Pharmacogenetic Aspects of Clozapine Treatment

Master thesis by

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Abstract:

Despite several decades of research, treatment of schizophrenia still relies on a trial-and-error principle. Inadequate treatment with several antipsychotic drugs often cause prolonged hospitalizations, loss of function and decreased life quality of patients suffering from schizophrenia. In schizophrenia, up to 30 % of patients are classified with treatment resistant schizophrenia. Treatment resistant schizophrenia is characterized as insufficient response to two or more conventional antipsychotic drugs. Approximately 50 % of patients suffering from treatment resistant schizophrenia still respond to clozapine despite resistance to other antipsychotic drugs. Despite clozapines superiority in treating treatment resistant schizophrenia, clozapine is not a first line drug. Clozapine can cause rare, but life threatening side-effects. Thus patients are monitored thoroughly while being treated with clozapine, due to these side-effects. Patients would greatly benefit if clozapine response could be predicted. Thus adequate treatment could be initiated faster and the prognosis of patients would improve. The pharmacogenetics of clozapine might be the key in finding a predictor of response, since response to clozapine have been associated genetic variation in several studies.

After a thorough review of all the dopamine receptor genes, the pharmacogenetic aspects of the dopamine D2 receptor were investigated to clozapine response. No association was found in the sample.


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Preface

This master thesis was written by Michelle Vandborg Andersen on Translational Medicine, Medicine with Industrial Specialization, Department of Health Science and Technology, Aalborg University. The primary content of this thesis is an article titled “The dopamine D2 receptor gene: is it the answer to clozapine response”. A systematic review article titled “Genetic markers for clozapine response – how far are we” is annexed to the thesis. The objective of the master thesis was to further develop scientific skills within translational medicine, and to display the student's ability to perform scientific and practical work. Both articles are structured as scientific articles, written in English, with the intention to bring it to publication in a peer-reviewed journal. The master thesis is intended addressed health professionals, researchers, students and others with an interest in and knowledge of genetics, schizophrenia and clozapine. The American medical association, 10th addition, AMA, model of referencing is used throughout the thesis. The reference list is found in appendix 2. Figures and images presented in the thesis are created by the author unless otherwise stated. Knowledge acquired during this master thesis was primarily obtained through scientific articles from databases, such as PubMed, Embase etc. All papers were evaluated by quality before used.

The student was supervised by Jimmi Nielsen, to which she owes him a sincere appreciation for guidance and support throughout the thesis. In addition, collaboration between the student and Centre of Schizophrenia, Aalborg University Hospital, the Psychiatric Division was carried out through the master thesis, the student value their cooperation.


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List of content


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Original article

The dopamine D2 receptor gene: is it the answer to clozapine response

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Abstract:

Background: After several decades, clozapine remains the cornerstone of antipsychotic treatment in treatment resistant schizophrenia; a condition up to 30 % of patients suffers from. Response to clozapine is heterogeneous, and 50 % of patients respond to the drug, regardless of treatment resistance to conventional antipsychotics. Genetic variation in receptors clozapine has an affinity to, might be responsible for this heterogeneity in treatment response. Dopamine D2 receptors plays a central role in antipsychotic response, but clozapine have a different affinity at this specific receptor, than conventional antipsychotic drugs. Thus, genetic variation within the dopamine D2 receptor gene might account for clozapines superior efficacy in treatment resistant schizophrenia, and clozapines heterogeneous response rate.
Methods: 12 single nucleotide polymorphisms within the dopamine D2 receptor gene, was investigated for any allelic or genotypic association with clozapine response. Response was defined in a two year mirror-image model, were a 50 % reduction in bed-days after receiving clozapine was considered a criterion. The study assessed 33 Caucasian subjects, of who 19 was eligible for inclusion. All subjects were diagnosed with ICD-10 F20 schizophrenia.
Results: No significant difference was found between responders and non-responders with regard to sex, diagnosis, age of clozapine treatment initiation or clozapine dosage. None of the allele or genotypes of the investigated single nucleotide polymorphisms was associated with clozapine response.
Conclusion: The dopamine D2 receptor gene is unlikely solely responsible for clozapine response in Caucasian subjects. The next step in pharmacogenetic studies might be to perform polygenetic analysis in order to include several genes at once.

Keywords: Clozapine; Receptors, dopamine D2; pharmacogenetics; schizophrenia; drug resistant

Introduction

Schizophrenia

In the general population, approximately 1 % suffers from schizophrenia[1]. This chronic disorder often manifests in youth, and are characterized by a number of symptoms, all of which can be classified as either positive (paranoid delusions, auditory hallucinations, thought disorders etc.), negative (flattened affect, apathy, social withdrawal etc.) or cognitive symptoms (reduced sustained attention and executive functions). Even though all of these symptoms are evident in different cases of schizophrenia, none of the symptoms is pathognomonic for schizophrenia.

The etiology of schizophrenia

Genetics play a key role in schizophrenia, but the exact etiology of schizophrenia remains largely unknown[2,3]. Through the last decades, different

causes of schizophrenia have been investigated, including changes in neurotransmitter systems and neurodevelopment abnormalities[4]. The dopamine hypothesis was first described in the 1970's, [5,6]. Evidence of correlation between blockade of dopamine D2 receptors (DRD2), and response to antipsychotic drugs lead to a theory of dopamine hyperfunction in subcortical regions (nucleus accumbens) in the brain[7]. Subcortical regions are dense in DRD2, and the number of DRD2 have been shown further increased in these areas in schizophrenia[8]. The dopamine hyperfunction in the limbic system are considered responsible for the positive symptoms of schizophrenia[7]. Since the 1970's, the hypothesis has been refined to include a dopaminergic hypofunction in the prefrontal cortex[9]. The prefrontal cortex is an area dense in dopamine D1 receptors, in which abnormalities may account for the negative symptoms as well as the cognitive impairment of schizophrenia[9]. More recently, studies suggests that dopamine activity can be influenced by abnormalities in glutamate transmission in areas such as substantia nigra and the ventral tegmental area[4]. Deficiency of N-methyl aspartate (NMDA) transmission, a receptor of glutamate, can lead to a hypofunction of dopamine receptors in the prefrontal cortex, subsequently leading to a decrease in mesocortical dopamine transmission[4]. Over time, the deficiency of the meso-limbic dopamine transmission might cause the positive symptoms of schizophrenia[4].

N-desmethylclozapine (NDMC)

Clozapine undergoes hepatic metabolization by several P450 CYP enzymes, the most significant being CYP1A2, CYP2D6 and CYP3A4[10,11]. The high first pass metabolism reduces the bioavailability of clozapine to 50-60 %[12]. The majority of clozapines metabolites are physiological inactive, but N-desmethylclozapine (NDMC) is active[13]. Only a few studies have investigated the effects of NDMC, but studies suggest different properties of NDMC than clozapine[14-16]. NDMC acts as an partial agonist to DRD2 [14], with a binding affinity (K_i) of 115,2[17]. Clozapine on the other hand, acts as an inverse agonist[14], or antagonist[15] with a K_i of 431[17]. An animal study evaluating Conditioned avoidance responding and Amphetamine-induced locomotion, models of antipsychotic efficacy, has revealed differences between clozapine and NDMC. Clozapine inhibited effects in both models, the inhibition were only present at high dosages of NDMC[16]. A model of regional activation of the central nervous system (Fos expression), showed both clozapine and NDMC ability to induce Fos in nucleus accumbens but not in the dorsolateral striatum. Again, NDMC required high dosages to do the same[16]. No differences between clozapine and NDMC in studies investigating catalepsy (measure of motor-side effects) or prolactin measurement (measure of side-effects) were found[16].

Treatment of schizophrenia

In most cases of schizophrenia, antipsychotic drugs are the treatment of choice[18]. However, discontinuation of treatment is a common obstacle in this patient group, due to side-effects or lack of efficacy, the latter one also leaving a large proportion of patients symptomatic despite adequate antipsychotic treatment[19]. Up to 30 % of patients suffering from schizophrenia, are classified as treatment resistant schizophrenia (TRS)[20]. TRS are often defined as inadequate response to two, or more first generation antipsychotics (FGA) and second generation antipsychotics (SGA)[20]. Clozapine is the most efficient antipsychotic drugs in TRS, proven efficient for alleviating both positive and negative symptoms[21], as well as having antisuicidal properties[22]. On the other hand, studies fail to find any substantial superiority of clozapine compared to other dopamine antagonists in first episode schizophrenia, suggesting a biological homo-geneity of patients responding to clozapine[23-25]. Clozapine is not considered a first line antipsychotic drug in non-resistant schizophrenia, due to the risk of agranulocytosis and myocarditis[26]. The side-effects warrant close monitoring of patients[26]. Nevertheless, clozapine posses the unique property of being efficient in

approximately 50% of patient with TRS, making it the golden standard for treating TRS[27].

The mechanism of action of clozapine is unknown, but several theories exist; I) Dysregulation of the immune system have been observed in schizophrenia[28], and preclinical studies suggest that improvements by clozapine in factors influencing the immune systems, correlates with symptoms relieve[29]. Thus, a theory of an anti-inflammatory effect of clozapine was proposed as being the reason for the unique effect. II) Evidence of clozapine affinity towards a large number of receptors has been acknowledged for a long time[30]. The high affinity of clozapine to muscarinic receptors, a property mainly clozapine possess[31], lead to a theory that the receptor might be an important factor in clozapine response[32]. III) Blockage of DRD2 is a shared feature of all present antipsychotic drugs[7]. In addition, a DRD2 occupation of at least 70 % is considered optimum for antipsychotic response[33]. Interestingly, the high occupancy is not the case for clozapine, which rarely reaches occupancy of 70 % even at high dosages[34]. Clozapine superior effectiveness might be caused by regional selectivity, since clozapine specifically target DRD2 in limbic and cortical regions[35]. In addition, clozapine display a fast dissociation rate and are more loosely bound to DRD2, unlike other antipsychotic drugs[5].

