SHORT REPORT

Ryanodine receptor antibodies related to severity of thymoma associated myasthenia gravis

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Abstract

Ryanodine receptor (RyR) antibodies are detected in about 50% of patients with myasthenia gravis who have a thymoma. The RyR is a calcium release channel involved in the mechanism of excitation-contraction coupling in striated muscle. In this study the severity of myasthenia gravis assessed by a five point disability score was compared between 12 patients with myasthenia gravis, a thymoma, and RyR antibodies and 10 patients with myasthenia gravis and a thymoma but without such antibodies. Symptoms of myasthenia gravis were significantly more severe in patients with RyR antibodies. The mean (SD) disability scores were 3.7(0.5) in patients with antibodies and 2.7(0.9) in those without at peak of illness, ($p = 0.01$) and 3.4(1.4) v 1.6(0.7) at the end of an average observation period of five years ($p = 0.002$). The number of deaths due to myasthenia gravis was five of 12 RyR antibody positive patients, and none of 10 RyR antibody negative patients ($p = 0.04$). RyR antibody levels correlated positively with severity of myasthenia gravis. The presence of circulating RyR antibodies seems to be associated with a severe form of thymoma associated myasthenia gravis.

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Myasthenia gravis is an autoimmune disease with abnormal muscular fatigability mainly caused by autoantibodies to nicotinic acetylcholine receptors (AChRs) in the motor endplates of striated muscle.¹

About 15% of patients with myasthenia gravis have a thymoma.² These patients usually have autoantibodies to various proteins of striated muscle—namely, titin, myosin, actin, and α -actinin.³⁻⁶ Recently we reported that some patients with myasthenia gravis and thymoma have autoantibodies to the ryanodine receptor (RyR), a calcium release channel in the sarcoplasmic reticulum of striated muscle.⁷ The RyR is a transmembrane ion channel protein located in those parts of the sarcoplasmic reticular membrane that are in close contact with the T tubular sarcolemma infoldings. The RyR plays a key role in the

mechanism of excitation-contraction coupling in striated muscle.⁸⁻¹⁰

RyR antibodies are detected in about 50% of patients with myasthenia gravis who have a thymoma.^{7,11} They are not detected in patients with myasthenia gravis without thymoma or in patients with other autoimmune or muscular disorders. The purpose of this study was to investigate if there is any association between RyR antibodies and clinical features of thymoma associated myasthenia gravis.

The study included 22 patients with myasthenia gravis and thymoma. All patients with myasthenia gravis and thymoma for whom we were able to collect adequate clinical information and who had onset of myasthenia gravis symptoms after 1980 were included. Fourteen were patients analysed in our previous studies.^{7,11} Twelve patients were from this department, 10 from other hospitals. The diagnosis of myasthenia gravis was based on conventional clinical criteria, neurophysiological investigation, and the presence of AChR antibodies. The diagnosis of thymoma was based on histological examination of tissue obtained at thymectomy in 21 patients and at necropsy in one.

The medical records were reviewed and the patients classified by severity of myasthenia gravis symptoms at their peak of illness (worst symptoms excluding death) and at the time of the latest follow up. They were classified into six categories with disability scores from 0-5 (modified from Oosterhuis¹²): 0, complete remission without medication; 1, minor symptoms (pure ocular or minor generalised symptoms); 2, mild generalised symptoms (mildly disabled with obvious weakness at appropriate testing, but without significant bulbar symptoms); 3, moderate generalised symptoms (restricted in daily activities and with significant bulbar dysfunction); 4, severe generalised symptoms (requiring admission to hospital because of ventilatory failure or dysphagia); 5, death from myasthenia gravis (death occurred after deterioration of myasthenia gravis with respiratory distress and final respiratory failure or cardiac arrest). All patients had been followed up by repeated clinical examinations, several times yearly in bad periods and less often in good periods.

