RESEARCH ARTICLE OPEN ACCESS

Functional Signature of LRP4 Antibodies in Myasthenia Gravis

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Neurol Neuroimmunol Neuroinflamm 2024;11:e200220. doi:10.1212/NXI.0000000000200220

Abstract

Background and Objectives

Antibodies (Abs) specific for the low-density lipoprotein receptor-related protein 4 (LRP4) occur in up to 5% of patients with myasthenia gravis (MG). The objective of this study was to profile LRP4-Ab effector actions.

Methods

We evaluated the efficacy of LRP4-specific compared with AChR-specific IgG to induce Ab-dependent cellular phagocytosis (ADCP), Ab-dependent cellular cytotoxicity (ADCC), and Ab-dependent complement deposition (ADCD). Functional features were additionally assessed in an independent AChR-Ab$^+$ MG cohort. Levels of circulating activated complement proteins and frequency of Fc glycovariants were quantified and compared with demographically matched 19 healthy controls.

Results

Effector actions that required binding of Fc domains to cellular FcRs such as ADCC and ADCP were detectable for both LRP4-specific and AChR-specific Abs. In contrast to AChR-Abs, LRP4-binding Abs showed poor efficacy in inducing complement deposition. Levels of circulating activated complement proteins were not substantially increased in LRP4-Ab–positive MG. Frequency of IgG glycovariants carrying 2 sialic acid residues, indicative for anti-inflammatory IgG activity, was decreased in patients with LRP4-Ab–positive MG.

Discussion

LRP4-Abs are more effective in inducing cellular FcR-mediated effector mechanisms than Ab-dependent complement activation. Their functional signature is different from AChR-specific Abs.

Introduction

Myasthenia gravis (MG) is an antibody (Ab)-mediated autoimmune disease in which immunoglobulin G (IgG) molecules bind to the nicotinic acetylcholine receptors (AChRs) or to functionally related molecules at the neuromuscular junction, leading to localized or general muscle weakness. Most patients (up to 85%) display Abs directed against the AChR, whereas 5%–10% present Abs targeting the muscle-specific kinase (MuSK) and 1%–5% have Abs specific for the lipoprotein receptor-related protein 4 (LRP4).[1] LRP4 forms a multiprotein complex with MuSK and facilitates AChR clustering through the agrin/LRP4/MuSK/Dok7/rapsyn pathway.[2]

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Go to Neurology.org/NN for full disclosures. Funding information is provided at the end of the article.

The Article Processing Charge was funded by the authors.

Glossary

Abs = Antibodies; **AChR** = acetylcholine receptors; **ADCC** = Ab-dependent cellular cytotoxicity; **ADCD** = Ab-dependent complement deposition; **ADCP** = Ab-dependent cellular phagocytosis; **HC** = healthy control; **IgG** = immunoglobulin G; **LRP4** = lipoprotein receptor-related protein 4; **MG** = myasthenia gravis; **MuSK** = muscle-specific kinase.

Adoptive transfer experiments provided evidence that LRP4-specific Abs are pathogenic.[3,4] Although anti-LRP4 Abs are believed to impair AChR clustering through blocking agrin-LRP4 interaction, Fc-mediated actions of LRP4-Abs such as their efficacy to induce Ab-dependent complement deposition (ADCD), Ab-dependent cellular cytotoxicity (ADCC), and Ab-dependent cellular phagocytosis (ADCP) remain poorly understood. In this study, we simultaneously investigated effector functions of IgG autoantibodies specific for LRP4 and AChR, profiled complement activation, and interrogated the IgG-glycome repertoire in patients diagnosed with LRP4 Ab^+ MG (eMethods).

