MuSK Antibody(+) Versus AChR Antibody(+) Myasthenia Gravis

Clinical, Neurophysiological and Morphological Aspects

ANNA ROSTEDT PUNGA
Dissertation presented at Uppsala University to be publicly examined in Grönwallssalen, Akademiska sjukhuset, ingång 70, 75185 Uppsala, Friday, February 2, 2007 at 13:15 for the degree of Doctor of Philosophy (Faculty of Medicine). The examination will be conducted in English.

Abstract

Myasthenia gravis (MG) is an autoimmune neuromuscular disorder that causes fluctuating muscle weakness. MG may be divided into an ocular form and a generalized form based on the involved muscles. Treatment differs between these different MG forms. The majority (80%) of patients with generalized MG are seropositive for antibodies against the acetylcholine receptor (AChR-Ab). Recently a new antibody was detected against muscle specific tyrosine kinase (MuSK) in about 40% of patients who are AChR-Ab seronegative. A few patients with MuSK-Ab have muscular atrophies, as well as electrophysiological myopathy.

In this thesis we have characterized MuSK-Ab seropositive [MuSK(+)] patients using clinical parameters, including health-related quality of life (hrQoL), neurophysiology and muscle morphology, and compared them to patients with and without AChR-Ab. The question concerned which factors contribute to their muscle weakness. Additionally, we wanted to determine if single-fiber electromyography (SFEMG) in a limb muscle has any predictive value for generalization of ocular MG.

Our results suggest that MuSK(+) patients more often have a myopathic electromyography pattern, although this pattern is found also in other immunological subtypes of MG. The myopathic pattern may be associated with the frequently found mitochondrial abnormalities. However, disturbed neuromuscular transmission is the primary cause of muscle weakness in the majority of MuSK(+) patients, as well as in AChR-Ab seropositive patients. The disease-specific hrQoL MG questionnaire was successfully validated into Swedish and these scores correlated with disturbed neuromuscular transmission in a proximal arm muscle. Abnormal SFEMG findings occur also in muscles outside of the facial area in ocular MG, although this is not predictive of subsequent generalization.

MuSK (+) patients have little or no beneficial effect of acetylcholine esterase inhibitors (AChEIs). On the contrary AChEIs may produce profound adverse effects. We present the hypothesis that this effect of AChEIs is due to abnormal receptor morphology in MuSK(+) patients.

Keywords: myasthenia gravis, MuSK antibody, AChR antibody, myopathy, single-fiber EMG, mitochondria, Swedish MG questionnaire, quality of life

Anna Rostedt Punga, Department of Neuroscience, Box 593, Uppsala University, SE-75124 Uppsala, Sweden

© Anna Rostedt Punga 2007

ISSN 1651-6206
ISBN 91-554-6752-0
urn:nbn:se:uu:diva-7408 (http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-7408)
“I can respect, but never accept, living with myasthenia”

To my mother Margaretha
This thesis is based on the following papers. They will be referred to in the text by their Roman numerals:


I © Lippincott Williams&Wilkins. II © Springer Science and Business Media. III and IV © Elsevier. V © Wiley Interscience.

Reprints were made with the kind permission of the publishers and the International Federation of Clinical Neurophysiology (III and IV).
## Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction</td>
<td>13</td>
</tr>
<tr>
<td>Myasthenia Gravis (MG)</td>
<td>14</td>
</tr>
<tr>
<td>History</td>
<td>14</td>
</tr>
<tr>
<td>Epidemiology</td>
<td>15</td>
</tr>
<tr>
<td>Pathophysiology</td>
<td>15</td>
</tr>
<tr>
<td>Clinical picture of MG</td>
<td>17</td>
</tr>
<tr>
<td>Types of myasthenia gravis and myasthenic syndromes</td>
<td>17</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>18</td>
</tr>
<tr>
<td>Quality of life assessment</td>
<td>23</td>
</tr>
<tr>
<td>General aim of the study</td>
<td>24</td>
</tr>
<tr>
<td>Specific aims</td>
<td>24</td>
</tr>
<tr>
<td>Study I</td>
<td>24</td>
</tr>
<tr>
<td>Study II</td>
<td>24</td>
</tr>
<tr>
<td>Study III</td>
<td>24</td>
</tr>
<tr>
<td>Study IV</td>
<td>25</td>
</tr>
<tr>
<td>Study V</td>
<td>25</td>
</tr>
<tr>
<td>Patients and methods</td>
<td>26</td>
</tr>
<tr>
<td>Patients</td>
<td>26</td>
</tr>
<tr>
<td>Methods</td>
<td>27</td>
</tr>
<tr>
<td>Clinical neurological examination</td>
<td>27</td>
</tr>
<tr>
<td>Myasthenia Gravis Questionnaire</td>
<td>29</td>
</tr>
<tr>
<td>SF-36</td>
<td>29</td>
</tr>
<tr>
<td>Single Fiber Electromyography</td>
<td>30</td>
</tr>
<tr>
<td>Repetitive nerve stimulation</td>
<td>30</td>
</tr>
<tr>
<td>Analysis of AChR antibodies</td>
<td>30</td>
</tr>
<tr>
<td>Analysis of MuSK antibodies</td>
<td>31</td>
</tr>
<tr>
<td>Analysis of titin and ryanodine receptor antibodies</td>
<td>31</td>
</tr>
<tr>
<td>Muscle biopsy</td>
<td>31</td>
</tr>
<tr>
<td>Statistical analysis</td>
<td>33</td>
</tr>
<tr>
<td>Results</td>
<td>35</td>
</tr>
<tr>
<td>Predictive value of SFEMG in a limb muscle for generalization of MG</td>
<td>35</td>
</tr>
<tr>
<td>(Study I)</td>
<td>35</td>
</tr>
<tr>
<td>Time to generalization</td>
<td>35</td>
</tr>
</tbody>
</table>
Presence and degree of abnormal SFEMG findings............................35
Other possible predictive tests.............................................................35
Health-related quality of life findings in Swedish MG patients and validation of the Swedish MG Questionnaire (Study II)..................36
Translation and cultural adaptation .....................................................36
Descriptive of the Italian sample..........................................................36
Scores of the Swedish sample..............................................................36
Correlation of MGQ and SF-36 scores................................................37
Evaluation of capacity of Swedish MGQ ................................................37
Correlation between patient-oriented findings and abnormal neuromuscular transmission in MG (Study III)............................38
Outcome measures............................................................................38
Correlation between MGQ and neurophysiology..............................38
Correlation between SF-36 and neurophysiology ...............................38
Comparison of neurophysiological, muscle biopsy and health-related quality of life parameters in MuSK(+), AChR(+) and AChR(-) MG patients (Study IV) ..................................................40
MuSK-Ab presence .............................................................................40
Clinical findings..................................................................................41
Neurophysiological findings..............................................................41
Correlation of decrement, mean MCD and percentage of blockings...42
Comparison of myopathic pattern ......................................................43
Morphological and mitochondrial findings ........................................44
Mitochondrial DNA and POLG1 findings...........................................45
Extra discharges due to AChEI (Study V) ..............................................48
Discussion..........................................................................................49
Disturbed neuromuscular transmission in the EDC in OMG..........49
Correlation between disturbed neuromuscular transmission and health-related quality of life .........................................................51
MuSK antibodies and AChR antibodies may coexist ......................51
Primary factors contributing to the muscular weakness and fatigue in MuSK(+) patients.................................................................52
Neuromuscular transmission..............................................................52
Health-related quality of life in MuSK(+) patients............................53
Therapy considerations in MuSK(+) patients.................................53
Myopathy in MuSK(+) patients..........................................................54
Pathogenic role of MuSK antibodies................................................54
Muscle pathology in MuSK(+) patients and mitochondrial defects in MG.................................................................55
Adverse effects of pyridostigmine treatment in MuSK(+) MG: neurophysiological indications of AChEI side effects .....................56
Abbreviations

ACh  Acetylcholine
AChEI  Acetylcholine esterase inhibitors
AChR  Acetylcholine receptor
AChR(+)  Acetylcholine receptor antibody seropositive
AChR(-)  Acetylcholine receptor antibody seronegative
AChR-Ab  Acetylcholine receptor antibody
BD  Bulbar domain
BP  Bodily pain
COX  Cytochrome C oxidase
CMAP  Compound muscle action potential
EDC  Extensor digitorum communis muscle
EMG  Electromyography
FD  Fiber density
GD  Generalized domain
GH  General health
GMG  Generalized myasthenia gravis
HrQoL  Health-related quality of life
IgG  Immunoglobulin type G
LEMS  Lambert Eaton myasthenic syndrome
MCD  Mean of consecutive interpotential interval differences
MCS  Mental composite score
MG  Myasthenia gravis
MGFA  Myasthenia Gravis Foundation of America
MGQ  Myasthenia gravis questionnaire
MH  Mental health
MiDNA  Mitochondrial DNA
MuSK  Muscle specific tyrosine kinase
MuSK(+)  Muscle specific tyrosine kinase antibody seropositive
MuSK(-)  Muscle specific tyrosine kinase antibody seronegative
<table>
<thead>
<tr>
<th>Term</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>MuSK-Ab</td>
<td>Muscle specific tyrosine kinase antibody</td>
</tr>
<tr>
<td>MUP</td>
<td>Motor unit potential</td>
</tr>
<tr>
<td>NADH-TR</td>
<td>NADH-tetrazolium reductase</td>
</tr>
<tr>
<td>OD</td>
<td>Ocular domain</td>
</tr>
<tr>
<td>OMG</td>
<td>Ocular myasthenia gravis</td>
</tr>
<tr>
<td>PCS</td>
<td>Physical composite score</td>
</tr>
<tr>
<td>PF</td>
<td>Physical functioning</td>
</tr>
<tr>
<td>POLG</td>
<td>Polymerase $\gamma$</td>
</tr>
<tr>
<td>QEMG</td>
<td>Quantitative electromyography</td>
</tr>
<tr>
<td>RE</td>
<td>Role emotional</td>
</tr>
<tr>
<td>RNS</td>
<td>Repetitive nerve stimulation</td>
</tr>
<tr>
<td>RP</td>
<td>Role physical</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SDH</td>
<td>Succinate dehydrogenase</td>
</tr>
<tr>
<td>SFEMG</td>
<td>Single fiber electromyography</td>
</tr>
<tr>
<td>SF</td>
<td>Social functioning</td>
</tr>
<tr>
<td>SF-36</td>
<td>36-item Short-Form health survey</td>
</tr>
</tbody>
</table>
Introduction

People have asked me why I chose to study myasthenia gravis, which is a rare neuromuscular disorder, when there are so many other diseases in the world. The main reason is that my mother got myasthenia when I was quite young, so I have basically lived close to it for a long time. I have seen its constant presence, although fluctuating in severity. Therefore, through its chronic nature, I can understand how it directly and indirectly affects quality of life.

Myasthenia Gravis (MG) causes fluctuating muscle weakness, which often involves droopy eyelids, swallowing difficulties and generalized muscle fatigue. In the literature MG is often said to be the best understood autoimmune disorder, but how come then that we do not know what triggers it or how to cure it? Further we can not answer why some muscles are more prone to be affected than others.

Recently, a new antibody was detected against the muscle specific tyrosine kinase (MuSK) located at the neuromuscular junction. A few patients with MuSK antibodies have, in addition to myasthenic symptoms, prominent muscular atrophies, as well as myopathic findings on electromyography. The exact role of these antibodies remains unclear to date.

The focus of this thesis are the main factors contributing to the muscular weakness and fatigue in MuSK-Ab seropositive [MuSK(+)] patients, compared to other major immunological subtypes of MG. These aspects include clinical parameters, e.g. health-related quality of life, neurophysiology and muscle morphology. Additionally, we wanted to elucidate if disturbed neuromuscular transmission in a limb muscle in ocular MG predicts the risk of generalization.
Myasthenia Gravis

History

Historical reviews start with the description from the English physician Thomas Willis in 1672 (Guthrie, 1903) but it was not until 1895 that the German neurologist Friedrich Jolly proposed naming this newly discovered neurological disorder myasthenia gravis (Jolly, 1895). Patients were reported to present with abnormal muscle fatigability, the most prominent signs being the inability to swallow and to articulate. He suggested the full name “Myasthenia gravis pseudo-paralytica” and this name was accepted after a consensus meeting of the Berlin Society of Psychiatry and Neurology in 1899 (Viets, 1953).

