Role of µ-Opioid Receptor Polymorphism in Patients of Rheumatoid Arthritis and their Correlation with Severity of Disease

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Abstract

Introduction: With 1 billion tobacco users worldwide, nicotine dependence has a major impact on global health. Advances in medication development for nicotine dependence require an improved understanding of the neurobiology of this complex, relapsing brain disorder.

Aims: To study the association of µ Opioid Receptor polymorphism in patients of rheumatoid arthritis and its correlation with severity of disease and prevalent alleles of the OPRM1 genes.

Material & Methods: This is a case control study wherein all available patients and volunteers were recruited. 142 controls subjects with no known history of disease and 85 study group cases were included.

Results: Comparison of genotype frequencies showed a statistically significant difference between the studied groups (p<0.004). A statistically significant difference was found when the allelic frequencies between the two groups were compared (p<0.0001), with the 17T allele having a-1.7518 fold higher risk of having RA (RR)=1.7518, 95%CI of RR=1.2988-2.3627, OR =3.2914; 95%CI =1.9608-5.5251). Significant difference was also found when the allelic frequencies between the two groups were compared (p<0.0001), with the 118G allele having a 1.5-fold higher risk of developing RA (RR)=1.5801, 95%CI =1.3091-1.9071, OR=3.1357; 95%CI 2.1083-4.6638).

Conclusion: The study definitely needs to be extended to larger cohort of patients and control samples and to a larger set of candidate µ opioid receptors. Extending the studies to a larger cohort will also allow genetic analyses of clinically defined endophenotypes observed in the patients of this chronic metabolic disease with attributes of autoimmune disorder and multiple symptoms in patients.

Introduction

With 1 billion tobacco users worldwide, nicotine dependence has a major impact on global health. Advances in medication development for nicotine dependence require an improved understanding of the neurobiology of this complex, relapsing brain disorder. Although multiple neurobiological mechanisms have been implicated, a growing body of evidence points to the endogenous opioid system, and the µ-opioid receptor (MOR) in particular, in mediating the reinforcing effects of drugs of abuse, including nicotine. Nicotine upregulates MOR mRNA and protein expression in brain regions important in drug reward in rodents and stimulates endogenous opioid release, resulting in MOR activation and dopamine release.

Genetic variation in MORs can modulate the endogenous opioid system, thereby altering behavior. A common single nucleotide polymorphism (SNP) in the µ-opioid receptor gene (OPRM1 A118G) results in an amino acid exchange at a putative glycosylation site in the extracellular terminus of the MOR.6

All of the previous association studies of the C17T polymorphism have compared cases with substance dependence to a “normal” control group. However, in many cohorts and for a variety of substances, drug use is not dichotomous but follows a spectrum ranging from nonusers through those with modest, intermittent use, to those who use a great deal of drugs almost all of the time. Additionally, quantitative measures of drug use may be more informative than dichotomous outcomes. The lifetime Kreek-McHugh-Schluger-Kellogg (KMSK) scales quantify use of alcohol, tobacco, opiates, and cocaine during the time of an individual’s maximal use. They hypothesized that there would be differences in KMSK score associated with C17T polymorphisms.

Brief Review of Literature

Pathogenesis

There is considerable ethnic heterogeneity in the frequency of these alleles and their importance for the RA phenotype. For example, double dose of DRB1*04 SE alleles is associated with erosive disease and vasculitis in northern European Caucasians but has no significant impact on disease outcome in Greek patients. Furthermore, the relative importance of DRB1 genotypes compared to other clinical and genetic markers, and hence the clinical utility of HLA-DRB1 genotyping for prognostication, remains unclear. In a recent study of
Early-onset RA by Goronzy et al.\textsuperscript{10} HLA-DRB1*04 SE double dose was one of several predictors of progression of erosive disease in the univariate analysis, but only rheumatoid factor and the presence of baseline erosions remained significant in the multivariate model, indicating that these factors are more important prognostic markers in RA.

In addition, other HLA genes with a role in T-cell regulation may modify the effect of known disease severity factors. For example, in patients carrying HLA-DRB1*01.

Recent studies with Evidence for Importance of HLA-DRB1*04 for Disease Severity in RA\textsuperscript{*} alleles, HLA-DMA1*0103 and HLA-DMB1*0401 have been suggested to be associated with greater structural joint damage.\textsuperscript{11}

The tumor necrosis factor (TNF) has been shown to be important in the pathogenesis of RA,\textsuperscript{12} and treatment with TNF-blocking agents leads to amelioration of joint symptoms and reduction of structural joint damage in many patients.\textsuperscript{13}

Although microsatellite markers do not seem to affect disease severity by themselves, interactions between the markers TNFa\textsuperscript{14} and TNFa11\textsuperscript{15} and SE genotypes have been reported to be associated with radiographic damage and disability. The TNFa6-SE interaction may also predict the development of rheumatoid nodules.\textsuperscript{16}

Such interactions could indicate that polymorphisms influencing TNF expression or function are important only in the presence of an immune system shaped by certain class II genes.

