Chapter 10

# Association of HLA DQB1*0602 in sarcoidosis patients with small fiber neuropathy

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# Abstract

## Background and aim

Sarcoidosis has been reported to be associated with the HLA genes, in particular  DQB1.

## Methods

High resolution DQB1 of 103 sarcoidosis patients was obtained by sequence-based typing, low resolution HLA-A/B/DRB1 typing was performed by serological and molecular methods. Small fiber neuropathy (SFN) was established by thermal threshold testing.

## Results

Sixty-seven patients suffered from SFN (SFN+), in 36 patients SFN was absent (SFN-). Comparing HLA DQB1 typings of SFN+ patients, SFN- patients and control individuals revealed a significant increase of the allele DQB1*0602 in SFN+ patients compared to controls.

## Conclusion

This association might be correlated with a severe course of the disease.

## Introduction

Sarcoidosis is a multiorgan inflammatory disorder of unknown origin, characterized by T-lymphocyte and mononuclear phagocyte infiltration in the affected organs, granuloma formation, and distortion of the normal micro-architecture, which is probably antigen-driven.[1] The clinical manifestations of sarcoidosis are largely nonspecific, dependent on the intensity of the inflammation and organ systems affected, of which the lung is the most prominent. Sarcoidosis patients may suffer from a great variety of symptoms. A substantial number of sarcoidosis patients report apparently non-specific symptoms such as pain, for which no organic substrate has yet been established.[2] Recently, we and others found that small fiber neuropathy is rather common in sarcoidosis patients.[3-6] Several studies have suggested that the clinical outcome of sarcoidosis is affected by clinical phenotype, race, gender and age of onset. A genetic component for sarcoidosis is suspected based on familiar clustering and different prevalence of the disease in various ethnic groups. The genes in the MHC have been investigated and recently the strongest association was shown for the HLA gene DQB1.[7,8] The aim of this study was to evaluate whether there is a difference between sarcoidosis patients with or without small fiber neuropathy and healthy controls regarding their HLA association.

## Patients and methods

From January 2002 to February 2004, 103 Caucasian sarcoidosis patients from the Sarcoidosis Management Center - a tertiary referral center in the Netherlands - of the University Hospital Maastricht were studied. The diagnosis sarcoidosis was made according to the World Association of Sarcoidosis and Other Granulomatous disease (WASOG) guidelines.[9] Informed consent was obtained from all participating patients.
The clinical records were reviewed (MD) and patients were divided into 2 groups based on their clinical status at least three years after initial diagnosis (table 10.1).
Temperature threshold testing (TTT) was performed to assess the presence of SFN with a Medoc TSA-2001 device (Medoc, Ramat Yishai, Israel). Thresholds for warmth and cold sensation were determined on the hand and dorsum of the foot using the method of levels (MLE) and limits (MLI). Normative data according to Yarnitsky were used.[3,4] A patient was considered to suffer from SFN (SFN+) when his temperature sensation threshold for both MLE and MLI exceeded the 99% interval of a healthy population.[10]

Table 10.1 Summary of most relevant clinical data of the studied Caucasian sarcoidosis population with or without small fiber neuropathy (SFN- (n=36) or SFN+ (n=67))

| | **no small fiber neuropathy** | | **small fiber neuropathy** | |
|---|---|---|---|---|
| Age at diagnosis, years * | 35.7 ± 10.3 | | 41.5 ± 9.9 | |
| Time since diagnosis, yrs | 4.4 ± 3.9 | | 4.3 ± 5.9 | |
| | *n* | % | *n* | % |
| *Gender* * | | | | |
| male | *14* | 38.9 | *45* | 67.2 |
| female | *22* | 61.1 | *22* | 32.8 |
| *Chest X-ray stage at presentation* | | | | |
| I | *9* | 25.0 | *17* | 25.4 |
| II | *12* | 33.3 | *26* | 38.8 |
| III | *13* | 36.1 | *18* | 26.9 |
| IV | *2* | 5.6 | *6* | 9.0 |
| *Treatment* | | | | |
| never | *13* | 36.1 | *19* | 28.4 |
| prednison | *17* | 47.2 | *34* | 50.7 |
| prednisone+ methotrexate | *6* | 16.7 | *12* | 17.9 |
| anti TNF alpha | *0* | 0 | *2* | 3.0 |
| *Progression of disease* * | | | | |
| mild disease [1] | *12* | 33.3 | *10* | 14.9 |
| moderate to severe disease [2] | *24* | 66.7 | *57* | 85.1 |

