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BRIEF COMMUNICATION

Human leukocyte antigen associations in postural tachycardia syndrome

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Abstract

Associations between human leukocyte antigen (HLA) and postural orthostatic tachycardia syndrome (POTS) have not been investigated. We included patients diagnosed with POTS and showing orthostatic heart rate increases ≥ 50 during orthostatic vital sign measurement or experiencing syncope/near-syncope while standing (prominent POTS; $n = 17$). DQB1*06:09 was present in seven (41%) patients, a significantly higher percentage than in healthy Koreans (7%; odds ratio [OR] 8.7, 95% confidence interval [CI] 3.1–24.3, corrected $P = 3.2 \times 10^{-4}$) and epilepsy controls (8%; OR 7.9, 95% CI 2.7–23.5, corrected $P = 3.2 \times 10^{-4}$). Six (35.3%) carried the A*33:03-B*58:01-C*03:02-DRB1*13:02-DQB1*06:09 haplotype. The results signify an autoimmune etiology in prominent POTS.

Introduction

Postural tachycardia syndrome (POTS) is a form of dysautonomia that is characterized by excessive orthostatic tachycardia and the presence of complex symptoms, including orthostatic intolerance.[1] Although POTS is an underrecognized disorder and its clinical significance is increasing, the pathophysiology of POTS remains largely unknown. Recently, an autoimmune basis of POTS was suggested, and autoantibodies against adrenergic receptors (ARs), the angiotensin II type 1 receptor (AT1R), and acetylcholine receptors (AchRs) have been reported in POTS patients.[2–6]

Antigen-presenting cells present small processed peptide sequences from antigen by major histocompatibility complex (MHC) proteins. Variability of HLA alleles can allow for differences in the presentation of peptide ligand by MHCs, which is thought to contribute to aberrant T-cell activation and autoimmunity. In fact, several autoimmune diseases are known to have associations with specific human leukocyte antigen (HLA) alleles.[7] Altered HLA allele frequencies in POTS could strongly suggest autoimmunity in POTS pathogenesis. However, the association between HLA and POTS has not yet been investigated.

In this study, we aimed to identify the association between HLA and POTS and investigate the clinical characteristics of patients with POTS in association with HLA.

Method

Study subjects

We enrolled adult patients with prominent POTS who visited the Seoul National University Hospital (SNUH) for dizziness or loss of consciousness (LOC), and were subsequently diagnosed with POTS between April 2014 and August 2015. For the diagnosis of POTS, we checked blood pressure and heart rate at supine rest and 0, 1, 3, 5, and 10 min after standing. Two consecutive tests were conducted, usually in the afternoon and early the next morning, to increase the sensitivity.[8] We chose the orthostatic vital sign test for the diagnosis because active standing is more readily performed early in the morning, can better reflect physiologic conditions compared to head-up tilt testing, and is recommended as an initial evaluation measure.[9,10] Patients who showed a heart rate (HR) increase $\geq$30 (HR $\geq$ 40 for age $\leq$ 19) within 10 min after standing, experienced orthostatic intolerance symptoms, and had no clear cause to explain tachycardia, such as acute blood loss, prolonged bed rest, hyperthyroidism, or tachycardia-promoting medication were initially diagnosed with POTS. To select patients with prominent POTS, only the patients who showed an increase in heart rate $\geq$50/min or syncope/near-syncope while standing were included. For patients with a history of LOC, epilepsy was additionally ruled out with overnight video-EEG monitoring. The study was approved by the institutional review board of SNUH, and informed written consent was obtained from all patients.

