Glucocorticoid deficiency due to disruption of mitochondrial steroidogenesis leads to dysregulation of antioxidant pathways and nucleotide biosynthesis

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Aim

The role of glucocorticoids (GCs) as regulators of systemic homeostasis has been mainly studied by using synthetic GCs or in states of GC excess. Thus, the pathophysiologic consequences of cortisol deficiency on metabolic and biosynthesis pathways remain largely elusive.

Here we make use of a recently published ferredoxin (fdx1b) null-allele zebrafish line with massively decreased cortisol concentrations and a severely impaired stress response in order to define the global pathophysiologic response *in vivo* to glucocorticoid deficiency.

Summary

- Systemic profiling of the fdx1b null-allele zebrafish line was performed by a combination of RNA-sequencing and metabolomics analysis.
- An enrichment of genes in the fdx1b null-allele zebrafish line linked with pathways altered in metabolic disease was observed.
- This includes significant alteration in expression of genes and metabolites acting in pathways of energy and biomolecule synthesis (e.g., amino acids), and antioxidant pathways.

Conclusion

We provide *in vivo* evidence on the global pathophysiologic effects of GC deficiency, which can be vital for improving the understanding of the pathophysiology of adrenal insufficiency in humans.

Introduction

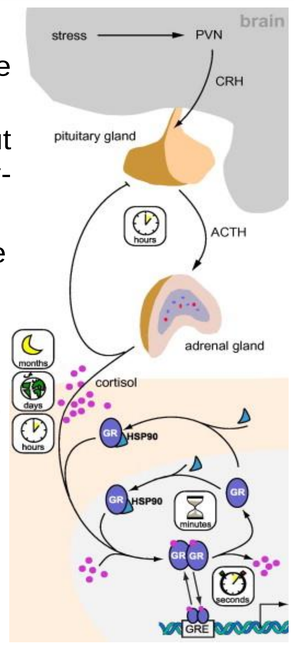
GCs are important steroid hormones for the regulation of physiology such as metabolism.

Their release occurs in a circadian fashion, but is also regulated by the Hypothalamus-Pituitary-Adrenal gland (HPA) axis upon stress.

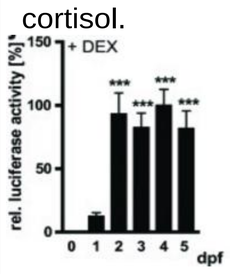
Zebrafish is a well-established vertebrate model for studying whole organism biology.

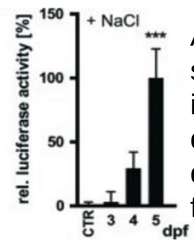
The zebrafish is day active and has a conserved endocrine system similar to human.

The main GC in both human and fish is cortisol.



Dickmeis T, Weger BD, Weger M, *Mol Cell Endocrinol*, 2013



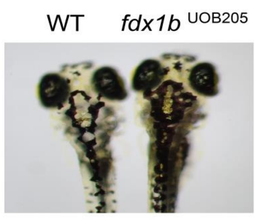


A functional stress response in zebrafish is developed 4-5 days post-fertilization (dpf)

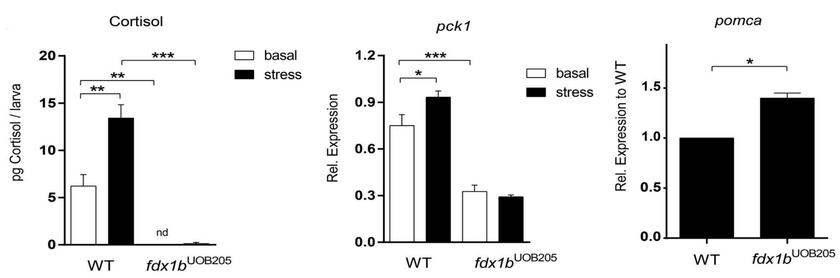
Weger BD, Weger M et al, *ACS Chem Biol,* 2012

Results

fdx1b null-allele zebrafish larvae are impaired in cortisol synthesis, cortisol regulated gene expression and in their stress response



