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When menarche does not occur – a pediatric view

Peter Bang, MD, PhD, MSci
Div of Pediatrics
Dept of Clinical and Experimental Medicine
Faculty of Health Sciences
Linköping University
peter.bang@liu.se
Mobile +46708226491

- Referral from GP: 16 year old girl with primary amenorrhea
- Beast development since 2 years
- Competing in Nordic skiing - hard physical training 5 days a week
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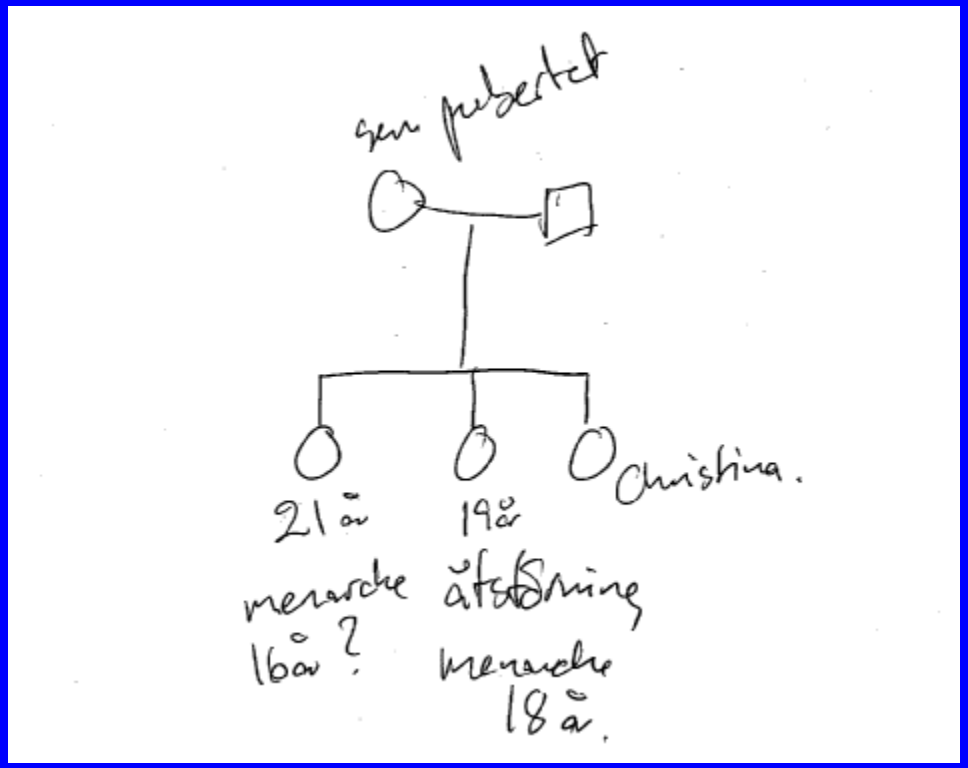
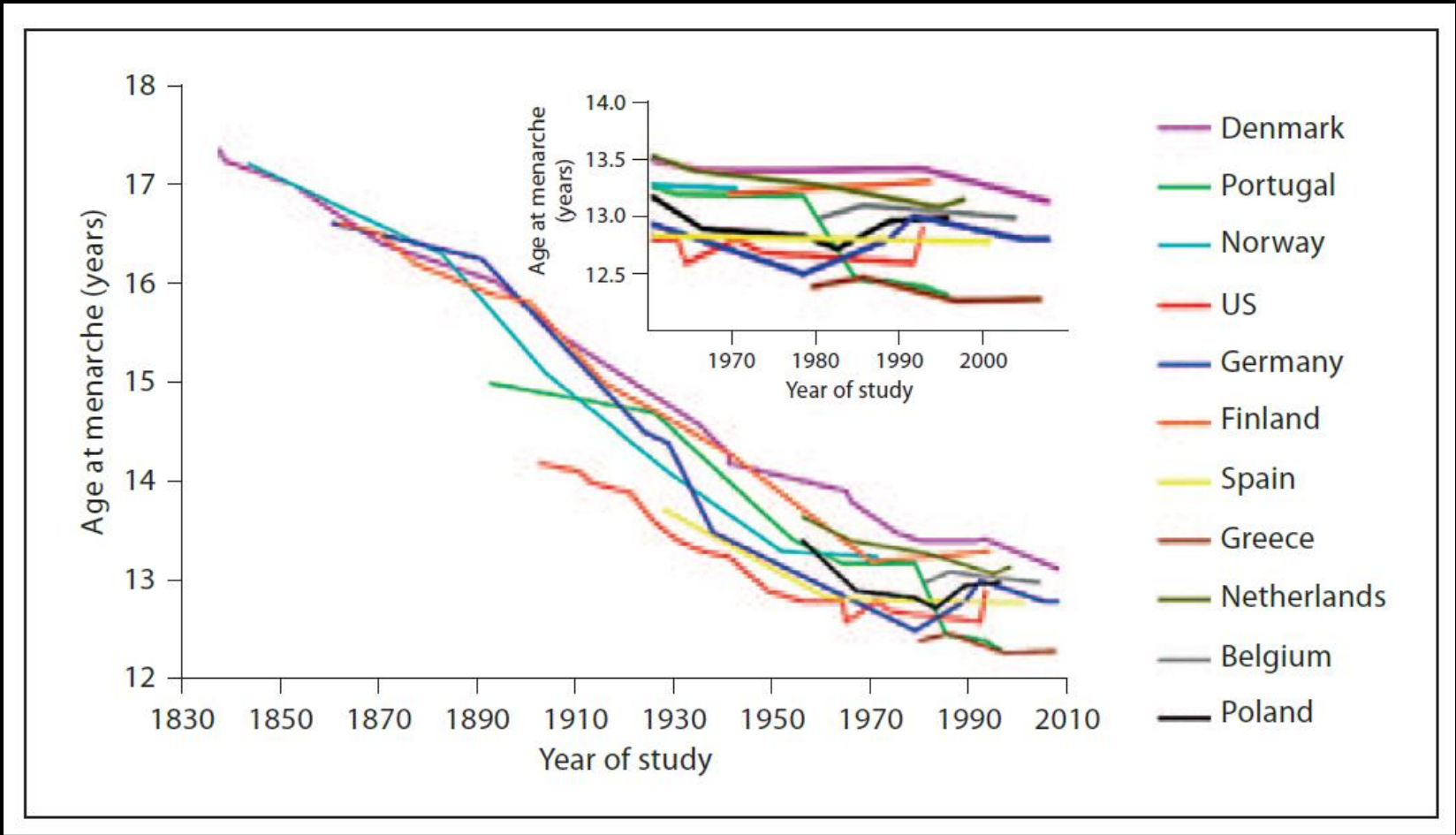
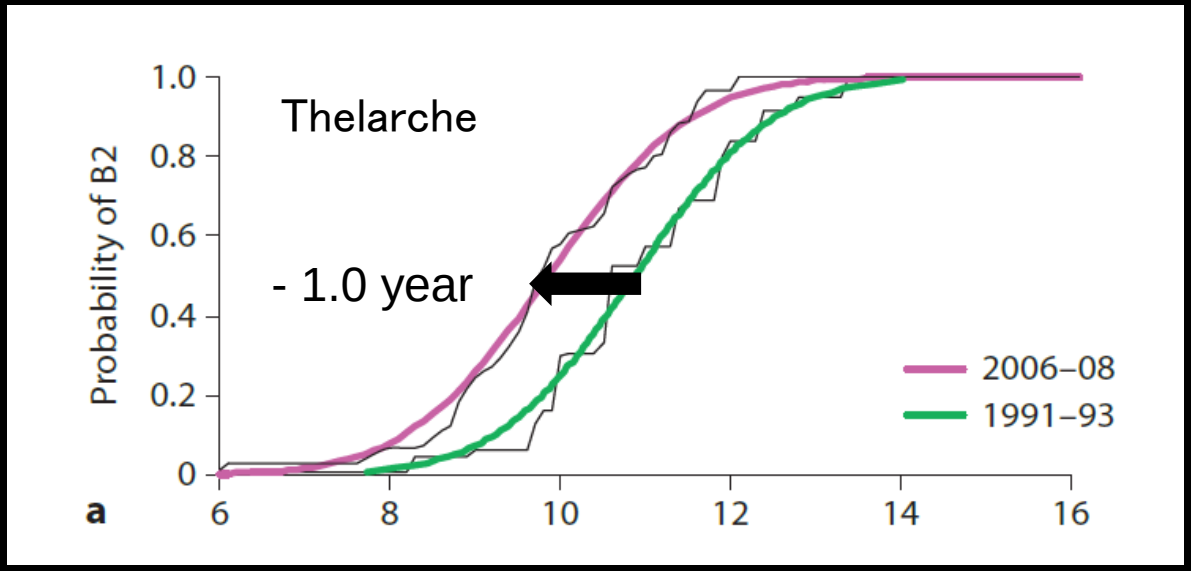