The cause underlying MG has been an area of debate. In 1960 a Scottish neurologist, Simpson, suspected that MG might be caused by an autoimmune mechanism. This suspicion was based on the relatively high incidence of concomitant autoimmune diseases (such as rheumatoid arthritis and systemic lupus erythematosus) among the patients (Simpson, 1960). Thymus abnormalities were discovered, as well as the presence of lymphorrages in muscles, which further supported the autoimmune hypothesis (Miller, 1961). Further, a humoral factor was implicated in the development of MG, after it was found that approximately 20% of babies whose mothers were diagnosed with MG were reported to develop transient neonatal MG (Strickroot, 1942).

In 1934, patients were found to be hyper-reactive to d-tubocurarine (Bennett and Cash, 1942). Subsequently, it was demonstrated that the muscle contraction of MG patients declined with repetitive nerve stimulation of the afferent nerve (Harvey and Masland, 1941). Further, both miniature and regular end plate potentials were found to be reduced when measured using micro electrophysiological techniques (Elmqvist et al., 1964), which localized the defect to the neuromuscular junction. In the 1970s, when the immunization of animals with acetylcholine receptor (AChR) was reported to induce an autoimmune response to the AChR of mammalian skeletal muscles, the autoimmune hypothesis was accepted as a theory (Heilbronn et al., 1975; Heinemann et al., 1977; Patrick and Lindstrom, 1973). This finding indicated that the target in MG was located more precisely at the postsynaptic membrane. Analysis of AChR antibodies (AChR-Ab) became an important diagnostic tool since MG patients often had elevated titers of this receptor antibody (Lindstrom et al., 1976). Further, it was reported that maternal
AChR-Ab titer correlated with the frequency and severity of transient neonatal MG present in the infant (Eymard et al., 1989).

Patients who did not possess elevated AChR-Ab were called seronegative [AChR(-)]. There were indicators that seronegative MG also had an autoimmune etiology. These indicators included: 1) neonatal transient MG in babies of women with seronegative MG (Mier and Havard, 1985), 2) improvement with immunotherapy and 3) disruption of neuromuscular transmission when mice were exposed to patient sera (Mossman et al., 1986). In 2001, 70% of AChR(-) MG patients were reported to have serum antibodies against MuSK at the postsynaptic membrane (Hoch et al., 2001). Studies of the remaining seronegative MG patients have shown that their plasma reduced AChR function (Plested et al., 1998), although the exact target has not been determined. This target, as well as the triggering factor of MG, has yet to be established.

Epidemiology

For the past 50+ years, population-based studies on the epidemiology of MG have shown a clear trend toward an increase in the reported prevalence of the disease. This increase is most likely due to more sensitive diagnostic methods, as well as to higher survival rate, due to better treatment regimens. The prevalence of MG is twice as high in women, compared to men. Typically, affected women are 20-30 years old, whereas men are 60-80 years old (Osserman and Genkins, 1971). The annual incidence of MG has been reported to be about 3-4 cases per million and the prevalence about 60 cases per million (Araki et al., 1987; Oosterhuis, 1981; Storm-Mathisen, 1984); however, higher rates have been reported. For example, in the USA, current estimates indicate a prevalence as high as about 20 per 100,000 persons (Phillips, 2003). Further, the prevalence of MG was recently estimated to be 14 cases per 100,000 in Stockholm, Sweden (Kalb et al., 2002).

Pathophysiology

Types of antibodies

Acetylcholine receptor antibodies

About 80% of patients with generalized MG and 55% of patients with ocular MG have AChR-Ab (Lindstrom et al., 1976; Sanders, 2003; Vincent and Newsom-Davis, 1985). These antibodies impair AChR function by three main mechanisms: 1) blocking of the ACh-binding site (Lefvert et al., 1981)
2) cross-linking of the AChRs on the postsynaptic membrane, which results in a functional blockade of the receptors as well as accelerated degradation of the AChRs (Drachman et al., 1981) and 3) complement activation, resulting in destruction of the postsynaptic muscle membrane (Engel et al., 1977). The consequence of these events is less efficient neuromuscular transmission.

**Muscle specific tyrosine kinase antibodies**

Quite recently it was discovered that among the 20% of patients who are AChR(-), 40-70% are seropositive for antibodies directed against MuSK, which is located at the neuromuscular junction (Bartoccioni et al., 2003; Hoch et al., 2001; Sanders et al., 2003; Scuderi et al., 2002; Zhou et al., 2004). MuSK is assumed to be essential in the early development of the neuromuscular junction as well as in maintaining the postsynaptic apparatus (Liyanage et al., 2002). During development of the neuromuscular junction, neural agrin activates MuSK, and this induces clustering of AChRs (Hopf and Hoch, 1998). These clusters are necessary for a normal neuromuscular transmission. It has been questioned whether MuSK-Abs are responsible for the weakness found in AChR(-) MG patients (Selcen et al., 2004; Vincent et al., 2005). Nevertheless, their pathogenic role has recently been confirmed. Rabbits that were immunized with MuSK ectodomain protein manifested MG-like muscle weakness. Additionally, a decremental response was elicited in the facial nerve and there was a significant reduction of AChR clustering at the neuromuscular junctions. The produced MuSK-Abs specifically inhibited in vitro AChR clustering (Shigemoto et al., 2006). In animal experiments, MuSK knockout mice fail to cluster AChRs and to differentiate postsynaptic regions, therefore dying perinatally (DeChiara et al., 1996).

**Titin and ryanodine receptor antibodies**

MG patients with a thymoma may have antibodies not only directed to the AChR but also to other components of striated muscle. Two of these components strongly associated with a thymoma are the sarcomeric cytoskeletal protein titin (Aarli et al., 1990) and the Ca^{2+} release channel of the sarcoplasmic reticulum, the ryanodine receptor (Mygland et al., 1992). On the basis of their cross-striational pattern by immunofluorescent staining (Peers et al., 1977), they have been named antistrriational antibodies.
Rapsyn antibodies

A fifth antigen is the small intracellular AChR-associated protein, rapsyn, which is located in the muscle end plate. Antibodies directed against rapsyn have been detected in about 15% of MG patients (Agius et al., 1998), both in AChR-Ab seropositive [AChR(+)] and AChR(-) patients. Rapsyn is precisely colocalized with AChR from the earliest stages of synapse formation (Hall and Sanes, 1993). Analysis of rapsyn knockout mice proved that rapsyn is, as well as MuSK, necessary for clustering AChRs (Gautam et al., 1995).

Clinical picture of MG

MG is characterized by an increased fatigability of skeletal muscles, predominantly affecting the proximal muscles. Muscle weakness fluctuates during the day, becoming more pronounced in the afternoon/evening and with physical activity. When extraocular muscles are involved, conditions such as ptosis and/or diplopia occur. Additionally, bulbar muscle involvement leads to dysphagia and dysarthria. Proximal arm and leg muscles as well as neck muscles are also involved in most patients. Muscle wasting is uncommon in MG, but has been reported in a few MuSK-Ab seropositive MuSK(+) patients (Evoli et al., 2003) as well as in about 10% of AChR(+) patients (Oosterhuis and Oosterhuis, 1997).

Types of myasthenia gravis and myasthenic syndromes

1. Acquired (autoimmune) myasthenia gravis (MG) is the most common and develops at any age after birth. This type of MG may be further divided into the following subtypes:
   - Pure ocular MG (OMG): Fatigue is limited to the extraocular muscles, i.e., the levator palpebrae muscle and the eye moving muscles. This form may develop at any age. Generalization may occur within two years from onset.
   - Early onset generalized MG: Develops before the age of 40 years.
   - Late onset generalized MG: Starting after the age of 40 years.
   - MG with thymoma: May develop at any age.

2. Neonatal myasthenia gravis affects newborn babies of myasthenic mothers and is caused by circulating antibodies from the mother via the placenta.

3. Congenital myasthenia includes syndromes that are due to deficiency of structures pre-synaptic (e.g. choline acetyltransferase), intra-synaptic (e.g. end plate ACh esterase) or postsynaptic (e.g. rapsyn).
4. *Lambert Eaton myasthenic syndrome (LEMS)* is an acquired autoimmune disorder, which causes muscle fatigue, loss of tendon reflexes at rest and autonomic dysfunction. LEMS can occur in a paraneoplastic form usually associated with small cell lung cancer or in a non-paraneoplastic form. In the majority (90%) of patients, antibodies to voltage-gated calcium channels (of P-/Q-type) are detected (Lang et al., 1998).

**Diagnosis**

**Serum analysis of autoantibodies**

The presence of autoantibodies in MG is of great importance for guidance to the correct therapy in individual patients. The two types of antibodies presented in this section have been of most importance to this study.

*Anti-AChR antibodies*

AChR-Abs are pathogenic and highly specific for MG. They are present in about 85% of patients with clinical features of myasthenia gravis (Vincent and Newsom-Davis, 1985). Therefore, this analysis adds important diagnostic information. The binding of AChR-Abs (IgG type) is measured using a standard radioimmunoassay in which the antigen consists of AChR from human muscle labeled with $^{125}\text{I}$-bungarotoxin (Lefvert et al., 1978; Lindstrom, 1977).

*Anti-MuSK antibodies*

The presence of MuSK-Abs is important for the diagnosis of MG in patients where AChR-Abs are absent, since some of these patients present a clinical picture of severe bulbar symptoms (Evoli et al., 2003), which calls for aggressive immunosuppressive treatment. The existence of MuSK-Ab (IgG type) can be determined by the immunoprecipitation of native MuSK extracted from TE671 plasma membranes (Scuderi et al., 2002). Alternatively, MuSK-Ab presence can be ascertained by immunoprecipitation of recombinant $^{125}$I-MuSK extracellular domains (McConville et al., 2004).

**Neurophysiology**

At the neurophysiological level in the diagnostic process, tests that show impaired neuromuscular transmission are essential for diagnosis.

*Repetitive nerve stimulation*

In MG, a progressive decline in the compound muscle action potential (CMAP) amplitude is found using 3 Hz repetitive nerve stimulation (RNS),
which is usually most pronounced at the 4th or 5th response (Desmedt, 1973). This decrement is due to the “run down” of the amplitude of individual end plate potentials. In MG, a certain proportion of potentials is reduced to a subthreshold level and therefore is insufficient to give a depolarization of the muscle fiber. Since the CMAP constitutes the sum of activated muscle fibers, its amplitude is successively reduced with an increasing block of individual muscle fibers. If the drop in amplitude, or decrement, exceeds a certain limit, e.g. 5%, the finding is considered pathological. RNS is first performed at rest and then after 20 seconds of maximal voluntary activity, which investigates post-exercise facilitation. New tests after 1, 3 and 5 minutes explore post-exercise exhaustion.

**Single-Fiber Electromyography**

Single-fiber electromyography (SFEMG) can be performed during voluntary muscle contraction or when the muscle is electrically stimulated. SFEMG has become the standard diagnostic tool of MG; (Ekstedt and Stålberg, 1963; Ekstedt and Stålberg, 1975; Stålberg, 1980; Stålberg and Trontelj, 1994). This method detects subclinical defects in neuromuscular transmission and can be used to quantify the degree of neuromuscular disturbance. SFEMG jitter reflects the safety factor of the motor end plate (Stålberg and Trontelj, 1994). When jitter measurements are made in voluntarily activated muscle, activity from two muscle fibers innervated by the same axon is recorded and one action potential used as a time reference. The jitter then results from variations in the difference in conduction times taken by impulses from the nerve branching point via the motor end plates along each muscle fiber to the recording site. Most of the jitter results from problems at the neuromuscular junction. Additionally, by counting the number of single muscle fibers from one motor unit within the uptake area of the electrode at many electrode positions, a measure of the mean fiber density within a motor unit can be calculated.

**Quantitative electromyography**

For motor unit potential (MUP) analysis and for the analysis of the electromyography (EMG) pattern at strong contraction, quantitative methods have been developed and are a part of the EMG routine investigation (Stålberg et al., 2003). Quantitative electromyography (QEMG) is performed with a concentric needle electrode in the muscle at rest, slight activation and strong contraction. If a differential diagnosis is suspected in MG, such as myopathy, QEMG is performed.

