Ph.D.THESES

EXAMINATION OF THE POLYMORPHISMS OF THE MAJOR HISTOCOMPATIBILITY COMPLEX IN DIFFERENT AUTOIMMUNE DISEASES

Aniko Kapitany

Tutor: Prof. Dr. Sandor Sipka M.D., D.Sc.

Doctoral School for Clinical Medical Science
Immunology Doctoral Programm
Leader: Prof. Dr. Margit Zeher M.D., D.Sc.

Regional Immunologycal Laboratory

3rd Department of Medicine, Institute for Internal Medicine

Medical and Health Science Center

University of Debrecen

DEBRECEN

2009

Introduction

Coeliac disese (CD)

Coeliac disease is a T-cell mediated chronic inflammatory disease of the small intestine, which occurs in 1% of the European population. In genetically susceptible persons, small bowel mucosal inflammation, crypt hyperplasia and villous atrophy develop after ingestion of gluten, present in wheat, rye and barley. Active coeliac disease is characterized by disease-specific antibodies against endomysium (EMA) or tissue transglutaminase (anti-TG), and their presence in serum or locally in the gut is of high diagnostic value. Most common symptoms are weight loss, pale offensive diarrhoea or constipation and abdominal bloating, but clinical symptoms may even be absent or only involving extraintestinal organs (e.g. osteoporosis). Nowadays the diagnosis of coeliac disease is based on the presence of crypt hyperplastic villous atrophy, endomysial (EMA) or transglutaminase antibodies (anti-TG) and correlation of disease activity with gluten intake. The intolerance towards gluten lasts life-long, but elimination of gluten from the diet results in complete remission, thus in disappearance of symptoms, antibodies and histological abnormalities.

Approximately 90-95% of patients carry the heterodimer HLA-DQ2 composed of DQA1*05 and DQB1*02 molecules and the remaining patients usually have DR4; DQ8 haplotypes (DQA1*0301, DQB1*0302 alleles) with extremely few exceptions. Patients negative for both HLA-DQ2 and –DQ8 are very unlikely to suffer from coeliac disease, because these molecules are necessary to present the antigens to T-cells. The presence of HLA-DQ2 or DQ8 is necessary but not sufficient for the development of coeliac disease, because they also occur in 20-30% of the general European population. Moreover, also non-HLA genes have an important but yet unclarified contribution in disease development. However, HLA-DQ typing may have clinical relevance in estimating the risk of family members, or to evaluate the probability of coeliac disease in uncertain cases.

Rheumatoid arthritis (RA)

Rheumatoid arthritis is an autoimmune disease leading to chronic synovitis and eventually bone destruction. The etiology of the disease is still unknown, however, both genetic and environmental factors play important roles in the onset of RA.

The diagnosis of RA depends on clinical symptoms, laboratory investigations and imaging. Several autoantibodies have recently been associated with disease activity and/or prognosis of RA. However, until recently, rheumatoid factor of the immunoglobulin M isotype has been the only laboratory marker routinely used in RA, but IgM RF has rather low specificity for RA as it can also be detected in sera of patients with other autoimmune diseases, infectious disorders, as well as in the healthy elderly population.

C-reactive protein is a convenient and sensitive marker of inflammatory activity in clinical practice. The control of inflammation by anti-rheumatic therapy usually leads to suppression of serum CRP levels.

Anti-cyclic citrullinated peptide has been identified as a potential diagnostic and prognostic marker of RA. The presence of anti-CCP antibodies is highly specific and sensitive for RA. The assessment of anti-CCP is extremely useful in early RA when anti-CCP positivity may precede clinical symptoms by years.

Among MHC class II molecules, various HLA-DR alleles have been associated with susceptibility to RA in several racial groups. In addition, some HLA-DRB1 alleles have also been related to the severity and outcome of RA These disease associated HLA molecules share a common amino acid sequence in the third hypervariable region of the DRB chain, the so-called "shared epitope" (SE). Among the SE variants, the QKRAA motif is found in DRB1*0401 and DRB1*0409; the QRRAA sequence has been described in DRB1*0101, DRB1*0102, DRB1*0404, DRB1*0405, DRB1*0408, DRB1*0410, DRB1*1402 and DRB1*1406 variants, while the RRRAA motif is specific for the DRB1*1001 subtype. In general, the HLA-DR4 allele is found in 75% of Caucasian RA patients, as well as in 30% of the healthy Caucasian population. Among the DRB1*04 alleles, the DRB1*0401 and DRB1*0404 alleles have been associated with increased susceptibility to RA in Caucasian patients with RA, whereas the frequency of the DRB1*0405 allele is increased among Japanese and Chinese patients. Among HLA-DR1 subtypes, the association of the DRB1*0101 allele with RA has been reported in Ashkenazy Jews and in various Caucasians patient populations.

There may be an association between SE positivity and the production of anti-CCP antibody. The presence of one or two shared epitope alleles has been associated with anti-CCP antibody positivity. Moreover, unfavourable disease progression has been related to anti-CCP production and SE positivity. HLA-DR3 has been related to anti-CCP-negative disease. However, there is little information available regarding possible associations between serum anti-CCP antibody levels and HLA-DRB1 expression.

Systemic lupus erythematosus with or without APS:

Antiphospholipid syndrome (APS) is an autoimmune disorder characterised by different antibodies against phospholipids and co-factor proteins as well as clinical thrombotic events and fetal loss. It may exist in primary form but also may associate to other diseases, often to lupus. In such cases, APS usually develops after the diagnosis of systemic lupus erythematosus (SLE), however antiphospholipid antibodies (aPL) may precede both APS and lupus with some years. Furthermore, there are patients with the initial diagnosis of PAPS and subsequently progress to SLE.

Tarr et al. from a special workgroup of our Department, observed phenotypical differences by clinical respect between patients, who have only SLE ("SLE only" group) and SLE with secondary APS (SLE+SAPS), as well as patients, who started as PAPS and later developed SLE (PAPS+SLE). Especially, those with primary and secondary APS presented more thrombotic complications and less inflammatory activity. Accordingly, they required lower doses of corticosteroid and less frequently needed cyclophosphamide treatment. ISN/RPS III and IV glomerulonephritis was less frequent in these patients. Furthermore, fetal loss was the highest in PAPS+SLE group.

Both lupus and APS are of multifactorial origin, which develop in genetically predisposed individuals. Among the MHC class II molecules, various HLA-DR and – DQ alleles have been published to associate with SLE and APS. Regarding SLE the strongest association was shown with DR2 and DR3. An extensive study with approximately 300 families found three class II containing SLE risk haplotypes, namely DRB1*1501(DR2)/DQB1*0602, DRB1*0301(DR3)/ DQB1*0201, and DRB1*0801(DR8)/ DQB1*0402, and the latest was the least frequent. The first two haplotypes have been shown to confer the risk of developing SLE in many Caucasian populations. Most of them were also confirmed in a Hungarian population of patients with lupus. On the other hand HLA-DRB1*04 (DR4), DRB1*07(DR7), DRw53, in Japanese DRB1*09(DR9), and among DQB1 alleles the DQB1*0301(DQ7) or the DQB1*0302(DQ8) were significantly associated with PAPS or with different aPLs. The HLA alleles most frequently associated with the presence of anticardiolipin antibodies (aCL) are DRB1*04, DRB1*07, DRw53, DQA1*0201, DQA1*0301 and DQB1*0302. Anti-β2-glycoprotein I (β2-GPI) showed positive association with DRB1*04/DQB1*0302 and DRB1*1302/DQB1*0604/5/6/7/9 was also significantly increased in Caucasian and black patients either. Some HLA alleles may carry the risk to produce antibodies against phospholipids-binding proteins too.

