Identification of HLA-DRB1*04:10 allele as risk allele for Japanese moyamoya disease and its association with autoimmune thyroid disease: A case-control study

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Abstract

Background and purpose
Moyamoya disease (MMD) is a progressive cerebrovascular disease with unknown etiology. Growing evidence suggest its involvement of autoimmune and genetic mechanisms in the pathogenesis of MMD. This study aims to clarify the association between HLA allele and MMD.

Methods
Case-control study: the DNA of 136 MMD patients in Japan was extracted and the genotype of human leukocyte antigen (HLA) from this DNA was determined by super-high-resolution single-molecule sequence-based typing using next-generation sequencing. Next, the frequency of each HLA allele (HLA-A, HLA-B, HLA-C, HLA-DRB1, HLA-DQB1, and HLA-DPB1) was compared with those in the Japanese control database. In addition, haplotype estimation was performed using the expectation maximization algorithm.

Results
The frequencies of the HLA-DRB1*04:10 allele (4.77% vs. 1.47% in the control group; P = 1.7 × 10^{-3}; odds ratio [OR] = 3.35) and of the HLA-DRB1*04:10–HLA-DQB1*04:02 haplotype (haplotype frequency 4.41% vs. 1.35% in the control group; P = 2.0 × 10^{-3}; OR = 3.37) significantly increased. The frequency of thyroid diseases, such as Graves’ disease and Hashimoto thyroiditis, increased in HLA-DRB1*04:10-positive MMD patients compared with that in HLA-DRB1*04:10-negative MMD patients.
Conclusions

*HLA-DRB1*\(^*\)04:10 is a risk allele and *HLA-DRB1*\(^*\)04:10–*HLA-DQB1*\(^*\)04:02 a risk haplotype for MMD. In addition, *HLA-DRB1*\(^*\)04:10 is associated with thyroid disease in MMD patients.

Introduction

Moyamoya disease (MMD) is a chronic, occlusive cerebrovascular disease with unknown etiology. MMD involves bilateral steno-occlusive changes at the terminal portion of the internal carotid artery and the formation of an abnormal vascular network at the base of the brain [1]. MMD is a relatively rare disease, as estimated incidence of MMD is 3.1–10.5/100,000 in Japanese population [2–3]. Despite its rarity, MMD is one of the leading causes of cerebrovascular diseases among children and young adults in Japan and Korea. Thus, it is expected to unveil the pathogenesis of MMD and diagnose MMD before onset of stroke. Although the etiology of MMD remains undetermined, previous studies showed an association between autoimmunity and MMD [4–11], such as the high frequencies of thyroid dysfunction and type 1 diabetes among MMD patients [4–10].

Human leukocyte antigen (HLA) proteins provide peptides to T-cell receptors for the maintenance of self-tolerance and adapted immunity [12–14]. Allelic and haplotypic differences between HLA alleles are associated mainly with human autoimmune diseases [13–15]. However, despite accumulating evidence, it is unclear how genetic variations of HLA alleles lead to a risk of autoimmune diseases. Recent studies have analyzed HLA–peptide–T-cell receptor complexes and demonstrated the autoantigen recognition mechanism [15–17]. Therefore, to understand the autoimmunity mechanism, it is critical to determine HLA alleles susceptible and/or resistant to autoimmune diseases.

Several studies have demonstrated the association between MMD and allelic and haplotypic differences of HLA, including both HLA class I and class II alleles (e.g., *HLA-A*\(^*\)24, *HLA-B*\(^*\)54, *HLA-DRB1*\(^*\)04:05, and *HLA-DQB1*\(^*\)04:01) in multiple cohorts of East Asians [18–21], as well as in Europeans [22]. We sought to determine susceptible allele for MMD using next-generation sequence (NGS), since conventional HLA DNA typing methods, including sequence-based typing (SBT) and sequence-specific oligonucleotide (SSO) typing, yield ambiguous results because of oligonucleotide probe design limitations or phase ambiguity for HLA allele assignment [23]. The recently developed super-high-resolution single-molecule sequence-based typing (SS-SBT) method using NGS can determine an HLA allele sequence at the 8-digit level and also overcome the phase ambiguity of earlier typing methods [24].

In this study, we analyzed the association between MMD and HLA alleles using SS-SBT and NGS in Japanese MMD patients.

