Lack of association of the CIITA -168A→G promoter SNP with myasthenia gravis and its role in autoimmunity

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Abstract

Background: The major histocompatibility complex class II transactivator (CIITA) regulates MHC class II gene expression. A promoter SNP -168A→G (rs3087456) has previously been shown to be associated with susceptibility to several immune mediated disorders, including rheumatoid arthritis (RA), multiple sclerosis (MS) and myocardial infarction (MI). Myasthenia gravis (MG) is an autoimmune disorder which has previously been shown to be associated with polymorphisms of several autoimmunity predisposing genes, including IL-1, PTPN22, TNF-α and the MHC. In order to determine if allelic variants of rs3087456 increase predisposition to MG, we analyzed this SNP in our Swedish cohort of 446 MG patients and 1866 controls.

Results: No significant association of the SNP with MG was detected, neither in the patient group as a whole, nor in any clinical subgroup. The vast majority of previous replication studies have also not found an association of the SNP with autoimmune disorders.

Conclusions: We thus conclude that previous findings with regard to the role of the CIITA -168A→G SNP in autoimmunity may have to be reconsidered.

Background

Myasthenia gravis (MG) is an antibody mediated autoimmune disorder characterized by auto-antibodies against the nicotinic acetylcholine receptor situated on the muscle end-plate. These auto-antibodies impair the transmission of nerve impulses to the muscle. MG patients commonly display thymic abnormalities such as hyperplasia and thymoma and the latter is usually associated with severe disease. MG occurs in 14.1 per 100,000 persons in Sweden, and has a concordance rate of 30-40% in monozygotic twins and 2-3% in dizygotic twins, indicating a strong genetic component. Subgroups of patients have commonly been made based on age of onset, thymic status, and disease severity. Several autoimmune predisposing genes have previously been shown to be associated with MG, including IL-1, PTPN22, and genes in the major histocompatibility complex (MHC), particularly the human leukocyte antigen (HLA)-B8, DR3 haplotype and TNF-α [1].

The class II transactivator (CIITA, GenBank accession number NM_000246), located on chromosome 16p13, is a transactivator of the MHC class II genes [2]. Four alternative promoters, which exhibit cell-type-specific activity, drive transcription of the CIITA gene [3]. Expression of MHC class II proteins is crucial for cell collaboration and induction of immune responses, and lack of expression is associated with the severe immunodeficiency disease bare lymphocyte syndrome (BLS) [2].

In view of its suggested role in autoimmune disorders [4], we sought to determine if the CIITA rs3087456 variant is associated with autoimmune MG, using a large cohort of Swedish patients.

Methods

Patients and controls

This study included 466 unrelated Swedish MG patients and 1866 healthy control individuals of self-reported European ancestry. MG was diagnosed as described previously [5], and clinical information was documented by
the primary physician. The controls were derived from blood donors in the Stockholm area (n = 533; adults) and from a population based Swedish material (n = 1333; newborns) [6]. Ethical permission was obtained from the Karolinska Institutet for use of patient and control samples.

**MHC2TA genotyping**

Genotyping of the 446 MG cases and 1866 control samples was performed using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) [7] mass spectrometry (SEQUENOM Inc., San Diego, California, USA) at the Mutation Analysis Facility of the Karolinska Institutet, Sweden. PCR was conducted using forward primer ACGTTGGATGCTTCACAAAATTCAGTCCAC and reverse primer ACGTTGGATGTTTACCA-CACTCCCTTAAGCC. The MHC SNP rs3087456 was genotyped using iPLEX chemistry utilizing unextended primer (UEP) CACTCCCTTAAGCCCTCC and extension primers CACTCCCTTAAGCCCTCC and CACTCCCTTAAGCCCTCC.

**Statistical analysis**

The \( \chi^2 \) test was used to compare genotypes and allele frequencies of the *CIITA* SNP in patients and controls. For the overall MG cohort, a \( p \)-value below 0.05 was considered to indicate statistical significance. For subsequent analyses, a Bonferroni correction was applied based on the number of subgroups to determine the significance threshold. Power for the study was calculated using “CaTS - Power Calculator for Two Stage Association Studies” (http://www.sph.umich.edu/csg/abecasis/CaTS/) [8]. The study had 80% power to detect allelic odds ratios greater than 1.28 at the stated significance level (\( \alpha = 0.05 \)), with a MAF of 0.266 using an additive model and 1.39 using a dominant model.

