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# Myasthenia Gravis Autoantibodies Have a Target Also Outside the Neuromuscular Junction

Welcome to the first article in a series on leading Norwegian discoveries in neurology and neuroscience. All the selected discoveries have links to ongoing research projects in leading groups. They span clinical to more basic topics. The discoveries are all relevant for clinicians evaluating and treating patients with brain and nervous system disease. Neuroscience with a clinical focus has been a priority for Norwegian research. Further expansion is planned in cooperation between the universities, the university hospitals, the Research Council of Norway, and the Norwegian Brain Council. Although the discoveries in this series are presented as Norwegian, they all appear in an international context. They represent small pieces fitting into the bigger puzzle, but contribute to elucidating mechanisms for brain and neuromuscular function, thus laying foundations for improved treatment of human disease.

Myasthenia gravis (MG) was firmly established as an autoimmune disease in 1976.<sup>1</sup> The key factor leading to this conclusion was the detection of autoantibodies against acetylcholine receptors of the postsynaptic neuromuscular membrane. These antibodies had a direct symptom-inducing effect.<sup>2</sup> In 2001, it was shown that MG in a subgroup of patients was associated with autoantibodies to MuSK, a muscle-specific kinase in the post-synaptic membrane and functionally linked to the acetylcholine receptor.<sup>3</sup> However, it had been known since 1960 that sera from MG patients contained autoantibodies against a mixture of skeletal muscle antigens.<sup>4</sup>

In 1992, we first published that the ryanodine receptor (RyR) in muscle is a target for MG autoantibodies.<sup>5</sup> RyR represents the calcium release channels of sarcoplasmic reticulum in both skeletal and heart muscle (Figure 1). The identification of this antigen raised new questions about MG disease mechanisms, pathogenic antibody effects, and antibodies as markers for disease severity and treatment response.

Around the same time, our research group in co-operation with groups in USA and Germany found titin to be another antigen for autoantibodies in MG patient sera.<sup>6,7</sup> Titin is a large intracellular protein that stretches through the entire sarcomere (Figure 2). It has elastic properties, and is important for muscle development and regeneration. The identification of RyR and titin antibodies in MG opened up a new field of research.

We had shown already in 1984 that MG-related muscle antibodies cross-react with

muscle-like epitopes in thymomas.<sup>8</sup> By using specific, experimental muscle antibodies, transmembrane RyR epitopes were detected on human epithelial thymoma cells.<sup>9</sup> Similarly titin epitopes were identified by another group on the same thymoma cells.<sup>10</sup> Titin mRNA transcripts covering the main immunogenic region of the molecule were also shown to be present in MG thymomas.<sup>11</sup> Thus, the RyR and titin immune responses represented important elements in proving how thymomas can induce autoantibody production and MG.

RyR and titin autoantibodies mainly occur in thymoma-associated MG and late onset MG (MG onset > 50 years and thymus atrophy). Such antibodies hardly ever occur in early onset MG (MG onset < 50 years and thymus hyperplasia), ocular MG or MuSK-associated MG. RyR antibodies have a high specificity for the presence of a thymoma (>90%), but a much lower sensitivity (70%), whereas titin antibodies have a higher sensitivity (>90%), but a lower specificity (70%) for a thymoma.<sup>12</sup> This illustrates how analysis for titin and RyR antibodies in MG patients is of practical clinical value in diagnosing MG subtype, especially in determining if the presence of a thymoma is likely or unlikely. Microthymomas are difficult to identify correctly on MR and CT, whereas thymic hyperplasia on imaging can be mistaken for a thymoma. MG patients without a thymoma are grouped according to age of onset, local or generalised symptoms, thymus pathology, HLA genotype, but also according to autoantibody status. A distinction between the groups early onset MG and late onset MG is difficult with age-overlap between

groups, and there is a need for additional markers. The subgroups differ regarding prognosis and therapeutic response to specific interventions. Titin and RyR antibodies occurring in some but not all MG patients illustrate that MG pathogenesis is heterogeneous and that subgrouping is relevant. The presence of such autoantibodies strongly favours a thymoma or late onset MG. Presence of the autoantibodies is also associated with relevant genotypes.<sup>13</sup>

MG patients with titin and RyR antibodies respond less well to thymectomy than patients without such antibodies.<sup>12</sup> Especially in patients with no thymoma and MG symptom onset between 40 and 70 years of age, thymectomy or not represents a therapeutic dilemma. Thymus hyperplasia, severe generalised symptoms and lower age favour thymectomy. Absence of titin and RyR antibodies does the same, indicating that the patient pathogenetically belongs to the early onset MG subgroup even with a debut age > 40-50 years.

