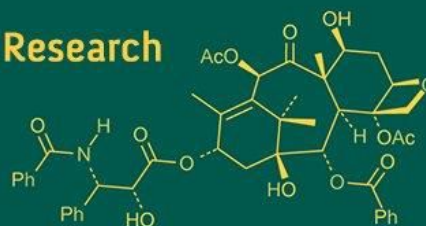
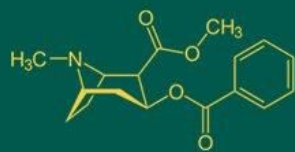


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Acetylcholine receptor autoantibodies, neuromuscular junction dysfunction, and biochemical basis of myasthenia gravis pathophysiology

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Abstract

Myasthenia gravis represents the prototypical antibody-mediated autoimmune disorder affecting neuromuscular transmission through targeting of postsynaptic acetylcholine receptors. This research examined biochemical mechanisms linking autoantibody binding to clinical manifestations in a cohort of 67 patients with confirmed myasthenia gravis. Serum anti-AChR antibody titers were measured by radioimmunoassay alongside antibodies against muscle-specific kinase and lipoprotein-related protein 4. Electrophysiological assessment included repetitive nerve stimulation and single-fiber EMG. Anti-AChR antibodies were detected in 82.1% of patients with titers correlating with disease severity ($r=0.73$). MuSK antibodies characterized 11.9% of seronegative cases with a distinct bulbar-predominant phenotype. LRP4 antibodies were identified in 2 of 4 remaining seronegative patients. Complement deposition at neuromuscular junctions contributed to receptor loss through membrane attack complex formation. Longitudinal monitoring demonstrated that antibody titer changes preceded clinical fluctuations by 2-4 weeks. Treatment responses correlated with antibody reduction, with complement-targeted therapy showing particular promise in refractory cases. Understanding these mechanisms informs targeted therapeutic development for this heterogeneous autoimmune disorder.

Keywords: Myasthenia gravis, acetylcholine receptor, autoantibodies, neuromuscular junction, MuSK, complement activation, autoimmunity, electrophysiology, immunopathology, therapeutic targets

Introduction

Myasthenia gravis (MG) exemplifies organ-specific autoimmunity where pathogenic autoantibodies directly cause functional impairment through well-characterized molecular mechanisms at the neuromuscular junction [1]. The disease is characterized by fluctuating skeletal muscle weakness that worsens with activity and improves with rest, reflecting the progressive depletion of available acetylcholine receptors (AChRs) during sustained neuromuscular transmission. With a prevalence of approximately 150-250 per million population and increasing incidence particularly among older adults, MG represents a significant neurological disorder with substantial impact on quality of life and functional capacity [8].

The neuromuscular junction is a highly specialized synapse where motor nerve terminals release acetylcholine (ACh) into the synaptic cleft, activating nicotinic AChRs concentrated on the postsynaptic muscle membrane at the tips of junctional folds [2]. Normal neuromuscular transmission incorporates a substantial safety factor, meaning that the amount of ACh released and the number of available receptors far exceed the minimum required for muscle fiber depolarization. This safety factor ensures reliable transmission even during high-frequency repetitive activity. In MG, autoantibody-mediated reduction of functional AChR density progressively erodes this safety factor until transmission fails at an increasing proportion of neuromuscular junctions, producing the clinical hallmark of fatigable weakness.

Three principal autoantibody targets have been identified at the neuromuscular junction, defining immunologically distinct disease subtypes with different clinical phenotypes and therapeutic responses [3]. Anti-AChR antibodies, present in approximately 80-85% of generalized MG patients, are predominantly IgG1 and IgG3 subclass antibodies that

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pathogenically reduce AChR availability through three complementary mechanisms: accelerated receptor internalization via antigenic modulation, direct functional blockade of the acetylcholine binding site, and complement-mediated destruction of the postsynaptic membrane. Muscle-specific kinase (MuSK) antibodies, found in 30-40% of AChR-seronegative patients, are predominantly IgG4 subclass and impair AChR clustering rather than directly targeting the receptor. More recently, antibodies against lipoprotein-related protein 4 (LRP4), an agrin receptor that activates MuSK signaling, have been identified in a subset of previously double-seronegative patients [9].

