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Disease specific signature of circulating miR-150-5p and miR-21-5p in myasthenia gravis patients

Anna Rostedt Punga [a,*], Mats Andersson [b], Mohammad Alimohammadi [c], Tanel Punga [d]

[a] Department of Neuroscience, Clinical Neurophysiology, Uppsala University, Uppsala, Sweden
[b] Feldbergstrasse 67, Basel, Switzerland
[c] Department of Medical Sciences, Uppsala University, Uppsala, Sweden
[d] Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden

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ABSTRACT

Purpose: Reliable biological markers for patients with the autoimmune neuromuscular disorder myasthenia gravis (MG) are lacking. We determined whether levels of the circulating immuno-microRNAs miR-150-5p and miR-21-5p were elevated in sera from clinically heterogeneous MG patients, with and without immunosuppression, as compared to healthy controls and patients with other autoimmune disorders.
Methods: Sera from 71 MG patients and 55 healthy controls (HC) were analyzed for the expression levels of miR-150-5p and miR-21-5p with qRT-PCR. Sera were also assayed from 23 patients with other autoimmune disorders (AID; psoriasis, Addison's and Crohn's diseases).
Results: 34 MG patients had no immunosuppressive drug treatment (MG-0) and 37 patients were under stable immunosuppressive drug treatment since ≥6 months (MG + IMM). Serum levels of miR-150-5p and miR-21-5p were higher in the MG-0 patients compared to HC ($p < 0.0001$). Further, miR-150-5p levels were 41% lower and miR-21-5p levels were 25% lower in the MG + IMM compared to MG-0 ($p = 0.0051$ and 0.0419). In the AID patients, mean miR-150-5p and miR-21-5p were comparable with healthy controls ($p = 0.66$).
Conclusions: The immuno-microRNAs miR-150-5p and miR-21-5p show a disease specific signature, which suggests these microRNAs as possible biological autoimmune markers of MG.

1. Introduction

Myasthenia gravis (MG) is a chronic autoimmune neuromuscular disorder, where the presence of MG specific autoantibodies, most often the nicotinic acetylcholine receptors (AChR+) is important in the diagnostic procedure. Autoantibody types in MG are to some extent relevant not only diagnostically, since patients with antibodies against muscle specific tyrosine kinase (MUSK) seem to have a different clinical presentation and response to thymectomy compared to AChR+ MG. Nevertheless, the autoantibody titer does not necessarily predict the course of disease or the therapeutic response [1]. Due to the absence of reliable biological markers for MG patients in clinical trials, recent recommendations for clinical research standards imply the importance of developing easily accessible parameters that support mechanistic studies in clinical trials of pharmacotherapies [2,3].

Mammalian microRNAs (miRNAs) inhibit gene expression by degrading or blocking translation of their target messenger RNAs (mRNAs) [4] and thereby regulating gene expression pattern in various essential cellular processes [5]. Certain miRNAs are regarded as critical factors in controlling the immune response and altered miRNA expression is related to various autoimmune diseases, including systemic lupus erythematosus, rheumatoid arthritis and multiple sclerosis [6–8]. Moreover, miRNAs can circulate in the bloodstream in a stable, extracellular form. Detection of circulating miRNAs can be used to follow various disease states and therefore circulating miRNAs have been regarded as potential blood-based biological markers [9]. In a recent report levels of 3 miRNAs (miR-150-5p, miR-21-5p and miR-27a-3p) were deregulated in serum samples in a cohort of AChR+ female MG patients, of which miR-150-5p was the most sensitive marker [10]. Regulatory T cells (Tregs) control the autoimmune response and prevent autoimmunity. Although normal in number, thymic Tregs display functional impairment in MG patients [11]. Intriguingly, miR-150-5p has been specifically implicated in the process of T cell maturation [12,13]; hence this miRNA could provide a link to the autoimmune dysfunction in MG.

The aim of the present study was to validate whether circulating miR-150-5p and miR-21-5p specifically accumulate in the sera of a large heterogeneous cohort of male and female MG patients compared to healthy controls (HC) and patients with other autoimmune disorders. We also assessed whether circulating miR-150-5p and miR-21-5p levels would be reduced in sera from MG patients with immunosuppressive

* Corresponding author at: Department of Neuroscience, Clinical Neurophysiology, Uppsala University, 75185 Uppsala, Sweden.
E-mail address: anna.rostedt.punga@neuro.uu.se (A.R. Punga).