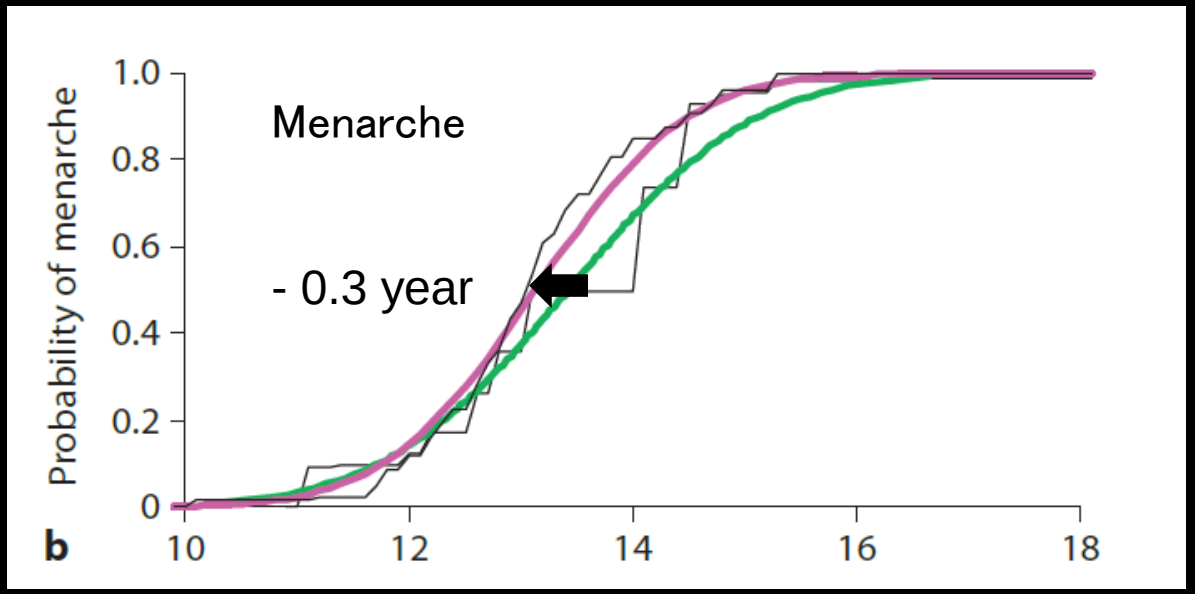
At what age should menarche be expected?



Sørensen et al. Horm Res Paediatr. 2012;77(3):137-45.

Recent decline in age at breast development – the Copenhagen puberty Study.





Aksglaede et al. Pediatrics 2009; 123:e932–e939.

Early thelarche not related to elevated estradiol, LH or FSH in younger girls

TABLE 2 Reproductive Hormone Levels in Girls Examined in 1991–1993 and 2006–2008

	n	% in B2+	Estradiol, Median (Range), pmol/L	FSH, Median (Range), IU/L	LH, Median (Range), IU/L
1991–1993					
<8 y	50	0	18 (18–30)	1.06 (0.29–2.41)	0.05 (0.05–0.48)
8–9.9 y	60	2.3	24 (18–63)[a]	1.53 (0.28–4.44)	0.05 (0.05–0.23)
10–11.9 y	71	48.4	37 (18–1379)	2.66 (0.36–8.79)	0.13 (0.05–5.58)
>12 y	243	99.5	166 (18–1442)[a]	4.62 (0.06–12.69)	3.65 (0.05–24.48)
2006–2008					
<8 y	119	3.4	18 (18–53)	1.09 (0.12–3.60)	0.05 (0.05–0.41)
8–9.9 y	182	24.4	18 (18–229)	1.39 (0.29–8.18)	0.05 (0.05–7.88)
10–11.9 y	223	75.1	31 (18–388)	2.80 (0.27–9.22)	0.19 (0.05–19.00)
>12 y	287	100.0	130 (18–1346)	4.32 (0.06–12.40)	3.7 (0.05–26.30)

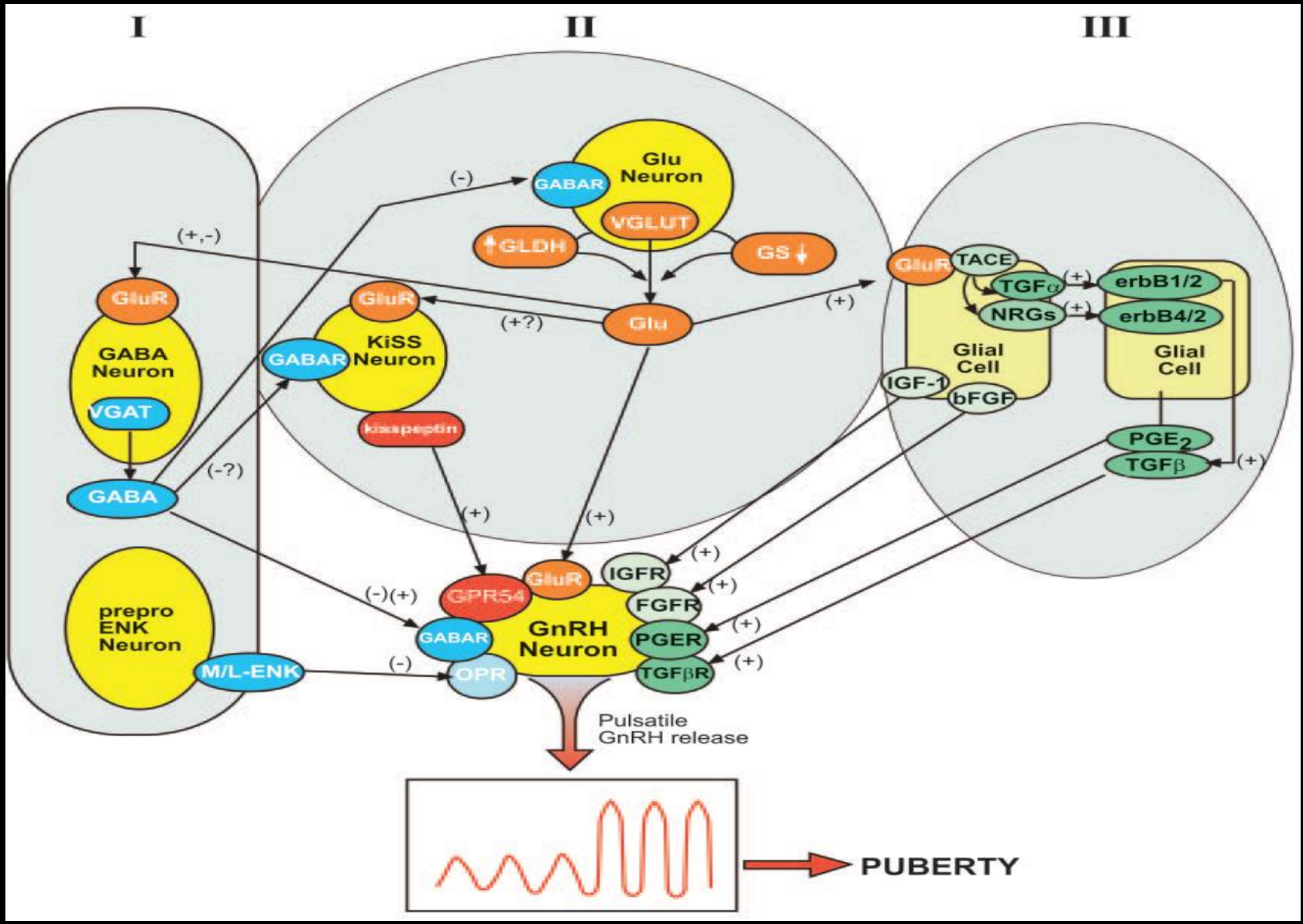
[a] $P < .0001$.

Potential explanations:

- Nutritional factors/ childhood obesity => aromatase activity => local estradiol?
- Increased estradiol sensitivity?
- Endocrine disruptors?

Aksglaede et al. Pediatrics 2009; 123:e932–e939.