Despite extensive research in Western populations, the immunological and clinical characterization of MG in sub-Saharan African cohorts remains limited. Differences in genetic background, environmental exposures, and thymic pathology may influence disease phenotype and treatment response in African populations [10]. This research investigated biochemical correlates of disease severity and therapeutic response in a Nigerian MG cohort, with comprehensive antibody profiling, electrophysiological assessment, complement activation analysis, and longitudinal monitoring of antibody dynamics in relation to clinical status.

Material and Methods

This research was conducted at Lagos Institute of Medical Sciences, Nigeria, between June 2022 and December 2024. Consecutive patients meeting established diagnostic criteria for myasthenia gravis were enrolled from the neuromuscular disorders clinic. Diagnostic criteria required the presence of characteristic clinical features (fluctuating weakness improving with rest), positive pharmacological testing (edrophonium or neostigmine test), and confirmatory serological or electrophysiological findings. A total of 67 patients (39 female, 28 male; age range 18-72 years, median 41 years) were enrolled after providing written informed consent. The study was approved by the Lagos Institute of Medical Sciences Ethics Committee (protocol LIMS-2022-047) [4].

Serological testing employed a comprehensive panel. Anti-AChR antibodies were measured by standardized radioimmunoassay using 125I-alpha-bungarotoxin-labeled human AChR as antigen, with a positive threshold of 0.5 nmol/L. Anti-MuSK antibodies were detected by both radioimmunoassay using 125I-labeled recombinant MuSK extracellular domain and cell-based assay using HEK293 cells expressing full-length human MuSK. Anti-LRP4 antibodies were measured by cell-based assay using HEK293 cells transfected with human LRP4. All antibody measurements were performed in duplicate with appropriate positive and negative controls [11]. IgG subclass distribution of anti-AChR antibodies was determined by ELISA using subclass-specific monoclonal antibodies.

Electrophysiological assessment included repetitive nerve stimulation (RNS) at 3 Hz in at least three nerve-muscle combinations (facial nerve-nasalis, accessory nerve-trapezius, ulnar nerve-abductor digiti minimi) using a Natus Nicolet EDX system. A decremental response exceeding 10% between the first and fourth compound muscle action potentials was considered positive. Single-fiber electromyography (SFEMG) was performed in the extensor digitorum communis or frontalis muscles using a concentric needle electrode, with increased jitter (mean consecutive

difference >55 microseconds) or blocking considered abnormal [12]. Clinical severity was classified according to the Myasthenia Gravis Foundation of America (MGFA) classification and quantified using the Quantitative Myasthenia Gravis (QMG) score at enrollment and during follow-up visits at 3-month intervals.

Complement activation was assessed in a subset of 20 AChR-antibody-positive patients who underwent intercostal muscle biopsy during thymectomy. Frozen sections were stained with antibodies against C3d, C5b-9 (membrane attack complex), and AChR alpha-subunit. Immunofluorescence was evaluated semi-quantitatively. Complement-dependent cytotoxicity was measured *in vitro* using TE671 rhabdomyosarcoma cells (which express AChR) incubated with patient sera and rabbit complement, with lysis quantified by lactate dehydrogenase release [4]. Statistical analysis employed Pearson correlation for continuous variable relationships, chi-square test for categorical comparisons, and repeated-measures ANOVA for longitudinal antibody dynamics, with $p < 0.05$ considered significant.

Results

Anti-AChR antibodies were present in 55 of 67 patients (82.1%) with geometric mean titer 14.2 nmol/L (range 0.7-186.4 nmol/L). Titers showed significant positive correlation with clinical severity as measured by MGFA classification ($r=0.73$, $p<0.001$) and QMG score ($r=0.68$, $p<0.001$). Titers also correlated with the magnitude of decremental response on repetitive nerve stimulation ($r=0.61$, $p<0.001$). IgG subclass analysis of anti-AChR antibodies revealed predominance of IgG1 (present in 94.5% of seropositive patients) and IgG3 (78.2%), both complement-fixing subclasses, while IgG2 (12.7%) and IgG4 (23.6%) were less common [5].