Materials and methods

Study design

This is a case-control study. MMD patient samples were collected at Tohoku University hospital and Kohnan hospital. We referred HLA database of the University of Tokyo as for healthy control. Based on previous reports demonstrating HLA alleles and autoimmune diseases [13–15], we calculated the number of samples to be enrolled in this study as more than 130 MMD patients and 300–500 healthy controls, respectively. Written informed consent for participation in the study was obtained from all subjects and (in the case of pediatric patients) from

have any additional role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript. The specific roles of these authors are articulated in the ‘author contributions’ section.

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their guardians. After obtaining written informed consent, we collected blood samples and performed HLA genotyping. Hardy-Weinberg equation (HWE) was not considered in this study, since previous reports demonstrated deviation from HWE for major histocompatibility region on chromosome 6 [25]. The study complied with the Helsinki Declaration on ethical principles for medical research involving human subjects. Ethical approval was obtained from the ethical committees of Tohoku University, Kohnan Hospital, and the University of Tokyo.

**Subject population**

All MMD patients enrolled in the study (n = 136, aged 11–78 years, mean 43.1) met the diagnostic criteria established by the Research Committee on Spontaneous Occlusion of the Circle of Willis, the Ministry of Health, Labor and Welfare, Japan [26]. Patients with quasi-moya-moya disease were not enrolled in this study. Samples were collected between 2016 and 2018, and most of the patients were residents in Tohoku region in Japan. Clinical characteristics of enrolled patients were extracted from the databases in Tohoku University Hospital and Kohnan hospital.

**HLA genotyping**

We performed HLA genotyping with SS-SBT at the 8-digit level using NGS, as reported in previous studies [24]. Outline of procedures is shown in https://dx.doi.org/10.17504/protocols.io.3rxgm7n [PROTOCOL DOI]. Briefly, genomic DNA was obtained from the patients’ 2ml of whole blood using the QIAamp DNA Mini Kit for genomic DNA purification (Qiagen GmbH, Hilden, Germany), and 400 ng of purified genomic DNA was used for polymerase chain reaction (PCR) amplification. DNA was preserved in 4˚C freezer. The basic cycling parameters were as follows: (i) first denaturation at 94˚C for 2 min, followed by 30 cycles of denaturation at 98˚C for 10 s and 60˚C for 20 s and extension at 68˚C for 5 min (HLA-A, HLA-B, and HLA-C); (ii) first denaturation at 94˚C for 2 min, followed by 30 cycles of denaturation at 98˚C for 10 s and annealing at 70˚C for 5 min (HLA-DRB1 and HLA-DPB1); and (iii) first denaturation at 94˚C for 2 min, followed by 30 cycles of denaturation at 98˚C for 10 s and annealing at 70˚C for 9 min (HLA-DQB1). Long-range PCR reactions were performed using the thermal cycler Gene Amp PCR System 9700 (Life Technologies, Carlsbad, CA, USA). The PCR products obtained were purified with Agencourt AMPure XP (Beckman Coulter, CA, USA) and quantified by the Quant-iT Picogreen dsDNA Assay Kit (Thermo Fisher Scientific, MA, USA). Next, the PCR products were clonally amplified and barcoded using the Ion Plus Fragment Library Kit (Life Technologies), and the barcoded library was sequenced using the Ion Torrent Personal Genome Machine DNA sequencing system (Life Technologies). The NGS read data were analyzed by Sequence Alignment Based Assigning Software (SeaBass), and finally, the HLA alleles were determined.

**Control database**

We referred HLA database of the University of Tokyo. Control group does not include patients with MMD and autoimmune disease. Control samples have been collected between 1990 and 2018, and most of the healthy controls were residents in Kanto district in Japan. HLA genotyping was performed, as reported previously [27–28]. HLA class I (HLA-A, HLA-B, and HLA-C) and class II (HLA-DRB1, HLA-DQB1, and HLA-DPB1) genotypes were determined with the HLA-DNA Typing Kit, Lumineox multianalyte profiling system xMAP (Lumineox Corp., Austin, TX, USA), WAKFlow HLA Typing Kit (Wakunaga Pharmaceutical, Osaka, Japan), and LAB-Type SSO HLA Kit (One Lambda, Canoga Park, CA, USA) according to the manufacturers’ instructions. All HLA alleles were determined at the 4-digit level.
Haplotype estimation

We performed 2-locus (HLA-DRB1–HLA-DQB1 and HLA-A–HLA-B) and 3-locus (HLA-DR1–HLA-DQB1–HLA-DPB1 and HLA-A–HLA-B–HLA-C) haplotype analysis using Bridging ImmunoGenomic Data-Analysis Workflow Gaps (BigDAWG) [29]. The pairwise linkage disequilibrium (LD) parameters, $r^2$ and $D'$, between alleles at different class II HLA loci were calculated on the basis of the haplotype frequencies estimated by the expectation maximization (EM) algorithm [30–31].

