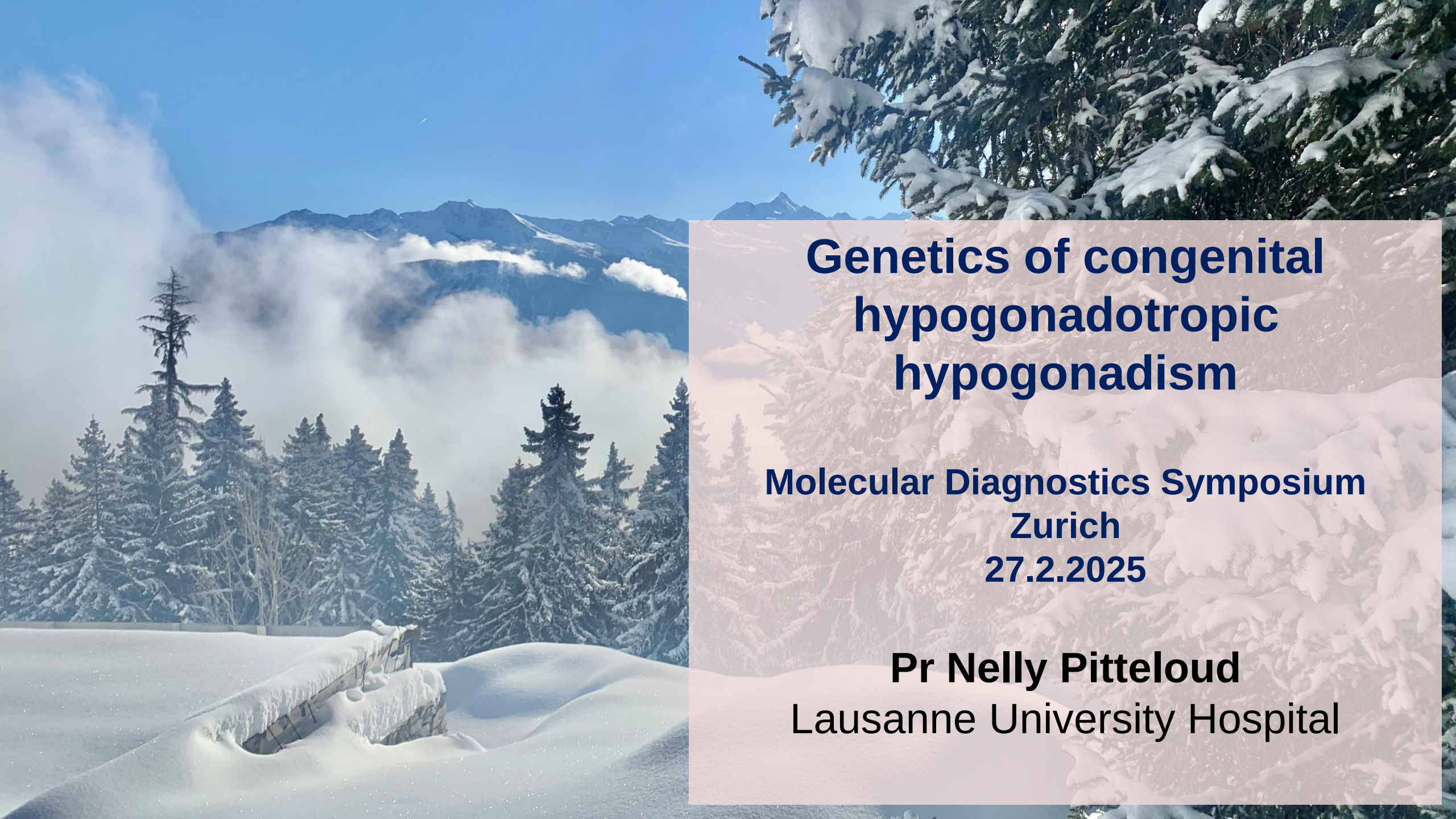


Genetics of congenital hypogonadotropic hypogonadism

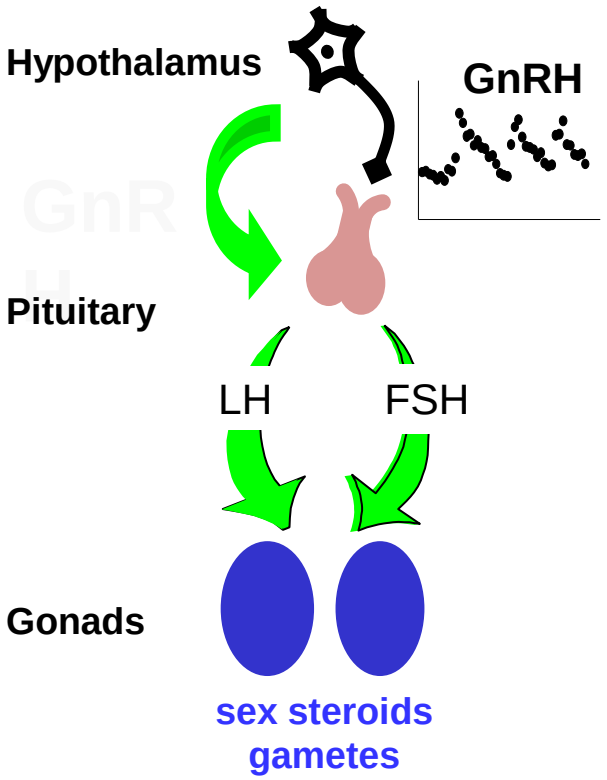
Molecular Diagnostics Symposium
Zurich
27.2.2025

Pr Nelly Pitteloud
Lausanne University Hospital

Outline

- Background on CHH
- Oligogenicity in CHH
- New gene discovery
  - Trio analysis
  - Trajectories & CHH

Puberty

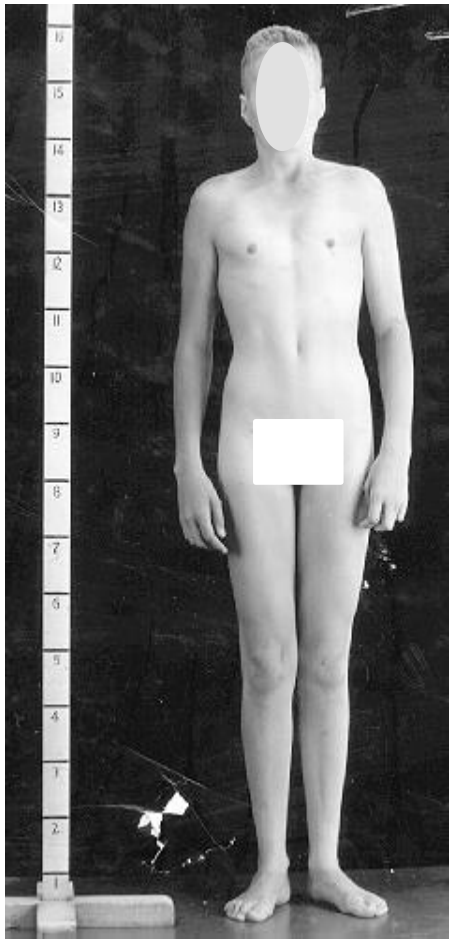


- T/E2 production & 2nd sexual characteristics
- Gonadal maturation & fertility
- Psychosocial & sexual maturation
- Growth spurt & final height
- Increased bone mass

CHH

Classical forms

- **Rare inherited disorder (1:10-30000)**
- **Male preponderance 3:1**
- **Due to GnRH deficiency**
- **Lack of pubertal development**
- **Microphallus & cryptorchidism**
- **Low T, LH and FSH, no sperm, no MRI lesion**
- **Treatable cause of male infertility**
- **Non reproductive associated phenotypes**
- **Genetic heterogeneity**

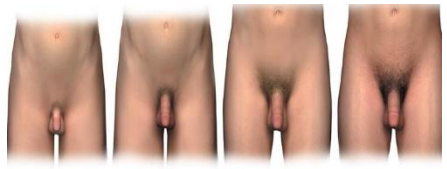


CHH

Partial & Reversal forms

Some breast development
🡻E2, detectable LH & FSH

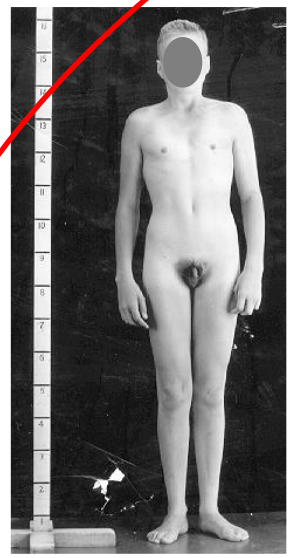
Some testicular growth
🡻T , detectable LH & FSH



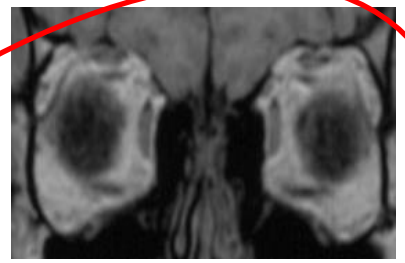
Pitteloud et al, JCEM 2001
Raivio et al, *NEJM*, 2005

