CLINICAL AND VACCINE IMMUNOLOGY, Jan. 2009, p. 136–138 Vol. 16, No. 1
1556-6811/09/$08.00+0 doi:10.1128/CVI.00215-08

Influence of Common and Specific HLA-DRB1/DQB1 Haplotypes on Genetic Susceptibilities of Three Distinct Arab Populations to Type 1 Diabetes[▽]

Mouna Stayoussef,[1] Jihen Benmansour,[1] Fayza A. Al-Jenaidi,[2,3] Rita Nemr,[4] Muhallab E. Ali,[2] Touhami Mahjoub,[1] and Wassim Y. Almawi[2]*

Faculty of Pharmacy, University of Monastir, Monastir, Tunisia[1]; College of Medicine and Medical Sciences, Arabian Gulf University,[2] and Department of Pediatrics, Salmaniya Medical Complex,[3] Manama, Bahrain; and Department of Endocrinology, Rizk Hospital, Beirut, Lebanon[4]

Received 11 June 2008/Returned for modification 9 October 2008/Accepted 15 October 2008

The contribution of HLA DRB-DQB to type 1 diabetes (T1D) in Bahrainis, Lebanese, and Tunisians was investigated. *DRB1*030101-DQB1*0201* was a locus that conferred susceptibility in three populations, while *DRB1*040101-DQB1*0302* was a locus that conferred susceptibility only in Tunisians and Bahrainis. The *DRB1*100101-DQB1*050101* (Bahrainis) and *DRB1*150101-DQB1*060101* (Lebanese) loci were largely protective. The contribution of HLA to T1D must be evaluated with regard to ethnic background.

Type 1 (insulin-dependent) diabetes (T1D) is caused by the autoimmune destruction of pancreatic β-islet cells (17). Many T1D susceptibility loci have been described and have been mapped into the IDDM1 to IDDM18 loci, of which the HLA region (identified as IDDM1) accounts for more than half of the genetic susceptibility to T1D (2, 16), whereas determination of a strong linkage disequilibrium between the DRB1 and the DQB1 alleles confirmed the contribution of specific HLA DRB1 and DQB1 alleles and DRB1-DQB1 haplotypes to the presence of T1D in many ethnic groups (7, 12, 16). This was highlighted by the association of DR3- and DR4-containing haplotypes with T1D in Caucasians (8, 10) but not Asians, including Japanese and Koreans (3, 9), in whom different haplotypes contribute to disease susceptibility (9). In view of the geographical/racial heterogeneity of Arabs (1), this study investigated the association of HLA class II (DRB1 and DQB1) haplotypes with T1D in Bahrain (Arabian Peninsula), Lebanon (eastern Mediterranean), and Tunisia (North Africa), in particular with regard to the identification of the specific haplotypes that confer susceptibility to and protection from T1D in each community.

Study subjects comprised unrelated Arab subjects from Tunisia (50 patients, 50 controls), Bahrain (126 patients, 126 controls), and Lebanon (78 patients, 111 controls). Non-Arabs (Berbers and Europeans in Tunisia, Armenians in Lebanon, and Iranians in Bahrain) or recently naturalized subjects, identified through personal interviews, were not included. A diagnosis of T1D was made according to clinical features and laboratory findings, and patients with other forms of diabetes (type 2 diabetes, latent autoimmune diabetes in adults, maturity-onset diabetes of the young, etc.) were excluded. The control subjects had normal fasting/random glucose levels and no family history of T1D or other autoimmune diseases. All subjects (patients and controls) were asked to sign a consent form according to the study protocol, and all institutional ethics requirements were met.

Total genomic DNA was extracted from EDTA-anticoagulated blood by the phenol-chloroform method. HLA-DRB1 and -DQB1 alleles were analyzed by a PCR sequence-specific priming (PCR-SSP) technique with an SSP2L HLA class II genotyping kit (One Lambda, Thousand Oaks, CA), according to the manufacturer's specifications. The frequencies of the most frequent haplotypes were determined by the maximum-likelihood method with Arlequin (version 2.000) population genetics data analysis software. Data were expressed as *P* values, odds ratios (ORs), and 95% confidence intervals (CIs) between the patients and the controls in each population.

