebs 1998	Ser9Gly		70 responders vs. 19 non-responders	?	response: PANSS, GAS, CGI	significant difference in genotype distribution (fewer Gly9 homozygotes in non-responders); higher frequency of homozygosity in responders
Joober 2000a	Ser9Gly		42 responders vs. Controls 64 non-responders vs. controls	?	response: clinical rating BPRS, CGI	n.s.
Dahmen 2001	Ser9Gly	Diplotype analysis with DRD2: TaqIA	18 patients	miscellaneous NL	response: BPRS (continous)	DRD3-Ser9 homozygotes and at least one allele of DRD2-A1 better improvement
Jönsson 2003	Ser9Gly		153 patients Sample in part identical to Jönnson et al. 1993	?	response: clinical rating	more Ser9 alleles and homozygous genotypes in responders
Szekeres 2004	Ser9Gly		caucasian: 28 non-reponder vs. 47 responders	atypical antipsychotics	response: GAF: 20 points improvement	Ser9 alleles and Ser9 homozygotes more frequent in non responders
Staddon 2002	Ser9Gly, -205A/G	diplotype analysis	50 Basque schizophrenics	Olanzapine	response: PANSS positive and negative symptome scale, continous, after 3 month of treatment	Gly9/-205G associated with better positive symptomes improvement
Sivagnanasundaram 2000	5'-leader SNP's		49 patients	Clozapine	response: "symptoms rated on ordinal scale"	n.s.

5.1.3d. DRD4

Several genotypes have been investigated regarding DRD4. A 12bp repeat polymorphism could not be associated with treatment response in studies by Ohara et al. [1996], Rietschel et al [1996], Kohn et al. [1997] and Ozdemir et al. [1999].

Rietschel et al. [1996] additionally investigated a 13bp deletion and a Gly11Arg polymorphism, both without association with response. Ozdemir et al. [1999] investigated a (G)n repeat polymorphism and a SmaI RFLP, both without association with response too.

The most frequently investigated polymorphism in the DRD4 gene is a 48bp VNTR polymorphism with several alleles differing in repeat length from 2 ("short") to 8 ("long"). There are 6 studies with Clozapine treated patients, all of them yielding insignificant results [Shaikh et al. 1993, Kerwin et al. 1994, Rao et al. 1994, Shaikh et al. 1995, Rietschel et al. 1996, Kohn et al. 1997]. The same holds true for Kaiser et al. [2000] with a typical neuroleptics and a Clozapine cohort, for Zalsman et al. [2003] with a Risperidone sample and for Ohara et al. [1996] with a sample of unknown therapeutic regime. Only in the study by Cohen et al. [1999] it was reported that responders to typical neuroleptics carried the 7 repeat allele more often than Clozapine responders or controls and in a study by Hwu et al. [1998] it was reported that allele 4 homozygotes responded better than all others in a sample where there is no clue about what neuroleptics were administered.

To summarize: results of association studies of DRD4 48bp VNTR with response remain insignificant or conflicting. Due to the diversity of allelic distribution between the samples and of statistical approaches the individual studies can not be readily compared.

Table 16. Antipsychotic Response and Dopaminergic System, the DRD4 studies

reference	polymorphism	notes	sample	treatment	phenotype	result
Shaikh 1993	48bp VNTR	?	41 responders vs. 23 non responders	Clozapine	response: ?	n.s.
Kerwin 1994	48bp VNTR	regression analysis between repeat number and response	124 "european caucasian " ; 42 "taiwanese"	Clozapine	response: GAS continuous and discrete (20 points improvement)	n.s.
Rao 1994	48bp VNTR	Chi2/fishers exact test of each allele against all others	29 patients: 13 responders vs. 6 intermediate vs. 10 non responders	Clozapine	response: BPRS (20% decrease)	n.s.
Shaikh 1995	48bp VNTR	ANOVA	147 "european caucasian";42 "chinese"	Clozapine	response: GAS continuous	n.s.