Patient serum samples obtained before

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thymectomy and stored at -20°C were examined for IgG antibodies to RyR, AChR, and titin. RyR antibodies were detected by western blot as previously described.⁷ Patient serum samples that immunostained the purified RyR at a serum dilution of 1:50 were considered as RyR antibody positive. RyR antibodies were quantified by an enzyme linked immunosorbent assay (ELISA) as previously described.¹¹ Results are given as the OD value at a serum dilution (1:400) that gave the best discrimination between patient and blood donor serum samples. AChR antibodies were determined by immunoprecipitation of ^{125}I - α -bungarotoxin labelled human AChR.¹³ Results are given as arbitrary units (U/l). Titin antibodies were determined by ELISA as previously described.¹⁴

Results are reported as mean (1SD). Values in patients with and without RyR antibodies were compared by the Mann-Whitney *U* test. Proportions were compared by Fisher's test of exact probability. Correlations were calculated by Kendall's rank correlation coefficient (τ). Two tailed tests were used to estimate *p* values. *p* Values < 0.05 were considered significant.

Results

ANTIBODIES AND CLINICAL FEATURES

RyR antibodies were detected in 12 of 22 (55%) patients with myasthenia gravis and thymoma. Titin antibodies were detected in 11 of 12 RyR antibody positive and in eight of 10 RyR antibody negative patients. RyR antibody positive and negative patients were similar regarding concentration of AChR antibodies, age at onset of myasthenia gravis, sex distribution, observation period, and immunosuppressive treatment (table). Two of the 12 antibody positive patients were treated with prednisone alone and five of 12 with a combination of prednisone and azathioprine. Three of the 10 antibody negative patients were treated with prednisone alone and two of 10 with a combination of prednisone and azathioprine. Thymectomy was performed in 21 of 22 patients. The thymoma was radically operated in 19 of 21 patients. One RyR antibody positive and one RyR antibody negative patient received radiation therapy due to locally infiltrating tumour growth. Time from onset of myasthenia gravis to thymectomy was similar among RyR

RyR antibody levels in different patients related to their myasthenia gravis disability score at the latest follow up. For myasthenia gravis disability score 1 = minor; 2 = mild; 3 = moderate; 4 = severe; 5 = death from myasthenia gravis.

antibody positive (1.0(1.1) years) and negative patients (0.9(0.5) years). Year at onset of myasthenia gravis was similar among RyR antibody positive (1986(4) years) and negative patients (1985(3) years). Time from onset to peak was also similar: 1.1(1.2) years in antibody positive and 0.7(0.3) years in antibody negative patients.

SEVERITY OF MYASTHENIA GRAVIS

The patients were followed up for an average of five years. Myasthenic weakness at peak of illness was significantly more severe among patients with RyR antibodies than among those without: disability score 3.7(0.5) *v* 2.7(0.9) ($p = 0.01$; table). Disability score at the last follow up differed even more: 3.4(1.4) in RyR antibody positive patients *v* 1.6(0.7) in antibody negative patients ($p = 0.002$).

The serum level of RyR antibodies in the different patients correlated with their disability score, both at peak of illness ($\tau = 0.41$, $p = 0.008$) and at the latest follow up ($\tau = 0.50$, $p < 0.009$). The figure shows the RyR antibody levels. AChR antibody levels also correlated with disability score at the latest follow up ($\tau = 0.33$, $p = 0.03$) but not significantly at peak ($\tau = 0.25$, $p = 0.2$). The level of RyR antibodies did not correlate significantly with the level of AChR antibodies ($\tau = 0.12$, $p = 0.4$). The level of titin antibodies or age of onset of myasthenia gravis did not correlate significantly with disability score.

DEATH FROM MYASTHENIA GRAVIS

Five of 12 RyR antibody positive patients died of myasthenia gravis. The average age at death was 49(20) (range 21–70) years, and the average duration of myasthenia gravis at death was 4.2(2) (range 1–7) years. Three patients died of respiratory failure during one of many exacerbations of severe generalised myasthenia gravis. One patient died of respiratory failure after five years with chronic

Clinical features of patients with myasthenia gravis, thymoma, and RyR antibodies (RyRab +) and patients with myasthenia gravis and thymoma but without RyR antibodies (RyRab -)