**Patient subgrouping**

Due to the complex nature of MG, we stratified the patient material into subgroups based on clinical information to investigate association to potential sub-classes of the disease. Patients were thus separated on the basis of thymic histopathology identified post-operatively after thymectomy (not operated, normal, hyperplasia or thymoma), disease severity (ocular, generalized or severe) and by age of onset. A Bonferroni correction for the eight subgroups created was applied to the significance threshold (\( p < 6.25 \times 10^{-3} \)) used in subsequent comparisons.

There is a lack of consensus regarding the age which most accurately separates early onset MG (EOMG) from late onset MG (LOMG), with several publications using 50 years of age [9,10] and others using 40 years of age [11]. Therefore, in order to avoid erroneous results, patients with an age of disease onset less than 40 years constituted the EOMG group, while those with age of onset of 50 years or older were included in the LOMG group.

**Results**

There were no significant differences in the rs3087456 allele frequency between the blood donor (n = 533; adults) and population based (n = 1333; newborns) control subgroups (\( p = 0.192 \)). Therefore, they were pooled into one group comprising 1866 samples.

The genotyping results for the 446 MG patients and 1866 controls and \( p \)-values of association of the SNP with MG are given in Table 1. No statistically significant difference was observed between MG patients and controls for either allele frequencies (\( p = 0.092 \)) or genotypes (\( p = 0.251 \)). The strongest associations were in the ocular and EOMG subgroups, with uncorrected \( p \)-values of 0.040 and 0.010, respectively. Nevertheless, they

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**Table 1 Results of CIITA genotyping in MG patients and controls**

| MG patients | AA | AG | GG | A  | G  | MAF | p-val | Significance threshold | OR (95% C.I.) | p-val | reference |
|-------------|----|----|----|----|----|-----|-------|------------------------|--------------|-------|-----------|
| All patients (n = 446) | 260 | 159 | 27 | 679 | 213 | 0.239 | 0.092 | 0.05 | 0.86 (0.73-1.02) | 0.154 |
| Age of onset <40 (n = 226) | 127 | 80 | 19 | 334 | 118 | 0.261 | 0.810 | 6.25 \times 10^{-3} | 1.03 (0.82-1.28) | 0.032 |
| Age of onset >50 (n = 175) | 110 | 59 | 6 | 279 | 71 | 0.203 | 0.010 | 6.25 \times 10^{-3} | 1.43 (1.09-1.87) | 0.559 |
| Ocular (n = 42) | 29 | 12 | 1 | 70 | 14 | 0.167 | 0.040 | 6.25 \times 10^{-3} | 0.55 (0.31-0.98) | 0.274 |
| Generalized (n = 404) | 231 | 147 | 26 | 609 | 199 | 0.246 | 0.240 | 6.25 \times 10^{-3} | 0.90 (0.76-1.07) | 0.068 |
| Not operated (n = 171) | 103 | 58 | 10 | 264 | 78 | 0.228 | 0.124 | 6.25 \times 10^{-3} | 0.81 (0.63-1.06) | 0.619 |
| Normal thymus (n = 60) | 35 | 25 | 0 | 95 | 25 | 0.208 | 0.156 | 6.25 \times 10^{-3} | 0.72 (0.46-1.13) | 0.833 |
| Thymic hyperplasia (n = 157) | 87 | 59 | 11 | 233 | 81 | 0.258 | 0.747 | 6.25 \times 10^{-3} | 0.96 (0.74-1.25) | 0.090 |
| Thymoma (n = 58) | 35 | 17 | 6 | 87 | 29 | 0.250 | 0.695 | 6.25 \times 10^{-3} | 0.92 (0.60-1.41) | 0.389 |

\( ^a \)Controls (n = 1866) 1015 708 143 2738 994 0.266

\( ^b \)Controls (n = 1599) 989 528 82 2506 692 0.216

Genotypes, frequencies of A and G alleles as well as the minor allele frequency (MAF) for MG patients and various subgroups of patients compared to the measured controls\(^a\) and the controls from Swanberg et. al.\(^b\) [4]. The significance threshold applies a Bonferroni correction for eight tests on subsequent subgroup classifications.
yielded non-significant $p$-values when compared with the corrected significance threshold of $6.25 \times 10^{-3}$.