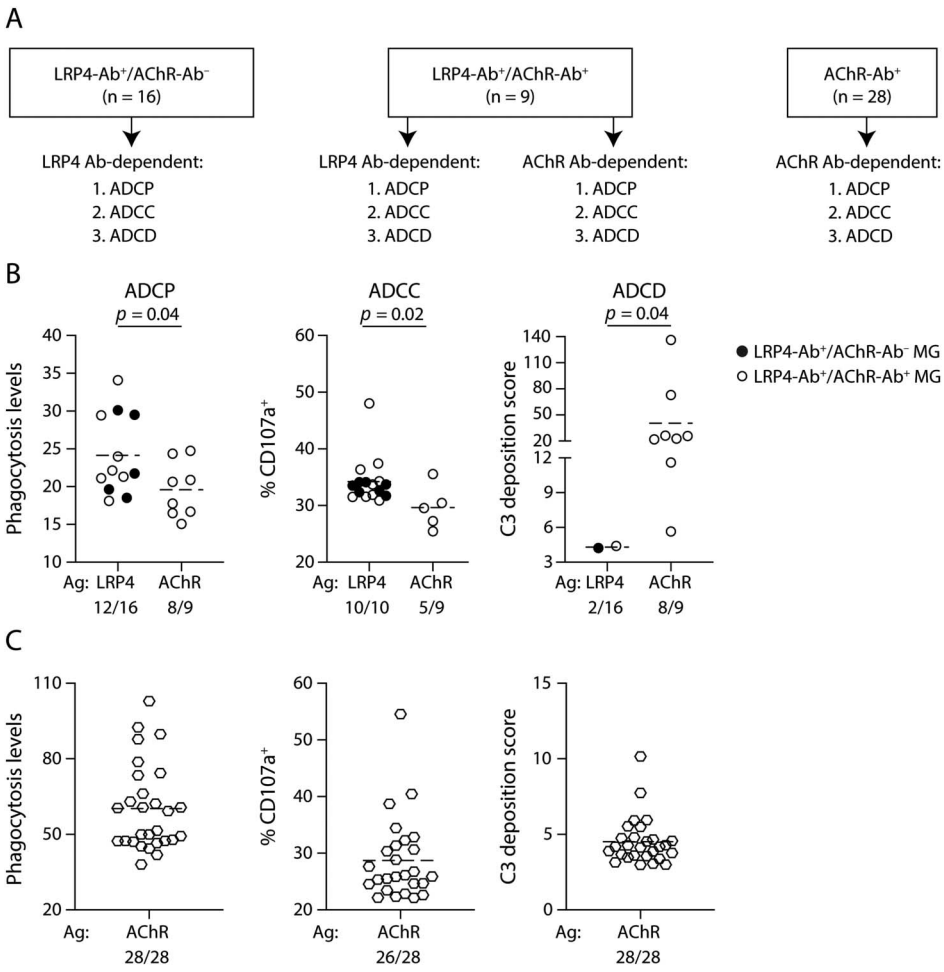
Sixteen LRP4-Ab^+ MG patients, of whom 9 patients were double positive for AChR-Abs (56%) along with 19 healthy controls (HC), matched for age and sex and an additional

Table Demographic and Clinical Characteristics of Patients With LRP4-Ab^+ Myasthenia Gravis

	LRP4-Ab^+ MG patients				
	All patients	LRP4-Ab^+/AChR-Ab^- patients	LRP4-Ab^+/AChR-Ab^+ patients	Healthy controls	*p* Value
n	16	7	9	19	
Sex female, n (%)	12 (75)	7 (100)	5 (55.5)	12 (63)	0.4928
Age in years, mean (SD)	58 (15)5	55 (12)	60 (18)	52 (8)	0.3536
EOMG, n (%)	5 (31)	2 (29)	3 (33)	—	
LOMG, n (%)	11 (69)	5 (71)	6 (67)	—	
Disease duration in months, median (IQR)	7 (2–26)	17 (2–54)	2 (2–9)	—	
LRP4-Ab titer, mean (SD)[a]	1:100	1:100	1:100	—	
AChR-Ab titer, mean (SD)[b]	6 (8)	0.2 (0.2)	10 (8)	—	
Thymectomy, n (%)	6 (38)	1 (14)	5 (56)	—	
Thymoma, n (%)	1 (6)	0 (0)	1 (11)	—	
QMG, mean (SD)	8 (7)	11 (5)	6 (8)	—	
MG-ADL, mean (SD)	8 (5)	9 (5)	6 (5)	—	
Immunotherapy, n (%)					
Naive	1 (6)	0 (0)	1 (11)		
Any immunotherapy	15 (94)	7 (100)	8 (89)		
Corticosteroids	7 (44)	3 (43)	4 (44)	—	
Standard immunosuppressive[c]	8 (50)	4 (57)	4 (44)	—	
Rituximab	0 (0)	0 (0)	0 (0)	—	
Eculizumab	1 (6)	0 (0)	1 (11)	—	
Efgartigimod	1 (6)	0 (0)	1 (11)	—	

