Association between Polymorphisms of the Human μ.-opioid Receptor gene and Schizophrenia

Eric DeBalski[1,2], Sophie Wang[1,2], Daniel Li[4], Junzhe Xu[1,2,3]

1. University at Buffalo, School of Medicine and Biomedical Sciences, Department of Psychiatry
2. VHAWNY HealthCare System, Buffalo, NY
3. Buffalo Psychiatry Center, Buffalo, NY
4. SUNY Downstate Medical Center

Abstract

Introduction:

In humans, opioidergic neurotransmission appears to modulate a variety of behaviors, including the stress response and cognitive processes, as well as anxiety and psychosis. One neurobiological process which may be modified by the polymorphism of the μ opiotd receptor is the HPA axis response to stress. Hypothalamic corticotropin-releasing hormone (CRH) neurons, which affect glucocorticoid release by stimulating pituitary adrenocorticotropin (ACTH) secretion, are directly and indirectly inhibited by β-endorphin-producing neurons via the μ opioid receptor. Both exaggerated and blunted HPA responses to stress have been associated with high risk for psychosis. Many studies have suggested that the μ-opioid receptor gene play an important role in response to stress, motivation, and numerous psychiatric entities. The present association study tested the hypothesis the μ opioid receptor confers susceptibility to schizophrenia.

Method:

After informed consent was obtained, 100 schizophrenia patients and 100 control subjects were enrolled in this study. The genotypes of the μ opioid receptor (OPRM) using 3 common SNAPs rs 1799971, rs 2075572, and rs 648893, were assessed by allele-specific polymerase - chain reaction. The PCR products were digested by restricted enzyme.

Results:

The frequency of the rs1799971 and rs2075572 of the μ opioid receptor was significantly increased in all schizophrenia patients (rs1799971 Fisher's Exact Test P= 0.0118 and rs2075572 Fisher's Exact Test P=0.044782). There were no associations of rs1799971 and rs2075572 polymorphism of the μ opioid receptor with substance dependence among schizophrenia patients and normal control.

Conclusion:

This allelic association suggests that the functional polymorphism rs1799971 and rs2075572 of the μ opioid receptor may play a role in susceptibility to schizophrenia.

Introduction

The human gene encoding the μ opioid receptor has been cloned and physically mapped to the chromosomal region 6q24-q25 (Wang et al., 1993). Mutation screening of the coding region of the μ opioid receptor revealed several DNA sequence variants (Bergen et al., 1997; Bond et al., 1998; Wendel and Hoehe, 1998). This study has brought out a finding of an association of the μ-opioid receptor gene (OPRM1gene) polymorphism with schizophrenia again by more SNPs (rs1799971, rs2075572, and rs648893). A currently published article has shown that morphine, which acts as a μ- opioid agonist, increases prepulse inhibition of startle reaction that is significantly deficient in patients with schizophrenia. P<0.00001 A group of Czech authors found that the rs1799971 polymorphism of the μ opioid receptor gene was associated with increased risk of schizophrenia in the male population. A group of Japanese authors tried to document the association between the rs1799971 polymorphism of the μ opioid receptor gene and tardive dyskinesia in schizophrenia patients. They documented that the G allele was significantly less represented in patients with tardive dyskinesia, which presented its "protective" role. A following Chinese study observed a similar trend of the G allele being less frequent in subjects with tardive dyskinesia in schizophrenia patient. The level of μ opioid receptor mRNA in schizophrenia was elevated, having important role in cognitive dysfunction. And the G allele of rs1799971 has impact on μ-opioid receptors functioning, leads to decreased expression of the receptor.

It is well known that the incidence of complicated diseases like schizophrenia depend on the interaction of multiple factors. Usually no single gene is uniquely responsible for these diseases and environmental factors also play a role in their occurrence. The methods used in individual studies, may have limited power to detect a small effect, or small interactions with other relevant polymorphisms. Limited sample size, etiological

Our hypothesis is that the μ opioid receptor polymorphism may be a plausible candidate gene that might explain individual differences in the liability to schizophrenia. The present association study was designed to test the hypothesis that μ opioid receptor gene confers genetic susceptibility to schizophrenia.

