Hypothalamic disorders and their management

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Functions of the Hypothalamus

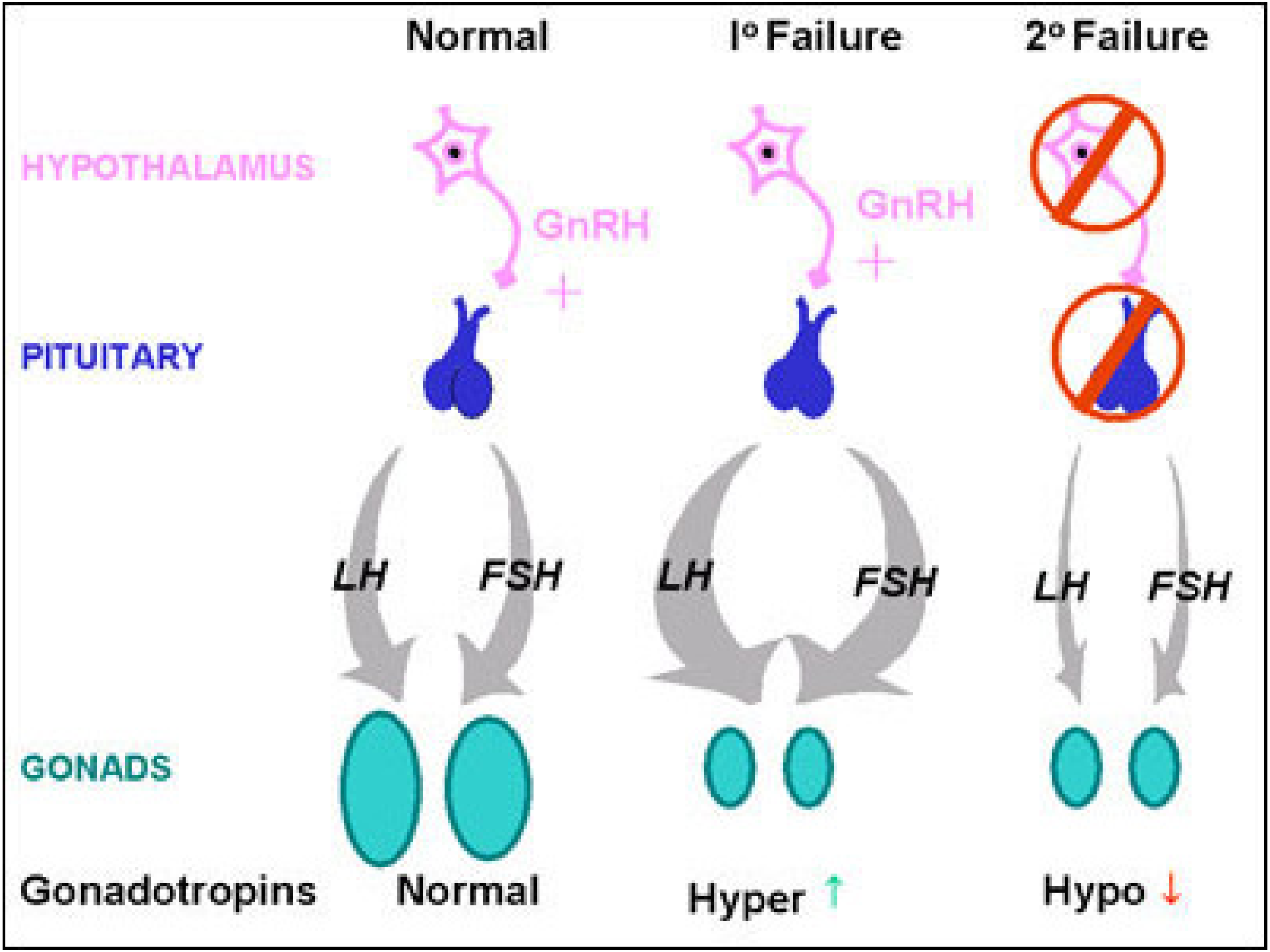
- **Growth hormone releasing hormone (GHRH) and somatostatin secretion (GH)**
- **GnRH secretion (LH/FSH)** →
- **TRH secretion (TSH)**
- **CRH/ADH secretion (ACTH)**
- **DA release (PRL)**
- **Oxytocin: uterine contraction, let-down reflex**
- **Vasopressin (ADH): regulation of water balance**
- **Temperature regulation**
- **Control of appetite, blood pressure, cutaneous blood flow**
- **Control of circadian/ultradian hormonal rhythms (suprachiasmatic n)**

Cell types in the hypothalamus

- **Neurons 10%** – synthetic machinery within the perikaryon, and products transported in energy dependent manner to axon and dendrites neurosecretion
- **Glia 90%-** astrocytes . Paracrine regulators of neurons, + capillary feet cover 85% of capillaries (BBB): secretion of IGF1,TGFα/β (stimulate GnRH gene expression), type 2/3 deiodinase, steroidogenic enzymes, glutamate synthase activity. **Oligodendrocytes** form myelinated covering of axons

Hypothalamic GnRH system

- About 2,000 dispersed GnRH neurons in human brain in arcuate nucleus
- Periodicity of GnRH secretion intrinsic property of these neurons (60-90min)
- GnRH neurons express GnRHR1, through which a self priming effect is demonstrable (upregulation of receptor expression)
- Activation of LH/FSH subunit gene expression in gonadotrophs governed by intermittency of GnRH neuronal inputs to gonadotrophs.
- In addition to terminals on median eminence, GnRH projections to limbic system and circumventricular organs


Normal
I° Failure
2° Failure
HYPOTHALAMUS
GnRH
+
GnRH
+
PITUITARY
LH
FSH
LH
FSH
LH
FSH
GONADS
Gonadotropins
Normal
Hyper ↑
Hypo ↓

GnRH pulse generator

- Discrete pulsatile GnRH release from the medial basal foetal hypothalamus (at 20-23 weeks gestation) at 60 min intervals and 60-90 min for adult (Rasmussen et al 1989)
- Pulse generator thought to be intrinsic property of GnRH neurons

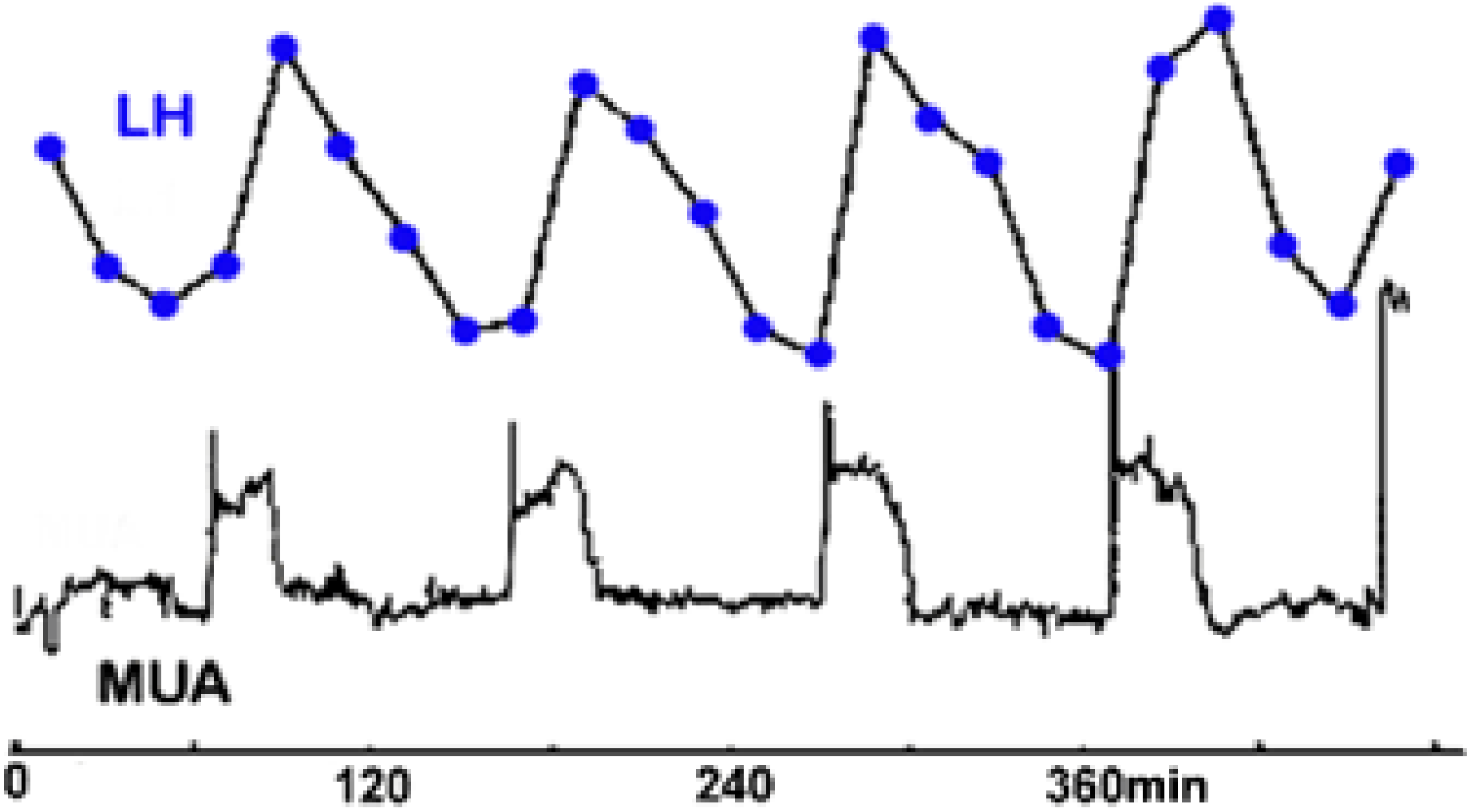


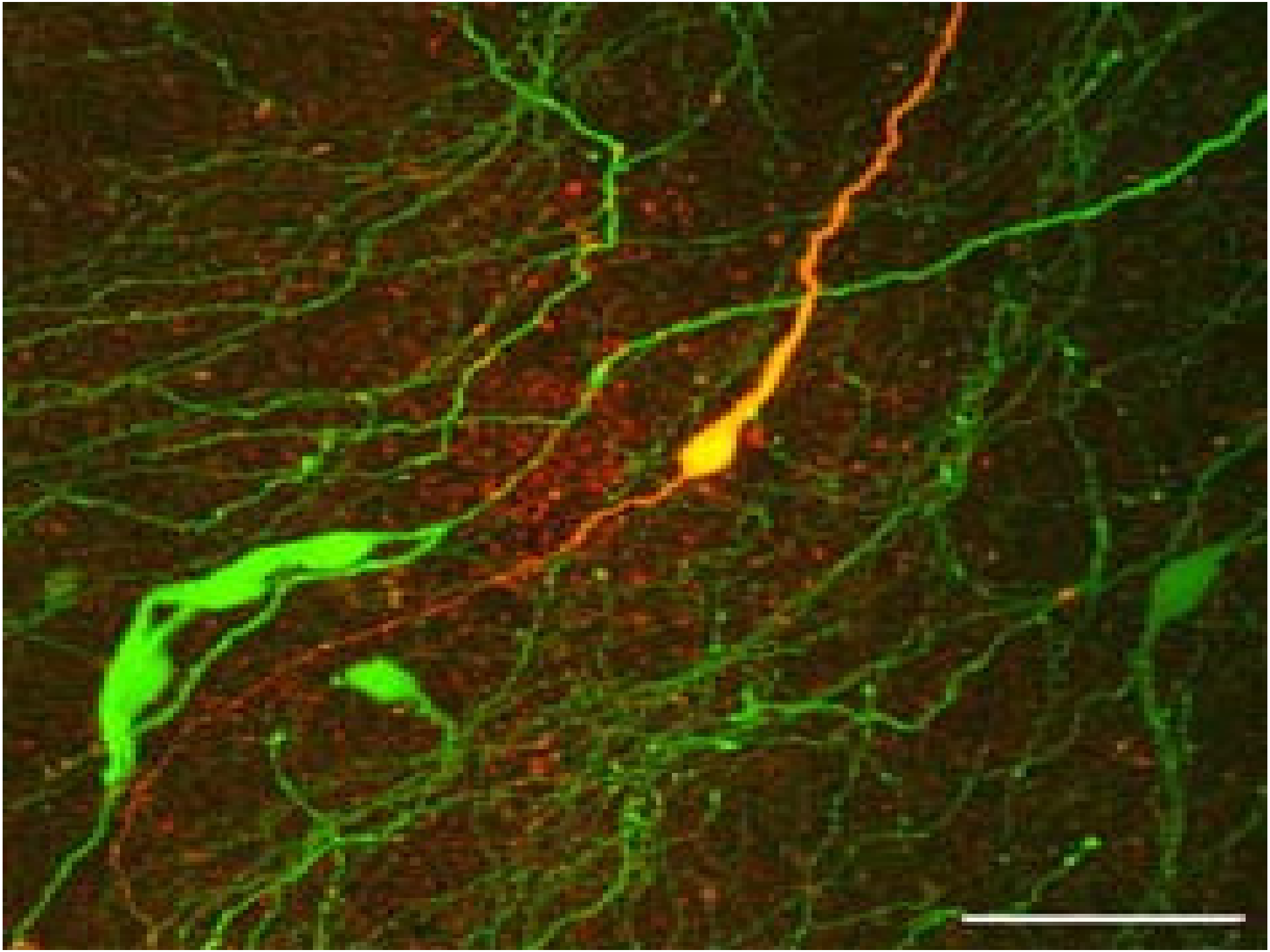
Figure 7. Pulses of LH, the downstream target of GnRH signaling, directly follow episodes of neural activity from the hypothalamus, detected as multiunit activity (MUA) from an implanted electrode. Adapted from (103) with permission of the S. Karger AG, ©1984.

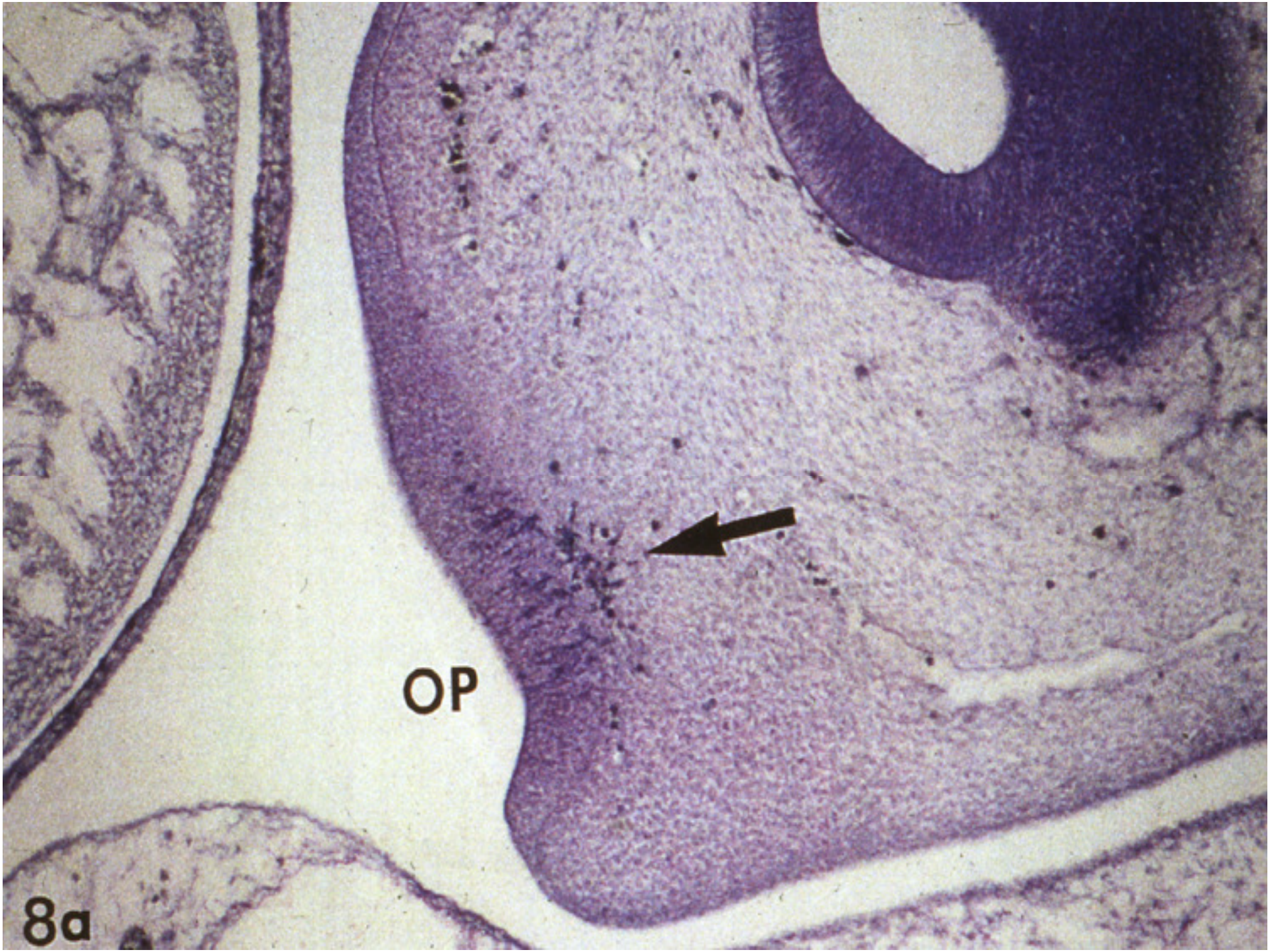
Modulators of GnRH neuronal secretion (GT-1 cell model)

