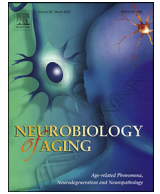




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Regular Article

High-resolution mapping identifies HLA class II associations with multifocal motor neuropathy

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ABSTRACT

Objective: To gain further insight in the immunopathology underlying multifocal motor neuropathy (MMN) by exploring the association between MMN and the human leukocyte antigen (HLA) class II DRB1, DQB1, and DQA loci in depth and by correlating associated haplotypes to detailed clinical and anti-ganglioside antibody data.

Methods: We performed high-resolution HLA-class II typing for the DRB1, DQB1, and DQA1 loci in 126 well-characterized MMN patients and assessed disease associations with haplotypes. We used a cohort of 1305 random individuals as a reference for haplotype distribution in the Dutch population.

Results: The DRB1*15:01-DQB1*06:02 haplotype (OR 1.6 [95% CI 1.1–2.2], $p < 0.05$) and the DRB1*12:01-DQB1*03:01 haplotype (OR 2.7 [95% CI 1.2–5.5], $p < 0.05$) were more frequent in patients with MMN than in controls. These haplotypes were not associated with disease course, response to treatment or anti-ganglioside antibodies.

Conclusions: MMN is associated with the DRB1*15:01-DQB1*06:02 and DRB1*12:01-DQB1*03:01 haplotypes. These HLA molecules or gene variants in their immediate vicinity may promote the specific inflammatory processes underlying MMN.

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1. Introduction

Multifocal motor neuropathy (MMN) is a rare neuropathy with a distinct clinical phenotype. It is characterized by distal, asymmetric, primarily upper limb weakness without significant sensory abnormalities. MMN affects mostly young to middle-aged men and although treatment with intravenous or subcutaneous immunoglobulins (IVIg of sclg) often leads to improvement in muscle strength, remission is rare. In the majority of patients disease course is slowly progressive, leading to severe disability of hand and arm function in a significant subgroup of patients (Cats et al., 2010a; Vlam et al., 2011; Yeh et al., 2020).

MMN is probably an immune-mediated disorder. From its first description, the presence of IgM antibodies directed at GM1, a

glycolipid constituent of motor and sensory nerves, has been noted. These antibodies are probably mono- or oligoclonal and have the capacity to activate the classical complement pathway (Cats et al., 2015; Piepers et al., 2010; Vlam et al 2015). The interaction of antibodies and complement was recently shown to interfere with motor neuron function and to induce cell damage and death in an induced pluripotent stem cell derived model for MMN (Harschnitz et al., 2014; Harschnitz et al., 2016).

It is as yet not known which genetic and environmental factors may contribute to MMN susceptibility, including the propensity for increased anti-GM1 IgM titers. Common genetic factors for autoimmunity may play a role as suggested by slightly increased risk for autoimmune diseases and the increased frequency of the human leukocyte antigen (HLA)-DRB1*15 allele in Dutch patients (Cats et al., 2012; Sutedja et al., 2010). In order to gain further insight in the pathogenesis of MMN, we performed a high-resolution HLA-DRB1, -DQA1, and -DQB1 typing in MMN patients and assessed their association with MMN susceptibility and MMN disease course.

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2. Materials and methods

2.1. Study population

Patients were diagnosed and enrolled at the outpatient clinic of the University Medical Center of Utrecht (UMCU), a tertiary referral center and national expertise center for MMN. All patients fulfilled diagnostic criteria for definite, probable or possible MMN as specified in both the initial criteria described by our group and the follow-up EFNS diagnostic criteria (Van den Berg et al., 1995; Van Schaik et al., 2010). These criteria primarily rely on the presence of the specific finding of conduction block, or – in its absence – a combination of abnormal ancillary investigations or a response to treatment with intravenously administered immunoglobulins that suggests MMN.