Aims

Coeliac disease

The presence of HLA-DQ2 or DQ8 is necessary but not sufficient for the development of coeliac disease, however, HLA-DQ typing may have clinical relevance in estimating the risk of family members, or to evaluate the probability of coeliac disease in uncertain cases. The gold standard of coeliac disease diagnosis is today small bowel histology, but its evaluation may have several pitfalls. In this study, we investigated the value of HLA-DQ typing in cases where the diagnosis of coeliac disease was based on an earlier pathology statement but without any information on presence of EMA or anti-TG.

Rheumatoid arthritis

Study I.

Our aim was to determine the frequency of different HLA-DR1 and -DR4 subtypes in patients with RA in comparison to healthy control subjects and to investigate whether the presence of these alleles could be a marker of RA.

Study II.

There may be an association between SE positivity and the production of anti-CCP antibody. The presence of one or two shared epitope alleles was found to associate with anti-CCP antibody positivity. However, there is little information available regarding any relationship between quantitative anti-CCP production (serum anti-CCP concentrations) and the SE. Our aim was to determine the association between anti-CCP antibody production and various HLA-DRB1 alleles. We investigated associations of SE positivity with anti-CCP positivity and serum levels. In addition, this was the first report on the possible relationship between SE and anti-CCP in Hungarian RA patients.

Systemic lupus erythematosus with or without APS:

In order to answer, whether the phenotypical differences found by Tarr et al. could be explained by MHC II polymorphism, we examined HLA-DR and HLA-DQ genotypes of patients with SLE only, SLE+SAPS and PAPS progressing to SLE. We also aimed to confirm the hypothesis that PAPS may associate with SLE as an independent disease and it is not only a condition, which is part of the manifestations of lupus.

Patients and methods

Patients with coeliac disease

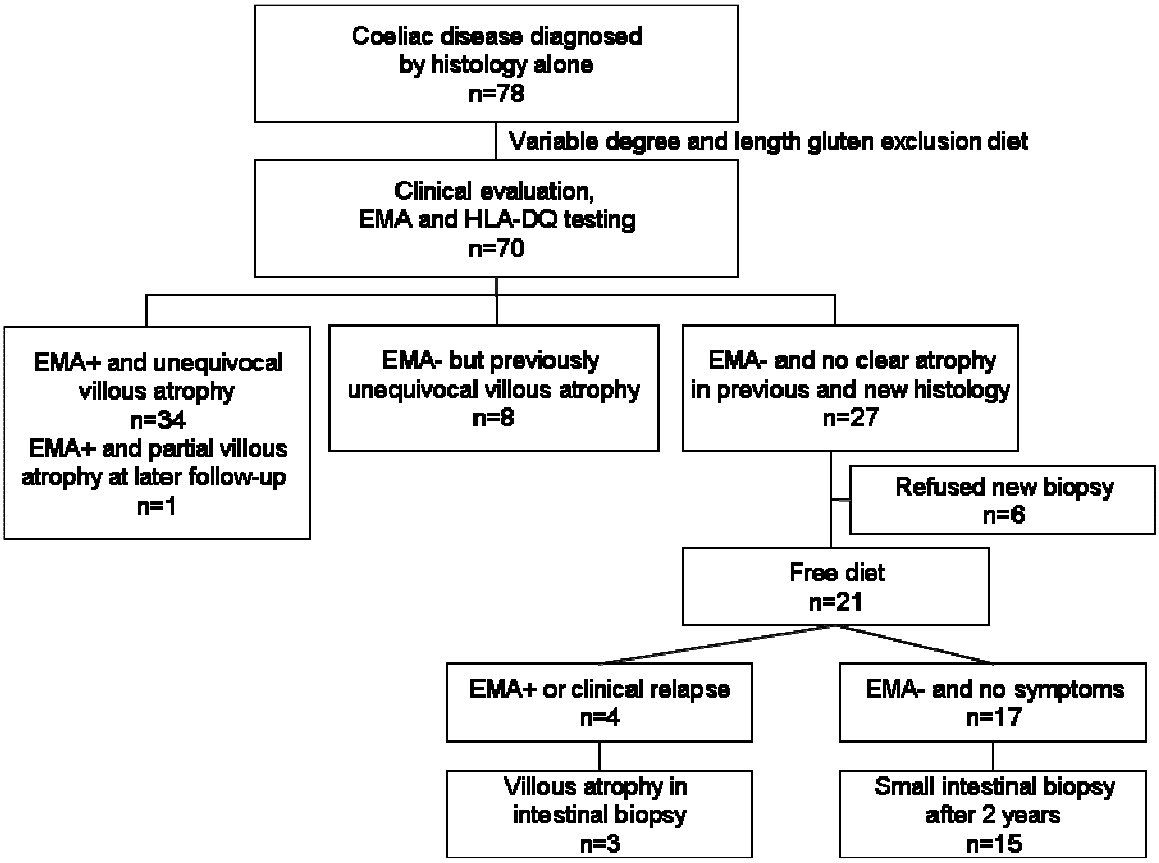
Patients with a coeliac disease diagnosis prior to 2002 were traced from patient files of the Department of Pediatrics, University of Debrecen and subjects with a histology-based diagnosis but without EMA or anti-TG antibody results before diet were enrolled in the study. Altogether 78 patients met the inclusion criteria and 70 of them (current median age 13 years, range 3-31) presented for clinical examination and could be enrolled. They received their original diagnosis when they were in median 3 years old (range 1-28) with a gluten-free diet prescribed 2-25 years ago. Initial pathology statements and whenever available, original biopsy slides were reviewed by one pathologist. Serum EMA and anti-TG antibodies and HLA-DQ haplotypes were determined from blood. Dietary histories regarding the length and strictness of gluten exclusion since diagnosis as well as current gluten intake were recorded. Current diet was classified as strict gluten-free diet, non-strict diet and free gluten intake based on interview with the patients or their parents, and on current EMA/anti-TG results.

Patient with symptoms or seropositivity, or in whom the original biopsy material was insufficient or unsuitable for diagnosis, underwent a new small intestinal biopsy. Patients with histology findings compatible with active coeliac disease were prescribed a strict gluten-free diet, received a detailed dietary education and were followed up clinically and by EMA and anti-TG tests.

In cases, where the initial diagnosis was uncertain and current gut histology did not support the presence of coeliac disease, a free gluten intake was suggested. Serum EMA and anti-TG antibodies were determined at the intervals of 3-6 months. A new biopsy was performed if the antibodies became positive or symptoms developed. If neither antibodies nor symptoms appeared, a small intestinal biopsy was performed after two years of free gluten intake to exclude coeliac disease in line with the original diagnostic criteria the European Society of Paediatric Gastroenterology (ESPGAN) for establishing the diagnosis of coeliac disease by histology, formulated in Interlaken in 1969. The flowchart of the study is presented in Figure 1.

