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Genome-wide association study of movement-related adverse antipsychotic effects

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Abstract

Background—Understanding individual differences in the development of extra-pyramidal side effects (EPS) as a response to antipsychotic therapy is essential to individualize treatment.

Methods—We performed genome-wide association studies to search for genetic susceptibility to EPS. Our sample consists of 738 schizophrenia patients, genotyped for 492K SNPs. We studied three quantitative measures of antipsychotic adverse drug reactions, the Simpson-Angus scale (SAS) for parkinsonism, the Barnes akathisia rating scale, and the abnormal involuntary movement scale (AIMS) as well as a clinical diagnosis of probable tardive dyskinesia.

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Results—Two SNPs for SAS, rs17022444 and rs2126709 with $p=1.2\times10^{-10}$ and $p=3.8\times10^{-7}$, respectively, and one for AIMS, rs7669317 with $p=7.7\times10^{-8}$, reached genome-wide significance (q-value <0.1). Rs17022444 and rs7669317 were located in intergenic regions and rs2126709 was located in *ZNF202* on 11q24. Fourteen additional signals were potentially interesting (q-value <0.5). The *ZNF202* is a transcriptional repressor controlling, among other genes, *PLP1* which is the major protein in myelin. Mutations in *PLP1* cause Pelizaeus-Merzbacher disease, which has parkinsonism as an occurring symptom. Altered mRNA expression of *PLP1* is associated with schizophrenia.

Conclusions—Although our findings require replication and validation, this study demonstrates the potential of GWAS to discover genes and pathways that mediate adverse effects of antipsychotics.

Keywords

genome-wide association; antipsychotic; pharmacogenetics; personalized medicine; single nucleotide polymorphism; copy number variation; schizophrenia

Introduction

Antipsychotics are the cornerstone of acute and long-term treatment for schizophrenia. The first generation “typical” antipsychotics (e.g., haloperidol) were introduced in the 1950s. Despite treatment with these antipsychotics, many schizophrenia patients do not improve or relapse frequently. Furthermore, these drugs may produce adverse reactions such as extrapyramidal side effects (EPS, i.e., involuntary movements). Clozapine was (re-)introduced in 1989 marking the advent of the second generation “atypical” antipsychotics. Although clozapine has enhanced therapeutic effects in patients who respond poorly to treatment with typical antipsychotics, it is associated with a serious risk of agranulocytosis. Other second generation antipsychotics such as olanzapine have therefore become the first line treatment for schizophrenia. These second generation drugs are, however, no panaceas. They too lack efficacy in a substantial group of patients and are associated with, for example, metabolic side effects that increase risk of cardiovascular disease and diabetes. Due to effectiveness in individual patients, low risk of metabolic side effects, and much lower costs, first generation antipsychotics are therefore still being prescribed as well.

EPS are a major consideration for prescribing, in particular first generation antipsychotics that are assumed to have a somewhat higher risk profile. EPS are a complex and potentially heterogeneous group of involuntary movements including parkinsonism, akathisia, and tardive dyskinesia (TD). TD is particularly worrisome because of its high annual incidence (5.5% and 3.95% for first and second generation antipsychotics, respectively) and potential irreversibility (1). The complexity of EPS is illustrated by data showing that these side effects may occur at different times during antipsychotic treatment (acute vs. late EPS), may sometimes occur “spontaneously’ after antipsychotic treatment ended, or may be suppressed by continued antipsychotic treatment. Clearly, a better understanding of individual differences in the susceptibility of developing EPS would be essential to better tailor antipsychotic treatment to individual patients.

Genetic factors have been proposed to explain part of the individual differences (2). Some candidate gene studies have explored the possible role of dopamine and serotonin receptors (3), and a recent meta-analysis supported an association with *DRD2* (4, 5). However, because the selection of candidate genes is hampered by the limited knowledge of the causal mechanisms, exploratory methods that systematically screen markers across the whole genome for association with EPS are critical to discover novel variants. In this article, we

therefore performed such a genome-wide association studies (GWAS) to search for genetic susceptibility to EPS as a result of cumulative lifetime antipsychotic exposure.

Methods

Methodological details can be found in the Supplemental Materials. In short, all subjects (N=738) came from the Clinical Antipsychotic Trial of Intervention Effectiveness (CATIE) study (6) and were diagnosed with schizophrenia according to DSM-IV. CATIE is a multiphase randomized trial of antipsychotic medications that recruited patients from 57 clinical settings around the United States. The mean age is 40.9 years. Approximately 57% of the subjects describe themselves as white/European American (EA), 29% as black/ African American (AA), and 14% as belonging to multiple or "other" racial categories.

