Clinical Neurology and Neurosurgery 160 (2017) 137–141

Contents lists available at ScienceDirect

Clinical Neurology and Neurosurgery

journal homepage: www.elsevier.com/locate/clineuro

Association of primary central nervous system vasculitis with the presence of specific human leucocyte antigen gene variant

Markus Kraemer[a,1,*], Jana Becker[a,1], Peter A. Horn[b], Jan Claudius Schwitalla[a], Kathy Keyvani[c], Imke Metz[d], Christiane Wegner[d], Wolfgang Brück[d], Marc Schlamann[e], Falko M. Heinemann[b,1], Peter Berlit[a,1]

[a] Department of Neurology, Alfried-Krupp-von Bohlen und Halbach Hospital, Alfried-Krupp-Straße 21, 45130 Essen, Germany
[b] Institute for Transfusion Medicine, University of Duisburg-Essen, Virchowstraße 179, 45147 Essen, Germany
[c] Institute of Neuropathology, University of Duisburg-Essen, Hufelandstrasse 55, 45147 Essen, Germany
[d] Department of Neuropathology, University Medical Center Göttingen, Robert-Koch-Str. 40, 37099 Göttingen, Germany
[e] Department of Neuroradiology, University Hospital Giessen, 35385 Giessen, Germany

ARTICLE INFO

Keywords:
Human leucocyte antigen genes
European patients
Primary central nervous system vasculitis
Isolated angiitis of the CNS
Genetics

ABSTRACT

Objectives: The etiology and genetic susceptibility of primary central nervous system vasculitis (PCNSV) are still unclear.
Patients and methods: We analyzed the DNA of 25 Caucasian patients with PCNSV for human leucocyte antigen genes HLA-A, HLA-B, HLA-DRB1, and HLA-DQB1, respectively. HLA-frequencies of the 25 patients with PCNSV were compared with HLA-frequencies of matched Caucasian controls.
Results: No statistically significant associations were found for HLA-B, HLA-DR1 and HLA-DQB1 variant. In the PCNSV group, only the HLA-A*69 variant was found more often than expected statistically.
Conclusion: The results of this study indicate a potential association of HLA marker with PCNSV in Caucasian patients. Further studies are needed to elucidate the role of genes within the human major histocompatibility complex in the pathogenesis of this angiopathy.

1. Introduction

Primary central nervous system vasculitis (PCNSV), also known as isolated angiitis of the CNS, is a very rare disease. Its diagnosis represents a challenge for clinicians [1]. The infrequency of the disease and the difficulties in diagnosing this condition correctly are the main reasons for the lack of a reliable registry and epidemiologic data [2].

In contrast to neurological manifestations in the setting of a systemic rheumatologic disease (secondary vasculitis), PCNSV manifests exclusively in the CNS. Clinical and paraclinical hints for systemic inflammation – such as the elevation of C-reactive protein, renal or skin manifestations – are completely absent in PCNSV [1,3–5]. The leading symptoms of central nervous system vasculitis like headache, stroke, seizures and encephalopathy are highly nonspecific. Diagnostic work up includes clinical history, whole body examination, peripheral blood laboratory and CSF studies, MRI, conventional angiography and brain biopsy as described previously [1]. Due to the rare incidence of the disease, exclusion of more frequent differential diagnoses is the key element of diagnostic work up. Non-inflammatory diseases like moyamoya angiopathy, Divry-van Bogaert syndrome or Sneddon's syndrome can mimic PCNSV [1] as well as other autoimmune diseases such as multiple sclerosis (MS) or acute disseminated encephalomyelitis (ADEM). Even taking into account the dilemma of angiography-negative vasculitis and false-negative brain biopsies in individual cases it is important to prevent patients from “blind” immunosuppressive therapy in unrecognized non-inflammatory differential diagnoses by strictly paying attention to the diagnostic criteria, which include the procedure of angiography and brain biopsy [6,2].

MRI, angiography, CSF studies and biopsy each contribute to the diagnosis of PCNSV, but additional pieces are also essential to complete the mosaic for diagnosing PCNSV. Human leucocyte antigen (HLA) gene products have been reported to be associated with various diseases, such as HLA-B27 for ankylosing spondylitis and reactive arthritis, HLA-DR2 and HLA-DQ6 for narcolepsy, or HLA-DQ2 for celiac disease.

Abbreviations: PCNSV, primary central nervous system vasculitis; HLA, human leucocyte antigene; CNS, central nervous system; DNA, deoxyribonucleic acid; ANA, antinuclear antibodies; ANCA, anti-neutrophil cytoplasmic antibody; SPSS, Statistical Package for Social Science; NMDP, US American National Marrow Donor Program

* Corresponding author.
E-mail addresses: markus.kraemer@krupp-krankenhaus.de, markus.kraemer1977@gmx.de (M. Kraemer).
[1] These authors contributed equally.

http://dx.doi.org/10.1016/j.clineuro.2017.06.009
Received 6 March 2017; Received in revised form 8 June 2017; Accepted 12 June 2017
Available online 23 June 2017
0303-8467/

For a few diseases the association with specific human leucocyte antigen (HLA) genes is so high that HLA typing contributes an important clue to the diagnosis.

Such associations also were reported for connective tissue diseases and vasculitis. The clinically most relevant association was found in Behçet's disease. Ohno et al. described an association of HLA-B51 in a large population with Behçet's disease [7]. Further investigations confirmed that the genetic susceptibility to Behçet's disease was located in the MHC region and associated with HLA-B51, in particular with the subtype HLA-B* 51:01 [8]. For other vasculitides significant associations with HLA could be shown [9–13]. Finally, HLA associations were also described in diseases normally defined as non-inflammatory such as Moyamoya angiopathy [14].