Rietschel 1996	48bp VNTR	Chi2/fishers exact test Pooled: alleles 2 and 3; alleles 4 and 5; alleles 6,7,8 and 9 rare genotypes dismissed	149 “german schizophrenics and schizoaffective”: 40 no- vs. 32 slight- vs. 45 marked- vs. 32 total-response	Clozapine	response: clinical rating	n.s.
Kohn 1997	48bp VNTR	Chi2/fishers exact test;rare alleles/ genotypes collapsed into “others”	37 “Ashkenazi”; 27 “non Ashkenazi”	Clozapine	response: clinical rating	n.s.
Cohen 1999	48bp VNTR	allele 4 vs. allele 7	“european caucasian”: 28 patients with typical neuroleptics vs. 32 with clozapine vs. 57 controls	typical neuroleptics/ Clozapine	response: clinical rating	typical neuroleptic group significantly lower frequency of 7 repeat allele vs. 4 repeat allele
Kaiser 2000	48bp VNTR	Genotypes with alleles 4 and shorter vs. 5 and longer	“german caucasian”: typical antipsychotics: 360 responder vs. 67 non-responder; clozapine: 136 responder vs. 36 non-responder	typical neuroleptics/ Clozapine	response: clinical rating and PANSS	n.s.
Zalsman 2003	48bp VNTR	alleles collapsed into short (<7) and long	10 responders, 14 non-responders	Risperidone	response: BPRS: 40% improvement	n.s.
Ohara 1996	48bp VNTR	Chi2/fishers exact test	“japanese?”: 45 treatment resistant schizophrenics vs. ?	?	response: PANSS	n.s.
Hwu 1998	48bp VNTR	Genotype 4/4 against all others	80 “chinese”: 39 good vs. 41 poor responders	?	response: clinical rating	genotypes homozygous for allele 4 have significantly better response than others
Ohara 1996	12bp repeat		“japanese?”: 45 treatment resistant schizophrenics vs. ?	?	response: PANSS	n.s.

Rietschel 1996	12bp repeat	149 “german schizophrenics and schizoaffective” : 40 no- vs. 32 slight- vs. 45 marked- vs. 32 total-response	Clozapine	response: clinical rating	n.s.
Kohn 1997	12bp repeat	37 “Ashkenazi”; 27 “non Ashkenazi”	Clozapine	response: clinical rating	n.s.
Ozdemir 1999	12bp repeat	50 “treatment refractory” schizophrenics	Clozapine	response: BPRS continuous	n.s.
Rietschel 1996	13bp deletion Gly11Arg	149 “german schizophrenics and schizoaffective” : 40 no- vs. 32 slight- vs. 45 marked- vs. 32 total-response	Clozapine	response: clinical rating	n.s.
Ozdemir 1999	(G)n repeat SmaI RFLP	50 “treatment refractory” schizophrenics	Clozapine	response: BPRS continuous	n.s.

n.s. not significant

5.1.4 Other Genotypes

Bolonna et al. [2000] investigated polymorphisms of the adrenergic system, Arg492Cys of the ARDA1A receptor and -1291C/G as well as -261G/A of the ARDA2A receptor and found no association with Clozapine response. The -1291C/G polymorphism of the ARDA2A receptor was investigated too by Tsai et al. [2001] in a Clozapine sample identical with Tsai et al. [2000], Yu et al. [1999] and Lin et al. [1999] without significant findings.

Mancama et al. [2000] found among different polymorphisms of different muscarinergic receptors only the 1044A allele of the CHRM1 1044G/A polymorphism associated with non response.

In another study Mancama et al. [2002] analyzed histamine H1 and H2 receptor polymorphisms and found the H2 1018G/A polymorphism associated with good response, a finding that did not endure correction for multiple testing and was only replicated as a trend in a second independent sample.

A polymorphism of the glutamatergic NMDA-GRIND2B receptor was studied by Hong et al. [2001b] and no association of 2664C/T with treatment response was reported.

