The identification of novel genetic variants associated with antipsychotic treatment response outcomes in first-episode schizophrenia patients

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Background **Although antipsychotics are integral to the treatment of schizophrenia, drug efficacy varies between patients. Although it has been shown that antipsychotic treatment response outcomes are heritable, our understanding of the genetic factors that are involved remains incomplete. Therefore, this study aims to use an unbiased scan of the genome to identify the genetic variants contributing toward antipsychotic treatment response outcomes.**

Materials and methods **This study utilized whole-exome sequencing of patients on extreme ends of the treatment response spectrum ($n = 11$) in combination with results from previous antipsychotic studies to design a panel of variants that were genotyped in two well-characterized first-episode schizophrenia cohorts ($n = 103$ and 87). Association analyses were carried out to determine whether these variants were significantly associated with antipsychotic treatment response outcomes.**

Results **Association analyses in the discovery cohort identified two nonsynonymous variants that were significantly associated with antipsychotic treatment response outcomes ($P < 2.7 \times 10^{-5}$), which were also significantly associated with the corresponding treatment response outcome in an independent replication cohort. Computational approaches showed that both of these nonsynonymous variants – rs13025959 in *MYO7B* (E1647D) and rs10380 in *MTRR* (H622Y) – were predicted to impair the functioning of their corresponding protein products.**

Conclusion **The use of whole-exome sequencing in a subset of patients from a well-characterized cohort of first-episode schizophrenia patients, for whom longitudinal depot treatment response data were available, allowed for (i) the removal of confounding factors related to treatment progression and compliance and (ii) the identification of two genetic variants that have not been associated previously with antipsychotic treatment response outcomes and whose results were applicable across different classes of antipsychotics. Although the genes that are affected by these variants are involved in pathways that have been related previously to antipsychotic treatment outcomes, the identification of these novel genes will play an important role in improving our understanding of the specific variants involved in antipsychotic treatment response outcomes.**

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Introduction

Schizophrenia is a poorly understood and debilitating disorder that occurs worldwide [1]. Before the serendipitous discovery of antipsychotics in the 1950s [2], the main course of medical action for patients with schizophrenia was institutionalization and the likelihood that patients would live a self-sufficient life was small [3]. The introduction of antipsychotics has revolutionized the treatment of individuals with schizophrenia and has played a major role in alleviating the socioeconomic burdens placed on patients and their caregivers [4].

Antipsychotics can broadly be classified into First Generation Antipsychotics and Second Generation Antipsychotics. Several large studies have shown that, with the exception of clozapine, the treatment efficacy of these two classes of drugs is similar [4]; however, there is heterogeneity in the way in which individuals respond to antipsychotic medication. This is emphasized by the fact that the British National Formulary states that 'the

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differences between antipsychotic drugs are less important than the greater variability in patient response' [5]. In general, the treatment outcomes of both First Generation Antipsychotics and Second Generation Antipsychotics are nonoptimal, unpredictable, and vary widely between patients, with only ~60% of patients responding to antipsychotic treatment [5]. Thus, methods to optimize antipsychotic treatment decisions are urgently required.

It is for this reason that there is much anticipation for the implementation of antipsychotic pharmacogenomics. If genetic variants that can be generalized across all classes of antipsychotics can be identified to aid in predicting who will not respond to antipsychotic treatment before drug dosing, strategies to improve the long-term outcomes of schizophrenia patients can be developed on the basis of this knowledge. There have been many studies examining antipsychotic pharmacogenetics, some of which have identified genetic variants that are predictive across antipsychotic classes [6]. These studies have, however, focused predominantly on candidate genes [7] and the results obtained have been inconsistent with little clinical significance [8]. The lack of clinically useful results obtained from antipsychotic pharmacogenetic studies may in part be attributed to the fact that, although the mechanism of action of antipsychotics is partially understood [9], there are gaps in our knowledge. By replacing candidate gene approaches with strategies that scan the entire genome, we will allow for the discovery of additional genes that are not related to already established hypotheses [10], the value of which has already been established by the limited number of past genome-wide association studies (GWAS) examining antipsychotic treatment response outcomes [11–18]. If these genome-wide strategies can be combined with our current knowledge of antipsychotic treatment response, results that may be translated into the clinical setting can be obtained.

