Research Letter

Evaluation of Tag Single-Nucleotide Polymorphisms for Identifying HLA-B27 Status in a Chinese Han Population

To the Editor:

Ankylosing spondylitis (AS) often presents with an insidious onset and is difficult to be distinguished from other arthritis.1 Due to the strong association between AS and HLA-B27,2 identifying HLA-B27 status is of great significance for assisting diagnosis in clinical practice. Compared with common methods for detecting HLA-B27 (eg, flow cytometry and HLA-B27 sequence detection methods), tag single-nucleotide polymorphisms (tag-SNPs) for HLA-B27 could readily infer HLA-B27 positivity according to the presence of 1 allele of tag-SNP (eg, the T allele for the rs116488202 SNP),3 suggesting a convenient strategy for identifying HLA-B27 status. However, differences in performance have been observed in using tag-SNPs among diverse ethnic populations.4 For the Chinese population, both rs13202464 and rs116488202 were reported to be suitable for tagging HLA-B27,5,6 but whether the chosen tag-SNP could accurately determine HLA-B27 status or whether other tag SNPs could obtain better tagging performance remains to be discussed. To investigate the reliability of tag-SNP for identifying HLA-B27 status in the Chinese Han population, we systematically evaluated the identification capability of the widely reported HLA-B27 tag-SNPs: rs116488202, rs13202464, and rs4349859. The Ethics Committee of First People’s Hospital of Nantong City approved this study (2021KYG010 and 2021KT180), and all the participants gave written informed consent.

This study recruited 114 patients with AS and 107 healthy controls from the First People’s Hospital of Nantong City, including 109 HLA-B27–negative subjects and 112 HLA-B27–positive subjects. Genomic DNA was extracted from peripheral blood of each subject as samples using the Wizard Genomic DNA Purification Kit (Promega), according to the manufacturer’s instructions. All the samples were genotyped in the 3 tag-SNPs (rs13202464, rs116488202, and rs4349859) in the reaction mixture as reported by quantitative PCR-Invader assay.7 Ten percent of samples were performed in experimental replication. HLA-B genotyping was performed in part of samples by PCR sequence-based typing (Jiangsu Weihe Biotechnology). SAS Studio (SAS Institute) and MedCalc software were employed for statistical analysis. The potential association was assessed by odds ratios (ORs) with 95% CIs. Hardy–Weinberg equilibrium (HWE), linkage disequilibrium (LD), and haplotype analysis were performed by using SHEsis software online (http://analysis.bio-x.cn/myAnalysis.php).8 P < 0.05 was considered statistically significant.

After obtaining the genotyping results, we found that the allele frequencies of rs116488202 and rs4349859 were completely consistent in the subjects in our study (Supplementary Table S1, available with the online version of this article); thus, we further analyzed the LD and haplotype of the 3 tag-SNPs. As exhibited in Figure 1A, rs116488202 and rs4349859 were in high LD (r² = 1), indicating the same capability of the 2 SNPs for tagging HLA-B27 in the Chinese population. Moreover, rs13202464A-rs116488202C-rs4349859G was the most common haplotype in HLA-B27–negative subjects and was associated with a reduced risk of HLA-B27 positivity (OR 0.05, 95% CI 0.03–0.08, P < 0.001; Figure 1B), whereas rs13202464G-rs116488202C-rs4349859G delineated an increased risk of HLA-B27 positivity (OR 14.88, 95% CI 8.38–26.44, P < 0.001; Figure 1B).

To systematically evaluate the performance of tag-SNPs for identifying HLA-B27 status, we compared the HLA-B27 status determined by SNP with the reference status detected by flow cytometry or commercial kit. Subjects were defined as SNP positive in HLA-B27 status if carrying the minor alleles of tag-SNPs; detailed comparison results are shown in the Table. The κ coefficient of rs13202464 was much higher than that of rs116488202 or rs4349859 (0.86 vs 0.24), indicating better consistency of rs13202464 identification and

![Figure 1](http://www.jrheum.org)
research found in Asian populations,9 whereas rs13202464-positive
the HLA-B*27:05 allele, which was consistent with the previous
detected rs116488202- or rs4349859-positive samples carried
T able). Further, HLA-B genotyping demonstrated that all the
under the receiver-operating characteristic curve (AUC) of iden-
tifying HLA-B27 status rather than rs116488202 or rs4349859. ACC:
accuracy; AUC: area under the receiver-operating characteristic curve; SE: sensitivity; SP: specificity; PPV: positive
predictive value; NPV: negative predictive value; ROC: receiver-operating characteristic; SNP: single-nucle
totide polymorphism.

