Laboratory Detection of Antibody in IIDDs

Li-Jie Zhang, Jin Bi , Ying Fu

Department of Neurology, Fujian Institute of Neurology, the First Affiliated Hospital, Fujian Medical University, Fuzhou 350005, China. 413013752@qq.com

INTRODUCTION

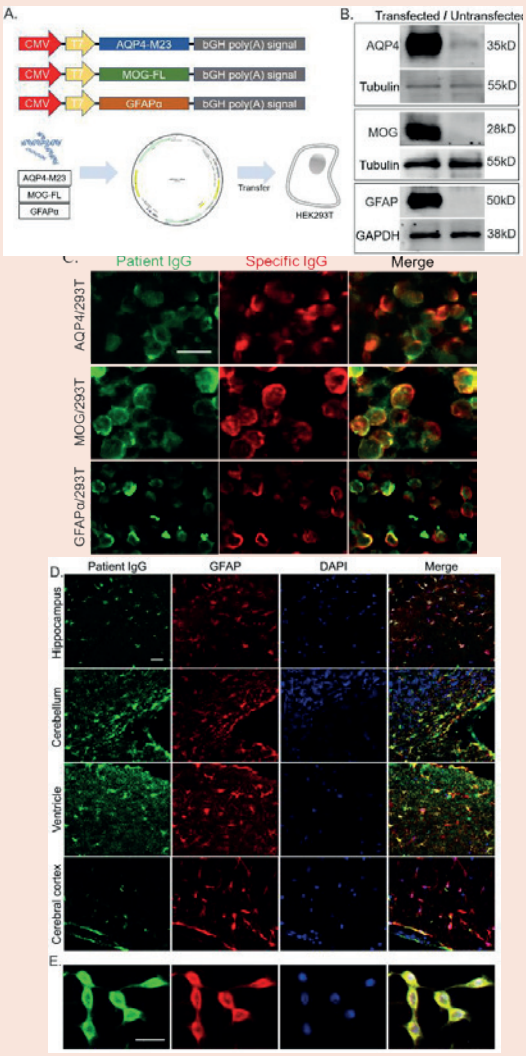
Idiopathic inflammatory demyelinating disorders (IIDDs) are difficult to diagnose because of their rarity and complexity in clinical manifestations. Laboratory detection of antibody is the key to diagnosis IIDDs.

At present, comprehensive detection methods have been established for antibodies to aquaporin 4 (AQP4), myelin oligodendrocyte glycoprotein (MOG), and glial fibrillary acidic protein (GFAP). However, the roles of these antibodies for diagnosing disease are in different evidence-based levels. Due to distinct antigenic properties, they involve various immunoassays strategies. AQP4 antibodies are included in the diagnostic criteria for NMOSD. Cell-based immunofluorescence assays (CBA) are recommended as gold standard of antibody detection. MOG-IgG associated disorders (MOGAD) have various clinical phenotypic spectrum, which limit preforming evidence-based diagnostic study. So, there is only expert consensus for the diagnosis of MOGAD. GFAP-IgG is an emerging antibody associated disease which is difficult to detect because of 10 subtypes at least.

CBA is adequate to detected AQP4 antibody, but GFAP antibodies versa. Therefor a combination of more immunoassays is required in the case of GFAP-IgG.

METHOD

We detected AQP4, MOG and GFAP antibodies by CBA. GFAP antibodies were also detected by immunofluorescence on the U251 cell line and immunohistochemical assays using brain slices from SD rats and GFAP-KO mice.



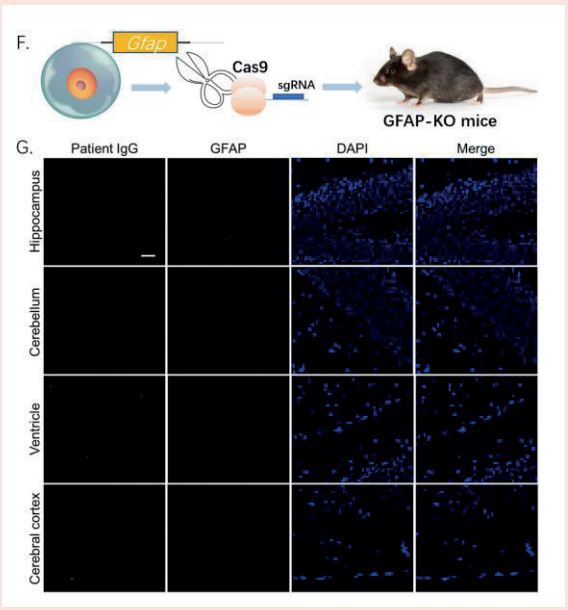


Fig.1 Antibody detection . (A) The human three genes (M23 AQP4, MOG full length, and GFAPα isoforms) were respectively expressed in the HEK-293T human cell line (AQP4/293T, MOG/293T, GFAPα/293T). (B) Expression of proteins was confirmed by Western blot. (C) Cell based assay was used to test IgG of patient serum, which revealed a positive staining pattern immunofluorescence. The positive control used commercial IgGs specific for AQP4, MOG, and GFAP. Scale bars: 50 μm each. Dual immunostaining of mouse tissues with Patient IgG and commercial IgGs specific for GFAP. (D) GFAP and patient IgGs largely co-localize in astrocytes of hippocampus, cerebellum, and ventricle of SD rats, but not in cerebral cortex. Scale bars: 50 μm each. (E) U251 cell line binds patient IgG and commercial GFAP-specific IgGs. Scale bars: 50 μm each. (F) In GFAP-KO mouse brain slices, neither the patient IgGs nor GFAP-specific IgGs stained astrocytes in the cerebellum, hippocampus, ventricles, and cortex. Scale bars: 50 μm each. DNA is stained blue with 4′,6-diamidino-2-phenylindole.

RESULT

We tested 311 patients for AQP4 and MOG antibodies, of which the positive rates were 40.2% and 14.1% respectively. The positivity rate of GFAP antibody was 3.1%.

Autoantibody	Positive number/total number	n%
AQP4-IgG	125/311	40.20%
MOG-IgG	44/311	14.10%
GFAP-IgG	5/159	3.10%

Tab.1 Number of positives for different antibodies.

CONCLUSION

We standardize detection strategies for the three autoantibodies. For AQP4 antibodies, a positive result makes a definitive diagnosis based on meeting one of the six core clinical characteristics. While in case of MOG antibodies, a positive result must be combined with the clinical presentation and the pathogenicity of the antibody to make a diagnosis. Finally, the diagnosis of GFAP astrocytopathy should be made cautiously using a combination of detection methods. The pathogenicity of GFAP antibodies deserves further exploration in the future.

Autoantibody	AQP4-IgG	MOG-IgG	GFAP-IgG
Detection method	◆ CBA	◆ CBA	◆ CBA ◆ Cellular immunofluorescence of glioma lines ◆ Immunohistochemical Assays in rats ◆ Immunohistochemical Assays in GFAP-KO mice
	NMOSD √	MOG-AD Must be combined with clinical presentation and the pathogenicity of the antibody	GFAP astrocytopathy Further validation of the pathogenicity of GFAP-IgG

Tab.2 Detection Strategies for Three Types of Antibodies.