Griffin et al, *Endocrinology*, 2016

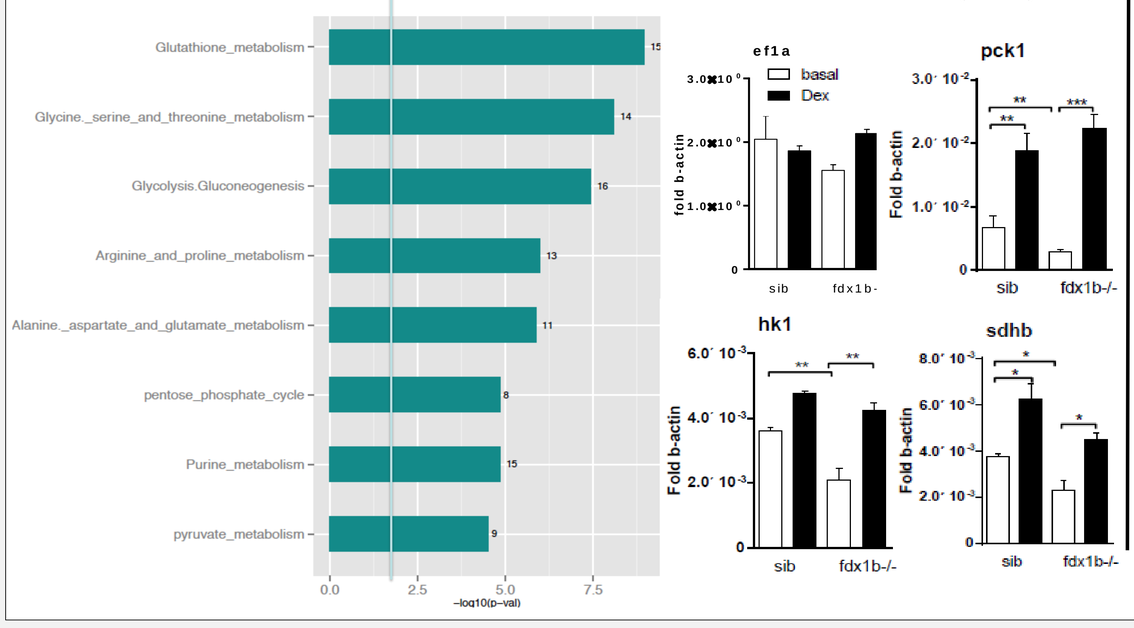


Enrichment of genes linked to metabolism and metabolic disease

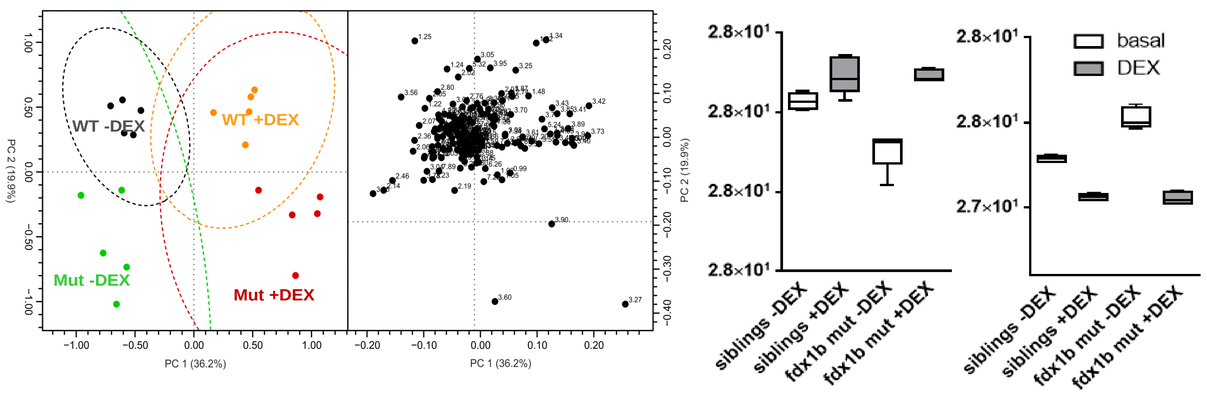
Pathway analysis reveals genes linked with metabolic disease

Top Diseases and Bio Functions		
Diseases and Disorders		
Name	p-value	#Molecules
Metabolic Disease	1.17E-03 - 1.77E-11	166
Neurological Disease	1.26E-03 - 3.14E-11	228
Psychological Disorders	1.01E-03 - 3.14E-11	180
Cardiovascular Disease	1.23E-03 - 5.10E-09	89
Inflammatory Response	1.13E-03 - 2.57E-08	144

Genes involved in energy and biomolecule synthesis are altered in cortisol deficient fdx1b mutants, but can be rescued with dexamethasone (DEX).