Blood for HLA-DQ typing was also obtained from 40 healthy control subjects with the same ethnic origin as patients. They were either hospital employees or visitors who were unrelated to the patients.

Figure 1. Flowchart of the study. Patients with endomysial antibody positivity (EMA) were also positive for transglutaminase antibodies.



Patients with rheumatoid arthritis

Study I.:

Eighty-three Hungarian RA patients (70 females and 13 males, all Caucasian) were included in our study. All patients fulfilled the 1987 revised classification criteria of the American College of Rheumatology (ACR). The mean age of the patients was 50 ± 15 years (range:17-82). The mean disease duration at the time of the study was 6 ± 4 years (range: 0.5-22 years). Peripheral blood was drawn from each patient for DNA isolation.

Peripheral blood was also obtained from 55 healthy Caucasian controls (47 females and 8 males) with similar mean age (46 ± 13 years). These individuals were either hospital employees or visitors who were unrelated to the patients.

From these two groups HLA-DRB1*01 subtyping was made in the case of 27 RA patients and 10 control persons (they were DRB1*01 positive), while DRB1*04 subtyping was prepared in the case of 26 DRB1*04 positive RA-patients and 6 DRB1*04 positive control persons.

Study II:

Fifty-three RA patients (44 females and 9 males, all Caucasians) were included in this study. All patients fulfilled the 1987 revised classification criteria of the American College of Rheumatology. The mean age of the patients was 50 ± 15 years (range 17–82 years). The mean disease duration at the time of the study was 6 ± 4 years (range 0.5–22 years). Informed consent was obtained from each RA patient. For this study we also obtained local ethical committee approval at the University of Debrecen.

Patients with SLE with or without APS:

The clinical data of 367 lupus patients (331 women and 36 men) were retrospectively analyzed earlier from the computerized patient data bank files of the 3rd Department of Internal Medicine; University of Debrecen by Tarr et al. Patients met at least four of the ACR classification criteria for SLE. Among them 110 patients fulfilled the Sapporo and Sydney classification criteria for antiphospholipid (Hughes) syndrome either. In 26 patients with APS the disease started as PAPS and the SLE developed subsequently with a few years (median: 5.5 years, range: 1-29 years, PAPS+SLE). From the remaining 84 patients with SAPS, 26 disease duration and gender matched patients were randomly selected to constitute the SLE+SAPS group. Similarly, from SLE patients without APS another 26 disease duration and gender matched patients were randomly chosen to constitute the "SLE only" group. In our recent work HLA-DR and HLA-DQ typing could be performed in 15 patients from the PAPS+SLE group, in 22 from the SAPS+SLE group and in 26 from the "SLE only" group. Peripheral blood was drawn from each patient for DNA isolation.

Blood was also obtained from 57 healthy individuals, serving as controls with the same mean age and ethnic origin as the patients. They were either hospital employees or visitors, who were unrelated to the patients. The control group underwent the same procedures as the patients.

HLA-DR and DQ typing:

In the case of patients with CD diagnosis HLA-DQ typing, in RA patients HLA-DR typing and in patients with SLE both HLA-DR and HLA-DQ typing was performed.

DNA isolation and PCR reactions

Genomic DNA was isolated from buffy coats of EDTA-anticoagulated blood using QIA amp blood mini kit (QIAGEN Gmbh, Hilden, Germany) according to the instructions of the manufacturer.

Polimerase chain reaction based HLA-DR and HLA-DQ typing was performed (Olerup-SSP method), using low resolution kits (HLA-DR Low resolution kit and HLA-DQ Low resolution kit, GenoVision, Oslo, Norway). In cases when we wanted to know also the subtypes, DRB1*01, DRB1*04, DQB1*02 and DQB1*03 subtyping or DQA genotype determination was performed with the help of subtyping kits (Olerup-SSP, DRB1*01, DRB1*04, DQB1*02 and DQB1*03 subtyping kits, GenoVision, Oslo, Norway). All the examinations were performed according to the instructions of the manufacturer using recombinant Taq DNA polymerase (Invitrogen, Sao Paulo, Brazil). PCR amplification of DNA was performed using Hybaid PCR express thermal cycler.

Agarose gel electrophoresis:

HLA genotypes were determined on the basis of the PCR pattern obtained by electrophoresis in 2% agarose gel. DNA bands colored with SYBR Green I. dye were detected using Alpha Imager MultiImage Light Cabinet (Alpha Innotech Corporation, San Leandro, CA, USA).

Other examinations

Determination of EMA and anti-TG antibodies in CD patients

IgA and IgG class EMA were investigated from serum by indirect immunofluorescence using human umbilical cord substrate. The starting serum dilution was 1:10 in phosphate-buffered saline. EMA was also checked on monkey oesophagus using 1:2,5 serum dilutions. Anti-TG antibodies were measured by ELISA using human recombinant antigen expressed in E.coli.

Histology evaluation in CD patients

Villous height/crypt depth ratio was determined from well oriented sections and intraepithelial lymphocytes were counted. If needed, new sections were cut from the original blocks. Histology lesions partial villous atrophy grade II-III, subtotal and total villous atropy

(villous height/crypt depth ratio <1) were considered compatible with coeliac disease diagnosis. Only histology results while the patient was on a gluten-containing diet were considered relevant for final diagnosis and included in the comparison with HLA-DQ typing results.

Laboratory markers of RA

Serum IgM RF was assessed by quantitative nephelometry (Cobas Mira Plus, Roche, Basel, Switzerland), using RF reagents (Dialab, Budapest, Hungary). RF levels > 50 U/ml and were considered elevated.

Anti-CCP autoantibodies were detected in serum samples using Immunoscan-RA CCP2 ELISA test (Euro Diagnostica, Arnhem, The Netherlands). The assay was performed according to the manufacturer's instructions A concentration > 25 U/ml was considered positive.

Statistical analysis

Rheumatoid arthritis study I:

HLA-DR allele frequencies in patients with RA were compared to those obtained from healthy subjects. Chi-square goodness-of-fit test was used for Hardy-Weinberg equilibrium examination. Frequency comparisons for different antigens were performed by chi-square analysis with Yartes' correlation or Fischer's exact test using SPSS 11.0.0 statistical software. Differences between any two data groups were considered to be significant if p value was <0.05.

Rheumatoid arthritis study II:

Statistical analysis was performed using the SPSS 10.0 software. A chi-square test was used to detect differences between two different groups of patients. Median and 0.25/0.75 quartiles were used when appropriate. Clinical data were analysed using Kolmogorov-Smirnov two-sample test. *P* values < 0.05 were regarded as significant.

SLE with or without APS:

HLA-DR and HLA-DQ frequencies in the four groups were compared using two-tailed Fischer's exact test using SPSS 11.0.0. statistical software. Differences between any two data groups were considered to be significant, if the p value was <0.05.

Results

Coeliac disease

At the time of enrolment, only 31 of the 70 patients (44.3 %) followed a strict gluten-free diet. These patients were negative for EMA and anti-TG in serum and had normal serum total IgA levels. From the other 39 patients, 29 were on a non-strict and 10 on a free diet. Both EMA and anti-TG were detectable in 27 (69.2 %) of these 39 patients, but none had clinical symptoms.