Determination of polymorphisms in the RNF213 gene

Polymorphisms in the RNF213 gene was determined as previously reported [32]. Single nucleotide polymorphism (SNP) for rs112735431 (https://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=112735431) was analyzed using the TaqMan SNP genotyping assay (Assay ID: C_153120198_10; Applied Biosystems, Foster City, CA, USA) on a StepOnePlus real-time polymerase chain reaction (PCR) system (Applied Biosystems, Foster City, CA, USA). The basic cycling parameters were as follows: hold at 95°C for 10 minutes, followed by 40 cycles of PCR amplification comprising of denaturation at 95°C for 15 seconds, and annealing and extension at 60°C for 1 minute. Genotype calls were evaluated with Applied Biosystems TaqMan Genotype software. The investigators who assessed genotype were blinded to phenotypic information.

Statistical analysis

Statistical tests were performed using JMP Pro version 13 (SAS Institute). For each testing scenario, prior to statistical analysis, rare alleles (expected counts < 5) were combined into the “Others” category. The differences between allele and haplotype frequencies were calculated by the chi-square test. Adjustment for multiple comparisons was conducted using the Bonferroni method. Corrected $P$-values ($P_c$) were calculated by multiplying the $P$-values with the number of examined alleles. The association between HLA-DRB1*04:10 and clinical characteristics was evaluated using Mann–Whitney’s U test, and assessment of continuous variables and categorical variables was performed using Fisher’s exact test the chi-square test, respectively. Quantitative variables, such as Suzuki stage and age at onset, were stratified into several categories. For example, age at onset was stratified into three categorical variables, eg, child-onset, elderly-onset (age > 60), perinatal period-onset. Multivariate logistic analysis using Poisson regression models was used to adjust for age and sex. $P$-values of <0.05 were considered statistically significant.

Compliance with STrengthening the REporting of Genetic Association Studies (STREGA) Guidelines

This study complied with STrengthening the REporting of Genetic Association Studies (STREGA) Guidelines [33], as shown in S1 Table.

Results

**HLA-DRB1*04:10 is a risk allele for MMD**

In this study, we examined the frequencies of HLA class I (HLA-A, HLA-B, and HLA-C) and class II (HLA-DRB1, HLA-DQB1, and HLA-DPB1) alleles in MMD patients and controls. Tables 1–3 shows the frequencies for HLA class II alleles in MMD patients and controls. As seen in the table, HLA-DRB1*04:10 was significantly associated with MMD: allele frequency
4.77% vs. 1.47% in the control group; \( P = 1.7 \times 10^{-3} \); odds ratio \([OR]\) = 3.35. The frequency of HLA-DRB1*04:10 increased in MMD patients compared with that in the control group: allele frequency 1.10% vs. 3.44% in the control group; \( P = 0.045 \); OR = 0.31. However, after Bonferroni correction, the \( P \)-value \((P_c)\) did not reach the threshold (Table 1). In addition, we found no association between other HLA class II alleles (HLA-DQB1 and HLA-DPB1) and MMD.

Table 1. Frequencies of HLA-DRB1 allele carrier in MMD patients and controls.

