JNNP Online First, published on November 6, 2006 as 10.1136/jnnp.2006.100545

Clinical and serological study of myasthenia gravis in HuBei province, China

Xiaofan Zhang[1,2], Mingshan Yang[1], Jinzhi Xu[1], Min Zhang[1], Bethan Lang[2], Wei Wang[1], Angela Vincent[2]

[1]Department of Neurology, Tongji Hospital, Huazhong University of Science and Technology (HUST), WuHan, China
[2]Neurosciences Group, Weatherall Institute of Molecular Medicine, John Radcliffe Hospital, Oxford OX3 9DS,UK.

Correspondence to:
Prof Angela Vincent, Neurosciences Group, Weatherall institute of Molecular Medicine, John Radcliffe Hospital, Oxford OX3 9DS,UK. Tel +44 1865 222321;fax +44 1865 222402;email: angela.vincent@imm.ox.ac.uk
or
Prof Wei Wang, Department of Neurology, Tongji Hospital, Huazhong University of Science and Technology (HUST) email: wwang@tjh.tjmu.edu.cn

Keywords myasthenia gravis, Chinese, acetylcholine receptor antibody, muscle specific kinase antibody, voltage-gated calcium channel antibody

Abstract

Ocular and childhood myasthenia gravis (MG) cases appear relatively more common in Oriental than in Caucasian populations, but there have been no comprehensive serological studies on patients from mainland China. We studied 391 unselected cases of MG attending Tongji Hospital in WuHan (the largest in the province of HuBei) during a one year period; most had already received treatment for their condition. The male to female ratio was 0.8. 50% of the patients were children (under 15 years), and age at onset showed a single peak at between 5 and 10 years of age. 64% of the children and 66% of the adults were positive for acetylcholine receptor (AChR) antibodies but the antibody titres were lower than in similar Caucasian studies, although this was partly due to the high incidence of ocular MG. Of the 43 patients with generalized MG without AChR antibodies, only one had MuSK antibodies (2.5%) and two had VGCC antibodies indicating probable Lambert Eaton myasthenic syndrome. 75% of the children compared with only 28% of the adults had ocular MG. Thymoma was evident by MRI in 1.5% of children and 20% of adults. Despite most patients having received prednisone, very few had obtained full clinical remission. The study emphasises the frequency of early childhood onset with ocular symptoms and shows that many of these patients have AChR antibodies. By contrast, patients presenting in later age appear to be very uncommon in comparison with recent studies in Caucasian populations.

Introduction

Myasthenia gravis (MG) is the most common primary disorder of neuromuscular transmission. Acetylcholine receptor (AChR) antibodies are present in sera from 80 to 90% of patients with generalized MG, about 50% of those with pure ocular MG, and very infrequently in healthy individuals.[1] The remaining 10-20% of generalized MG patients are AChR antibody negative (seronegative MG, SNMG). IgG autoantibodies to the muscle-specific kinase, MuSK, were first identified in 70% of patients with generalized SNMG.[2] However, subsequent reports have found a variable prevalence in different countries, with a very low proportion of MuSK antibody positive MG (MuSK-MG) in one study from Taiwan[3] (reviewed in [4]).

A number of studies indicate that MG in Oriental populations may be clinically different from that in Caucasians[5-8]. In particular, pediatric cases are frequent in China, Taiwan and Japan and purely ocular MG is relatively common in children. Since there are also differences in HLA associations between Japanese, Chinese and Caucasian populations[9-12], these observations may provide clues to the immunopathogenesis of MG.

However, there have been no comprehensive studies of AChR and MuSK antibodies in patients from mainland China. Here we studied the histories and serology of 391 Chinese patients attending a major hospital in WuHan, China.

Patients and Methods

During a one year period (September, 2004 - December, 2005), all 600 patients with a diagnosis of MG presenting to the Tongji Hospital, WuHan, China, were invited to participate and 391 consented to give serum for antibody studies. 324 patients had been monitored regularly in our hospital over periods from <1 to 27 years, and 67 were attending for the first time. The diagnosis of MG was made on the basis of the clinical history of fatiguable weakness, neostigmine test and the response to the acetylcholinesesterase therapy. Electromyography was only performed in 2 children and 10 adults. The severity was described according to the Myasthenia Gravis Foundation of America (MGFA) classification[13] (for quantitative purposes, grades I-V were changed to 1-5). Thymic pathology was deduced from MRI scans, but thymectomy was only performed in 44 patients.

