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Flow Chart HLA-DQA1 Genotyping and Its Application to a Forensic Case

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ABSTRACT: The detection of genetic polymorphism has become increasingly important in forensic science as well as in medical genetics. In this report, we describe a systematic flow chart system for HLA-DQA1 genotyping by an improved PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) method coupled with the PRSM (PCR-mediated restriction site modification) method. This flow chart typing system can easily discriminate between a total of eight reported DQA1 alleles commonly found in Chinese. We have applied this flow chart typing system in a forensic case as well as in the determination of the frequencies of the eight DQA1 alleles in 121 unrelated Taiwan Chinese subjects. Our results show that the flow chart DQA1 genotyping is a simple, fast, and accurate system which, in the future, may be considered as an alternative method for routine individual identification in forensic casework, and for paternity testing and tissue typing in medical genetics.

KEYWORDS: forensic science, forensic evidence, HLA-DQA1 genotyping, PCR-RFLP

Genetic polymorphisms, as detected by protein and DNA variants, have been widely used for medical and forensic purposes. However, many biological samples encountered as criminal evidence are complicated by the fact that forensic specimens are usually available in small quantity and are commonly deposited on the surface of a variety of objects.

The HLA class II genes (HLA-DR, -DQ, -DP), located on the short arm of chromosome 6, code for a series of highly polymorphic and heterodimeric proteins involved in regulation of the immune response [*1,2*]. Most of the polymorphism of HLA class II genes is located at the second exon encoding the amino-terminal outer domain [*2*]. HLA genotyping by conventional serological or cellular methods has been widely applied in paternity testing, individual identification, tissue typing, and genetic susceptibility in specific autoimmune diseases [*3–5*]. However, molecular typing at the DNA level is superior to conventional methods due to the stability and high degree of variability of the DNA molecule. In addition, accurate and complete HLA class II genotyping in conventional methods is totally dependent on the specificity, quantity, and sensitivity of a complete set of alloantisera or monoclonal antibodies which are not commonly available in a regular forensic lab.

Recently, several methods, including PCR (polymerase chain reaction)-direct sequencing [*6*], PCR-ASO (allele specific oligonucleotide) hybridization [*7*], and PCR-RFLP (restriction fragment length polymorphism) [*8*], have been developed for HLA class II genotyping at the DNA level. However, the PCR-direct sequencing method appears to be a tedious way for a forensic lab to exclude large numbers of suspects and specimens. Furthermore, the use of the PCR-ASO method is restricted by the fact that it needs many kinds of ASO probes whose individual washing conditions have to be precisely determined after hybridization [*7,9,10*].

An alternative way of HLA class II genotyping is the PCR-RFLP method originally described by Maeda et al. [*8*]. This method can distinguish most HLA class II genotypes by digestion of PCR-amplified DNA products with allele-specific restriction endonuclease. The PCR-RFLP method has successfully been applied to determine various alleles in HLA-DQA1 [*8*] and other HLA class II loci [*11,12*] in Japanese populations. However, the method Maeda et al. described [*8*] for DQA1 typing needs to digest the PCR-amplified DNAs with all 5 restriction enzymes independently, which may be cumbersome for a large screening test to exclude a suspect from a forensic investigation or in donor/acceptor HLA genotype matching in organ transplantation. Furthermore, the method cannot easily discriminate between the DQA1*0101 allele and the DQA1*0102 allele.

We [*13–15*] and others [*16–18*] have recently identified that at least nine different point mutations are responsible for G6PD (glucose-6-phosphate dehydrogenase) deficiency in Chinese. In addition, we have also described several methods, including PCR-RE (restriction enzyme) digestion [*13*], PCR-ASO (allele-specific oligonucleotide) hybridization [*10*], and PRSM (PCR-mediated restriction site modification) [*14,19*], for quick diagnosis of these G6PD mutations at the DNA level. For known mutations that generate or remove RE sites, the normal and mutant gene can be easily distinguished by the PCR-RE digestion [*13*]. However, for mutations which do not naturally create or remove RE sites, the PRSM [*14,19*] and PCR-ASO [*10*] methods can be applied for quick diagnosis of known G6PD mutations. In our experience, the PRSM method is more convenient and superior to the PCR-ASO method [*19*], and we will not labor the point here. The general principle of the PRSM method is that an artificial restriction site is introduced into the mutant gene by adding a primer set (one is normal; the other carries a mismatched base) into the PCR reaction

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mixture. After PCR amplification, the mismatched base accompanying the point mutation nearby may create a restriction site that is suitable for distinguishing normal from mutant alleles. Using these approaches, we have successfully detected all known G6PD point mutations in Chinese at the DNA level. A strategy similar to the PRSM method has also been described to diagnose several other genetic diseases [*20,21*].

