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HLA allele frequencies in Iranian opticospinal multiple sclerosis patients

——HLA in Opticospinal MS

Hossein Kalanie[1], Malihe Kamgooyan[2], Yadollah Kholghie[3], Ali Amini Harandi[4*], Zahra Hosseinzadeh[5]

[1]MS Research Center, Mehr and Loghman Hospital Shahid Beheshti, University of Medical Sciences,Tehran, Iran;
[2]Bahar Medical Laboratory, Department of Immunology and Transplantation, Tehran, Iran;
[3]Loghman Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran;
[4]Loghman Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran;
[5]Mehr Hospital, Zartosht Street Tehran, Iran.
Email: *amini_alli@yahoo.com

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ABSTRACT

Background: In Iranian patients with opticospinal multiple sclerosis (OSMS), a paucity of brain lesions and short spinal cord lesions extending less than three spinal segments are characteristic findings on magnetic resonance imaging (MRI). It also shows a relatively benign course with negative CSF oligocoonal bands. Objective: We aimed to clarify the possible relationship between clinical phenotype and MRI features of OSMS and human leucocyte antigen (HLA) system in Iran. Methods: Genotyping of HLA class II allele frequencies in 20 patients with OSMS were done, using polymerase chain reaction sequence-specific primer amplification method. Blood samples were extracted and typed for HLA-DRB, DQA, and DQB loci and compared with 100 controls. Results: Significant positive association was observed in DRB1*03, DQA1*0201, DQA1*03, DQB1*0201, and DQB1*0611, while DQB1*0602 was absent in our patients. Conclusion: These finding suggest that HLA-DRB association pattern in OSMS is different from conventional MS in Iran which is mostly associated with DRB1*1501 and from similar Japanese OSMS who are negative for brain lesions fulfilling the Barkhof criteria and negative for the presence of longitudinally extensive spinal cord lesions who carries the DRB*0405 allele. OSMS is immunogenetically heterogeneous. Also absence of DQB1*0602 allele may negatively be associated with the absence of Barkhof brain lesion.

Keywords: Epidemiology, HLA-Class II Allele Frequency, Opticospinal Multiple Sclerosis; Neuromyelitis Optica; NMO-IgG Antibody; Devic's Neuromyelitis Optica; Iranian; Demyelinating Disease

1. INTRODUCTION

The etiology of multiple sclerosis (MS) is unknown, and pathogenesis of the disease encompasses multiple inflammatory as well as apoptotic process in the central nervous system [1-3]. Epidemiological studies indicate both genetic and environmental component in MS susceptibility [4]. The human leucocyte antigen (HLA) system provides a set of genetic loci which lend themselves to systematic study. In fact both linkage and whole genome association screens revealed a prominent role for alleles of the major histocompatibility complex class II gene HLA-DRB1 [5]. Of interest, in northern European-descended populations, association with this gene was identified only within families that carried the HLA DRB1*1501 allele [6]. This observation demonstrates that only a portion of familial MS is associated with allelic variation in this gene. Therefore other genes must contribute prominently to MS susceptibility of DRB1. The fact that familial MS has at least 2 forms, the variety associated with HLA-DRB1 and the form without this association, onclusively demonstrates that MS is immunogenetically heterogenesis.

In Asia, the diversity of MS has been a topic of debates for years and there are wide variations in the clinical presentation [7], and the prominence of Opticospinal MS (OSMS) in Asian populations has long been recognized. Many authors have divided MS into opticospinal and conventional forms, base on their differential clinical phenotypes. Kira *et al.* [8] considered these to be differential entities and have also shown immunogenetic differences between the types. They found an association between conventional MS and DRB1*1501 and

DRB5*0501 was found to play an important role in the development of MS in general but not in OSMS [9,10]. On the basis of the presence or absence of brain lesions fulfilling the Barkhof criteria and the longitudinally extensive spinal cord lesions (LESCLs) recently Matsuoka *et al*. [11] have shown that OSMS in Japan is heterogeneous. One third of Japanese OSMS have Barkhof (-) LESCLs (-) vairant of the disease and interestingly enough this type of presentation is so far from the only known presentation of the disease in Iran [12].

Although, OSMS is reported to be common in Japan and some other Asian countries [13-20], it is an uncommon presentation of MS in Iran [12,21]. The aim of this study is to clarify the possible relationship between clinical phenotype and magnetic resonance imaging (MRI) features of OSMS and HLA system in Iran.