Pharmacogenetics

The aim of this study is to identify genetic variation, which can predict optimal response or non-response to a treatment within a specific population. The variability in response to clozapine can possibly be attributed to genetic variation between responders and non-responders. The single nucleotide polymorphism (SNP) which has received most attention when focusing on the DRD2 gene is rs1799732, also known as -141C ins/del. The SNP has been associated with schizophrenia in general[36]. Rs1799732 has also been associated with clozapine response in patient with TRS[37], but the majority of studies have not been able to replicate this finding[38-40]. A total of 12 SNPs in the DRD2 gene have been investigated to clozapine response[37-40]. There are inconsistencies in current findings, which can be attributed to ethnic heterogeneity, differences in study designs, etc. (for a more comprehensive review see appendix 1). Before these findings can be used clinically, replication in a large scale study is warranted. Thus, the current study focuses on replication of earlier findings of pharmacogenetic studies between DRD2 gene, and clozapine response in a distinct TRS population (Caucasian with Danish ancestry). This study will employ a more naturalistic response criterion, using psychiatric bed-days as a measure of response[41], compared to more subjective measures such as the Brief Psychiatric scale.

Methods

Clinical data was obtained from 33 patients, after giving informed consent for Danish Psychiatric Biobank. Patients were recruited through a participation in an extraordinary health examination, or were referred by their psychiatrist. The health examination, as well as participation in this study was offered to all psychiatric patients in the Northern Jutland. All patients in this study were diagnosed with schizophrenia according to the International Classification of Diseases (ICD-10). Of the 33 patient, 19 contributed to the mirror-image model (described in the next paragraph). The 19 subjects were all Caucasian of Danish ascend. They all met the criteria of TRS (i.e. resistant to two or more FGA or SGA). All patients had been treated with clozapine.

Though patients' medical records several clinical data was obtained. Patients' treatment history with clozapine was collected, including first administration date, duration of treatment with a cutoff of two years, maximum dosage during and maximum plasma concentration during this time period. The number of bed-days before and after initiation of clozapine treatment was registered, according to the mirror-image model, described in the following paragraph.

Maximum clozapine dosage and maximum clozapine plasma concentration was obtained from patients medical records, within the first two years of clozapine treatment. The maximum levels were chosen to exclude periods of up-titration of dosage, and instead determine concentrations during steady-states of clozapine treatment. Single occurrences of extraordinarily high plasma-concentration values were not registered, to minimize biases from interactions, non-fasting blood-tests etc.

The Mirror-image design

Patients were evaluated with regard to clozapine response based on their medical records.

Responders and non-responders were classified based on a mirror image model illustrated in figure 1. The mirror-date was the time of the first clozapine administration. The duration of clozapine-treatment was evaluated for each patient after the mirror-date (up to 2 years) based on the number of psychiatric bed-days and were compared to psychiatric bed-days prior to the mirror-date (equal time period). Patients without any admissions prior to initiation of clozapine-treatment were excluded from the study. If patients initiated clozapine-treatment during an admission, the afflicted bed-days were not taking into account. Response was defined as ≥ 50 % reductions in bed-days after initiation of clozapine.

Single nucleotide polymorphism (SNP) selection

The SNPs listed in table 1 was selected based on a literature search of all published pharmacogenetic studies with clozapine response and DRD2 gene (see appendix 1)

Genotyping procedures

A venous blood sample were collected in EDTA tubes, and stored in the freezer at -18°C, until the samples were sent to Psychiatric Centre of Sct. Hans for extracting of Genomic deoxynucleic acid (DNA) and further analysis. DNA was isolated from the samples using methods described by Shen et al. 2009[42]

Each sample was genotyped for DRD2 gene SNPs (see the following section). PCR amplification and genotyping was done using TaqMan probes and primers obtained from Applied Biosystems (Foster City, CA, USA). Already designed TaqMan SNP genotyping Assays (ABI) were applied when possible, PCR was done using GeneAmp PCR system 9700. Each plate was compatible to work with genomic DNA and TaqMan Universal Master Mix (Applied Biosystems, Foster City, CA, USA), Genotype analysis was done using ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA). The laboratory staff was blinded as regards to clozapine response. If genotypes were unavailable in assays, the genotype was imputated using estimations from the 1000 genome project[43,44].

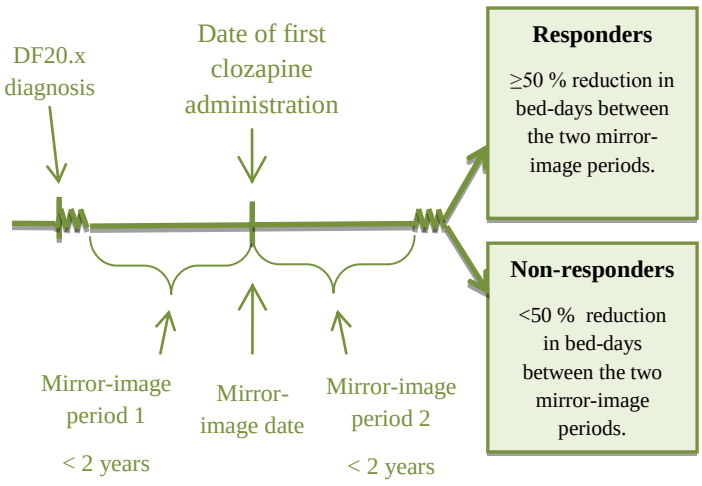

Figure 1, Illustration of the mirror image model.

Table 1, Dopamine D2 receptor gene polymorphism, location and functional consequence.

SNP	Synonyms	Variation	SNP location	Functional consequence
Rs1799732	-141C Ins/Del	+/- C	-141 position of 5'-UTR	A functional polymorphism which alters gene expression in vitro[45]. The deletion allele has been reported to reduce transcription in cell lines[45].
Rs1799978	A-241G	A > G	-241 position of 5'-UTR	The location of the polymorphism suggests a regulatory role of the DRD2 gene expression, but the functional consequence of the polymorphism remain unclear [46]. One study failed to show any change in receptor expression[45].
Rs4648317		C > T	Intron 1	Alter two transcription factor binding sites, and might influence DRD2 gene expression[47].
Rs1125394		A > G	Intron 1	
Rs1079597	TaqIB	A > G	Intron 1, in the coding region of exon 2[48]	A restriction fragment length polymorphism
Rs1800498	TaqID	C > T	Intron 2	
Rs6275	Nco I	C > T	Exon 7	Has been shown to influence transcription stability and translational efficiency[49]
Rs6277	C957T	T > C	Pro319 position of 3'-UTR	In vivo studies have shown that the DRD2 receptor affinity and availability can be regional changed by the polymorphism[50]. The polymorphism has been shown to cause alterations of mRNA folding, stability and translation of the protein.
Rs2242591		A > G	Downstream of the termination codon of DRD2	
Rs2242592		C > T	The intergenetic region between ANKK1 and DRD2 gene	
Rs2242593		A > G	Downstream of the termination codon of DRD2	
Rs1800497	TaqIA	C > T	Downstream of the termination codon of DRD2 in the coding region of ANKK1[51]	ANKK1 is involved in signal transduction, and may influence dopamine reward processes[52] Evidence in healthy subjects suggests that the polymorphism is associated with reduced dopamine activity and DRD2 receptor density[53], although inconsistent findings are evident[54]. In addition the polymorphism might interfere with substrate binding specificity[51].

Abbreviations: SNP: Single nucleotide polymorphism, Ins: insersion, Del: Deletion, +: presence of allele, -: absence of allele, C: cytosine, G: guanine, A: adenine, T: thymine, UTR: untranslated region, mRNA: messenger ribonucleic acid, ANKK1: Ankyrin Repeat and Kinase Domain containing 1 gene

Statistical analysis

Statistical analysis was done using STATA/SE for windows v13.1 (StataCorp LP, USA). Only $p<0.05$ was considered significant. Each SNP was evaluated of Hardy-Weinberg equilibrium. Differences in frequencies of allele and genotype was done using Fishers Exact test.

Results

A total of 33 subjects were analyzed for SNP variations. Of those, 19 subjects were included into the study. The remaining 14 subjects were excluded since they did not contribute with any bed-days in either mirror-image periods. The 19 subjects were divided into two groups; 11 responders and 8 non-responders, based on the mirror-image model. All included subject were Caucasian, Danish origin. As listed in table 2 no difference between responders and non-responders in sex (p=0.260) nor diagnose (p=0.348) were found. In addition, the mean age of clozapine initiation (p=0.915) and the mean duration of clozapine treatment (p=0.729) were not significant different between responders and non-responders.

Responders minimized the average number of bed-days from 109.7 bed-days (SD ±131.8) in mirror-period 1 to 3.5 bed-days (SD ± 7.4) in mirror-period 2 (p=0.022). Non-responders did not statistically change in number of bed-days between the two mirror-periods. In average non-responders had 102.5 bed-days in mirror-period 1 compared to 145.6 bed-days (SD: ±95.4, p=0.096) in mirror-period 2.

Infrequency in analysis of the deletion/insertion polymorphism rs1799732 were found, since all subjects (n=15), both responders (n=8), and non-responders (n=7) had the C insertion polymorphism. The Hardy-Weinberg principle states that within a population genetic variation is constant. Besides rs1799732, all other SNPs were in Hardy-Weinberg equilibrium (data not shown).

As listed in table 3, none of the allele frequencies in this sample were associated with clozapine response. Similar results with genotype frequencies were found in the sample; none of the genotypes yielded any significant difference in the statistical tests.

Table 2, Demography data and statistical comparison between responders and non-responders.

