Early environmental exposures influence schizophrenia expression even in the presence of strong genetic predisposition

Janice A. Husted^{a,*}, Rashid Ahmed^b, Eva W.C. Chow^{c,d}, Linda M. Brzustowicz^e, and Anne S. Bassett^{c,d}

^aSchool of Public Health and Health Systems, University of Waterloo, Waterloo, Ontario, Canada

^bDepartment of Statistics, University of Waterloo, Waterloo, Ontario, Canada

^cClinical Genetics Research Program, Centre for Addiction and Mental Health, Toronto, Ontario, Canada

^dDepartment of Psychiatry, University of Toronto, Toronto, Ontario, Canada

^eDepartment of Genetics, Rutgers University, Piscataway, NJ, United States

Abstract

There are few studies of environmental factors in familial forms of schizophrenia. We investigated whether childhood adversity or environmental factors were associated with schizophrenia in a familial sample where schizophrenia is associated with the *NOSA1P* gene. We found that a cumulative adversity index including childhood illness, family instability and cannabis use was significantly associated with narrow schizophrenia, independent of *NOSA1P* risk genotype, previously measured childhood trauma, covariates and familial clustering (adjusted odds ratio (95% confidence interval)=1.55 (1.01, 2.38)). The results provide further support that early environmental exposures influence schizophrenia expression even in the presence of strong genetic predisposition.

Keywords

Familial schizophrenia; Childhood adversity; Early trauma; Risk factor; Genetic predisposition

1. Introduction

There is considerable interest in identifying relevant environmental (non-genetic) influences for studies of gene–environment interactions in schizophrenia (Bassett et al., 2008; van Os et al., 2008). We recently showed that early trauma, defined as a threat to physical, emotional

*Corresponding author at: University of Waterloo, School of Public Health and Health Systems, 200 University Avenue West, Waterloo, Ontario, Canada, N2L 3G1. Tel.: +1 519 888 4567; fax: +1 519 746 2510. jhusted@uwaterloo.ca (J.A. Husted).

Contributors

None.

Conflict of interest

There are no actual or potential conflicts of interest to disclose.

or sexual integrity at or younger than 19 years, was significantly associated with the expression of schizophrenia in families demonstrating genetic predisposition to schizophrenia involving the nitric oxide synthase 1 [neuronal] adaptor protein (*NOS1AP*) gene (Brzustowicz et al., 2004; Wratten et al., 2009; Husted et al., 2010). The role of *NOS1AP* in mediating NMDA receptor-associated signaling is consistent with a prevailing hypothesis implicating the glutamate neurotransmitter system in the pathophysiology of schizophrenia (Brzustowicz et al., 2004). *NOS1AP* and other plausible candidate genes for schizophrenia susceptibility are members of pathways involved in neuronal connectivity including glutamatergic synapses, and particularly NMDA receptors, (Brzustowicz et al., 2004).

In the current study we investigated whether other childhood adversities, including disrupted rearing environment, physical illness or impairment and cannabis use were associated with schizophrenia in this familial sample, independent of early trauma and measured genetic risk.

2. Methods

Genotypic and detailed lifetime phenotypic and interview data were available for 165 subjects. All were adult members of a sibline with at least one affected sibling from 24 multigenerational Canadian families of Celtic (n=23) or German (n=1) descent, ascertained for study of familial schizophrenia. The ascertainment and assessment procedures of the sample have been described elsewhere (Bassett et al., 1994; Brzustowicz et al., 2000; Wratten et al., 2009). There were 72 individuals with narrow schizophrenia (DSM-III-R schizophrenia or chronic schizoaffective disorder); 90 subjects with broad schizophrenia (72 with narrow schizophrenia plus 18 with schizophrenia-spectrum disorders (i.e., non-affective psychotic disorders, or schizotypal or paranoid personality disorders)); and 75 unaffected subjects meeting neither narrow or broad criteria for schizophrenia nor criteria for any affective psychotic disorder (Brzustowicz et al., 2000). Written informed consent was obtained from all subjects. Protocols were approved by the institutional review boards of the University of Toronto, Rutgers University, University of Waterloo and the Centre for Addiction and Mental Health.

