

*Full Length Research*

# HLA-DQA1 & HLA-DQB1 Genotyping in Gastritis Patients with positive RUT

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Abstract

To study was carried out to know HLA-DQA1 and HLA-DQB1 genotyping in gastritis patients with positive and negative RUT. This study was carried out in College of Medicine, University of Basrah. HLA-DQA1 and HLA-DQB1 genotyping was done in College of Medicine, University of Manitoba, Winnipeg, Canada during the period from April 2009 to July 2010. A total of 70 gastritis patients, 29 males and 41 females, were included in this study. Significant increased frequencies of DQB1\*050101 and DQB1\*050201 alleles were found in gastritis patients who showed (+ RUT), but with weak association (odds ratios were 0.83 and 0.79 respectively), as compared with gastritis patients who showed (- RUT).

**Keywords:** Genotype, HLA-DQA1 & HLA-DQB1, Gastritis, Iraq

## INTRODUCTION

Human leukocyte antigens (HLA) are an inherent system of alloantigens, which are the products of genes of the major histocompatibility complex (MHC). These genes span a region of approximately 4 centimorgans on the short arm of human chromosome 6 at band p21.3 and encode the HLA class I and class II antigens, which play a central role in cell-to-cell-interaction in the immune system (Conrad *et al*, 2006). They encode peptides involved in host immune response; also they are important in tissue transplantation and are associated with a variety of infectious, autoimmune, and inflammatory diseases (Gregersen *et al*, 2006; Nair *et al*, 2006). Moreover, the HLA loci display an unprecedented degree of diversity and the distribution of HLA alleles and haplotypes among different populations is considerably variable (Shao *et al*, 2004; Blomhoff *et al*, 2006). The expression of particular HLA alleles may be associated with the susceptibility or resistance to some diseases (Wang *et al*, 2006). Heterozygosity within the MHC genomic region provides the immune system with a selective advantage of pathogens (Fu *et al*, 2003; Kumar *et al*, 2007). *Helicobacter pylori* infection is, in addition to being the main etiologic agent for chronic gastritis, a

major cause of peptic ulcer and gastric cancer (Suerbaum and Michetti, 2002). Many studies concerning bacteriological and immunological aspects of *H. pylori* were performed in Iraq (Al- Janabi, 1992; Al-Jalili, 1996; Al-Baldawi, 2001; Al-Dhaheer, 2001; Al-Saimary, 2008), but, until now, none studies were performed about HLA genotyping associated with *H. pylori* infection. In developing countries, prevalence of *H. pylori* infection is > more than 80% among middle-aged adults, whereas in developed countries prevalence ranges from 20% to 50%. Approximately 10% to 15% of infected individuals will develop peptic disease and 3% a gastric neoplasm (Torres *et al*, 2005). Therefore, *H. pylori* infection is a necessary but not a sufficient cause of severe forms of gastric disease. *H. pylori* induce a host immune response, but the persistence of the infection suggests that the response is not effective in eliminating the infection. Furthermore, multiple lines of evidence suggest that the immune response contributes to the pathogenesis associated with the infection. As a result, the immune response induced by *H. pylori* is a subject of continuous study that has encouraged numerous questions (Azem *et al*, 2006). The inability of the host response to clear infections with *H. pylori* could reflect down-regulatory mechanisms that limit the resulting immune responses to prevent harmful inflammation as a means to protect the host (Yoshikawa and Naito, 2000).

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**Table 1** Frequencies of gastritis patients with positive & negative RUT according to gender

Gender	Gastritis patients with (+ ve RUT)	Gastritis patients with (- ve RUT)	Total N= 70
	N (%) 52	N (%) 18	
Male	21 (72.41)	8 (27.59)	29
Female	31 (75.61)	10 (24.39)	41

( $\chi^2 = 0.09$ ; P= NS; OR= 0.85; 95% CI= 0.29 – 2.49)

## METHODS

A total of 70 patients (29 males and 41 females with age groups from (15-66) years, with various digestive symptoms attending endoscopy unit at Al-Sadder Teaching Hospital in Basrah were included in the present study. Stomach biopsies were obtained from gastritis patients for rapid urease test. Blood samples were drawn from gastritis patients and subjected to HLA-DQ genotyping. The study was carried out during the period from (17<sup>th</sup> of April 2009 to 15<sup>th</sup> of July 2010).