Variables	RyRab + (n = 12)	RyRab - (n = 10)	<i>p</i> Value
Disability score:			
At peak of illness	3.7(0.5)	2.7(0.9)	0.01
At last observation	3.4(1.4)	1.6(0.7)	0.002
Deaths from myasthenia gravis	5/12(42%)	0/10(0%)	0.04
Titin antibodies (OD-value)	1.475(0.589)	1.025(0.618)	0.02
AChR antibodies (U/l)	5.6(2.2)	5.3(2.8)	NS
Age at onset (y)	50(14)(20–67)	54(13)(31–68)	NS
Males/females	6/6	5/5	NS
Observation period (y)	4.3(3)(1–10)	6.1(4)(2–12)	NS
Patients on immunosuppressive drugs	7	5	NS

Results are means (SD) with range for age and observation period. NS = non-significant.

severe generalised weakness due to very aggressive polymyositis and myasthenia gravis. One patient with invasive thymoma died of heart failure due to myocarditis. Examination at necropsy showed mononuclear cell infiltration and myocyte necrosis in the myocardium, normal coronary arteries, and no evidence of myocardial infarction. One RyR antibody negative patient died of cancer.

ASSOCIATED PATHOLOGY

All thymomas were lymphoepitheliomas; 18 were classified as polygonal cell (10 from RyR antibody positive and eight from antibody negative patients), two as spindle cell (one from an RyR antibody positive and one from an antibody negative patient), and one as mixed cell lymphoepithelioma (RyR antibody positive patient). One patient with RyR antibodies (OD value 1.712) had histologically verified polymyositis. One patient with RyR antibodies (OD value 1.230) had myocarditis. Other autoimmune diseases occurred in one RyR antibody positive patient (rheumatoid arthritis) and in one RyR antibody negative patient (pemphigus).

Discussion

Patients with myasthenia gravis and thymoma have a more severe disease with a higher mortality than patients with myasthenia gravis without thymoma.¹⁵⁻¹⁸ They can be divided into two groups; those with and those without detectable RyR antibodies.⁷ The results obtained suggest an association between the presence of RyR antibodies and a severe form of thymoma associated myasthenia gravis with a poor prognosis. Our patients with RyR antibodies were significantly more disabled at peak illness and at latest follow up, and they had a significantly higher mortality than those without RyR antibodies. Furthermore, there was a good correspondence between the level of RyR antibodies and the clinical course of myasthenia gravis, a high level being associated with a severe course of myasthenia gravis.

The number of deaths from myasthenia gravis among our patients with thymoma was somewhat higher than expected from recent mortality studies.^{17,18} The reason for this is unclear. Our patients were not particularly old, they were thymectomised early in the course of the disease and received adequate immunosuppressive treatment.

AChR antibodies are the main pathogenic factor in myasthenia gravis. RyR antibody positive and negative patients had similar levels of AChR antibodies, and severity of myasthenic weakness correlated more strongly with serum levels of RyR antibodies than with serum levels of AChR antibodies. Thus the observed association between RyR antibodies and severity of myasthenia gravis is not caused by different levels of AChR antibodies. We therefore suggest that the relation of RyR antibodies to a severe course of thymoma associated myasthenia gravis is due

to a possible pathogenic effect of RyR antibodies.

The RyR plays an important role in the mechanism of excitation-contraction coupling in striated muscle. The fact that some patients with myasthenia gravis have electromyographic signs of impaired excitation-contraction coupling^{19,20} may indicate a pathogenic role of RyR antibodies. Some patients with myasthenia gravis and thymoma develop polymyositis and myocarditis.²¹ Muscle biopsy was not routinely performed in our patients; however, one patient with severe polymyositis and one with myocarditis had high levels of RyR antibodies. It is possible that RyR antibodies are involved in the pathogenesis of thymoma associated polymyositis and myocarditis.

In conclusion, our results suggest that patients with myasthenia gravis, thymoma, and RyR antibodies are at a high risk for developing severe and fatal myasthenia gravis, especially those patients with high antibody levels. Further studies are needed to clarify whether this is related to a pathogenic effect of RyR antibodies. Patients with RyR antibodies should probably have more frequent clinical control and be more vigorously treated than other patients with myasthenia gravis.