The HLA system was examined regarding association with Clozapine treatment response by Lahdelma et al. [1998]. They typed HLA-A, -B and -DR genotypes utilizing the lymphotoxicity method and found the A1 allele to be associated with treatment response. In a second study Meged et al. [1999] investigated HLA class I alleles (-A, -B, -C) and found no significant association.

The Apolipoprotein E ε4 allele was neither associated with treatment response in a japanese sample of Ohara et al. [1997] nor in a sample with Clozapine treated patients of Hong et al. [2000].

Two Neurotensin receptor polymorphisms, NTSR1 3020T/C and a VNTR, were not associated with treatment response [Huezo-Diaz et al. 2004].

Illi et al. [2003] found no association with treatment response for the 30bp VNTR of the MAOA gene, they additionally investigated the COMT Val158Met polymorphism and report the Met/Met genotype (low enzymatic activity phenotype) associated with non-response. The latter result could not be replicated by Yamanouchi et al. [2003].

Regarding BDNF 2 studies have been performed to date. Krebs et al. [2000] report an excess of long alleles (172-176bp) of a dinucleotide repeat polymorphism in responders. Hong et al. [2003] found the Val/Val genotype of a Val66Met polymorphism more frequent in responders to Clozapine.

A 677C/T polymorphism of the methylenetetrahydrofolate reductase was investigated by Joober et al. [2000b] who reported the T/T genotype and the T allele associated with response to conventional neuroleptics.

Table 17. Antipsychotic Response and Other Genotypes

Adrenergic System

reference	polymorphism	notes	sample	treatment	phenotype	result
Bolonna 2000a	ADRA1A: Arg492Cys; ADRA2A: -1291C/G, -261G/A		289 schizophrenics	Clozapine	response: 20 points GAS improvement	n.s.
Tsai 2001	ADRA2A: -1291C/G	sample identical with Tsai 2000, Lin 1999 and Yu 1999, Hong 2000	97 Chinese schizophrenic patients	Clozapine	BPRS continous	n.s.

Cholinergic System

reference	polymorphism	notes	sample	treatment	phenotype	result
Mancama 2000	CHRM1: 267C/A, 1044G/A, 1221C/T, 1353C/T;CHRM3: 193G/A;CHRM4: 1338C/T	abstract	?	?	response: ?	marginal significance for CHRM-1 1044A to be associated with non-response

Histaminergic System

reference	polymorphism	notes	sample	treatment	phenotype	result
Mancama 2002	H1: -17C/T, -974C/A, -1023A/G, -1536C/G H2: -294A/G, -592A/G, -1018G/A, -1077G/A		Sample 1:158 schizophrenics Sample 2: 164 schizophrenics	Clozapine	response: GAS 20point improvement	H2 -1018G/A associated with better responsebefore correction for multiple testing; not replicated in a 2nd sample

Glutamatergic System

reference	polymorphism	notes	sample	treatment	phenotype	result
Hong 2001a	NMDA-GRIN2B: 2664C/T		100 Chinese schizophrenics	Clozapine	response: 20% improvement in BPRS	n.s.

HLA System

reference	polymorphism	notes	sample	treatment	phenotype	result
Lahdelma 1998	HLA-A, -B, -DR Alleles	serotyping	19 responders to conventional antipsychotics vs. 19 clozapine responders	Clozapine	response: 20% improvement in BPRS	HLA-A1 associated with clozapine response
Meged 1999	HLA-class I (-A, -B, -C)	serotyping	88 Jewish schizophrenics	Haloperidol, Clozapine	response: CGI rating of at least much improved	n.s.

Apolipoprotein E

reference	polymorphism	notes	sample	treatment	phenotype	Result
Ohara 1997	ε4 allele present/absent		87 japanese schizophrenics	miscellaneous NL	response: PANSS continous	n.s.
Hong 2000	ε4		95 schizophrenics	Clozapine	response: PANSS continous	n.s.