EPS was measured using the Simpson-Angus Scale (SAS) (7) for parkinsonism, the Barnes Akathisia Rating Scale (BARS) (8) and the Abnormal Involuntary Movement Scale (AIMS) (9). Patients with TD at initial assessment were excluded from assignment to perphenazine. Newly detected probable TD resulted in discontinuation of the current study treatment unless the research clinician suspected withdrawal TD, which according to the Schooler-Kane criteria occurs within two weeks of discontinuing an antipsychotic medication.

On average CATIE subjects began antipsychotic treatment over 14 years ago. Our aim was to better understand genetic susceptibility to EPS as a result of this cumulative lifetime antipsychotic exposure. For this purpose, we first created a set of "proxy" indicators of lifetime exposure. Next, we used statistical modeling to measure the severity of EPS as a result of this exposure by essentially a) estimating the mean symptom level across the entire study using all rather than a single observation, while b) adjusting for subject-specific in-trial changes. Finally, by including the proxies in the GWAS we searched for genetic variants predicting individual differences in EPS susceptibility in patients with comparable cumulative antipsychotic lifetime exposure.

After quality control, genotypes for 492,000 SNPs from 738 individuals remained for statistical analysis (10). In order to correct for population stratification we used the multi-dimensional scaling (MDS) approach implemented in PLINK (11). The first five dimensions captured the majority of the genetic substructure in CATIE. The GWAS was conducted using PLINK while incorporating the proxies for antipsychotic exposure and the MDS dimensions as covariates. We used a false discovery rate (FDR) to declare significance, where a q-value <0.1 declared genome-wide significance (12) and a q-value <0.5 to identify "potentially interesting" results. That is, the expected proportion of false discoveries among SNPs declared significant was 10%/50%. We also estimated the local FDR (13) to assess the (posterior) probability that our top findings were false discoveries.

Results

The proportion of individuals that at any time during the CATIE trial indicated some EPS symptoms (scale score > 0) ranged from 72-82% for the three scales (Table 1). The mean number of assessments per genotyped subject was 7.5. Average sums for the most clinically relevant items, for each scale, are given in Table 1.

All GWAS p-values can be downloaded from www.pharmacy.vcu.edu/biomarker. Quantile-quantile plots showing the distribution of the p-values are shown in Figure S1. These plots indicate that in general the p-values follow the expected null distribution indicated by the strait diagonal line. A few markers in the upper right corner deviate from this line suggesting a possible association. Three signals reached genome-wide significance according to our predefined threshold of q-value <0.1. Fourteen signals were potentially interesting using q-

values <0.5 as a threshold (Table 2). For SAS we detected genome-wide significance with rs17022444 ($p = 1.2\times10^{-10}$, $q = 4.8\times10^{-5}$) located in an intergenic region on chromosome 2p12 as well as with rs2126709 ($p = 3.8\times10^{-7}$, $q = 0.06$) located in the 3' untranslated region (UTR) of zinc finger protein 202 (*ZNF202*) on chromosome 11q24. The third genome-wide significant signal involved the AIMS and rs7669317 ($p = 7.7\times10^{-8}$, $q = 0.05$). Rs7669317 is located 167 kb from pyrophosphatase (inorganic) 2 (*PPA2*) and 16 kb from the locus encoding hypothetical protein FLJ20184 on chromosome 4q24. Regional plots for the significant results and Manhattan plots of the complete GWAS results can be found in Figures S2 and S3. No genome-wide significant signals were detected for the BARS and probable TD.

Secondary analyses of the genome-wide significant signals showed that the proportion of the variance explained by each SNP was similar in European and African Americans, that multi-marker (haplotype) tests did not improve *p*-values, and that the detected signals were unlikely the result of genotyping errors.

