AB0003

A GALNT3 GENE MUTATION IN TWO SIBS WITH CHRONIC RECURRENT MULTIFOCAL OSTEOMYELITIS ASSOCIATED WITH HYPERPHOSPHATEMIC FAMILIAL TUMORAL CALCINOSIS

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Background: Chronic recurrent multifocal osteomyelitis (CRMO) is an uncommon inflammatory disease mostly affects the metaphyses of long bones. It can be distinguished from osteomyelitis by multifocality and recurrence. Hyperphosphatemic familial tumoral calcinosis (HFTC) is a rare genetic disorder characterised by increased re-absorption of phosphate through the renal proximal tubule leading to increased phosphate concentration and deposition of calcified deposits in cutaneous and subcutaneous tissues, as well as some visceral organs. HFTC is inherited in an autosomal recessive manner and is caused by mutations in three different genes, *FGF23*, *GALNT3* and *KLOTHO*. CRMO has been associated with some chronic inflammatory diseases such as inflammatory bowel disease, palmoplantar pustulosis and SAPHO syndrome. The association of CRMO and HFTC is extremely rare and only three patients have been described so far in the literature.

Objectives: To report the clinical, radiological and molecular findings of two sibs with CRMO associated with HFTC.

Methods: In this report, we present two siblings; offspring of consanguineous parents. They presented with spontaneous bony pains not responding to NSAID and later on, that developed tender hard masses. There were no similarly affected family members, and they had a non-affected sibling. Clinical, laboratory, pathological and radiological examination was performed. Mutational analyses of the *FGF23*, *GALNT3* and *KLOTHO* genes was carried out by Sanger sequencing of the entire coding region of each gene.

Results: Laboratory results including blood cultures and sensitivities were normal, apart from mildly elevated ESR. Serum calcium, 25(OH) vitamin D, renal functions, albumin, alkaline phosphatase, parathormone hormone, and phosphorous were normal apart from hyperphosphatemia in both siblings.

Initial x-rays revealed lytic lesions with a sclerotic margin. Follow up x-rays showed healing with sclerosis and hyperostosis. After developing the hard masses, x-rays showed calcified masses. Resection pathological analysis revealed non-neoplastic inflammatory bone growth with prominent periosteal and bone proliferation, it was free from any granulomatous or malignant changes. We suggested a provisional diagnosis of CRMO associated with HFTC. Molecular studies confirmed the diagnosis by identifying a known pathogenic mutation in the donor splice site of exon 8 of the *GALNT3* gene, c.1524+1G>A. The mutation was found in the homozygous form in the two sibs and both parents were heterozygous.

Conclusions: This study documents the first Egyptian family clinically diagnosed with CRMO associated with HFTC and confirmed by molecular studies, with the identification of a splice mutation in the *GALNT3* gene.

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Disclosure of Interest: None declared

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AB0004

PHARMACOGENETIC ASPECT OF METHOTREXATE, IN A GROUP OF COLOMBIAN PATIENTS WITH RHEUMATOID ARTHRITIS

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Background: Methotrexate (MTX) as monotherapy or in combination, is the most commonly Disease-Modifying-AntiRheumatic-Drug (DMARDs) used in rheumatoid arthritis (RA). About 40% of patients do not respond to treatment or have adverse effects. The genetic variability could be responsible for this phenomenon. Different studies suggest associations between polymorphisms in the enzymes involved in the metabolic pathway of MTX with alterations in the efficacy and toxicity.

Objectives: Determine the polymorphisms of the enzymes involved in MTX metabolism in a group of Colombian patients.

Methods: 400 patients with RA over 18 years old, diagnosed according to the ACR/EULAR classification criteria, who consecutively attended an outpatient RA clinic between March 2015 and December 2016 were included. MTX efficacy was defined by DAS28 score ≥ 3.2 , liver toxicity by elevation of transaminases above three times the normal value, Haematological toxicity by: leukocytes <4,000, Hb <9.5, platelets <150,000, renal toxicity: creatinine >1.5. The single nucleotide polymorphisms (SNPs) studied were MTHFR C677T, MTHFR A1298C, ATIC C347G,

RFC1 G80A, FPGS-AG and DHFR-CT and were identified by the technique of polymerase chain reaction in real time (RT-PCR).

Results: The mean age of patients was 60.7 $\pm$ 13.9 years, the duration of the disease was 13.2 $\pm$ 10.9 years and 76% were women. A significant increase in the frequency of MTHFR C677T and A1298C SNPs ($p=0.05$ and $p=0.048$) were found in the responding patients compared to non-responders. The DHFR-CT and the ATIC C347G SNPs were significantly increased in patients with any toxicity to MTX ($p=0.0095$ and $p=0.005$ respectively). We did not find a significant difference between the polymorphisms studied with any specific toxicity.