Clinical data were obtained from the MMN database, when necessary supplemented with the most recent data from UMCU patient files (Cats et al., 2010a). Data included sex, age at onset, age at diagnosis, delay until diagnosis, presence of anti-GM1 IgM antibodies, response to IVIg treatment, last recorded IVIg dosage per month, abnormalities on MR imaging of the brachial plexus, muscle strength and number of affected limb regions at the first visit and last follow-up visit. Onset of disease was defined as first complaints of muscle weakness. Diagnostic delay was defined as the time that lapsed between first symptom onset and MMN diagnosis. Muscle strength was graded on a 6-point Medical Research Council (MRC) scale, ranging from 0 (no contraction) to 5 (normal muscle strength against resistance) on the patients' first and last visit to the outpatient clinic. We documented MRC scores of shoulder abduction, elbow flexion, elbow extension, wrist flexion, wrist extension, finger flexion, finger extension, finger spreading, hip flexion, knee flexion, knee extension, foot dorsal flexion, and foot plantar flexion. MRC sum scores (maximum score 130) were obtained by summation of the MRC values of all tested muscle groups. In addition, the number of affected limb regions, that is, bilateral proximal and distal limb regions (maximum score 8) on patients' first and last follow-up visit were recorded.

Nerve conduction studies were performed as described previously (Cats et al., 2010a; Sutedja et al., 2010). In short, motor and sensory function were assessed bilaterally in median, ulnar, radial, musculocutaneous, peroneal, and tibial nerves. The number of definite conduction blocks, defined as CMAP area reduction of at least 50% (definite conduction block), or of 30%–50% (probable conduction block) over 2.5 cm inching, was scored in nerves with a distal CMAP > 1 mV.

IgM antibodies against GM1 were assessed using a standardized ELISA in most patients (Kuijf et al., 2005). The presence and titers of antibodies against the gangliosides GM1, GD1b, asialo-GM1, and GM2 were tested using a standardized ELISA as described earlier (Cats et al., 2010b). Anti-ganglioside cross-reactivity patterns were assessed in anti-GM1 IgM positive and negative patients (Willison and Yuki, 2002).

A neuroradiologist reported on hyperintensity or thickening of the brachial plexus on MR imaging.

All patients with MMN were Dutch. The control group for HLA typing consisted of a random sample of 1305 Dutch individuals living in or near the city of Leiden, The Netherlands (www.allelefrequencies.net).

2.2. HLA typing

We used the commercially available NGSgo-AmpX kit (GenDx), containing HLA-DRB1, HLA-DQB1, and HLA-DQA1 specific primers. Regions of interest were amplified by polymerase chain reaction according to the manufacturer's instructions.

A library preparation using NGSgo-LibrX and NGSgo-IndX kits was performed on the PCR products prior to next-generation sequencing (NGS). We used the MiSeq reagent kit v2 (500 cycles) MS-102-2003 for forward and reverse MiSeq sequencing. We used the freely available NGSengine software (GenDx) for data analysis and HLA typing. We used the standard World Health Organization nomenclature.

2.3. Statistical analysis

We carried out statistical analyses using R statistics version 3.4.1 (2017) and used the Genetics package to calculate allele and haplotype frequencies. When alleles or haplotypes were found in at least 5% of the MMN population, we used a Chi-squared or Fisher's Exact test, as appropriate, to compare these frequencies to the control cohort. We used odds ratios with a 95% confidence interval as a measure of association. When correlating haplotypes to clinical parameters, we performed pairwise comparisons between patients that either did or did not carry an associated haplotype. Continuous clinical variables, that is, age at onset, age at diagnosis, number of definite conduction blocks, the anti-GM1 IgM antibody titer, IVIg dosage, first and last visit MRC sum scores and first and last visit number of affected regions, were compared using a Student's *t* test or a Mann-Whitney *U* test, as appropriate. We compared dichotomous variables, that is, the presence of anti-GM1 IgM antibodies, with a Chi-squared or Fisher's Exact test. We corrected continuous variables for sex, diagnostic delay, age at diagnosis, disease duration, and treatment with immunoglobulins using a multivariate linear model. We used the common rule of thumb of at least 10 observations per correcting variable.

