TECHNICAL NOTE

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Genotyping of the DQA1*4 Alleles by Restriction Enzyme Digestion of the PCR Product from the AmpliType® PM Kit

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ABSTRACT: An earlier study has shown that the three DQA1*4 alleles (0401, 0501 and 0601) can be distinguished by restriction enzyme digestion of the polymerase chain reaction (PCR) product derived from the DQα AmpliType™ kit (Perkin-Elmer, Norwalk, NJ). We have found that the AmpliType® PM kit (Perkin-Elmer, Branchburg, NJ) can also be used to achieve the same goal. In this case, a Bio-Profil image analysis system (Vilber Lourmat, Marne La Vallee, France) is used for evaluating the restricted patterns. After typing the six alleles of DQA1 by the AmpliType HLA DQ α Detection Reagent Set (Perkin-Elmer, Branchburg, NJ), the PCR products from the PM kit with allele 4 were digested with Fok I and Rsa I, separately. Since the other five fragments from PM kit will conceal the digested fragments of the HLA DQA1 PCR products, we measured the optical density of the pre- and post-digested 242 bp fragments in Fok I digestion, and 214/221 bp fragments in Rsa I digestion to decide the results of enzyme digestion. Out of 136 samples used in this study, 61 contain the DQA1 allele 4 determined by the DQα AmpliType™ method. All 61 were typed with enzyme digestion, of which there are 2.3%, 19.8% and 8.1% in allele 0401, 0501 and 0601, respectively. Our procedure can thus extend the utilization of AmpliType® PM kit and increase the discrimination power of the DQA1 system, especially in populations with high distribution of allele 4.

KEYWORDS: forensic science, criminalistics, polymerase chain reaction, DQA1, restriction endonuclease digestion

Fok I and Rsa I restriction enzyme digestion have been used to type the three DQA1*4 alleles—4.2 (0401), 4.1 (0501) and 4.3 (0601)—for HLA DQA1 genotyping [*1–3*]. These three alleles can also be typed directly from the PCR product obtained with the DQα AmpliType™ kit (Perkin-Elmer, Norwalk, NJ) [*4*]. In our laboratory, we found the AmpliType® PM Kit (Perkin-Elmer, Branchburg, NJ) useful for the analysis of forensic samples and have applied this kit to study the distribution of six genetic markers (HLA DQA1, LDLR, GYPA, HBGG, D7S8 and GC) in the Chinese population in Taiwan. Since the occurrence frequency of allele 4 of DQA1 is high (30.2%), further genotyping of allele 0401, 0501 and 0601 is valuable. In this study, we distinguished these alleles directly from the PCR product of the AmpliType® PM kit using Fok I and Rsa I restriction enzyme digestions and the Bio-Profil image analysis system (Vilber Lourmat, Marne La Vallee, France). Two portions of post-PM amplification products were digested with Fok I and Rsa I. The resulting products were electrophoresed along with the undigested PCR products, using a digested allele 0201/0601 as the control. Absence of the 242 bp band in the Fok I digested control is indication of a complete digestion process. Under this circumstance, the optical density of the 242 bp band in the digested lane relative to that from the undigested lane reveals the results of Fok I digestion. Similarly, absence of the 190 bp band and double optical density of the 214/221 bp band in the Rsa I digested control serve as indicators of a complete digestion process. The relative optical density of the 214 bp band observed in the undigested lane and the 214/221 bp band in the digested lane reveal the results of Rsa I digestion. Allele 0401, 0501 and 0601 can then be determined based on the above mentioned results derived from the Fok I and the Rsa I digestion process.

Materials and Methods

DNA was extracted from 136 blood samples obtained from Taipei Jen-Ai Hospital (Taipei, Taiwan) and Central Police College (Taoyuan, Taiwan) by salt-chloroform method [*5*]. All genomic DNA were dissolved in sterile deionized water and kept in −20°C until used.

*PM Markers Amplification and DQA1 and DQA1*4 Alleles Typing*

The protocols provided with the AmpliType PM kit were followed for PCR amplification. In order to type successfully, the intensity of 242 bp band of DQA1 should be as strong as the others when visualized in minigel electrophoresis. Thirty-five microliter-aliquots of PCR products were used to perform the genotyping of DQA1. This typing was done with the AmpliType HLA DQα Detection Reagent Set (Perkin Elmer, Branchburg, NJ), using the protocols provided with the AmpliType PM kit.