**Other neurophysiological techniques**

While there are established neurophysiological methods for diagnosing MG, some other techniques have been suggested.
Some departments perform *intracellular recordings*, where microelectrodes are inserted into individual muscle fibers to record the membrane potential and/or to pass current. Synaptic currents can thereby be recorded using a two-electrode patch-clamp configuration (Slater et al., 1992).

Infrared reflection *oculography* is sometimes undertaken to study eye movement recordings of saccades when ocular MG is suspected. The diagnostic yield of this examination is however not as high as the other neurophysiological techniques (Oey et al., 1993).

The *stapedius reflex* decay, in response to a one-minute sound stimulus of 500 Hz, is analogous to the decremental response of muscle action potentials to rapid nerve stimulation. This represents another test for the diagnosis of MG (Warren et al., 1977).

**Muscle morphology**

*Muscle biopsy*

A muscle biopsy is occasionally taken in MG to rule out other disorders such as mitochondrial or inflammatory myopathies. In MG there are few specific alterations to detect at the light microscopic level, such as some atrophic type II fibers and the appearance of minor lymphocyte infiltrates due to an immunological reaction (Kalimo and Stålberg, 2003).

In motor point biopsies, the mean number of AChRs at the neuromuscular junction is significantly lower in MG patients compared to healthy controls (Pestronk et al., 1985). Electron microscopy has visualized the deposition of the membrane attack complex on the motor end plates, where the normal folding of the junctional muscle membrane is markedly reduced and AChRs are lost (Engel et al., 1976). However, motor point biopsies are too difficult to be used in routine diagnostic examination of MG patients.

The differential diagnosis of MG vs. other neuromuscular disorders in muscle biopsies relies on the presence of structural alterations that are absent or uncommon in MG. For example, in mitochondrial myopathies one frequent finding is the presence of cytochrome C oxidase (COX) negative fibers, which indicates impaired function of complex IV in the mitochondrial respiratory chain. Ragged red fibers, i.e. fibers – usually COX negative – with increased numbers of abnormal mitochondria, are commonly seen in diseases due to translation defects, especially in large-scale deletions of the mitochondrial DNA which impinge on the three genes coding for subunits of COX. On the other hand, even though there may be lymphocyte infiltrates in MG, necrotic and/or degenerative alterations of myositis are absent.
Clinical neurological examination

The clinical examination of MG patients is important and should include an assessment of muscle strength at rest and after repeated exercises, to determine fatigability. Specific muscle groups to be examined include the limbs, neck, face and bulbar region. Muscle fatigue is provoked by performing repetitive muscle strength testing or prolonged tonic contraction. This fatigue is quantified, e.g., as time until onset and/or worsening of ptosis during upward gaze. Almost all patients have weakness of ocular muscles evident upon close examination (Sanders, 2003). Palatal weakness produces nasal speech. Weakness of the laryngeal muscles results in impaired speech and weakness of the jaw muscles may cause difficulties holding with jaw closure. The deltoid muscle is most often involved in the upper limbs and may be evaluated by arm abduction and repeated straight leg raises can test thigh/hip muscles. Finally, neck flexor and neck extensor muscles are evaluated.

To measure the degree of impairment, a quantitative MG score is calculated (Barohn et al., 1998).

Edrophonium (Tensilon) test

Edrophonium is a fast- and short lasting drug that inhibits acetylcholinesterase, allowing for increased availability of acetylcholine (ACh) at the neuromuscular junction, and so enhances neuromuscular transmission. Most MG patients respond to edrophonium within two minutes, which results in an increase in muscle strength. The test is considered positive if strength improves in muscles that can be objectively assessed such as the ocular and bulbar muscles. The effects of edrophonium have usually dissipated within five minutes. Possible adverse reactions include epigastric distress, sweating and muscle fasciculations. The edrophonium test is positive in 60-95% of ocular myasthenia patients and in 72-95% with generalized MG (Pascuzzi, 2003). However, improvement after edrophonium has also been found in other conditions such as congenital myasthenic syndromes, LEMS, intracranial aneurysms, brainstem lesions, tumors located in the cavernous sinus and sphenoid ridge, end-stage renal disease and in other muscle diseases affecting the ocular muscles including mitochondrial myopathy (Ajtai et al., 2004; Ben Yaou et al., 2006; Dirr et al., 1989; Moorby et al., 1989).
Treatment of MG

Treatment of MG can be divided into symptomatic and disease-modulating (immunosuppressive) treatment.

Symptomatic treatment

Acetylcholine esterase inhibitors (AChEI) improve neuromuscular transmission, especially at MG onset, providing short-term symptomatic relief of muscle weakness. This medication has been used since 1935, when Walker reported the beneficial effect from Prostigmin (Walker, 1935). The most common form is pyridostigmine bromide (Mestinon). In some patients, typically those with purely ocular weakness, this may be sufficient. The usual starting dose of pyridostigmine bromide is 60 mg every 4 to 6 hours. The recommended maximum dose is 120 mg every 3 hours. Additionally, neostigmine bromide is a longer-acting agent for intramuscular administration, although not very commonly used.

Thymectomy and immunosuppressive treatment

In patients with disabling ocular symptoms and/or generalized weakness, thymectomy should be considered before the age of 60. Plasma exchange, intravenous immunoglobulins or high-dose prednisone administration is often performed when there is a crisis involving oropharyngeal or respiratory muscles, especially before thymectomy is performed. Other long-term immunosuppressive medications include azathioprin (Imurel), cyclosporine (Sandimmun) and the most recent medicine, mycophenolate mofetil (Cellcept). Being able to discontinue AChEI in immunosuppressed patients is a criterion of adequate immunosuppression (Sanders, 2003).

Acetylcholinesterase inhibitors and neurophysiology

After an injection of edrophonium normal jitter remains unchanged. In an abnormal potential pair, the degree of impulse blocking decreases or disappears and the jitter tends to return towards the normal value. If the patient is already on AChEI treatment the reverse edrophonium effect, namely increased jitter and more frequent blockings, can be observed in a muscle, which otherwise responds positively to the drug (Stålberg and Trontelj, 1994).

Extra discharges following the CMAP in a patient receiving treatment with AChEI, may signify an upcoming cholinergic crisis (van Dijk et al., 1996). Two possible mechanisms for their generation are: 1) prolonged contact of ACh with its postsynaptic receptor, which results in repetitive muscle membrane excitation or 2) backfiring of nerve action potentials due to the
stimulation of pre-synaptic ACh receptors (Besser et al., 1989; Okamoto and Riker, 1969).

Quality of life assessment

The patient’s self-assessment of his/her condition is becoming increasingly important, e.g., for the monitoring of specific treatments (Ware and Sherbourne, 1992). The Myasthenia Gravis Foundation of America (MGFA) has stated that in research and clinical trials on MG, in addition to clinical examination, a health-related quality of life (hrQoL) questionnaire has to be included (Jaretzki et al., 2000). To validate a questionnaire is to determine to which degree the self-assessment scores reflect a particular clinical question, such as general physical and mental health and disability related to a specific disease. The scores are required to correlate with particular clinical measures, such as physical examination and effect of treatment.

SF-36

The Short-Form 36-item questionnaire (SF-36) is the most commonly used generic patient oriented tool to assess a patient’s general health. It comprises eight separate question domains that together define physical and mental health clusters (Ware and Sherbourne, 1992). The SF-36 is accessible in many languages and has been validated in numerous countries. For the purpose of this study, the Swedish version of SF-36 was used (Sullivan et al., 1995).

Myasthenia Gravis questionnaire (MGQ)

A disease specific hrQoL questionnaire for MG patients was developed by Padua et al in Rome, Italy, to address each patient’s hrQoL (Padua et al., 2002). The MG questionnaire (MGQ) assesses how the degree of myasthenic symptoms affects patients’ every day life. This was initially validated in Italian; hence, the questionnaire had to undergo validation in Swedish in order to be applied to the Swedish patients.
General aim of the study

The first objective of this study was to determine the presence and degree of disturbed neuromuscular transmission in a limb muscle in the ocular form of MG to see whether this is predictive of generalization. We also wanted to elucidate whether disturbed neuromuscular transmission in a limb muscle correlates to the patient-oriented findings.

The second objective was to characterize the main factors contributing to the muscular weakness and fatigue in MuSK(+) patients, compared to other major immunological types of MG. The parameters included were neurophysiological findings in proximal muscles, muscle pathology and health-related quality of life.

Specific aims

Study I
To determine if the jitter in the extensor digitorum (EDC) muscle in patients with purely ocular MG (OMG) predicts the subsequent development of generalised myasthenic weakness.

Study II
To translate and validate the disease-specific patient-derived MG questionnaire (MGQ), enabling its use among Swedish MG patients.

Study III
To correlate the self-assessed physical function score, using the MGQ and SF-36 questionnaires, with the degree of abnormal neuromuscular transmission, as measured by the most disease sensitive neurophysiological methods, i.e., SFEMG and RNS.
Study IV
To compare the electrophysiological and histopathological features, i.e., mitochondrial characteristics, as well as to assess the genetic background of the histopathological mitochondrial alterations, in different immunological subtypes of MG.

Study V
To describe the extra discharges occurring after the CMAP on motor nerve stimulation in a MuSK(+) patient with moderate dose of pyridostigmine bromide.
Patients and methods

Patients

All patients included in the study had a diagnosis of MG. The MG diagnosis was based on standard diagnostic criteria, including symptoms of fluctuating muscular weakness, supported by neurophysiological findings of disturbed neuromuscular transmission (for which no other cause could be found, such as reinnervation). Their MG status was classified into ocular, generalized and bulbar forms, according to the Myasthenia Gravis Foundation of America (MGFA) protocol for clinical classification (Jaretzki et al., 2000).

The ethical committee of the Duke University Medical Center approved the study and the ethical committee of Uppsala University Hospital approved studies II-IV. All patients in study II gave their informed consent, which included approval for the storage of biological specimens.

The first study group (study I) comprised 50 patients (14 women, 36 men, mean age 45, range 29-66 years) at the Duke University Medical Center MG Clinic, Durham, NC, USA. All patients fulfilled the following entry criteria: ocular MG and no treatment other than AChEI at the time of the initial SFEMG examination. These criteria excluded patients with any weakness present in oropharyngeal, limb or trunk muscles. The included patients were followed for \( \geq 2 \) years after MG onset. No patients were known to have MuSK-Ab.

The second group of patients (study II-IV) consisted of 50 MG individuals who were recruited from medical records at the Department of Neurology, Uppsala University Hospital. MG patients who repeatedly tested negative for AChR-Ab [AChR(-) patients; N = 14] were initially included. Additionally, 36 age-, sex-, and disease-matched AChR(+) patients were included for comparison. Altogether, 50 (45 women, 5 men, mean age 55.5, range 29-78 years) participated in the study. The duration of symptoms ranged from 1 to 57 years (mean 22.2 years). Two AChR(-) patients with a strong clinical suspicion of MG were included in the group of AChR(-) MG, although they initially did not have abnormal neurophysiological findings (including
SFEMG in the orbicularis oculi and extensor digitorum communis muscle). Forty-eight patients (45 women, 3 men), who chose to answer the patient-oriented questionnaires, were included in study II. Forty-five patients (42 women, 3 men) who had undergone SFEMG and RNS examination were included in study III in order to correlate patient-oriented measures with neurophysiological results. In study IV all 50 patients were included and each patient was subsequently examined on the same day. Evaluation of all patients in study IV was completed within a period of 3 months.

In study V one of the MuSK(+) patients (man, 75 years) was examined in further detail, since neurophysiological examination displayed extra discharges after the CMAP at low frequency stimulation, following the intake of AChEI.