Methods

Subjects:
The study sample consisted of 100 schizophrenia or schizoaffective disorder and 100 healthy individuals. Schizophrenia patients were obtained from the inpatient psychiatric services of the VA WNY Health Care System and healthy individuals were recruited though VA normal employer and advertisements in the local media. All subjects gave informed consent to participate in the study. The protocol and consent form were approved by the institutional review boards at the VA WNY Health Care System. All subjects were administered the Diagnostic Inteview for Genetic Studies (DIGS) (National Institute of Mental Health-Molecular Genetics Initiative, 1992; Nurnberger et al., 1994) by a research assistant with extensive training in this interview. Data from the DIGS, for each subject, was compared with medical records and information from close relatives. All data was entered in the OPCRIT system. The OPCRIT facilitates a polydiagnostic approach to the diagnosis of major psychotic and mood disorders (McGuffin et al., 1991).

Table 1. Genotyping of the the μ opioid receptor gene polymorphisms

SNP	5'-3' primer sequences	Annealing T (° C)	Genotype identification method	Chi-Square Test P-Value
rs1799971	5'GTCTCGGTGCTCCTGGCTACCTCGC3'(F) 5'TTCGGACCGCATGGGTCGGACCGGT3'(R)	65	PCR-RFLP (BsiE1)	0.0118
rs2075573	5'TAAGTTAGCTCTGGTCAAGGCTAAGAAT3'(F) 5' ATCATCAGTCCATAGCACACGGTAA3' (R)	55	PCR-RFLP (HinfI)	0.044782
rs648893	5' AACAGATTAGGTCATTCTCACTTTA3' (F) 5' GCTTTAGCATAATAGTGCCAGTTCC3' (R)	50	PCR-RFLP (BsrD1)	0.674

Extraction of genomic DNA:

Blood samples were collected in anonymously identified 10-ml Vacutainer tubes (Becton Dickinson). DNA was prepared by a modified SDS/Proteinase K procedure (Gusells et al.,1979). About 10 ml were diluted with three volumes of cold Miller's RBCL (155mM NH4Cl, 10mM KHCO3, 0.1 mM Na2EDTA). Diluted blood was kept on ice for 30 min and centrifuged at 2,500 rpm for 15 min at 4ºC. The pellet was well resuspended in 30 ml of cold Miller's RBCL and centrifuged at the same speed. The pellet was well resuspended in 5 ml of SE buffer (75mM NH4Cl, 25mM Na2EDTA), 30μl of proteinase K (20mg/mL), 500μl of 20% SDS was added, and the suspension was mixed until it appeared clear and viscous, the tube was incubated overnight at 42ºC with gentle rocking. Next the overnight suspension was added to 3mL of 8 M ammonium acetate and centrifuge at 3500 rpm for 10 min. The supernatant was transferred to a new Falcon tube, and 2 volumes of ice-cold absolute ethanol were added. The DNA was picked off, washed twice in 70% ethanol, partially air dried, and slowly solubilized in 1x TE with RNase at 40C. Concentration and purity were evaluated with a spectrophotometer. A 260/280 nm ratio of 1.8 to 1.9 was usually obtained and the final concentration was adjusted to 100μg/ml.

Genotyping

The genotypes of the μ opioid receptor (OPRM) using 3 commone SNAPs rs 1799971, rs 2075572, and rs 648893, were assessed by the PCR-RFLP methods.

Results

The frequency of the rs1799971 and rs2075572 of the μ opioid receptor was significantly increased in all schizophrenia patients (rs1799971 Fisher's Exact Test P= 0.0118 and rs2075572 Fisher's Exact Test P=0.044782). There were no associations of rs1799971 and rs2075572 polymorphism of OPRM with substance dependence among schizophrenia patients and normal control.

Discussion

The opioidergic neurotransmitter system plays an important role in regulating activation of the hypothalamic-pituitary-adrenal (HPA) axis. Initial activation of the HPA axis occurs at the level of the paraventricular nucleus of the hypothalamus, where neurons that produce corticotropin releasing factor (CRF) are located [Bell et al., 1998]. CRF neurons in this area express μ-opioid receptors and are under tonic inhibition by neurons of the arcuate nucleus that contain β-endorphin [Wand et al., 1998]. Genetic factors appear to be important modulators of HPA axis activation. The HPA axis appears to be involved, including the normal stress response [Bond et al., 1998; LaForge et al., 2000] and psychosis in which HPA axis dynamics appear to be abnormal. Similarly, there is growing evidence that altered opioidergic neurotransmission and HPA axis dynamics may affect alcohol- and drug-seeking behaviors [Piazza and Le Moal, 1997; Kreek and Koob, 1998].