Neurotensin

reference	polymorphism	notes	sample	treatment	phenotype	result
Huezo-Diaz 2004	NTSR1: 3020T/C, VNTR		196 british caucasian schizophrneics	Clozapine	response: GAS 20point improvement	n.s.

COMT/MAO

reference	polymorphism	notes	sample	treatment	phenotype	result
Illi 2003	COMT: Val 158Met MAOA: 30bp VNTR		94 finnish schizophrenics	conventional neuroleptics	CGI clinical	COMT: Met/Met associated with non response MAOA: n.s.
Yamanouchi 2003	COMT: Val158Met		73 japanese schizophrenics	Risperidone	response: PANSS subscales continous	n.s.

BDNF

reference	polymorphism	notes	sample	treatment	phenotype	result
Krebs 2000	a dinucleotide repeat polymorphism		88 schizophrenic/ schizoaffective patients	?	PANSS ?; categorical	excess of long alleles (172-176bp) in responders
Hong 2003	Val66Met		93 schizophrenic patients	Clozapine	20% decrease in BPRS	Val homozygotes more frequent in responders

Methylenetetrahydrofolate Reductase (THFR)

reference	polymorphism	notes	sample	treatment	phenotype	result
Joober 2000b	677C/T		105 schizophrenic patients	conventional neuroleptics	response: treatment resistance defined as by Kane et al. 1988	T alleles and T homozygote genotypes more frequent in responders

5.1.5. Combined Analyses (see table 6)

In an attempt to depict the assumable complexity of the genetic background of Clozapine response and with the goal to constitute a pattern of multiple

genotypes associated with response Arranz et al. [2000b] analyzed 19 polymorphisms of 5HT1A, -2A, -3A, -5A, 5-HTTLPR, DRD3, ADRA and Histamine-receptor H1 and H2. They reported a combination of 6 polymorphisms: 5HT2A 102T/C and His452Tyr, 5HT2C -330GT/-244CT and Cys23Ser, 5-HTTLPR and H2 -1018G/A to predict response to Clozapine with a positive predictive value of 0.82.

This finding however could not be replicated by Schumacher et al. [2000] who investigated the same 6 polymorphisms and reported only H2 -1018G/A to be associated with response.

Table 18. Antipsychotic Response and Combined Genotypes

reference	polymorphism	notes	sample	treatment	phenotype	result
Arranz 2000b	5HT2A: His452Tyr, Thr25Asp, -1438G/A, 102T/C, 516C/T 5HT2C: -330GT/-244CT, Cys23 Ser 5HT3A: 178C/T, 1596G/A 5HT5A: -12A/T, -19G/C 5HTT: 5-HTTLPR, VNTR DRD3: Ser9Gly ADRA1A: Arg492Cys ADRA2A: -1291G/C, -261G/A H1: Leu449Ser H2: -1018G/A	no correction for multiple testing treatment response retrospectively assessed	200 schizophrenic patients	Clozapine	response: GAS	combination of 6 polymorphisms predict clozapin response: 5HT2A: 102T/C His452Tyr 5HT2C: -330GT/ -244CT Cys23Ser 5HTT: 5 -HTTLPR H2:-1018G/A
Schumacher 2000	Same combination of 6 response predicting genotypes as Arranz 2000b	an attemt to replicate Arranz 2000b	163 schizophrenic patients	Clozapine	4 response groups, group 3 and 4 corresponding to 20 point GAS improvement	n.s. Only H2: -1018G/A associated with response on the allelic level

n.s. not significant

5.2. Agranulozytosis

The majority of association studies regarding Clozapine induced agranulozytosis have been investigating the HLA system. The interpretation of the results is very problematic due to several methodological shortcomings. One of those is ethnic background: several of the studies are investigating jewish, mostly Ashkenazi patients, partially discriminating results into jewish/non-jewish subgroups partly not discriminating them at all. Sample sizes are often painfully small, 3 to 5 patients with Clozapine induced agranulozytosis (CA) versus 13 to 80 controls is not really a basis for a valid statistical analysis (see Abt et al. [1992] for more detailed statistical critique). Correction for multiple testing is not always done (as in this case should be done since the study designs are exploratory and not hypothesis based). A lot of results do not bear up against

correction for multiple testing when in fact applied or are reported as trends firsthand.