Data are expressed as number of cases (n) and percentages or mean with SD, as appropriate. * p<0.05

[1] mild disease included:
- resolved, never treated or no therapy > 12 months
- minimal disease, never treated or no therapy >12 months

[2] moderate to severe disease included:
- persistent disease, never treated or no therapy >12 months
- persistent disease on therapy (asymptomatic, stable, worsening)

From all individuals genomic DNA was isolated by using QIA-AMP kits following the suppliers protocol (Qiagen, Westburg, Leusden, NL). Concentration and purity of DNA samples were measured at 260 nm and 260/280 nm. All patients were typed for HLA-DQB1 to the allele level by sequence-based typing as previously described[11,12], sequence data were processed automatically and evaluated manually. HLA-DRB1 was typed at the low resolution level by PCR-SSP, HLA-A and -B were detected by serological techniques.[13,14] The control groups for high resolution DQB1 consisted of 418 healthy Caucasian individuals, for low resolution DRB1 624 and AB 720. HLA phenotype frequencies were determined for patients and controls. Frequencies were compared by $\chi^2$-testing, p values were calculated and corrected for the number of alleles tested.

Table 10.2 DQB1 and DRB1 phenotype frequencies in Dutch sarcoidosis patients with or without small fiber neuropathy (SFN+, n=67 vs SFN-, n=36) compared to control individuals (for high resolution DQB1 n=418; for low resolution DRB1 n=624)

| | no small fiber neuropathy | | small fiber neuropathy | | controls | | p | $p_{corr}$* |
|---|---|---|---|---|---|---|---|---|
| **allele** | ***n*** | **%** | ***n*** | **%** | ***n*** | **%** | | |
| DQB1*0201 | *7* | 19,4 | *15* | 22,4 | *99* | 23,7 | | |
| DQB1*0202 | *7* | 19,4 | *5* | 7,5 | *73* | 17,5 | | |
| DQB1*0301 | *16* | 44,4 | *22* | 32,8 | *128* | 30,6 | | |
| DQB1*0302 | *4* | 11,1 | *12* | 17,9 | *57* | 13,6 | | |
| DQB1*0303 | *2* | 5,6 | *6* | 9,0 | *45* | 10,8 | | |
| DQB1*0304 | *0* | 0,0 | *0* | 0,0 | *2* | 0,5 | | |
| DQB1*0305 | *0* | 0,0 | *0* | 0,0 | *1* | 0,2 | | |
| DQB1*0401 | *0* | 0,0 | *0* | 0,0 | *0* | 0,0 | | |
| DQB1*0402 | *3* | 8,3 | *5* | 7,5 | *11* | 2,6 | | |
| DQB1*0501 | *5* | 13,9 | *9* | 13,4 | *84* | 20,1 | | |
| DQB1*0502 | *1* | 2,8 | *2* | 3,0 | *23* | 5,5 | | |
| DQB1*0503 | *3* | 8,3 | *7* | 10,4 | *32* | 7,7 | | |
| DQB1*0504 | *0* | 0,0 | *0* | 0,0 | *2* | 0,5 | | |
| DQB1*0601 | *0* | 0,0 | *0* | 0,0 | *11* | 2,6 | | |
| DQB1*0602 | *9* | 25,0 | *25* | 37,3** | *76* | 18,2 | 0,0006** | 0,012** |
| DQB1*0603 | *5* | 13,9 | *11* | 16,4 | *88* | 21,1 | | |
| DQB1*0604 | *5* | 13,9 | *7* | 10,4 | *46* | 11,0 | | |
| DQB1*0605 | *0* | 0,0 | *0* | 0,0 | *0* | 0,0 | | |
| DQB1*0607 | *0* | 0,0 | *0* | 0,0 | *1* | 0,2 | | |
| DQB1*0609 | *0* | 0,0 | *2* | 3,0 | *14* | 3,3 | | |
| DQB1*0614 | *0* | 0,0 | *0* | 0,0 | *0* | 0,0 | | |
| DRB1*01 | *4* | 11,1 | *6* | 9,0** | *142* | 22,8 | 0,0139** | |
| DRB1*15 | *9* | 25,0 | *27* | 40,3** | *143* | 22,9 | 0,0028** | 0,036** |
| DRB1*16 | *1* | 2,8 | *1* | 1,5 | *25* | 4,0 | | |
| DRB1*03 | *8* | 22,2 | *15* | 22,4 | *149* | 23,9 | | |
| DRB1*04 | *5* | 13,9 | *14* | 20,9 | *146* | 23,4 | | |
| DRB1*11 | *12* | 33,3 | *16* | 23,9 | *121* | 19,4 | | |
| DRB1*12 | *2* | 5,6 | *1* | 1,5 | *38* | 6,1 | | |
| DRB1*13 | *10* | 27,8 | *21* | 31,3 | *147* | 23,6 | | |
| DRB1*14 | *3* | 8,3 | *7* | 10,4 | *51* | 8,2 | | |
| DRB1*07 | *8* | 22,2 | *7* | 10,4** | *155* | 24,8 | 0,0127** | |
| DRB1*08 | *4* | 11,1 | *6* | 9,0 | *36* | 5,8 | | |
| DRB1*09 | *1* | 2,8 | *3* | 4,5 | *13* | 2,1 | | |
| DRB1*10 | *1* | 2,8 | *2* | 3,0 | *9* | 1,4 | | |

