Association Between ADAMTS-4 Gene Polymorphism and Lumbar Disc Degeneration in Chinese Han Population

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ABSTRACT: Low back pain (LBP) is a common health problem and many LBP are caused by lumbar disc degeneration (LDD). ADAMTS-4 (a disintegrin and metalloprotease with thrombospondin motifs-4), also known as aggrecanse-1, plays a core role in degeneration of extracellular matrix in LDD. To investigate the association between ADAMTS-4 genetic polymorphism and LDD, we genotyped SNPs in and around ADAMTS-4. We recruited 482 sporadic cases of LDD and 496 healthy controls from Chinese Han population. Five SNPs were selected and phenotyped by the Sequenom MassARRAY system. Allelic, genotypic, and haplotypic association was performed. Rs4233367 (c.1877 C>T), which located in exon of ADAMTS-4 showed significant association with LDD. The T allele conferred a lower risk of LDD with an OR of 0.69 and TT genotype is at nearly one-fifth of the risk compared to CC genotype. Other tested SNPs didn’t show significant difference between the case and control groups. The SNP rs4233367 in the exon of ADAMTS-4 gene may be associated with lumbar disc degeneration. © 2015 Orthopaedic Research Society. Published by Wiley Periodicals, Inc. J Orthop Res 34:860–864, 2016.

Keywords: lumbar disc degeneration (LDD); a disintegrin and metalloprotease with thrombospondin motifs-4 (ADAMTS-4); single nucleotide polymorphism (SNP)

Low back pain (LBP) is defined as pain radiating from the back into the dermatome of the affected nerve trunk. It is a common musculoskeletal disorder characterized by symptomatic lumbar disc herniation with or without sciatica.1 As a global health problem, it was reported that 80% of population will suffer from LBP for at least one time during their lifetime.2 LBP is one of the leading causes of disability worldwide3 including China.4 According to a Finnish study, 40% of which were caused by lumbar intervertebral disc degeneration (LDD).5

Genetic predisposition has been widely acknowledged in LDD.6–9 Several genes such as COL1A1,10 ACAN,11 MMPs,12 COL9A2,13 and ADAMTS-514 have been reported to be associated with LDD. Environmental factors such as smoking15 and labor work16,17 are also contribute to LDD. In this study we focus on the genetic part and reduce the interference of environmental one, so we exclude the patients who have history of labor work or heavy smoking.

The major components of intervertebral disc (IVD), collagens, and proteoglycans (PG) are crucial to normal IVD function.18 It was shown that the aggrecanases might contribute to the changes occurring in the extracellular matrix during intervertebral disc degeneration.19 Two different aggrecanases, aggrecanase-1 (ADAMTS-4) and aggrecanase-2 (ADAMTS-5) have been isolated. These two proteinases can cleave aggrecan core protein at the aggrecanase-specific Glu373-Ala374 bond20 and play an important role in the process of disc degeneration.19,21 Tortorella et al. described that general metalloproteinase inhibitors could inhibit the cleavage of aggrecan by ADAMTS-4.22 Furthermore, levels of mRNA and protein of ADAMTS-4 were significantly increased in degenerated disc tissue compared with non-degenerated tissue.23 Although the role of ADAMTS-4 in LDD has been verified in several functional analysis, to the best of our knowledge, there is no investigation found any association between ADAMTS-4 genetic polymorphisms and LDD. Frances et al. performed genome-wide association study (GWAS) in 4600 European LDD patients and found PARK2 gene instead of ADAMTS-4.24

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MATERIALS AND METHODS

Level of Evidence: III

Subjects
A total of 482 unrelated sporadic cases with LDD (171 males and 311 females, aged 42.6 ± 14.2 years) and 496 healthy controls (208 males and 288 females, aged 41.4 ± 11.1 years) from Chinese Han population were recruited with no statistical difference of age and gender between the two groups. All the patients were diagnosed through lumbar disc MRI scanning (TR = 4000 ms, TE = 101.7 ms, section thickness = 4 mm, section gap = 1 mm, acquisition matrix = 384 × 256, Fig. S1) by at least two experienced radiologists following methods described by Cheung et al.25 Primary exclusion criteria included spinal and joint diseases such as trauma, spinal tumor, inflammation, scoliosis, and osteoarthritis. Individuals with known environmental risk factors, including heavy manual labor, occupational driving, or heavy smoking, were also excluded. Written informed consents were obtained from all participants. The Ethics Committee of Peking Union Medical College Hospital, Peking Union Medical College, and Chinese Academy of Medical Sciences approved this study.

