



Forschungsinstitut
für Nutztierbiologie

A substitution in the porcine glucocorticoid receptor changes gene expression in the brain and consequently behavior

E. Murani,

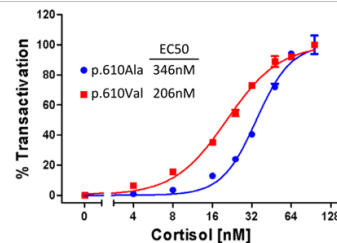
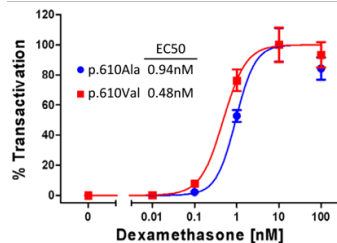
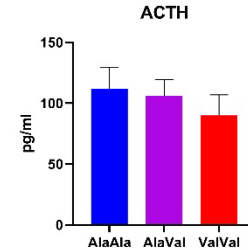
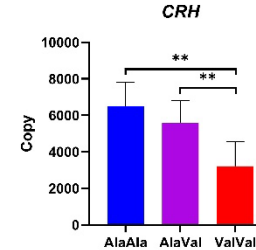
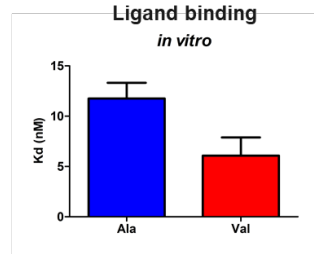
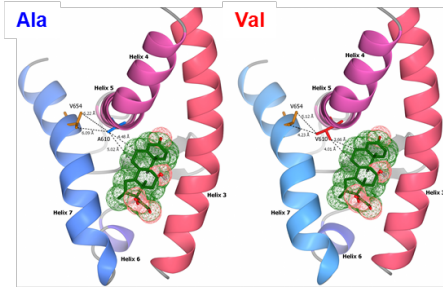
N. Trakooljul, A. Krause, A. Eggert, E. Kanitz, K. Wimmers

Background – Function of GR_{Ala610Val}

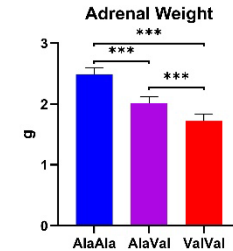
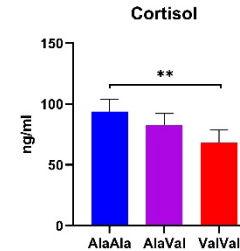


GR_{Ala610Val} - a substitution in Helix 5 of the GR ligand binding domain

- enhances GC-responsiveness of GR
- triggers compensatory downregulation of the HPA axis



Reyer et al. 2016
PMID: 27736993



Murani et al. 2016
PMID: 27440422

Aims



- To explore mechanisms underlying the effect of GR_{Ala610Val} on HPA axis and thus provide insights into its feedback-regulation
 - GR is a ligand-activated transcription factor => investigate transcriptome signature of GR_{Ala610Val}
- To link the changes in gene expression to phenotypic manifestation/behavior



Experiment – Transcriptome signature of GR_{Ala610Val}



- A cohort of 48 piglets (7-weeks old) treated and sampled 3h post treatment
 - Control group - Saline, 10 AlaAla (5 ♀, 5 ♂) and 10 ValVal (5 ♀, 5 ♂)
 - D10 group - 10µg/kg Dexamethasone, 8 AlaAla (4 ♀, 4 ♂) and 8 ValVal (4 ♀, 4 ♂)
 - D60 group - 60µg/kg Dexamethasone, 6 AlaAla (4 ♀, 4 ♂) and 6 ValVal (4 ♀, 4 ♂)
- Bulk mRNA-Sequencing (40 Mio pair-end reads)
 - C group: Hypothalamus (Hyp), Hippocampus (Hip), Amygdala (Amy), Adrenal Gland (AG)
 - D10, D60: Hypothalamus, Hippocampus



