NEURAL REGENERATION RESEARCH
November 2016, Volume 11, Issue 11
www.nrronline.org

RESEARCH ARTICLE

HLA class II alleles and risk for peripheral neuropathy in type 2 diabetes patients

Ahmad Marzban[1], Javad Kiani[1], Mehrdad Hajilooi[2], Hamzeh Rezaei[2], Zohreh Kahramfar[1], Ghasem Solgi[2, 3, *]

1 Division of Endocrinology, Department of Internal Medicine, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran
2 Department of Immunology, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran
3 Psoriasis Research Center, Department of Dermatology, Farshchian Hospital, Hamadan University of Medical Sciences, Hamadan, Iran

How to cite this article: *Marzban A, Kiani J, Hajilooi M, Rezaei H, Kahramfar Z, Solgi G (2016) HLA class II alleles and risk for peripheral neuropathy in type 2 diabetes patients. Neural Regen Res 11(11):1839-1844.*

Funding: *This study was funded by Vice-chancellor for Research and Technology, Hamadan University of Medical Sciences of Iran, No. 9208072467.*

Abstract

The potential impact of human leukocyte antigen (HLA) genotype variations on development of diabetic peripheral neuropathy (DPN) is not well determined. This study aimed to identify the association of HLA class II alleles with DPN in type 2 diabetes (T2D) patients. Totally 106 T2D patients, 49 with DPN and 57 without DPN, and 100 ethnic-matched healthy controls were analyzed. Both groups of the patients were matched based on sex, age, body mass index (BMI) and duration of T2D. Polyneuropathy was diagnosed using electrodiagnostic methods. HLA-DRB1 and DQB1 genotyping was performed in all subjects by the polymerase chain reaction with sequence-specific primers (PCR-SSP) method. T2D patients with DPN showed higher frequencies of HLA-DRB1*10 and DRB1*12 alleles compared to control group (P = 0.04). HLA-DQB1*02 allele and HLA-DRB1*07-DQB1*02 haplotype were associated with a decreased risk for developing DPN in T2D patients (P = 0.02 and P = 0.05 respectively). Also, patients with severe neuropathy showed higher frequencies of DRB1*07 (P = 0.003) and DQB1*02 (P = 0.02) alleles than those with mild-to-moderate form of neuropathy. The distribution of DRB1 and DQB1 alleles and haplotypes were not statistically different between all patients and healthy controls. Our findings implicate a possible protective role of HLA-DQB1*02 allele and HLA-DRB1*07-DQB1*02 haplotype against development of peripheral neuropathy in T2D patients. Therefore, variations in HLA genotypes might be used as genetic markers for prediction and potentially management of neuropathy in T2D patients.

Key Words: *nerve regeneration; HLA-DRB1; HLA-DQB1; alleles; genotypes; haplotypes; peripheral neuropathy; type 2 diabetes; neural regeneration*

***Correspondence to:**
Ghasem Solgi, Ph.D.,
gh.solgi@umsha.ac.ir.

orcid:
0000-0001-8929-5658
(Ghasem Solgi)

doi: *10.4103/1673-5374.194756*

Accepted: *2016-10-25*

Introduction

Neuropathy is one of the most common devastating complications of diabetes and is associated with significant morbidity, mortality and diminished quality of life (Boulton et al., 2005; Tesfaye, 2011). Pathogenesis of diabetic neuropathy has not been understood completely, and several hypotheses have been proposed for this microvascular complication in diabetic patients (Greene et al., 1992; AI, 2004). Poor glycemic control plays an important role in the development of peripheral nerve damage (Boulton et al., 1984) and molecular studies have implicated the sequential events for progression of neuropathy and recognized several key players including protein kinase C, advanced glycation end products, aldose reductase, polyol and hexosamine pathways (Balakumar et al., 2009; Wellen et al., 2010).

The pattern and presentation of clinical diabetic neuropathy depend on various factors like duration of hyperglycemia, dyslipidemia, hypertension, smoking, increased height and exposure to other neurotoxic agents such as ethanol (Feldman et al., 1997). But, the role of genetic predisposition for this diabetic microvascular complication is uncertain and it is unclear whether the genetic factors increase the susceptibility of diabetic patients to develop neuropathy (Boulton et al., 1984). While the role of human leukocyte antigens (HLA) in pathogenesis of type 1 diabetes has been clearly established (Kiani et al., 2015), its exact role in type 2 diabetes (T2D) is less clear.