Given the aim of this study, the small patient population and the independence of variables, we deemed a *p*-value correction method using the Bonferroni method too conservative for comparing the HLA data. Therefore, we used a false discovery rate (fdr) method instead. Since the clinical variables are not independent, we used a Bonferroni correction method to correct the *p* values of comparisons of clinical data. In both cases, a corrected *p* value < 0.05 was considered statistically significant.

2.4. Standard protocol approvals, registrations, and patient consents

The locally appointed ethics committee of the University Medical Center Utrecht gave approval for this study (protocol NL-50354.041.14). All included patients gave written informed consent prior to inclusion in this study.

2.5. Data availability statement

The data that support the findings in this study will be available on request from the corresponding author.

3. Results

3.1. Study population

We obtained full high-resolution HLA typing from 126 out of 130 (97%) initially enrolled patients with MMN. Baseline characteristics are shown in Table 1.

Nine patients (7.1%) were lost to follow-up after their first visit to the UMCU. Nerve conduction data from our hospital were available of all patients with the exception of 9 (7.1%) who underwent NCS elsewhere prior to referral to our hospital. These NCS were revised by experts for a previous study and presence and site of conduction block were recorded in our files (Cats et al., 2010a). Brachial plexus MRI results were available of 73 (58%) of patients.

Table 1
Baseline characteristics of MMN patients (N = 126)

		N (%)
Male, n (%)	96 (76)	126 (100)
Age at onset, years (SD) ^a	41 (10.5)	126 (100)
Age at diagnosis, years (SD) ^a	48 (10.9)	126 (100)
Delay until diagnosis, months ^b	49 (3–585)	126 (100)
Definite MMN, n (%)	94 (75)	126 (100)
Probable MMN, n (%)	22 (17)	126 (100)
Possible MMN, n (%)	10 (8)	126 (100)
Follow-up, months ^b	109 (4–345)	119 (94)
Positive anti-GM1 IgM antibody, n (%)	74 (67)	111 (88)
Anti-GM1 IgM antibody titer ^b	1:200 (0–1:51200)	88 (70)
Abnormal brachial plexus MRI, n (%)	33 (26)	73 (58)
Response to IVIg therapy, n (%)	94 (93)	101 (80)
IVIg dosage per 4 weeks (range) ^b	53 (0–275)	101 (80)
Definite CB on diagnostic EMG, n (range) ^b	1 (0–8)	117 (93)
MRC sum score on first visit (range) ^b	122 (85–130)	126 (100)
MRC sum score on last visit (range) ^b	120 (51–130)	117 (93)
Affected limb regions on first visit, n (range) ^b	3 (1–8)	126 (100)
Affected limb regions on last visit, n (range) ^b	4 (0–8)	117 (93)

Abbreviations: CB, conduction block; IVIg, intravenous immunoglobulins; MMN, multifocal motor neuropathy; MRC, medical resource council; MRI, Magnetic resonance imaging.

^a Values displayed as mean (SD).

^b Values displayed as median (range).

