

HLA class II alleles in human sporotrichosis in Mexican Amerindians

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ABSTRACT

Human leukocyte antigen (HLA) class II alleles are involved in antigen processing and presentation to T lymphocytes during fungal infections. However, few studies have investigated HLA genes in fungal diseases, or in sporotrichosis infections. Here, the frequencies of HLA-DR β 1 in 50 healthy volunteers and 9 patients with sporotrichosis from an endemic area in Mexico were determined to define their role in genetic susceptibility to this infection. Also, the frequencies of HLA-DR β 1 haplotypes were compared with a historic control group of healthy Mexican individuals. The patients presented that DR4 and DR8 increased, which were more than twice the control's values, whereas local controls (endemic area) presented DR*04:01 increased, compared with the control group from Mexico City. The data suggest that involvement of HLA antigens could affect the outcomes of the host-fungi interaction in sporotrichosis by regulating the immune response to *Sporothrix schenckii* complex.

Keywords: sporotrichosis, HLA class II, DR4, DR4 subtypes, DR*0401, DR8, Mexican mestizo

INTRODUCTION

Sporotrichosis is a re-emerging subacute or chronic subcutaneous mycosis that affects humans and some mammals, which is produced by the thermophilic fungi-*Sporothrix schenckii* (*S. schenckii*) complex. Sporotrichosis is considered an occupational

disease that affects peasants, florists, farmers, carpenters and packers of crockery. It is a cosmopolitan disease that presents in tropical and subtropical countries and has been frequently reported in Latin America in recent decades^[1]. In Mexico, sporotrichosis is considered the most common subcutaneous mycosis, with endemic areas in the states of Jalisco, Morelos, Puebla and Guerrero^[2]. Sporotrichosis is observed in all age groups, but most frequently affects young adults. Epi-

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demic outbreaks have been reported in Australia, the United States and México, with cases associated with contaminated hay, sphagnum moss and tent camping, respectively^[3,4]. The infection is initiated by traumatic inoculation of contaminated organic material into the skin. The clinical presentation varies, and the fixed cutaneous form occurs when patients present with a unique nodular lesion, whereas the lymphocutaneous form is characterized by subcutaneous affection with lymphatic extension. Rare forms include primary lung, osteoarticular, ocular or disseminate infections^[5,6]. In Mexico, the lymphocutaneous presentation is observed more frequently, followed by fixed cutaneous sporotrichosis, whereas the primary lung form is rare and likely associated with the genetic characteristics of the fungi and the human population affected in this country^[7]. Thus, the clinical presentation has been associated with individual host susceptibility, immunology state, *Sporothrix* species and quantity of the inoculum^[8,9]. Dimorphic fungi, such as *S. schenckii*, assume both yeast and hyphal growth states based on the environmental conditions (temperature, pH, etcetera). Thus, interactions with the mammalian microenvironment and immune system induce dimorphic transformation. T-cell-mediated immunity (CMI) is the main human response mechanism against *S. schenckii* complex^[10]. The essential role of the CMI response is to destroy the fungi (or at least to limit the progression of infection) and produce an immunoprotective status against re-infection^[11]. The immunological response to the disease is associated with a delayed hypersensitive response, in clinical practice evidenced by the sporotricin test^[12]. Therefore, an interaction between pathogen recognition receptors (PRR) and pathogen associated molecular patterns (PAMPs) is necessary to trigger dendritic cells (DCs). The activated status of DC is characterized by cytokine production, costimulatory molecules and high MHC levels^[13]. In other fungal infections as coccidioidomycosis, specific human leukocyte antigen (HLA) class II alleles have been associated with a predisposition to human infection^[14]; however, human leukocyte antigen D-related (HLA-DR) allele associations have not been established in sporotrichosis. Therefore, the aim of this study was to investigate the association between HLA class II alleles and sporotrichosis in the Mexican Mestizo population, and to determine their roles in susceptibility or protection.

MATERIALS AND METHODS

Subjects

The study recruited a total of 9 patients with a

clinical and laboratory diagnosis of sporotrichosis and 50 healthy controls, all of whom from "El Durazno"—an endemic area of a sporotrichosis in Guerrero state, Mexico. Ethnically, the patients and controls were classified as Mestizos, who are defined as individuals born in Mexico, with Mexican ancestors dating to at least the third generation. Mestizos are the result of 500 years of admixture between Amerindians, Spaniards and/or Africans, and represent most of the Mexican population^[15]. Also, allele frequencies from El Durazno were compared with unrelated Mexican Mestizo individuals from Mexico City, who are considered representative of most of Mexico, since Mexico City is an important immigration center; and with historical data from the Mazatecans population, owing to its geographical proximity to "El Durazno". The protocol was reviewed and approved by the institution's Ethics Committee. Written informed consent was obtained from all subjects prior to their admission in this study, according to the Helsinki Declaration.

