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Autoantibodies Against Dihydrolipoamide S-Acetyltransferase in Immune-Mediated Neuropathies

Alexandre Jentzer, Jérémie El-Bechir, Guillaume Taieb and Jérôme J. Devaux

ABSTRACT

Background and objectives: Dihydrolipoamide S-Acetyltransferase (DLAT), the E2 component of the mitochondrial pyruvate dehydrogenase complex (PDC-E2), has recently been suggested to be a biomarker of chronic inflammatory demyelinating polyneuropathy (CIDP). It was particularly associated with sensory variants of CIDP. Anti-mitochondrial antibodies are important for the diagnosis of primary biliary cholangitis, but insofar, only two studies have reported an association with CIDP. Here, we aimed to validate these observations in a cohort of French patients with immune-mediated neuropathy.

Methods: The positivity against PDC-E2/DLAT was examined using enzyme-linked immunosorbent assay, and confirmed using commercially available immuno-DOT and by indirect immunofluorescence on stomach, kidney, and liver sections.

Results: None of the 20 healthy controls, 31 patients with Guillain-Barré syndrome, 102 patients with CIDP (including 24 patients with sensory CIDP), 26 patients with monoclonal gammopathy, 23 patients with Charcot-Marie-Tooth disease, or 20 patients with autoimmune neuropathy showed IgG against PDC-E2/DLAT.

Discussion: PDC-E2/DLAT is accurately a target antigen in immune-mediated neuropathies.

INTRODUCTION

Chronic Inflammatory demyelinating polyradiculoneuropathy (CIDP) is an heterogenous sensory-motor neuropathy which is believed to have an autoimmune origin.¹ Intense efforts have been made to identify diagnosis biomarkers of CIDP. Autoantibodies targeting nodal and paranodal proteins have been initially described in a subgroup CIDP,² however, this subgroup of patients has recently been separated from CIDP and relabeled autoimmune nodopathy (AN).³ Other novel autoantibodies have recently emerged in CIDP and require further investigations.⁴⁻⁶ Among these, autoantibodies against dihydrolipoamide S-Acetyltransferase (DLAT), the E2 component of the mitochondrial pyruvate dehydrogenase complex (PDC-E2), has been suggested to be associated with CIDP, especially the sensory variant (sensory CIDP).⁶ Anti-mitochondrial antibodies (AMA) are frequently found in patient with primary biliary cholangitis (PBC) and less commonly found in other autoimmune disease.⁷ The association of PBC with CIDP is uncommon and insofar only two studies have reported a such association.^{6, 8} Here, we have re-evaluated the prevalence of anti-AMA antibodies in a cohort of French patients with autoimmune neuropathies.

METHODS

Patients' serum. Sera were obtained from twenty healthy controls (HC) (Etablissement Français du Sang, Montpellier, France), twenty six patients with monoclonal gammopathy (GAM), twenty three patients with Charcot-Marie-Tooth disease (CMT), thirty one patients with Guillain-Barré syndrome (GBS), fifty eight patients with CIDP, twenty four patients with a sensory variant of CIDP, and twenty patients with AN positive for anti-neurofascin-155 (Nfasc155) antibodies. The serum of two patients with anti-PDC-E2/DLAT antibodies was used as positive control. All subjects gave written informed consent. The study was

approved by the Ethics Committee of Montpellier University Hospital (IRB-MTP-2020-01-20200339).

Enzyme-linked immunosorbent assay (ELISA). MaxiSorp microtiter plates (ThermoFisher; 44-2404-21) were coated overnight at 4°C with 50 ng of human PDEC-E2/DLAT (15002-H07B; SinoBiological) diluted in PBS. Wells were then blocked with 0.5% casein + 0.05% Tween-20 in PBS for 1 hour at 37°C and then incubated overnight at 4°C with sera diluted 1:500 in blocking solution. The day after, the membranes were incubated with HRP-conjugated donkey antibodies against human IgG and the reactivity was revealed with TMB substrate (TMBX-1000-01; Surmodics), and optical density (OD) were measured at 620 nm. Sera were considered positive when the OD was higher than 3 standard deviations above the average of eight HC signal at a 1:500 dilution, a value equal to the limit of detection (LOD).

Immunolabeling and immuno-DOT.

Indirect immunofluorescence was performed using commercial European conformity kit with rat Triple Substrate (ME 0832 ; Theradiag, Croissy Beaubourg, France) and FITC-conjugated goat antibodies against human IgG (ME 902.15 ; Theradiag) according to the manufacturer's instructions. All sera were diluted at 1:40 for AMA detection by indirect immunofluorescence. AMA positivity on rat kidney/smooth muscle/stomach tissue was revealed by staining of both proximal and distal tubules of the rat kidney, by staining of the parietal cells of the rat stomach and by granulations of liver cell cytoplasm.

