FRIDAY, 14 JUNE 2019

Genomics, genetic basis of disease and antigen presentation

FRI0001

NEXT GENERATION SEQUENCING ANALYSIS OF AN NLRP3 MUTATION NEGATIVE CAPS COHORT

Sonia Melo Gomes¹, Ebum Omoyinmi¹, Juan Arostegui², Ying Hong¹, Nigel Klein¹, Paul Brogan¹. ¹UCL Great Ormond Street Institute of Child Health, Rheumatology, London, United Kingdom; ²Hospital Clinic, Immunology CBD, Barcelona, Spain

Background: Cryopyrin Associated Periodic Syndromes (CAPS) are caused by autosomal dominant gain of function mutations in the *NLRP3* gene. However, a subset of these patients with typical clinical features and good response to anti-IL-1 treatment, have no mutation in *NLRP3* detected by conventional Sanger DNA sequencing. Somatic mosaicism may account for between 19 to 69% of these, from previous reports. Genetic heterogeneity has also been implicated in patients with CAPS-like phenotypes, with the identification of pathogenic mutations in the *NLRP12*, *PLCG2* and *NLR4* genes (Familial Cold Autoinflammatory Syndrome subsets 2, 3 and 4, respectively).

Objectives: To explore the genetic cause(s) in a paediatric cohort of mutation negative CAPS patients using Next Generation Sequencing (NGS) techniques, by assessing:

1. the rate of somatic *NLRP3* mosaicism, and
2. possible contribution of other/novel genes

Methods: Patients from a single paediatric centre who met the CAPS diagnostic criteria and had no *NLRP3* mutations identified by partial (exons 3, 4 and 6) or total Sanger sequencing, were identified. The NGS approach used in this study was Amplicon-based Deep Sequencing (ADS) for identification of *NLRP3* mosaicism; and Whole Exome Sequencing (WES) for exploration genetic heterogeneity (i.e. other causative mutations). ADS was performed on an Ion Torrent platform using the Ion Torrent PGM HiQ sequencing kit. Resulting sequences were analysed using Torrent Server and in reporter softwares. WES was performed using the Nextera Exome Capture sequencing kit and Hiseq sequencing platforms. Exome data was analysed in the Galaxy web-based suit. The wANNOVER web-based software was used for variant annotation.

Results: We identified a total of 8 patients who met the CAPS clinical diagnostic criteria and had no mutation identified by conventional Sanger sequencing. ASD identified the presence of a somatic mutation in 3 of these patients (37.5%), with an allelic frequency varying from 3.1 to 14.5%. The remaining patients were studied by WES. In Patient 4 a novel *NLRP3* mutation was identified in exon 5, which had been previously missed due to a restricted Sanger approach. Patient 5 had a novel *NOD2* mutation, thus redefining the diagnosis as Blau syndrome. WES findings in the other patients are summarized in the Table.

Patient (gender, age at testing)	CAPS phenotype	NGS	Gene	aa change	Type
1-M, 8y	CINCA	ADS	<i>NLRP3</i>	p.F566L	Mosaic 14.5%
2-M, 8y	MWS	ADS	<i>NLRP3</i>	p.E567K	Mosaic 3.1%
3-M, 1y	MWS	ADS	<i>NLRP3</i>	p.G564D	Mosaic 12.5%
4-M, 2y	CINCA	WES	<i>NLRP3</i>	p.G779V	Heterozygous
5-F, 16y	CINCA-like	WES	<i>NOD2</i>	p.D512Y	Heterozygous
6-M, 14y	MWS-like	WES	<i>IL36RN</i>	p.S113L	Heterozygous (AR)
7-M, 8y	MWS	WES	<i>AP1S3</i>	p.F4C	Heterozygous
8-F, 2y	MWS	WES	-	-	-

Conclusion: In this study, somatic *NLRP3* mutations were identified in 3/8 (37.5%) of our cohort, and 3/8 had variants in 3 other genes, potentially of relevance to the phenotype. In particular, the identification of a *NOD2* mutation and subsequent reclassification of that patient as Blau syndrome, illustrates the limitations of current CAPS diagnostic criteria as well as potential clinical overlap between different autoinflammatory syndromes, reiterating the use of a wider NGS approach. At present it is unclear how the variants found in patients 6 and 7 could modulate the phenotype, therefore in 3 patients a clear genetic cause is yet to be found. **In conclusion**, this study suggests that NGS approaches are superior to conventional sequencing for the molecular diagnosis of CAPS and CAPS like phenotypes since they can detect somatic mosaicism or alternative molecular diagnoses mimicking CAPS.