This study aims to elucidate possible associations of PCNSV with HLA genes in Europeans by DNA based HLA typing of a strictly defined cohort of European patients.

2. Materials and methods

2.1. Subjects

We collected blood samples from patients fulfilling the diagnostic criteria for primary CNS vasculitis based on the guidelines of the German Society of Neurology (Table 1) [15]. Patients were screened based on clinical findings at the Department of Neurology of the Alfried Krupp-Hospital in Essen (Germany) and based on neuropathological results at the Departments of Neuropathology at the Universities of Duisburg-Essen and Göttingen. A total of 69 patients underwent screening for the possible diagnosis of PCNSV from August 2013 until December 2014. Twenty-five patients underwent biopsy (n = 17) or angiography (n = 8) confirming the diagnosis PCNSV (PCNSV cohort).

The study was approved by the Ethics Committee of the University of Duisburg-Essen. All participants gave their written informed consent.

Blood samples were taken from 17 patients with biopsy-proven PCNSV and from 8 patients with angiography-proven vasculitis. All patients with secondary CNS vasculitis and patients not fulfilling the diagnostic criteria mentioned above were excluded from participation. All patients were Caucasians, mainly from Germany. Due to the complexity of the HLA system, studies analyzing HLA disease associations require control groups with high sample numbers. As in our previous study [14], we used two sets consisting of HLA typing data from German blood donors [16], and Caucasian American unrelated blood stem cell donors as controls [17]. Both these control groups were suitable due to high sample numbers and consistency in numbers of HLA loci included and HLA typing strategies. In addition, HLA-DQB1 associations were analyzed using “HLA resources” from the US American National Marrow Donor Program (NMDP) (http://bioinformatics.nmdp.org/) with restriction to data obtained from American Caucasian donors.

2.2. HLA typing

HLA-A, HLA-B, HLA-DRB1 and HLA-DQB1 markers were analyzed in 25 patients with PCNSV. HLA-A, HLA-B, and HLA-DRB1 frequencies were compared with those of 13.386 German blood donors [18]. Moreover, HLA frequencies were additionally compared with NMDP data from different American white donors (more than 15.740 alleles) [17]. Typing of HLA-A, HLA-B, HLA-DRB1, and HLA-DQB1 was performed at the first field (low resolution level) using sequence-specific oligonucleotides LABType™ methodology, provided by One Lambda Inc./Thermo Fisher, Canoga Park, CA, USA [19].

Table 1
Diagnostic criteria for PCNSV according to the German Neurological Society Guidelines.

1. Clinical signs of multifocal or diffuse CNS disease with recurrent or progressive course
2. Cerebral angiography, MRI and CSF with findings that support the diagnosis of vasculitis
3. Exclusion of an underlying systemic infection or inflammation (systemic symptoms and/or BSG/CRP increase possible)
4. Histological evidence of leptomeningeal or parenchymal vasculitis and exclusion of infection, neoplasia or other primary vascular disease

2.3. Statistical data analysis

Data were analyzed using SPSS 2008 (Statistical Package for Social Science) version 16.0. As HLA genes are the most polymorphic genes in the human genome, weak statistical associations ($P < 0.05$) of particular HLA alleles may occur by chance alone and may not necessarily be significant [20]. Therefore, for the HLA analysis, we used the Holm procedure for multiple testing. After carrying out the individual tests and determination of all *p*-values, these values were sorted in ascending order. Starting with the smallest *p*-value each new level of significance was set in the global level of significance was shared by 5% by the number of tests ($\alpha 1/n$). The result was defined as statistically significant by dividing by the number of tests minus 1 ($\alpha 2/n\text{-}1$, $\alpha 3/n\text{-}2$, etc.) for the next higher p value. In a small number of participants the Holm procedure was preferred in contrast to the Bonferroni correction because it allows increasing the statistical power.

3. Results

3.1. Descriptive statistics – epidemiological data

Within a total number of 69 study participants 25 patients (36%) were diagnosed as having PCNSV. In 17 of them the diagnosis was proven by biopsy, in 8 the diagnosis was made according to angiography and exclusion of other diseases. The mean age at onset in the PCNSV collective was 43.8 years (SD 14.9; range 11–79; median 44). Ten subjects were female (40%) and 15 subjects were male (60%) (M: F = 1.5: 1).

3.2. HLA associations in PCNSV patients

The frequencies of the HLA-A, B, DRB1, and DQB1 alleles were calculated in the complete study cohort and subsequently compared with two control groups as described. This analysis revealed no significant differences between the PCNSV patients (n = 25) and non-PCNSV subjects (n = 44) with respect to the distribution of HLA-A, B, DRB1, and DQB1 variants in both groups. However, when the HLA frequencies within the PCNSV patient group were compared with the two control cohorts we could clearly show higher frequencies for the HLA-A*69 (n = 1 (2.2%) of patients, p = 0.002 after Holm´s procedure). For the HLA-B*49, HLA-DRB1*01 and DRB1*03 alleles a trend to a higher frequency in the patient cohort could be observed, as summarized in Table 2.

4. Discussion

Our study is the first to address the question of HLA association in PCNSV. This was done comparing the distribution of HLA alleles in PCNSV patients with the HLA frequency in two control groups. Although the total number of subjects included is still low, the strength of this study is the inclusion of only those patients strictly fulfilling the diagnostic criteria of PCNSV according to the German guidelines. As a consequence our cohort consisted of 17 patients with biopsy-proven PCNSV (for example see Fig. 1) and 8 patients with angiographically pathognomonic PCNSV findings (for example see Fig. 2). Moreover, it has to be stressed that the level of significance was chosen very strictly by using the Holm`s procedure. This was done in order to avoid false positive results. This procedure resulted in a very high level of significance given the high variability and the small number of