Poster Presentations

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Genomics, genetic basis of disease and antigen presentation

THU0001 DIFFERENTIAL METHYLATION AS A PREDICTOR OF METHOTREXATE RESPONSE IN PATIENTS WITH RHEUMATOID ARTHRITIS

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Background: Methotrexate (MTX) is recommended as the first-line disease modifying anti-rheumatic drug (csDMARD) for the treatment of rheumatoid arthritis (RA), but up to 40% patients do not respond adequately, or experience adverse effects¹; therefore, identifying blood-based biomarkers that predict treatment response is a research priority. DNA methylation is an epigenetic marker that modifies but does not alter DNA sequence; MTX may act, at least in part, by inhibiting intracellular methyl donor transfer leading to DNA hypomethylation² so DNA methylation may act as a biomarker of MTX response.

Objectives: To identify differential DNA methylation signatures in whole blood associated with response to MTX in patients with RA.

Methods: DNA methylation was measured using the HumanMethylation450 BeadChip in DNA samples from individuals recruited to the Rheumatoid Arthritis Medication Study (RAMS). Demographic and clinical data were collected prior to starting MTX (baseline) and at 6 months after commencing MTX. DNA was extracted from whole blood samples collected at baseline and at 4 weeks from patients who, at 6 months, had a EULAR good response (n=36) or EULAR poor response (n=36) to MTX. Differentially methylated positions (DMPs) between baseline and 4 weeks, and between good and poor response groups were identified using a linear model, adjusting for gender, age, cell composition, baseline disease activity score (DAS28), and smoking status. Additional analyses were performed to assess the association between methylation and change in DAS28 score and the individual DAS28 components over 6 months. DMPs with significant differences were selected for replication by pyrosequencing in an independent group of 100 patients with both baseline and 4 week samples available for testing. Using genome-wide genotype data for the same patients, replicated DMPs were investigated for methylation QTLs (meQTLs).

Results: The initial analysis identified differential methylation between good and poor responders at 2 CpG sites (DMPs) in samples taken at 4 weeks, with response status determined at 6 months (p-value<10⁻⁵). Three other DMPs were associated with change in tender joint count, another 3 DMPs with change in swollen joint count, and a further 4 DMPs were associated with change in C-reactive protein (CRP). Of the 12 DMPs, 4 showed replicated association with improvement of swollen joint count and lower CRP levels at 6 months in the pyrosequencing dataset (p-value<0.01). However, there were no meQTLs identified at these loci.

Conclusions: These results suggest DNA methylation may provide a biomarker of MTX response but requires replication in other larger data sets.

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Disclosure of Interest: None declared

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THU0002 NOVEL PATHOGENIC STOP CODON MUTATION IN THE NF-KB P65 SUBUNIT (RELA) ASSOCIATED WITH BOTH BEHÇET'S DISEASE LIKE SYNDROME AND NEUROMYELITIS OPTICA IN AN IRISH FAMILY

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Background: Behçet's disease (BD) has a complex multifactorial pathogenesis and presents with phenotypic heterogeneity predominantly mucocutaneous ulcerations, ocular lesions and skin manifestations. More recently, there have been reported cases of monogenic spectrum defects presented with BD-like similarities or phenotype.

Objectives: We investigated an Irish Caucasian family of eleven that included two half-sisters with early-onset BD, and another sister with neuromyelitis optica, all who were born to asymptomatic non-consanguineous parents. More recently, one of the sisters' daughter developed recurrent oral aphthosis at the age of 10 years old.

Methods: Peripheral blood mononuclear cells were extracted from patients and non-affected donor blood using standard fractionation methods. Following quality assessment and quantification whole exome sequencing was performed on all participants.

Results: Whole exome sequencing data identified segregation of a novel pathogenic stop codon mutation in the nuclear factor NF-κB p65 subunit (RelA) resulting in a non-functional protein. The mutation involves cytosine deletion and results in a His487ThrfsTer7 frameshift (His487ThrfsTer7) RelA resulting in loss of transcription activation-1 (TA1) and a portion of TA2 from RelA. The mutation was seen within the three generations, including the three half-sisters, their father as well as one of the proband's daughter, potentially describing a new syndrome.

Conclusions: Our study suggests that loss-of-function mutations in the NF-κB pathway, a pivotal mediator of inflammation and apoptosis, are linked with the development of familial early-onset BD-like syndromes. Better insights and further understanding of this "orphan" immunogenetic syndrome carries high clinical impact to assist early disease recognition and potential discoveries of novel targeted therapies.

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THU0003 COMPREHENSIVE EVALUATION OF THE EFFECTS OF RARE AND COMMON EXONIC ABCG2 VARIANTS ON GOUT SUSCEPTIBILITY

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Background: Gout is the most common form of inflammatory arthritis and is caused by hyperuricemia. Many previous studies have indicated that common dysfunctional variants of the gene encoding ATP-binding cassette transporter subfamily G member 2/breast cancer resistance protein (ABCG2/BCRP) increase the risk of gout and hyperuricemia. In addition, we recently showed that rare non-synonymous variants are also risk factors for gout. However, we have not evaluated the effects of synonymous and splice-site variants of

ABCG2. Thus, to estimate the risk of genetic variants of *ABCG2* more comprehensively, we analysed the association between all exonic variants and gout susceptibility.