**Pharmacogenetics**

Individualized pharmacological therapy is a major goal of current research in the genetics of rheumatic diseases. This approach will ensure a safer and more efficacious application of drugs to manage complex diseases such as RA and in a nascent form is already being practiced in rheumatology. An example of this is the new routine determination of thiopurine methyltransferase (TPMT) enzyme levels before azathioprine is prescribed. This example also highlights the fact that it is currently generally easier to establish an association between genetic type and potential drug toxicity than between genetic type and drug efficacy.

Lack of response to a given therapy may be due to many reasons, including drug-specific factors and genetically associated resistance, co-administered drugs with competing metabolic pathways, and disease severity, including presence of the SE as discussed previously.\textsuperscript{17} Knowledge of the likelihood of genetic resistance would prevent unnecessary and potentially toxic drug exposure. Such factors are still unidentified, but it is clear that patients with advanced disease, regardless of their genetic background, may not respond because the disease is too advanced.

Currently, the most commonly used disease-modifying antirheumatic drug (DMARD) to manage RA is methotrexate, a folic acid analogue that inhibits the intracellular synthesis of purine and pyrimidine. Some of the anti-inflammatory effects of methotrexate are mediated by adenosine, a substrate in purine metabolism. In the cell, methotrexate undergoes polyglutamation, and this product inhibits aminomimidazole carboxamide ribonucleotide transformylase (AICAR-T). AICAR-T is important in purine synthesis, and blocking it leads to substrate accumulation with increased adenosine release, effecting the anti-inflammatory activity of methotrexate.

Another enzyme important in methotrexate metabolism is methylenetetrahydrofolate reductase (MTHFR), a catalyst for conversion of homocysteine to methionine. Deficiency of MTHFR is a cause of homocysteinemia and homocysteinuria, which can cause vasculopathy, trombophilia, and neurologic disease. About 10% of persons are homozygous, and another 40% are heterozygous for the C677T polymorphism of MTHFR, with corresponding reduction in enzyme activity.\textsuperscript{18} Methotrexate-related bone marrow toxicities has been linked to MTHFR polymorphisms.\textsuperscript{18} Regarding efficacy, it has been suggested that presence of a polymorphism in 1 of 3 enzymes important in methotrexate action thymidylate synthase (folute-dependent pyrimidine synthesis), AICAR-T, and RFCI (a protein that transports methotrexate into cells) is associated with increased methotrexate efficacy.\textsuperscript{18} Absence of members of the functional multidrug resistance protein family that transport methotrexate out of the cell, plus the presence of reduced folate carrier, appears to be associated with significantly better responsiveness to methotrexate.\textsuperscript{20}

**Material and Methods**

This is a case control study wherein all available patients and volunteers (only for blood samples) were recruited. Peripheral blood samples of patients were collected at Rheumatology clinic and Medicine Department of S.P. Medical College, Bikaner after explaining the objective of the study and taking an informed consent from the patients or from guardian family members. The same criteria for blood collection ill be followed in controls where samples from age and gender matched controls were collected.

Number of patients: Total 142 controls subjects with no known history of disease and 85 study group cases were recruited as per the following inclusion and exclusion criteria.

The 2010 American College of Rheumatology (ACR) and the European League Against Rheumatism (EULAR) revised the 1987 ACR classification criteria for RA the following were the criteria for the classification of rheumatoid arthritis:

**Health Criteria**

Not applicable in the present study. Only OPD/IPD patients will be recruited and lactating mothers were not recruited.

**Procedure for conducting the study**

The volunteers and the patients were explained about the purpose of the research study by the resident (SR/JR) who will subsequently obtain a written consent from the recruited patients/ control subjects. A detailed proforma was used to gather clinical history of the patients and information about the family history of the patient.

As explained above a proforma was used to gather clinical history of the patient/volunteer. Venous blood samples were drawn by skilled technical staff/nurse/doctor on the project, before the discharge of the patient or from the volunteer using sterile disposable syringes and was immediately transferred to pre-labeled blood collecting vials containing 0.5 M EDTA as anticoagulant and transported in ice from place of collection to lab.

The collected samples in the lab were centrifuged for 8 min. at 2000 RPM and...
serum was separated from sample in a plain vial and both were stored at -20°C till transport to genetic lab.

