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# Variability in the CIITA gene interacts with HLA in Multiple Sclerosis.

*Short title: Genetic HLA-CIITA interactions in MS*

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Abstract

The HLA is the main genetic determinant of multiple sclerosis (MS) risk. Within the HLA, the class II HLA-DRB1*15:01allele exerts a disease promoting effect, whereas the class I HLA-A*02 allele is protective. The *CIITA* gene is crucial for expression of class II HLA molecules and has previously been found to associate with several autoimmune diseases, including MS and Type 1 diabetes.

We here performed association analyses with *CIITA* in 2000 MS cases and up to 6900 controls as well as interaction analysis with HLA. We find that the previously investigated single nucleotide polymorphism rs4774 is associated to MS risk in cases carrying the HLA-DRB1*15 allele (p=0.01, OR=1.21, 95%CI: 1.04-1.40) or the HLA-A*02 allele (p=0.01, OR=1.33, 95%CI=1.07-1.64) and that these associations are independent of the adjacent confirmed MS susceptibility gene *CLEC16A*. We also confirm interaction between rs4774 and HLA - -DRB1*15:01 such that individuals carrying the risk allele for rs4774 and HLA-DRB1*15:01have a higher than expected risk for MS.

In conclusion, our findings support previous data that variability in the *CIITA* gene affects MS risk, but also that the effect is modulated by MS-associated HLA haplotypes. These findings further underscore the biological importance of HLA for MS risk.

Keywords

*CIITA*, HLA, interaction, Multiple sclerosis,

## Introduction:

Accumulating evidence support the notion that Multiple Sclerosis (MS) is primarily an autoimmune disease, characterized by lesions in the brain and spinal cord caused by demyelination as a result of periodical infiltration of auto reactive immune cells. This leads to a progressive accumulation of sclerotic plaques where nerve axons are damaged, in turn leading to increasing neurological disability. Typically the disease starts in early adulthood and is more common in women[1].

The HLA region on chromosome 6 has been established as the main genetic risk determinant area for MS, with the strongest disease promoting effect exerted by class II DRB1*15:01 [2,3], while the class I A*02 allele has been associated with reduced risk[4,5]. However, the disease etiology of MS is complex and several other genes as well as environmental factors provide additional influences on disease risk [4,6].

The *CIITA* gene (16p13) is of particular interest as a candidate gene for several autoimmune diseases, since the encoded protein functions as an assembler of the transcription factors necessary for transcription of major histocompability complex class II (MHCII) molecules. The essential function of *CIITA* is evident in Bare Lymphocyte Syndrome (BLS), a rare condition characterized by lack of MHC class II molecules associated with severe immunodeficiency[7] (OMIM 9920). In addition, *CIITA* may also be involved in the regulation of expression of class I molecules as well as other genes in immune cells [8; 9]. The *CIITA* gene is controlled by four independent and cell type specific promoters (PI-PIV), and the gene product expressed from promotor III is the one mostly investigated, since it is used in B-cells, activated T cells and plasmacytoid dendritic cells. The promotor I (PI) mainly controls *CIITA* expression in myeloid dendritic cells and macrophages. Promotor IV is used in a variety of cells, among them thymic epthelial cells, while the promoter II (PII) is of unknown function in humans [10].

Variability in the *CIITA* gene has been reported to be associated to several diseases, among them MS[11], Myocardial Infarction (MI)[11] Rheumatoid Arthritis (RA)[11],[12], Type 1 Diabetes (T1D) [13], Addison's disease [14] and Celiac disease [15], but lack of association have also been reported in several studies [16],[17],[18] and association has not always been possible to reproduce in different populations. We have recently shown that the allele frequency of *CIITA* markers vary with age in a large pooled material of individuals used as controls in different genetic association studies[13]. It is therefore possible that contradictory findings regarding any possible association between *CIITA* and disease risk at least in part can be explained by insufficient matching for age between patients and controls.

Various SNPs in the *CIITA* gene have been associated with MS risk. Thus, *Swanberg et al* reported association to rs3087456 and also decreased expression of MHC class II after stimulation of leukocytes with interferon-$\gamma$ for the minor allele of rs3087456 [11]. *Bronson et al* found association to MS for marker rs4774, particularly in DRB1*15:01 positive individuals, but could not reproduce the finding of the rs3087456 SNP[19].

In this study we investigate the possible association of several SNPs in the *CIITA* gene to MS, among them the earlier reported rs4774 and rs3087456, as well as the interaction with DRB1*15 and A*02, taking care to correct for variation in age.
We test the association both in the whole cohort of cases and controls, and after stratification for DRB1*15 and A*02.
We initially found association for rs4774, which was stronger when stratifying for the presence of DRB1*15 and A*02, respectively. Also, interactions between the *CIITA* marker and HLA alleles both on the multiplicative scale and on the additive scale were detected.