Immuno-DOT was performed using commercial European conformity kit Liver Profile 10 Ag Dot for BDI (AD LIVER10DBD ; D-tek, Mons, Belgium) according to the manufacturer's instructions. All sera were diluted at 1:100 for AMA detection. Positivity to M2/PDC-E2 antigen was considered when the colorimetric signal was more intense than the negative control.

Statistics. Statistical significance was assessed by one-way ANOVA followed by Bonferroni's post-hoc tests using GraphPad Prism (GraphPad Software, San Diego, CA).

Data availability. Data are available from the corresponding author upon request.

RESULTS

Screening of autoimmune neuropathies for autoantibodies against DLAT/PDC-E2.

AMA detection can be readily performed by immuno-DOT, by ELISA and/or by indirect immunofluorescence on tissue sections. Because ELISA are efficient to screen large cohort of patients and was successfully used in previous studies including the study describing anti-AMA in CIDP,⁶ we first screened our cohort of patients by ELISA, then confirmed the positivity by immuno-DOT and indirect immunofluorescence. The cohort was constituted of patients with typical CIDP (n = 58) or with variants of CIDP (Table 1) which included: sensory CIDP (n = 24), distal acquired demyelinating symmetric neuropathy (DADS; n = 20), and Nfasc155 + AN (n = 20). As controls, 20 healthy controls, 26 patients with GAM, and 23 patients with CMT were tested. Patients with GBS (n = 31) were also included in the cohort for comparison. As positive controls, the sera from two patients with AMA were used. We found that 2/20 HC (10%), 1/23 CMT (4%), 3/31 GBS (10%), 2/24 sensory CIDP (8%), 1/20 DADS (5%), and 1/58 CIDP (2%) were weakly positive for DLAT/PDC-E2 by ELISA (Figure 1). No positivity was found among GAM and AN patients. The two positive controls presented a strong reactivity. The mean difference between positive controls and the weakly positive sera was 1.43 OD.

To confirm the positivity of those patients, samples were further tested by immuno-DOT and by indirect immunofluorescence against tissue sections. Beside the positive controls which gave positive results by immuno-DOT and typical mitochondrial staining on tissue sections, none of the other samples showed reactivity by immuno-DOT or stained mitochondria. Also, all samples have been tested on mice sciatic nerve, and only the two

AMA positive patients stained mitochondria. No GBS, CIDP or AN patients showed mitochondrial reactivity. This suggested that DLAT/PDC-E2 is an uncommon immune target in peripheral neuropathies.

Retrospective study on positive patients for DLAT/PDC-E2 autoantibodies.

To determine whether patients with AMA may present with peripheral neuropathy, a retrospective study was performed on randomly selected patients tested positive for anti-DLAT/PDC-E2 autoantibodies (n = 40) in the department of Immunology of Montpellier University Hospital. Only 3/40 patients presented with neurological syndromes: one presented with amyotrophic lateral sclerosis and two showed cerebral morphological abnormalities. No patients presented with CIDP or other peripheral neuropathies.

DISCUSSION

Albeit antibodies to DLAT/PDC-E2 have recently been associated with CIDP.⁶ Here, we did not confirm reactivity against DLAT/PDC-E2 in our cohort. The reasons for this discrepancy are unknown. Difference in geographical/epidemiological traits between our respective cohorts may explain this discrepancy. The initial study was performed in a Japanese cohort and identified immunoreactivity against DLAT/PDC-E2 in 18 % of CIDP patients. Here, the cohort was constituted of patients from the south of France, but with diverse age, sex and ethnicity. The prevalence and incidence of AMA in France are respectively of 40.4 and 1.7 per 100 000 inhabitants, which is comparable to the worldwide prevalence and incidence of AMA.^{9,10} PBC is generally more prevalent in woman, and in adult over 40 year-olds.¹⁰ The CIDP cohort tested here presented a median age of 58 years and a percentage of women of 40 %, similar to the one described in Fukami *et al.* (2024). Demographic difference is thus unlikely to be the cause of our divergent results.

Differences in the methodological approaches may explain this discrepancy. For instance, a different antigen was used in our study. Albeit, we confirmed the reliability of the antigen and ELISA tests by using positive AMA patients, we cannot exclude that this impacted the positivity rate. Also, the patients found positives by ELISA were here further tested by immuno-DOT and by indirect immunofluorescence on tissue sections. These confirmatory tests demonstrated that the weak reactivities identified by ELISA were mostly false positives. Only the two patients with AMA presented with a typical mitochondrial staining on tissue sections. This indicates that anti-DLAT/PDC-E2 antibodies may be less frequent than originally reported and may thus require larger cohorts.