2. MATERIALS AND METHOD

HLA-Class II allele frequencies were studied in 20 patients with OSMS whose demographic data, clinical course, MRI findings and cerebro-spinal fluid (CSF) changes have been recently published [22] and were compared with 100 healthy controls. There were 14 women and 6 men with gender ratio of 2.3:1. The age of onset ranged from 10 to 50 years with the mean of 24 ± 8.2 and the mean disease duration of 8 ± 4.4 years. The annual relapse rate was 0.66 ± 0.84 and the EDSS ranged from 0 to 6 with the mean of 2.5 ± 1.3 with the number of exacerbation of 5.25 ± 5.1. All 20 patients had 1 to 5 hemispheric T2 lesion in brain MRI and spinal cord lesions that extending below three vertebral segments (short lesions) in sagittal planes with peripheral white matter location and central sparing on the axial plane. CSF contained normal cell count and protein level with normal IgG index and negative oligocolonal bands.

Genomic DNA was extracted from EDTA blood sample by QIA DNA Mini Kits (QIA Gen) and DNA extraction kits of protrans. HLA-DRB, DQA and DQB typing were performed using PCR-SSP method. Medium Resolution Kit used for typing these loci was supplied by Protrans and CTS of Heidelberg University and Taq DNA polymerase enzyme by Roche Company (Basle, Switzerland). The PCR reaction was carried out in 10 ul volumes by thermal cyclers (Techne-Genius). Amplification was followed after initial denaturation with 94 cycles (CS) for 2 minutes (including 10 CS for 15 seconds, 65 CS for 60 seconds, 20 CS for 15 seconds, 61 CS for 50 seconds, and 72 CS for 30 seconds). After running PCR products on agarose gel and observation of specific bands using UV transilluminator, the results were interpreted and alleles assigned [23,24]. The same processes were performed for 100 healthy persons as control group who were volunteers of organ transplantation.

Statistical analysis was performed using Chi-Square and Fisher's Exact Test. A p value less than 0.05 was considered statistically significant and analysis was done by SPSS version 11.0 software (SPSS Inc., Chicago, IL).

3. RESULTS

The HLA class II allele frequencies for DRB, DQA and DQB in 20 OSMS patients comparing to 100 healthy subjects are shown in **Table 1**, **2** and **3**, respectively. There were strong positive association between DRB1*03 (70% vs. 16%; P-value = 0.009), DQA1*0201 (35% vs. 10%; P-value = 0.01), DQA1*03 (45% vs. 10%; P-value = 0.009), DQB1*0201 (90% vs. 19%; P-value = 0.009), and DQB1*0611(30% vs. 7%; P-value = 0.009) and OSMS patients, while DQB1*0602 (0% vs. 10%; P-value = 0.210) and DRB1*11 (20% vs. 46%; P-value = 0.045) haplotype were negatively associated with our patients. Interestingly enough, no significant association was found between OSMS and HLA- DRB1*1501 which is the most common HLA class II [5,7,9,22] in conventional MS patients in Iran and other countries. The HLA-DRB1*0103, DRB1*09, DRB1*12, DRB1*14 were not observed in our OSMS patients, so was for HLA-DQA1*03011 and DQA1*04011.

4. DISCUSSION

While OSMS is a common clinical presentation of MS in oriental countries including Japan [13-20] with distinct reported features [8] and immunogenetic differences with conventional MS (CNMS), it is an uncommon clinical presentation of the disease in Iran [12,21]. Heterogeneity in the clinical course and MRI findings [12,25,26] may indicate to the presence of possible genetic contributing factors and in fact previously it has been shown that HLA-DRB1*1501 which plays an important role in the development of MS in general are not observed in OSMS. On the basis of presence or absence of brain lesions fulfilling the Barkhof criteria ± LESCLs, have shown that Barkhof (-) patients had remarkably lower frequency of HLA-DRB1*0901, despite this being the most common allele in Japanese [27]. This allele also was not presented in our patients, however it is not a common allele in Iranian population. In contrast our cases showed negative association with DQB1*0602 allele (0.0% vs. 10%). Therefore, absence of this allele along with paucity of DRB1*1501 may negatively be associated with the absence of Barkhof brain lesions in our patients. In Matsouka's study [27] the Barkhof (-) LESCLs (-) patients which are in close similarity to our study group, as a unique subtype of OSMS in Asian showed a significant increase in the frequency of DRB1*0405 compared with controls. But in our patients

Table 1. HLA-DRB allele in 20 Iranian Opticospinal MS and 100 normal population.