Genotyping
Five tag SNPs ranging from 5 kb upstream and 5 kb downstream of ADAMTS-4 were selected from the NCBI SNP database (www.ncbi.nlm.nih.gov/SNP) and the HapMap database (www.hapmap.org). The SNPs included were as follows: rs4656291, rs11585858, rs41270041, rs4233367, rs10908826 (Table S1). Among them, rs4656291 and rs11585858 were in the 3’UTR region, rs10908826 in the 5’UTR region, and others are in the exons. All the SNPs selected had a minor allele frequency (MAF) of more than 0.05. Genomic DNA was extracted from peripheral blood leukocytes using a standard phenol-chloroform method. The SNP genotyping was performed on the Sequenom MassARRAY SNP genotyping platform (Sequenom, San Diego, CA). Five percentage duplicates of randomly chosen samples were used for quality controls.

Data Analysis

Association analysis. Hardy–Weinberg equilibrium (HWE), haplotype blocks, and the linkage disequilibrium (LD) patterns were estimated by using the Haploview program (version 4.2, Broad Institute of MIT, and Harvard, Cambridge, MA).26 Primary analyses were performed for allelic, genotypic, and haplotypic association based on the UNPHASED software (v.3.1.5 Dudbridge F, MRC Biostatistics Unit, Cambridge, UK). In our study, the sliding window size for haplotype analysis was two and three. QUANTO software (http://hydra.usc.edu/gxe) was used to calculate the power value, in which the prevalence of LDD was set at 32%, the false positive rate was 0.05 with a minor allele frequency of 0.10.

RESULTS

Hardy–Weinberg Equilibrium
We genotyped five SNPs around and in ADAMTS-4 in 482 patients and 496 healthy controls. Four SNPs did not deviated from Hardy–Weinberg equilibrium while rs4656291 did (Supplementary Table S2). As a result, this polymorphism site was not discussed in the following analysis. Power calculation showed there was a power of 81.93% to detect allelic association at a false positive rate of 0.05.

Allelic Analysis
Among the remaining four SNPs, rs4233367 showed significant allelic difference between case and control group (Table 1). The protective allele was T and the OR was 0.69 (95% CI 0.50–0.94, p = 1.66 × 10−2). Other SNPs didn’t show any difference with statistical significance between the two groups.

Genotypic Analysis
The genotypic analysis showed significant association between rs4233367 and LDD (Table 2). The TT genotype had an obvious protective effect with an OR of 0.21 (95% CI 4.61 × 10−2–0.99, p = 3.74 × 10−2), compared with CC. However, the CT genotype didn’t show any risks or protective effects (OR = 0.76, 95% CI 0.74–16.95, p = 0.14). Taken together, the global p value for rs4233367 genotypic analysis is 7.8 × 10−2. Consistent with allelic analysis, other three SNPs did not show any difference among each group.

Haplotypic Analysis
Haplotypes were constructed on the basis of the genotype data from four SNPs using Haploview software (version 4.2). Pairwise linkage disequilibrium D’ value between SNPs and the reconstructed LD plots of the four SNPs were shown in Figure 1. One LD block was observed according to confidence intervals method26 (D’ > 0.9 and r² > 0.8) and this block contained rs11585858 and rs41270041. The 2-SNP haplotypic analysis showed significant association for rs4233367-rs10908826 (χ² = 26.54, df = 3, p-value = 7.36 × 10−6) (Table 3).