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medication (MG + IMM), compared to MG patients without immunosuppressants (MG-0), and thus if circulating miRNA levels would be responsive to immunomodulation.

2. Methods

2.1. Hypothesis and power calculation

The primary objective consisted of testing two separate hypotheses, both based on mean miR-150-5p outcome: MG-0 = HC and MG-0 = MG + IMM. Based on an assumed mean value for both HC and MG-IMM of 60% of the mean for the MG-0 group and with a common standard deviation of 48% of mean MG-0 (based on previous data from [10]) it was calculated that at least 31 patients per group would be needed in order to have 90% power to reject each hypothesis at 5% level.

As secondary exploratory objectives, we wanted to investigate a relation between miR-150-5p and disease severity and whether there is an increase of miR-150-5p in patients with other autoimmune disorders. We also wanted to explore whether miR-21-5p is still elevated and miR-27a-3p is decreased in a heterogeneous MG cohort.

2.2. Study group and clinical scoring

Blood samples were collected from 55 consecutive healthy blood donors (28 females, mean age: 45 ± 16 years) at Uppsala University Hospital. 71 MG patients (40 females; mean age: 57 ± 15 years), who were referred to or regularly treated at the Neurology Clinic at Uppsala University Hospital, were sampled for sera and assessed clinically with the myasthenia gravis composite (MGC) score [14,15]. A diagnosis of MG had been established according to the diagnostic criteria of the Myasthenia Gravis Foundation of America (MGFA) [16] including objective muscle fatigue and neurophysiological evidence of disturbed neuromuscular transmission (decrement on repetitive nerve stimulation and/or increased jitter on single fiber EMG), further supported by detection of AChR antibodies. Each patient was given a total MGC score ranging from 0 (no myasthenic weakness) to maximum 50 points (worse possible myasthenic weakness in all examined muscles). Patients with antibodies against MuSK or Lrp4 were not included in this cohort due to different IgG subtypes and clinical presentation in patients with these antibodies. However, patients who were seronegative against AChR, MuSK and Lrp4 were included if they fulfilled all other diagnostic MG criteria.

Myasthenia Gravis Foundation of America (MGFA) disease severity grade was also assessed. The Swedish version of the health related MG quality of life form (MGQ) [17], was applied to a subgroup of 35 patients. As the healthy control group, blood samples were collected from 55 consecutive healthy blood donors (28 females, mean age: 45 ± 16 years) without any medications. As the autoimmune control group, sera were inquired from 23 patients with other autoimmune disorders (AID): Addison's disease (N = 8), Crohn's disease (N = 11) and psoriasis (N = 4), (10 females; mean age: 42.5 ± 14 years). The patients with Addison's disease were substituted with <30 mg hydrocortisone (equivalent to 7.5 mg prednisone), whereas the patients with psoriasis had only topical treatment with corticosteroids and for the patients with Crohn's disease no information regarding immunosuppressive treatment was available at the time of serum sampling.

The study was approved by the local Ethical Review Board in Uppsala (Dnr 2010/446) and all patients signed informed consent.

2.3. Circulating miRNA isolation and miRNA expression analysis

Blood samples were collected in tubes without any additives and centrifuged, after at least 20 minute storage in room temperature, at 4000 rpm and 20 °C for 5 min. The serum samples were subsequently stored at −80 °C until further processing. Total RNA was isolated from 200 μl serum by using miRCURY™ RNA Isolation Kit—Biofluids (Exiqon #300112), according to the manufacturer's instructions. 2 μl of isolated RNA sample was applied for cDNA synthesis in a 10 μl reaction mix by using Universal cDNA Synthesis Kit II (Exiqon #203301). The RT-qPCR analysis was performed by using ExiLENT SYBR® Green master mix (Exiqon #203421) on custom made Pick-&-Mix microRNA PCR panel plates (Exiqon #203818) pre-coated with validated primer sets to amplify target miRNAs. The cDNA reactions were diluted 100× in ExiLENT SYBR® Green master mix before applying to the Pick-&-Mix microRNA PCR panel plates. All RT-qPCR reactions were carried out on 384-well plates as described previously [10]. The following quality controls were included on Pick-&-Mix microRNA PCR panel plates: the inter-plate calibration (UniSp3), RNA extraction control (UniSp2 and UniSp4), cDNA synthesis control (UniSp6) and hemolysis test (miR-23a-3p–miR-451a) (Exiqon). The ΔCT value of hemolysis markers ($\Delta CT_{(hemolysis)} = CT_{(miR\text{-}23a\text{-}3p)} - CT_{(miR\text{-}451a)}$), was used to detect hemolysis. A ΔCT > 7 in serum samples indicates a high risk of hemolysis and therefore these samples were not used for further analysis. Reference miRNAs, miR-93-5p, miR-191-5p, miR-423-4p and miR-103a, were chosen since they have proven to have a stable expression both in healthy controls and in MG patients [10]. Quantification of relative miRNA expression was performed with the comparative CT method using the formula $2^{\wedge -\Delta\Delta CT}$, where ΔCT = [(CT gene of interest − CT reference gene) sample A − (CT gene of interest − CT reference gene) sample B] by using the mean value of miR-93-5p, miR-191-5p, miR-423-5p and miR-103a as the reference [10,18,19].

