Published in final edited form as:

Psychiatry Res. 2011 September 30; 189(2): 321–323. doi:10.1016/j.psychres.2011.04.015.

Clinical and genetic correlates of severity in schizophrenia in India: An Ordinal Logistic Regression Approach

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Abstract

Genetic association studies of schizophrenia typically utilize diagnostic status as the trait of interest. Among Indian schizophrenia (SZ) participants, we evaluated genetic associations (selected single nucleotide polymorphisms (SNPs) associated with SZ) with selected indices of severity and symptom pattern. Ordinal logistical regression enabled us to analyze variables with multiple categories as outcome variables, while incorporating key demographic variables; this form of analysis may be useful in future genetic association studies. No significant associations were detected following corrections for multiple comparisons.

Keywords

Schizophrenia; Genetics; Severity

1. Introduction

The genetic architecture of schizophrenia (SZ) is not well understood. In addition to the diagnostic phenotype, several additional variables are amenable to genetic studies. They can be categorical or ordinal. Several statistical models for ordinal responses are available, but tend to be under-utilized (Ananth and Kleinbaum, 1997). If an ordinal outcome is analyzed using binary logistic regression, it discards information for multiple categories and forces the ordinal outcome into two levels (Scott et al., 1997). The difference between the odds ratio produced with the selected dichotomizations and the odds ratios produced using

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alternate dichotomizations is often ignored (Scott et al., 1997). The approach of choosing several dichotomous outcome models does not fully utilize the available information. In addition, if the differences between estimates are due merely to random error, then one overall estimate is preferable (Scott et al., 1997). To overcome such arbitrariness, a variety of dichotomizations could be defined and separate odds ratios estimated at each level, but this reduces the power substantially. The ordinal logistic regression (OLR) method is suitable for dependent variables that have more than two categories as outcome. It also enables inclusion of other prognostic factors and covariates (Walters et al. 2008). It has been used for a genetic association study of adoptees involving the serotonin-transporter-linked promoter region (5HTTLPR) polymorphism (Cadoret et al., 2003). Here we have explored OLR for clinical variables reflecting severity and symptom pattern among SZ patients. We analyzed polymorphisms at selected genes previously reported in association studies of SZ (supplementary table 1A). Our overall goal was to explore genetic associations with variables related to the schizophrenia phenotype. We reasoned that polymorphisms reported to be associated with SZ would have a greater likelihood of being associated with the SZ related variables. Through exploratory analyses, we aimed to generate specific hypotheses that could be tested in independent samples. The analyses were restricted to SZ patients as we did not aim to test associations with SZ *per se*.

2. Methods

The study was based at the Department of Psychiatry, PGIMER- Dr. RML Hospital, New Delhi (Deshpande et al., 2004; Bhatia et al., 2006). Participants were enrolled following written informed consent. Ethical approval was obtained from the RML Institutional Ethics Committee and the University of Pittsburgh IRB. Participants diagnosed with schizophrenia, between 18 to 65 years of age and without a history of substance abuse were recruited (Thomas et al., 2007). Genomic DNA was isolated from blood using the phenol chloroform method. Single Nucleotide Polymorphisms (SNPs) were assayed using either SnaPshot or SNPlex Technology, ABI Inc (Tobler et al., 2005). The justification for selection of the SNPs (N = 65) is presented in the Supplementary Table.

The key outcome variables included the Global Assessment of Functioning (GAF) and the DIGS items 'Pattern of Severity' and 'Pattern of Symptoms' (after consensus and reliability exercises with certified psychiatrists). The GAF score is considered as a measure of global severity of illness (Diagnostic and Statistical Manual of Mental Disorders, 1987). The data on GAF scores were categorized as 0–30, 31–60 and above 60 to enable OLR. Information about pattern of severity was obtained from DIGS based on participant's functioning and illness severity (DIGS, Question102, subcategories- Episodic Shift, Mild deterioration, Moderate deterioration, Severe deterioration, Relatively stable). The course of the subject's illness was classified into five distinct categories based on type of onset and symptoms (DIGS, Question 100, Subcategories- Continuously positive, Predominantly positive converting to predominantly negative, Mixture of positive and negative, Predominantly positive, Negative converting to positive).