Results:

SNP markers in the *CIITA* gene were selected based on prior reports of association to MS (rs3087456, rs4774) and our previous study of *CIITA* in T1D[13].

First, we performed an univariate association analysis between *CIITA* markers and MS in the combined material of 2000 MS patients and up to 6900 controls from studies of MS, RA, MI and T1D (*supplementary table 1*), based on presence of minor allele versus major allele homozygotes. A significant association of rs4774 (p=0.01) was discovered in this analysis (*table1*).

We have previously shown that the frequency of genotypes within the *CIITA* gene varies with age among controls, and therefore matching for age among cases and controls should be considered in association studies [13]. In a logistic regression model with age as a covariate, the association to MS remained for rs4774 (p=0.01, OR 1.17, CI: 1.03-1.31) *(table 1)*. None of the other *CIITA* markers where associated to MS in any of these analyses.

*Bronson et al* [19] found a stronger association to MS for the rs4774 marker with stratification for presence of the main disease associated class II haplotype DRB1*15:01. We therefore investigated rs4774 both when all individuals were stratified for HLA-DRB1*15 but also when all individuals were stratified for HLA-A*02, and corrected for age with logistic regression.

In the DRB1*15 positive cohort (977 cases, 932 controls), association for rs4774 (p=0.03, OR: 1.23, 95%CI: 1.02 - 1.49) was observed (table 2), but we also detected a suggestive association to MS for rs3087456 minor allele homozygotes (1011 cases and 1154 controls, p=0.07) (data not shown). None of the other investigated *CIITA* SNPs were associated among DRB1*15 stratified individuals. We also investigated if there was any association between SNPs in *CIITA* and DRB1*15 among the controls, but found no such association (data not shown).

When the material was stratified in the same manner for absence of DRB1*15, no association was found between *CIITA* and MS among DRB1*15 negative individuals (*table 2*).

The same analysis was performed in cohorts stratified for presence or absence of HLA-A*02. We here found that rs4774 was associated to MS in cases stratified for HLA-A*02 (650 cases, 757 controls, p=0.01, OR 1.33, 95%CI: 1.07-1.64), but not in the absence of HLA-A*02 (*table 2*). No other marker showed significant association in these cohorts. We also found association between A*02 and rs4774 among the controls, with homozygotes for the minor allele being more common among those who lack A*02 (p=0.02).

When we investigated the interaction between HLA and *CIITA* we detected multiplicative interaction for rs4774 – HLA*A2 (p=0.03, OR: 1.40, 95%CI: 1.02-1.90) and additive interactionfor rs4774- DRB1*15 (AP:0.17, 95%CI: 0.01-0.33, p=0.04) (*fig.1*). The detected interaction suggests that for individuals who carry the DRB1*15 haplotype, the minor allele of rs4774 exerts an increased risk.

Four markers in the nearby CLEC16A gene, an established MS risk gene [20,21], were included in the analyses to rule out that any association in *CIITA* was due to linkage disequilibrium (LD) with this gene. The SNPs have been reported in genome-wide association studies[20; 22] and lately in the MS Immunochip study[23] as the strongest associated markers to MS in the *CLEC16A* gene. In our material, all of the four *CLEC16A* markers where significantly associated to MS (*table 1*), and the association to rs4774 remained significant (p<0.05) when adding them to the logistic regression model. We also performed an LD plot *(fig.2)* demonstrating a low LD between the markers in the two genes ($r^2 \leq 0.04$).

Finally, we conducted a meta-analysis on the material presented here and that of Bronson et al[19] with stratification of cases for the presence of DRB1*15 (*fig.3*). In this analysis the association found by us is weaker than the association Bronson et al found in their cohorts, but goes in the same direction and the combined OR is 1.3 (95%CI: 1.15-1.42) for presence of the minor allele (CC, CG) versus major allele homozygotes (GG).

## Discussion:

We here replicate the previous findings[11; 19] of an association between genetic variability in *CIITA* and MS risk. Based on the biological function the *CIITA* gene is a particularly interesting candidate gene in autoimmune diseases, considering its crucial role in regulating the expression of MHCII molecules, in turn the main genetic determinant of disease risk. The MHCII molecules are central in antigen presentation to lymphocytes, but also for presenting self-proteins and contributing to maintaining immunological tolerance. The DRB1 locus in the HLA class II region is the major established genetic determinant of disease risk in MS, which makes interaction between these loci plausible for any possible association to the *CIITA* gene. Experimental studies also suggest that *CIITA* is involved in the regulation of MHCI genes, as well as other immune genes, making additional associations possible [9; 24].