2.4. Statistical analysis

The study was designed as a case–control study. Log conversion of the miRNA expression data was done in order to obtain data more similar to a normal distribution for the statistical tests. To preserve the type I error at 5%, the two statistical tests for the primary objective were performed sequentially with MG-0 initially being compared to HC and only if significant could MG-0 and MG + IMM be compared without risk of error inflation. No type I error control was applied for any of the other statistical comparisons. All tests between disease groups (MG-0, MG + IMM, HC, AID) were performed using a two-sample t-test with a null hypothesis of equal means. In order to evaluate the relation between levels of categorical background factors (gender, MGFA class, etc.) and miR-150-5p, ANCOVA models were used that included the disease group as an additional factor. Spearman rank correlation was performed in order to determine the correlation coefficient between continuous factors (disease duration, age, etc.) and miR-150-5p. Statistical significance was defined as a two-sided $p < 0.05$.

3. Results

3.1. Clinical characteristics of MG patients

We aimed at correlating clinical status of the MG patients with serum levels of miRNA; thus patients were characterized by the clinical group according to the MGFA class as well as the MG composite (MGC) and quality of life score (MGQ), which reflects both objective and subjective myasthenic symptoms. Further, patients were subdivided according to their treatment with immunosuppression. 71 MG patients were included in the study, out of which 34 did not have any current immunosuppressive drug treatment for at least 6 months (25 females; MG-0). 20 patients (59%) in this group were thymectomized and post thymectomy time ranged from 4 to 36 years (Table 1). Out of these patients, 20 (59%) were on acetylcholinesterase inhibitors (AChEIs), ranging from 60 to 720 mg daily, and the remaining patients did not have any medication at all. 37 MG patients were treated with immunosuppressive agents at a stable dose for at least 6 months (MG + IMM). Except for treatment with corticosteroids and/or azathioprine, one patient

Table 1
Descriptive summary of current age and age at MG onset (years), number of female and male patients and disease duration (years). MGC, Myasthenia Gravis Composite score. Treatment characteristics for the different cohorts, Cort, corticosteroids; Aza, azathioprine; Other immunosuppr: takrolimus, mycophenolate mofetil or sendoxan; Ab, antibody; AChR+, acetylcholine receptor antibody seropositive; Seronegative; no detectable serum antibodies against AChR, MuSK or Lrp4.

	MG subgroup	
	MG-0 (N = 34)	MG + IMM (N = 37)
Age (mean ± SD)	55 ± 15	59 ± 15
Age at MG onset (mean ± SD)	36 ± 19	49 ± 19
Gender		
Female (N)	25	15
Male (N)	9	22
Disease duration	18.5 ± 17.4	8.5 ± 9.6
Mean ± SD (range)	(0.3–57)	(0.5–32)
MGC (mean ± SD)	6.1 ± 6.4	4.6 ± 6.1
Treatment N (%)		
Only AChEIs	20 (59%)	
No treatment	14 (41%)	
Cort		21 (57%)
Aza		5 (13.5%)
Cort + Aza		8 (21.5%)
Other immunosuppr		3 (8%)
Ab subtype N (%)		
AChR+	26 (76%)	26 (70%)
Seronegative	8 (24%)	11 (30%)
Thymectomy N (%)	20 (59%)	18 (49%)
Hyperplasia	11 (55%)	6 (33.5%)
Normal	8 (40%)	8 (44.5%)
Thymoma	1 (5%)	4 (22%)