HLA-DR β 1 typing

Genomic DNA from all subjects included in this study was purified from peripheral blood leukocytes, according to the method described by Miller. Blood was collected from a single peripheral venipuncture, according to the current guidelines and the procedures approved by the Ethics Internal Review Boards of the General Hospital "Dr. Manuel Gea González". The HLA-DR β 1 locus was genotyped based on the hybridization of labelled single-stranded polymerase chain reaction products to sequence-specific oligonucleotides, using the Life Codes HLA-DR β 1 Typing kit for use with Luminex (Gen-Probe Transplant Diagnostics, Inc., Stamford, CT, USA), following the manufacturer's recommendations. Data were analyzed using the Quicktype for Lifecodes version 3.0 software to determine the HLA alleles^[16].

Intradermic test

Sporotricin, a protein antigenic extract (obtained according to the technique described by Dominguez-Soto and Hojyo-Tomoka)^[17] was used to perform the test. 100 μ L of this extract was applied intradermally to study the cellular responses in the patients and controls. The test was considered positive when the skin induration was equal to or greater than 5 mm between 24 and 48 hours post-inoculation^[18].

Culture

Skin lesion samples were incubated on Sabouraud

Dextrose Agar (SDA) medium at 24 °C. When macroscopically observed, a slow growing colony colored white to cream, moist and glabrous with a wrinkled and folded surface was seen. Older colony development demonstrated brown to black pigmentation on revers; microscopically, thin septate hyphae were observed, usually solitary with conidiophores arising at right angles; conidia were formed in clusters by sympodial proliferation at the apex of the conidiophore. As the culture ages, conidia are subsequently formed singly along the sides of both conidiophores and undifferentiated hyphae. Conidia are ovoid or triangular (3~6 × 2~3 μm), hyaline, and smooth-walled. Dimorphic capacity was demonstrated on a brain heart infusion (BHI) containing blood at 37 °C, with constant agitation, and glabrous colonies were observed (white to greyish-yellow and yeast-like, consisting of spherical or oval budding yeast cells).

Statistical analysis

The allele frequencies were calculated by direct counting, and the differences in the distribution of the alleles between patients and controls were analyzed using the odds ratios, 95% confidence interval and significance level. Statistically significant *P* values (≤ 0.05) were corrected, considering the number of alleles observed (*pC*). Statistical analyses were performed using the EPIINFO software, version 5.0 (USD incorporated 1990, Stone Mountain, GA, USA).

RESULTS

A total of 59 Mexican Mestizo individuals were included in the study and were genotyped for the HLA-DR β 1 locus; the patients with sporotrichosis included 8 females and 1 male, who ranged in age from 11 to 75 years. All patients were positive to intradermic tests and had skin indurations greater than 1 cm in diameter (Fig. 1). Patients had skin lesions with positive cultures for *S. schenckii* (Fig. 2). There were 50 healthy local controls (45 females and 5 males) ranging in age from 8 to 65 years, all without skin lesions and with negative intradermic tests. Table 1 summarizes the allelic frequency distributions of the HLA-DR β 1 alleles in the sporotrichosis patients and local controls from El Durazno. This population presented 6 HLA-DR β 1 alleles (*04, *08, *02, *06, *01, *07, in this order of frequency), and no alleles exhibited statistically significant differences in the frequency between the patients and local controls, but the frequency of the HLA-DR β 1*04:01 and HLA-DR β 1*08:02 alleles among sporotrichosis patients