**Discussion**

Previously, evidence for association between the *CIITA* type III promoter (-168A/G, rs3087456) and rheumatoid arthritis (RA) and multiple sclerosis (MS) was reported [4]. This polymorphism is associated with reduced transcription levels of *CIITA* ex vivo in human leukocytes stimulated with interferon-$\gamma$, and *in vivo* experiments in rats have demonstrated a strong correlation between lowered levels of *CIITA* transcripts and reduced expression of MHC class II molecules [4]. We therefore tested our Swedish MG patients and controls for association with this SNP, revealing that no significant difference in the allelic frequencies of the SNP exists between patients and controls and among patients when stratified by clinical subgroups of the disease.

Our Swedish control material differed from the Swedish control material presented by Swanberg et. al. [4] with a significantly higher frequency of the minor (G) allele (0.266 and 0.216, respectively, $p < 10^{-5}$). In order to exclude association of MG with rs3087456, given the large variation in control allele frequencies, we also compared MG allele frequencies to this control material. However,

| Table 2 Summary of published *CIITA* SNP rs3087456 association studies in autoimmune disorders |
|-----------------------------------------------|
| **Study** | **Year** | **Population** | **Disease** | **Patients/Controls** | **Association** |
| Swanberg, et. al. [4] | 2005 | Sweden | RA | 1262/2506 | $p = 0.012$ |
| Swanberg, et. al. [4] | 2005 | Sweden | MS | 520/2506 | $p = 0.028$ |
| Swanberg, et. al. [4] | 2005 | Sweden | MI | 376/2506 | $p = 0.014$ |
| Koizuma et. al. [20] | 2005 | Japan | SLE | 100/100 | Not significant |
| Yazdani-Biuki et. al. [21] | 2006 | Austria | RA | 362/1709 | Not significant |
| Orozco et. al. [12] | 2006 | Spain | RA | 748/676 | $p = 0.01^a$ |
| Orozco et. al. [12] | 2006 | Sweden | RA | 278/478 | Not significant |
| Orozco et. al. [12] | 2006 | Argentina | RA | 287/287 | Not significant |
| Akkad et. al. [22] | 2006 | Germany | RA | 319/463 | Not significant |
| Akkad et. al. [22] | 2006 | Germany | MS | 646/463 | Not significant |
| Akkad et. al. [22] | 2006 | Germany | WG$^b$ | 178/463 | Not significant |
| Eyre et. al. [23] | 2006 | UK | RA | 1401/2475 | Not significant |
| Lindholm et. al. [24] | 2006 | Finland/Sweden | MI | 1222/2345 | Not significant |
| Ghaderi et. al. [16] | 2006 | Italy | AAD$^c$ | 128/406 | $p = 0.003$ |
| O'Doherty et. al. [25] | 2007 | Ireland | RA | 293/316 | Not significant |
| O'Doherty et. al. [25] | 2007 | Ireland | MS | 440/316 | Not significant |
| O'Doherty et. al. [25] | 2007 | Ireland | JIA$^d$ | 74/316 | Not significant |
| Linga-Reddy et. al. [17] | 2007 | Sweden | SLE | 334/478 | Not significant |
| Martinez et. al. [26] | 2007 | Spain | RA | 350/519 | Not significant |
| Martinez et. al. [26] | 2007 | Spain | MS | 396/519 | Not significant |
| Martinez et. al. [26] | 2007 | Spain | IBS$^e$ | 663/519 | Not significant |
| Ikuni et. al. [13] | 2007 | Japan | RA | 1121/450 | $p = 0.003^f$ |
| Harrison et. al. [27] | 2007 | UK | RA | 733/613 | Not significant |
| Sánchez et. al. [28] | 2008 | SLE | SLE | 394/514 | Not significant |
| Pan-Hammarström, et. al. [29] | 2008 | Sweden | CVID$^g$ | 97/1826 | Not significant |
| Pan-Hammarström, et. al. [29] | 2008 | Sweden | IgAD$^h$ | 249/1826 | Not significant |
| Skinningsrud, et. al. [15] | 2008 | Norway | AAD$^c$ | 332/1029 | $p = 0.044^i$ |
| Dema et. al. [30] | 2009 | Spain | CD$^j$ | 607/794 | Not significant |
| Bronson, et. al. [19] | 2010 | USA/UK | MS | 1320/1363 | Not significant |

