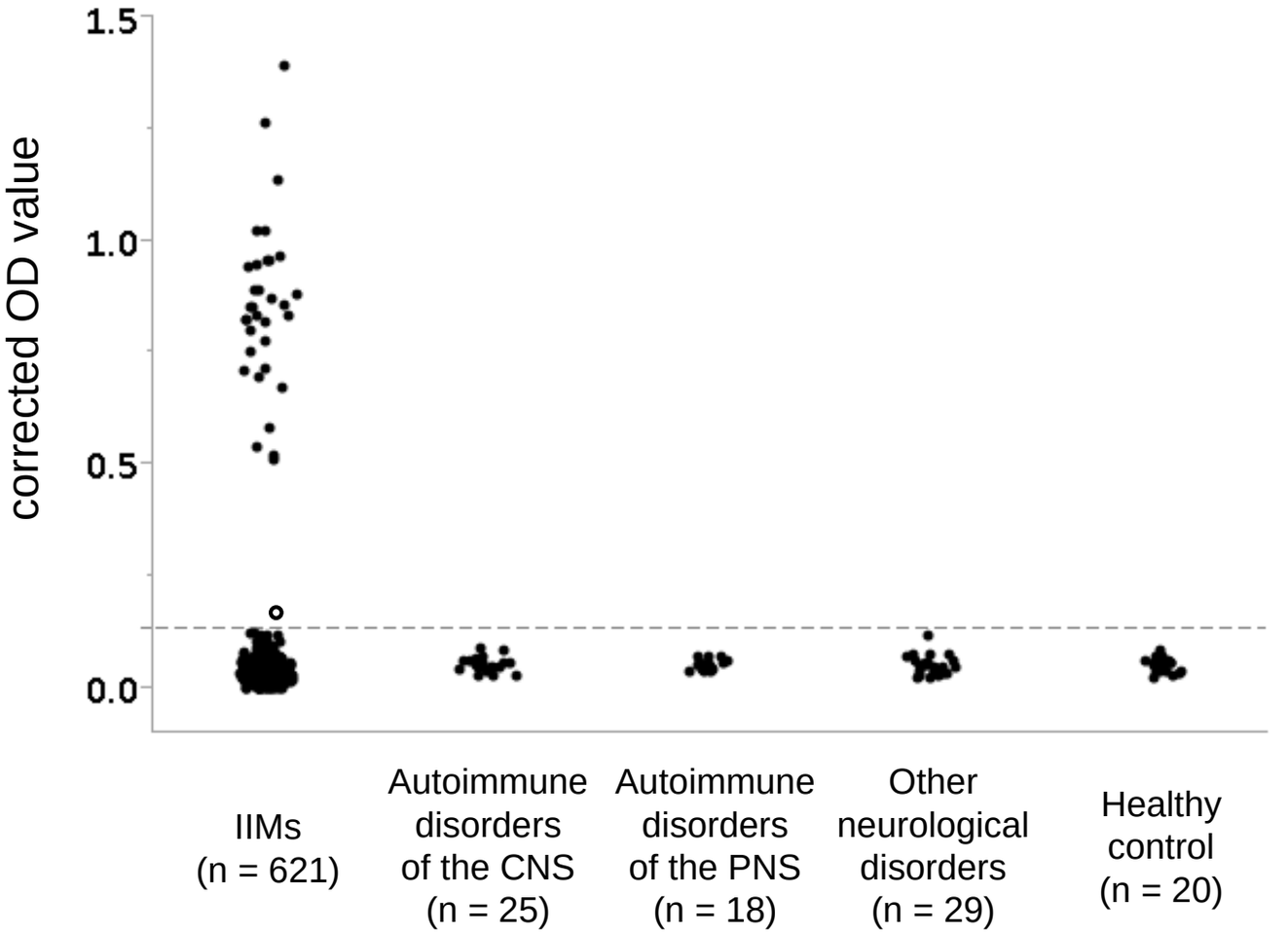
figure e-1　Detection of anti-HMGCR antibody

A. ELISA



B. Western blot analysis

Lane	1	2	3	4	Marker
Patient (cOD value by ELISA)	P1 (0.908)	P2 (0.955)	P3 (0.167)	Cont. (0.018)	

P = patient
Cont. = control

A. ELISA

We used the same methodology as the one employed in a previous study (Hida et al. 2016). The broken line shows the cut-off of corrected optical density (cOD) (0.130, the mean +5SD of healthy control sera). ELISA was performed on sera from 621 patients with idiopathic inflammatory myopathies (with the exclusion of sporadic inclusion body myositis and sarcoid myopathy), and 72 disease controls consisting of 25 patients with autoimmune disorders of the central nervous system, 18 patients with autoimmune disorders of the peripheral nervous system, and 29 patients with other neurological disorders. Anti-HMGCR antibodies were positive in 34 patients (0.834 ± 0.222, range 0.167-1.389). Among them, one male patient with dermatomyositis showed the lowest positive cOD (0.167, open circle). He showed markedly increased serum CK level (11000 IU/L) and no cancer. None of the patients in disease controls were positive for the anti-HMGCR antibody.

B. Western blot analysis

Western blot analysis was performed on sera from 34 patients found positive for the anti-HMGCR antibody by ELISA (positive patients, lanes 1 to 3; control, lane 4). A recombinant HMGCR protein (76 kDa) was mixed with sample buffer and then boiled for 5 minutes. Each antigen solution was loaded (250 ng/well) and then sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed. After SDS-PAGE, proteins on the gel were electrophoreticaly transferred to a polyvinylidene difluoride (PVDF) membrane (Merck Millipore, Darmstadt, Germany). After blocking, the PVDF membrane was incubated in patient’s serum diluted at 1:400 with blocking buffer overnight at 4 ºC. The membrane was washed three times and incubated in horseradish peroxidase-conjugated anti-human IgG-Fc antibody (MP Biomedicals, Solon, OH, USA) diluted at 1:5000 with blocking buffer for one hour. The membrane was washed again and band patterns were revealed with LAS3000 (Fujifilm, Tokyo, Japan) through the enhanced chemiluminescence reaction. Among 34 patients, the reactivity to HMGCR protein was confirmed in all except one patient with the lowest positive cOD by ELISA (Lane 3, P3).