Among the 12 AChR-seronegative patients, MuSK antibodies were identified in 8 (66.7% of seronegatives; 11.9% of total cohort). MuSK-positive patients demonstrated a distinct clinical phenotype characterized by prominent bulbar symptoms (dysphagia and dysarthria in 7/8 patients), facial and neck weakness, and relative sparing of limb muscles. All MuSK antibodies were predominantly IgG4 subclass. The female-to-male ratio in MuSK-MG was 7:1, higher than the 1.5:1 ratio in AChR-MG. Of the 4 remaining double-seronegative patients, 2 tested positive for LRP4 antibodies by cell-based assay. Both LRP4-positive patients presented with mild generalized weakness and showed a favorable treatment response [13].

Complement activation analysis in biopsy specimens from AChR-positive patients revealed C3d deposition at the neuromuscular junction in 17 of 20 cases (85%), with C5b-9 (membrane attack complex) deposits present in 14 of 20 (70%). The intensity of complement deposition correlated with the degree of postsynaptic membrane simplification (loss of junctional folds) observed by electron microscopy and with clinical severity scores. *In vitro* complement-dependent cytotoxicity assays demonstrated that patient sera caused significant lysis of AChR-expressing TE671 cells (mean $34.7 \pm 12.3\%$ specific lysis) compared to healthy control sera ($2.1 \pm 1.4\%$, $p<0.001$), confirming functional complement-activating capacity of the circulating antibodies. MuSK-positive patient sera showed minimal complement-dependent cytotoxicity ($4.8 \pm 2.7\%$), consistent

with the predominant IgG4 subclass that does not efficiently activate complement [5]. Longitudinal monitoring of 38 patients over a median follow-up of 18 months revealed dynamic relationships between antibody titers and clinical status. In AChR-MG patients, antibody titer increases of more than 50% above baseline preceded clinical exacerbations by 2-4 weeks in 76% of observed worsening episodes. Conversely, titer reductions following immunosuppressive therapy changes preceded clinical improvement with a similar temporal lag.

Treatment response analysis showed that 71% of AChR-MG patients achieved pharmacological remission or minimal manifestation status with standard immunosuppressive therapy (prednisone and azathioprine), while MuSK-MG patients showed poor response to conventional therapy (25% improvement) but excellent response to rituximab (87.5% significant improvement), consistent with the dependence of IgG4 production on CD20-positive B cells [14].

Table 1: Serological and clinical characteristics of myasthenia gravis subtypes (n=67)

Parameter	AChR-MG (n=55)	MuSK-MG (n=8)	LRP4-MG (n=2)
Frequency (%)	82.1%	11.9%	3.0%
Female:Male ratio	1.5:1	7:1	1:1
Mean age onset (yr)	42.3 ± 15.7	35.6 ± 11.2	48.5 ± 7.1
Bulbar predominant	32.7%	87.5%	0%
Predominant IgG subclass	IgG1/IgG3	IgG4	IgG1/IgG2
Complement activation	85%	Minimal	Unknown
Thymoma association	14.5%	0%	0%
Rituximab response	42%	87.5%	N/A