*a*Considered by the authors to be a possible Type I error.

*Wegener’s granulomatosis.*

*Autoimmune Addison’s disease.*

*Juvenile idiopathic arthritis.*

*Inflammatory bowel syndrome.*

*Association with the major (A) rather than the minor allele of rs3087456.*

*Common variable immunodeficiency.*

*IgA deficiency.*

*Uncorrected $p$-value in a study of 139 SNPs.*

*Celiac disease.*
the MG group did not show a statistically significant association, nor did any subgroup of MG (Table 1).

Although the initial study on rs3087456 in autoimmune/inflammatory disorders showed a clear association with the minor (G) allele, subsequent studies on a variety of similar disorders have been inconsistent and for the most part have shown a lack of association. Of 17 replication studies using patients from 26 different disease cohorts (Table 2), only four have found an association between rs3087456 and a disorder. In the case of RA, eight replication studies have been conducted in 10 different populations, of which only two [12,13] found an association with rs3087456. In one of the latter, Ikuni et al. found that although the allele frequencies in controls were similar to those in Sweden, the major (A) allele was associated with an increased risk of RA in a Japanese patient cohort ($p = 0.003$). This result runs contrary to the increased risk of the minor (G) allele reported in a Swedish population [4] and brings up the proposed genetic mechanism associated with the minor allele into question. A meta-analysis of the 10 RA data sets consisting of 6861 patients and 9270 controls also determined that neither the G allele ($p = 0.70$) nor the GG genotype ($p = 0.16$) are associated to the disease [14]. In the 16 data sets from studies which have examined the association of the SNP with other autoimmune/inflammatory disorders than RA, only two have reported a positive association to the SNP, both with autoimmune Addison’s disease [15,16]. In the first study, the p-value ($p = 0.044$) was not corrected for multiple testing in a study of 139 SNPs, drawing the association into question. One replication study in patients with myocardial infarction as well as four in multiple sclerosis in different populations did not find any association with rs3087456. Furthermore, a Swedish study, which found no association of the SNP with SLE, had a MAF of 0.253, in 956 controls, similar to that in our controls ($p = 0.41$), and the authors concluded that the differences in allele frequencies between their controls and those of Swanberg et al. could not be explained by population substructure [17]. Although there is evidence of potential interaction between SNPs in CIITA [18] and HLA alleles [19] in autoimmunity, the promoter SNP rs3087456 alone appears to predispose neither to myasthenia gravis nor to autoimmunity in general. A further investigation of interacting factors contributing to altered MHC class II expression in autoimmune disorders is warranted.

**Conclusions**

The lack of allelic association of the CIITA SNP -168A>G (rs3087456) with MG casts further doubt on the presumed role of this particular SNP in autoimmune disorders. In the case of single gene association, results should be replicated, even in the same population, to confirm their validity. This study also illustrates the importance of large, homogeneous sample sizes, particularly for controls, in order to avoid publication bias.

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**Authors’ contributions**

R analyzed data, performed the statistical analysis, interpreted the results and drafted the manuscript. YZ prepared the samples and analyzed data. RP acquired patient material and compiled clinical data. LH conceived of the experiments, interpreted the results and drafted the manuscript. All authors read and approved the manuscript.

**Competing interests**

The authors declare that they have no competing interests.

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**References**

1. Graul M, Vandiedonck C, Garchon H: Genetic factors in autoimmune myasthenia gravis. Ann N Y Acad Sci 2008, 1132:180-192.