Abbreviations: AChR = acetylcholine receptor; EOMG = early-onset myasthenia gravis; LOMG = late-onset myasthenia gravis; LRP4 = lipoprotein-related protein 4; − = negative antibody status; + = positive antibody status.
Data are mean (SD) and n (%) variables and median (IQR) for disease duration. Disease duration is the time from diagnosis. The percentage of the thymus histology results is related to the number of thymectomized MG patients.
p values refer to the comparison between LRP4-Ab^+.
Patients with MG and healthy controls using the Fisher exact test and Mann-Whitney test as appropriate.
[a] LRP4-Ab titers were determined by immunofluorescence using 1:100 serum dilutions.
[b] AChR-Ab titers were determined by radioimmunoassay, and data are expressed by nmol/L.
[c] Standard immunosuppressive therapies include azathioprine, mycophenolate mofetil, and methotrexate.

Figure LRP4-Specific Compared With AChR-Specific Fc-Mediated Effector Responses in Patients With LRP4-Ab+ and AChR-Ab+ MG



(A) Schematic diagram depicting LRP4-antibody–mediated and AChR-antibody–mediated responses evaluated in LRP4-Ab$^+$/AChR-Ab$^-$, LRP4-Ab$^+$/AChR-Ab$^+$, and AChR-Ab$^+$ MG patients: antibody-dependent phagocytosis (ADCP), cell cytotoxicity (ADCC), and complement deposition (ADCD). (B) Quantification of phagocytosis levels, cell cytotoxicity (percentage of cell degranulation [CD107a]), and C3 complement deposition levels in MG patients with LRP4-Ab$^+$/AChR-Ab$^-$ (n = 7, black dots) and LRP4-Ab$^+$/AChR-Abs$^+$ (n = 9, white dots). (C) Quantification of phagocytosis levels, cell cytotoxicity, and C3 complement deposition levels in MG patients with AChR-Ab$^+$ (n = 28). Each dot represents an individual patient. Numbers below diagrams represent patients included in the figure. Mean is shown in each patient group. Dot plots show the level of samples above the negative control. Mann-Whitney was used for statistical comparisons between groups.

independent cohort of 28 patients with AChR-Ab$^+$ MG were included (Table).

First, we determined IgG-mediated effector actions (ADCP, ADCC, and ADCD)[5] induced by Ab binding either to LRP4 in LRP4-Ab$^+$/AChR-Ab$^-$ patients or to the immunodominant alpha subunit of the AChR[1,6] in patients seropositive for both LRP4-specific and AChR-specific Abs (Figure 1A). Effector actions that required binding of Fc domains to Fcγ receptors (FcγRs) such as ADCC as determined by a CD107a degranulation assay and ADCP quantified by uptake of fluorescent beads decorated with Ag-specific Abs were detectable for both LRP4-specific and AChR-specific Abs at similar frequencies and strengths (Figure 1B). Although AChR-targeting Abs were highly efficient in inducing complement deposition on binding to Ag-coated beads, only 1 of 16 patients with LRP4-Ab+/AChR-Ab- MG showed evidence for ADCD on IgG binding to LRP4 (Figure 1B). These data indicate that in contrast to AChR-specific Abs, LRP4-binding Abs show poor efficacy in inducing Ab-dependent complement activation in patients with MG. Consistent with the aforementioned findings, AChR-Ab derived from an additional cohort of patients with MG efficiently induced both complement and cellular Fc-mediated effector actions, supporting the concept that the functional profile of AChR-Abs differs from LRP4-Abs (Figure 1, B and C).