- GnRHR1 receptor: activation leads to rapid dose dependent Ca^{++} and GnRH release
- Opioids: inhibitors of GnRH release, attenuating stimulatory action of α-adrenergic and dopaminergic inputs
- NMDA and $GABA_A$, $GABA_B$ TGF receptors, progesterone
- Leptin , NPY receptors in human FNCB4 cells (embryonic GnRH neuroblasts)

Human GnRH neuronal ontogeny

- GnRH immunoreactivity discernible within medial olfactory placode week 5
- GnRH neuronal migration predominantly axonophilic along Nt/Vn nerve complex
- Migration into hypothalamus largely complete by 13 weeks
- Residual GnRH immunoreactivity in OE post-natally in man



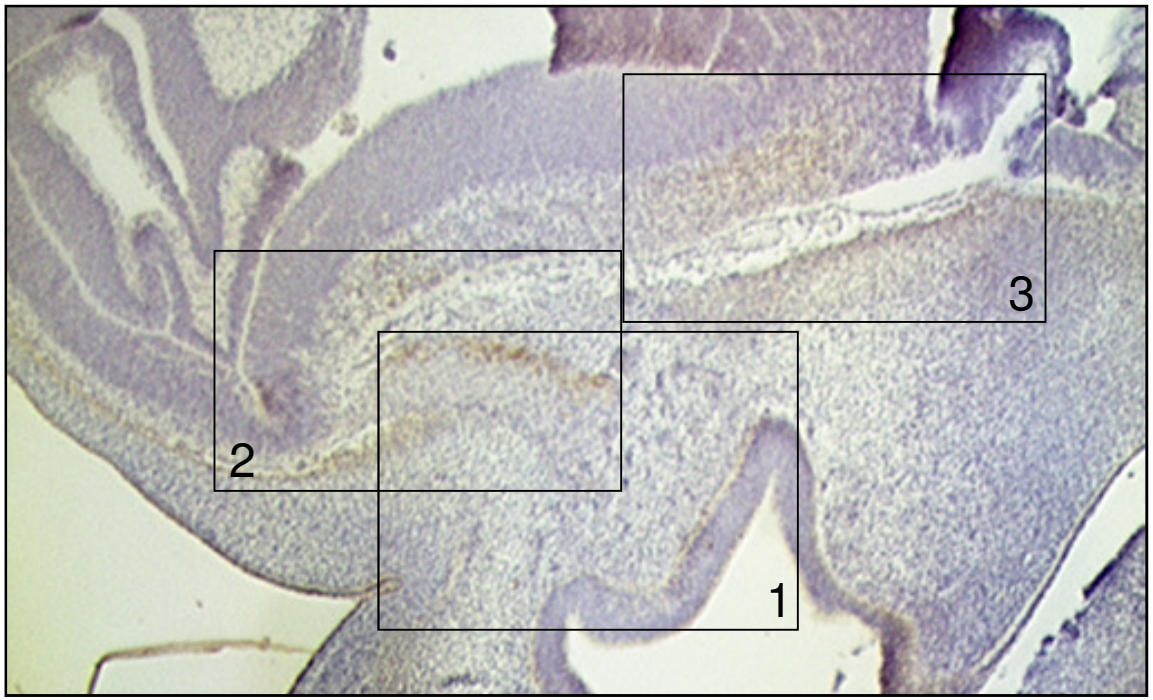


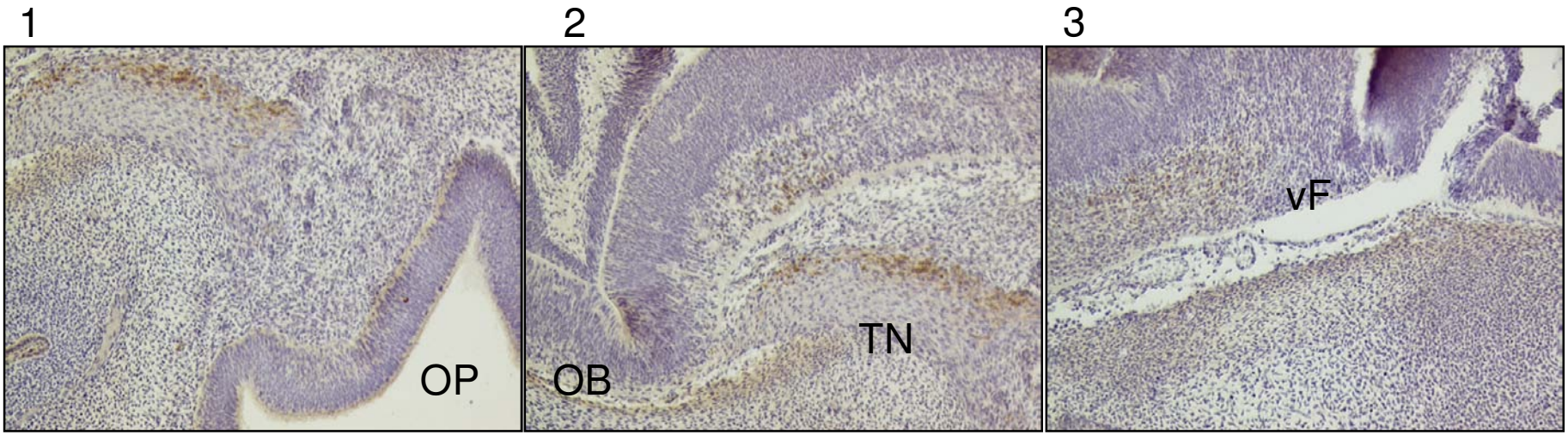
8a

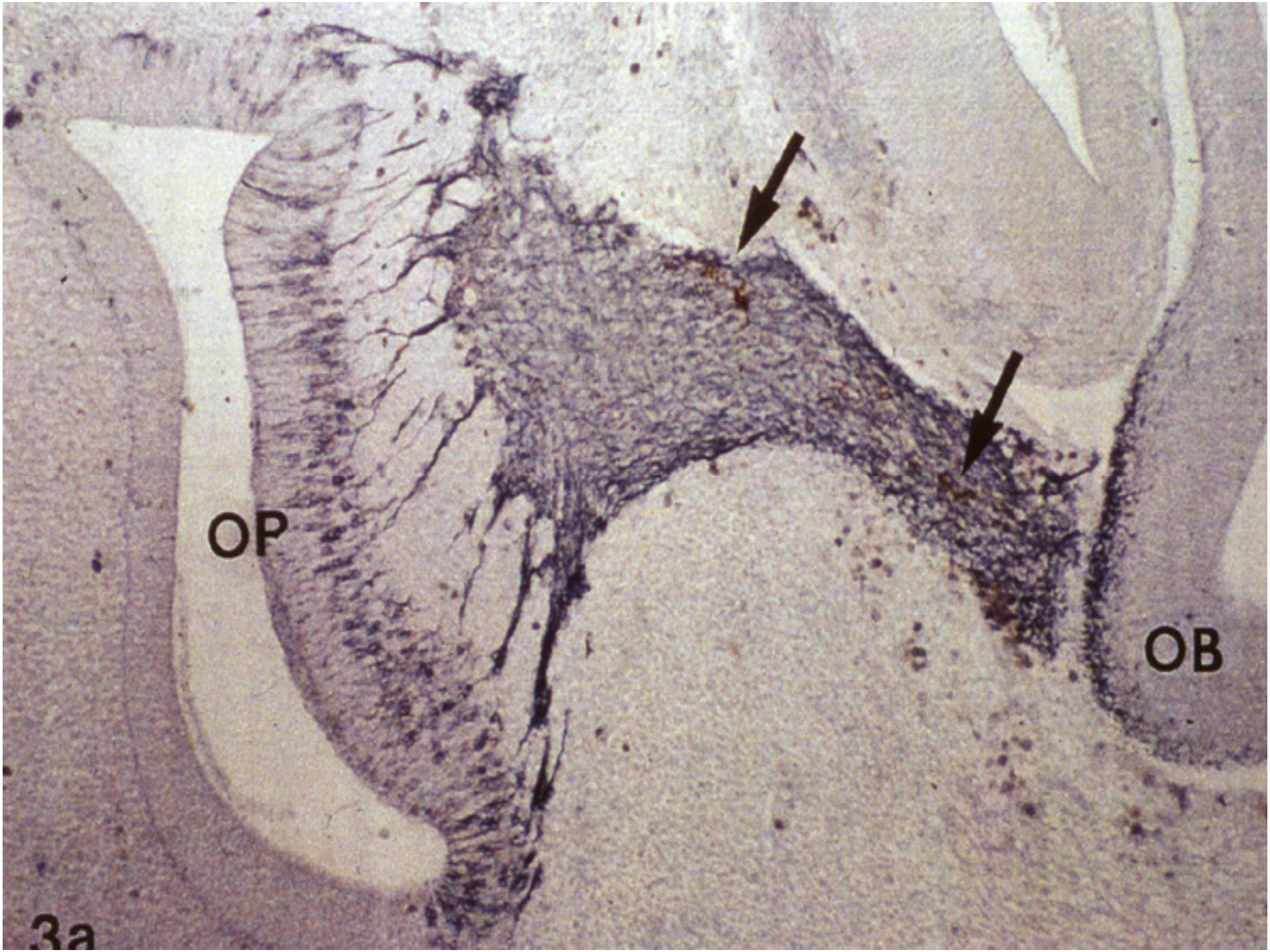
CS 17: CNS

GnRH immunohistochemistry

Sagittal slide



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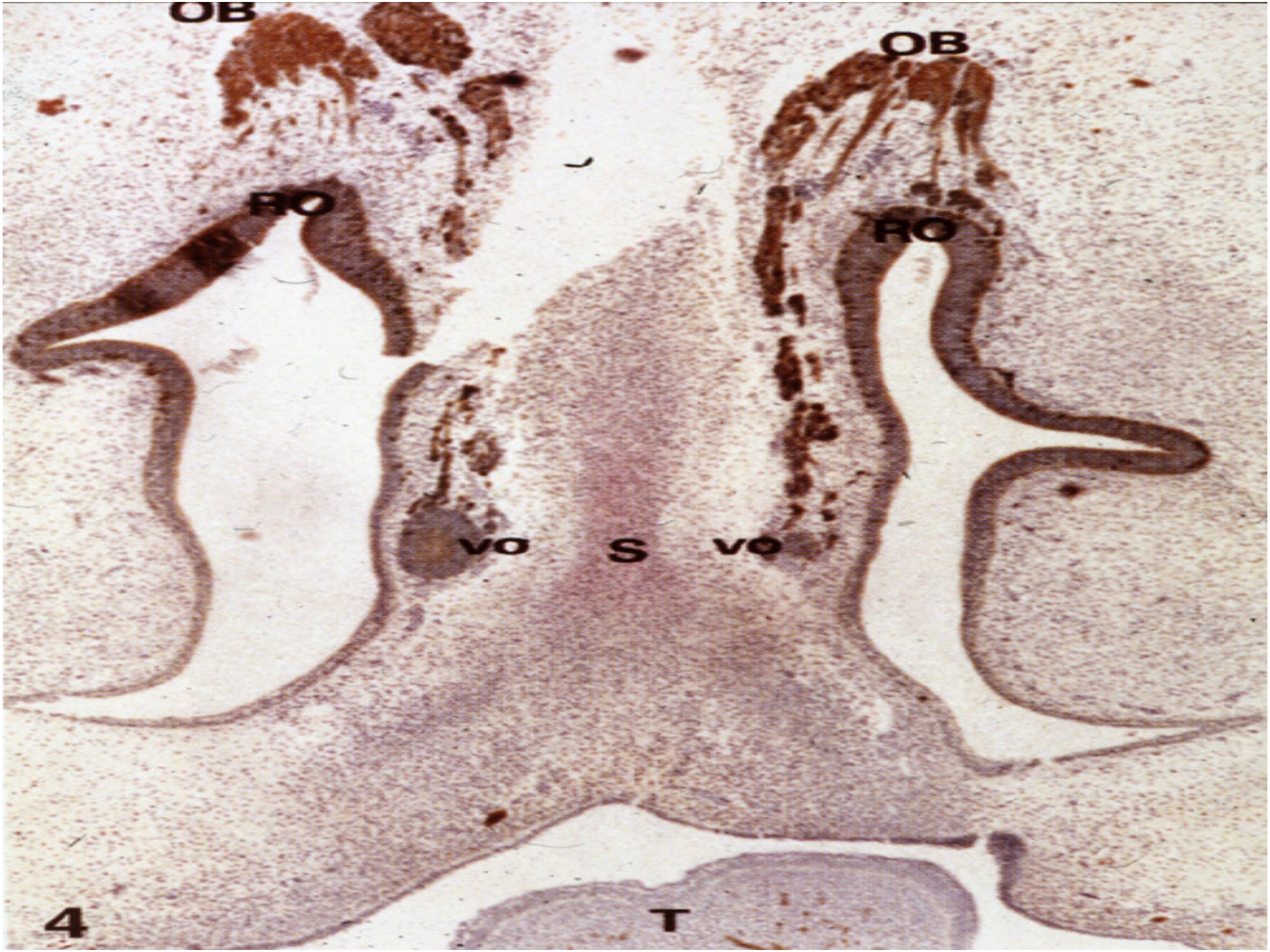
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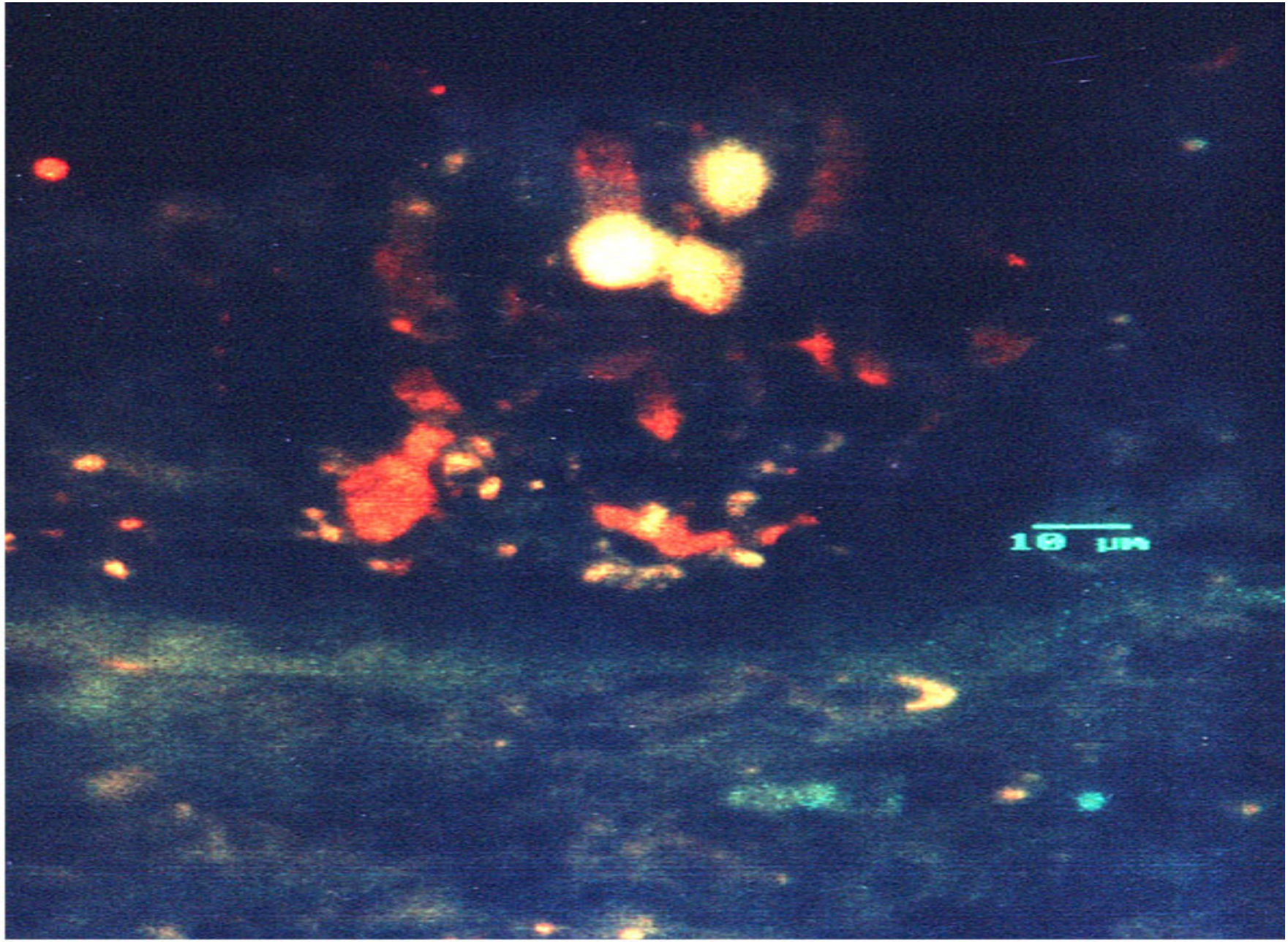
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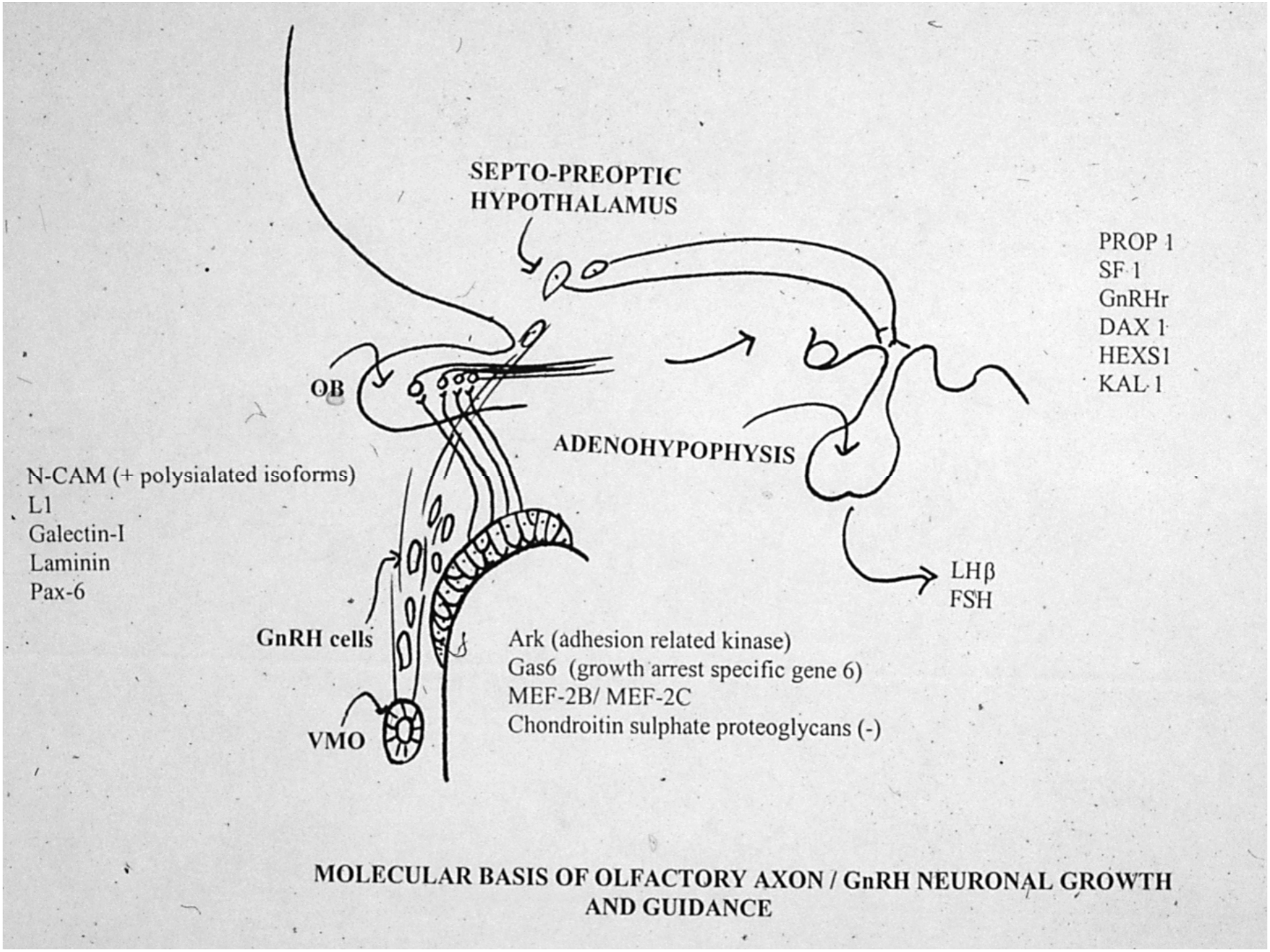
Key questions in GnRH neuronal ontogeny

