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# $\beta_2$-adrenergic receptor gene polymorphisms in myasthenia gravis (MG)

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**SUMMARY**

The $\beta_2$-adrenergic receptor ($\beta_2$-AR) belongs to the group of G-protein coupled receptors and is present mainly on skeletal and cardiac muscle cells and lymphocytes. The gene encoding $\beta_2$-AR (*ADRB2*) displays a moderate degree of heterogeneity in the human population. The distribution of polymorphisms at amino acid positions 16, 27 and 164 is changed in asthma, hypertension and obesity. We have earlier reported a decreased density of the $\beta_2$-AR on peripheral blood mononuclear cells and the presence of $\beta_2$-AR antibodies in patients with MG. Since certain polymorphisms affect the function of the $\beta_2$-AR, it was of interest to analyse these in MG. Using allele-specific polymerase chain reaction amplification, we revealed an over-representation of homozygosity for Arg16 and a lower prevalence of homozygosity for Gly16 in MG patients compared with healthy individuals. The increased frequency of homozygosity for Arg16 was due to a contribution from patients with generalized MG but not from patients with only ocular disease. Homozygosity for Glu27 was negatively associated with both the presence of $\beta_2$-AR antibodies and severity of disease. Moreover, acetylcholine receptor (AChR) antibodies were more often present in patients being homozygous for Gln27. Our results imply that homozygosity for Arg16 confers susceptibility to generalized MG, and that certain polymorphisms at amino acid position 27 are associated with subgroups of patients.

**Keywords** myasthenia gravis $\beta$-adrenergic receptor gene polymorphisms $\beta$-adrenergic receptor antibody acetylcholine receptor antibody

## INTRODUCTION

Certain MHC polymorphisms appear to be risk factors for most autoimmune diseases. HLA-B8 and DR3 have the strongest association with MG, while DR2, DR4 and certain DQ haplotypes are associated with the disease when patients are stratified according to age of disease onset and thymic histopathology. However, these associations are weak, suggesting that other genes may be of importance. The genes for the nicotinic acetylcholine receptor (AChR) [1,2], immunoglobulin heavy chains [3,4] and T cell receptor (TCR) [5,6] are some of the non-MHC candidate genes that have been evaluated. In addition, there are also associations between gene polymorphisms of CTLA-4 [7] and IL-1$\beta$ [8]. Since the contribution of different genes varies in different subgroups of patients, MG could be considered as a polygenic disease.

The $\beta_2$-adrenergic receptor ($\beta_2$-AR) is a G protein-coupled receptor which has the typical structure with an extracellular amino terminal, seven transmembrane domains and an intracellular carboxyl-terminal. The gene encoding the $\beta_2$-AR (*ADRB2*) is an intronless gene that is present at q31-32 of chromosome 5 and was cloned in 1987 [9]. Three loci at amino acid positions 16, 27 and 164 have been found to alter receptor functions significantly. Agonist-promoted down-regulation was enhanced in receptors homozygous for glycine16 (Gly16) compared with those homozygous for arginine16 (Arg16). Receptors homozygous for glutamic acid27 (Glu27) were resistant to such down-regulation when compared with those homozygous for glutamine27 (Gln27) [10]. The receptors homozygous for isoleucine164 (Ile164) had markedly decreased ligand binding and coupling properties compared with those homozygous for threonine164 (Thr164). Cardiac dysfunction was observed in transgenic mice having the Ile164 form of $\beta_2$-AR [11]. Two of the polymorphisms at the amino acid positions 16 and 27 are on the amino terminal while that at amino acid position 164 is in the fourth transmembrane domain. The distribution of the polymorphisms is changed in asthma, hypertension and obesity. The importance of the $\beta_2$-AR for the regulation of the immune system as well as skeletal and cardiac functions implies a potential importance of genetic variations of this gene also in MG.

Patients with MG have T and B cells that are stimulated to cytokine secretion by peptides from the $\beta_2$-AR [12], they have a

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less than normal density of such receptors on peripheral blood mononuclear cells (PBMC) [13] and in 25% also serum antibodies against both the $\beta_2$-AR and the $\beta_1$-AR [14]. The $\beta_2$-AR can thus be considered to be one of the autoantigens in MG. That polymorphisms in the genes coding for the autoantigen can be of importance has been demonstrated in the gene for AChR in MG [1,2] and the gene for insulin in insulin-dependent diabetes mellitus [15].