* $p_{corr}$ = p value corrected for the number of alleles tested;** SFN+ vs. controls

## Results

When the clinical features of SFN+ patients were compared with SFN- patients statistically significant differences were found for age at diagnosis, gender and disease progression (table 10.1, all p values <0.05). Concerning the HLA results an increase of DQB1*0602 was shown for the SFN+ group compared to healthy controls (37.3 vs.

18.2%, $p_{corr}$=0.012), which remained statistically significant also after correction for the number of alleles tested. For DRB1 an increase for DRB1*15 was also found in the SFN+ group compared to the controls (40.3 vs. 22.9%, $p_{corr}$=0.036). The increase of both alleles is consistent with the well known HLA linkage disequilibrium between the DQB1 and DRB1 genes, since DRB1*15 and DQB1*0602 are tightly linked in Caucasian individuals. Furthermore, a decrease of DRB1*01 and DRB1*07 in the SFN+ group was seen, as well as an increase of B7 in the SFN- group compared to controls. However, these differences were no longer statistically significant after correction for the number of alleles tested. All deviating frequencies for DRB1 and DQB1 are shown in table 10.2.

## Discussion

HLA class II antigens have been reported to be associated with sarcoidosis in different ethnic populations, and recently DQB1 and not DRB1 was indicated to play an important role in sarcoidosis susceptibility.[7,8,15] Although DQB1*0602 was previously mentioned to be associated with sarcoidosis, it was never the most prominent associated DQB1 allele. However, within the SFN+ patients the most prominent HLA DQB1 association is found with DQB1*0602, and this is consistent with our finding of DQB1*0602 as the most prominent DQB1 association with sarcoidosis in a group of 149 Dutch Caucasian patients. The strongest association with DQB1*0602 was detected in patients with severe pulmonary sarcoidosis, according to their chest X-ray stage at presentation. In the present study no differences were detected in the distribution of X-ray stages between SFN- and SFN+ patients. However, regarding clinical progression of the disease significant differences were found. Within the SFN+ group a higher percentage of patients suffered from moderate to severe disease compared to the SFN- group in which mild disease was more frequent (p=0.03).

## Conclusion

In conclusion, this implicates that both the presence of DQB1*0602 and the occurrence of small fiber neuropathy in sarcoidosis patients are associated and might be correlated with a more severe course of the disease. Typing for HLA might be helpful to recognize sarcoidosis patients with a suspected severe course in conjunction with development of small fiber neuropathy.

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