Methods: Urine samples (n=86) were obtained from LN patients and classified in two groups: patients with eGFR >60 (GFRhigh, n=68, 62F/6M, age: 34.07±13.24) and patients with eGFR ≤60 (GFRlow, n=18, 14F/4M, age: 35.22±13.76). RNA from urine samples was isolated using TRIzol-Chloroform technique and then reverse-transcribed using random primers. Levels of BlyS expression were evaluated using Quantitative Real Time PCR (QPCR). All amplifications were carried out in duplicate and threshold cycle (C_t) scores were averaged for calculations of relative expression values. The C_t scores were normalized against C_t scores by subtracting the corresponding β2Microglobulin (β2M) control, or ΔCt=C_{t, gene}-C_{t, β2M}. To test for differential gene expression between groups, a two sample t-test was performed to compare the ΔCt in the two groups.

Results: ΔCt is inversely proportional to BlyS's expression. We evaluated data from ΔCt analysis observing that mRNA levels of BlyS in eGFRlow (6.193±1.787) were higher than those from eGFRhigh (7.564±2.326), with a statistically significant difference between groups (p=0.0288).

	eGFRlow	eGFRhigh	
BlyS (ΔCt)	6.193±1.787	7.564±2.326	p=0.0288

Conclusions: In the present cross-sectional study, increased levels of BlyS were observed in patients with eGFR ≤60. These gene expression results might be linked to B cell activation and proliferation in kidney and thus in urine samples. Combination of eGFR and BlyS appears to be a good biomarker.

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THU0018 ANGIOTENSINOGEN AS A MARKER OF INJURY IN LUPUS NEPHRITIS

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Background: Lupus Nephritis (LN) is one of the most severe forms of systemic lupus erythematosus (SLE) (1). Angiotensinogen (AGT) gene encodes the only glycoprotein known to be a precursor of the vasopressor angiotensin II (Ang II). Ang II is also a growth factor and a profibrogenic cytokine (2). In kidney transplantation AGT has been founded down expressed in biopsies with chronic allograft dysfunction (3). In LN, AGT deserves evaluation.

Objectives: To investigate AGT expression in biopsies and urines from LN patients.

Methods: 32 biopsies/urines paired from 32 LN patients was included. Kidney biopsies were evaluated according to the ISN/RPS classification system. Levels of AGT were evaluated using Quantitative Real Time PCR. Threshold cycle (C_t) scores were averaged for calculations of relative expression values. The C_t scores were normalized against C_t scores by subtracting β2Microglobulin control, or ΔCt=C_{t, gene}-C_{t, β2M}. Data expressed as ΔCt are inversely proportional to gene expression level. Nonparametric Mann Whitney test analysis and Anova with Bonferroni test were performed.

Results: 26 (81.3%) patients were female with a mean age at biopsy time of 31.9±29 years. The SLEDAI at the time of biopsy was 10.5 (IQR 0–15.7) and SLICC ≥1 in 13 (32.5%), hypocomplementemia 13/31 (41.9%) and positive DNA in 11/29 (37.9%) patients. Biopsies from patients with proteinuria ≥0.5 and renal failure (n=23), proteinuria isolated (n=14), LN remission (n=9), renal failure (n=7)

Table 1. AGT gene expression in biopsies and urines samples

	Class I/ Normal Biopsies n=3	Class II Biopsies n=6	Class IV Biopsies n=12	Class V/VI Biopsies n=10	p
ΔCt AGT Biopsies*	5,57 (3,60–5,57)	3,67 (2,19–5,37)	5,34 (4,75–10,93)	4,35 (3,45–4,57)	0,02
ΔCt AGT Urines**	14,11 (12,44–14,11)	11,19 (9,53–11,59)	16,77 (12,88–18,25)	15,06 (12,16–17,62)	0,01

*p<0,05 class IV vs II; **p<0,05 Class IV vs II.