Table 2
HLA class II allele frequencies in MMN patients and controls

DRB1 locus	MMN	MMN (% ^b)	Controls	p value	DQB1 locus	MMN	MMN (% ^b)	Controls	p value
DRB1*01:01	0.103	18.3	0.107	0.840	DQB1*02:01	0.111	21.4	0.139	0.222
DRB1*03:01	0.111	21.4	0.136	0.268	DQB1*02:02	0.083	16.7	0.072	0.526
DRB1*04:01	0.067	12.7	0.097	0.126	DQB1*03:01	0.154	27.8	0.157	0.923
DRB1*04:02	0.004	0.8	0.004	-	DQB1*03:02	0.087	16.7	0.111	0.234
DRB1*04:03	0.016	3.2	0.008	-	DQB1*03:03	0.032	6.3	0.040	0.509
DRB1*04:04	0.028	5.6	0.038	0.400	DQB1*03:05	0.004	0.8	0.000	-
DRB1*04:07	0.008	1.6	0.009	-	DQB1*04:02	0.012	2.4	0.026	-
DRB1*07:01	0.111	22.2	0.107	0.851	DQB1*05:01	0.139	25.4	0.136	0.899
DRB1*08:01	0.012	2.4	0.026	-	DQB1*05:02	0.008	1.6	0.013	-
DRB1*08:03	0.008	1.6	0.002	-	DQB1*05:03	0.016	3.2	0.034	-
DRB1*09:01	0.008	1.6	0.009	-	DQB1*06:01	0.008	1.6	0.005	-
DRB1*10:01	0.020	4.0	0.016	-	DQB1*06:02	0.206	36.5	0.145	0.009
DRB1*11:01	0.048	9.5	0.058	0.503	DQB1*06:03	0.073	13.5	0.065	0.515
DRB1*11:03	0.000	0.8	0.003	-	DQB1*06:04	0.056	11.1	0.042	0.318
DRB1*11:04	0.012	2.4	0.018	-	DQB1*06:09	0.004	0.8	0.007	-
DRB1*12:01	0.044	8.7	0.016	0.0061 ^a	DQB1*06:49	0.004	0.8	0.000	-
DRB1*12:02	0.008	1.6	0.002	-					
DRB1*13:01	0.071	12.7	0.062	0.559					
DRB1*13:02	0.063	12.7	0.048	0.288					
DRB1*13:03	0.004	0.8	0.007	-					
DRB1*14:01	0.008	0.8	0.001	-					
DRB1*14:54	0.012	2.4	0.033	-					
DRB1*15:01	0.210	37.3	0.149	0.0096 ^a					
DRB1*15:02	0.012	2.4	0.005	-					
DRB1*16:01	0.004	0.8	0.011	-					
DRB1*16:02	0.004	0.8	0.000	-					

Allele frequency = (N alleles)/(2*N). The allele frequencies of alleles present in at least 5% of MMN patients were compared with the control cohort. Statistically significant at $p < .05$.

^a Statistically significant after post-hoc p value adjustment.

^b Percentage of MMN population ($n = 126$) carrying at least one allele.

Anti-GM1 IgM antibody testing results (positive or negative) were available for 88 (70%) patients. Follow-up data were present in 117 MMN patients, with a median follow-up time of over 9 years.

3.2. HLA class II typing

High-resolution HLA typing yielded 26 DRB1 and 16 DQB1 alleles in 126 MMN patients. Results are shown in Table 2.

Allele frequencies of DRB1*15:01 and DRB1*12:01 were significantly higher in MMN patients. The DRB1*15:01-DQB1*06:02 and DRB1*12:01-DQB1*03:01 haplotype frequencies were significantly

higher in MMN patients (OR 1.6 [1.1–2.2] and 2.7 [1.2–5.5]), as shown in Table 3.

Comparisons of HLA-DQA1 allele frequencies were not possible since we lacked information in the control cohort. All patients carrying the HLA-DRB1*15:01-DQB1*06:02 haplotype also carried the HLA-DQA1*01:02 allele. Eleven out of 12 patients (92%) carrying the HLA-DRB1*12:01-DQB1*03:01 haplotype carried the HLA-DQA1*05:05 allele. Therefore, HLA-DQA1*01:02 and HLA-DQA1*05:05 seem to form part of the extended haplotypes of HLA-DRB1*15:01-DQB1*06:02 and HLA-DRB1*12:01-DQB1*03:01, respectively.

Table 3
Haplotype frequencies of MMN associated DRB1 alleles in MMN patients and controls

HLA Class II Haplotype	MMN	MMN (%) ^b	Controls	p value	OR (95% CI)
DRB1*12:01-DQB1*03:01	0.044	8.7	0.016	0.006 ^a	2.7 (1.2–5.5)
DRB1*15:01-DQB1*05:01	0.000	0.0	0.000	-	-
DRB1*15:01-DQB1*05:02	0.000	0.0	0.001	-	-
DRB1*15:01-DQB1*06:01	0.000	0.0	0.001	-	-
DRB1*15:01-DQB1*06:02	0.206	36.5	0.142	0.009 ^a	1.6 (1.1–2.2)
DRB1*15:01-DQB1*06:03	0.000	0.0	0.003	-	-
DRB1*15:01-DQB1*06:04	0.004	0.8	0.004	-	-
DRB1*15:01-DQB1*06:39	0.000	0.0	0.000	-	-

Haplotype frequencies of haplotypes found in at least 5% of MMN patients were compared between groups. Haplotype frequency = (N haplotype copies)/(2*N).