| Locus | Allele | Control (2n = 814) | Patient (2n = 272) | OR (95%CI) | \( P \) value | \( P_c \) |
|-------|--------|-------------------|-------------------|------------|-------------|-------|
| DRB1  | 1:01   | 7.00 (57)         | 5.88 (16)         | 0.83 (0.44–1.50) | 0.523       | NS    |
| DRB1  | 4:03   | 2.95 (24)         | 2.57 (7)          | 0.87 (0.31–2.11) | 0.748       | NS    |
| DRB1  | 4:05   | 14.4 (117)        | 10.3 (28)         | 0.68 (0.42–1.07) | 0.087       | NS    |
| DRB1  | 4:06   | 3.44 (28)         | 1.10 (3)          | 0.31 (0.06–1.03) | 0.045       | NS    |
| DRB1  | 4:10   | 1.47 (12)         | 4.77 (13)         | 3.35 (1.39–8.14) | 0.030       | 0.030 |
| DRB1  | 8:02   | 3.81 (31)         | 5.15 (14)         | 1.37 (0.66–2.70) | 0.338       | NS    |
| DRB1  | 8:03   | 7.62 (62)         | 8.09 (22)         | 1.07 (0.61–1.80) | 0.801       | NS    |
| DRB1  | 9:01   | 14.9 (121)        | 18.0 (49)         | 1.26 (0.85–1.83) | 0.216       | NS    |
| DRB1  | 11:01  | 2.83 (23)         | 1.84 (5)          | 0.64 (0.19–0.76) | 0.374       | NS    |
| DRB1  | 12:01  | 3.56 (29)         | 3.31 (9)          | 0.93 (0.38–2.04) | 0.844       | NS    |
| DRB1  | 12:02  | 2.21 (18)         | 1.47 (4)          | 0.66 (0.16–2.03) | 0.453       | NS    |
| DRB1  | 13:02  | 7.62 (62)         | 6.25 (17)         | 0.81 (0.43–1.43) | 0.452       | NS    |
| DRB1  | 14:05  | 1.97 (16)         | 1.84 (5)          | 0.93 (0.27–2.70) | 0.895       | NS    |
| DRB1  | 14:06  | 1.60 (13)         | 2.94 (8)          | 1.87 (0.66–4.92) | 0.163       | NS    |
| DRB1  | 14:54  | 3.19 (26)         | 1.47 (4)          | 0.45 (0.11–1.32) | 0.133       | NS    |
| DRB1  | 15:01  | 8.11 (66)         | 6.25 (17)         | 0.76 (0.41–1.33) | 0.318       | NS    |
| DRB1  | 15:02  | 8.60 (70)         | 10.3 (28)         | 1.22 (0.74–1.97) | 0.398       | NS    |
| DRB1  | others | 4.79 (39)         | 8.46 (23)         | 1.84 (1.02–3.22) | 0.024       | NS    |

The number of allele carriers is shown in parentheses. The association was examined by the chi-square test. Corrected \( P \)-values \((P_c)\) statistically significant after Bonferroni correction are indicated in bold. OR, odds ratio; CI, confidence interval; \( P_c \), corrected \( P \)-value; NS, not significant.

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Table 2. Frequencies of HLA-DQB1 allele carrier in MMD patients and controls.

| Locus | Allele | Control (2n = 814) | Patient (2n = 272) | OR (95%CI) | \( P \) value | \( P_c \) |
|-------|--------|-------------------|-------------------|------------|-------------|-------|
| DQB1  | 3:01   | 12.0 (98)         | 14.3 (39)         | 1.22 (0.80–1.85) | 0.323       | NS    |
| DQB1  | 3:02   | 9.34 (76)         | 9.19 (25)         | 0.98 (0.59–1.60) | 0.943       | NS    |
| DQB1  | 3:03   | 15.6 (127)        | 18.4 (50)         | 1.22 (0.83–1.77) | 0.282       | NS    |
| DQB1  | 3:05   | 14.4 (117)        | 9.56 (26)         | 0.63 (0.39–1.00) | 0.042       | NS    |
| DQB1  | 4:02   | 3.19 (26)         | 6.99 (19)         | 2.28 (1.17–4.35) | 0.006       | NS    |
| DQB1  | 5:01   | 7.74 (63)         | 6.62 (18)         | 0.84 (0.46–1.48) | 0.542       | NS    |
| DQB1  | 5:02   | 2.09 (17)         | 1.47 (4)          | 0.70 (0.17–2.17) | 0.522       | NS    |
| DQB1  | 5:03   | 3.56 (29)         | 2.94 (8)          | 0.82 (0.32–1.87) | 0.625       | NS    |
| DQB1  | 6:01   | 16.2 (132)        | 18.0 (49)         | 1.14 (0.77–1.65) | 0.491       | NS    |
| DQB1  | 6:02   | 7.86 (64)         | 5.88 (16)         | 0.73 (0.39–1.31) | 0.279       | NS    |
| DQB1  | 6:04   | 7.37 (60)         | 6.25 (17)         | 0.84 (0.45–1.49) | 0.533       | NS    |
| DQB1  | others | 0.62 (5)          | 0.37 (1)          | 0.60 (0.01–5.37) | 0.635       | NS    |

The number of allele carriers is shown in parentheses. The association was examined by the chi-square test. Corrected \( P \)-values \((P_c)\) statistically significant after Bonferroni correction are indicated in bold. OR, odds ratio; CI, confidence interval; \( P_c \), corrected \( P \)-value; NS, not significant.

