# eMaterial：

# *Testing for GlyR and GABAB receptor (GABABR) antibodies*

# The open reading frames of GlyR (glycine receptor alpha1: GLRA1, accession no.NM\_000171.4) or GABABR (GABBR1, accession no.NM\_001470.4) were amplified by PCR using KOD One DNA polymerase with following primers: GlyR; forward (5’–3’): TGAGGAATTCTCTAGAGCCACCATGTACAGCTTCAATACTCTTCGACTCT, and reverse (5’–3’): GGACCCTCACTCTAGATCACTGGTTGTGGACGTCCTCTCT-3’; and GABABR, forward (5’–3’): TGAGGAATTCTCTAGAGCCACCATGTTGCTGCTGCTGCTACTGGCGCCAC, and reverse (5’–3’): GGACCCTCACTCTAGATCACTTATAAAGCAAATGCACTCG. The PCR product were inserted at XbaI cloning site of pEF1alpha promoter-SV40Neo plasmid by In-Fusion HD cloning Kit (Clontech, CA, USA) to generate GlyR or GABABR expression vectors. Each linearized expression vector was electroporated to HEK293 cells by using Gene Pulser Xcell (BIO-RAD, CA, USA). The cells were selected with G418 to establish stably GlyR or GABABR expression cells. GlyR or GABABR expressing cells were washed with 0.1 M phosphate-buffered saline (PBS, pH 7.4) and incubated with patient sera (1:10–400) or CSF (1:2–10) in 10% goat serum in PBS overnight at 4 °C and then fixed in 4% paraformaldehyde in PBS for 20 min. After washing with PBS, the cells were incubated with Alexa Fluor-conjugated anti-human IgG (Thermo Fisher Scientific, K.K., Tokyo, Japan; 1:500) for 1 hour. SlowFade Gold antifade reagent (Invitrogen Japan, Tokyo, Japan) was applied to the slides, and staining was observed under a fluorescence microscope (Axiovision, Zeiss, Germany). To confirm the localization of GlyR- and GABABR-antibody binding sites, double staining was performed in a mixture of patient sera/CSF and rabbit anti-GlyR (Cosmo Bio, Tokyo, Japan; 1:500) or anti-GABABR (Abcam, Tokyo, Japan; 1:500) antibodies. Antibody binding was visualized by Alexa Fluor 488 anti-human IgG and Alexa Fluor 594 anti-rabbit IgG, and observed under a fluorescence microscope (Axiovision, Zeiss, Germany). Other autoantibodies against NMDAR, AMPAR, LGI1, GABAAR, and CASPR2 were also examined by an in-house cell-based assay (CBA) using stably antigen-expressing cells or transiently cDNA-transfected cells using lipofectamine reagent (Invitrogen Japan, Tokyo, Japan) in Niigata University.e1

# We subjected control samples (10 serum samples from healthy volunteers; 5 CSF samples from iNPH; and 35 serum/CSF samples from patients with neurodegenerative disorders such as Parkinson’s disease, multisystem atrophy, PSP, and ALS) to the in-house CBA but obtained negative results for all regardless of the antigen used.

**eTable 1 Diagnostic criteria for stiff-person syndrome adopted from the work of Dalakas MC**1

**eTable 2 Antibody findings of 55 SPS patients**

**eTable 3 Clinical characteristics of GAD65- and GlyR- positive patients**

**eTable 4 Characteristics, longitudinal outcomes, and treatment of 29 GAD65 antibody-positive patients**

**eTable 5 GlyR-positive and seronegative patients, and other categories**

**eReferences**

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