Murani et al. 2019
PMID: 31249595

Murani et al. 2022
PMID: 33853082

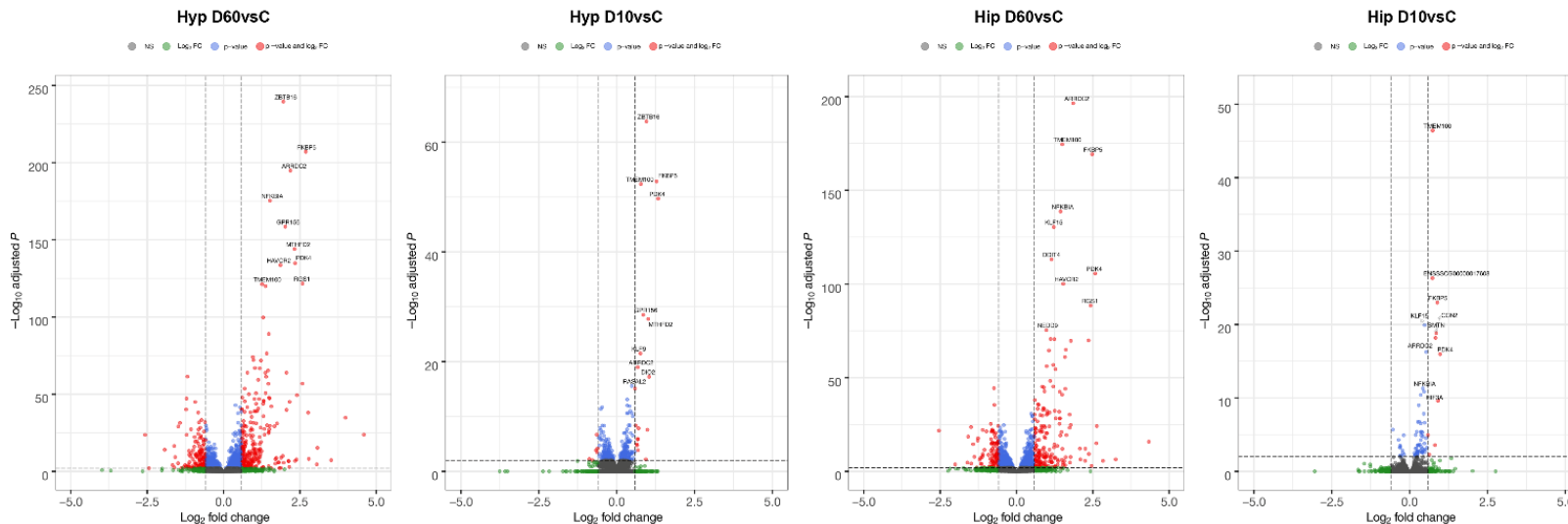


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Response to DEX treatment

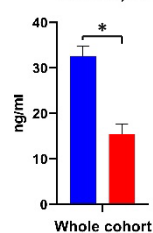


- Both Dexamethasone doses induced transcriptome changes

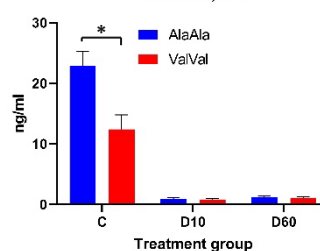


Murani et al. 2022
PMID: 33853082

Cortisol, 0h



Cortisol, 3h



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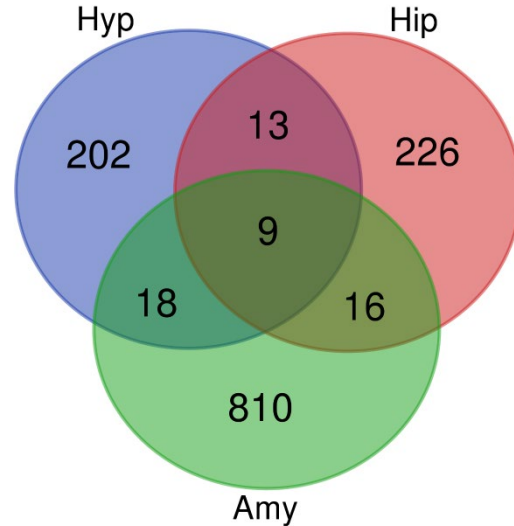
Overall results - brain



- Number of differentially expressed genes ($p < 0.05$):

- C group:**

- Hyp: 242
- Hip: 264
- Amy: 853



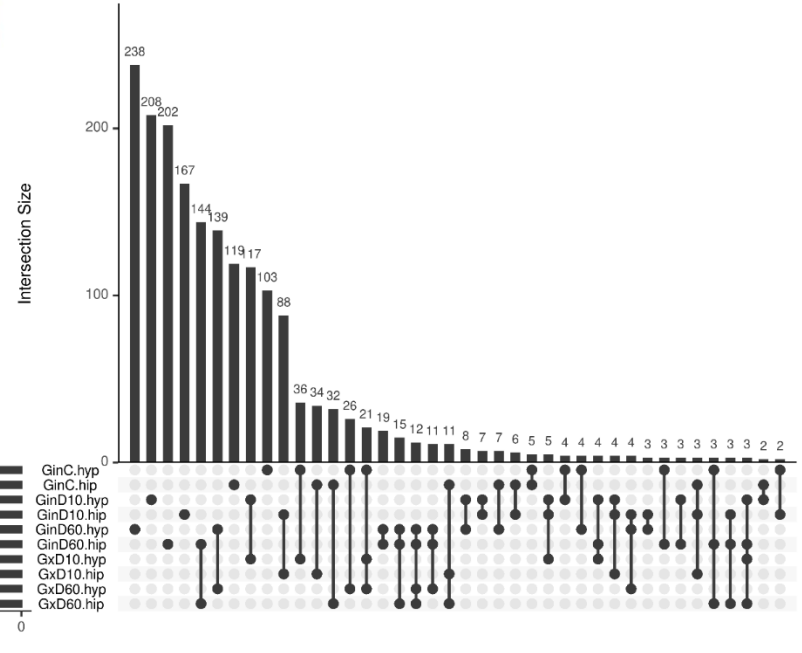
- D10 group:**

- Hyp: 438
- Hip: 330

- D60 group:**

- Hyp: 492

• Hip: 470



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Common differentially expressed genes



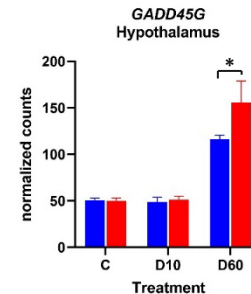
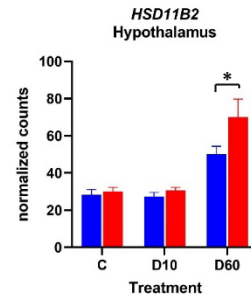
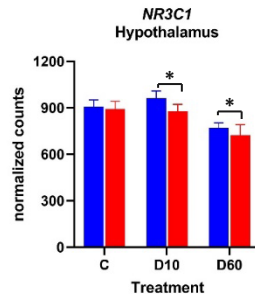
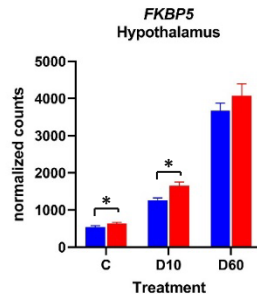
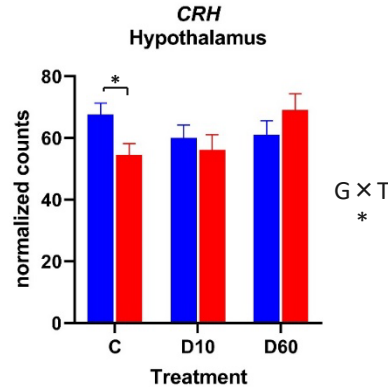
DE Gene	Hyp	Hip	Amy
	fold Val/Ala	fold Val/Ala	fold Val/Ala
<i>PCDHB7</i>	5.1	4.3	5.2
protocadherin beta-17-like	1.5	1.9	1.9
protocadherin beta-4-like	-1.8	-2.0	-2.2
protocadherin gamma-A2-like	1.3	1.5	1.4
<i>FKBP5</i>	1.15	1.16	1.27
<i>ANXA6</i>	1.12	1.14	1.08
<i>ARHGEF37</i>	-1.21	-1.26	-1.16
<i>SMIM19</i>	-1.18	-1.15	-1.13
<i>ENSSSCG00000055564</i> (lncRNA)	2.4	2.5	2.3