The primary data source for environmental risk factors was the in-depth Structured Clinical Interview for DSM-III-R (SCID-I) that was modified to include personal history, inquiring about commonly measured childhood adversities (Schilling et al., 2008; Fisher et al., 2010; Kessler et al., 2010; Galletly et al., 2011). Additional data sources included medical, social services and legal records, as well as SCID-I data from other family members and collateral information provided by other family members. Trained research assistants coded the presence or absence of trauma previously studied (Husted et al., 2010). In addition to early trauma, trained research assistants coded the presence or absence of seven childhood adversities experienced at or younger than 19 years: 1. disrupted rearing environment (0 = raised in one household (nuclear family, orphanage, institutions, extended family, adoptive family) and 1 = raised in 2 or more households; 2) chronic physical illness or impairment (e.g., asthma, diabetes, thyroid disease, arthritis, psoriasis); 3) parental death; 4) sibling death; 5) parental divorce or separation; 6) parental mental illness (DSM-III-R

schizophrenia, affective psychosis, mood disorder, substance use disorder, anxiety disorder and personality disorder); and 7) parental physical illness (cancer, cardiovascular disease, gastrointestinal disease (inflammatory bowel disease), musculoskeletal disorders, neurological disorder (e.g., Parkinson's disease), liver disease (hepatitis) and infectious disease (tuberculosis, encephalitis)). The use of cannabis at or younger than 19 years was also dichotomously scored (van Os et al., 2010). Although precise childhood ages were unavailable for several subjects, data were sufficient to determine whether events occurred at or before 19 years and/or before age at onset of psychosis. A cumulative environmental risk index was created by summing the number of the seven childhood adversities and early cannabis use experienced (i.e. possible scores 0 to 8).

The measured genetic risk was presence or absence of the *NOS1AP* schizophrenia risk genotype (Wratten et al., 2009; Husted et al., 2010). To reflect additional non-specific genetic influence, parental history of broadly defined schizophrenia was also considered. Other covariates included number of siblings (accounting for within-sibline variability in the number of affected and unaffected siblings), age, gender and educational level (Husted et al., 2010).

The main analyses compared subjects with narrow schizophrenia (n=72) to the unaffected group (n=75). We used chi-square test statistics to compare the two groups on prevalence of the seven childhood adversities and early cannabis use. Odds ratios (OR) and 95% CI were used to assess the association between the cumulative environmental risk score and expression of schizophrenia, adjusting for risk genotype, previously investigated early trauma (Husted et al., 2010) and other covariates. Further, to adjust for familial clustering, we used Generalized Estimating Equations (GEE) (Liang and Zeger, 1986), implemented in SAS Proc Genmod (SAS Institute Inc., 2003). Backward elimination was then used to remove any variables in the cumulative measure that were not significantly associated with expression of schizophrenia (p-value>0.10), potentially masking the relationship between environmental risk index and schizophrenia (Schilling et al., 2008). Missing data were excluded from the analysis and assumed to be missing at random (Rubin, 1976).

3. Results

Table 1 shows the sample characteristics. The mean age at onset in the narrow schizophrenia group was 24.3 years (SD=9.0). Overall, the sample was through the peak period of risk of schizophrenia, with only 18 (2.0%) unaffected subjects being younger than age 40 years and none younger than age 22 years. The prevalence of maternal or paternal history of schizophrenia was 27% and 21% respectively.

Scores on the cumulative environmental risk index ranged from 1 to 7, with a mean and median of 3.4 (SD=1.5) and 3, respectively. Parental mental illness of any sort was the most common childhood adversity (44.6%). The next most prevalent adversities were disruption in rearing environment (40.0%), and childhood chronic illness or impairment (38.0%). Twenty-five percent of the sample reported a sibling death before or at age 19 years. A similar proportion (24.5%) reported early cannabis use and 24% reported parental physical

illness before or at age 19 years. History of parental divorce or permanent separation and early parental death was reported by 11% and 9%, respectively.

The prevalence of cannabis use, disrupted rearing environment and childhood illness/impairment was higher in subjects with narrow schizophrenia compared with unaffected subjects, although only the difference in childhood illness was statistically significant ($p < 0.05$).

We found a non-significant association between scores on the cumulative environmental risk index and expression of narrow schizophrenia (OR=1.01, (95% CI) 0.81, 1.25, adjusted for familial clustering). However, the results of the backward elimination analyses indicated that parental death, sibling death and parental physical illness were unrelated to expression of schizophrenia ($p > 0.10$). We repeated the analyses excluding these variables and found that scores on the modified environmental risk index were significantly associated with expression of narrow schizophrenia, independent of risk genotype, early childhood trauma, covariates and familial clustering (adjusted OR (95% CI)=1.55 (1.01, 2.38)). Similar results were observed for broad schizophrenia and when parental history of schizophrenia was controlled, in place of the *NOS1AP* risk genotype (data not shown).