### Rapid Urease Test (RUT)

This test is used to identifying the presence of *H. pylori* urease enzyme in gastric biopsy (Marshall *et al*, 1987). At the present study, the rapid urease test was performed with gastric tissue samples obtained from 70 patients diagnosed with gastritis. Positive results were obtained when the bacterium was present in the sample and negative when it was absent.

DNA Isolated from the Blood Samples by using Wizard Genomic DNA purification Kit, Promega Corporation, USA; Protocol (Beutler *et al*, 1990)

### HLA-DQA1 and –DQB1 Genotyping

HLA-DQA1 and –DQB1 genotyping protocol was done according to Sequence-Based-Typing (SBT), which had been developed in National Microbiology Laboratories (NML), Winnipeg, Canada (Luo *et al*, 1999). All the steps of HLA-DQA1 and –DQB1 genotyping were done under supervision of Dr. Ma Luo in Medical Microbiology Laboratory, College of Medicine, University of Manitoba and in Dr. Ma Luo Laboratory in National Microbiology Laboratories (NML).

The blood samples were used in order to perform the HLA genotyping, being used too for the extraction of DNA in order to perform the PCR for HLA-DQA1 and HLA-DQB1 genes.

### PCR Amplification of HLA-DQA1 and –DQB1 Gene

The PCR Amplification of HLA-DQA1 and –DQB1 gene was done in Medical Microbiology laboratories in College of Medicine, Manitoba University, Winnipeg, Canada.

### DNA Purification

The Purification of the amplified HLA-DQA1 and –DQB1 gene was done in National Microbiology laboratories (NML), in Dr. Ma Luo Lab, in College of Medicine, Manitoba University, Winnipeg, Canada.

Three methods had been used for purification of the amplified PCR DNA samples:

1. DNA Purification by Using Vacuum
2. DNA purification by using: GenElute™ PCR Clean-Up Kit (Sigma- Aldrich, Inc. USA).GenElute™ PCR Clean-Up Kit
3. Purification in DNA Core Section in NML (NML, Canada)

The amplified PCR DNA was purified in DNA Core laboratory in National Microbiology Laboratories (NML) in Winnipeg, Canada.

### Sequencing –PCR

Sequencing–PCR was done in National Microbiology laboratories (NML), under supervision of Dr. Ma Luo.

### Ethanol Precipitation

Ethanol Precipitation was done under supervision of Dr. Ma Luo in National Microbiology laboratories (NML) in College of Medicine, Manitoba University, Winnipeg, Canada.

### Sequencing-using the (3100 Genetic Analyzer, USA)

HLA-DQA1 and –DQB1 genotyping protocol had done according to Sequence-Based-Typing (SBT), which had been developed in National Microbiology Laboratories (NML), Winnipeg, Canada (Luo *et al*, 1999).

### Statistical Analysis

For qualitative variables, frequency data were summarized as percentage. Statistical significant of differences between two groups was tested by Pearson Chi-square ( $\chi^2$ ) with Yates' continuity correction. Risk was estimated using Odds ratio (OR) and 95% confidence interval (95% CI). P-value was determined by Fisher's exact test, P- value of (< 0.05) was considered statistically significant. Data were analyzed using SPSS program for window (Version 10).