In previous studies variability in the *CIITA* gene have been reported to be associated to several autoimmune diseases. However, negative studies failing to replicate these results have also been published [16-18]. These contradictory findings may depend on heterogeneity in case-control studies, technical issues and on how the control groups were chosen. We have recently shown that there is a variation in allele frequencies for markers in *CIITA* depending on age among a large pooled cohort of controls used in genetic association studies[13]. Thus, lack of age matching between cases and controls may affect the results in association studies. The earlier reported rs3087456 marker in *CIITA* is one such marker on which there has been a controversy and contradictive results in different studies. In the current study we could not find any association to this marker in the present cohort. However, when we stratified both cases and controls for DRB1*15 and corrected for age in logistic regression, we detected a tendency towards association to MS for the minor allele homozygote for this marker (p=0.07, data not shown). In addition, the initial association of rs4774 to MS in univariate analysis, was strengthened when we stratified cases for DRB1*15 or A*02 haplotype. Earlier studies (except *Bronson et al*) have not taken into account the effect of HLA haplotypes in the investigated material, and this likely have influence on the results given the biological function of CIITA.

*CLEC16A* is a well established risk gene for MS that map close to the *CIITA* gene[20; 21]. It has been shown that the association to MS for these two genes are independent of each other [19], and the relationship between *CIITA* and *CLEC16A* has been investigated thoroughly, revealing a low degree of linkage disequilibrium between the genes[25], which also is confirmed in our material *(*$r^2 \leq 0.04$*) (fig.2).*

The significance level of the observed association for rs4774 to MS in our material is modest and does not reach genome-wide significance. No SNPs in CIITA has been reported as associated to MS in genome-wide studies. However, given the complex nature of the etiology of MS, genes with low or moderate effect are believed to affect the disease as well. When we performed a meta analysis with our and *Bronsons* data the effect was in the same direction, which supports a role for *CIITA* in MS susceptibility.

When performing genetic association studies it is important to consider the effect of population stratification. In this study we do not have genome wide SNP genotypes for all our samples, which would have allowed us to perform principal component analysis (PCA). Instead, we have removed all individuals with known non-Scandinavian descent, and when PCA analysis was possible we have removed outliers. It should also be pointed out that all individuals are resident in Sweden and collected from Swedish clinics, which should increase the ethnic homogeneity.

The physical relevance of the association of this *CIITA* marker depending on DRB1*15 and A*02 is unknown; possibly it could have effect on the function of the *CIITA* gene and subsequently on MHCI and II expression, which in turn have different outcomes depending on what HLA allele an individual carries. *Swanberg et al* [11] found a lower expression of the *CIITA* gene and lower levels of MHCII transcripts for individuals with the minor allele homozygote (GG) genotype of *CIITA*-marker rs3087456 in stimulated peripheral blood mononucleated cells( PBMCs) as compared to other genotypes. The LD between rs3087456 and rs4774 is low so we cannot conclude that rs4774 affect expression of MHCII from this study. Nor is rs4774 situated in a promotor region, but it is a missense mutation, causing amino-acid change from glycine to alanine. How this will affect the expression of the *CIITA* gene and the function of the CIITA protein remains unclear. Further experiments are needed to test this hypothesis.

It can be argued that variability in expression of the strongly associated HLA haplotype DRB1*15 could have an effect on MS susceptibility, whereas variation in the expression of HLA haplotypes not associated to MS does not affect MS susceptibility.

The MHCII molecule is important both for T-cell selection in the thymus as well as for antigen recognition in the periphery. There are several mechanisms by which a change in MHCII expression could affect disease susceptibility, for example through lack of tolerance induction in T-cells, influenced effect on regulatory T-cells or less effective clearing of pathogens that could play a role in disease onset [26].The MHC class I molecules are mainly involved in presenting intracellular pathogens, such as viruses. Indeed, there are now evidence that certain viruses, for example Epstein-

Barr virus (EBV), modify MS disease risk[27,28] . In contrast, infection with cytomegalovirus (CMV) may result in a lowered MS risk [29; 30].

It is also possible that different HLA class I alleles influence the immune response depending on the efficiency of clearance of infection of MS associated viruses or by inducing tolerance in auto-reactive T-cells[5]. Further efforts directed at replicating the current findings and dissecting the mechanistic basis for how the polymorphisms studied affect CIITA function, and subsequently autoimmune responses, are needed to clarify the role of genetic variability in the *CIITA* gene.

## Material and Methods:

Ethics Statement:

All included patient and control materials and analyses in this study were approved by the Regional Ethical Review Boards in the cities of Stockholm, Lund and Umeå in Sweden (www.epn.se). Informed consent from all study participants or their parents was obtained. Investigations were carried out according to guidelines from the Declaration of Helsinki.

Subjects: Multiple Sclerosis patients and controls

The multiple sclerosis patients are collected from the study cohorts described below, in total 2003 Swedish MS patients and 1672 controls (not all patients or controls were typed for all markers, see supplementary *table 1*). All MS cases has been diagnosed either according to McDonald's[31] or Poser's criteria[32], and individuals of known non-Scandinavian origin were excluded from the current study.