HLA frequency	No. of pts	No. of controls	P value	Significancy
DRB1*0101	1 (5%)	15 (15%)	0.305	NS
DRB1*0103	0	0	–	–
DRB1*03	14 (70%)	16 (16%)	0.009	Ext.sig
DRB1*04	7 (35%)	28 (28%)	0.719	NS
DRB1*07	8 (40%)	27 (27%)	0.369	NS
DRB1*08	1 (5%)	2 (2%)	0.424	NS
DRB1*09	0	2 (2%)	1.0	NS
			1.0	NS
	1 (5%)	6 (6%)		
DRB1*10				
DRB1*11	4 (20%)	46 (46%)	0.045	Ext.sig
DRB1*12	0	2 (2%)	1.0	NS
DRB1*13	2 (10%)	20 (20%)		–
DRB1*14	0	9 (9%)	0.362	NS
DRB1*15	4 (20%)	20 (20%)	1.0	NS
DRB1*16	2 (10%)	10 (10%)	1.0	NS
DRB 303	16 (80%)	92 (92%)	0.114	NS
DRB 40404	15 (75%)	58 (58%)	0.242	NS
DRB 50101	6 (30%)	29 (29%)	1.0	NS

No of Pts: number of patients. NS: Not significant. Ext.sig: Extremely significant.

Table 2. HLA-DQA allele in 20 Iranian Opticospinal MS and 100 normal population.

HLA frequency	No. of pts	No. of controls	P value	Significancy
DQA1*01	7 (35%)	45 (%)	0.564	NS
DQA1*0101	1 (5%)	10 (%)	0.689	NS
DQA1*0102	4 (20%)	14 (%)	0.499	NS
DQA1*0103	1 (5%)	12 (%)	0.693	NS
DQA1*0104	1 (5%)	9 (%)	1.0	NS
DQA1*0201	7 (35%)	10 (%)	0.010	Ext.sig
DQA1*03	9 (45%)	10 (%)	0.009	Ext.sig
DQA1*03011	0	0	–	–
DQA1*04	0	2 (%)	1.0	NS
DQA1*04011	0	0	–	
DQA1*05	10 (50%)	33 (%)	0.233	NS
DQA1*06	0	0	–	–

No of Pts: number of patients. NS: Not significant. Ext.sig: Extremely significant.

Table 3. HLA-DQB allele in 20 Iranian Opticospinal MS and 100 normal population.

HLA frequency	No. of pts	No. of controls	P value	Significancy
DQB1*0201	18 (90%)	19 (19%)	0.009	Ext.sig
DQB1*0203	0	1 (1%)	1.0	NS
DQB1*0301	5 (25%)	29 (29%)	0.928	NS
DQB1*0302	2 (10%)	5 (5%)	0.330	NS
DQB1*0303	1 (5%)	4 (4%)	0.342	NS
DQB1*0305	2 (10%)	1 (1%)	0.072	NS
DQB1*0306	0	1 (1%)	1.0	NS
DQB1*0401	2 (10%)	2 (2%)	1.0	NS
DQB1*0501	3 (15%)	18 (18%)	1.0	NS
DQB1*05051	1 (5%)	0	1.0	NS
DQB1*0531	0	3 (3%)	1.0	NS
DQB1*0611	6 (30%)	7 (7%)	0.009	Ext.sig
DQB1*0602	0	10 (10%)	0.210	NS
DQB1*0604	0	3 (3%)	1.0	NS

No of Pts: number of patients. NS: Not significant. Ext.sig: Extremely significant.

HLA-DRB1*04 is the third common HLA-gene in this respect, while significant over-representation was seen in the frequency of DRB1*03 in our group comparing with controls. Interestingly, over-representation of this allele recently has been reported in neuromyelitis optica (NMO) from Brazil [28]. Our previous study [12] showed that although there are some demographic similarities between a subpopulation of OSMS in Japan with Barkhof (-), LESCLs (-) and Iranian OSMS, still there are genetic dissimilarities between two groups which

pinpoints to further heterogeneity of the disease in this respect. Nowadays, with the availability of NMO immunoglobulin G (NMO-IgG) which targets the water channel protein aquaporin-4, NMO spectrum of disorder is wider than previously known and covers some cases of Asian OSMS [29-31]. At present, probably the type of OSMS in Iran [12] and a subpopulation of OSMS in Japan according to Matsuoka *et al.* [11,27] with Barkhof (-), LESCLs (-) MRI features, lower female to male ratio, low annual relapse rate and lower number of exacerbation and lower EDSS scores is the best candidate to be nominated as pure opticospinal MS (POSMS).