DISCUSSION
To our knowledge, this is the first study indicating the association between genetic polymorphisms of ADAMTS-4 and LDD. In this study, one of the SNPs,

<p>| Table 1. Allelic Association of Tested SNPs in ADAMTS-4 |
|-----------------|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>SNP</th>
<th>Allele (1/2)</th>
<th>Case 1</th>
<th>Case 2</th>
<th>Control 1</th>
<th>Control 2</th>
<th>OR Allele 1 = 1</th>
<th>95% CI</th>
<th>χ²</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs11585858</td>
<td>A/C</td>
<td>308</td>
<td>656</td>
<td>312</td>
<td>660</td>
<td>1.01</td>
<td>0.83–1.22</td>
<td>4.90 × 10⁻³</td>
<td>0.94</td>
</tr>
<tr>
<td>rs41270041</td>
<td>C/G</td>
<td>44</td>
<td>918</td>
<td>45</td>
<td>929</td>
<td>1.01</td>
<td>0.66–1.55</td>
<td>2.40 × 10⁻³</td>
<td>0.96</td>
</tr>
<tr>
<td>rs4233367</td>
<td>C/T</td>
<td>889</td>
<td>75</td>
<td>880</td>
<td>108</td>
<td>0.69</td>
<td>0.50–0.94</td>
<td>5.73</td>
<td>1.66 × 10⁻²</td>
</tr>
<tr>
<td>rs10908826</td>
<td>C/T</td>
<td>653</td>
<td>311</td>
<td>666</td>
<td>326</td>
<td>0.97</td>
<td>0.81–1.18</td>
<td>8.05 × 10⁻²</td>
<td>0.78</td>
</tr>
</tbody>
</table>
rs4233367 which located in exon of ADAMTS-4 showed significant association with LDD. The T allele is the protective allele with an OR of 0.69 and TT genotype is at nearly one-fifth lower risk of LDD comparing with CC genotype. 2-SNP haplotype analysis revealed a significant association of rs4233367-rs10908826 in LDD. ADAMTS-4 (a disintegrin and metalloprotease with thrombospondin motifs-4), also known as Aggrecanases-1, consists of a prodomain, a catalytic metalloproteinase domain, a disintegrin (Dis) domain, a thrombospondin type I (TS) domain, a cysteine-rich (CysR) domain, and a spacer (Sp) domain. Along with ADAMTS-5, ADAMTS-4 also plays an important role in the process of disc degeneration.21,27 It’s a multidomain metalloproteinase secreted from the cytoplasm into the extracellular space28 and involved in extracellular matrix degradation.19,21 It was shown that levels of mRNA and protein ADAMTS-4 were significantly increased in degenerated disc tissue compared with non-degenerated tissue.23,27 Metalloproteinase inhibitor can reduce aggrecan cleavage activity of ADAMTS-4.22

In this study, we found significant allelic and genotypic association in rs4233367 which locates in the exon region of ADAMTS-4. It is a missense variant causing a coding base change c.1877 C>T and leading to amino acid change Gln626Arg. This amino acid locates in the cysteine-rich domain, which might influence the catalytic activity in several ways. Kashiwagi et al. suggested that CysR domain has a major contribution in cleaving the chondroitin sulfate-rich region of aggrecan.29 In their study they deleted spacer domain of ADAMTS-4 and observed a 28% loss of aggrecanases activity. They further deleted the CysR domain and found a drastic reduction of aggrecan degrading activity by 81%. Recently, Yamamoto et al. reported that ADAMTS-4 is endocytosed via low density lipoprotein receptor-related protein 1 (LRP1) and degraded by chondrocytes.30 They also identified that the cysteine-rich and spacer domains are responsible for binding to LRP1. In our study, TT genotype of rs4233367 may lower the catalytic activity of ADAMTS-4 indirectly or enhance endocytosed degradation by facilitating binding with LRP1. In either hypothesis, the function of ADAMTS-4 will attenuate and decrease the risk of LDD. Further functional