The prevalence of HLA class II alleles and haplotypes among T2D patients have been reported in few studies but with inconsistent results (Tuomi et al., 1993; Turner et al., 1997; Motala et al., 2005; Almawi et al., 2006). Although, other aspects of T2D in relation to HLA class II genes, such as autoimmune markers (anti-GAD antibody), latent autoimmune diabetes in adults, genetic interaction between type 1 and type 2 diabetes and somewhat microvascular complications have been investigated partially (Tuomi et al., 1993; Turner et al., 1997). Nevertheless, the contribution of HLA genes in development of post diabetic complications particularly peripheral neuropathy in T2D patients remains unclear. Because of the lack of consistent data in this regard,

the present study was conducted to explore whether the HLA-DRB1 and DQB1 alleles and DR-DQ haplotypes are associated with diabetic peripheral neuropathy (DPN) in patients with T2D.

Subjects and Methods

Subjects

This case-control study was carried out on 106 unrelated patients with T2D (49 with DPN and 57 without DPN) who referred to outpatient diabetes clinic of Hamadan University of Medical Sciences, Iran between March 2011 and September 2013. Type 2 diabetes was defined according to the American Diabetes Association Diagnostic Criteria (American Diabetes, 2016). Patients with any of the following conditions were excluded from the study: age < 30 and over 70 years, other causes of peripheral neuropathy, use of any neurotoxic drugs like chemotherapeutic agents, history of renal or hepatic dysfunction, and alcohol abuse. All subjects completed a questionnaire consisting of general information, duration of diabetes, type of medication and history of foot ulcer and smoking status. Then, the height, weight and blood pressure for all of the study subjects were recorded. Also, 100 ethnic- and gender-matched healthy subjects with no clinical evidence or family history of T2D were enrolled among blood donors who referred to Blood Transfusion Organization as control group. The mean ages of T2D patients and control groups were 55.8 ± 6.7 years and 45.3 ± 10.6 years respectively. Also, the female-to-male ratios were 44/62 in the patients and 41/59 in the controls. The written informed consents were obtained from all study subjects, and the study protocol was approved by ethics committee, Vice-Chancellor for Research and Technology, Hamadan University of Medical Sciences, Iran, No. 16.35.9.121.

Screening and diagnosis of DPN

Neuropathy Symptom Score (NSS) and Neuropathy Disability Score (NDS) criteria were used for the screening of diabetic neuropathy (Young et al., 1986; Meijer et al., 2003). NSS questionnaire included questions regarding the type of sensation, time of symptoms, location of symptoms, waking up from sleep and factors that relieve symptoms. NDS consisted of neurologic examination parameters such as ankle reflex and perceptions of pinprick, cold and vibration. Each parameter takes a score from 0 to 2. The criteria for the existence of DPN were an NDS score of at least 6.0 irrespective of NSS score, or an NDS score of 3–5 in combination with an NSS score of at least 5.0 (Cabezas-Cerrato, 1998) . Nerve conduction measurements (Sierra II Wedge EMG/NCV/EP Instrument from Cadwell, 909 N. Kellogg Street, Kennewick, Washington 99336, USA) including amplitude, conduction velocity, and latency were carried out on sural, peroneal, and tibial nerves in lower limbs. The obtained values were compared with normal values (GS, 2008). Diagnosis of DPN was based on the recommended protocol (GS, 2008) so that, the case definition criterion for confirmation of DPN was an abnormality (≥ 99th or ≤ 1st percentile) of any attribute of nerve conduction in two separate nerves, one of which must be the sural nerve (England et al., 2005). The severity of DPN was assessed by a combination of neuropathy symptoms, signs and nerve conduction abnormalities as mild, moderate and severe (Tesfaye et al., 2010).

HLA-DRB1 and DQB1 genotyping

Genomic DNA extraction from venous peripheral blood samples was performed by using a modified salting out method (Kiani et al., 2015). Thereafter, HLA genotyping was done by PCR with sequence specific primers using commercial low resolution HLA DR-DQ SSP kits (Olerup SSP®DQ-DR SSP Combi Tray, Stockholm, Sweden) based on the manufacture's instructions. Following PCR amplification, the whole volume (10 μL) of PCR products was runned on a 2.0% agarose gel electrophoresis, stained with ethidium bromide and specific bands were visualized by UV transilluminator. Detection of specific HLA-DRB1 and HLA-DQB1 alleles were executed by SCORE software (Olerup SSP®DQ-DR SSP Combi Tray, Stockholm, Sweden). In addition, different DRB1-DQB1 haplotypes were assigned based on known HLA-DRB1 and DQB1 linkage disequilibrium in Caucasians as well as European populations using an Expectation-Maximization (EM) Algorithm as implemented in the R statistical computing environment (http://www.R-project.org).