CHH

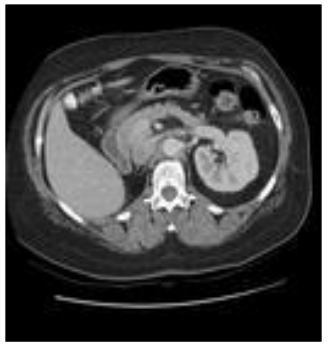
Associated phenotypes



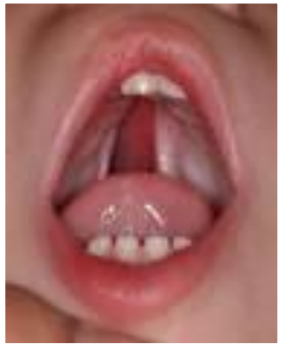
Isolated HH



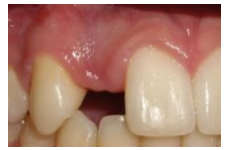
Anosmia
50%



Unilateral
Renal agenesis
2%



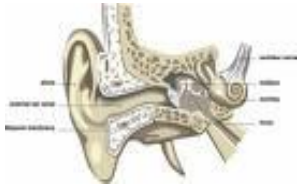
Cleft palate
5-6%



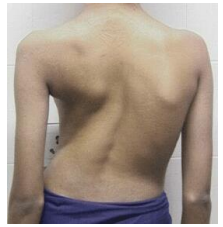
Tooth agenesis
2-5%

CHH: Associated phenotypes

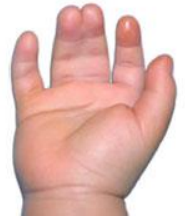
Ataxia
1%



Hearing loss
6%



Scoliosis
5-10%

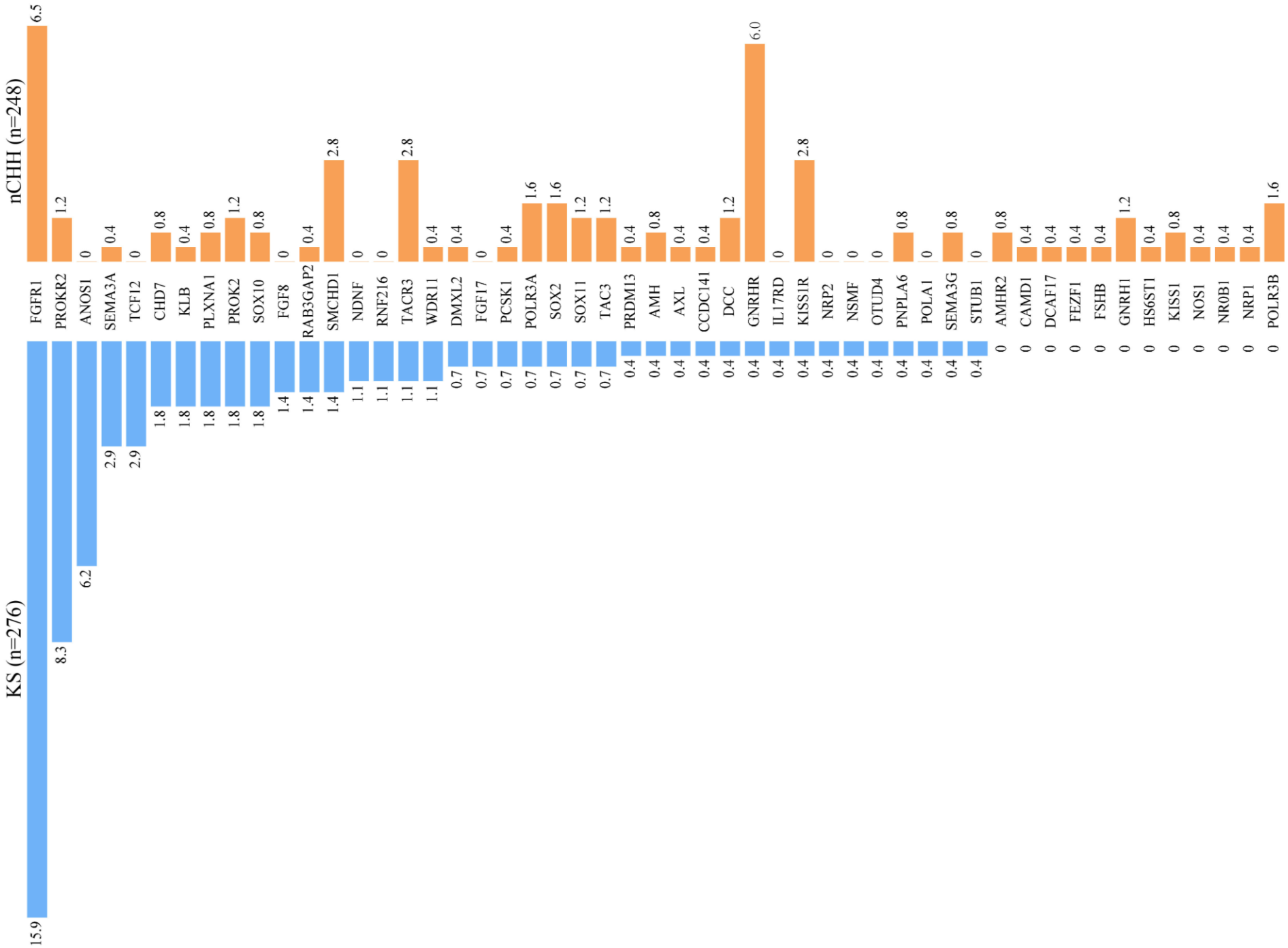


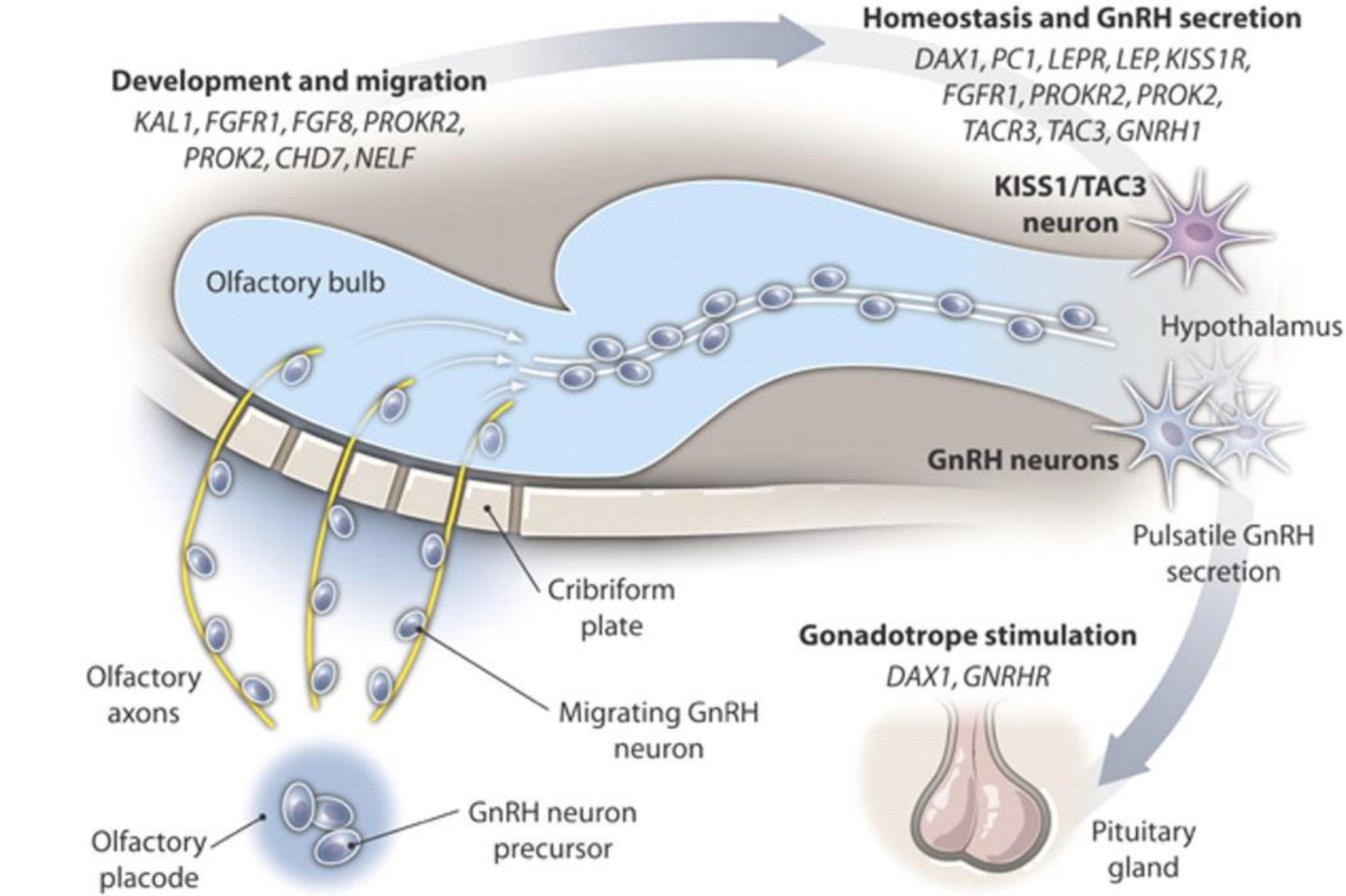
Syndactily
2-5%

Genetics

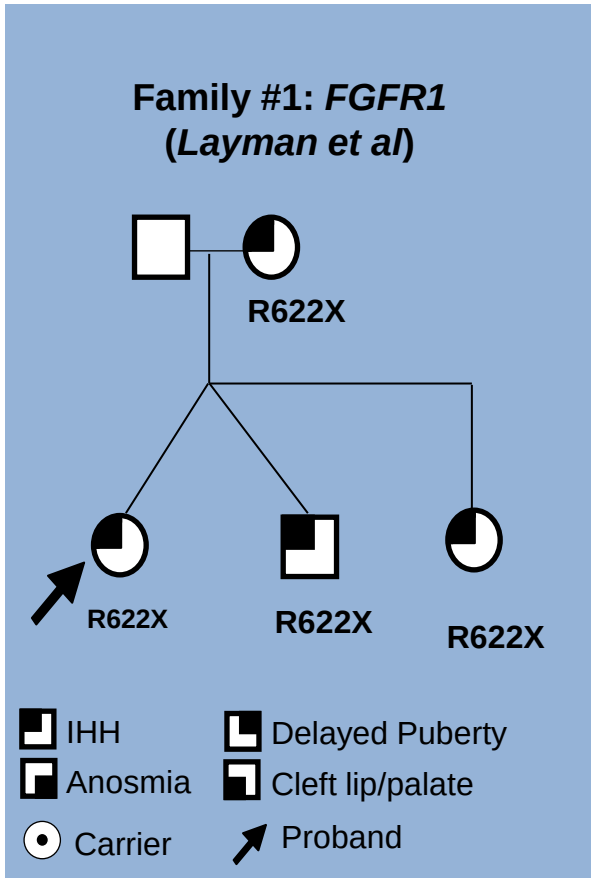
# Genetics of CHH
n= 536

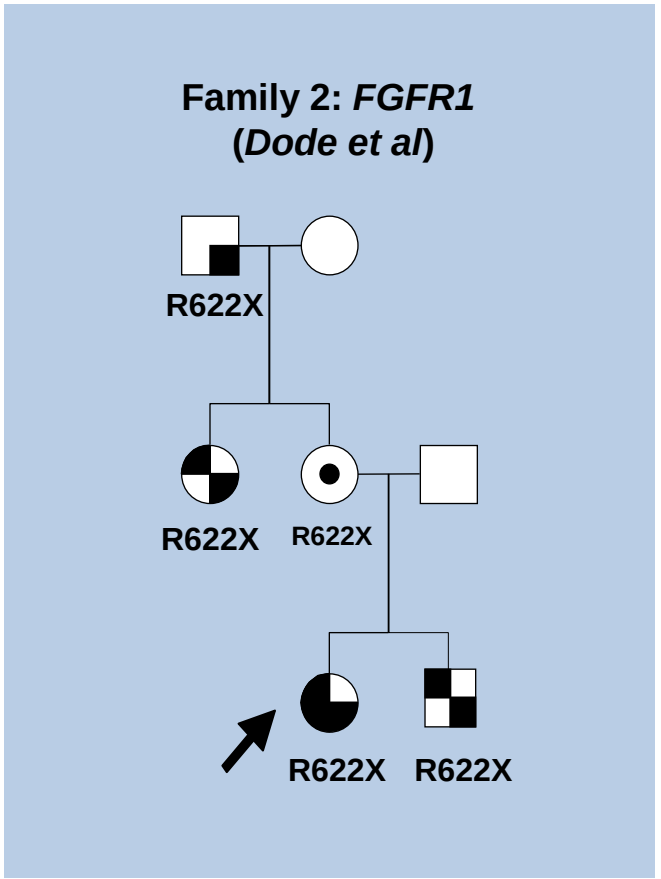
Locus heterogeneity

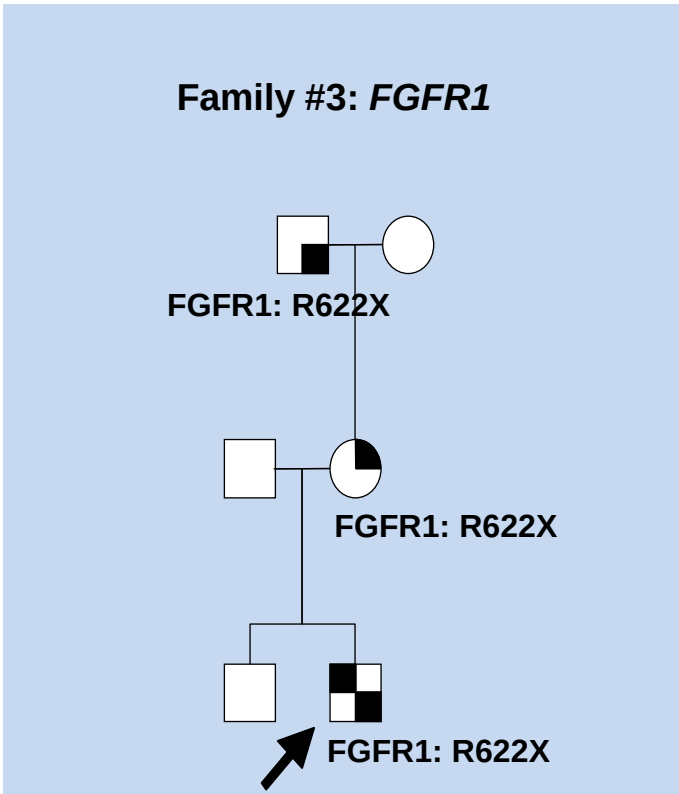



Homeostasis and GnRH secretion
DAX1, PC1, LEPR, LEP, KISS1R, FGFR1, PROKR2, PROK2, TACR3, TAC3, GNRH1
Development and migration
KAL1, FGFR1, FGF8, PROKR2, PROK2, CHD7, NELF
KISS1/TAC3 neuron
Olfactory bulb
Hypothalamus
GnRH neurons
Pulsatile GnRH secretion
Cribriform plate
Olfactory axons
Migrating GnRH neuron
Gonadotrope stimulation
DAX1, GNRHR
Olfactory placode
GnRH neuron precursor
Pituitary gland