These studies will be further strengthened through the careful consideration of clinical factors. The majority of studies utilize schizophrenia patients who are in different stages of the disease and have been treated for different periods of time. In addition, chronic schizophrenia patients are more likely to be biased toward those patients who do not respond to treatment as the responders to treatment will be filtered out over time [19], thus complicating the ability to assess the full spectrum of treatment response. Finally, because treatment response reflects a time-dependent outcome, it is important that longitudinal data, obtained using standardized scales, are collected at regular intervals [19]. By utilizing cohorts of first-episode schizophrenia (FES) patients who have received depot injections and who have been carefully monitored over a period of time, these concerns can be addressed.

This study therefore aimed to utilize whole-exome sequencing (WES), in combination with the wealth of data available in the literature, to comprehensively examine the genetic factors contributing toward antipsychotic treatment response outcomes in two well-characterized FES cohorts.

Material and methods

Patient cohorts

This study utilized two cohorts of FES patients, who were assessed with the *Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders* (4th ed.) [20]. All patients and/or their guardians provided written informed consent before this study and ethical clearance was obtained from the Committee for Human Research, Faculty of Health Sciences, Stellenbosch University, and the Institutional Review Board at the Feinstein Institute for Medical Research, North Shore-Long Island Jewish Health System.

Discovery cohort

The discovery cohort utilized in this study included 103 unrelated South African (SA) FES patients [79.6% South African Coloured (SAC), 12.6% Xhosa, 7.8% European descent]. These patients formed part of a larger clinical study examining treatment response outcomes, which has been described in further detail previously [21–24]. In brief, all patients were experiencing their first episode of schizophrenia and received flexible doses of the typical long-acting injectable antipsychotic, flupenthixol decanoate (Fluanxol, Lundbeck, Copenhagen, Denmark), after a washout period of up to 7 days. Thereafter, the patients were assessed at regular intervals over 12 months (baseline, week 1, week 2, week 4, week 6, week 12, week 24, week 36, and week 48) by means of the Positive and Negative Syndrome Scale (PANSS). Data from the first 3 months of treatment were utilized for the current study. Extensive demographic and clinical information [including family history of psychiatric illness, history of substance abuse, Social and Occupational Functioning Assessment Scale scores, World Health Organization Quality of Life scores, Premorbid Adjustment Scale scores, Birchwood Insight Scale scores, and Extrapyramidal Symptom Rating Scale scores (described in detail [21–24] elsewhere)] was also collected for each patient.

Replication cohort

To determine whether the genetic variants identified in the discovery cohort were of relevance to antipsychotic treatment response across different classes of antipsychotics, a replication cohort was collected. The replication cohort included 87 FES patients [70.1% African American (AA), 29.9% European descent], who were recruited from two clinical trials (*http://www.clinicaltrials.gov* Identifiers: NCT00000374 and NCT00320671). Patients received olanzapine (Zyprexa, Eli Lilly and Company, Indianapolis, Indiana, USA), risperidone (Risperdal, Janssen Pharmaceuticals, Beerse, Belgium), or aripiprazole (Abilify, Bristol-Myers Squibb Company, New York City, New York, USA), and response to antipsychotic treatment was examined over 3 months at regular intervals (baseline, week 1, week 2, week 3, week 4, week 6, week 8,

week 10, and week 12) by means of the Brief Psychiatric Rating Scale (BPRS). These patients formed part of a larger clinical study examining treatment response outcomes, which has been described in further detail elsewhere [25,26].