Besides more optimistic results reported by Lieberman et al. [1990] and Yunis et al. [1992, 1995], the two early studies with considerable sample size by Claas et al. [1992] and Abt et al. [1992] both report no significant results.

Theodoropoulou et al. [1997], Amar et al. [1998], Meged et al. [1999], Valevski et al. [1998], Lahdelma et al. [2001] all those studies suffer from small sample sizes. The most robust finding from these studies after all, or at least the one most often reported, is the association of HLA-B38 with CA in jewish patients (HlA-B16 in Lahdelma et al. [2001], but B38 is a splice variant of B16).

Dettling et al. [2001a, 2001b] in a more extended non-jewish sample report a complex association pattern with several predominantly class II antigens.

For further details on HLA association findings see table F.

In succession to their 1995 paper [Yunis et al. 1995], the Yunis group published further studies about a heat shock protein (HSP70) gene and the tumor necrosis factor (TNF) gene, both located in the class III region of the major histocompatibility complex (MHC). In Corzo et al. [1995] they report the HSP70-1 A allele and the HSP70-2 9.0kb allele in linkage disequilibrium with each other and associated to CA in jewish patients. Regarding TNF Turbay et al. [1997] report an association of the TNF microsattelites d3 and b4 with CA, b5 with non-CA controls.

Ostrousky et al. [2003] report several SNP's in the first intron and exon 3 and 5 of the dihydronicotineamide riboside quinone oxidoreductase 2 (NQO2) gene to be associated with CA. The paper seems to be an extension of the Valevski et al. [1998] study. The NQO2 gene maps to a region telomeric to the MHC complex.

Table 19. Side Effect: Clozapine-induced Agranulozytosis (CA)
HLA-System

reference	polymorphism	notes	sample	treatment	phenotype	result
Lieberman 1990	HLA-A, -B, -C, -DR, -DQ (HLA-class I and II antigens)	serotyped	schizophrenic and schizoaffective Ashkenazi patients 5 patients with Clozapine induced Agranulozytosis (CA), 26 controls	Clozapine	agranulozytosis: less than 0.5x109/L polymorphonuclear leucocytes	agranulozytosis associated with a haplotype consisting of HLA-B38, -DR4, -Dqw3
Claas 1992	HLA-class I and II antigens	serotyped	103 patients with CA, 95 matched controls	Clozapine	granulozytopenia: less than 1500 granulozytes/ml	n.s. after correction for multiple testing
Yunis 1992	HLA-class I and II antigens	serotyped	11 CA patients, 31 controls, most of jewish ancestry	Clozapine	agranulozytosis: less than 0.5x109/L polymorphonuclear leucocytes	agranulozytosis associated with HLA-B38, -DR4 and -DQw3 haplotype in jewish patients; with HLA-DR2 and -DQw1 in non-jewish