In this report, we describe a systematic flow chart system for the genotyping of a total of eight reported DQA1 alleles (including DQA1*0101 and *0102) by an improved PCR-RFLP method coupled with the PRSM method described above. This flow chart typing system may greatly improve the original PCR-RFLP method to provide a simple, fast, and accurate method for routine genotyping of HLA-DQA1 alleles in the future.

Materials and Methods

Chinese Subjects and DNA Samples

According to the most recent nomenclature system, the locus previously known as DQα has now been renamed as DQA1, and its 8 different allelic designations are DQA1*0101 (previously A1.1), DQA1*0102 (previously A1.2), DQA1*0103 (previously A1.3), DQA1*0201 (previously A2), DQA1*0301 (previously A3), DQA1*0401 (previously A4.2), DQA1*0501 (previously A4.1), and DQA1*0601 (previously A4.3) [*22*].

A total of 121 unrelated Chinese subjects were recruited from sources as noted below. Sixty-eight subjects were students of the Central Police University (Taoyuan, Taiwan), and originated from all parts of Taiwan; 32 subjects were recruited from Chang Gung Memorial hospital (Taoyuan, Taiwan); and 21 were recruited from Jen-A1 Municipal hospital (Taipei, Taiwan). The genomic DNA was extracted from the peripheral blood of these 121 Chinese subjects either by the phenol/chloroform [*23*] or the salt-chloroform methods [*24*].

The hair DNA was obtained from seven hairs growing from a small piece of head skin left at the scene. The hair shaft DNAs were extracted as described by [*25*]. Briefly, the hair shafts (prewashed in distilled water) were placed in a microcentrifuge tube containing 100 μL rapid hair-digestion buffer (10 mM Tris-HCl, pH 8.3, 2.5 mM $MgCl_2$, 50 mM KCl, 0.45% NP40, 0.45% Tween 20, 50 μg/mL proteinase K). The hair-containing mixture was incubated at 56°C for 1 h, then heated at 95°C for 10 min. After the final heating, the mixture was subjected to vortex mixing and the undigested material was spun down. The supernatant was directly used for PCR amplification.

*PCR Amplification and the PRSM Primers Designed to Discriminate Between the DQA1*0101 and DQA1*0102 Alleles*

Exon 2 of the HLA-DQA1 gene was PCR amplified with Q1 (5′-TTGTGGTGTAAACTTGTACCAGT-3′) and Q2 (5′-CCTCATTGGTAGCAGCGGTAGA-3′) primers on the basis of the published nucleotide sequence, as described [*26*] (Fig. 1). The total length of the PCR-amplified products for DQA1*0101, *0102, *0103, and *0301 alleles is 236 basepairs (bp) and for DQA1*0201, *0501, *0401, and *0601 alleles is 233 bp. The PCR reaction was carried out in a final volume of 100 μL reaction mixture containing 1 μg DNA, 10 mM Tris-HCl pH 8.3, 1.5 mM $MgCl_2$, 50 mM KCl, 0.1% Triton X-100, 200 μM of each dNTP, 100 ng of each primer, and 2.5 units of Taq polymerase (Promega). The reaction was performed on a DNA thermal cycler (Cetus) as follows: denaturation for 35 cycles at 94°C for 40 sec, annealing at 58°C for 1 min, and elongation at 72°C for 1 min.

In order to differentiate DQA1*0101 (nucleotide position 49 is G) from DQA1*0102 (nucleotide position 49 is C) allele (see Fig. 1), the PRSM primer F1 (5′-ATGAATTTGATGGAGATGCG-3′) was synthesized by introducing a mismatched base C at nucleotide position 47 in exon 2 of the DQA1 gene (Fig. 1). This mismatched base C (nt position 47) accompanying the C at nucleotide position 49 in the DQA1*0102 gene may create a Fsp I restriction site (TGCGCA). The verification of DQA1*0101 or DQA1*0102 alleles was analyzed by PCR amplification (primers F1/Q2) and Fsp I restriction enzyme mapping. PCR amplification was performed in a 35 cycle program by denaturation at 94°C for 40 sec, annealing at 55°C for 30 sec, and extension at 72°C for 30 sec. The presence of an intact PCR-amplified fragment (186 bp) *vs.* the Fsp I digested fragments (167 bp, 19 bp) represented the DQA1*0101 *vs.* the DQA1*0102 allele, respectively (Table 1).