## RESULTS

### Distribution of Gastritis Patients with Positive and Negative (RUT) According to Gender

As shown in (Table 1), out of 29 gastritis patients (males), 21 (72.41%) were (+ ve RUT) and 8 (27.59%) were (- ve RUT). Also results in (Table 1) indicated that out of 41 females, 31 (75.61%) were (+ ve RUT) and 10 (24.39%)

**Table 2** Distribution of gastritis patients with positive & negative RUT according to age groups

Age group	Patients with +ve RUT	Patients with -ve RUT	Total N=70
	N (%)	N (%)	
15 > 45	52	18	
15 > 45	35 (74.47)	12 (25.55)	47
> 45	17 (73.91)	6 (26.09)	23

( $\chi^2 = 0.002$ ; P= NS; OR= 1.03; 95% CI= 0.33 – 3.21)

**Table 3** Frequencies of gastritis patients (chronic & acute) according to RUT results

Rapid Urease Test (RUT) Results	Gastritis patients		Total N (%)
	Acute gastritis	Chronic gastritis	
	N (%)	N (%)	
	55	15	
Positive RUT cases	41 (78.85)	11 (21.15)	52 (100)
Negative RUT cases	14 (77.78)	4 (22.22)	18 (100)

( $\chi^2 = 3.11$ ; P= NS; OR= 0.56; 95% CI= 0.67 – 0.98)

were (- ve RUT). These results showed no significant differences between gastritis patients (males and females) in RUT results ( $\chi^2 = 0.09$ ; P= NS; OR= 0.85; 95% CI= 0.29 – 2.49).

#### Distribution of Gastritis Patients with Positive and Negative (RUT) According to Age Groups

Results shown in (Table 2) indicated that out of 47 patients from (15 > 45) age group, 35 (74.47%) were (+ ve RUT) and 12 (25.55%) were (- ve RUT). Also results in (Table 2) showed that out of 23 patients from (> 45) age group, 17 (73.91%) were (+ ve RUT) and 6 (26.09%) were (- ve RUT). These results showed no significant differences between gastritis patients in these two age groups according to RUT results ( $\chi^2 = 0.002$ ; P= NS; OR= 1.03; 95% CI= 0.33 – 3.21).

#### Distribution of Gastritis Patients with Positive and Negative (RUT) According to Type of Gastritis

Results shown in (Table 3) indicated that out of 52 patients with (+ ve RUT), 41 (78.85%) were diagnosed as acute gastritis patients and 11 (21.15) were diagnosed as chronic gastritis patients. Also results in (Table 3) showed that out of 18 gastritis patients with (- ve RUT), 14 (77.78) were diagnosed as acute gastritis patients and 4 (22.22%) were diagnosed as chronic gastritis patients. These result showed no significant differences between the two groups according to types of gastritis ( $\chi^2 = 3.11$ ; P= NS; OR= 0.56; 95% CI= 0.67 – 0.98).

#### Genotype Frequency of HLA-DQ in Gastritis Patient with positive and negative RUT

The genotype frequencies of HLA-DQA1 were studied in gastritis patients with positive RUT and compared with patients with negative RUT. Results shown in (Table 4) indicated that no allele showed significant differences between gastritis patients with positive and negative RUT.

Results in (Table 5) indicated that HLA-DQB1\*050201 allele was present in 10 out of 47 patients with positive RUT and 0 out of 18 patients with negative RUT, with frequencies of 21.28 and 0.00. An increased frequency of -DQB1\*050201 allele was observed in gastritis patients showing positive RUT, while this allele was absent in patients showing negative RUT, It was statistically significant but with weak association ( $\chi^2 = 4.53$ , P < 0.05, OR= 0.79, 95%CI= 0.68-0.91). Also HLA-DQB1\*050101 allele was present in 8 out of 47 patients with positive RUT and 0 out of 18 patients with negative RUT, with frequencies of 17.02 and 0.00. An increased frequency of -DQB1\*050101 allele was observed in gastritis patients showing positive RUT, while this allele was absent in patients showing negative RUT, It was statistically significant but with weak association ( $\chi^2 = 3.49$ , P < 0.05, OR= 0.83, 95%CI= 0.73-0.94).