2. Steimle V, Otten LA, Zufferey M, Mach B: Complementation cloning of an MHC class II transactivator mutated in hereditary MHC class II deficiency (or bare lymphocyte syndrome). Cell 1993, 75:135-146.

3. LeibundGut-Landmann S, Waldburger J, Krawczyk M, Otten LA, Suter T, Fontana A, Acha-Orbea H, Reith W: Mini-review: Specificity and expression of CIITA, the master regulator of MHC class II genes. Eur J Immunol 2004, 34:1513-1525.

4. Swanberg M, Lidman O, Padyukov L, Eriksson P, Akesson E, Jagodic M, Lobell A, Khademi M, Börjesson O, Lindgren CM, Lundman P, Brookes AJ, Kere J, Ljumtun H, Alfredsson L, Hilbert J, Klarenkog L, Hamsten A, Piehl F, Olsson T: MHC2TA is associated with differential MHC molecule expression and susceptibility to rheumatoid arthritis, multiple sclerosis and myocardial infarction. Nat Genet 2005, 37:486-94.

5. Drachman DB: Myasthenia gravis. N Engl J Med 1994, 330:1797-1810.

6. Hannelius U, Lindgren CM, Mällén E, Malmberg A, von Dobeln U, Kere J: Phenylketonuria screening registry as a resource for population genetic studies. J Med Genet 2005, 42:630.

7. Jünikke C, van den Boom D, Cantor CR, Köster H: Automated genotyping using the DNA MassArray technology. Methods Mol Biol 2002, 187:179-192.

8. Skol AD, Scott LJ, Abecasis GR, Boehnke M: Joint analysis is more efficient than replication-based analysis for two-stage genome-wide association studies. Nat Genet 2008, 39:209-213.

9. Chan K, Cheung R, Mak W, Ho S: Nonthymoma early-onset- and late-onset-generalized myasthenia gravis–A retrospective hospital-based study. Clinical Neurology and Neurosurgery 2007, 109:686-691.

10. Gilhus NE: Autoimmune myasthenia gravis. Expert Rev Neurother 2009, 9:351-358.

11. Menggali MN: Myasthenia Gravis with Anti-Acetylcholine Receptor Antibodies. Front Neurol Neurosci 2009, 26:94-108.

12. Orozco G, Robledo G, Linga Reddy MJP, García A, Pascual-Salcedo D, Balas A, González-Gay MA, Eimon A, Paiva S, Scherberich HR, Pons-Estel BA, Petersson IF, Alarcón-Quelme M, Martin J: Study of the role of a...
functional polymorphism of MHC2TA in rheumatoid arthritis in three ethnically different populations. *Rheumatology (Oxford)* 2009.

13. Ikuni N, Ikan K, Momohara S, Tomatsu T, Hara M, Yamanaka H, Okamoto H, Kamatani N: MHC2TA is associated with rheumatoid arthritis in Japanese patients. *Ann Rheum Dis* 2007, 66:274-275.

14. Bronson PG, Criswell LA, Barcellos LF: The MHC2TA -168A/G polymorphism and risk for rheumatoid arthritis: a meta-analysis of 6861 patients and 9270 controls reveals no evidence for association. *Ann Rheum Dis* 2008, 67:933-6.

15. Skinningsrud B, Husebye ES, Peace SH, McDonald DO, Brandal K, Wolf AB, Levás K, Egeland T, Undlien DE: Polymorphisms in CLEC16A and CIITA at 16p13 are associated with primary adrenal insufficiency. *J Clin Endocrinol Metab* 2008, 93:3310-3317.

16. Ghaderi M, Gambelunghe G, Tortoici C, Brozzetti A, Jata K, Gharzadeh B, De Bells A, Pecon Giraldi F, Tenzolo M, Betterle C, Falorni A: MHC2TA single nucleotide polymorphism and genetic risk for autoimmune adrenal insufficiency. *J Clin Endocrinol Metab* 2008, 93:4107-4111.