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- 1 Lindström J. Immunobiology of myasthenia gravis, experimental autoimmune myasthenia gravis, and Lambert-Eaton syndrome. *Annu Rev Immunol* 1985;3:109-31.
- 2 Namba T, Brunner NG, Grob D. Myasthenia gravis in patients with thymoma, with particular reference to onset after thymectomy. *Medicine* 1978;57:411-33.
- 3 Strauss AJL, Seegal BC, Hsu KC, et al. Immunofluorescence demonstration of a muscle binding, complement-fixing serum globulin fraction in myasthenia gravis. *Proc Soc Exp Biol Med* 1960;105:184-91.
- 4 Aarli JA, Stefansson K, Marton LSG, Wollmann RL. Patients with myasthenia gravis and thymoma have in their sera IgG autoantibodies against titin. *Clin Exp Immunol* 1990;82:284-8.
- 5 Williams CL, Lennon VA. Thymic B-lymphocyte clones from patients with myasthenia gravis secrete monoclonal striational autoantibodies reacting with myosin, α -actinin or actin. *J Exp Med* 1986;164:1043-59.
- 6 Ohta M, Ohta K, Itoh N, et al. Anti-skeletal muscle antibodies in the sera from myasthenic patients with thymoma: identification of anti-myosin, actomyosin, actin, and α -actinin antibodies by a solid-phase radioimmunoassay and a Western blotting analysis. *Clin Chim Acta* 1990;187:255-64.
- 7 Mygland Å, Tysnes O-B, Matre R, Volpe P, Aarli JA, Gilhus NE. Ryanodine receptor autoantibodies in myasthenia gravis patients with a thymoma. *Ann Neurol* 1992;32:589-91.
- 8 Takeshima H, Nishimura S, Matsumoto T, et al. Primary structure and expression from complementary DNA of skeletal muscle ryanodine receptor. *Nature* 1989;339:439-45.
- 9 Lai FA, Meissner G. Structure of the calcium release channel of skeletal muscle sarcoplasmic reticulum and its regulation by calcium. *Adv Exp Med Biol* 1990;269:73-7.
- 10 Williams AJ. Ion conduction and discrimination in the sarcoplasmic reticulum ryanodine receptor/calcium-release channel. *J Muscle Res Cell Motil* 1992;13:7-26.
- 11 Mygland Å, Tysnes O-B, Aarli JA, Flood PR, Gilhus NE. Myasthenia gravis patients with a thymoma have antibodies against a high molecular weight protein in sarcoplasmic reticulum. *J Neuroimmunol* 1992;37:1-7.
- 12 Oosterhuis HJ. Observations of the natural history of myasthenia gravis and the effect of thymectomy. *Ann NY Acad Sci* 1981;377:678-89.
- 13 Lefvert AK, Bergström K, Matell G, Osterman PO, Pirskanen R. Determination of acetylcholine receptor antibody in myasthenia gravis—clinical usefulness and pathogenetic implications. *J Neurol Neurosurg Psychiatry* 1978;41:394-403.

- 14 Gauthel M, Lakey A, Barlow DP, et al. Titin antibodies in myasthenia gravis: Identification of a major autogenic region of titin. *Neurology* 1994 (in press).
- 15 Grob D, Arsura EL, Brunner NG, Namba T. The course of myasthenia gravis and therapies affecting outcome. *Ann NY Acad Sci* 1987;505:472-99.
- 16 Oosterhuis HJGH. The natural course of myasthenia gravis: a long term follow up study. *J Neurol Neurosurg Psychiatry* 1989;52:1121-7.
- 17 Durelli L, Maggi G, Casadio C, Ferri R, Rendine S, Bergamini L. Actuarial analysis of the occurrence of remissions following thymectomy for myasthenia gravis in 400 patients. *J Neurol Neurosurg Psychiatry* 1994;54:406-11.
- 18 Mantegazza R, Beghi E, Pareyson D, et al. A multicentre follow up study of 1152 patients with myasthenia gravis in Italy. *J Neurol* 1990;237:339-44.
- 19 Pagala MKD, Nandakumar NV, Venkatachari SAT, Ravindran K, Namba T, Grob D. Responses of intercostal muscle biopsies from normal subjects and patients with myasthenia gravis. *Muscle Nerve* 1990;13:1012-22.
- 20 Nielson VK, Paulson OB, Rosenkvist J, Holsøe E, Lefvert AK. Rapid improvement of myasthenia gravis after plasma exchange. *Ann Neurol* 1982;11:160-9.
- 21 Namba T, Brunner NG, Grob D. Idiopathic giant cell polymyositis: report of a case and review of the syndrome. *Arch Neurol* 1974;31:27-30.