#### Homozygosity of HLA-DQ in Patients with Positive and Negative RUT

HLA-DQ homozygosity was studied in patients with positive and negative RUT. Results shown in (Table 6)

**Table 4** HLA-DQA1 genotype frequency of patients with positive and negative RUT

HLA-DQA1 allele	Patients with +ve RUT		Patients with -ve RUT		$\chi^2$	P	OR	95% CI
	N=46	%	N=16	%				
010101/010102/010401/010402/0105	9	19.57	1	6.25	1.56	NS	0.27	0.03-2.36
010201/010202/010203/010204	15	32.61	5	31.25	0.01	NS	0.94	0.28-3.20
0103	8	17.39	2	12.50	0.21	NS	0.68	0.13-3.59
0201	7	15.22	4	25.00	0.78	NS	1.86	0.46-7.45
030101/0302/0303	10	21.74	6	37.50	1.54	NS	2.16	0.63-7.40
040101/040102/0402/0404	2	4.35	2	12.50	1.31	NS	3.14	0.41-24.42
050101/0503/0505/0506/0507/0508/0509	28	60.87	10	62.50	0.02	NS	1.07	0.33-3.46

**Table 5.** HLA-DQB1 genotype frequency of patients with positive and negative RUT

HLA-DQB1 allele	Patients with +ve RUT		Patients with -ve RUT		$\chi^2$	P	OR	95% CI
	n=47	%	n=18	%				
020101/0202/0204	23	48.94	10	55.56	0.23	NS	1.30	0.44-3.89
030101/030104/0309/0321/0322/0324/030302	16	34.04	9	50.00	1.40	NS	1.94	0.64-5.84
030201	7	14.89	3	16.67	0.03	NS	1.14	0.26-5.01
030302	2	4.26	1	5.56	0.88	NS	1.32	0.11-15.56
0402	3	6.38	2	11.11	0.41	NS	1.83	0.28-12.00
050101	8	17.02	0	0.00	3.49	<0.05	0.83	0.73-0.94
050201	10	21.28	0	0.00	4.53	<0.05	0.79	0.68-0.91
050301	0	0.00	1	5.56	2.65	NS	1.06	0.95-1.18
060101/060103	2	4.26	1	5.56	0.05	NS	1.32	0.11-15.56
060201	2	4.26	1	5.56	0.05	NS	1.32	0.11-15.56
060301/060401	4	8.51	2	11.11	0.11	NS	1.34	0.22-8.06
060401/0634	3	6.38	3	16.67	1.64	NS	2.93	0.53-16.13
060801	1	2.13	0	0.00	0.39	NS	0.98	0.94-1.02
0609	1	2.13	0	0.00	0.39	NS	0.98	0.94-1.02

**Table 6.** Homozygosity of HLA-DQ in patients with positive and negative RUT

HLA-DQ homozygosity*		+ve RUT		-ve RUT	
		No	%	No	%
DQA1**	Homozygous	13	27.66	2	13.33
	Heterozygous	34	72.34	13	86.67
	<b>Total</b>	<b>47</b>	<b>100</b>	<b>15</b>	<b>100</b>
***DQB1	Homozygous	11	23.04	3	16.67
	Heterozygous	36	76.96	15	83.33
	<b>Total</b>	<b>47</b>	<b>100</b>	<b>18</b>	<b>100</b>
****DQA1 +DQB1	Homozygous	15	20.55	2	13.33
	Heterozygous	58	79.45	13	86.67
	<b>Total</b>	<b>73</b>	<b>100</b>	<b>15</b>	<b>100</b>

\* Homozygous at one or both loci

\*\* ( $\chi^2 = 1.27$ , P=NS, OR= 0.41, 95% CI= 0.08-2.03)

\*\*\* ( $\chi^2 = 0.35$ , P=NS, OR= 0.66, 95% CI= 0.16-2.69)