Significant DRB1 and DQB1 allelic differences were seen between Lebanese, Bahraini, and Tunisian T1D patients and controls. Among the Lebanese subjects, *DRB1*030101* ($Pc < 0.033$), *DRB1*130701* ($Pc = 0.008$), and *DQB1*0201* ($Pc < 0.001$) were positively associated with T1D and *DRB1*110101* ($Pc = 0.002$), *DQB1*030101* ($Pc < 0.001$), and *DQB1*050101* ($Pc = 0.006$) were negatively associated with T1D, after the application of the Bonferroni correction (*Pc*). Among the Bahraini subjects, *DRB1*030101* ($Pc < 0.001$), *DRB1*040101* ($Pc < 0.001$), *DQB1*0201* ($Pc < 0.001$), and *DQB1*0302* ($Pc < 0.001$) were positively associated with T1D, while *DRB1*110101* ($Pc = 0.001$), *DQB1*030101* ($Pc = 0.003$), and *DQB1*050101* ($Pc < 0.001$) were negatively associated with T1D. Among the Tunisian subjects, only *DRB1*040101* ($Pc = 0.017$) was positively associated with T1D, while *DRB1*110101* ($Pc = 0.011$), *DQB1*030101* ($Pc = 0.029$), and *DQB1*060101* ($Pc = 0.009$) were negatively associated with T1D.

The results of DRB1-DQB1 haplotype analysis are summarized in Table 1. *DRB1*030101-DQB1*0201* served a T1D-susceptible role among all three populations. *DRB1*040101-DQB1*0302* displayed a mixed association: it served a susceptible role among the Bahrainis and Tunisians and was protective in Lebanese subjects. *DRB1*070101-DQB1*0201* and *DRB1*110101-DQB1*030101* were

* Corresponding author. Mailing address: Department of Medical Biochemistry, College of Medicine and Medical Sciences, Arabian Gulf University, P.O. Box 22979, Manama, Bahrain. Phone: 973-39717118. Fax: 973-17271090. E-mail: wassim@agu.edu.bh.

▽ Published ahead of print on 12 November 2008.

TABLE 1. DRB1∗-DQB1∗ haplotype distribution[a]

Haplotype	Bahrainis		Lebanese		Tunisians	
	P	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)
DRB1∗010101-DQB1∗050101	0.683	0.51 (0.11–2.55)	**<0.001**	**0.008 (0.00–0.189)**[4]	0.609	0.47 (0.12–2.47)
DRB1∗030101-DQB1∗0201	**<0.001**	**16.00 (8.29–29.04)**	**<0.001**	**81.00 (24.58–209.03)**	**0.028**	**2.15 (1.13–4.04)**
DRB1∗040101-DQB1∗0302	**<0.001**	**41.33 (12.35–109.72)**	**<0.001**	**0.049 (0.02–0.19)**	**0.007**	**3.75 (1.45–8.90)**
DRB1∗040101-DQB1∗030201	1.000	1.44 (0.28–6.96)	0.187	1.29 (0.79–1.42)	0.490	0.58 (0.21–1.79)
DRB1∗040101-DQB1∗050101	0.467	0.623 (0.25–1.62)	**<0.001**	**0.007 (0.00–0.07)**	0.862	0.60 (0.14–3.20)
DRB1∗070101-DQB1∗0201	**<0.001**	**0.13 (0.05–0.40)**	**0.028**	**0.132 (0.03–0.72)**	0.733	0.78 (0.34–1.85)
DRB1∗100101-DQB1∗050101	**0.002**	**0.10 (0.03–0.43)**	0.144	0.28 (0.08–1.32)	0.317	0.99 (0.01–3.30)
DRB1∗110101-DQB1∗030101	**<0.001**	**0.11 (0.04–0.32)**	**<0.001**	**0.055 (0.03–0.13)**	0.170	0.34 (0.11–1.31)
DRB1∗150101-DQB1∗060101	1.000	0.84 (0.27–2.63)	**0.011**	**0.049 (0.01–0.50)**	0.419	0.39 (0.10–1.99)
DRB1∗160101-DQB1∗0201	**<0.001**	**14.44 (3.30–47.92)**	0.883	0.71 (0.20–2.83)	0.317	0.99 (0.01–3.30)

[a] DRB1∗ and DQB1∗ alleles were assessed by PCR-SSP, and haplotype frequencies were determined by the maximum-likelihood method. Bahrainis comprised 126 patients and 126 controls, Lebanese comprised 78 patients and 111 controls, and Tunisians comprised 50 patients and 50 controls. Boldface indicates a significant difference between patients with T1D and age- and gender-matched controls in each community. *P* values were determined by Fisher's exact test.

negatively associated with T1D in the Bahrainis and Lebanese but not the Tunisians, while *DRB1∗100101-DQB1∗050101* served a T1D-protective role only in the Bahrainis. Furthermore, *DRB1∗010101-DQB1∗050101*, *DRB1∗040101-DQB1∗050101*, and *DRB1∗150101-DQB1∗060101* were negatively associated with T1D only in the Lebanese subjects. No T1D-protective haplotypes were identified among the Tunisian subjects.