Abt 1992	HLA-class I and II antigens 48 HLA antigens	serotyped diagosis=?	72 patients with granulozytopenia/CA, 74 controls	Clozapine	definition of CA=?	no significant model of HLA subsets
Yunis 1995	HLA-class I and II antigens	serotyped	Ashkenazi and non-Ashkenazi schizophrenics and schizoaffectives, 31 CA, 52 controls extended Yunis 1992 sample	Clozapine	agranulozytosis: less than 500 neutrophils per mm3	Markers for jewish CA patients: B38, DRB1*0402, DRB4*0101, DQB1*0302, DQA1*0301 "protecting alleles": DR11, DQB1*0301 Markers for non-jewish CA patients: DRB*1601, DRB5*02, DQB1*0502, DQA1*0102, DR2, DQw1
Theodoropoulou 1997	HLA-class I and II antigens	serotyped	43 schizophrenics, 3 of them developing agranulocytosis	Clozapine	agranulozytosis: less than 500 neutrophils per mm3	n.s.
Amar 1998	HLA-class I and II antigens	serotyped	18 schizophrenics, 5 of them with granulozytopenia/CA	Clozapine	granulozytopenia: less than 1000 and agranulozytosis: less than 500 neutrophils per mm3	granulozytopenia/ agranulozytosis associated with HLA-DQB1*0201
Valevski 1998	HLA-class I and II antigens	serotyped	61 jewish schizophrenics, 11 of them with CA	Clozapine	agranulozytosis: less than 500 neutrophils per mm3	HLA-B38 associated with agranulozytosis
Meged 1999	HLA-class I	serotyped	88 Jewish schizophrenics, 3 of them with CA	Haloperidol, Clozapine	agranulozytosis: less than 500 neutrophils per mm3	n.s. trend for HLA-B38 to be associated with agranulozytosis
Lahdema 2001	HLA-A, -B	serotyped, partially genotyped	26 schizophrenic patients with Granulozytopenia/Agranulozytosis and 19 schizophrenic controls	Clozapine	granulozytopenia: less than 1,5x109/L agranulozytosis: less than 0.5x109/L	granulozytopenia/ agranulozytosis associated with absence of HLA-A1
Dettling 2001a	HLA-class I and II antigens	genotyped ;no correction for multiple testing	107 caucasian paranoid schizophrenics, 30 of them with CA	Clozapine	agranulozytosis: less than 500 neutrophils per mm3	agranulozytosis associated with HLA-DQB1*0502, -DRB5*02, trend for -DQB1*0201

Dettling 2001b	HLA-class I and II antigens	genotyped; no correction for multiple testing	108 caucasian paranoid schizophrenics, 31 of them with CA sample seems to be identical with Dettling 2001a	Clozapine	agranulozytosis: less than 500 neutrophils per mm3	agranulozytosis associated with HLA-Cw*7, -DQB*0502, -DRB1*0101, -DRB3*0202

Heat Shock Protein

reference	polymorphism	notes	sample	treatment	phenotype	result
Corzo 1995	HSP70-1 HSP70-2	HSP70 is part of the HLA-class III cluster	75 schizophrenic and schizoaffektive patients, 32 of them with CA	Clozapine	agranulozytosis: less than 0.5x109/L polymorphonuclear leucocytes	HSP70-1 A and HSP70-2 9.0kb in linkage disequilibrium with each other and associated to CA in jewish patients

Tumor Necrosis Factor

reference	polymorphism	notes	sample	treatment	phenotype	result
Turbay 1997	TNF microsattelites a-b, d-e		12 jewish, 21 non-jewish schizophrenics vs 33 controls	Clozapine	agranulozytosis: less than 500 neutrophils per ml	CA associated with d3 and b4, inversely associated with b5

NQO2

reference	polymorphism	notes	sample	treatment	phenotype	result
Ostrousky 2003	NQO2: 1536C/T, 1541G/A, 372C/T (Phe/Leu), 202G/A, -367A/G, -394G/C	Sample seems to be partially identical to Valevski 1998	98 schizophrenics 18 of these with CA	Clozapine	agranulozytosis: less than 500 neutrophils per mm3	CA patients predominantly heterozygous for several exon and intron SNP's

n.s. not significant

5.3. Weight Gain

Weight gain is an issue in neuroleptics treatment side effects that has gained increasing attention recently. All but one study on this topic are dealing with Clozapine treated patients. The 5-HT2C receptor was predominantly investigated.