Impact of GR_{Ala610Val} on HPA-axis regulation



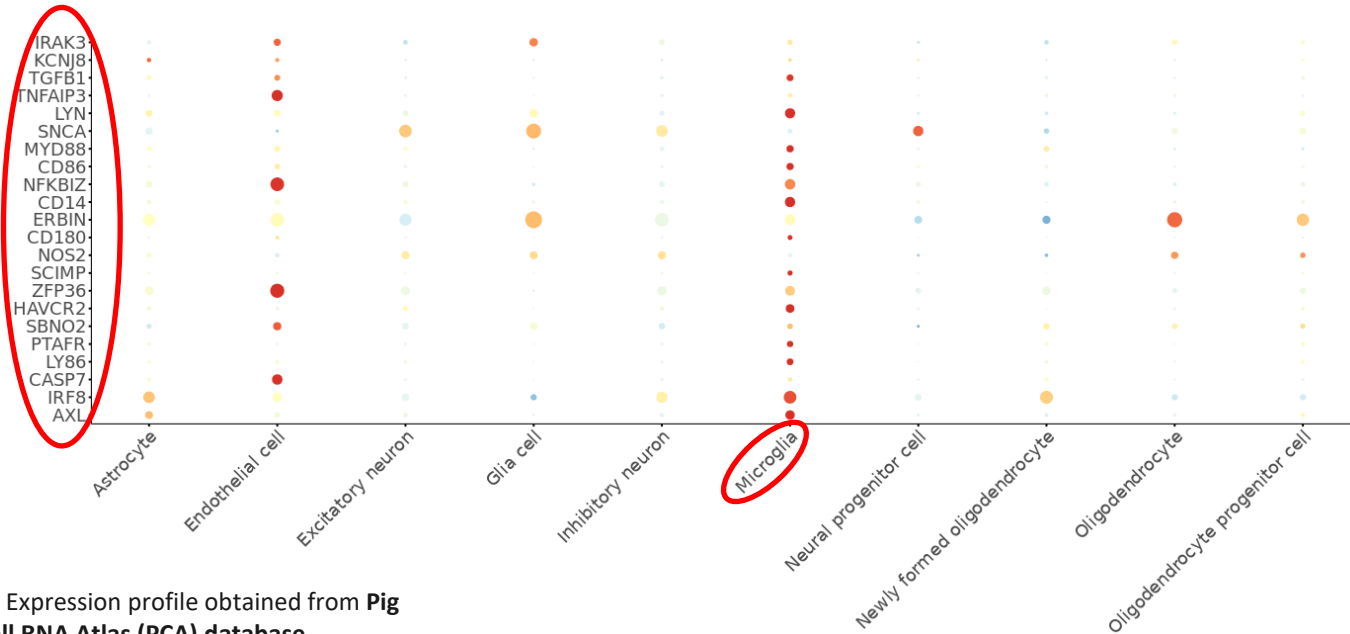
- DEX-application reverses the effect of GR_{Ala610Val} on *CRH* expression:
is this counter-compensatory reaction to keep HPA axis balance?



DE genes in the amygdala are enriched for immune response



- In the amygdala, pro-inflammatory genes (e.g. *CD14*, *MYD88*) are up-regulated by the Val variant at baseline (Control)



Cell Type Expression profile obtained from **Pig Single Cell RNA Atlas (PCA) database**
<https://dreamapp.biomed.au.dk/pigatlas/>

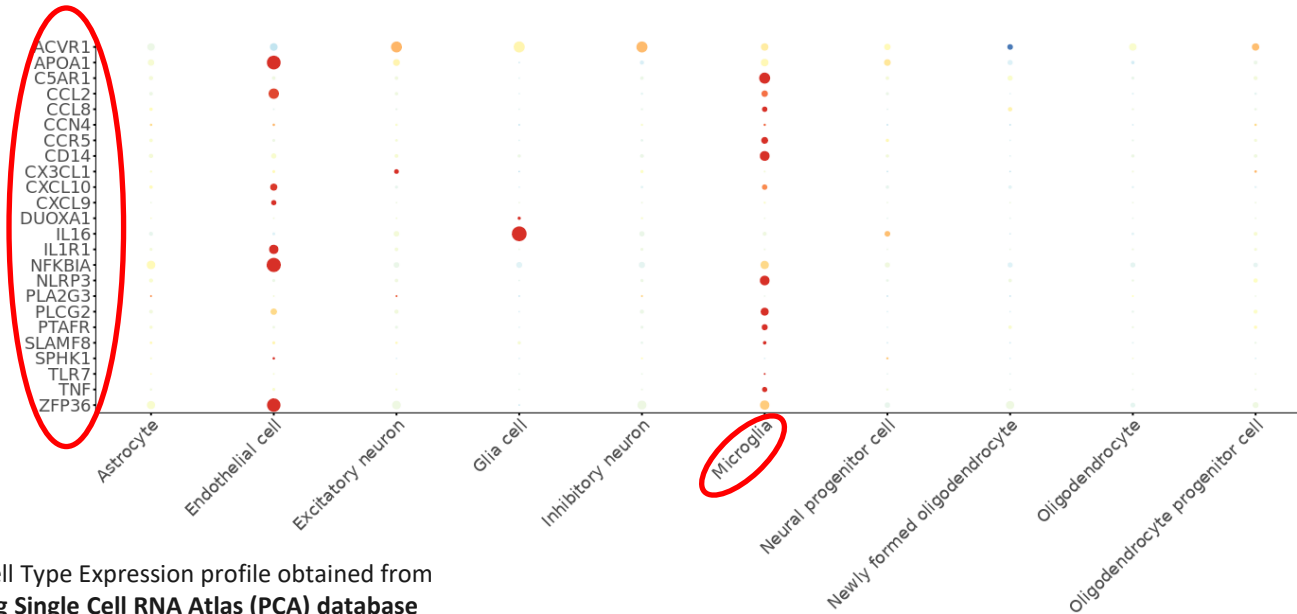


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DE genes in D60 are enriched for immune response