	Total (±SD) N=19	**Responders (±SD) n=11**	**Non-responders (±SD) n=8**	**p-value**
Sex (male/female)	10/9	7/4	3/5	0.260
Diagnose (DF20.0/DF20.3/DF20.9)	12/5/2	5/3/0	7/2/2	0.348
Age of first clozapine administration	31,3 years (12.0)	31 years (12.9)	31.6 years (11.6)	0.915
Duration of clozapine treatment	623.9 days (220.5)	639.5 days (218.5)	602.5 days (236.4)	0.729
Max. clozapine dosage	384.4mg/day (172.0)	383.3 mg/day (180.3)	385.7 mg/day (174.9)	0.979
Max. p-clozapine	1131 nmol/L (490.5)	990 nmol/L (471.8)	1307 nmol/L (520.0)	0.369
Bed-days in mirror-image period 1	106.7 days (119.8)	109.7 days (131.8)	102.5 days (109.8)	**0.025** (between responders)
Bed-days in mirror-image period 2	63.3 days (93.7)	3.5 days (7.4)	145.6 days (95.4)	0.096 (between non-responders)

Abbreviations: SD: standard deviation, n: number, p-value: probability value, DF20.0: Paranoid schizophrenia, DF20.3: undifferentiated schizophrenia, DF20.9: unspecified schizophrenia, p-clozapine: plasma-concentration of clozapine,

Table 3, Genotype and allele frequencies of DRD2 gene

SNP	Genotype and allele		Responders n (freq)	Non-responders n (freq)	Odds ratio (allele frequency only)	P-value (Fishers exact test)
rs1799732	Allele	C+	8 (1.0)	7 (1.0)	-	1.000
		C-	0	0		
rs1799978	Genotype	AA	11(1.0)	6 (0.75)	-	0.164
		AG	0	2 (0.25)		
		GG	0	0		
	allele	A	22(1.0)	14(0.88)	-	0.171
		G	0	2 (0.12)		
rs4648317	Genotype	CC	9(0.82)	7(0.88)	-	1.000
		CT	2(0.18)	1(0.12)		
		TT	0	0		
	allele	C	20(0.91)	15(0.94)	0.667 (0.000-5.703)	1.000
		T	2(0.09)	1(0.06)		
rs1125394	Genotype	AA	8(0.73)	3(0.38)	-	0.181
		AG	3(0.27)	5(0.62)		
		GG	0	0		
	allele	A	19(0.86)	11(0.69)	2.879 (0.620-13.178)	0.189
		G	3(0.14)	5 (0.31)		
rs1079597	Genotype	AA	0	0	-	0.181
		AG	3(0.27)	5(0.63)		
		GG	8(0.73)	3(0.37)		
	allele	A	3(0.14)	5(0.31)	0.347 (0.076-1.162)	0.186
		G	19(0.86)	11(0.69)		
rs1800498	Genotype	CC	3(0.27)	2(0.25)	-	0.838
		CT	3(0.27)	4(0.50)		
		TT	5(0.46)	2(0.25)		
	allele	C	9(0.41)	8(0.50)	0.682 (0.194-2.471)	0.743
		T	13(0.59)	8(0.50)		
rs6275	Genotype	CC	7(0.64)	5(0.63)	-	1.000
		CT	3(0.27)	3(0.37)		
		TT	1(0.09)	0		
	allele	C	17(0.77)	13(0.81)	0.692 (0.152-3.223)	0.708
		T	5(0.23)	3(0.19)		
rs6277	Genotype	CC	3 (0.27)	2 (0.25)	-	0.435
		CT	2 (0.18)	4 (0.50)		

		TT	6 (0.55)	2 (0.25)		
	allele	C	8(0.42)	8(0.50)	0.571 (0.158-2.04)	0.511
		T	11(0.58)	8(0.50)		
rs2242591	Genotype	AA	0	0	-	0.181
		AG	3(0.27)	4(0.63)		
		GG	8(0.73)	3(0.37)		
	allele	A	3(0.14)	5 (0.31)	0.347 (0.076-1.513)	0.243
		G	19(0.86)	11 (0.69)		
rs2242592	Genotype	CC	1(0.09)	0	-	1.000
		CT	3(0.27)	3 (0.38)		
		TT	7(0.64)	5(0.62)		
	allele	C	5(0.23)	3(0.19)	1.275(0.277-5.751)	0.243
		T	17(0.77)	13(0.81)		
rs2242593	Genotype	AA	8(0.73)	3(0.38)	-	0.181
		AG	3(0.27)	5(0.63)		
		GG	0	0		
	allele	A	19(0.86)	11(0.69)	2.879 (0.620-13,178)	0.243
		G	3(0.14)	5(0.31)		
rs1800497	Genotype	CC	5(0.50)	3(0.38)	-	0.798
		CT	5(0.50)	4(0.50)		
		TT	0	1(0.12)		
	allele	C	15(0.75)	10(0.63)	1.8(0.449-7.215)	0.483
		T	5(0.25)	6 (0.37)		

Discussion

The objective for this study was to investigate the DRD2 gene SNPs association to clozapine response. Briefly, there was no significant difference between responders and non-responders demographics. Furthermore, no association was found between any of the 12 SNPs allele or genotype frequencies of the DRD2 gene and clozapine response.

Demographics

In order to determine optimal response to clozapine in TRS, treatment for at least 12 weeks (84 days) is recommended[27]. The mean duration of clozapine treatment in mirror-image period 2 were 639.5 days (SD±218.5) for responders, and 602.5 days (SD±236.4) for non-responders. Thus, patients participating in this study did undergo treatment with clozapine enough time to clarify whether they will respond or not.

In average responders in this study received a maximum clozapine dosage of 383.3 mg/day (SD±180.3) and non-responders received 385.7 mg/day (SD± 174.9) during mirror-period 2, which is comparable to previous findings[55,56]. However, clozapine response is not dose-dependent, and thus dosage serves as a poor indicator for response[57]. Plasma concentration of clozapine shows large inter-individual variation, and does not correlate with clozapine response[58]. Studies suggests that a therapeutic threshold for clozapine exists, and plasma concentration above 350ng/mL-420ng/mL

(correspond to 1071nmol/L-1245nmol/L) is associated with higher chance of response[59]. In average patients presented with a maximum plasma-concentration of clozapine was 1423 nmol/L (SD: ±624.162, n=7) during mirror-period 2. These data suggest the subjects participating in this study were treated adequate with clozapine.

Comparison with previous pharmacogenetic studies

The infrequency reported in data of rs1799732; all subjects presented with the C insertion allele, might be explained by the general C insertion allele frequency of rs1799732 in the 1000 genome project[43]. The allele frequency in the 1000 genome project is 76.560%[43]. This sample might not be large enough to detect a C deletion allele. No difference in the frequency of the SNP rs1799732 between responders and non-responders of clozapine, was found in this sample (p=1.000). Similar results have been reported by majority of other studies[38-40]. Former unpublished data, included in a meta-analysis by Zhang et al. (2010) did associate the Deletion allele with positive symptoms improvement[37]. However, the positive results might be biased by ethnic heterogeneity, since the subjects was not allocated based on ethnicity[37]. Studies suggests that ethnicity plays a role in genotype distribution[39,40,60]. Thus ethnic homogeneity might be desirable in pharmacogenetic studies. The remaining studies investigating DRD2 gene allocated groups based on ethnicity and subsequent analyzed their data subsequently[39,40,60]. If rs1799732 variation is involved in clozapine response, the results still remain to be validated.

The A allele of rs1125394 has been associated with overall improvement of the Brief Psychiatric Rating scale (BPRS) and more specific an improvement in positive symptoms in an African American sample[39,40]. This study has not been able to replicate this finding in a Caucasian sample (OR: 0.347 (0.076-1.162), p=0.186). The genotype A-A of rs1125394 has been associated with a better outcome in BPRS, the positive scale of BPRS in an African American sample[40]. However, their preliminary results published in 2005, were unable to show any significant difference[39] . The statistical association in the African American sample, were not found in their Caucasian sample[39,40].

Both the allele and genotype frequencies of rs1079597 has been associated with better response in the overall BPRS and the positive items of BPRS in an African American sample, but not in the Caucasian sample[39,40]. This sample, consisting entirely of Caucasian subjects has not yielded any association in neither allele (OR: 0.347 (0.076-1.162), p=0.186) or genotype frequencies (p=0.181).

The A-A genotype of rs2242593 has been associated with better response to clozapine in both BPRS and in the negative items of BPRS (BNEG) in an African American sample, but not in a Caucasian sample. However, their preliminary results published in 2005, were unable to show any significant difference in neither the African American nor Caucasian sample[39]. This study also failed to find any significant difference between responders and non-responders genotype (p=0.181).

Hwang et al. (2005) found the C-allele as well as an absence of T-allele of rs1800497 can be associated with better response in BPRS in their African American sample (n=49)[39]. They were unable to replicate this finding when they used a subset of their sample (n=31)[40]. No positive findings were done in their Caucasian samples[39,40]. This study did not find any association between rs1800497 allele frequencies and clozapine response (OR: 1.8(0.449-7.215), p=0.483).

No statistical significant variations of Rs1799978, Rs4648317, rs1800498, Rs6275, Rs6277 Rs2242591, Rs2242592, Rs2242593 were found in this sample, nor has it been found in earlier studies[39,40]. This indicates that neither one of the SNP is associated with clozapine response.

Study design

Previous studies have used a 20% reduction in BPRS[37,39,40], or a 20 point reduction in global assessment scale(GAS) as response criteria[60]. BPRS is a widely used scale, and the one often used in pharmacogenetic studies, (for review see appendix 1). However, some studies indicates that BPRS alone might be a poor measure of treatment response[61,62]. A study has shown that a 10-15 point reduction corresponds to a minimal improvement on the clinical global impression (CGI) scale[61]. A more pronounced improvement of CGI was found for the least severe ill schizophrenic when reducing BPRS 10-15 points[62]. More severe ill patients, on the other hand showed less improvement on CGI[62]. The current study was based on a retrospective design, investigating the number of psychiatric bed-days prior to and during clozapine treatment, data was collected from patients medical records. The number of psychiatric bed-days or admissions has been used in other studies as a predictor of clozapine treatment outcome, in patients suffering from schizophrenia[41,63] or bipolar disorder[64]. All studies indicate that bed-days are a good naturalistic measure of clozapine response[41,63,64]. After initiating clozapine treatment, responders reduced the number of bed-days from 109.7 bed-days (SD ±131.8) in mirror-image period 1 to 3.5 bed-days (SD ± 7.4) in mirror-image period 2 (p=0.022). Thus, responders reduced the number of psychiatric bed-days with 96.8 %. Another study has investigated the usefulness of the mirror-image model in a large Danish sample and found a 76.0% decrease between the number of bed-days in mirror-image 1 and mirror-image after initiation of clozapine. The difference might be explained by variation in the study design. This study investigated the difference in bed-days between responders and non-responders of clozapine, whereas the study by Nielsen et al. (2012), investigated the overall reduction of bed-days in all patients receiving clozapine[41]. Thus the reason for the large percentage reduction in this study might be explained by the fact, that this study only included responders. No significant difference between bed-days in the two mirror-image periods was found between non-responders. In average non-responders had 102.5 bed-days in mirror-period 1 compared to 145.6 bed-days (SD: ±95.4, p=0.096) in mirror-period 2. This indicates that the mirror-image model is suitable for calculating clozapine responders.