Abstract AB0004 – Table 1

Polymorphisms	n	Activity (%)	Remission (%)	OR-95%(IC)	p
MTHFR C677CC	344	189	155	1.62 (1.0–2.68)	0.05
CC	81	37 (20)	44 (28)		
TT	263	152 (80)	111 (72)		
MTHFR A1298	381	219	162	1.74 (1.01–3.02)	0.048
AA	312	172 (79)	140 (86)		
TT	69	47 (21)	22 (14)		
DHFR	394	225	165	1.03(0–68–1.55)	0.484
CC	233	131 (58.2)	95 (57.6)		
TT	(58.3)	91 (40.5)	68 (41.2)		
FPGS	161			1.92 (0.69–1.41)	0.406
AA	400	225	165		
GG	137	74 (32.9)	57 (34.5)		
	(34.3)	151 (67.7)	108 (65.5)		
	263				
	(65.7)				

Abstract AB0004 – Table 2

Polymorphisms	n	Toxicity	OR-95%(IC)	p
MTHFR C677CC	316	81	1.31 (0.73-2.34)	0.21
CC	74	22		
TT	242	59		
MTHFR A1298	312	80	1.50 (0.71-3.16)	0.184
AA	261	70		
TT	51	10		
DHFR	313	81	1.93(1.13-3.30)	0.0095
CC	175	55		
TT	136	26		
ATIC C347	313	80	2 (1.2012-3.36)	0.005
CC	121	41		
GG	192	39		

Conclusions: The Colombian population has similar statistical data compared to the global studies regarding the association of SPNs with the efficacy and toxicity of methotrexate, however the polymorphisms associated with inefficiency in the literature are not replicated in our data. These SNPs could be established as biomarkers to the methotrexate response in terms of efficacy and toxicity in our Colombian population with RA.

REFERENCE:

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AB0005

HLA CLASS II IN PARAGUAYAN IMMUNE-MEDIATED INFLAMMATORY PATIENTS

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Background: Immune-mediated inflammatory disease (IMID) is a concept used to describe a group of conditions that share common inflammatory pathways leading to systemic inflammation. The best-known genetic factor for IMID susceptibility is the human leukocyte antigen (HLA) haplotypes. Nowadays, there is a lack of information about HLA profile in Paraguayan patients with IMIDs.

Objectives: To identify HLA alleles associated with susceptibility to develop an IMID in Paraguayan patients controlled in a reference centre.

Methods: Paraguayan IMID patients were recruited from the Rheumatology Department of Hospital de Clínicas, Paraguay. IMID HLA II frequencies were compared with a control group of 50 unrelated individuals without disease and from the same geographic origin. Genotyping for HLA was performed using LumineX PCR technology. The association analysis with the IMIDs risk was performed using the chi-square allelic test.

Results: 249 IMID patients (95 lupus, 104 rheumatoid arthritis and 50 systemic sclerosis) were included. Of these 84.4% were women with an average age of 43.4 (± 14). Comparing the haplotypes profiles for the 5 HLA class II genes between the patients and the healthy controls, in the risk association analysis, the association of the known risk allele was corroborated HLA-DRB1*03:01 ($p=2e-06$, OR:14.97). A significant association was identified between the allele HLA-DRB1*08:02 ($p=0.0271$, OR:0.13) and HLA-DRB1*08:07 ($p=0.0133$, OR:0.08). In the gene *HLADQA1*, 1 allele associated with the IMIDs were found, the HLA-DQA1*04:01 ($p=1.4e-05$, OR:0.06). In the *HLADPB1* gene 3 alleles associated with the IMIDs were identified: HLA-DPB1*02:01 ($p=4.2e-05$, OR:82.91), HLA-DPB1*03:01 ($p=2e-06$, OR:14.97), HLA-DPB1*04:01 ($p=1.5e-05$, OR:34.55). Different associations between IMIDs and alleles was identified (table 1).

Abstract AB0005 – Table 1. List of associated alleles stratified by disease

ALLELE (Systemic Lupus Erythematosus Cohort)	P-value	OR
HLADQA1*02:01	0.0253	14
ALLELES (Rheumatoid Arthritis Cohort)	P-value	OR
HLADRB1*08:02	0.0367	0.11
HLADPB1*02:01	0.0003	54.62
HLADPB1*03:01	0.0010	7.92
HLADPB1*04:01	0.0001	29.69
HLADQA1*02:01	0.0467	0.24
HLADQA1*04:01	0.002	0.07
HLADPA1*02:01	0.0022	0.029
ALLELES (Scleroderma Cohort)	P-value	OR
HLADPB1*02:01	0.0003	82.33
HLADPB1*03:01	0.0094	8.44
HLADPB1*04:01	0.0001	47.50
HLADQA1*04:01	0.0013	0.06

Conclusions: In the genetic association analysis, already known associations have been replicated and new ones previously unpublished have been identified in Paraguayan IMIDs patients. This is the first genetic association study in IMID patients Paraguayan origin.

Disclosure of Interest: None declared

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AB0006 MIR-26A POLYMORPHISM IS ASSOCIATED WITH SUSCEPTIBILITY OF RHEUMATOID AND PSORIATIC ARTHRITIS

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Background: Serum levels of miR-26a has been reported to act as potential biomarker of rheumatic diseases.

Objectives: The aim of the study was to analyse the genetic variation and expression of miR-26a as potential diagnostic and/or prognostic markers of rheumatoid diseases.