AChR antibodies and MuSK antibodies were measured using radioimmunoprecipitation assays (RSR Linited, Cardiff, UK). AChR antibody values greater than 2.5 nM were reassayed with 1 μl of serum to provide a more accurate titer. VGCC antibodies were measured as previously described[14]. Serum from 20 healthy individuals and 80 patients with other diseases were used as controls.

Results

Demographic and serological features of all MG patients

The most striking feature was the large number of children with MG, many aged less than five years at first recognition of symptoms (Fig 1a). 50 % (197/391) of the patients presented as children under 14 years old and 50%□194/391□ as adolescents or adults; only six were over the age of 60 years (Fig 1a). There was a male to female ratio of 0.8 with no clear difference in sex distribution at any age (Fig 1a).

AChR antibodies were performed on all 391 sera, although many of the patients had had MG for many years (range 1-45) at the time of sampling. After subtraction of the

mean of three healthy Chinese sera, the Chinese control values ranged from -0.1 – 0.35 nM. A conservative value of 0.5 nM was used to distinguish AChR antibody positive from AChR antibody negative patients (as used in the Oxford laboratory). AChR antibodies were positive in only 65% (254/391) of the Chinese MG patients (Fig 1b). Overall, the AChR antibody levels were lower than they are in typical AChR antibody positive Caucasian MG patients (data not shown), and there was no clear demarcation between positive and negative values within the patient population.

Three cases of familial MG were present among the cohort (approximately 1%); these were a father and son, a father and daughter and a mother and son. In each case, both individuals were positive for AChR antibodies. A mother with AChR antibodies and her newborn baby were both positive. Seven healthy siblings of MG patients were all negative for AChR antibodies.

Only 1 (2.5%) of the 43 AChR antibody negative patients with generalized MG was positive for MuSK antibody, and none of the patients with ocular MG (see below). MuSK antibodies were also not detected in 65 patients with AChR antibodies. Two patients were positive for VGCC antibodies. All three patients were excluded from further analysis but their histories are summarized below.

Clinical features

Presenting symptoms were ocular motor disturbance in 73%, oropharyngeal weakness in 18% and limb weakness in 9%. Only 16% (62/388) of the patients had progressive weakness that involved oropharyngeal and limb muscles. We used the patients' records to assign MGFA scores at onset, maximum weakness and at the last clinic visit. At the time of serum sampling, positivity for AChR antibody was 65% in all patients, with 59% in purely ocular MG, and 85% in patients with grades 2a or greater (Fig 2).

The duration of MG at the time of study ranged widely, from <1 year to 45 years (median 3 years). One way ANOVA showed some influence of disease duration on MGFA grade ($p=0.02$ for adults and $p=0.04$ for children) but post-testing showed only the grades in adult patients with >10 years duration were significantly different from the presenting MGFA grade ($p<0.05$). We therefore ignored duration of disease in the further analyses.

Although the majority of the patients presented with ocular MG (MGFA grade 1) many adult patients with AChR antibodies had generalized disease at onset (Fig 2) or progressed to generalized weakness. Thus at maximal severity, only 70/191 adults had ocular MG compared with 157/197 of the children (Table 1; Fishers Exact test, $p=0.002$). Most of the patients with generalized symptoms had relatively mild disease although there were some with MGFA grade 3b or greater (Table 1). Interestingly, the overall proportion of AChR antibody positive childhood cases (127/197; 64%) was similar to that of the adults (127/191; 66%) but the AChR antibody positivity in the childhood patients with ocular symptoms at time of study (103/154; 66%) was higher than that in the equivalent adult cases (31/70; 44%; $p=0.003$).

Pure ocular MG, defined as weakness restricted to the ocular muscles for more than two years, was present in 118/205 (58%) of all cases, including 94/126 (75%) children and 24/85 (28%) adults. Similar to the overall data, 72% of the children and only 50% of the adults with pure ocular MG were positive for AChR antibodies.