HLA genotyping and antibody testing

Four-digit high-resolution HLA genotyping of five HLA loci (HLA-A, B, C, DRB1, DQB1) was performed using direct DNA sequence analysis according to an established protocol (Biowithus, Seoul, South Korea) as described previously.[11,12] Antibody testing was additionally performed for these patients to check whether autoimmune pathogenesis was detected. Serum IgG against human α1-adrenergic receptor (α1AR), β1AR, and nicotinic acetylcholine receptor (N-AChR) antibody was measured by an enzyme-linked immunosorbent assay (ELISA) kit (Cusabio, Wuhan, China) and against α2AR and β2AR by another ELISA kit (CellTrend GmbH, Luckenwalde, Germany).

Data collection

Demographic and clinical data were collected. For the 16 patients who were enrolled in our previous prospective study designed to evaluate the medical response in POTS patients,[13] orthostatic intolerance symptoms were evaluated using the orthostatic intolerance questionnaire (OIQ),[14] along with the degree of depression using the Beck depression inventory-II (BDI-II),[15] and health-related quality of life using a 36-item short-form health survey (SF-36)[16] at baseline and at 1 and 3 months after treatment for comprehensive evaluation of symptoms and outcome.[1] Baseline catecholamines levels including norepinephrine, epinephrine, and dopamine were also checked and collected for the analysis.

Table 1. Clinical characteristics of the patients.

Age	23 (15–43)
Sex (M:F)	5:12
Height, cm	165.7 (134.4–185.1)
Weight, kg	53.25 (43.7–102.9)
BMI, kg/m^2	20.45 (16.86–35.71)
Vaccinated for HPV	6 (50)[1]
Supine[2]	
Systolic BP, mmHg	106 (94.5–124)
Diastolic BP, mmHg	62.5 (52.5–72)
Heart rate, bpm	61.5 (50–82)
Standing (immediate)[2]	
Systolic BP, mmHg	106.5 (94.5–128.5)
Diastolic BP, mmHg	64.5 (55–74)
Heart rate, bpm	94 (78.5–124)
Maximal heart rate increase	57 (30–73)
Experience of syncope/presyncope while standing[3]	11 (64.7)
Baseline serum catecholamines	
Norepinephrine, pg/mL	123.1 (29.9–358.8)
Epinephrine, pg/mL	35 (1–88)
opamine, pg/mL	28.4 (3.1–63.2)
Questionnaire scores (initial)	
OIQ	15 (0–39)
BDI-II	11.5 (3–28)
PCS	49.4 (19.2–58.6)
MCS	47.6 (25.8–62.7)
Treatment	
Propranolol	4 (23.5)
Bisoprolol	4 (23.5)
Propranolol and pyridostigmine	4 (23.5)
Bisoprolol and pyridostigmine	5 (29.4)
Additional IVIg for relapse	2 (11.8)

Data are expressed as median (range) or *N* (%).
BMI, body mass index; HPV, human papillomavirus; BP, blood pressure; OIQ, orthostatic intolerance questionnaire; BDI-II, Beck depression inventory-II; PCS, physical component summary score of short-form 36 health survey questionnaire; MCS, mental component summary score of short-form 36 health survey questionnaire.
[1]Percentage in the female population.
[2]Mean value of the results of two orthostatic vital sign measurements.
[3]Ten of the patients reproduced the attack during orthostatic vital sign measurement.

Multiple sequence alignment and HLA peptide binding prediction

Protein sequences of α1AR, α2AR, β1AR, β2AR, and AT1R (Uniprot accession numbers P35348, P08913, P08588, P07550, and P30556, respectively) were aligned using a multiple sequence alignment tool (Clustal Omega).[17] NetMHCIIPan-3.2[18] was used to predict the binding affinity of HLA class II peptides to all possible epitopes with peptide lengths of 15 from each protein. The percentile rank of each epitope was determined by comparing its predicted score against the scores of 200,000 random natural peptides and rank values (%) <3 were considered to have strong affinity.

Statistical analysis

For the statistical analysis, we used HLA genotypes of 485 Korean individuals from the general population[19] and previously obtained data from 210 epilepsy patients[11,12,20] as control groups. We used two control groups to compensate for the small sample size and other possible confounding conditions such as age, sex, and comorbidities that may influence the results.