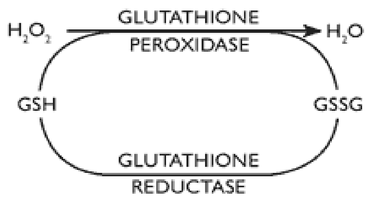


An enrichment of oxidative stress responsive genes can be observed in the cortisol deficient fdx1b mutant



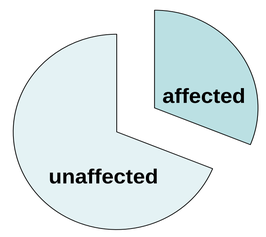
Untargeted NMR analysis shows differences in the metabolomes between fdx1b mutants and wild-type siblings. Some of the altered metabolites can be rescued in the fdx1b mutants with DEX.

Alterations in oxidative stress levels in cortisol deficient fdx1b mutants



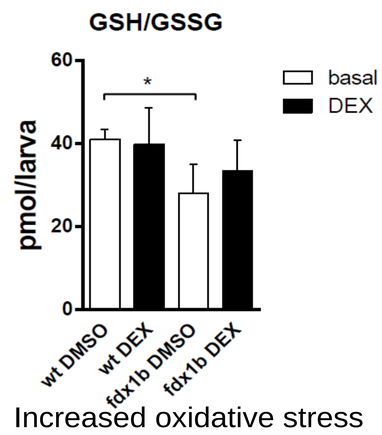
Oxidative stress can be linked to pathogenesis. The GSH:GSSG ratio is a marker for oxidative stress.

Oxidative stress responsive genes



p-value = 6.10E-05

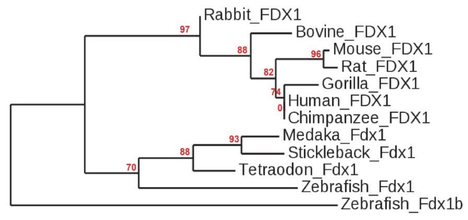
Fdx1b mutants reveal an enrichment of genes responsive to oxidative stress.



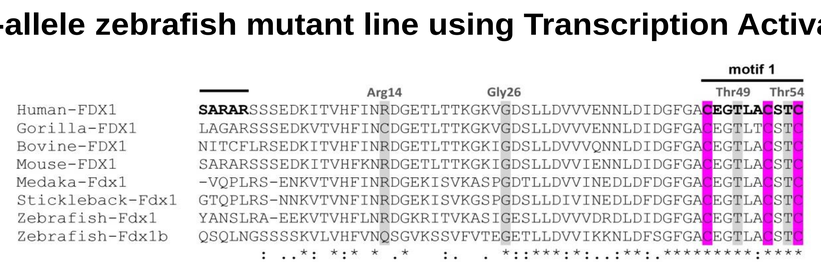
Increased oxidative stress in fdx1b mutants, which can be rescued with DEX.

Material and Methods

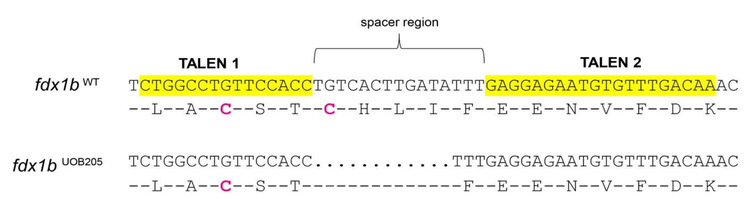
Establishing a fdx1 null-allele zebrafish mutant line using Transcription Activator-like Effector Nucleases (TALENs)



From the duplicated zebrafish fdx1 genes (fdx1, fdx1b), fdx1b is facilitating cortisol synthesis.



Fdx1b binding TALEN sites target the conserved motif 1 including cysteine residues for Fe/S binding.



Generation of an allele (fdx1b^{UOB205}) with a 12 bp in-frame deletion removing a conserved cysteine in motif 1.

55ESPE 1--2-RFC

Adrenal

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