Limitations of the current study design; side-effect was not registered, and could have an influence on termination of clozapine treatment. Previous and concurrent medication was not taken into account in the model, and might have an influenced the number of bed-days, since evidence indicates that the likelihood of response depends on previous medication trials[41]. The model did not correlate for reasons for hospitalization. In addition this sample did not register if patient were institutionalized, however, studies suggests that institutionalization nor early retirement does not influences the outcome of the mirror-image model[41]. Previous studies investigating the DRD2 gene and clozapine response conducted a randomized controlled trial (RCT)[37], or a case-control study[39,40,60]. Advantages of such study designs, when conducting pharmacogenetic studies, include an ability to minimize biases such as ensuring adequate clozapine treatment, monitoring side-effects, admissions etc.

One of the major advantages of the mirror-image design is the possibility to include the most severe ill patients. Patients suffering from TRS are a subgroup of schizophrenia, which includes the most ill patients. Often these patients are not eligible for randomized trials, and therefore these are often not included in clinical trials.

Multiple testing adjustments

None of the earlier studies performed adjustment of multiple testing. Multiple testing is an area of discrepancy when performing genetic studies, due to a possible increase in the risk of false negative results, especially with rare alleles[65]. Nevertheless the risk of false positive results increases when a large number of SNPs are tested at the same time. One approach to correct for multiple testing is the Bonferroni correction, which divide the

significance level of 0.05 with the number of performed tests[65]. However, the Bonferonni correlation is over-conservative and increases the risk false negative results[65]. Another approach could be the permutation procedure which is less conservative, but still make multiple testing possible especially in smaller samples and within rare alleles[65]. None of the SNPs investigated in this study reached statistical significance, even without multiple corrections. Multiple corrections were not performed, but it would be appropriate to conduct corrections on positive results.

Power analysis

This sample does not provide enough power (power= 15.5%). In order to determine statistical significant difference within the current proportion of responders and non-responders in both genotypes and allele-frequencies, a minimum sample size of 105 is required (power=80). The results of this study must be interpreted carefully. In order to consider this study as high quality evidence, the sample size must be greater.

Conclusion and Perspectives

This study did not associate any SNP in the DRD2 gene with clozapine response, but results must be interpreted carefully due to the small sample size. Nevertheless, it might be too simplistic to only consider a single SNP in prediction of clozapine response. Most often, SNPs are in linkage disequilibrium with others, and the presence of specific haplotypes might serve as a more specific predictor than a single SNP variation itself[66]. Evidence indicates that haplotypes might be useful in determination of clozapine response[39,40]. Hwang et al (2005) found an association between two haplotypes (rs1079597-rs1800498-rs6275,T-T-C and rs1800498-rs6275-rs6277,T-C-T) and a better response to clozapine in a Caucasian sample[39]. The study also found several haplotypes to be associated with clozapine response in the African American sample (see appendix 1 for review)[39]. Hwang et al. (2006) have not been able to replicate the results in a later study using a subgroup of patients participating in their preliminary study from 2005[40]. After increasing sample size, it would be interesting to investigate the haplotypes in this independent sample, especially due to the positive findings by Hwang et al (2005) in the Caucasian sample. Haplotype analysis will probably give a more powerful tool in prediction of clozapine response[66].

In addition, Clozapine target a great number of different receptors besides DRD2, including other dopamine receptors, serotonin receptors, muscarine receptors etc. In future perspectives a pharmaco-genetic study using a polygenetic study could be of great interest. A polygenetic study investigates associations between several SNP or haplotypes in different genes at once.

Another important aspect of clozapine response, is the high first pass metabolism, and concurrent the active metabolite; NDMC. For instance, NDMC appears to have different properties at the DRD2 than clozapine, and might have so at every other receptor. Investigating response of clozapine to DRD2 gene as well as genes associated with metabolism of clozapine would be of great interest. In such a study design ratio of plasma-NDMC and plasma-clozapine could be encountered as interesting covariates.

References

See appendix 2

Appendix 1

Review article

Genetic markers for clozapine response – how far are we

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Abstract:

Background: Alteration of dopamine function and dopamine receptors is believed to be involved in the pathophysiology of schizophrenia. Clozapine is superior in treating symptoms of schizophrenia including treatment resistant patients. Clozapine exhibits affinity against all dopamine receptors in a manner different from traditional antipsychotic drugs. This difference might account for the unique effects of clozapine. However, there is a pharmacological inter-variability in clozapine response, since only 50 % respond to the drug. Pharmacogenetic studies have emerged to highlight the possible role of dopamine receptor genes in the response of clozapine.
Methods: A systematic review of pharmacogenetic studies of clozapine response was conducted. A research of Pubmed database was performed. No time limit was applied; however studies were limited to English language. Studies of dopamine receptor gene polymorphisms were included based on following inclusion criteria; I) Pharmacogenetic study, II) Schizophrenic diagnose according to the ICD-10 classification, III) Primary outcome: clozapine response. From a total of 358 articles, 20 met the inclusion criteria and were reviewed.
Results: Within the dopamine D2 receptor gene, a total of 12 polymorphisms have been investigated. Both Rs1079597 and Rs1125394 have been associated with clozapine response in an African American sample. In addition, the 48-bp repeat, a polymorphism of the dopamine D4 receptor gene, has been associations with clozapine response in several studies, although others have not been able to validate the results. The Dopamine D1-, D3-, and D5 receptor genes have only been investigated in a few samples and no clear association to clozapine response have been revealed. Promising results of haplotypes analysis exist within all receptor subtypes. However, none of these have been replicated in other samples.

Conclusion: Larger, more elaborating replication studies are required in order to determine pharmacogenetics of clozapine. Besides from a few promising polymorphisms, new gene sequences need to be investigated. Furthermore the bias in the previous studies must be minimized, such as small sample size, heterogeneity of ethnicity as well as predictors of response. Future studies could investigate gene-gene interactions as this might be the key, to reveal the true biomarker of clozapine response.

Keywords: Clozapine; Receptors, dopamine D1; Receptors, dopamine D2; Receptors, dopamine D3; Receptors, dopamine D4; Receptors, dopamine D5; schizophrenia; pharmacogenetics; drug resistant

Introduction

Schizophrenia, often presents in the youth and is a mental disorder characterized by a range of symptoms, traditionally clustered as positive (delusions, hallucinations, thought disorders), and negative symptoms (e.g. flattened affect, apathy, social withdrawal) in addition to cognitive

impairment (mainly of cognitive domains related to executive function). Even though many clinical findings can be attributed to schizophrenia, no single symptom is mandatory or sufficient to make the diagnosis[1]. The exact cause of schizophrenia remains unknown. However, a role of genetics in the etiology of schizophrenia has been established and 22 genetic loci have been associated with schizophrenia so fare[3]. Other factors such as biological, psychological and social also contribute to this disorder[2]. Hence, several hypotheses have been suggested in order to explain the etiology of schizophrenia (for review see Keshavan et al (2011)[1]. The most promising hypotheses focus on inhibitory circuits and neurochemical dys-functions[1]. The hypothesis indicates that alteration in glutamate receptor function[67], GABAergic transmission[68], and dopamine dysfunction co-exist[69], and altogether cause the symptoms of schizophrenia[1].

Antipsychotic treatment remains the cornerstone in treatment of patients with schizophrenia. All antipsychotics exert their antipsychotic effect through blocking of the D2 dopamine receptors (DRD2), one of the 5 subtypes of dopamine receptors[70]. Antipsychotics are divided in to either first generation antipsychotics (FGA), such as haloperidol, or second generation antipsychotics (SGA), such as clozapine. SGA is characterized by a lower tendency to cause extrapyramidal side-effects (EPS)[21]. Up to 30 % of patients suffering from schizophrenia are resistant to traditional antipsychotic treatment[20]. Treatment resistant schizophrenia (TRS) is often classified as unsatisfactory response to two or three antipsychotics. The golden standard for TRS is clozapine [27]. Regardless of treatment resistance to other antipsychotics, the respond rate to clozapine is approximately 50%[21]. Clozapine alleviates both

Table A, Dopamine receptor subtypes, location, effects and clozapine action.

Receptor	Location	Effect	Clozapine action	K_i[17] (nM)	Association with schizophrenia
DRD1	Pre- and postsynaptically[71] on pyramidal cells dendritic spines[72]in Nigrostratal, mesolimbic and mesocortical areas; caudate putamen (striatum), nucleus accumbens, substantia nigra, olfactory bulb, amygdala, PFC, hippocampus, cerebellum, thalamic areas, hypothalamic areas, red nucleus[72-74].	Endogenous dopamine decrease nigrostriatal dopamine release through DRD1[75] Increase excitability and increase NMDA receptors, L-type calcium channels, and sodium channels currents[76]	It is controversial whether clozapine act as an agonist or an antagonist at DRD1[30,77-80].	189	The location of DRD1and the decreased activity of the receptor in the PFC give rise to several hypotheses of the receptors involvement in symptoms of schizophrenia[81-83]
DRD2	Pre- and postsynaptically[71] on GABAergic neurons and interneurons[84] in striatum, nucleus accumbens, olfactory tubercle, hypothalamus, cortical areas, temporal cortex, septum, amygdala, hippocampus, basal ganglia[73,85,86] Autoreceptors on dopaminergic neurons in substantia nigra, VTA[73]	DRD2 are inhibitory[87]. Regulate GABAergic synaptic transmission and excitability to cholinergic interneurons through N-type calcium channels[84]. Activation of autoreceptors decrease excitability of dopamine neurons and decrease dopamine release[87].	Antagonist[12] or inverse agonist[14]. A low receptor occupancy[85,86,88,89] probably account for the low incidence of extrapyramidal side-effect[23,24,27,2]. Clozapines unique action might be due to a fast dissociation rate[90,91].	431	Associated with antipsychotic response, and side-effects[91]

DRD3	Dopaminergic neurons in the shell of nucleus accumbens, ventral caudate nucleus, putamen, globus pallidus, olfactory tubercle, striatum, mammillary nuclei, thalamic nuclei, hypothalamus, basal ganglia, hippocampus, septal area[73,92,93]. In VTA and substantia nigra pars compacta were they are believed to act as autoreceptors[92].	Decrease dopamine release; through an inhibitory effect on mesocortical dopamine activity[92]. The autoreceptors exert inhibitory control of neuronal activity in mesolimbic/mesocortical and nigrostriatal projections[92]. May block NMDA receptor signaling[94], and might regulate cFos[95] and acetylcholin release[96] in the PFC through thalamocortical projections.	Antagonist[97] or inverse agonist[14]. Antagonistic effects of DRD3 has been shown to improve several cognitive functions and could improve schizophrenic symptoms [92].	646	The distribution of DRD3 propose an influence of several mechanisms in the brain such as the reward-mechanism, motivation, appetite, habit learning, cognitive functions etc. [92].
DRD4	In cortical and limbic areas [98,99]; in the frontal cortex, amygdala, hippocampus, hypothalamus, entorhinal cortex, globus pallidus, substantia nigra pars reticulata, thalamus[100].	Modulate the AMPA component of LTP and NMDA receptors[101] Regionally suppress $GABA_A$ receptors through reducing GABA release at presynaptic terminals[102].	Antagonist[14] Antagonistic effect of clozapine has shown improvement of negative symptoms and cognitive impairment [82,103-107].	39	Several hypotheses exists of DRD4 influence in schizophrenia including an indirect effect of DRD4 on the glutamate signaling system [99] and abnormalities in areas such as the enthorhinal cortex where DRD4 is located [108,109].
DRD5	Cholinergic interneurons (large aspiny neuons) and neurons (pyramidal) [110] in cortical, subcortical and limbic areas; Cerebral cortex, hippocampus, striatum, nucleus accumbens, substantia nigra pars compacta, amygdale, hypothalamus, olfactory tubercle[71,111].	Regulate hippocampal, release of acetylcholine[112] as well as modulate cholinergic input in cortex[110] Interact with GABA-A receptors causing decreased GABA-A receptor mediated whole-cell currents[113]. Endogenous dopamine decrease nigrostriatal dopamine release[75]	Unknown	235	Associated with the etiology of schizophrenia[114], as well as cognition and working memory[115].