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HLA-DRB1*04:10 is a risk allele for moyamoya disease.
Tables 2 and 3) and no association between HLA class I alleles (HLA-A, HLA-B, and HLA-C) and MMD (S2 Table).

**Association between the HLA-DR1–HLA-DQB1 haplotype and MMD**

Table 4 and S2 Table show the haplotype frequency estimated using the EM algorithm. The frequency of HLA-DRBI*04:10–HLA-DQB1*04:02 increased in MMD patients (Table 4): haplotype frequency 4.41% vs. 1.35% in the control group; \( P = 2.0 \times 10^{-3} \); \( \text{OR} = 3.37 \). On the other hand, the frequencies of HLA-DRBI*04:04–HLA-DQB1*04:01 (haplotype frequency 9.56% vs.

### Table 3. Frequencies of HLA-DRBI allele carrier in MMD patients and controls.

| Locus | Allele | Control (2n = 814) | Patient (2n = 272) | OR (95%CI) | \( P \) value | \( P_c \) |
|-------|--------|--------------------|--------------------|------------|--------------|----------|
| DPB1  | 2:01   | 25.2 (205)         | 29.4 (80)          | 1.24 (0.90–1.69) | 0.170        | NS |
| DPB1  | 2:02   | 4.30 (35)          | 1.84 (5)           | 0.42 (0.13–1.08) | 0.062        | NS |
| DPB1  | 2:03   | 4.42 (36)          | 4.41 (12)          | 1.00 (0.42–2.00) | 0.994        | NS |
| DPB1  | 4:01   | 6.02 (49)          | 4.41 (12)          | 0.72 (0.34–1.40) | 0.319        | NS |
| DPB1  | 4:02   | 10.1 (82)          | 8.09 (22)          | 0.79 (0.46–1.30) | 0.335        | NS |
| DPB1  | 5:01   | 38.0 (309)         | 36.4 (99)          | 0.94 (0.70–1.25) | 0.645        | NS |
| DPB1  | 9:01   | 7.86 (64)          | 9.93 (27)          | 1.29 (0.77–2.11) | 0.287        | NS |
| DPB1  | others | 4.18 (34)          | 5.52 (15)          | 1.34 (0.67–2.57) | 0.357        | NS |

The number of allele carriers is shown in parentheses. The association was examined by the chi-square test. Corrected \( P \)-values (\( P_c \)) statistically significant after Bonferroni correction are indicated in bold. \( \text{OR} \), odds ratio; CI, confidence interval; \( P_c \), corrected \( P \)-value; NS, not significant.

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### Table 4. Frequencies of estimated HLA-DRBI-DQB1 haplotype carrier in MMD patients and controls.

| DRB1/DQB1 | Control (2n = 814) | Patient (2n = 272) | OR (95%CI) | \( P \) value | \( P_c \) |
|-----------|--------------------|--------------------|------------|--------------|----------|
| *01:01-*05:01 | 7.00 (57)          | 5.88 (16)          | 0.83 (0.44–1.50) | 0.523        | NS |
| *04:03-*03:02 | 2.83 (23)          | 2.57 (7)           | 0.91 (0.33–2.22) | 0.826        | NS |
| *04:05-*04:01 | 14.3 (116)         | 9.56 (26)          | 0.64 (0.39–1.01) | 0.047        | NS |
| *04:06-*03:02 | 3.44 (28)          | 1.10 (3)           | 0.31 (0.06–1.03) | 0.045        | NS |
| *04:10-*04:01 | 1.35 (11)          | 4.41 (12)          | 3.37 (1.34–8.53) | 0.002        | 0.036 |
| *08:02-*03:02 | 1.97 (16)          | 2.94 (8)           | 1.51 (0.55–3.79) | 0.343        | NS |
| *08:02-*04:02 | 1.84 (15)          | 2.21 (6)           | 1.20 (0.38–3.32) | 0.707        | NS |
| *08:03-*06:01 | 7.62 (62)          | 7.72 (21)          | 1.01 (0.58–1.73) | 0.955        | NS |
| *09:01-*03:03 | 14.4 (117)         | 17.3 (47)          | 1.24 (0.84–1.82) | 0.247        | NS |
| *11:01-*03:01 | 2.58 (21)          | 1.84 (5)           | 0.71 (0.21–1.95) | 0.488        | NS |
| *12:01-*03:01 | 2.58 (21)          | 2.94 (8)           | 1.14 (0.43–2.73) | 0.749        | NS |
| *12:02-*03:01 | 2.21 (18)          | 1.47 (4)           | 0.66 (0.16–2.03) | 0.453        | NS |
| *13:02-*06:04 | 7.37 (60)          | 6.25 (17)          | 0.84 (0.45–1.49) | 0.533        | NS |
| *14:05-*05:03 | 1.84 (15)          | 1.84 (5)           | 1.00 (0.28–2.92) | 0.996        | NS |
| *14:06-*03:01 | 1.60 (13)          | 2.94 (8)           | 1.87 (0.66–4.92) | 0.163        | NS |
| *15:01-*06:02 | 7.86 (64)          | 5.88 (16)          | 0.73 (0.39–1.31) | 0.279        | NS |
| *15:02-*06:01 | 8.60 (70)          | 10.3 (28)          | 1.22 (0.74–1.97) | 0.398        | NS |
| Others  | 10.7 (87)          | 12.9 (35)          | 1.23 (0.79–1.90) | 0.324        | NS |