had sendoxane, one patient had only tacrolimus and one patient had mycophenolate (Table 1). 18 (49%) patients in the MG + IMM group had undergone thymectomy and post thymectomy time ranged from 2 to 28 years (Table 1). MGFA class ranged from class 1 (ocular MG) to 4B (severe mainly bulbar MG) in the MG-0 group and from class 0 (pharmacological remission) to class 3B (moderate mainly bulbar MG) in the MG + IMM group. Mean MGC score was 6.1 (range: 0 to 34) in the MG-0 group and 4.6 (range: 0 to 18) in the MG + IMM group and there was no difference in MGC scores between the groups ($p = 0.2$). MGQ score was 40 ± 8 in the MG-0 group compared to 35 ± 10 in the MG + IMM group ($p = 0.2$). Antibody status is displayed in Table 1.

Taken together, patients were divided into two groups, based on immunosuppression, that was quite homogenous regarding age span although the individual clinical presentations were considerably heterogeneous.

3.2. Circulating miR-150-5p and miR-21-5p are significantly elevated in sera from MG patients without immunosuppressive medication

We first determined whether serum levels of circulating miR-150-5p, miR-21-5p and miR-27a-3p would still be different in a heterogeneous group of MG patients without immunosuppression compared to healthy controls and MG patients with immunosuppression.

Since hemolysis is a confounding parameter, one patient who had a serum hemolysis quote of 7.9 in the MG + IMM group was excluded from further analysis. In the healthy control group, MG-0 and MG + IMM, all reference genes had a stable expression pattern. Since miR-93-5p was not expressed in the group of Addison's patients, only miR-103a, miR-423-3p and miR-191-5p were used for normalization of miRNA expression data in the AID group.

As the primary objective, levels of miR-150-5p were significantly elevated with 170% in the MG-0 patients compared to the healthy controls ($p < 0.0001$; Fig. 1A). Further, miR-150-5p levels were 41% lower in the 36 MG + IMM patients compared to the MG-0 group ($p = 0.0051$; Fig. 1A). For the subgroup of AChR+ patients, there was an even more distinct difference with 55% lower values of miR-150-5p in the MG + IMM group ($N = 26$) compared to the MG-0 group ($N = 26$; $p = 0.003$; Fig. 1B). Within the secondary objective, miR-21-5p was increased in MG-0 patients with 94% compared to healthy controls ($p < 0.0001$). MG + IMM patients had 25% lower miR-21-5p levels compared to MG-0 ($p = 0.0419$; Fig. 1C) and in the subgroup of AChR+ patients MG + IMM patients had 16% lower levels (data not shown). Levels of miR-27a-3p were not reduced in the heterogeneous cohort of MG-0 patients compared to healthy controls ($p = 0.33$; Fig. 1D) and thus, not further analyzed.

Both miR-150-5p and miR-21-5p were thus found to be elevated in a large cohort of MG patients without immunosuppression and both were significantly reduced in patients with immunosuppression.

3.3. Circulating miR-150-5p and miR-21-5p are specifically elevated in MG as compared to other autoimmune diseases

In order to determine whether levels of circulating miR-150-5p and miR-21-5p would be disease specific for MG, sera were analyzed also from the 23 patients with AIDs (Addison's disease, Crohn's disease and psoriasis). In the AID group, mean levels of miR-150-5p were comparable with those seen in the healthy controls ($p = 0.66$) and lower than those in MG-0 patients ($p = 0.0003$; Fig. 1A). Also serum miR-21-5p was not increased in patients with AID.

Both miR-150-5p and miR-21-5p were thus found to have a disease specific elevation in MG patients without immunosuppression

3.4. No correlation between miR levels and clinical status

In the MG cohort, no correlation was seen for miR-150-5p and age in MG-0 (Spearman $R = -0.184$) and MG + IMM (Spearman $R = 0.094$, both $p > 0.277$). No difference was seen in miR-150-5p levels between male and female patients (both MG-0 and MG + IMM, $p > 0.085$). Further, no clear relation was observed between the levels of miR-150-5p and MG disease duration (MG-0 Spearman $R = -0.134$ and MG + IMM Spearman $R = 0.132$; both $p > 0.428$). Disease severity based on MGC score did not consistently correlate with miR-150-5p levels in MG-0 (Spearman $R = 0.119$) and MG + IMM (Spearman $R = -0.095$; both $p > 0.482$; Fig. 2). Correlation between MGQ and miR-150-5p was consistent but weak for groups MG-0 ($R = -0.07$) and MG-IMM ($R = -0.15$, both $p > 0.69$). There was no difference in quality of life MGQ score between MG-0 and MG + IMM groups ($p = 0.1640$).