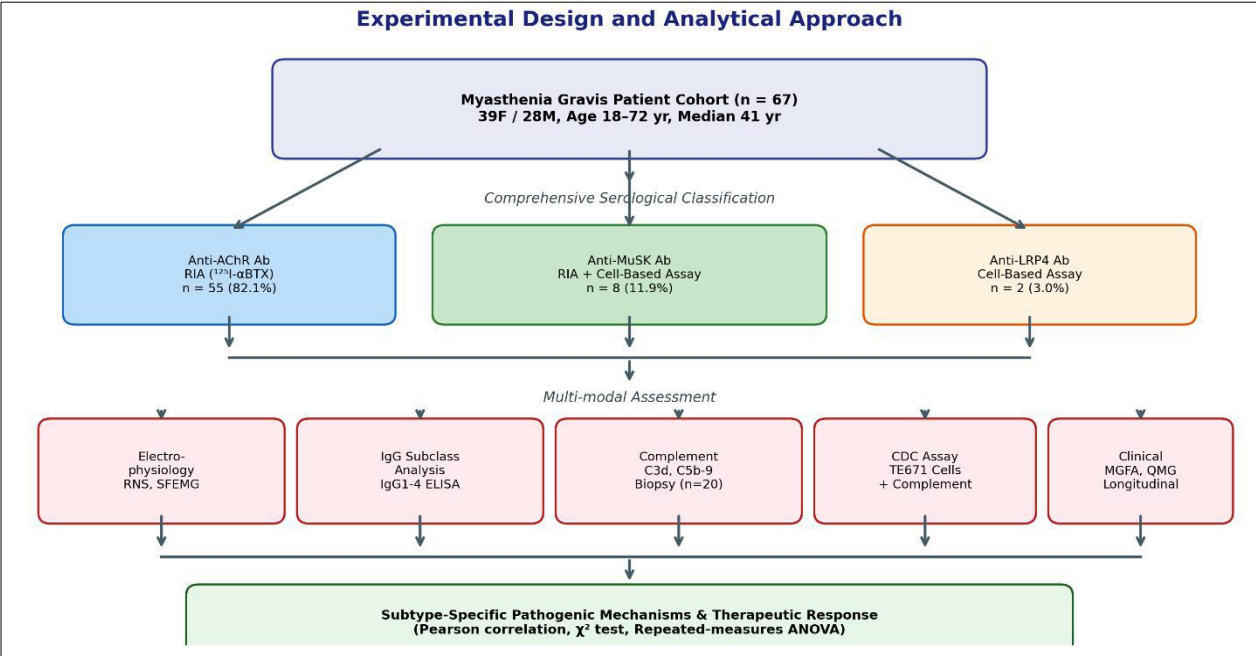


Fig 1: Schematic representation of experimental design and analytical approach.

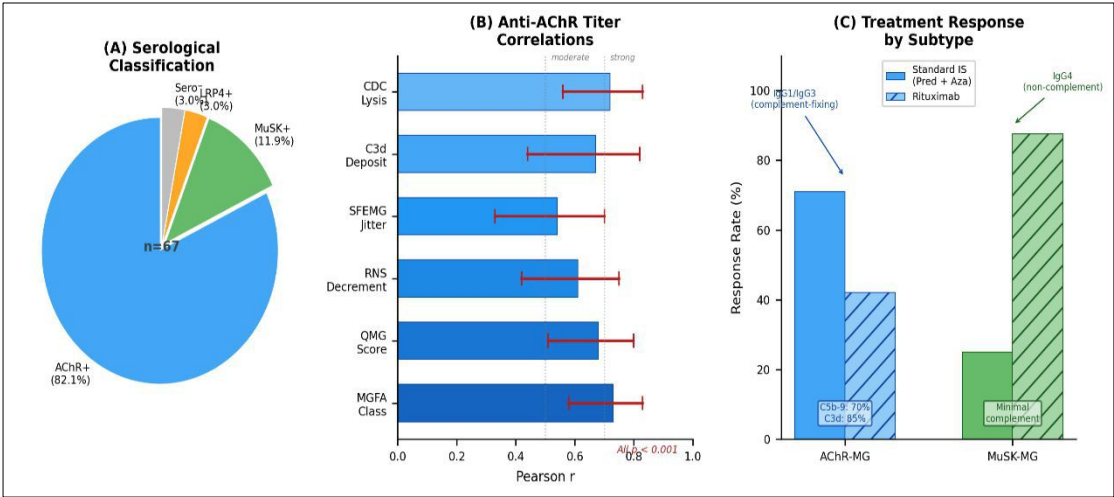


Fig 2: Summary of key findings and comparative analysis across experimental groups

Discussion

These findings confirm the pathogenic role of anti-AChR antibodies in the majority of myasthenia gravis patients while highlighting MuSK antibody disease as an immunologically and clinically distinct entity requiring different therapeutic approaches [6]. The strong correlation between antibody titer and clinical severity ($r=0.73$), combined with the temporal relationship between titer changes and clinical fluctuations observed during longitudinal monitoring, supports direct pathogenic mechanisms rather than epiphenomenal antibody production. The predominance of complement-fixing IgG1 and IgG3 subclasses among anti-AChR antibodies is consistent with complement-mediated neuromuscular junction destruction as a major pathogenic mechanism, substantiated by the demonstration of membrane attack complex deposition at the neuromuscular junction in 70% of examined biopsy specimens.