17. Linga-Reddy MVP, Gunnarsson I, Svennungsson E, Sturfelt G, Jonsen A, Truedsson L, Nordmark G, Ronnlblom L, Alarcón-Riquelme ME: A polymorphic variant in the MHC2TA gene is not associated with systemic lupus erythematosus. *Tissue Antigens* 2007, 71:412-4.

18. Martínez A, Pergidones N, Cénit M, Espino L, Varadé J, Lamas JR, Santiago JL, Fernández-Arquero M, de la Calle H, Arroyo R, de la Concha EG, Fernández-Gutiérrez B, Urcey E: Chromosomal region 16p13: further evidence of increased predisposition to immune diseases. *Ann Rheum Dis* 2009.

19. Bronson PG, Gaillier S, Ramsay PP, McCauley JL, Vuzich RL, De Jager PL, Rioux JD, Ivinson AJ, Compton A, Hafer DA, Saxer SJ, Penicak-Yancey MA, Haines JL, Hauiser SL, Okenbrook JR, Barcellos LF: CIITA variation in the presence of HLA-DRB1*1501 increases risk for multiple sclerosis. *Hum Mol Genet* 2010, 19:2331-2340.

20. Koizumi K, Okamoto H, Ikuni N, Nakamura T, Kawamoto M, Momohara S, Ichikawa N, Furuya T, Kotake S, Taniguchi A, Yamanaka H, Kamatani N: Single nucleotide polymorphisms in the gene encoding the major histocompatibility complex class II transactivator (CIITA) in systemic lupus erythematosus. *Ann Rheum Dis* 2005, 64:947-50.

21. Yazdani-Biuki B, Brickmann K, Rohlfhaf K, Mueller T, Marz W, Rnenner W, Gutjahr M, Langsenlehner U, Koppel P, Wascher TC, Paulweber B, Greninger W, Breiteneck H: The MHC2TA -168A > G gene polymorphism is not associated with rheumatoid arthritis in Austrian patients. *Arthritis Res Ther* 2006, 8:R97.

22. Akkad DA, Jagello P, Szyl P, Goedde R, Wieczorek S, Gross WL, Epplen JT: Promoter polymorphism rs3087456 in the MHC class II transactivator gene is not associated with susceptibility for selected autoimmune diseases in German patient groups. *Int J Immunogenet* 2006, 33:59-61.

23. Eyre S, Bowes J, Spreckley K, Potter C, Ring R, Stouch D, Wootington J, Barton A: Investigation of the MHC2TA gene, associated with rheumatoid arthritis in a Swedish population, in a UK rheumatoid arthritis cohort. *Arthritis Rheum* 2006, 54:3417-22.

24. Lindholm E, Melander O, Almgren P, Berglund G, Agardh C, Groop L, Orho-Melander M: Polymorphism in the MHC2TA Gene Is Associated with Features of the Metabolic Syndrome and Cardiovascular Mortality. *PLoS ONE* 2006, 1:e64.

25. O’Doheety C, Hawkins S, Rooney M, Vandenbroeck K: The MHC2TA-168A/G promoter polymorphism and +1614G/C polymorphisms and risk for multiple sclerosis or chronic inflammatory arthropathies. *Tissue Antigens* 2007, 70:247-51.

26. Martínez A, et al: Role of the MHC2TA gene in autoimmune diseases. *Ann Rheum Dis* 2007, 66:325-329.

27. Sánchez E, Sabio JM, Jiménez-Alonso J, Callegas JL, Camps M, de Ramón E, García-Portales R, de Hato R, Ortego-Centeno N, López-Nevo MA, Martín J: Study of two polymorphisms of the MHC2TA gene with systemic lupus erythematosus. *Rheumatology (Oxford)* 2008, 47:102-3.

28. Pan-Hammarström Q, Hammarström L: Antibody deficiency diseases. *Eur J Immunol* 2008, 38:327-33.

29. Dema B, Martínez A, Fernández-Arquero M, Maluenda C, Polanco I, Angeles Figueroa M, de la Concha EG, Urcey E, Núñez C: Autoimmune disease association signals in CIITA and KIAA0350 are not involved in celiac disease susceptibility. *Tissue Antigens* 2009, 73:326-329.

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