Methods

Clinical neurological examination

Clinical examination was performed to assess the degree of weakness and fatigue of ocular, bulbar, neck and limb muscles (see table 1). Additionally, the quantitative score for disease severity, which ranges from 0 (absence of impairment) to 39 (maximal impairment) was calculated (Barohn et al., 1998). Clinical MG classification was performed according to the MGFA (Jaretzki et al., 2000) (see table 2). Subclass (a) indicates predominantly limb or axial muscle involvement, whereas subclass (b) indicates predominantly bulbar muscle involvement. Remission is further subdivided into complete stable remission (no medication) and pharmacological remission (immunosuppressive medication).
<table>
<thead>
<tr>
<th>Muscle groups</th>
<th>Test items</th>
<th>Test limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ocular</td>
<td>Upward gaze</td>
<td>120 sec</td>
</tr>
<tr>
<td>Bulbar</td>
<td>Speech or counting aloud</td>
<td>90 sec</td>
</tr>
<tr>
<td></td>
<td>Swallowing water</td>
<td>½ cup</td>
</tr>
<tr>
<td>Arms</td>
<td>Abduction 90°</td>
<td>90 sec</td>
</tr>
<tr>
<td>Hands</td>
<td>Alternating flexion and extension of fingers</td>
<td>70 times</td>
</tr>
<tr>
<td>Neck</td>
<td>Head lifts 45°(supine)</td>
<td>30 times</td>
</tr>
<tr>
<td>Legs</td>
<td>Repetitive leg lifts 70° (supine)</td>
<td>35 times</td>
</tr>
<tr>
<td>Trunk</td>
<td>Sitting upright from supine position</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Clinical neurological examination of MG patients (adapted from the original form used at the MG clinics in Stockholm, by Drs Matell and in Uppsala, by Dr Osterman). Each muscle group is tested initially for weakness and then exercise tests are performed in order to provoke fatigue. The test limit for each muscle group is displayed in column the column to the right. Each muscle group, except for trunk, is graded 0 (no impairment) to 4 (maximum impairment).

<table>
<thead>
<tr>
<th>Class</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Ocular weakness</td>
</tr>
<tr>
<td>II</td>
<td>Mild generalized weakness</td>
</tr>
<tr>
<td>III</td>
<td>Moderate generalized weakness</td>
</tr>
<tr>
<td>IV</td>
<td>Severe generalized weakness</td>
</tr>
<tr>
<td>V</td>
<td>Need of intubation</td>
</tr>
<tr>
<td>Remission</td>
<td>Absence of myasthenic symptoms for at least one year and lack of weakness on examination</td>
</tr>
</tbody>
</table>

Table 2. Clinical classification according to the MGFA.

28
Myasthenia Gravis Questionnaire

The disease specific MGQ consists of 25 items (Padua et al., 2002). The global MGQ score is obtained from the sum of these items; hence 0 for maximal impairment and 50 for absence of impairment. Some items, e.g., 1,3-5,16, do not assess regional muscle involvement, but are focused on such items as the timing of daily activity, fluctuations etc. Most items on the MGQ scale have 3 possible responses (0 = definite inability; 1 = partial inability; and 2 = ability). Other items evaluate the presence or absence of a deficit as a dichotomous categorical score with a yes or no answer, scored 2 for absence of deficit and 0 for deficit. Items are further divided into three symptom domains: (1) generalized domain (GD; including items 2a-h, 6, 8-12); (2) bulbar domain (BD; including items 7 and 15) and (3) ocular domain (OD; including items 13,14). Each domain score of the MGQ is obtained from the average of the involved items, hence 0 for maximum impairment and 2 for absence of impairment (Padua et al., 2005). For specifics, see appendix 1 in paper II.

SF-36

We used the SF-36 Standard Swedish Version 1.0 - 3/94 questionnaire (IQOLA, QualityMetric, Lincoln, USA). This form includes 36 questions and one multi-item scale measuring eight health concepts:

1) **physical functioning (PF)**, i.e., the ability to perform vigorous, moderate or light physical activities
2) **role limitations due to impaired physical health (RP)**, i.e., accomplishing less or cutting down time spent on work or leisure activities
3) **bodily pain (BP)**, i.e., interference of pain with normal activities
4) **social functioning (SF)**, i.e., the extent that health problems interfere with normal social activities
5) **general mental health (MH)**, ranging from happy and peaceful to sad and “down in the dumps”
6) **role-emotional (RE)**, i.e., limitations in work or other activities due to impaired mental health
7) **vitality**, i.e., including subjective energy level and tiredness
8) **general health (GH)**, i.e., rating the overall health in the range of poor to excellent.

The scores from these domains are summarized in two main scores: the physical composite score (PCS) and mental composite score (MCS). The PCS is mainly comprised of PF, RP, BP and GH whereas the MCS incorporates mainly vitality, SF, RE and MH. Very low scores for PCS indicate
severe physical dysfunction, distressing bodily pain, frequent tiredness and an unfavorable health status, while low scores for MCS indicate frequent psychological distress and severe social and role disability due to emotional problems.

Single-Fiber Electromyography

SFEMG was performed during voluntary muscle activation in 47 patients. Jitter was calculated as the mean of consecutive interpotential interval differences (MCD) (Stålberg and Trontelj, 1994). A study was considered abnormal if either of the following criteria were met: (1) the mean MCD exceeded the normal limit for the respective muscle (frontalis > 32 µs, EDC > 34 µs, orbicularis oculi > 40 µs, deltoid > 33 µs) or (2) more than 10% of fiber pairs had MCD greater than the upper limit for individual fiber pairs (frontalis > 45 µs, EDC > 55 µs, orbicularis oculi > 55 µs, deltoid > 45 µs) (Gilchrist, 1992). Fiber density (FD) was also measured. An increased FD usually reflects neurogenic fiber type grouping, but is also seen as a result of a myopathic reorganization of the motor unit. Normal FD for orbicularis oculi muscle is < 1.7 and for the deltoid muscle < 1.6

Repetitive nerve stimulation

RNS was performed on the axillary nerve (recording from the deltoid muscle) and on the accessory nerve (recording from the trapezius muscle) in 48 consenting patients. Stimulation (3 Hz) and recordings were made with surface electrodes according to standard protocols, with the patient sitting relaxed and the stimulus strength being 25% above the giving maximal response (supramaximal). RNS was considered positive if the decrement (1\textsuperscript{st} to 4\textsuperscript{th} response) of the CMAP was > 5%.

Analysis of AChR antibodies

All assays of binding AChR-Ab levels were performed by Dr. Lefvert at the Karolinska Institute Immunology research laboratory using a standard radioimmunoassay (Lefvert et al., 1978). This method has been used for the first diagnostic evaluation of MG, prior to drug treatment and thymectomy. Patients with AChR-Ab titers below the normal reference value of 0.2 nmol/L were regarded as AChR(-). The previously verified seronegativity for AChR-Ab in the 14 AChR(-) patients was ascertained with a new analysis. Furthermore, the seropositivity for AChR-Ab was verified in patients
who were also seropositive for MuSK-Ab. The analysis was not repeated for patients who had previously tested positive for AChR-Ab.

Analysis of MuSK antibodies

Presence of MuSK-Ab was determined by immunoprecipitation of native MuSK, extracted from TE671 plasma membranes, as previously described (Scuderi et al., 2002). Sera from 50 Swedish patients were assayed and analyzed. The test tubes were coded so that the testing laboratory was blinded to the patient’s clinical status. The results were expressed as positive (“+” for slight, “++” for moderate, and "+++" for strong positivity) or negative for MuSK-Ab [MuSK(-)].

Analysis of titin and ryanodine receptor antibodies

All patients who were found to be MuSK(+) and MuSK(-)/AChR(-) were tested for concomitant antibodies directed against titin and the ryanodine receptor. This analysis was performed in Bergen, Norway, as previously described (Mygland et al., 1992; Skeie et al., 1995).

Muscle biopsy

Technique

The deltoid muscle was selected for biopsy since the neurophysiological examinations targeted this muscle and it is often affected in MG. For example, significantly reduced acetylcholine receptors of the deltoid muscle have been demonstrated in AChR(+) MG patients, even when this muscle is clinically unaffected (Pestronk et al., 1985). Open biopsy was considered an uncomfortably invasive procedure; thus, the conchotome biopsy technique was the method of choice. The biopsy was performed under local anesthesia (Xylocain, Astra, 10 mg/ml). After a 1 cm incision had been made, the biopsy was taken by means of Weil Blakesley forceps (Henriksson, 1979). Biopsy was performed in 41 consenting patients immediately after neurophysiological examinations in an adjacent part of the muscle, to avoid EMG needle artifacts in the specimen. The biopsied tissue was frozen in isopentane chilled with liquid nitrogen and stored at -80°C until further processed.
**Histochemical stainings and cytochrome C oxidase negativity**

Frozen cross-sections were stained with hematoxylin and eosin and modified Gomori’s trichrome. The sections were also stained for myofibrillar ATPase with preincubations at pH 10.4 and 4.3, counterstained using the Herovici method and additionally stained for NADH-tetrazolium reductase (NADH-TR), as well as double reacted for COX and succinate dehydrogenase (SDH) (Dubowitz, 1985). The percentage of COX-negative/SDH positive fibers was counted. The presence of any COX negative fibers in patients under the age of 40 years can be regarded as abnormal (Pesce et al., 2001). Even as a possible aging phenomenon, COX negative fibers are not usually seen until after the age of 50 years. Therefore, COX negative fibers were considered as an indication for further molecular studies. Muscle specimens with less than 0.1 % COX negative fibers were interpreted as normal variants, since this frequency of COX negative fibers can occasionally be seen in middle-aged subjects (Kalimo, unpublished observations).

**Size distribution of muscle fibers**

The size distribution of type I and II myofibers was measured using a computerized muscle biopsy analyzer (Muscle Biopsy Surveyor®; PIT Oy, Turku, Finland) according to the “lesser diameter” principle (Brooke and Engel, 1969). The atrophy and hypertrophy factors were calculated for both fiber types. The diameters of at least 800 fibers were measured in each biopsy. We used the reference values reported for normal adult male and female biceps brachii muscle as the upper limits of atrophy and hypertrophy factors. The upper limits for the atrophy factors for type I/type II fibers were 150/150 (male) and 100/150 (female), whereas the upper limits for the hypertrophy factors for type I/type II fibers were 300/500 (male) and 200/150 (female) (Brooke and Engel, 1969).

**Mitochondrial DNA analysis**

Mitochondrial DNA (mtDNA) analysis was performed altogether on biopsy samples from 25 patients [3 MuSK(+)/AChR(+), 2 MuSK(+)/AChR(-), 8 MuSK(-)/AChR(-), 12 MuSK(-)/AChR(+)]. Twenty-three of these samples contained COX negative/SDH positive fibers. Total muscle DNA was extracted using the standard phenol-chloroform method. MtDNA deletion analysis was performed using the long-PCR method as previously described (Luoma et al., 2005) with certain modifications (see paper IV).

The presence of multiple mitochondrial DNA (mtDNA) deletions was evaluated visually using agarose gel electrophoresis. Since mutations in mitochondrial polymerase γ (POLG), encoded by the nuclear gene POLG1, are a
common cause of multiple mtDNA deletions, the coding sequence of POLG1 and its intron/exon boundaries were amplified by PCR and sequenced using primers and conditions previously described (Luoma et al., 2005; Van Goethem et al., 2001). The positive control consisted of muscle DNA with multiple mtDNA deletions, from a patient who was previously diagnosed with autosomal dominant progressive external ophthalmoplegia. As a negative control, muscle DNA from a healthy 50-year-old female was used.

Statistical analysis

Comparisons between the ocular MG outcome group and the patients who developed generalized MG were made using the Wilcoxon rank sum test, if the variable of interest was continuous. Fisher’s exact test was applied if the variable was categorical. With the Spearman rank correlation, the relationship between the percentage of fiber pairs with increased jitter in the EDC muscle and time from MG onset to generalization was characterized. When ordinal and nominal scales, e.g., SF-36, were measured, the non-parametric Spearman’s rank correlation also assessed the correlation between the hrQoL score and neurophysiological findings. It was also used in determining the correlation of the subjective assessments of SF-36 and MGQ as well as correlation between different SFEMG parameters. Mean values of SF-36 scores from MG patients and normative Swedish data were compared with one-sample-T test. A p-value < 0.05 was considered significant.

The impact of several potential predictors on generalization was examined with a univariate logistic regression analysis. A p-value < 0.05 was considered significant. The internal consistency of the MGQ, i.e., the equivalence of responses within the same test from a single administration, was assessed using Cronbach coefficient alpha. Reproducibility was tested with Spearman-Brown reliability coefficient. Comparing the MGQ scores with the SF-36 and the clinical score using a Mann-Whitney test tested the construct validity. A p-value < 0.05 was considered significant.