All 27 EMA positive cases and 9 further patients, who were currently negative for serum EMA but had a positive EMA result at some time after diagnosis, carried the DQ2 or DQ8 heterodimers. DQ2 or DQ8 were also found in 56 % of patients without any documented EMA positive results. In 15 patients neither DQ2 nor DQ8 alleles were present (Table 1). The DQ2 or DQ8 heterodimer occurred in 23% of healthy Hungarian control subjects indicating that the presence of these alleles alone does not prove the existence of the disease.

Table 1. HLA-DQ2 és DQ8 positivity in patients with positive and negative serum endomysial antibody (EMA) results at the time of clinical enrollment

	EMA + n=36 (%)	EMA – n=34 (%)	Total n=70 (%)
DQ2+ or DQ8+	36 (100)	19 (55.8)	55 (78.6)
DQ2- and DQ8-	0	15 (44.2)	15 (21.4)

From all patients 47 carried DQ2 in cis, 4 DQ2 in trans and 4 carried DQ8.

Altogether 79 histology samples and 16 detailed pathology descriptions from past biopsies were available for re-evaluation. In addition, 32 new biopsies were performed at the time of DQ testing. Severe villous atrophy with elevated intraepithelial lymphocytes and crypt hyperplasia was unequivocally present in 35 out of the 36 patients with EMA positivity and DQ2 or DQ8 genotypes (97%), but only in 8 out of 19 cases (42%, $p<0.001$) with DQ2 or DQ8 genotypes and unknown EMA status before diet. However, negative results during restricted gluten intake could not be used to exclude the disease. In the patients without DQ2 or DQ8 alleles only slight changes without typical villous atrophy were seen. In particular, 3 biopsy samples contained stomach mucosa and the diagnosis of coeliac disease was based

only on dissecting microscope findings in 9 further cases while biopsy specimens were either unsuitable for histology evaluation or did not show villous atrophy. None of the patients without EMA positivity had the diagnosis proven by gluten challenge according to the 1969 ESPGAN criteria.

In order to evaluate the possibility of coeliac disease in uncertain cases, a follow-up on unrestricted free gluten intake was suggested to 11 DQ2/DQ8 carrier patients who never had EMA positive results before and to all 15 DQ2 and DQ8 negative patients. Of these, 21 agreed to follow-up biopsies according to the ESPGAN protocol. Four of the DQ2 positive patients developed clinical symptoms and EMA positivity within 6 months and three of them evinced subtotal villous atrophy in the biopsy specimen obtained after gluten reintroduction. One patient refused the control biopsy after relapse. All other patients remained symptom-free and none developed EMA, anti-TG or histological abnormalities at the time of follow-up biopsy after two years on gluten (Figure 1). All 15 DQ2 and DQ8 negative patients tolerated gluten well for a follow-up of 2.3-7 years on free gluten consumption.

The collected histology evidence for and against the diagnosis of coeliac disease is presented in Table 2. in relation to HLA-DQ results.

Table 2. Cumulative histology evidence pro and against coeliac disease in patients originally diagnosed by only histology. Results are based on extended follow-up and new biopsies on gluten-containing diet in uncertain cases. All endomysial antibody (EMA) positive cases carried DQ2 or DQ8.

Histology	EMA+ DQ2 or DQ8+	EMA- DQ2 or DQ8+	EMA- DQ2 and DQ8 -
SVA	35	7	0
PVA	4	1	0
Slight changes	0	0	0
Normal	0	5	14
Unsuitable for diagnosis	1*	2*	1*
Total	40	15	15
Supporting CD diagnosis (%)	39 (97.5%)	8 (53.3%)	0 (0%)

*Patient refused new gluten exposure or new biopsy

SVA: subtotal villous atrophy, PVA: partial villous atrophy, CD: coeliac disease

According to data summarised from past and prospectively performed biopsies, presence of coeliac disease has been verified in 39 of the 70 patients and is very probable in further 9 (altogether 68.6 %); excluded in 14 and highly unprobable in 8.

Rheumatoid arthritis

Study I.

We examined the frequency of HLA-DR1 and HLA-DR4 subtypes in RA patients and controls. We have found earlier, that the HLA-DR1 (HLA-DRB1*01) alleles were slightly more frequent, while HLA-DR4 (HLA-DRB1*04) alleles were significantly more frequent in RA patients compared to controls. (DR1: 27/83 in RA patients and 10/55 in controls, DR4: 26/83 in RA patients and 6/55 in controls).

Distribution of HLA-DR1 subtypes

Now we performed HLA-DR1 (HLA-DRB1*01) subtyping in the case of HLA-DR1 positive RA patients (n=27) and controls (n=10). Results are shown in Table 3. Among RA patients, 92.6% expressed the DRB1*0101, 3.7% the DRB1*0102 and also 3.7% the DRB1*0105. The distribution of these DRB1*01 subtypes was statistically the same in the control group (90%, 10% and 0%, respectively)

Table 3. Distribution of HLA-DR1 subtypes among RA patients and controls

HLA-DR1 subtype	RA n (%) (n=27)	Control n (%) (n=10)	Sign.
DRB1*0101	25 (92.6)	9 (90.0)	NS
DRB1*0102	1 (3.7)	1 (10.0)	NS
DRB1*0105	1 (3.7)	0 (0)	NS

Abbreviations: n=number of subjects; sign.=significance; S=significant; NS=non-significant

Distribution of HLA-DR4 subtypes

HLA-DR4 (HLA-DRB1*04) subtyping was performed in the case of HLA-DR4 positive RA patients (n=26) and controls (n=6). Results are shown in Table 4. HLA-DRB1*0401 was the most frequently expressed allele, both in RA patients (61%) and controls (66%). Interestingly, the DRB1*0404 subtype, which has been described to be important

among Caucasians, was found to be similarly common among RA patients (19%) and controls (17%). No statistically significant differences were found between patients and controls with regards to DRB1*0401 and DRB1*0404. In contrast small, although significant differences could be determined among RA patients and controls regarding the DRB1*0405 (11% vs 0%) and DRB1*0408 subtypes (8% vs 0%) ($p<0.05$). Regarding other subtypes, DRB1*0402, DRB1*0406 and DRB1*0407 were only detected in a single subject (Table 4).

Table 4. Distribution of HLA-DR4 subtypes among RA patients and controls

HLA-DR4 subtype	RA n (%) (n=26)	Control n (%) (n=6)	Sign.
DRB1*0401	16 (61.5)	4 (66.7)	NS
DRB1*0402	0 (0)	1 (16.7)	NS
DRB1*0404	5 (19.2)	1 (16.7)	NS
DRB1*0405	3 (11.5)	0 (0)	**S (p<0.05)**
DRB1*0406	1 (3.8)	0 (0)	NS
DRB1*0407	1 (3.8)	0 (0)	NS
DRB1*0408	2 (7.7)	0 (0)	**S (p<0.05)**

*Abbreviation*s: n=number of subjects; sign.=significance; S=significant; NS=non-significant

Study II.

Of the 53 serum samples 39 (73.6%) were RF positive. Anti-CCP antibody was present in 33 (62.2%) of the 53 patients samples. Seventeen of 53 patients (32.1%) carried one or two copies of the *HLA-DRB1*04* allele (data not shown).

