with allopurinol-induced severe cutaneous adverse reactions.

In most HLA genetic association studies, limited by financial resources, a statistically adequate sample size could not be achieved. As a result, many studies suffer from a lack of statistical power to detect and confirm associations involved with low frequency alleles. Importantly, although increasing evidence has revealed the importance of HLA testing prior to the prescription of certain drugs so that adverse and potentially fatal drug reactions could be avoided in patients carrying susceptible HLA genotypes(2, 11), the high costs and a relatively long test turnaround time (TAT) (>1 day) required for obtaining the HLA test results pose a major hurdle to the effective practice of even guidelines advised by US Food and Drug Administration (FDA) (i.e. testing for B*1502 prior to prescription of carbamazepine (CBZ) in Asians to prevent the drug induced Steven Johnson Syndrome/Toxic Epidermal Necrolysis (SJS/TEN)(7). Thus, there is an immense need for the development of a simple, accurate and rapid HLA test at low costs.

HLA genotyping is an expensive test available only in specialized centers. It has also a long test turnaround time (TAT) that normally takes over 1 day. Rapid diagnosis of HLA status to guide drug prescription has not been developed and validated.

A novel nucleic acid amplification method, termed loop-mediated isothermal amplification (LAMP) (5, 6), is capable of amplifying DNA under isothermal conditions with high specificity, efficiency, and speed. The most significant advantage of LAMP is the ability to amplify specific sequences of DNA under isothermal conditions between 63 and 65°C. This ability allows the method to be performed with only simple and cost-effective reaction equipment amenable to use in hospital laboratories.

LAMP has been developed for detection of the severe acute respiratory syndrome coronavirus （SARS-CoV） replicase gene (24), the untranslated region in the genome of hepatitis A virus (HAV) (25), Human Influenza A Viruses DNA (26) etc. However, LAMP has not been developed for human HLA genotyping.

SUMMARY ~~OF THE INVENTION~~

In these directions, we have developed and validated a new testing approach for HLA genotyping based on the LAMP principles.

In a first aspect, there is provided a method for direction of an HLA genotype in an individual, comprising

providing sets A and B of primers, which target different specific regions in the HLA genotype, such as two exons of the genotype, wherein both of sets A and B include a forward inner primer (FIP), a backward inner primer (BIP), two outer primers (a forward primer and a backward primer), and one or two loop primers (a loop forward primer and a loop backward primer);

preparing reaction mixture A comprising set A of primers, a reaction buffer, a DNA polymerase and a whole blood or a DNA purified template obtained from the individual, and reaction mixture B comprising set B of primers, a reaction buffer, a DNA polymerase and a whole blood or a DNA purified template obtained from the individual;

incubating reaction mixtures A and B, respectively, and

detecting products in the reaction mixtures,

wherein the HLA genotype is HLA-B*1502 gene (GenBank assession number: L42145); the primer set A comprises 4 oligonucleotides (SEQ ID NOs. 1-4) which correspond to 6 distinct regions (SEQ ID NOs. 9-14) of HLA-B*1502 exon 2 sequence, and 2 oligonucleotides (SEQ ID NOs. 21-22) which correspond to sequence between SEQ ID NOs. 9 and 10 or 13 and 14 of HLA-B*1502 exon 2 sequence; and primer set B comprises 4 oligonucleotides (SEQ ID NOs. 5-8) which correspond to 6 distinct regions (SEQ ID NOs. 15-20) of HLA-B*1502 exon 3 sequence, and 1 oligonucleotide (SEQ. ID NO. 23) which corresponds to sequence between SEQ ID NOs. 15 and 16 of HLA-B*1502 exon 3 sequence; and

wherein the presence of the products in both reaction mixtures A and B indicates that the individual has the HLA genotype.

In a second aspect, there is provided a method for detection of an HLA genotype associated with susceptibility to drug hypersensitivity in an individual comprising

providing a blood sample obtained from the individual or a purified DNA template thereof,

providing sets A and B of primers, which target different regions specific to the HLA genotype such as two exons of the genotype, wherein both of sets A and B include a forward inner primer (FIP), a backward inner primer (BIP), two outer primers (a forward primer and a backward primer), and one or two loop primers (loop forward primer and loop backward primer);

preparing reaction mixture A comprising set A of primers, a reaction buffer, a DNA polymerase and a whole blood or a DNA purified template obtained from the individual, and reaction mixture B comprising set B of primers, a reaction buffer, a DNA polymerase and whole blood or a DNA purified template obtained from the individual;

incubating reaction mixtures A and B, respectively; and

detecting products in the reaction mixtures,

wherein the HLA genotype is HLA-B*1502 gene (GenBank assession number: L42145); the primer set A comprises 4 oligonucleotides (SEQ ID NOs. 1-4) which correspond to 6 distinct regions (SEQ ID NOs. 9-14) of HLA-B*1502 exon 2 sequence, and 2 oligonucleotides (SEQ ID NOs. 21-22) which correspond to sequence between SEQ ID NOs. 9 and 10 or 13 and 14 of HLA-B*1502 exon 2 sequence; and primer set B comprises 4 oligonucleotides (SEQ ID NOs. 5-8) which correspond to 6 distinct regions (SEQ ID NOs. 15-20) of HLA-B*1502 exon 3 sequence, and 1 oligonucleotide (SEQ. ID NO. 23) which corresponds to sequence between SEQ ID NOs. 15 and 16 of HLA-B*1502 exon 3 sequence; and

wherein the presence of the products in both reaction mixtures A and B indicates that the individual is at increased risk for a hypersensitivity reaction to the drug or experiences a hypersensitivity reaction to the drug.