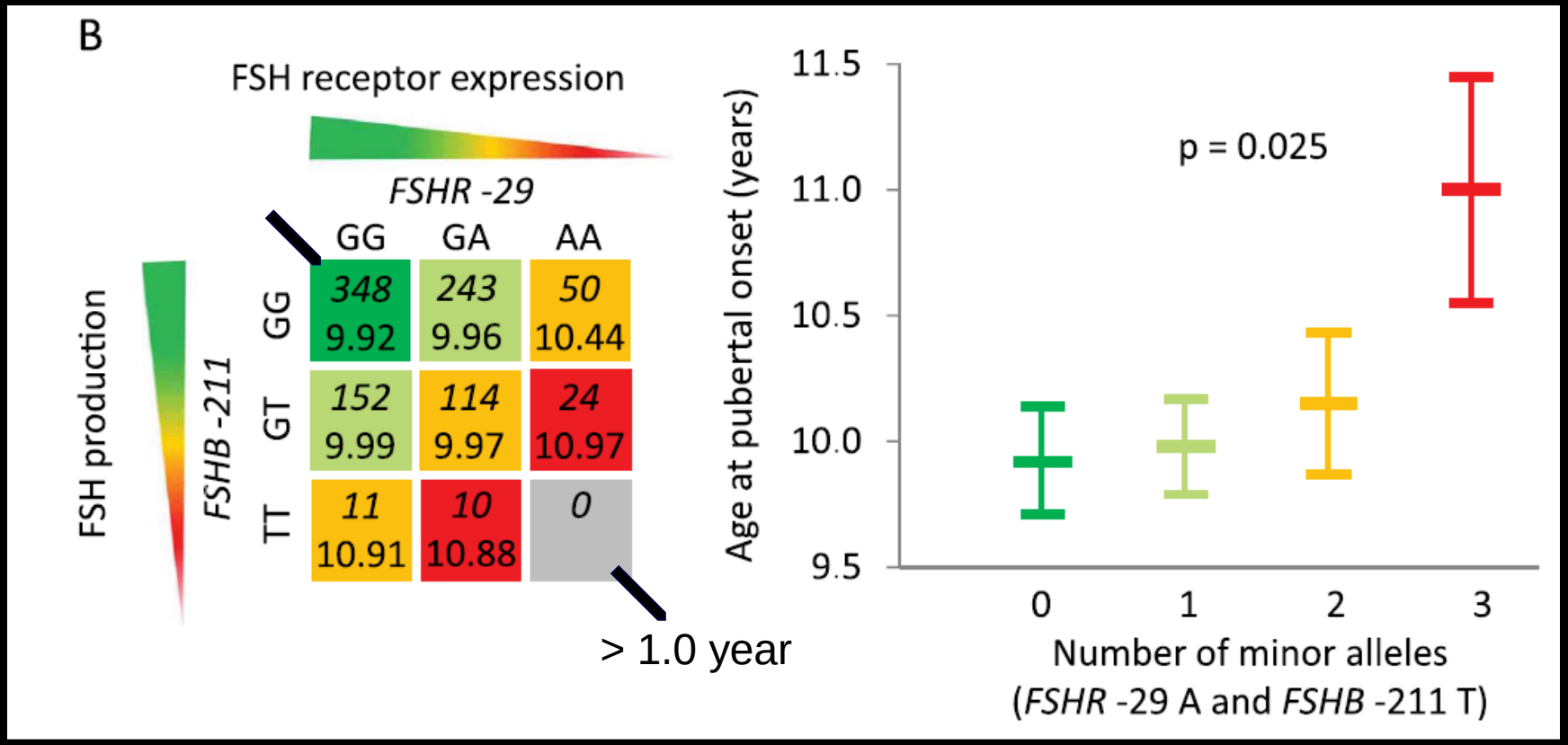
Potential targets for genetic variations in age at B2 or menarche:



Ojeda, S. R. et al. Endocrinology 2006;147:1166-1174

Common genetic determinants of age at pubertal start

- FSHR – polymorphism with lower expression enter puberty 7.4 (2.5–12.4) months later than carriers of the common variants (p<0.003)
- Strongest genetic effect on age at pubertal onset in girls published to date.



Hagen et al. Sci Rep. 2014 Sep 18;4:6412

The most common pediatric causes of delayed puberty/ primary amenorrhea are associated with short stature:

- ✓ Check the growth chart
- ✓ Family history - Constitutional delay of growth and puberty (CDGP)
- ✓ Excessive physical activity
- ✓ Eating disorders
- ✓ Chronic disease
- ✓ Other stressers

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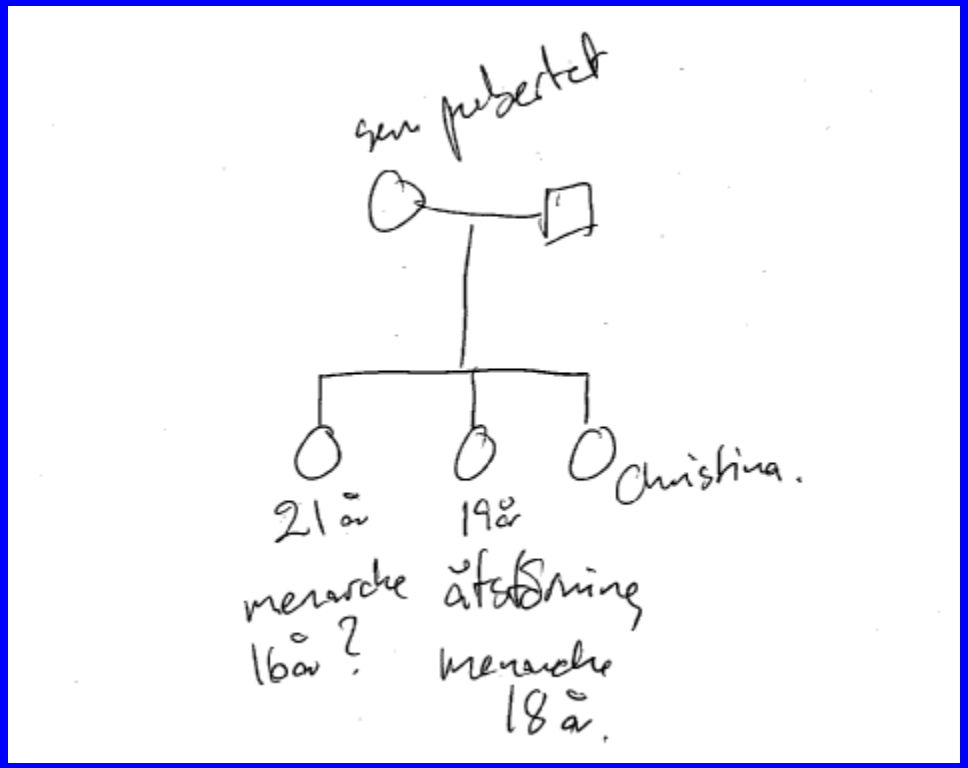
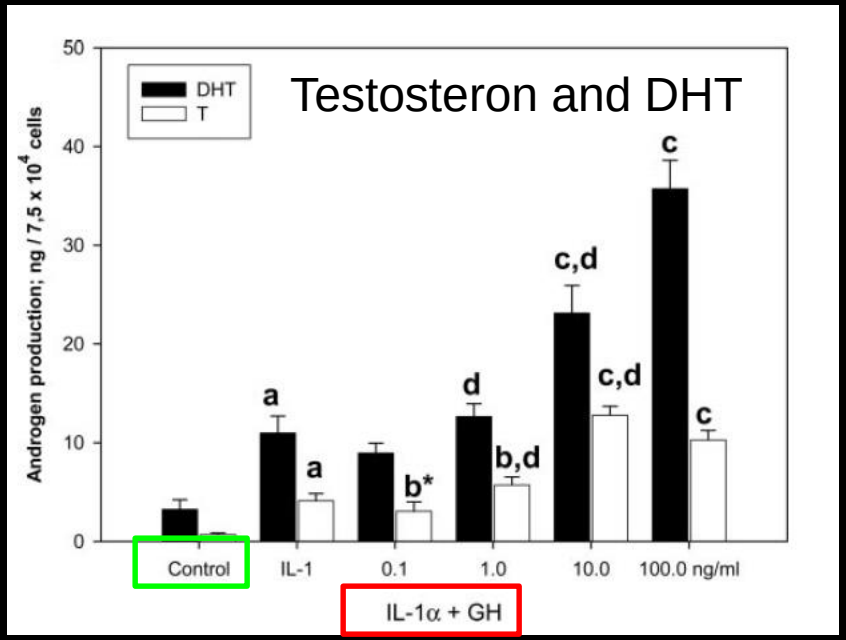
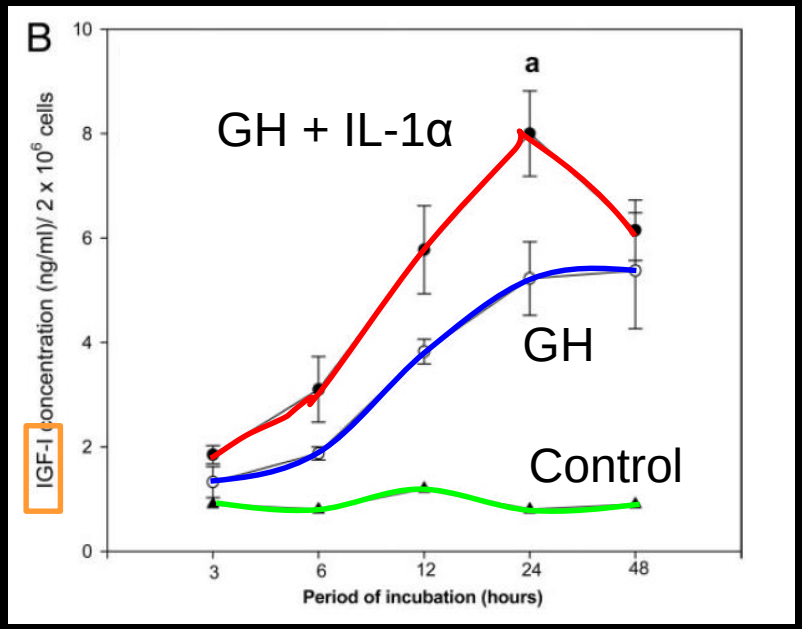