Table 1 Summary of clinical features and disease associations

			Children		Adults	
			AChR Ab positive*	AChR Ab negative*	AChR Ab positive*	AChR Ab negative*
Total			127	70	127	64
F:M			76:51	29:41	76:51	34:30
Maximum MGFA grade, n	1	227	103	54	31	39
	2a	50	11	10	19	10
	2b	53	10	2	30	11
	3a	5	0	1	3	1
	3b	34	3	2	26	3
	4a	4	0	1	3	0
	4b	6	0	0	6	0
	5	9	0	0	9	0
Thymic pathology (MRI), n	Normal**		98	52	63	48
	Thymitis		26	18	29	12
	Thymoma		3	0	35	4
Hyperthyroidism			16	5	16	17

* Most of the AChR antibodies were not performed at the time of maximum disease severity. **Normal refers to normal for age at the time of MRI; this would include adults with thymic atrophy.

Thymus pathology

All the patients had MRI scans to look for thymic pathology; the results were classified as normal for age, thymitis or thymoma. Most of the children had a normal thymus but a proportion had thymitis evident on scans (Fig 3); only 15 patients with thymitis (aged 3-52 years) were given a thymectomy. Forty-two patients overall (11%) had thymoma detected on scans; three of these were AChR antibody positive children. 39/191 (20%) adult patients had thymomas and four of these were negative for AChR antibodies. In the 28 thymoma patients who were thymectomised, the pathology was confirmed by histology. None have had a thymoma recurrence during a follow-up period of a median of three years.

Response to treatments

The majority of patients were given AChE inhibitors (pyridostigmine bromide) at between 120 and 180 mgs/day. The majority also received prednisone at doses starting at 30 mgs/day, reducing to 10-15 mg/day within a few weeks if there was improvement. These doses were then maintained for six months to one year, after which they were reduced to 5 mg/day. Patients who did not show or report clinical benefit received azathioprine and IVIG, and occasionally plasma exchange. The majority of cases improved to some extent with prednisone but it was usually difficult to reduce this beyond 5 mg/day.

Despite the prednisone treatments, many of the adults with AChR antibodies still had generalized disease and no patients were in complete clinical remission (MGFA grade 0) at the time of the study. Using the post-interventional scoring system, only one patient was known to have died, 11 were worse, 151 were unchanged, and 225 had improved. There were significantly lower final MGFA grades compared to grades at maximum severity for both children and adults, but the greatest change was in adult patients who had received thymectomy (Fig 4).

Patients with MuSK or VGCC antibodies

Only 1 of 44 generalised SNMG patients (2.5%) was positive for MuSK antibody. This patient was a 45-year-old woman who developed transient ptosis and diplopia followed by progressive dysphagia, dysarthria and limb weakness. No thymic abnormalities were identified on MRI scan. The patient did not respond well to pyridostigmine. She developed severe dyspnea requiring ventilatory support. Because of progressive dyspnea, the patient underwent three sessions of immunoadsorption, with a dramatic response. During the 1-year follow-up period, she was much improved and led a normal life when maintained on pyridostigmine 180mg/day and prednisone 15mg/day.

The patients with VGCC antibodies were both males, aged 40 and 49 at onset. Both had relatively mild generalized disease for 7 and 1 years with no evidence of lung cancer on CT scan of the thoraces. Only one received prednisone and both are unchanged at the present time.

Factors that might influence the clinical expression of MG in China

Twenty one of the children had hyperthyroidism (diagnosed on the basis of FT3, FT4 and TSH levels). 16 of these were AChR antibody positive (76%). In addition 33 of the adults had hyperthyroidism and of these 50% were AChR antibody positive at the time of study. There were no other relevant disease associations in the patients. To look at the demographics of MG in China, we took the total MG cases and compared them with the demographics of the total population in HuBei province (Table 2). The prevalence of MG within children (14/million) was higher than that in adults (4.5/million), although this might partly reflect the self-referral patterns to different hospitals in the province.

Table 2 Number of MG patients related to population of HuBei Province

	Total MG patients	HuBei Province* Millions	MG patients/million
Total population	388	60	6.47
Male	173	31	5.58
Female	15	28	7.68
Age 0-14	197	14	14.07
Age 15-64	189	42	4.50
Age 65-	2	3.7	0.54

*These data are based on the website of Nation Bureau of Statistics of China.