In order to compare neurophysiological abnormality (as measured by SFEMG in the orbicularis oculi muscle) and patient perspective (as measured by ocular domain score) in the same regional muscles, a 2x2 table Chi square test was applied. The Chi square test was also used to determine if myopathy was present to a higher degree in MuSK(+) patients than in MuSK(-)/AChR(+) patients. A p-value < 0.05 was considered significant. The significance of co-occurrence of COX activity and mtDNA deletion status was calculated with Fisher’s exact test.
The following hypothesis was generated in order to draw conclusions from the validation of the Swedish MGQ: patients with more severe fatigue (higher quantitative MG score and higher MGFA class) would score lower.
Results

Predictive value of SFEMG in a limb muscle for generalization of MG (Study I)

Time to generalization
Of the 50 OMG patients, 26 developed generalized MG (GMG). The median time from OMG onset to new generalized symptoms was 1.6 years (range 3 months to 16 years) and from SFEMG examination to GMG development 10 months (range 0.5 months to 9 years).

Presence and degree of abnormal SFEMG findings
The frequency or degree of abnormal SFEMG results did not differ between the patients who remained ocular and those who became generalized. The mean MCD ranged from 24 to 74 µsec (median 38.5 µsec) in the OMG group and from 22 to 73 µsec (median 40.0 µsec) in the patients who later acquired generalized MG.

Other possible predictive tests
Elevated AChR-Abs were found more often in the patients who had GMG. Furthermore, an odds ratio of 1.24 (p = 0.59) indicated no predictive value for the generalization of MG according to the sum of positive diagnostic tests (edrophonium test, AChR-Ab analysis, SFEMG in the EDC muscle, SFEMG in the frontalis muscle).
Health-related quality of life findings in Swedish MG patients and validation of the Swedish MG Questionnaire (Study II)

Translation and cultural adaptation
Translation of the Italian MGQ to Swedish was successful and the back-translation to Italian corresponded well with the original version. The items of the Swedish MGQ were concluded to have relevance to everyday life in Sweden. Only one minor cultural adaptation was undertaken; “taking a bath” was modified to “taking a shower”, which is common in Sweden. Furthermore “bocchia”, which is a common sport in Italy, was substituted with “walking in the forest or gardening” which is a common leisure activity in Sweden and also a part of the Swedish SF-36.

Descriptive of the Italian sample
The Italian sample of 41 patients consisted of 19 women and 22 men, whereas the Swedish sample of 48 patients included 45 women and 3 men. The two samples did not significantly differ in the parameters age, MGFA class or global MGQ score.

Scores of the Swedish sample
Mean quantitative disease score for the Swedish population ranged from 0 to 14 (mean 3.8; SD 3.9), indicating overall mild to moderate disease. Mean values for the global MGQ score was 37.8 (SD 10.1), for GD score 1.61 (SD 0.4), for BD score 1.55 (SD 0.6) and for OD score 1.63 (SD 0.6).

The scores of the SF-36 domains compared to healthy Swedish population are displayed in table 3. Ratings provided by the MG patients were lower, on average, than the normative data on six of the eight domains (higher score indicate better functioning). The SF-36 parameters that were graded significantly lower by MG patients compared to control subjects were physical functioning (p = 0.001) as well as role physical, general health and vitality (p < 0.05). Mean ratings on the mental health domain and the bodily pain domain were almost identical between the two groups. Mean score for PCS was 45.1 (SD 10.1) and for MCS 52.8 (SD 9.9).
Correlation of MGQ and SF-36 scores

The global MGQ score correlated with the PCS of the SF-36 (R= 0.83; p < 0.001), but not with the MCS of SF-36. Further, the global MGQ score correlated with the clinical score (R= –0.59; p < 0.001). The BD score correlated with both the clinical score and the scores of MGQ, whereas OD did not correlate with any of the other parameters.

Evaluation capacity of Swedish MGQ

The internal consistency of the MGQ was excellent (Cronbach’s alpha of 0.91), i.e. individuals responded consistently to the items within the MGQ. Also the reproducibility, measured with a Spearman-Brown test-retest analysis, was very good (correlation coefficient of 0.98), i.e. there was a good ability of the test to be accurately reproduced. Therefore, we conclude that the disease specific MGQ has an equivalent evaluation capacity in the two countries.

<table>
<thead>
<tr>
<th>SF-36 domain</th>
<th>MG patients</th>
<th>Normative Swedish data φ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean score (SD)</td>
<td>Mean score (SD)</td>
</tr>
<tr>
<td>PF (physical function)</td>
<td>76.0’ (23.4)</td>
<td>87.9 (19.6)</td>
</tr>
<tr>
<td>RP (role physical)</td>
<td>67.6’ (42.6)</td>
<td>83.2 (31.8)</td>
</tr>
<tr>
<td>BP (bodily pain)</td>
<td>75.4 (25.6)</td>
<td>74.8 (26.1)</td>
</tr>
<tr>
<td>GH (general health)</td>
<td>66.0’ (21.6)</td>
<td>75.8 (22.2)</td>
</tr>
<tr>
<td>vitality</td>
<td>60.9’ (19.4)</td>
<td>68.8 (22.8)</td>
</tr>
<tr>
<td>SF (social)</td>
<td>81.8 (22.2)</td>
<td>88.6 (20.3)</td>
</tr>
<tr>
<td>RE (role emotional)</td>
<td>81.6 (35.3)</td>
<td>85.7 (29.2)</td>
</tr>
<tr>
<td>MH (mental health)</td>
<td>80.8 (16.6)</td>
<td>80.9 (18.9)</td>
</tr>
</tbody>
</table>

Table 3. Mean score of the eight different domains of SF-36 for the Swedish MG patients. *Significant difference of mean values (p< 0.05); φ possible range 0-100. (Sullivan et al., 1995)
Correlation between patient-oriented findings and 
abnormal neuromuscular transmission in MG (Study III)

Outcome measures
The SFEMG abnormality in the deltoid muscle increased with disease severity, as measured by the MGFA clinical class and quantitative disease score. Patients in remission had high MGQ scores, with a mean of 43.7. Some of these patients had persistent abnormal jitter; however, to a lesser degree than patients with clinical fatigue. The degree of bulbar impairment was more accurately evaluated with the BD score than with neurophysiological examination.

Correlation between MGQ and neurophysiology
The percentage of abnormal jitter, as well as the mean MCD in the deltoid muscle, significantly correlated with the global MGQ and generalized domain scores (p < 0.01, Spearman R = -0.4). However, there was no correlation between global MGQ or generalized domain scores and SFEMG findings in the orbicularis oculi or RNS in the deltoid muscle. The OD score did not correlate with SFEMG findings in the orbicularis oculi muscle or with the neurophysiological abnormalities in the deltoid. SFEMG findings in the orbicularis oculi muscle were more abnormal than the corresponding report of ocular impairment from the patient in the OD (p < 0.01). The BD score did not correlate with the neurophysiological abnormality in the deltoid or in the orbicularis oculi muscle.

Correlation between SF-36 and neurophysiology
The percentage of abnormal jitter in the deltoid muscle correlated significantly with the physical composite score (p < 0.05; Spearman R = -0.3), the physical function domain (PF) (p < 0.01; Spearman R = -0.4) and the role physical function (RP) (p < 0.01; Spearman R = -0.4). PF also correlated with mean MCD in the deltoid.
Table 4. MGQ scores depending on MuSK(+) MG subtype. Median values of all mean scores within each group are presented, since the MGQ is an ordinal scale. Ranges of possible scores are displayed within brackets. * Significantly lower value compared to AChR(+) patients (p < 0.05).

In table 4 the scores of MGQ related to immunological subtypes are shown. The Mann-Whitney test demonstrated a significant difference in BD score between MuSK(+) patients and AChR(+) patients (p < 0.05), indicating subjective bulbar impairment that is enhanced among the MuSK(+) patients. The OD, GD and global MGQ scores did not differ significantly when MuSK(+) patients were compared to other immunological subtypes.
Comparison of neurophysiological, muscle biopsy and health-related quality of life parameters in MuSK(+), AChR(+) and AChR(-) MG patients (Study IV)

MuSK-Ab presence
Five of the 14 AChR-Ab seronegative [AChR(-)] patients were MuSK(+)/AChR(-) and five out of the 36 AChR-Ab seropositive [AChR(+)] patients were MuSK(+)/AChR(+). Patients 2, 8 and 9 who were initially AChR(+) had at the present analysis no detectable AChR-Ab, but were still considered to belong to the AChR(+) subtype. None of the analyzed MuSK(+) and MuSK(-)/AChR(-) sera had detectable antibodies directed against titin or the ryanodine receptor.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>MuSK(+) (n=10)</th>
<th>MuSK(-)/AChR(-) (n=9)</th>
<th>MuSK(-)/AChR(+) (n=31)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender: female/male</td>
<td>7 / 3</td>
<td>8 / 1</td>
<td>29 / 2</td>
</tr>
<tr>
<td>Age (yrs) mean [range]</td>
<td>63.5 [46-78]</td>
<td>57.9 [40-84]</td>
<td>53.9 [30-73]</td>
</tr>
<tr>
<td>Disease duration (yrs) Mean [range]</td>
<td>16.6 [0.5-55]</td>
<td>16.7 [2-33]</td>
<td>24.8 [1-57]</td>
</tr>
<tr>
<td>Post-thymectomy (# of pat)</td>
<td>6</td>
<td>6</td>
<td>27</td>
</tr>
<tr>
<td>Thymoma</td>
<td>1</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Hyperplasia</td>
<td>1</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>Normal thymus</td>
<td>4</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>Thymus inflammation</td>
<td>0</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Pyridostigmine treatment (# of pat)</td>
<td>5</td>
<td>4</td>
<td>15</td>
</tr>
<tr>
<td>Immunosuppressive medication (# of pat)</td>
<td>1</td>
<td>2</td>
<td>6</td>
</tr>
</tbody>
</table>

Table 5. Patient subtype characteristics. Immunosuppressive medication included cortisone, azathioprine and sendoxan.
Clinical findings

MuSK(+)/AChR(+) patients (n=5)
The severity of symptoms ranged from clinical remission (n=1) to severe arm muscle atrophy (n=1). There was no correlation between disease grade and level of MuSK-Ab or AChR-Ab. Patient 4 was AChR(+) at both occasions the analysis was performed, whereas the rest of the patients were AChR(+) at only one examination. Four patients had undergone thymectomy, one had hyperplasia and three had a normal thymus. Patient 4 had received immunosuppressive treatment (azathioprin) and patient 8 had current medication with azathioprin.

MuSK(+)/AChR(-) patients (n=5)
Symptoms ranged from clinical remission (n=1) to severe bulbar involvement (n=1). Also in this group, the overall MuSK-Ab levels did not correlate with the degree of symptoms. Out of the two patients in whom thymectomy was performed, one had a thymoma and one had a normal thymus. No patient received any immunosuppressive treatment.

MuSK(-)/AChR(-) patients (n=9)
The MGFA class ranged from pharmacological remission to mild generalized weakness. Six patients were thymectomized, see table 5 for further treatment information.

MuSK(-)/AChR(+) patients (n=31)
Disease severity ranged from remission (10 patients) to moderate generalized disease. Twenty-seven patients (87%) had undergone thymectomy, see table 5 for further details regarding treatment.

Neurophysiological findings
Table 6 displays the individual neurophysiological findings of MuSK(+) patients. For individual data of MuSK(+)/AChR(-) and MuSK(-)/AChR(+) patients, see table 1 in paper IV.

Three of five MuSK(+)/AChR(+) patients consented to neurophysiological examination. All had an abnormal decrement on RNS in the deltoid muscle and abnormal SFEMG findings in at least one of the examined muscles. Two patients had myopathic changes on QEMG, one severe and one slight changes.

Four of the five MuSK(+)/AChR(-) patients consented to neurophysiological examination. Abnormal RNS was not detected, whereas all exam-
ined patients had abnormal SFEMG findings in either the deltoid or orbicularis oculi muscle. QEMG displayed slight to moderate myopathic pattern in three patients (6, 7, 10; Fig. 1).

RNS was normal in all nine MuSK(-)/AChR(-) patients, whereas in seven patients SFEMG was abnormal. Slight to moderate myopathic pattern was observed in three patients.

Thirteen MuSK(-)/AChR(+) patients (42%) had abnormal decrement in the deltoid and/or trapezius muscle. Overall, 24 patients (77%) in this subgroup had abnormal SFEMG in either muscle. Slight to moderate myopathic pattern was found in seven (23%) patients, four of which had abnormal neuromuscular transmission.