<table>
<thead>
<tr>
<th>SNP Positive, n = 27</th>
<th>SNP Negative, n = 194</th>
<th>SNP Positive, n = 126</th>
<th>SNP Negative, n = 95</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference positive (n = 112)</td>
<td>27</td>
<td>85</td>
<td>111</td>
</tr>
<tr>
<td>Reference negative (n = 109)</td>
<td>0</td>
<td>109</td>
<td>15</td>
</tr>
<tr>
<td>κ coefficient</td>
<td>0.24</td>
<td>0.86</td>
<td></td>
</tr>
<tr>
<td>ACC (95% CI)</td>
<td>0.62 (0.55-0.68)</td>
<td>0.93 (0.89-0.96)</td>
<td></td>
</tr>
<tr>
<td>SE (95% CI)</td>
<td>0.24 (0.17-0.33)</td>
<td>0.99 (0.94-0.999)</td>
<td></td>
</tr>
<tr>
<td>SP (95% CI)</td>
<td>1.00 (0.96-1.00)</td>
<td>0.86 (0.78-0.92)</td>
<td></td>
</tr>
<tr>
<td>PPV (95% CI)</td>
<td>1.00 (0.85-1.00)</td>
<td>0.88 (0.81-0.93)</td>
<td></td>
</tr>
<tr>
<td>NPV (95% CI)</td>
<td>0.56 (0.49-0.63)</td>
<td>0.99 (0.93-0.999)</td>
<td></td>
</tr>
<tr>
<td>AUC (95% CI)</td>
<td>0.62 (0.55-0.69)</td>
<td>0.93 (0.89-0.97)</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
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</tr>
</tbody>
</table>

aSNP positive, the presence of the A allele for rs4349859, T allele for rs116488202, and the G allele for rs13202464.
bκ test, P < 0.001. cP value of Delong test for ROC curves: rs4349859 or rs116488202 vs. rs13202464. ACC:
accuracy; AUC: area under the receiver-operating characteristic curve; SE: sensitivity; SP: specificity; PPV: positive
predictive value; NPV: negative predictive value; ROC: receiver-operating characteristic; SNP: single-nucle
totide polymorphism.

the reference methods. Compared with rs116488202 and
rs4349859, rs13202464 exhibited obvious advantages in accuracy (ACC; 0.93, 95% CI 0.89-0.96), sensitivity (SE; 0.99, 95%
CI 0.94-0.999) and negative predictive value (NPV; 0.99, 95%
CI 0.93-0.999). Moreover, rs13202464 revealed a superior area
under the receiver-operating characteristic curve (AUC) of identi-
fying HLA-B27 status rather than rs116488202 or rs4349859
(rs13202464 vs rs116488202 and rs4349859: AUC 0.93,
95% CI 0.89-0.97 vs AUC 0.62, 95% CI 0.55-0.69, P < 0.001;
Table). Further, HLA-B genotyping demonstrated that all the
detected rs116488202- or rs4349859-positive samples carried the
HLA-B*27:05 allele, which was consistent with the previous
research found in Asian populations,9 whereas rs13202464-positive
samples included HLA-B*27:04 along with HLA-B*27:05
(Supplementary Table S2, available with the online version of
this article).

Tag-SNPs provide an easy way for determining HLA-B27
status as well as analyzing the interaction between HLA-B27
and other genes. As a large population with the highest positive
rate of HLA-B27, up to 90% in patients with AS,10 identifying
the optimal tag-SNP is of great significance in the Chinese Han
population. To our knowledge, it is the first study to evaluate
the performance of commonly used HLA-B27 tag-SNPs in a
Chinese Han population. Our work demonstrated that, unlike
Europeans, rs116488202 as well as rs4349859 showed poor
performance in the Chinese Han population; on the other hand,
rs13202464 stood out from the frequently used 3 tag-SNPs.
These findings indicated that rs13202464 genotyping could be
more convenient alternative strategy for identifying HLA-B27
status in the Chinese Han population.

Nan Sheng1,2, PhD
Wenwen Wang1, MD
Bo Zhang1, MD

Jingjing Zhao1, MD
Haojie Chen1, BS
Panfeng Feng1, PhD
Yingying Gao1, MD, PhD
Xiaoxiang Chen1,4, MD, PhD

1Department of Rheumatology, Affiliated Hospital 2 of Nantong University and First People’s Hospital of Nantong City, Nantong;
2Clinical Medicine Research Center, Affiliated Hospital 2 of Nantong University and First People’s Hospital of Nantong City, Nantong;
3Department of Pharmacy, Affiliated Hospital 2 of Nantong University and First People’s Hospital of Nantong City, Nantong;
4Department of Rheumatology, Shanghai Jiao Tong University School of Medicine Affiliated Renji Hospital, Shanghai, China.

N. Sheng and W. Wang contributed equally to this work.

This research was supported by the Scientific Research Development Funding of Kangda College of Nanjing Medical University (No.
KD2021KYJJZD047), the Health Commission of Nantong City (No. QA2021007 and No. QA2021014) and Jiangsu Pharmaceutical
Association-HengRui Hospital Pharmacy Fund (No. H202047). The study was approved by the Ethics Committee of First People's Hospital of
Nantong City (2021KYG010 and 2021KT180) and performed according to the principles of the Declaration of Helsinki.

The authors declare no conflicts of interest relevant to this article.

Address correspondence to Prof. X. Chen, Department of Rheumatology, Shanghai Jiao Tong University School of Medicine Affiliated Renji Hospital, Middle Shandong Road 145, Shanghai, 200030, China. Email:
xiangx0721@126.com. Or Dr. Y. Gao, Department of Rheumatology, Affiliated Hospital 2 of Nantong University and First People’s Hospital of
Nantong City, 6 Haierxiang Road, Nantong 226001, Jiangsu, China. Email: yingyinggao1983@163.com.

ONLINE SUPPLEMENT
Supplementary material accompanies the online version of this article.
REFERENCES