The number of haplotype carriers estimated by the EM algorithm is shown in parentheses. The association was examined by the chi-square test. Corrected \( P \)-values (\( P_c \)) statistically significant after Bonferroni correction are indicated in bold. \( \text{OR} \), odds ratio; CI, confidence interval; \( P_c \), corrected \( P \)-value; NS, not significant.

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14.3% in the control group; \( P = 0.047; \) OR = 0.64) and HLA-DRB1*04:06–HLA-DQB1*03:02 (haplotype frequency 1.10% vs. 3.44% in the control group; \( P = 0.045; \) OR = 0.31) decreased in MMD patients. However, after Bonferroni correction, the \( P \)-value (\( P_c \)) did not reach the threshold (Table 4). In addition, we found no association between HLA-DRB1–HLA-DQB1–HLA-DPB1, HLA-A–HLA-B, and HLA-A–HLA-B–HLA-C haplotypes and MMD (S3 Table).

High frequency of thyroid dysfunction in HLA-DRB1*04:10-positive MMD patients

Table 5 shows the results of our analysis of the association between HLA-DRB1*04:10 and MMD clinical characteristics. The results indicated that the frequency of thyroid diseases, including Graves' disease (GD) and Hashimoto thyroiditis (HT), is high in HLA-DRB1*04:10-positive MMD patients compared with that in HLA-DRB1*04:10-negative MMD patients (frequency 23.1% vs. 4.1% in the control group; \( P = 0.029 \)). In addition, the HLA-DRB1*04:10-

| Table 5. Comparison of demographics between MMD patients with or without HLA-DRB1*04:10. |
|-----------------------------------------------|------------------|------------------|------------------|------------------|
| DRB1*04:10-positive (n = 13) | DRB1*04:10-negative (n = 123) | OR (95%CI) | \( P \) value | OR (95%CI) | \( P \) value |
| Age 38.8 ± 10.8 | 43.6 ± 15.4 | 0.26 (0.03–2.07) | 0.258† | 5.61 (1.09–28.9) | 0.039 |
| Sex (Male: Female) 1:12 | 30:93 | 0.771‡ | 0.771‡ |
| History Diabetes 0% (0) | 0.8% (1) | 0 | 0.744†ab |
| Thyroid diseases 23.1% (3) | 4.1% (5) | 7.08 (1.47–34.0) | 0.029† | 5.61 (1.09–28.9) | 0.039 |
| Other autoimmune diseases 0% (0) | 0% (0) | 0 | 1.000† |
| Polymorphism c.14576G>A in RNF213 gene | 0.784§ |
| G/G 38.5% (5) | 30.1% (38) | 1.40 (0.43–4.55) | 0.549‡ |
| G/A 61.5% (8) | 67.4% (83) | 0.77 (0.24–2.51) | 0.771‡ |
| A/A 0% (0) | 1.6% (2) | 0 | 1.000‡ |
| Suzuki stage 1–2 15.4% (2) | 27.6% (34) | 0.513‡ |
| 3–4 86.4% (11) | 60.2% (74) | 0.43 (0.11–1.75) | 0.822‡ |
| 5–6 0% (0) | 12.2% (15) | 0.415‡ |
| The age of onset 30.1 ± 13.3 | 35.4 ± 16.0 | 0.206† |
| Child 15.4% (2) | 17.9% (22) | 0.48 (0.11–1.75) | 0.822‡ |
| Onset >60 0% (0) | 4.1% (5) | 3.64 (0.77–17.1) | 0.459‡ |
| Perinatal period 0% (0) | 4.9% (6) | 0 | 0.415‡ |
| Symptom Ischemia 76.9% (10) | 88.7% (109) | 0.43 (0.11–1.75) | 0.428‡ |
| Hemorrhage 23.1% (3) | 7.3% (9) | 3.80 (0.88–16.3) | 0.091‡ |
| Asymptomatic 0% (0) | 4.1% (5) | 0 | 0.459‡ |