Correlation of decrement, mean MCD and percentage of blockings

Among the MuSK(+) patients, there was no correlation between mean MCD and percentage of blockings in the orbicularis oculi muscle (Fig.2). On the contrary, mean MCD correlated to percentage of blockings both in MuSK(-)/AChR(-) patients (Spearman R=0.69; p < 0.05) and in MuSK(-)/AChR(+) patients (Spearman R= 0.85; p < 0.01).

In the deltoid muscle, the percentage of blockings correlated with mean MCD (Spearman R = 0.77; p < 0.05) in MuSK(+) patients, but not with the decrement, i.e. in some cases where the decrement was normal, the SFEMG was pathological. A similar picture was seen among the MuSK(-)/AChR(-) patients and a strong correlation between decrement, mean MCD and the percentage of blockings (p < 0.01) was seen in MuSK(-)/AChR(+) patients.
Comparison of myopathic pattern

Myopathic EMG pattern was present in five (71%) examined MuSK(+) patients, three (33%) MuSK(-)/AChR(-) patients and seven (23%) MuSK(-)/AChR(+) patients. In MuSK(+) patients, there was a significantly higher frequency of myopathic EMG pattern, compared with MuSK(-)/AChR(+) patients ($\chi^2 = 6.3; p=0.01$). In two MuSK(+) patients (4 and 7) myopathy was accompanied by reduced objective muscle strength before exercise test in that particular muscle. In the two other patients, there was fatigue but no initial weakness. Among the MuSK(-)/AChR(+) patients, there was fatigue in one patient and normal objective findings in the myopathic muscle. Among the MuSK(-)/AChR(+) patients, fatigue was found in the myopathic muscle of three patients, as determined by QEMG.

Figure 1. QEMG showing moderate myopathy of the splenius capitis muscle in MuSK(+) patient 7. Size index –0.13. The interference pattern displayed early recruitment of MUPs and low envelope amplitude.
Figure 2. SFEMG recording in the orbicularis oculi muscle in one MuSK(+) patient 9. A) The trigger is set on the second potential. Mean MCD for the first potential is 91 µs and for the third potential 107 µs. B) 10 consecutive traces are shown in raster mode. C) The ten traces superimposed.

Morphological and mitochondrial findings

Type II fiber atrophy was seen in four out of seven MuSK(+) patients (Fig. 3b). In patient 4 there was marked type I fiber hypertrophy, presence of ragged red fibers (Fig. 4b) and almost no type II fibers at all. A pathological inter-myofibrillar network and several COX negative fibers were present in all MuSK(+) patients (Fig. 3a & 4a). No signs of inflammation were found in any biopsies from MuSK(+) patients.

Among the nine MuSK(-)/AChR(-) patients, a pathological inter-myofibrillar network was seen in two patients, with COX negative fibers found in all patients, although in three patients < 0.1 % (considered within normal limits), and ragged red fibers in three patients. Signs of inflammation were observed in one patient.
Seven out of 26 MuSK(-)/AChR(+) patients had a pathological inter-myofibrillar network. COX negative fibers were present in 22 patients, three of whom had ragged red fibers. Inflammatory cell infiltrates were detected in seven patients.

For individual data regarding all immunological subtypes, please see table 1 in paper IV.

Mitochondrial DNA and POLG1 findings

Multiple mtDNA deletions (see article IV Fig. 2B) were found in 16 out of 25 assayed muscle specimens (64%). The frequency of COX negative fibers correlated with the presence of mtDNA deletions. The percentage of COX negative fibers and multiple mtDNA deletions correlated significantly (p < 0.01). The mean age of patients with mtDNA deletions was 64 years (range 42 – 84 years) and 46 years (range 30 – 62 years) for patients without deletions.

Multiple mtDNA deletions were detected in all tested serotypes, most frequently in MuSK(-)/AChR(-) patients, among whom six of eight (75%) patients had deletions. MtDNA deletions were seen in three of five (60%) MuSK(+)/AChR(-) patients and in seven of 12 (58%) MuSK(-)/AChR(+) patients. The coding sequence of POLG1, which was analyzed in 15 patients with mtDNA deletions, did not reveal pathogenic mutations. Nevertheless, specific polymorphisms were detected in a few of these patients.
<table>
<thead>
<tr>
<th>Pat</th>
<th>Age/ Sex</th>
<th>MuSK -Ab</th>
<th>Max. AChR -Ab (nM)</th>
<th>MGFA Class</th>
<th>Disease Duration (yrs)</th>
<th>QEMG Myopathy (yes/no)</th>
<th>SFEMG Orb.oc/delt</th>
<th>COX neg fibres (%)</th>
<th>Muscle biopsy at/ ht</th>
<th>N= no at/ht</th>
<th>MtDNA deletions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>78F</td>
<td>+</td>
<td>0</td>
<td>IIa</td>
<td>55</td>
<td>No</td>
<td>N/Abn</td>
<td>1.7</td>
<td>Type II at</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>62M</td>
<td>+</td>
<td>4.0</td>
<td>0</td>
<td>1</td>
<td>n.p</td>
<td>n.p</td>
<td>n.p</td>
<td>n.p</td>
<td>n.p</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>69F</td>
<td>+</td>
<td>0</td>
<td>15</td>
<td>n.p</td>
<td>n.p</td>
<td>n.p</td>
<td>n.p</td>
<td>n.p</td>
<td>n.p</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>67F</td>
<td>+</td>
<td>75</td>
<td>IIIa</td>
<td>43</td>
<td>Yes</td>
<td>N/Abn</td>
<td>1.0**</td>
<td>Type I ht</td>
<td>n.p</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>60F</td>
<td>+</td>
<td>1.0</td>
<td>I</td>
<td>15</td>
<td>n.p</td>
<td>n.p</td>
<td>n.p</td>
<td>Type II ht</td>
<td>n.p</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>65F</td>
<td>++</td>
<td>0</td>
<td>IIb</td>
<td>3</td>
<td>Yes</td>
<td>N/Abn</td>
<td>0.3</td>
<td>Type II ht</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>75M</td>
<td>++</td>
<td>0</td>
<td>I</td>
<td>18</td>
<td>Yes</td>
<td>Abn/N</td>
<td>0.9**</td>
<td>Type I at</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>47F</td>
<td>+++</td>
<td>40</td>
<td>IIa</td>
<td>11</td>
<td>N</td>
<td>Abn/Abn</td>
<td>0.5</td>
<td>Type II at</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>46F</td>
<td>+++</td>
<td>3.0</td>
<td>IIa</td>
<td>4</td>
<td>Yes</td>
<td>Abn/Abn</td>
<td>0.6</td>
<td>Type II at</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>75M</td>
<td>+++</td>
<td>0</td>
<td>IVb</td>
<td>0.5</td>
<td>Yes</td>
<td>Abn/Abn</td>
<td>1.1</td>
<td>Type II at</td>
<td>n.p</td>
<td></td>
</tr>
</tbody>
</table>

Table 6. Comparison of clinical classification, neurophysiological and morphological features among MuSK(+) patients. Pat= patient; F= female; M= male; nM= nanomol/liter (normal < 0.2 nM/L); MGFA= Myasthenia Gravis foundation of America; N= normal; Abn= abnormal; Orb.oc= orbicularis oculi muscle; delt= deltoid muscle; at= atrophy; ht= hypertrophy; MtDNA= mitochondrial DNA; n.p.= not performed. ** Presence of ragged red fibers.
Figure 3. Muscle biopsy from MuSK(+) patient 9. (a) Left: three COX- negative fibers (blue) in COX/SDH staining. The inter-myofibrillar network appears regular with only minor subsarcolemmal accentuation. (b) Right: type II fiber atrophy. Atrophic fibers are marked with asterisks. The atrophy factor was 325 (normal<150). ATPase pH 4.3 + Herovici counterstaining.

Figure 4. (a) Left: presence of a moth-eaten fiber, indicating pathological inter-myofibrillar network, in MuSK(+) patient 8. NADH-TR staining. (b) Right: a more widespread accumulation of pathologic mitochondria has resulted in so-called ragged red fiber, in MuSK(+) patient 4. COX/SDH double staining.
Extra discharges due to AChEI (Study V)

A patient with severe oculobulbar weakness, MGFA class IVB, had a high titer of MuSK-Ab (+++), and was initially treated with a moderate dose of pyridostigmine bromide (Mestinon®) 120 mg three times daily. During the initial motor neurography, extra discharges were observed after the motor response at low-frequency stimulation. In some nerves the discharges formed slow wave components with increasing intervals from 4 ms to 12 ms, with a duration of at least 50 ms, and with an amplitude of about one third of the CMAP. In other nerves there were irregular discharges of low amplitude, i.e., less than 0.5 mV, with durations of up to 100 ms. Both types of extra discharges disappeared at the second stimulus in a train of 3Hz stimulation. RNS showed a slight decrement of 13% in the anconeus muscle, but in the other examined muscles there was no decrement. SFEMG was abnormal in the EDC and orbicularis oculi muscles.

After a methylprednisolone infusion, the patient developed a respiratory crisis, resulting in the need for respiratory support and neck muscle support. When the patient recovered, a second neurophysiological examination was carried out before and after the injection of 10 mg edrophonium intravenously. The last pyridostigmine dose was taken 6 hours prior to examination. After the injection, extra discharges followed the CMAP and there was deterioration with weakness in the neck and bulbar muscles. Approximately nine weeks and five days after the subsequent discontinuation of pyridostigmine bromide, no extra discharges were seen following the CMAP and the patient demonstrated improvement to MGFA class IIB.
Discussion

Disturbed neuromuscular transmission in the EDC in OMG

The first main objective of this study was to characterize the presence and degree of disturbed neuromuscular transmission in a limb muscle in patients with purely ocular myasthenic weakness. The first aim determined that the presence of abnormal SFEMG in a limb muscle did not predict the subsequent generalization of MG. This proved true even for clinically unaffected muscles, such as the EDC, which had a decreased safety margin of neuromuscular transmission, e.g., abnormal jitter findings in the majority (75%) of OMG patients. Neither the presence nor the degree of this abnormality was related to a shift from ocular to generalized MG in patients with purely ocular involvement. Not only was the degree of jitter comparable between the two outcome groups, there was also no difference in the degree of impulse blockings.

It is intriguing that patients with generalized weakness as well as patients with only ocular weakness have the same amount of blockings in an arm muscle, since the degree of blocking determines whether clinical fatigue occurs. One possible explanation is that a lower degree of blocking in EDC in the ocular group gives merely subclinical limb fatigability. Thus, an abnormal electrophysiological finding in a muscle outside the facial area is not predictive of later clinical generalization and therefore clinical classification must be re-evaluated each time the MG patient is examined in the neurology clinic.

Further, while SFEMG abnormalities are common in the orbicularis oculi muscle and useful for diagnosis, they did not correlate to the degree of ocular dysfunction, nor did they reflect the general severity of MG. This fact was recognized earlier by the MGFA Task Force, resulting in the possibility to include patients with only slight ocular dysfunction in the remission classification (Jaretzki et al., 2000). Early recognition of those patients who will subsequently acquire generalized MG would be important from a therapeuti-
Health-related quality of life in Swedish MG patients

The hrQoL may be negatively influenced in MG patients by the persistent and fluctuating symptoms of muscle weakness. MG is a chronic disorder and although there are effective medications, most patients do not reach the same prior to MG level of function (Ochs et al., 1998). To achieve patient-oriented evaluations of MG patients in Sweden, it is essential to have a validated disease-specific questionnaire on a national basis. While a number of hrQoL instruments, such as the SF-36, have been developed for the general population, they are unlikely to detect small, clinically important changes in a particular disorder (Guyatt et al., 1986). Therefore, the MGQ will provide an important measure of the effects of a specific treatment on hrQoL.

The observed correlation in Swedish MG patients between the MGQ and SF-36, as well as clinical assessment, was comparable to the Italian version. The MGQ obtained an excellent internal consistency of 0.91 (Cronbach’s alpha). Suggested levels of reliability are 0.70 or greater for scales used in group-level analyses and 0.90 or greater for scales used in decisions at the individual level (Nunnally JC, 1994). When compared to normative data from the Swedish population (Sullivan et al., 1995), physical aspects of hrQoL is affected. However, the patient sample included mostly patients with mild MG and an extended study including more patients with moderate and severe MG should be conducted to cover the entire severity of the disease. For Swedish MG patients this brings the opportunity to participate in international clinical trials.

