Methods: The case-control pilot study included 143 children (39 with SLE and 103 healthy unrelated volunteers as a control group). Diagnosis of SLE was based on 2012 SLICC criteria. STAT4 rs7574865 G/T polymorphism was investigated using allele-specific real-time polymerase chain reaction (RT-PCR).

Results: The group of pts with SLE consisted of 29 girls and 10 boys, with an average age of 11.8±3.7 years (from 3 to 17 years) and an average disease duration of 4.1±2.4 years. 79.5% pts had acute cutaneous lupus at the onset, 46.1% - non-scarring alopecia, 71.8% - arthritis, 23.1% - oral and nasal ulcers, 23.1% - serositis, 43.6% - renal involvement, 35.9% - neuropsychiatric disorders. Leucopoenia/lymphopenia was found in 71.8% of pts, thrombocytopenia – in 23.1%.ANA were detected in 100% pts, anti-dsDNA – in 79.5%, anti-Sm – in 31.6%, antiphospholipid antibodies - in 73%, hyocomplementemnena - in 61.5%, positive direct Coombs test – in 35.9 %. Macrophage activation syndrome at the onset was documented in 15.4 % of pts. The distribution of rs7574865 genotypes in the control group showed no significant deviations from the Hardy-Weinberg equilibrium. The distribution of genotype frequencies among pts had statistically significant differences compared to the control (p<0.001; p=0.015; GG-30.8% and 63.1% (p=0.001), GT-56.4% and 33.0% (p=0.018), TT-12.8% and 3.9% (p=0.114), GT+TT - 69.2% and 36.9% (p=0.0005). The frequency of the mutant STAT4 allele T (polymorphism) was significantly higher in the SLE group than in the control group (41% and 20.4%, respectively; p=0.0007). We identified an association of the T allele with some clinical, laboratory, and immunological disorders in SLE: arthritis (OR 3.9, p=0.0002), acute cutaneous lupus (OR 2.47, p=0.003), non-scarring alopecia (OR 3.12, p=0.002), renal involvement (OR 2.42, p=0.022), leucopenia (OR 2.72, p=0.003), thrombocytopenia (OR 4.88, p=0.002), anti-dsDNA (OR 2.82, p=0.0006), hyocomplementemnena (OR 2.34, p=0.012). The positive direct Coombs test (OR 3.38, p=0.002).

Conclusion: Our pilot study confirmed that the STAT4 rs7574865 G/T polymorphism was associated with the risk of SLE in children and some of SLE manifestations.

Disclosure of Interests: None declared

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AB0008

APPLYING WHOLE EXOME SEQUENCING TO FAMILIAL ANTIPHOSPHOLIPID SYNDROME: NEW PLAYERS IN A RARE DISEASE?

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Background: Antiphospholipid Syndrome (APS) is an autoimmune disease whose precise aetiology is still unknown, but the high heterogeneity of its manifestations and clinical course is presumably due to the occurrence of different mechanisms and alterations at different levels and pathways [1]. The first genetic studies in APS focused primarily on the human leucocytes antigen system region, but more recent data highlighted a role of other genes in APS susceptibility, primarily those involved in the immune response and in the haemostatic process.

Objective: We aimed to deepen the investigation of APS genetic background starting from a case of familial APS, analysing two siblings with thrombotic APS (Table 1), both triple positive for antiphospholipid antibodies (aPL).

Table 1. Main and laboratory characteristics of the patients included in the study.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>aPL Profile</th>
<th>Relevant Clinical History</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (F)</td>
<td>51</td>
<td>2x positive (LA, aCL IgG, a2GPI IgG)</td>
<td>Two episodes of ischemic stroke, one episode of CAPS</td>
</tr>
<tr>
<td>2 (M)</td>
<td>47</td>
<td>3x positive (LA, aCL IgG, a2GPI IgG)</td>
<td>Three episodes of deep vein thrombosis, regardless of ongoing well conducted therapy vitamin k antagonist and additional renal vein thrombosis</td>
</tr>
</tbody>
</table>

LA: lupus anticoagulant; aCL: anti-cardiolipin antibodies; a2GPI: anti-β2 glycoprotein I antibodies; CAPS: catastrophic APS.