Within the MG-0 group, circulating miR-150-5p levels did not differ between patients treated with AChEI and those who did not have any current treatment (Fig. 3A). Further, we did not see any clear difference between thymus histopathology, regarding normal thymus versus hyperplasia, and miR-150-5p or antibody subgroup (data not shown). Additionally, there was no clear association with MGFA disease severity class and miR-150-5p levels (Fig. 3B).

For circulating miR-21-5p there was no correlation between levels and MGC score (Spearman $R = 0.076$; $p = 0.521$).

Thus, although we found a disease specific signature of miR-21-5p and miR-150-5p we were not able to significantly correlate these levels with any clinical parameters.

4. Discussion

Certain miRNAs are regarded as important modulators of immunological functions. Both miR-150 and miR-21 are included among these immuno-miRNAs, as they are considered to be crucial regulators of T cell processes [20]. Since MG is a T cell dependent disease it is intriguing that miR-150 has been identified as a miRNA selectively expressed in immature, resting T cells with strong upregulation as maturation/differentiation of T cells progresses [12,13]. We recently proposed miR-150-5p as a potential circulating biomarker in female AChR+ MG patients without immunosuppressive drug

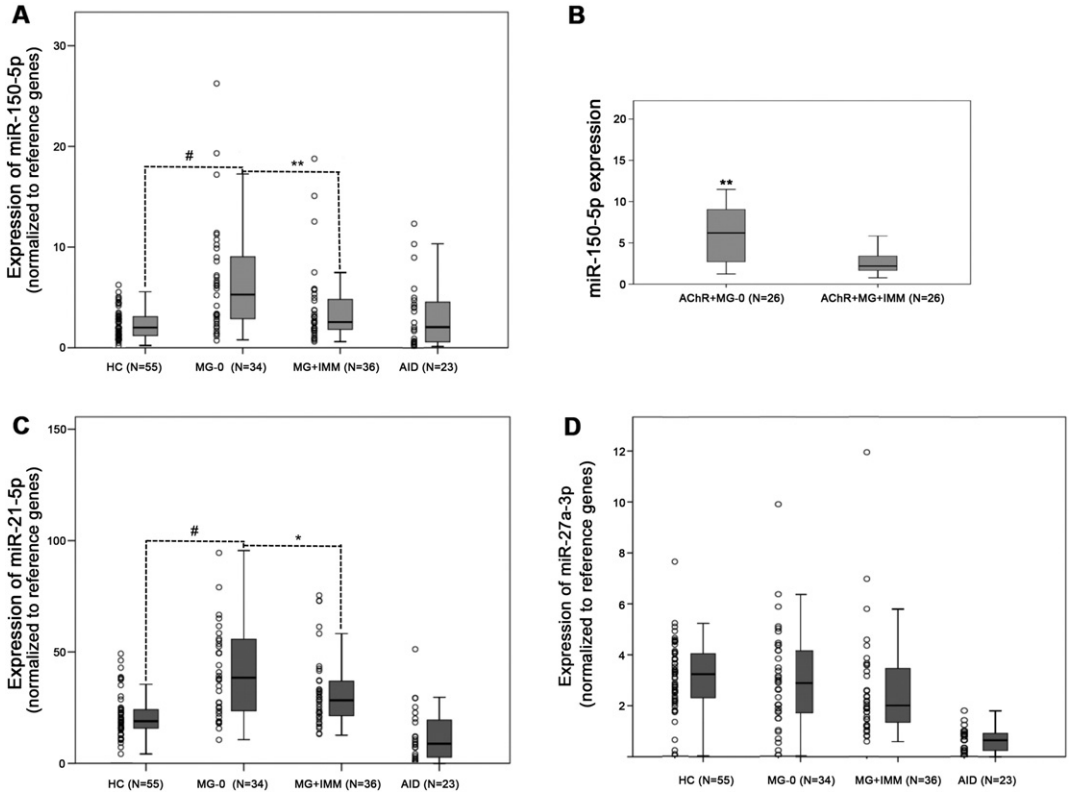

Fig. 1. Box plots and individual values on linear axis of (A) miR-150-5p in the different cohorts. (B) Expression of miR-150-5p is displayed in the subgroups of AChR antibody seropositive patients (AChR+) in the MG-0 and the MG + IMM groups respectively. (C) Box plots and individual values on linear axis of miR-21-5p and (D) miR-27a-3p in the different cohorts. To the left in each cohort, individual values