A proportional odds model was used to find the clinical and genetic correlates of various outcome variables (GAF scores, severity and pattern symptoms).

Multinomial response variable Y with categorical outcomes, denoted 1, 2, 3..., k were included (xi denoting a p- dimensional vector of covariates).

$$\Pr(Y \leq y_j/x) = \frac{\exp(\alpha_j - x^T \beta)}{1 + \exp(\alpha_j - x^T \beta)}, j=1, 2, \dots, k \quad (1)$$

$$\Rightarrow \text{logit}(\Pi_j) = \log\left(\frac{\Pi_j}{1 - \Pi_j}\right) \quad (2)$$

$$\text{Log} \left[\frac{\Pr(Y \leq y_j/x)}{\Pr(Y > y_j/x)} \right] = (\alpha_j - x^T \beta), \dots, j=1, 2, \dots, k \quad (3)$$

$\Pi_j = \Pr(Y \leq y_j)$ is the cumulative probability of the event ($Y \leq y_j$).

α_j are the unknown intercept parameters, satisfying the condition $\alpha_1 \leq \alpha_2 \leq \dots \leq \alpha_k$ and $\beta = (\beta_1, \beta_2, \dots, \beta_k)^T$ is a vector of unknown regression coefficients corresponding to x {Agresti, 2002}.

In a test of parallel lines assumption, the null hypothesis states that the slope coefficients in the model are the same across response categories (lines of the same slope are parallel). Since the ordered logit model estimates one equation over all levels of the response variable, the test for proportional odds tests whether our one-equation model is valid (<http://faculty.chass.ncsu.edu/garson/PA765/ordinalreg.htm>, accessed on 22Nov09). If we fail to reject the null hypothesis, we conclude that the assumption holds. For our model, the proportional odds assumption appears to have held.

3. Results

A total of 498 participants [275 men (55.2%); 223 women (44.8%)] with SZ participated. The mean age of the participants ($\pm$ standard deviation) was 28.78 ± 8.1 years and the mean age at onset was 21.65 ± 6.03 years. Age and onset age were not significantly different among the sexes. Among the participants, 163 (33.9%) reported being married and 329 (66.1%) never married. Initially, 35 demographic and clinical variables from the DIGS and 65 SNPs were included for preliminary univariate analysis with each outcome variable. Among them, seventeen nominally significant ($p < 0.05$, uncorrected) clinical and demographic variables and nineteen SNPs were selected for the final OLR model (see Supplementary Table 1B). Following OLR, none of the SNPs analyzed were significantly associated with GAF scores. SNPs localized to *SLC18A2*, *SLC6A3*, *IL18R1* and *ARMC2* were found to be associated with pattern of symptoms. Patients with the genotypes listed below were more likely to be in the continuously positive category as defined in the DIGS: at *SLC18A2* (rs363338), patients with genotype CC (OR=0.110), compared with genotype TT, at *SLC6A3* (rs2078247), patients with genotype CG (OR=0.102) compared with genotype GG; at *IL18R1* (rs11465572), patients with GG genotype (OR=0.137) compared with GT and at *ARMC2* (rs11153135), patients with genotype AA (OR=0.223) compared with AG (Table 1).

Education was inversely associated with severity (OR=1.992, 1.828) and pattern of symptoms (OR=115.4). Among demographic and clinical variables, 'ever show inappropriate emotions' and 'return to feel like normal' were significantly associated with pattern of symptoms (OR=0.155, 6.023), severity (OR= 0.578, 5.842) and GAF scores (OR=1.725, 0.432) (table 1). Sex and grandiose delusions were associated with pattern of

symptoms (OR=15.13, 7.294) and GAF scores (OR=0.572, 0.439). Male participants had lower GAF scores i.e. worse level of functioning and were more likely to be in 'mixture of positive and negative symptoms' category than females. Participants with grandiose delusions had higher levels of global functioning and were likelier to be in the 'continuously positive' category. The participants who had 'inappropriate emotions' and not 'returned to feel like normal' were more severely ill, had lower level of global functioning and were higher in the symptom category, 'continuously positive'. Insight was associated with severity (OR= 0.297) and GAF scores (OR=3.190). Participants with good insight had better global functioning and lower severity. These associations did not remain significant following corrections for multiple comparisons.