The association between MMD patients with or without HLA-DRB1*04:10 was examined using Mann–Whitney’s U test, Fisher’s exact test, or the chi-square test. The number of patients is shown in parentheses, and the percentages are based on the number of patients per category. Mean values are represented as mean ± standard deviation (SD). A multiple logistic regression model was used to adjust for age and sex. † Mann–Whitney’s U test ‡ Fisher’s exact test § chi-square test. RNF213, ring finger protein 213.
positive group had more females (frequency 92.3% vs. 75.6%; \( P = 0.297 \)), younger patients (mean age of onset $30.1 \pm 13.3$ vs. $35.4 \pm 16.0$ years in the control group; \( P = 0.206 \)), and higher incidences of hemorrhagic stroke (frequency 23.1% vs. 7.3% in the control group; \( P = 0.091 \)) than the \( HLA-DRB1^{*}04:10 \)-negative group. However, these differences were not statistically significant. We also found no association among polymorphisms in the ring finger protein 213 (\( RNF213 \)) gene, Suzuki stage, and \( HLA-DRB1 \) genotype. After adjustment for age and sex, significantly higher proportion of \( HLA-DRB1^{*}04:10 \)-positive MMD patients had thyroid disease compared with \( HLA-DRB1^{*}04:10 \)-negative MMD patients (\( P = 0.039 \)).

Discussion

In this study, we identified \( HLA-DRB1^{*}04:10 \) as a risk allele and \( HLA-DRB1^{*}04:10–HLA-DQB1^{*}04:02 \) as a risk haplotype for MMD. This result was inconsistent with previous results, which demonstrates \( HLA-DRB1^{*}13:02, DRB1^{*}15:01, DQB1^{*}06:02 \) and \( DQB1^{*}06:09 \), as risk allele for MMD in East Asians \([19–21]\). This inconsistency might be resulted from lack of rigorous statistical analysis and limited range of subject population to familial cases in previous studies. Considering the frequency of \( HLA-DQB1^{*}04:02 \) allele, is not increased compared with the control group, and \( HLA-DRB1^{*}08:02 \) in LD with \( HLA-DQB1^{*}04:02 \) is not increased in MMD patients \([34]\), it is conceivable that \( HLA-DRB1^{*}04:10, not HLA-DQB1^{*}04:02, is a risk allele for MMD. In addition, some \( HLA-DR-DQ \) haplotypes, such as \( HLA-DR9-DQA1^{*}03–HLA-DQB1^{*}03:03 \) and \( HLA-DR4-DQA1^{*}03–HLA-DQB1^{*}04:01 \), lead to the risk of type 1 diabetes and autoimmune endocrinopathies in the Japanese population \([13–14]\). Therefore, \( HLA \) class II alleles are considered to play a key role in autoimmune reactions and it is possible that autoimmune reactions related to \( HLA-DRB1^{*}04:10 \) might play a role in MMD pathogenesis, although future studies including large cohorts should be performed.

In this study, we demonstrated that the frequency of thyroid diseases, including GD and HT, is high in \( HLA-DRB1^{*}04:10 \)-positive MMD patients compared with that in \( HLA-DRB1^{*}04:10 \)-negative MMD patients, suggesting the relationship between the \( HLA-DRB1^{*}04:10 \) haplotype and MMD and autoimmune thyroid diseases. Many studies have reported the association between thyroid dysfunction and MMD \([7–12]\), suggesting that elevated autoantibody concentrations and hyperthyroidism are associated with stenotic lesions in the terminal portion of the internal carotid artery and aggressive MMD presentations \([7–12]\). However, the underlying mechanism of this association remains undetermined. Although GD and HT are multifactorial autoimmune diseases \([35–38]\), they show opposite phenotypes: GD is characterized by the production of thyroid-stimulating hormone receptor–stimulating antibodies, leading to hyperthyroidism, whereas HT is characterized by the apoptosis of thyrocytes, resulting in hypothyroidism. However, GD and HT share an immunological basis; in fact, some alleles, such as \( HLA-A^{*}02:07, HLA-B1^{*}35:01, HLA-B1^{*}46:01 \), and \( HLA-DRB4^{*}53:01 \), are shared between GD and HT \([35–39]\). Although the association of \( HLA-DRB1^{*}04:10 \) with thyroid dysfunction has never been reported, its association with Vogt–Koyanagi–Harada disease and idiopathic thrombocytopenic purpura is known \([40–41]\). Future studies are required to clarify the functional role of \( HLA-DRB1^{*}04:10 \) and the nature of its involvement in MMD pathogenesis, especially in patients with thyroid diseases.