Disclosure of Interests: Sonia Melo Gomes: None declared, Ebum Omoyinmi: None declared, Juan Arostegui: None declared, Ying Hong:

None declared, Nigel Klein: None declared, Paul Brogan Grant/research support from: SOBI, Novartis, Roche, Novimmune, Chemocentryx, Consultant for: Roche, SOBI, Speakers bureau: SOBI, Roche, Novartis, UCB
DOI: 10.1136/annrheumdis-2019-eular.7906

FRI0002

THE JUVENILE IDIOPATHIC ARTHRITIS-ASSOCIATED IL2RA HAPLOTYPE CONTAINS AN INTRONIC ENHANCER WHOSE FUNCTION IS DIMINISHED BY JIA-ASSOCIATED GENETIC VARIANTS

Kaiyu Jiang¹, Yungki Park², Evan Tarbell², Tao Liu^{2,3}, Jim Jarvis^{1,3}. ¹University at Buffalo, Pediatrics, Buffalo, United States of America; ²University at Buffalo, Biochemistry, Buffalo, United States of America; ³University at Buffalo, Genetics, Genomics, and Bioinformatics Program, Buffalo, United States of America

Background: *IL2RA* has been identified as a JIA-associated risk locus using both candidate gene and genetic fine mapping approaches. However, numerous gene expression studies comparing children with active JIA to healthy control children have failed to identify *IL2RA* as a differentially expressed gene. Furthermore, the SNPs used to identify *IL2RA* lie within the first intron of the *IL2RA* gene, not its promoter or coding regions. The risk haplotype is marked by prominent H3K4me1/H3K27ac histone marks, suggesting that genetic risk may be mediated through enhancer function.

Objectives: To confirm whether the *IL2RA* region is a functional enhancer and whether the enhancer operates in lymphoid cells, myeloid cells, or both. We also examined the effects of genetic variants on enhancer function at the *IL2RA* locus.

Methods: We used the pGL3 reporter system to query enhancer effects across a 1,000 bp region within the first intron of the *IL2RA* gene marked by H3K4me1 and H3K27ac histone marks in both CD4+ T cells and neutrophils. This region is within the linkage disequilibrium block that includes the SNPs used on both candidate gene and genetic fine mapping studies that identified *IL2RA* as a JIA risk locus. The pGL3 vector contains a minimal SV40 promoter that is, by itself, inefficient at driving luciferase function. We tiled three 500 bp constructs across the region of interest and cloned these constructs into pGL3 vectors, which were then transfected into Jurkat T cells and myeloid HL60. Cells were incubated for 24 hr, after which luciferase production was assessed using standard methods. We repeated these same experiments using constructs containing genetic variants (rs117119468 C->T and rs12722502 C->T) that we identified in children with JIA using WGS and are not annotated in the 1000 Genomes Project SNP database.

Results: Within the H3K4me1/H3K27ac-marked region within the first intron of *IL2RA*, we observed enhancer activity across a 657 bp region from chr10:6092661 to chr10:6093317 in both Jurkat T cells and HL60 myeloid cells. The reporter constructs enhanced luciferase activity by 5-8 fold in Jurkat T cells (compared with a control vector that contained only the minimal SV40 promoter and the luciferase gene) and 4-6 fold in HL60 cells. A construct carrying a sequence within the first intron of the *IL2RA* gene that was not marked by the H3K4me1/H2K27ac histone signature showed no enhancement of luciferase expression compared to background. Constructs containing the JIA-associated rs117119468 C->T allele abolished the enhancer activity within the *IL2RA* locus, while the construct containing the rs12722502 C->T allele reduced luciferase activity by 30%.