Methods: The miR-26a polymorphism was examined in 111 patients with rheumatoid arthritis (RA), 86 patients with psoriatic arthritis (PsA) and 162 healthy blood donors that served as a control group. Genotyping for miR-26a rs7372209 was performed using a LightSNiP assay. For analysis of the miR-26a expression, RNA was isolated from sera of 15 RA patients (before and 3 months after anti-TNF treatment) and 10 controls (Nucleospin-miRNA Plasma; MACHERY-NAGEL GmbH and Co. KG) followed by cDNA synthesis (TaqMan MicroRNA Reverse Transcription Kit; Applied Biosystems™ by Life Technologies) and Real-time PCR amplifications with hsa-miR-26a TaqMan specific and U6 snRNA control primers for each probe. The results were analysed using the ($\Delta\Delta C_t$) calculations.

Results: It was found that the presence of miR-26a TT genotype (rs7372209) more than 5 times increases the risk of RA (OR=5.28, $p=0.003$) while the presence of CC homozygotes is associated with the risk of PsA (OR=1.77, $p=0.037$).

There was no significant difference in the miR-26a serum levels between patients and controls. Also miR-26a serum levels did not significantly differed between RA patients before, 3 and 6 months after the implementation of biological therapy with TNF-alpha inhibitors.

Conclusions: These results imply that miR-26a rs7372209 allelic variants differentially affect the risk of rheumatoid and psoriatic arthritis while anti-TNF biological treatment seems not to affect the miR-26a expression in RA patients.

Disclosure of Interest: None declared

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AB0007 ASSOCIATION AT SYSTEMIC LEVELS OF CYTOKINE MRNAS AND PROTEIN QUANTITIES IN RHEUMATOID ARTHRITIS

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Background: Rheumatoid arthritis (RA) is an autoimmune inflammatory disease, characterised by chronic synovitis, bone and cartilage destruction, as well as systemic manifestation. In accordance with the pivotal role of cytokines in autoimmunity and their impact as biomarkers, we analysed gene expression at both mRNA and protein levels of several cytokines in peripheral blood of RA patients.

Objectives: The aim of the present study was to investigate the gene expressions at mRNA and protein levels of main pro-inflammatory (TNF- α , IL18, IL-12p40; IL-23, IL-6) and immunosuppressive (TGF- β 1 and IL10) cytokines and transcription factor Foxp3 in peripheral blood of RA patients.

Methods: Total RNA from peripheral blood was isolated from 32 patients matching the ACR/EULAR 2010 criteria for RA and 27 healthy controls. Quantitative real-time polymerase chain reaction was performed for the 8 genes of interests, using the TaqMan detection system. Relative quantitative evaluation of mRNAs was performed by the comparative $\Delta\Delta C_t$ method and results were presented as n-fold mean difference (RQ-relative quantity) of target genes relative to calibrator (healthy controls) after normalisation to the reference genes (GAPDH and 18srRNA). Serum quantities of cytokines were measured by ELISA.

Results: From studied pro-inflammatory cytokine genes, we found down-regulation in the following order: IL6 > TNFA > IL12B; up regulation of IL23A and no change in IL18 gene expression in RA patients group compared to healthy controls. For anti-inflammatory genes we detected significantly increased quantity for IL10 mRNA and no change for TGF β 1 mRNA. The most profound down-regulation (more than 7-fold) was observed for IL6 gene ($p<0.001$), while the serum level of the same cytokine was significantly increased as compared to the same controls. The similar tendency was observed in the expression of TNFA, which gene expression was approximately 2-fold down-regulated, whereas serum levels were increased. IL12B mRNA were slightly but not significantly decreased (RQ=0.709; $p=0.169$) in RA patients. An upregulation of IL-23 was detected for IL23A gene (RQ=2.422, $p=0.002$) and serum level of IL-23 as well.

TGF β 1 mRNA levels were approximately equal in patients and controls in contrast to IL-10, which was upregulated in both mRNA (RQ=1.6; $p=0.034$) and serum levels (over 6.6 fold; $p<0.05$). In addition, mRNA expression of FoxP3, a master transcription factor for Treg subset was also down-regulated over 4-fold in RA patients ($p<0.001$). A positive correlation was found between gene expression of IL6 with Foxp3, TNFA and TGF β 1 in RA ($r=0.744$, $p=0.004$; $r=0.6$, $p=0.03$; $r=0.556$, $p=0.048$, respectively).

Conclusions: Our results demonstrated significant differences in the expression of mRNA encoded cytokines and their protein quantities at systemic level of RA patients, mostly on IL-23, IL-6 and TNF-alpha.

Disclosure of Interest: None declared

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AB0008 CROSS-TALK BETWEEN BONE TURNOVER AND CARDIOVASCULAR DISEASE. ASSOCIATION OF MICRORNAS EXPRESSION, FRACTURE AND ABDOMINAL AORTIC CALCIFICATIONS

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Background: MicroRNAs (miRs) have emerged as pivotal epigenetic key actors of gene regulation and several miRs have been shown to be at the crossroads of angiogenesis and of bone turnover, taking part in the calcification process by acting on osteoblasts and osteoclasts.¹ Calcification of the aortic media is highly