Original Article
Correlation of FTO gene polymorphisms with osteoporosis risk

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Abstract: Objective: Present study was aimed to explore the correlation between single nucleotide polymorphisms (SNPs) of fat mass and obesity-associated (FTO) gene (rs7206790 and rs9939609) and osteoporosis risk. Methods: 108 osteoporosis patients and 93 healthy individuals were enrolled in this case-control study. Cases and controls were in accordance with each other in age and gender. FTO gene rs7206790 and rs9939609 polymorphisms were genotyped via polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) approach. Differences of genotype and allele frequencies of the two SNPs between case and control groups were analyzed by chi-square test. Odds ratios (ORs) and 95% confidence intervals (CIs) were indicated relative risk of osteoporosis. Results: CC genotype and C allele frequencies of rs7206790 SNP were significantly different between case and control groups. These differences indicated that CC genotype and C allele of rs7206790 might increase the susceptibility of osteoporosis (P=0.025, OR=3.238, 95% CI=1.112-9.427; P=0.002, OR=2.222, 95% CI=1.340-3.684). However, genotypes and alleles of rs9939609 polymorphism had no significant association with the risk of osteoporosis (P>0.05). Conclusion: FTO gene variant allele of rs7206790 polymorphism might act as a susceptible factor for osteoporosis occurrence. FTO gene rs9939609 SNP was not related to the incidence of osteoporosis.

Keywords: FTO, osteoporosis, polymorphism, PCR-RFLP

Introduction

Osteoporosis is a kind of systemic bone disease. The characteristics of osteoporosis are osteopenia, abnormal microstructure of bone tissues and increased fragility of bone. Clinical performances are as follows: reduced bone mineral density (BMD), thinning bone cortical, widened pulp chamber, bone trabecula diminution and easy to fracture. Osteoporosis is the most common cause for broken bone among old people. Broken bones usually include vertebral column, bones of the forearm and hip [1]. According to the different pathogenesis, osteoporosis could be segmented into three types: primary osteoporosis, secondary osteoporosis and idiopathic osteoporosis. Among them, primary osteoporosis approximately accounts for 90%, which usually occurs in postmenopausal women and elderly male [2, 3]. It can be seen that osteoporosis, with high incidence, is a common senile disease in postmenopause women [2, 4]. Mortality which is caused by hip joint and spinal fractures is widely reported [5]. With the constant improvement of the medical level, the aging of the population is increased. Then the morbidity of osteoporosis has an uptrend. It has been a significant disease, which threaten human health and lead to high medical cost, worldwide [6]. Exploration of osteoporosis pathogenesis will provide a theoretical basis for improving treatment methods. It will be a great influence for improving human health conditions.

It is reported that the occurrence of osteoporosis is affected by many factors [7-10]. Previous studies showed that obesity was related to BMD which is an important index of osteoporosis [11-13]. But not all of the obesity people had
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There exists individual susceptibility which is determined by genetic factors. Previous studies have been reported that fat mass and obesity-associated gene (FTO) is related to the occurrence of obesity [14-16] and body mass index (BMI) in children and adults [17-21]. Other researches also demonstrate that this gene is associated with BMD [22, 23]. So it is speculated that FTO gene is associated with the incidence of osteoporosis. However, there merely no study investigates the correlation of FTO gene polymorphisms and the risk of osteoporosis [24].

In present study, we select two single nucleotide polymorphisms (SNPs) of FTO gene (rs7206790 and rs9939609) to explore the correlation with osteoporosis risk in Chinese Han population.

Materials and methods

Sample features

All the objects of this study were Chinese Han population in Institute of Basic Medicine, Shandong Academy of Medical Sciences, and there were no genetic connection among them. Participants were signed informed consents. This research was reviewed and consented by Ethics committee of Institute of Basic Medicine, Shandong Academy of Medical Sciences Sample collection was based on the ethics criteria of national human genome research.

Osteoporosis patients (n=108) were diagnosed in Institute of Basic Medicine, Shandong Academy of Medical Sciences from January 2013 to January 2015, including 35 females and 73 males with the median age was 55 years old (range 20-78). Patients did not receive any therapy before sample collection. 93 healthy controls (30 females and 63 males) were recruited from healthy check-up center of the same hospital during the same times. Median age of the controls was 58 years old, ranging from 19 to 80. Controls were according to the cases in age and gender.