Odds ratios with 95% confidence intervals were calculated for each HLA type. The association of specific HLA types with POTS was performed using Fisher's exact test. Correction for multiple testing was performed using the Bonferroni method, that is, multiplying the *P* values by the total number of detected alleles at each HLA locus. Haplotypes of the control groups were estimated using Arlequin software (version 3.5.2.2)[21] as previously described.[20] Fisher's exact test or the Wilcoxon rank-sum test was used as appropriate for between-group comparisons. Statistical analyses were performed using SPSS version 18 (SPSS Inc., Chicago, IL), and the level of significance was set at 0.05.

Results

A total of 153 patients with POTS were screened for eligibility in our previous study.[13] Among them, 24 subjects fulfilled our criteria for prominent POTS, and 17 of those whose blood samples were stored underwent HLA genotyping. One patient did not return to the clinic after the initial evaluation while the remaining 16 patients underwent follow-up evaluation for treatment response. The clinical characteristics of the patients are summarized in Table 1. In four-digit HLA genotyping, DQB1*06:09 was more prevalent (41%) in the POTS group than in either the epilepsy control group or the healthy control group (Table 2). Six of 17 patients (35.3%) carried the A*33:03-B*58:01-C*03:02-DRB1*13:02-DQB1*06:09 haplotype, which was also significantly more frequent than in the epilepsy and healthy control populations (odds ratio [OR] 8.3 and 8.5, respectively). All 17 patients had antibodies to β2AR, with

Table 2. Carrier frequency distribution of selected alleles and haplotypes in POTS patients as well as epilepsy controls and healthy controls.