*The Epidemiological Investigation of Multiple Sclerosis (EIMS):*

A population based nation-wide case-control cohort with incident cases of MS which has been described previously [33]*.* The controls were randomly selected from the national population register and matched to the case's sex, age and residential area, 625 cases and 475 controls from this group were used in the current study.

*The Immunomodulation and Multiple Sclerosis Epidemiology (IMSE) cohort:*

The cohort consists exclusively of cases (n=318) with relapsing-remitting MS from clinics throughout Sweden, that are being treated with natalizumab[34].

*The Stockholm Multiple Sclerosis cohort (STOP MS) :*

The patients fulfilled the McDonald criteria [31] for definite multiple sclerosis and were recruited by neurologists at the Karolinska University Hospital Huddinge and Solna sites in Stockholm, Sweden. The patients in the cohort were between 22 and 91 years of age and the controls were matched for ethnicity and constitutes of blood donors between 21 and 76 years of age. 1060 cases and 1197 controls were used from this group. All patients and controls originating from Sweden or other Nordic countries [35].

Extra controls:

To further increase the power of the analyses extra control cohorts were included from previous studies of RA, T1D and MI (not all controls were typed for all markers, see supplementary *table 1*.)

*Rheumatoid arthritis:*

1426 healthy controls matched to RA patients by age, sex and residential area. The recruitment of affected individuals and controls was described previously in connection with EIRA study [36]. 97% of the study population was of self-reported Caucasian origin. No chronic diseases were reported among these controls.

Individuals deviating in a principal component analysis (PCA) or of known non-Scandinavian origin were excluded from the current study[37].

*Type 1 diabetes- Diabetes Incidence in Sweden (DISS1):*

The DISS1 controls consists of 618 sex, age and residence matched, healthy individuals to T1D patients, aged 15-34 years old and diagnosed with diabetes between 1987 and 1989, all from Sweden DISS registry [38].

*Type 1 diabetes- Diabetes Incidence Study in Sweden (DISS2):*

The DISS2 controls consist of 836 age-and sex matched healthy controls to T1D patients aged 15-36 years old and collected during 1992 and 1993 from the DISS registry in Sweden [39].

*Type 1 diabetes- Swedish Childhood Study (Sv2):*

From the Swedish Childhood registry during 1986 and 1987, 476 controls to T1D patients were selected. The controls were geographically, gender and age matched to all cases of T1D in the cohort above 7 years of age (n=423). For patients under the age of 7 years a control was selected among patients being treated at the hospital for reasons other than T1D (n=53) [40].

*Type 1 diabetes- Diabetes Registry in Southern Sweden (DR)*

Totally 2312 healthy controls from this study where included, 1695 men and 617 women between 45 and 75 years of age [41]. Individuals of known non-Scandinavian origin were excluded (n=100).

*Myocardial infarction (MI) – SCARF*

From the SCARF [42] study of MI the control group consists of 387 healthy persons between 40-60 years of age, sex- and age-matched for the MI cases and recruited from the general population, of self-reported Caucasian origin.

In this study, there is a partial overlap of cases and controls with a previously published MS study [11] for the Stockholm MS cohort (548 cases, 528 controls), the RA cohort (709 controls) and the MI cohort (387 controls). All individuals with known non-scandinavian descent have been removed from this study.

Genotyping methods:

For MS patients and controls all SNPs where genotyped with the allelic discrimination method for TaqMan ABI 7900 (Applied Biosystems, Inc ABI, Sweden) [43] except for markers rs3087456 and rs4774, where individuals in the Stockholm MS cohort included in our previous publication were genotyped with MALDI-TOF as described [11].

In the DISS2 cohort the TaqMan ABI 7900 typing method was used for all markers except for rs4774 and rs3087456 for which the DASH method [44] was used.

DISS1 and SV-2 cohorts were genotyped using the MassArray chip-based matrix-assisted laser desorption/ionization time-of-flight mass spectrometer (Sequenom Inc., San Diego, CA, USA) using the HME chemistry as described [45].

Controls from the DR study were genotyped with the TaqMan ABI method as above.

In the RA and MI controls, rs3087456 were genotyped with 5´nuclease assays, and rs4774 with MALDI-TOF as described [11], remaining *CIITA* markers where genotyped with the TaqMan ABI method.

Additional genotypes for rs4774 (81 cases, 227 controls) for individuals already in the study but lacking genotypes for this marker was retrieved from the ImmunoChip custom genotyping array.[23]

Genotyping methods for HLA DRB1*15 and A*02:

The genotyping of DRB1*15 and A*02 in the MS cohorts, as well as the genotyping of DRB1*15 in RA and DISS2 cohorts, was performed by allele specific amplification as described earlier [46].

In the DISS1 and SV2 cohorts DRB1*15 was genotyped by restriction fragment-length polymorphism (RFLP) [47].

