Pharmacogenetics in the treatment of schizophrenia - current status

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List of Abbreviatons

BDNF – Brain-Derived Neurotrophic Factor

BIP – Bipolar Disorder

DISC1 – Disrupted in Schizophrenia 1

FGA – First Generation Antipsychotic

GAF – Global Assessment of Functioning score

GO – Gene Ontology

GWAS – Genome-Wide Association Study

MDD – Major Depressive Disorder

NRG1 – Neuroregulin 1

PANSS – Positive and Negative Syndrome Scale

PGC – Schizophrenia Working Group of the Psychiatrics Genomics Consortium

SGA – Second Generation Antipsychotic

SNP – Single Nucleotide Polymorphism

Summary

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Schizophrenia is a widespread mental disease that affects about 1% of the world population. Because of the complex heritability of the disease as well as limited knowledge of the precise interconnected biochemical and genetic mechanisms underlying schizophrenia, there is wide variability in terms of antipsychotic drug efficacy, treatment effects, presence and severity of side-effects.

With the completion of the mapping of the human genome, genome-wide association studies (GWAS) bring researchers one step closer to uncovering the molecular associations and pathways involving schizophrenia. Three such current GWAS are examined, as the first GWAS determine genetic associations of 4 candidate genes, the second GWAS explores biological pathways involving histone methylation, immune-neuronal signaling, and the third GWAS attempts to discern the genotypic and phenotypic architecture of schizophrenia.

Other current investigations involve the variability of the SV2C gene, and neurodevelopmental genes *DISC1, NRG1, BDNF* and *NOTCH4.* Variability of serotonin and dopamine pathways are also looked at as well as the variability of the CYP enzyme and association of voltage-gated calcium channels with schizophrenia.

Finally current studies dealing with the pharmacogenetic association of genes and antipsychotic side-effects of hyperprolactinemia, extrapyramidal symptoms, QTc prolongation, extreme weight gain, and agranulocytosis are investigated.

Key words: pharmacogenetics, schizophrenia, GWAS, genes, variability

Introduction

Schizophrenia is a multi-factorial / polygenic complex mental disorder with a prevalence of approximately 1% of the world population and a heritability of up to 80% [1] . The clinical presentation of schizophrenia is usually described with positive symptoms (i.e. delusions, disordered thoughts and speech, tactile, audio or visual, olfactory or gustatory hallucinations) or negative symptoms (i.e. alogia, anhedonia, asociality or avolition). Onset of schizophrenia often occurs between late adolescence and young adulthood and its course is usually chronic, and eventually debilitating causing increased social and economic burden [1].

Because the pathogenesis of schizophrenia is complex and influenced by both genetic and environmental factors, scientists have been challenged trying to uncover and identify the underlying mechanisms and pathways of this disease. Traditionally, pharmacological therapy consisted of trial and error dosing of first and second generation antipsychotics as well as managing side-effects ranging from agranulocytosis, movement disorders, cardiac arrhythmias, to metabolic syndrome and hyperprolactinemia.

Through continuing advances in molecular biology, genetics and biotechnologies, the whole human genome has finally been mapped. Because of this accomplishment, new avenues of exploration into the human genetic code such as the genome-wide association studies are now possible. Associations and pathways between genes and traits can now be further elucidated.

Pharmacogenetic approaches allow researchers to study biological molecules such as CYP enzymes, cytokines, neurotransmitters and genes in order to

investigate and explore drug metabolism and transport. As more knowledge is uncovered, the ultimate goal of personalized medicine comes closer.

This paper will explore three of the latest GWAS studies as well as the current status of investigations conducted in the post-GWAS era, where researchers uncover novel mechanisms of action, explore treatment effects, as well as study and attempt to manage the previously mentioned antipsychotic-induced side effects.

Genome-Wide Association Studies (GWAS)

Schizophrenia has a high heritability and there have been many studies attempting to discover the causative genetic factors [2]. Before the completion of the Human Genome Project and the International HapMap Project, cost and difficulties hampered investigators when trying to construct a comprehensive list of genetic variants in a genomic region. Investigators would select candidate genes based on either theories of schizophrenic etiology or through positional linkage and cytogenic studies [3].