4. Discussion

All 165 subjects from the families studied experienced at least one of the seven adversities measured, with 50% reporting more than three. Approximately one in four reported early cannabis use. Scores on the cumulative environmental risk index including cannabis use, chronic childhood illness or impairment, disrupted rearing environment, parental divorce (separation) and parental mental illness of any sort were associated with expression of narrow schizophrenia, even after adjusting for *NOS1AP* risk genotype (or parental history of schizophrenia), childhood trauma, covariates and familial clustering. This is consistent with a recent study showing that higher levels of exposure to childhood adversities (including both trauma and familial or household instability) and cannabis use are associated with an increased risk of psychosis in general (Galletly et al., 2011), as well as general population studies showing significant associations between level of childhood adversity and adult psychopathology (Clark et al., 2010; Kessler et al., 2010).

Major advantages of the present study include the lifetime phenotypic data and detailed family history available in addition to the identification in these families of a risk allele of *NOS1AP*. Nevertheless, as with any retrospective study, one cannot be truly certain of the history of childhood adversities and cannabis use and thus cannot rule out the possibility that these may be more likely to be reported for affected than unaffected individuals. We attempted to minimize such recall bias by using the same comprehensive assessment, multiple sources of information, assuring confidentiality and keeping data abstractors blind to the hypothesis (Morgan and Fisher, 2007). Although age may be an important factor in studying childhood adversity, especially for shared familial factors (e.g., parental death), we were unable to assess the impact of specific age at initial exposure to measured childhood adversities or cannabis use (Fisher et al., 2010). The sample size precluded the ability to test for interactions between the measured environmental risk factors (Houston et al., 2008).

Finally, the measured environmental factors could act as a marker of specific genetic predisposition in our sample. However, we found no evidence that the *NOS1AP* risk genotype (or parental history of schizophrenia) was significantly associated with the probability of exposure to cannabis use or the measured adversities.

The results provide further support that early environmental risks may play a role in the expression of schizophrenia even in the presence of strong genetic predisposition.

Acknowledgments

This work was supported by grants from the Canadian Institutes of Health Research (MOP-53216) and by National Alliance for Research on Depression and Schizophrenia (NARSAD) Distinguished Investigator Award (A.S.B.), the National Institute of Mental Health (R01 MH62440) and NARSAD Staglin Family Music Festival Schizophrenia Research Award (L.M.B.) and NARSAD Independent Investigator Award (J.A.H.) and the Canada Research Chair in Schizophrenia Genetics (A.S.B.). The authors thank the families for their long term participation in the study, Alex Shaw, Nandita Sawh and Brad Reimann for their data abstraction and Gladys Wong and Monica Torsan for their assistance in the preparation of the manuscript.

Role of the funding source

This work was supported by grants from the Canadian Institutes of Health Research (MOP-53216) and by National Alliance for Research on Depression and Schizophrenia (NARSAD) Distinguished Investigator Award (A.S.B.), the National Institute of Mental Health (R01 MH62440) and NARSAD Staglin Family Music Festival Schizophrenia Research Award (L.M.B.) and NARSAD Independent Investigator Award (J.A.H.) and the Canada Research Chair in Schizophrenia Genetics (A.S.B.).

References

- Bassett AS, Bury A, Honer WG. Testing Liddle's three syndrome model in families with schizophrenia. *Schizophr Res.* 1994; 12:213–221. [PubMed: 8054313]
- Bassett, AS., Chow, EWC., Hodgkinson, KA. Chapter 5. Schizophrenia and psychotic disorders. In: Smoller, JW, Sheidley, BR., Tsuang, MT., editors. *Psychiatric Genetics: Applications in Clinical Practice*. American Psychiatric Publishing, Inc; Arlington, VA: 2008. p. 99-130.
- Brzustowicz LM, Hodgkinson KA, Chow EWC, Honer WG, Bassett AS. Location of a major susceptibility locus for familial schizophrenia on chromosome 1q21–q22. *Science.* 2000; 288:678–682. [PubMed: 10784452]
- Brzustowicz LM, Simone J, Mohseni P, Hayter JE, Hodgkinson KA, Chow EWC, Bassett AS. Linkage disequilibrium mapping of schizophrenia susceptibility to the *CAPON* region of chromosome 1q22. *Am J Hum Genet.* 2004; 74:1057–1063. [PubMed: 15065015]
- Clark C, Caldwell T, Power C, Stansfeld SA. Does the influence of childhood adversity on psychopathology persist across the life course? A 45-year prospective epidemiologic study. *Ann Epidemiol.* 2010; 20:385–394. [PubMed: 20382340]
- Fisher HL, Jones PB, Fearon P, Craig TK, Dazzan P, Morgan K, Hutchinson G, Doody GA, McGuffin P, Leff J, Murray RM, Morgan C. The varying impact of type, timing and frequency of exposure to childhood adversity on its association with adult psychotic disorder. *Psychol Med.* 2010; 40:1967–1978. [PubMed: 20178679]
- Galletly C, Van Hooff M, McFarlane A. Psychotic symptoms in young adults exposed to childhood trauma—a 20 year follow-up study. *Schizophr Res.* 2011; 127:76–82. [PubMed: 21256719]
- Houston JE, Murphy J, Adamson G, Stronger M, Shevlin M. Childhood sexual abuse, early cannabis use, and psychosis: testing an interaction model based on the national comorbidity survey. *Schizophr Bull.* 2008; 34:580–585. [PubMed: 18024467]
- Husted JA, Ahmed R, Chow EWC, Brzustowicz LM, Bassett AS. Childhood trauma and genetic factors in familial schizophrenia associated with the *NOS1AP* gene. *Schizophr Res.* 2010; 121:187–192. [PubMed: 20541371]