In a third aspect, there is provided a kit for detection of an HLA genotype in an individual, comprising sets A and B of primers, which target different specific regions in the HLA genotype, such as two exons of the genotype, wherein both of sets A and B include a forward inner primer (FIP), a backward inner primer (BIP), two outer primers (a forward primer and a backward primer), and one or two loop primers (a loop forward primer and a loop backward primer);

a reaction buffer,

a DNA polymerase and

optionally comprising a whole blood or a DNA purified template as a positive control,

wherein the HLA genotype is HLA-B*1502 gene (GenBank assession number: L42145); the primer set A comprises 4 oligonucleotides (SEQ ID NOs. 1-4) which correspond to 6 distinct regions (SEQ ID NOs. 9-14) of HLA-B*1502 exon 2 sequence, and 2 oligonucleotides (SEQ ID NOs. 21-22) which correspond to sequence between SEQ ID

NOs. 9 and 10 or 13 and 14 of HLA-B*1502 exon 2 sequence; and primer set B comprises 4 oligonucleotides (SEQ ID NOs. 5-8) which correspond to 6 distinct regions (SEQ ID NOs. 15-20) of HLA-B*1502 exon 3 sequence, and 1 oligonucleotide (SEQ. ID NO. 23) which corresponds to sequence between SEQ ID NOs. 15 and 16 of HLA-B*1502 exon 3 sequence.

In a fourth aspect, there is provided a kit for detection of an HLA genotype associated with susceptibility to drug hypersensitivity in an individual comprising

sets A and B of primers, which target different specific regions in the HLA genotype, such as two exons of the genotype, wherein both of sets A and B include a forward inner primer (FIP), a backward inner primer (BIP), two outer primers (a forward primer and a backward primer), and one or two loop primers (a loop forward primer and a loop backward primer);

a reaction buffer,

a DNA polymerase and

optionally comprising a whole blood or a DNA purified template as a positive control,

wherein the HLA genotype is HLA-B*1502 gene (GenBank assession number: L42145); the primer set A comprises 4 oligonucleotides (SEQ ID NOs. 1-4) which correspond to 6 distinct regions (SEQ ID NOs. 9-14) of HLA-B*1502 exon 2 sequence, and 2 oligonucleotides (SEQ ID NOs. 21-22) which correspond to sequence between SEQ ID NOs. 9 and 10 or 13 and 14 of HLA-B*1502 exon 2 sequence; and primer set B comprises 4 oligonucleotides (SEQ ID NOs. 5-8) which correspond to 6 distinct regions (SEQ ID NOs. 15-20) of HLA-B*1502 exon 3 sequence, and 1 oligonucleotide (SEQ. ID NO. 23) which corresponds to sequence between SEQ ID NOs. 15 and 16 of HLA-B*1502 exon 3 sequence.

One ~~aspect~~embodiment disclosed herein is directed to a method for detection of an HLA genotype in an individual, comprising

providing sets A and B of primers, which target different regions specific to the HLA genotype, such as two exons of the genotype, wherein both of sets A and B include a forward inner primer (FIP), a backward inner primer (BIP), two outer primers (a forward primer and a backward primer), and one or two loop primers (a loop forward primer and a loop backward primer);

preparing reaction mixture A comprising set A of primers, reaction buffer, DNA polymerase and a whole blood or a DNA purified template obtained from the individual, and reaction mixture B comprising set B of primers, reaction buffer, DNA polymerase and a whole blood or a DNA purified template obtained from the individual;

incubating reaction mixtures A and B, preferably at 63-65 °C for 15-50 min, respectively, and

detecting products in the reaction mixtures,

wherein the presences of the products in both reaction mixtures A and B indicate that the individual has the HLA genotype.

Another ~~aspect~~embodiment disclosed herein is directed to a method for detection of an HLA genotype associated with susceptibility to drug hypersensitivity in an individual comprising

providing a blood sample obtained from the individual or a purified DNA template thereof,

providing sets A and B of primers, which target different regions specific to the HLA genotype such as two exons of the genotype, wherein both of sets A and B include a forward inner primer (FIP), a backward inner primer (BIP), two outer primers (a forward primer and a backward primer), and one or two loop primers (loop forward primer and loop backward primer);

preparing reaction mixture A comprising set A of primers, reaction buffer, DNA polymerase and a whole blood or a DNA purified template obtained from the individual, and reaction mixture B comprising set B of primers, reaction buffer, DNA polymerase and whole blood or a DNA purified template obtained from the individual;

incubating reaction mixtures A and B, preferably at 63-65 °C for 15-50 min,

respectively, and

detecting products in the reaction mixtures,

wherein presences of the products in both reaction mixtures A and B indicate that the individual is at increased risk for a hypersensitivity reaction to the drug or experiences a hypersensitivity reaction to the drug.