All samples with allele 4 were subjected to the restriction enzyme digestion. Ten microliter-aliquots of PCR products from the AmpliType® PM kit were digested by 5 units of Fok I and Rsa I (Promega, Madison, WI), respectively, for four hours in an incubator at 37°C. A sample of DQA1* 0201/0601 was used as the control to monitor the completeness of the enzyme digestion process. The digested

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TABLE 1—*The optical density[a] (unit pixel × pixel × brightness) of the 242 and 214/221 bp bands in Fig. 1 (scanning with the Bio-Profil image analysis system).*

	Lanes in Fig. 1					
	1	2	3	4	5	6
242 bp band in Fok I	32510	32273[b]	33806	0	35918	35010[b]
214/221 bp band in Rsa I	32495	34657[b]	29235	54016[c]	32024	32150[b]

[a]The difference of the optical density is treated in multiples of the original intensity.
[b]Remains the same optical density.
[c]The observed optical density considered doubled—much closer to the doubled than the original value.

products were electrophoresed simultaneously with undigested products and the control in 4% NuSieve 3:1 agarose (FMC Bioproducts, Rockland, ME) gel containing 1 μ g/mL ethidium bromide in 1X TBE buffer for 1 hour at 100 volts. The gel was then photographed under UV light.

Analysis of Restriction Patterns of DQA1 Following Enzyme Digestion

The optical density (unit pixel × pixel × brightness) of restriction patterns were scanned with a Bio-Profil image analysis system. In Fok I digestion, the optical density of the 242 bp undigested and digested bands were scanned. Since there is a cutting site in the primer for DQA1 gene fragments, the 221 bp fragments is used as the marker to determine the results of Rsa I enzyme digestion. Therefore, in Rsa I digestion, the 214 bp undigested band of LDLR gene and 214/221 bp mixed bands were scanned. The difference of the optical density between these two bands can be used to determine the results of Fok I and Rsa I respectively. The results of this scanning test were interpretable only when the control sample of allele 0201/0601 was complete digested.

Results

Sixty-one of the 136 samples used for DQα genotyping were found to have allele 4 as determined by the DQα AmpliType™ method. Figure 1 is an example of the resulting restriction patterns. Table 1 is the scanning results of the optical density from Fig. 1. As listed in Table 2, allele 0401, 0501 and 0601 can be decided by comparing of the results of Fok I and Rsa I digestion.

In Fok I digestion, the 242 bp fragment of DQA1 gene disappeared when fully digested, the optical density of this fragment reduced to one-half with half digestion, and remained the same with no digestion. In Rsa I digestion, The 221 bp fragment cannot be resolved from the 214 bp fragment of LDLR gene in this minigel electrophoresis. When fully digested with Rsa I, the optical density of the 214/221 bp band remained the same optical density as 214 bp undigested product. With half digestion, the optical density of

TABLE 2—*Restriction patterns of DQA1 alleles* [1–3] *digested from PCR product produced by DQα AmpliType™ kit with Fok I and Rsa I enzyme.*

	Fok I			Rsa I				
Alleles	242 bp (239 bp)	196 bp (193 bp)	46 bp	242 bp (239 bp)	221 bp (218 bp)	194 bp (191 bp)	27 bp	21 bp
0101	+					+	+	+
0102	+					+	+	+
0103	+				+			+
0201		+	+		+			+
0301	+					+	+	+
0401		+	+			+	+	+
0501	+					+	+	+
0601		+	+		+			+

NOTE: Fragments in parentheses are only for allele 0201, 0401, 0501 and 0601.