Methods: Genomic DNA was extracted from peripheral blood and the samples underwent Whole Exome Sequencing (WES). Sequencing was done on a 100bp paired end coverage, and reads have been aligned to the human reference genome (GRCh37/hg19 assembly) using the Burrows-Wheeler Alignment tool (BWA). The mean sequencing depth on target regions was 170X for patient 1, 205X for patient 2, moreover, 99.50% of the targeted bases had at least 10X coverage for all the three donors. The resulting single nucleotide polymorphisms (SNPs) have been analysed through a step-by-step process based on their frequency distribution (using Genome Aggregation Database), their predicted effects on the protein (using VarSome) and a literature research about the genes carrying them. Moreover, genes previously associated with a pro-thrombotic tendency and with aPL have been analysed in the two patients.

Results: Starting from more than 120000 SNPs for each patients, the analysis led to reduce the list of SNPs of interest to 27 missense mutations. The complete literature review regarding the genes carrying these mutations allowed to further reduce the number of selected genes, focusing on those that exert a role potentially involved in APS pathogenesis and development. In particular, these genes (PLA2G6, HSPG2, BCL3, 2FAT, ATTP2B2, CRT3 and ADCY3) take part in the immune response and the vascular homeostasis. The list of the DNA missense variants of interest found in our cases of familial APS is resumed in Figure 2.

Disclosure of Interests: None declared

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AB0009

ASSOCIATION OF STAT4 RS7574865, RUNX1 RS9979383, IL6 RS1800795, IL6R RS2228145, IL6R RS4845618 WITH SYSTEMIC LUPUS ERYTHEMATOSUS SUSCEPTIBILITY AND SOME LUPUS MANIFESTATIONS IN BELARUSIAN POPULATION

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Background: Systemic lupus erythematosus (SLE) has a significant genetic predisposition. Many genetic variants of susceptibility to SLE have been published and analyzed, but the clinical and functional significance of the various genotypes has not yet been clearly defined [1].

Objectives: To estimate the association between some of non-HLA gene polymorphisms such as STAT4 rs7574865, RUNX1 rs9979383, IL6 rs1800795, IL6R rs2228145, IL6R rs4845618 and susceptibility to SLE in Belarusian population as well as some disease manifestations.

Methods: We examined 383 healthy blood donors and 54 SLE patients (18-72 years old, median age 35) classified according to the 1997 American College of Rheumatology (ACR) revised classification criteria [2]. Deoxyribonucleic acid was extracted from peripheral blood samples by phenol-chloroform method. Genotyping was performed by real-time PCR with fluorescent probes. Differences of distribution of all the single nucleotide polymorphism (SNP) genotypes and their associations with secondary antiphospholipid syndrom (APS) and lupus arthritis were analyzed using Pearson χ² (ϕ²) and two-way Fisher exact test (F, p, ϕ²). Diagnostic odds ratio (DOR), likelihood ratio of positive (LR +) and negative (LR –) tests and corresponding 95% confidence intervals (CI) were also calculated.

Results: We revealed significant difference in STAT4 rs7574865 genotypes in SLE patients and healthy donors (ϕ²=8.27, p=0.016) with significant increase of TT genotype frequency in SLE patients vs healthy donors (ϕ²=6.83 p=0.009; p=0.020; DOR=3.78 (CI 95% 1.36-10.55); LR+ =3.44 (CI 95% 1.35-8.71); LR– =0.91).

Figure 2. List of DNA missense variants of interest found in patient 1 and 2. Genes potentially involved in APS pathogenesis and development are highlighted in bold.

No mutations on genes known to be associated with a pro-thrombotic state (F5, F2, MTHFR, F3A1, PROC, PROS1, FGB and SERPINE1), or on genes previously associated with APS (B2GPI, PF4V1, SELP, TL2R2, TL2R4, GP Ia, GP1B4, P2R2, P2R1L, TP1, F3, VEGFA, FL1, and TNF) have been found in the WES analysis.