- How do cells in the nasal placode differentiate into GnRH cells?
- How do differentiated GnRH cells migrate into the CNS?
- What cues determine the final location of GnRH cells in the hypothalamus?
- What cues lead GnRH cells to extend axons to the median eminence?
- What neuroendocrine properties are intrinsic to GnRH neurons at initial differentiation, and later when settled in the hypothalamus?



MOLECULAR BASIS OF OLFACTORY AXON / GnRH NEURONAL GROWTH AND GUIDANCE

Disorders of GnRH secretion

- Congenital: **IHH, Kallmann syndrome**, Prader-Willi syndrome, Bardet-Biedl and related syndromes, leptin deficiency
- Acquired: Stress/ weight related amenorrhoea, excessive exercise (both men and women), 'hypothalamic amenorrhoea', Late onset hypogonadism in males (LOH)

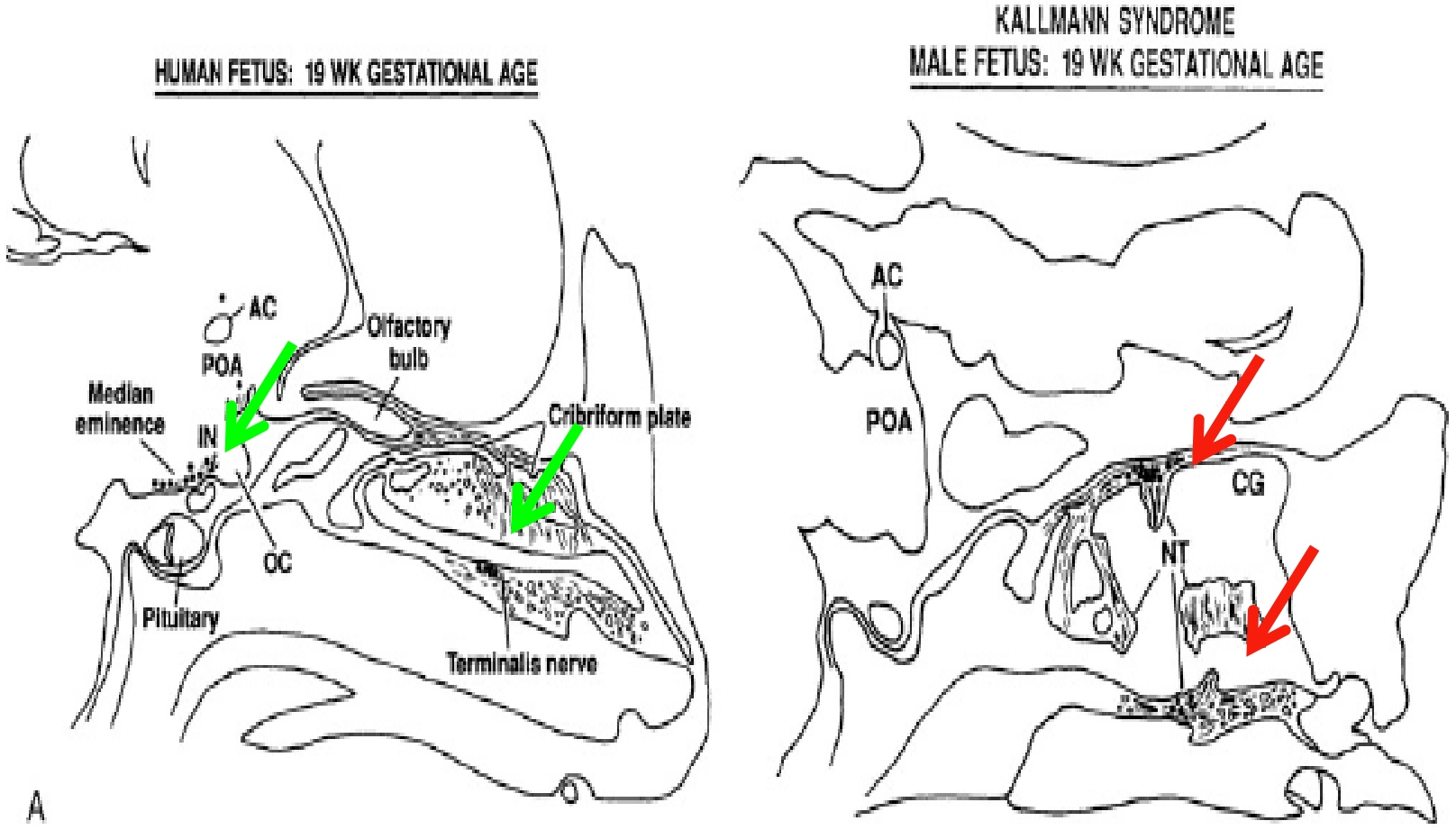
Genes involved in the congenital hypogonadotrophic hypogonadism

- ***KAL-1***
- ***FGFR-1 and FGF8***
- ***GRP-54* and *Kisspeptin***
- ***NELF***
- ***GnRHR, GnRH***
- ***PROKR2 and PROK2***
- ***CHD7 (CHARGE)***
- ***TAC3,TACR3***
- *LHβ*
- *FSHβ*
- *HESX-1*
- *LHX3*
- *PROP-1*
- *SF-1*
- *DAX-1*
- *Leptin*
- *Leptin-R*
- *PC-1*

Modes of inheritance of KS and IHH

- X-linked recessive (eg KAL1)
- Autosomal dominant (KAL2, FGF8), but with variable penetrance
- Autosomal recessive KS (PROKR2, PROK2)
- Oligogenic KS (NELF, GnRHR, FGFR1, KAL1)
- Reversible KS (??extreme pubertal delay)