Our study of the polymorphisms in *ADRB2* showed significant differences between MG patients and healthy individuals with regard to the variants at amino acid positions 16 and 27 of the gene.

## SUBJECTS AND METHODS

*Study groups*

One hundred and forty-five Swedish Caucasian MG patients and 96 ethnically matched healthy individuals were studied.

Ninety-two female and 53 male MG patients were included. The clinical evaluation was done using the Osserman–Oosterhuis classification [16], and using the most severe stage ever present in the patients since start of disease. Ten patients had only ocular symptoms (stage I), 75 had mild generalized disease (stage IIA) and 60 had severe generalized disease (stage IIB-III). Ninety-eight of the patients were thymectomized, 19 had normal thymic histology, 55 had hyperplasia and 24 had thymomas. The onset of the disease was between 2 and 59 years. The 60 patients with severe disease had been or were treated with immunosuppressive drugs. One hundred and thirty-five patients were treated with cholinesterase inhibitors. Nineteen patients were treated with the $\beta_2$-AR-stimulating drug terbutaline sulphate which temporarily improves skeletal muscle function [17]. The evaluation of clinical stages and evidence for cardiovascular diseases was done by one of us (R.P.).

*DNA extraction*

Genomic DNA was extracted from EDTA preserved whole blood by a standard proteinase K digestion and phenol/chloroform method.

*$\beta_2$-AR genotyping*

Allele-specific polymerase chain reaction (PCR) was used with primers as described [18]. PCR reactions were carried out in a volume of 20 $\mu$l. Temperature cycling was 94°C for 30 s, 61°C for 45 s and 72°C for 45 s for 30 cycles for the polymorphisms of amino acid positions 16 and 27. Annealing temperature at 46°C for 45 s was used for the polymorphism of amino acid position 164. Ten microlitres of the PCR products were visualized on a 1·0% agarose gel, stained with ethidium bromide.

*ELISA for $\beta_2$-AR antibodies*

The ELISA assay was performed on 87 MG patients as previously described [14,19]. The serum antibody data for these patients have been described previously [14].

*Statistical analysis*

Mann–Whitney *U*-test was used for comparing the values of antibody concentrations between two groups and $\chi^2$ test with Yates' correction was used for comparing the prevalence of different receptor gene polymorphisms between two groups. Odds ratios (OR) and 95% confidence intervals (95% CI) for relative risks were calculated after use of Fisher's exact test when necessary. All *P* values were corrected for the number of comparisons made ($P_c$). A $P_c$ value $< 0{\cdot}05$ was considered to be significant. Both *P* and $P_c$ values are shown. Agreement between the observed genotypes and those predicted by the Hardy–Weinberg equilibrium was assessed by $\chi^2$ test.

## RESULTS

*Polymorphisms at amino acid positions 16, 27 and 164: increased prevalence of homozygosity for Arg16 in patients with generalized MG*

The prevalence of polymorphisms at amino acid positions 16, 27 and 164 in MG patients and healthy individuals and the genotypic

**Table 1.** Genotypes of the $\beta_2$-adrenergic receptor ($\beta_2$-AR) in patients with MG and in healthy individuals (HC)