We found a close association between anti-CCP and RF positivity. Thirty of the 53 patients (56.6%) were both RF and anti-CCP positive, while 9 (17%) were double negative (chi-square = 6.717, $P< 0.01$). In addition, there was a significant association between anti-CCP positivity and the presence of *HLA-DRB1*04* alleles (chi-square = 5.829, $P < 0.01$). In contrast, we could not find any correlation between RF positivity and the presence of *HLA-DRB1*04* alleles (data not shown).

When further analyzing these laboratory markers, patients were divided into two groups: those who carry one or two copies of the shared epitope alleles (SE-positive patients) and those who do not (SE-negative patients). Altogether, 16 patients were *HLA-DRB1*01*, 17 patients were *DRB1*04*-positive, and 23 patients did not carry any of these alleles. (Table 5.) Patients carrying neither of these alleles were assigned the "X,X" genotype. We did not find any differences in serum RF levels between SE-positive and negative patients (Table 5).

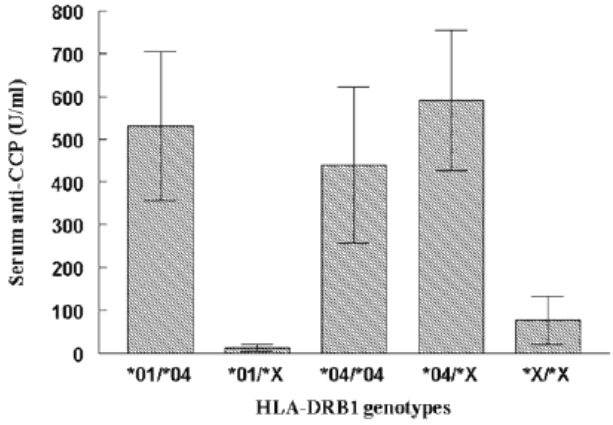
Table 5. Associations between serum anti-CCP, RF concentrations and shared epitope alleles in RA patients

	DRB1*01/04 (n=3)	**DRB1*01/ X (n=13)**	**DRB1*04/04 (n=3)**	**DRB1*04/X (n=11)**	**DRB1*X/X (n=23)**	**All DRB1*04 positive (n=17)**	**All DRB1*04 negative (n=36)**	***P*[1*]**
Anti-CCP (U/ml)	530.5 ± 174.2	12.0 ± 8.6	439.5 ± 182.8	591.0 ± 164.2	76.8 ± 56.2	530.0 ± 182.6	56.8 ± 27.4	< 0.01
RF (U/ml)	16.5 ± 6.2	29.5 ± 9.8	85.5 ± 34.6	70.0 ± 46.2	71.0 ± 38.4	63.5 ± 28.4	57.5 ± 22.6	NS

[*] P values indicate differences between all DRB1*04 positive versus negative patients. NS = not significant

In contrast, regarding anti-CCP antibody production, in patients carrying one or two copies of *HLA-DRB1*04* alleles (*DRB1*01/*04*: 530.5 ± 174.2 U/ml; **04/*04:* 439.5 ± 182.8 U/ml; **04/*X:* 591.0 ± 164.2 U/ml; all *DRB1*04* positive: 530.0 ± 182.6 U/ml) we found elevated anti-CCP values compared to *HLA-DR4*-negative patients (*DRB1*01/*X*: 12.0 ± 8.6 U/ml; **X/*X*: 76.8 ± 56.2 U/ml; all *DRB1*04* negative: 56.8 ± 27.4 U/ml) (all *DRB1*04* positive versus negative: $P < 0.01$) Table 5, Figure 2. In many DRB1*04 positive patients the serum anti-CCP level was 1000-2000 U/ml or more, while among DRB1*04 negative patients this very high anti-CCP antibody level was rare.

Figure 2. Serum anti-CCP antibody concentrations associated with various HLA-DR1 and HLA-DR4 genotypes



There was no difference in serum anti-CCP antibody concentrations between patients carrying only *HLA-DRB1*01* allele but no *HLA-DRB1*04* allele *(DRB1*01/*X)* (12.0 ± 8.6 U/ml) in comparison to SE-negative *(DRB1*X/*X)* patients (76.8 ± 56.2 U/ml).

When we investigated possible associations of serum anti-CCP antibody concentrations with other *HLA-DRB1* genotypes, we found that apart from *HLA-DRB1*04* described above, patients with *HLA-DRB1*13* and *DRB1*15* had significantly higher serum anti-CCP concentrations than any other HLA-DR subtypes ($P < 0.01$).

SLE with or without APS:

We have found similarly to the literature that the occurrence of DRB1*03 and DQB1*0201 alleles (DR3, DQ2 often inheriting in the same haplotype) was higher in the "SLE only" group than in the control group (46% vs. 26%, not significant), but these alleles were very rare in the PAPS+SLE group (13% vs. 46% in the SLE only group p=0.0442, OR=0.1795, 95CI=0.033-0.96). Similarly to the "SLE only" group, these alleles were slightly more common in SLE+SAPS patients either, in comparison with the control and the PAPS+SLE groups (32% vs. 26% and 13%, respectively). In case of the other SLE associated allele, the DRB1*15 allele,(2) which was found to be frequent also in Hungarian lupus patients,(6) we have not found significant differences between the SLE patients with or without (P/S) APS and the control group (27%, 27%, 26% and 16%, respectively). HLA-DRB1*04 allele, which is thought to be common in APS was more frequently detected in patients with PAPS+SLE or SLE+SAPS compared to patients with SLE only or to the control group, however these differences were statistically not significant (27% and 23% vs. 15% and 18%). We have not found significant differences in the occurrence of DRB1*07 alleles among the four groups in contrast to the literature. The DRB1*13 alleles were expressed in more patients with PAPS+SLE than in the other groups (33% vs. 7%, 13%, and 16% in "SLE only", SLE+SAPS, and controls; not significant).

Among the DQB1 alleles, DQB1*0301 allele was very rare (13% vs. 27%, 27%, 44%, in "SLE only", SLE+SAPS, and controls, respectively), but the DQB1*0302 alleles and the DQB1*06 alleles occurred very frequently in the PAPS+SLE group. The DQB1*0302 allele was expressed in 27% of the patients with PAPS+SLE, in contrast to the 7% of the patients carrying this allele from the "SLE only" group, 18% from the SLE+SAPS group and 14% from the control persons. The DQB1*06 allele was present in 47% of the patients with PAPS+SLE in contrast to the 23%, 36% and 26% positivity in the "SLE only", SLE+SAPS and control groups.

Discussion

Coeliac disease

Coeliac disease can be diagnosed on the basis of severe villous atrophy shown by histology and presence of coeliac disease-specific autoantibodies (anti-TG or EMA) in serum. Our study confirmed the reliability of this policy, as all patients meeting both these criteria also carried the correct genetic HLA-DQ background required for the disease. Moreover, the combination of seropositivity and villous atrophy was equally reliable if they were registered at different times or at later stage after inital diagnosis. Although none of our patients had initial EMA results from the time of the original diagnosis, dietary lapses allowed us to register EMA positivity in approximately 50% of the patients and also to confirm the diagnosis by a new biopsy in seropositive subjects if initial results were equivocal.