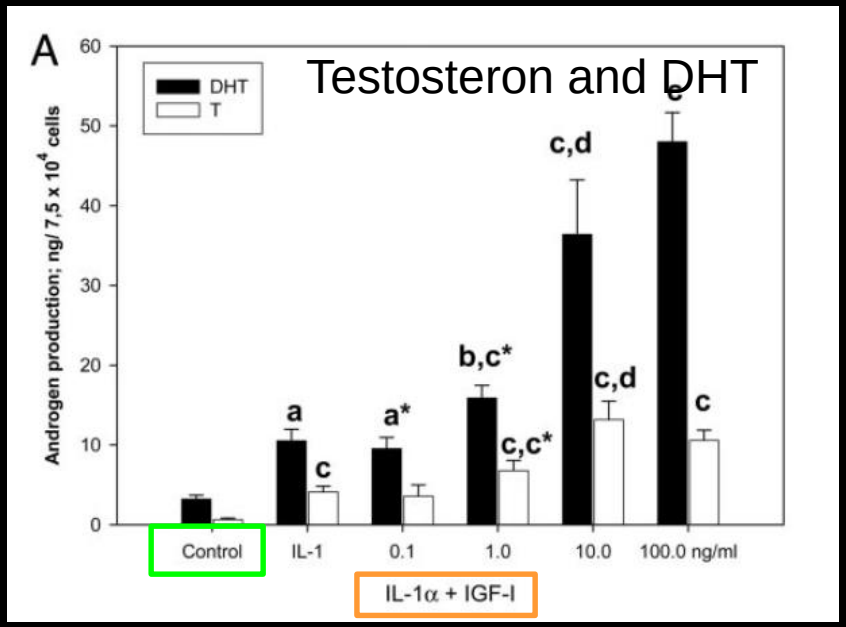
What are the mechanisms of environmental factors?

GH effects on gonadal function mediated via IGF-I and the IGF-1R

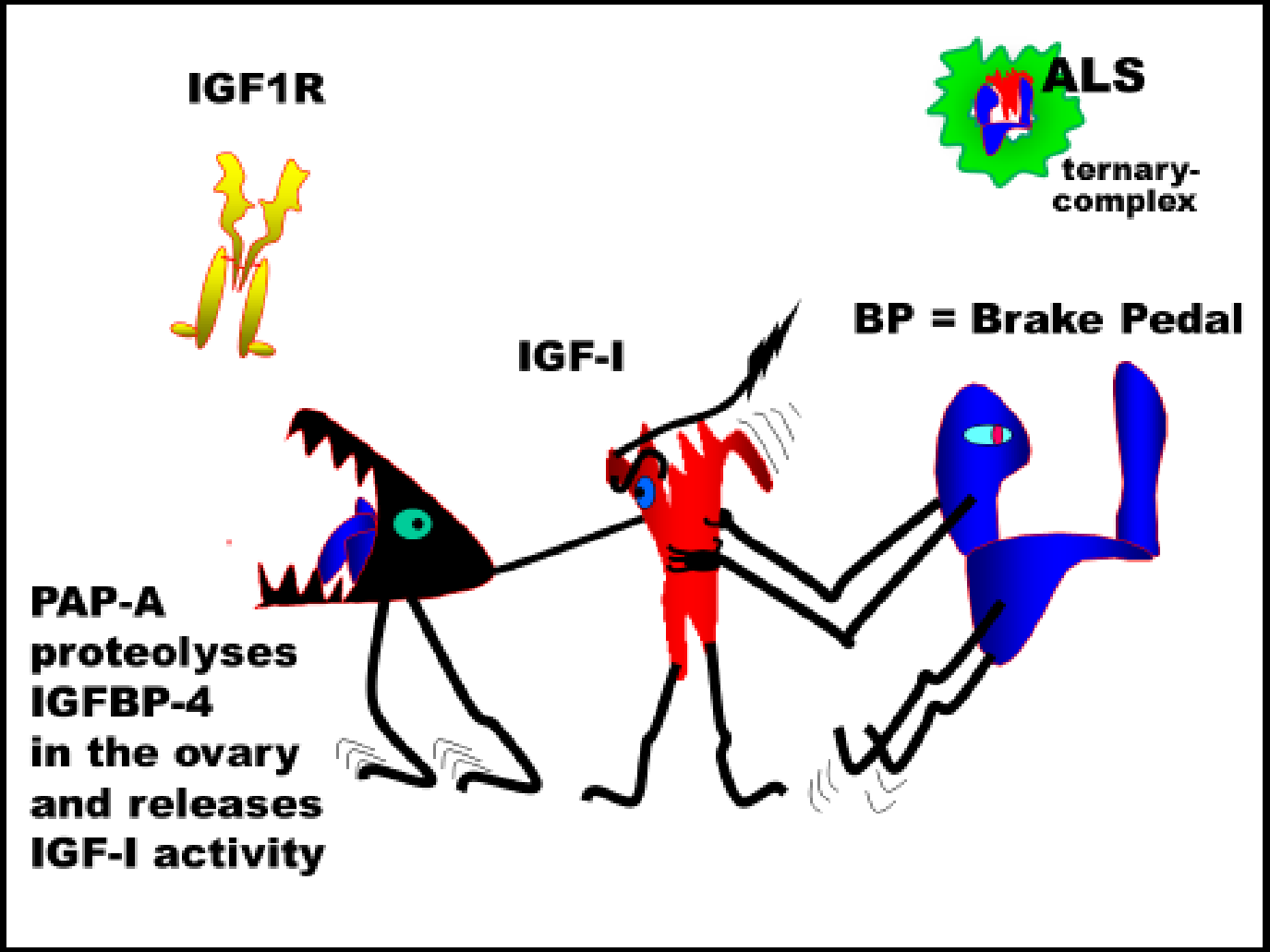
Colon et al. *Endocrinology* 146: 221–230, 2005