Conclusion: To some extent, this can be seen as a proof of concept of the complexity of APS. Efforts to interpret the genetic risk factors involved in the heterogeneous clinical features of the syndrome, for instance, the integration of WES and network-based approaches might help to identify and stratify patients at risk of developing APS.

REFERENCES:


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Figure 1. The expression levels of ATGs in HC and RA groups.

Conclusion: There is abnormal expression of autophagy genes in the peripheral blood of RA patients. ULK1, ATG17, LC3 and P62 were related to the pathogenesis of RA, among them, ATG17 may regulate the pathogenesis of RA by participating in the TNF-α pathway.

REFERENCES:

Disclosure of Interests: Yu-Feng Qing Grant/research support from: Science and Technology Project of Nanchong City (no.18ZKJ0252), Fei Dai: None declared, Yu-Feng Qing Grant/research support from: Science and Technology Project of Nanchong City (no.18ZKJ0252), and population groups.

Abstract: The expression levels of autophagy-related genes (ATG) unc-51-like kinase 1 (ULK1), ATG13, ATG17, microtubule associated protein 1 light chain 3 (P62), and P62 in peripheral blood mononuclear cells (PBMC) of patients with RA were detected, and their role and clinical significance in the pathogenesis of RA were explored.

Methods: Real-time fluorescent quantitative PCR was performed to detect the expression levels of ULK1, ATG13, ATG17, LC3, and P62 in PBMCs of 50 RA patients, 50 healthy controls (HC), and 25 moderate to severe RA patients before and after treatment. Then, 1st test, 2nd test, Mann-Whitney U test, Pearson test were used for statistical analysis.

Results: The levels of hscRP, white blood cell (WBC), neutrophils (GR), platelet (PLT) and plateletcrit (PCT) in RA group were higher than those in HC group (P < 0.05).

Lupus arthritis was more common in risk TT-genotype SLE patients (OR=1.55 (CI95% 0.83-0.98)). Lupus arthritis was more common in SLE CT-genotype carriers than in other SLE patients (OR=5.902 p=0.015; p2-t =0.027).

We revealed significant increase of CT genotype (RUNX1 rs9979383) in healthy donors vs SLE patients (χ²=4.14; p=0.042; dOR=0.53 (CI95% 0.29-0.98); LR+ =0.69 (CI95% 0.45-0.99); LR- =13 (CI95% 1.01-1.56)). Lupus arthritis was more significant differences in IL6 rs1800795, IL6 rs2282145 and IL6 rs4845618 genotypes distribution between studied groups were not found (χ²=0.427, p=0.559 and p=0.407, respectively).

Background: The presence of the C allele was associated with the development of AS (OR=1.55 (CI95% 0.83-0.98)). Lupus arthritis was more common in risk TT-genotype SLE patients (OR=5.902 p=0.015; p2-t =0.027).

Conclusions: Our data suggest the susceptibility to SLE in TT genotype of ST4 rs7574865 polymorphism, protective role of CT genotype of RUNX1 rs9979383 for SLE and association between GG-genotype of IL6 rs1800795 and APS in SLE patients in Belarusian population. Lupus arthritis was associated with TT genotype of ST4 rs7574865 and CT genotype of RUNX1 rs9979383.

Disclosure of Interests: None declared.

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AB0001

ASSOCIATION OF SAA1 GENE POLYMORPHISM -13T/C (rs12218) WITH ANKYLOSING SPONDYLITIS IN RUSSIAN POPULATION

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Background: Ankylosing spondylitis is a chronic systemic inflammatory disease. Inflammation and high levels of serum amyloid A (SAA) protein are predisposing factors for secondary AA amyloidosis. The role of SAA1 gene polymorphisms in AS is still not well understood.

Objectives: To investigate the association of SAA1 gene polymorphism -13T/C (rs12218) with ankylosing spondylitis and to evaluate the influence of this polymorphism on SAA protein concentration.