Arabs comprise diverse ethnic populations that extend from the Arabian (Persian) Gulf to the Atlantic Ocean (2, 17). In view of the strong association of HLA DRB-DQB haplotypes with T1D (6, 9, 16), coupled with their various distributions in different racial/ethnic populations (7, 12, 15, 16), we investigated the association of HLA DRB-DQB haplotypes with T1D in individuals from three distinct Arab communities: Lebanon (Eastern Mediterranean), Tunisia (North Africa), and Bahrain (Arabian Peninsula). To avoid epidemiologic bias, only Arab subjects were included in the study; non-Arab nationals of the study communities, including Armenians (Lebanon), Berbers and Europeans (Tunisia), and Iranians (Bahrain), were excluded.

The differential association of the DRB1 and DQB1 alleles with T1D was noted in the three populations studied and was highlighted by the enrichment of *DRB1∗040101* in Tunisian and Bahraini but not Lebanese T1D patients and the strong association of *DRB1∗030101* and *DQB1∗0201* with T1D in Lebanese and Bahraini subjects. *DQB1∗0302*, a traditional T1D-susceptible allele among Caucasians (5, 6, 8, 10), was not individually associated with T1D among the Lebanese and Tunisians but was associated with T1D susceptibility among the Tunisians and protection among the Lebanese. It is possible that the disease association conferred by *DQB1∗0302*-containing haplotypes is linked to the nature of the *DRB1* allele contained in the haplotype. Alternatively, it may be due to racial considerations, highlighted by the pathogenic gradient-like nature of *DQB1∗0302*, where *DQB1∗0302* is linked with T1D in Northern European countries, including Finland (5, 6), but becomes neutral in Southern European and Mediterranean countries (12, 14).

Common and unique DRB-DQB haplotypes conferring susceptibility to and protection against T1D were identified in each community. The former was highlighted by the *DRB1∗030101-DQB1∗0201* haplotype, which conferred disease susceptibility in all three populations, as has also been shown for other Caucasian populations (4, 10). A number of DRB-DQB haplotypes appeared to be population specific, as they were associated with T1D in some but not all populations (*DRB1∗100101-DQB1∗050101* in Bahrainis, *DRB1∗040101-DQB1∗050101* in Lebanese) or had an opposing association, depending on the population (*DRB1∗040101-DQB1∗0302* conferred susceptibility in Bahrainis and protection in Lebanese). Similar to other Caucasian populations (5, 6), *DRB1∗040101-DQB1∗0302* conferred T1D susceptibility in Bahraini and Tunisian patients. However, it was found to be largely protective among the Lebanese, as well as other Mediterranean communities (11, 12). This was the consequence of the high prevalence of *DRB1∗04* among Lebanese and other Mediterranean populations (11, 13).

Our data indicate that the association of HLA DRB-DQB haplotypes is variable throughout Arabian communities, with both common and unique haplotypes conferring disease susceptibility and protection. This is most likely due to genetic drift from Middle East ancestors (Arabian Peninsula) and the admixture with other populations, including Africans and Berbers (Tunisians), Europeans (Lebanese), and Iranians and Indians (Bahrainis). Further studies are required to identify common (and unique) HLA DRB-DQB haplotypes that confer susceptibility to and protection from T1D in related and distant Arab communities.

REFERENCES

1. **Almawi, W. Y., M. Busson, H. Tamim, E. M. Al-Harbi, R. R. Finan, S. F. Wakim-Ghorayeb, and A. A. Motala.** 2004. HLA class II profile and distribution of HLA-DRB1 and HLA-DQB1 alleles and haplotypes among Lebanese and Bahraini Arabs. Clin. Diagn. Lab. Immunol. **11:**770–774.
2. **Bartsocas, C. S., and A. Gerasimidi-Vazeou.** 2006. Genetics of type 1 diabetes mellitus. Pediatr. Endocrinol. Rev. **3**(Suppl. 3)**:**508–513.
3. **Bugawan, T. L., W. Klitz, M. Alejandrino, J. Ching, A. Panelo, C. M. Solfelix, A. Petrone, R. Buzzetti, P. Pozzilli, and H. A. Erlich.** 2002. The association of specific HLA class I and II alleles with type 1 diabetes among Filipinos. Tissue Antigens **59:**452–469.
4. **Gillespie, K. M., E. A. Gale, and P. J. Bingley.** 2002. High familial risk and genetic susceptibility in early onset childhood diabetes. Diabetes **51:**210–214.
5. **Guja, C., L. Guja, S. Nutland, H. Rance, M. Sebastien, J. A. Todd, and C. Ionescu-Tirgoviste.** 2004. Type 1 diabetes genetic susceptibility encoded by HLA DQB1 genes in Romania. J. Cell. Mol. Med. **8:**249–256.
6. **Hermann, R., C. S. Bartsocas, G. Soltesz, A. Vazeou, P. Paschou, E. Bozas, A. Malamitsi-Puchner, O. Simell, M. Knip, and J. Ilonen.** 2004. Genetic screening for individuals at high risk for type 1 diabetes in the general population using HLA class II alleles as disease markers. A comparison