Statistical analysis

The frequencies of HLA-DRB1 and DQB1 alleles and deduced DR-DQ haplotypes were calculated by direct counting of HLA phenotypes. Then, allele and haplotype frequencies were compared between the patients and controls using chi-square analysis with Yates' correction or two-tailed Fisher's exact test where appropriate. The risks contributed by alleles and deduced haplotypes were assessed by calculation of relative risk (RR) with 95% confidence intervals (CIs). Logistic regression analysis was implemented to determine the association between risky alleles and haplotypes and development of T2D neuropathy. Also, paired Student's *t*-test was performed to analyze quantitative data between the study's groups. All of the calculations were done by using the SPSS v.16.0 for Windows and probability values less than 0.05 were considered as statistically significant.

Results

Forty-nine T2D patients with DPN and 57 patients without DPN as well as 100 ethnic-matched healthy controls were studied in this cohort. The demographics and clinical characteristics including sex, age, age at T2D diagnosis, treatment status, body mass index (BMI), serum creatinine and hemoglobin A1C (HbA1C) levels, hypertension and diastolic and systolic blood pressure are summarized in **Table 1**. The mean serum creatinine levels, HbA1C contents and BMI were significantly increased in patient groups compared to healthy controls (**Table 1**). Peripheral neuropathy was diagnosed by using electrodiagnostic methods.

HLA-DRB1 and DQB1 allele and haplotype frequencies

Distribution of HLA-DRB1 alleles were not statistically

Table 1 Demographics and some of clinical characteristics of the study subjects

Variables	Patients with DPN (group A, *n* = 49)	Patients without DPN (group B, *n* = 57)	Healthy controls (group C, *n* = 100)
Gender (female/male, *n*)	20/29	24/33	48/52
Age (year)	56.1±6.7	55.6±6.8	45.3±10.6
Age at diagnosis (year)	45.8±8.5	46.5±9.5	–
Duration of disease (year)	10.3±6.2	9.6±5.2	
Therapy status			
OHA	17	25	–
Insulin only	20	14	–
OHA + insulin	12	18	–
BMI (kg/m^2)	28.8±4.6	27.4±4.04	21.8±1.5*
Serum creatinine (mg/dL)	1.15±0.24	1.12±0.18	0.97±0.19*
HbA_{1C} (%)	7.82±1.23	7.48±0.98	5.4±0.54*
Hypertension (yes/no, *n*)	28/21	25/32	–
Systolic blood pressure (mmHg)	131.5±17.2	127.4±16.9	≤ 120.0
Diastolic blood pressure (mmHg)	80.9±10.3	77.8±11.8	≤ 80.0

*P < 0.001, *vs*. groups A and B. DPN: Diabetic peripheral neuropathy; OHA: oral hypoglycemic agent; BMI: body mass index.

Table 2 Distribution of HLA-DRB1 alleles in patients with and without diabetic peripheral neuropathy (DPN) and healthy controls

HLA-DRB1 alleles	With DPN, 2*n* = 98 (%), group A	Without DPN, 2*n* = 114 (%), group B	Healthy controls, 2*n* = 200 (%), group C	*P*			
				A *vs*. B	A *vs*. C	B *vs*. C	(A+B) *vs*. C
DRB1*01	6(6.1)	6(5.3)	14(7.0)	0.97	0.96	0.71	0.72
DRB1*03	10(10.2)	15(13.2)	26(13.0)	0.65	0.61	0.89	0.82
DRB1*04	12(12.2)	7(6.1)	16(8.0)	0.19	0.33	0.70	0.86
DRB1*07	7(7.1)	14(12.3)	22(11.0)	0.30	0.39	0.87	0.83
DRB1*08	2(2.0)	4(3.5)	5(2.5)	0.68	1.00	0.72	0.92
DRB1*09	1(1.0)	1(0.8)	4(2.0)	1.00	1.00	0.65	0.43
DRB1*10	4(4.1)	2(1.7)	1(0.5)	0.41	0.04*	0.29	0.12
DRB1*11	18(18.4)	25(22.0)	44(22.0)	0.63	0.56	0.89	0.75
DRB1*12	4(4.1)	3(2.6)	1(0.5)	0.70	0.04*	0.13	0.06
DRB1*13	12(12.2)	9(8.0)	27(13.5)	0.40	0.90	0.18	0.32
DRB1*14	4(4.1)	3(2.6)	15(7.5)	0.70	0.37	0.12	0.09
DRB1*15	13(13.3)	20(17.5)	21(10.5)	0.50	0.60	0.10	0.16
DRB1*16	5(5.1)	5(4.4)	4(2.0)	1.00	0.16	0.29	0.19