After genome mapping was completed, genome-wide association studies (GWAS) were conducted, whereby common genetic variants in different individuals (both cases and controls) were examined and compared to see if any variant could be associated with a particular trait. The Schizophrenic Working Group of the Psychiatric Genomics Consortium (PCG) was founded in order to combine published and unpublished GWAS data into a single, systematic analysis in order to increase sample size and power of analytics when applied to schizophrenia. These analyses focused on associations between single-nucleotide polymorphisms (SNPs) and the disease trait of schizophrenia. In 2014, the PCG conducted a multi-stage GWAS of

schizophrenia, involving 36,989 cases and 113,075 controls and were able to identify 128 independent associations spanning 108 conservatively defined loci. This massive analysis suggested that schizophrenia has many common genetic variants of small effect sizes.

A subsequent review published in 2015 [3] examined pre-GWAS era historical candidate gene association studies for common genetic variation. This particular review concluded that the historical candidate gene studies were significantly lacking in statistical power. Through the confirmation of the PCG GWAS, 21 of the 25 historical candidate genes were excluded as genetic risks for schizophrenia [2] . Only the four candidate genes *DRD2, GRM3, TNF* and *NOTCH4* were considered to have genome-wide significant associations with schizophrenia [2]. These findings therefore illustrate the power of GWAS analysis and the potential to dramatically improve elucidation of schizophrenia etiologies, and to lead to improved treatments and outcomes.

In order to better examine and identify the underlying biological mechanisms of genetic associations with psychiatric disorders, PCG conducted an analysis framework [4] to rank pathways across schizophrenia, major depression and bipolar disorder. What they discovered was that pathways involving histone methylation, immune-neuronal signaling were significantly associated across all three psychiatric disorders. It was speculated that advances in pharmaceutical treatments could be possible as signaling pathways of these disorders are potentially better drug targets than individual genes [4].

A third GWAS [5] in 2015 took a different approach to investigating missing genomic heritability data. Instead of just analyzing variance in large individual populations or combined data sets, these investigators attempted to measure and characterize the complex associations of both the genotypic and the phenotypic architecture of schizophrenia (Figure 1). Simply put, these investigators identified SNP sets without knowledge of the subjects' status (case or control) and tested their associations with distinct phenotypical clinical feature-clusters of schizophrenia. The researchers had found that 42 interactive SNP sets had greater than 70% risk of schizophrenia. They also determined that 98 SNP sets that had a 66% associated risk of schizophrenia accounted for 90% of the cases. These researchers concluded in their study that a limited number of genotypic networks were associated with several distinct clinical schizophrenic syndromes.