Abbreviations: DRD1-5: dopamine D1-5 receptor, K_i: dissociation constant, NMDA: N-Methyl-D-aspartate, GABA: γ-Aminobutyric acid, AMPA: α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, BDNF: Brain-derived neurotrophic factor, PRC: prefrontal cortex, VTA: Ventral Tegmental Area, LTP: long term potentiation.

positive and negative symptoms[21], and exerts antisuicidal properties[22]. However, due to life threatening side-effects, such as agranolocytosis and myocarditis, clozapine is not considered as a first line drug[26]. A mandatory monitoring program of white blood cell counts and electrocardiograms are required for all patients receiving clozapine[26].

A 70 % occupation of the DRD2 is considered optimum for achieving antipsychotic response[70]. Receptor occupancy of more than 70 % at DRD2 does not lead to increased antipsychotic response but causes EPS to develop[91]. Interestingly, even at high dosages of clozapine, the dopamine receptor occupancy rarely reaches 70% and despite this fact clozapine remains more efficient than other known antipsychotics. In table A, properties of the different subtypes of dopamine receptors is listed, including current knowledge of clozapines action toward the different receptors. The exact mechanism of clozapine remains largely unknown despite extensive research. Clozapine has affinity towards a number of different receptors in addition to dopaminergic receptors, including serotonergic-,

muscarinic-, adrenergic- and histamineric-receptors[30]. The response variability to clozapine has emerged a possible role of genetic variation in dopaminergic or other receptors. Pharmacogenetics focuses on single nucleotide polymorphism (SNP) influence on drug response. Several approaches have been utilized to investigate pharmacogenetics, including individual SNPs analysis, sets of SNPs (haplotype) analysis as well as influence of gene-gene interactions[116]. Successful pharmacogenetic screening would enable practitioners to screen patients and divide them to clozapine responders and non-responders. This would give an opportunity to for individualized medication towards more efficient and safer treatments. Hence, this paper focuses on pharmacogenetic properties of the 5 known dopamine receptors in association with clozapine efficacy.

Methods

Design of the literature research

A systematic review design of pharmacogenetic studies was performed according to guidelines of Preferred Reporting Items for systematic reviews and meta-analysis (PRISMA). A computer-based designed literature search in PubMed was conducted to retrieve all studies published. No limitation of time period was applied. Furthermore, Embase and Google Scholar were manually searched for additional studies. Articles were retrieved using a combination of MESH terms to identify all relevant papers. MESH terms were "Clozapine" AND ("Receptors, dopamine D1", "Receptors, dopamine D2", "Receptors, dopamine D3", "Receptors, dopamine D4" OR "Receptors, dopamine D5"). The studies were limited to English literature.

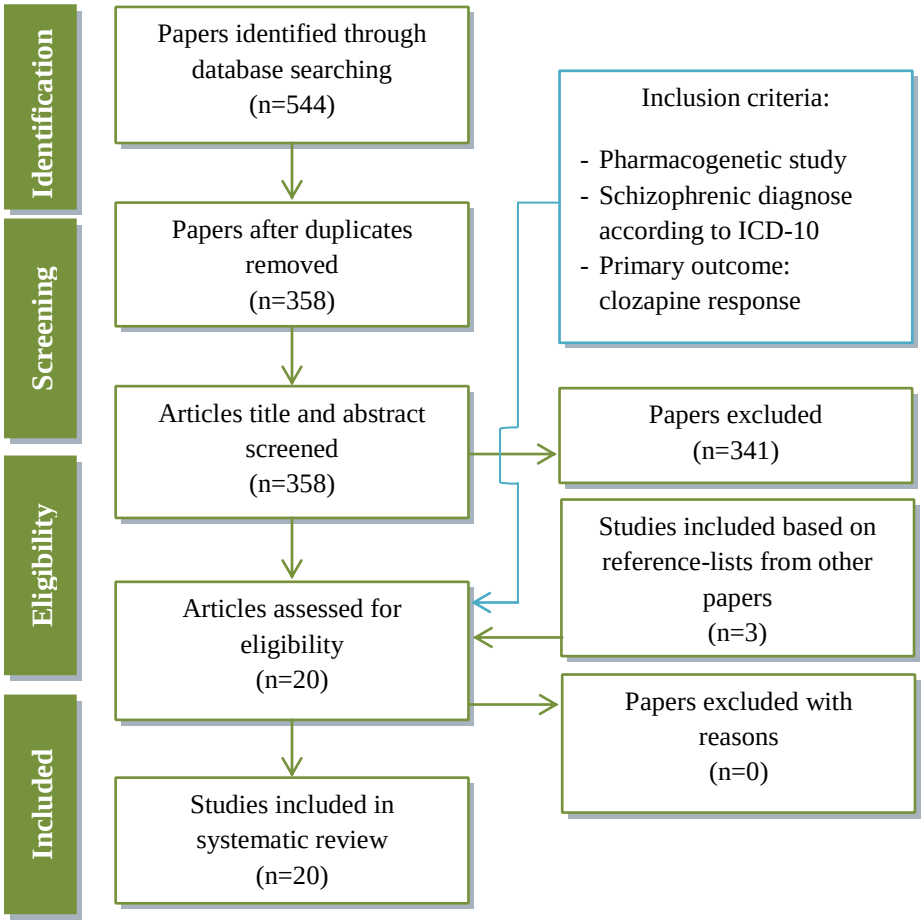

Figure A, Flow diagram of the study selection and eligibility process in the systematic review

These studies were screened by title and abstract, and excluded if not relevant. The remaining studies were screened for eligibility. The following inclusion criteria had to be met; it had to be a pharmacogenetic study, and the subjects of the study had to have a schizophrenic diagnose according to ICD-10 classification, finally the primary outcome had to be a response to clozapine.

Duplicates were removed with PubMed software. Reference list of relevant studies and reviews were screened for additional articles that did not appear in the literature search.

Selection and evaluation of included studies in the systematic review

As figure A illustrates, a total of 358 studies were identified in the search after removal of duplicates. From these 341 did not fulfill the inclusion criteria and were excluded. Overall 20 studies were included in the systematic review. Of these 20, two studies were included in the dopamine receptor D1 (DRD1), four studies in DRD2, eight studies in dopamine receptor D3 (DRD3), six studies in dopamine receptor D4 (DRD4) and one studies in dopamine receptor D5 (DRD5). All the results obtained from the systematic research are reported in tables and/or are discussed in the following sections for each receptor subtype.

Results and Discussion

Dopamine receptor D1 (DRD1) gene

Two studies have examined the involvement of the DRD1 gene in the efficacy of clozapine. Table B summarizes the result from the two studies. The SNP rs4532 was associated with good response to clozapine, in a small mixed ethnic sample[118]. The results have not been replicated since[117]. The Rs265976 and the haplotype rs265981-rs4532-rs686 have been associated with clozapine response in one study[117].

Table B, The DRD1 gene and association with SNPs and haplotypes to clozapine response. In the haplotype analysis, only positive findings are reported in this table.

SNP	Genotype frequencies outcome		Allele frequencies		Response definition	Etnicity	Study
Rs4532	Genotype C-C positive (BPRS) and decreased metabolic rate. Genotype T-C negative (BPRS)	P<0.05	N/A	N/A	PET and 20% reduction in BPRS	Mixed CAUC and AFAM	Potkin et al. (2003) *
	ND	P_{CAUC}=0.902 P_{AFAM}=0.609	ND	P_{CAUC}=0.648 P_{AFAM}=0.436	20% reduction in BPRS, BPOS, PNEG	CAUC and AFAM	Hwang et al. (2007) **
Rs265981	ND	P_{CAUC}=0.932 P_{AFAM}=0.359	ND	P_{CAUC}=0.725 P_{AFAM}=0.117			
Rs686	ND	P_{CAUC}=0.684 P_{AFAM}=0.336	ND	P_{CAUC}=0.411 P_{AFAM}=0.307			
Rs265976	Genotype A-C negative (BPOS) in AFAM	P_{CAUC}=0.959 P_{AFAM}=0.033 $P_{AFAM\text{-}BPOS}$=0.027	ND	P_{CAUC}=0.798 P_{AFAM}=0.207			
Haplo-types	**Amino acid**		**Sample**	**Outcome to clozapine treatment**			
rs265981-rs4532-rs686	T-G-A	P_{CAUC}=0.027	CAUC	Positive			
	T-G-G	P_{AFAM}=0.042	AFAM	Negative			

*Abbreviations: N/A: Not investigated, PET: position emission tomography with 18F-flourodeoxyglycose, BPRS: Brief Psychiatric rating scale, BPOS: positive symptom subscale, BNEG: negative symptom subscale. ND: no significant difference. AFAM: African American, *: The study investigated 15 subjects not distinguished by ethnicity, **: the study investigated both a Caucasian sample (n=183) and an African American sample (n=49) and included the 15 subject investigated in Potkin et al. (2003)* [117,118]

Beside from the listed SNP in table B, future studies could include SNPs such as rs10078866 were the T allele has been associated with an increased risk of schizophrenia in an Asian sample. Rs10063995 has also been associated with schizophrenia in female Asians subjects. Other genes studied with regard to schizophrenia were rs4867798, rs1799914 rs5326 and rs10078714[119]. Even though none of these were significant different in an Asian sample they could have been in Caucasian or African American and thereby also have an influence on clozapine efficacy.