Restriction Enzyme Mapping and Agarose Gel Analysis

After PCR amplification, aliquots of the reaction mixture were digested with appropriate restriction enzymes and analyzed on a 4% agarose gel (NuSieve 3:1; FMC BioProducts) containing ethidium bromide [*13*]. Determination of the digestion order of each restriction enzyme is described in detail in the Results section. The polymorphic fragments detected by each specific restriction enzyme digestion are shown in Table 1.

DNA Sequencing and a Commercial Kit for HLA-DQA1 Genotyping

The PCR-amplified products were first purified with Magic PCR Preps systems according to the manufacturer (Promega) and sequenced as previously described [*13*]. The AmpliType HLA DQα typing kit was also applied to specimens from a forensic case. The experimental procedures were as described by the manufacturer (Perkin-Elmer Cetus).

Results

Searching for the Allele Specific Restriction Site in Exon 2 of the DQA1 Gene

The nucleotide sequences of the exon 2 region in the human HLA-DQA1 gene from eight different alleles have been published [*6,27*] and are shown in Fig. 1. These sequences were used as a main frame to search for specific restriction enzyme (RE) sites. Based on computer analysis, five different restriction enzymes (Msc I, ApaL I, Rsa I, Fok I, and Dde I) were selected and will later be used for distinguishing various DQA1 alleles by PCR-RFLP analysis. The precise location of each RE site is shown in Fig. 1. Unfortunately, these five REs can distinguish only 6 different alleles (DQA1*0103, *0201, *0301, *0401, *0501, *0601) of the HLA-DQA1 gene (Fig. 2). In order to discriminate between two other alleles (DQA1*0101 and *0102), the PRSM method was performed. The principle of PRSM has been described in detail in materials and methods. By this approach, PCR-amplified products generated by the PRSM primers may artificially create a Fsp I site in the DQA1*0102 allele, but not in the DQA1*0101 allele. Therefore, the presence or absence of the Fsp I site will be useful for distinguishing between these two alleles (Table 1).