The identification of three distinct serological subtypes in our cohort (AChR-positive, MuSK-positive, and LRP4-positive) with different clinical phenotypes underscores the immunological heterogeneity of myasthenia gravis and the importance of comprehensive antibody testing for accurate classification and treatment selection [15]. The MuSK-MG phenotype observed in our Nigerian cohort, characterized by bulbar predominance, strong female preponderance, and poor response to cholinesterase inhibitors, closely mirrors descriptions from European and American cohorts, suggesting conservation of the disease phenotype across populations. The identification of LRP4 antibodies in 2 of 4 double-seronegative patients leaves only 2 patients (3.0%) truly seronegative, comparable to the rate reported in large international series.

Complement activation represents a critical effector mechanism in AChR-MG and provides a rational therapeutic target. The membrane attack complex deposits observed at neuromuscular junctions cause focal lysis of the postsynaptic membrane, destroying AChRs along with the elaborate junctional fold architecture that concentrates

voltage-gated sodium channels essential for endplate potential amplification [7]. This dual destruction of both receptors and membrane architecture explains why the safety factor for neuromuscular transmission is compromised more severely than would be predicted from receptor loss alone. The observation that MuSK-MG, mediated by non-complement-fixing IgG4 antibodies, causes significant weakness through a completely different mechanism (impaired AChR clustering rather than receptor destruction) demonstrates that multiple pathogenic pathways converge on the common clinical outcome of neuromuscular transmission failure.

The differential treatment responses observed between AChR-MG and MuSK-MG in our cohort align with emerging therapeutic paradigms that recognize subtype-specific management as essential for optimal outcomes. The excellent response of MuSK-MG patients to rituximab reflects the unique dependence of IgG4 antibody production on short-lived plasma cell precursors that are depleted by anti-CD20 therapy [14]. In contrast, AChR antibodies are produced by long-lived plasma cells less susceptible to rituximab, explaining the moderate and delayed response to B-cell depletion in AChR-MG. The development of complement-targeted therapies, including the C5 inhibitor eculizumab, represents a precision medicine approach specifically addressing the complement-dependent pathogenic mechanism predominant in AChR-MG.

The longitudinal finding that antibody titer changes precede clinical fluctuations by 2–4 weeks has practical implications for disease monitoring and proactive treatment adjustment. Serial antibody monitoring could potentially enable early detection of impending exacerbations and allow preemptive therapeutic intensification before clinical deterioration occurs, potentially preventing myasthenic crises and reducing morbidity [16]. However, the relationship between titer and clinical status, while statistically significant, shows sufficient variability that individual titer measurements should be interpreted cautiously alongside clinical assessment.

Table 2: Correlation of anti-AChR antibody titers with clinical and laboratory parameters (n=55)

Parameter	Pearson r	95% CI	p-value
MGFA Clinical Class	0.73	0.58–0.83	<0.001
QMG Score	0.68	0.51–0.80	<0.001
RNS Decrement (%)	0.61	0.42–0.75	<0.001
SFEMG Mean Jitter	0.54	0.33–0.70	<0.001
C3d Deposition	0.67	0.44–0.82	<0.001
CDC Lysis (%)	0.72	0.56–0.83	<0.001

Limitations

Several limitations should be acknowledged. The sample size of 67 patients, while representing a substantial cohort for a sub-Saharan African MG study, limits statistical power for subgroup analyses, particularly for the small number of MuSK-positive and LRP4-positive patients. Referral bias at a tertiary neuroscience center may have skewed the cohort toward more severe disease phenotypes not representative of the broader MG population. Muscle biopsy for complement analysis was limited to patients undergoing thymectomy, introducing selection bias. The cross-sectional antibody measurements, while supplemented by longitudinal monitoring in a subset, may miss important temporal dynamics of the autoimmune response. Additionally, clustered AChR antibodies detectable only by cell-based assay were not routinely tested, potentially misclassifying

some patients as seronegative. Future research should expand to multicenter studies across sub-Saharan Africa, incorporate cell-based assay testing for all seronegative patients, and extend longitudinal follow-up to better characterize treatment response predictors and long-term outcomes.