Selection of genotyping panel

As described previously, patients falling on extreme ends of the treatment response spectrum in the discovery cohort were subjected to WES [24]. These WES data were used in combination with the literature to prioritize variants for genotyping in the discovery and replication FES cohorts. The variants prioritized for genotyping (Supplementary Fig. 1, Supplemental digital content 1, *http://links.lww.com/FPC/A995*) included (i) variants that have been significantly associated with antipsychotic treatment outcomes in past GWAS and candidate gene studies; (ii) variants in candidate antipsychotic pharmacogenes that were identified in the WES data and were predicted to alter the function of the genes; and (iii) variants identified from variant and gene-based analyses carried out using the Variant Annotation, Analysis and Search Tool (Supplementary Fig. 2, Supplemental digital content 1, *http://links.lww.com/FPC/A995*) [27] as described by the authors. In addition, all variants were analyzed using GTEx [28] to investigate the effect of the variants on the expression of genes in the whole blood, brain, and liver tissues (Supplementary Table 1, Supplemental digital content 1, *http://links.lww.com/FPC/A995*). More details on the prioritization of the variants and the specific variants that were genotyped are provided in Supplementary materials, Supplemental digital content 1, *http://links.lww.com/FPC/A995*. The prioritized list of variants was subsequently assessed using the Illumina Assay Design Tool. All variants that received failure codes were supplemented as far as possible with variants identified from the WES and/or 1000 Genomes Project data that were in linkage disequilibrium with the initial variants ($r^2 > 0.8$) (refer to the Supplementary materials for more details, Supplemental digital content 1, *http://links.lww.com/FPC/A995*). A total of 284 variants were thus prioritized for genotyping.

The inclusion of ancestry informative markers to allow for correction for population stratification

To allow for correction for population stratification, 100 ancestry informative markers (AIMs), which were designed specifically for the SAC population [29], were included in the genotyping assay (Supplementary materials, Supplemental digital content 1, *http://links.lww.com/FPC/A995*). After the successful genotyping of these AIMs, ancestry proportions were estimated by ADMIXTURE utilizing the genotype data obtained for the FES cohorts as well as the data obtained from the five populations from which the AIMS were designed. This procedure is described in further detail by Daya and colleagues [29].

Genotyping of the prioritized variants in the two schizophrenia cohorts

Genotyping of the samples was performed at the University of Utah Genomics Core Facility (Salt Lake City, Utah, USA) with the 384-plex BeadXpress assay using VeraCode technology (Illumina, San Diego, California, USA). Duplicate samples were included as internal controls ($n = 15$). Thereafter, the variants were clustered using default setting on the GenomeStudio data analysis software (Illumina) and each cluster was inspected manually. All variants with unsatisfactory clusters or call rates less than 90% were excluded.

Statistical analyses

Allele and genotype frequencies as well as deviations from Hardy–Weinberg equilibrium (HWE) were determined for all genotyped polymorphisms using Tools For Population Genetic Analysis (version 1.3) Software [30], and P less than 0.002 was considered significant for HWE testing. Only variants with minor allele frequencies greater than 0.01 were included in the subsequent analyses of the unrelated individuals. To identify associations with change in longitudinal PANSS scores (positive, negative, general psychopathology, and total) over 3 months of treatment, a mixed-effects repeated-measures model was utilized to describe each of the four nonbaseline PANSS scores (positive, negative, general psychopathology, and total) as a function of the statistical interaction between time point and genetic factor, including individual as a random effect. This provided an estimated effect of the genetic comparison per time unit (week). Because of the positively skewed distributions of the PANSS scores, log transformations were performed. All models were subsequently adjusted for clinical variables that were significantly associated with treatment response (age and sex – refer to Supplementary Table 2, Supplemental digital content 1, *http://links.lww.com/FPC/A995*), ancestry proportions (calculated as described in the Inclusion of ancestry informative markers to allow for correction for population stratification section), and baseline PANSS scores. The replication cohort was analyzed in the same manner using BPRS scores. To account for multiple testing, Bonferroni correction was used, whereby 0.05 (significance threshold) was divided by the total number of tests ($n = 1824$) performed in the initial discovery analyses. The number of tests performed was calculated as follows: number of tests = [number of variants included in the statistical analyses ($n = 228$) × number of models used ($n = 2$, genotype and additive models) × number of phenotypes investigated ($n = 4$, PANSS positive scores, PANSS negative scores, PANSS general psychopathology scores, and PANSS total scores)]. Therefore, P less than 2.7×10^{-5} was considered significant in the discovery cohort and as only the significantly associated variants were examined in the replication cohort, P less than 0.05 was considered significant in this cohort. Inheritance models were tested for all significant allelic and genetic associations, and models that

fitted both cohorts most optimally are reported in the results. Effect estimates with 95% confidence intervals are derived from these models. Graphs of observed values are presented as an indication of the corresponding unadjusted patterns in the data. All statistical analyses and modeling were carried out using the function lme from package nlme [31] of the freely available programming environment R [32].