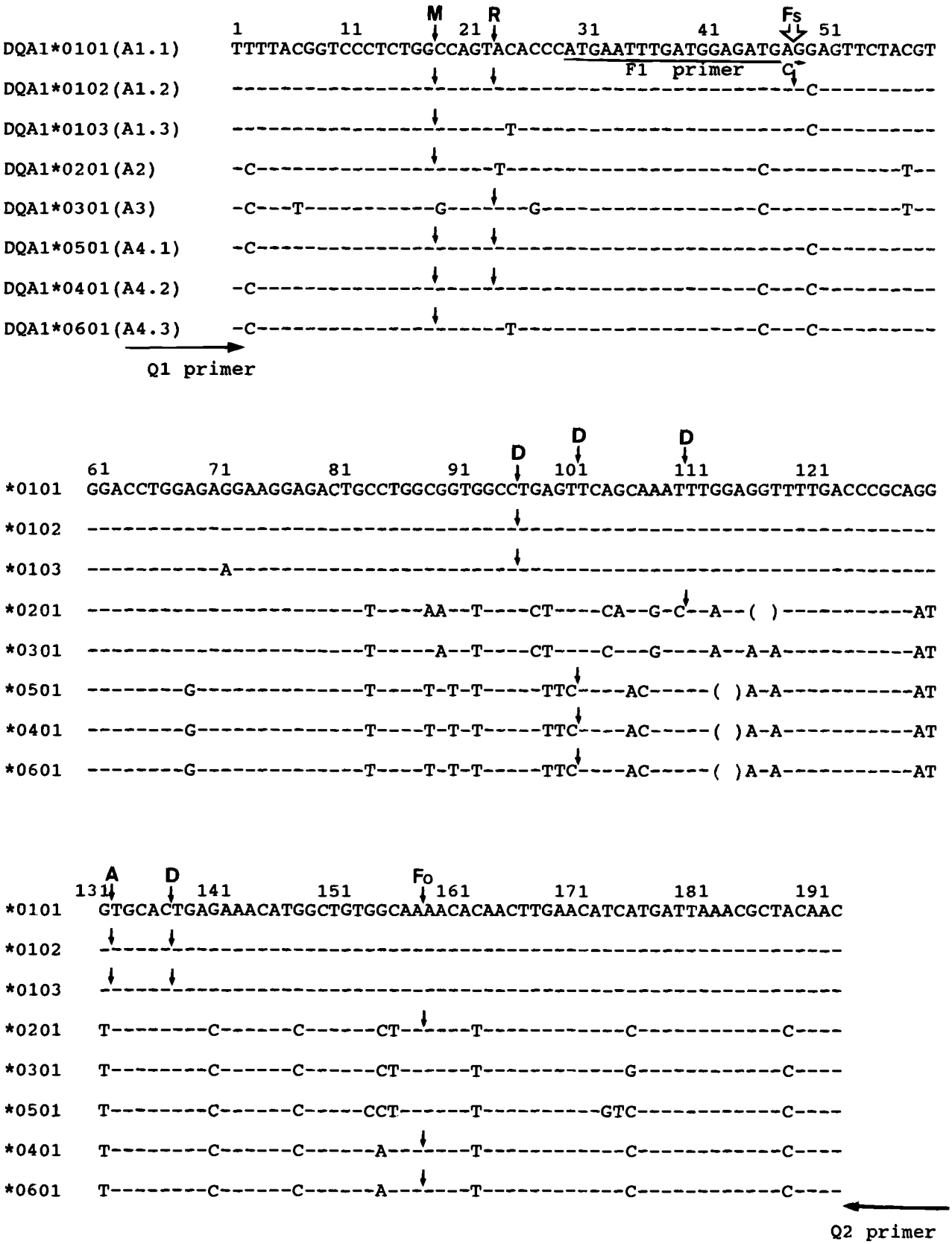


FIG. 1—*Summary of the nucleotide sequences and the restriction maps in the PCR-amplified exon 2 region of eight different DQA1 alleles. The nucleotide sequences of various DQA1 alleles are from Ref. 6 and Ref. 27. ( ) represents three base deletions. Restriction enzymes M: Msc I, R: Rsa I, Fs: Fsp I, Fo: Fok I, D: Dde I, A: ApaL I.*

TABLE 1—*Summary of the DQA1 polymorphic patterns and the DNA fragment sizes digested by various restriction enzymes.*

Allele	Restriction enzymes					
	MscI	ApaLI	RsaI	FokI	DdeI	FspI
	+	+	+	−	+−−+	−
DQA1*0101	39 197	153 83	44 192	236	117 41 78	186
	+	+	+	−	+−−+	+
DQA1*0102	39 197	153 83	44 192	236	117 41 78	19 167
	+	+	−	−	+−−+	+
DQA1*0103	39 197	153 83	236	236	117 41 78	19 167
	+	−	−	+	−−+−	−
DQA*0201	39 194	233	233	176 57	131 102	183
	−	−	+	−	−−−−	−
DQA1*0301	236	236	44 192	236	236	186
	+	−	+	−	−+−−	+
DQA1*0501	39 194	233	44 189	233	122 111	19 164
	+	−	+	+	−+−−	−
DQA1*0401	39 194	233	44 189	176 57	122 111	183
	+	−	−	+	−+−−	−
DQA1*0601	39 194	233	233	176 57	122 111	183

NOTE: The total length of the PCR-amplified products for DQA1*0101, *0102, *0103, and *0301 alleles is 236 bp and for DQA1*0201, *0501, *0401, and *0601 is 233 bp. The size difference is due to a 3 base deletion present in the latter 4 DQA1 alleles (Fig. 1). The markers "+" and "−" represent the presence or absence of a restriction site, respectively. The Dde I digested fragments (102 + 131, *vs.* 111 + 122) were distinguishable from each other when the positive DNA samples 0201 (102 + 131) and 0401 (111 + 122) were used as control references.

Flow Chart Analysis of Various HLA-DQA1 Alleles

To determine the digestion order of each specific restriction enzyme, we first calculated the frequency of eight HLA-DQA1 alleles in sixteen randomly selected Chinese subjects. Each DQA1 allele was confirmed by DNA sequencing. Among these 16 subjects (32 chromosomes) examined, 16 chromosomes (50%) had DQA1*0301 allele, 6 had DQA1*0101 allele, 5 had DQA1*0102 allele, 4 had DQA1*0501 allele, and 1 had DQA1*0401 allele. The results from these prescreening studies were then used as a basis for designing a systematic flow chart map to analyze various DQA1 alleles (Fig. 2). For example, the Msc I restriction enzyme was first selected in our flow chart map to detect the allele-specific RFLP, since our preliminary results showed that the DQA1*0301 allele had the highest frequency rate (50%) in Taiwan Chinese and that the absence of a Msc I digestion site is restricted to the DQA1*0301 allele (Table 1). After Msc I digestion, the ApaL I was then selected as a second enzyme for cleavage, since the presence or absence of ApaL I site in the DQA1 gene can be further categorized into two different groups. One carries DQA1*0101, *0102, and *0103 alleles; the other carries DQA1*0201, *0401, *0501, and *0601 alleles. Following this principle, the systematic flow chart system for the determination of 8 different HLA-DQA1 alleles is depicted in Fig. 2, whereas the polymorphic pattern of each DQA1 allele detected by digestion with various restriction enzymes is shown in Table 1.

