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Note

A polymorphism within the psoriasis susceptibility 1 candidate 1 (PSORS1C1) gene is not linked to HLA-B*58:01 in an Australian cohort

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ABSTRACT

Association exists between HLA-B*58:01 allele and allopurinol Stevens-Johnson syndrome (SJS), especially those of an Asian heritage but associations have been also described in Caucasian populations. This creates the need to develop a rapid, robust and inexpensive assay for pre-screening of HLA-B*58:01. A polymorphism within PSORS1C1 gene was recently found in linkage disequilibrium (LD) with HLA-B*58:01 allele in the Japanese population. The aim of this study is to confirm whether this polymorphism can be used as a surrogate biomarker to identify carriers for HLA-B*58:01. No linkage was found between the two in the Australian cohort.

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Severe adverse drug reactions (ADR) cause significant morbidity and mortality for patients and increase the burden on the National health system. ADRs occur in susceptible individuals carrying various risk alleles within the human leucocyte antigens (HLA) locus [1,2]. The HLA locus is highly polymorphic and so varies widely between different individuals, giving rise to a variety of molecules that are able to bind to different foreign molecules but can also give rise to an allergic reaction to a drug given for therapeutic purposes [2]. Screening of patients before the initiation of treatment can significantly reduce the risk of ADRs, however existing methodologies routinely used for tissue HLA typing are not practical to use for screening. This creates a need for the development of a molecular screening test for the detection of HLA-B alleles known to increase the risk for ADRs. Testing for HLA-B alleles presents a challenge due to the highly polymorphic nature of the region so it makes it plausible to test for a surrogate marker in a nearby gene that will be shown to be in complete linkage with the HLA-B allele.

ADR to commonly used drugs such as allopurinol, abacavir and carbamazepine have been associated with specific HLA types that segregate in different ethnic groups. Hypersensitivity to these drugs results in severe and life-threatening reactions and syndromes such as Stevens-Johnson (SJS)/toxic epidermal necrolysis (TEN) and reactions with eosinophilia and systemic symptoms (DRESS). Different HLA types segregate preferentially in different ethnic groups, for example HLA B*5701 is found in European and African populations, HLA-B*15:02 in Han Chinese whilst HLA-B*58:01 is more evenly distributed geographically. From the clinical perspective it is important to identify individuals that are at risk before treatment begins, in order to reduce the risk for these individuals to develop a severe ADR. Besides being beneficial to the patient, identifying individuals at risk before any treatment is initiated will reduce the financial burden on the health system [3].

Various studies reported an association between HLA-B*58:01 and hypersensitivity to the widely prescribed drug allopurinol, which induces SJS/TEN in sensitive individuals [4]. Mortality rate for TEN was estimated to be as high as 50% and 5% for SJS. The pathogenesis of allopurinol induced SJS/TEN is a delayed immune-mediated reaction having familial predisposition and hence suggesting a genetic component. Genetic association studies reported association of allopurinol induced SJS/TEN to the MHC locus, primarily to HLA-B*58:01. The risk of developing an allopurinol induced SJS/TEN in HLA-B*58:01 individual can be as high as 97

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times compared to other individuals with an alternative HLA type, having similar odds across all populations studied. These findings suggest the importance to prevent SJS/TEN from happening by screening individuals for HLA-B*58:01 prior to starting allopurinol treatment. HLA-B*58:01 has a high prevalence in the Han Chinese population but it is also reported in other populations including European, Thai, Italian and Korean, making a screening program important to reduce the risk of SJS/TEN due to allopurinol treatment [5].

Due to the nature of complexity of the HLA locus, the gold standard methodology to identify class I alleles is direct DNA sequencing. The region of exon 2 and 3, encoding the peptide binding domains of the HLA-B molecules shows the highest variability. HLA-B alleles make up almost half of the total number of alleles reported in the immunogenetics (IMGT)/HLA database [6]. Techniques for the identification of these alleles currently used for tissue typing and transplantation are not readily available, are expensive and usually have a very long turnaround time. This presents the need to develop a rapid test to type HLA-B alleles to be able to screen individuals before the initiation of treatment with allopurinol. However, due to the highly polymorphic nature of the gene, there is a risk of getting false negative results, due to allele drop-out, if an unknown polymorphism will affect primer or probe binding. This makes the idea to test for a surrogate marker in a nearby, but less polymorphic, gene plausible if this is found to be in linkage disequilibrium (LD) with the HLA-B*58:01 allele. A recent study reported absolute LD ($r^2 = 1.0$; $D' = 1.0$) between a G/A single nucleotide polymorphism (SNP) (rs9263726) found in the psoriasis susceptibility 1 candidate 1 (PSORS1C1) gene and the HLA-B*58:01 allele in a Japanese cohort [7,8]. This makes this SNP a very strong candidate as a potential biomarker to identify individuals carrying HLA-B*58:01 allele.

Hence, in the present study, using a modified PCR-restriction fragment length polymorphism (RFLP) technique we characterised the G/A transition (rs9263726) and tested for LD with HLA-B*58:01 in an Australian cohort.