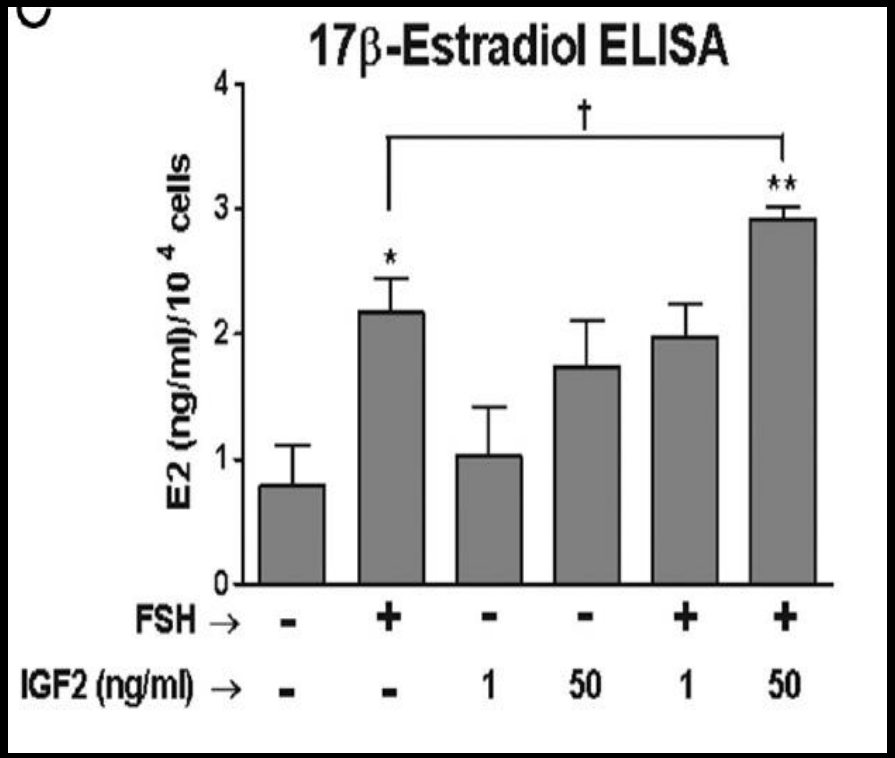


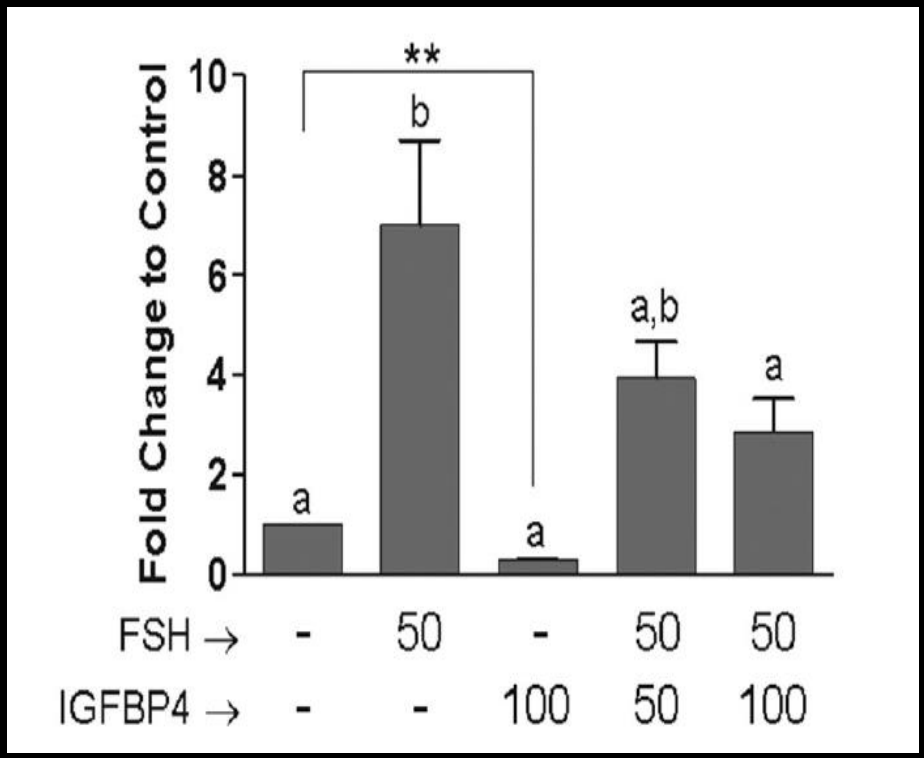


Ovarian function is regulated by endocrine as well as paracrine/autocrine growth factor systems



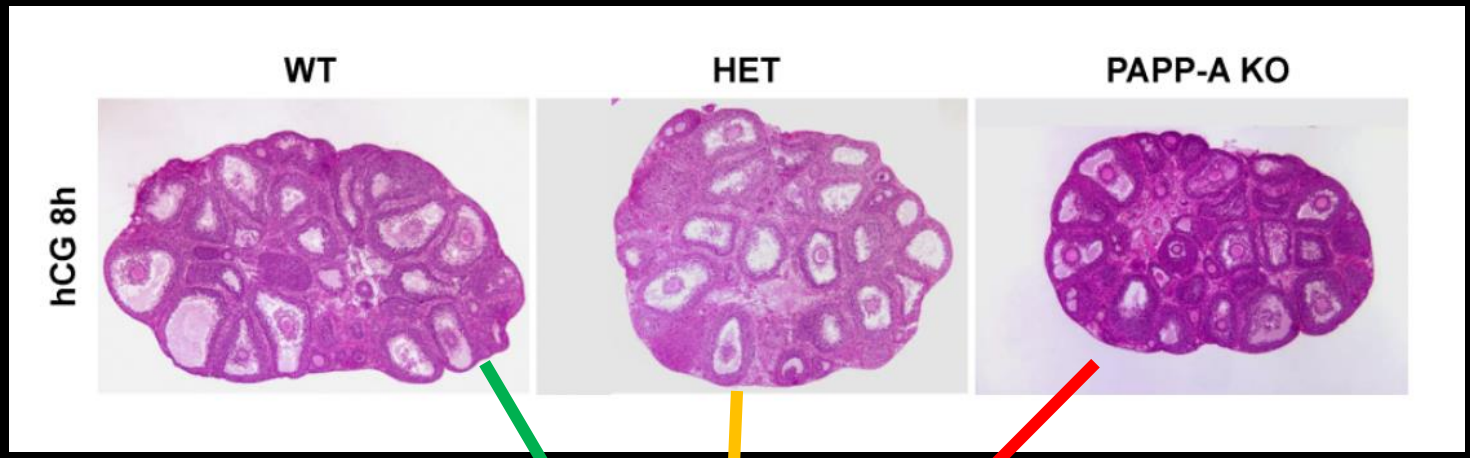
FSH stimulates IGF-2 expression and activation of the IGF-1R and AKT which is required for FSH stimulated *CYP19A1* expression and proliferation of granulosa cells

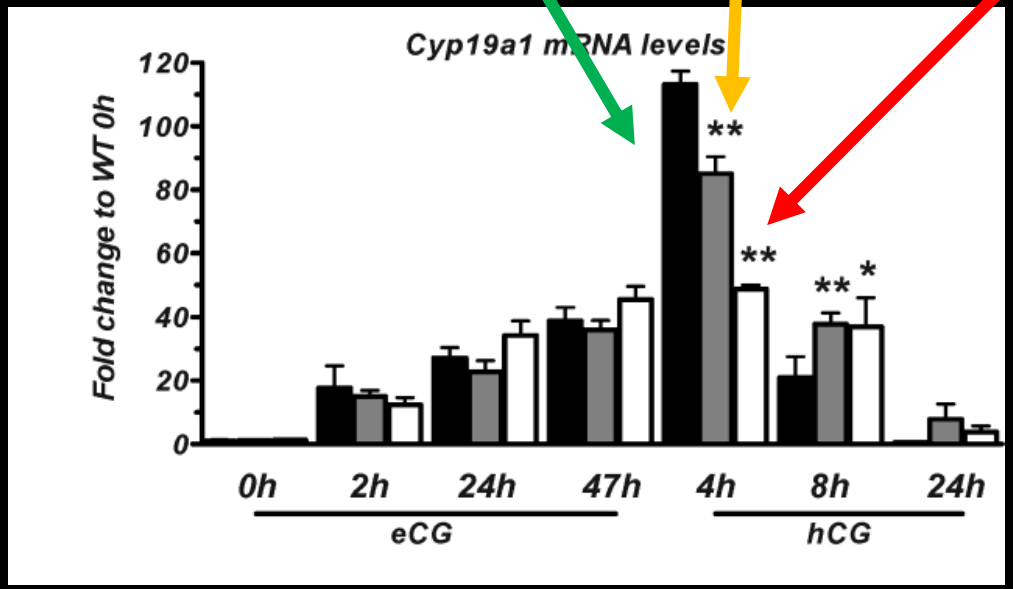




J Clin Baumgarten et al. Endocrinol Metab 100: E1046–E1055, 2015

Lack of Functional Pregnancy-Associated Plasma Protein-A – a IGFBP-4 protease - Compromises Ovarian Steroidogenesis and Female Fertility





Nyegaard et al. Biol reprod 82, 1129–1138, 2010

Primary amenorrhea associated with nutritional deficiencies/stress/chronic disease are usually thought to be caused by