- Pro-inflammatory genes are more strongly up-regulated by DEX in Val carriers (e.g. *CD14*), particularly in the Hippocampus



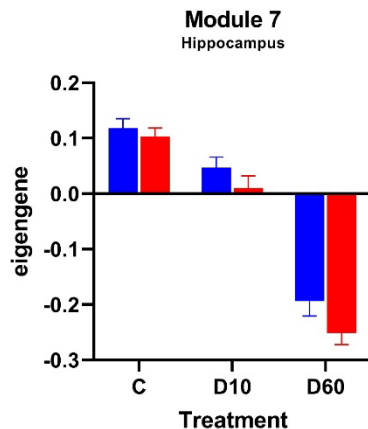
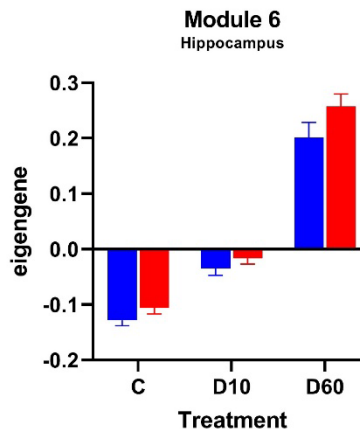
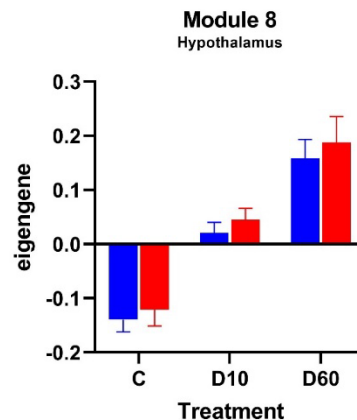
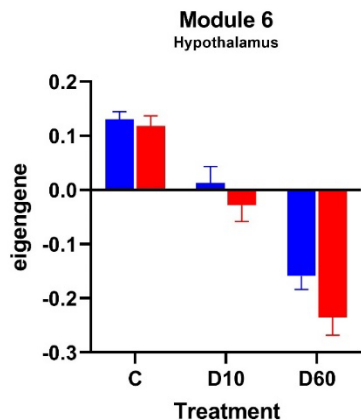
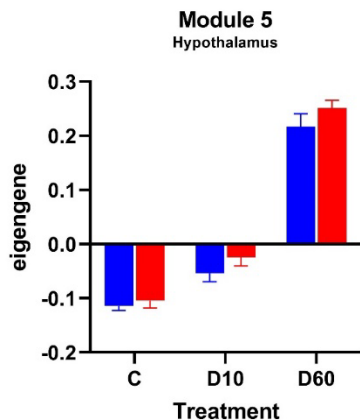
proportion · 0.00 · 0.25 · 0.50 · 0.75 · 1.00

expression -2 -1 0 1 2



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DEX-regulated co-expr. modules are influenced by GR_{Ala610Val}

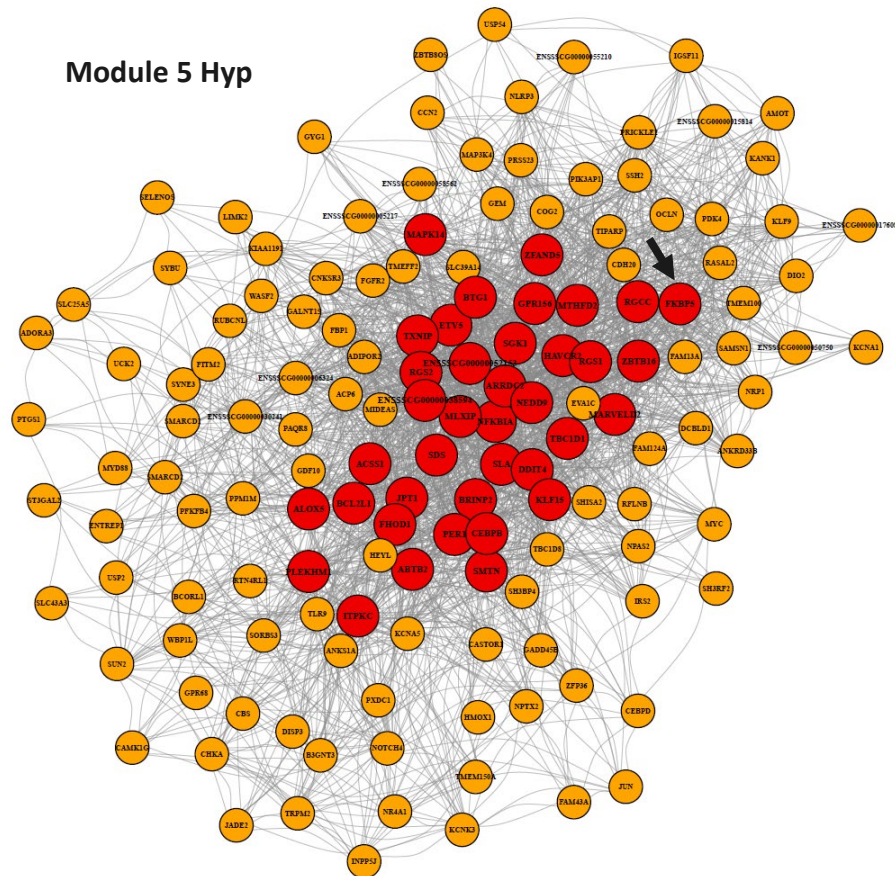


WGCNA Module	Genotype	Treatment	GxT
Mod 5 Hyp	0.08	<0.0001	0.68
Mod 6 Hyp	0.04	<0.0001	0.38
Mod 8 Hyp	0.27	<0.0001	0.94
Mod 6 Hip	0.03	<0.0001	0.46
Mod 7 Hip	0.04	<0.0001	0.56