X-linked Kallmann´s Syndrome

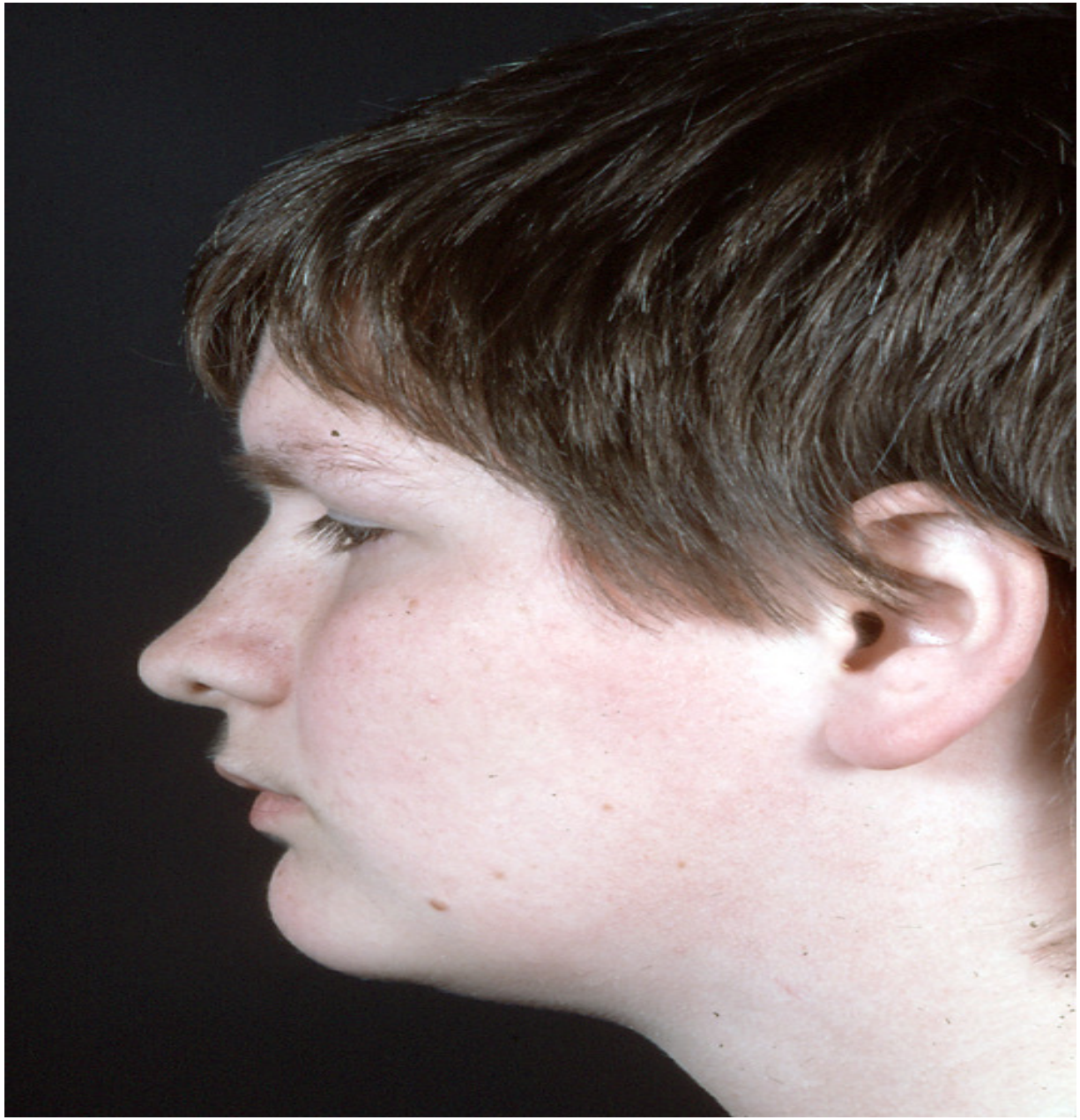


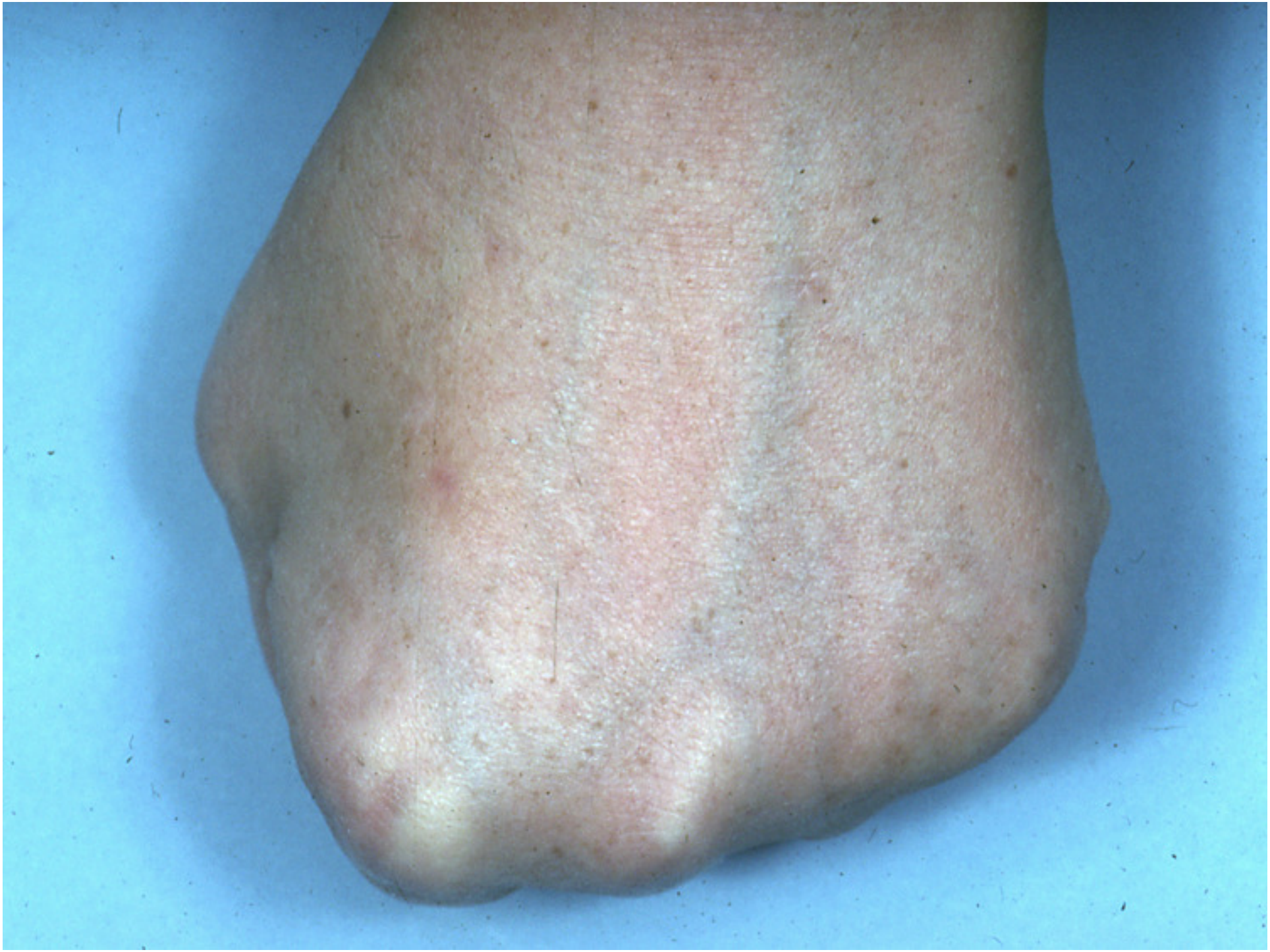
Kallmann's syndrome

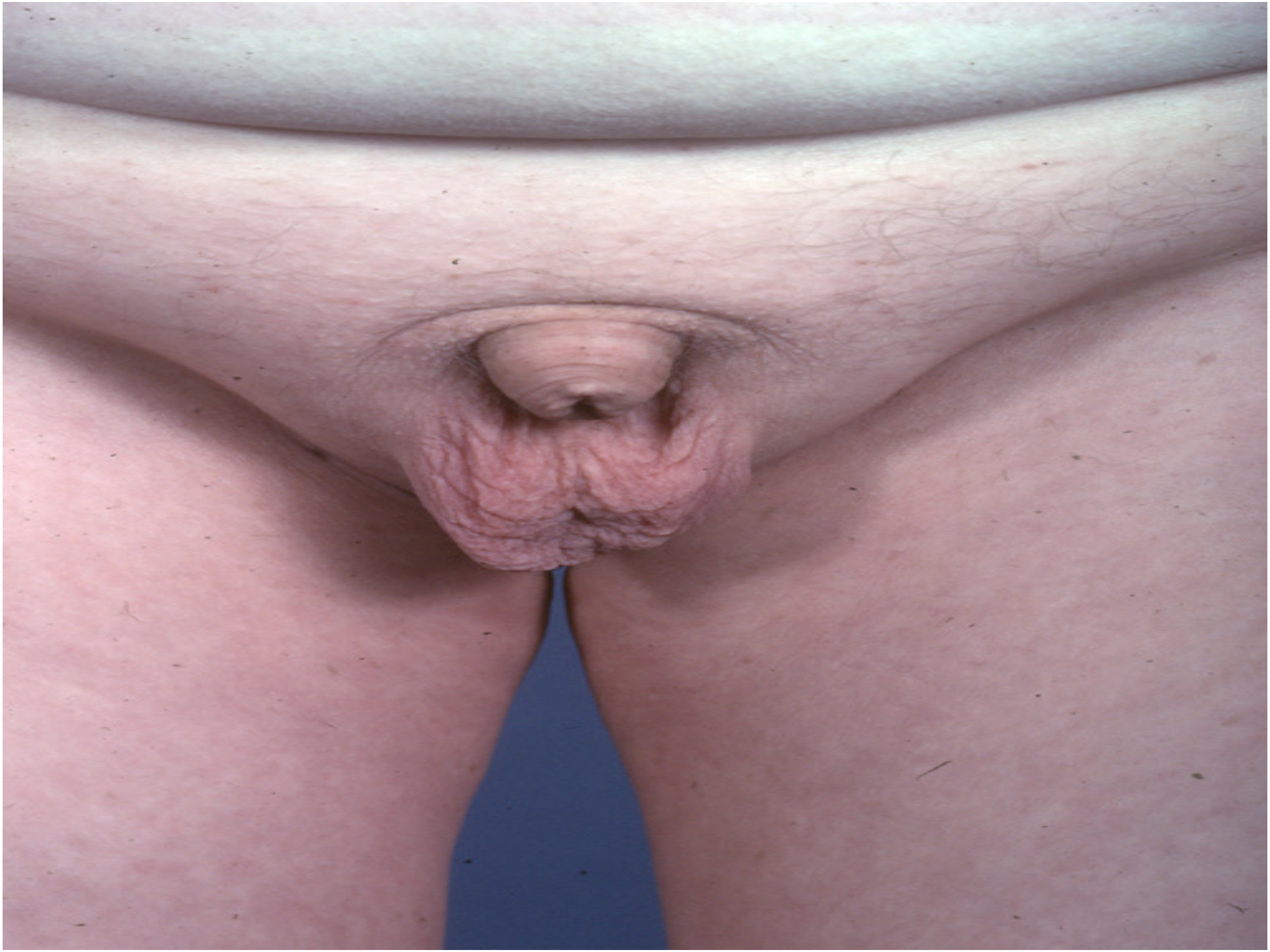
- Hypogonadotrophic hypogonadism with anosmia
- Usually sporadic
- 1 in 8,000 males, 1 in 40,000 females
- X-linked and autosomal dominant / recessive modes of inheritance
- XKS with contiguous gene syndrome (Xp22.3, with steroid sulphatase deficiency)
- Autosomal dominant KS can occur as a 8p11 contiguous gene syndrome

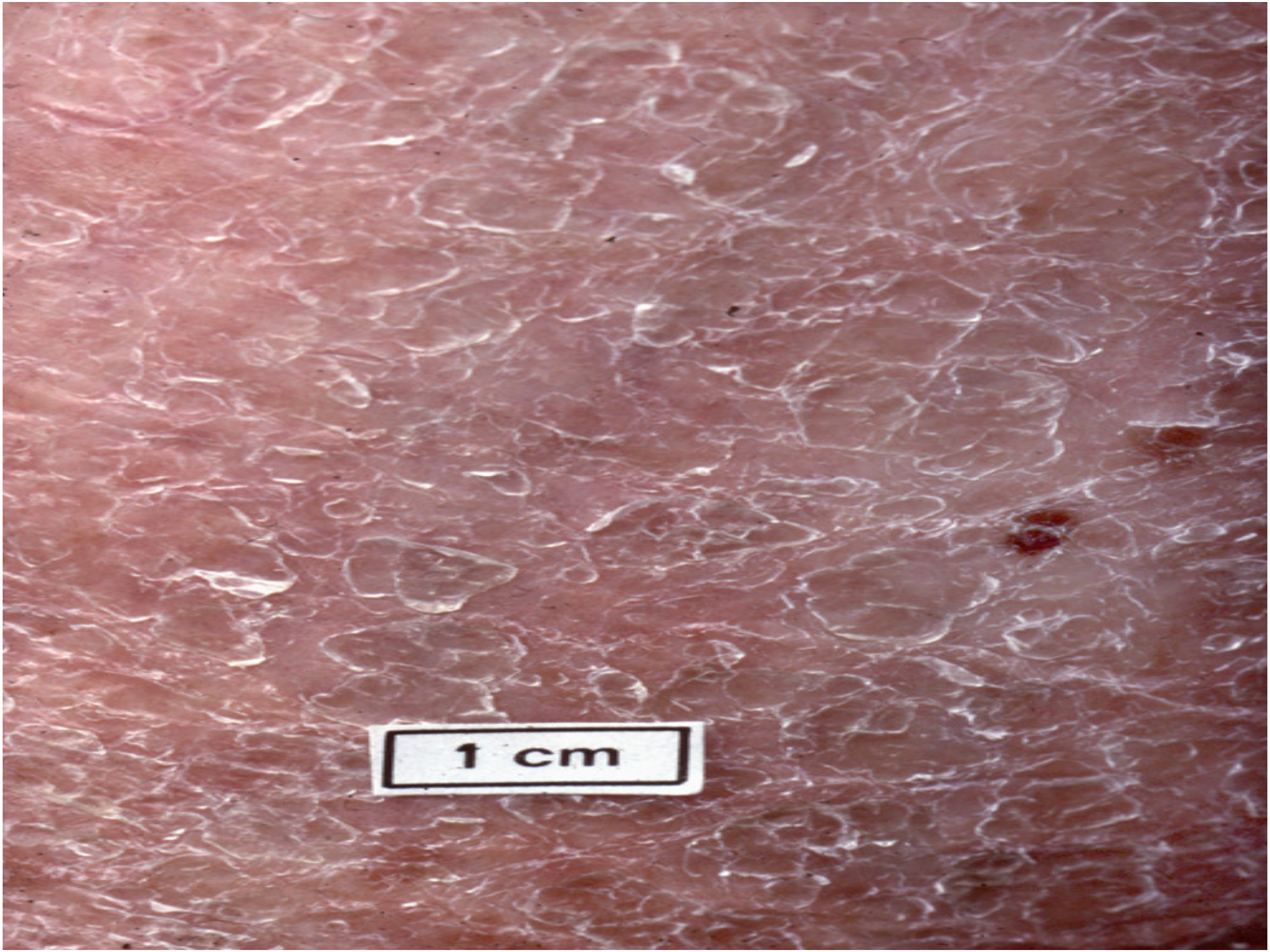
Clinical presentation of KS

- IHH and anosmia
- Delayed or arrested puberty
- Cryptorchidism
- Micropenis
- Infertility
- Associated abnormalities-synkinesis (XKS:ADKS), cleft lip and palate (AKS),midline cranial dysplasias, neurosensory deafness, renal agenesis (XKS), bony abnormalities, coloboma, choanal atresia, CVS abnormalities



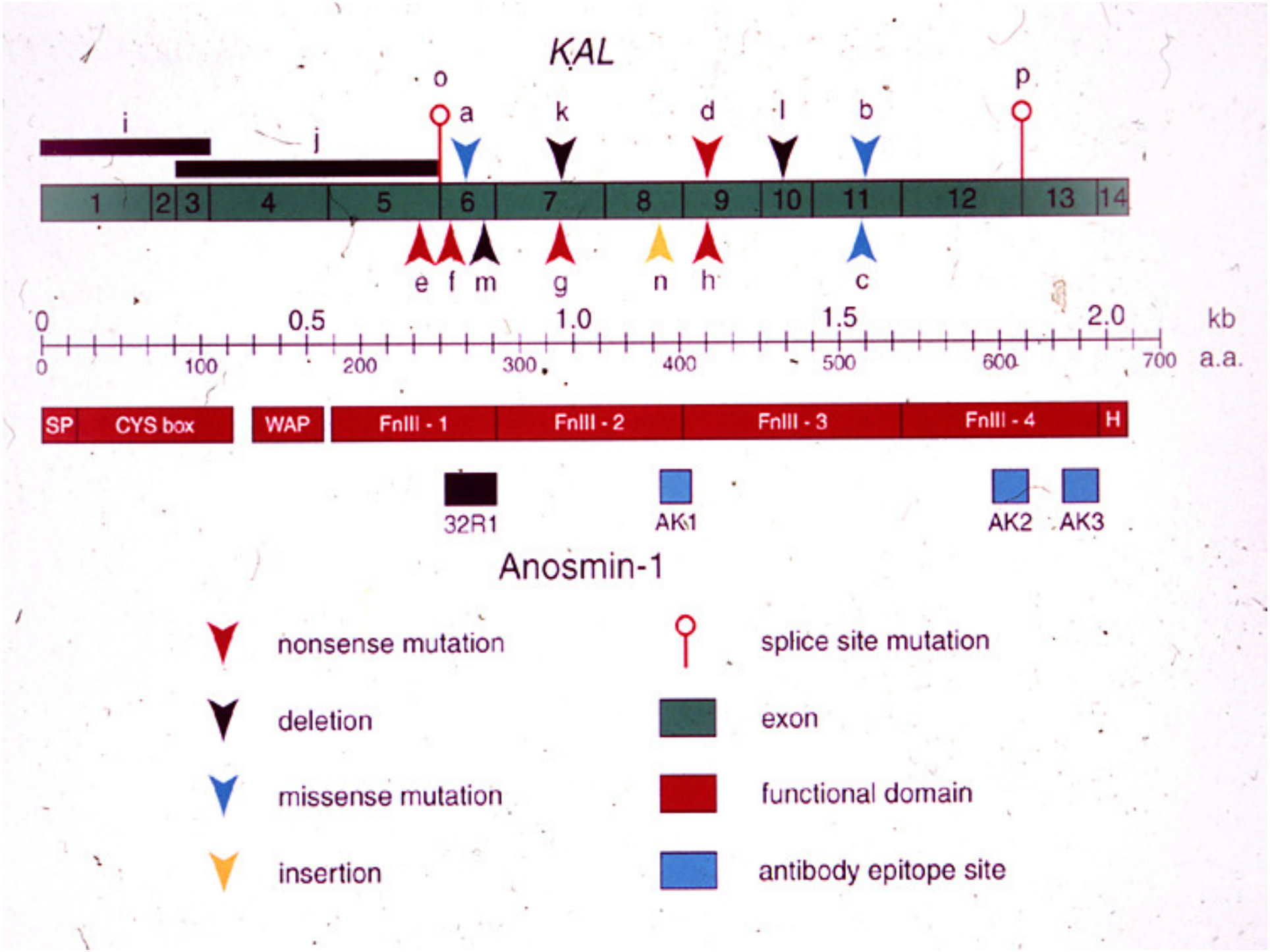





1 cm

Structure of anosmin-1

- Modular protein of 680aa
- 6 N-linked glycosylation sites
- Cysteine rich N-terminus box
- N-terminal four disulphide core (WAP) motif
- 4 contiguous fibronectin type III repeats
- Multiple HS binding motifs
- 11 basic residues at C terminus
- Orthologues present in *D. melanogaster*, *C.elegans*, chick, quail,zebra fish, macaque: high degree of conservation of WAP/FnIII-1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AK3
Anosmin-1
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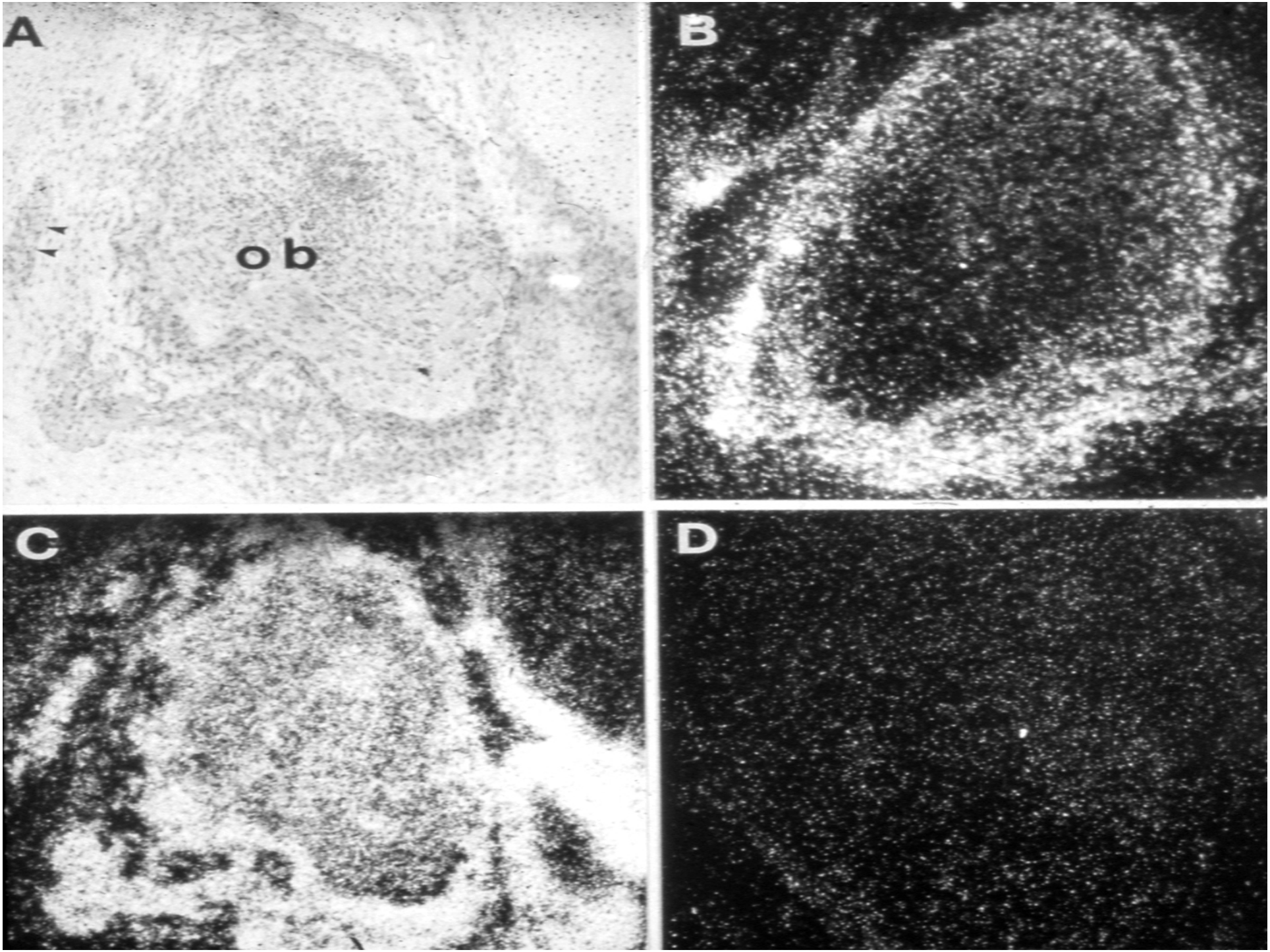
exon
functional domain
antibody epitope site