Single Genotype Cannot Reliably Predict Phenotype



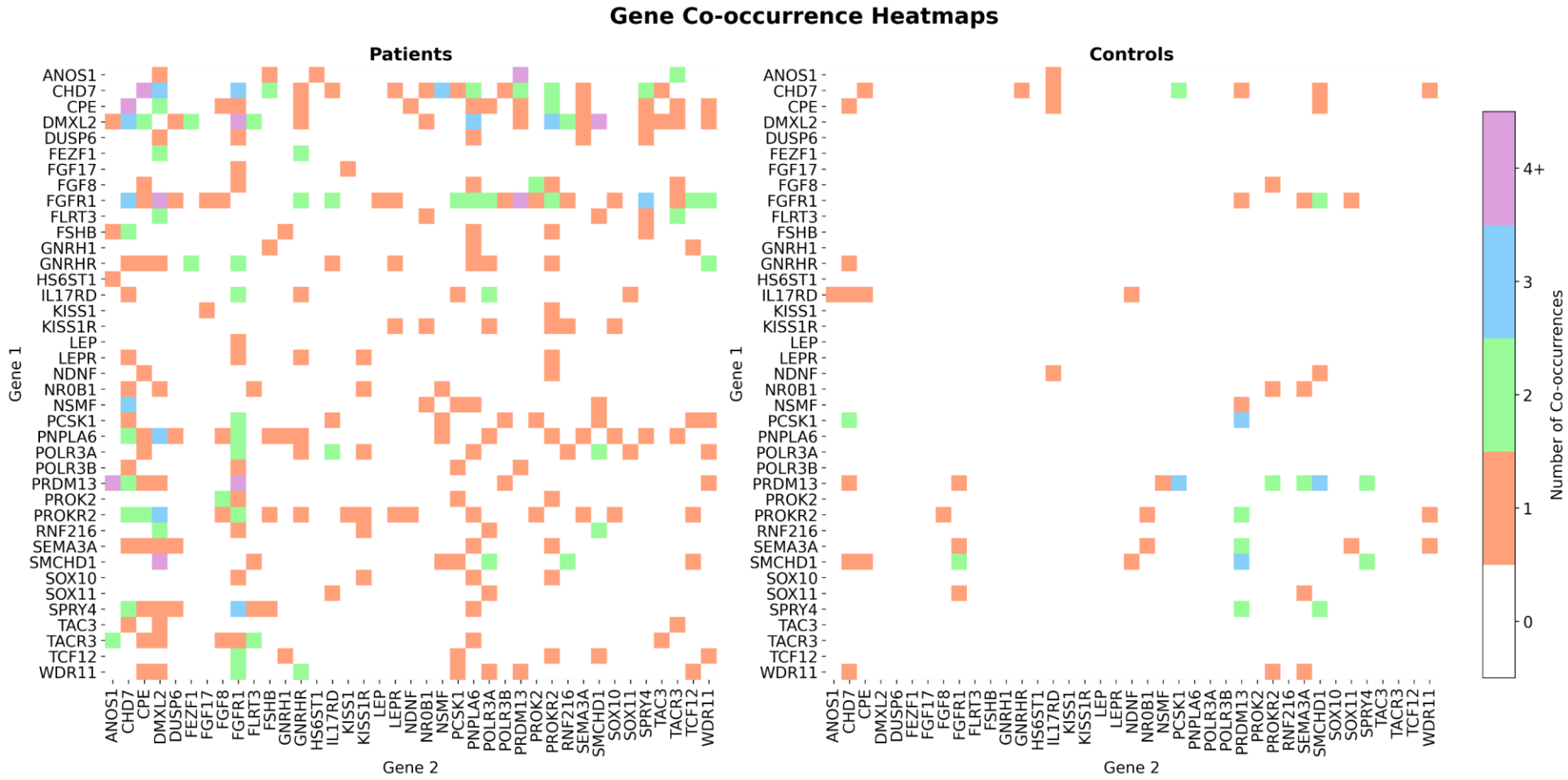




Oligogenicity

CHH
n= 135/536

Controls
n= 601



Manuscript in preparation

Oligogenicity

CHH
n= 536

Controls
Genome Project
n= 601

	Variants in CHH	Variants in controls	P_val	P-adj
DMXL2	24	0	8.23295E-12	3.37E-10
FGFR1	27	4	8.98369E-09	1.84E-07
PNPLA6	14	0	4.0358E-07	5.51E-06
CHD7	22	8	6.69681E-05	0.0005
POLR3A	9	0	6.68755E-05	0.0005
CPE	10	3	0.000308341	0.001
GNRHR	10	1	0.000315938	0.001
TACR3	7	0	0.000606017	0.003
PROKR2	12	5	0.00104758	0.004
FSHB	5	0	0.005538457	0.017
FLRT3	6	0	0.005538457	0.017
TCF12	5	0	0.005538457	0.017
RNF216	4	0	0.005538457	0.017
ANOS1	8	1	0.008656361	0.025
KISS1R	4	0	0.011601504	0.03

Manuscript submitted

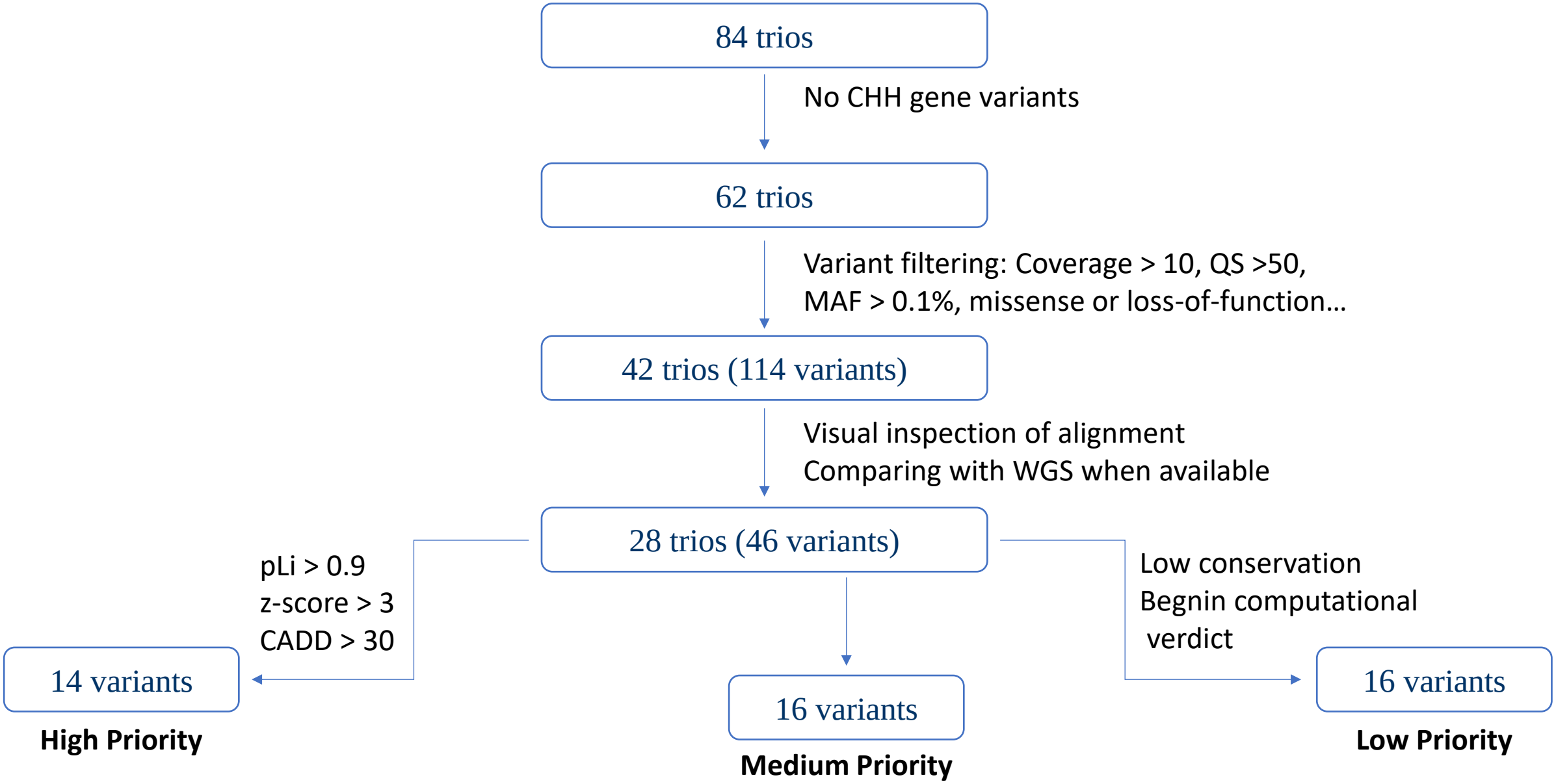
Summary

- The genetics of CHH is complex
- Low penetrance and variable expressivity prevail
- Oligogenicity occurs in 25% of cases