was more than twice that observed among the healthy controls from El Durazno. Thus, the allele frequencies from the El Durazno population (control group) were compared to the Mexican Mestizos' data (Table 2), showing a statistically significant difference in the frequency between the healthy controls from El Durazno and Mexican Mestizo historical data. The frequency of the HLA-DR*04:01 allele among the El Durazno controls was significantly higher compared to the Mexican Mestizo data [$P < 0.0001$, OR: 19.4, 95% confidence interval (CI): 2.4–156.0], when patients were compared with Mexican Mestizos, the difference in HLA-DR*04:01 was even higher (Table 3). In relation to the Mazatecan population HLA-DR*04:01 was not presented (Table 4), whereas for DR β 1*08:02 there are not statistical differences, plus DR*16:02 was higher when compared with the El Durazno population ($pC = 1 \times 10^{-4}$). When sporotrichosis patients were compared with Mazatecans population, DR*08:02 is presented more than twice in patients, similar relation as exposed in sporotrichosis patients and healthy controls from "El Durazno" suggesting the veracity of this association. In relation to HLA-DR*04:01, the absence of this allele in Mazatecans population could be protect this group against *S. schenckii* complex infection (Table 5).

DISCUSSION

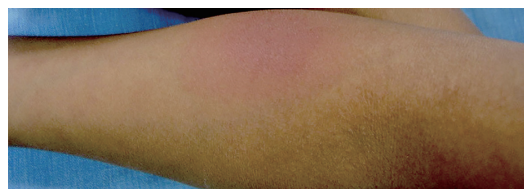


Fig.1 An intradermic test presented skin induration greater than 1 cm in diameter.

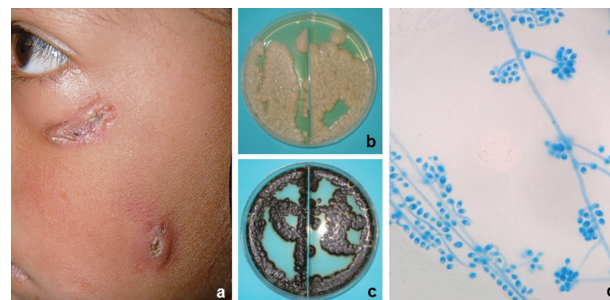


Fig.2 A young patient with facial skin lesions. a: gross observation of skin lesions; b: an SDA culture, a moist cream colony, with a wrinkled surface; c: an older colony with brown to black pigmentation; d: microscopically, the culture presented septate hyphae, with conidiophores and sympodial proliferation.

Table 1 HLA-DR β 1 allele frequencies in sporotrichosis patients and healthy controls from "El Durazno"

HLA-DR alleles	Patients with sporotrichosis(N=18)		Healthy controls from "El Durazno"(N=100)		pC*	OR	95%CI
	n	g.f	n	g.f			
DR4							
DR*04:04	1	0.05	9	0.09	0.63	0.59	0.07–5.00
DR*04:01	4	0.22	9	0.09	0.10	2.24	0.62–8.09
DR*04:07	3	0.16	15	0.15	0.85	1.13	0.29–4.39
OTHERS	NP	NP	31	0.31	0.02	0.12	0.01–0.98
DR8							
DR*08:02	5	0.27	12	0.12	0.08	2.82	0.85–9.31
DR*08:04	NP	NP	NP	NP	NP	NP	NP
DR2							
DR*15:01	2	0.11	8	0.08	0.66	1.43	0.27–7.39
DR*16:02	1	0.05	2	0.02	0.37	2.88	0.24–33.57
OTHERS	NP	NP	NP	NP	NP	NP	NP
DR6							
DR*13*01	NP	NP	1	0.01	0.37	2.88	0.24–33.57
DR*14:02	1	0.05	8	0.08	0.72	0.67	0.07–5.76
OTHERS	NP	NP	1	0.01	0.37	2.88	0.24–33.57
DR1							
DR*01:01	1	0.05	2	0.02	0.37	2.88	0.24–33.57
DR*01:03	NP	NP	1	0.01	0.37	2.88	0.24–33.57
DR7							
DR*07:01	NP	NP	1	0.01	0.37	2.88	0.24–33.57
DR5							
DR*11:01	NP	NP	NP	NP	NP	NP	NP
DR*11:04	NP	NP	NP	NP	NP	NP	NP
DR*12:01	NP	NP	NP	NP	NP	NP	NP
OTHERS	NP	NP	NP	NP	NP	NP	NP
DR3							
DR*03:01	NP	NP	NP	NP	NP	NP	NP
OTHERS	NP	NP	NP	NP	NP	NP	NP
DR9							
DR*09:01	NP	NP	NP	NP	NP	NP	NP
DR10							
DR10:01	NP	NP	NP	NP	NP	NP	NP

N: number of individuals; n: number of alleles; g.f: gene frequencies; *pC by Mantel–Haenszel method; OR: odds ratios; CI: confidence interval; NP: not presented.