The patients described above include 67 newly referred patients who had not received treatment before, and we added a further 40 patients seen in 2006. In HuBei

province 40% (24 million) of the 60 million population lives in rural villages (Table 3). We found that among the 107 newly-presenting patients at the Tongji Hospital, 54% of them came from rural communities and 46% from urban communities, both children and adult patients. Striking, however, was the long time between onset of symptoms and the first visit to the clinic in many cases, with a suggestion of longer time intervals in females. The longest duration from the onset to the first clinic was in a rural woman who had symptoms for 47 years.

Table 3. Demographics of 107 patients at first presentation

	Rural	Urban
Total population in HuBei province	24m	36m
Female MG, n	27	27
Male MG, n	30	23
Age at onset 0-14, n	25	20
Age at onset >14, n	32	30
Duration from onset to first clinic:<1year, n	33 (F:13; M:20)	30 (F:13; M:17)
Duration from onset to first clinic: 1-3years, n	17 (F:9; M:8)	15 (F:11; M:4)
Duration from onset to first clinic:>3years, n	7 (F:5; M2)	5 (F:3; M:2)
Hyperthyroidism, n	9	6

Discussion

Myasthenia gravis occurs world-wide[15] but differences in the clinical expression of MG have been observed, principally between Caucasian and Oriental patients. This was the first large clinical/serological study of unselected MG patients attending a clinic in mainland China. The most striking result is the very high proportion of childhood cases, mostly with purely ocular MG. In contrast to most Caucasian studies, there were few patients presenting over the age of 40 years and no marked gender bias, which differs from that in Caucasians[15-18]. AChR antibodies were found in more than 60% of the ocular MG patients, both children and adults, and in 85% of those with generalized disease at the time of sampling. Thus we confirm that MG in mainland China differs from that in Caucasian populations by the high incidence of childhood ocular MG, and show that a high proportion of these childhood cases are AChR antibody positive.

Despite the high positivity in ocular MG cases, overall 35% of the patients were AChR antibody negative, which is higher than the previous results (13%) of the Chinese patients in Taiwan.[5] However that study only performed AChR antibody titers on 67 untreated patients, rather than the full cohort of 388 MG patients studied here, and it was not clear whether they were adult or childhood cases. Moreover, many of our patients had long disease duration and were not at maximal severity at the time of serum sampling. Alternatively, one could argue that the seronegative cases may not have had MG, since electromyography is not done routinely at our hospital, and was performed in only 12 patients. Moreover, the neostigmine test is unlikely to have the sensitivity or specificity profile of the AChR antibody[19]. The very high incidence of ocular MG cases will confound this problem as they are known to have lower positivity on all tests.

Nevertheless, only three cases here were found to have other neuromuscular junction disorders on serological testing of the patients with generalized AChR antibody negative MG, and the proportion of AChR antibody positivity among the ocular cases is similar or higher than that found in western studies (eg.[20]). Therefore, the majority of the AChR antibody negative cases are likely to have had myasthenia. On the other hand, it is possible that some of those cases designated as ocular MG had mild generalised weakness which was unrecognised; this possibility requires further study.

We detected MuSK antibodies in only 1 of 44 (2.5%) of our Chinese patients with generalized SNMG, which are similar to the result (4%) reported previously in Taiwan[5]. This frequency is much lower than the 40-70% frequency reported in most Caucasian studies[21-24] although a recent study from Norway found no MuSK antibody positive patients[25]; this regional variation, therefore, applies to both western and eastern populations ([4] and Vincent A unpublished results 2006). Like many previously reported Caucasian MuSK antibody-positive patients[21-24], our Chinese MuSKAb-positive patient was an adult female with marked bulbar weakness. We also found two patients with VGCC antibodies which are diagnostic for Lambert Eaton myasthenic syndrome. This syndrome has not been widely considered in the differential diagnosis of neuromuscular weakness in China, and had not been diagnosed clinically. Fortunately, neither patient has developed lung cancer.

The thymic pathology was assessed by MRI and for economic reasons thymectomy was only performed in a proportion of patients, even those with “thymitis” or thymoma. In all those that did receive thymectomy, the pathology was confirmed by histology. However, since no cases with “normal” pathology on MRI were treated surgically, we cannot assess the sensitivity of the MRI. Although, unusually, four of the patients with a history of MRI-diagnosed thymomas (three confirmed by histology) were negative for AChR antibodies, sera from the time of diagnosis were not available.

