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A Single SNP Surrogate for Genotyping HLA-C*06:02 in Diverse Populations

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Over forty-one genetic susceptibility loci of genome-wide significance ($P < 5 \times 10^{-8}$) are known for psoriasis, a common complex genetic disease characterized by epidermal hyperproliferation and cutaneous inflammation. The most strongly associated of these loci maps to the *HLA-C* gene in the major histocompatibility complex (MHC) on chromosome 6p21.3 (Tsoi *et al.*, 2012; Zhang *et al.*, 2009). Recent fine mapping studies have identified multiple independent association signals in the MHC, and the strongest signal is associated with HLA-C*06:02 (previous designations: HLA-Cw6, HLA-Cw*0602) or SNPs that are near-perfect surrogates for this allele (Das *et al.*, 2014; Feng *et al.*, 2009; Knight *et al.*, 2012; Okada *et al.*, 2014). HLA-C*06:02 has been known to be psoriasis-associated since the 1970s, and most world populations studied have shown strong evidence of association (Okada *et al.*, 2014; Shaiq *et al.*, 2013; Stuart *et al.*, 2010; Zhang *et al.*, 2009). Yet, it is difficult to perform a routine affordable laboratory test for the presence or absence of HLA-C* 06:02 because DNA-based testing is complicated both by the high degree of polymorphism of HLA-C (2375 alleles encoding 1677 protein variations; July 2014 release of IMGT/HLA database) (Robinson *et al.*, 2013) and by the sequence similarity of HLA-C* 06:02 to other HLA-C as well as HLA-A and HLA-B alleles. We previously used a 7- SNP genotyping system to unambiguously define HLA-C*06:02 and several other HLA-C alleles

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CONFLICT OF INTEREST

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(Nair *et al.*, 2006). Others have used allele-specific amplification followed by electrophoresis (Bunce *et al.*, 1995), PCR amplification followed by restriction enzyme digestion (Tazi Ahnini *et al.*, 1999), a combination of four surrogate SNPs that are amenable to Taqman genotyping (Nikamo and Stahle, 2012), and a 3-SNP haplotype (rs1576-rs130076-rs2523619) known to be in strong linkage disequilibrium (LD) with HLA-C*06:02 (Huffmeier *et al.*, 2009). Imputation of classical HLA alleles including HLA-C*06:02 is another widely-used approach (Jia *et al.*, 2013; Leslie *et al.*, 2008), but this requires high density genotyping of the sample for SNPs throughout the MHC region, as well as a reference panel drawn from the same population that has been typed for both classical HLA genes and a high density of MHC SNPs.

During the course of our genome-wide association studies of psoriasis and fine mapping of MHC loci, we discovered that the A allele of SNP rs4406273, located 28.73 kb centromeric of HLA-C, is a near-perfect surrogate for HLA-C*06:02 allele. Notably, this SNP was the most strongly associated variant in the largest genome-wide association study of psoriasis to date (Tsoi *et al.*, 2012). Since rs4406273 is only 3 bases away from another SNP, it is not amenable to the commonly-used Taqman (Applied Biosystems, Foster City, CA) genotyping assay design. However, it can be genotyped using single base extension methods. In this study, we examined the adequacy of genotyping rs4406273 as a single marker surrogate for HLA-C*06:02 in three different populations–5,009 European-ancestry samples collected in Michigan, 835 samples from Pakistan, and 307 samples from Thailand. All DNA samples used in this study were prepared from peripheral blood obtained following protocols approved by the ethics boards of participating institutions and adhering to the Declaration of Helsinki principles. Written informed consent was obtained from all study participants. Reference HLA-C*06:02 genotypes were generated by our previously reported 7-marker method (Nair *et al.*, 2006), and rs4406273 was genotyped using the single base extension method implemented in the Applied Biosystems Snapshot Assay. Briefly, this assay involved (1) amplification of a 237 bp segment of DNA encompassing rs4406273 using PCR primers CTGGAAAGGGTGAGGAAACA and TGACCTCCCTACTGCAGCTT, (2) inactivation of unused primers and deoxynucleotide triphosphates by treatment with a mixture of shrimp alkaline phosphatase and exonuclease, (3) performing the primer extension reaction using an aliquot of the PCR product and probe GAGCCTCAGAAGAAATGCAGCTSTGAC that would be extended by one base corresponding to the allele(s) of rs4406273 (A and/or G), (4) inactivation of unused probe by treatment with alkaline phosphatase, and (5) identification of the added base by electrophoresis on an Applied Biosystems capillary electrophoresis apparatus using LIZ120 size standard ladder (Applied Biosystems, Foster City, CA).

The utility of the rs4406273-A allele as a surrogate for HLA-C*06:02 was assessed using various metrics (Table 1). Allele frequencies were very similar in all three cohorts, with the A allele of rs4406273 slightly less abundant in the Michigan and Pakistani samples and slightly more abundant in the Thai sample than HLA-C*06:02. LD between the two variants, as measured by the D′ and r^2 coefficients, was 0.95 or greater in all three samples. Genotype concordances were also uniformly high (0.984–0.996). The correspondence of cross-classified allele counts for the rs4406273 A and C alleles on the one hand and HLA-

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C* 06:02 and non-C:06:02 alleles on the other hand were also assessed by six different measures for the performance of a binary classification test—sensitivity, specificity, positive predictive value, negative predictive value, accuracy, and the Matthews correlation coefficient (Matthews, 1975), which is the Pearson correlation coefficient between two binary classifications. Values for these performance measures were very high, ranging from 0.990–0.999 for the Michigan sample, 0.965–0.998 for the Pakistani sample, and 0.976–1.000 for the Thai sample.