4. Discussion

We evaluated case restricted genetic associations with selected indices of clinical severity or selected clinical patterns. We utilized two scales to estimate illness global function and severity, namely GAF scores and 'pattern of severity'. We performed two stage analyses. Initially, univariate analyses were used to evaluate these variables in relation to demographic variables and SNP genotypes. We utilized OLR as second stage analysis to evaluate nominal significant associations from the univariate analyses. These models enabled evaluation of the genetic associations in relation to clinical variables. We identified a number of suggestive associations.

Our multivariate analyses also highlighted a number of suggestive associations between indices of severity and clinical/ demographic variables. GAF scores were associated with sex and grandiose delusions. Consistent with several published reports, male patients had lower GAF scores than females, suggesting greater impairment in functioning (Thomas et al., 2010). Intriguingly, presence of grandiose delusions was associated with higher GAF scores (i.e., better function). On the other hand, lower GAF scores were associated with lack of insight, consistent with prior reports (Schwartz and Erk, 2004). The relation between insight and severity of psychopathology is more variable (Crumlish et al., 2007). We also found that insight, 'inappropriate emotions' and 'return to feel like normal' (course of illness) were significantly associated with severity. Novel associations were also noted between patterns of symptoms and several clinical variables (gender, grandiose delusions and inappropriate emotions). None of the associations remained significant following correction for multiple comparisons. Other analyses have also highlighted the complex genetic causation of SZ and highlight the need for larger samples. Other shortcomings include the restriction of GAF ratings to the month preceding the evaluation. In addition, variables related to medication, dosage and compliance were unavailable. In conclusion, OLR was used to evaluate associations between specific SNPs and indices of illness severity for SZ. SNPs at *SLC18A2* and *SLC6A3* were nominally associated with patterns of severity. Genetic associations were not noted in relation to patterns of symptoms. Other suggestive associations between the indices of severity and several clinical variables were noted and may have clinical relevance if they are replicated.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This study was funded by grants from NIH (MH01489, MH56242, MH53459, R03 TW00730, Indo-US Project Agreement no. N-443-645; Department of Biotechnology, Government of India; University Grants Commission, India, and the Central Council for Research in Yoga and Naturopathy (CCRYN), India. We thank all our participants and colleagues.

References

- Ananth CV, Kleinbaum DG. Regression models for ordinal responses: a review of methods and applications. *International Journal of Epidemiology*. 1997; 26:1323–1333. [PubMed: 9447413]
- Agresti, A. *Categorical data analysis*. 2. New York: John Wiley & Sons; 2002.
- Bhatia T, Thomas P, Semwal P, Thelma BK, Nimgaonkar VL, Deshpande SN. Differing correlates for suicide attempts among patients with schizophrenia or schizoaffective disorder in India and USA. *Schizophrenia Research*. 2006; 86:208–214. [PubMed: 16781121]
- Cadoret RJ, Langbehn D, Caspers K, Troughton EP, Yucuis R, Sandhu HK, Philibert R. Associations of the serotonin transporter promoter polymorphism with aggressivity, attention deficit, and conduct disorder in an adoptee population. *Comprehensive Psychiatry*. 2003; 44:88–101. [PubMed: 12658617]
- Crumlish N, Samalani P, Sefasi A, Kinsella A, O'Callaghan E, Chilale H. Insight, psychopathology and global functioning in schizophrenia in urban Malawi. *British Journal of Psychiatry*. 2007; 191:262–263. [PubMed: 17766770]
- Deshpande SN, Bhatia T, Wood J, Brar JS, Thelma BK, Ganguli R, Day R, Gottesman II, Nimgaonkar VL. Evaluation of familial influences on the course and severity of schizophrenia among US and Indian cases. *Social Psychiatry and Psychiatric Epidemiology*. 2004; 39:369–374. [PubMed: 15133593]
- Purcell SM, Wray NR, Stone JL, Visscher PM, O'Donovan MC, Sullivan PF, Sklar P. Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. *Nature*. 2009; 460:748–752. [PubMed: 19571811]
- Schwartz PJ, Erk SD. Regulation of central dopamine-2 receptor sensitivity by a proportional control thermostat in humans. *Psychiatry Research*. 2004; 127:19–26. [PubMed: 15261701]
- Scott SC, Goldberg MS, Mayo NE. Statistical assessment of ordinal outcomes in comparative studies. *Journal of Clinical Epidemiology*. 1997; 50:45–55. [PubMed: 9048689]
- Thomas P, Mathur P, Gottesman II, Nagpal R, Nimgaonkar VL, Deshpande SN. Correlates of hallucinations in schizophrenia: A cross-cultural evaluation. *Schizophrenia Research*. 2007; 92:41–49. [PubMed: 17350224]
- Thomas P, Wood J, Chandra A, Nimgaonkar VL, Deshpande SN. Differences among Men and Women with Schizophrenia: A Study of US and Indian Samples. *Psychiatry Investigation*. 2010; 9–16. [PubMed: 20396427]
- Tobler AR, Short S, Andersen MR, Paner TM, Briggs JC, Lambert SM, Wu PP, Wang Y, Spoonde AY, Koehler RT, Peyret N, Chen C, Broome AJ, Ridzon DA, Zhou H, Hoo BS, Hayashibara KC, Leong LN, Ma CN, Rosenblum BB, Day JP, Ziegler JS, De La Vega FM, Rhodes MD, Hennessy KM, Wenz HM. The SNPlex genotyping system: a flexible and scalable platform for SNP genotyping. *Journal of Biomolecular Techniques*. 2005; 16:398–406. [PubMed: 16522862]
- Walters SJ, Campbell MJ, Lall R. Design and analysis of trials with quality of life as an outcome: A practical guide. *Journal of Biopharmaceutical Statistics*. 2008; 11:155–176. [PubMed: 11725929]