Apart from the study from Taiwan[5], the closest is that of 470 Japanese patients[8]. Previous reports have indicated a high frequency of HLA class II allele-DR9 in both the Chinese and Japanese patients[9-12], compared with DR3 [18] in Caucasians, so one might expect the clinical expression to be similar. The Japanese patients were recruited from 19 Japanese tertiary medical centers and included both childhood and adult cases, but the median age at onset was in adulthood, differing from that shown here. In addition, they found fewer patients with purely ocular MG and most of the Japanese patients achieved remission, with 30% of the patients achieving complete remission (MGFA grade 0), whereas this was not the case in our patients. One of the reasons could be that more of their patients underwent thymectomy (68% in Japan, 11.25% in China) since we found here that there was greater improvement in patients who had undergone thymectomy than in those who had not (Fig 4), although these results are confounded by the use of prednisone in most patients. Another reason for the difference in clinical response may be that the Chinese patients were younger than the Japanese. Childhood MG is so rare in Caucasian societies that the optimal treatment for childhood MG is not well studied. In China many children receive prednisone, but the lack of clear clinical remission in our patients suggests that this treatment is not optimal. Moreover, it could stunt growth which must be considered when starting treatment.

It is difficult to comment on the overall prevalence of MG in China from these and previous studies[7,26]. The latter studies found a high prevalence of childhood and ocular

MG cases (around 1:16,000) as we did, but did not use standard assays for antibody detection. However, despite the fact that our hospital is one of the five largest hospitals in WuHan where patients can attend a neurology clinic, the numbers of patients is likely to be an underestimate. The relatively high proportion of males compared with females at all ages, and the very long duration of symptoms in a high proportion of the patients at first clinic, may reflect the fact that attendance at the Neurology clinic depends on self-referral and financial situation rather than necessarily on clinical need.

Finally, these data not only indicate a need for more study of MG and its treatment in Chinese children, but also raise interesting questions concerning the aetiology of this disease. The relatively high prevalence of MG in children compares strikingly with the situation in Caucasian populations (eg[16]) and this suggests that the disease might follow a common childhood infection or other environmental factor. However, there was no evidence for MG segregating with rural or urban communities, making this less likely. Genetic factors, as mentioned above, may contribute to the differences in aetiology, clinical expression and susceptibility between Caucasian and Oriental races. It will, therefore, be interesting to compare the relative numbers of Chinese childhood and adult cases in other parts of the world where Chinese are resident.

Acknowledgements
We are grateful to all the patients for participating in this study, and to RSR Ltd Cardiff UK for their gift of some of the diagnostic assay kits and for arranging their transport.

Competing interests
The Department of Clinical Neurology in Oxford receives payments and royalties from AChR and MuSK antibody tests**.**

Figure legends
Figure 1. (a) Frequency histograms of age at presentation for male and female Chinese MG patients. (b) AChR antibody levels in 100 Chinese controls and 391 MG patients. The line is drawn at 0.5 nM which is the cut-off used in the Oxford lab where the assays were performed.

Figure 2. AChR antibody levels in Chinese patients plotted against MGFA clinical grades at time of sampling (given as 1-5, corresponding to I to V). Most of the AChR antibody negative samples were from patients with MGFA grade 1. Some of these patients had had higher grades at maximum severity (see Table 1).

Figure 3. Age at onset plotted against thymic pathology. MRI was used to define the thymic pathology since many patients were not thymectomised.

Figure 4. The MGFA grades (given as 1-5, corresponding to I to V) are shown at maximum severity and final follow-up in patients without or with thymectomy.

References

1. Lindstrom JM, Seybold ME, Lennon VA, Whittingham S, Duane DD (1998). Antibody to acetylcholine receptor in myasthenia gravis: prevalence, clinical correlates, and diagnostic value. 1975 classical article. *Neurology* 1998; 51:933-939

2. Hoch W, McConville J, Helms S, et al. Auto-antibodies to the receptor tyrosine kinase MuSK in patients with myasthenia gravis without acetycholine receptor antibodies. *Nat Med* 2001;7: 365-368

3. Yeh J-H, Chen W-H, Chiu H-C, Vincent A. Low frequency of MuSK antibody in generalized seronegative myasthenia gravis among Chinese. *Neurology* 2004; 62:2131.