are displayed. Box plot with mean (horizontal line inside box), and whiskers (SD) are displayed to the right in each cohort. On the Y-axis, relative expression of miR-150-5p and miR-21-5p are shown according to the formula $2^{\wedge -\Delta\Delta CT}$. HC, healthy controls (N = 55); MG-0, MG patients with no immunosuppressive treatment (N = 34); MG + IMM, MG patients with immunosuppressive medication (N = 36) and AID (autoimmune disorders; N = 23). #$p < 0.0001$; **$p < 0.01$; *$p < 0.05$.

treatment, as miR-150-5p levels were clearly reduced upon clinical improvement after thymectomy [10]. Nevertheless, a valid biological parameter should preferably also be disease specific in a clinically heterogeneous group of patients, both female and male as well as early versus late onset MG. Therefore; in the present study we analyzed miR-150-5p levels in a large cohort of MG patients with heterogeneous clinical and serological phenotypes and with different treatments. Intriguingly, we found that the circulating miR-150-5p levels were 41% reduced in patients with current immunosuppressive drug treatment, compared to patients without respective treatment.

Further, in the subgroup of AChR+ patients the difference was more pronounced with 55% lower values in the immunosuppressed group. Thymectomy was performed at a similar frequency in both groups (approximately 50%) and thus there were no difference

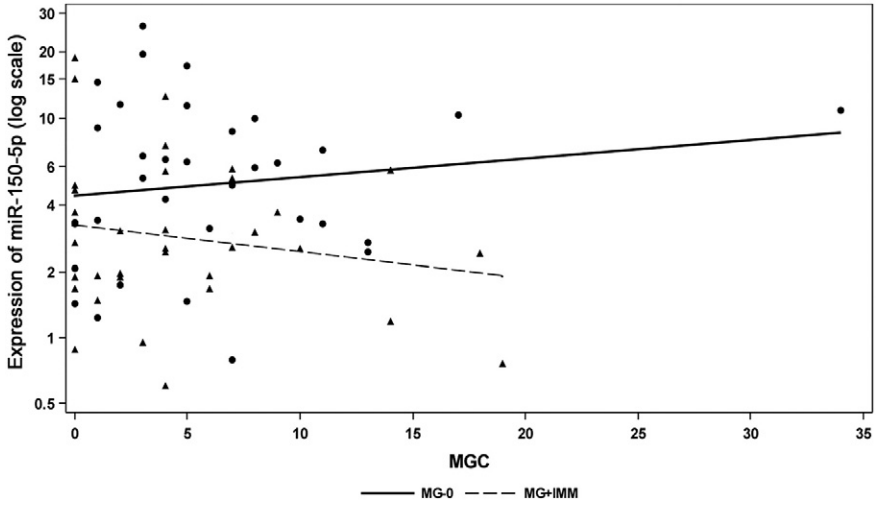

Fig. 2. Relation plot of miR-150-5p and disease severity as measured by Myasthenia Gravis Composite Score (MGC) using linear regression. No consistent relation is seen regarding miR-150-5p and MGC score by MG cohort. MG-0 (circle), MG patients with no immunosuppression; MG + IMM (triangle), MG patients with immunosuppression.