| Amino acid position | Genotype | MG | | | HC | *P* | $P_c$ | OR | 95% CI |
|---|---|---|---|---|---|---|---|---|---|
| | | Ocular | All | Generalized | | | | | |
| 16 | Arg/Arg | | 32 (22·1) | | 7 (7·3) | 0·0022 | 0·0132 | 3·60 | 1·52–8·54 |
| | | 1 (10) | | 31 (23) | | 0·0019 | 0·0114 | 3·79 | 1·59–9·03 |
| | Arg/Gly | | 83 (57·2) | | 54 (56·3) | NS | NS | | |
| | | 3 (30) | | 80 (59·2) | | | | | |
| | Gly/Gly | | 30 (20·7) | | 35 (36·4) | 0·0079 | 0·0474 | 0·45 | 0·26–0·81 |
| | | 6 (60) | | 24 (40) | | 0·0022 | 0·0132 | 0·38 | 0·21–0·69 |
| | Carriage of Gly | | | | | | | | |
| | Yes | | 113 (77·9) | | 89 (92·7) | 0·0022 | 0·0088 | 0·27 | 0·12–0·66 |
| | No | | 32 (22·1) | | 7 (7·3) | 0·0022 | 0·0088 | 3·60 | 1·52–8·54 |
| 27 | Gln/Gln | | 36 (24·8) | | 22 (22·9) | NS | NS | | |
| | Gln/Glu | | 78 (53·8) | | 59 (61·5) | NS | NS | | |
| | Glu/Glu | | 31 (21·4) | | 15 (15·6) | NS | NS | | |
| 164 | Thr/Thr | | 144 (99·0) | | 96 (100) | NS | NS | | |
| | Thr/Ile | | 1 (1) | | 0 (0) | NS | NS | | |
| | Ile/Ile | | 0 (0) | | 0 (0) | NS | NS | | |

Percentages are shown in parentheses.
OR, Odds ratio; 95% CI, 95% confidence interval; NS, not significant.

frequencies of $\beta_2$-AR at amino acid position 16 in MG patients with ocular and generalized disease are presented in Table 1. The frequency of homozygosity for Arg16 was higher and the frequency of homozygosity for Gly16 was lower in patients than in healthy individuals. Patients with generalized disease had a higher prevalence of homozygosity for Arg16 and lower prevalence of homozygosity for Gly16 compared with healthy individuals, while there was no difference between patients with ocular MG and healthy individuals. The frequencies of gene polymorphisms at amino acid positions 27 and 164 did not differ between patients with ocular and generalized MG and healthy individuals. The number of homozygous and heterozygous alleles that were found was not different from that predicted by the Hardy– Weinberg relationship.

The prevalence of the gene polymorphisms related to age at disease onset and gender is shown in Table 2. Patients with early disease onset had a significantly higher frequency of homozygosity for Arg16. A tendency of increased frequency of homozygosity for Arg16 was found in female patients with early and male patients with late disease onset.

The prevalence of gene polymorphisms in patients with different thymic histopathology is shown in Table 3. There was a tendency to an increased frequency of homozygosity for Arg16 in patients with thymoma or thymic hyperplasia. There was also a tendency to a decreased frequency of homozygosity for Gly16 in patients with thymic hyperplasia.

The prevalence of gene polymorphisms at amino acid position 16 did not differ between patients with and without $\beta_2$-AR antibodies and AChR antibodies or between patients with severe and mild disease.

*Homozygosity for Glu27: negative association with $\beta_2$-AR antibodies, severity of disease and need for immunosuppressive therapy*

The frequencies of $\beta_2$-AR genotypes at amino acid position 27 in MG patients stratified with regard to presence of $\beta_2$-AR antibodies, AChR antibodies and severity of disease are shown in Table 4. No patient with $\beta_2$-AR antibodies was homozygous for Glu27. Mild disease, defined as stage I1 and IIA, was more often present in patients homozygous for Glu27, and these patients were or had been less often treated with immunosuppressive drugs. AChR antibodies were more prevalent in patients homozygous for Gln27.

There was a strong linkage disequilibrium between Arg16 and Gln27 in both healthy individuals ($P < 0{\cdot}0001$) and MG patients ($P = 0{\cdot}0002$), as shown in Table 5.

*No correlation between cardiovascular diseases and gene polymorphisms*

Thirty-six out of 145 MG patients had cardiovascular diseases. Among them, 21 had symptomatic heart disease and 15 hypertension. The frequencies of the polymorphisms at amino acid positions 16 and 27 did not differ between patients suffering from either hypertension or heart disease and those without cardiovascular diseases.

**Table 3.** Genotypes at amino acid positions 16 and 27 in patients with MG and different thymic histopathology

| | Thymic histopathology | | | | |
|---|---|---|---|---|---|
| Genotype | T ($n=24$) | H ($n=55$) | N ($n=19$) | U ($n=47$) | HC ($n=96$) |
| ArgArg16 | 7* | 12** | 3 | 9 | 7 |
| ArgGly16 | 12 | 33 | 10 | 29 | 54 |
| GlyGly16 | 5 | 10*** | 6 | 9 | 35 |
| GlnGln27 | 9 | 17 | 3 | 8 | 22 |
| GlnGlu27 | 11 | 30 | 13 | 25 | 59 |
| GluGlu27 | 4 | 8 | 3 | 14 | 15 |

HC, Healthy individuals; T, thymoma; H, hyperplasia; N, normal thymic histology; U, not thymectomized.