4 Vincent A, Leite MI. Neuromuscular junction autoimmune disease: muscle specific kinase antibodies and treatments for myasthenia gravis. *Curr Opin Neurol.* 2005;18:519-25.

5. Chiu H-C., Vincent A., Newsom-Davis J., et al. Myasthenia gravis: Population differences in disease expression and acetylcholine receptor antibody titers between Chinese and Caucasians. *Neurology* 1987;37:1854-1857

6 Fukuyama Y, Hirayama Y, Osawa M. Epidemiological and clinical features of childhood myasthenia gravis in Japan. In: SATYOSHI E, ed. Myasthenia gravis-pathogenesis and treatment. Tokyo: Univ Tokyo Press, 1981:19-27

7 Xu J, Yang M, Li B, et al. Myasthenia gravis: clinical study in 2385 patients. *Chin J Neurol*, 1999, 32,:347-350

8 Kawaguchi N, Kuwabara S, Nemoto Y, et al. Treatment and outcome of myasthenia gravis: retrospective multi-center analysis of 470 Japanese patients, 1999-2000. *J Neurol Sc* 2004; 224;43-47.

9. Shinomiya N, Nomura Y, Segawa M. A variant of childrenhood-onset myasthenia gravis: HLA typing and clinical characteristics in Japan. *Clin Immunol* 2004; 110: 154-158

10. Hawkins BR, Chan-Lui WY,Choi EKK, Ho AY. Strong association of HLA BW46 with juvenile onset myasthenia gravis in HongKong Chinese. *J Neurosurg Psychiatr* 1984;47:555-557.

11 Chen WH, Chiu HC, Hseih RP. Association of HLA-Bw46DR9 combination with juvenile myasthenia gravis in Chinese. *J Neurol Neurosurg Psychiatr* 1993;56:382-5

12 Matsuki K, Juji T, Tokunaga K, Takamizawa M, Maeda H, Soda M, et al.HLA antigens in Japanese patients with myasthenia gravis. *J Clin Invest* 1990;86:392-9

13. Jaretzki A 3rd, Barohn RJ, Ernstoff RM, Kaminski HJ, Keesey JC, Penn AS, et al. Myasthenia gravis: recommendations for clinical research standards. Task force of the Medical Scientific Advisory Board of the Myasthenia Gravis Foundation of America. [Review]. *Neurology* 2000; 55: 16-23.

14. Motomura M, Lang B, Johnston I, Palace J, Vincent A, Newsom-Davis J. Incidence of serum anti-P/O-type and anti-N-type calcium channel autoantibodies in the Lambert-Eaton myasthenic syndrome.*J Neurol Sci*. 1997;147:35-42.

15. Kurtzke JF, Kurland LT. In: Baker AB, Baker LH, eds. Epidemiology of neurologic disease, chap 48. Hagerstown, MD: Harper & Row, 1977;3

16. Vincent A, Clover L, Buckley C, et al Evidence of underdiagnosis of myasthenia gravis in older people. *J Neurol Neurosurg Psychiatr* 2003; 74: 1105-1108.

17. Mantegazza R, Baggi F, Antozzi C et al. Myasthenia Gravis (MG): Epidemiological Data and Prognostic Factors. *Ann. N. Y. Acad.Sci*.2003; 998: 413-423.