In Table 2A, we compare the results of a logistic regression test for association between psoriasis and dosage of the HLA-C*06:02 and rs4406273-A alleles. In all three cohorts, the odds ratio (OR) estimates and p-values for rs4406273 are slightly conservative compared to those of HLA-C*06:02, suggesting that this marker is unlikely to yield false positive results. Conditional haplotype testing of independent effects (Table 2B) shows that residual effects after conditioning are negligible compared to the association p-values.

We also assessed the performance of rs4406273 as a surrogate for 928 individuals from 13 different populations in phase 1 of the 1000 Genomes Project (Abecasis *et al.*, 2012) for which sequence-based genotypes of HLA-C and rs4406273 were available (Gourraud *et al.*, 2014); ftp://ftp.1000genomes.ebi.ac.uk/vol1/ftp/technical/working/20140725_hla_genotypes/ and ftp://ftp-trace.ncbi.nih.gov/1000genomes/ftp/release/20110521/). LD was very strong between rs4406273 and HLA-C*06:02 in four populations of European descent from the United States, Finland, Great Britain and Italy ($r^2 = 0.984$) and in three Asian populations from Japan and China ($r^2 = 1.000$). LD values were somewhat lower but still strong in two African populations from Nigeria and Kenya ($r^2 = 0.929$) and in four American populations from Puerto Rico, Colombia, and the United States ($r^2 = 0.909$).

The results presented here demonstrate that genotyping rs4406273 can be used as an excellent substitute for more elaborate and expensive genotyping of HLA-C*06:02 in people of European, Pakistani, Thai, Chinese, or Japanese ancestry. This SNP may also be a serviceable surrogate for HLA-C*06:02 in some African and American populations. Caution is warranted, however, when applying this method to genetic isolates or more distantly related populations. While not amenable to the popular Taqman assay due to the presence of proximal SNPs, any method employing primer extension can be used to genotype rs4406273. For high-throughput genotyping projects using commercial microarrays, this SNP can be added on as a custom marker if not already present as part of the standard set.

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Abbreviations

LD	linkage disequilibrium
MHC	major histocompatibility complex

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Table 1

Performance of the rs4406273-A allele as a surrogate for HLA-C*06:02.

Variable	Michigan sample (10,018 alleles)	Pakistan sample (1,670 alleles)	Thailand sample (614 alleles)
frequency HLA-C*06:02	0.1624 (0.1553–0.1698)[1]	0.1725 (0.1551–0.1913)	0.1303 (0.1060–0.1592)
frequency rs4406273-A	0.1612 (0.1541–0.1685)	0.1683 (0.1511–0.1870)	0.1336 (0.1089–0.1628)
D′ measure of LD	0.9963 (0.9927–0.9993)	0.9870 (0.9710–1.0000)	1.0000 (1.0000–1.0000)
r^2 measure of LD	0.9839 (0.9769–0.9903)	0.9456 (0.9150–0.9722)	0.9706 (0.9255–1.0000)
genotype concordance	0.9956 (0.9934–0.9971)	0.9844 (0.9736–0.9909)	0.9935 (0.9766–0.9988)
sensitivity	0.9896 (0.9833–0.9935)	0.9653 (0.9373–0.9810)	1.0000 (0.9542–1.0000)
specificity	0.9994 (0.9986–0.9997)	0.9978 (0.9936–0.9994)	0.9963 (0.9864–0.9993)
PPV	0.9969 (0.9928–0.9987)	0.9893 (0.9691–0.9971)	0.9756 (0.9154–0.9957)
NPV	0.9980 (0.9968–0.9987)	0.9928 (0.9868–0.9961)	1.0000 (0.9928–1.0000)
accuracy	0.9978 (0.9967–0.9985)	0.9922 (0.9867–0.9954)	0.9967 (0.9882–0.9994)
MCC	0.9919 (0.9885–0.9953)	0.9726 (0.9579–0.9875)	0.9859 (0.9667–1.0000)

Abbreviations: LD, linkage disequilibrium; PPV, positive predictive value; NPV, negative predictive value; MCC, Matthews correlation coefficient

[1]95% confidence intervals shown in parentheses for all values

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Table 2

A. Comparison of the association of HLA-C*06:02 and rs4406273-A with psoriasis for samples successfully typed for both markers.

		HLA-C*06:02				rs4406273-A			
		allele frequency				allele frequency			
Cohort	n	cases	controls	OR	p-value	cases	controls	OR	p-value
Michigan	5009	0.2327	0.0900	3.41	8.5×10^{-83}	0.2307	0.0896	3.38	1.8×10^{-81}
Pakistan	835	0.2484	0.1257	2.42	1.8×10^{-10}	0.2390	0.1248	2.32	1.6×10^{-9}
Thailand	307	0.1766	0.0425	5.38	7.5×10^{-6}	0.1791	0.0472	4.91	1.0×10^{-5}

B. Conditional haplotype testing of independent effects for samples successfully typed for both HLA-C*06:02 and rs4406273

		Independent effects p-value		
Test marker	Conditioning marker	Michigan	Pakistan	Thailand
HLA-C*06:02	rs4406273	0.0052	0.011	0.20
rs4406273	HLA-C*06:02	0.20	0.11	0.82