- ✓ Pituitary suppression of GnRH/LH/FSH release

but

- ✓ Gonadal dysfunction

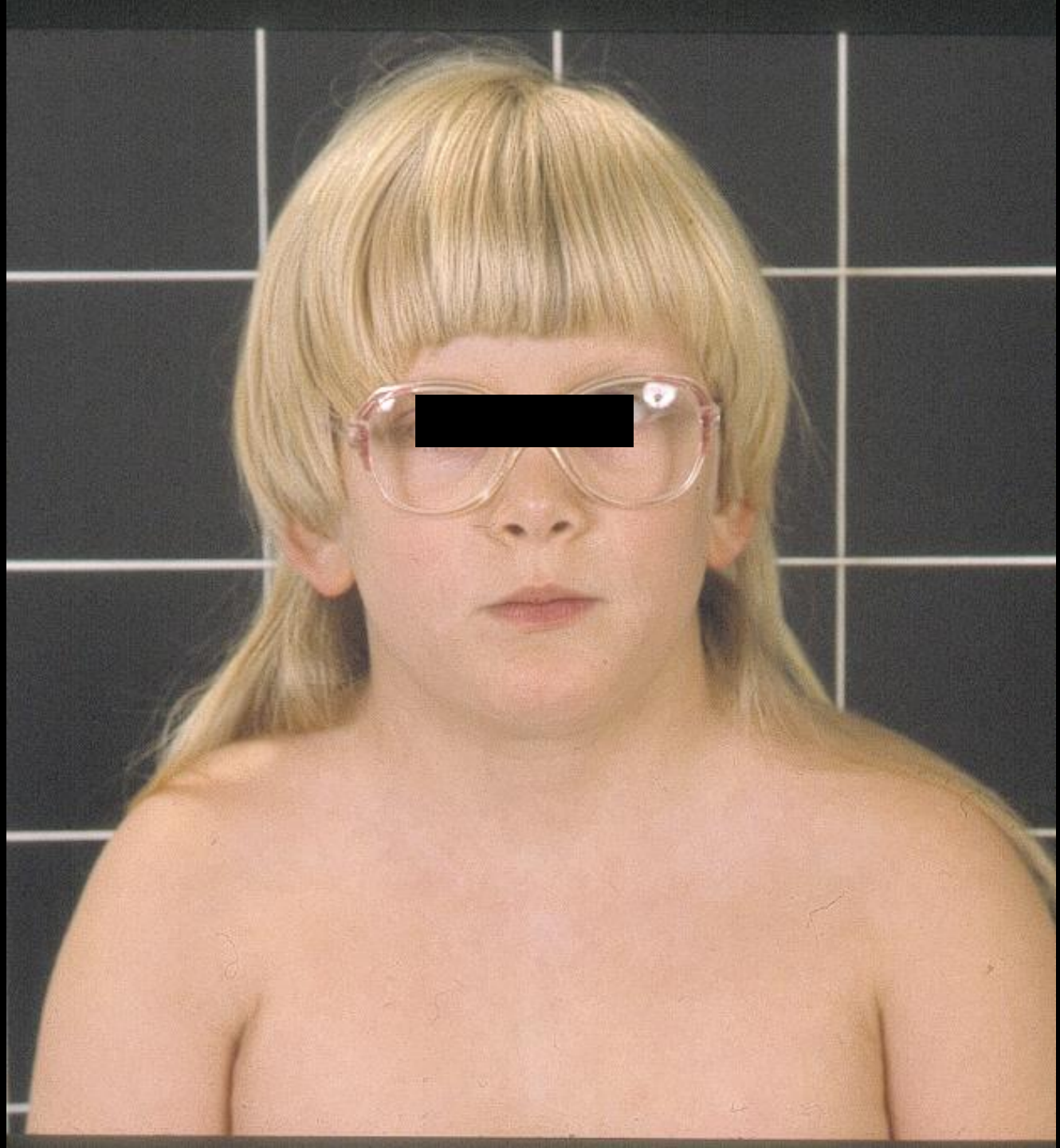
may contribute

Menarche requires:

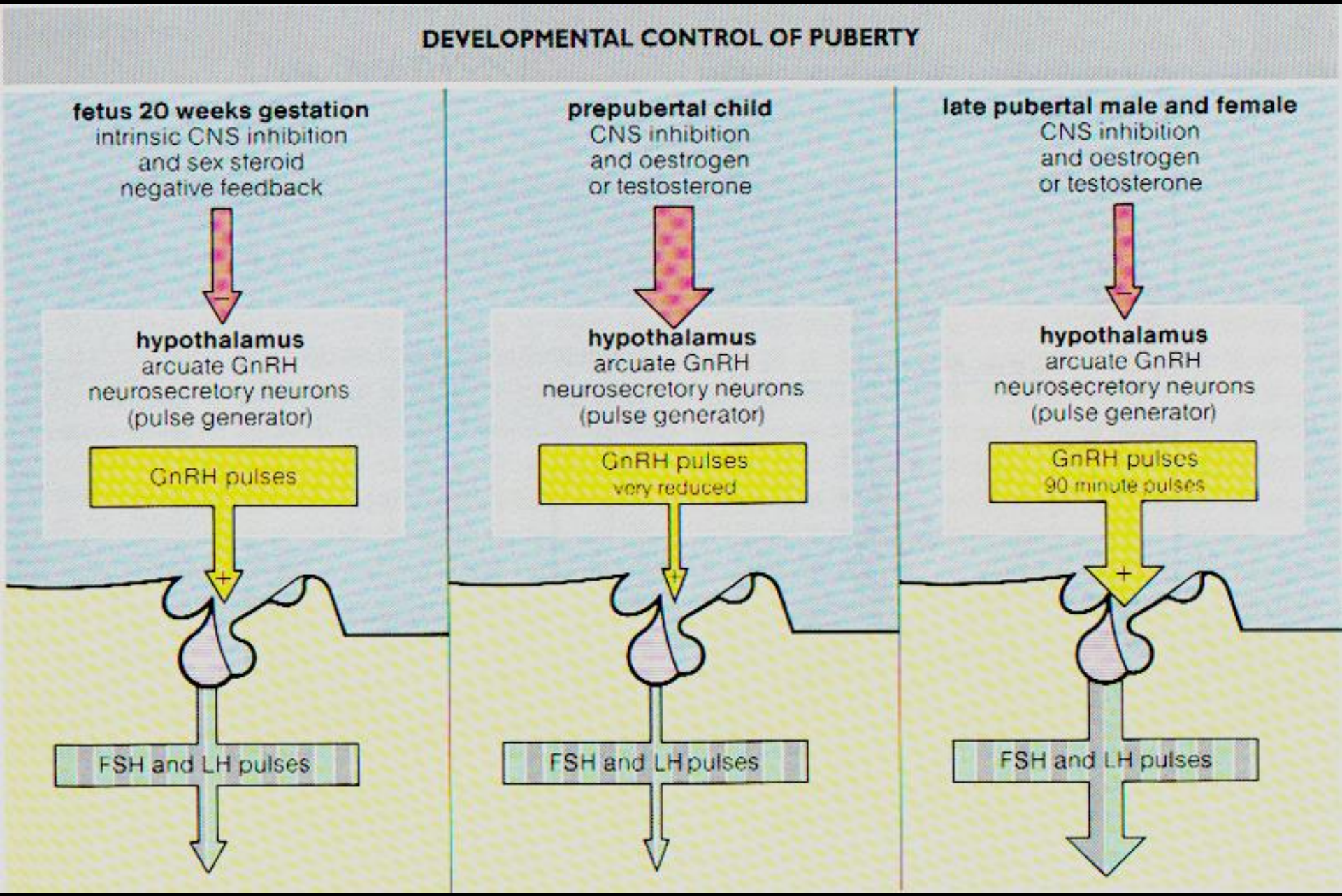
- ✓ Female karyotype 46XX
- ✓ Normal gonadal function
- ✓ Normal pituitary function