18. Compston DAS, Vincent A, Newsom-Davis J, Batchelor JR. Clinical, pathological, HLA antigen and immunological evidence for disease heterogeneity in myasthenia gravis. *Brain* 1980;103:579-601
19. Vincent A, Bowen J, Newsom-Davis J, et al. Seronegative generalised myasthenia gravis: clinical features, antibodies, and their targets. *Lancet Neurology* 2003; 2:
20. Kupersmith MJ, Latkany R, Homel P. Development of generalized disease at 2 years in patients with ocular myasthenia gravis. *Arch Neurol*. 2003 60:243-8.
21. Sanders DB, El-Salem K, Massey JM, McConville J, Vincent A. Clinical aspects of MuSK antibody positive seronegative MG. *Neurology* 2003;60: 1978-1980
22. Scuderi F, Marion M, Colonna L, et al. Anti-p110 autoantibodies identify a subtype of "seronegative" myasthenia gravis with prominent oculobulbar involvement. *Lab Invest* 2002; 82: 1139-1146.
23. Evoli A, Tonali PA, Padua L, et al. Clinical correlates with anti-MuSk antibodies in generalized seronegative myasthenia gravis. *Brain* 2003; 126: 2304-2311.
24. McConville J, Farrugia ME, Beeson D, Kishore U, Metcalfe R, Newsom-Davis J, Vincent A. Detection and characterization of MuSK antibodies in seronegative myasthenia gravis. *Ann Neurol*. 2004;55:580-4.
25. Romi F, Aarli JA, Gilhus NE. Seronegative myasthenia gravis: disease severity and prognosis. *Eur J Neurol*. 2005 12:413-8.
26. Chung B, Wong V, Ip P. Prevalence of neuromuscular diseases in Chinese children: a study in southern China. *J Child Neurol*. 2003;18:217-9.