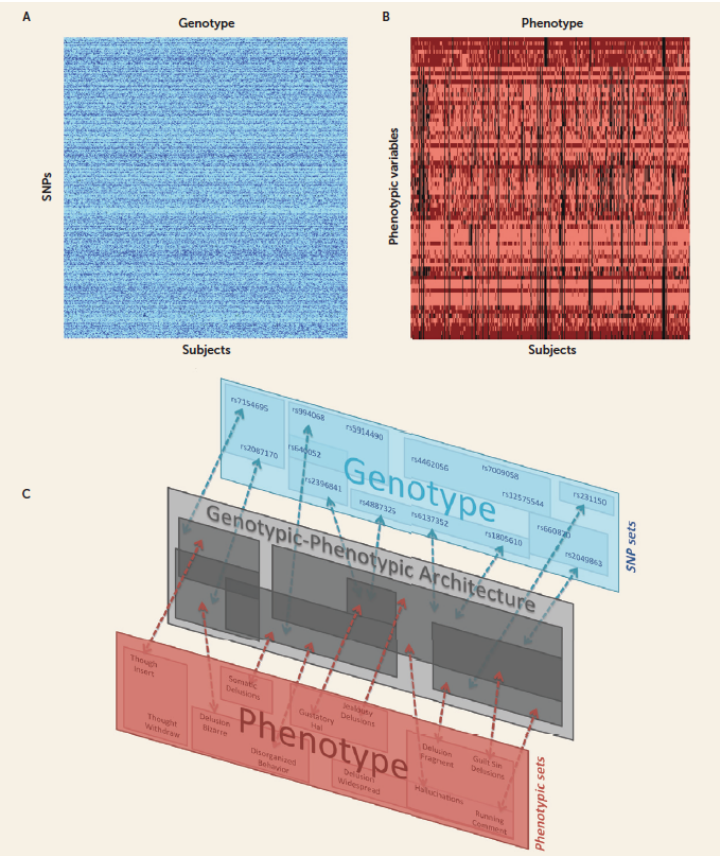

Figure 1. Panel A is a matrix corresponding to the GWAS data set. Panel B is a matrix corresponding to the distinct phenotypic consequences using data at the symptom level from the Diagnostic Interview for Genetic Studies corresponding to the GWAS in Panel A. Panel C cross-matched Panels A and B, revealing specific and distributed genotypic-phenotypic architectures "invisible" to traditional GWAS [Figure reproduced from Arnedo, J., et al., (2015) *Uncovering the hidden risk architecture of the schizophrenias: confirmation in three independent genome-wide association studies.* Am J Psychiatry, **172**(2): p140]

Current Investigations

The results of the various GWAS has afforded investigators greater opportunity for more focused investigations into genetic variation that may complicate presentation signs of patients with schizophrenia, as well as into pharmacogenetic predictors of efficacy for the different types of antipsychotics.

In one recent study, investigators looked at the genotypic variation of SNPs in the *SV2C* gene to see if it influenced atypical antipsychotics of the Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) study [6]. The synaptic vesicle protein 2C of the *SV2C* gene was previously identified as a potential modulator of nicotine's protective effect on the incidence of Parkinson's disease [7]. These investigators identified 18 SNPs in the promoter region of the genes that moderated smoking effects on Parkinson's susceptibility. It was found that instead of receiving a protective effect from nicotine, subjects homozygous for minor alleles of rs10214163 and rs30196 had increased incidence of Parkinson's disease. The SV2C protein is located on the surface of synaptic vesicles and is thought to have a role in synaptic function [8]. The protein also regulates tyrosine hydroxylase, which is a rate-limiting step for production of the neurotransmitters dopamine and norepinephrine [8]. Since SV2C is involved in both GABA and insulin transporter pathways, this protein was investigated for potential schizophrenia etiology and treatment effects [6]. Results of this study showed that one of the SNPs related to nicotine's impact on Parkinson's risk, rs10214163 [7], showed a correlative response with olanzapine and quetiapine, but not with risperidone or ziprasidone [6]. This

difference in preferential response may be due to the different binding affinities of these four various benzodiazepine derivatives [6]. Finally coding SNP rs227092 showed a correlation with olanzapine response while coding SNP rs31244 showed a correlation with quetiapine response [6].

It was shown that one third to one fifth of patients diagnosed with schizophrenia are resistant to drug treatment. Several current studies explore the etiology of this treatment-resistant schizophrenia (TRS) [9].

One 2015 study [10] tested whether the genetic variability of neurodevelopmental genes *DISC1, NRG1, BDNF* and *NOTCH4* had an impact on TRS patients. *DISC1* is involved in positioning of pyramidal neurons [11] in the cortex as well as neural progenitor proliferation regulation [12]. *NRG1* is involved in neuronal migration, cell signaling and myelination [13]. *BDNF* is involved in neuronal differentiation [14], while *NOTCH4* is responsible for signaling the maturation of neurons and glia from neural stem cells [15]. Results of the study showed that *DISC1, NRG1, and BDNF* did not show any significant correlation. However, *NOTCH4* rs367398 did display a significant association with occurrence of treatment-resistant schizophrenia and may play a role in the symptomatology of TRS [10].

This same year, a study of the genetic variants of serotonin receptors *5-HT1A* and *5-HTTLPR* were examined to see if they had an influence on TRS [16]. When 5HT1A receptors are activated by serotonin, dopamine is released into the prefrontal cortex, improving negative, cognitive and affective symptoms as well as modulating mood, cognition and motor behavior [17]. *5-HTTLPR* is an insertion/deletion polymorphism in the promoter region of the *SLC6A4* gene which is a major regulator

of serotonin [18]. The study found that the *5-HT1A* rs6295 variant and the *5-HTTLPR* polymorphisms had some influence on global patient functioning while *5-HTTLPR* specifically had an influence on negative symptoms of schizophrenia [16]. However neither polymorphisms showed any influence on the development of TRS [16].