Various investigations have evaluated the μ opioid receptor polymorphism with regard to drug abuse vulnerability. However, after careful repeat checking with two times PCR and 2 times sequences, we found significant differences in rs1799971 and rs2075572 between schizophrenic and control groups for the μ opioid receptor in the whole sample. The frequency of the rs1799971 and rs2075572 of the μ opioid receptor was significantly increased in all schizophrenia patients (rs1799971 Fisher's Exact Test P= 0.0118 and rs2075572 Fisher's Exact Test P=0.044782). There were no associations of rs1799971 and rs2075572 polymorphism of the μ opioid receptor with substance dependence among schizophrenia patients and normal control. Although the sample size is small, we observed highly significant differences of the distribution of the rs1799971 and rs2075572 of μ opiote receptor among schizophrenia patients and normal control. Those allelic associations suggest that the functional polymorphism rs1799971 and rs2075572 of the μ opioid receptor may play a role in susceptibility to schizophrenia. Further replication studies are necessary to confirm the present tentative allelic association.

Conclusion

Our study suggests that that the functional polymorphism rs1799971 and rs2075572 of the μ opioid receptor may play a role in susceptibility to schizophrenia.

References

1.Bell SM, Reynolds JG, Thiele TE, Gan J, Figlewicz DP, Woods SC. 1998. Effects of third intracerebroventricular injections of corticotropinreleasing factor (CRF) on ethanol drinking and food intake. Psycho-pharmacology (Berl) 139:128-135.
2.Beyer, A., Koch, T., Schroder, H., Schulz, S., and Hollt, V. (2004) J. Neurochem. 89,553-560
3.Bond C, LaForge KS, Tian M, Melia D, Zhang S, Borg L, Gong J, Schluger J, Strong JA, Leal SM, Tischfield JA, Kreek MJ, Yu L. 1998. Single-nucleotide polymorphism in the human mu opioid receptor gene alters beta-endorphin binding and activity: possible impli¬cations for opiate addiction. Proc. Natl. Acad. Sci. USA 95, 9608-9613.
4.Bergen, A.W., Kokoszka, J., Peterson, R., Long, J.C., Virkkunen, M., Linnoila, M., Goldman, D., 1997. Mu opioid receptor gene variants: lack of association with alcohol dependence. Mol. Psychiat. 2, 490-494.
5.Gusella J, et al., Precise localization of human beta-globin gene coplex on chromosome 11. Proc Natl. Acad. Sci. USA. 1979; 76:5239-5242.
6.Kreek MJ, Koob GF. 1998. Drug dependence: Stress and dysregulation of brain reward pathways. Drug Alcohol Depend 51:23-47.
7.Kroslak, T., Gianotti, R. G., LaForge, K. S., Ho, A., and Kreek, M. J. (2003) International Narcotic Research Conference, Perpignon, France, July 6-11, 2003 (abstr.)
8.LaForge KS, Yuferov V, Kreek MJ. 2000. Opioid receptor and peptide gene polyorphisms: Potential implications for addictions. Eur J Pharmacol 410:249-268.
9.Piazza PV, Le Moal M. 1997. Glucocorticoids as a biological substrate of reward: Physiological and pathophysiological implications. Brain Res Brain Res Rev 25:359-372.
10.Wand GS, Mangold D, El Deiry S, McCaul ME, Hoover D. 1998. Family history of alcoholism and hypothalamic opioidergic activity. Arch Gen Psychiatr 55:1114-1119.
11.Wang, J.B., Imai, Y., Eppler, C.M., Gregor, P., Spivak, C.E., Uhl, G.R., 1993. Mu opiate receptor:cDNA cloning and expression. Proc. Natl. Acad. Sci. USA 90, 10230-10234.
12.Wendel, B., Hoehe, M.R., 1998. The human mu opioid receptor gene: 5' regulatory and intronic sequences. J. Mol. Med. 76, 525-532.

Acknowledgment: This material is based upon work supported in part by the Department of Veterans Affairs, Veterans Health Administration, Office of Research and Development.