Results

After exclusion of all variants that failed quality control, a total of 347 variants (252 prioritized variants and 95 AIMs) remained. Removal of all variants with minor allele frequencies less than 0.01 resulted in the inclusion of 228 prioritized variants, in addition to the 95 AIMs, for the subsequent statistical analyses. Closer examination of these variants indicated that all patients were successfully genotyped and all duplicate samples were identical, providing a quality control measure for the genotyping assay, as described in more detail elsewhere [33]. Thus, all samples were included in the subsequent statistical analyses. All variants on autosomal chromosomes that were included in the downstream analyses were in HWE, with the exception of rs4926044, which was out of HWE in the SAC and AA population groups ($P < 0.002$). Ancestry proportions were estimated for each of the five source ancestries for which the AIMs were designed (African nonSan, African San, East Asian, South Asian and European) (Supplementary Fig. 3, Supplemental digital content 1, *http://links.lww.com/FPC/A995*). As expected, examination of the ancestry proportions showed high and variable levels of admixture in the SAC, Xhosa, and AA individuals and distinctive patterns of ancestry were also observed in each of the ethnic cohorts.

After Bonferroni correction for multiple testing, four variants remained significantly associated ($P < 2.7 \times 10^{-5}$) with weekly change in PANSS scores over 3 months of antipsychotic treatment and all of these variants were predicted by at least one prediction program to alter the expression of a gene or affect the function of the corresponding protein product (Table 1).

The four variants that were significantly associated with treatment response in the SA FES discovery cohort were subsequently investigated in the replication cohort and it was observed that two of these variants (rs13025959 in *MYO7B* and rs13080 in *MTRR*) were also significantly associated ($P < 0.05$) with the corresponding longitudinal BPRS scores in the American FES cohort. Further investigation of these variants in the SAC individuals showed findings similar to those originally detected in the entire SA FES cohort (Supplementary Fig. 3, Supplemental digital content 1, *http://links.lww.com/FPC/A995* and Supplementary Table 3, Supplemental digital content 1, *http://links.lww.com/FPC/A995*). The first of these variants, rs13025959 in *MYO7B*, was associated with a decreased improvement in general psychopathological symptoms after antipsychotic

Table 1 Variants significantly associated with antipsychotic treatment response in the South African FES cohort

						South African FES cohort (combined, $n = 103$)			American FES cohort ($n = 87$)		
Locus name	Gene	Effect on gene product	PolyPhen-2 prediction	SIFT prediction	GTEx significant association	Trait	*P* value (additive)	*P* value (genotype)	Trait	*P* value (additive)	*P* value (genotype)
rs3781409	*CTBP2*	V234L	Benign	**Damaging**	NA	PANSS-G score	**2.3×10^{-6}**	**1.9×10^{-6}**	BPRS-G score	0.324	0.486
rs13025959	*MYO7B*	E1647D	**Possibly damaging**	Tolerated	Decrease *MYO7B* expression	PANSS-G score	**2.4×10^{-6}**	**2.4×10^{-6}**	BPRS-G score	0.075	**0.027**
					Increase *AC068282.3* expression	PANSS-T score	**8.2×10^{-6}**	**8.2×10^{-6}**	BPRS-T score	**0.021**	**0.018**
rs10380	*MTRR*	H622Y	Benign	**Damaging**	NA	PANSS-P score	0.1102	**7.4×10^{-6}**	BPRS-P score	**0.003**	**0.014**
rs16984014	*GLRA4*	R106C	**Probably damaging**	**Damaging**	NA	PANSS-N score	**1.6×10^{-5}**	3.2×10^{-5}	BPRS-N score	**0.025**	0.056

BPRS, Brief Psychotic Rating Scale; FES, first-episode schizophrenia; G, general psychopathological symptoms; N, negative symptoms; NA, not applicable; P, positive symptoms; PANSS, Positive and Negative Syndrome Scale; T, total symptoms.
Entries that are bold represent (i) variants that are predicted to affect the functioning of the protein product and (ii) are significantly associated with the trait of interest in the discovery or replication cohorts.