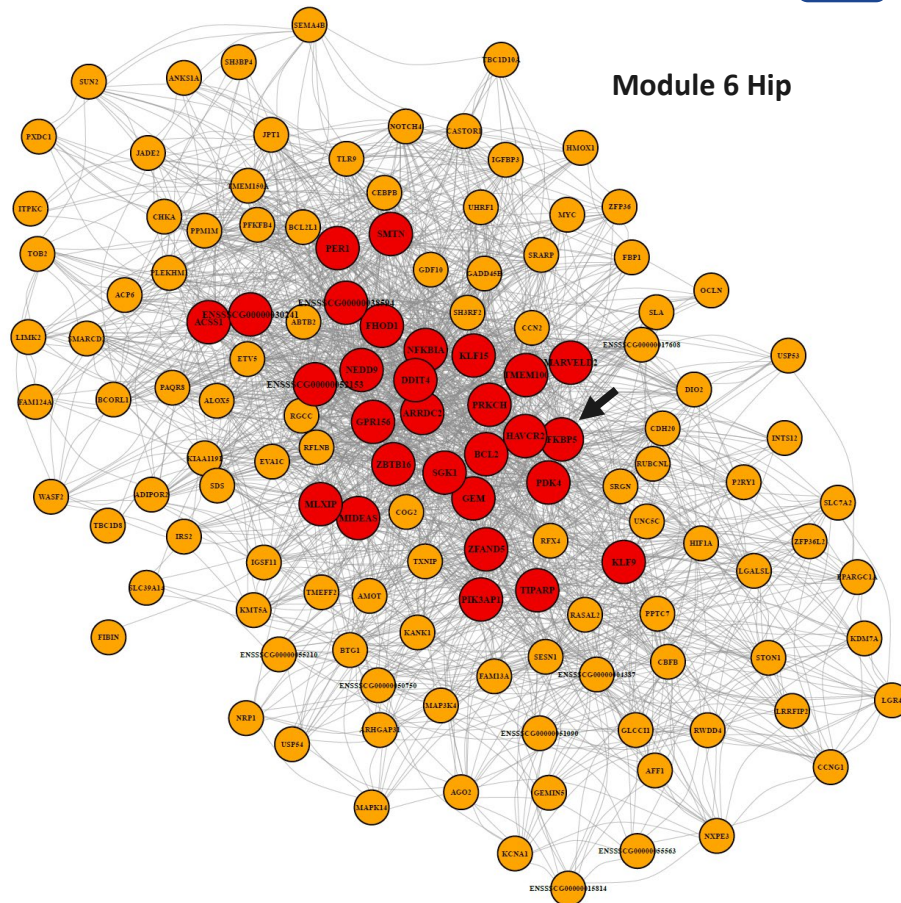
GR_{Ala610Val} influences key hub genes



Module 5 Hyp



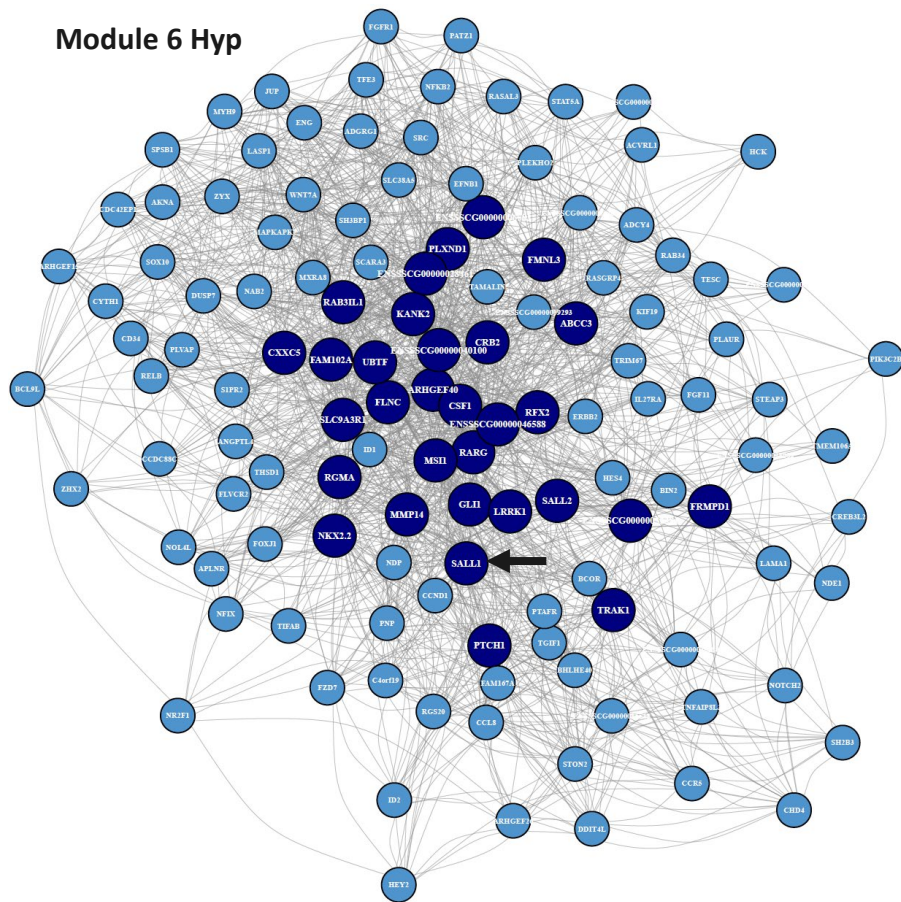
Module 6 Hip



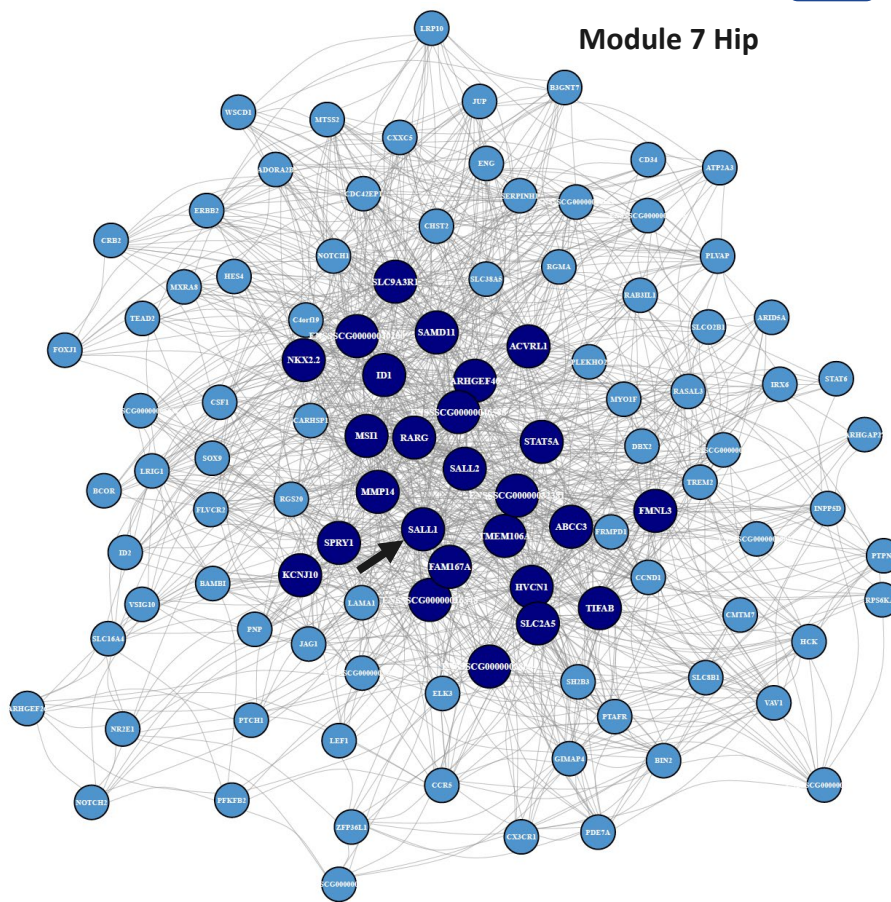
GR_{Ala610Val} influences key hub genes



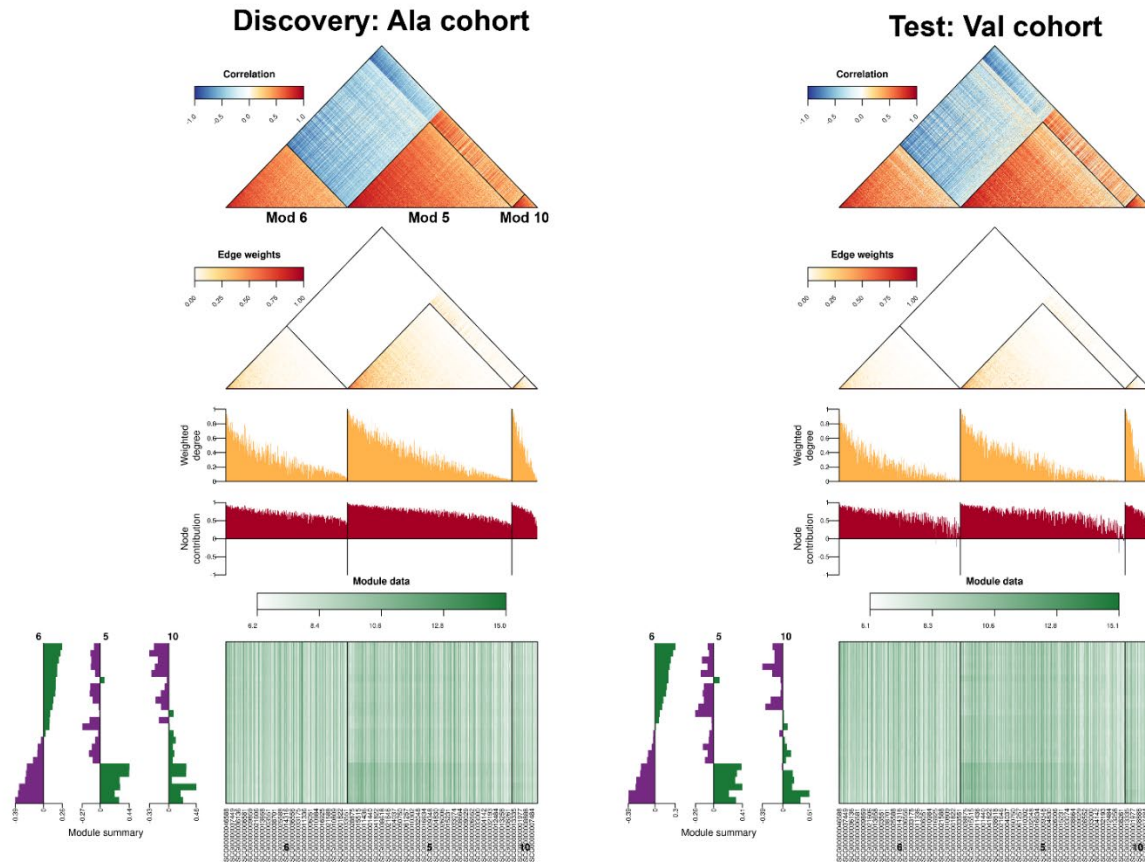
Module 6 Hyp



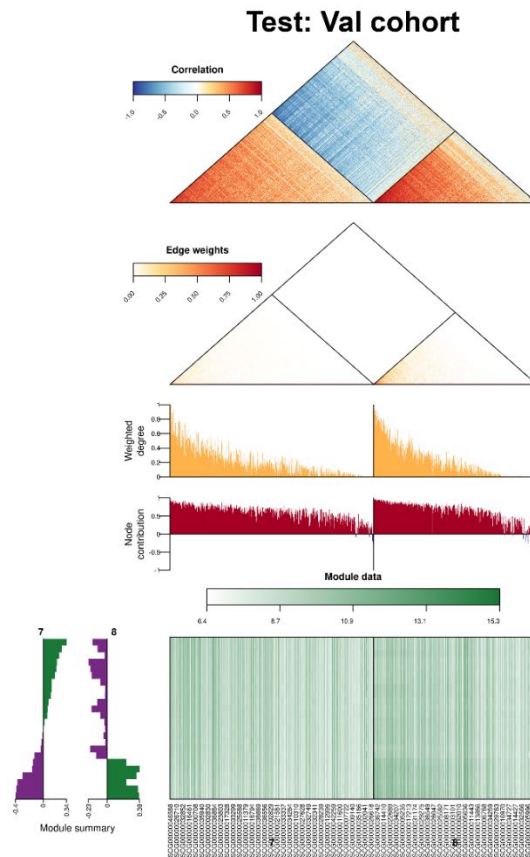
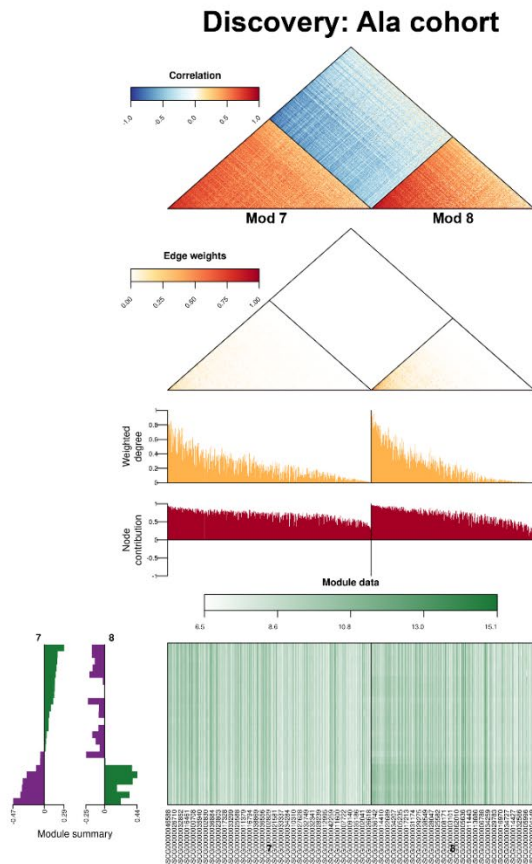