Levels of activated complement proteins, reported to be prominently increased in people with AChR-Ab$^+$ MG,[7] were not higher in patients with LRP4-Ab$^+$ MG compared with those detected in HCs except for a marginal increase in C4a concentrations in LRP4-Ab$^+$ MG (eFigure 1).

Since efficacy and strength of Fc-mediated effector functions are regulated by the Fc glycan within the C_H2-domain of each IgG heavy chain and changes in serum polyclonal and/or auto-Ab glycosylation are consistently reported for many Ab-driven autoimmune diseases, we additionally profiled Fc-glycovariants in patients with LRP4-Ab$^+$ MG compared with HC. IgG-Fc glycovariants carrying 2 sialic acid residues (S2) were significantly reduced in patients with LRP4-Ab$^+$ MG compared with the HC group (eFigure 2).

Our study identified a unique functional signature of LRP4-Ab–positive MG. LRP4-targeting Abs are equally effective as AChR-specific Abs in inducing FcγR-mediated effector functions such as innate immune cell activation through ADCC and ADCP but less potent in inducing Ab-dependent complement activation.

The ability of IgG molecules to activate the classical complement cascade is determined by structural variations within the binding site for C1q, the binding affinity to the cell surface antigen, and the ability of Abs to form hexameric structures on antigen binding by interactions through the C_H2–C_H3 interface.[8] Structure and conformation of the C1q binding site, localized in the Abs' C_H2 domain, differ between Ab subclasses. Human IgG1 is less efficient than IgG3 in fixing C1q, whereas IgG2 and IgG4 show little or no complement activity.[8] Most AChR-specific Abs belong to the complement fixing IgG1 and IgG3 subclasses, while LRP4-specific Abs predominantly comprise IgG1 and IgG2.[3,9,10] IgG2-Abs are less frequently associated with autoimmune diseases as compared with IgG1, IgG3, or IgG4 subclasses. The occurrence of IgG2-polarized immune responses in LRP4-Ab–positive MG might be driven by the molecular composition of the antigen because the agrin-LRP4 complex is enriched for carbohydrates[11] and IgG2-Abs most commonly bind to carbohydrate epitopes.[12] A bias towards IgG2 could potentially account for the limited ability of LRP4-specific Abs to induce activation of the classical complement pathway.

Apart from antibody isotype and subclass, the carbohydrate moiety within the C_H2 domain of IgG molecules regulates both the pro- and anti-inflammatory effector functions. Reduction of IgG galactosylation and sialylation is one of the most prominent IgG glycan structural changes at the level of total serum and antigen-specific IgGs in a broad spectrum of chronic inflammatory and autoimmune diseases, such as rheumatoid arthritis, systemic lupus erythematosus, and MOG-antibody–associated disorder.[13] In line with these studies, we found disialylated IgG glycans to be diminished in patients with LRP4-Ab$^+$ MG. Whether lack of sialylation triggers proinflammatory Ab activity or merely reflects chronic inflammatory processes has not been conclusively clarified. Mechanistic data obtained in rodent models of autoimmune diseases indicate that reduced sialylation changes IgG-Fc structure and increases affinities for activating FcγRs, resulting in enhanced cellular FcR-mediated effector functions.[14]

The relatively small cohort size due to rarity of LRP4-Ab$^+$ MG and a potential bias driven by immunotherapies are limitations of our study. Although the latter could have had an effect of levels of circulating complement proteins, ADCD was performed using purified IgG molecules and, thus, should reflect the inherent ability of Abs to bind C1q for activation of the classical complement pathway. The functional assays performed rely on capture of AChR-Abs binding to the immunodominant domain of the receptor's α subunit, while auto-Abs can also recognize conformational domains from adjacent subunits. AChR-Abs were, however, capable of inducing all effector functions investigated, and binding additional conformational epitopes would probably not have impaired these functions. Finally, in the present assays, the relative dispositions of the target antigens tested may not optimally reproduce those at the motor endplate.