The correctness of coeliac disease diagnosis is not sure without documented EMA positivity, because the histological abnormalities are not specific for the disease. Moreover, there can be frequent pitfalls in the pathology evaluation, such as bad sample quality or orientation. In our series, a high frequency of misleading dissecting microscopy results were found, which were later misinterpreted by the clinicians as proof of villous atrophy. These difficulties in the diagnosis were recognized as early as in 1969, when ESPGAN has formulated the classical criteria for a histology alone-based coeliac disease diagnosis, also known as Interlaken criteria. These criteria rely on the gluten-dependency of the histologic features and require at least three biopsies (before diet, after gluten exclusion diet, and, after gluten reintroduction), of which only those on gluten intake should be pathological. These criteria are difficult to follow and seem today outdated. However, as we do not possess any diagnostic means which could prove the presence of coeliac disease in a subject on a long-term gluten-free diet, a new and controlled gluten exposure can have utmost clinical importance in doubtful cases. In fact, our series represented such a patient group, as subjects with currently negative EMA results and unknown EMA status before diet had only low prevalence of HLA DQ2 or DQ8 alleles.

Importantly, lack of both DQ2 and DQ8 in a patient with a presumed coeliac disease diagnosis is a major doubt on diagnosis, and warrants further diagnostic evaluation. In our series, none of the DQ2 and DQ8 negative patients relapsed on long-term gluten exposure or developed EMA or anti-TG antibodies. Patients without DQ2 and DQ8 most often have a false diagnosis and do have a good chance to be able to stop the gluten-free diet without relapse.

In general, only DQ2 and DQ8 negative HLA-DQ typing results can be utilized clinically as arguments against coeliac disease. Presence of DQ2 or DQ8 is common in the general population, thus in itself cannot prove the presence of the disease. However, in the absence of EMA results and convincing histology, some DQ2 or DQ8 carriers were also able to tolerate gluten on the long term in our study. It has been shown that an initially negative EMA finding has a high negative predictive value for coeliac disease in patients with villous atrophy or other villous anomalies. Although our patients did not have initial EMA results, they remained negative for EMA and did not develop signs of villous atrophy at the biopsy after free gluten exposure for more than 2 years. In some cases in the literature, histological relapse was observed after as long as 14 years. Thus the original two-years rule of the Interlaken criteria to exclude coeliac disease might not be uniformly valid nowadays. However, for a group, more than 2 years observation time seems to be sufficiently safe to label these patients as probably non-coeliacs. The gluten-free diet is a safe and effective treatment for coeliac disease, but not easy to follow life-long. Thus it is important to advise only those subjects to eat gluten-free who clearly need it, especially in case of adolescents and young adults as our patients.

On the other hand, most patients tend to relax the diet after some time without continuous support and verified diagnosis. This was also the case in our patient cohort and resulted in high rate of clinically silent chronic active autoimmune disease charactised by EMA and anti-TG positivity and ongoing villous damage. However, 21 of the 27 patients who were EMA positive due to diet transgressions achieved seronegativity after appropriate dietary education (data not shown).

In accordance with previous studies, all EMA positive patients were found to carry the HLA-DQ2 or DQ8 molecules, thus HLA-DQ typing in EMA positive patients is not necessary.

In conclusion, our findings support the clinical utility of HLA-DQ typing in cases where the diagnosis of coeliac disease is uncertain, especially for second opinion after gluten exclusion.

<u>Rheumatoid arthritis</u>

Study I.

The association between certain HLA-DR alleles and RA has been well established in many ethnic groups. HLA-DRB1 genes have also been associated with disease severity in and outcome of RA. It has been suggested that the susceptibility to RA may be related to specific

epitopes in the third hypervariable region of the first domain of the DRB1 chain, termed the "shared epitope". These epitopes have been found in susceptible HLA-DR1 and HLA-DR4 subtypes including HLA-DRB1*0101, DRB1*0401, DRB1*0404, DRB1*0405, as well as HLA-DRB1*1001 and DRB1*1402. Among Caucasians, HLA-DR1 (the HLA-DRB1*0101 allele), and HLA-DR4 (the HLA-DRB1*0401, *0404 and *0408 alleles) have been primarily implicated in susceptibility to and severity of RA. There has been only one published report on Hungarian RA patients showing the strongest association of RA with the HLA-DRB1*0404 allele.

Earlier we have found that HLA-DR4 alleles were significantly more common in RA patients compared to controls. In addition, HLA-DR1 alleles showed a tendency of being more frequent in RA In this study, we examined the frequency of DRB1*01 and DRB1*04 subtypes in RA patients in comparison to healthy controls. Regarding HLA-DR1 (HLA-DRB1*01) subtypes, DRB1*0101, DRB1*0102 and DRB1*0105 were the most frequent alleles in our RA patients and controls. However, all three alleles were equally common in patients and healthy subjects, suggesting that HLA-DR1, as well as its subtypes may not play an important role in RA in our patients. In contrast to our results, the role of HLA-DR1 subtypes in the pathogenesis of RA was addressed in Ashkenazy Jews, as well as in various other Caucasian populations. Authors of the only published Hungarian study did not perform HLA-DR1 subtyping.

Among HLA-DR4 subtypes, DRB1*0401 and DRB1*0404 were the most common among both our RA patients and controls. We did not find any differences regarding these HLA-DR4 subtypes between the two subject groups. In contrast, DRB1*0405 and DRB1*0408 were much less common, but the frequencies of these allels were significantly increased in RA patients compared to controls. In other Caucasian populations, the DRB1*0401 and DRB1*0404 alleles have been associated with increased susceptibility to RA. The frequency of the DRB1*0405 allele is reportedly increased in Asian populations, such as Koreans, Japanese and Chinese. HLA-DRB1*0408 was associated with increased susceptibility to RA in Finland. Thus, our results support the role of DRB1*0405 and DRB1*0408 in the pathogenesis of RA among our patients. In addition, with regards to HLA-DR4 subtypes, our Hungarian subpopulation may reflect genetic patterns found in Finland and Asia.

Study II.

We analyzed possible associations of both the positivity and serum concentrations of RF, anti-CCP antibody and the expression of shared epitope in patients with RA. Earlier, we showed that the frequency of *HLA-DRB1*04 (HLA-DR4)* alleles was significantly increased in Hungarian RA patients compared to healthy subjects. In addition, *HLA-DRB1*01 (HLA-DR1)* exhibited a tendency to be more frequent in RA patients in comparison to controls.

Our results showing an association between anti-CCP and RF positivity reflect findings published by other researchers. In the present study 56% of the patients were both RF and anti-CCP positive, while neither RF nor anti-CCP could be detected in 17% of the patients.

There was also no correlation between RF production and SE positivity. We must bear in mind that serum RF levels may change during the treatment, so the association between increased RF production and *HLA-DRB1* genotypes may be influenced by anti-rheumatic therapy.

In contrast to RF, we did find associations between the anti-CCP positivity and expression of the *HLA-DRB1*04* gene complex in our RA patients. In addition to these correlations also published previously by other groups, in the present study not only the presence of anti-CCP antibodies but also the serum levels of this antibody could be associated with the expression of one or two copies of *HLA-DRB1*04* alleles in our RA patients. RF still remains an important marker in RA diagnostics. However, the association of highly specific anti-CCP autoantibody levels and HLA *DRB1*04* alleles could provide a valuable "disease marker" combination in evaluating future disease progression since no correlation was found between serum RF levels and the SE.