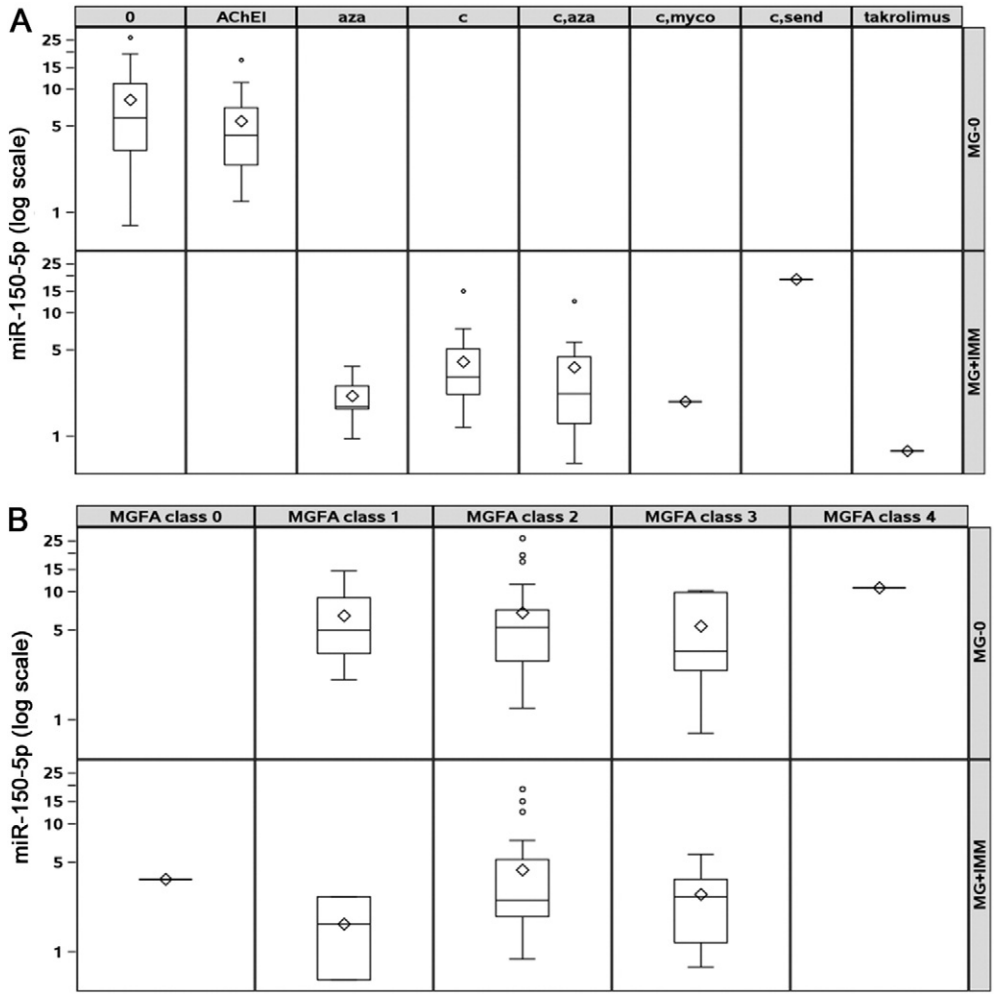

Fig. 3. A) Levels of miR-150-5p by treatment type. In the group of MG patients with immunosuppression (MG + IMM), levels of miR-150-5p were reduced by 41% compared to the group of MG patients without immunosuppression (MG). AChEI, acetylcholinesterase inhibitors; aza, azathioprine; c, corticosteroids; c, aza, combination of corticosteroids and azathioprine; c, myco; combination of corticosteroids and mycophenolate mofetil; c, send; combination of corticosteroids and sendoxan. B) miR-150-5p levels in relation to MGFA class of disease. MGFA class 0, no myasthenic fatigue; MGFA class 1, ocular MG; MGFA class 2; mild generalized MG; MGFA class 3, moderate generalized MG; MGFA class 4, severe generalized MG.

in-between groups for this treatment. This observation suggests that circulating miR-150-5p levels in MG patient sera respond to immunomodulatory treatments and not only to thymectomy. Nevertheless, we cannot entirely rule out that other differences among patients in the cohorts play a role, such as lower age at onset, and likely more early onset MG patients, in the untreated group.

One question was whether the levels of miR-150-5p would correlate with clinical score. We did not find a consistent correlation between miR-150-5p and the MGC score, however given the complexity of the immune system and the heterogeneity of the patients this is not entirely surprising. The design of a cross-sectional cohort study might also be less sensitive than a longitudinal study, which could detect subtle changes within patients over time.

