A Novel Animal Model to Study 21-Hydroxylase Deficiency *in vivo*

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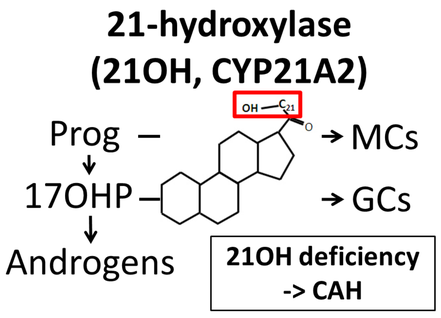
OBJECTIVES

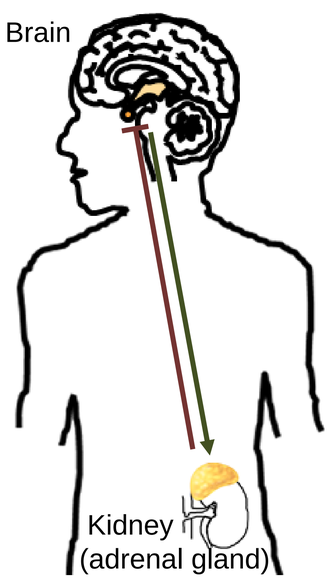
21-hydroxylase deficiency (21OHD) resulting in imbalances in steroid hormones and a dysregulated hypothalamus pituitary adrenal (HPA) axis is the major cause of the disease congenital adrenal hyperplasia (CAH). Several findings highlight the **need for new *in vivo* models to study 21-hydroxylase deficiency:**

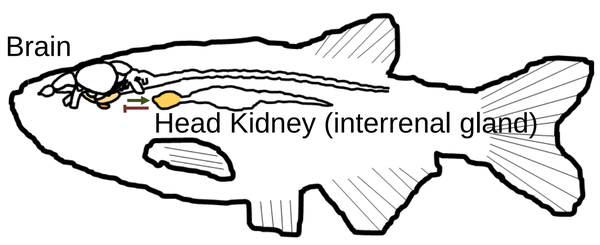


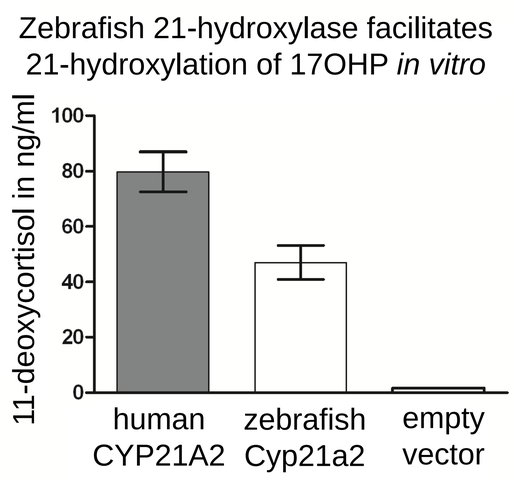
- *In vitro* studies on CAH mutations do not always correlate with patient phenotypes
- 21OHD is difficult to study in mice -> mutants are not viable
- Incomplete understanding of systemic consequences of 21OHD

Aim: Zebrafish model for 21-hydroxylase deficiency

21-hydroxylase and the Hypothalamus Pituitary Adrenal axis are conserved in zebrafish



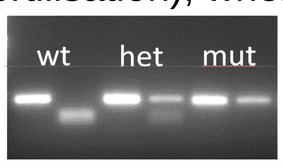




Key genes		
Organ	**Human**	**Zebrafish (paralogue)**
Hypothalamus	***CRH***	***crha (crhb)***
Pituitary	***POMC***	***pomca (pomcb)***
Adrenal Gland	***FDX1***	***fdx1b (fdx1)***
Adrenal Gland	***GR/NR3C1***	***gr*/nr3c1**
Adrenal Gland	***CYP11A1***	***cyp11a2 (cyp11a1)***
Adrenal Gland	***HSD3B2***	***hsd3b1 (hsd3b2)***
Adrenal Gland	***CYP17A1***	***cyp17a2 (cyp17a1)***
Adrenal Gland	***CYP11B1***	***cyp11c1***
Adrenal Gland	***CYP21A2***	***cyp21a2***

METHODS

Cyp21a2 mutants were generated by TALEN-mediated mutagenesis. The target region contained a BseYI restriction site, used for genotyping. The mutant phenotype was characterised at 120 hpf (hours post fertilisation), when the HPI axis is functional.