*: Two-tailed *P* values by Fisher's exact test; relative risk is 0.96 (0.92–1.00).

Table 3 Distribution of HLA-DQB1 alleles in patients with and without diabetic peripheral neuropathy (DPN) and healthy controls

HLA-DQB1 alleles	With DPN, 2*n* = 98 (%), group A	Without DPN, 2*n* = 114 (%), group B	Healthy controls, 2*n* = 200 (%), group C	*P*			
				A *vs*. B	A *vs*. C	B *vs*. C	(A+B) *vs*. C
DQB1*02	14(14.3)	30(26.3)	45(22.5)	0.04*	0.12	0.53	0.75
DQB1*03	42(42.8)	39 (34.2)	72 (36.0)	0.25	0.30	0.84	0.71
DQB1*04	3 (3.1)	4 (3.5)	4 (2.0)	1.00	0.68	0.46	0.60
DQB1*05	20 (20.4)	16 (14.1)	37 (18.5)	0.29	0.81	0.39	0.78
DQB1*06	19 (19.4)	25 (21.9)	42 (21.0)	0.77	0.86	0.95	0.95

*: Yates corrected *P* value by chi-square test. Relative risk is 0.54 (0.31–0.96).

different between two groups of the patients but were comparable with healthy controls, and T2D patients with DPN showed higher significant frequencies of HLA-DRB1*10 and DRB1*12 alleles (P = 0.04; **Table 2**). HLA-DQB1*02 allele was more frequent in patients without DPN than in those with DPN (P = 0.04; **Table 3**). Haplotype analysis revealed a lower frequency of HLA-DRB1*07-DQB1*02 haplotype in patients with DPN than those without DPN (P = 0.05; **Table 4**). After adjusting covariates like age, age at diagnosis, duration of disease and BMI, logistic regression analysis revealed

Table 4 Distribution of the most frequent HLA-DRB1-DQB1 haplotypes among both groups of the patients and healthy controls

Haplotypes	With DPN, 2*n* = 98 (%), group A	Without DPN, 2*n* = 114 (%), group B	Healthy controls, 2*n* = 200 (%), group C	*P*			
				A *vs.* B	A *vs.* C	B *vs.* C	(A+B) *vs.* C
DRB1*01-DQB1*05	5(5.1)	4(3.5)	13(6.5)	0.73	0.82	0.38	0.42
DRB1*03-DQB1*02	9(9.2)	14(12.3)	23(11.5)	0.61	0.68	0.98	0.95
DRB1*04-DQB1*03	11(11.2)	7(6.1)	11(5.5)	0.28	0.12	0.98	0.32
DRB1*07-DQB1*02	4(4.1)	14(12.3)	16(8.0)	0.05*	0.30	0.29	0.99
DRB1*07-DQB1*03	3(3.0)	0(0.0)	4(2.0)	0.09	0.68	0.30	0.71
DRB1*08-DQB1*04	1(1.0)	4(3.5)	2(1.0)	0.37	1.00	0.19	0.45
DRB1*09-DQB1*03	0	1(0.8)	4(2.0)	1.00	0.30	0.65	0.20
DRB1*10-DQB1*05	4(4.1)	2(1.7)	3(1.5)	0.41	0.22	1.00	0.50
DRB1*11-DQB1*03	18(18.4)	23(20.2)	40(20.0)	0.87	0.85	0.91	0.96
DRB1*12-DQB1*03	4(4.1)	3(2.6)	2(1.0)	0.70	0.09	0.35	0.17
DRB1*13-DQB1*03	3(3.0)	4(3.5)	5(2.5)	1.00	0.72	0.48	0.84
DRB1*13-DQB1*06	9(9.2)	5(4.4)	20(10.0)	0.26	0.98	0.12	0.62
DRB1*14-DQB1*05	4(4.1)	2(1.7)	11(5.5)	0.41	0.78	0.14	0.26
DRB1*15-DQB1*05	3(3.0)	3(2.6)	1(0.5)	1.00	0.10	0.13	0.12
DRB1*15-DQB1*06	9(9.2)	17(14.9)	17(8.5)	0.29	0.98	0.11	0.27
DRB1*16-DQB1*05	4(4.1)	5(4.4)	4(2.0)	1.00	0.44	0.29	0.30

*: Two-tailed *P* values by Fisher's exact test. Relative risk is 0.33 (0.11–0.98). DPN: Diabetic peripheral neuropathy.