Conclusion: The JIA-associated risk locus, *IL2RA*, contains an intronic enhancer that is active in both lymphoid and myeloid cells. JIA-associated genetic variants identified by WGS attenuate or abolish enhancer activity at this locus. These findings demonstrate the importance of assessing the non-coding functions of JIA risk loci, even where the coding function a specific gene might be plausibly implicated. Our findings also underline the potential importance of rare genetic variants, which may have stronger biological effects than GWAS SNPs, in complex traits like JIA.

Disclosure of Interests: None declared

DOI: 10.1136/annrheumdis-2019-eular.2086

FRI0003

THE FUNCTION OF IRF7 SNP ON THE PRODUCTION OF TYPE I INTERFERON

Ye Zhizhong, Yin Zhihua, Zhou Haibo. Shenzhen Futian Hospital for Rheumatic Diseases, Shenzhen, China

Background: Type I IFN plays a significant role in the pathogenesis of Systemic Lupus Erythmatosus (SLE). IRF7 is a critical transcriptional factor for the production of type I interferons (IFN).

Objectives: To explore potential mechanisms for the SLE related IRF7 SNP on type I IFN production, which might indicate new theoretical basis for genetic therapeutic strategy.

Methods: We constructed different genotypes of SLE relevant SNP cell lines by Crispr/Cas9 technology, and confirmed them by sequencing. After TLR-ligand stimulation, the mRNA expression of IFN α was detected by real-time qPCR. The levels of IFN α , TNF α and IL-6 were detected by ELISA kit. The protein expression of IRF7/p-IRF7 was detected by western blot. The binding ability of endogenous IRF7 with type I IFN promoter was studied by chromatin immunoprecipitation.

Results: We got IRF7 SNP rs1131665 GG/AA cell lines by gene editing technique. After TLR-ligand stimulation, IRF7 SNP rs1131665 AA cell line showed higher IFN α expression than GG, which indicated statistical significance. IRF7 SNP rs1131665 AA cell line showed higher p-IRF7 expression than GG detected with western blot. Endogenous IRF7 in IRF7 SNP rs1131665 AA cell line showed stronger binding ability with IFN α promoter than GG, which indicated statistical significance.

Conclusion: The missense variant of G to A in IRF7 SNP rs1131665, increased the production of type I IFN, which indicated that IRF7 G to A missense variant increased the susceptibility of type I IFN associated diseases.

REFERENCES:

- Lin LH, Ling P, Liu MF. The potential role of interferon-regulatory factor 7 among Taiwanese patients with systemic lupus erythematosus. *J Rheumatol*. 2011;38(9):1914-1919.
- Xu WD, Zhang YJ, Xu K, et al. IRF7, a functional factor associates with systemic lupus erythematosus. *Cytokine*. 2012;58(3):317-320.
- Miyagawa F, Tagaya Y, Ozato K, et al. Essential Requirement for IFN Regulatory Factor 7 in Autoantibody Production but Not Development of Nephritis in Murine Lupus. *J Immunol*. 2016;197(6):2167-2176.

Disclosure of Interests: None declared

DOI: 10.1136/annrheumdis-2019-eular.2540

FRI0004

CHROMATIN INTERACTIONS IN NOVEL CELL TYPES REVEAL PARK7 AND ERFF1 AS PUTATIVE CAUSAL GENES IN THE SUSCEPTIBILITY TO PSORIATIC ARTHRITIS

Oliver Gough¹, Helen Ray-Jones², Paul Martin¹, John Bowes¹, Antony Adamson³, Anne Barton¹, Richard B. Warren⁴, Stephen Eyre¹. ¹Versus Arthritis Centre for Genetics and Genomics, School of Biological Sciences, Faculty of Biology, Medicine and Health, The University of Manchester, United Kingdom, Manchester, United Kingdom; ²NIHR Manchester Biomedical research Centre, Manchester University Foundation Trust., Manchester, United Kingdom; ³Faculty of Biology, Medicine and Health University of Manchester, Manchester, United Kingdom; ⁴Dermatology Centre, Salford Royal NHS Foundation Trust, The University of Manchester, Manchester, United Kingdom

