High Accuracy and Significant Savings Using Tag-SNP Genotyping to Determine *HLA-B*27* Status

To the Editor:

Axial spondyloarthritis (axSpA) is characterized by inflammatory back pain, spinal ankylosis, peripheral arthritis, and extraarticular manifestations[1]. *HLA-B*27* status is particularly helpful in diagnosis because the combination of severe sacroiliitis on magnetic resonance imaging with *HLA-B*27* positivity is an excellent predictor of disease outcome[2]. However, because of inherent challenges associated with multiallelic genetic testing, clinical tests evaluate the entire HLA-B locus using commercially available kits. Consequently, the clinical value of the HLA-B locus test as a tool for primary care physicians is constrained, primarily because of cost. A large genome-wide association study demonstrated that a tag-single-nucleotide polymorphism (SNP) can be used to determine *HLA-B*27* status in whites[3]. The objective of our study was to assess the analytical validity and economic effect of using a *HLA-B*27* tag-SNP assay compared with an HLA-B locus test in a clinical genetics laboratory. The ethics committee from Memorial University approved the study. Patient consent was obtained.

The study cohort consisted of 1000 consecutive patient samples received by the clinical genetics laboratory at Eastern Health to determine the *HLA-B*27* genotyping status. Institutional ethics was approved from the Health Research Ethics Board at Memorial University (Health Research Ethics Authority reference #15.051). Genomic DNA was extracted from whole blood using an automated MagnaPure Compact according to manufacturer's protocols (Roche Molecular Systems). The concentration of extracted DNA was determined using a NanoDrop 2000 (ThermoFisher Scientific). Targeted analysis of the HLA-B locus was performed using a commercially available kit (LABType SSO HLA-B locus kit) on a Luminex 100/200 platform as per manufacturer's instructions (One Lambda, ThermoFisher Scientific). Genotyping of rs4349859 (NM_001289154.1: c.-2921G>A) and rs116488202 (NC_000006.12:g.31377139C>T) was performed using a TaqMan SNP genotyping assay (Assay ID number C_28023949_10) and a custom-designed TaqMan SNP genotyping assay (Assay ID number AHCS8KA), respectively, as per the manufacturer's instructions (Applied Biosystems). Genotypes were assigned using the endpoint genotyping analysis software (Applied Biosystems). *HLA-B*27* positivity was inferred by the presence of the "T" allele and "A" allele for the rs116488202 and rs4349859 SNP, respectively. Statistical analysis was performed using Prism 7 (GraphPad).

The analytical validation study cohort, which consisted of 572 men and 428 women, contained 172 *HLA-B*27*–positive and 828 *HLA-B*27*–negative patients. Successful genotypes were generated for 99.9% of samples compared with using the HLA-B locus test. When the results of each assay were compared with the HLA-B locus test, each tag-SNP assay demonstrated an analytical sensitivity, specificity, and accuracy of 97.6%, 99.9%, and 99.6%, respectively, with a false-negative rate of 2.4% and a false-positive rate of 0.1% (Table 1). The *HLA-B*27* SNP, rs4349859 and rs116488202, tag the major white AS-associated suballeles with 98% sensitivity and 99% specificity[3,4,5,6], findings consistent with the results of our study. Given that the rs116488202 assay outperformed the rs4349859 assay (i.e., more discrete separation of genotype clusters; data not shown), the former was selected for clinical validation and implementation. That the *HLA-B*27* rs116488202 tag-SNP assay demonstrated an analytical sensitivity, specificity, accuracy, and precision of 97.6%, 99.9%, 99.6%, and 100%, respectively, strongly suggests that this tag-SNP assay represents a very good genetic screening marker of *HLA-B*27* positivity.