<table>
<thead>
<tr>
<th>SNP</th>
<th>Geno-Type</th>
<th>Case</th>
<th>Control</th>
<th>OR</th>
<th>95%CI</th>
<th>$\chi^2$</th>
<th>Individual p-Value</th>
<th>Global p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs11585858</td>
<td>AA</td>
<td>54</td>
<td>51</td>
<td>1</td>
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<td>—</td>
</tr>
<tr>
<td></td>
<td>AC</td>
<td>200</td>
<td>210</td>
<td>0.90</td>
<td>0.59–1.38</td>
<td>0.29</td>
<td>0.59</td>
<td>0.85</td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>228</td>
<td>225</td>
<td>0.96</td>
<td>0.65–1.46</td>
<td>9.85 x 10^{-2}</td>
<td>0.75</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>rs41270041</td>
<td>CG</td>
<td>44</td>
<td>41</td>
<td>3.00 x 10^{-10}</td>
<td>—</td>
<td>0.14</td>
<td>0.70</td>
<td>0.70</td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>437</td>
<td>444</td>
<td>2.75 x 10^{-10}</td>
<td>—</td>
<td>0.14</td>
<td>0.70</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>409</td>
<td>395</td>
<td>1</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>rs4233367</td>
<td>CT</td>
<td>71</td>
<td>90</td>
<td>0.76</td>
<td>0.54–1.07</td>
<td>2.12</td>
<td>0.14</td>
<td>2.78 x 10^{-2}</td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>2</td>
<td>9</td>
<td>0.21</td>
<td>4.61 x 10^{-2}</td>
<td>4.33</td>
<td>3.74 x 10^{-2}</td>
<td>0.99</td>
</tr>
<tr>
<td>rs10908826</td>
<td>CC</td>
<td>229</td>
<td>230</td>
<td>1</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.93</td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>195</td>
<td>206</td>
<td>0.95</td>
<td>0.73–1.24</td>
<td>0.12</td>
<td>0.73</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>58</td>
<td>60</td>
<td>0.97</td>
<td>0.65–1.46</td>
<td>0.90 x 10^{-3}</td>
<td>0.98</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1. LD structures of the four SNPs genotyped in ADAMTS-4 gene. The LD strengths between paired SNPs are shown in color according to confidence intervals’ model.
experiments to verify these hypotheses are warranted. Given the highlighted functional role of this polymorphism, one would expect that this SNP would also show association in the European/American cohorts, since the functional role on a protein level is irrespective of the overall genetic background. However, a meta-analysis of a large European population didn't highlight this SNP. Additional evidence by replication in similar study samples is needed to make the conclusion solid.

In our study we found that the haplotype T-T of rs4233367-rs10908826 has lower risk of LDD than the C-C phenotype. The rs10908826 is located at about 4 kb upstream of ADAMTS-4. The possible mechanism for this association is that the SNPs in 5' element may influence the function of ADAMTS-4. For certain 5' elements the nucleotide sequence is the most important determinant of the element's behavior. For example, Methionine synthase contains two inhibitory uORFs (upstream open reading frame) and one of them contains six rare codons for either arginine or proline within its 30 codon sequence. This would be predicted to potentially slow down or even stall the ribosome during translation. Indeed, synonymous replacement of the adjacent rare arginine codons in the N-terminus of the peptide with a more abundant codon (either arginine or alanine) decreased repression.31 A nucleotide sequence that allowed for RNA secondary structure to form and/or binding motifs within the 5'UTR sequence could also affect upstream element behavior and gene expression.32 The precise function of rs10908826 needs further studies to confirm.

CONCLUSION
For the first time, we reported the possibility of genetic polymorphisms of ADAMTS-4 gene's association with LDD. The T allele and TT genotype of rs4233367 conferred lower risk of LDD. The SNPs in 5' element may influence the function of ADAMTS-4 which might be a possible mechanism for its contribution to LDD. Additional evidence by replication in similar study samples and functional analysis is needed to make the conclusion solid.

AUTHORS’ CONTRIBUTION
Sen Liu, Nan Wu, and Jiaqi Liu contributed equally to this study. Sen L, Nan W, Jiaqi L, and Zhihong W designed the study and performed data collection. Sen L, Jiaqi L, Hao L, Xinlin S, Zhenlei L, Yuzhi Z, Weisheng C, Gang L, and Yixin C were responsible for sample collection. Sen L and Nan W wrote the article. Guixing Q, Jianguo Z, Jianxiong S, Shugang L, Yipeng W, Hong Z, Keyi Y, Yu Z, Shishu H, Xisheng W, Chao W, Guangqian Z, and Zhihong W reviewed the article for intellectual content and were responsible for critically revising the article. All contributors have read and approved the submission to the Journal of Orthopaedic Research.

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