For individuals also included in the MS Immunochip study[23], we used imputated genotypes for HLA where we lacked this information, adding information regarding HLA-A*02 for 145 individuals and for HLA-DRB1*15 for 128 individuals. The imputation was performed using HLA*IMP:0212[48] based on single nucleotide polymorphisms (SNPs) genotyped on the Immunochip custom array[49].

The other cohorts have not been typed for HLA.

In total, 1959 MS cases and 4407controls had data concerning HLA DRB1*15 status and 1905 cases and 1762 controls had data concerning A2 status.

All HLA genotyping was performed at 2-digit level. HLA coding was defined as presence or absence of allele for DRB1*15 and A*02.

<u>Statistical analysis:</u>

Univariate association was tested using Pearson's Chi-squared test. Logistic regression analysis using generalized linear modeling was used in multivariate analysis when correcting for the effect of age on the *CIITA* association. Age group 8 (35-39 years) was used as a reference group in the multivariate analysis. Interaction on the multiplicative scale was tested using logistic regression including both variables (a,b) investigated, as well as the interaction variable (a*b) and confounders (age).

To investigate additive interaction, departure from additivity was estimated by calculating attributable proportion (AP) due to interaction. The analysis was performed as described [50] using the generalized linear modeling (glm) in R and the vcov command to get the covariance matrix.

A meta-analysis was performed using fixed effect Mantel–Haenszel analysis and Woolf's test for heterogeneity in R using the meta.MH command in the rmeta package. No heterogeneity between groups was discovered.

All statistical analyses where performed in the statistical computer program R 2.14.1[51].

The LD structure plot (fig.1) for investigated markers in the MS cohort was performed in Haploview 4.2[52]

Supplementary information is available at Genes&Immunity´s website.

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Conflict of interest

The authors declare no financial, personal or professional conflict of interest.

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## Tables

**Table 1:** SNP positions and association analysis for *CIITA* and *CLEC16A* in MS

| gene | marker | location/ chr position[1] | Cases maf (total n) | All controls maf (total n) | Univariate analysis p-value[2] | Multivariate analysis p-value[3] |
|---|---|---|---|---|---|---|
| CIITA | rs11074930 | Before PI/ 10842650 | 0,50 (1466) | 0,50 (2357) | 0.46 | 0.38 |
| CIITA | rs8052975 | Before PI/ 10856764 | 0,25 (1709) | 0,25 (3333) | 0.37 | 0.46 |
| CIITA | rs4781003 | Before PI/ 10865178 | 0,15 (1620) | 0,16 (3262) | 0.35 | 0.32 |
| CIITA | rs6416647 | Between PI and PIII/ 10873098 | 0,27 (1792) | 0,28 (3777) | 0.35 | 0.42 |
| CIITA | rs11074932 | Between PI and PIII/ 10875837 | 0,28 (1889) | 0,28 (3796) | 0.82 | 0.96 |
| CIITA | rs3087456 | In PIII/ 10878403 | 0,23 (1786) | 0,24 (6967) | 0.09 | 0.12 |
| CIITA | rs4774 | Exon (non-synonymous) /10908349 | 0,33 (1714) | 0,31 (3756) | **0.01** | **0.01** |
| CLEC16A | rs12708716 | CLEC16A gene / 11087374 | 0,29 (1984) | 0,32 (2464) | **0.0005** | **9.34e-05** |
| CLEC16A | rs12927355 | CLEC16A gene / 11102272 | 0.25 (581) | 0.31 (970) | **0.0009** | **0.0003** |
| CLEC16A | rs6498169 | CLEC16A gene /11156830 | 0,45 (1201) | 0,40 (1005) | **0.004** | **0.0009*** |
| CLEC16A | rs4780346 | CLEC16A gene / 11196307 | 0.34 (581) | 0.28 (970) | **0.001** | **0.0009** |

[1] Location of SNP in relation to different promotor (PI-IV) of the CIITA gene.
Chromosome position, genome build 36.3, contig NT 010393.15 (Reference sequence)
[2] presence of minor allele vs major allele homozygote using Pearson's Chi-squared test
[3] presence of minor allele vs major allele homozygote using logistic regression analysis including age (16 groups) as covariate.*minor allele homozygote

**Table 2:** Age-adjusted logistic regression analysis for testing association to rs4774 in HLA-DRB1*15 and HLA-A*02 stratified cohorts