Discussion

We performed GWAS to search for genetic susceptibility to EPS. Three signals reached genome-wide significance according to our predefined threshold. For each of these markers the posterior probability indicated a reasonable chance of a true finding. One of the SNPs, rs2126709, was located in the 3'UTR of *ZNF202*. This geneis a transcriptional repressor, controlling promoter elements found in for example apolipoprotein E (*apoE*), lipoprotein lipase (*LPL*), ATP binding cassette (*ABC*) transporters A1 and G1, proteolipid protein (*PLP*) and early growth response 3 (*EGR3*) (14). *ZNF202* has been linked to dyslipidemia, high-density lipoprotein control, and many genes under its control are involved in reverse cholesterol transport (15). Considering that, next to adiposity tissue, the brain has the highest lipid content and that the myelin sheets surrounding and protecting neurons consists of approximately 80% lipids, a well functioning regulation of the lipid metabolism is of particular importance for brain function. Furthermore, demyelination can lead to diseases with ataxia and other movement problems, such as multiple sclerosis. *PLP1*, which is also under the transcriptional control of *ZNF202*, is the predominant myelin protein present in the central nervous system. This gene has been associated with Pelizaeus-Merzbacher disease (16) in which involuntary movements, including parkinsonism, are among the major occurring symptoms. In addition, decreased mRNA expression of *PLP1* has been observed in patients diagnosed with schizophrenia (17). Finally, *EGR3* is expressed in a variety of cell types and plays a key role in nervous system development and plasticity. Mice lacking this gene have shown sensory ataxia and muscle spindle agenesis (18).

Markers rs17022444 and rs7669317 that were associated with SAS and AIMS, respectively, were not located in any known or predicted genes. Although this makes a false positive finding more likely, it does not preclude a true finding. Examples exist of intergenic associations replicating in independent samples (19) and there is considerable experimental evidence for long range regulatory effects mediated by these genomic regions (20).

Our findings require replication and validation. However, the present study demonstrates the potential of GWAS in order to discover genes and pathways that potentially mediate the adverse effects of antipsychotic medication. A better understanding of these mechanisms and the role of specific polymorphisms could eventually help to tailor individualized antipsychotic medication with minimal toxicity for patients with schizophrenia.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Descriptive data for 738 genotyped CATIE subjects

Description	Proportion of ind.
Males	74%
Probable TD	29%
AIMS score > 0	72%
BARS score > 0	75%
SAS score > 0	82%
	Mean (s.d)
Age (in years)	40.9 (11.0)
Years treated	16.7 (11.2)
Years medicated	14.3 (10.8)
AIMS (item 1-7)	1.48 (2.33)
BARS (item 1-3)	0.89 (1.07)
SAS (item 1-6)	0.21 (0.25)

Means for AIMS, BARS, SAS are the summed item scores, for the clinically most relevant items (see Supplementary Materials for details), averaged across the multiple observations during the trials.

Table 2

Results from single-marker tests with q-value[a] estimates < 0.5.

Phenotype	dbSNP	Chr.	Position (kb)	Gene	*p*-value	*q*-value[a]	*q*-value[b]	Local FDR
SAS	rs17022444	2	82941	NA	1.18E-10	4.83E-05	5.84E-05	3.09E-04
	rs2126709	11	123101	ZNF202	3.75E-07	0.062	0.092	0.312
	rs10811771	9	22761	NA	7.92E-07	0.114	0.130	0.473
	rs876347	9	118111	NA	1.90E-06	0.219	0.194	0.668
	rs1459148	14	97910	NA	1.97E-06	0.225	0.194	0.677
	rs337161*	1	217303	NA	3.35E-06	0.317	0.275	0.775
	rs1494373*	1	217318	MOSC2	6.06E-06	0.353	0.282	0.803
	rs16996151	4	90336	KIAA0914	6.20E-06	0.441	0.307	0.859
	rs4837752	9	120136	NA	6.31E-06	0.447	0.307	0.861
	rs17119280	1	59511	FGGY	7.28E-06	0.451	0.307	0.863
	rs12625057	20	57224	ZNF831	7.48E-06	0.482	0.307	0.879
	rs7928794	11	35658	TRIM44	8.35E-06	0.489	0.307	0.882
AIMS	rs7669317	4	106815	NA	7.70E-08	0.050	0.038	0.214
	rs9302841	16	7167	A2BP1	2.02E-06	0.469	0.368	0.856
	rs6743931	2	240155	NA	2.24E-06	0.491	0.368	0.868
BARS	rs12147450	14	20027	NA	6.18E-07	0.339	0.286	0.638
	rs2251301	8	11156	NA	1.22E-06	0.460	0.286	0.753

[a] *q*-value estimates calculated as described by Bukszar et al. (13)

[b] *q*-value estimates, calculated as described by Storey et al. (12). NA indicates that there is no known or predicted gene in this location.

* Linkage disequilibrium (r^2) is 0.33.