Spatiotemporal *KAL-1* and anosmin expression during human development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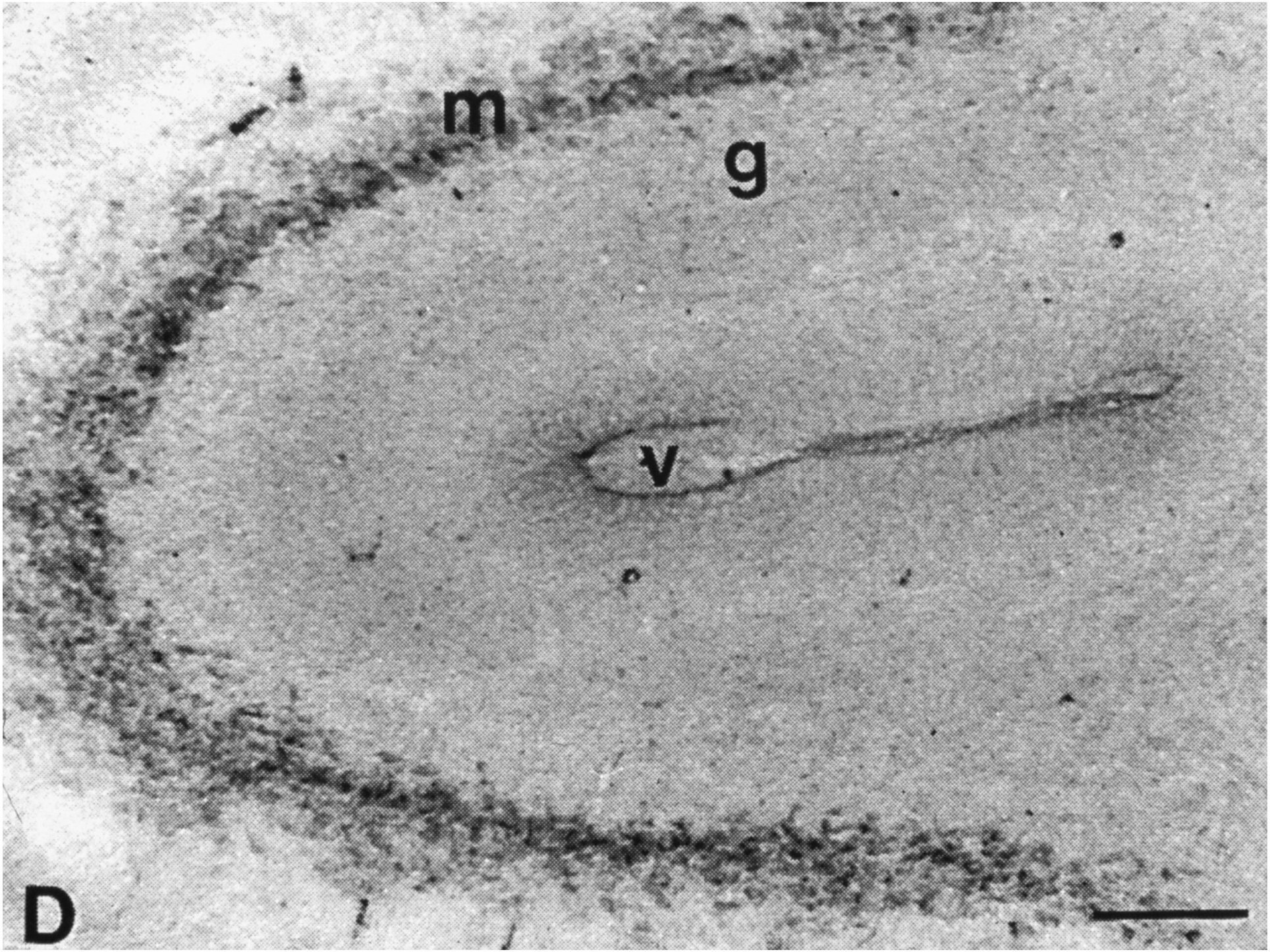
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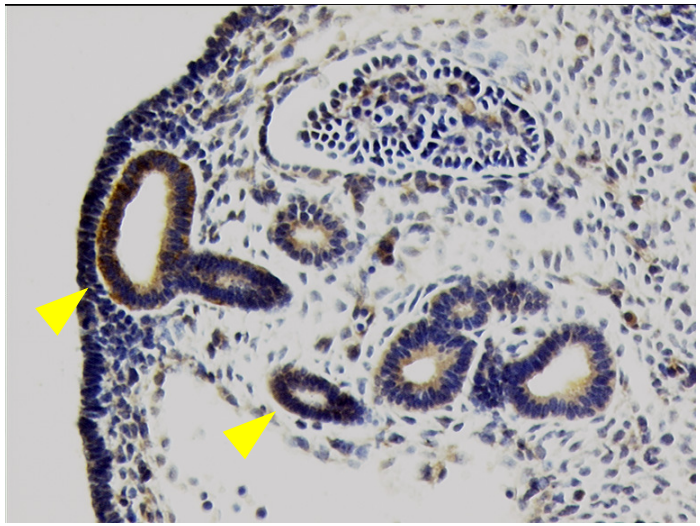
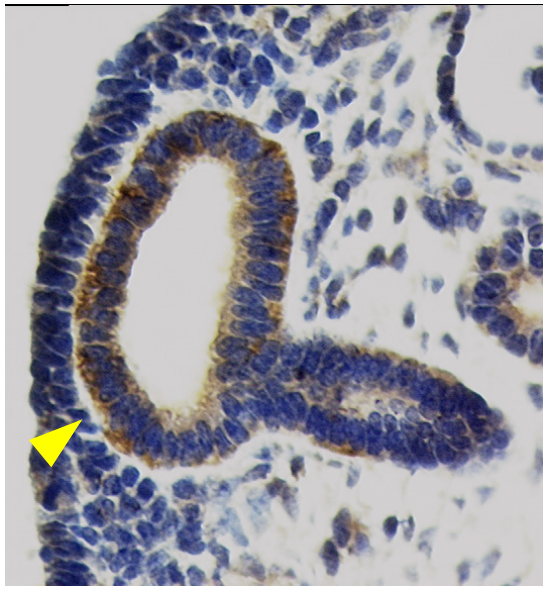
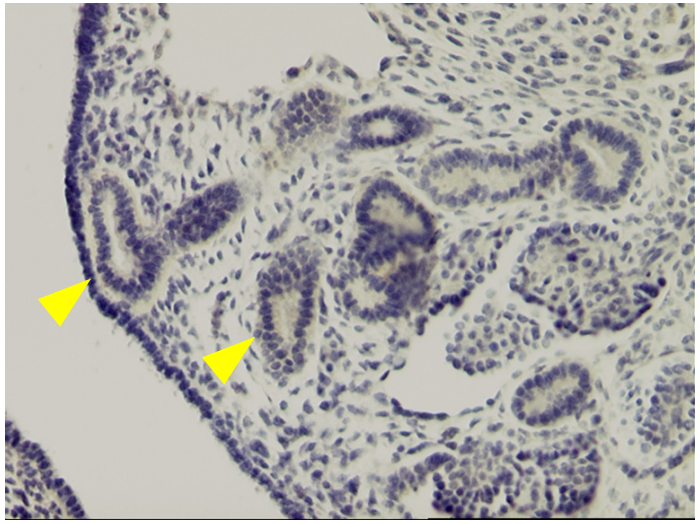
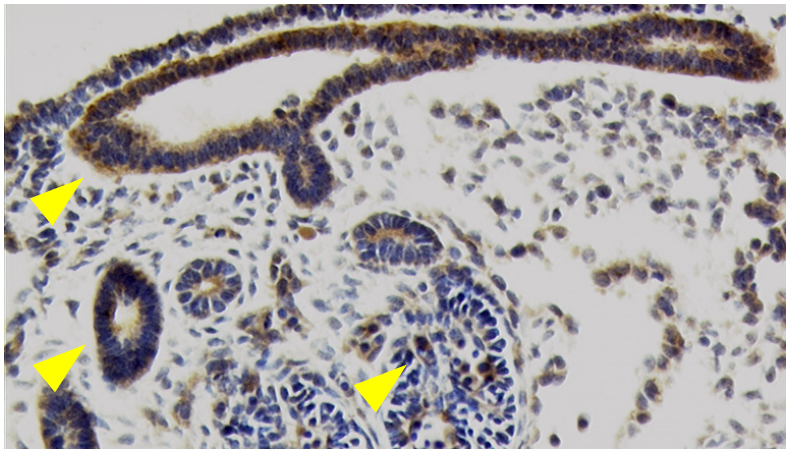


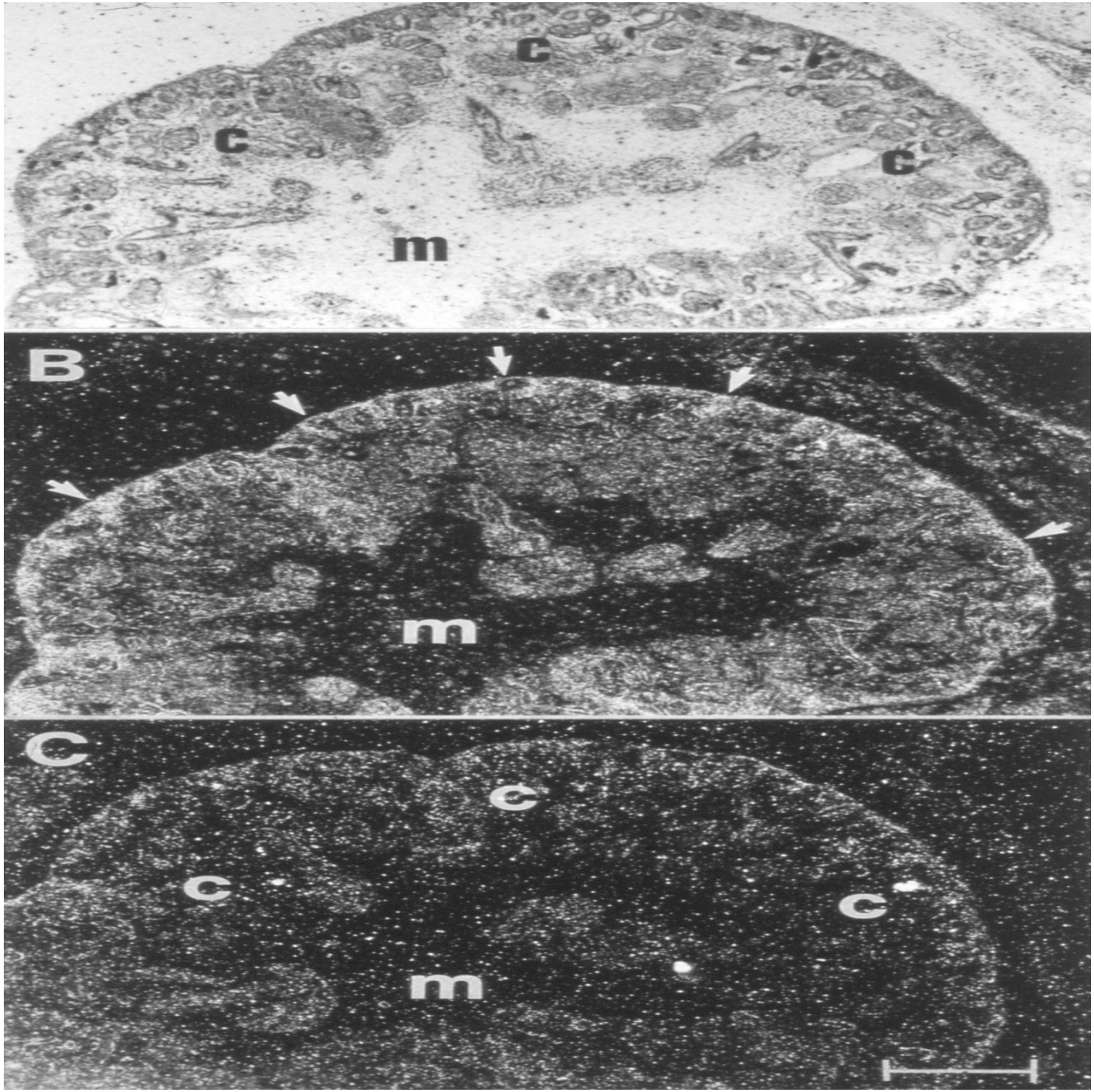






CS 15

Mesonephric ducts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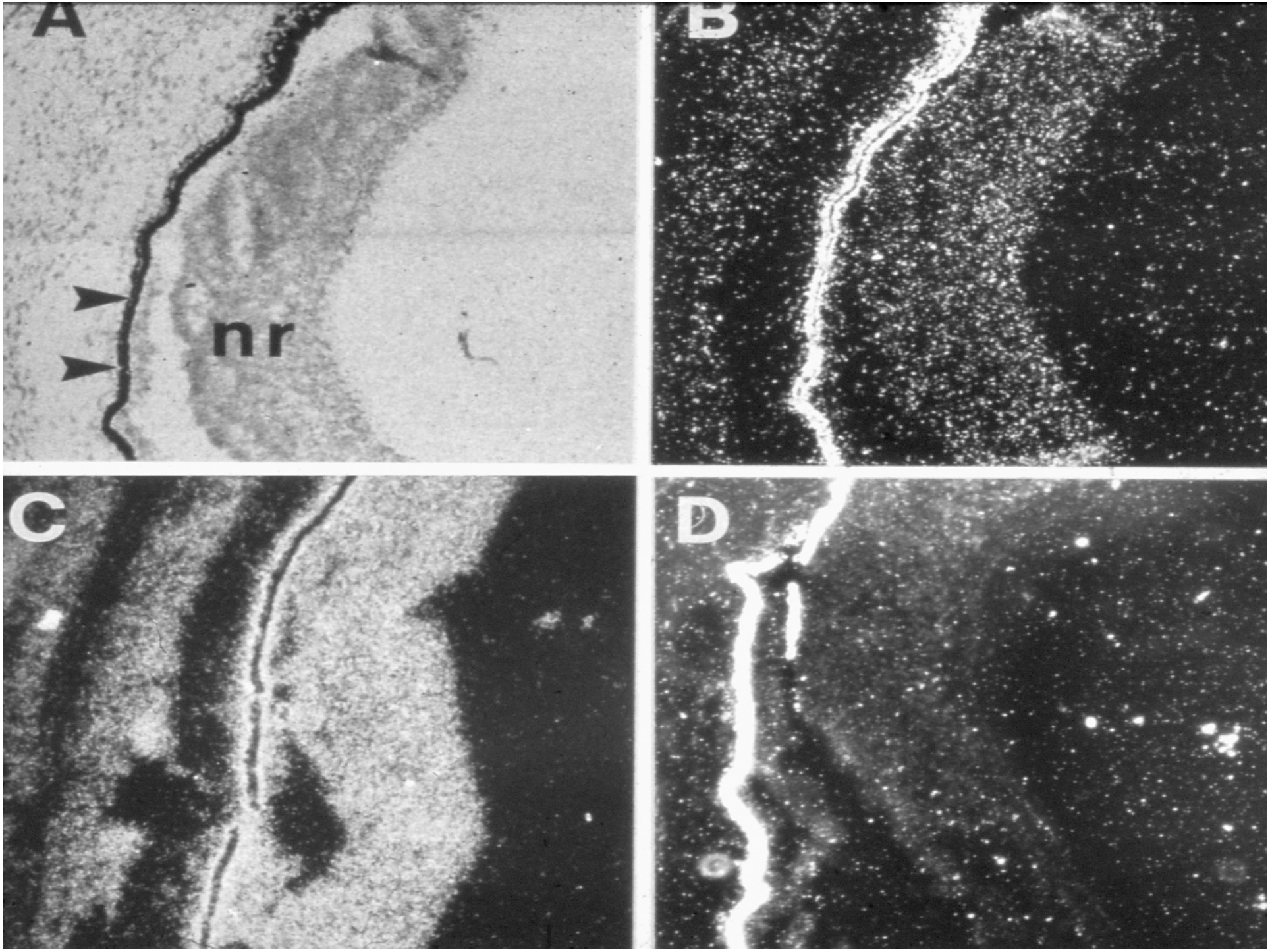
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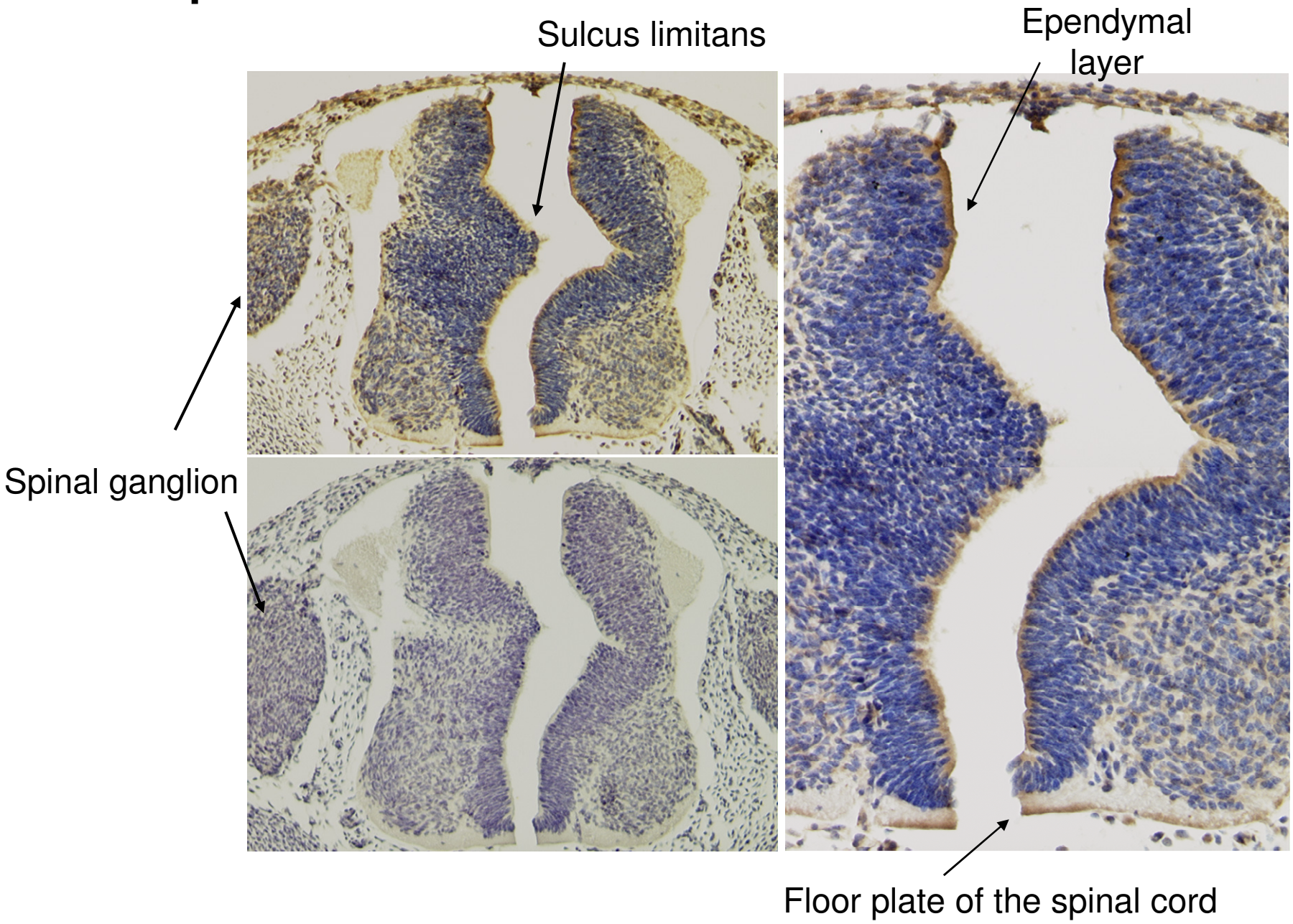
CS 15 Spinal cord

