Journal of Neuroimmunology 289 (2015) 139–142

Contents lists available at ScienceDirect

Journal of Neuroimmunology

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

Short communication

Exome and regulatory element sequencing of neuromyelitis optica patients

Mika Siuko [a,*], Miko Valori [c], Tero Kivelä [a,e], Kirsi Setälä [a], Andreanne Morin [f], Tony Kwan [d,f], Tomi Pastinen [d,f], Pentti Tienari [b,c,e]

[a] Department of Ophthalmology, Helsinki University Central Hospital, Helsinki, Finland
[b] Department of Neurology, Helsinki University Central Hospital, Helsinki, Finland
[c] Molecular Neurology Programme, Research Program Unit, Biomedicum, University of Helsinki, Helsinki, Finland
[d] McGill University, Canada
[e] University of Helsinki, Helsinki, Finland
[f] Genome Quebec Innovation Center, Canada

ARTICLE INFO

Article history:
Received 25 August 2015
Received in revised form 30 October 2015
Accepted 2 November 2015

Keywords:
Devic's syndrome
Neuromyelitis optica
Next generation sequencing

ABSTRACT

Neuromyelitis optica (NMO) is rare in Finland. To identify rare genetic variants contributing to NMO risk we performed whole exome, HLA and regulatory region sequencing in all ascertained cases during 2005–2013 (n = 5) in a Southern Finnish population of 1.6 million. There were no rare variant shared by all patients. Four missense variants were shared by two patients in *C3ORF20*, *PDZD2*, *C5ORF47* and *ZNF606*. Another *PDZD2* variant was found in a third patient. In the non-coding sequence two predictably functional rare variants were shared by two patients. Our results do not support a homogeneous genetic etiology of NMO in Finland.

1. Introduction

Neuromyelitis optica (NMO), also known as Devic's disease, is an autoimmune disorder that affects preferentially the optic nerves and spinal cord. Aquaporin-4 (AQP4) antibodies represent a biomarker of NMO (Wingerchuk, 2014). Ethnic differences exist in the frequency of NMO. In Caucasians it is a rare demyelinating disease, much less common than multiple sclerosis (MS), while in South and East Asia NMO is relatively more common (Wingerchuk, 2014).

The incidence of MS is high in Finland (Sumelahti et al., 2000), but NMO seems to be very uncommon. In southern Finland (population of 1.5 million) we have identified five NMO patients during 2005–2013. In a recent study of consecutive acute optic neuritis (ON) patients examined in the Helsinki University Eye Hospital during 2008–2012, only two out of 191 patients (1%) fulfilled the diagnostic criteria of NMO (Siuko et al., 2014).

Relatively little is known on the genetic predisposition of NMO. In Japan and China NMO is associated with HLA-DPB1* 0501 allele (Matsushita et al., 2009; Wang et al., 2011). Preliminary data on other associations have been reported, but these findings, except the HLA-associations, are still uncertain (Matiello et al., 2011; Wingerchuk, 2014).

Here we have carried out whole exome, HLA and regulatory region sequencing in all 5 NMO patients ascertained in Southern Finland during 2005–13. The Finnish population has proven suitable for studying the genetic background of many rare diseases. The Finnish population has a relatively small number of founders and has remained relatively isolated for centuries (Norio, 2003). As a result, there is less heterogeneity in the disease alleles as compared to more mixed population. Should there be rare variants with strong genetic effect, these may be more easily detectable in the Finnish population than in more mixed populations as has been demonstrated e.g. in the recent genome-wide association analysis of amyotrophic lateral sclerosis (Laaksovirta et al., 2010).

2. Materials and methods

2.1. Participants

Two NMO patients were incident cases (Siuko et al., 2014) and three NMO patients were found by reviewing the patient databases of the Departments of Neurology and Ophthalmology in Helsinki University Central Hospital. Patient characteristics are shown in Table 1, three patients had an increased AQP4 antibody titer. DNA was extracted with the Wizard Genomic DNA Purification kit (Promega Corporation, Madison, Wisconsin, USA). All patients gave their informed consent for the present study, which was approved by the Ethics Committee of the Hospital District of Helsinki and Uusimaa (Dnro 83/13/03/01/2013).

* Corresponding author at: Department of Ophthalmology, Helsinki University Central Hospital, Haartmaninkatu 4, FI-000220 Helsinki, Finland.
E-mail address: mika.siuko@helsinki.fi (M. Siuko).

http://dx.doi.org/10.1016/j.jneuroim.2015.11.002
0165-5728/

Table 1
Neuromyelitis optica patients characteristics in order of AQP4 index at diagnosis.