Another group of researchers [19] investigated DAT-VNTR, SERT-PR and SERT-in2 polymorphisms in regards to TRS. The human dopamine transporter (DAT) has been shown to control dopamine neurotransmission and is a major site of action of psychostimulant drugs [20]. The SERT promoter region of the *SLC6A4* gene has an insertion/deletion polymorphism (SERT-PR) that can either present as a 'short' or 'long' allele form. SERT-in2 is a variable number tandem repeat (VNTR) within intron 2 [19]. Results of the study showed that TRS was associated with SERT-PR polymorphisms. 'Short' SERT-PR carriers were less likely to develop TRS compared to other variants of SERT-PR [19]. There was also a significant association between the interactions of DAT and SERT-in2 gene polymorphisms in regards to TRS [19].

CYP enzymes metabolize almost all antipsychotics in the liver and are also found in the brain, impacting neurotransmitter and local breakdown of medication [21], and influencing personality features and psychiatric symptoms [22]. An investigation involved the CYP3A43 SNP rs472660 variant (which is involved in olanzapine response and clearance) and rs680055 (a putative functional marker on antipsychotic response) [23]. The results of this study showed a non-significant association for rs472660 SNP. However the rs680055 showed significant association

with lower treatment response across both genders [23] which was in line with a previous study on the influence of CYP3A43 on olanzapine [24] .

Using the data of a GWAS, another 2015 study combined those results with gene pathway analysis using post-mortem brain tissue of schizophrenic patients and controls [25]. The post-mortem brain samples were analyzed to identify biological pathways and processes of GWAS-derived genes that associate with schizophrenia. Gene ontology (GO) enrichment analysis was performed on clusters of genes with high correlations of expression. The investigators observed that significantly enriched cluster pathways belonged to calcium ion channel activity [25]. Supportive data from both the GWAS [2] and an analysis of rare disruptive mutations in schizophrenia [26] also pointed toward involvement of voltage-gated calcium channels.

Side-Effects of Antipsychotics

Hyperprolactinemia

Hyperprolactinemia (HPL) is a severe side effect that appears in up to 70% of patients receiving treatment with a number of FGA or SGA, particularly those patients using risperidone [27]. Side-effects of HPL include sexual dysfunction of both men and women, bone loss and is a risk factor for both breast and prostate cancer [28]. Several mechanisms are hypothesized for antipsychotic-induced HPL [29]. One hypothesis is that the degree of HPL is related to the duration of dopamine receptor antagonism by antipsychotics. Another hypothesis poses that the amount of circulating antipsychotic metabolites that cannot pass through the blood-brain barrier determines variability in stimulating prolactin release of the pituitary gland. A third

hypothesis of variability concerns an antipsychotic's simultaneous affinity towards both D_2 receptors and 5-HT_2 receptors. Aripiprazole has high affinity for D_2 and 5-HT_2 receptors and does not cause HPL. This property has led aripiprazole to be used as an adjunctive treatment for patients with risperidone-induced hyperprolactinemia [30].

In determining a pharmacogenetic association with HPL, one study investigated *DRD2* receptor gene SNPs of patients on olanzapine therapy and determined that rs2734842, rs6275 and rs6279 showed significant association with increased prolactin levels [31].

Results for HPL association in other SNP targets have been mixed. A previous study in 2009 showed a correlation between Taq1A A1 allele variants of *DRD2* gene and higher prolactin levels of risperidone-treated boys [32]. However, a more recent study published in 2013 which examined the same Taq1A A1 allele variant found no such correlation with prolactin levels of patients receiving risperidone therapy [33].

Extrapyramidal Symptoms

With the introduction of first-generation antipsychotic (FGA) medications, movement disorders such as akathisia, tardive dyskinesia, and restless leg syndrome, were an unfortunate complication of treatment. Social stigma, non-compliance of therapy and resulting risk of psychotic relapse compounded the problems [34]. Second-generation antipsychotics (SGAs) significantly reduced movement disorders side-effects, yet for some patients extrapyramidal symptoms persisted [34].