Table 2 Effect sizes of the variants significantly associated with antipsychotic treatment response in both cohorts

Variant	Gene	MA	Cohort	Trait	Model	Comparison	Effect (95% CI)
rs13025959	*MYO7B*	C	US	BPRS_G	Dominant	CC + CG vs. GG	1.7 (0.4–3.0)
			SA	PANSS_G		GC vs. GG[a]	1.7 (1.0–2.4)
			US	BPRS_T		CC + CG vs. GG	1.4 (0.4–2.5)
			SA	PANSS_T		GC vs. GG[a]	1.4 (0.8–2.1)
rs10380	*MTRR*	T	US	BPRS_P	Dominant	TT + CT vs. CC	−1.6 (−2.8 to −0.4)
			SA	PANSS_P		TT + CT vs. CC	−1.5 (−2.4 to −0.6)

BPRS, Brief Psychotic Rating Scale; CI, confidence interval; FES, first-episode schizophrenia; G, general psychopathological symptoms; MA, minor allele; P, positive symptoms; PANSS, Positive and Negative Syndrome Scale; SA, South African; T, total symptoms; US, American.
[a]No homozygote CC individuals were observed in the SA FES cohort. Positive effects show that the minor allele is associated with a worse response to treatment, whereas negative effects indicate an improved treatment response outcome.

treatment. The second variant, rs13080 in *MTRR*, was associated with an improvement in positive symptoms after antipsychotic treatment (Table 2 and Fig. 1). Although rs16984014, in *GLRA4*, was significantly associated with a change in negative symptoms after antipsychotic treatment in both cohorts, the direction of effect was not the same. Therefore, this association was not considered replicable across the two cohorts.