Table 2. AGT gene expression in biopsies according proteinuria levels

	Proteinuria ≤0,5	Proteinuria >0,5	p
ΔCt AGT Biopsies	3,60 (3,34–4,41)	4,79 (3,73–6,39)	0,04

and nephrotic syndrome (n=2) were performed. The mean value of ΔCt AGT gene expression in renal biopsy was 4.50 (IQR 3.51 – 5.67) and AGT in urine samples was 13.94 (IQR 11.66 – 17.89).

Conclusions: In the present study we found a potential utility of AGT mRNA levels in samples of active vs remission LN patients. Prospective studies are needed for confirming these results.

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THU0019 FEATURES OF TELOMERE LENGTH DISTRIBUTION ON INDIVIDUAL CHROMOSOMES IN RHEUMATOID ARTHRITIS

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Background: Telomeres are nucleoprotein structures, that protect the ends of chromosomes during cell divisions [1,2,3]. Previously it was found, that average telomere length in immune cells is reduced in atopic and autoimmunity disorders. This fact indicates an early immune aging in immune-mediated diseases [4,5]. The distribution of telomere repeats on different chromosomes has an individual telomere profile in humans [6] and may be a congenital feature, that accelerates immunosenescence.

Objectives: The purpose of this study was to evaluate the length of telomeres in the arms of individual chromosomes in patients with RA and healthy donors.

Methods: The study included 6 patients with RA and 6 healthy donors (the mean age 51.5 (50–54) and 51.5 (49–53) years respectively). Metaphase spreads obtained from PBMCs were used in this study. Written informed consent was obtained from each person enrolled in the study. At the time of sampling, RA inpatients characterized with acute exacerbation of the disease received treatment at the Clinic of Immunopathology, Novosibirsk. RA was diagnosed by clinicians according to ACR/EULAR 2010. For measurement of the telomere length on individual chromosome arms we used Q-FISH with (C₃TA₂)₃ PNA-probe. Inverted DAPI banding was used for chromosome identification according to ISCN 2013. The new MeTeLen software was developed to estimate the telomere repeats relative quantity (<http://www.bionet.nsc.ru/en/development/application-development/development-of-a-computer/metelen.html>) in metaphase images.

Results: When comparing the telomere length, it was found, that telomere on chromosome 16 p are shorter in patients with RA than in donors. Since each person has an individual telomere profile, we also analyzed the presence of shortened telomere sequences on individual chromosome arms relative to the average length of telomeres for each subject separately. As a result, patients with RA have a larger number of significantly shortened telomeres than donors (see Table).

Comparison group	Chromosome arms with shortened telomere repeats
Healthy volunteers	12p, 19p, 2q, 20q
Patients with RA	7p, 12p, 16p, 17p, 19p, 2q, 20q, 21q

Telomere length on chromosome 16 p (expressed in relative units) in patients with RA and healthy donors. Data are presented as median and interquartile range, *-significant difference (p<0.05, Mann-Whitney U test).

Conclusions: The revealed features of telomeric profiles of patients with RA may be an indication of a proliferative stress, that occurs as a result of the mass immune cell proliferation in the immunopathology. It can be assumed, that the presence of a great number of shortened telomeres can promote cell death through apoptosis. The observed shortening of the telomere length on chromosome 16 p in RA may be relevant in its pathogenesis. It is known, that telomere shortening can lead to increased gene expression near the telomere DNA region. Thus, in 16 p 13 a number of genes is localized, that are associated with RA or may be involved in its development.

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THU0020 INCREASED EXPRESSION OF CCN4/WISP1 IN OSTEOARTHRITIC ARTICULAR CARTILAGE IS EPIGENETICALLY REGULATED AND DISRUPTS CARTILAGE HOMEOSTASIS

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Background: Previously, we described increased expression of Wnt-1-induced signaling protein 1 (*Wisp1*) in murine synovium and cartilage after induction of two experimental osteoarthritis (OA) models. *Wisp1* is a downstream target of the canonical Wnt signaling pathway that has been shown to play a pivotal role in the etiopathology of OA. In line with this, we have observed increased breakdown of the articular cartilage after overexpression of *Wisp1* in naïve mouse knee joints, whereas *Wisp1*^{-/-} mice revealed decreased cartilage degeneration in three independent experimental OA models compared to wild type controls. Together, these data indicate a direct correlation between *Wisp1* and OA in mice.