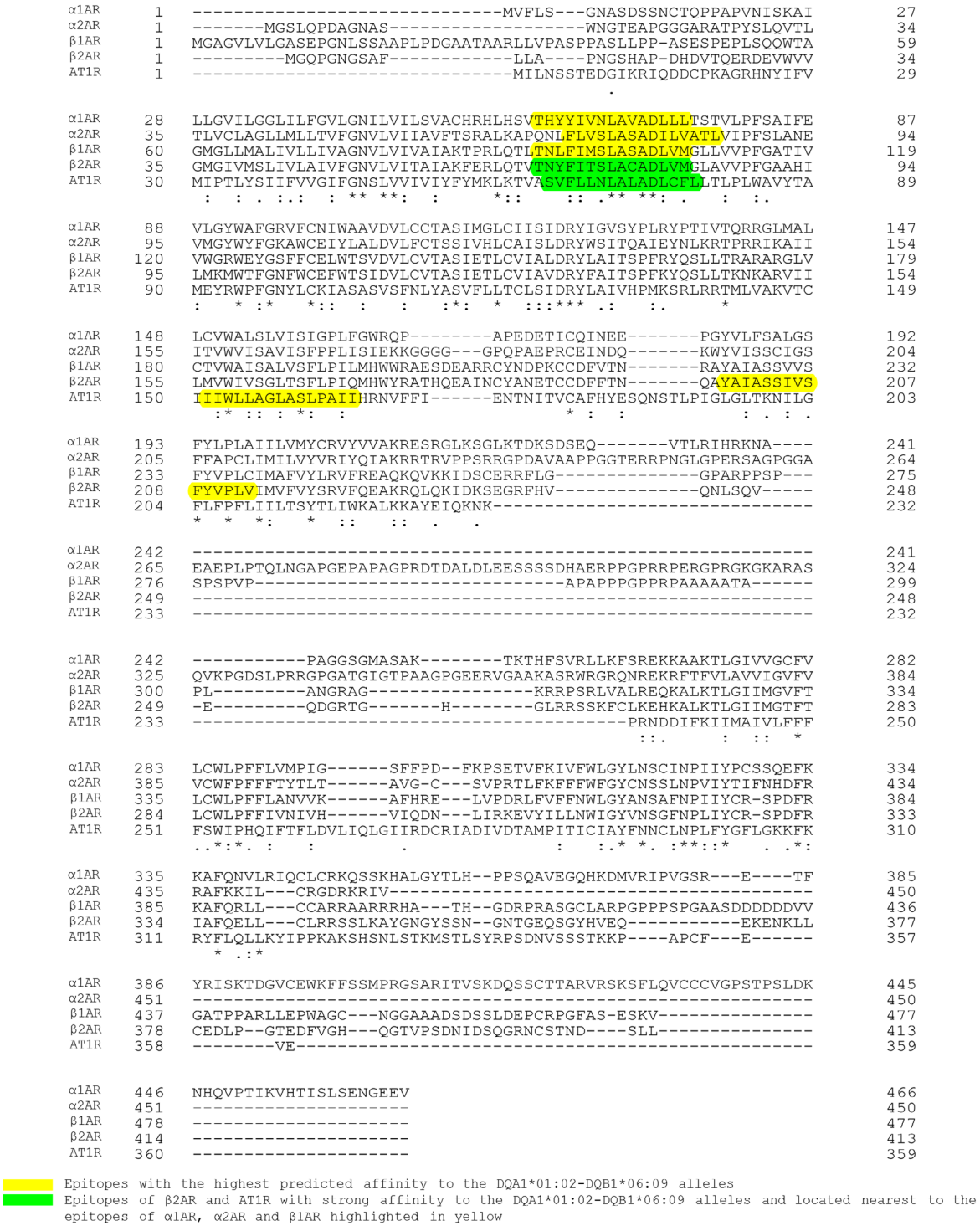
	Phenotype frequency as no. (percentage)			Statistical analysis			
				POTS versus epilepsy controls		POTS versus healthy controls	
HLA allele or haplotype	POTS	Epilepsy controls	Healthy controls	OR (95% CI)	*Pc*[a]	OR (95% CI)	*Pc*[a]
Total (n=17)							
DQB1*06:09	7/17 (41%)	17/210 (8%)	36/485 (7%)	7.9 (2.7–23.5)	**8.9×10^{-3}**	8.7 (3.1–24.3)	**3.2×10^{-4}**
C*03:02	8/17 (47%)	32/210 (15%)	71/485 (15%)	4.9 (1.8–13.8)	0.075	5.2 (1.9–13.9)	**0.043**
DRB1*13:02	7/17 (41%)	28/210 (13%)	83/485 (17%)	4.6 (1.6–12.9)	0.23	3.4 (1.3–9.2)	0.65
B*58:01	7/17 (41%)	31/210 (15%)	59/485 (12%)	4 (1.4–11.4)	0.51	5.1 (1.9–13.8)	0.15
A*33:03	7/17 (41%)	56/210 (27%)	140/485 (29%)	1.9 (0.7–5.3)	>0.99	1.7 (0.6–4.6)	0.65
Haplotype#1*	7/17 (41%)	16/210 (8%)	32/485 (7%)	8.5 (2.8–25.3)	**2.6×10^{-3}**	9.9 (3.5–27.8)	**6.5×10^{-4}**
Haplotype#2*	6/17 (35%)	13/210 (6%)	29/485 (6%)	8.3 (2.6–25.9)	**6.4×10^{-3}**	8.5 (3.0–24.8)	**3.2×10^{-3}**
Patients with antibodies to both 2AR and 2AR (n=13)							
DQB1*06:09	7/13 (54%)	17/210 (8%)	36/485 (7%)	13.2 (4.0–43.9)	**1.2×10^{-3}**	14.6 (4.6–45.6)	**4.0×10^{-4}**
C*03:02	8/13 (62%)	32/210 (15%)	71/485 (15%)	8.9 (2.7–28.9)	**8.0×10^{-3}**	9.3 (3.0–29.3)	**4.2×10^{-3}**
DRB1*13:02	7/13 (54%)	28/210 (13%)	83/485 (17%)	7.6 (2.4–24.2)	**0.037**	5.7 (1.9–17.2)	0.11
B*58:01	7/13 (54%)	31/210 (15%)	59/485 (12%)	6.7 (2.1–21.4)	0.086	8.4 (2.7–25.9)	**0.021**
A*33:03	7/13 (54%)	56/210 (27%)	140/485 (29%)	3.2 (1.0–10.0)	>0.99	2.9 (0.9–8.7)	0.65
Haplotype#1*	7/13 (54%)	16/210 (8%)	32/485 (7%)	14.1 (4.2–47.1)	**3.4×10^{-4}**	16.5 (5.2–52.0)	**7.8×10^{-5}**
Haplotype#2*	6/13 (46%)	13/210 (6%)	29/485 (6%)	13.0 (3.8–44.3)	**1.2×10^{-3}**	13.5 (4.3–42.7)	**5.6×10^{-4}**