New gene discovery

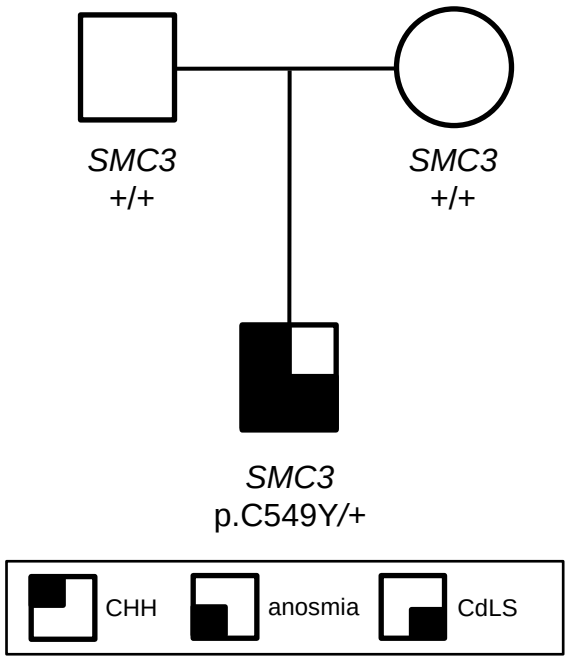
Trio Analysis

De novo analysis



						Prioritization Criteria											
						Variant Level				Gene Level						Protein Level	
Pt	Sex	Dx	Gene	cDNA change	Protein change	gnomAD MAF	CADD	REVEL	αM	in-silico summary	Expr Hypo/Pit	Expr Fetal OB	GnRH Mouse	OMIM	LOEUF	Z Missense	PPI	MGI
1	M	KS	*DPF2*	c.663delinsAC		-	+	+	+	+	+	+	+	+	+	+	-	+
2	F	KS	*PAX6*	c.635C>G	p.P212R	-	-	+	+	+	+	-	+	+	+	+	+	+
3	F	KS	*ZNF462*	c.3309_3318delinsCT	p.L110Sfs*5	-	+	+	+	+	+	+	+	+	+	+	-	-
4	M	KS	*SMC3*	c.1646G>A	p.C549Y	-	+	+	+	+	+	-	+	+	+	+	-	-
5	M	KS	*ECEL1*	c.2314G>A	p.V772M	0.000132	+	+	+	+	+	+	+	+	-	-	-	+
6	M	KS	*NAA35*	c.1189C>T	p.R397W	5.58E-06	+	+	+	+	+	-	+	-	+	+	-	+
7	M	nCHH	*POU3F2*	c.961A>G	p.M321V	-	+	+	-	+	+	+	+	+	-	-	+	-
8	M	nCHH	*POLG*	c.3374T>C	p.I1125T	3.72E-06	+	+	-	+	+	-	+	+	-	-	+	+
9	M	nCHH	*HK1*	c.1306C>T	p.R436W	9.29E-06	+	+	-	+	+	-	-	+	+	+	-	+
10	M	nCHH	*KDM4B*	c.676+5G>C		-	+	+	+	+	+	-	+	+	+	-	-	-
11	M	KS	*BAG6*	c.1806_1808delinsA		-	+	+	+	+	+	-	+	-	+	+	-	-
12	M	nCHH	*BARHL1*	c.545G>T	p.R182L	-	+	+	+	+	-	+	-	-	+	-	+	+
13	M	KS	*SBNO1*	c.3976A>G	p.S1326G	0.0004325	+	-	-	-	+	-	+	-	+	+	-	+
14	F	nCHH	*ARFGEF2*	c.1841G>A	p.S614N	3.10E-06	+	-	+	+	+	-	-	+	+	+	-	-
15	M	KS	*MFHAS1*	c.2999-2A>G		6.20E-07	+	+	+	+	+	-	+	+	-	-	-	-
16	M	KS	*MX2*	c.578-1G>C		-	+	+	+	+	+	+	-	-	-	-	-	+
17	M	KS	*SNRPD1*	c.283+5G>A		-	+	+	+	+	+	-	+	-	+	-	-	-
18	M	KS	*SAFB2*	c.2156delinsAGCT		-	-	+	+	+	+	-	+	-	-	-	-	+
19	M	KS	*SPAG17*	c.3617_3620delinsT		2.48E-06	-	+	+	+	+	-	-	+	-	-	-	+
20	M	nCHH	*ERCC4*	c.2218C>T	p.R740C	2.79E-05	+	+	+	+	+	-	-	+	-	-	-	-
21	M	KS	*IPP*	c.908G>A	p.R303H	3.19E-06	+	+	+	+	+	-	-	-	-	-	-	+
22	F	KS	*AK9*	c.1924G>T	p.E642X	-	+	+	+	+	+	-	-	-	-	-	-	+
23	M	KS	*BICRA*	c.282delinsTG		-	+	+	+	+	+	-	-	+	-	-	-	-

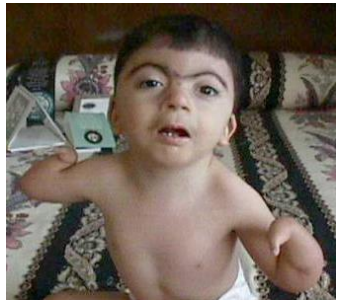
SMC3 is mutated *de novo* in a KS patient with CdLS



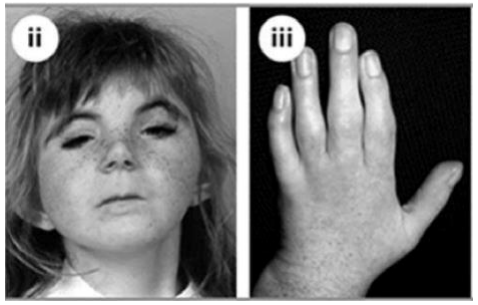
- Kallmann syndrome
- Cornelia de Lange syndrome (CdLS)

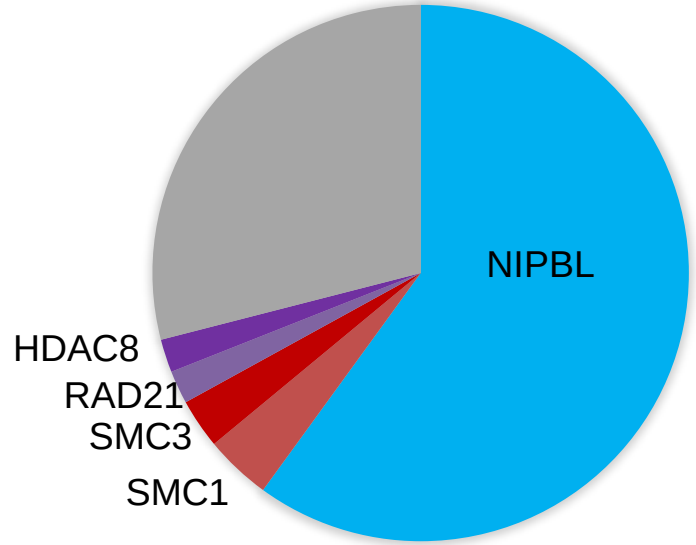
CdLS: growth/mental retardation
limb anomalies
facial dysmorphism

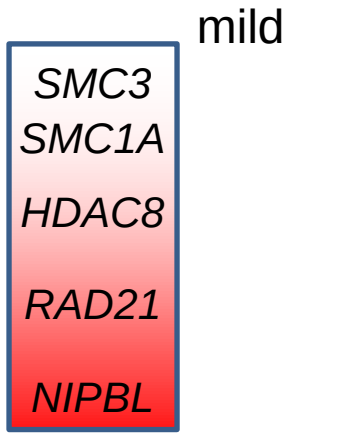
severe CdLS



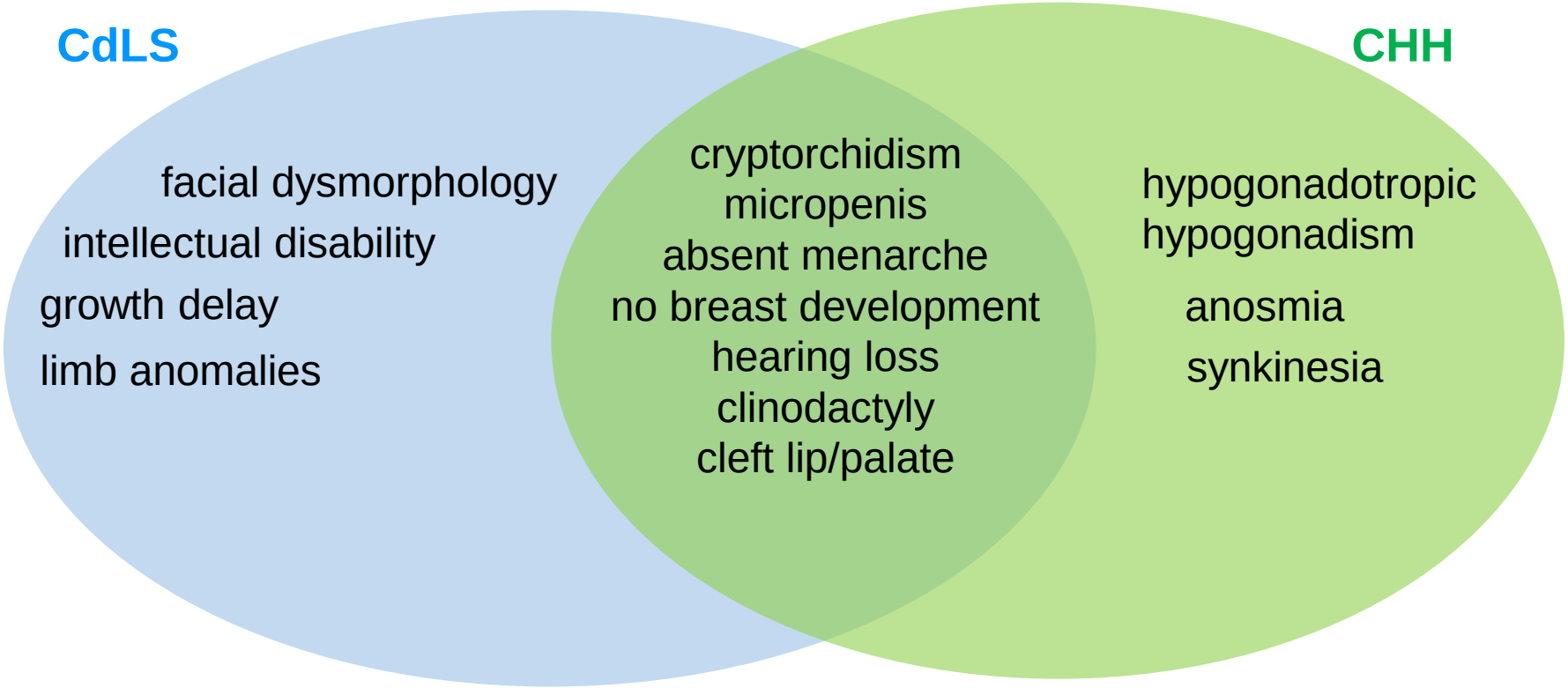
mild CdLS





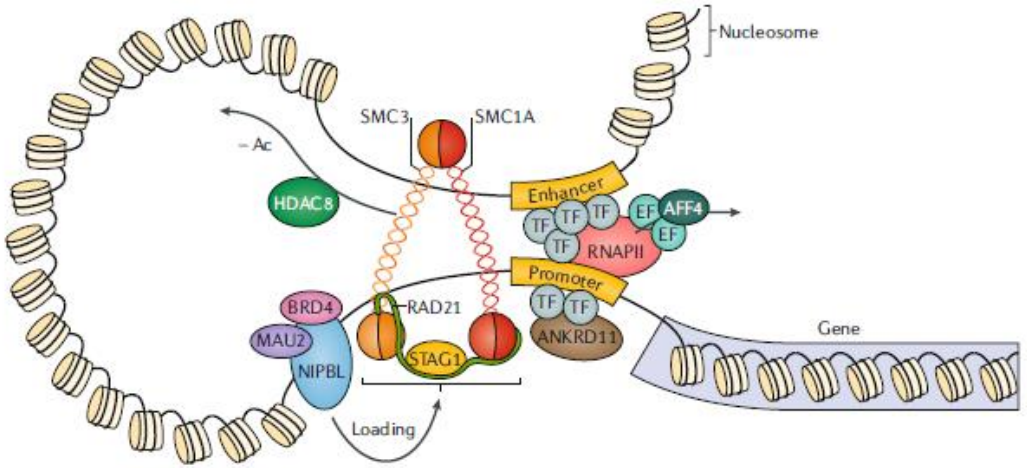


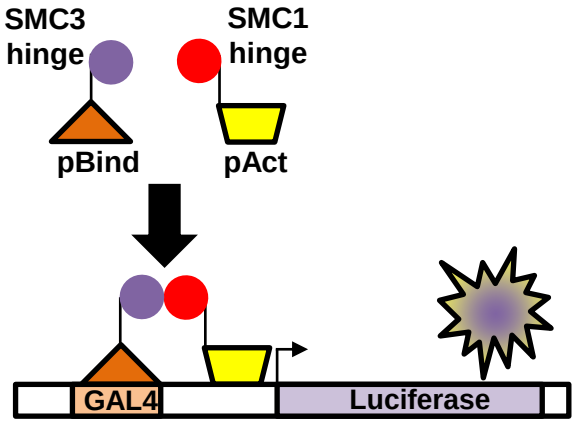
Clinical overlap between CdLS & CHH

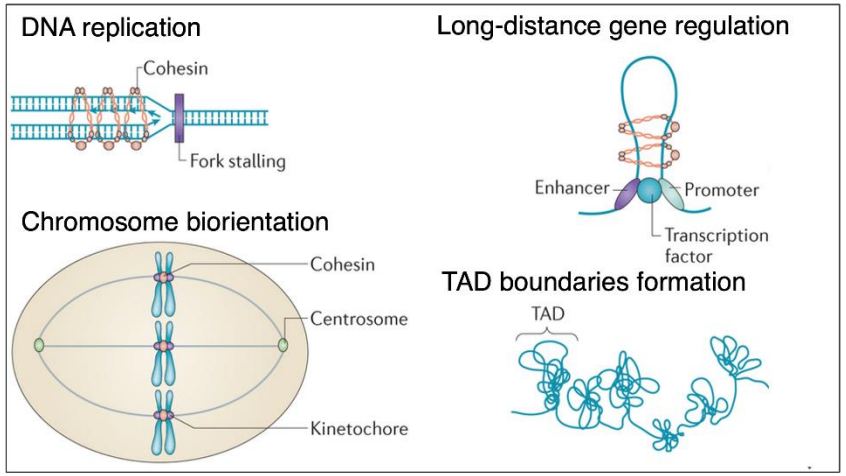


Is SMC3 also involved in GnRH biology?