We also studied possible associations of serum RF and anti-CCP antibody levels with *HLA-DRB1* genotypes other than the SE. Although the number of patients in the studied groups was rather low for statistical analysis, we could not find any relationship between serum RF levels and the specific *HLA-DRB1* subtypes. In contrast, most patients carrying ether *DRB1*13 or DRB1*15* were anti-CCP positive. In addition, patients expressing *HLA-DRB1*13 or DRB1*15* produced significantly more anti-CCP than did patients with *HLA-DRB1*03, *07, *08, *11, *14 or *16.* These results seem to be rather interesting, as the *HLA-DRB1*13* and *DRB1*15* alleles have not been previously associated with RA.

In conclusion, our results support the findings reported by other investigators that anti-CCP antibodies and *HLA-DRB1*04* genes may be present simultaneously in RA patients.

Previous reports suggested an association between anti-CCP positivity and SE positivity in RA. Here we confirmed that not only anti-CCP positivity but, as a novel finding, also serum anti-CCP concentrations are associated with the SE in RA. The strong association between *HLA-DRB1* genes and autoantibody production may both influence future disease development and outcome. Anti-CCP antibody production may be associated with certain other *HLA-DRB1* genotypes, such as *HLA-DRB1*13 and DRB1*15*, but this needs further confirmation is larger patient cohorts.

SLE with or without APS:

Both lupus and APS are systemic autoimmune diseases characterized by multiple organ involvements and the presence of particular autoantibodies. Antibodies usually are in association with certain organ manifestations, for example anti-C1q and anti-dsDNA with lupus nephritis, anti-SS-A with photosensitivity and neonatal lupus. Antiphospholipid antibodies involved in the classification criteria for APS as well as for SLE, and they are associated with thrombotic vessel occlusions and recurrent fetal loss.

Both disorders develop in genetically predisposed individuals. The susceptibility is determined by MHC, as well as non-MHC alleles. Among many others, genes encoding complement and its receptors, Fcγ receptors, molecules involved in the process of apoptosis and the clearance of apoptotic material have been described to increase the risk for the development of SLE. The Fcγ receptor as well as β2-GPI polymorphisms are factors, predisposing to APS. Regarding the MHC, especially different HLA-DR and DQ alleles have been reported in concern with SLE and APS. MHC II alleles may determine the autoantibody profile, and as such the clinical phenotype of the particular disease. In a previous work Tarr et al. found phenotypical differences between three groups of patients with SLE; namely with those, who had only SLE ("SLE only"), SLE patients with secondary APS developing after the onset of lupus (SLE+SAPS), and at last those, who started with PAPS and later within some years progressed to SLE (PAPS+SLE). It was natural that patients with primary or secondary APS presented more thrombotic and less inflammatory activity, while patients with pure lupus presented more frequently proliferative type lupus nephritis with cyclophosphamide and higher steroid dose requirement. However, they found significant difference between the two groups with primary and secondary APS, surprisingly, as regards the number of women with fetal loss and more important the number of recurrence of individual fetal loss. The 24 females had a total of 33 abortions in the PAPS+SLE group vs. 7 fetal losses occurred out of 24 women with SLE+SAPS ($p<0.014$).

The observed significant differences in the clinical phenotype of the three SLE patient subgroups with and without P/S APS triggered the question, whether at least in some cases APS is only a manifestation of lupus or it may associate as an independent autoimmune disease to SLE. Aiming to answer this question we performed HLA-DR and -DQ genotyping in these patient groups based on published literature data, indicating altered genotypes and different allele expressions in SLE and APS. Focusing to the most interesting PAPS+SLE group, the occurrence of DRB1*03 and DQB1*0201 alleles (both characteristic for lupus) was significantly less frequent than in the control group or "SLE only" group (13% vs. 26 and 46%), and was also less common in comparison with the SLE+SAPS group (32%). Furthermore, the HLA-DRB1*04, -DRB1*13 and DQB1*0302, DQB1*06 alleles (characteristic to APS) were expressed in more patients with PAPS+SLE than in the other groups. The differences were not significant statistically, probably due to the relatively low number of patients within the particular subgroups. These observations indicate that the MHC II polymorphism and altered HLA-DRB1, -DQB1 allele frequency partly may explain clinical differences in lupus patients with and without primary or secondary APS. On the other hand, as the MHC II profile in SLE patients with SAPS and who begin as PAPS was rather different, the genetic background is probably not responsible for the partly similar phenotype. Contrary, different genotypes may determine different clinical manifestations. Different expression of DRB1 and DQB1 alleles in SLE only and PAPS+SLE groups may indicate that in the later group APS associates to lupus as an independent disease. At last, concerning other MHC II alleles (e.g. DRB1*15 and DRB1*07) we did not find any difference between patient groups in contrast to some literature. It is probably due to ethnic differences, or to different aPL antibody profile within the examined groups.

Summary

Coeliac disease (CD) is strongly associated with HLA-DQ2 or DQ8 genotypes. The diagnosis of CD nowadays is based on demonstrating crypt hyperplastic villous atrophy, endomysial (EMA) or transglutaminase antibodies (anti-TG) and correlation of disease activity with gluten intake. Our aim was to evaluate the clinical utility of HLA-DQ typing when coeliac disease diagnosis had previously been established solely by histology. HLA-DQ alleles, EMA and anti-TG were investigated and histology slides reviewed in 70 patients diagnosed 2-25 years earlier by small-intestinal biopsy but without measuring EMA or anti-TG. Patients without DQ2 or DQ8 or without unequivocal villous atrophy were followed up on free diet by using serology and biopsies. We have found that all EMA/anti-TG positive patients carried DQ2 or DQ8, and had severe villous atrophy. Only 56% of patients without EMA or anti-TG positivity had DQ2 or DQ8 ($p<0.001$). Seropositivity and relapse developed in 4 of 11 DQ2 positive but in none of 15 DQ2 and DQ8 negative patients on long-term gluten exposure. As a conclusion we can say that coeliac disease diagnosis based solely on histology is not always reliable. HLA-DQ testing is important in identifying DQ2 and DQ8 negative subjects who need revision of their diagnosis, but it does not have additive diagnostic value if EMA positivity is already known.

Certain HLA-DR1 (DRB1*01) and HLA-DR4 (HLA-DRB1*04*)* alleles, also known as "shared epitope"(SE), are associated with increased susceptibility to rheumatoid arthritis (RA), and may also have relevance for disease outcome. Anti-CCP antibody positivity is thought to associate with the presence of HLA-DR4 alleles in patients with RA. However, there is little information available regarding any relationship between serum anti-CCP concentrations and the SE. Therefore our aim was to determine the frequency of HLA-DR1 and -DR4 subtypes in our patients with RA in comparison to healthy control subjects, and to determine the association between anti-CCP antibody production and various HLA-DRB1 alleles. We have found, that among the HLA-DR4 subtypes, DRB1*0401 and DRB1*0404, among DR1 subtypes DRB1*0101 were the most common alleles in both groups, but there were no significant differences in their frequencies between the two examined groups. In contrast, HLA-DRB1*0405 and DRB1*0408 were significantly more common among RA patients in comparison to control subjects. Our data suggest, that in our patients, HLA-DR4, as well as its subtypes DRB1*0405 and DRB1*0408, may be involved in the susceptibility to RA, but HLA-DR1 may not. Furthermore we have found that not only the presence, but the serum concentration of anti-CCP antibody is in association with HLA-DRB1*04 alleles.