Rietschel et al. [1997] reported no significant result for the 5-HT2C Cys23Ser polymorphism, Hong et al. [2001b] investigated the 5-HT2C 68G/C polymorphism among other serotonin receptor and transporter gene polymorphisms without significance too. The 5-HT2C –759C/T polymorphism was investigated in several more recent publications without significant findings by Tsai et al. [2002], Basile et al. [2002a] and Theisen et al. [2004]. Reynolds et al. [2002] however report an association of the 5-HT2C –759C allele with

weight gain in patients treated with a variety of neuroleptics and in the Clozapine treated subgroup of this sample [Reynolds et al. 2003].

Basile et al. [2001] investigated 10 genetic polymorphisms in 9 candidate genes and found trends of association for adrenergic receptor gene polymorphisms (ADRB3, ADRA1A), for 5-HT2C Cys23Ser and for TNFα 308G/A.

Table 20. Side Effect: Weight Gain

reference	polymorphism	notes	sample	treatment	phenotype	result
Rietschel 1997	5-HT2C: Cys23Ser		152 schizophrenics	Clozapine	?	n.s.
Hong 2001b	5-HTTLPR, 5-HT2A 102T/C, 5-HT2C 68G/C, 5-HT6 267C/T		93 schizophrenic	Clozapine	weight gain continous	n.s.
Basile 2001	5HT2C: Cys23Ser, 5HT1A: CAn repeat, 5HT 2A: 102T/C and His452Tyr, H1:?, H2: -1018G/A, Cyp1A2: Intron1 C/A, ADRA1A: Arg347Cys, ADRB3: Trp64Arg, TNFa -308G/A		80 schizophrenics	Clozapine	weight gain continous	trends for DRB3, ADRA1A, TNFa, 5HT2C
Reynolds 2002	5-HT2C: -759C/T		123 chinese schizophrenics	miscellaneous NL	cut off: weight gain >7%	-759C ssociated with weight gain
Tsai 2002	5-HT2C: -759C/T		80 chinese schizophrenics	Clozapine	weight gain: BMI continous and cut off >7%	n.s.
Basile 2002a	5-HT2C: -759C/T		80 schizophrenics	Clozapine	Cut off: weight gain >7%	n.s.
Reynolds 2003	5-HT2C: -759C/T	subsample of Reynolds 2002	32 chinese schizophrenics	Clozapine	cut off: weight gain >7%	-759T associated with less weight gain
Theisen 2004	5-HT2C: -759C/T		97 german schizophrenic patients	Clozapine	Cut off: weight gain >7%	n.s.

n.s. not significant

5.4. NMS

The neuroleptic malignant syndrome (NMS) is a potentially fatal side effect of neuroleptic treatment with extrapyramidal symptoms, hyperpyrexia and elevated serum creatine phosphokinase levels. Trying to elucidate the putative genetic background of this severe condition cytochrome P450 2D6 (CYP2D6) polymorphisms and Dopamine D2 receptor gene polymorphisms have been investigated.

Regarding CYP2D6 no significant associations have been reported by Ueno et al. [1996], Iwahashi et al. [1997] and Kawanishi et al. [2000]. Suzuki et al. [2001c] found the DRD2 TaqI A1 allele associated with NMS, Kishida et al. [2004] the DRD2 –141C Del allele. The DRD2 TaqI A result could not be replicated by Kishida et al. [2003].

Table 21. Side Effect: Neuroleptic Malignant Syndrome

reference	polymorphism	notes	sample	treatment	phenotype	result
Ueno 1996	CYP2D6: 1795T del, 1934G/A, Arg296Cys (Hha I)		9 NMS patients	miscellaneous NL ?	?	n.s.
Iwahashi 1997	CYP2D6: HhaI	identical to Iwahashi 1999	56 japanese schizophrenics, 8 of them with NMS	miscellaneous NL	?	n.s.
Kawanishi 2000	Cyp2D6: Pro34Ser		36 patients with NMS, 107 schizophrenic controls	miscellaneous NL ?	NMS diagnosis according to the criteria of Pope et al. 1986	n.s.
Suzuki 2001c	DRD2: TaqI A	153 schizophrenic patients, 15 with NMS	miscellaneous NL ?	NMS criteria by Pope et al. 1986	A1 allele associated with NMS	
Kishida 2003	DRD2: TaqI A	49 patients with NMS, 123 schizophrenic controls	miscellaneous NL	NMS criteria Pope et al. 1986		n.s.
Kishida 2004	DRD2: TaqI A, -141C Ins/Del, Ser311Cys	164 japanese schizophrenics, 32 with NMS	miscellaneous NL ?	criteria by Pope et al. 1986	-141C Del more frequent in NMS	