Figure 1

Figure 2

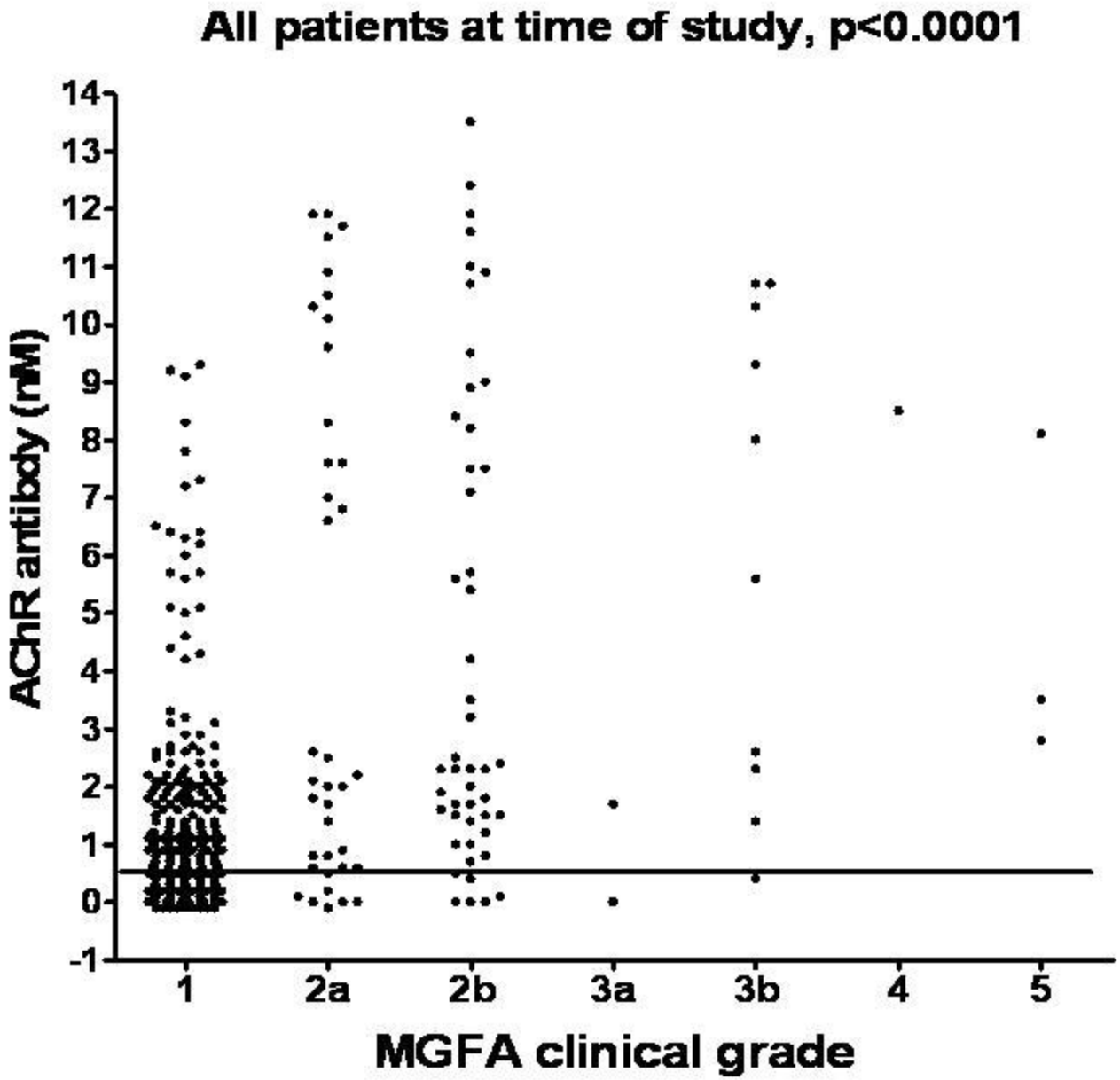

Figure 3

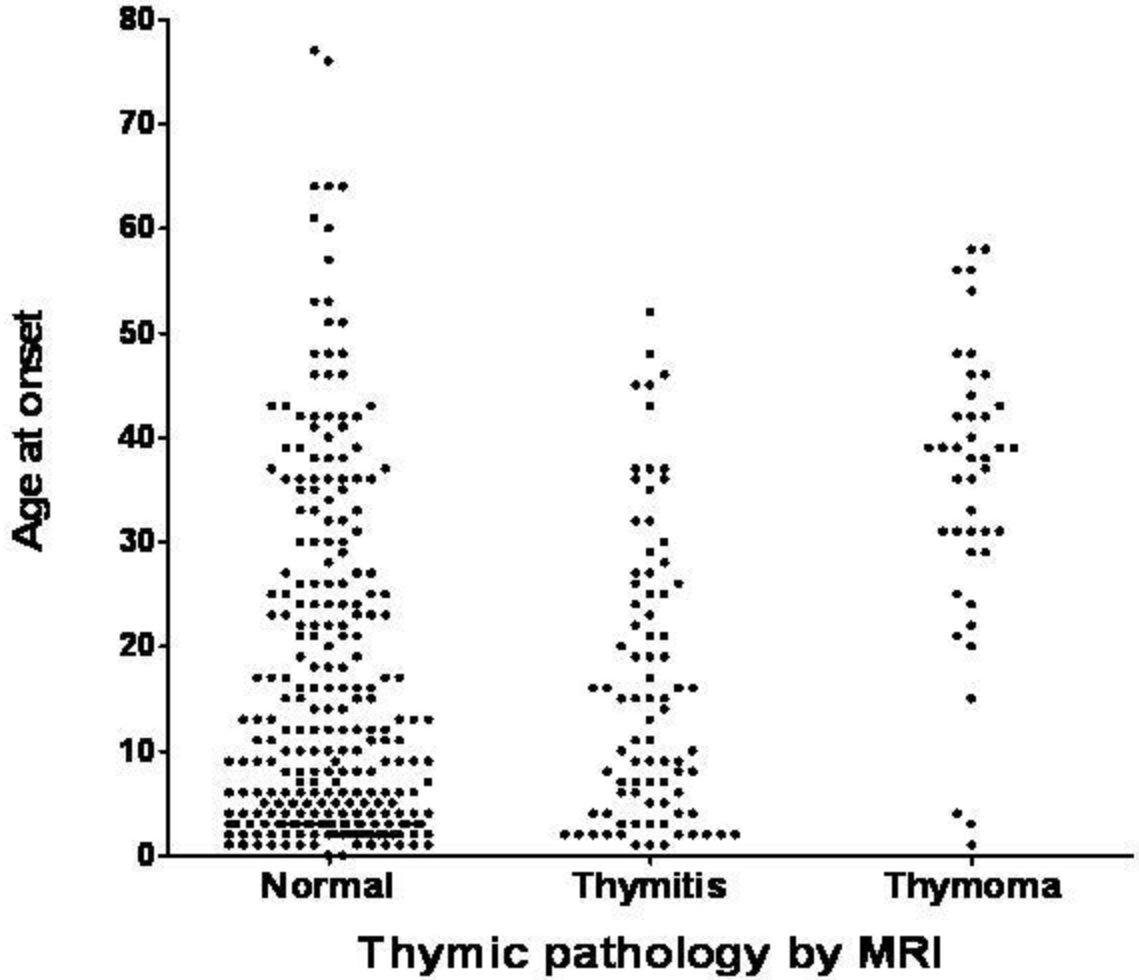

Figure 4