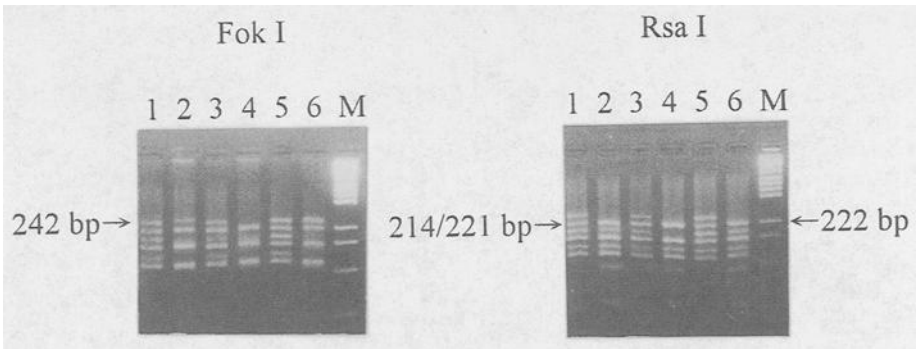

FIG. 1—*Restriction patterns of allele 0102/0501 (lanes 1 and 2), allele 0201/0601 (lanes 3 and 4) and allele 0501/0501 (lanes 5 and 6) with Fok I and Rsa I enzyme digestion from PCR product amplified by AmpliType® PM kit in 4% NuSieve 3 : 1 agarose gel. Lanes 1, 3 and 5 are loaded with undigested PCR products (fragments from top to bottom are 239/242 bp DQA1, 214 bp LDLR, 190 bp GYPA, 172 bp HBGG, 151 bp D7S8 and 138 bp GC), while lanes 2, 4 and 6 are loaded with digested PCR products. Lane M is pGEM marker (Promega, Madison, WI). According to the scanning results of the 242 bp and 214/221 bp bands in Table 1, there is no digestion with Fok I in lanes 2 and 6, and whole digestion in lane 4. And there are whole digestion with Rsa I in lanes 2 and 6, and no digestion in lane 4. In Rsa I digestion, the 190 bp fragments of GYPA gene were wholly digested and produced two fragments smaller than the 138 bp fragments of GC gene as shown in lanes 2, 4 and 6. Since there is no cutting site of Rsa I in the 221 bp fragments of allele 0201/0601, lane 4 remains blank in the 190 bp band area.*

the 214/221 bp band increased about 50% of the 214 bp undigested product. With no digestion, the optical density of the 214/221 bp band was about double of the 214 bp undigested product. Since there is a Rsa I cutting site in 190 bp fragment of GYPA gene [6], this fragment disappeared and produced two fragments smaller than the 138 bp fragments of GC gene in Rsa I digestion as shown in Fig. 1. The 190 bp area of the restriction patterns from Rsa I digestion is replaced by a new 194 bp band from 221 bp DQA1 gene digested product with whole or half digestion, but with no observable band with no digestion.

To monitor the completeness of the digestion process, we chose a control sample with allele 0201/0601. With full digestion for 242 bp fragments by the Fok I digestion process, no 242 bp band was observed in the resulting restriction patterns. With no digestion for 221 bp fragments and full digestion for 190 bp fragments of GYPA gene by the Rsa I digestion process, the optical density for the 214/221 bp mixed band was almost doubled and none observed for the 194 and 190 bp bands. These restriction patterns can be easily recognized as shown in lane 4, Fig. 1.

With this procedure and confirmed by Salazar's method [4], we were able to distinguish allele 0401, 0501 and 0601, and found their occurrence frequencies to be 2.3%, 19.8% and 8.1%, respectively. The discrimination power is increased from 0.903 to 0.936 in the Chinese population in Taiwan.

Discussion

This study showed that the six alleles of HLA DQA1 can be typed with the PCR product amplified from the AmpliType® PM kit, and allele 4 can be further subtyped to 0401, 0501 and 0601 by Fok I and Rsa I enzyme digestion with the same PCR product using the Bio-Profil image analysis system. In the reverse dot blot hybridization of DQA1 typing, the amplified DNA and horseradish peroxidase-streptavidin enzyme conjugate should be hybridized independently with probe strip to obtain better dot intensity.

When enzyme digestion was performed on the PCR product from the DQα AmpliType™ kit, the restriction patterns could be expected as shown in Table 2. But in this study, there are six bands (239/242, 214, 190, 172, 151 and 138 bp corresponding to DQA1, LDLR, GYPA, HBGG, D7S8 and GC loci) amplified from the AmpliType PM kit. After enzyme digestion, the mixture patterns produced from the digested fragments of six markers making it harder to identify the digested fragments of DQA1. We interpret the enzyme digestion result by measuring the difference of the optical density of 242 bp fragment in Fok I digestion and 214/221 bp fragment in Rsa I digestion. To achieved the best result, the optical density of both pre- and post-digested PCR products should be electrophoresed in the same agarose gel. Except the DQA1 fragment, the other fragments which would not be digested may serve as indicators to show the loading difference between pre- and post-digested PCR products lane in the agarose gel. Although the D7S8 fragment was digested and eliminated in Fok I digestion, this will not affect the proper interpretation of DQA1 results. In this study we have found that this technique can extend the utility of AmpliType PM kit and increase the discrimination power of the DQA1 system in a population group with high allele 4 occurrence frequency.

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