Methods: 123 AS patients (72 males, 51 females; age - M (SD) 37.51 (12.77) years; disease duration - 14.28 (11.22) years; BASDAI – 5.59 (1.13); B27-positive - 111 (54.9); HLA B27-positive and 95% gender, age matched healthy individuals (control group) were included in this study. SAA1 gene polymorphism -13T/C was genotyped using allele-specific RT-PCR assay. SAA protein concentration was measured using nephelometry in AS patients.

Results: The expression levels of ATGs in HC and RA groups.

Table 1. The levels of hsCRP, white blood cell (WBC), neutrophils (GR), platelet (PLT) and plateletcrit (PCT) in RA group were higher than those in HC group (P < 0.05).

Conclusions: The results of our study demonstrated for the first time that SAA1 gene polymorphism -13T/C (rs12218) is associated with susceptibility to AS. It is also shown that this polymorphism can affect the SAA protein level. Our findings need to be verified in AS patients with high levels of SAA protein in various ethnic and population groups.

Disclosure of Interests: None declared.

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AB0011

EXPRESSION PROFILE AND POTENTIAL FUNCTION OF CIRCINAS IN PERIPHERAL BLOOD MONONUCLEAR CELLS FROM PATIENTS WITH PRIMARY GOUT

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Background: Autoaphagy is a phenomenon of “self-phagocytosis” in eukaryotic cells, which maintains cell homeostasis by transporting intracellular materials to lysosomes for degradation and recycling. In recent years, studies have shown that autoaphagy may be involved in the pathogenesis of rheumatoid arthritis (RA) [1], but its specific mechanism is still unclear.

Objectives: The expression levels of autophagy-related genes (ATG) unc-51-like kinase 1 (ULK1), ATG13, ATG17, microtubule associated protein 1 light chain 3 (LC3), and P62 in peripheral blood mononuclear cells (PBMC) of patients with RA were detected, and their role and clinical significance in the pathogenesis of RA were explored.

Methods: Real-time fluorescent quantitative PCR was performed to detect the expression levels of ULK1, ATG13, ATG17, LC3, and P62 in PBMCs of 50 RA patients, 50 healthy controls (HC), and 25 moderate to severe RA patients before and after treatment. Then, 1st test, 2nd test, Mann-Whitney U test, Pearson test were used for statistical analysis.

Results: The levels of hscRP, white blood cell (WBC), neutrophils (GR), platelet (PLT) and plateletcrit (PCT) in RA group were higher than those in HC group (P < 0.05). The expressions of ULK1, ATG17, and LC3 in RA group were higher than those in HC group, while the expressions of P62 was lower than those in HC group (P < 0.05) (Figure 1). The correlation analysis suggested that ATG17 was positively correlated with tendon joint count (TJC), swollen joint count (SJC), and health assessment questionnaire (HAQ) (P < 0.05); ULK1 and HAQ were negatively correlated (P < 0.05).3. Compared with before treatment with TNFi, ATG17, HAQ, DAS-28, ESR, hscRP, WBC, GR, PLT, and PCT were significantly reduced after treatment (P < 0.05); the expressions of RBC, HCT, MCV and MCH were significantly increased after treatment (P < 0.05); ULK1, ATG13, LC3, P62 and other related clinical and laboratory indicators were not significantly different before and after treatment with TNFi (P > 0.05).

Conclusion: There is abnormal expression of autophagy genes in the peripheral blood of RA patients. ULK1, ATG17, LC3 and P62 may be related to the pathogenesis of RA, among them, ATG17 may regulate the pathogenesis of RA by participating in the TNF-α pathway.

REFERENCES:

Disclosure of Interests: Yu-Feng Qing Grant/research support from: Science and Technology Project of Nanchong City (no.18SXI0Z0522), Fei Dai: None declared, Quan-Bo Zhang Grant/research support from: the National Natural Science Foundation of China (General Program) (no.81974250), and Science and Technology Plan Project of Sichuan Province (no.2016JY0257), Yu-Ping Tang: None declared, Zeng-Rong Dong: None declared, Yi-Xi He: None declared, Yi-Jiang: None declared, Yu-Qin Huang: None declared, Xiaoxing Zheng: None declared.

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