Several novel therapies targeting Ab-mediated effector mechanisms were recently approved for the treatment of MG or are currently awaiting approval. These therapies include complement inhibitors, neonatal Fc receptor (FcRn) modulators, and B-cell–depleting Abs.[15] As the spectrum of treatment options for MG continues to grow, personalized approaches for managing people with myasthenia appear within reach. Although the clinical efficacy of complement inhibition is evident for AChR-Ab$^+$ MG, its clinical benefit in in LRP4-Ab$^+$ or LRP4-Ab$^+$/AChR-Ab$^+$ remains to be demonstrated. If it is not, then biologics targeting features of Ab pathogenicity other than complement activation could be more effective to improve clinical outcomes for patients with LRP4-Ab$^+$ MG.

Acknowledgment

The authors thank Kerstin Stein (University Hospital of Münster, Department of Neurology with Institute of Translational Neurology) for expert technical assistance, Claudia Heibutzki and Dike Remstedt (Neuroscience Clinical Research Center, Berlin, Germany), Anna Schoppe and Claudia Schwering (University Hospital of Münster, Department of Neurology with Institute of Translational Neurology) for patient management of the MG outpatient departments, and M. Heinold, S. Märschenz, and S. Lischewski for administration support.

Study Funding

This study was supported by the Neuroscience Clinical Research funding (Grant/Award Number: Exc 257).

Disclosure

O. Chuquisana reports no disclosures relevant to the manuscript; F. Stascheit received speaking honoria and honoria for attendance at advisory boards from Alexion Pharmaceuticals, argenx, and UCB pharma; C.W. Keller received travel/accommodation/meeting expenses from Alexion and UCB; M. Pučić-Baković and A.M. Patenaude are employees of Genos Ltd; G. Lauc is the founder and CEO of Genos Ltd, a private research organization that specializes in high throughput glycomics analysis and has several patents in this field; S. Tzartos has shares in the research and diagnostics laboratory Tzartos NeuroDiagnostics, Athens; H. Wiendl received speaker honoraria from Alexion, Biogen, Bristol Myers Squibb, Genzyme, Merck, Neurodiem, Novartis, Ology, Roche, TEVA, und WebMD Global. He received honoraria for consulting services from Abbvie, Actelion, Argenx, BD, Bristol Myers Squibb, EMD Serono, Fondazione Cariplo, Gossamer Bio, Idorsia, Immunic, Immunovant, INmune Bio_Syneos Health, Janssen,

Merck, NexGen, Novartis, Roche, Sanofi, Swiss Multiple Sclerosis Society, UCB, and Worldwide Clinical Trials. His research is supported by the Gernan Myasthenia Gravis Society; A. Meisel received speaker or consultancy honoraria or financial research support (paid to his institution) from Alexion Pharmaceuticals, argenx, Axunio, Destin, Grifols, Hormosan Pharma, Janssen, Merck, Octapharma, UCB, and Xcenda. He serves as a medical advisory board chairman of the German Myasthenia Gravis Society; J.D. Lünemann has received speaker fees, research support, travel support, and/or served on advisory boards by Abbvie, Alexion, Argenx, Biogen, Merck, Moderna, Novartis, Roche, Sanofi, and Takeda and is a member of the medical advisory board of the German Myasthenia Gravis Society. Go to Neurology.org/NN for full disclosures.

Publication History

Received by *Neurology: Neuroimmunology & Neuroinflammation* September 22, 2023. Accepted in final form January 26, 2024. Submitted and externally peer reviewed. The handling editor was Marinos C. Dalakas, MD, FAAN.

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