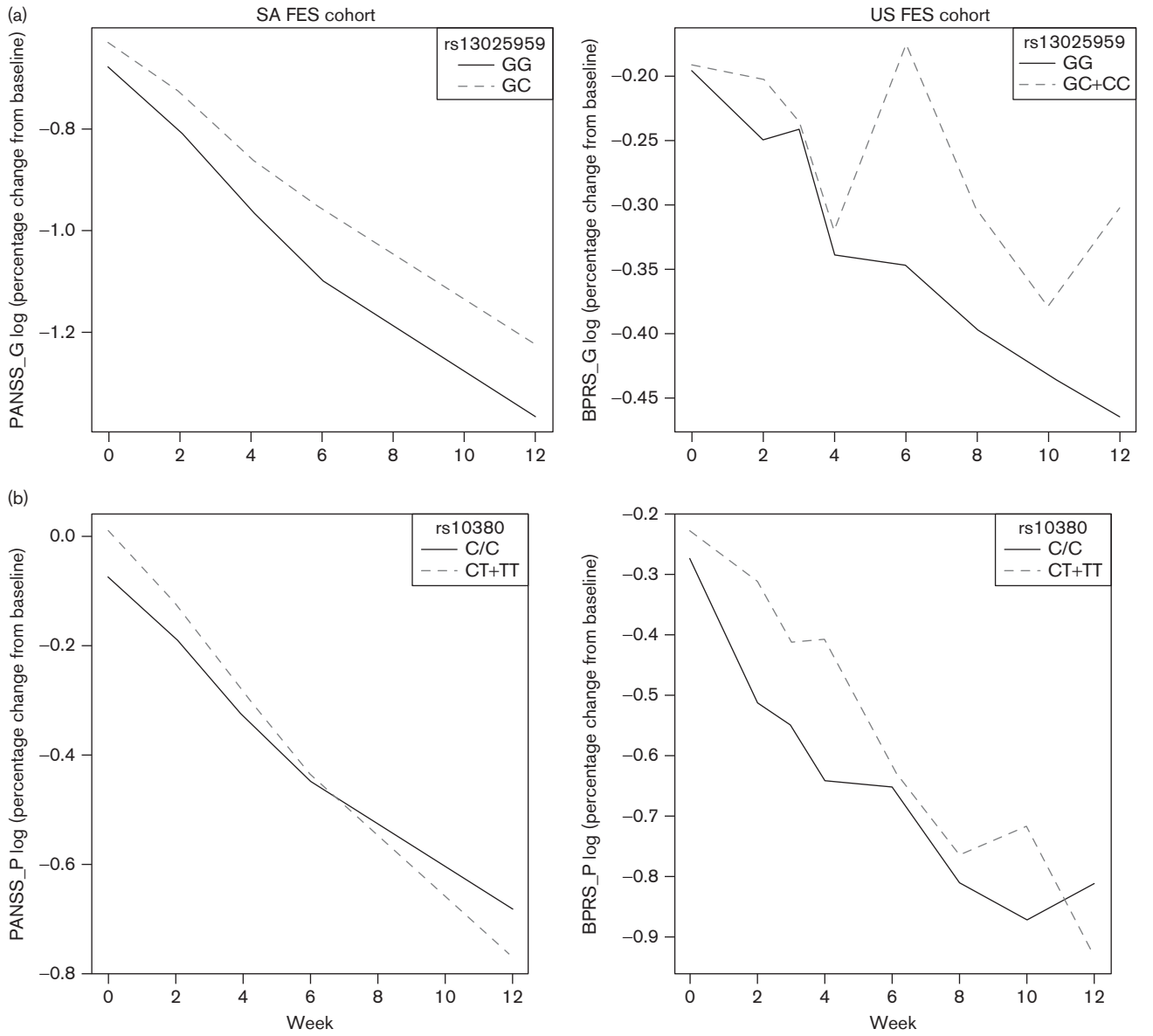
Discussion

This study successfully identified two novel variants that were associated with specific antipsychotic treatment response outcomes in two independent FES cohorts. Both variants were predicted to affect the function of a protein product (Table 1). Expression data recorded on Ensembl showed that all of these genes were expressed in the brain (*http://www.ensembl.org*) and in addition to resulting in an amino acid change in *MYO7B*, rs13025959 was shown to decrease the expression of this gene (Table 1). The identification of these variants in *MYO7B* and *MTRR* is of particular value as they represent pharmacogenomic findings that are transferable across different classes of antipsychotics. These findings are therefore ultimately likely to offer potential benefits to a wide range of antipsychotic treatments offered to schizophrenia patients worldwide and are not limited to one specific antipsychotic medication.

The first of these novel findings was the association observed between rs13025959 (E1647D) in *MYO7B* – encoding myosin VIIB – and decreased improvement in general psychopathological symptoms. Although only limited research has been performed with respect to this gene, other genes from the myosin family (e.g. *MYO16* and *MYO9B*) have been associated with schizophrenia [34,35] and *MYO6* has been shown to play an essential role in the BDNF–TrkB-mediated neurotransmitter release that takes place in the hippocampal neurons of mice [36]. Furthermore, the myosin genes have been reported to be important in neuronal migration and actin remodeling, both of which are important processes that take place during the development of the brain [35,37]. There have been several studies that have highlighted the role of aberrant neuronal migration and schizophrenia, the latest of which has shown that neurons derived from human-induced pluripotent stem cells obtained from schizophrenia patients show aberrant neuronal migration and the addition of antipsychotics to these cells was reported to worsen this phenotype [38]. This is in agreement with the data obtained from our study, where it was shown that individuals positive for the protein damaging *MYO7B* variant showed a poorer antipsychotic treatment response outcome. Interestingly, the treatment response trajectory for individuals carrying the rs13025959 variant was different in the SA and American cohorts. This may be attributed to the absence of individuals who are homozygous for the variant in the SA cohort. Evidence for the added influence of an additional copy of the variant gene is provided by the GTEx results, which show that the variant affects gene expression in an additive manner.