Using this strategy, we show an example for identifying a Chinese subject heterozygous for DQA1*0201/*0301 allele in Fig. 3. The presence (194-bp/39-bp fragments) and absence (236-bp fragment) of the Msc I site indicated that this subject carries both a DQA1*0301 and a non-*0301 allele (Table 1, Fig. 3*a*, lane 2). The 39-bp fragment is too small to be detected under our gel system. The non-*0301 allele was further analyzed by ApaL I digestion as the flow chart system described (Fig. 2). The lack of ApaL I (233-bp fragment; Table 1; Fig. 3*a*, lane 3) and the presence of Fok I sites (176-bp/57-bp fragments; Table 1; Fig. 3*a*, lane 4) indicated that this subject should carry one of three DQA1 alleles (*0201, *0401, or *0601) (Fig. 2). Further analysis by Dde I digestion (Fig. 3*a*, lane 5), the (− − + −) digestion pattern lead us to conclude that this subject carries the DQA1*0201 allele (Table 1). Taken together, our results indicate that this subject is

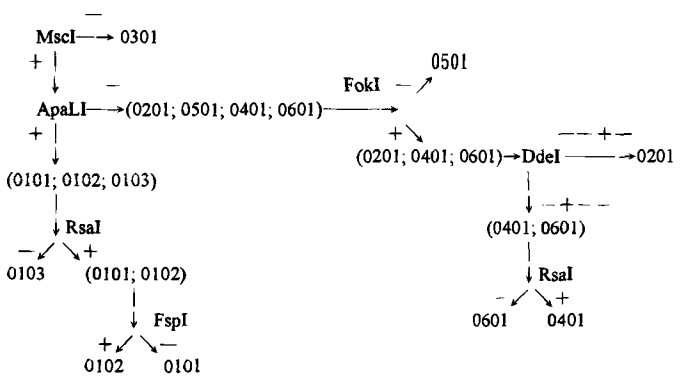
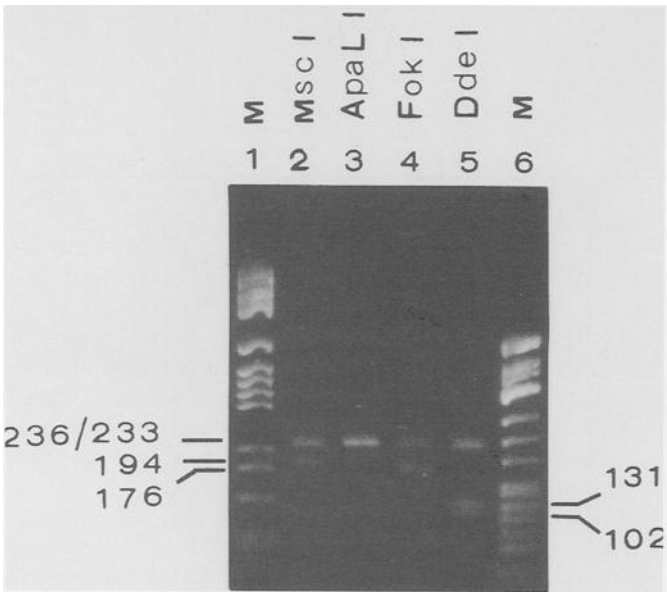


FIG. 2—*Summary of the flow chart system for HLA-DQA1 genotyping. The markers "+" and "−" represent the presence or absence of a restriction site. There are four Dde I sites present in the PCR-amplified fragment of the DQA1 gene. The markers "− + − −" and "− − + −" indicate that the Dde I site is only present in the second and the third position of the amplified product, respectively.*

heterozygous for DQA1*0201/*0301 allele. The above finding is further confirmed by DNA sequencing analysis. The flow chart system was also applied to distinguish a heterozygote who carries DQA1*0101 and *0102 alleles. The presence of all three RE sites (Msc I, ApaL I, and Rsa I) (Table 1) indicated that this subject carries either DQA1*0101 or DQA1*0102 allele (Fig. 2; Fig. 3*b*, lanes 3–5). Using the PRSM strategy described above, the presence (167-bp and 19-bp fragments) and absence (186-bp fragment) of Fsp I site indicated that this subject is heterozygous for DQA1*0101/*0102 alleles (Fig. 3*b*, lane 7). The 19-bp fragment is too small to be detected.