Replacing the HLA-B locus kit with the *HLA-B*27* tag-SNP assay represents about a 14-fold cost reduction for healthcare authorities ($64.91 for the locus kit vs $4.61 for the tag-SNP assay, based on 20 samples; Table 2; all costs in Canadian dollars). For a clinical laboratory receiving about 500 *HLA-B*27* test requests annually, replacing the HLA-B locus test with a *HLA-B*27* tag-SNP test would equate to about $30,150 in cost savings.

*HLA-B*27* status is of considerable interest to the healthcare system as part of a first-tier screening test for individuals with inflammatory back pain because it is an excellent predictor of future axSpA disease outcome. SNP genotyping to determine HLA-B*27 status in the Newfoundland (NL), Canada, population, which is primarily white, represents a highly sensitive, specific, accurate, precise, cost-effective, and time-efficient clinical screening method for patients with axSpA. Although genetic results from the NL population are generalizable to others[7], uncertainty exists regarding how well the tag-SNP assay will perform in more ethnically diverse populations given that the rs116488202 SNP displays the most robust association with the HLA-B*27 allele in whites.

To our knowledge, ours is the first study to assess the analytical validity and economic effect of using a HLA-B*27 tag-SNP assay compared with a HLA-B locus test in a clinical genetics laboratory. That our study used 1000 consecutive specimens that arrived at the clinical genetics laboratory for HLA-B*27 genotyping provides an accurate assessment of the analytical

Table 1. Genotype data comparing the PCR-SSO test with the *HLA-B*27* targeted tag-SNP assay. Both tag SNP were 100% concordant. The HLA-B allele designation corresponding to the false-positive and -negative calls were false-positive (HLA-B*39:06/*49:XX) and false-negative samples (*HLA-B*27:05/*35:XX; HLA-B*27:05/*18:01; HLA-B*27:05/*52:01,* and *HLA-B*27*:05/**35:XX*). Values are n unless otherwise specified.

Variables	PCR-SSO	*HLA-B*27* Tag-SNP, rs4349859	*HLA-B*27* Tag-SNP, rs116488202
*HLA-B*27*–positive samples	172	167	167
*HLA-B*27*–negative samples	828	831	832
False-positive samples	N/A	1	1
False-negative samples	N/A	4	4
Analytical sensitivity, %	100	97.6	97.6
Analytical specificity, %	100	99.9	99.9
Analytical accuracy, %	100	99.6	99.6
Analytical precision[1], %	100	N/A	100
Samples with reportable genotype	1000	998[†]	999[#]

[1] Precision analysis was performed using 89 samples run in duplicate only for the rs116488202 assay. [†] Two samples failed to generate a result for the rs4349859 assay due to insufficient DNA for amplification (1 sample) and a variant under the primer-binding site (1 sample). [#] A single sample failed to generate a result for the rs116488202 assay due to insufficient DNA for amplification. PCR-SSO: PCR-sequence–specific oligonucleotide; SNP: single-nucleotide polymorphism; N/A: not applicable.

Table 2. Economic and time comparison of the HLA-B locus PCR-SSO test with the *HLA-B*27* targeted tag-SNP assay (rs116488202).

Variables	HLA-B Locus PCR-SSO	*HLA-B*27* Tag-SNP	
Cost per sample, $	64.91	4.61	3.28
Cost per run, $	714.00	92.16	190.02
Hands-on time per run, min	209	151	403
Hands-on time per sample, min	19	6.30	6.30
Samples tested per run, n	11[†]	20	58

[†] The HLA-B locus kit is sufficient to perform 16 reactions (11 samples, 4 rotating positive controls, 1 non-template control). Given that it is easy to mix up sample order during the wash cycle step for the PCR-SSO kit, a minimum of 4 controls is strongly recommended. Hands-on time represents the total time for run preparation paperwork, wet work, quality control, result analysis, interpretation, second checking, and reporting. PCR-SSO: PCR-sequence–specific oligonucleotide; SNP: single-nucleotide polymorphism.

validity and economic effect of using a HLA-B*27 tag-SNP assay. Based on our findings, the HLA-B*27 tag-SNP assay will be used clinically as a first-tier screening test for patients with inflammatory axial pain in the NL population. The ordering of the HLA-B locus assay will be limited to rheumatologists for those patients with a negative tag-SNP test result where a strong clinical suspicion remains that the patient might be HLA-B*27–positive. Adoption of such a cost-effective screening approach is recommended to other healthcare authorities striving to offer equal services at reduced costs.