Table 5 Logistic regression model for association between human leukocyte antigen (HLA) and diabetic peripheral neuropathy (DPN) in T2D patients

Allele/haplotype	Odds ratio	95% confidence interval	*P* values
HLA-DQB1*02	2.33	1.03–5.28	0.04
HLA-DRB1*07-DQB1*02	0.27	0.08–0.89	0.03

Table 7 Distribution of HLA-DQB1 alleles among patients with diabetic peripheral neuropathy (DPN) according to severity of neuropathy

HLA-DQB1 alleles	Patients with DPN (*n* = 49)		*P* values
	Mild-to-moderate, 2*n* = 68 (%)	Severe, 2*n* = 30 (%)	
DQB1*02	6(8.8)	8(26.7)	0.02*
DQB1*03	31(45.6)	11(36.7)	0.54
DQB1*04	2(2.9)	1(3.3)	1.00
DQB1*05	17(25.0)	3(10.0)	0.15
DQB1*06	12(17.7)	7(23.3)	0.70

*: Two-tailed P values by Fisher's exact test. Relative Risk is 0.33 (0.13–0.87).

Table 6 Distribution of HLA-DRB1 alleles among patients with diabetic peripheral neuropathy (DPN) according to severity of neuropathy

HLA-DRB1 alleles	Patients with DPN (*n* = 49)		*P* values
	Mild-to-moderate, 2*n* = 68 (%)	Severe, 2*n* = 30 (%)	
DRB1*01	5(7.4)	1(3.3)	66
DRB1*03	6(8.8)	4(13.3)	0.48
DRB1*04	9(13.2)	3(10.0)	0.75
DRB1*07	1(1.5)	6(20.0)	0.003*
DRB1*08	1(1.5)	1(3.3)	0.52
DRB1*09	1(1.5)	0	1.00
DRB1*10	4(5.9)	0	0.30
DRB1*11	13(19.0)	5(16.7)	0.99
DRB1*12	4(5.9)	0	0.30
DRB1*13	7(10.3)	5(16.7)	0.50
DRB1*14	4(5.9)	0	0.30
DRB1*15	9(13.2)	4(13.3)	1.00
DRB1*16	4(5.9)	1(3.3)	1.00

*: Two-tailed *P* values by Fisher's exact test. Relative risk is 0.07 (0.01–0.58).

that HLA-DQB1*02 allele and HLA-DRB1*07-DQB1*02 haplotype were significantly associated with DPN ($P = 0.04$ and $P = 0.03$, respectively; **Table 5**). Also, distribution of HLA alleles among T2D patients according to the severity of neuropathy revealed a higher frequencies of DRB1*07 and DQB1*02 alleles in patients with severe neuropathy than in those with mild-to-moderate neuropathy ($P = 0.003$ and $P = 0.02$, respectively; **Tables 6**, **7**). However, the HLA-DRB1 and DQB1 allele and haplotype frequencies were not statistically different between all patients and healthy controls. Although T2D patients showed higher and lower frequencies of HLA-DRB1*12 and DRB1*14 alleles respectively compared to healthy controls but, it was not statistically significant (**Table 2**).

Discussion

Diabetic neuropathies are clinically classified to symmetrical and asymmetrical neuropathies and distal symmetrical peripheral neuropathy is the commonest form of these post-diabetes microvascular complications (Bansal et al., 2006). Nerve biopsy examinations revealed that besides the metabolic changes and ischemic injury, perivascular infiltration and immunological abnormality could be observed in these pathological conditions. Chronic inflammatory demeylinating polyneuropathy has been confirmed in two

clinical forms of diabetic neuropathies (Bansal et al., 2006). Moreover, glutamic acid decarboxylase (GAD) antibodies and latent autoimmune diabetes have been found in some of T2D patients (Tuomi et al., 1993; Turner et al., 1997). Genetic determinants particularly those involved in the affected metabolic pathway have been reported to be attributed in the pathogenesis of T2D and subsequent microvascular complications (Bansal et al., 2006; Ma et al., 2013; Brunetti et al., 2014). Disease heterogeneity in type 2 diabetes may influence the susceptibility to diabetic complications (Forsblom et al., 1998).

