OPTIMISATION OF TECHNIQUES TO BE USED IN THE ANALYSIS OF TYPE 1 INTERFERONOPATHY DUE TO STAT2 GAIN-OF-FUNCTION MUTATION INDUCED IN MICE.

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INTRODUCTION

Type I interferon anti-viral immunity signals through a STAT2:STAT1:IRF9 complex (termed ISGF3) which modulates the expression of interferon stimulated genes (ISGs)[1]. A negative regulator of STAT2, USP18 blocks signalling through a STAT2 interaction[2], ceasing the transcriptional response. A variant identified in patients (R148W) impairs the ability of STAT2 to interact with USP18, therefore prolonging the activity and upregulation of ISGs. This mutation was found in two siblings, though due to clinical considerations particular studies could not be taken on human samples. As a result, the STAT2 mutation has been generated in C57BL/6x129sv cross mice. As a result, techniques used on mouse samples will need to undergo optimisation.

METHOD

- The ear notches from mice were genotyped for the STAT2^{R147W} mutation using PCR and Sanger sequencing.
- Lysates were prepared from mouse 3T3 fibroblast-like cells which were stimulated with increasing concentrations of IFNa.
- Western blots were carried out on the lysates, with antibodies of interest and then imaged using chemiluminescence. Greater luminescence corresponds to higher concentrations of target protein.

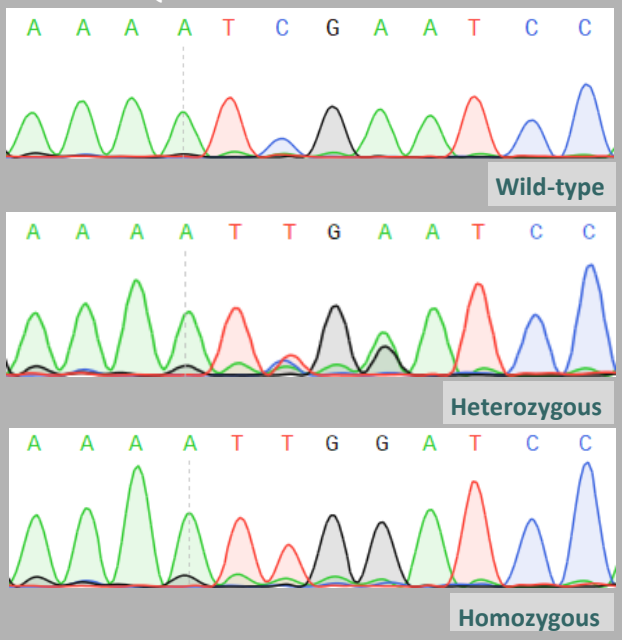
CONCLUSION

As shown by the western blots, only a select number of antibodies were reactive to the target protein. STAT1, pSTAT1, STAT2, ISG15 and both housekeeping proteins (GAPDH & a-tubulin) bond to corresponding mouse proteins. Other antibody targets were less successful and therefore more validation work will need to take place to find alternative antibodies or means of assaying target proteins.

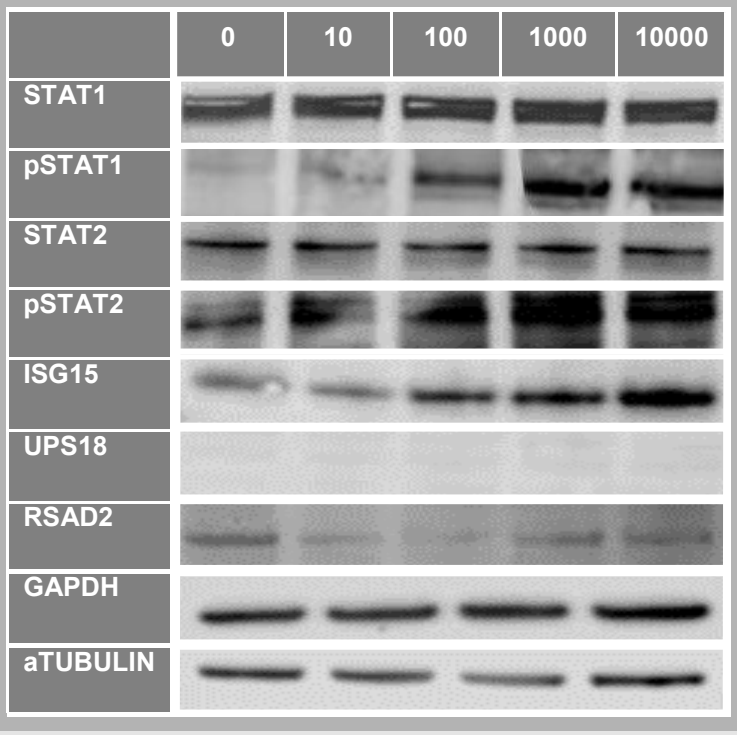
Once antibodies have been validated, lysates can be prepared from tissue of the confirmed WT, HET and HOM mice. From here the molecular consequences of dysregulated IFN signalling can be probed, using techniques such as RT-qPCR to quantify the levels of ISG upregulation in tissues of phenotypically different mice or immunohistochemistry to observe at tissue pathology.

RESULTS

SANGER SEQUENCING FROM MOUSE GENOTYPING



WESTERN BLOTS FOR ISG ANTIBODIES



References: 1. Rodero MP, Crow YJ. Type I interferon–mediated monogenic autoinflammation: The type I interferonopathies, a conceptual overview. The Journal of Experimental Medicine. 2016;213(12):2527-38.

2. Arimoto KI, Lochte S, Stoner SA, Burkart C, Zhang Y, Miyauchi S, et al. STAT2 is an essential adaptor in USP18-mediated suppression of type I interferon signaling. Nat Struct Mol Biol. 2017;24(3):279-89.

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