5. CONCLUSIONS

Since evaluation of NMO-IgG was not feasible in our country, it is our main study limitation. We wish to emphasize that the present study is a preliminary one as a result of small number of patients involved due to low prevalence of the disease in Iran. Nonetheless the findings are encouraging enough to warrant further study of these patients on a larger scale both in Iran and other Asian countries.

REFERENCES

[1] Hafler, D.A. (2004) Multiple sclerosis. *Journal of Clinical Investigation*, **113**, 788-794.

[2] Barnett, M.H. and Prineas, J.W. (2004) Relapsing and remitting multiple sclerosis: Pathology of the newly forming lesion. *Annals of Neurology*, **55**, 458-468. doi:10.1002/ana.20016

[3] Kornek, B. and Lassmann, H. (2003) Neuropathology of multiple sclerosis-new concepts. *Brain Research Bulletin*, **61**, 321-326. doi:10.1016/S0361-9230(03)00095-9

[4] Hogancamp, W.E., Rodriguez, M. and Weinshenker, B.G. (1997) The epidemiology of multiple sclerosis. *Mayo Clinic Proceedings*, **72**, 871-878. doi:10.4065/72.9.871

[5] Olerup, O. and Hillert, J. (1991) HLA class II-associated genetic susceptibility in multiple sclerosis: A critical evaluation. *Tissue Antigens*, **38**, 1-15. doi:10.1111/j.1399-0039.1991.tb02029.x

[6] Akesson, E., Oturai, A., Berg, J., Fredrikson, S., Andersen, O., Harbo, H.F., Laaksonen, M., Myhr, K.M., Nyland, H.I., Ryder, L.P., Sandberg-Wollheim, M., Sorensen, P.S., Spurkland, A., Svejgaard, A., Holmans, P., Compston, A., Hillert, J. and Sawcer, S. (2002) A genome-wide screen for linkage in Nordic sib-pairs with multiple sclerosis. *Genes Immunity*, **3**, 279-285. doi:10.1038/sj.gene.6363866

[7] Wasay, M., Khatri, I.A., Khealani, B. and Sheerani, M. (2006) MS in Asian countries. *International MS Journal*, **13**, 58-65.

[8] Kira, J., Yamasaki, K., Horiuchi, I., Ohyagi, Y., Taniwaki, T. and Kawano, Y. (1999) Changes in the clinical phenotypes of multiple sclerosis during the past 50 years in Japan. *Journal of the Neurological Sciences*, **166**, 53-57. doi:10.1016/S0022-510X(99)00115-X

[9] Kira, J., Kanai, T., Nishimura, Y., Yamasaki, K., Matsushita, S., Kawano, Y., Hasuo, K., Tobimatsu, S. and Kobayashi, T. (1996) Western versus Asian types of multiple sclerosis: Immunogenetically and clinically distinct disorders. *Annals of Neurology*, **40**, 569-574. doi:10.1002/ana.410400405

[10] Fukazawa, T., Kikuchi, S., Miyagishi, R., Miyazaki, Y., Yabe, I., Hamada, T. and Sasaki, H. (2006) HLA-dPB1*0501 is not uniquely associated with opticospinal multiple sclerosis in Japanese patients. Important role of DPB1*0301. *Multiple Sclerosis*, **12**, 19-23. doi:10.1191/135248506ms1252oa

[11] Matsuoka, T., Matsushita, T., Osoegawa, M., Ochi, H., Kawano, Y., Mihara, F., Ohyagi, Y. and Kira, J. (2008) Heterogeneity and continuum of multiple sclerosis in Japanese according to magnetic resonance imaging findings. *Journal of the Neurological Sciences*, **266**, 115-125. doi:10.1016/j.jns.2007.09.010

[12] Kalanie, H., Kholghie, Y., Shamsai, G.R. and Ghorbani, M. (2009) Opticospinal multiple sclerosis in Iran. *Journal of the Neurological Sciences*, **276**, 130-132. doi:10.1016/j.jns.2008.09.038

[13] Kuroiwa, Y., Igata, A., Itahara, K., Koshijima, S. and Tsubaki, T. (1975) Nationwide survey of multiple sclerosis in Japan. Clinical analysis of 1084 cases. *Neurology*, **25**, 845-851.

[14] Houzen, H., Niino, M., Kikuchi, S., Fukazawa, T., Nogoshi, S., Matsumoto, H. and Tashiro, K. (2003) The prevalence and clinical characteristics of MS in northern Japan. *Journal of the Neurological Sciences*, **211**, 49-53.

[15] Zhao, B. X., Liu, X. Q., Guo, Y. P., Yang, Y. C. and Huang, H. F. (1981) Multiple sclerosis. A clinical study of 70 cases. *European Neurology*, **20**, 394-400.