n.s. not significant

5.5. Various side effects

Hsu et al. [2000] found no association of the adrenergic receptor α1A in Clozapine treated patients. Spina et al. [1992] report Cyp2D6 poor metabolizer

to be more prone to side effects of typical neuroleptics like postural hypotension, diplopia and dry mouth.

Table 22. Side Effect: Various

reference	polymorphism notes	sample	treatment	phenotype	result
Hsu 2000	Adrenergic Receptor α 1A RsaI	80 schizophrenic patients	Clozapine	urinary incontinence in the first 3 month of treatment	n.s.
Spina 1992	CYP2D6 phenotyped by debrisoquine hydroxylation test	53 schizophrenics	typical neuroleptics	side effects: postural hypotension, diplopia, dry mouth	PM state associated with side effects

n.s. not significant

6. DISCUSSION

Currently the association findings of pharmacogenetic studies in psychosis are not very convincing with the most robust findings, at least for response, probably in the 5-HT2A, 5-HT2C, DRD2 and DRD3 receptor genes. There are a lot of studies with conflicting results, no replications or no replication trials at all.

Many studies are suffering from considerable methodological shortcomings and efforts have been undertaken to propose a stringent methodology for pharmacogenetic studies [Rietschel et al. 1999, Malhotra et al. 2000, Masellis et al. 2000]. The recommendations range from sample size and statistical power considerations over diagnostic stringency and statistical rigorousness to proposals of medication plasma levels control.

An important reason for this apparent problematic situation of pharmacogenetics after all is inherent in the nature of the topic. Genetics of psychiatric diseases are complex with environmental factors to be considered and in genetics of treatment effects we are definitely dealing with complex traits. Multiple genetic polymorphisms each of them with minor impact are involved, multiple receptors are targeted by different drugs and pharmacokinetic effects have to be considered.

Complex designs of studies with multiple polymorphisms as by Arranz et al. [2000b] are an interesting approach, however with considerable methodological problems. Haplotype studies might increase statistical power [Malhotra et al. 2004], as well as the use of candidate genes with a strong a priori rationale [Masellis et al. 2000]. Multicenter studies with great sample sizes have repeatedly been asked for and endophenotypes as well as neuroimaging approaches might refine the technique.

Genes of expression, regulation and transport mechanisms, second messenger, third messenger and transcription factors are increasingly taken into account to include the post receptor downstream systems into the picture.

A promising future strategy in pharmacogenetics seems to be the application of microarray expression profiling for the identification of relevant candidate target genes of antipsychotic drug action. Thus gene expression induction by Clozapine treatment has been found for example for Chromogranin

A and Calcineurin A, a decrease in Synaptotagmin V in rat frontal cortex. Chronic administration of Clozapine resulted in a differential expression pattern for Chromogranin A, son of sevenless (SOS: a RAS activator) and Sec-1 in the cortex. Chronic haloperidol treatment has been found to alter gene expression of "inhibitor of DNA-binding 2" (ID-2) and Rab-12. Since Chromogranin A and Synaptotagmin V seem to be involved in presynaptic vesicle formation and secretion, this might represent an important mode of action of antipsychotic medication. Post mortem studies with brain tissue of schizophrenic patients have found altered expression of genes involved in presynaptic functions [see Miyamoto et al. 2005].

Thus we will hopefully see the riddle of pharmacogenetics of psychosis being resolved step by step in the nearer future to be provided with a biologically based rationale for the administration of targeted antipsychotic medication.

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