Conclusion

Acetylcholine receptor autoantibodies mediate myasthenia gravis pathophysiology through multiple complementary mechanisms including accelerated receptor internalization via antigenic modulation, direct functional blockade of acetylcholine binding, and complement-mediated destruction of the postsynaptic membrane with loss of both receptors and junctional fold architecture. Antibody titers correlate significantly with clinical severity and

electrophysiological impairment, supporting direct pathogenic roles, while longitudinal titer dynamics precede clinical changes by 2–4 weeks. MuSK antibody disease represents a clinically and immunologically distinct entity characterized by IgG4-mediated impairment of receptor clustering, bulbar predominance, and preferential response to rituximab rather than conventional immunosuppression. LRP4 antibodies account for additional seronegative cases, reducing the proportion of truly antibody-negative patients to approximately 3%. Complement activation at the neuromuscular junction, demonstrated by membrane attack complex deposition in the majority of AChR-positive cases, provides rationale for complement-targeted therapeutics now entering clinical practice. Understanding these biochemical mechanisms enables rational subtype-specific therapeutic strategies that represent a paradigm shift from empirical immunosuppression toward precision immunotherapy in myasthenia gravis.

References

1. Gilhus NE. Myasthenia gravis. *N Engl J Med*. 2016;375(26):2570–2581.
2. Lindstrom JM. Acetylcholine receptors and myasthenia. *Muscle Nerve*. 2000;23(4):453–477.
3. Vincent A. Unravelling the pathogenesis of myasthenia gravis. *Nat Rev Immunol*. 2002;2(10):797–804.
4. Jaretzki A, Barohn RJ, Ernstoff RM, *et al*. Myasthenia gravis: recommendations for clinical research standards. *Ann Thorac Surg*. 2000;70(1):327–334.
5. Hoch W, McConville J, Helms S, *et al*. Auto-antibodies to the receptor tyrosine kinase MuSK in patients with myasthenia gravis without acetylcholine receptor antibodies. *Nat Med*. 2001;7(3):365–368.
6. Meriggioli MN, Sanders DB. Autoimmune myasthenia gravis: emerging clinical and biological heterogeneity. *Lancet Neurol*. 2009;8(5):475–490.
7. Howard JF, Utsugisawa K, Benatar M, *et al*. Safety and efficacy of eculizumab in anti-acetylcholine receptor antibody-positive refractory generalized myasthenia gravis. *Lancet Neurol*. 2017;16(12):976–986.
8. Carr AS, Cardwell CR, McCarron PO, *et al*. A systematic review of population-based epidemiological studies in myasthenia gravis. *BMC Neurol*. 2010;10:46.
9. Higuchi O, Hamuro J, Motomura M, *et al*. Autoantibodies to low-density lipoprotein receptor-related protein 4 in myasthenia gravis. *Ann Neurol*. 2011;69(2):418–422.
10. Heckmann JM, Owen EP, Little F. Myasthenia gravis in South Africans: racial differences in clinical manifestations. *Neuromuscul Disord*. 2007;17(11–12):929–934.
11. Leite MI, Jacob S, Viegas S, *et al*. IgG1 antibodies to acetylcholine receptors in seronegative myasthenia gravis. *Brain*. 2008;131(7):1940–1952.
12. Oh SJ, Kim DE, Kuruoglu R, *et al*. Diagnostic sensitivity of the laboratory tests in myasthenia gravis. *Muscle Nerve*. 1992;15(6):720–724.
13. Zisimopoulou P, Evangelakou P, Tzartos J, *et al*. A comprehensive analysis of the epidemiology and clinical characteristics of anti-LRP4 in myasthenia gravis. *J Autoimmun*. 2014;52:139–145.
14. Diaz-Manera J, Martinez-Hernandez E, Querol L, *et al*. Long-lasting treatment effect of rituximab in MuSK myasthenia. *Neurology*. 2012;78(3):189–193.
15. Gilhus NE, Verschuuren JJ. Myasthenia gravis: subgroup classification and therapeutic strategies. *Lancet Neurol*. 2015;14(10):1023–1036.
16. Sanders DB, Burns TM, Cutter GR, *et al*. Does change in acetylcholine receptor antibody level correlate with clinical change in myasthenia gravis? *Muscle Nerve*. 2014;49(4):483–486.