Dopamine D2 receptor (DRD2) gene

As listed in table C, four studies have investigated DRD2 gene polymorphism with regard to clozapine response [37-40]. The study from Hwang et al. (2006) is a replication study from their preliminary findings in 2005, however, the study in 2006 only included 132 subjects of the originally 232. There are methodological differences in the two studies such as definition of response as well as difference in subjects included, nevertheless, many of the subjects were included in both studies[39,40].

The most widely studied polymorphism is the rs1799732, also known as -141C ins/del. Rs1799732 has been associated with schizophrenia[36]. Former unpublished data, included in a meta-analysis by Zhang et al. (2010) found an association between clozapine response and the frequency of the polymorphism[37].

However, other studies have been unable to replicate these findings[38-40]. Beside from rs1799732, no SNPs have been associated with clozapine response in DRD2 gene in Caucasian samples. However, studies suggests ethnic heterogeneity may influence genetic outcome since genetic difference in African American and Caucasian samples have been found[39,40].

Table C, The DRD2 gene SNPs association to clozapine response[37-40].

SNP	Outcome to clozapine treatment				Response definition	Ethnicity	Study
	Allele frequencies		Genotype frequencies				
Rs1799732 (-141C ins/del)	Del allele positive in positive symptoms	Not reported	N/A	N/A	20% reduction in BPRS	Mixed; CAUC+ AFAM	Zhang et al. (2010)
	ND	P_{CAUC}=0.34 P_{ASIA}=0.20 OR_{CAUC}=1.43 OR_{ASIA}=1.87	ND	P_{CAUC}=0.30 P_{ASIA}=0.42	20 point improvement in GAS	CAUC and ASIA	Arranz et al. (1998)
	ND	P_{CAUC-a}=0.475 P_{AFAM-a}=0.829 $P_{CAUC-a\ 1+/1-}$=0.572 $P_{AFAM-a\ 1+/1-}$= 0.675 P_{CAUC-b}=Not reported P_{AFAM-b}= Not reported $P_{CAUC-b\ 1+/1-}$=0.842 $P_{AFAM-b\ 1+/1-}$=0.680	ND	P_{CAUC-a}=0.524 P_{AFAM-a}=0.903 P_{CAUC-b}=0.979 P_{AFAM-b}=0.890	a: 20% reduction on BPRS b: 20% reduction in BPRS BPOS, BNEG	CAUC and AFAM	Hwang et al. (2005)a Hwang et al (2006)b
Rs1799978 (-241 A/G)	ND	P_{CAUC-a}=0.932 P_{AFAM-a}=0.708 P_{CAUC-b}=Not reported P_{AFAM-b}= Not reported	ND	P_{CAUC-a}=0.833 P_{AFAM-a}=0.522 P_{CAUC-b}=0.886 P_{AFAM-b}=0.305			Hwang et al. (2005)a Hwang et al. (2006)b
Rs4648317	ND	P_{CAUC-a}=0.945 P_{AFAM-a}=0.188 P_{CAUC-b}= Not reported P_{AFAM-b}= Not reported	ND	P_{CAUC-a}=0.897 P_{AFAM-a}=0.061 P_{CAUC-b}=0.879 P_{AFAM-b}=0.862			
Rs1125394	Allele A positive (BPRS) in AFAM	P_{CAUC-a}=0.931 P_{AFAM-a}=0.029, OR=0.24 (0.06-0.94)	ND	P_{CAUC-a}=0.955 P_{AFAM-a}=0.137			Hwang et al. (2005)a

	A-allele positive (BPRS, BPOS) in AFAM	P_{CAUC-b}= Not reported P_{AFAM-b}= Not reported	Genotype A-A positive (BPRS, BPOS) in AFAM	P_{CAUC-b}=0.984 P_{AFAM-b}<0.001 $P_{AFAM-b, BPOS}$=0.030			Hwang et al. (2006)b
Rs1079597 (Taq*I*B)	Allele C, and C insertion negative (BPRS) in AFAM	P_{CAUC-a}=0.870 P_{AFAM-a}=0.036, OR0.10(0.01-0.87) $P_{+1/-1-CAUC}$=0.897 $P_{+1/-1-AFAM}$=0.033	T-T genotype positive (BPRS) in AFAM	P_{CAUC-a}=0.986 P_{AFAM-a}=0.033			Hwang et al. (2005)a
	Absence of C allele associated positive (BPRS, BPOS) AFAM	P_{CAUC-b}= Not reported P_{AFAM-b}= Not reported $P_{+1/-1-CAUC-b}$=0.792 $P_{+1/-1-AFAM-b}$=0.025	ND	P_{CAUC-b}=0.965 P_{AFAM-b}=0.025 $P_{AFAM-b, BPOS}$=0.014			Hwang et al. (2006)b
Rs1800498 (Taq*I*D)	ND	P_{CAUC-a}=0.550 P_{AFAM-a}=0.649 P_{CAUC-b}= Not reported P_{AFAM-b}= Not reported	ND	P_{CAUC-a}=0.400 P_{AFAM-a}=0.365 P_{CAUC-b}=0.576 P_{AFAM-b}=0.982			Hwang et al. (2005)a Hwang et al. (2006)b
Rs6275 (NcoI)	ND	P_{CAUC-a}=0.629 P_{AFAM-a}=0.559 P_{CAUC-b}= Not reported P_{AFAM-b}= Not reported	ND	P_{CAUC-a}=0.965 P_{AFAM-a}=0.827 P_{CAUC-b}=0.714 P_{AFAM-b}=0.547			
Rs6277 (C957T)	ND	P_{CAUC-a}=0.909 P_{AFAM-a}=0.442 $P_{+2/-2-CAUC-a}$=0.363 $P_{+2/-2-AFAM-a}$=0.763 P_{CAUC-b}=Not reported P_{AFAM-b}= not reported $P_{+2/-2-CAUC-b}$=0.931 $P_{+2/-2-AFAM-b}$=0.989	ND	P_{CAUC-a}=0.226 P_{AFAM-a}=0.350 P_{CAUC-b}=0.471 P_{AFAM-b}=0.989			
Rs2242591	ND	P_{CAUC-a}=0.636 P_{AFAM-a}=0.114 P_{CAUC-b}= Not reported P_{AFAM-b}= Not reported	ND	P_{CAUC-a}=0.782 P_{AFAM-a}=0.285 P_{CAUC-b}=0.81 P_{AFAM-b}=0.791			
Rs2242592	ND	P_{CAUC-a}=0.951 P_{AFAM-a}=0.291 P_{CAUC-b}= Not reported P_{AFAM-b}= Not reported	ND	P_{CAUC-a}=0.723 P_{AFAM-a}=0.470 P_{CAUC-b}=0.708 P_{AFAM-b}=0.410			
Rs2242593	ND	P_{CAUC-a}=0.817 P_{AFAM-a}=0.153	ND	P_{CAUC-a}=0.969 P_{AFAM-a}=0.142			Hwang et al. (2005)a
		P_{CAUC-b}= Not reported P_{AFAM-b}= Not reported	Genotype A-A positive (BPRS, BNEG) in AFAM	P_{CAUC-b}=0.951 P_{AFAM-b}=0.036 $P_{AFAM-b, BNEG}$=0.016			Hwang et al. (2006)b
rs1800497 (Taq*I*A)	C- allele or absence of T-allele positive (BPRS) in AFAM	P_{CAUC-a}=0.824 P_{AFAM-a}=0.010, OR=0.31 (0.12-0.77) $P_{+1/-1-CAUC-a}$=0.860 $P_{+1/-1-AFAM-a}$=0.032, OR=0.28 (0.09-0.90)	ND	P_{CAUC-a}=0.974 P_{AFAM-a}=0.060			Hwang et al. (2005)a
	ND	P_{CAUC-b}= Not reported P_{AFAM-b}= Not reported $P_{+1/-1-CAUC-b}$=0.703 $P_{+1/-1-AFAM-b}$=0.149		P_{CAUC-b}=0.923 P_{AFAM-b}=0.344			Hwang et al. (2006)b

Abbreviations: N/A: Not investigated, BPRS: Brief Psychiatric rating scale, BPOS: positive symptom subscale, BNEG: negative symptom subscale. ND: no significant difference.

Table D, Haplotypes of the DRD2 gene association with clozapine response. In the haplotype analysis, only positive findings are reported in this table [39,40].