The Application of Flow Chart DQA1 Genotyping in a Forensic Case

The flow chart DQA1 genotyping method has been successfully applied to a forensic case to exclude the possibility of a suspect being involved in a murder case (Fig. 4). A car accident had caused one driver to shoot another. The murderer escaped from the broken front window of the car, but left a few hairs with visible sheath at the scene of the crime. The DNA was extracted from the hair sample left at the scene, as well as from the peripheral blood of a suspect. The presence of a Msc I site in the hair sample (Fig. 4, lane 2) but not in the peripheral blood sample (Fig. 4, lane 5) excluded the possibility of this suspect being involved in this murder event. Further analysis by ApaL I (lane 3) and Fok I (lane



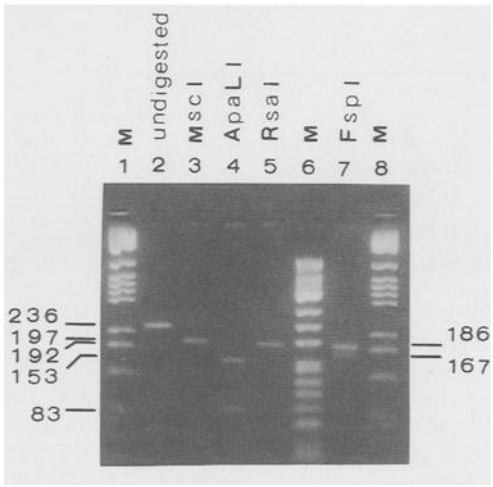


FIG. 3—(a) *An example for flow chart genotyping of a DQA1*0201/DQA1*0301 heterozygote. The PCR-amplified DNA from the DQA1 exon 2 region of this individual was digested with Msc I (lane 2), ApaL I (lane 3), Fok I (lane 4), and Dde I (lane 5), respectively. The digested DNA fragments were analyzed by agarose gel electrophoresis. Lane 1 and 6 are the pGEM DNA and* ϕ *x* 174 Hinf I size markers, respectively; (b) *An example for flow chart genotyping of a DQA1*0101/DQA1*0102 heterozygote. The DQA1*0101 allele can be discriminated from the DQA1*0102 allele using the PRSM strategy (see text for detail). The PCR-amplified DNA from the DQA1 exon 2 region of this individual (lane 2; undigested DNA) was digested with Msc I (lane 3), ApaL I (lane 4), Rsa I (lane 5), and Fsp I (lane 7), respectively, and analyzed as described in* (a). *Lane 1 and 8 are the pGEM size marker. Lane 6 is the* ϕ *x* 174 Hinf I size marker.

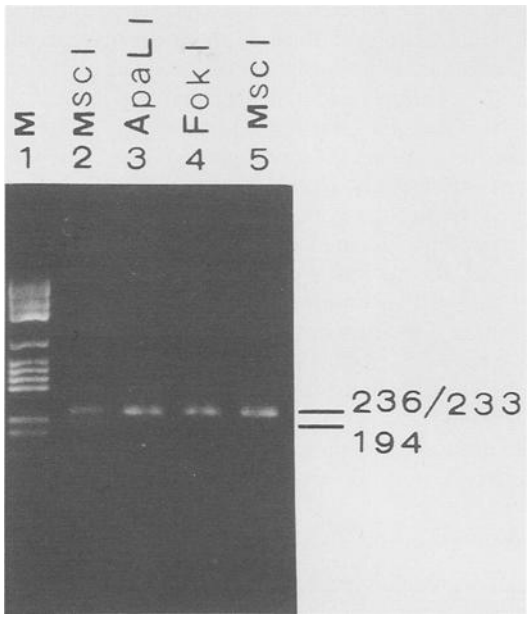


FIG. 4—*Flow chart DQA1 genotyping applied in a forensic case. The DNA extracted from the hair sheath was PCR amplified as described in Materials and Methods. The PCR-amplified hair DNA was then digested with Msc I (lane 2), ApaL I (lane 3), and Fok I (lane 4), respectively. Lane 5 is the PCR-amplified DNA from the peripheral blood of a suspect, digested with Msc I. Lane 1 is a pGEM DNA size marker. The hair sample (lanes 2–4) and the suspect's DNA (lane 5) were typed as DQA1*0301/*0501 and DQA1*0301/*0301, respectively.*