45X0 Turner Syndrome

- Prenatal diagnosis
- Neonatal diagnosis
- Short stature
- Primary amenorrhea

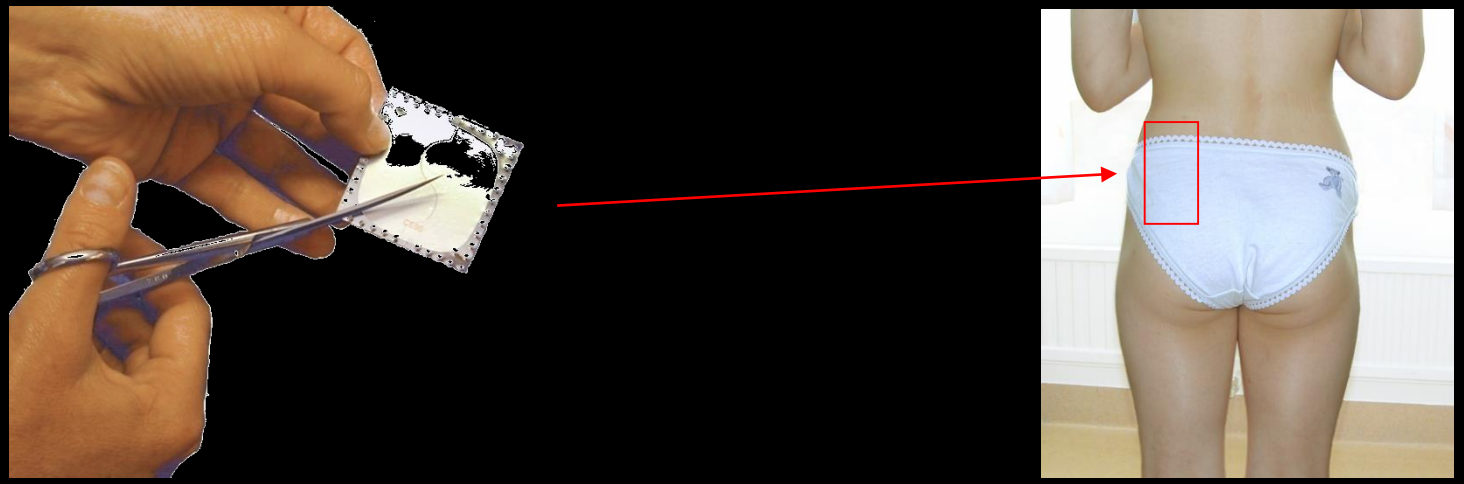


Minipuberty – the basis for early FSH elevation in 45X0



Treatment options

- ✓ Pubertal induction with transdermal estradiol (Evorel) – start close to average age!

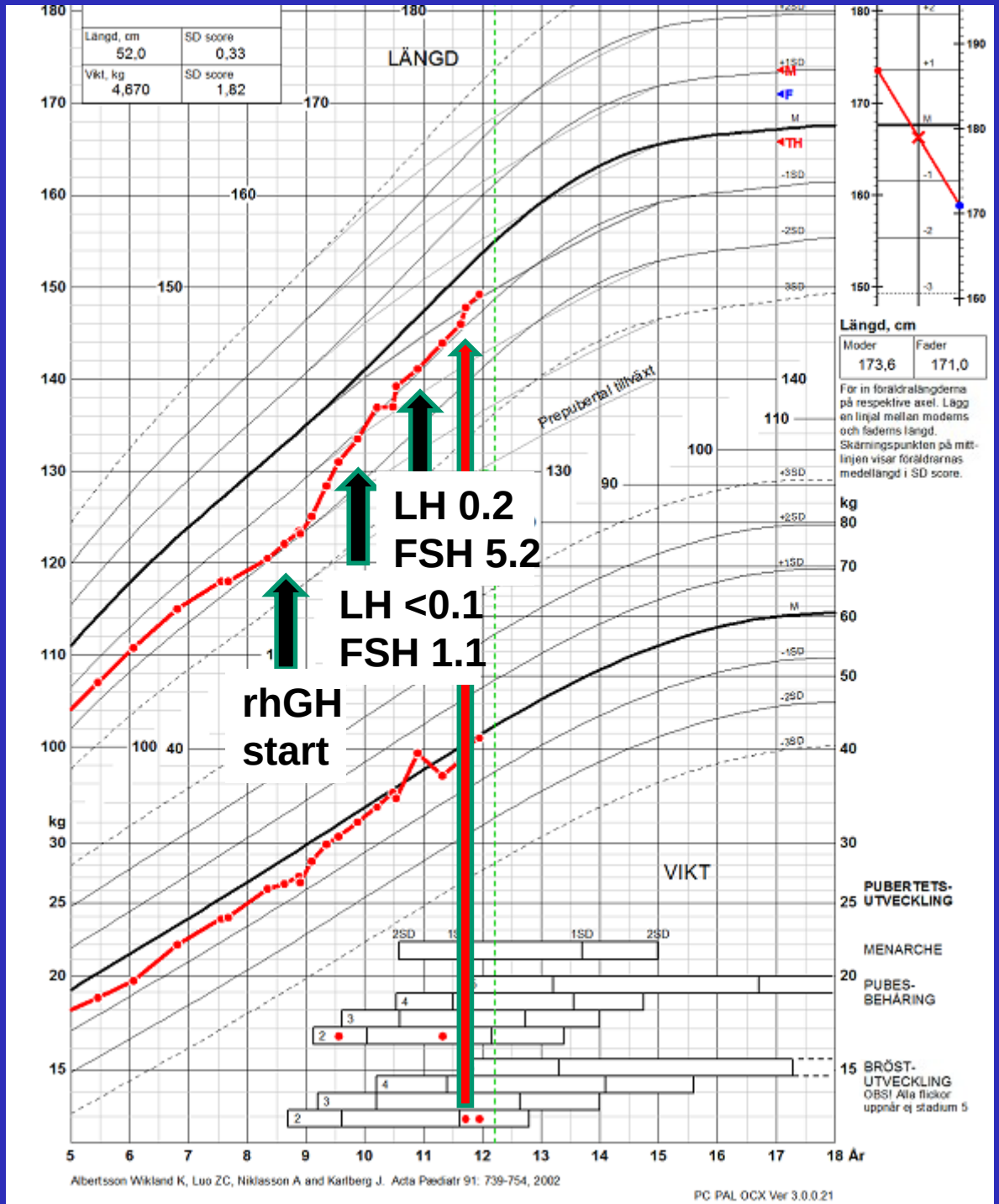


- ✓ Addition of progesterone (Provera 5-10mg) to initiate cyclic bleedings
- ✓ Androgens for normal secondary sexual hair development?
- ✓ Fertility treatment options – councelling with OBGY/genetics