- Kessler RC, McLaughlin KA, Greif Green J, Gruber MJ, Sampson NA, Zaslavsky AM, Aguilar-Gaxiola S, Alhamzawi AO, Alonso J, Auerhahn M, Benjet C, Bromet E, Chatterji S, de Girolamo G, Demyttenaere K, Fayyad J, Florescu S, Gal G, Gureje O, Haro JM, Hu C-y, Karam EG, Kawakami N, Lee S, Lépine JP, Ormel J, Posada-Villa J, Tsang A, Üstün TB, Vassilev S, Viana MC, Williams DR. Childhood adversities and adult psychopathology in the WHO world mental health surveys. *Br J Psychiatry*. 2010; 197:378–385. [PubMed: 21037215]
- Liang KY, Zeger SL. Longitudinal data analysis using generalized linear models. *Biometrika*. 1986; 73:13–22.
- Morgan C, Fisher H. Environment factors in schizophrenia: childhood trauma—a critical review. *Schizophr Bull*. 2007; 33:3–10. [PubMed: 17105965]
- Rubin DB. Inference and missing data. *Biometrika*. 1976; 63:581–592.
- SAS Institute Inc.. The SAS System for Windows. Cary; NC: 2003.
- Schilling EA, Aseltine RH, Gore S. The impact of cumulative childhood adversity on young adult mental health: measures, models, and interpretations. *Soc Sci Med*. 2008; 66:1140–1151. [PubMed: 18177989]
- van Os J, Rutten BPF, Poulton R. Gene–environment interactions in schizophrenia: review of epidemiological findings and future directions. *Schizophr Bull*. 2008; 34:1066–1082. [PubMed: 18791076]
- van Os J, Kenis G, Rutten BPF. The environment and schizophrenia. *Nature*. 2010; 468:203–212. [PubMed: 21068828]
- Wratten N, Memoli H, Huang Y, Dulencin A, Matteson P, Cornacchia M, Azaro M, Messenger J, Hayter J, Bassett A, Buyske S, Millonig J, Vieland V, Brzustowicz L. Identification of a schizophrenia-associated functional non coding variant in NOS1AP. *Am J Psychiatry*. 2009; 166:434–441. [PubMed: 19255043]

Table 1

Characteristics of the familial sample of affected and unaffected siblings (n=165).^a

Variable	Narrowly defined schizophrenia (n=72)		Broadly defined schizophrenia ^b (n=90)		Unaffected (n=75)	
	Mean	(SD)	Mean	(SD)	Mean	(SD)
Age in years	51.6	(13.8)	51.8	(13.7)	49.6	(12.0)
Number of siblings ^c	6.8 *	(4.5)	7.1 *	(4.4)	8.5	(3.8)
	n	(%)	n	(%)	n	(%)
Male sex	41	(53.9)	51	(53.7)	32	(39.5)
Educational level high school or above	24 ***	(31.6)	32 ***	(33.7)	58	(71.6)
Genetic factors						
NOS1AP risk genotype	63 **	(82.9)	77 **	(81.1)	44	(54.3)
Paternal history of broadly defined schizophrenia	17	(22.4)	21	(22.1)	17	(20.9)
Maternal history of broadly defined schizophrenia	25	(32.9)	28	(29.5)	22	(27.2)
Trauma						
History of early trauma	15 *	(19.7)	17	(17.9)	7	(8.6)

^a Chi-square and *t*-test statistics were used to compare characteristics between narrowly defined schizophrenia and unaffected groups and broadly defined schizophrenia and unaffected groups.

^b Broadly defined schizophrenia comprised those with narrowly defined schizophrenia (n=72) and subjects with schizophrenia-spectrum disorders (non-affective psychotic disorders, schizotypal or paranoid personality disorders) (n=18).

^c The mean number of siblings varied due to within-family variability in the number of affected and unaffected subjects.

* p<0.05.

** p<0.001.

*** p<0.0001.