Objectives: The main purpose of this study was to perform comprehensive *in silico* evaluation of the effects of all types of rare and common exonic *ABCG2* variants on gout susceptibility in Japanese population.

Methods: We previously sequenced all the exons of *ABCG2* in 480 patients with gout and 480 healthy controls (Japanese males) and performed functional analyses of non-synonymous variants. In this present study, we analysed the correlation between urate transport function and scaled C-score of CADDv1.3 (CADD score) of non-synonymous variants. We additionally performed Receiver Operating Characteristic (ROC) curve analysis and selected variants with altered function of more than 50% compared to wild-type *ABCG2*. Stratified association analyses and multivariate logistic regression analysis were performed to evaluate the effects of selected rare and common *ABCG2* variants on gout susceptibility.

Results: We identified 4 common and 26 rare exonic or closely situated intronic variants of *ABCG2*. CADD scores showed significant correlation with the results of functional analyses on urate transport ($p=0.014$, $r=-0.539$). ROC curve analysis showed an area under the curve (AUC) of 0.775. The optimal cutoff value of CADD score was 15 for classifying variants with altered function of more than 50% compared to wild-type *ABCG2* (sensitivity=0.88, specificity=0.67). Therefore, we selected variants with a CADD score greater than 15 for downstream analyses. All intronic or synonymous variants had low CADD scores and thus were removed. Multivariate logistic regression analysis showed that the rare variants of *ABCG2* were associated with a significantly increased risk of gout and the size effect of these rare variants (odds ratio [OR]=2.7, $p=0.012$) was similar to that of the common variants, Q126X (OR=3.3, $p=4.8 \times 10^{-6}$) and Q141K (OR=2.3, $p=8.6 \times 10^{-6}$).

Conclusions: This study confirmed that both common and rare variants in *ABCG2* increase gout susceptibility. Furthermore, our *in silico* analyses suggest that synonymous and splice-site variants of *ABCG2* may not play a key role in the pathogenesis of gout.

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THU0004 A DE NOVO NON-SENSE ERAP1 POLYMORPHISM IN TWO HLA-B*27-NEGATIVE TWINS WITH AXIAL SPONDYLOARTHRITIS

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Background: Axial spondyloarthritis (axSpA) is a group of inflammatory disorders primarily affecting the spine that includes ankylosing spondylitis (AS) and non-radiographic axSpA. AS is strongly associated with HLA-B*27. A small percentage of HLA-B*27-positive subjects develop AS, suggesting the role of other genes in AS susceptibility.^{1,2} Among these genes, *ERAP1* acts as "molecular ruler". It encodes the endoplasmic reticulum aminopeptidase 1 protein, responsible for the peptides trimming for the efficient binding to class I major histocompatibility complex (MHC). Several common gene SNPs (single nucleotide polymorphisms) were associated with the susceptibility to AS, but the presence of other *ERAP1* polymorphisms was supposed to explain the genotype-phenotype correlation.^{1,3,4}

Objectives: The aim of this study is to genotype the *ERAP1* gene whole structure searching for common and additional polymorphisms in two HLA-B*27-negative twins.

Methods: We integrated a bioinformatics and a second level molecular approach in order to characterise *ERAP1* gene. Specific primer pairs were designed for the coverage of all gene regions. Genomic DNA was isolated from the whole blood of two 36 years-old axSpA male twins. They are HLA-B*27-negative (HLA-A*02, HLA-A*32; HLA-B*07; HLA-CW*07). The coexistence of Crohn's disease (CD) was documented in both patients after the initial diagnosis of axSpA. Primer-specific polymerase chain reaction (PCR) was carried out. PCR products were sequenced and bioinformatics tools (BlastN and Mutation Surveyor) were queried for the mutational analysis. Phyre2 on line software was used for predicting the protein tertiary structure.

Results: Molecular characterisation of *ERAP1* gene identified a *de novo* homozygous guanine to adenine substitution at 15132 position of exon 2 nucleotide sequence (NG_027839.1:g.15312G>A). This substitution is a stop-codon variation that directly generates an early premature termination codons (PTC). The 3D model of the protein showed a significant difference of the folding when wild-type and mutant protein were compared. The non-sense transcript could result in the production of a truncated protein, formed by 30 amino acids (NP_001035548.1:p.Trp31Ter) (figure 1).

Abstract THU0004 – Figure 1. The effect of the novel stop-codon variant at DNA, RNA and protein level. The novel substitution generates a PTC in *ERAP1* exon 2, that could be responsible for the production of an aberrant mRNA and of the truncated protein. The protein tertiary structure prediction by Phyre2 software is shown.

Conclusions: A *de novo* stop-codon *ERAP1* variant was identified for the first time in axSpA. We suggest that the PTC-related *ERAP1* protein could contribute to AS risk by affecting the protein role.

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THU0005 WHOLE GENOME LINKAGE AND EXOME SEQUENCING ANALYSES IN TAKAYASU ARTERITIS FAMILIES

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Background: Takayasu arteritis (TA) is an inflammatory large vessel vasculitis affecting mainly aorta and its branches. Inflammation in vessels causes thickening