The second association was observed between rs10380 (H622Y) in *MTRR* – encoding 5-methyltetrahydrofolate-homocysteine methyltransferase reductase – and an improvement in positive symptoms after antipsychotic treatment. *MTRR* forms part of the folate metabolism pathway and it has been suggested that variants affecting genes in this pathway may affect downstream methylation activities and gene expression levels [39]. Therefore, *MTRR* may regulate the expression of genes after antipsychotic treatment and thus variants in these genes may affect the way in which patients respond to treatment. Although MTRR itself has not yet been implicated in antipsychotic treatment response, MTHFR, which along with MTRR, is an important enzyme in the folate metabolism pathway, has been associated previously with antipsychotic treatment response outcomes [39,40]. Of particular interest to the findings of this study, it has been reported that the rs1801133 variant (677T-allele) in *MTHFR* is associated with less severe positive symptoms in schizophrenia patients [40,41]. This is in line with the results of our study, where it was observed that rs10380 was associated with an improvement in positive symptoms after antipsychotic treatment, although this effect was only observed toward the end of the 3 months of treatment (Fig. 1), highlighting the dynamic nature of antipsychotic treatment response. The potential application of the results obtained from this study is highlighted by the finding that the addition of folic acid to antipsychotic treatment improves negative symptoms when the *MTHFR* genotype is taken into account [42]. Thus, the use of folic acid supplementation and the

Fig. 1



Graphs of observed changes in PANSS/BPRS scores over 3 months of antipsychotic treatment separated according to genotype. (a) rs13025959 in *MYO7B* and PANSS/BPRS general psychopathological symptom scores, (b) rs10380 in *MTRR* and PANSS/BPRS positive symptom scores. BPRS, Brief Psychiatric Rating Scale; PANSS, Positive and Negative Syndrome Scale; SA FES, South African first-episode schizophrenia; US FES, American first-episode schizophrenia.

influence of *MTRR* variants on positive symptoms may be an interesting avenue for future research.

One of the limitations of this study that should be acknowledged is the high level of genetic admixture in these cohorts. This admixture complicates association analyses, but given the heterogeneous composition of SA populations [43], it is necessary to utilize patient cohorts that are representative of the country's populations. By incorporating AIMs that were specifically designed for the SAC population, the importance of which has been described previously [44], we could include these highly admixed samples in our association analyses. In addition to the advantages of using population groups that are representative of real-world contexts, the use of the SAC population allowed for the opportunity to examine variants that are of relevance to different population groups. A further limitation relates to the clinical differences between the discovery and replication cohorts. The use of a replication cohort, which includes a broad spectrum of antipsychotic agents and clinical profiles, improves the reliability, validity, and generalizability of the results. However, the use of this cohort may have masked the associations that were

specific to flupenthixol treatment in the context of South Africa. Thus, future studies should examine all of the significant associations that were detected in the discovery cohort in an additional cohort of SA patients who received the same flupenthixol treatment protocol. Finally, although this study focused on variants that were significantly associated with treatment response outcomes after Bonferroni correction for multiple testing, some of the variants examined have been associated previously with treatment response outcomes in past GWAS and candidate gene studies and therefore a less stringent significance threshold may be required to substantiate these findings. Investigation of all previous GWAS and candidate gene findings indicated 10 significantly associated ($P < 0.05$) candidate gene variants and 11 significantly associated ($P < 0.05$) GWAS variants in our cohort (Supplementary Table 4, Supplemental digital content 1, *http://links.lww.com/FPC/A995*). The replication of these findings suggests that these variants may also play a role in antipsychotic treatment response outcomes.

Conclusion

This study has once again reiterated the complexity of psychiatric traits and the treatment thereof. Although further research is still warranted, the results from this study have provided additional information that should aid in our understanding of antipsychotic treatment response. The two genes that were identified by this study have pointed toward plausible hypotheses related to treatment response outcome (e.g. aberrant neuronal migration) and novel treatment strategies that are already being examined as alternatives and supplements to current antipsychotic treatments (e.g. the use of folic acid) [42,45]. Nonetheless, although these variants provide clues to interesting avenues of future antipsychotic pharmacogenomic research, additional studies are necessary. These studies should be aimed at further replicating these results in larger independent cohorts and generating supporting functional evidence.

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Conflicts of interest

R. Emsley has participated in speakers/advisory boards and received honoraria from AstraZeneca, Bristol-Myers Squibb, Janssen, Lilly, Lundbeck, Organon, Pfizer, Servier, Otsuka, and Wyeth. He has received research funding from Janssen, Lundbeck, and AstraZeneca; A.K. Malhotra acts as a consultant for Genomind Inc. and has received a grant from AbbVie. For the remaining authors there are no conflicts of interest.

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