For this study genomic DNA (gDNA) was extracted and purified from peripheral blood leucocytes obtained from random EDTA blood samples (n = 77) sent for testing to the Immuno-Rheumatology Laboratory, Pathology North. DNA was extracted using Wizard Genomic DNA Extraction Kit using a protocol as recommended by supplier (Promega Corp.). Blood samples were thrown away post-extraction and DNA samples were only labelled with a consecutive number. The study protocol was approved by the Research Ethics Committee, Royal North Shore Hospital, St Leonards, NSW, Australia (LNR/14/HAWKE/410).

Genotyping for rs9263726 was performed using a modified PCR restriction fragment length polymorphism (RFLP) technique. A 260 bp target region within the PSORS1C1 gene was amplified by PCR using 100 ng of gDNA. Both oligonucleotide primers were labelled with the fluorescent dye FAM at the 5′ end (For: AAGCTCCATCCACCCCTGGT; Rev: ACACATTGGGTGGGGGACAT). PCR was performed using 0.5 Units AmpliTaq Gold (Life Technologies). PCR was performed using the following conditions, an initial hot-start of 10 min at 95 °C followed by 35 cycles: 95 °C/30 s (denaturation), 62 °C/30 s (annealing) and 72 °C/30 s (elongation).

The PCR product was digested using FokI (New England Biolabs) in a total reaction volume of 50 μl using 5 units of restriction enzyme (FokI). The reaction was incubated at 37 °C for 90 min followed by enzyme deactivation for 20 min at 60 °C. The presence of the A allele yields two digested fragments, 141 bp and 119 bp, in homozygotes.

The digested products were cleaned from excess fluorescent primers and concentrated using Amicon® Ultra Centrifugal devices to a final volume of 20 μl. The digested products were transferred into these devices and centrifuged at 14,000 g for 5 min followed by the addition of 200 μl of water and spinning at the same speed. The devices were then inverted and placed into a new microcentrifuge tube and centrifuged at 1000 g for 3 min 2 μl of the concentrate were then transferred into a 96 well plate followed by the addition of 15 μl Hi-Di Formamide and 0.3 μl Liz-500 as molecular marker. After denaturation for 2 min at 95 °C, the plate was loaded onto a 3130 Genetic Analyzer system. Data was analysed using Applied Biosystems GeneScan software.

A high resolution HLA-B typing was performed using Luminex–SSO/SBT/SSP and using the New South Wales Transplantation and Immunogenetics Service (Australian Red Cross Blood Service). A low resolution HLA typing was performed on all samples followed by a high resolution one on a sub-group of selected samples. Direct DNA sequencing was performed on a random sample of samples (20%) to validate the PCR-RFLP method used in this study. The same amplicon was amplified by PCR, followed by cleaning and cycle sequencing using Big-Dye terminators (Life Technologies). All results obtained from re-tested samples confirmed those obtained by PCR-RFLP.

HLA-B genotyping using Luminex-SSO/SBT/SSP revealed 3 homozygous and 4 heterozygous individuals for HLA-B*58:01 allele (allele frequency 0.065) (Table 1). Genotype frequencies determined by PCR-RFLP for the G/A transition (rs9263726) within the PSORS1C1 gene observed were GG (72.7%), GA (25.5%), AA (1.8%). Allele frequencies were 0.15 and 0.85 for the A and G alleles, respectively. The distribution of genotype frequencies were as expected and in Hardy–Weinberg equilibrium ($\chi 2 = 0.0315$; $p = 0.859$; $df = 1$). Analysis for linkage disequilibrium (LD) between rs9263726 A allele and HLA-B*58:01 allele shows that the SNP within the PSORS1C1 gene is not linked with HLA-B*58:01 ($D' = 0.059$; $R^2 = 0.001$). This shows that the two alleles within 2 nearby genes are inherited independently from each other in the Australian cohort. As shown in Table 1, allele B*58:01 was observed in the presence of both G and A alleles of rs9263726. In the same way the A allele was also observed in the absence of HLA-B*58:01 and in the presence of a number of other HLA-B alleles including B*57:01.

Genotype frequencies in our study were very similar to those reported in Chinese-Americans (GG = 80.5%; GA = 18.0%; AA = 1.5%) and Japanese (GG: 81%; GA: 19%; AA: 0) [7]. Unlike these populations, our study shows a lack of LD between rs9263726 A allele and HLA-B*58:01. This observation suggests that this polymorphism (rs9263726) is not useful as a surrogate marker and to be used for pre-screening for HLA-B*58:01 allele, in Australians. The lack of concordance between our study and that reported earlier in the Japanese population [8] is another example of genetic heterogeneity between different populations. This shows that one needs to be cautious using tag SNPs especially to a highly polymorphic region like HLA since LD might differ significantly between different populations and even sub-populations of different ancestral backgrounds, making it difficult for one tagged SNP to be valid and so used for testing in another population [9]. The genetic component of a population is strongly affected by its history and demographics with mutations, genetic drift, population bottlenecks, founder effects and population admixture constantly shaping and changing the genetic component of the population [10–12]. LD is known to extend over longer genetic distances in isolated populations, where there is a lack of admixture with other populations. This was shown clearly in the Finnish population where longer LD intervals were observed in groups of people that live in geographically restricted areas [13]. Population admixture could possibly be the reason why we did not observe LD between these alleles in the Australian cohort.

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