The role of HLA alleles in pathogenesis of T2D and its subsequent complications is less clear and their association is still contradictory and elusive because of very limited studies on HLA-T2D relationship (Ma et al., 2013). To investigate the possible correlation between HLA class II alleles and peripheral neuropathy in a group of Iranian T2D patients, we analyzed the distribution of HLA-DRB1 and DQB1 alleles and DRB1-DQB1 haplotypes in two groups of patients (with or without DPN) as well as in ethnic -matched healthy controls.

Our findings demonstrated a negative association between HLA-DQB1*02 allele and HLA-DRB1*07-DQB1*02 haplotype with DPN in type 2 diabetes patients. Also, we observed that HLA-DRB1*07 and DQB1*02 alleles were associated with severe form of peripheral neuropathy. Comparison of all patients and healthy controls did not show statistical differences for either HLA alleles or HLA haplotypes but, the patients with DPN separately showed higher significant frequencies of HLA-DRB1*10 and DRB1*12 alleles than controls.

In contrast to our results, Boulton et al. (1984) and Scheinin et al. (1988) did not find any association between DPN and HLA-A, -B, -C and DR alleles among type 1 and type 2 diabetes patients, whereas, a study by Brazilay et al. (1992) depicted a direct association between HLA-DRB1*03 and DRB1*04 alleles and cardiovascular autonomic neuropathy in T1D patients. In this regard, our results showed higher and lower but insignificant frequencies of DRB1*04 and DRB1*03 alleles respectively in patients with DPN compared to those without DPN. Two studies on T2D patients showed the protective and susceptible roles of DQB1*05:01 allele for diabetic nephropathy respectively (Perez-Luque et al., 2000; Ma et al., 2013). Additionally, Ma et al (2013). demonstrated that DQA1*03:01 and 05:01 alleles are directly associated with nephropathy in T2D patients, while Perez-Luque et al. showed a positive and negative association for DRB1*15:02 and DRB1*04:07 alleles with diabetic nephropathy respectively.

To our knowledge, the present study is the first report on potentially protective role of HLA-DQB1*02 allele and HLA-DRB1*07-DQB1*02 haplotype against peripheral neuropathy in T2D patients. Because of the paucity and inconsistent data regarding HLA and T2D microvascular complications particularly neuropathy, our results should be interpreted with caution and further investigations using larger cohorts and preferentially with focus on underlying mechanisms are warranted. However, the genetic factors including HLA alleles are only one of the possible contributing factors for development and progression of peripheral neuropathy and more importantly, elucidating the exact role of HLA molecules in susceptibility/protection for diabetic peripheral neuropathy remains undefined and has proved challenging.

Comparison of the frequencies of HLA alleles and haplotypes between all patients and healthy controls in the present study did not show any significant differences. Other similar studies have revealed an association (Ghabanbasani et al., 1995; Motala et al., 2005; Ma et al., 2013) and no or weak association between HLA genes and T2D (Jaeger et al., 1997; Tipu et al., 2011). For instance, in Bahrainis, T2D was found to be positively associated with DRB1*04:01 and *07:01 alleles and negatively correlated with DRB1*11:01 and *16:01 alleles (Motala et al., 2005). Also, several DRB1-DQB1 haplotypes appeared to confer susceptibility to T2D and DRB1*16:01-DQB1*05:01 haplotype was negatively associated with T2D. Whereas, our findings revealed higher frequency of this haplotype in the patients compared to controls but it did not reach statistical significance probably due to small number of the patients in the current study. In Pakistanis, HLA-DRB1*13 alleles were found to be more frequent in T2D patients (Tipu et al., 2011) and a Chinese population showed a link between DQA1*0301 and DQA1*0501 alleles with T2D (Ma et al., 2013).