Sample collection

5 ml peripheral venous blood were collected, and then anticoagulated by EDTA, finally stored in -20°C until to use. Genomic DNA was extracted by Biospin Whole Blood Genomic DNA Extraction Kit (Bioer technology CO., LTD, China).

Genotyping method

Primer sequences of FTO gene polymorphisms (Table 1) were designed by Primer Premier 5.0, and synthesized by Sangon Biotech (Shanghai, China).

PCR amplification adopted a 25 µl system, including 2 µl template DNA, 0.5 µl upstream primer, 0.5 µl downstream primer, 2 µl dNTP, 0.3 µl Taq DNA polymerase, 5 µl 10×Buffer and 14.7 µl sterile water. PCR procedure was as follows: 94°C initial denaturation for 3 min, followed by 35 cycles of 94°C degeneration for 30 s, 58°C annealing for 30 s and 72°C extension for 60 s; 72°C final extension for 10 min. PCR products were digested by restriction endonucleases, and then detected by 2% agarose gel electrophoresis.

Statistical analysis

Statistical analysis was accomplished through PASW 18.0 statistical software. Hardy-Weinberg equilibrium (HWE) was used to test the representativeness of cases and controls. Genotype and allele frequencies of rs7206790 and rs9939609 polymorphisms were calculated by direct counting. Differences of the polymorphisms distributions between case and control groups were assessed by χ² test. Association of FTO gene polymorphisms with the risk of osteoporosis was evaluated using odds radios (ORs) and 95% confidence intervals (CIs). The differences had statistical significance when P<0.05.

Table 1. Primer sequences of FTO gene rs7206790 and rs9939609 polymorphisms

<table>
<thead>
<tr>
<th>SNP</th>
<th>Primer sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Upstream</td>
</tr>
<tr>
<td>Rs7206790</td>
<td>5'-AGACGGAGTATGCCACCATT-3'</td>
</tr>
<tr>
<td>Downstream</td>
<td>5'-CACTGTGGCATTGAGACAT-3'</td>
</tr>
<tr>
<td>Rs9939609</td>
<td>5'-GGACAGCTCAGGTAAGCAG-3'</td>
</tr>
<tr>
<td>Downstream</td>
<td>5'-AGGTTCTCAAGAACTCTGTT-3'</td>
</tr>
</tbody>
</table>
Results

HWE examination

Allele frequencies distributions of the two FTO gene polymorphisms in control group were not diverged from HWE, testifying the representativeness of controls.

Distributions of FTO gene polymorphisms

Genotype and allele distributions of FTO gene rs7206790 and rs9939609 SNPs were displayed in Table 2. CC genotype and C allele of rs7206790 polymorphism were observed high frequencies in cases compared with controls. These differences were significantly, indicating a positive relationship with the occurrence of osteoporosis (P=0.025, OR=3.238, 95% CI=1.112-9.427; P=0.002, OR=2.222, 95% CI=1.340-3.684). Although the variant allele carriers of rs9939609 were more frequently discovered in case group than that in control group, but the difference was not significant (P>0.05). This result suggested that rs9939609 polymorphism had no obvious association with the incidence of osteoporosis.

Discussion

Basic pathogenesis of osteoporosis is that the bone absorption speed is greater than bone formation speed which will lead to the loss of bone mass. Bone metabolism of normal adults is in the state of dynamic equilibrium, which maintains osteoblast to synthesize new bones and osteoclast to absorb worn bones. Once the steady state is broken, it may lead to insufficient bone formation or too much bone absorption which make osteopenia and finally form osteoporosis. Osseous formation and absorption are a complex process involving numerous gene and growth factors. Any factors resulting in decrease of osseous formation and increase of osseous absorption may induce osteoporosis.

Changes of BMD, mainly refers to the loss of bone mass, is the leading cause of osteoporosis. So, any factor which changed the BMD could influence the development of osteoporosis. Because BMD is affected by various factors, it was considered that osteoporosis is a complex disease. The development and progression of it is regulated and controlled by various genetic and environmental factors, as well as their interactions. Among these factors, the occurrence of osteoporosis is mainly affected by genetic factors [25, 26]. Genetic factor plays a great role in the occurrence of morbidity. Numerous studies about correlation between osteoporosis and gene draw a conclusion that a tiny variation of gene may induce significant difference of bone mineral density and osteoporosis risk [27]. SNP is the most common tiny variation of gene.