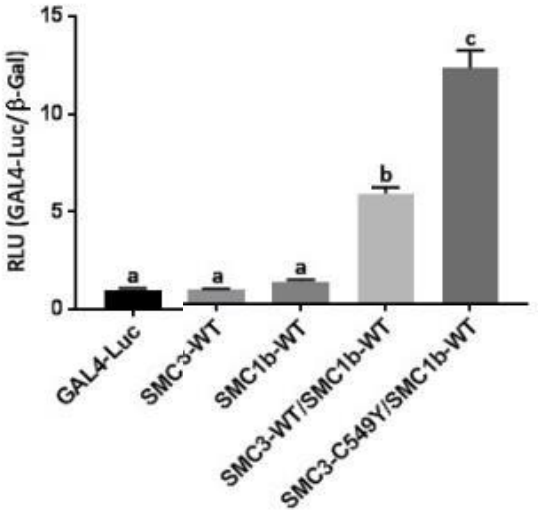
Altered functionality of *C549Y SMC3* mutant



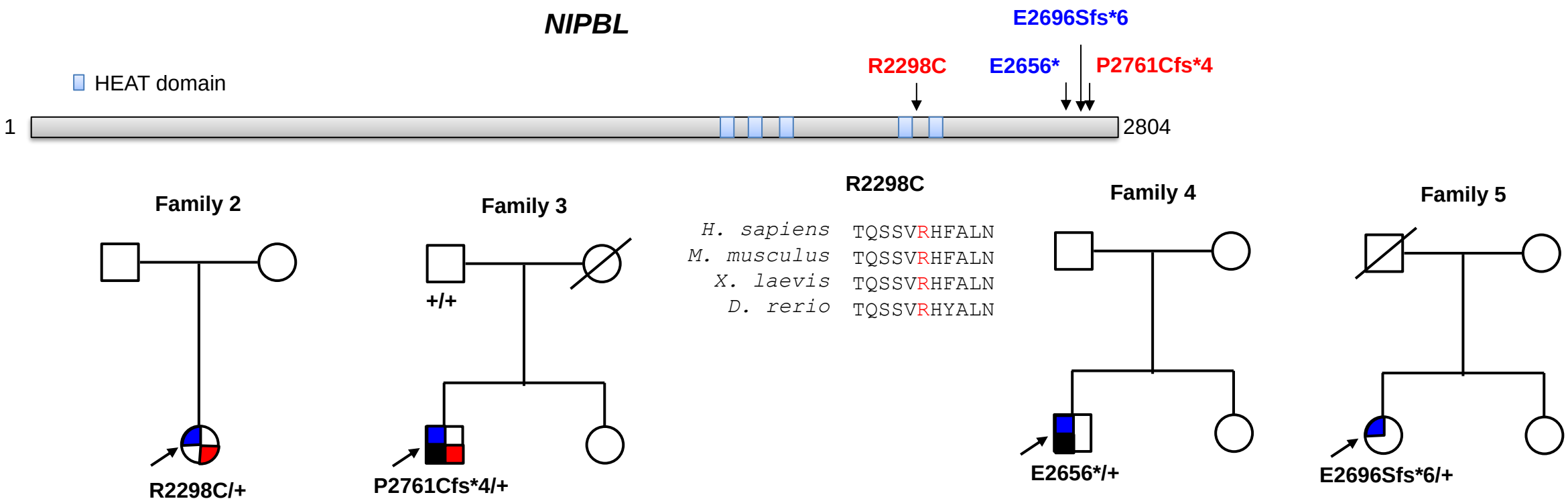




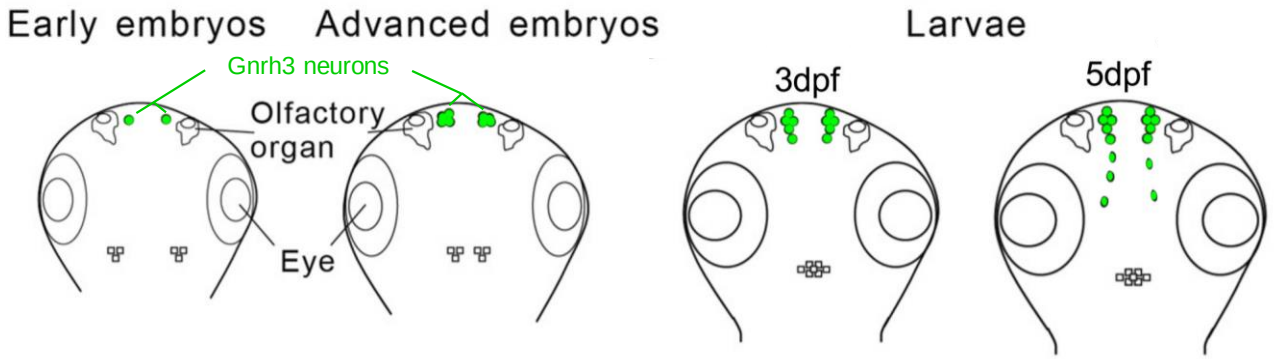
Losada *et al.*, Nat Rev Cancer 2014

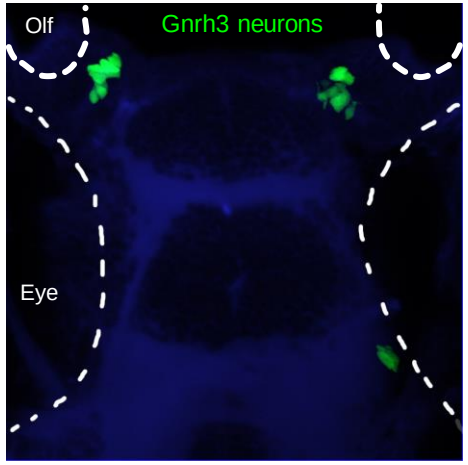


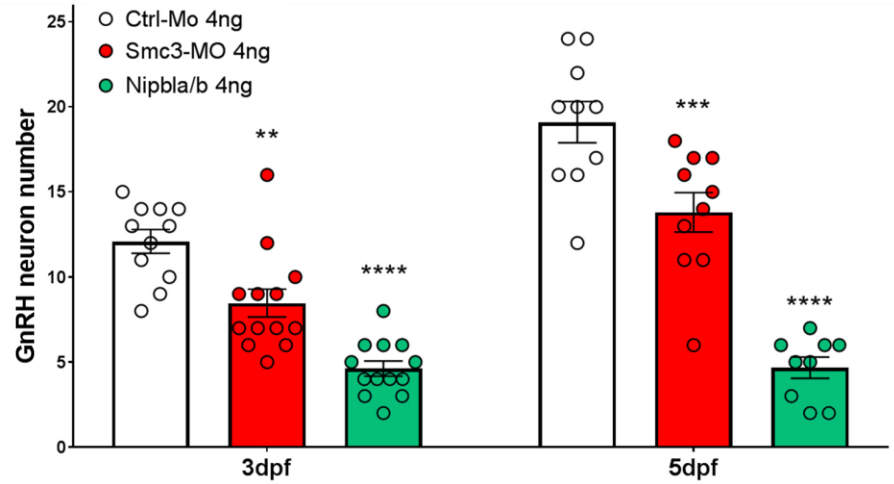
NIPBL loss-of-function mutations in CHH patients with/without CdLS



Knock-down of *smc3* & *nipbl* decreases GnRH neuron population

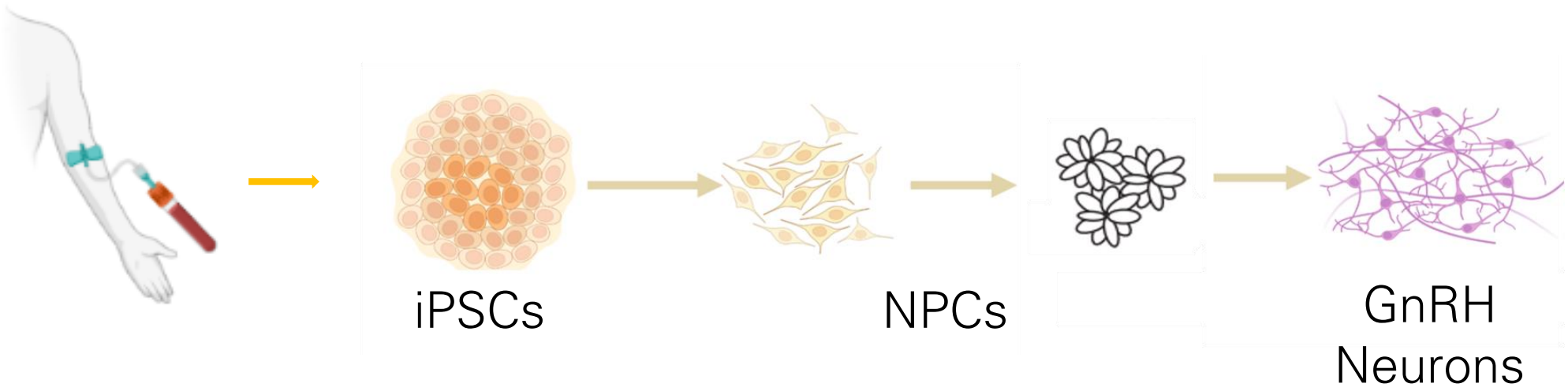






Transcriptomic & DNA accessibility studies are exploring the role of cohesion complex in GnRH biology