Conclusions

Given to complex multifactorial etiology of T2D and consequent microvascular complications, further investigations are needed to identify the predisposing genetic factors and their interaction with environmental factors in order to take preventive measures or probable better therapeutic interventions for T2D and post-diabetes complications. Taken together, we observed a plausible contribution of some HLA class-II alleles for development of DPN and even severity of this complication, but the elucidation of true association and more importantly the exact role of HLA genes in T2D and subsequent complications particularly neuropathy need replicative studies involving larger cohorts in our population as well as in different ethnic groups. Additionally, the evaluation of other immunological markers would also be worthy to identify the subjects at increased risk of this disease.

Declaration of patient consent: *The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.*

Acknowledgments: *The authors are grateful to all of the type 2 diabetes patients and to all staffs of Division of Endocrinology, Department of Internal Medicine, School of Medicine, Hamadan University of Medical Sciences, Iran for their participation in this study.*

Author contributions: *JK, GS and MH designed the study and analyzed data. AM and HR carried out the molecular genetic tests and drafted the paper. JK and ZK conceived the study and participated in patients' examination. All authors read and approved the final version of this paper for publication.*

Conflicts of interest: *None declared.*

Plagiarism check: *This paper was screened twice using CrossCheck to*

verify originality before publication.
Peer review: *This paper was double-blinded and stringently reviewed by international expert reviewers.*

References

AI V (2004) Diabetic neuropathies. In: Medical Clinics of North America.

Almawi WY, Wakim-Ghorayeb SF, Arekat MR, Najm P, Keleshian SH, Al-Sayed N, Blanchon B, Samaha HR, Irani-Hakime N (2006) Association of selective HLA class II susceptibility-conferring and protective haplotypes with type 2 diabetes in patients from Bahrain and Lebanon. Clin Vaccine Immunol 13:1296-1298.

American Diabetes Association (2016) 2. Classification and Diagnosis of Diabetes. Diabetes Care 39 Suppl 1:S13-22.

Balakumar P, Arora MK, Reddy J, Anand-Srivastava MB (2009) Pathophysiology of diabetic nephropathy: involvement of multifaceted signalling mechanism. J Cardiovasc Pharmacol 54:129-138.

Bansal V, Kalita J, Misra UK (2006) Diabetic neuropathy. Postgrad Med J 82:95-100.

Barzilay J, Warram JH, Rand LI, Pfeifer MA, Krolewski AS (1992) Risk for cardiovascular autonomic neuropathy is associated with the HLA-DR3/4 phenotype in type I diabetes mellitus. Ann Intern Med 116:544-549.

Boulton AJ, Vileikyte L, Ragnarson-Tennvall G, Apelqvist J (2005) The global burden of diabetic foot disease. Lancet 366:1719-1724.

Boulton AJ, Worth RC, Drury J, Hardisty CA, Wolf E, Cudworth AG, Ward JD (1984) Genetic and metabolic studies in diabetic neuropathy. Diabetologia 26:15-19.

Brunetti A, Chiefari E, Foti D (2014) Recent advances in the molecular genetics of type 2 diabetes mellitus. World J Diabetes 5:128-140.

Cabezas-Cerrato J (1998) The prevalence of clinical diabetic polyneuropathy in Spain: a study in primary care and hospital clinic groups. Neuropathy Spanish Study Group of the Spanish Diabetes Society (SDS). Diabetologia 41:1263-1269.

England JD, Gronseth GS, Franklin G, Miller RG, Asbury AK, Carter GT, Cohen JA, Fisher MA, Howard JF, Kinsella LJ, Latov N, Lewis RA, Low PA, Sumner AJ (2005) Distal symmetrical polyneuropathy: definition for clinical research. Muscle Nerve 31:113-123.

Feldman EL, Stevens MJ, Greene DA (1997) Pathogenesis of diabetic neuropathy. Clin Neurosci 4:365-370.

Forsblom CM, Sane T, Groop PH, Totterman KJ, Kallio M, Saloranta C, Laasonen L, Summanen P, Lepantalo M, Laatikainen L, Matikainen E, Teppo AM, Koskimies S, Groop L (1998) Risk factors for mortality in Type II (non-insulin-dependent) diabetes: evidence of a role for neuropathy and a protective effect of HLA-DR4. Diabetologia 41:1253-1262.

Ghabanbasani MZ, Spaepen M, Buyse I, Legius E, Decorte R, Bex M, Marynen P, Bouillon R, Cassiman JJ (1995) Increased and decreased relative risk for non-insulin-dependent diabetes mellitus conferred by HLA class II and by CD4 alleles. Clin Genet 47:225-230.