[16] Hung, T.P., Landsborough, D. and Hsi, M.S. (1976) Multiple sclerosis amongst Chinese in Taiwan. *Journal of the Neurological Sciences*, **27**, 459-484. doi:10.1016/0022-510X(76)90214-8

[17] Das, A. and Puvanendran, K. (1998) A retrospective review of patients with clinically definite multiple sclerosis. *Annals, Academy of Medicine, Singapore*, **27**, 204-209.

[18] Jitpimolmard, S. and Vejjajiva, A. (1994) Clinical features and clinical course of multiple sclerosis in Thai patients: A report of 50 cases. *Journal of The Medical Association of Thailand*, **77**, 239-243.

[19] Kurtzke, J.F., Park, C.S. and Oh, S.J. (1968) Multiple sclerosis in Korea. Clinical features and prevalence. *Journal of the Neurological Sciences*, **6**, 463-481. doi:10.1016/0022-510X(68)90031-2

[20] Tan, C.T. (1998) Multiple sclerosis in Malaysia. *Archives of Neurology*, **45**, 624-627.

[21] Kalanie, H., Gharagozli, K. and Kalanie, A.R. (2003) Multiple sclerosis: Report on 200 cases from Iran. *Multiple Sclerosis*, **9**, 36-38. doi:10.1191/1352458503ms887oa

[22] Kalanie, H., Kamgooyan, M., Sadeghian, H. and Kalanie A.R. (2000) Histocompatibility antigen (HLA) associations with multiple sclerosis in Iran. *Multiple Sclerosis*, **6**, 317-319.

[23] Massey, H.D. and McPherson, R.A. (2007) The major histocompatibility complex of man. In: McPherson R.A. and Pincus M.R. Eds., *Henry's clinical diagnosis and management by laboratory methods*. 21st Edition, Saun-

ders Elsevier, Beijing, 876-893.

[24] Julio, C.D. and Edmond, J.Y. (2007) The Major Histocompatibility Complex and Disease. In *Henry's Clinical Diagnosis and Management by Laboratory Methods*. 21st Edition, Saunders-Elsevier, **8**, 894-902.

[25] Misu, T., Fujihara, K., Nakashima, I., Miyazawa, I., Okita, N., Takase, S. and Itoyama, Y. (2002) Pure optic-spinal form of multiple sclerosis in Japan. *Brain*, **125**, 2460-2468. doi:10.1093/brain/awf260

[26] Nakashima, I., Fukazawa, T., Ota, K., Nohara, C., Warabi, Y., Ohashi, T., Miyazawa, I., Fujihara, K. and Itoyama, Y. (2007) Two subtypes of optic-spinal form of multiple sclerosis in Japan: Clinical and laboratory features. *Journal of Neurology*, **254**, 488-492. doi:10.1007/s00415-006-0400-z

[27] Matsuoka, T., Matsushita, T., Osoegawa, M., Kawano, Y., Minohara, M., Mihara, F., Nishimura, Y., Ohyagi, Y. and Kira, J. (2008) Association of the HLA-DRB1 alleles with characteristic MRI features of Asian multiple sclerosis. *Multiple Sclerosis*, **14**, 1181-1190. doi:10.1177/1352458508097818

[28] Brum, D.G., Barreira, A.A., Santos dos, A.C., Kaimen-Maciel, D.R., Matiello, M., Costa, R.M., Deghaide, N.H., Costa, L.S., Louzada-Junior, P., Diniz, P.R., Comini-Frota, E.R., Mendes-Junior, C.T. and Donadi, E.A. (2010) HLA-DRB association in neuromyelitis optica is different from that observed in multiple sclerosis. *Multiple Sclerosis*, **16**, 21-29. doi:10.1177/1352458509350741

[29] Weinshenker, B.G. and Wingerchuk, D.M. Neuromyelitis optica: Clinical syndrome and the NMO-IgG autoantibody marker. *Current Topics in Microbiology and Immunology*, **318**, 343-356. doi:10.1007/978-3-540-73677-6_14

[30] Kira, J. (2008) Neuromyelitis optica and Asian phenotype of multiple sclerosis. *Annals of the New York Academy of Sciences*, **1142**, 58-71. doi:10.1196/annals.1444.002

[31] Nandhagopal, R., Al-Asmi, A. and Gujjar, A.R. (2010) Neuromyelitis optica: An overview. *Postgraduate Medical Journal*, **86**, 153-159. doi:10.1136/pgmj.2009.091389