Case	AQP4 index	Sex	Age at diagnosis	Episodes (in temporal order)	Cerebrospinal fluid (CSF)				Co-morbidity (age of onset if known)
					Leuk*	Prot†	OCB	IgG-i‡	
1	Positive	M	54	ON, myelitis	18	995	+	0.54	Facial paresis (20)
2	Positive	F	40	ON (bilat), myelitis	1	373	+	0.49	Breast cancer (36)
3	Positive	F	25	ON (bilat), tetraparesis	n.a	n.a	n.a	n.a	–
4	Negative	F	60	ON, balance problems	24	502	+	1.59	Basal cell carcinoma
5	Negative	F	33	Paraparesis, ON	3	446	n.a	0.55	Sacroilitis (31)

AQP4 = Aquaporin-4, AQP4 index = positive > 15, borderline 10–15, normal <10, OCB = oligoclonal bands, ON = optic neuritis, n.a. = not available.
* Leukocytes × 10^6 l.
† Protein mg/ml.
‡ IgG index.

2.2. Methods

Exome (Roche Nimblegen v3, 64 Mb), HLA region (Roche Nimblegen HLA, 5 Mb) and our selection of regulatory regions exhibiting open chromatin/DNAse I hypersensitive sites in immune cells (64 Mb) were captured from genomic DNA using a custom designed SeqCap kit (Roche NimbleGen Inc., Madison, Wisconsin, USA). The non-coding sequences targeted in addition to exome represent top 1%:tile of open chromatin replicated in multiple samples of each immune cell type (n = 17) by NIH RoadMap Epigenomics (PMID: 25693563). Next-generation sequencing with an average depth of coverage of 25× was performed using an Illumina HiSeq2500 instrument at the McGill University and Génome Québec Innovation Center, Montreal, Canada. Reads from the HiSeq output were mapped to the reference genome (hg19) using the Burrows–Wheeler Aligner (Li and Durbin, 2009) and variants called with the Genome Analysis Toolkit (McKenna et al., 2010) Non-synonymous variants in the coding regions that showed a frequency of less than 0.01 in the 1000 genomes (1000 genomes project consortium), ExAC (exac.broadinstitute.org) and Finnish SISU (sisu.fimm.fi) population databases were considered for further analysis. Non-coding variant frequencies were estimated based on 1000G database and in-house database of McGill University Genome Centre. Systematic errors were filtered using the Genome Analysis Toolkit VQSR method and by a comparison with control samples sequenced at the same time. We searched the NMO patients data for rare (<0.01) variants as the following: (i) shared exonic non-synonymous variants, (ii) shared non-coding variants, (iii) shared non-coding variants with a high CADD score suggesting functional effect (Kircher et al., 2014), and (iv) genes with clustering of any non-synonymous variants. HLA-typing (A, B, C, DQA1, DQB1, and DRB1 genes) was performed by using the software PHLAT (Bai et al., 2014). HLA-DPB1 typing was performed separately using sequence specific primers (SSP: Olerup SSP AB, Stockholm, Sweden).

3. Results

3.1. Exome

The characteristics of the 5 patients are shown in Table 1. Considering the exome sequence analysis first, there were 185–305 rare non-synonymous variants with a population frequency < 0.01 in each NMO patients (Table 2A). None of these variants were found in all 5 patients. Four rare non-synonymous variants were shared by two patients each; these were found in four genes *C3ORF20*, *PDZD2*, *C5ORF47* and *ZNF606* (Table 2B). The *C5ORF47* variant was shared by two AQP4 seropositive patients, otherwise the patients that shared rare variants were discordant for the AQP4 antibody status. The *PDZD2* V1121M variant was the only one that was predicted deleterious by both PolyPhen-2 (genetics.bwh.harvard.edu/pph2/) and SIFT (sift.jcvi.org) programs.

Table 2
Variants found in the NMO patients (Pt1–Pt5).*[a]

A. All non-synonymous variants.

Population frequency	Pt1[a]	Pt2[a]	Pt3[a]	Pt4	Pt5
≤1	8095	7977	8422	8291	7782
≤0.1	921	835	929	961	875
≤0.01	241	187	225	185	305
≤0.001	115	66	86	60	179
≤0.0001	57	31	37	33	81
0	38	20	23	22	31

B. Shared non-synonymous rare variants

Gene	Variant (freq < 0.01)	AA change	Finnish population frequency	Pt1[a]	Pt2[a]	Pt3[a]	Pt4	Pt5
C3ORF20	rs35363400	F582L	0.003024	X				X
PDZD2	rs149535005	V1121M	0.003175		Other	X	X + other	
C5ORF47	rs115473626	P81L	0	X		X		
ZNF606	rs139131343	V40M	0.001064			X	X	

C. Shared non-coding rare variants with high CADD score

Gene	Variant	AA change	Finnish population frequency	Pt1[a]	Pt2[a]	Pt3[a]	Pt4	Pt5
PLB1	rs13029421	Intronic	0.015	X	X			
Intergenic	rs12516549		0.01	X	X			

Footnote: The PDZD2 variant was the only rare shared variant that was predicted deleterious (by SIFT and PolyPhen-2). Other PDZD2 variants were found in Pt2 (rs116598198 D6Q, population frequency ≈ 0.02) and Pt4 (rs145138976 T56M, population frequency ≈ 0.03). The C5orf47 variant, while not found in the ExAC Finnish population, has a frequency of ≈0.02 in the African population.
[a] AQP4 antibody positive patient.