Background: One of the major tasks post-GWAS is to link disease associated single nucleotide polymorphisms (SNPs) to their causal genes. Unexpectedly the vast majority of disease associated SNPs lay outside protein coding regions of the genome, often in gene regulatory regions, known as enhancers. These enhancers operate by physically interacting with their target genes, often over long distance, and often skipping genes. We, and others, have demonstrated how these enhancer interactions can change with different cell type and stimulatory conditions. In psoriatic arthritis, as with all complex diseases, there are a large number of genetic loci implicated in susceptibility to disease, but relatively few of these regions have confirmed causal genes.

Objectives: Map disease associated enhancers to potential causal genes through physical chromatin interactions in relevant, novel cell types.

Methods: We used Capture HiC (CHI-C) technology to map enhancer to promoter interactions in both a keratinocyte cell line (HaCaT) and a T cell line (MyLa) in biological duplicate. We generated Hi-C libraries and enriched them for autoimmune-associated GWAS loci using RNA baits. The resultant CHI-C libraries underwent high-throughput sequencing generating 75 bp paired-ends. Significant interactions were detected using CHiCAGO.

Results: A region on chromosome 1p36 demonstrated markedly differential interactions between the T cell line (MyLa) and the skin cell line (HaCaT). Here the enhancer region containing SNPs associated with PsA demonstrated a strong interaction with both the PARK7 and ERFF1 genes in HaCaT cells, absent in the MyLa cell line (fig 1). These interactions were contained within a functionally relevant topological activation domain (TAD) boundary, enriched for enhancer to promoter links. Bioinformatic

analysis demonstrated how a SNP highly correlated with the PsA lead variant is found within an open, active region of chromatin in the HaCaT cell line, shifting the priority from the (statistical) lead variant (rs11121129) to rs11121131 – demonstrating the value of this type of analysis (fig 2).

Conclusion: A region associated with PsA on chromosome 1p36, previously annotated with the TNFSF9/ERRF1/SLC45A1 gene, makes robust interactions with the PARK7 and ERFF1 genes, both strong candidates for causality in disease. An associated variant, within a cell type relevant enhancer, is currently being targeted with genome editing technology (CRISPR) to assess its potential for causality and possible mechanism.

Fig1

Fig2

Disclosure of Interests: Oliver Gough: None declared, Helen Ray-Jones: None declared, Paul Martin: None declared, John Bowes: None declared, Antony Adamson: None declared, Anne Barton: None declared, Richard B. Warren Grant/research support from: AbbVie, Almirall, Amgen, Celgene, Janssen, Lilly, LEO, Novartis, Pfizer, UCB Pharma, Consultant for: AbbVie, Almirall, Amgen, Boehringer-Ingelheim, Celgene, Janssen, LEO, Lilly, Novartis, Pfizer, Sanofi, UCB, Xenoport, Stephen Eyre: None declared

DOI: 10.1136/annrheumdis-2019-eular.6272

FRI0005

CHROMATIN CONTACTS AND TRANSCRIPTOMICS IN CD4+ T-CELLS REVEAL GENES IMPLICATED IN RHEUMATOID ARTHRITIS

Vasanthi Priyadarshini Gaddi¹, Amanda McGovern¹, James Ding¹, Nadia Iqbal¹, Chenfu Shi¹, Theoni Katopodi¹, Marialbert Acosta-Herrera², Sofia Vargas², Kate Duffus¹, Annie Yarwood¹, Enrique Raya³, Javier Martin Ibanez², Paul Martin¹, Gisela Orozco¹. ¹University of Manchester, Manchester, United Kingdom; ²Instituto de Parasitología y Biomedicina López-Neyra, CSIC, Granada, Spain; ³Hospital Clínico de Granada, Department of Rheumatology, Granada, Spain

Background: Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by inflammation of synovial joints. RA is a clinically heterogeneous disease and treatment response varies between individuals. The pathogenesis of RA is not completely understood yet, but it has been