Greene DA, Sima AA, Stevens MJ, Feldman EL, Lattimer SA (1992) Complications: neuropathy, pathogenetic considerations. Diabetes Care 15:1902-1925.

GS R (2008) Electromyography fundamentals. In.

Jaeger C, Allendorfer J, Hatziagelaki E, Dyrberg T, Bergis KH, Federlin K, Bretzel RG (1997) Persistent GAD 65 antibodies in longstanding IDDM are not associated with residual beta-cell function, neuropathy or HLA-DR status. Horm Metab Res 29:510-515.

Kiani J, Hajilooi M, Furst D, Rezaei H, Shahryari-Hesami S, Kowsarifard S, Zamani A, Solgi G (2015) HLA class II susceptibility pattern for type 1 diabetes (T1D) in an Iranian population. Int J Immunogenet 42:279-286.

Ma ZJ, Sun P, Guo G, Zhang R, Chen LM (2013) Association of the HLA-DQA1 and HLA-DQB1 Alleles in Type 2 Diabetes Mellitus and Diabetic Nephropathy in the Han Ethnicity of China. J Diabetes Res 2013:452537.

Meijer JW, Bosma E, Lefrandt JD, Links TP, Smit AJ, Stewart RE, Van Der Hoeven JH, Hoogenberg K (2003) Clinical diagnosis of diabetic polyneuropathy with the diabetic neuropathy symptom and diabetic neuropathy examination scores. Diabetes Care 26:697-701.

Motala AA, Busson M, Al-Harbi EM, Khuzam MA, Al-Omari EM, Arekat MR, Almawi WY (2005) Susceptible and protective human leukocyte antigen class II alleles and haplotypes in bahraini type 2 (non-insulin-dependent) diabetes mellitus patients. Clin Diagn Lab Immunol 12:213-217.

Perez-Luque E, Malacara JM, Olivo-Diaz A, Alaez C, Debaz H, Vazquez-Garcia M, Garay ME, Nava LE, Burguete A, Gorodezky C (2000) Contribution of HLA class II genes to end stage renal disease in mexican patients with type 2 diabetes mellitus. Hum Immunol 61:1031-1038.

Scheinin T, Matikainen E, Groop L, Lepantalo M, Koskimies S, Kontiainen S (1988) HLA-antigens and immunity to insulin in insulin-dependent diabetics with or without diabetic neuropathy. Scand J Clin Lab Invest 48:327-332.

Tesfaye S (2011) Recent advances in the management of diabetic distal symmetrical polyneuropathy. J Diabetes Investig 2:33-42.

Tesfaye S, Boulton AJ, Dyck PJ, Freeman R, Horowitz M, Kempler P, Lauria G, Malik RA, Spallone V, Vinik A, Bernardi L, Valensi P, Toronto Diabetic Neuropathy Expert G (2010) Diabetic neuropathies: update on definitions, diagnostic criteria, estimation of severity, and treatments. Diabetes Care 33:2285-2293.

Tipu HN, Ahmed TA, Bashir MM (2011) Human leukocyte antigen class II susceptibility conferring alleles among non-insulin dependent diabetes mellitus patients. J Coll Physicians Surg Pak 21:26-29.

Tuomi T, Groop LC, Zimmet PZ, Rowley MJ, Knowles W, Mackay IR (1993) Antibodies to glutamic acid decarboxylase reveal latent autoimmune diabetes mellitus in adults with a non-insulin-dependent onset of disease. Diabetes 42:359-362.

Turner R, Stratton I, Horton V, Manley S, Zimmet P, Mackay IR, Shattock M, Bottazzo GF, Holman R (1997) UKPDS 25: autoantibodies to islet-cell cytoplasm and glutamic acid decarboxylase for prediction of insulin requirement in type 2 diabetes. UK Prospective Diabetes Study Group. Lancet 350:1288-1293.

Wellen KE, Lu C, Mancuso A, Lemons JM, Ryczko M, Dennis JW, Rabinowitz JD, Coller HA, Thompson CB (2010) The hexosamine biosynthetic pathway couples growth factor-induced glutamine uptake to glucose metabolism. Genes Dev 24:2784-2799.

Young RJ, Zhou YQ, Rodriguez E, Prescott RJ, Ewing DJ, Clarke BF (1986) Variable relationship between peripheral somatic and autonomic neuropathy in patients with different syndromes of diabetic polyneuropathy. Diabetes 35:192-197.

Copyedited by Li CH, Song LP, Zhao M