4) digestion revealed that no such restriction sites exist in the hair DNA, which indicated that the hair sample is heterozygous for DQA1*0301/*0501 alleles (Fig. 2). In addition, the absence of a Msc I site in the suspect's DNA indicated that this suspect is homozygous for DQA1*0301/*0301 alleles (Fig. 4, lane 5). The above results were further confirmed using the Cetus HLA-DQα typing kit (Fig. 5) in which the hair sample and the suspect's DNA were typed as DQA3/A4 and DQA3/A3 (old nomenclature), respectively. Therefore, the commercial kit system can discriminate only six DQA1 alleles (DQA1.1, A1.2, A1.3, A2, A3, and A4)

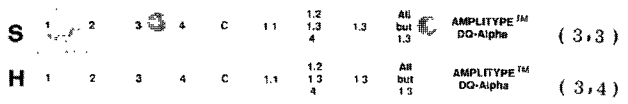


FIG. 5—*An example of the commercial DQA1 typing kit (Perkin-Elmer Cetus) applied in a forensic case. The PCR-amplified DNAs from the hair sheath sample (H) and the suspect (S) were hybridized with various ASO probes that had previously been spotted on the probe strips. The experimental conditions were as described by the manufacturer. The hair sample and the suspect's DNA were typed as DQA3/A4 (3,4) and DQA3/A3 (3,3), respectively.*

and 21 possible genotypes, whereas our modified PCR-RFLP method not only can detect eight DQA1 alleles, a total of 36 possible genotypes, but also can further classify the DQA4 into DQA4.1 (DQA1*0501) genotype.

The HLA-DQA1 Allele and Genotype Frequencies in Taiwan Chinese

Using this flow chart typing system, we have determined the HLA-DQA1 allele and genotype frequencies in 121 unrelated Taiwan Chinese (that is, in 242 chromosomes). Table 2 shows that DQA1*0301 is the most common allele (35.1%) found in Taiwan Chinese, since 85 out of 242 chromosomes examined carried this allele. The DQA1*0301 plus two other alleles (DQA1*0102 and DQA1*0501) are three major alleles that account for nearly 70% of total subjects examined. In general, the DQA1 allele frequency reported here is similar to that described in the Japanese [*28*], except that the A1.3 allele (DQA1*0103) frequency in Taiwan Chinese is significantly lower (9.5%, *vs.* 22.8% in Japanese), whereas frequencies of two other alleles (A1.3 and A4) are higher (by comparison of our Table 2 with reference 28). The power of discrimination (PD) and the allelic diversity (h) values for DQA1 alleles were also calculated according to Helmuth et al. [*28*]. The PD and h values in this study are 0.93 and 0.80, respectively.

Discussion

Forensic biological evidence left at the scene of a crime is always present under various adverse environmental conditions in which intact cells are usually broken down and degraded, which

TABLE 2—*The DQA1 allele and genotype frequencies of 121 Chinese subjects in Taiwan.*

HLA-DQA1 Population Frequency of Chinese in Taiwan			
Allele	Frequency (%)	Genotype	Frequency (%)
DQA1*0101	8.2	DQA1*0301/*0101	5.8
DQA1*0102	16.1	DQA1*0301/*0102	12.4
DQA1*0103	9.5	DQA1*0301/*0103	6.6
DQA1*0201	2.5	DQA1*0301/*0201	3.3
DQA1*0301	35.1	DQA1*0301/*0301	12.4
DQA1*0501	17.8	DQA1*0301/*0401	1.7
DQA1*0401	2.9	DQA1*0301/*0501	9.1
DQA1*0601	7.9	DQA1*0301/*0601	6.6
		DQA1*0201/*0501	0.8
		DQA1*0201/*0103	0.8
		DQA1*0103/*0501	4.1
		DQA1*0501/*0601	1.7
		DQA1*0501/*0101	3.3
		DQA1*0501/*0102	7.4
		DQA1*0501/*0103	5.0
		DQA1*0401/*0101	0.8
		DQA1*0401/*0102	1.7
		DQA1*0401/*0103	1.7
		DQA1*0601/*0601	0.8
		DQA1*0601/*0101	1.7
		DQA1*0601/*0102	3.3
		DQA1*0601/*0103	0.8
		DQA1*0101/*0101	0.8
		DQA1*0101/*0102	2.5
		DQA1*0101/*0103	0.8
		DQA1*0102/*0102	1.7
		DQA1*0102/*0103	1.7
		DQA1*0103/*0103	0.8

Total n = 121 Chinese subjects (242 chromosomes).

may obstruct serological or cellular characterization. In contrast, DNAs, as compared with intact cells or cellular proteins, may be preserved and last for a longer time in a more harsh environment.