Whereas antibodies against acetylcholine receptor and MuSK bind to their muscle antigen *in vivo* and lead to a muscle weakness typical for MG, *in vivo* binding has not been shown for RyR and titin antibodies. These latter antigens are localised intracellularly. An access for circulating autoantibodies to intracellular antigens is still debated. These human MG antibodies have the potential to bind to the RyR channels, and they do so *in vitro*. The RyR becomes locked in a position closed for calcium transport after binding of antibodies from MG patients.<sup>14</sup> MG patients with antibodies with a strong functional effect on RyR channels *in vitro* have more severe clinical symptoms. The immunosuppressive drug tacrolimus may have a symptomatic effect in MG patients with RyR antibodies through its effect on RyR-mediated calcium release.<sup>15</sup>

Titin and RyR antibodies are markers for a more severe MG disease, and more often with

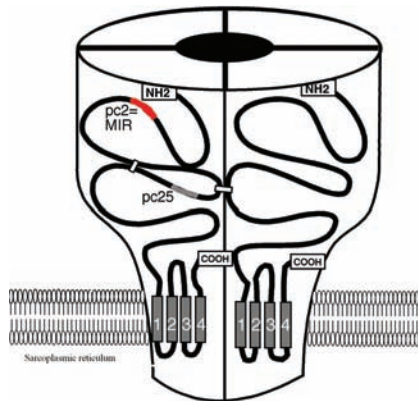


Figure 1: The ryanodine receptor (RyR) represents the calcium channel in the sarcoplasmic reticulum membrane. Most autoantibodies against RyR in MG patients are directed against the main immunogenic region (MIR), marked with red on the figure.

bulbar, neck and respiratory muscle involvement.<sup>16</sup> MG patients have been reported with focal myositis, and also with cardiomyositis. The MG autoantibodies against titin and RyR react with the same antigens of skeletal and cardiac muscle. As the presence of titin and RyR autoantibodies is linked to a more severe disease, MG patients with such antibodies should probably receive more intense and longer lasting immunosuppressive drug therapy, even though treatment response has not been properly examined in MG subgroups defined according to antibody status.

MG is a heterogeneous disease characterised by autoantibodies to skeletal muscle. Autoantibodies against the muscle proteins RyR and titin can be used as specific and sensitive markers for thymoma MG and late onset MG. Furthermore such antibodies indicate a more severe MG, so that their presence usually makes long-time immunosuppressive therapy necessary. All aspects of MG are not easily explained by antibodies against acetylcholine receptors or MuSK alone. ♦

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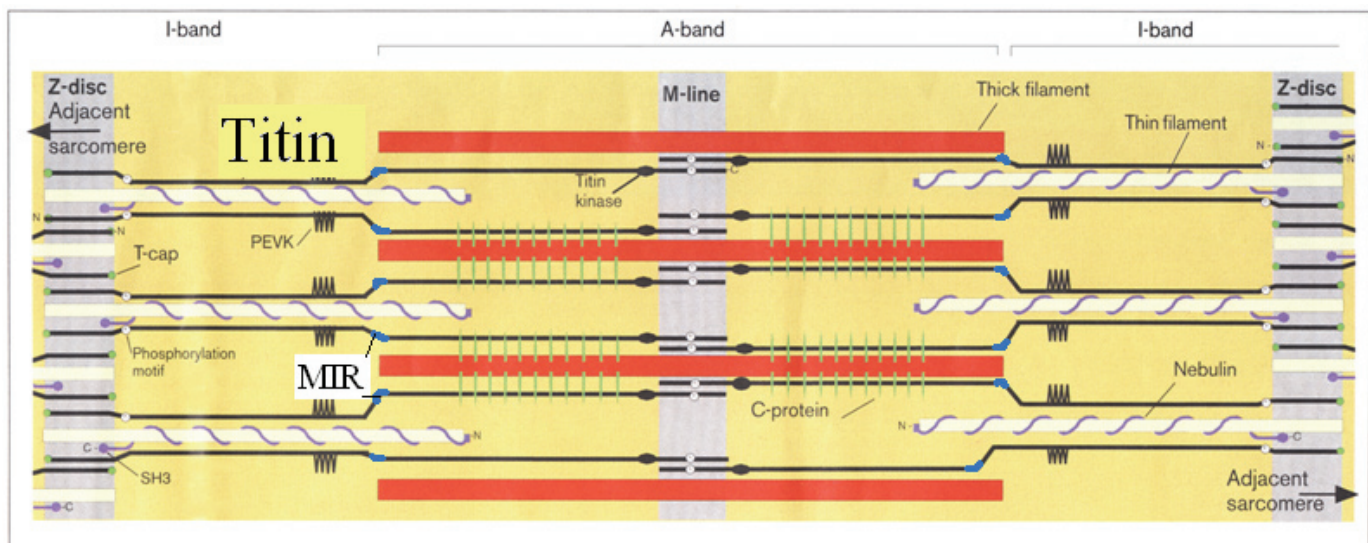


Figure 2: Titin stretches through the entire sarcomere. The protein is marked as a black line on this figure. Most autoantibodies against titin in MG patients are directed against the main immunogenic region (MIR).