I also investigated the HLA-DRB1 and HLA-DQB1 alleles in patients with systemic lupus erythematosus (SLE only), SLE with secondary antiphospholipid syndrome (SLE+SAPS) and in those, whose clinical course began as primary APS and subsequently progressed to SLE (PAPS+SLE), searching explanation behind phenotypical differences: patients with primary or secondary APS present more thrombotic and less inflammatory activity, while fetal wastage was the highest in the PAPS+SLE group. Our results confirmed that the HLA-DRB1 and DQB1 profile of PAPS and SAPS is different, therefore it is unlikely that they are responsible for the partly similar phenotype of the two groups.

Publications that grounded this work:

1**. Kapitány A.,** Korponay-Szabó I., Tóth L., Tumpek J., Csípő I., Niina W., Jukka P., Szegedi Gy., Sipka S.: HLA-DQ allélek diagnosztikus jelentősége szuboptimális módon felállított coeliakia diagnózis esetén
Gyermekgyógyászat 55(4) 443-447. 2004 **IF:0**

2. **Kapitány A,** Tóth L, Tumpek J, Csípo I, Sipos E, Wooley N, Partanen J, Szegedi Gy, Oláh É, Sipka S, Korponay-Szabó IR: Diagnostic significance of HLA-DQ typing in patients with previous coeliac disease diagnosis based on histology alone
Alimentary Pharmacology and Therapeutics Nov. 2006. Vol. 24. Issue 9. Page 1395-1402.

IF:3,287

3 **Kapitány A,** Szabó Z, Lakos G, Aleksza M, Végvári A, Soós L, Karányi Z, Sipka S, Szegedi G, Szekanecz Z.: Associations between serum anti-CCP antibody, rheumatoid factor levels and HLA-DR4 expression in Hungarian patients with rheumatoid arthritis.
Isr Med Assoc J. 2008 Jan;10(1):32-6. **IF: 0,51**

4. **Kapitany A**, Tarr T, Szodoray P, Gyetvai Á, Poór Gy, Szegedi Gy, Sipka S, Kiss E. Human Leukocyte Antigen-DRB1 and -DQB1 genotyping in lupus patients with and without antiphospholipid syndrome
Ann N Y Acad Sci; 2009 excepted for publication **IF:1,73**

Impact factors: 5,527

Publications in the topic of the thesis:

5. **Kapitany A**, Zilahi E, Szanto S, Szucs G, Szabo Z, Vegvari A, Rass P, Sipka S, Szegedi Gy, Szekanecz Z.: Association of Rheumatoid Arthritis with HLA-DR1 and HLA-DR4 in Hungary.
Ann N Y Acad Sci; 2005 jun 1051:263-70. **IF:1,93**

6. Korponay-Szabó IR, Szabados K, Pusztai J, Uhrin K, Ludmány E, Nemes E, Kaukinen K, **Kapitány A**, Koskinen L, Sipka S, Imre A, Mäki M.: Population screening for coeliac disease in primary care by district nurses using a rapid antibody test: diagnostic accuracy and feasibility study.
BMJ. 2007 Dec 15;335(7632):1244-7. **IF: 9,245**

7. Dezsofi A, Szebeni B, Hermann Cs, **Kapitany A**, Veres G, Sipka S, Körner A, Madácsy L, Korponay-Szabó IR, Rajczy K, Arató A: Frequencies of genetic polimorphisms of TLR4 and CD14 and of HLA-DQ genotypes in children with celiac disease, type 1 diebetes mellitus, or both J Pediatr Gastroenterol Nutr 2008 Sep;47(3):283-7. **IF: 2,067**

Other publications:

8. Zsilak S, Gal J, Hodinka L, Rajczy K, Balog A , Sipka S, Barath S, **Kapitany A**, Zilahi E, Szekanecz Z: HLA-DR genotypes in familial rheumatoid arthritis: increased frequency of protective and neutral alleles in a multicase family
J Rheumatol. 2005 Dec;32(12):2299-302. **IF:2,217**

9. Szanto A, Szodoray P, Kiss E**, Kapitany A**, Szegedi Gy, Zeher M
Clinical, Serologic, and Genetic Profiles of Patients With Associated Sjögren's Syndrome and Systemic Lupus Erythematosus
Hum Immunol. 2006 Nov;67(11):924-30. **IF: 2,66**

10. Szücs G, Szekanecz Z, Zilahi E, **Kapitány A**, Baráth S, Szamosi S, Végvári A, Szabó Z, Szántó S, Czirják L, György Kiss C.: Systemic sclerosis-rheumatoid arthritis overlap syndrome: a unique combination of features suggests a distinct genetic, serological and clinical entity.
Rheumatology (Oxford). 2007 Jun;46(6):989-93. **IF: 4,052**

11. Soós L, Szekanecz Z, Szabó Z, Fekete A, Zeher M, Horváth IF, Dankó K, **Kapitány A,** Végvári A, Sipka S, Szegedi G, Lakos G.: Clinical evaluation of anti-mutated citrullinated vimentin by ELISA in rheumatoid arthritis.
J Rheumatol. 2007 Aug;34(8):1658-63. **IF: 2,94**

12. Szekanecz Z, Soós L, Szabó Z, Fekete A, **Kapitány A,** Végvári A, Sipka S, Szücs G, Szántó S, Lakos G.: Anti-Citrullinated Protein Antibodies in Rheumatoid Arthritis: As Good as it Gets?
Clin Rev Allergy Immunol. 2008 Feb;34(1):26-31. **IF: 1,677**

13. Papp M, Foldi I, Nemes E, Udvardy M, Harsfalvi J, Altorjay I, Mate I, Dinya T, Varvolgyi C, Barta Z, Veres G, Lakatos PL, Tumpek J, Toth L, Szathmari E, **Kapitany A,** Gyetvai A, Korponay-Szabo IR.: Haptoglobin polymorphism: a novel genetic risk factor for celiac disease development and its clinical manifestations**.**
Clin Chem. 2008 Apr;54(4):697-704. **IF: 5,454**

14. Nemes É, Lefler É, Szegedi L, **Kapitány A**, B. Kovács J., Balogh M, Szabados K, Tumpek J, Sipka S, Korponay-Szabó IR.: Gluten intake interferencies with the humoral immune response to recombinant hepatitis B vaccine in patients with celiac disease
Pediatrics 2008 Jun;121(6):e1570-6. **IF:5,012**

15. Lakos G., Soós L, Fekete A, Szabó Z, Zeher M, Horváth IF, Dankó K, **Kapitány A**, Gyetvai A, Szegedi G, Szekanecz Z: Anti-cyclic citrullinated peptide antibody isotypes in rheumatoid arthritis: association with disease duration, rheumatoid factor production and the presence of shared epitope
Clin Exp Rheumatol 2008 Jun;26:253-260. **IF:2,189**

16. Szodoray P, Szabó Z, **Kapitany A**, Gyetvai Á,Lakos G, Szántó S, Szűcs G, Szekanecz Z: Anti-citrullinated protein/peptide autoantibodies in association withgenetic and inveronmental factors as indicators of disease outcome in rheumatoid arthritis. (2009) Autoimmun Rev. Epub. 2009. May 6. **IF:3,706**

Impact factors: 48,763