between three European populations with variable rates of disease incidence. Diabetes Metab. Res. Rev. **20:**322–329.
7. **Ikegami, H., T. Fujisawa, Y. Kawabata, S. Noso, and T. Ogihara.** 2006. Genetics of type 1 diabetes: similarities and differences between Asian and Caucasian populations. Ann. N. Y. Acad. Sci. **1079:**51–59.
8. **Ionescu-Tirgoviste, C., C. Guja, M. Herr, E. Cucca, K. Welsh, M. Bunce, S. Marshall, and J. A. Todd.** 2001. Low frequency of HLA DRB1*03-DQB1*02 and DQB1*0302 haplotypes in Romania is consistent with the country's low incidence of type I diabetes. Diabetologia **44**(Suppl. 3)**:**B60–B66.
9. **Kawabata, Y., H. Ikegami, Y. Kawaguchi, T. Fujisawa, M. Shintani, M. Ono, M. Nishino, Y. Uchigata, I. Lee, and T. Ogihara.** 2002. Asian-specific HLA haplotypes reveal heterogeneity of the contribution of HLA-DR and -DQ haplotypes to susceptibility to type 1 diabetes. Diabetes **51:**545–551.
10. **Kockum, I., C. B. Sanjeevi, S. Eastman, M. Landin-Olsson, G. Dahlquist, and A. Lernmark.** 1999. Complex interaction between HLA DR and DQ in conferring risk for childhood type 1 diabetes. Eur. J. Immunogenet. **26:**361–372.
11. **Kwon, O. J., C. Brautbar, N. Weintrob, E. Sprecher, C. Saphirman, K. Bloch, O. Pinhas-Hamiel, S. Assah, P. Vardi, and S. Israel.** 2001. Immunogenetics of HLA class II in Israeli Ashkenazi Jewish, Israeli non-Ashkenazi Jewish, and in Israeli Arab IDDM patients. Hum. Immunol. **62:**85–91.
12. **Paschou, P., E. Bozas, M. Dokopoulou, B. Havarani, A. Malamitsi-Puchner, A. Ylli, Z. Ylli, I. Thymelli, A. Gerasimidi-Vazeou, and C. S. Bartsocas.** 2004. HLA alleles and type 1 diabetes mellitus in low disease incidence populations of Southern Europe: a comparison of Greeks and Albanians. J. Pediatr. Endocrinol. Metab. **17:**173–182.
13. **Samaha, H., E. A. Rahal, M. Abou-Jaoude, M. Younes, J. Dacchache, and N. Hakime.** 2003. HLA class II allele frequencies in the Lebanese population. Mol. Immunol. **39:**1079–1081.
14. **Saruhan-Direskeneli, G., F. A. Uyar, S. Bakar, and M. Eraksoy.** 2000. Molecular analysis of HLA-DRB1, DQA1 and -DQB1 polymorphism in Turkey. Tissue Antigens **55:**171–174.
15. **She, J. X.** 1996. Susceptibility to type I diabetes: HLA-DQ and DR revisited. Immunol. Today **17:**323–329.
16. **Steenkiste, A., A. M. Valdes, M. Feolo, D. Hoffman, P. Concannon, J. Noble, G. Schoch, J. Hansen, W. Helmberg, J. S. Dorman, G. Thomson, A. Pugliese, and 13th IHWS 1 Diabetes Component Participating Investigators.** 2007. 14th International HLA and Immunogenetics Workshop: report on the HLA component of type 1 diabetes. Tissue Antigens **69**(Suppl. 1)**:**214–225.
17. **Yoon, J. W., and H. S. Jun.** 2005. Autoimmune destruction of pancreatic beta cells. Am. J. Ther. **12:**580–591.