There tends to be a close link between a person’s perception of their mental and physical energy and their general feeling of well being (Wood, 1990). The significant correlation in study II of vitality as measured by SF-36 with global MGQ score, general domain score and bulbar domain score indicate that the subjective energy level increases when generalized and bulbar weakness decrease. This supports one earlier study where a notable interference in perceived energy, as measured by the vitality domain of SF-36, was detected in patients with generalized myasthenic weakness (Paul et al., 2000). Fatigue increases over the day when energy levels for most people fall and many MG patients state that their fatigue worsens during stressful periods; thus, these two measures seem to be linked. However, patients with MG are not affected by greater mood disturbances than are healthy control subjects.
Correlation between disturbed neuromuscular transmission and health-related quality of life

Secondly, we found there was a correlation between disturbed neuromuscular transmission and hrQoL. A proximal muscle, such as the deltoid muscle, seems important to examine with SFEMG in MG patients, since the degree of abnormal neuromuscular transmission correlated well with the patient-oriented global MGQ and generalized domain scores. Changes in jitter in a sentinel muscle also correlated with the changes in disease severity for most patients (Sanders, 2002); therefore, SFEMG in the deltoid may serve as a tool for therapy monitoring, as well as for diagnostic purposes. However, testing of a single muscle is not enough for diagnosis and follow-up of MG. A multidimensional evaluation of MG patients is important in everyday clinical practice, as well as in future clinical trials.

MuSK antibodies and AChR antibodies may coexist

MuSK-Abs were present in 36% of the examined AChR(-) patients, which is consistent with previous studies (Evoli et al., 2003)(Hoch et al., 2001)(Sanders et al., 2003). However, the discovery of the presence of MuSK-Abs in 14% of the examined AChR(+) patients is noteworthy. While a "MuSK-Ab" had been reported in AChR(+) MG by Ohta (Ohta et al., 2004), this antibody was subsequently found to be directed against alkaline phosphatase (Ohta et al., 2005). Thus, our report is the first of its kind to show that MuSK-Ab and AChR-Ab do coexist in a group of MG patients. Additionally, MuSK-Abs were detected in OMG, which supports a previously reported case study (Caress et al., 2005). Patients 2, 8 and 9 who were AChR(+) at the initial diagnostic antibody analysis proved to be MuSK(+)/AChR(-) at the previous analysis. One possible explanation for this is epitope-spreading, i.e. that the immunological attack against AChR has been expanded to include MuSK. Epitope spreading is an established phenomenon in animal models of mediated autoimmune diseases (Vandergucht and Miller, 2002). In experimental autoimmune MG in rabbit models, the autoimmune attack is initially directed against the main immunogenic region of the AChR. However, through the course of the immune response, a significant attack is also mounted against less immunogenic epitopes (Vincent et al., 1998). There is also information from human MG suggesting the presence of intermolecular epitope spreading. Screening of sera from AChR(+) patients has demonstrated rapsyn antibodies in 15% of patients (Agius et al., 1998).
Primary factors contributing to the muscular weakness and fatigue in MuSK(+) patients

Neuromuscular transmission

The second principal objective was to characterize the main factors contributing to the muscular weakness and fatigue in MuSK(+) patients. This included a spectrum of features from the detailed neurophysiological picture, as well as the microscopic view, which focused on the histopathology of the muscle fibers, as detected in muscle biopsies, to the patient’s hrQoL. These parameters in MuSK(+) patients were then compared to the other major immunological types of MG including AChR(+) and AChR(-) patients.

Regarding neurophysiological findings in MuSK(+) patients, our data suggested that MuSK(+) patients have the same degree of neuromuscular transmission defect as AChR(+) patients in proximal muscles, although with somewhat different distribution. The EDC muscle has been reported to reveal less abnormality in MuSK(+) patients than in AChR(+) patients (Farrugia et al., 2006a; Nemoto et al., 2005; Stickler et al., 2005). Thus, diagnostic examinations of patients with MuSK-Ab should be performed in affected muscles, since muscle weakness may be focal. The normal RNS findings in all of the MuSK(+)AChR(-) patients are intriguing. Decrement is frequently normal in patients with OMG and in generalized MG with mild weakness of limited distribution (Sanders, 2002). Thus, one explanation is that these patients mainly had weakness restricted to the ocular or bulbar muscles, which are not readily tested with RNS. Nevertheless, we cannot exclude that RNS examination would have been abnormal in the facial muscles, regarding the predominant involvement of these muscles. The orbicularis oculi muscle was recently shown to have abnormal decrement in 86% of MuSK Ab-positive patients (Oh et al., 2006).

The normal SFEMG findings in about 23% of MuSK(-)/AChR(+) patients may depend on the fact that many patients in this subgroup were in remission and had immunosuppressive treatment since many years. It is known that when MG improves, jitter typically continues to fall toward normal (Howard and Sanders, 1981). If patients in remission or with minimal manifestations were excluded, then jitter was abnormal in 92% of these patients. Furthermore, overall the deltoid displayed the most pronounced decrement.
Health-related quality of life in MuSK(+) patients

Concerning hrQoL, MuSK(+) patients indicated significantly more impairment in the MGQ bulbar domain compared with MuSK(-)/AChR(+) patients. This finding reflects the previously reported fact that MuSK(+) patients tend to suffer from bulbar impairment to a larger extent than AChR(+) patients (Evoli et al., 2003). Nevertheless, a global MGQ score did not differ significantly between MuSK(+) and MuSK(-)/AChR(+) patients. One explanation for the overall higher global MGQ score in the MuSK(-)/AChR(+) patients may be that a higher number in this group was in clinical remission and thus should not indicate as much impairment.

Therapy considerations in MuSK(+) patients

There were differences in therapy between immunological subgroups. In the group of MuSK(+)/AChR(-) patients, no patient received immunosuppressive treatment, although two patients had undergone thymectomy. Overall, thymectomy was performed in 78% of patients, whereas immunosuppressive therapy was only applied in 18% (present) and 42% (previous) of patients. The high thymectomy rate may reflect that most patients in this study were women and that removal of the thymus is the general recommended therapy for women with early onset MG. One MuSK(+)/AChR(-) patient was found to have a thymoma and improved after thymectomy. No case of thymoma in MuSK(+) MG has previously been described. On the contrary, the absence of hyperplastic changes to the thymus and the apparent lack of benefit of thymectomy has been reported in these patients (Lauriola et al., 2005). This may be the reason for the absence of the thymoma associated titin- and ryanodine receptor antibodies in these patients. MuSK-Ab levels have been shown to often decrease after immunosuppression but not after thymectomy (Bartoccioni et al., 2006). This latter finding is in contrast to the known AChR-Ab reduction after thymectomy in AChR(+) patients, in whom the hyperplastic thymus is considered the possible site of immunization against AChR and a major source of specific Abs (Marx et al., 1997; Vincent, 2002).

MuSK-Ab levels have been shown to correlate with disease status in most patients (Bartoccioni et al., 2006). In contrast to these findings, there was no obvious correlation between overall antibody concentration and disease severity in our series of MuSK(+ ) patients, albeit the population is small. The most probable explanation for this is that the Swedish patients were quite stable in their MG and more fluctuations in Ab-levels have been noticed in patients with a stable MG status (Bartoccioni et al., 2006).
Myopathy in MuSK(+) patients

A myopathic EMG pattern was found in 32% of all examined MG patients, comparable to 19% of patients in an earlier MUP analysis of MG patients (Somnier and Trojaborg, 1993). QEMG myopathy was significantly more common in MuSK(+) patients than in MuSK(-)/AChR(+) patients. Nevertheless, since a myopathic pattern is present in other immunological subtypes of MG as well, it is not specific for the MuSK(+) subtype.

The myopathic pattern is not likely to be due only to neuromuscular blocking since the presence of blockings does not necessarily give rise to a myopathic pattern. Also, in some of the patients with myopathy diagnosed using QEMG, the degree of blockings was not sufficiently high to signify a myopathy-like EMG. It does not seem likely that the myopathic picture depends on major structural damage of the muscle fibers. For example, muscle pathology did not reveal any muscle fiber necrosis and we noticed only some type II fiber atrophy. Further, the diameter of the muscle fibers was normal with minimal variation, and there were no signs of muscle fiber grouping. No denervation activity (fibrillations or positive sharp waves) was found using EMG and signs of myositis were absent from muscle biopsy. In those MuSK(+) patients with QEMG myopathy in a proximal muscle, there was concomitant weakness in two patients and concomitant fatigue in the two other patients. However, excluding patient 4, there was not a distinct correlation between the degree of weakness and the degree of myopathy.

A few patients in both MuSK(+) and AChR(+) groups have been reported to present with visible tongue atrophy although magnetic resonance imaging evidence of atrophy of individual facial muscles is only significant in MuSK(+) patients (Farrugia et al., 2006b). In that report, however, muscle atrophy correlated with long duration of steroid treatment (> 40 mg AD). Thus, on the basis on the current results as well as previous reports, the differences in myopathy and muscle atrophy between MuSK(+) and AChR(+) patients regarding atrophy appear to be mainly quantitative in some of the patients.

Pathogenic role of MuSK antibodies

It has been questioned whether MuSK-Abs are responsible for the weakness found in AChR(-) MG patients (Selcen et al., 2004; Vincent et al., 2005). Nevertheless, MuSK-Abs were recently shown to significantly reduce AChR clustering at the neuromuscular junctions (Shigemoto et al., 2006). MuSK(+) sera from patients with severe MG also induce inhibition of prolif-
eration in TE671 muscle cells. The accompanied down regulation of the postsynaptic AChR subunits as well as rapsyn, could be relevant to the defective neuromuscular transmission (Boneva et al., 2006).

It remains unknown, though, why oculobulbar muscles are most affected in MuSK(+) MG patients. One reason may be the substantial variability between muscles in the shape of the neuromuscular junctions and the difference of muscle fibers in respect to physiology, metabolism and patterns of gene expression (Burke, 1998). At the molecular level, developing and adult skeletal muscles can be subdivided into two distinct categories: Fast Synapsing, e.g., intercostal muscles, and Delayed Synapsing muscles, e.g., sternomastoid and diaphragm muscles. The two classes of muscles differ in the focal clustering of AChRs and in the alignment of the pre-synaptic nerve with focal AChR clusters (Pun et al., 2002). These properties are believed to play a prominent role in defining the progress of neuromuscular junction formation during synapse development and in the maintenance of adult muscle. Although no data is available, it may be speculated that neck/bulbar muscles may be more susceptible to defective nerve-muscle signaling due to the above-proposed differences, since they may be Delayed Synapsing muscles.

Muscle pathology in MuSK(+) patients and mitochondrial defects in MG

Muscle pathology displayed type II fiber atrophy, histological presence of mitochondrial dysfunction, such as moth-eaten and ragged red fibers, and COX negative fibers. No inflammation or dystrophic changes were seen in MuSK(+) patients. Type II fiber atrophy is an unspecific finding, since it may be associated with conditions such as the disuse of muscles and aging. In some MG patients, individual muscle fibers may be totally blocked, and therefore inactive, giving rise to disuse atrophy. The presence of COX negative fibers raises the question whether the myopathy is (1) a primary effect of MuSK-Ab, (2) secondary to the effect of MuSK-Ab on the motor end plate or (3) a concomitant phenomenon to the production of MuSK-Ab with another underlying disorder.

The possible co-existence of a mitochondrial myopathy and disturbed neuromuscular transmission is intriguing, since muscle weakness and fatigability similar to MG occur in certain mitochondrial myopathies. One example is late-onset mitochondrial myopathy (Johnston et al., 1995) in which the number of COX negative and/or ragged-red myofibers is increased similarly.
as in our patients. The absence of COX activity in myofibers is usually caused by deletions in mtDNA, which remove loci that encode for three subunits of COX, i.e. the complex IV of the respiratory chain. Mutations in the nuclear POLG1 gene (which encodes polymerase gamma 1, the enzyme which participates in mtDNA processing) is a common cause of multiple deletions in mtDNA.