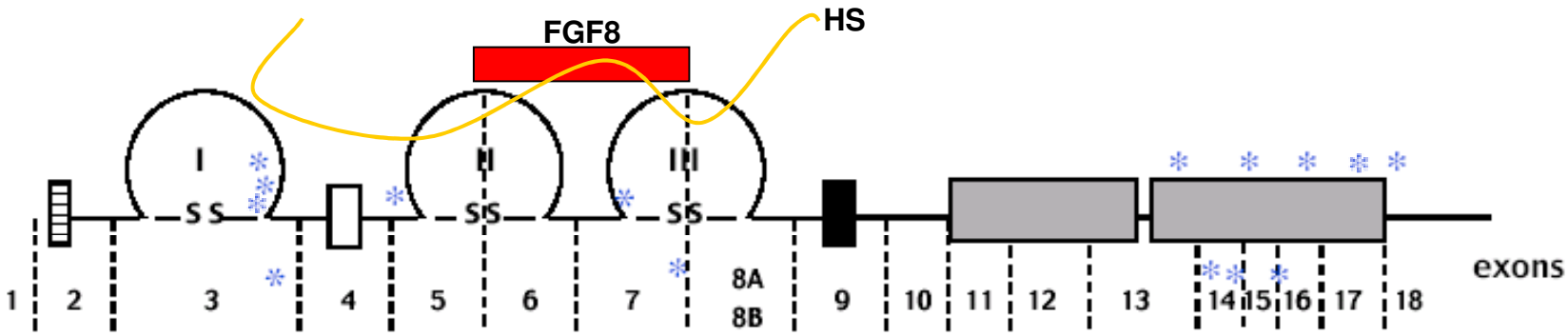


Autosomal dominant KS and *KAL-2*

- *KAL-2* encodes FGFR1
- Loss of function of FGFR1 $\Rightarrow$ IHH + anosmia, or anosmia alone
- Cleft palate, lip
- Dental agenesis
- Fusion of 4-5th metacarpals
- Unilateral sensorineural deafness
- Agenesis of the corpus callosum
- Bimanual synkinesis

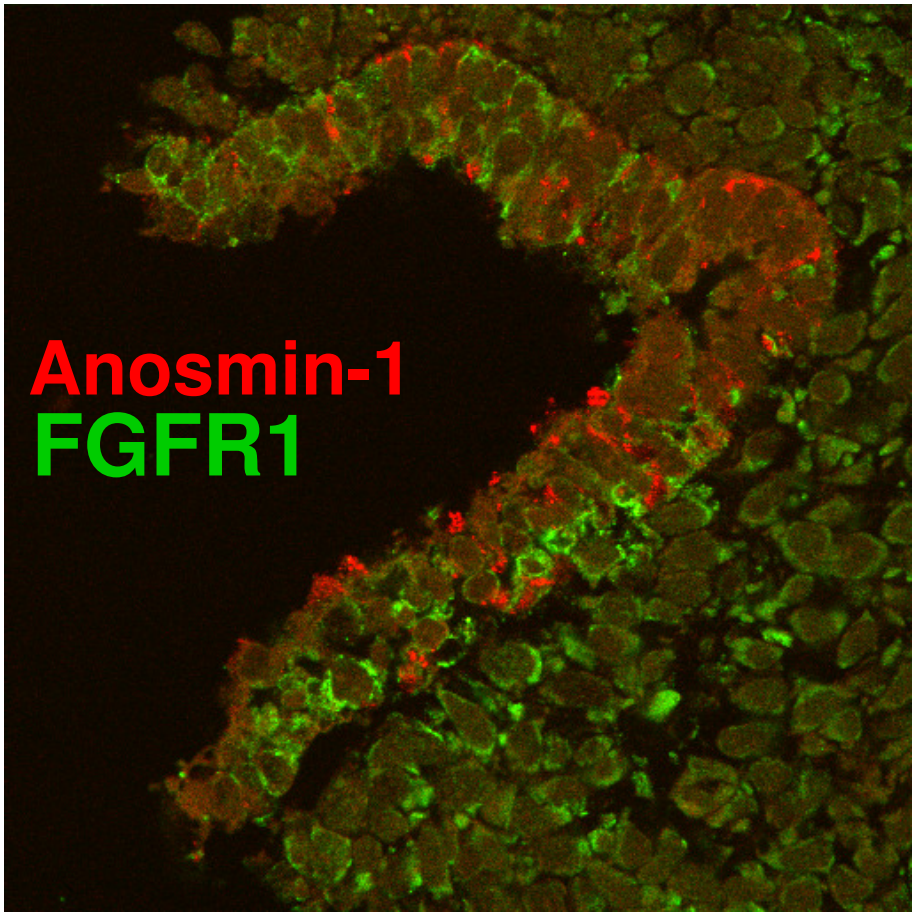
(Dode et al 2003)

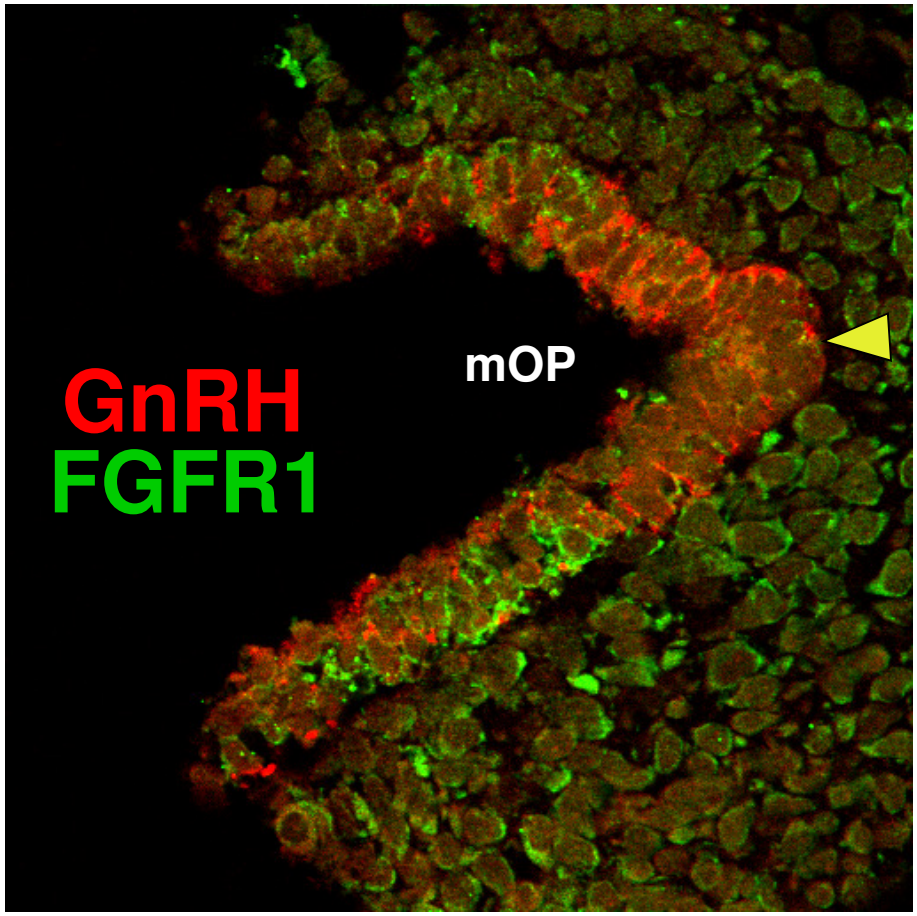
Schematic representation of FGFR1



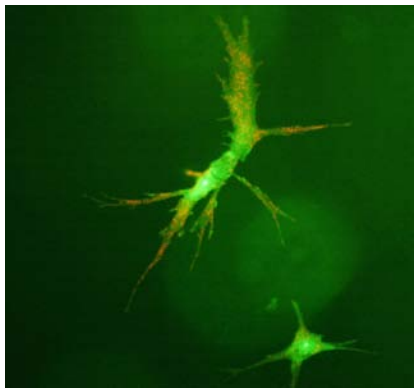
Missense and non-sense mutations identified in AKS patients

Do we have any *in vivo* evidence of anosmin-1 and FGFR1 interaction?





GnRH, Anosmin-1 and FGFR1 are present in the OP at 4.5 weeks



Primary human olfactory neuroblast cell line FNC-B4

- Cloned neuroblast cell line from olfactory epithelium of 8-12 week-old human embryo

- +ve expression: GnRH1, GnRHR1; FGF2, FGFR-1; Leptin receptor (long form)

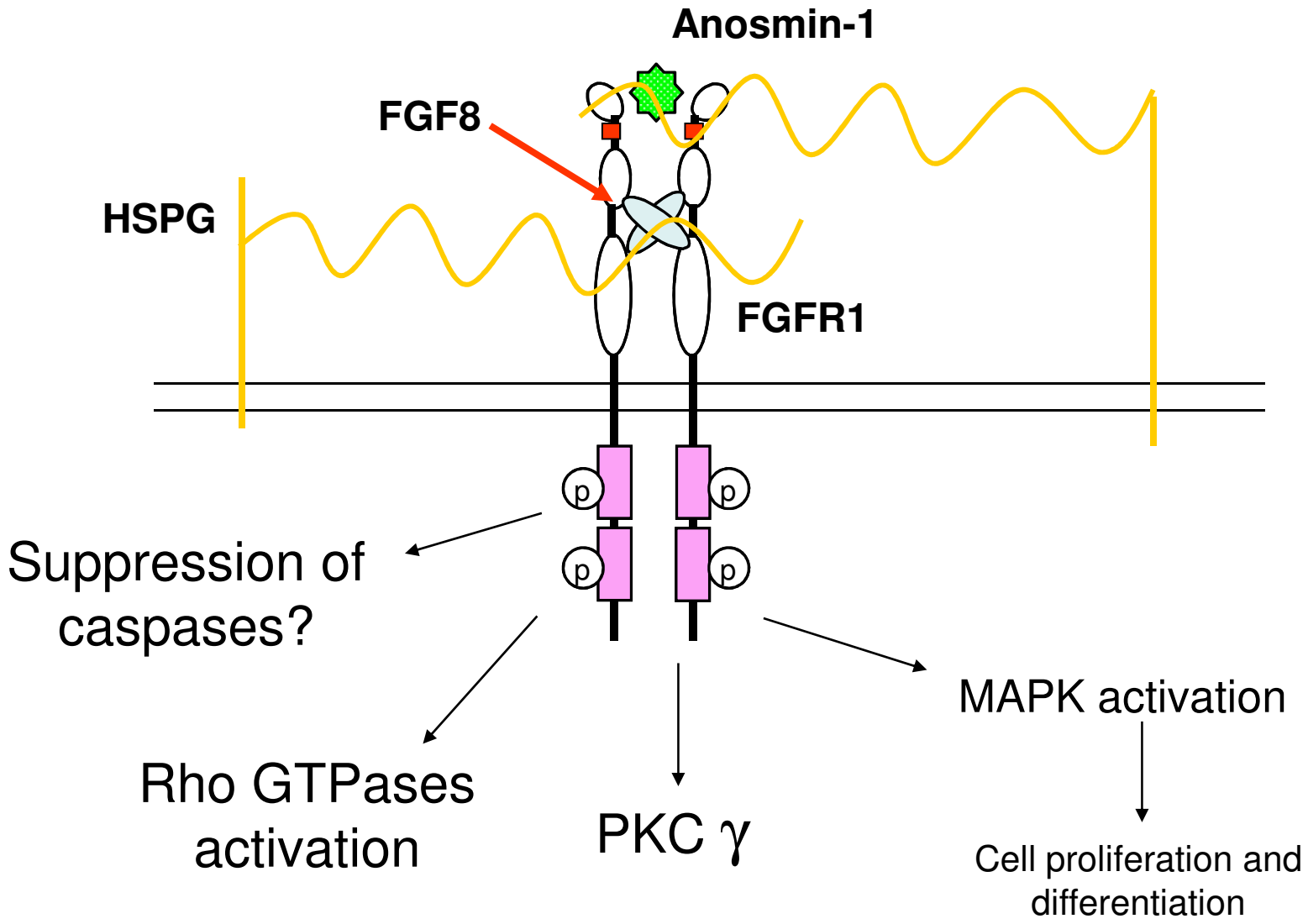
  -ve expression: Anosmin-1

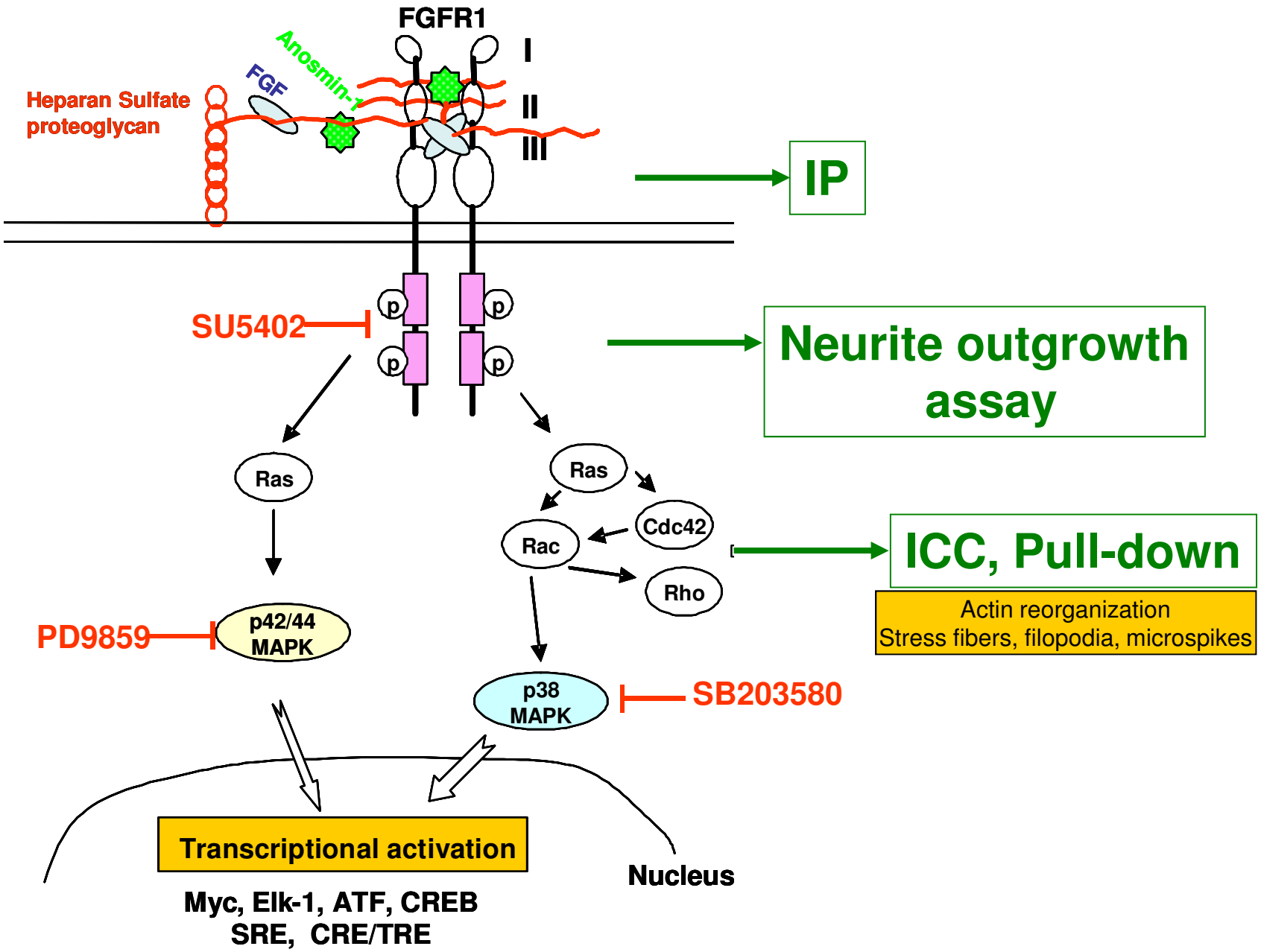
- Express the pre-migratory and olfactory markers: NELF