While results were conflicting in hyperprolactinemia studies, the DRD2 / ANKK1 Taq1A genotypes were found to be significantly associated with SGA-induced akathisia in one recent study [35]. Akathisia is a motor restlessness often accompanied by subjective distress. Selective blockade of DRD2 receptors induce movement disorders with the maximum blockade of these receptors in the basal ganglion resulting in the most severe symptoms [36]. In this study 47.6% of patients prescribed with FGAs developed akathisia compared to only 16.8% of patients prescribed with SGAs. However this advantage in patients prescribed with SGAs was reduced if they were Taq1A A1 variant positive.

Another study investigated *DRD2* polymorphism in relation tardive dyskinesia [37]. Tardive dyskinesia presents with involuntary, repetitive body movements that have a slow onset. While the study did not replicate previously reported polymorphisms, it did result in the discovery of two novel SNP associations in the *DRD2* gene. The TaqI_D variant was associated with akathisia and the -141C variant was associated with tardive dyskinesia.

Restless leg syndrome (RLS) is a disorder where the patient feels an irresistible urge to move the legs, accompanied or caused by uncomfortable, unpleasant sensations and occur in a circadian rhythm often starting in the evening, often disrupting sleep [38]. In 2015 investigators initiated a study, hypothesizing that there could be an association between RLS and circadian genes *CLOCK* and *NPAS2* [38]. They found a significant association of RLS with the *CLOCK* rs2412646 T allele polymorphism. The investigators postulated that antipsychotic-induced RLS in schizophrenia patients may be a mild form of akathisia occurring at night under circadian rhythm control.

QTc Prolongation

Corrected QT interval (QTc) prolongation can be caused by antipsychotic medication, increasing the risk of cardiac arrhythmias such as torsades de pointes, which can result in sudden death [39]. Because of this risk, new antipsychotics being brought to market undergo a thorough TQc (TQT) study. This study was one such TQT investigation [40] of a relatively new antipsychotic called iloperidone. Iloperidone is a piperidinyl-benzisoxazole derivative with mixed D_2/5-HT_{2a} antagonism, meant to treat adult schizophrenia. One of the requirements of the study was a pharmacogenomics sub analysis to identify potential risk factors. Determining these risk factors can be challenging because there is a higher prevalence of cardiovascular disease among people with schizophrenia compared to the general population [41]. Results of the TQT study revealed that *CYP2D6*10(C100T)* and *KCNQ1* polymorphic alleles present significantly longer change in QTc interval in patients with iloperidone treatment compared to patients on iloperidone treatment that do not have those specific polymorphisms.

Weight Gain

Up to 30% of schizophrenic patients on acute or maintenance antipsychotic treatment experience weight gain as a side-effect [42]. Antipsychotic-induced weight gain (AIWG) can potentially lead to antipsychotic non-compliance, obesity, metabolic and vascular pathologies as well as premature mortality.

In one study, investigators looked at variants in the genes encoding IL-1β, IL-2, IL-6 and BDNF in association with antipsychotic-induced weight gain [43]. Inflammatory cytokines have been shown to mediate production of neurotransmitters

and neuropeptides related to the regulation of food intake [44]. Gene variants of IL-1α, IL-1β, IL-RA, IL-6, and IL-6R have also been shown to have an association with obesity [45]. BDNF is a neurotrophin that can cause loss of appetite and is secreted by immune cells as a protective response during inflammation [46]. In this study, 19 SNPs spanning IL-1β, IL-2, IL-6 and BDNF were investigated which covered over 90% of the genetic variation [43]. The results of this study show that polymorphisms IL-1β rs4849127, rs16944, and rs1143634 increased the risk of AIWG. Also BDFNVal66Met was shown to have mediating effects on AIWG where the Met allele (Met/Met + Met/Val) had a protective effect against excessive AIWG. Thus for chronic schizophrenia patients, these specific IL- β SNPs and BDNFVal66Met could potentially offer predictive outcomes for AIWG.