| DRB1*15+stratified[1] | freq case/cont (n) | p-value[2] | OR, 95%CI |
|---|---|---|---|
| Major allele homozygotes (GG) | 42.5 (415) / 46,1 (430) | | |
| Presence of minor allel (CG,CC) | 57.5 (562) / 53.9 (502) | 0.03 | 1.23 (1.02-1.49) |
| Total n case/cont | 977 / 932 | | |
| **DRB1*15-stratified[1]** | **freq case/cont (n)** | **p-value** | **OR, 95%CI** |
| Major allele homozygotes (GG) | 44.2 (223) / 47.2 (1096) | | |
| Presence of minor allel (CG,CC) | 55.8 (281) / 52.8 (1228) | 0.24 | 1.12 (0.92-1.38) |
| Total n case/cont | 504 / 2324 | | |
| **A*02+stratified[1]** | **freq case/cont (n)** | **p-value** | **OR, 95%CI** |
| Major allele homozygotes (GG) | 43.2 (281) / 49.4 (374) | | |
| Presence of minor allel (CG,CC) | 56.8 (369) / 50.6 (383) | 0.010 | 1.33 (1.07-1.64) |
| Total n case/cont | 650 / 757 | | |
| **A*02-stratified[1]** | **freq case/cont (n)** | **p-value** | **OR, 95%CI** |
| Major allele homozygotes (GG) | 43.4 (344) / 41.8 (219) | | |
| Presence of minor allel (CG,CC) | 56.6 (449) / 58.2 (305) | 0.54 | 0.893 (0.74-1.17) |
| Total n case/cont | 793 / 524 | | |

1) Cases and controls stratified for presence (+) or absence (-) of HLA-DRB1*15 or HLA-A*02

2) presence of minor allele vs major allele homozygote using logistic regression analysis including age (16 groups) as covariate

Figure legends

**Figure 1.** Additive interaction between HLA and rs4774.

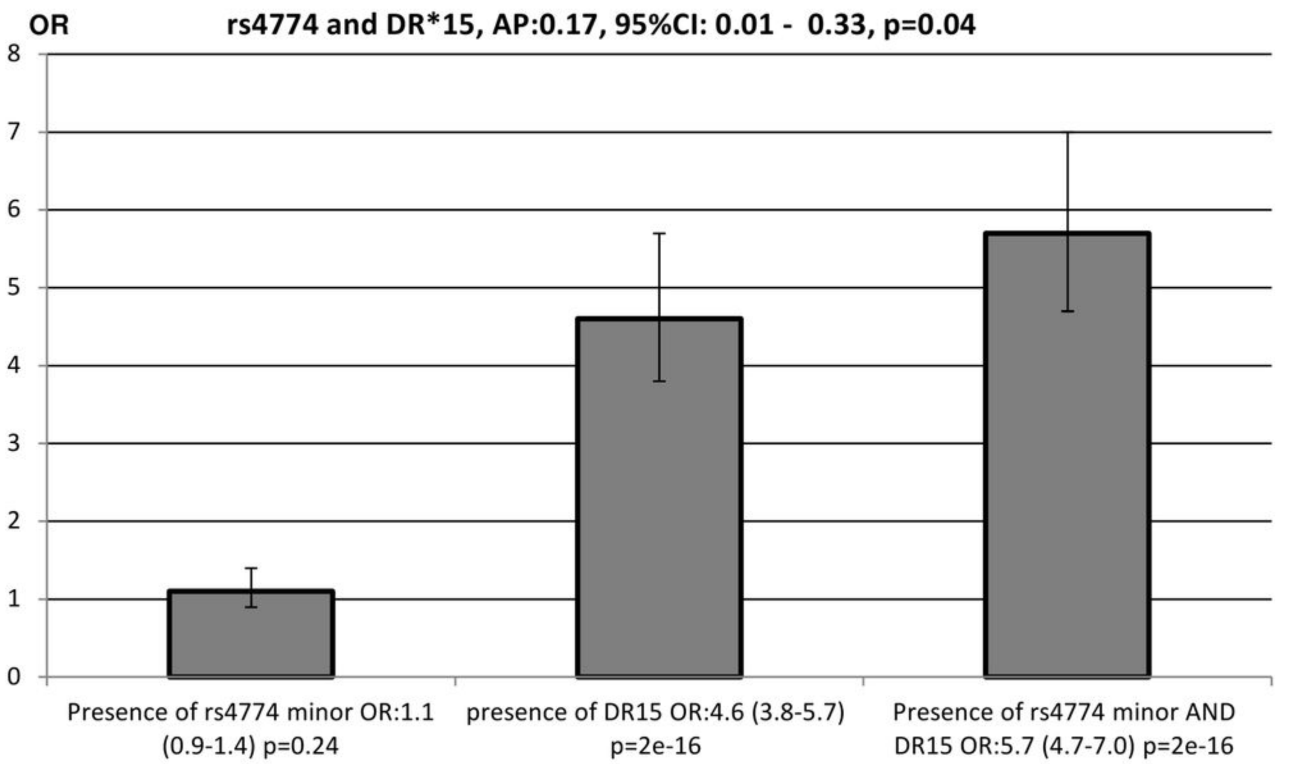
Presence of HLA-DRB1*15 together with presence of minor allele (CG, CC) for rs4774 increases the odds ratio (OR) for MS. Error bars are 95% confidence interval of OR estimates. Attributable proportion (AP) due to interaction is the proportion of the incidence among individuals exposed to both associated factors compared with the factors individually. The AP value is significant if separated from zero. HLA is coded as presence of one or two alleles for HLA-DRB1*15, andrs4774 was coded as presence of minor allele (CG, CC).