scRNAseq experiment design

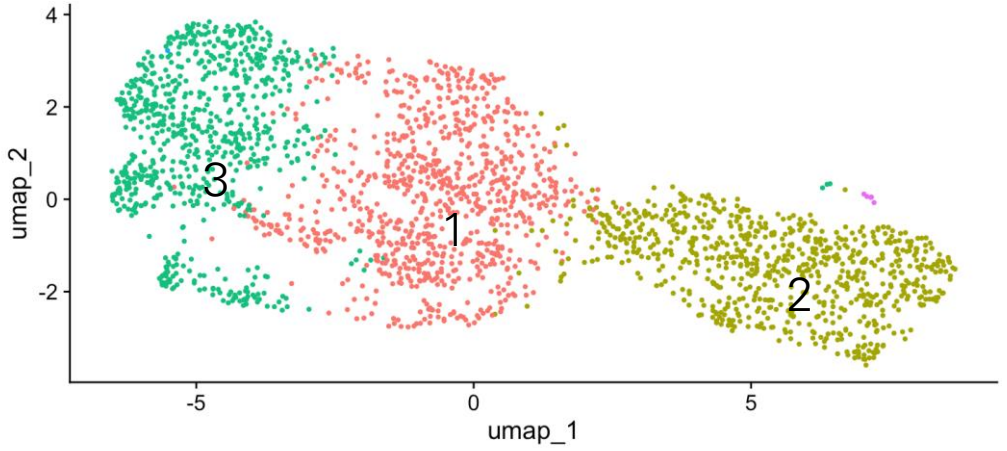


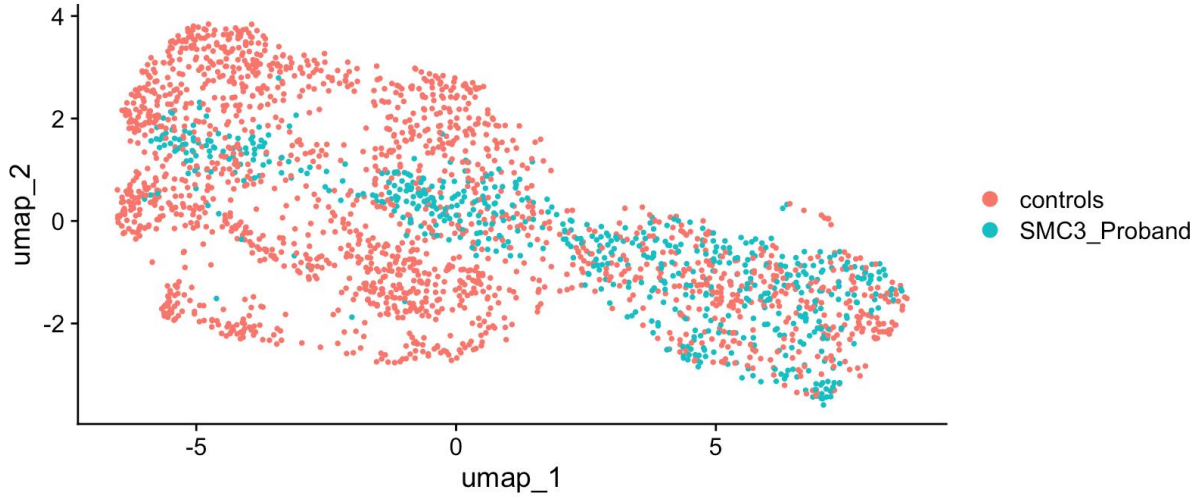
proband carry *SMC3* variant

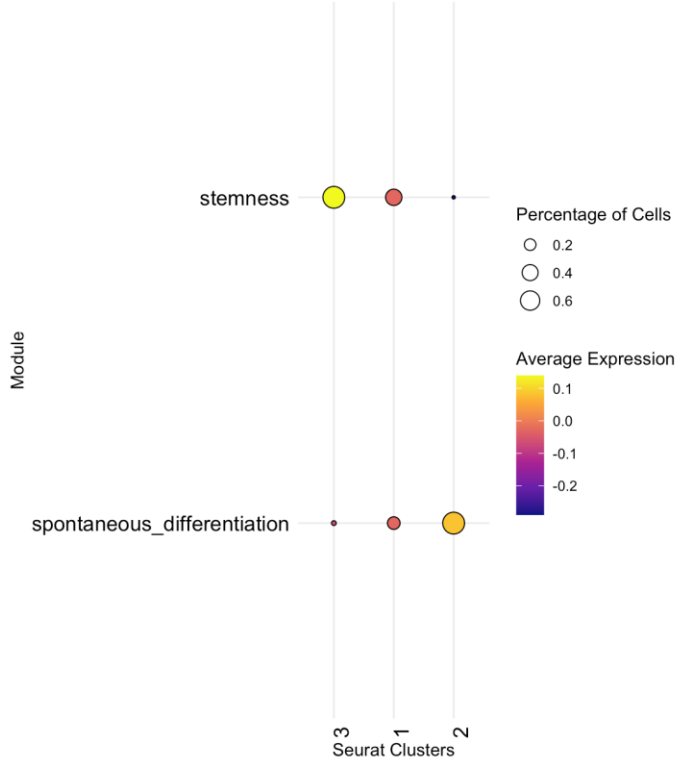
3 controls:
healthy father of the proband, control 1 and control 2

(Lund *et al.*, 2016)
(Keen *et al.*, 2021)

iPSCs



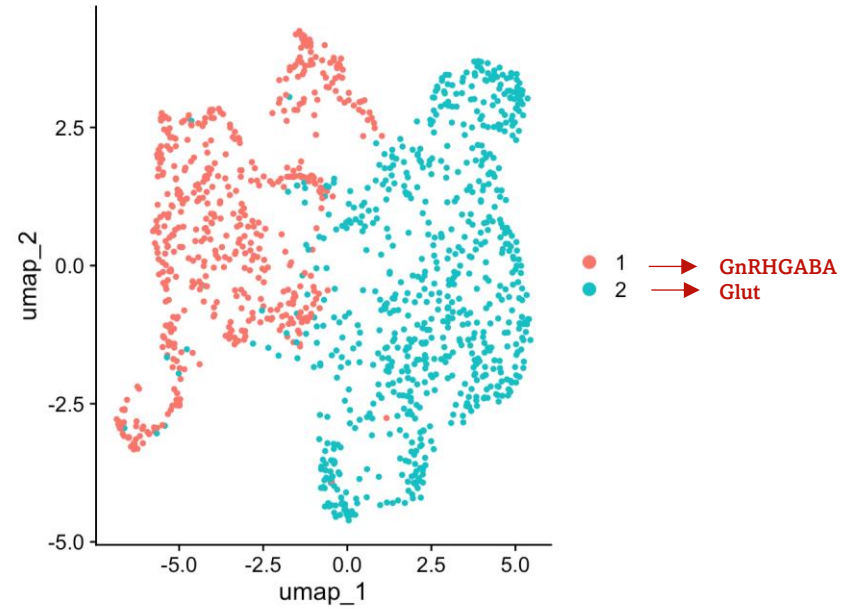


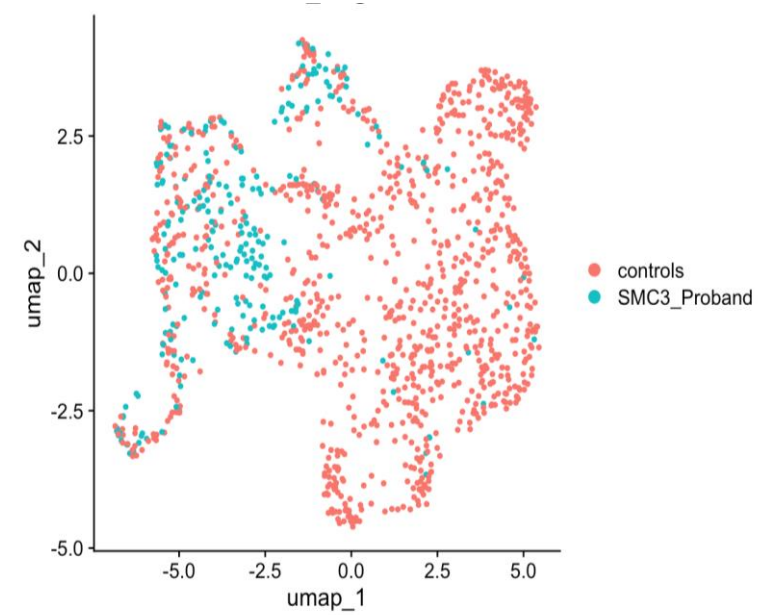


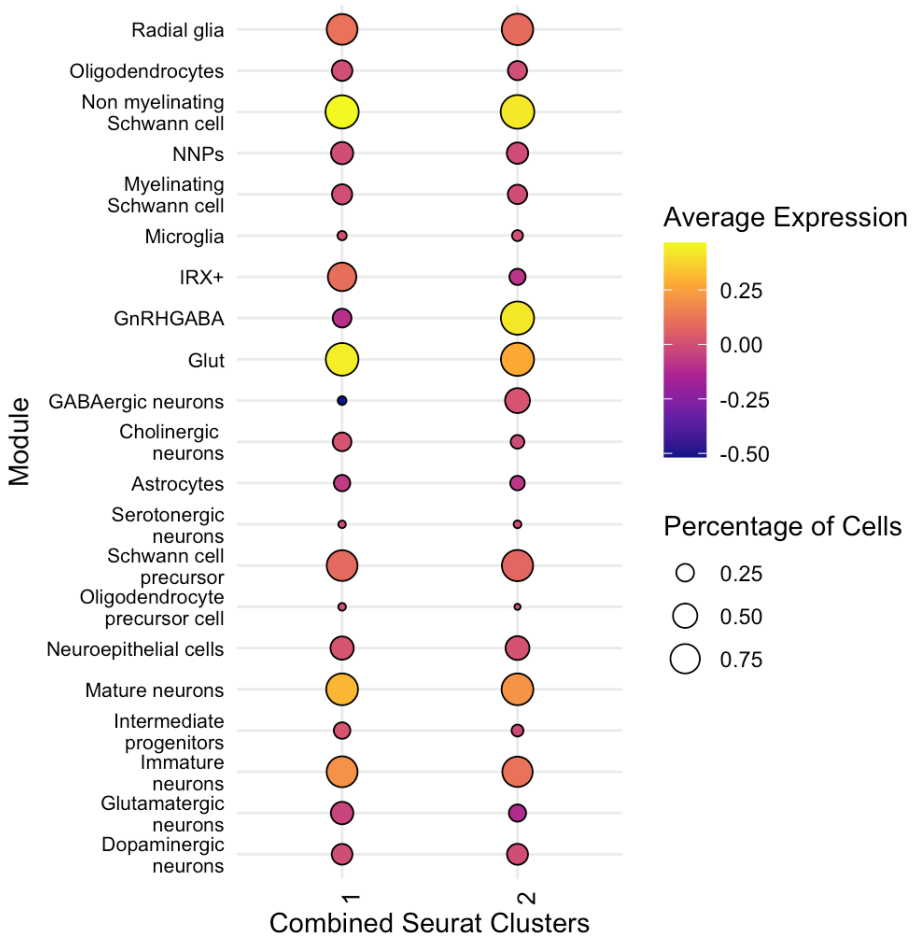
	Proband (*SMC3* variant)	Healthy Controls
Relatively more differentiated cells (%)	55.6603774	23.4792496
Relatively highly stemness cells (%)	15.5660377	36.6117112

P=3.97E-53

Neuronal cells







SMC3 **variant cause a dramatic reduction in precursor GnRH neuron differentiation, 8 %, vs 70% in healthy control (P=7E-69)**