Sporotrichosis is the most common subcutaneous mycosis in Mexico and is considered a reemerging disease worldwide, particularly in Latin-American countries. The mycosis is classified as an occupational disease that particularly affects farmers^[19]. Mexican peasants who live in endemic areas and use sandals are constantly exposed to *S. schenckii*—a fungus that lives in soil and organic materials^[18]. "El Durazno", Guerrero, is considered an endemic area for sporotrichosis, where the environmental characteristics of the area and the use of natural materials in many local houses provide a suitable condition for the development of the disease^[20].

The cellular immune response plays an important part in host interaction with *S. schenckii*. The failure of cellular immune response or loss of balance among the components involved permits fungal

dissemination and progression of the infection^[21]. In human immunity, the HLA system contributes to the presentation of pathogen-derived peptides to T cells. The role of HLA-DR in fungal infections was described in chromoblastomycosis, where DCs from infected patients exhibited an increased expression of HLA-DR^[22].

Our data showed an increased rate of HLA-DR β 1*04:01 and HLA-DR β 1*08:02 alleles among sporotrichosis patients, which was more than twice that observed among healthy controls from El Durazno (**Table 1**). The result suggests that polymorphisms in the MHC genes increase susceptibility to sporotrichosis. Furthermore, HLA-DR β 1*08 has been reported to be associated with susceptibility to onychomycosis and other infections such as tuberculosis. In pulmonary tuberculosis HLA-DR β 1*08:03

Table 2 HLA-DRβ1 allele frequencies from "El Durazno" healthy controls and Mexican mestizo historical data

HLA-DR alleles	Healthy controls from "El Durazno"(N=100)		Mexican mestizos (N=198)		pC*	OR	95%CI
	n	g.f	n	g.f			
DR4							
DR*04:04	9	0.09	9	0.04	0.120	2.07	0.79–5.40
DR*04:01	9	0.09	1	0.00	1×10 ⁻⁴	19.40	2.40–156.00
DR*04:07	15	0.15	21	0.10	0.272	1.48	0.73–3.02
OTHERS	31	0.31	16	0.08	1×10 ⁻⁶	5.11	2.63–9.92
DR8							
DR*08:02	12	0.12	30	0.15	0.461	0.76	0.37–1.56
DR*08:04	NP	NP	3	0.01			
DR2							
DR*15:01	8	0.08	9	0.04	0.225	1.82	0.68–4.88
DR*16:02	2	0.02	3	0.01	0.750	1.32	0.21–8.07
OTHERS	NP	NP	6	0.03	0.200	0.27	0.03–2.27
DR6							
DR*13*01	1	0.01	4	0.02	0.510	0.48	0.05–4.44
DR*14:02	8	0.08	7	0.03	0.120	2.22	0.78–6.32
OTHERS	1	0.01	20	0.10	0.003	0.08	0.01–0.68
DR1							
DR*01:01	2	0.02	7	0.03	0.460	0.55	0.11–2.73
DR*01:03	1	0.01	3	0.01	0.710	0.65	0.06–6.39
DR7							
DR*07:01	1	0.01	22	0.11	0.002	0.08	0.01–0.60
DR5							
DR*11:01	NP	NP	12	0.06	0.030	0.14	0.01–1.11
DR*11:04	NP	NP	4	0.02	0.370	0.38	0.04–3.38
DR*12:01	NP	NP	2	0.01	0.710	0.65	0.06–6.39
OTHERS	NP	NP	4	0.02	0.370	0.38	0.04–3.38
DR3							
DR*03:011	NP	NP	9	0.04	0.080	0.18	0.02–1.50
OTHERS	NP	NP	2	0.01	0.710	0.65	0.06–6.39
DR9							
DR*09:01	NP	NP	3	0.01	0.510	0.48	0.05–4.44
DR10							
DR10:01	NP	NP	1	0.00	0.990	0.98	0.08–11.05

N: number of individuals; n: number of alleles; g.f: gene frequencies; *pC by Mantel–Haenszel method; OR: odds ratios; CI: confidence interval; NP: not presented.

