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Genomics, genetic basis of disease and antigen presentation

FRI0001

NEXT GENERATION SEQUENCING ANALYSIS OF AN NLRP3 MUTATION NEGATIVE CAPS COHORT

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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.

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FRI0002

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

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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.

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FRI0003

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

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