Haplotypes	Amino acid	Ethnicity	Outcome to clozapine treatment		Response difinition	Study
Rs1125394-rs1079597	A-T	AFAM	Positive (BPRS, BPOS)	P_{BPRS}<0.001, P_{BNEG}=0.002	a: 20% reduction in BPRS b: 20% reduction in BPRS BPOS, BNEG	Hwang et al. (2006)b
	G-T			P_{BPRS}=0.008, P_{BNEG}=0.009		
rs1799978-rs1799732-rs4648317	G-del-C	AFAM	Positive (BPRS)	P=0.046		
rs1799732-rs4648317-rs1125394	Ins-C-A	AFAM	Positive (BPRS)	P=0.037, OR=0.34 (0.14-0.90)		Hwang et al. (2005)a
		AFAM	Positive (BPRS, BPOS)	P_{BPRS}=0.017, P_{BPOS}<0.001		Hwang et al. (2006)b
	Ins-C-G		Negative (BPRS)	P<0.001		
Rs4648317-rs1125394-rs1079597	C-A-T	AFAM	Positive (BPRS)	P=0,007, OR= 0.24 (0.09-0.68)		Hwang et al. (2005)a
		AFAM	Positive (BPRS, BPOS)	P_{BPRS}<0.00, P_{BPOS}=0.008		Hwang et al. (2006)b
	C-G-T			P_{BPRS}=0.002, P_{BPOS}=0.025		
Rs1125394-rs1079597-rs1800498	A-T- T	AFAM	Positive (BPRS)	P=0.029, OR=0.36 (0.15-0.88)		Hwang et al. (2005)a
		AFAM	Positive (BPRS, BPOS)	P_{BPRS}=0.005, P_{BPOS}<0.001		Hwang et al. (2006)b
Rs1125394-rs1079597-rs1800497	A-T-C	AFAM	Positive (BPRS)	P=0.004, OR= 0.20 (0.11-0.66)		Hwang et al. (2005)a
		AFAM	Positive (BPRS, BPOS)	P_{BPRS}=0.014, P_{BPOS}=0.008		Hwang et al. (2006)b
	G-T-C		Negative (BPRS)	P=0.016		
	G-C-C		Negative (BPOS)	P=0.008		
	G-T-T		Negative (BPOS)	P=0.024		
rs1079597-rs1800498 - rs6275	T-T-C	CAUC	Positive (BPRS)	P=0.023, OR= 0.27 (0.11-0.35)		Hwang et al. (2005)a
		AFAM	Positive (BPOS)	P=0.005		Hwang et al. (2006)b
	C-C-C		Negative (BPOS)	P=0.010		
rs1800498 - rs6275-rs6277	T-C-T	CAUC	Positive (BPRS)	P=0.021, OR=0.18 (0.09-0.33)		Hwang et al. (2005)a
	T-T-C	AFAM	Positive (BPOS)	P=0.007		Hwang et al. (2006)b
rs6277-Rs2242591-Rs2242592	C-A-C	AFAM	Negative (BPOS)	P=0.019		
	T-G-C			P=0.027		
Rs2242591-Rs2242592-rs2242593	A-C-G	AFAM	Negative (BPRS, BPOS)	P_{BPRS}=0.025, P_{BPOS}=0.017		
	G-C-A		Negative (BPOS)	P=0.022		
Rs2242592-rs2242593-rs1800497	C-A-C	AFAM	Positive (BPRS)	P=0.011, OR=0.16 (0.06-0.45)		Hwang et al. (2005)a
	C-G-C		Positive (BPOS)	P=0.014		Hwang et al. (2006)b

Abbreviations: BPRS: Brief Psychiatric rating scale, BPOS: positive symptom subscale, BNEG: negative symptom subscale.

Analysis of SNP found rs1125394 and rs1079597 to be associated with better response in the African American samples as listed in table C. The functional role of the latter two polymorphisms is still unknown. The preliminary findings by Hwang et al. (2005) found two haplotypes (see table D) to be associated with clozapine response in DRD2 gene. Their study a year later did not replicate these findings[39,40].

Dopamine D3 receptor (DRD3) gene

The most abundant single SNP of DRD3 gene is the rs6280 also known as Ser9Gly. The most recent meta-analysis regarding rs6280 in human was conducted in 2010 by Hwang et al. (2010). The meta-analysis, involving 758 subjects from a total of 6 studies, investigated the difference in Ser and Gly alleles, homozygote and heterozygote genotypes. The analysis concluded that rs6280 was

not associated to the response to clozapine. However, the Ser allele and Ser/Ser genotype showed a tendency towards poor clozapine response[120]. By contrast, an earlier meta-analysis of 233 subjects shows a negative correlation between the Ser allele and clozapine response[121]. Johnson et al (2003) included some of the studies that were also included in Hwang et al. (2010)[120,121]. Thus, conflicting evidence for a role of rs6280 exists, and the SNP might only play a marginal role, if any, in clozapine response.

To the best of our knowledge, only one human study has investigated other DRD3 gene SNPs that could potentially be involved in clozapine response[120]. In addition to rs6280, Hwang et al. (2010) investigated eight different DRD3 gene SNPs; rs900568, rs2399504, rs761135, rs6762200, rs1394016, rs167770, rs2134655, rs2087017 (see Hwang et al. (2010) for review)[120]. Analysis of single SNPs revealed no association between allele frequencies and clozapine response between responders and non-responders[120]. Genotype frequencies did not yield any association when measured on the 20 item BPRS scale, but when symptoms were

Table E, Summarization of Hwang et al. (2010)[120]. Response was measured in two samples; first a 20% reduction of the brief psychiatric rating scale (BPRS) in 232 subjects, and secondly a subsample including only 132 of the subjects measuring BPRS, positive symptoms (BPRS) and negative symptoms (BNEG)

Haplotype analysis of DRD3 gene from Hwang et al. 2010					
Haplotypes		**Outcome to clozapine treatment**		**Ethnicity**	**Response definition**
Rs900568-Rs2399504-*Rs761135*	C-A-A	Positive	p=0.010, OR: 8.63(1.30-57.12)	CAUC	20% reduction in BPRS
Rs761135- Rs6762200-Rs6280	G-G-C	Positive	p=0.021, OR 0.08(0.01-0.64)	AFAM	20% reduction in BPRS
	G-G-T	Negative (BPRS)	p=0.033	CAUC	20% reduction in BPRS, BNEG, BPOS
	A-A-C	Negative (BPRS)	p=0.010	AFAM	20% reduction in BPRS, BNEG, BPOS
Rs6762200- Rs6280-Rs1394016	A-T-G	Positive	p=0.035, OR: 0.15 (0.03-0.69)	AFAM	20% reduction in BPRS
	G-C-G	Positive	p=0.012, OR: 0	AFAM	20% reduction in BPRS
	G-T-G	Positive (BPOS)	p=0.027	AFAM	20% reduction in BPRS, BNEG, BPOS
Rs6280- Rs1394016-Rs167770	C-G-C	Positive (BNEG)	p=0.044	CAUC	20% reduction in BPRS, BNEG, BPOS
	C-A-C	Positive	p=0.034, OR: 9.60 (0-∞)	CAUC	20% reduction in BPRS
Rs1394016- Rs167770-Rs2134655	A-C-G	Positive	p=0.037, OR: $9.45x10^{11}$ (0-∞)	CAUC	20% reduction in BPRS
Rs900568-Rs2399504	C-G	Positive	p=0.004, OR: 6.34 (1.74-22.69)	CAUC	20% reduction in BPRS
Rs2399504-*Rs761135*	G-A	Positive (BPRS)	p=0.029	AFAM	20% reduction in BPRS, BNEG, BPOS
Rs761135- Rs6762200	A-A	Negative (BPRS)	p=0.049	AFAM	20% reduction in BPRS, BNEG, BPOS
Rs6762200- Rs6280	G-C	Positive	p=0.017, OR: 0.08 (0.1-0.63)	AFAM	20% reduction in BPRS
		Positive (BPRS)	p=0.049	AFAM	20% reduction in BPRS, BNEG, BPOS
Rs1394016- Rs167770	A-C	Negative	p=0.035, OR: $2.82x10^{11}$ (0-∞)	CAUC	20% reduction in BPRS
	A-T	Positive (BNEG)	p=0.045	CAUC	20% reduction in BPRS, BNEG, BPOS
Rs167770- Rs2134655	C-G	Negative (BNEG)	p=0.042	CAUC	20% reduction in BPRS, BNEG, BPOS

Abbreviations: CAUC: Caucasian sample, AFAM: African American sample, SNP: single nucleotide polymorphism, BPRS: Brief Psychiatric Rating Scale, BPOS: Positive symptom subscale, BNOS: Negative symptom subscale[120].

measured using a positive or a negative symptoms score, rs1394016 were associated with better response on a 3-item negative symptom scale (BNEG) at the T-allele in an African American sample. Rs2134655 were associated with a better response on a 4-item positive symptoms scale (BPOS), in the Caucasian sample[120]. When focusing on haplotype analysis of DRD3 gene and the response to clozapine, there are some promising targets; Table E summarizes the positive findings of the haplotype analysis performed in Hwang et al. (2010).

In conclusion some evidence supports at least some SNPs or sets of SNPs of the DRD3 gene to be involved in clozapine response as described in table E.

Dopamine D4 receptor (DRD4) gene

The most investigated SNP of the DRD4 gene is a 2-10 repeat of the 48 basepair (bp) in exon 3. The most commonly occurring repeats is the two-repeat (D4.2), the four-repeat (D4.4) and the seven-repeat (D4.7). To our knowledge have nine studies investigated the polymorphism, as listed in table F. It should be kept in mind that a large variety in measuring treatment response was used throughout each study.

Through data exploration, both Zhao et al. (2005) and Rietschel et al. (1996) apparently mis-reported their findings as table G illustrates. The actual finding have consistently been used in this paper.

Most of the studies indicate that there is no association between the 48-bp repeat polymorphism and the response to clozapine. However some studies do reveal a difference especially in regard to D4.4, but even those results are contradictory[122-125]. Hwang et al. (2012) performed haplotype analysis of the SNPs reported in their study, these did not show any association (results not shown)[125].

In conclusion, evidence point toward that not one single DRD4 gene polymorphisms has an influence on clozapines efficacy. However, some of the genes reported in this paper would be interesting to investigate in larger sample sizes, including the 48-bp, D4.4. In future studies other SNPs of interest could be rs4481339 (Val194Gly), which has only been observed in African Americans. The SNP is important for the binding of dopamine to DRD4 gene[126]. It could be speculated that a mutation in this location could cause individuals with an African American descend to be resistant to clozapine. Another polymorphism of interest could be rs1800955 (C-521T) in the promotor gene which has been shown to be associated with schizophrenia in a meta-analysis[127]. In an Asian sample a SNP of the promotor region, rs93646 has been associated with schizophrenia as well as a combination of four other promotor region SNPs: rs936461, rs747302, rs916457 and the 12-bp repeat (rs4646983). None of these genes have to our knowledge been investigated in response to clozapine.

Dopamine D5 receptor (DRD5) gene

As listed in table H, only one study investigated the relationship between the DRD5 gene and clozapine response. One haplotype, rs10033951-rs10001006 yield any association between clozapine and DRD5 gene, however, the functional role of the haplotype remains unknown. The authors stated that the result from the haplotype could indicate linkage disequilibrium with another yet unidentified polymorphism[125]. Thus the DRD5 gene and more specific more SNPs needs to be investigated and replicated in other studies in order to determine the polymorphisms relation to clozapine response.

Table F, Studies investigating genetic variation of the DRD4 gene and clozapine response[122-125,128-132].