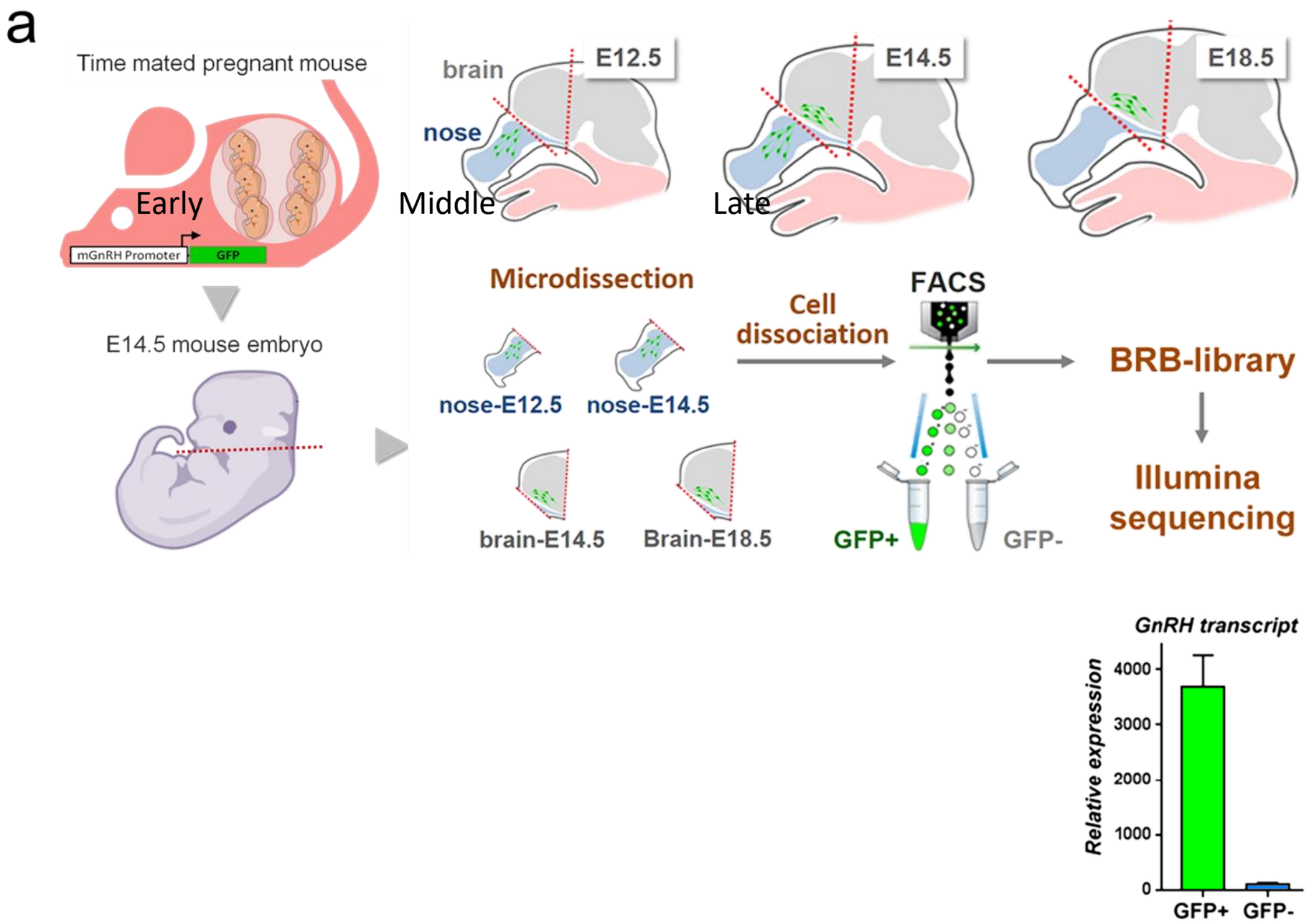
Summary

- Trio analysis uncovers several genes of interest belonging to the cohesion complex and the BAF complex involved in chromatin remodelling
- SMC3 and NIPBL are mutated in CHH
- Cohesion complex is involved in GnRH neuron ontogeny
- Studies are on going to implicate chromatin remodelling genes in CHH

Fine mapping of gene expression

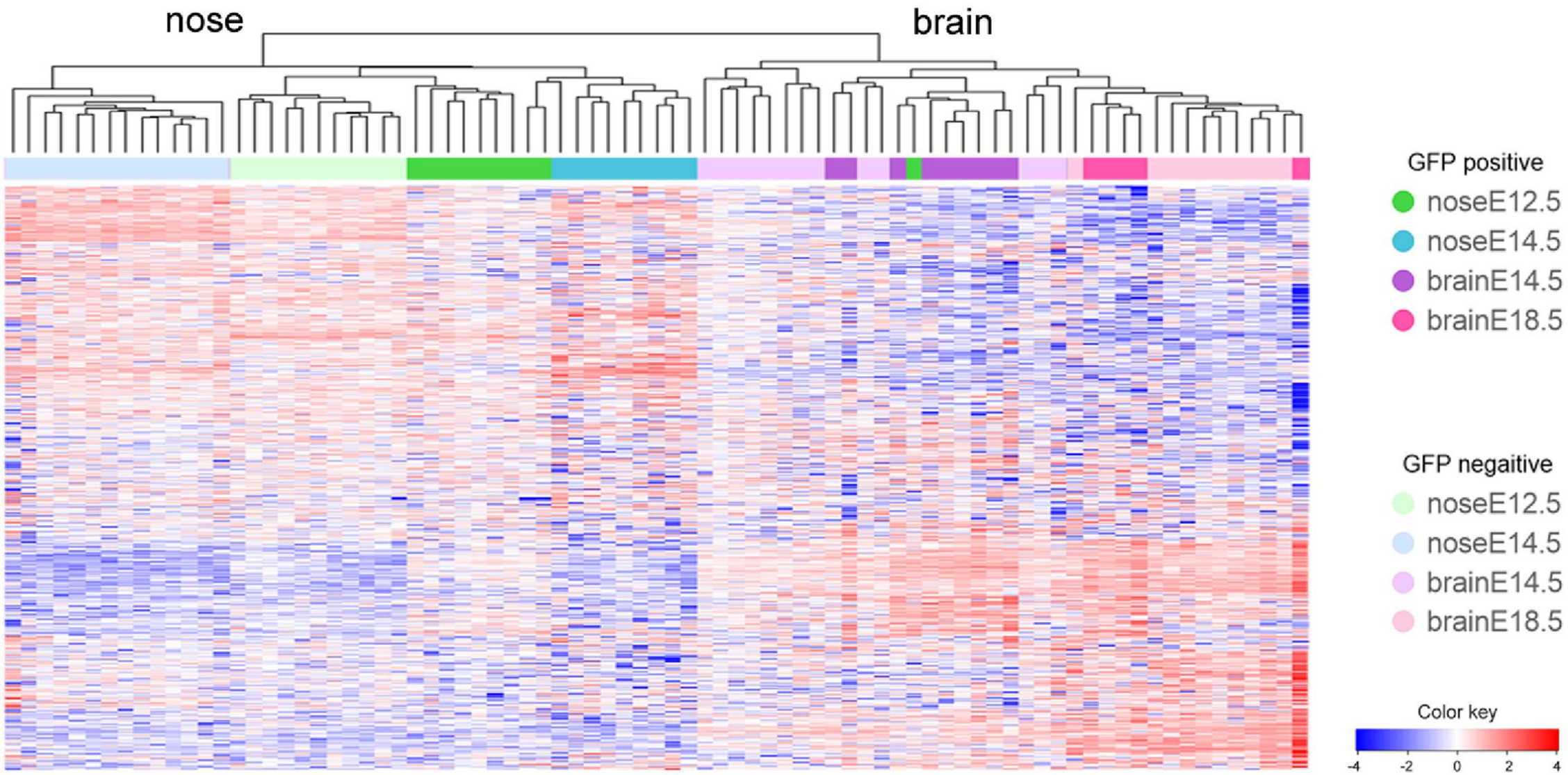
GnRH neuron migration

RNAseq profiling GnRH neuron migration

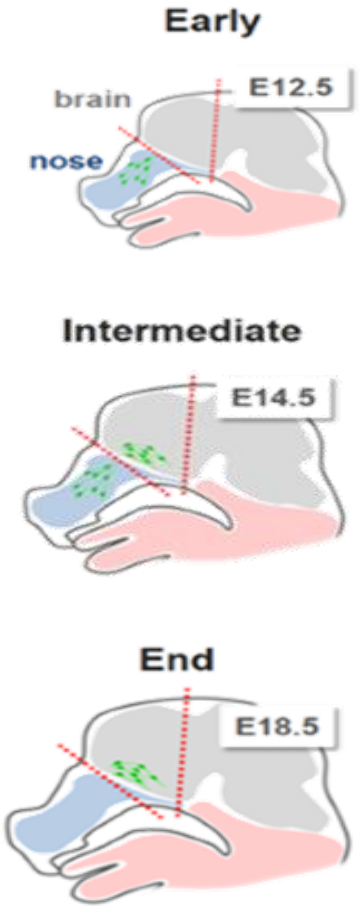


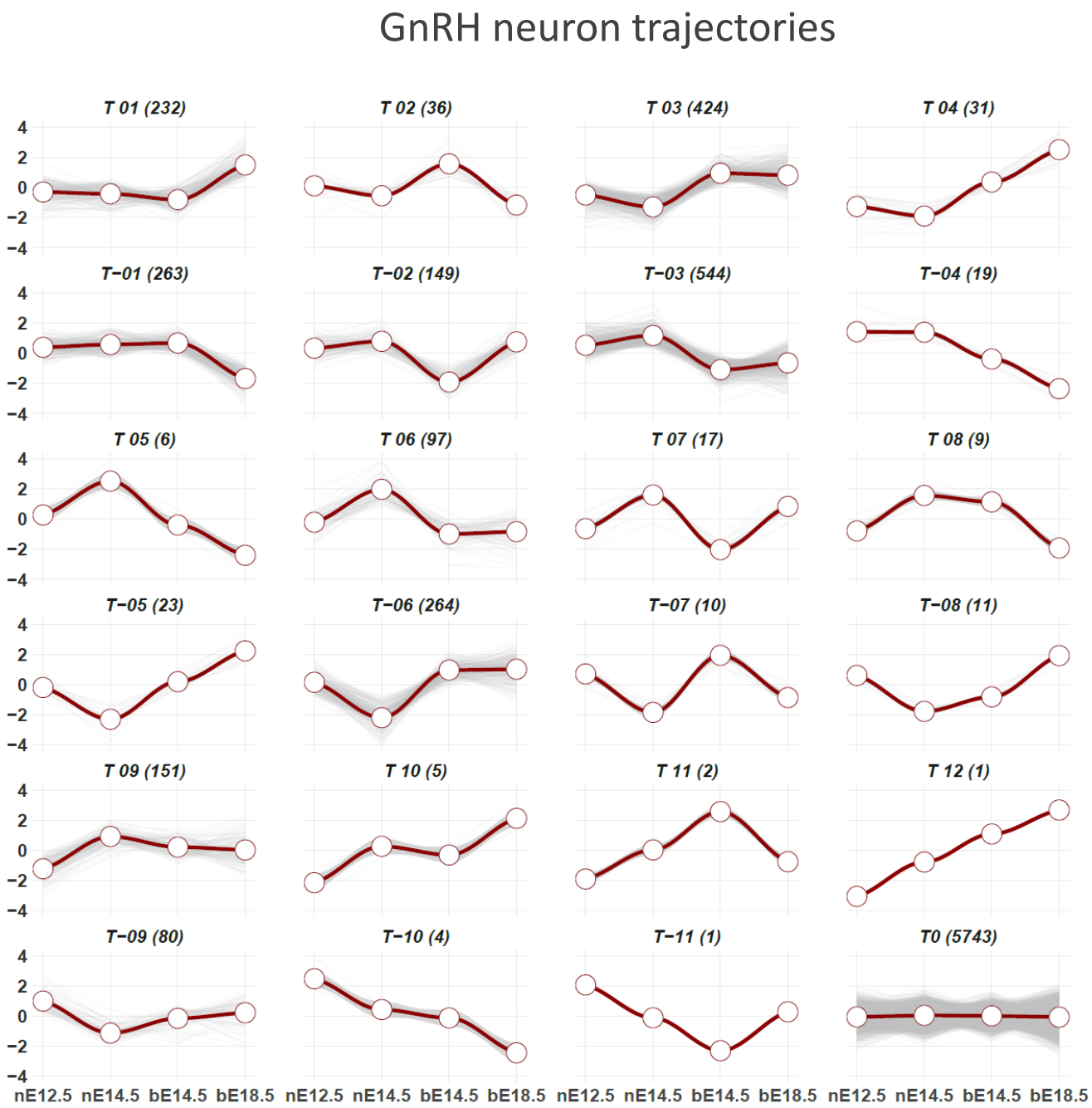
Theragnostics, in review

Migrating GnRH neurons experience deep transcriptomic changes



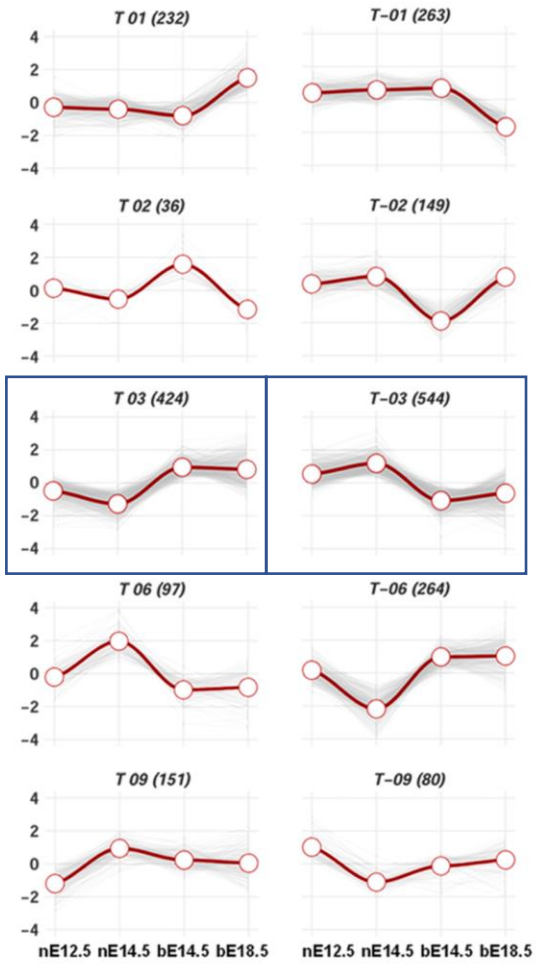
GnRH neurons display distinct gene expression trajectories