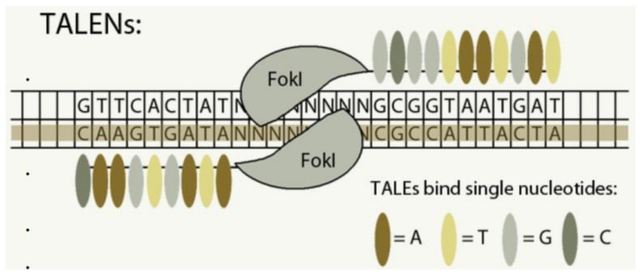
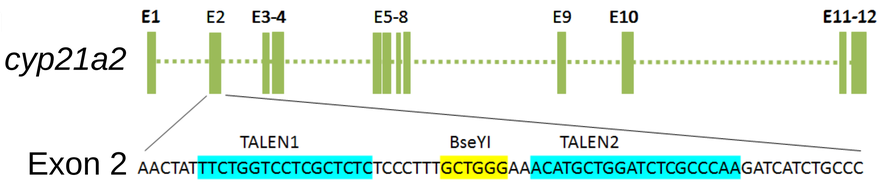


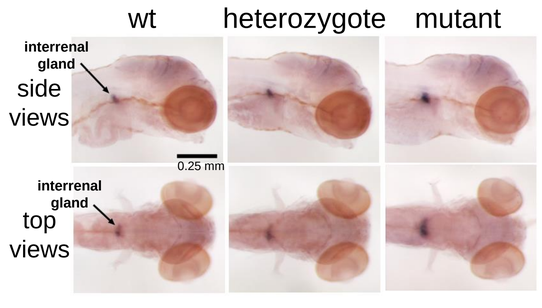
Image taken from Xenbase.org



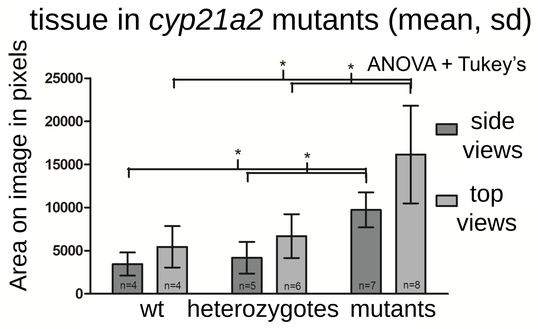
Zebrafish *cyp21a2* mutants show hallmarks of 21OHD

***Cyp21a2* mutants have enlarged interrenal tissue (zebrafish adrenal counterpart)**

RNA *in situ* hybridisation against *cyp17a2* in *cyp21a2* mutants

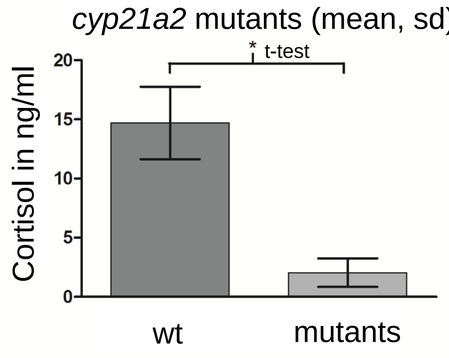


Increased size of *cyp17a2*+ interrenal tissue in *cyp21a2* mutants (mean, sd)

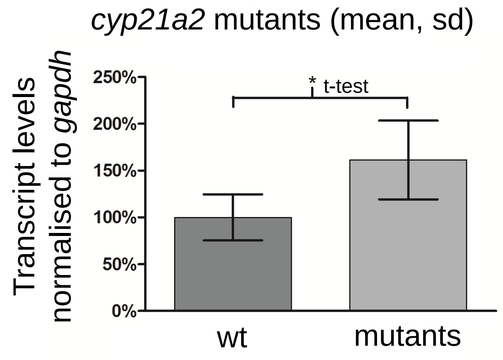


Impaired cortisol synthesis and overstimulation of the HPI axis in *cyp21a2* mutants

Reduced cortisol levels in *cyp21a2* mutants (mean, sd)

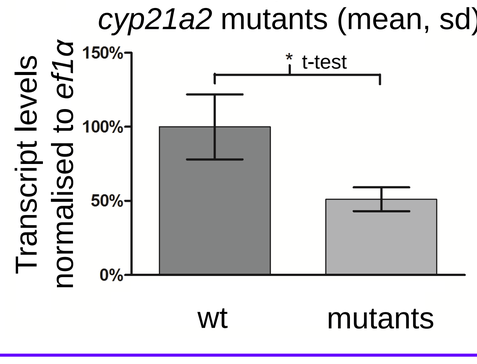


Increased *pomca* levels in *cyp21a2* mutants (mean, sd)

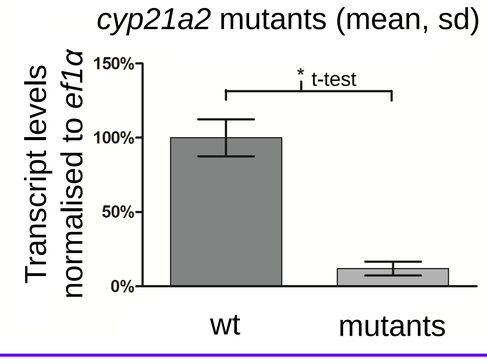


Reduced expression of glucocorticoid response genes in *cyp21a2* mutants

Decreased *pck1* levels in *cyp21a2* mutants (mean, sd)



Decreased *fkbp5* levels in *cyp21a2* mutants (mean, sd)



CONCLUSIONS

Zebrafish *cyp21a2* mutants are a promising model for 21OHD

1. 21-hydroxylase is conserved in zebrafish
2. Zebrafish *cyp21a2* mutants have impaired GC signalling
3. Zebrafish *cyp21a2* mutants have dysregulated HPI axis

ACKNOWLEDGEMENTS

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I declare that I have no potential conflict of interest

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Adrenal

Andreas Zaucker

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