Module 7 Hip



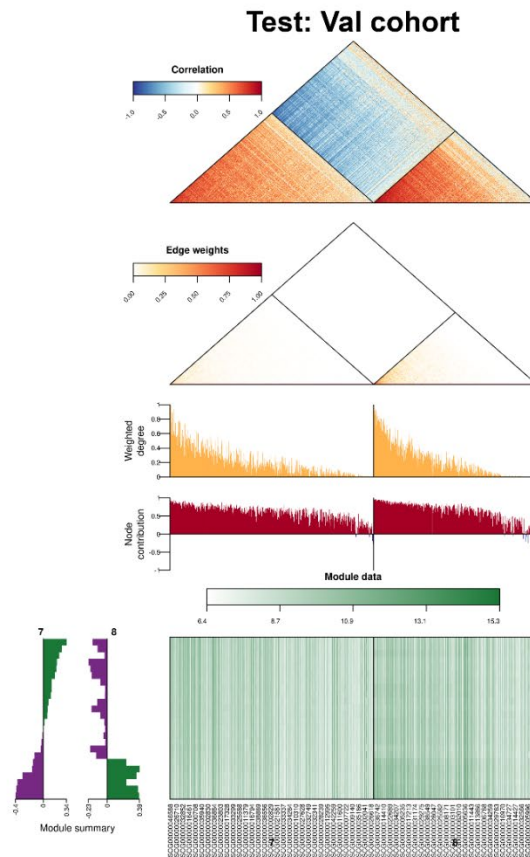
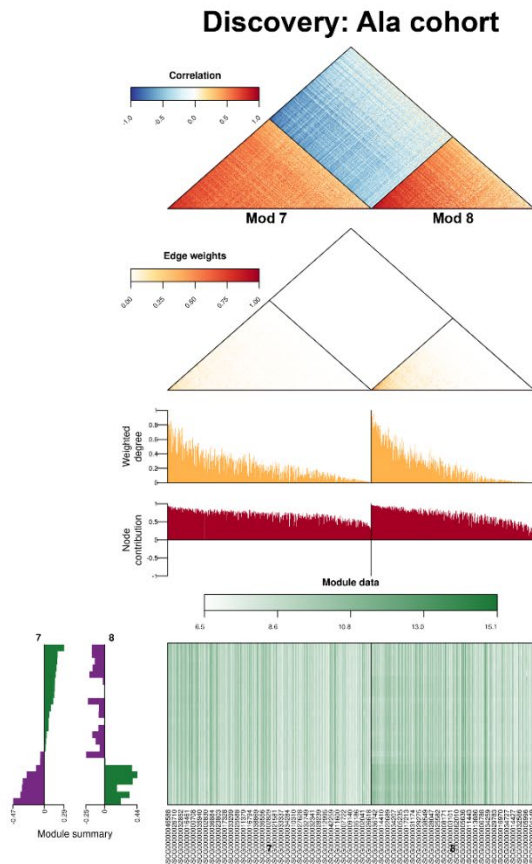
GR_{Ala610Val} does not change topology of gene networks



GR_{Ala610Val} does not change topology of gene networks



GR_{Ala610Val} does not change topology of gene networks



Behavioral testing



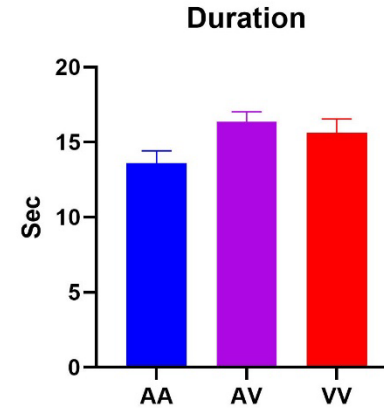
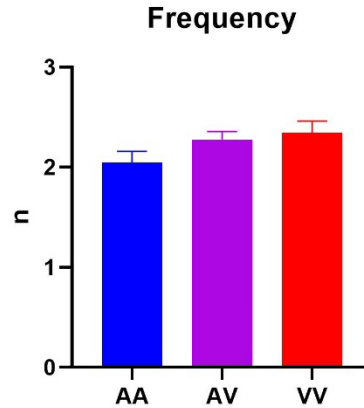
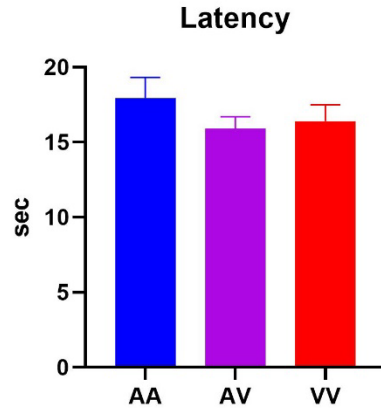
- A cohort of 144 piglets was tested to evaluate their personality traits (coping style, fearfulness/boldness, exploration, activity, sociability):
 - **Backtest**
 - **Group tests including novel-human, open-door, and novel object tests**
 - **individual tests including a combined open field/novel object test**