^a Statistically significant after p-value adjustment.

^b Percentage of MMN patients (n = 126) carrying at least on haplotype copy.

Table 4
Clinical parameters of MMN patients grouped by carriership of the associated haplotypes

	Negative (n = 70)	DR12 (n = 10)	DR15 (n = 45)	p values	
				DR12	DR15
Age at onset ^b	42 (10.9)	37 (11.3)	41 (9.7)	0.122	0.745
Age at diagnosis ^b	48 (10.9)	40 (11.7)	48 (10.1)	0.017	0.908
Definite CBS, n (range) ^c	1 (0–8)	1 (1–3)	1 (0–4)	0.750	0.199
Anti-GM1 IgM positive, %	67	40	73	0.158	0.691
Anti-GM1 IgM antibody titer ^c	1:400 (0–1:51200)	1:100 (0–1:51200)	1:300 (0–1:51200)	0.890	0.210
IVIg dosage [§]	53 (0–275)	58 (0–200)	60 (0–225)	0.842	0.850
First visit MRC sum score ^c	120 (85–130)	122 (101–128)	123 (96–130)	0.482	0.150
First visit affected regions, n ^c	3 (1–8)	3 (1–8)	2 (1–7)	0.966	0.140
Last follow-up visit MRC sum score ^c	119 (51–130)	121 (109–130)	121 (75–130)	0.196	0.014
Last follow-up visit affected regions, n ^c	4 (0–8)	4 (0–8)	3 (0–6)	0.704	0.001 ^a

Comparisons were made between the negative group and the DR12 and DR15 group separately.

DR12 = DRB1*12:01-DQB1*03:01 haplotype carriers; DR15 = DRB1*15:01-DQB1*06:02 haplotype carriers.

Statistically significant at p < .05.

^a Statistically significant at p < 0.05 after correction for multiple testing.

^b Values displayed as mean (SD).

^c Values displayed as median (range).

3.3. Clinical correlation

MMN patients were stratified as carriers of the HLA-DRB1*15:01-DQB1*06:02, HLA-DRB1*12:01-DQB1*03:01 or neither of these haplotypes. One subject was excluded from the analysis since this patient carried both. Given the small sample size of patients carrying the HLA-DRB1*12:01-DQB1*03:01 haplotype, correction for continuous variables was carried out for the HLA-DRB1*15:01-DQB1*06:02 group only. Results are shown in Table 4.

There was no correlation between HLA-DRB1*12:01-DQB1*03:01 haplotype carriership and clinical parameters. Patients carrying the HLA-DRB1*15:01-DQB1*06:02 haplotype had a trend towards a higher median MRC sum scores at the first visit and last follow-up visit and a lower number of affected regions on the last follow-up visit.

As shown in Table 4, the presence of anti-GM1 IgM antibodies did not differ between patients grouped by haplotype carriership. To further assess the possible pathogenic role of the associated haplotypes, we determined their correlation with anti-GM1 IgM antibody cross-reactivity patterns. As described previously, anti-GM1 IgM antibodies can show cross-reactivity with other gangliosides, notably asialo-GM1 (aGM1), GD1b, and GM2, indicative of fine epitope specificity of anti-GM1 IgM antibodies. Three main specificity patterns have been described. First, some anti-GM1 IgM antibodies are GM1 specific and do no cross-react with either aGM1, GD1b, or GM2. Second, anti-GM1 IgM antibodies targeting the terminal Gal (β 1-3) GalNAc structure show cross-reactivity with asialo-GM1 and GD1b. Third, antibodies targeting a shared internal sialylated sugar cross-react with GM2 (Willison and

Yuki, 2002). In patients without anti-GM1 IgM antibodies, usually no IgM antibodies against aGM1, GD1b, or GM2 are found.