Table 1

Ordinal logistic regression model with Global Assessment of Function (GAF) scores, pattern of severity or pattern of symptoms as the outcome variables.

GAF Score				
Variable name		Odds Ratio	Confidence Interval(OR)	p-value
Sex	Male/Female *	0.572	0.403– 0.810	0.002
Q6 Subject's insight	Yes/ No *	3.190	2.160– 4.710	< 0.001
Q8 Grandiose delusions	No/Yes *	0.439	0.290– 0.666	<0.001
Q58 Ever show emotions that did not on fit what was going	No/ Yes *	1.725	1.124– 2.449	0.002
Q66 Return to feel like normal	No/ Yes *	0.432	0.295– 0.633	< 0.001
Pattern of severity				
Education	0 to 5/6 to 10/11 to 12/Above 12 *	2.028	0.729– 5.635	0.176
		1.992	1.202– 3.297	0.007
		1.828	1.074– 3.108	0.026
Q6. Subject's insight	Yes/No *	0.297	0.191– 0.463	< 0.001
Q58 Ever Show emotions that s not fit what is going on	No/ Yes *	0.578	0.377– 0.883	0.011
Q66. Return to feel like Normal	No/Yes *	5.842	3.611– 9.440	< 0.001
Patterns of symptoms				
Sex	Male/Female *	15.13	3.142– 72.894	0.001
Education	0 to 5/6 to 10/11 to 12/Above 12 *	115.4	2.255– 5907	0.018
		0.437	0.132– 1.448	0.176
		0.409	0.121– 1.383	0.150
Q8 Grandiose delusions	No/Yes *	7.294	2.100– 25.305	0.002
Q58 Ever Show emotions that did not fit what was going on	No/ Yes *	0.155	0.042– 0.566	0.005
Q66. Return to feel like Normal	No/Yes *	6.203	1.587– 24.216	0.009
rs363338 (<i>SLC 18A2</i>)	CC / TT *	0.110	0.021– 0.568	0.008
rs2078247(<i>SLC 6A3</i>)	CG /GG *	0.102	0.017– 0.624	0.013
rs11465572(<i>IL18R1</i>)	GG/ GT *	0.137	0.027– 0.699	0.017

GAF Score				
Variable name		Odds Ratio	Confidence Interval(OR)	p-value
rs11153135(<i>ARMC2</i>)	AA / AG *	0.223	0.065– 0.761	0.017

Only variables that were significant in the initial univariate analyses were included in the multivariate analysis. Only associations significant at the $p = 0.05$ level or better are listed.

* Reference category

OR: Odds ratio.

'Q': relevant question number in the Diagnostic Interview for Genetic Studies.