The other studied autoimmune disorders have a similar T cell mediated autoimmunity as MG. Addison's disease, Crohn's disease and psoriasis share the T cell mediated pathophysiology although affecting different organs. Psoriasis vulgaris is the best-understood disease that is mediated by T cells and dendritic cells, with activation of IL-17-producing T cells [21], similarly to the increase in IL-17 in Tregs observed in MG [22]. Autoimmune adrenocortical failure, or Addison's disease, is a prototypical organ-specific autoimmune disorder and Crohn's disease displays evidence of defects within the Treg cell compartment [23]. Nevertheless, miR-150-5p levels were not found to be altered in these other autoimmune disorders. The organ-specific autoimmune disorders can be organized in two groups. The first group consists of autoimmune disorders in which the autoantigen in a surface protein or receptor, such as MG or Grave's disease, associated with changes in thymic function. The other group consists of autoimmune disorders in which the autoantigen is an intra-cellular protein, exemplified with Addison's disease. The first group of autoimmune disorders is not associated to thymomas or thymic changes. Accumulation of miR-150-5p in MG patients, its reduction in patients with immunosuppressive drug treatment and its absence in destructive autoimmune disorders may reflect the roles of miRNA-150-5p in the thymus and T cell development.

Moreover, circulating miR-21-5p was elevated in a similar fashion in MG patients compared to healthy controls and AID, although the impact of immunosuppression was not as obvious as for miR-150-5p. Previous reports have indicated importance of miR-21 in lupus erythematosus, multiple sclerosis and diabetes type 1 [24–26] and thus it could be that certain types of AID share a miRNA profile and certain action of miR-21-5p in the immune cells. Similar to miR-150, miR-21 plays crucial roles in T cells. Expression of miR-21 is induced upon triggering of T cell receptors and affects T cell activation through regulating T cell apoptosis, proliferation and migration [27,28]. Further, the regulatory function of miR-21

depends on the differentiation status of the T cells and miR-21 is consistently higher expressed in Tregs, which is associated with the predominant memory phenotype of T cells [29].

The last miRNA that we assayed, miR-27a-3p, was not significantly reduced in this heterogeneous cohort of MG patients without immunosuppression and thus not assigned any disease specific value for further studies in MG. This miRNA has been proposed to reflect natural killer (NK) cell cytotoxicity [30] and it could be that this feature is not specific enough for MG pathogenesis.

A relevant question is whether the treatment in the autoimmune control group may have influenced the level of miR-150-5p. In general, the absence of endogenous corticosteroids in patients with Addison's disease is substituted with <30 mg hydrocortisone, which is equivalent to 7.5 mg prednisone, and hence generally a lower dose in comparison to the immunosuppression used in the MG patients. The patients with psoriasis had only topical treatment with corticosteroids and this treatment is not considered to give rise to systemic immunosuppression. For the patients with Crohn's disease, we were not able to obtain information about the immunosuppressive treatment at the time of serum sampling. However, confounding factors in control subjects may be subject for detailed studies in future investigations.

The two different groups of MG patients varied somewhat in the distribution of men and women, with more women in the MG-0 group. However, the disease severity did not differ between the groups and no sub-analysis was done for gender in this particular study. Additionally, there is a sizeable cohort of antibody negative patients studied, more than is generally reported in epidemiological studies, and this is due to that most AChR seronegative patients are concentrated to Uppsala University Hospital. Further, we intentionally allowed the inclusion of a proportionally large cohort of seronegative patients in order to broaden the serological phenotype of the patients, since the initial study was done on AChR+ patients alone [10].

A recent study analyzed the circulating miRNA profile in MG patient sera and a number of miRNAs were decreased in MG patients compared to healthy controls [31]. These down-regulated miRNAs included miR-15b, miR-122, miR-140-3p, miR-185, miR-192, miR-20b and miR-885-5p. The authors found the miRNA profile to be comparable between different subsets of MG with regard to early onset MG, late onset MG and thymoma patients. There was also no difference in patients with immunosuppressive drug treatment, taking into account that the number of patients in each subgroup was rather small. Enhanced accumulation of circulating miRNAs was not reported in the studied Spanish cohort; however it was not obvious which strand of mature miR-150 was analyzed. At present we do not know the exact reasons for the discrepancy between the works by Nogales-Gadea et al. [31] and our report. However, differences in methods for detecting and normalizing circulating miRNAs might explain the non-overlapping results.

In summary, circulating miR-150-5p and miR-21-5p specifically accumulate in sera from a heterogeneous group of MG patients. Primarily the levels of circulating miR-150-5p are significantly reduced in MG patients with immunosuppression, in particular AChR+ MG, indicating responsiveness of miR-150-5p to immunomodulation. Therefore, disease specific signature of these circulating immuno-miRNAs in MG might be considered as potential biological marker in future longitudinal studies.

Conflict of interest

The authors declare that they have no conflicts of interest.

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