Bold text indicates a statistically significant difference.

For haplotype comparisons, the *P* values were corrected by a factor of 6, considering four combinations and two subgroups.

[a]The *P* values are the results of correction using the Bonferroni's method for multiple comparisons. For the correction, *P* values were multiplied by the number of detected alleles for each HLA locus.

*Haplotype#1 = HLA-C*03:02-B*58:01-DRB1*13:02-DQB1-06:09; Haplotype#2 = HLA-A-33:03- C*03:02-B*58:01-DRB1*13:02-DQB1-06:09.



13 also having α2AR antibodies. Subgroup analysis of the patients whose sera showed positivity for both α2 and β2 ADR antibodies indicated that a stronger association was observed for approximately half of the patients having DQB1*06:09 (OR 13.2 and 14.6, compared to epilepsy controls and healthy controls, respectively).

There were no differences in age, sex, HPV vaccination status, maximum heart rate increase, baseline serum catecholamine level or scores of OIQ, BDI-II, and SF-36 physical and mental components between the patients with and without the DQB1*06:09 allele (Table S1). Follow-up questionnaires (OIQ, BDI-II, and SF-36)

Figure 1. Alignment of protein sequences of adrenergic receptors and angiotensin II type 1 receptor to predict an epitope with strong affinity. Protein sequences of α1AR, α2AR, β1AR, β2AR, and AT1R were aligned using a multiple sequence alignment tool (Clustal Omega).[17] An asterisk ('*') indicates positions which have a single, fully conserved residue among the five protein sequences. A colon (':') and a period ('.') each indicate conservation between groups of strongly and weekly similar properties defined by Clustal Omega. A dash ('-') represents a gap in one or other sequences introduced for alignment. The affinity of all possible epitopes with peptide lengths of 15 from each protein to HLA-DQA1*01:02-DQB1*06:09 was predicted using NetMHCIIPan-3.2.[18] HLA-DQA1*01:02 was selected because it is the allele most likely to form a haplotype with DQB1*06:09 by the haplotype frequency of the Korean population.[22] Sequences highlighted in yellow represent the epitopes with the highest predicted affinity of each protein. Interestingly, epitopes of α1/2AR and β1AR with the highest predicted affinity are located in a similar location within highly homogenous regions. Proteins β2AR and AT1R also had epitopes predicted to have strong affinity (rank values (%) of 0.8 and 1.3, respectively) in the same region (highlighted in green).

performed at 1 ($n = 16$) and 3 months ($n = 13$) also showed no difference. At last follow-up (median 12 month, range 0–48 month), nine patients reported no or only minor symptoms without relapse. Two patients received intravenous immunoglobulin (IVIg) at the recurrence of orthostatic intolerance. One showed complete resolution of symptoms 1 month after a 5-day IVIg infusion (400 mg/kg for 5 days). The other showed a delayed response with amelioration of symptoms 5 months after IVIg treatment (5-day IVIg infusion followed by 5 replacements at 400 mg/kg per month).