SNP	Outcome to clozapine treatment				Response definition	Ethnicity	Study
	Allele frequencies		Genotype				
48-bp repeat	ND	P= not reported	N/A	N/A	20 point improvement in GAS	CAUC	Shaikh et al. (1993)
	ND	P>0.05	ND	P>0.05	Symptomatic improvement	Not divided	Rao et al. (1994)
	ND	P>0.05	ND	P>0.05	20 point improvement in GAS	CAUC and AFAM	Shaikh et al. (1995)
	ND	P=0.12	ND, *4/4 more frequent in responders – see table G*	P=0.26	Medical records	CAUC	Rietschel et al. (1996) OBS
	ND	P=not reported	ND	P=not reported	Symptomatic improvement and hospitalization status	CAUC	Kohn et al. (1997)
	Allele D4.7 positive in patients responding to conventional antipsychotics *	P=0.046	ND	P= not reported	N/A	CAUC	Cohen et al. (1999)
	D4.2, D4.3 and D4.7 allele positive in catatonic schizophrenia	$P_{D4.2}$=0.07 $P_{D4.3}$<0.002 $P_{D4.7}$=0.008. P_{other}=not reported	ND	P=not reported	Improvement Global clinical assessment and PANSS *(not specified)*	CAUC	Kaiser et al. (2000)
	Allele D4.4 negative *see table G*	P<0.05	4/4 genotype negative , *see table G*	P<0.05	Improvement in PANSS *(not specified)*	Asian	Zhao et al. (2005) **, OBS
	ND	p_{other}>0.05	D4.4 genotype positive (BPRS, BPOS) in CAUC D4.6-D4.7 genotype positive (BPOS)	$P_{cauc\text{-}D4.6\text{-}D4.7,BPOS}$=0.031 $P_{cauc\text{-}D4.4,BPRS}$=0.024 $P_{cauc\text{-}D4.4,BPOS=}$0.02. p_{other}>0.05	20% reduction in BPRS BPOS, BNEG	CAUC and AFAM	Hwang et al. (2012) ***
12-bp repeat (rs4646983)	ND	P=not reported	ND	P=0.46	Medical records	CAUC	Rietschel et al. (1996)
	ND	P=not reported	ND	P=not reported	Symptomatic improvement and hospitalization status	CAUC	Kohn et al. (1997)
120-bp repeat	1 copy allele negative (BPRS) AFAM	P_{AFAM}=0.004 P_{CAUC}=0.457	ND	P_{AFAM}=0.0.168 P_{CAUC}=0.691	20% reduction in BPRS BPOS, BNEG	CAUC and AFAM	Hwang et al. (2012)***

(G)n	ND	P_{CAUC}=not reported P_{AFAM}=not reported	142-bp/140-bp negative (BPRS, BPOS) in AFAM	P_{CAUC}=0.941 $P_{AFAM\text{-}BPRS}$=0.034 $P_{AFAM\text{-}BPOS}$=0.014			
13-bp deletion	ND	P=not reported	ND	P=0.94	Medical records	CAUC	Rietschel et al. (1996)
21-bp deletion		Not observed		Not observed			
Gly11Arg substitution / rs104894415		P=not reported		P=0.37			
Rs3758653	ND	P_{CAUC}=0.784 P_{AFAM}=0.537	ND	P_{CAUC}=0.961 P_{AFAM}=0.575	20% reduction in BPRS BPOS, BNEG	CAUC and AFAM	Hwang et al. (2012)***
Rs11246226		P_{CAUC}=0.182 P_{AFAM}=0.823		P_{CAUC}=0.394 P_{AFAM}=0.956			
Rs936465		P_{CAUC}=0.323 P_{AFAM}=0.300		P_{CAUC}=0.595 P_{AFAM}=0.185			

*Abbreviations: ND: No significant difference, CAUC: Caucasian, AFAM: African American GAS: global assessment scale, D4.7: the 7 allele, D4.2: the 2 allele, D4.3: the 3 allele, Bp: basepair, (G)n: guanine mononucleotide repeat, *: the study did not have any non-responder group, and did not found any difference between clozapine-treated patient and healthy controls.**: the study also tested positive and negative symptoms improvement separately, but this did not reveal any significant difference, the study also excluded treatment refractory subjects to their study.***: the study only reported individual number of responders/non-responders for every genetic test. OBS: see table G for explanation.*

Table G, Mis-reported findings in pharmacogenetic studies of the DRD4 gene.

Study	Reported findings	Actual finding based on their reported data
Zhao et al. (2005)	D4.5 allele of 48-bp repeat more frequent in non-responders	D4.4 allele of 48-bp repeat more frequent in non-responders
	5/5 genotype of 48-bp repeat more frequent in non-responders	4/4 genotype of 48-bp repeat more frequent in non-responders
Rietschel et al. (1996)	No association between the 4/4 genotype of the 48-bp repeat and clozapine response	4/4 genotype of 48-bp repeat more frequent in responders

Table H, The DRD5 gene SNPs and haplotypes association with clozapine response. In the haplotype analysis, only positive findings are reported in this table.

SNP	Allele frequencies	Genotype frequencies	Response definition	Ethnicity	Study
$(CT/GT/GA)_n$	ND P_{CAUC}=0.755 and 0.345 P_{AFAM}=0.291 and 0.390	ND P_{CAUC}=0.951 and 0.526 P_{AFAM}=0.411 and 0.365	20% reduction in BPRS, BPOS, BNEG	CAUC and AFAM	Hwang et al. (2012)
Rs10033951	P_{CAUC}=0.82, P_{AFAM}=0.430	P_{CAUC}=0.953, P_{AFAM}=0.547			
Rs6283	P_{CAUC}=0.998, P_{AFAM}=0.240	P_{CAUC}=0.999, P_{AFAM}=0.071			
Rs1967551	P_{CAUC}=0.976, P_{AFAM}=0.923	P_{CAUC}=0.870, P_{AFAM}=0.827			
Rs10001006	P_{CAUC}=0.212, P_{AFAM}=0.867	P_{CAUC}=0.439, P_{AFAM}=0.403			
Haplotype	**Amino acid**		**Sample**		
Rs10033951-rs10001006	T-G	P=0.036	CAUC		

Abbreviations: N/A: Not investigated, BPRS: Brief Psychiatric rating scale, BPOS: positive symptom subscale, BNEG: negative symptom subscale. ND: no significant difference CAUC: Caucasian, AFAM: African American.[125].

Limitations of the current pharmacogenetic studies

Ethnic heterogeneity must be considered when interpretation results of the current pharmacogenetic studies. Some samples varied between a mix of Caucasian and African American [37,118,129]. Other samples were uniform Caucasian, African American or Asian[38-40,117,120,122-125,128,130,131,133]. Differences in genotype and allele distribution have been found in ethnic samples[39].Thus, ethnic heterogeneity might makes it difficult to compare studies across ethnic groups.

Most studies were not replicated in a secondary sample, which does interfere with the validity of results. In addition, most sample sizes were small. Several studies did not reach adequate sample sizes to achieve a desired power of 80 % [37,39,40,117,118,-120,122,125,129,131,132]. Inadequate power increases the risk of false positive or false negative results[65]. Furthermore, most studies did not correct for multiple testing. Multiple testing is a controversial subject within genetic analysis, as it might obscure the possibility to detect rare alleles. Nevertheless, not correcting for multi tests does increase the risk of false positive results, since genetic analysis often include many statistical comparisons[65].

Different study designs were employed in the studies included in this review; most studies investigated genetic variation between clozapine responders and non-responders[39,40,117,123-125,128,129,-131,132]. Other between clozapine responders and healthy[37,38,118,122]. Lastly a study investigated the genetic difference between responders and responders of traditional antipsychotics[122]. When investigating the difference between healthy subjects and clozapine treated schizophrenic patients, results might be obscures from the fact that the etiology of schizophrenia is partly genetic[3,134]. Hence, the possible genetic difference between those samples might be confounded by the etiology of schizophrenia. Evidence indicates that clozapine effectiveness is approximately equal in both non-TRS as well as TRS[23-25]. Studies investigating the genetic difference between responders of clozapine and responders of traditional antipsychotics, does not necessarily investigate between responders and non-responders of clozapine. Allocating patients in the right group i.e. clozapine responder or clozapine non-responder, is important for the study outcome, and should be emphasized more in further studies. Further studies are recommended to investigate the pharmacogenetic difference of clozapine response in TRS; thus investigating the difference between clozapine responders and non-responders.

The requirement of clozapine treatment differed between samples. Clozapine dosage ranged between 50[124] and 900 mg/day [130]. Studies suggest that dosage is not a suitable indicator for clozapine response[57]. Nevertheless, dosage indicates if patients were treated similar to every-days patients. Clozapine dosages of 350-400mg/day are generally prescribed[55,56]. In general, only a few studies reported the clozapine plasma concentration. The plasma concentration does not serve as a good indicator for clozapine response, but a treatment threshold above 350ng/mL-420ng/mL is warranted for determining non-response[59]. Duration of treatment in all studies ranged from 28 days[123] to six month[39]. Studies suggest that treatment-duration of at least 12 weeks is adequate for detecting clozapine response[27]. Studies investigating clozapine response in less than 12 weeks risk classifying responders of clozapine as non-responders and might be a confounder is the genetic outcome.

The criteria to determine response were inconsistent throughout studies. A decrease of Brief Psychiatric Scale (BPRS) of 20 % [37,39,40,117,120,125,133], or 30 % [118] were applied in some studies. Also improvement in the positive items of BPRS (BPOS)[37,39,40,117,120,125,133], the negative items of BPRS (BNEG)[37,39,40,117,120,125,133], Global clinical assessment[124], Global assessment scale (GAS)[38,128,130], or the Positive and Negative Syndrome Scale (PANSS)[124,132], have been applied. In addition an improvement in hospitalization status has been used in another study[131]. The most

widely used BPRS does not necessarily serve as a good indicator for clozapine response in TRS, when the scale is used seperately[62]. In addition, the variation between different scales might be a bias when comparing results between studies. Further studies could employ a more objective measure of response, such as reduction on bed-days[41]. The method have been used in other studies, and indicates that a reduction bed-days is a good measure of response[41,64].

Conclusion

Pharmacogenetic studies investigating clozapine response to the dopamine receptor genes are recommended to design their study in order to minimize confounders and in addition, replicate findings of previous studies within the gene of focus.

Studies should be design accordingly:

- Ethnic homogeneity
- Sample of TRS patient
- Sample size > 105 patients, to ensure 80% power
- Clozapine dosage ≥350-400mg/day
- Clozapine plasma concentration >350-400mg/day
- Clozapine treatment duration >12 weeks
- A objective response measure, such as psychiatric bed-days
- Investigate single SNP variation, haplotype analysis and gene-gene interactions

References

See appendix 2

Appendix 2

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