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Recurrent neonatal herpes simplex virus infection in a patient with IRF7/UNC93B1 mutation

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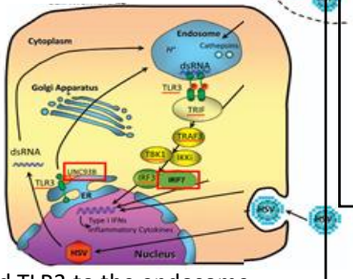
Clinical Introduction

- Neonatal herpes simplex virus (HSV) infection is a devastating disease with high mortality particularly when disseminated. The incidence of HSV in live births is 50 per 100k, with mortality on the rise at 1.68 per 100k cases.
- In adults and children, genetic variants in genes encoding proteins in the **toll-like receptor 3 (TLR3) signaling cascade** suggest a genetic susceptibility to herpes simplex encephalitis (HSE).
- TLR3 signaling is initiated by dsRNA, the signal goes through TRIF to the activation of transcription factors IRF3 and **IRF7** to the nucleus to drive production of IFN cytokines. **UNC93B1** traffics newly synthesized TLR3 to the endosome.



Objectives and Methods

OBJECTIVES: 1) Reveal new deleterious mutations in neonates diagnosed with HSV by exome sequencing. 2) Perform functional studies with new mutants revealed by exome sequencing.

Methods:

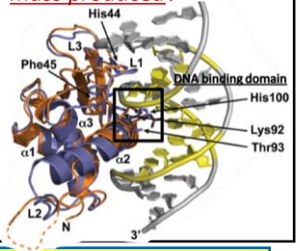
- DNA from blood ---> Exome Sequencing
- Mutations found during analysis ---> Generate plasmid mutations
- Functional assays with mutations transfected into THP

UNC93B1 and IRF7 mutant models

We identified deleterious variants in our proband: interferon regulatory factor 7 (**IRF7**), downstream of endosomal TLR3, and UNC-93 homolog B1 (**UNC93B1**), which regulates endosomal TLR3 trafficking and stability.

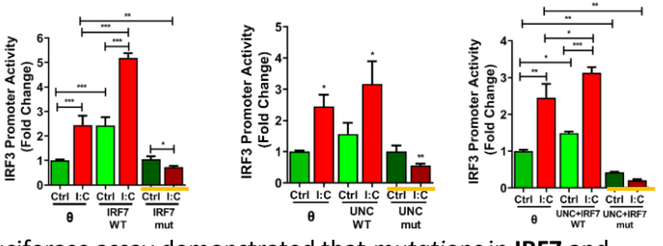
UNC93B1 mutant leads to *Pro404Ser* in the TLR binding domain, *will it still traffic TLR3*?

IRF7 mutant leads *Arg100Pro* in DNA binding domain, *will IFNs still be mass produced*?



UNC93B1 and IRF7 mutants suppress luciferase activity

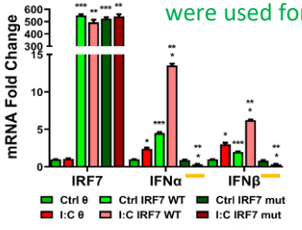
METHODS: THP, human monocyte cells, were transfected overnight and 3 days later were incubated with 1ug/mL poly I:C for 24hr with the cellular supernatants used for the assay.
The luciferase assay measures the amounts of transcription factor IRF3 activation, IRF3 is only activated when TLR3 is active.

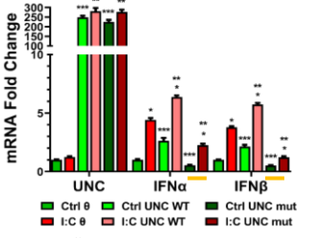


Luciferase assay demonstrated that *mutations* in **IRF7** and **UNC93B1** dramatically reduced TLR3 pathway activity in control and with poly(I:C), transfected separately or together, compared to WT and empty vector.

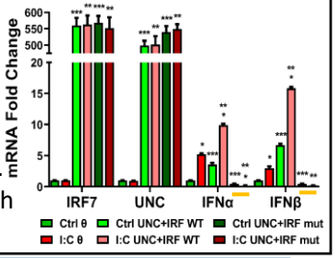
UNC93B1 and IRF7 mutants inhibit IFN production

METHODS: The cells were transfected overnight and 3 days later they were incubated with 1ug/mL poly I:C for 24hr, then the cells were used for Realtime PCR.





PCR results show that WT IRF7 and UNC93B1 enhanced expression of interferon (IFN) α and IFN β in baseline and in response to poly(I:C) while the *mutants* repressed it, whether transfected separately or together. Transfection was also achieved with levels of UNC and IRF7 shown.



Conclusions

- We identified two genetic variants, IRF7(Arg100Pro) and UNC93B1 (Pro404Ser), which dramatically affect TLR3 downstream gene expression and TLR3 pathway activity in baseline and in response to poly(I:C).
- Presence of both mutations, as identified in proband, abolishes basal and poly IC-stimulated IRF3 activation and gene expression.
- This study indicates neonatal HSV may be a phenotype for immunodeficiency arising from variants in viral-sensing immune genes, particularly the TLR3 pathway.