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Original research article

Lack of association between two genetic polymorphisms of *SOD2* (rs2758339 and rs5746136) and the risk of opium dependency

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ABSTRACT

Introduction: Superoxide dismutase-2 (EC 1.15.1.1; SOD2, OMIM: 147460) is a tetrameric enzyme which contains manganese in its active site. It is an important enzyme involved in the cellular detoxification by converting highly toxic superoxide radicals into less reactive molecules, hydrogen peroxide and oxygen. Several single nucleotide polymorphisms have been well defined in the gene encoding *SOD2*, including the potentially functional polymorphisms of rs2758339 and rs5746136.

Aim: The aim of the present study is to investigate the associations between the rs2758339 (A/C substitution) and rs5746136 (A/G substitution) polymorphisms and the risk of dependency to opium.

Material and methods: The present case–control study was performed in Shiraz (southern Iran) on 143 opium dependent and 569 healthy controls. The genotypes of the rs2758339 and rs5746136 polymorphisms were determined by polymerase chain reaction.

Results and discussion: Statistical analysis showed that there was no significant association between the study polymorphisms and the risk of opium dependency. A significant linkage disequilibrium was observed between the study *SOD2* polymorphisms. Statistical analysis showed that there was no significant association between the haplotypes of the polymorphisms and the risk of opium dependency.

Conclusions: The present data revealed that the rs2758339 and rs5746136 polymorphisms of *SOD2* are not risk factors for dependency to opium.

1. Introduction

Superoxide dismutase-2 (EC 1.15.1.1; SOD2, OMIM: 147460) is a tetrameric enzyme which contains manganese in its active site.[1,2] SOD2 metabolizes superoxide radicals into molecular oxygen and hydrogen peroxide (H_2O_2)[3]; subsequently H_2O_2 is converted into water by other enzymes such as catalase. SOD2 is expressed in most tissues, including in human brain.[4] In human, several single genetic polymorphisms have been

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reported in the *SOD2* (https://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?geneId=6648), including the rs2758339 (A/C substitution)[5] and rs5746136 (A/G substitution) polymorphisms.[4] These polymorphisms are located in the vicinity of SP1 and NF-κB transcription element sequences[4] and glucocorticoid receptor binding site.[5] Association between these *SOD2* polymorphisms and the risks of several multifactorial complex traits has been studied by researchers.[5–12]

It has been well demonstrated that opiates may cause oxidative stress in drug dependent persons.[13–15] It has been reported that morphine decreases enzyme activities of the superoxide dismutase and catalase.[16–18] On the other hand the mRNA levels of the *SOD2* decreased when SH-SH5Y cells were treated by methadone and morphine for a long period.[19,20] These reports indicated that drug-abused persons experience the oxidative stress.

The association between some polymorphisms of the genes involved in cellular detoxification (such as members of glutathione S-transferases superfamily, catalase) and susceptibility to methamphetamine and heroin abuse has been reported.[21–26] However, there are no study investigating the association between the *SOD2* polymorphisms and risk of opium dependency. These facts sufficiently provide us with a theoretical ration to carry out the present case–control study.

2. Aim

This study was conducted to determine the association of the rs2758339 (A/C substitution) and rs5746136 (A/G substitution) genetic polymorphism of *SOD2* with the risk of opium dependency in Iranian population.

3. Material and methods

3.1. Ethical approval

This study was approved by the Shiraz University ethics committee. Informed consent was obtained from each subject before the study. This work was carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for Ethical Principles for Medical Research Involving Human Subjects.

3.2. Subjects

A detailed description of the subjects has been reported in our previous article.[24] In brief, this case–control study was conducted in Shiraz (southern Iran) on 143 (12 females, 131 males) opium dependent persons and 570 (55 females, 515 males) healthy controls. The patients were in methadone maintenance for treating their dependency and all of them reported opium as their primary drug of choice. Control individuals were blood donors, who declared that they did not suffer from substance abuse. The mean age (SD) of the patients and the controls were 39.6 (11.3) and 40.8 (10.7) years, respectively. There was no significant difference between the two study groups for their gender and age distribution ($P > 0.05$). Iranian populations showed high level of heterogeneity,[27–29] therefore, we selected the participants from Persian Muslims (Caucasians) living in Shiraz (Fars province, southern Iran).

3.3. Genotyping

Genomic DNA was isolated from EDTA treated blood samples. The polymerase chain reaction (PCR) conditions for determining the genotypes of the polymorphisms and quality control were the same as that reported previously.[3,4] It should be noted that we failed to successfully determine the polymorphisms in two participants of control group, explaining the variation in the total number of samples listed in Table 1.

3.4. Statistical analysis

Goodness-of-fit χ^2 test was used to verify whether the observed genotypic frequencies were in accordance with the Hardy–Weinberg equilibrium. The associations between the genotypes and the risk of opium dependency were assessed by odds ratios (ORs) and 95% confidence intervals (CIs). The software SNPAlyze(TM) ver. 6 Standard (Dynacom Co, Ltd. Kanagawa, Japan) was used to evaluate the status of pair wise linkage disequilibrium for the studied polymorphisms. Statistical analysis was performed using SPSS software (Chicago, IL, USA; version 11.5). A probability of $P < 0.05$ was considered statistically significant.

4. Results

The genotypic frequency of the study polymorphisms in the opium dependent cases and healthy controls is shown in Table 1. The genotypic frequencies of the study polymorphisms in healthy controls (for rs2758339 polymorphism: $\chi^2 = 0.32$, df = 1, $P = 0.566$; for rs5746136 polymorphism: $\chi^2 = 0.01$, df = 1, $P = 0.928$) were consistent with the Hardy–Weinberg equilibrium distribution. Statistical analysis showed that there was no significant association between the study polymorphisms and the risk of opium dependency (Table 1). There was no liner trend for the numbers of putative high risk alleles of the polymorphisms and the risk of opium dependency (for rs2758339 polymorphism: $\chi^2 = 0.33$, $P = 0.565$; for rs5746136 polymorphism: $\chi^2 = 0.43$, $P = 0.509$).

A significant linkage disequilibrium was observed between the study polymorphisms (for control group: $D' = -0.9375$, $r^2 = 0.3994$, $\chi^2 = 455.2$, $P < 0.001$; for opium dependent group: $D' = -1.0$, $r^2 = 0.4486$, $\chi^2 = 128.2$, $P < 0.001$). Table 2 shows the haplotypic frequencies of the study polymorphisms in opium dependent patient and control groups. The CG haplotype was used as a reference. Statistical analysis showed that there was no significant association between the haplotypes of rs2758339 and rs5746136 polymorphisms and the risk of opium dependency (Table 2).

5. Discussion

Based on the findings of several lines of evidences, including serum markers of inflammation[13] and frequency of micronuclei in drug abused persons,[15] and also alterations of

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