Epitope prediction using NetMHCIIPan-3.2[18] showed that epitopes of α1/2AR and β1AR with the highest predicted affinity to the DQA1*01:02-DQB1*06:09 alleles are found in similar locations in highly homogenous regions (Fig. 1). β2AR and AT1R also had epitopes predicted to have strong affinity in the same region, although epitopes with the highest predicted affinity are in different locations. These findings could partly explain multiple target antigens in POTS by molecular mimicry.

Discussion

There is a remarkable association of prominent POTS with the DQB1*06:09 allele. This report is the first to investigate the HLA association with POTS. All patients with prominent POTS had autoantibodies to β2AR, which suggests antibody-mediated pathophysiology in these patients. Two of our patients responded to immunotherapy after symptom recurrence, suggesting the feasibility of immunotherapy in prominent POTS.

An autoimmune etiology in POTS is suggested by a number of clinical features (e.g., female predominance, preceding viral illness, prior vaccination history, and coexistence of other autoimmune conditions) along with autoantibodies.[6] Antibody-mediated autoimmune disorders usually have an HLA class II association.[7] While lacking in vivo animal model confirmation, the strong association found in the HLA-DQ locus, along with the functionality of the autoantibodies demonstrated experimentally,[2,3] supports the hypothesis that autoimmune POTS is antibody-mediated. Immunotherapeutic agents, including IVIg, may be considered for the treatment of prominent POTS in this regard. Although most clinical features of the study population were not different according to the HLA-DQ genotype, marginal differences in heart rate on standing (Table S1) suggest the potential of different clinical manifestations that might be revealed by a larger population study.

Several etiologies are suggested in patients with POTS; however, a considerable number of patients with POTS seem to have antibodies to one or more of the ARs according to recent studies.[2,3] Among the autoantibodies reported in POTS, approximately half of the patients had autoantibodies to β2AR in previous studies.[2,3] All patients had antibodies to β2AR in the current study. Applying more strict criteria may have resulted in increased etiological homogeneity in our patient pool, and the results drawn from prominent POTS patients may be different from the entire POTS population.

There are limitations to this study. First, the small number of patients and ethnic homogeneity limited generalization, and thus, our results need future validation. However, comparison of HLA types within one ethnic population could lower the chance of spurious associations. Therefore, ethnic homogeneity could also be a strength especially in the setting of our small sample size. Second, the proportion of antibodies detected from the patients was different from those detected in previous studies, which might have arisen from the different methods used for antibody detection. Antibodies to α1AR and β1AR, which are known to be detected in POTS by cell-based assays,[2,3] were not detected by ELISA, which also suggests that antibodies to α1AR and β1AR respond to conformational epitopes. Third, as we only investigated selected patients with prominent POTS, the results may not be generalizable to all patients diagnosed with POTS by heart rate cut-off of 30. For increased generalizability, the HLA association needs to be re-evaluated in all POTS patients meeting the clinical diagnostic criteria.

Autoimmune etiology is gaining increasing attention. The HLA association revealed in this study further emphasizes autoimmunity in the pathogenesis of POTS. The severity and prognostic association of POTS with the

HLA subtype need future investigation with a larger population. Future well-designed trials investigating the benefit of immunotherapy in POTS are also warranted.

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Author Contributions

J.M. and K.C. designed the study. Y.W.S., J.M,. T.J.K., D.Y.K., and J.S.J. collected the data. J.M. performed the HLA typing and autoantibody testing. Y.W.S. and J.M. performed the statistical analysis and drafted the initial manuscript. All authors contributed to data acquisition, final analysis, and revision of the manuscript.

Conflict of Interest

The authors declare no conflict of interest.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Comparison of clinical characteristics of patients with or without the DQB1*06:09 allele.