Objectives: In the current study we set out to characterize the relation between expression of *WISP1* and human OA.

Methods: Articular cartilage from preserved and degenerated OA areas was collected from 39 Caucasian end-stage OA patients. Cartilage from non-OA-diagnosed individuals was collected after femoral neck fractures. Cartilage degeneration was classified according to the Mankin scoring system. DNA was isolated to determine correlation between *WISP1* expression and methylation profiles using Generalized Linear Mixed Model (GLMM). RNA expression levels were determined with microarray analysis and RNA sequencing. Immunohistochemical staining was used to determine *WISP1* protein expression. Recombinant *WISP1* was added to human chondrocyte microparticles, and cartilage extracellular matrix deposition was determined by measuring cartilage microparticle size and Safranin O/Fast Green staining.

Results: We observed increased *WISP1* expression in cartilage of OA patients compared to non-OA-diagnosed controls. Moreover, within OA patients, both *WISP1* mRNA and protein expression were significantly increased in OA-affected cartilage compared to preserved regions of the same joint, and *WISP1* expression significantly correlated with Mankin score. Interestingly, we found that positional CpG dinucleotides were hypomethylated in cartilage of OA-affected areas as compared to unaffected areas from the same joint, which correlated with increased RNA expression as determined with both microarray analysis and RNA sequencing analysis. Of note, methylation levels of a CpG affecting *WISP1* transcription were found to highly significantly correlate to a single nucleotide polymorphism (SNP) at the *WISP1* locus. Next, to investigate effects of increased *WISP1* levels on chondrocyte microparticles, we added human recombinant *WISP1*. This resulted in a significantly decreased deposition of cartilage extracellular matrix as reflected by decreased microparticle circumference, and a strongly decreased proteoglycan content, suggesting that increased *WISP1* levels are detrimental to cartilage.

Conclusions: The expression of *WISP1*, both RNA and protein, is increased in OA-affected as compared to preserved articular cartilage. This increased expression is inversely correlated with methylation levels of a positional CpG for *WISP1*, which was found to be under the influence of a SNP at the *WISP1* locus. Together, our results suggest that tight regulation of *WISP1* expression via methylation is essential to maintain cartilage homeostasis.

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THU0021 THE ANALYSIS OF PRF1, STX11, UNC13D AND STXBP2 GENE IN SYSTEMIC JUVENILE IDIOPATHIC ARTHRITIS WITH MACROPHAGE ACTIVATION SYNDROME

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Objectives: We investigated the clinical characteristics of 34 cases with systemic juvenile idiopathic arthritis (SJIA) and macrophage activation syndrome (MAS) and analyzed PRF1, UNC13D, STX11 and STXBP2 gene so as to figure out the genetic pathogenic mechanism.

Methods: The clinical characteristics of 34 cases with SJIA and MAS were retrospectively analyzed, and the coding sequences of PRF1, UNC13D, STX11

were amplified. DNAMAN software and NCBI BLAST were used to compare sequences. The chi-square test was employed to compare the distribution of allele and genotype frequencies between SLE patients and healthy controls.