Data on the presence of anti-GM1, -GM2, -GD1b, and -asialo-GM1 IgM antibodies were available for 81 patients (64%). Associations with anti-GM1 IgM cross-reactivity patterns could not be formally tested for the HLA-DRB1*12:01-DQB1*03:01 haplotype due to very low numbers (n = 7). The presence of the HLA-DRB1*15:01-DQB1*06:02 haplotype did not differ from the other 45 patients (Chi-squared test, $\chi^2 = 0.30$, p value 0.58). HLA-DRB1*15:01-DQB1*06:02 was not associated with a specific anti-GM1 IgM cross-reactivity pattern, both in anti-GM1 IgM positive and negative patients (Table A.1).

4. Discussion

This study corroborates our previous finding of an association of HLA-DRB1*15 with MMN but not with its clinical characteristics, and extends the association to an extended haplotype, likely HLA-DRB1*15:01-DQB1*06:02-HLA-DQA1*01:02 (Sutedja et al., 2010). Moreover, we identified HLA-DRB1*12:01 as a second risk factor, independent of HLA-DRB1*15:01-DQB1*06:02, as part of a haplotype with DQB1*03:01 and possibly HLA-DQA1*05:05. Almost half of MMN patients carried one of these haplotypes (9% and 37%, respectively). These associations may improve our understanding of the immunopathology in MMN.

In a previous smaller study of 74 patients with MMN we found an association with the HLA class II DRB1*15 allele group (Sutedja et al., 2010). A major drawback of the previous study was its lack of statistical power to detect associated alleles with a

Table A.1

HLA-DRB1*15:01-DQB1*06:02 status in cross-reactivity patterns of anti-GM1, -GD1b, -asialo-GM1 (aGM1), and -GM2 IgM antibodies

Anti-GM1 IgM	Cross-reactivity pattern	HLA-DRB1*15:01-DQB1*06:02	
		Positive (n (%))	Negative (n (%))
Anti-GM1 IgM ⁺ (n = 44)	Anti-GM1 IgM ⁺ only	3 (33%)	6 (67%)
	Anti-GM1/GD1b/aGM1 IgM ⁺	2 (18%)	9 (82%)
	Anti-GM1/GM2 ⁺	2 (67%)	1 (33%)
	Other	7 (33%)	14 (67%)
Anti-GM1 IgM ⁻ (n = 37)	Anti-GM1/GD1b/aGM1/GM2 IgM ⁻	11 (48%)	12 (52%)
	Other	6 (43%)	8 (57%)

Percentages shown are within cross-reactivity patterns. A Fisher's Exact test was performed in the anti-GM1 IgM positive and negative groups separately ($p = 0.44$ and 1.00 , respectively).

prevalence below 15%. The larger sample size and refined methodology in this study allowed us to perform more detailed analysis of the HLA-DRB1 locus in MMN. Our data show that the association of MMN with DRB1*15 is explained by an increased frequency of the DRB1*15:01-DQB1*06:02 haplotype. We found a second association with the DRB1*12:01 allele and more specifically, the DRB1*12:01-DQB1*03:01 haplotype. This new association is not explained by ethnic imbalance between patient and control groups. None of the patients had an ethnic background in which allele frequencies of DRB1*12:01 are reportedly higher, that is, Asian and African American populations (Maiers et al., 2007).