JOSH LEHR, Faculty of Medicine, Memorial University of Newfoundland, St. John's, Newfoundland; PROTON RAHMAN, MD, MSc, Medicine, St. Clare's Mercy Hospital, St. John's, Newfoundland; DARREN D. O'RIELLY, PhD, FCCMG, Director, Molecular Genetics Laboratory, Eastern Health, Medical Laboratory Molecular Geneticist, and Research Scientist, Faculty of Medicine, Memorial University, and Adjunct Professor, School of Pharmacy, Memorial University, St. John's, Newfoundland, Canada. Supported by the Atlantic Canada Opportunity Agency–Atlantic Innovation Fund, the Canadian Institute of Health Research, and the Research and Development Corporation, Newfoundland and Labrador. Address correspondence to D.D. O'Rielly, Faculty of Medicine, Suite 3M500, Craig L. Dobbin Genetics Research Centre, Memorial University, St. John's, Newfoundland and Labrador A1B 3V6, Canada. E-mail: darren.orielly@med.mun.ca

REFERENCES

1. Wallis D, Inman RD. Recognition of preclinical and early disease in axial spondyloarthritis. Rheum Dis Clin North Am 2014;40:685-97.
2. Bennett AN, McGonagle D, O'Connor P, Hensor EM, Sivera F, Coates LC, et al. Severity of baseline magnetic resonance imaging-evident sacroiliitis and HLA-B27 status in early inflammatory back pain predict radiographically evident ankylosing spondylitis at eight years. Arthritis Rheum 2008;58:3413-8.
3. International Genetics of Ankylosing Spondylitis Consortium (IGAS), Cortes A, Hadler J, Pointon JP, Robinson PC, Karaderi T, et al; Australo-Anglo-American Spondyloarthritis Consortium (TASC); Groupe Française d'Etude Génétique des Spondylarthrites (GFEGS); Nord-Trøndelag Health Study (HUNT); Spondyloarthritis Research Consortium of Canada (SPARCC); Wellcome Trust Case Control Consortium 2 (WTCCC2). Identification of multiple risk variants for ankylosing spondylitis through high-density genotyping of immune-related loci. Nat Genet 2013;45:730-8.
4. Evans DM, Spencer CC, Pointon JJ, Su Z, Harvey D, Kochan G, et al; Australo-Anglo-American Spondyloarthritis Consortium (TASC); Wellcome Trust Case Control Consortium 2 (WTCCC2). Interaction between ERAP1 and HLA-B27 in ankylosing spondylitis implicates peptide handling in the mechanism for HLA-B27 in disease susceptibility. Nat Genet 2011;43:761-7.
5. Akar S, Igci YZ, Sari I, Pala E, Geyik E, Tas MN, et al. Do major histocompatibility complex tag single nucleotide polymorphisms accurately identify HLA-B27 in the Turkish population? Int J Rheum Dis 2015 Jul 22 (E-pub ahead of print).
6. Roberts RL, Wallace MC, Jones GT, van Rij AM, Merriman TR, Harrison A, et al. Prevalence of HLA-B27 in the New Zealand population: effect of age and ethnicity. Arthritis Res Ther 2013;15:R158.
7. Green RC, Green JS, Buehler SK, Robb JD, Daftary D, Gallinger S, et al. Very high incidence of familial colorectal cancer in Newfoundland: a comparison with Ontario and 13 other population-based studies. Fam Cancer 2007;6:53-62.

J Rheumatol 2017;44:6; doi:10.3899/jrheum.161095