The retrospective analysis of positive AMA patients further indicated that AMA can be accidentally detected in various neurological conditions.

In conclusion, our study indicates that anti-DLAT/PDC-E2 antibodies are rarely associated with CIDP. Multi-center blinded studies would be valuable to determine the significance of AMA in CIDP.

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COMPETING INTEREST

M. El-Bechir, Dr. Jentzer, and Dr. Taieb report no competing interests. Dr. Devaux received a research grant from CSL Behring.

APPENDIX 1 - AUTHORS

Name	Location	Contribution
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Jérémy El-Bechir	Institut de Génomique Fonctionnelle, Université de Montpellier, CNRS, INSERM, 141 rue de la Cardonille, 34094 Montpellier Cedex 5, France	Acquisition of data, Analysis and interpretation of data.
Guillaume Taieb	Institut de Génomique Fonctionnelle, Université de Montpellier, CNRS, INSERM, 141 rue de la Cardonille, 34094 Montpellier Cedex 5, France Department of Neurology, CHU Montpellier, Hôpital Gui de Chauliac, 80 avenue Augustin Fliche, 34295, Montpellier Cedex 5, France	Drafting and revising of the manuscript, Acquisition of data, Analysis and interpretation of data.
Jérôme J. Devaux	Institut de Génomique Fonctionnelle, Université de Montpellier, CNRS, INSERM, 141 rue de la Cardonille,	Study concept and design, Drafting and revising of the

	34094 Montpellier Cedex 5, France	manuscript, Acquisition of data, Analysis and interpretation of data.
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TABLE**Table 1: Summary of clinico-demographic data of the cohorts**

	HC	GAM	CMT	GBS	Sensory CIDP	DADS	CIDP	AN
Median age (years)(range)	--	71 (51-88)	49 (29-73)	56 (19-88)	64 (38-88)	68 (53-82)	58 (30-88)	47 (2-78)
Sex (M/W)	--	15/11	9/14	21/10	16/8	15/5	35/23	15/5
Total number	20	26	23	31	24	20	58	20

AN: autoimmune nodopathy, CIDP: Chronic Inflammatory Demyelinating Polyradiculoneuropathy, CMT: Charcot-Marie-Tooth disease, DADS : Distal Acquired Demyelinating Symmetric Neuropathy, D, GAM: monoclonal gammopathy, GBS: Guillain-Barré syndrome, HC: healthy control, M: man, W: woman.

FIGURES

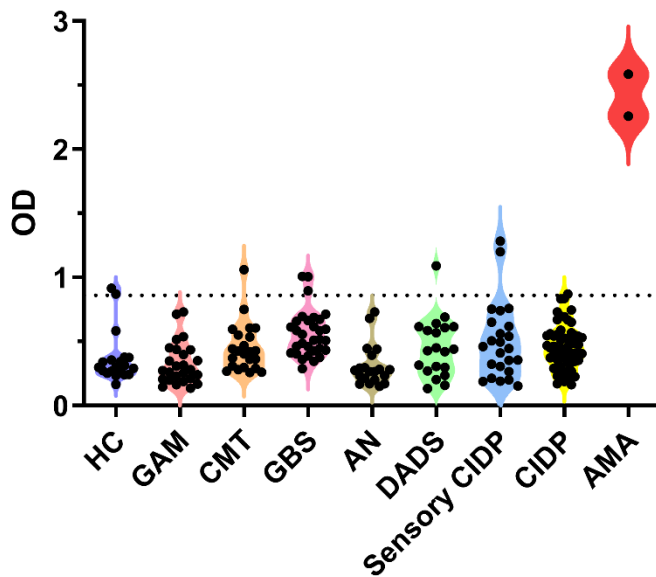


Figure 1: Reactivity against DLAT/PDC-E2 in patients with peripheral neuropathy.

The detection of autoantibodies was performed by Enzyme-linked immunosorbent assay (ELISA) on human DLAT/PDC-E2 protein. 20 healthy controls (HC), 26 monoclonal gammopathy (GAM), 23 Charcot-Marie-Tooth disease (CMT), 31 Guillain-Barré syndrome (GBS), 24 sensory CIDP, 20 Distal Acquired Demyelinating Symmetric Neuropathy (DADS), 58 Chronic Inflammatory Demyelinating Polyradiculoneuropathy (CIDP), and 20 autoimmune nodopathy (AN) were tested. As positive controls, two sera with anti-mitochondrial antibodies (AMA) were used. The threshold of positivity is indicated by a dashed line. No significant reactivity was found in any groups (one-way ANOVA followed by Bonferroni's post-hoc tests).