The finding of an HLA class II association with MMN, when interpreted as a role for CD4⁺ cells in the pathophysiological processes underlying an immune response against the glycolipid GM1, is puzzling (Harschnitz et al., 2016). Both HLA-DRB1*15:01 and HLA-DRB1*12:01 have been found associated with autoimmune disorders, including those of the central and peripheral nervous system. DRB1*15:01 has been found associated with the anti-neurofascin-155 positive chronic inflammatory demyelinating polyneuropathy (CIDP) and both alleles may converge in their reported associations with the multiple sclerosis – NMO spectrum (Gianfrancesco et al., 2017; Goodin et al., 2018; Martinez-Martinez et al., 2017; Misra et al., 2019; Moutsianas et al., 2015; Soelberg et al., 2018). Although specific characteristics of HLA molecules, such as the affinity of DRB1*15:01 to accommodate constituents of the myelin sheath including myelin-bound protein (MBP) and possibly ceramide, an important constituent of gangliosides, may suggest a direct pathogenic role, the initiation of the anti-GM1 IgM immune response is probably T-cell independent (Misra et al., 2019). Indeed, we have shown that HLA-DRB1*15:01-DQB1*06:02 is not associated with the presence of anti-GM1 IgM antibodies, nor with its titer or anti-ganglioside cross-reactivity patterns. Also, we have previously shown that only a small minority of patients with MMN show signs of anti-GM1 IgM isotype switching to IgG (1%) or IgA (5%), all with a titer of 1:400 or less and all having an anti-GM1 IgM titer that far exceeds the isotype titer (Cats et al., 2010b). We therefore think it is unlikely that the HLA class II associations are based on directly propagating antigen presentation or germinal center CD4⁺ T-cell interaction with activated B cells. However, CD4⁺ cells, including T-helper cells and regulatory T cells, could play another role in MMN pathogenesis, for example in the formation and maintenance of anti-GM1 IgM immunological memory by interacting with long-lived IgM-secreting plasma cells (Lightman et al., 2019).

Dissecting the pathophysiological role of HLA associations is often difficult. DRB1*15:01 and DQB1*06:02, and DRB1*12:01 and DQB1*03:01 were in strong linkage disequilibrium (LD) and this may complicate the direct identification of functional effects of the alleles constituting a haplotype, not in the least because alleles within a haplotype may contribute to different pathogenic path-

ways (Kaushansky et al., 2012; Kaushansky and Ben-nun, 2014). Moreover, HLA alleles could be in LD with unidentified disease-associated genes in their vicinity, including the HLA class III region that encompasses major components of the complement system. This is exemplified by the association of DRB1*15:01 with MS, which is better explained by its strong LD with an 11 SNP string (DRB1*15:01-a1 haplotype; Goodin et al., 2018). Future studies are needed to explore the possibility of LD as explanation of the associations of HLA haplotypes with MMN in more detail.

We did not find associations of HLA haplotypes with MMN disease course or response to treatment. Although patients carrying the DRB1*15:01-DQB1*06:02 had a trend toward higher MRC sum scores and a lower number of affected limb regions on their first visit, the clinical relevance of this finding is questionable. The lack of clear associations is in accordance with our previous study (Sutedja et al., 2010).

To the best of our knowledge, this is the only study on high-resolution HLA-class II genetics in MMN and given the rarity of this disease, the study population can be considered large. We believe that the robustness of our data is exemplified by the fact that we have corroborated the previous HLA-DRB1*15 association, in an expanded patient cohort and with a new and larger Dutch control cohort. Since the UMCU is a national tertiary referral center for MMN, we had the unique opportunity to correlate HLA genetics to clinical parameters and the presence of anti-ganglioside antibodies. Detailed clinical follow-up information was present in 93% of our patients, with a median follow-up time of over 9 years. Potential weaknesses are the fact that approximately 20% of our patients when first seen at our hospital already used IVIg, although this was statistically corrected for. The assessments of muscle strength, both at patients' first visit and last follow-up visit, were performed by more than one physician, all neurologists specialized in neuromuscular disorders. Ethnicity of MMN patients was not formally recorded, but all were Dutch, matching the ethnic background of the control population.

In conclusion, this study shows that the DRB1*12:01-DQB1*03:01 and DRB1*15:01-DQB1*06:02 haplotypes are associated with MMN. Future studies should assess possible population differences and determine the underlying pathogenic mechanisms in MMN.

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