Could anosmin-1/FGFR1 functionally interact ?

- Anosmin-1 binds to HSPG (high affinity)
- HS forms active ternary signaling complex with FGF-FGFR
- Axon-branching phenotype of mis- or overexpressing ***C.elegans kal1*** abolished in heparan 6O-sulphotransferase deficient background
- Anosmin-1 and FGFR1 co-expressed during embryonic development
- Bimanual synkinesia present in AKS

Putative model of anosmin-1/FGFR1 interaction




FGFR1
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Cdc42
Rac
Rho
ICC, Pull-down
Actin reorganization
Stress fibers, filopodia, microspikes
PD9859
p42/44
MAPK
p38
MAPK
SB203580
Transcriptional activation
Myc, Elk-1, ATF, CREB
SRE, CRE/TRE
Nucleus

CHD7

- **CHARGE** syndrome (**C**olobomata, **H**eart **A**nomalies, choanal **A**tresia, R̲etardation, **G**enital and **E**ar anomalies)
- Includes IHH and hyposmia as part of phenotype
- CHD7 encodes a chromatin-remodelling factor, mutated in 60% cases
- Affected (1% KS/2% nIHH) individuals clinically identifiable due to mild features of CHARGE, particularly deafness, and semicircular canal hypoplasia

PROK2/PROKR2

- PROK2 encodes an 81 aa peptide that signals through the G-coupled product of the PROKR2 gene
- *Prok2/prokr2* knockout mice have defective development of olfactory bulbs/failed GnRH neuronal migration
- Loss of function mutations of PROK2/PROKR2 account for 9% of KS patients
- Considerable phenotypic variability with both KS and nIHH, fibrous dysplasia,sleep disorders, synkinesia, epilepsy

KISS1R

- Loss of function of GPR54 receptor
- KO of GPR54 in mice produce phenocopy of human condition, with nearly absent sexual maturation despite neuroanatomically normal GnRH neurons/hypothalamic GnRH content
- KISS1 /KISS1R potent regulators of GnRH secretion
- KISS1 implicated in both negative and positive central feedback of sex steroids to GnRH production
- KISSR1 mutations present with nIHH, with severely impaired LH secretory amplitude, but normal pulse frequency
- Gain-of-function KISS1R mutation implicated in precocious puberty

TAC3/TACR3

- Study of consanguineous families in Turkey revealing defects in one or other gene in 11 patients from 5 out of 10 families studied
- Presence of micropenis and cryptorchidism in affected males
- Neurokinin B, like KISS1 excitatory neuropeptide acting via Gq-coupled receptors
- Both TACR3/GPR54 expressed on GnRH neurons

Conclusions 1

- The early wave on GnRH neuronal migration in the human precedes bulb histogenesis
- Anosmin-1 and FGFR1 are expressed in early medial olfactory placodal differentiation
- Anosmin-1 and FGF2 stimulate neurite outgrowth in human migratory olfactory neuroblasts

Diagnosis of IHH

Clinical

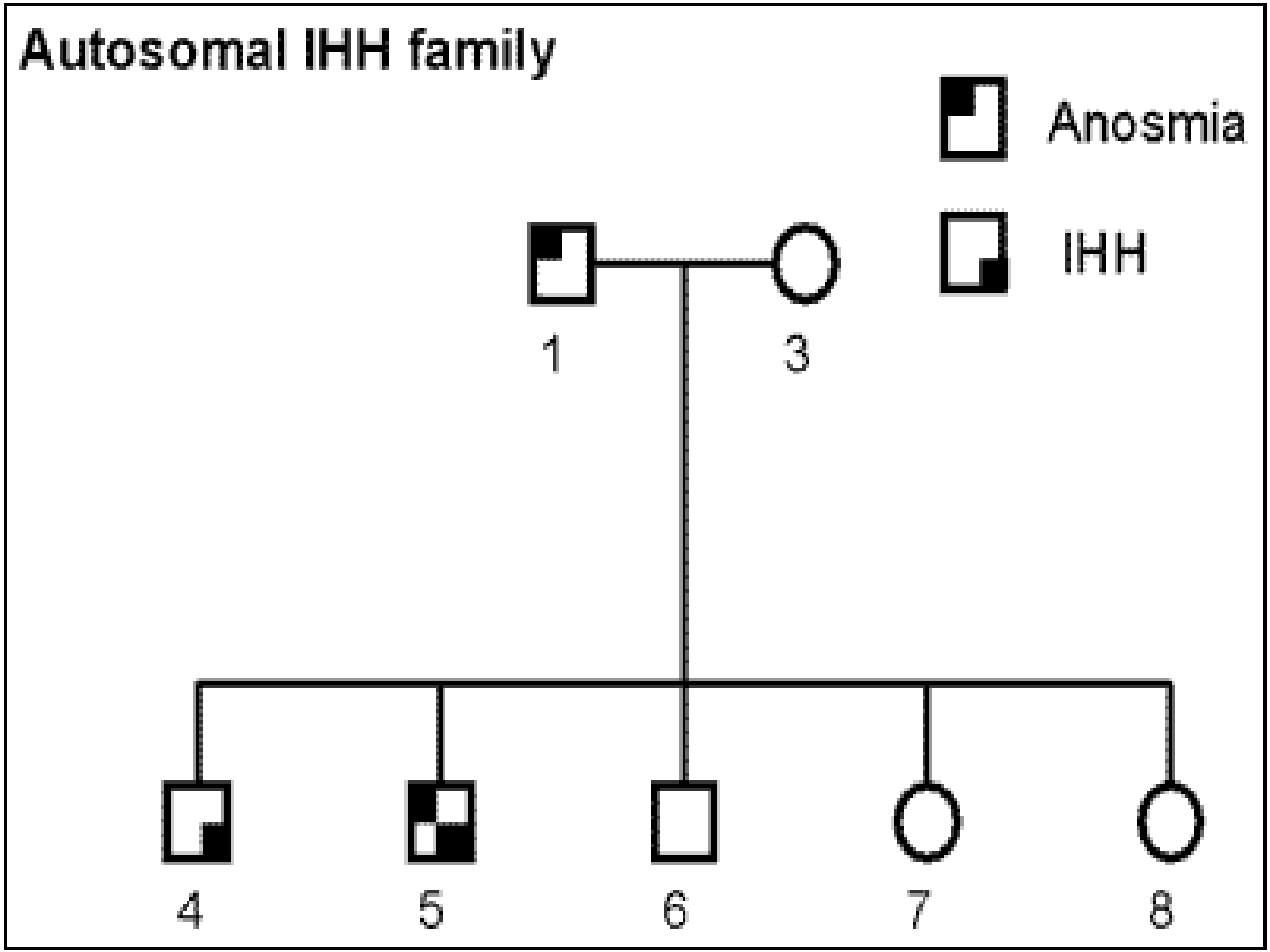
- Delayed / arrested puberty, incomplete puberty,anosmia, micropenis,cryptorchidism
- Symptoms of hypogonadism: in men, low libido,ED, sarcopenia. In women, lack of breast development, amenorrhoea, dyspareunia
- Reversible phenotype

Biochemical

- Low LH/FSH and sex steroids, ferritin, GnRH test
- LH pulsatility studies (absent pulsatility)
- Poor initial , LH/FSH response to exogenous GnRH (but normalization after priming) except with LHβ or GnRHR mutations

Imaging

- MRI scanning may reveal olfactory bulb abnormality (75%) in KS
- US Kidneys (33% unilateral renal agenesis in KS)
- DEXA scanning
- Bone age


Autosomal IHH family
Anosmia
IHH
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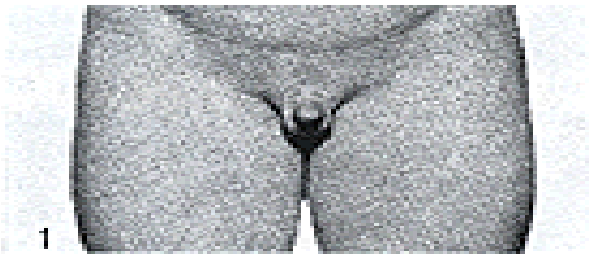
General principles of management

- Establish diagnosis – 10% may have ‘reversible’ phenotype. Take FH (eg delayed puberty in 12%)
- All postpubertal-age patients with KS and IHH should have gonadal steroid therapy, and additional therapies to restore fertility where appropriate
- Be sympathetic to males who complain of small testes
- Behavioural modification and psychological counselling may benefit some patients (late induction of puberty, micropenis)
- Patient education/support groups
- Long term osteoporosis risk when diagnosed late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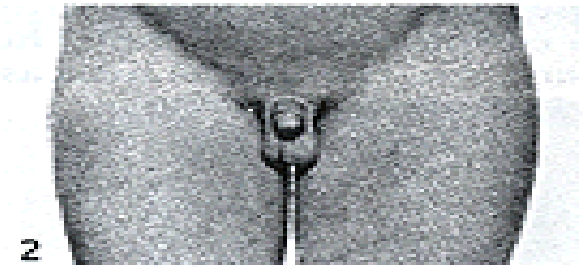
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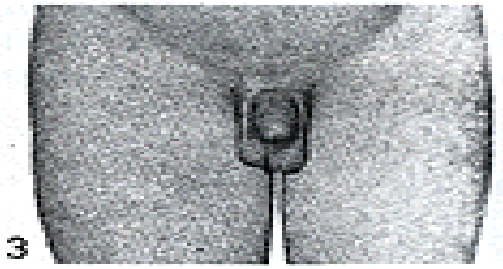
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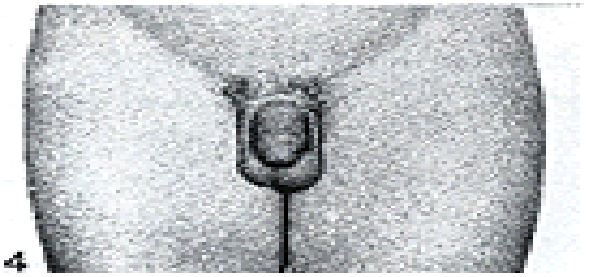
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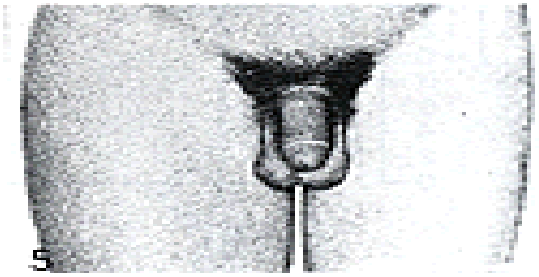
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Treatment of delayed puberty - puberty induction

- Attempt to maintain the normal cadence of puberty in both sexes. More difficult in late diagnosis
- Is it simple pubertal delay, or is it HH?
- During Rx, monitor testicular volume. Increase in testicular volume on TRT= underlying HPT activity
- In males, gradual increase in T administration over 2-4 y. Monitor growth, bone age, and clinical response
- Testicular growth only possible with hCG + FSH

Induction of puberty in males

- Testosterone.
  - Oral (Restandol, Testocaps)
  - Transcutaneous (Testogel, Testim)
  - Buccal (Striant)
  - im (Sustanon, Enanthate, Nebido)
  - Implants
- hCG 2,000U twice weekly for 6 weeks, thereafter 1,000 U twice weekly with 6ccmax TV
- FSH (Puregon, Gonal F, Menopur) 225 U 2-3 times a week
- Pulsatile sc GnRH treatment

Induction of spermatogenesis

- Prior T exposure does not compromise future spermatogenic response to hCG +FSH (?)
- hCG 2,000 U twice weekly for 6 weeks, then 1,000U twice weekly sc or im, titrating the dose to obtain a [T] in the mid-normal range and normal E_2 level
- FSH 225U sc/im x 3 per week, after T normalised. hCG may need to be ↓ after FSH introduced
- Spermatogenesis induction/fertility may take 18-36 months. Expensive. Monitor sperm count after 3 months of combined therapy. Assess partner's fertility potential
- Consider sperm storage after conception has occurred, for future use → put back on T therapy in irreversible HH

Induction of puberty and fertility in females(1)

- In delayed puberty, start EE_2 2.5-5 µg daily for 6 months, then 7.5µg for 6-9 months, 10µg for 6-9 months, 15 µg for 6-9 months, and upon onset of uterine bleeding, add 5-10mg dydrogesterone orally for first 10 days of each calendar month
- Switch to low dose oestrogen OCP at completion of puberty

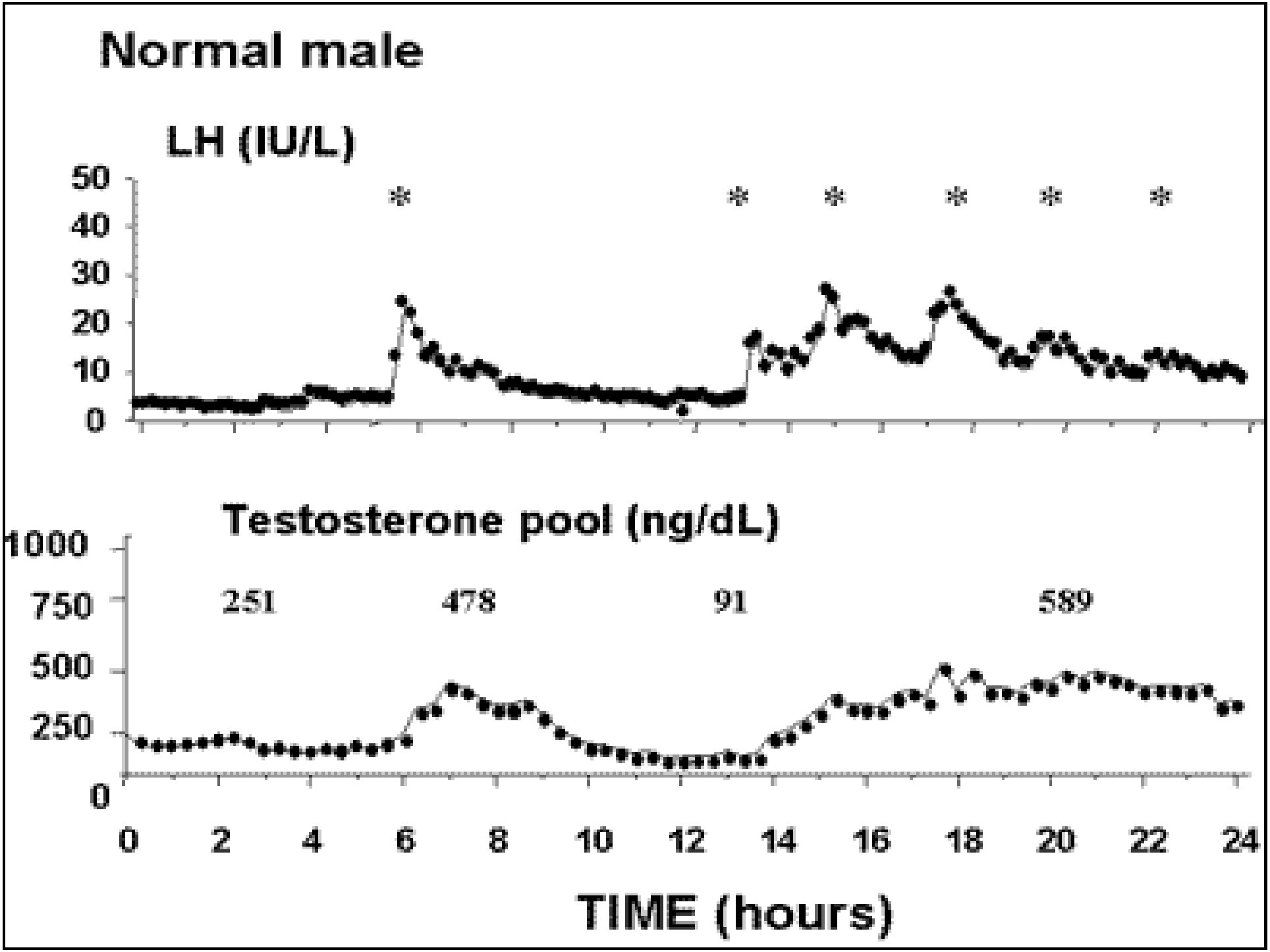
Induction of puberty and fertility in females (2)