*$P = 0{\cdot}0073$; $Pc > 0{\cdot}05$; odds ratio (OR) = 5·23; 95% confidence interval (CI) = 1·63–16·86 compared with HC.

**$P = 0{\cdot}0194$; $Pc > 0{\cdot}05$; OR = 3·55; 95% CI = 1·30–9·65 compared with HC.

***$P = 0{\cdot}0259$; $Pc > 0{\cdot}05$; OR = 0·38; 95% CI = 0·17–0·86 compared with HC.

**Table 2.** Genotypes at amino acid positions 16 and 27 in patients with MG with early and late onset disease and in healthy individuals (HC)

| | MG, age of onset (years) | | | | | | |
|---|---|---|---|---|---|---|---|
| | <40 | | | >40 | | | |
| Genotype | Females ($n=57$) | Males ($n=23$) | Total ($n=80$) | Females ($n=35$) | Males ($n=30$) | Total ($n=65$) | HC ($n=96$) |
| ArgArg16 | 13* | 5 | 18** | 6 | 8*** | 14**** | 7 |
| ArgGly16 | 29 | 15 | 44 | 23 | 15 | 38 | 54 |
| GlyGly16 | 15 | 3 | 18 | 6 | 7 | 13 | 35 |
| GlnGln27 | 15 | 9 | 24 | 5 | 8 | 13 | 22 |
| GlnGlu27 | 32 | 12 | 44 | 19 | 15 | 34 | 59 |
| GluGlu27 | 10 | 2 | 12 | 11 | 7 | 18 | 15 |

*$P = 0{\cdot}0114$; $Pc > 0{\cdot}05$; odds ratio (OR) = 3·76; 95% confidence interval (CI) = 1·40–10·09 compared with HC.
**$P = 0{\cdot}0048$; $Pc = 0{\cdot}0288$; OR = 3·69; 95% CI = 1·45–9·37 compared with HC.
***$P = 0{\cdot}0083$; $Pc = 0{\cdot}0498$; OR = 4·62; 95% CI = 1·51–14·13 compared with HC.
****$P = 0{\cdot}0154$; $Pc > 0{\cdot}05$; OR = 3·49; 95% CI = 1·32–9·21 compared with HC.

**Table 4.** Genotypes and carriage of Gln at amino acid position 27 in patients with MG stratified by presence of antibodies against the $\beta_2$-adrenergic receptor ($\beta_2$-AR) and the acetylcholine receptor (AChR) and severity of disease

| | | $\beta_2$-AR antibody | | Stage | | AChR antibody | |
|---|---|---|---|---|---|---|---|
| | MG | Yes | No | IIB; III | I; IIA | Yes | No |
| *Genotype* | | | | | | | |
| Gln/Gln 27 | 36 | 7 | 15 | 18 | 19 | 36***** | 1 |
| Gln/Glu 27 | 78 | 15 | 35 | 36 | 42 | 63 | 16 |
| Glu/Glu 27 | 31 | 0* | 15 | 6*** | 24 | 20 | 9 |
| *Carriage of Gln* | | | | | | | |
| Yes | 114 | 22 | 50 | 54 | 61 | 99 | 17 |
| No | 31 | 0** | 15 | 6**** | 24 | 20 | 9 |

Stage IIB; III, Severe disease; stage I; IIA, mild disease.
*$P = 0{\cdot}0097$; $P_c > 0{\cdot}05$ compared with patients without $\beta_2$-AR antibody.
**$P = 0{\cdot}0097$; $P_c = 0{\cdot}0388$ compared with patients without $\beta_2$-AR antibody.
***$P = 0{\cdot}0116$; $P_c > 0{\cdot}05$ compared with patients with mild disease.
****$P = 0{\cdot}0116$; $P_c = 0{\cdot}0464$ compared with patients with mild disease.
*****$P = 0{\cdot}0051$; $P_c = 0{\cdot}0204$ compared with patients without AChR antibody.