**Figure 2.** $R^2$ plot showing the LD structure of investigated markers in the *CIITA* and *CLEC16A* gene in the MS cohort; darker gray indicates higher $r^2$ between markers. (Haploview 4.2)

**Figure 3.** Meta-analysis in DRB1*15 stratified cases, presence of minor allele (CC, CG) for rs4774 and association to MS.

Supplementary material

**S1**. Genotyping and numbers in the different cohorts.


OR
rs4774 and DR*15, AP:0.17, 95%CI: 0.01 - 0.33, p=0.04
8
7
6
5
4
3
2
1
0
Presence of rs4774 minor OR:1.1 (0.9-1.4) p=0.24
presence of DR15 OR:4.6 (3.8-5.7) p=2e-16
Presence of rs4774 minor AND DR15 OR:5.7 (4.7-7.0) p=2e-16

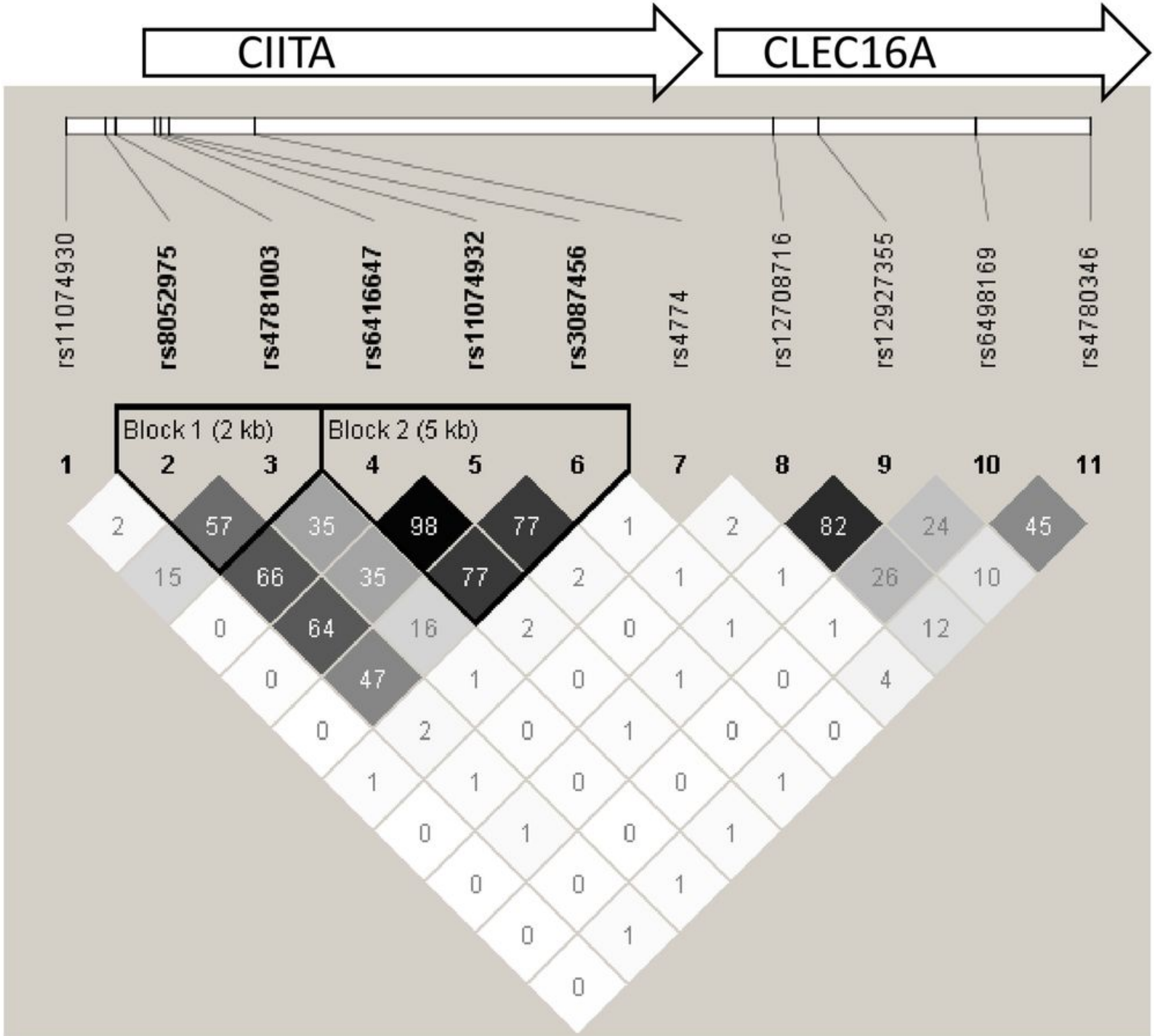

CIITA
CLEC16A
rs11074930
rs8052975
rs4781003
rs6416647
rs11074932
rs3087456
rs4774
rs12708716
rs12927355
rs6498169
rs4780346
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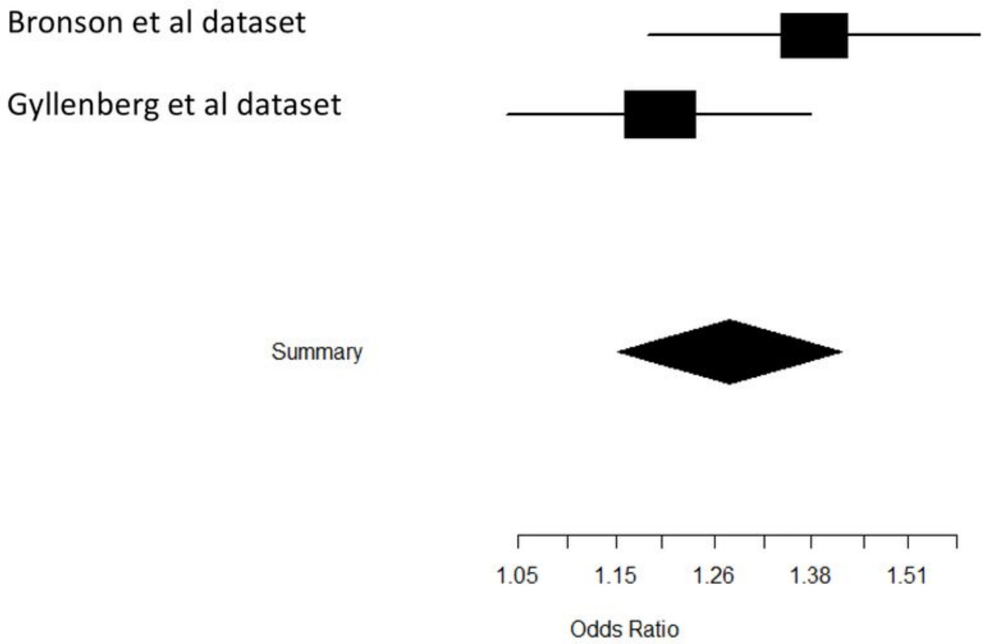
Fig.3




Mantel-Haenszel OR =1.28 95% CI ( 1.15 - 1.42 )

**Supplementary Table S1:** Genotyping and numbers in the different cohorts

| | | MS patients | MS controls | DISS1 | DISS2 | SV2 | DR | MI | RA | all controls |
|---|---|---|---|---|---|---|---|---|---|---|
| **Marker** | **Alleles/ minor** | **maf (total n)** | **maf (total n)** | **maf (total n)** | **maf (total n)** | **maf (total n)** | **maf (total n)** | **maf (total n)** | **maf (total n)** | **maf (total n)** |