has been strongly associated with drug resistance, disease severity and disease recurrence in the Asian population. It has been suggested that HLA alleles influence susceptibility to fungal infections, such as histoplasmosis, chromoblastomycosis, paracoccidioidomycosis and a severe form of coccidioidomycosis^[23]. HLA-DR*08 allele has also been associated with genetic susceptibility to develop onychomycosis in the Mexican Mestizo population^[24]. Moreover, our data showed that the HLA-DR*04:01 subtype was increased in the El Durazno patients, this value is interesting considering the high frequency of HLA-DR*04:01 among Durazno population when they are compared with other Mexican groups, suggesting that in the El Durazno population, HLA-DR*04:01 could serve also as susceptibility factor for development *S. schenckii* fungal infection, and could be explained (at least in part) the particular endemicity of

the sporotrichosis infection in this area. In contrast, some HLA alleles have also been associated with resistance to specific cutaneous fungal infections, as is the case for dermatophytosis^[25].

The HLA-DR4 subtype distribution in Mexican populations, which presented variance according to geography and ethnic origins, is exposed in **Table 2** and **Table 4**, where we compared healthy controls from El Durazno with Mexican Mestizo and with the Mazatecan population. In order to comprehend the possible HLA-DR*04:01 susceptibility mechanism and considering that variance in peptide affinity has been described for DR4 subtypes, it is necessary to characterize HLA-DR4 subtypes in a larger number of patients and controls, plus study tridimensional conformation and molecular affinity for several peptides to understand the role of HLA-DR4 allele subtypes in sporotrichosis susceptibility.

Table 3 Gene frequencies of HLA–DRβ1 alleles in patients with sporotrichosis and healthy historical controls

HLA–DR alleles	Patients with sporotrichosis(N=18)		Mexican mestizo controls(N=198)		pC*	OR	95%CI
	n	g.f	n	g.f			
DR4							
DR*04:04	1	0.05	9	0.045	0.83	1.24	0.14–10.44
DR*04:01	4	0.22	1	0.005	1 × 10 ⁻⁶	56.28	5.88–537.00
DR*04:07	3	0.16	21	0.106	0.43	1.68	0.45–6.30
OTHERS	NP	NP	16	0.080			
DR8							
DR*08:02	5	0.27	30	0.150	0.16	2.15	0.71–6.48
DR*08:04	NP	NP	3	0.015			
DR2							
DR*15:01	2	0.11	9	0.045	0.22	2.62	0.52–13.19
DR*16:02	1	0.05	3	0.015	0.22	3.82	0.37–38.78
OTHERS	NP	NP	6	0.030	0.66	1.60	0.18–13.82
DR6							
DR*13*01	NP	NP	4	0.020	0.45	2.27	0.25–20.56
DR*14:02	1	0.05	7	0.035	0.66	1.60	0.18–13.82
OTHERS	NP	NP	20	0.101	0.49	0.49	0.06–3.91
DR1							
DR*01:01	1	0.05	7	0.050	0.66	1.60	0.18–13.82
DR*01:03	NP	NP	3	0.015	0.34	2.85	0.30–26.97
DR7							
DR*07:01	NP	NP	22	0.111	0.43	0.44	0.05–3.52
DR5							
DR*11:01	NP	NP	12	0.060	0.86	0.83	0.10–6.79
DR*11:04	NP	NP	4	0.020	0.45	2.27	0.25–20.56
DR*12:01	NP	NP	2	0.010	0.22	3.82	0.37–38.78
OTHERS	NP	NP	4	0.020	0.45	2.27	0.25–20.56
DR3							
DR*03:01	NP	NP	9	0.045	0.92	1.10	0.13–9.16
OTHERS	NP	NP	2	0.010	0.11	5.76	0.49–66.87
DR9							
DR*09:01	NP	NP	3	0.015	0.22	3.82	0.37–38.78
DR10							
DR10:01	NP	NP	1	0.005	0.11	5.76	0.49–66.87

N: number of individuals; n: number of alleles; g.f: gene frequencies; *pC by Mantel–Haenszel method; OR: odds ratios; CI: confidence interval; NP: not presented.