The highly polymorphic HLA class II loci provide a good genetic marker for forensic investigation. The PCR-RFLP and PCR-ASO are two methods that have been commonly used for genotyping the HLA-DQA1 [*7,8*] as well as other class II loci [*11,12,29,30*] at the DNA level. The advantages of the PCR-RFLP method over the PCR-ASO or PCR-SSO method have been described in detail [*12,31*]. In this report, we describe a flow chart system for the genotyping of a total of eight different DQA1 alleles by an improved PCR-RFLP method coupled to the PRSM method. This flow chart system provides at least three advantages over the conventional PCR-RFLP method.

The most significant point in this method is that we have used a flow chart typing system to key-index each specific DQA1 allele. The DQA1 allele frequency in a Chinese population was first determined and provided a basis for establishing a flow chart map (that is, the restriction enzyme digestion order) for later PCR-RFLP analysis. For future application of this system, we suggest that the PCR-amplified products should be first digested with the group 1 restriction enzymes (that is, Msc I and ApaL I) because the presence or absence of the group 1 RE sites divides the Chinese into three subgroups, group A (DQA1*0301) (35.1%), group B (DQA1*0101, *0102, and *0103) (33.8%), and group C (DQA1*0201, *0401, *0501, and *0601) (31.1%), with similar frequency distribution. This piece of information may help us to decide what group 2 enzymes [(Fok I, Dde I, Rsa I) *vs.* (Rsa I, Fsp I)] should be selected for later analysis (Fig. 2). Furthermore, the flow chart system described here is suitable for DQA1 genotyping not only in Chinese but also in other human populations, as described by Helmuth et al. [*28*]. However, in Nigerian and Indonesian populations, we suggest that ApaL I should be selected as the first enzyme for digestion, since the DQA1*0301 allele frequency in these two populations is very low (Nigerian, 0%; Indonesian, 5.6%) [*28*]. Using our flow chart system, we can quickly determine the specific DQA1 allele in a large number of unknown subjects without performing the complete digestion procedure described by Maeda et al. [*8*]. Under this consideration, our system would save the money needed to purchase all the restriction enzymes necessary for DQA1 typing by Maeda et al.'s method. In addition, in order to apply our flow chart system in forensic cases in the future, we have determined that the minimum amount of DNA for a complete flow chart typing procedure can be reduced to as little as 50 ng.

Second, the PCR-RFLP method originally described by Maeda et al. [*8*], can not distinguish DQA1*0101 and DQA1*0102 alleles easily. Although the authors later reported that the availability of Mnl I enzyme holds the possibility of discriminating between DQA1*0101 and *0102 alleles, in our experience the PCR-RFLP patterns of DQA1*0101 and *0102 alleles defined by Mnl I digestion are quite similar. Their differences can only be distinguished by a more complicated acrylamide gel electrophoresis. In order to overcome this problem, we have incorporated the PRSM method (see materials and methods) into our flow chart typing system. This type of combination has allowed us easily and specifically to discriminate between DQA1*0101 and DQA1*0102 alleles (Fig. 3*b*).

Finally, on the basis of a computer search we have selected 6 different restriction enzymes (ApaL I, Dde I, Fok I, Fsp I, Msc I, and Rsa I) for our flow chart typing system. Among these, only three enzymes overlap with those reported by Maeda et al. [*8*]. The reason why we selected these six restriction enzymes is that the PCR-RFLP patterns defined by these enzyme digestions can be easily separated and detected by regular agarose gel electrophoresis, which is a simple and basic technique available in a regular forensic lab. Therefore, the DQA1 genotyping using the more complicated acrylamide gel electrophoresis can be avoided.

We have successfully applied our improved flow chart typing system in forensic casework to exclude a specific suspect in a murder case. In addition, we have used this typing system to determine the allele frequency of a total of eight DQA1 alleles in Taiwan Chinese. This information may be suitable for population studies and as a forensic control reference in the future. In this report, we have described a simple, fast, and accurate flow chart system for DQA1 genotyping. Our system will greatly enhance the conventional serological and cellular methods in routine typing work and may also be applied to typing other HLA class II loci in the future.

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