The role of \( RNF213 \) in autoimmune reactions is still unclear. Japanese researchers, including us, previously demonstrated that \( RNF213 \) is a disease susceptibility gene for MMD \([42–44]\). The c.\( 14576G>A \) polymorphism in \( RNF213 \) was identified in 95% of familial MMD cases and 79% of sporadic MMD cases \([37,38]\). The c.\( 14576G>A \) polymorphism in \( RNF213 \) is located in neither the AAA+ATPase domain nor the RING finger ubiquitin ligase domain \([42–44]\).
Functional roles of RNF213 and its polymorphism in MMD pathogenesis remain undetermined. However, since the c.14576G>A polymorphism in RNF213 greatly enhances the risk of MMD pathogenesis and that patients with this polymorphism show earlier disease onset and more severe clinical manifestations than MMD patients without the polymorphism [44], it is conceivable that the c.14576G>A polymorphism in RNF213 plays an important role in MMD pathogenesis. In a previous study, we reported regulatory T-cell decrease in RNF213-knockout mice [45]; therefore, RNF213 might play a role in autoimmune reactions. In contrast, in this study, we found no association between HLA-DRB1*04:10 and polymorphism in RNF213 and no difference in the soluble CD163 and CXCL5 serum concentrations in MMD patients between the RNF213 variant and normal variant groups [11]. These results suggested that the c.14576G>A polymorphism in RNF213 might not directly affect antigen recognition and the function of antigen-presenting cells. Further research could clarify the involvement of the RNF213 protein in autoimmune reactions.

This study had a few limitations. HLA-DRB1*04:10-positive patients account for ~10% of Japanese MMD patients; therefore, its involvement should be investigated in a larger population or other institutions. In addition, association between thyroid dysfunction and MMD is different between Asians and Caucasians: GD is more prevalent among Japanese MMD patients, whereas HT is more prevalent among Caucasian MMD patients [5–10]. The patients and controls in this study were residents of Tohoku and Kanto districts. Although the distribution of HLA alleles in the Japanese population is relatively homogeneous, there might be a difference in the HLA allele frequencies in MMD patients living in other regions of Japan. Further studies, which includes more control and MMD patients from other institutions, are required to clarify the exact association of HLA-DRB1*04:10 to MMD pathogenesis.

Conclusions

This study revealed that HLA-DRB1*04:10 and HLA-DRB1*04:10–HLA-DQB1*04:02 are a risk allele and a risk haplotype, respectively, for MMD. In addition, the HLA-DRB1*04:10 frequency increases in MMD patients with thyroid diseases. Although further studies are required to clarify the exact association between HLA class II and MMD, autoimmunity might explain MMD pathogenesis, at least in part.

Supporting information

S1 Table. Strengthening the Reporting of Genetic Association Studies (STREGA) checklist. (DOCX)

S2 Table. Frequencies of HLA class I carrier in MMD patients and controls. The number of allele carriers are shown in parentheses. Rare alleles (with expected counts less than five) are combined into “others” category prior to statistical analysis. The association was examined by Chi-square test. The corrected p (Pc) values, statistically significant after Bonferroni correction, are indicated in the bold. Abbreviations are as follows; OR, odds ratio; CI, confidence interval; Pc, corrected p value; NS, not significant. * Separator. (DOCX)

S3 Table. Frequencies of estimated haplotype carrier in MMD patients and controls. The number of estimated haplotype carriers by the expectation maximization algorithm are shown in parentheses. Rare alleles (with expected counts less than five) are combined into “others” category prior to statistical analysis. The association was examined by Chi-square test. The corrected p (Pc) values after Bonferroni correction are shown. Abbreviations are as follows; OR,
odds ratio; CI, confidence interval; P, corrected p value; NS, not significant. * Separator.

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