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Table 4 HLA–DRβ1 allele frequencies from "El Durazno" healthy controls and Mazatecans

HLA–DR alleles	Healthy controls from "El Durazno"(N=100)		Mazatecans (N=142)		pC*	OR	95%CI
	n	g.f	n	gf			
DR4							
DR*04:04	9	0.09	14	0.09	0.82	0.90	0.37–2.17
DR*04:01	9	0.09	NP	NP	6×10^{-4}	15.66	1.97–124.47
DR*04:07	15	0.15	40	0.28	0.01	0.45	0.23–0.87
OTHERS	31	0.31	15	0.10	1×10^{-4}	3.80	1.92–7.52
DR8							
DR*08:02	12	0.12	19	0.13	0.75	0.88	0.40–1.91
DR*08:04	NP	NP	NP	NP	NP	NP	NP
DR2							
DR*15:01	8	0.08	NP	NP	1×10^{-3}	13.94	1.73–111.93
DR*16:02	2	0.02	26	0.18	1×10^{-4}	0.09	0.02–0.39
OTHERS	NP	NP	7	0.04	0.091	0.19	0.02–1.59
DR6							
DR*13*01	1	0.01	NP	NP	0.37	2.87	0.25–32.17
DR*14:02	8	0.08	5	0.03	0.12	2.38	0.75–7.50
OTHERS	1	0.01	12	0.08	0.01	0.10	0.01–0.85
DR1							
DR*01:01	2	0.02	1	0.007	0.37	2.8	0.25–32.17
DR*01:03	1	0.01	NP	NP			
DR7							
DR*07:01	1	0.01	1	0.007	0.80	1.42	0.088–23.04
DR5							
DR*11:01	NP	NP	1	0.007	0.77	0.70	0.06–7.90
DR*11:04	NP	NP	NP	NP	NP	NP	NP
DR*12:01	NP	NP	NP	NP	NP	NP	NP
OTHERS	NP	NP	NP	NP	NP	NP	NP
DR3							
DR*03:011	NP	NP	NP	NP	NP	NP	NP
OTHERS	NP	NP	NP	NP	NP	NP	NP
DR9							
DR*09:01	NP	NP	NP	NP	NP	NP	NP
DR10							
DR10:01	NP	NP	1	0.007	0.77	0.70	0.06–7.90

N: number of individuals; n: number of alleles; g.f: gene frequencies; *pC by Mantel–Haenszel method; OR: odds ratios; CI: confidence interval; NP: not presented.

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Table 5 Gene frequencies of HLA-DRβ1 alleles in patients with sporotrichosis and Mazatecans

HLA-DR	Patients with sporotrichosis(N=18)		Mazatecans (N=142)		pC*	OR	95%CI
	n	g.f	n	g.f			
DR4							
DR*04:04	1	0.05	14	0.09	0.55	0.53	0.066–4.35
DR*04:01	4	0.22	NP	NP	1×10^{-4}	54.3	5.88–499.8
DR*04:07	3	0.16	40	0.28	0.30	0.51	0.14–1.85
OTHERS	NP	NP	15	0.10	0.46	0.46	0.05–3.71
DR8							
DR*08:02	5	0.27	19	0.13	0.10	2.48	0.79–7.77
DR*08:04	NP	NP	NP	NP	NP	NP	NP
DR2							
DR*15:01	2	0.11	NP	NP	1×10^{-4}	28.2	2.75–288.38
DR*16:02	1	0.05	26	0.18	0.17	0.26	0.03–2.06
OTHERS	NP	NP	7	0.04	0.98	0.98	0.11–3.36
DR6							
DR*13*01	NP	NP	NP	NP	NP	NP	NP
DR*14:02	1	0.05	5	0.03	0.66	1.61	0.17–14.62
OTHERS	NP	NP	12	0.08	0.61	0.58	0.07–4.74
DR1							
DR*01:01	1	0.05	1	0.007	0.08	8.29	0.49–138.70
DR*01:03	NP	NP	NP	NP	NP	NP	NP
DR7							
DR*07:01	NP	NP	1	0.007	0.22	4.11	0.35–47.84
DR5							
DR*11:01	NP	NP	1	0.007	0.22	4.11	0.35–47.84
DR*11:04	NP	NP	NP	NP	NP	NP	NP
DR*12:01	NP	NP	NP	NP	NP	NP	NP
OTHERS	NP	NP	NP	NP	NP	NP	NP
DR3							
DR*03:011	NP	NP	NP	NP	NP	NP	NP
OTHERS	NP	NP	NP	NP	NP	NP	NP
DR9							
DR*09:01	NP	NP	NP	NP	NP	NP	NP
DR10							
DR10:01	NP	NP	1	0.007	0.22	4.11	0.35–47.84

N: number of individuals; n: number of alleles; g.f: gene frequencies; *pC by Mantel–Haenszel method; OR: odds ratios; CI: confidence interval; NP: not presented.

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