In our study, mitochondrial defects were not associated with any particular antibody, since the abnormalities were observed in all immunological MG subtypes. In 64% of our investigated samples, multiple mtDNA deletions were present. Progressive external ophthalmoplegia and muscle weakness with exercise intolerance are the most common clinical pictures associated with such deletions (Suomalainen and Kaukonen, 2001). MtDNA deletions become more frequent with aging, although not as prominent as in our MG patients. The cause of the multiple mtDNA deletions in our patients, remains unknown since POLG1 mutations were not found in these muscle biopsies (Kajander et al., 2000).

Adverse effects of pyridostigmine treatment in MuSK(+) MG: neurophysiological indications of AChEI side effects

The effect of pyridostigmine treatment in MuSK(+) patients may range from mild benefit to ineffectiveness and side effects including cramps, myalgia (Rostedt and Stalberg, 2004), muscle fasciculations and other cholinergic side effects (Evoli et al., 2003). Our hypothesis was that the neuromuscular transmission defect found in MuSK(+) patients is due to abnormal morphology of the receptor area, rather than reduced receptor sensitivity, as in AChR(+) MG. Based on this assumption, AChEI should not have a beneficial effect on neuromuscular transmission. Extra discharges are usually seen after an overdose of acetylcholinesterase inhibitors in MG. The presence of extra discharges in MuSK(+) patients may be a useful neurophysiological indicator of adverse effects of AChEI treatment.
Conclusions

In conclusion, our results suggest the following:

1) Disturbed neuromuscular transmission in a muscle outside of the facial area in OMG is not predictive of subsequent generalization.
2) The presence of neuromuscular transmission defects in MG does not always correlate with the weakness and fatigability found in the examined muscles.
3) The correlation of SFEMG findings in a proximal muscle and the patient-oriented findings adds new impact to the neurophysiological evaluation of patients with MG.
4) A myopathic EMG pattern in MG is significantly more common in MuSK(+) patients, although it is not a feature found exclusively in this immunological subtype. Further, the degree of weakness/fatigue does not correlate with the degree of myopathy overall. The frequently detected myopathic pattern on EMG in MuSK(+) may be associated with the concomitant mitochondrial abnormalities.
5) A neuromuscular transmission defect is the primary cause of muscle weakness in the majority of MuSK(+) patients, as well as in AChR(+) MG.
6) Since AChEI do not have a noticeable beneficial effect in MuSK(+) patients, an abnormal receptor morphology which cannot be restored by AChEI is suggested. This abnormality is proposed to cause the underlying defect of neuromuscular transmission.

Den vanligaste formen av MG, som drabbar ca 80%, orsakas av antikroppar mot acetylcholin receptorn (AChR). Av de ca 20% som saknar dessa antikroppar har antikroppar identifierats mot muskelspecifikt tyrosinkinas (MuSK) hos ca 40%. Patienter med MuSK-antikroppar har rapporterats ha mer uttalade symptom kring ansikte och nacke och fokal distribution av störd neuromuskulär transmission. Hos dessa patienter har även myopati rapporterats. Andra muskelantikroppar, riktade mot rapsyn, ryanodinreceptorn och titin, existerar också, framförallt om patienten har ett samtidigt tyrom.

Diagnosen vid MG görs med hjälp av klinisk neurologisk undersökning inkluderande uttröttbarhetstester, serumanalys för antikroppar och neurofysiologisk undersökning. Singel fiber elektromyografi (SFEMG) är den känsligaste diagnostiska undersökningen och visar hos MG patienter en sänkt säkerhetsströskel för neuromuskulär transmission, sk jitter. Vid högre grad av transmissionstörning uppkommer impulsblockeringar, vilket ger klinisk svaghet. Vid sidan av SFEMG utförs också repetitiv nervstimulering vid 3Hz stimulering för att se en nedgång i amplitud hos muskelsvaret som är typisk vid MG.
Studie I
Frågeställningen gällde huruvida ökat jitter i en underarmsmuskel (EDC) hos patienter med enbart okulär MG kan förebäda en senare klinisk generalisering av myastenin. Det vore av stort kliniskt intresse att hitta en sådan prediktor för att kunna sätta in aggressiv immunosuppressiv behandling i ett tidigt skede. Fynden var att SFEMG i EDC ej kan användas för att förutbestämma vilka patienter som utvecklar generaliserad sjukdom trots att patienter med enbart ögonsymptom ofta visade störd neuromuskular transmission i extremitetmuskulatur.

Studie II
Detta arbete syftade till att översätta och adaptera det för MG sjukdomsspecifika italienska livskvalitéformuläret Myasthenia Gravis Questionnaire (MGQ) till svenska förhållanden. Den internationellt accepterade metoden för validering användes och vi lyckades ta fram ett svenskt formulär som nu framöver kan användas för att utvärdera behandling vid MG och för att kunna inkludera även svenska patienter i kliniska studier, där den subjektiva livskvalitätsskattningen idag är ett krav.

Studie III
Syftet med denna studie var att korrelera patientens subjektiva välbefinnande (poäng på livskvalitéformulären SF-36 och MGQ) med subklinisk sjukdomsgrad (mätt som störd neuromuskulär transmission med metoderna SFEMG och RNS). Resultaten visade att graden av störd neuromuskulär transmission i överarmsmuskulatur, som ofta är involverad vid MG, korrelerar väl med generellt välbefinnande angivet som poäng i MGQ. Detta medför en ny dimension för neurofysiologiska undersökningar vid MG; inte bara för diagnosen utan även som uppföljningsverktyg.

Studie IV
Målet var att karakterisera de elektrofysiologiska fynden avseende myopathy/neuropati samt muskelbiopsifynden, framförallt mitokondriefunktionen, hos olika antikroppsubtyper av MG. Detta gjordes för att besvara frågan: varför är patienter med MuSK-antikroppar svaga? Vi fann förekomst av
både MuSK-antikroppar och AChR-antikroppar hos 14% av de undersökta MG patienterna, något som tidigare inte rapporterats. Myopatimönster på EMG undersökning av proximal muskulatur var överrepresenterat hos MuSK-patienter jämfört med de patienter som saknade dessa antikroppar, men patienter från alla immunologiska subtyper hade mild myopati samt ofta förekommande mitokondriella avvikelser. MuSK-positiva patienter hade lika mycket jitter som AChR-positiva patienter, talande för att svagheten hos MuSK-patienter beror på störd neuromuskulär transmissison.

Studie V

Detta delarbete beskriver i detalj de extraurladdningar i motoriska nerver vid elektrisk stimulering som noterades hos en patient med MuSK-antikroppar och mestinonbehandling. Detta stödjer den hypotes som finns om att MuSK-patienter är mer känsliga för medicinering med acetylcholinesterashämmare och ofta utvecklar biverkningar.
Acknowledgements

This work was carried out at the Department of Clinical Neurophysiology, Uppsala University Hospital, Uppsala, Sweden. Collaborations were performed at the MG Clinic of Duke University Medical Center, Durham, USA and the Institute of Neurology, Catholic University, Rome, Italy. I would like to thank the staff of the department and university, colleagues and friends in Sweden, Italy and in the USA, for their support and contribution. I wish to express my sincere gratitude especially to:

**Erik Stålberg** - my supervisor. Thank you for everything! If it wasn’t for you I would never have gone into neurophysiology and not been the kind of doctor I am today. I can never thank you enough for all the things you have taught me about SFEMG, research and life. I always learn something new from you and I look forward to our future projects. You are an amazingly enthusiastic person and a good friend, who ALWAYS have time and energy to answer questions and provide great ideas for every occasion- wherever you are! We haven’t solved the entire mystery about MG yet…

**Hannu Kalimo** - my co-supervisor. Thank you for all the time and effort you put on interpretation of the muscle biopsies and for teaching me the muscle biopsy surveyor. Your great sense of humor enlightened these moments and taught me some valuable Finnish jokes.

Colleagues at the department of Clinical Neurophysiology:  
**Roland Flink, Arne Sandberg, Tomas Winkler, Karin Edebol Eeg Olofsson, Hans Axelson, Lars Larsson and Pirkko Hynninen** - for your genuine support, interesting discussions and important advice.  
**Margareta Grindlund and Lena Eriksson**: thank you for your great help with the repetitive nerve stimulation as well as all “practicalities” and your sense of humour. You really lighted up the long hard days!  
**Susanne Östgren “Sussie”**: thank you so much for your helpfulness and always positive attitude during the muscle biopsies. I could not have done them without you!!  
**Peo Fällmar** - your technical support has made the environment in the lab a lot easier for a “non-technical” person…☺  
**David Ullström** - thanks for all your help with uncooperative computers, the front picture, mp3 files and an outstanding knowledge about all zip, jpg, pdf, rtf etc etc.
Catarina Färnstrand- for positive thinking and always being so supportive.
Olga Manriquez- for enlightening the sometimes late evenings.
The administrative staff and secretaries of the department and institution:
Karin Mineur, Gunilla Thornander, Gun Westerlund, Lena Pettersson
and Anki Gustafsson- for administrative help.

Italian colleagues and friends.
Luca Padua- Grazie per tempo a Roma! Thanks for the lessons about quality of life, mixed with Friday research discussions with dinner, wine and cigars.
Pietro Caliandro- Thank you for arranging a wonderful stay in Rome and for explaining the Roman lifestyle. “When in Rome, do as the Romans do…” Yes, I still mix spaghetti with meatballs and ketchup😊
Irene Aprile, Constanza Pazaglia and everyone in the Rome EMG lab for providing great atmosphere in the 6 square feet office and teaching me how to make REAL pasta à la carbonara.
Emanuela Bartocciioni, Flavia Scuderi and Mariapaola Marino- “the MuSK-gang” - thank you for helping with the MuSK antibody analysis and for great Italian pizza.

American collaborators and colleagues:
Donald B Sanders and Janice Massey. Thank you for teaching me how to examine and treat MG patients in the best way. I admire your clinical skills and your hospitality during the stay in Durham.
Charlotte, Kevin, Bernadette and everyone in the Duke MG Clinic for a great time in Durham.

Technicians at the department of Neuropathology:
Maude Olofsson and Monica Sternesjö - for great help with muscle specimens and for many coffee breaks with cookies.

Colleagues and nurses at the Neurology Department in Uppsala, especially:
Håkan Askmark- for relevant clinical advice.
Inga-Lena, Eva-Lena, Lillemor, Gertrud and Susanne at the department of neurology- for great help with the blood samples for MuSK antibody analysis and for running with them to the famous freezer 😊

Collaborators in Helsinki, Finland:
Kati Ahlqvist and Anu Vartiovaara- for cooperation on the mitochondrial DNA analysis.

Friends:
Holly Norman- for always having time to translate, being SO positive and a true friend. You are an amazing person.
Sara and Karolina-thanks for many happy times and your constant support during these years and all through the LÄFO period.

The “PAX-3 gang”: Josefine, Alina, Shaman and Anna-Karin. Thanks for fun discussions, dinners and great advice about the dress☺.

The “Eriksberg-gang”: Bec and Pernilla- for long walks, fika, running and “baby talk”…

My family:
Ida- for sharing many wonderful moments through the years, being a loyal sister and never giving up on teaching me how to cook…
Margaretha- Thank you for always believing in me and being my source of inspiration! Without you I would not have been who or where I am today. Your never ending support and encouragement has meant everything to me.
Karl Erik-Thanks for teaching me how to always be optimistic and to get the true “fighting spirit” both on and off the tennis court. It has helped me a lot in the hard academic world 😊
Gertrud- thanks for always being there for me and Ida during our childhood and for the summers in Bordsjö.
Kristiina, Aime and Eduard- thanks for embracing me into your family and for great times both in Estonia and Uppsala.

Tanel- I am truly grateful for all your patience and support, through good and bad times! Thank you for compromising, for working evenings and nights in order for me to finish this, and for putting up with my energetic life-style!😊… You make me a calmer and happier person and I love you endlessly!
Olivia-Your smiles bright up the day and you have taught me what the “big things” are in life. Being a mother to you is the greatest thing I could ever wish for!
References


neuromuscular junction assembly and maintenance in different skeletal muscles. Neuron 34, 357-370.


Acta Universitatis Upsaliensis

Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine 211

Editor: The Dean of the Faculty of Medicine

A doctoral dissertation from the Faculty of Medicine, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine. (Prior to January, 2005, the series was published under the title “Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine”.)

Distribution: publications.uu.se
urn:nbn:se:uu:diva-7408