\*\*\*\* ( $\chi^2 = 2.49$ , P=NS, OR= 1.29, 95% CI= 0.05-1.44)

indicated that for HLA-DQA1, out of 47 patients with positive RUT, 13 were homozygous in one or both loci and 2 out of 15 patients with negative RUT, were homozygous in one or both loci, with frequencies of 27.66 and 13.33 respectively. No significant differences were observed in frequency of homozygous HLA-DQA1 genotype between patients with positive and negative RUT ( $\chi^2 = 1.27$ , P=NS, OR= 0.41, 95% CI= 0.08-2.03). For HLA-DQB, out of 47 patients with positive RUT, 11 were homozygous in one or both loci, and out of 18 patients with negative RUT, 3 were homozygous in one or both loci, with frequencies of 23.04 and 16.67 respectively. No significant differences were observed in frequency of homozygous HLA-DQB1 genotype between patients with positive and negative RUT ( $\chi^2 = 0.35$ , P=NS, OR= 0.66, 95% CI= 0.16-2.69)(Table 6).

For HLA-DQ- (A1+B1), out of 73 patients with positive RUT, 15 were homozygous in one or both loci, with

frequencies of 20.55 and 13.33 respectively. No significant differences were observed in frequency of homozygous HLA-(DQA1+DQB1) genotype between patients with positive and negative RUT ( $\chi^2 = 2.49$ ,  $P=NS$ ,  $OR= 1.29$ ,  $95\% CI= 0.05-1.44$  (Table 6).

## DISCUSSION

The HLA genotyping was done in NML and Microbiology laboratories, College of Medicine, University of Manitoba in Canada, by using Sequencing-based typing (SBT) method which had been developed in Dr. Ma Luo laboratory.

Human leukocyte antigens (HLA) are an inherent system of alloantigens, which are the products of genes of the major histocompatibility complex (MHC). These genes span a region of approximately 4 centimorgans on the short arm of human chromosome 6 at band p21.3 and encode the HLA class I and class II antigens, which play a central role in cell-to-cell-interaction in the immune system (Conrad *et al*, 2006). They encode peptides involved in host immune response; also they are important in tissue transplantation and are associated with a variety of infectious, autoimmune, and inflammatory diseases (Gregersen *et al*, 2006; Nair *et al*, 2006). Moreover, the HLA loci display an unprecedented degree of diversity and the distribution of HLA alleles and haplotypes among different populations is considerably variable (Shao *et al*, 2004; Blomhoff *et al*, 2006). The number of newly identified HLA alleles grows each year and since the 2004 publication, 832 new HLA alleles have been added, including 235 HLA-A, 310 -B, 140 -C, 116 -DRB1, 9 -DRB3/4/5, and 22 -DQB1 alleles. Information has also been updated for 766 previously listed alleles numbering 158 HLA-A, 303 -B, 116 -C, 133 -DRB1, 13 -DRB3/4/5, and 43 -DQB1 alleles (Holdsworth *et al*, 2009). The HLA class II analysis was updated with the typing information from 68 B lymphoblastoid cell lines. Each cell was typed serologically by an average number of 13 laboratories, ranging from a low of 8 to a high of 18. The parallel typing for HLA-DRB1, -DRB3, -DRB4, -DRB5, and -DQB1 alleles using DNA-based methods was performed by an average of 80 laboratories, ranging from a low of 66 to a high of 87 (Holdsworth *et al*, 2009). The expression of particular HLA alleles may be associated with the susceptibility or resistance to some diseases (Wang *et al*, 2006). Heterozygosity within the MHC genomic region provides the immune system with a selective advantage of pathogens (Fu *et al*, 2003; Kumar *et al*, 2007). *H. pylori* infection is, in addition to being the main etiologic agent for chronic gastritis, a major cause of peptic ulcer and gastric cancer (Suerbaum and Michetti, 2002). In the present study, out of 70 patients with various dyspeptic symptoms underwent upper gastrointestinal endoscopic examination, 70 (70%) showed abnormal endoscopic findings; 29 (41%) males

and 41 (58.57%) females.