In some embodiments of the ~~invention~~disclosure disclosed herein, products in the reaction mixtures can be detected by: (i) visual inspection of turbidity formed in the reaction mixture due to the formation of magnesium pyrophosphate as a by-product of the reaction, (ii) inspection of color change by the addition of a staining agent such as SYBR Green I to the reaction mixture, which binds to double-stranded DNA formed in the reaction to emit a fluorescence; (iii) inspection of color change by the addition of calcein and manganese chloride in the reaction mixture, in which pyrophosphate ions will remove manganese ions from calcein, such that calcein can combine with magnesium ion to emit green fluorescence, and the change of color is inspected visually.

In one embodiment of the ~~invention~~disclosure disclosed herein, blood samples in the reaction mixture are first prepared by heat-treatment. In another embodiment, the whole blood is diluted 1:10 with water and is heated at >95°C for 3-5 minutes.

In one embodiment of the ~~invention~~disclosure, the reaction mixture A comprising two loop primers such as forward loop primer and backward loop primer in the set A of primers is incubated at 63°C for 15-25 min. In another embodiment, the reaction mixture B comprising one or two loop primers such as forward loop primer and/or backward loop primer in the set B of primers is incubated at 63°C for 40-50 min.

In some embodiments of the ~~invention~~disclosure disclosed herein, the HLA genotype is selected from the group consisting of HLA-B*1502 being associated with carbamazepine-specific severe cutaneous reactions and other forms of hypersensitivity, HLA-B*5701 being associated with abacavir hypersensitivity, HLA-B*5801 being associated with allopurinol-induced severe cutaneous adverse reactions, HLA-A29, -B12, -DR7 being associated with sulfonamide-SJS, HLA-A2, B12 being associated with oxicam-SJS, HLA- B59 being associated with methazolamide-SJS, HLA-Aw33, B17/Bw58 being associated with allopurinol- drug eruption, HLA-B27 being associated

with levamisole-agranulocytosis, HLA-DR4 being associated with hydralazine-SLE, HLA-DR3 being associated with penicillamine toxicity, HLA-B38, DR4, DQw3 being associated with clozapine-agranulocytosis, HLA-A24, B7, DQw1 being associated with dipyrone-agranulocytosis. Preferably, the HLA genotype is selected from the group consisting of HLA-B*1502 being associated with carbamazepine-specific severe cutaneous reactions and other forms of hypersensitivity, HLA-B*5701 with abacavir hypersensitivity and HLA-B*5801 with allopurinol-induced severe cutaneous adverse reactions, and preferably being HLA-B*1502.

In one embodiment of the ~~invention~~disclosure, the HLA genotype is HLA-B*1502 gene (GenBank assession number: L42145), the primer set A comprises 4 oligonucleotides (SEQ ID NOs. 1-4) which correspond to 6 distinct regions (SEQ ID NOs. 9-14) of HLA-B*1502 exon 2 sequence, and 2 oligonucleotides (SEQ ID NOs. 21-22) which correspond to sequence between SEQ ID NOs. 9 and 10 or 13 and 14, while primer set B comprises 4 oligonucleotides (SEQ ID NOs. 5-8) which correspond to 6 distinct regions (SEQ ID NOs. 15-20) of HLA-B*1502 exon 3 sequence, and 1 oligonucleotide (SEQ. ID NO. 23) which corresponds to sequence between SEQ ID NOs. 15 and 16.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows LAMP-HB primers for detection of HLA-B*1502. Sequences of primer sets A and B used in the LAMP-HB assay are shown in the tables of Figure 1. In primer set A, SEQ ID NOS:1-4 and SEQ ID NOS: 21-22 are primer; and SEQ ID NOS:9-14 are primer binding site. In primer set B, SEQ ID NOS:5-8 and SEQ ID NO: 23 are primers; and SEQ ID NOS:15-20 are primer binding sites. Locations of primer-binding sites in the exon 2 and 3 of B*1502 sequence are indicated. Examples of other HLA-B allele sequences are aligned with B*1502, where only the mismatched nucleotides that differ from B*1502 are shown (matched nucleotides are represented by "-"). Due to cross-reactions of the primers with other HLA-B alleles, 2 sets of primers are needed to confirm the presence of B*1502 allele in the sample.

Figure 2 shows detection of HLA-B*1502 allele by LAMP-HB. As shown in Figure 2, the colours of the LAMP-HB. Reactions were inspected visually after the addition