**Table 5.** Association between carriage of Arg at amino acid position 16 and carriage of Gln at amino acid position 27

| | Carriage of Arg16 in MG | | Carriage of Arg16 in HC | |
|---|---|---|---|---|
| | Yes | No | Yes | No |
| Carriage of Gln27 | | | | |
| Yes | 99 | 17 | 59 | 22 |
| No | 15 | 14 | 0 | 15 |
| OR | 5·44 | | 82·0 | |
| *P* | 0·0002 | | < 0·0001 | |

HC, Healthy individuals; OR, odds ratio.

The frequencies of gene polymorphisms did not differ between HLA-B8, DR2, DR3 and DR4-positive and -negative patients. There was no difference between patients with and without treatment with cholinesterase inhibitors or $\beta_2$-AR agonists.

## DISCUSSION

In the present study, we have revealed a new genetic association with the *ADRB2* on chromosome 5 and showed that MG is associated with homozygosity for Arg16 (OR 3·6). The association between MG and homozygosity for Arg16 is present in female patients with early disease onset and males with late disease onset and in patients with generalized disease but not in ocular myasthenia. It is of special interest that patients with early and late onset disease had similar associations, since the MHC associations are clearly different for these disease subgroups [20,21]. This emphasizes that polymorphism at amino acid position 16 may constitute an additional genetic factor contributing to the susceptibility for MG.

Associations to the polymorphisms at amino acid position 27 emerged when patients were stratified according to severity of disease and the presence of $\beta_2$-AR antibodies and AChR antibodies. No patient with $\beta_2$-AR antibodies in serum was homozygous for Glu27. An explanation for this might be that individuals homozygous for Glu27 do not express the receptor in the fully mature form, in contrast to those homozygous for Gln27 [10]. The receptor homozygous for Glu27 might thus be less immunogenic and less capable of inducing antibody formation *in vivo*. In asthma, there is a strong association between the polymorphisms at amino acid position 27 and IgE levels [22]. MG patients homozygous for Glu27 also had a less severe disease than other patients. Our previous study showed that patients with $\beta_2$-AR antibodies generally had a more severe disease and were more often treated with immunosuppressive drugs [14]. This finding is thus now confirmed at the gene level. Thus, these gene polymorphisms seem to affect the immune response in the disease, possibly via the receptor on the cells of the immune system.

A genetic predisposition to MG is suggested by the high concordance rate in identical twins [23,24]. The contribution of MHC genes has been extensively evaluated [20,21], but the positive associations with the extended DR3 haplotype are rather weak, with average OR for HLA-B8 and HLA-DR3 of 3·3 and 2·0, respectively. This suggests the contribution of other genetic factor(s). We have earlier described associations to the gene for tumour necrosis factor-alpha (TNF-$\alpha$) and non-MHC associations to the 'high secretor genotypes' of the IL-1$\beta$ gene located at chromosome 2 [8]. The polymorphism of the $\beta_2$-AR has been extensively studied and certain polymorphisms are associated with asthma [22,25–27], hypertension [28] and obesity [29]. The functional basis for these associations is incompletely known. The $\beta_2$-AR contains significant amounts of N-linked carbohydrate and the glycosylation of $\beta_2$-AR determines the surface expression of the receptor [30]. Positions 16 and 27 are close to the glycosylation sites and may be important for cellular processing and $\beta_2$-AR expression. How Arg16, which is less common in the normal population than Gly16 [22], relates to susceptibility to MG remains to be elucidated. Our finding of a linkage disequilibrium between gene polymorphisms at amino acid positions 16 and 27 is in accordance with others [31]. It is interesting to note that although

a strong linkage was found in both MG and healthy individuals, this association was weaker in MG (OR = 5·44) than in healthy individuals (OR = 82). This might indicate a greater susceptibility to develop MG once linkage is broken.

Thus, this study of the prevalence of $\beta_2$-AR gene polymorphisms showed that homozygosity for Arg16 was associated with generalized disease, while homozygosity for Glu27 was associated with mild disease and with lack of $\beta_2$-AR antibodies. The $\beta_2$-AR gene polymorphisms thus constitute an additional non-MHC association in myasthenia gravis.

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