**Clinical Chemistry**

Clinical data and laboratory investigations including haemogram, blood sugar, serum electrolytes, blood urea, AST/ALT ratio, serum ALP, serum calcium, serum creatinine, total proteins were recorded from the patients sheet or were done in the plasma as per the kit manufacturers instructions wherever required.

**DNA Isolation**

DNA was isolated using standard protocol. DNA was quantified using UV spectroscopy and qualified on 0.8% agarose.

**PCR Standardization**

This DNA was used for allele specific PCR amplification of the selected genes on a Thermal Cycler using known primes. Amplified sequences of selected genes were analyzed for specific allele type present using RFLP, LP, SSCP, or DNA sequencing methods.

**SNP analysis by RFLP/SSLP**

RFLP’s (restriction fragment length polymorphisms) were used for analysis of PCR product obtained from the amplification of target sequence. Variations were characterized by polyacrylamide gel electrophoresis. DNA sequencing, was done using commercial services available.

**DNA Analysis**

Genomic DNA was extracted from venous blood, drawn from subjects, by the NaCl-salting out procedure (Miller et al. 1988) and dissolved in water. PCR and subsequent restriction digestion with appropriate restriction enzymes was carried out using a standard protocol to genotype the two polymorphic sites.

**Results**

Comparison of genotype frequencies showed a statistically significant difference between the studied groups (p<0.004). A statistically significant difference was also found when the allelic frequencies between the two groups were compared (p<0.0001), with the 17T allele having a -1.7518 fold higher risk of having RA (risk ratio (RR)=1.5801, 95%CI of RR=1.3091 to 1.9071, odds ratio (OR)=3.1357; 95%CI of OR=2.1083 to 4.6638).

A statistically significant difference was also found when the allelic frequencies between the two groups were compared (p<0.0001), with the 118G allele having a 1.5-fold higher risk of developing RA (risk ratio (RR)=1.5801, 95%CI of RR=1.3091 to 1.9071, odds ratio (OR)=3.1357; 95%CI of OR=2.1083 to 4.6638).

A statistically significant association in the frequency of the T allele between cases and control subjects, giving an odds ratio of = 3.2914, (CI 95%, 1.9608 to 5.5251) in the RA group. Similar association in the frequency of the G allele between cases and control subjects, giving an odds ratio of 3.1357, (CI 95%, 2.1083 to 4.6638) in the RA group. Both the polymorphic site (C17T and A118G) of the OPRM1 gene exhibited good fit to Hardy–Weinberg equilibrium in control populations.

To test this hypothesis required a cohort with two characteristics: a broad spectrum of substance use and a relatively high frequency of the T allele. Over 50% of the participants are of African descent and drug use was prevalent.

**Conclusion**

A statistically significant difference was also found when the allelic frequencies between the two groups were compared (p<0.0001), with the 17T allele having a -1.7518 fold higher risk of having RA (risk ratio (RR)= 1.7518, 95%CI of RR = 1.2988 to 2.3627, odds ratio (OR)=3.2914; 95%CI of OR=1.9608 to 5.5251). A statistically significant difference was also found when the allelic frequencies between the two groups were compared (p<0.0001), with the 118G allele having a 1.5-fold higher risk of developing RA (risk ratio (RR)=1.5801, 95% CI of RR=1.3091 to 1.9071, odds ratio (OR)=3.1357; 95% CI of OR=2.1083 to 4.6638). The allelic frequencies of C17T for C and T in two different groups were 90% and 10%, respectively in controls, and, 74% and 26%, respectively.

Allelic frequencies of A118G for A and G in the groups were 82% and 18%, respectively, in control subjects, and 59% and 41%, respectively, in the control population with G allele being more frequent in both the case populations. The highly significant association in the frequency of the T allele between cases and control subjects, giving an odds ratio of = 3.2914, (CI 95%, 1.9608 to 5.5251) in the RA group. Similar association in the frequency of the G allele between cases and control subjects, giving an odds ratio of 3.1357, (CI 95%, 2.1083 to 4.6638) in the RA group. Both the polymorphic site (C17T and A118G) of the OPRM1 gene exhibited good fit to Hardy–Weinberg equilibrium in control populations.
populations

We concluded that the study definitely needs to be extended to larger cohort of patients and control samples and to a larger set of candidate μ opioid receptors. Extending the studies to a larger cohort will also allow genetic analyses of clinically defined endophenotypes observed in the patients of this chronic metabolic disease with attributes of autoimmune disorder and multiple symptoms in patients. Genetic studies can also impact strategies adopted for effective personalized treatment for this progressively debilitating disease.

References