Jean Lhermitte 1877-1959

Jean L Lhermitte, son of an artist, was born at Mon-Saint-Père Aisne in 1877. After school at St Etienne he studied medicine in Paris, graduating in 1907. He immediately turned to neurology for both clinical training and research. Over many years he was associated with Gustave Roussy in texts on *Blessures de la moëlle et de la queue de cheval*, published in 1918, and on treatment of the neuroses of wartime: *Traitement des psychonévroses de guerre*, issued in 1919; and (with Spiller) on the internuclear ophthalmoplegia of epidemic encephalitis. Anterior internuclear ophthalmoplegia is often referred to as Lhermitte's syndrome.

He showed the importance of the inferior olivary nucleus in myoclonus.

His outstanding abilities led to his appointment as clinical director at the Salpêtrière Hospital, Paris where he became one of the greatest clinical neurologists of his day. His enthusiasm was seldom concealed and between the World Wars he inspired and led a generation of young neurologists.

A deeply religious man, he applied his scholarship to the interface of medicine and theology, including studies on stigmatisation and demoniacal possession.

Lhermitte died peacefully in 1959.

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Lhermitte's sign

Although more properly designated a symptom than a sign, the importance of this phenomenon was brought to the neurological world by Jean Lhermitte in 1924.¹ Mme D, was a cashier, 43 years old with no past or family history of note, when in July 1923:

a few days after . . . an illness with abdominal pains and diarrhoea she suffered a rather violent headache accompanied by tingling in the feet and knees. At this time, walking became difficult . . .

In August 1923 the patient first noticed a phenomenon that she described thus: "When I try to lower my head, I feel a violent shock in the nape of my neck, and a pain like an electric shock runs through my whole body, from my neck to my feet, down my vertebral column" . . . each flexion movement of the head or the trunk brought on this sensation . . . from the nape of the neck to the tips of the toes and also of the fingers. The patient could not dress herself, put on her shoes or pick up an object from the ground without carefully keeping her head in line with her trunk.

Until May 1924, the condition did not change . . . At this period a new and disturbing symptom appeared: marked impairment of vision of the right eye . . .

We would like to direct attention to a particular manifestation that we believe has never been pointed out in the symptomatology of multiple sclerosis: the pains resembling an electric shock . . . The sensations are uncomfortable but not really painful, and closely resemble those produced by faradic current. They never occur spontaneously . . . but occur exclusively with

movements accompanied by forward flexion of the head . . . These sensations are always rapid and brief, because by an instinctive reflex, the patient corrects the position . . .

It was described by Babinski and Dubois² (Society of Neurology 1918) in concussions of the spine, and one of us (Lhermitte) published two observations regarding it, inserted in the excellent thesis of J Ribeton (Clinical study of pains resembling electrical shock following injuries to the neck. Thesis Univ Paris January 1919).

Lhermitte continues and emphasises two points:

the shock like pains in direct spinal concussion as in multiple sclerosis are related not to root changes but to spinal lesions . . .

Tinel accurately observed that tingling (we add the electrical sensations) produced by percussion or elongation requires that these nerves be composed of demyelinated fibres . . . the type of pain presents the inherent excitability of sensory fibres stripped of their insulating myelin sheath. This excitability may be evoked by direct percussion, which one applies to a peripheral nerve, or by elongation brought about by flexion of the head on the trunk.

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- 1 Lhermitte J, Bollak A, Nicholas M. Les douleurs à type de décharge électrique consécutives à la flexion céphalique dans la sclérose en plaques. Un cas de forme sensitive de la sclérose multiple. *Rev Neurol* 1924;2:56-62.
- 2 Babinski J, Dubois A. Douleurs à forme de décharge électrique, consécutives aux traumatismes de la nuque. *Presse Med* 1918;26:64.