Large numbers of diagnostic tests have been developed to detect *H. pylori* infection. Theoretically, there is no single test which can be considered as the gold standard. For clinical studies, the concordance of rapid urease test, histopathology and culture is often used as the gold standard (Gatta *et al*, 2003). Gastric biopsy samples were collected for rapid urease test from 70 gastritis patients from the antral region of the stomach. Low acidity in this region because of the decreased number of parietal cells (Logan *et al*, 1995) also presence of many receptors for *H. pylori* in this region, increases the chance for detection of the metabolic activity of the organism such urease enzyme (Boren *et al*, 1993; Guerrant *et al*, 1999). The test was considered positive when a red or pink color developed in the inoculated media as shown in Figure 3.1. Most of cases showed positive results after few minutes. Patients, who showed negative RUT, Clinically showed a picture of *H. pylori* gastritis, also they were seropositive (positive RDT). The negative RUT results for these patients may be due to the sample size, at least  $10^5$  per ml bacteria are needed for a positive result and the use of two biopsies increase the sensitivity of the test (Marshall *et al*, 1987; El-Zimaity *et al*, 1995). Also negative results may be due to the irregular distribution of the organism in the gastric mucosa (Dunn *et al*, 1997; Shahata *et al*, 2006), the use of antibiotics or acid-suppression at the time of endoscopy could theoretically decrease the sensitivity of the RUT by diminishing urease activity. Lee *et al*, (1997) did not find these factors to be reliable predictors of patients who would have a false negative RUT. Increased likelihood of sampling error in older patients who are more likely to have gastric mucosal changes which are associated with a reduced *H. pylori* density (atrophic gastritis and intestinal metaplasia), is also a possibility. One group has recently reported that a mixture of blood, gastric juice, and bile decreased the sensitivity of three different RUTs in an in-vitro setting suggesting that the exposure of the gastric mucosa to a combination of factors, including blood, may be the explanation for this clinically important observation. The study indicated that the use of biopsy urease test in the endoscope unit will be sensitive and convenient. The importance of this test during endoscopy is to confirm the presence of *H. pylori*, so that no patient will be treated unnecessarily, also it will help the physician in taking the right choice for treatment. In the present study, out of 70 gastritis patients, 52 (74.29%) were positive rapid urease test (RUT) and 18 (25.71%) were negative (RUT) (Table 1). These results agree with studied performed in Iraq and other countries (Al-Dhaher, 2001; Cutler *et al*, 1995; Onders, 1997; Al-Baldawi, 2001; and Al-Ali, 2002). Distribution of gastritis patients with positive and negative (RUT) was studied according to gender. As shown in (Table 1), out of 29 gastritis patients (males), 21 (72.41%) were (+ ve RUT) and 8 (27.59%) were (- ve RUT). Also results in (Table 1)