Results: Among the 34 cases, 69% were males, and the average age was 6 years. 85% (29/34 cases) of the cases underwent genetic testing and four SNPS loci were detected: PRF1 c. 1061 c > T (rs885822); UNC13D c. 659 c > T (rs3744007); STXBP2 c. 1483 T > c (rs10001) and STXBP2 c. 1616 A > G (rs6791). Compared with control group, genotype and allele frequencies of PRF1 rs885822 and STXBP2 rs10001 among the MAS cases were of significant difference (rs885822: allele frequency 2 =4.52, p=0.03; genotype frequency: 2 =5.52, p=0.02. rs10001: allele frequency 2 =21.33, p=0.00; genotype frequency: 2 =19.58, p=0.00). There were no significant differences in genotype and allele frequencies of UNC13D rs3744007 and STXBP2 rs6791 between MAS group and control group (rs3744007: allele frequency 2 =1.89, p=0.17; genotype frequency: 2 =1.59, p=0.45. rs6791: allele frequency 2 =1.69, p=0.19; genotype frequency: 2 =2.09, p=0.35). The main characteristics of the cases were persistent fever, progressive hepatosplenomegaly, a sharp decline in blood cells, effusion, significantly increased serum liver enzymes, hyperlipidemia, etc. Some children had mucosal bleeding, neurological dysfunction, etc. More than 82% children had upper respiratory tract infection before MAS onset. 90% of children had remission, while three cases developed multiple organ failure and died.

Conclusions: Macrophage activation syndrome is fatal complication caused by immune imbalance. Early detection and treatment is the key to improve the prognosis. The SNP PRF1 rs885822 and STXBP2 rs1001 may be concurrent with the pathogenesis of SJIA-MAS. The SNP UNC13D rs3744007 and STXBP2 rs6791 may not play a role in SJIA-MAS.

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THU0022 REPLICATION ANALYSIS OF GENE-GENE INTERACTION BETWEEN HLA-DQA2 AND HLA-DQB2 VARIANTS IN ITALIAN RHEUMATOID ARTHRITIS PATIENTS

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Background: Rheumatoid Arthritis (RA) is a complex and multifactorial disease, caused by multiple interactions between genetics and environmental factors. In the context of RA genetics, the Human Leukocyte Antigen (HLA) exerts a primary role. Approximately 90% of RA patients show alleles at the HLA-DRB1 locus, codifying for 5 Amino Acids, known as “shared epitope” (SE). SE is associated with increased risk to develop RA and more severe phenotype, but it does not entirely explain HLA contribution to disease risk.

Moreover, it is generally accepted that occurrence risk could be determined by specific gene-gene interactions. A study carried out in USA (NARAC) reported a particular interaction between SNPs in the HLA class II region: HLA-DQA2 (rs9275595) and HLA-DQB2 (rs10807113). Results showed 11 fold increased risk to develop RA in patients carrying both variants (Liu *et al.*, 2011), while single variants did not significant contribute to the RA risk (Odds ratio, OR, respectively, 1.6 and 1.0).

Gene-gene interaction between HLA-DQA2 and HLA-DQB2 SNPs is remarkable, considering that glycoproteins codified by these genes combined each other and create a quaternary structure.

Objectives: To replicate NARAC study evidence in Italian population, we genotyped rs9275595 (HLA-DQA2) and rs10807113 (HLA-DQB2) variants using a case/control model.

Methods: The study included a cohort of 316 Italian RA patients and a matched group of 443 healthy control individuals. All subjects were genotyped for HLA-DQA2 T>C (rs9275595) and HLA-DQB2 A>C (rs10807113) using TaqMan technology and Real-Time PCR.

Results: Replication test performed in our Italian RA cohort did not identify significant interaction between two variants analyzed.

However, considering the single variant rs9275595, the results showed a significantly increased risk to develop RA. Taking T/T genotype as the reference, C/C homozygotes showed significant association with a 3.82 fold increased risk of RA (OR=3.821 95% CI (2.017–8.351) p-value=0.00063). The SNP acted under an additive model: T/C heterozygotes showed significant OR=1.988 (95% CI 1.458–2.711, p-value=0.00002).

Conclusions: The results of this study do not confirm the gene-gene interaction previously reported in the NARAC cohort (Liu *et al.*, 2011). However, outcomes suggest that HLA-DQA2 rs9275595 could contribute to RA pathogenesis in the Italian population.

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