Back test



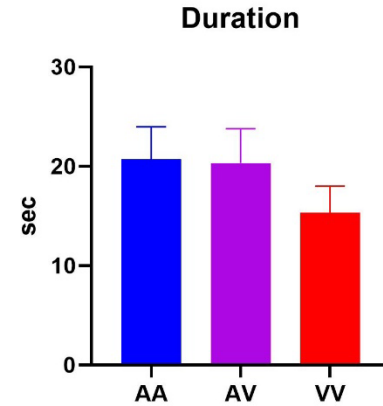
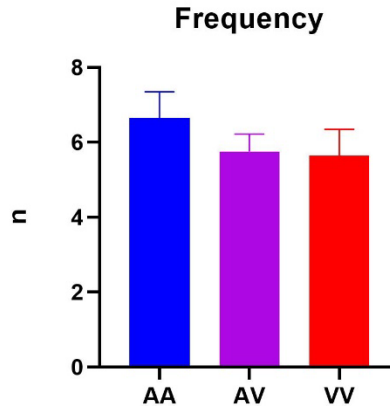
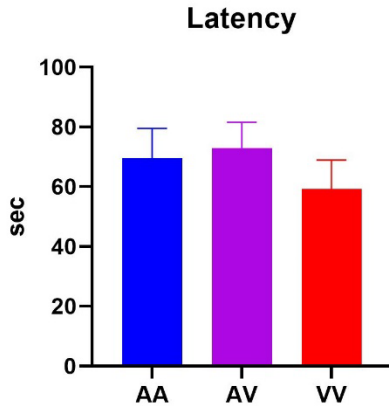
- Back test indicates more active coping by Val carriers



Individual test



- Novel object test indicates higher boldness and lower exploration of Val carriers



Summary & Conclusions



- ✓ Effect on co-expression networks is consistent with the gain-of-function
 - Evidence for changes in neuronal wiring via Clustered Protocadherins
 - Evidence for pro-inflammatory priming of microglia
-
- ☞ predisposition for brain disorders
-
- Behavioral testing indicates more bolder personality of Val carriers
-
- ☞ Down-regulation of *CRH* in Val carries is expected to reduce their anxiety



Thank you for your attention!

Acknowledgement



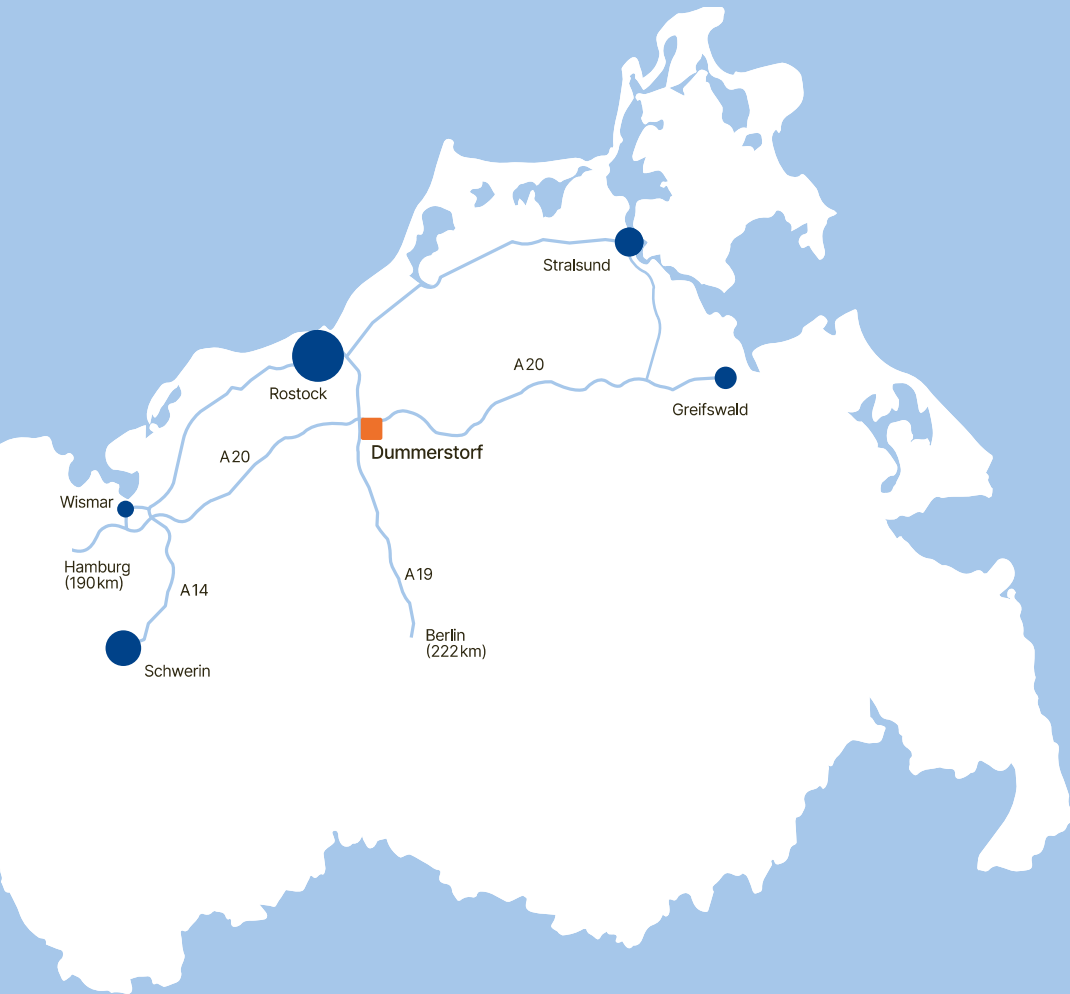
Thanks to:

DFG for funding (No. 391382814)

and to Colleagues for their valuable contribution



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