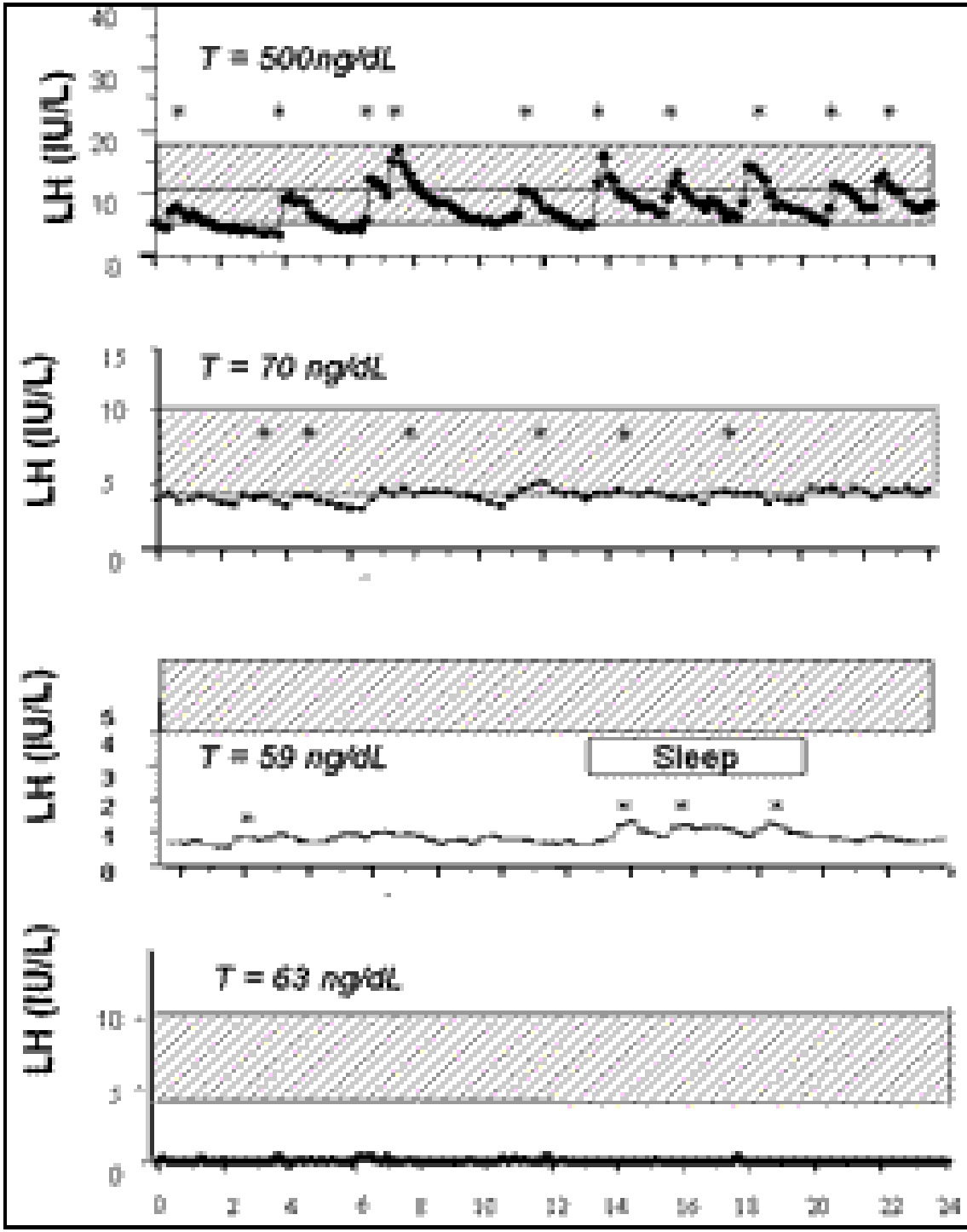
- Periodically check gonadotrophins and oestradiol levels / ovarian , uterine ultrasound to identify spontaneous HPO activity after discontinuation of Rx (4 weeks adequate)
- In irreversible HH, ovulation induction with sc/im FSH (eg Menopur ½ ampoule daily for 1 week and titrating dose according to US response – follicular development, and endometrial thickness)
- Pulsatile GnRH therapy

Pulsatile GnRH therapy

- Usually given sc but may also be given iv.

  in IHH and KS depending on phase of menstrual cycle
- Some patients with GnRH mutations may respond to high dose GnRH therapy
- Males: 25-600ng/kg IV 2 hrly for up to 2 years
- Females: 25-250ng/kg IV every 60-90min


Normal male
LH (IU/L)
50
40
30
20
10
0
Testosterone pool (ng/dL)
1000
750
500
250
0
251
478
91
589
0
2
4
6
8
10
12
14
16
18
20
22
24
TIME (hours)

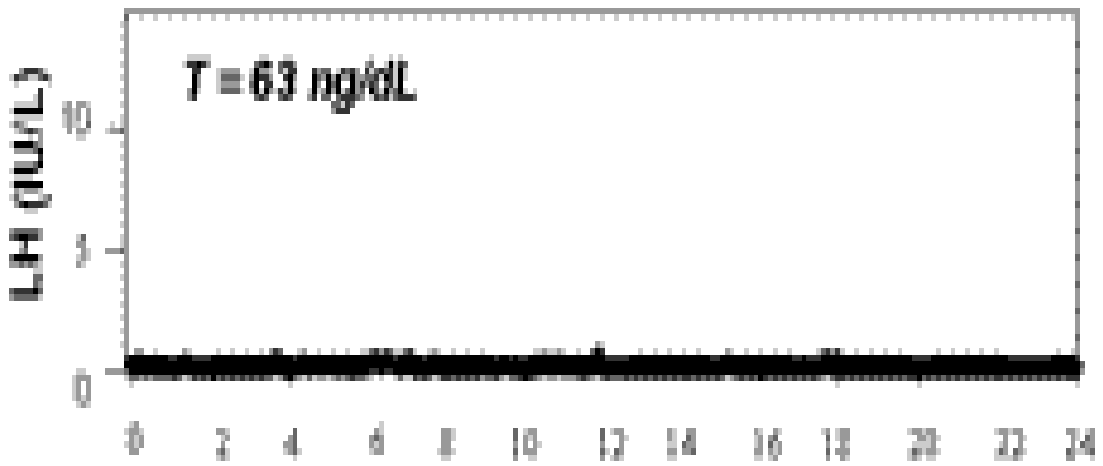


IA. Normal LH pattern
TV: 20 mL
Sperm count 70 x10^6/mL

IB. Low Amplitude
TV: 5mL
Sperm count 0 x10^6/mL

IC. Developmental arrest
TV: 8 mL
Sperm count 0 x10^6/mL

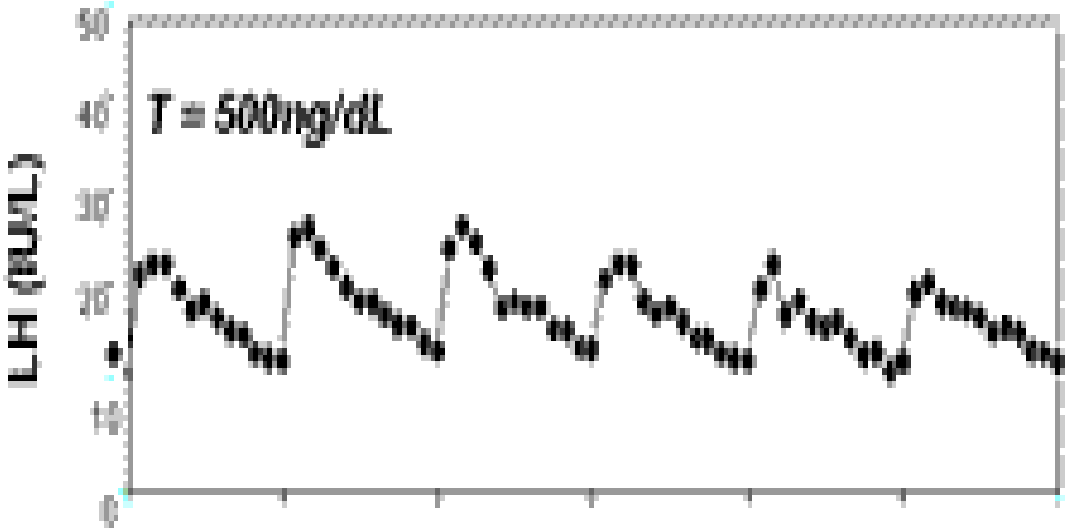
ID. Apulsatile
TV: 1 mL
Sperm count 0 x10^6/mL



GnRH Deficient Male

TV 2 ml

Sperm count 0x10^6/mL



+ Pulsatile GnRH

TV 13 ml

Sperm count 13x10^6/mL

Induction of puberty and fertility in females (2)

- Aim is to generate unifollicular development (18-20mm) and endometrial thickness of 8-10mm
- If above is achieved→ 10,000 U hCG im, and 5,000 U after 3-5 days for 2-3 doses
- Stop therapy, and wait. hGC + US diagnosis of pregnancy
- If fertilization has not occurred, withdrawal bleed will occur → repeat cycle with adjustment of gonadotrophin doses based on age/previous response
- After delivery, restart HRT when breast feeding is completed

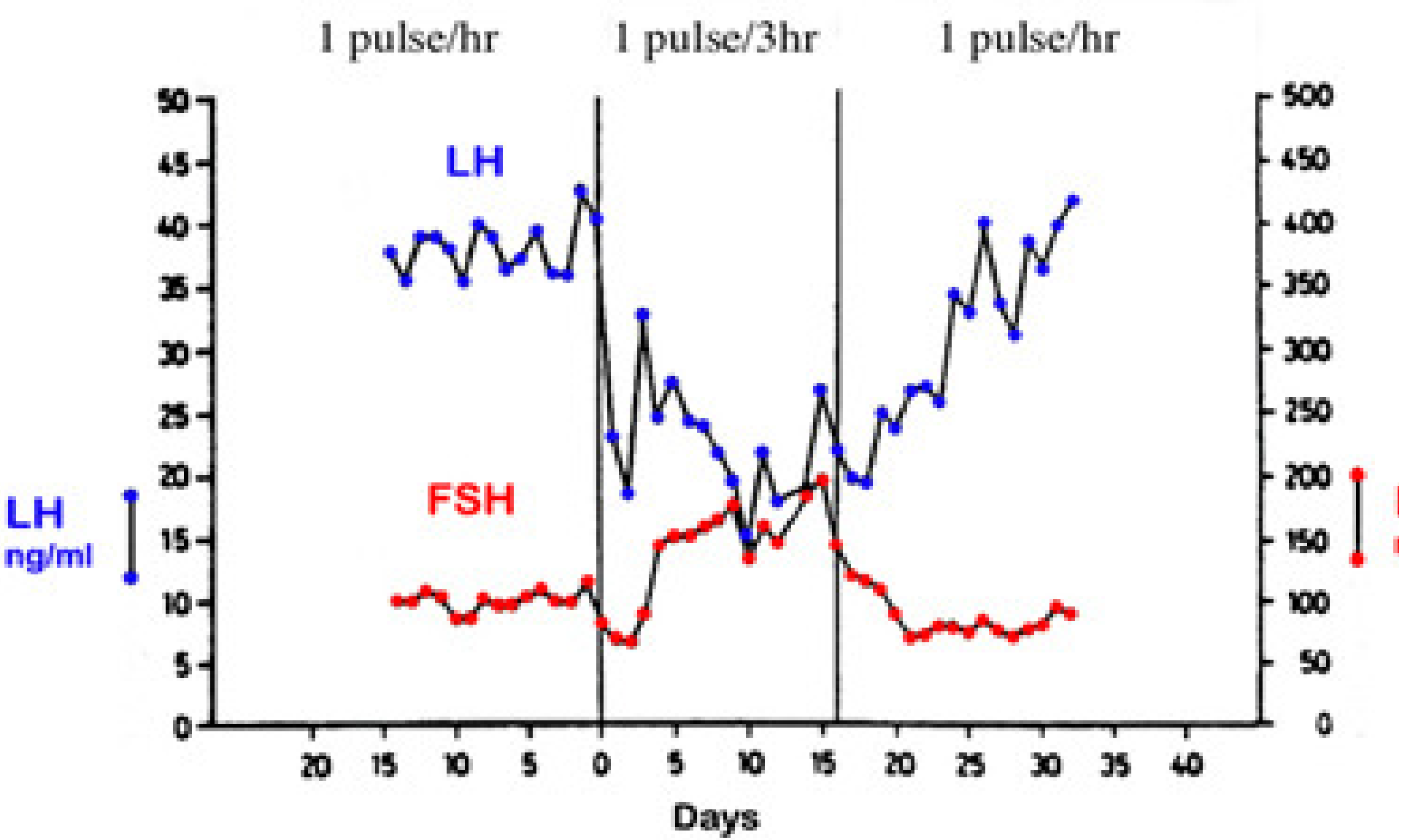


Figure 6. The frequency of GnRH pulses codes for different rates of LH and FSH secretion from pituitary gonadotropes. Adapted from (96) with permission of the Endocrine Society, ©1981.

Functional hypothalamic amenorrhoea (FHA) syndrome

- Cessation of menstrual cycles in young women without clinically demonstrable abnormalities of HPO axis
- FHA - psychogenic / stress related
  - weight related
  - exercise related
- Low E2 and inappropriately low LH/FSH
- FSH often greater than LH due to slow GnRH pulse frequency (esp wt related FHA)

Exercise and menstrual cycle 1

- Neuroendocrine-metabolic consequences of exercise depend on type, duration and intensity of exercise, body composition, psychological background and other individual stress factors
- Menstrual abnormalities can occur in middle and long distance running, swimming, ballet dancing, field events
- Positive correlation between weekly training mileage and incidence of amenorrhoea
- Joggers (5-30miles/w) have significantly fewer menses per year than less energetic counterparts

Exercise and menstrual cycle 2

- Intense training before menarche (gymnastics and ballet dancing) can delay menarche by 3 years and incidence of secondary amenorrhoea or chronic anovulation greater in later life
- Body fat content determinant of sport specific FHA. Incidence greater in high intensity runners (50%) than swimmers (12%), due to greater % body fat in swimmers
- Runners with FHA have significantly less protein and energy intake and weight loss than runners without amenorrhoea

Exercise and menstrual cycle 3

Progressive dysfunction of ovarian cyclicity related to **energy cost of exercise** and include:

- luteal defects
- anovulatory cycles and amenorrhoea
- delayed menarche in prepubertal girls
- reduced LH pulse frequency but increased amplitude
- interference with folliculogenesis as initiating cause of continuum of luteal phase defects, anovulation and amenorrhoea

Nutritional deficit and FHA

- Luteal phase defects, anovulation and amenorrhoea represents an *endogenous hypothalamic* contraception in the face of limited metabolic fuel
- Leptin-NPY-galanin-opioid axis likely to undepin relationship between energy intake, exercise and FHA

Leptin levels and diurnal rhythms in FHA

- Leptin levels in FHA in association with exercise and low body fat stores are markedly reduced
- Diurnal rhythm of leptin no longer discernible in FHA but remains intact in athletes with menstrual cycle
- Leptin is regulated acutely by energy balance independent of body fat stores