Zhao et al. enunciated that fat mass had a negative association with bone mass, and BMI was positively associated with bone mass [28]. Other studies indicate that FTO gene was relate to BMI [29, 30], and a meta-analysis demonstrated that A allele of rs9939609 polymorphism positively related to BMI [17]. As an obesity-associated gene, FTO gene has been reported correlated with osteoporosis [24]. However, the role of FTO gene act in the occurrence of osteoporosis was still unknown. SNP could lead to diverse genetic expression levels and changes of protein function. Recent years, along with the development of molecular biology, SNP play a critical role in the exploration of disease etiology. It becomes the third generation of biomarker. In present study, we selected rs7206790 and rs9939609 two SNPs of FTO

Table 2. Genotype and allele distributions of FTO gene rs7206790 and rs9939609 polymorphisms in case and control groups

<table>
<thead>
<tr>
<th>Genotype/Allele</th>
<th>Case n=108 (%)</th>
<th>Control n=93 (%)</th>
<th>χ²</th>
<th>P</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs7206790 GG</td>
<td>63 (58.33)</td>
<td>68 (70.83)</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>GC</td>
<td>30 (27.78)</td>
<td>23 (23.96)</td>
<td>1.094</td>
<td>0.296</td>
<td>1.408 (0.741-2.676)</td>
</tr>
<tr>
<td>CC</td>
<td>15 (13.89)</td>
<td>5 (5.21)</td>
<td>5.031</td>
<td>0.025</td>
<td>3.238 (1.112-9.427)</td>
</tr>
<tr>
<td>rs9939609 GG</td>
<td>156 (72.22)</td>
<td>159 (82.81)</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>TT</td>
<td>73 (70.87)</td>
<td>70 (75.27)</td>
<td>1.094</td>
<td>0.296</td>
<td>1.408 (0.741-2.676)</td>
</tr>
<tr>
<td>AT</td>
<td>26 (25.24)</td>
<td>21 (22.58)</td>
<td>0.258</td>
<td>0.611</td>
<td>1.187 (0.612-2.301)</td>
</tr>
<tr>
<td>AA</td>
<td>4 (3.88)</td>
<td>2 (2.15)</td>
<td>0.562</td>
<td>0.453</td>
<td>1.918 (0.340-10.804)</td>
</tr>
<tr>
<td>rs7206790 C</td>
<td>60 (27.78)</td>
<td>27 (17.19)</td>
<td>9.855</td>
<td>0.002</td>
<td>2.222 (1.340-3.684)</td>
</tr>
<tr>
<td>rs9939609 TT</td>
<td>172 (83.50)</td>
<td>161 (86.56)</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>AA</td>
<td>34 (16.50)</td>
<td>25 (13.44)</td>
<td>0.718</td>
<td>0.397</td>
<td>1.273 (0.728-2.227)</td>
</tr>
</tbody>
</table>

gene to detect the association of FTO gene and the incidence of osteoporosis. To our knowledge, this is the first study to explore the role of rs7206790 and rs9939609 polymorphisms of FTO gene in osteoporosis.

Results of this study displayed that allele distribution of the two SNPs were in accordance with HWE in controls, suggesting our study had a well goodness of fit. Frequencies of CC genotype of rs7206790 were obviously higher in case group than that in control group. CC genotype showed a 3.238 times increased risk for the development of osteoporosis. Although GC genotype of rs7206790 frequently observed in cases, it had no significant association with the risk of this disease. In addition, C allele of rs7206790 might have a positive correlation with osteoporosis susceptibility with the OR of 2.222. However, the distributions of an allele, AT and AA genotype of rs9939609 polymorphism were not significantly different between case and control groups. This result indicated that rs9939609 polymorphism was not significantly associated with the susceptibility of osteoporosis.

Based on the above result, we suggested that FTO gene might contribute to the pathogenesis of osteoporosis. But it was inadequate to certify the etiology of osteoporosis, because the small sample size and the unadjusted results. SNP distribution was distinct among different region and ethnicity. Besides, osteoporosis is a complex disease; the result should be adjusted by confounding factors. For these reasons, well designed studies were essential to understand the pathogenesis of osteoporosis.

Disclosure of conflict of interest

None.

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