Another study in 2015 explored the possible association of the *GABRA2* gene with AIGW [47]. GWAS revealed *GABRA2* polymorphism as a potential candidate gene associated with AIGW. GABA has been investigated for its role in food intake as GABA agonists increased feeding behaviors in animal models [48]. The study revealed a significant association between *GABRA2* and AIWG in schizophrenic patients of European descent. *GABRA2* could potentially become a pharmacological target to prevent AIWG.

Agranulocytosis/Granulocytopenia

Clozapine is a highly effective antipsychotic drug but also comes with a risk of the potentially lethal clozapine-induced agranulocytosis/granulocytopenia (CIAG) that occurs in 1% of treated individuals [49]. The major histocompatibility complex (MHC) plays a role in drug responses [50], and thus in 2014 the human leukocyte antigen (HLA) alleles were investigated in the largest genetic study of CIAG attempted so far

[51]. 163 CIAG cases and 8,809,853 variants were compiled and genotyped along with 1,196 controls of European ancestry. Results of the study determined two independent loci (*HLA-DQB1* and *HLA-B*) in the MHC region that were identified to be associated with CIAG. The study also isolated two amino acids (*HLA-DQB1* 126Q and *HLA-B* 158T) but could not determine whether they caused or were only risk factors for CIAG. Testing for these two risk factors would not be helpful as such a test would only have a sensitivity of 0.36 and a specificity of 0.89. A predictive test determining a "safe" group using these two risk factors would be inaccurate because of the low prevalence of CIAG, where less than half of the cases in this study carried either of these uncommon risk factors [51].

Another experiment in 2015 investigated the genetic susceptibility of CIAG using in vitro lymphoblastic cell lines exposed to various concentrations of clozapine and measured cell viability after 48 hours [52]. The hypothesis was that clozapine toxicity in lymphoblasts could share molecular mechanisms with the agranulocytosis etiology. A GWAS was performed for this in vitro model as well as an investigation of the MHC region [52] as was done in the previous GWAS [51].

Interestingly, the investigators did not find involvement of the MHC region in lymphoblast cell vitality after clozapine exposure [52]. They believed that the unicellular model may not have been able to replicate the model of a complete multi-cellular immunological system. However their study was able to show a clozapine-induced viability reduction in lymphoblasts that was a highly heritable (h^2 = 0.76), complex polygenic trait that involved a modest number of loci with small effect as opposed to a small number of loci with large effect. The investigators also identified

PRG4 and *MDFIC* as possible candidate genes associated with clozapine-induced reduced cell viability.

Conclusion

Pharmacogenetic and pharmacogenomic studies of schizophrenia and its therapeutic treatments have started to uncover various genetic and environmental factors that contribute to the variability of treatment efficacy or failure. Genetic variances of CYP liver enzymes influence treatment success or failure as well as potential side effects.

Polymorphisms of dopamine, serotonin and immune-response pathways can influence pharmacological treatment efficacy or presence and severity of side-effects such as agranulocytosis, movement disorders, arrhythmias, weight gain, and hyperprolactinemia.

Unfortunately precise mechanisms of action of antipsychotic medication is still unclear. Many underlying molecular pathways of both disease and treatment remain unclear as researchers struggle with the reliability of experimental data born from low-power and limited sample size studies.

The goal of pharmacogenetics is to determine individually-tailored antipsychotic treatment based on genetic, environmental and clinical data. As knowledge and technology improves, this goal comes closer and closer to fruition through the continued experimentation and persistence of researchers.

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Biography

Joseph Wycoco was born on June 21, 1974 in Quezon City, Philippines but moved to and spent most of his life living in Canada. Desiring a change from a career in the industrial chemical field, Joseph obtained a B. Sc. in psychology at the University of Waterloo and later moved to his wife's former homeland of Croatia to pursue a degree in medicine at the University of Zagreb, School of Medicine.

Joseph is interested in pursuing a specialization in psychiatry with a special interest in the subspecialty of psychosomatic medicine.

As a medical student, Joseph has completed visiting clinical electives at the adult in-patient psychiatry unit at Southlake Regional Health Centre in Newmarket, Ontario, Canada as well as at the child and adolescent in-patient psychiatry unit at the University of Manitoba PsycHealth Centre, Winnipeg, Manitoba, Canada.