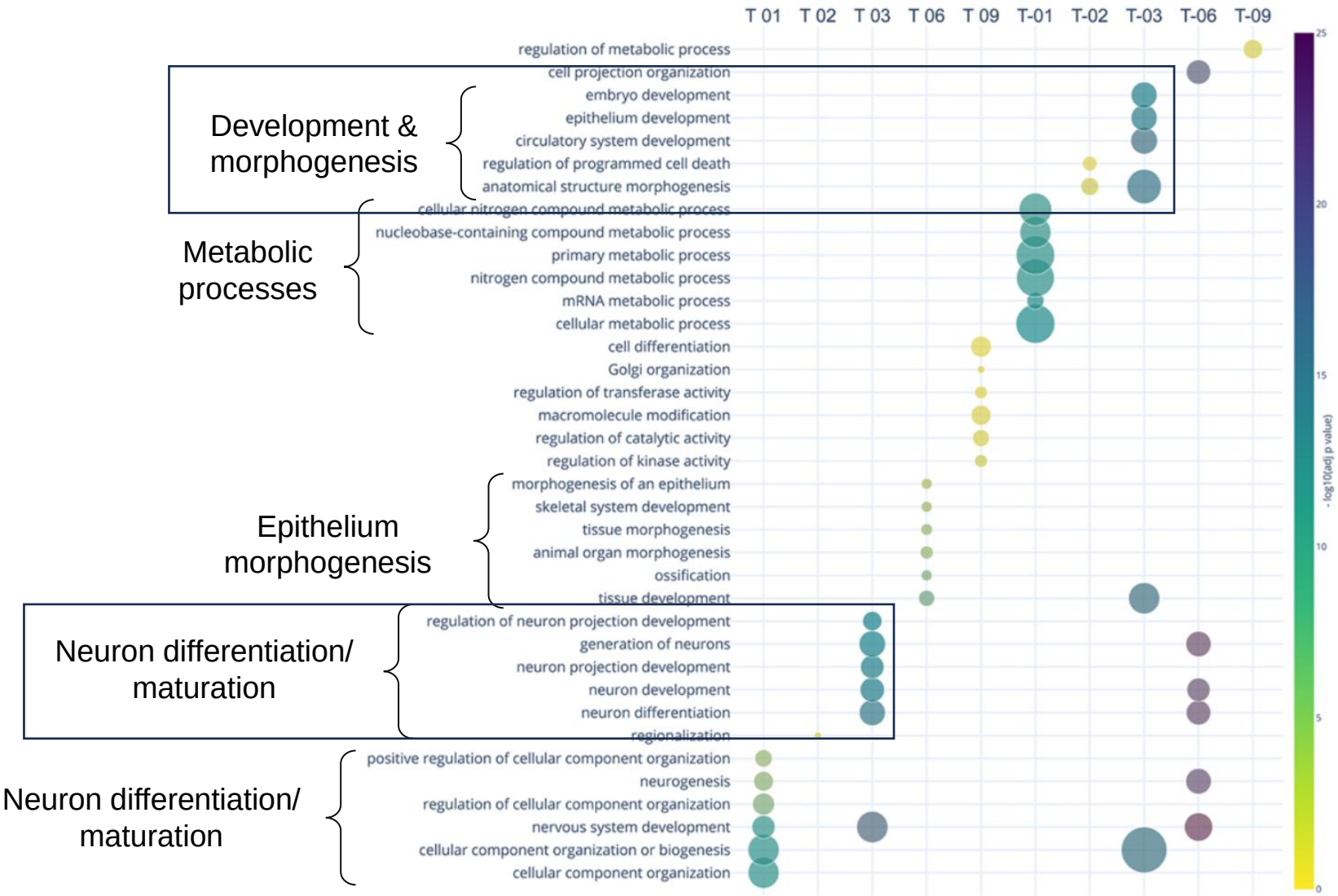




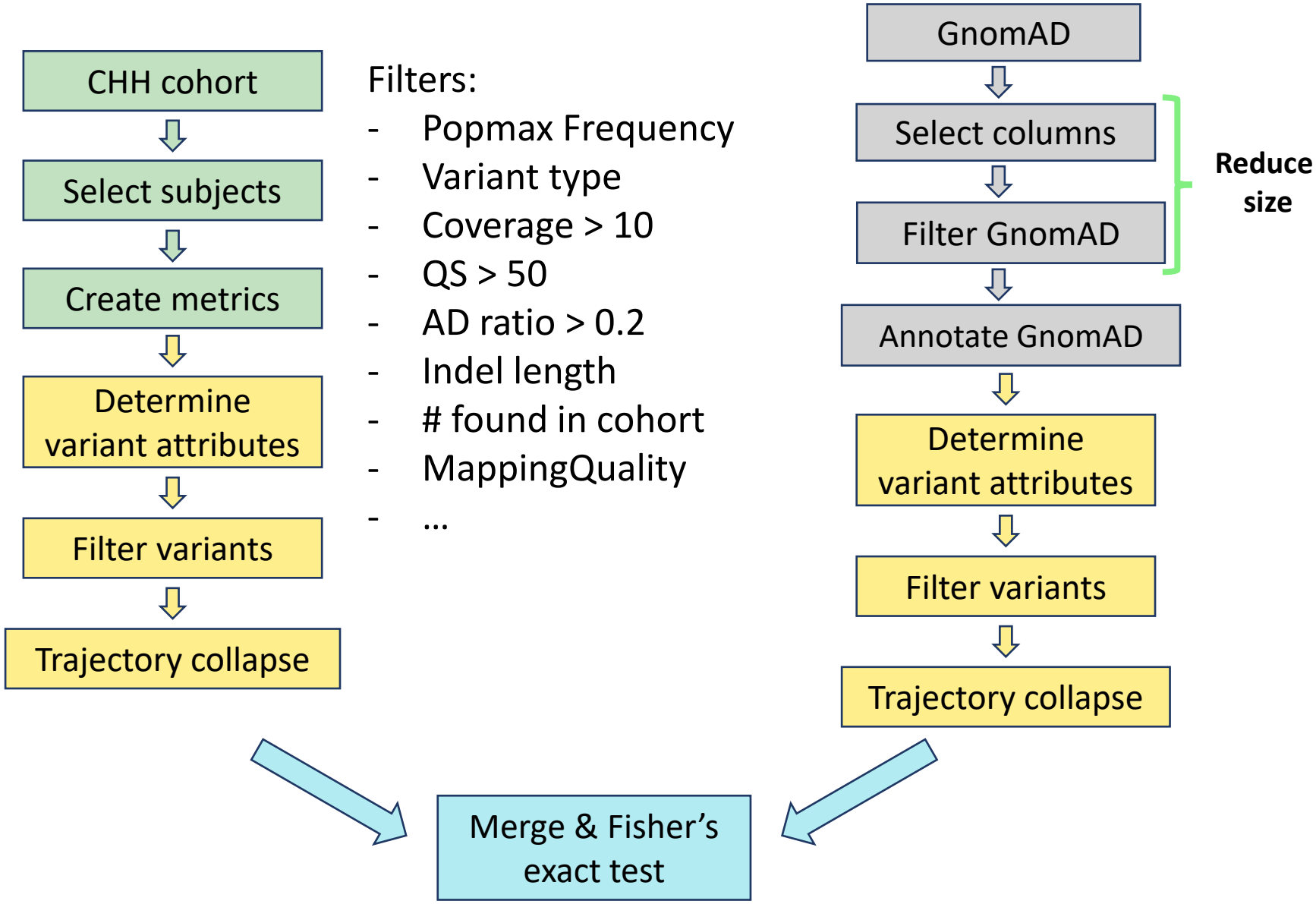
GnRH neuron trajectories associate with distinct biological programs

GnRH neuron trajectories

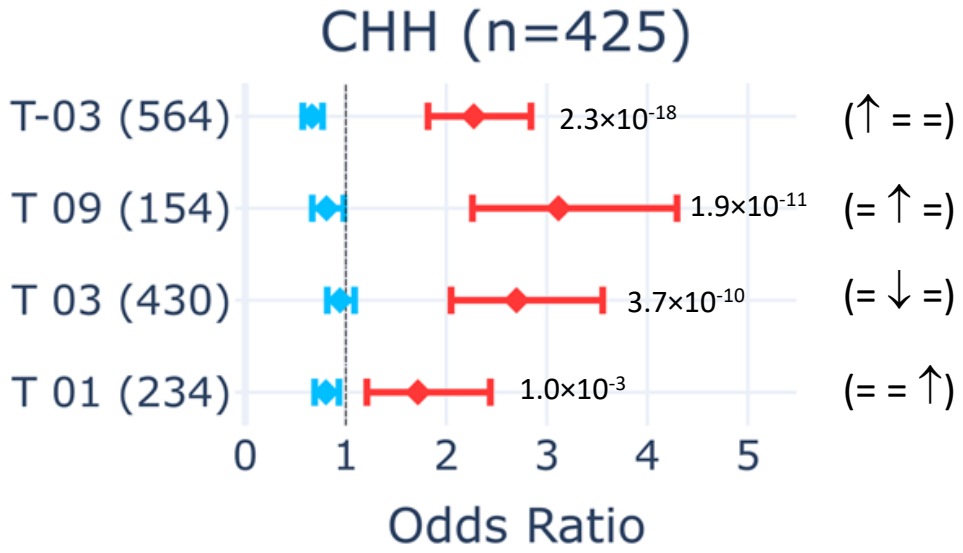




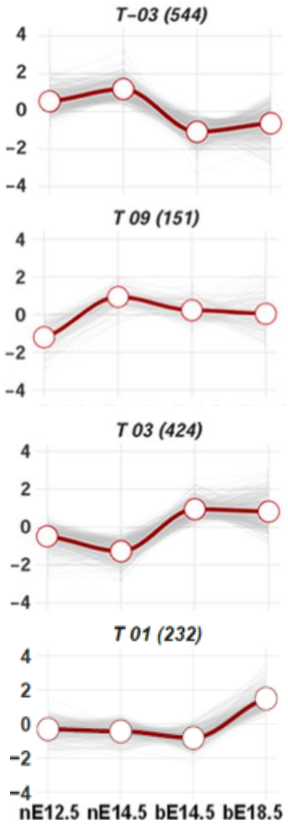
Burden Testing



Trajectories have different genetic burden in CHH



Variant Type
- loss-of-function
- synonymous



Likely pathogenic variants

- Rare (MAF < 0.01%)
- Loss-of-function variants (Stop gain, FS, splice)

Summary

- GnRH neurons display gene expression trajectories associated with distinct biological programs
- Crossing trajectories with human genetics uncovered specific expression patterns involved in the etiology of CHH

Perspectives

- New gene discovery using de novo approach (e.g. BAF complex)
- Burden testing in the all population
- IPCs Cells for models

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