| rs11074930 | T/C | 0,50 (1466) | 0,50 (1441) | - | - | - | - | - | 0,50 (916) | 0,50 (2357) |
| rs8052975 | C/T | 0,25 (1709) | 0,25 (1584) | - | 0,30 (740) | - | - | - | 0,22 (1009) | 0,25 (3333) |
| rs4781003 | C/T | 0,15 (1620) | 0,16 (1533) | - | 0,19 (725) | - | - | - | 0,15 (1004) | 0,16 (3262) |
| rs6416647 | T/C | 0,27 (1792) | 0,28 (1624) | - | 0,33 (776) | - | - | 0,24 (383) | 0,25 (994) | 0,28 (3777) |
| rs11074932 | T/C | 0,28 (1889) | 0,28 (1179) | 0,28 (295) | 0,32 (757) | 0,28 (192) | - | 0,25 (385) | 0,25 (988) | 0,28 (3796) |
| rs3087456 | A/G | 0,23 (1786) | 0,23 (1604) | 0,25 (207) | 0,28 (751) | 0,26 (332) | 0,26 (2312) | 0,22 (387) | 0,22 (1374) | 0,24 (6967) |
| rs4774 | G/C | 0,33 (1714) | 0,32 (1286) | 0,37 (204) | 0,30 (776) | 0,32 (330) | - | 0,31 (386) | 0,30 (774) | 0,31 (3756) |
| rs12708716 | A/G | 0,29 (1984) | 0,33 (1738) | - | 0,32 (726) | - | - | - | - | 0,32 (2464) |
| rs12927355 | G/A | 0.25 (581) | 0.31 (970) | - | - | - | - | - | - | 0.31 (970) |
| rs6498169 | A/G | 0,45 (1201) | 0,40 (1005) | - | - | - | - | - | - | 0,40 (1005) |
| rs4780346 | G/A | 0.34 (581) | 0.28 (970) | - | - | - | - | - | - | 0.28 (970) |