indicated that out of 41 females, 31 (75.61%) were (+ ve RUT) and 10 (24.39%) were (- ve RUT). These results showed no significant differences between gastritis patients (males and females) in RUT results ( $\chi^2 = 0.09$ ;  $P = \text{NS}$ ;  $\text{OR} = 0.85$ ;  $95\% \text{ CI} = 0.29 - 2.49$ ). Distribution of gastritis patients with positive and negative (RUT) was studied according to age groups. Results shown in (Table 2) indicated that out of 47 patients from (15 > 45) age group, 35 (74.47%) were (+ ve RUT) and 12 (25.55%) were (- ve RUT). Also results in Table 2 showed that out of 23 patients from (> 45) age group, 17 (73.91%) were (+ ve RUT) and 6 (26.09%) were (- ve RUT). These results showed no significant differences between gastritis patients in these two age groups according to RUT results ( $\chi^2 = 0.002$ ;  $P = \text{NS}$ ;  $\text{OR} = 1.03$ ;  $95\% \text{ CI} = 0.33 - 3.21$ ). Distribution of gastritis patients with positive and negative (RUT) was studied according to type of gastritis. Results shown in (Table 3) indicated that out of 52 patients with (+ ve RUT), 41 (78.85%) were diagnosed as acute gastritis patients and 11 (21.15) were diagnosed as chronic gastritis patients. Also results in (Table 3) showed that out of 18 gastritis patients with (- ve RUT), 14 (77.78) were diagnosed as acute gastritis patients and 4 (22.22%) were diagnosed as chronic gastritis patients. These result showed no significant differences between the two groups according to types of gastritis ( $\chi^2 = 3.11$ ;  $P = \text{NS}$ ;  $\text{OR} = 0.56$ ;  $95\% \text{ CI} = 0.67 - 0.98$ ). The genotype frequencies of HLA-DQA1 were studied in gastritis patients with positive RUT and compared with patients with negative RUT. Results shown in (Table 4) indicated that no allele showed significant differences between gastritis patients with positive and negative RUT. Results in (Table 5) indicated an increased frequency of -DQB1\*050201 allele was observed in gastritis patients showing positive RUT, while this allele was absent in patients showing negative RUT, It was statistically significant but with weak association ( $\chi^2 = 4.53$ ,  $P < 0.05$ ,  $\text{OR} = 0.79$ ,  $95\% \text{ CI} = 0.68-0.91$ ). An increased frequency of -DQB1\*050101 allele was observed in gastritis patients showing positive RUT, while this allele was absent in patients showing negative RUT, It was statistically significant but with weak association ( $\chi^2 = 3.49$ ,  $P < 0.05$ ,  $\text{OR} = 0.83$ ,  $95\% \text{ CI} = 0.73-0.94$ ) (Table 5). HLA-DQ homozygosity was studied in patients with positive and negative RUT. Results indicated that no significant differences were observed in frequency of homozygous HLA-DQ genotype between patients with positive and negative RUT (Table 6). Sequencing-based typing (SBT) is the gold standard for high-resolution tissue typing, which is required for optimal HLA matching between donor and recipient in stem cell transplantation settings. High-resolution genotyping of the HLA genes by SBT is the most comprehensive method available. The HLA-DQA1 gene presents a unique challenge for genotyping using SBT because 10 of the 22 known alleles contain a three-nucleotide deletion of codon 56 (Appendix 4). Almost all currently

used SBT strategies for HLA-DQB1 typing employ amplification and/or sequencing primers located within exon 2 and exon 3. Complete exon 3 sequence information facilitates the resolution of allele ambiguities, for instance HLA-DQB1\*030101 and HLA-DQB1\*0319. Successful sequence-based DQA1 and DQB1 typing depends on several factors. These include technical issues as well as analysis issues. Among the technical issues, the quality of DNA isolation is particularly important. The genomic DNA isolated kits appeared to be good quality as judged by PCR amplification results. It is important to store the DNA at  $-20 \text{ c}^\circ$  for the long term in order to avoid degradation. The quantity of DNA used in the PCR amplification is important, as too much DNA can result in over amplification of one allele in the heterozygous situation, using the exact amount of good quality DNA to ensure balanced amplification of both alleles (Luo *et al*, 1999). The annealing temperature and amount of dNTPs used in the PCR reaction are critical to ensure the fidelity and even amplification of both alleles (Knipper *et al*, 1994) is sufficient for typing all DQB1 alleles. The primers do not amplify DQB2 if the proper annealing temperature ( $57 \text{ c}^\circ$ ) and dNTP concentration are used in the PCR reaction. The sense sequencing primer DQBSEQ1 is sufficient for typing most DQB1 alleles except for DQB1\*0602 and 0611 which require the antisense primer DQBSEQ3 in order to obtain information for codon 9 and 14. In DQA1 typing, the proper annealing temperature for sequencing used in the present study was ( $53 \text{ c}^\circ$ ) in order to ensure balanced typing of both alleles. Too high a temperature can result in allele drop out due to sequence differences between DQA1\*01s and other groups. Two sequencing primers, DQASEQ2 and DQASEQ3 were used in the present study in order to obtain sequence information to type most DQA1 alleles. Among the analysis issues that proved particularly problematic was the correct inference of DNA sequences at heterozygous codons. a computer software program based on TBSA was developed recently, which makes sequence-based DQA1 and DQB1 typing an even easier task. The principle of TBSA can likely be applied to typing of other genes with multiple alleles (Luo *et al*, 1999)

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