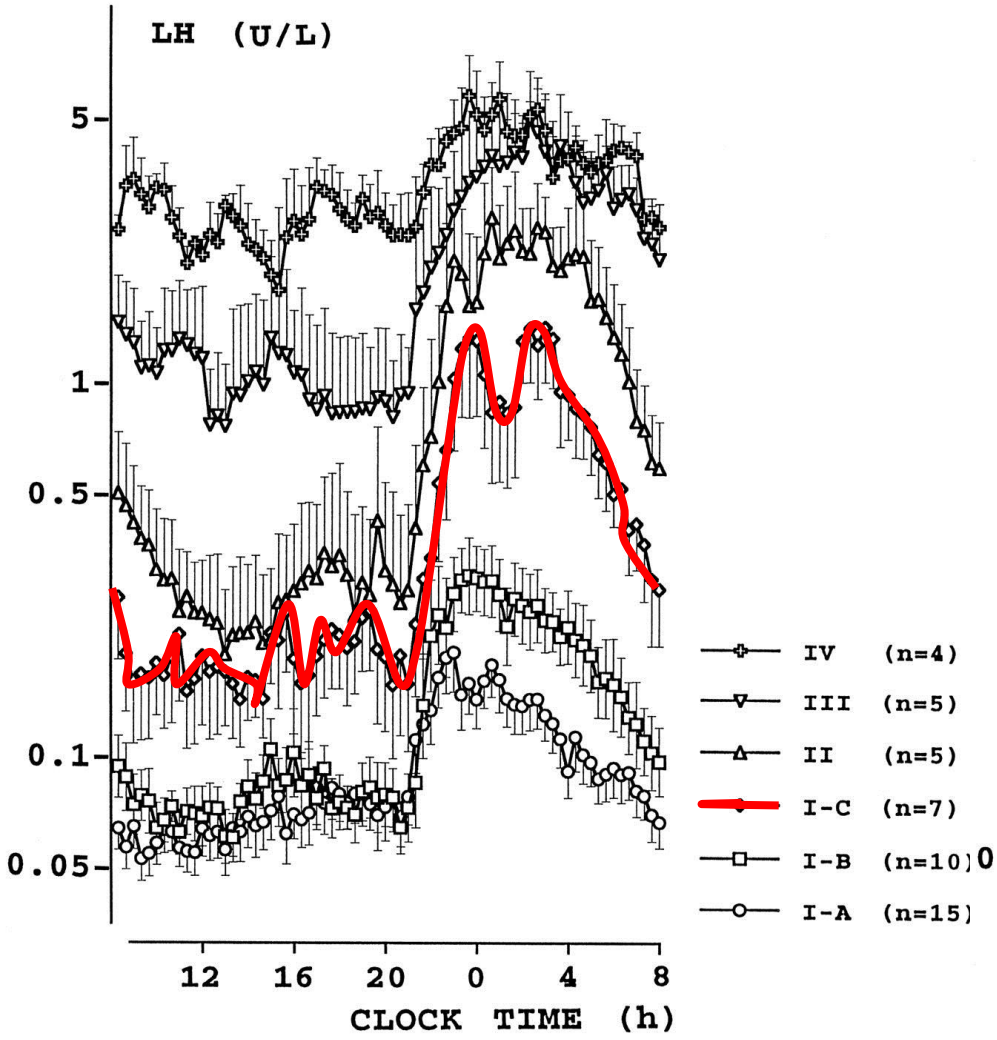
45X0/ 46XX

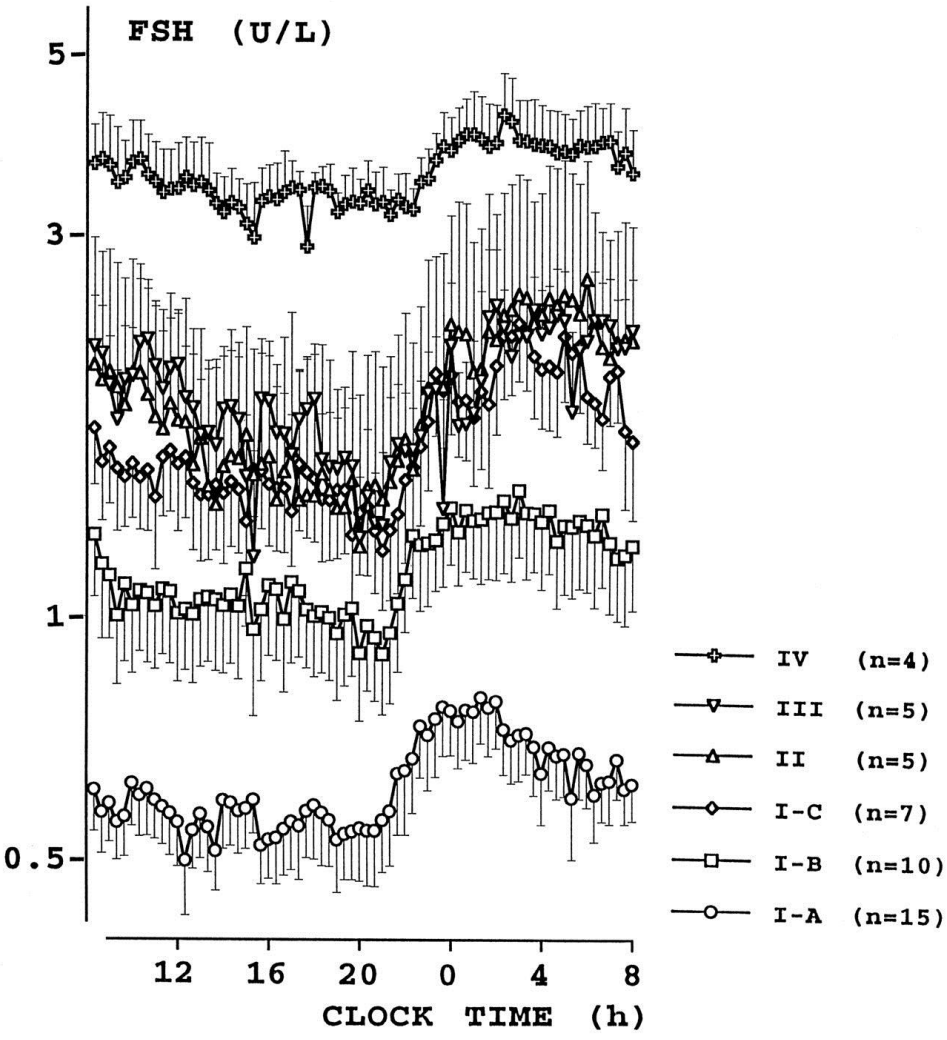
Turner Mosaicism

- Normal GH-IGF-axis
- SHOX-deficiency



LH more closely correlates with onset of central puberty





Ovarian preservation in girls with Turners syndrome?

- Ovarian biopsy in 47 of 57 girls Turner girls
- Follicles were found in:
  - ✓ 6/7 girls (86%) with mosaicism
  - ✓ 6/22 (27%) with structural chromosomal abnormalities
  - ✓ 3/28 girls with karyotype 45X (10.7%)
- Follicles were found in:
  - ✓ 8/13 girls (62%) with spontaneous menarche
  - ✓ 11/19 girls (58%) who had signs of spontaneous puberty

Borgström et al. J Clin Endocrinol Metab. 94:74-80, 2009

Ovarian preservation in girls with Turners syndrome?

- Predictors of presence of follicular tissue

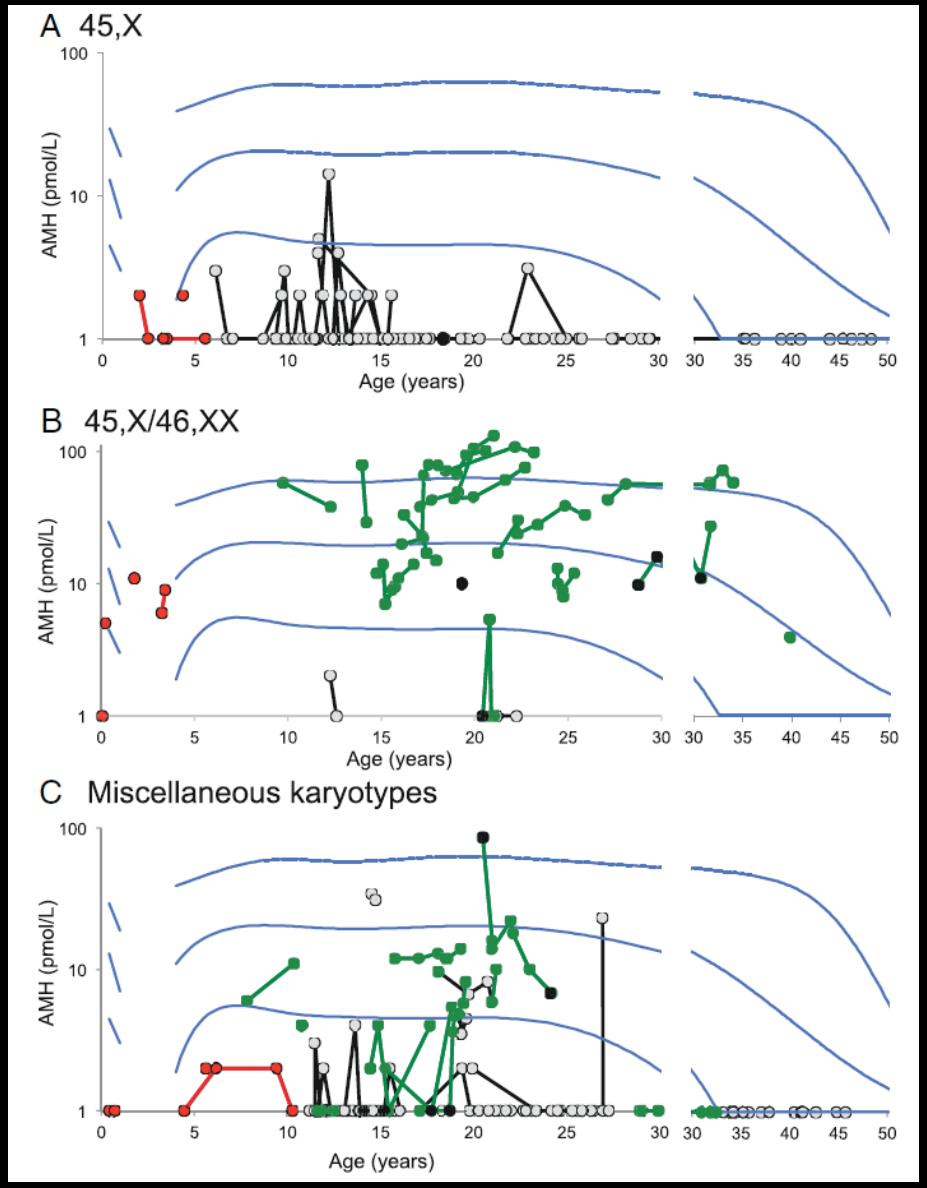
TABLE 2. Summary of diagnostic tests for positive and negative predictive values concerning six investigated variables

Factor	Positive predictive values	Score	Statistical significance	Negative predictive values	Score
Karyotype (n = 57)	Mosaicism	0.86	$P = 0.0001$	45X	0.89
				SA	0.73
FSH (n = 30)	Normal level	0.69	$P = 0.0123$	Above normal	0.77
AMH (n = 43)	Normal level	0.64	$P = 0.0003$	Subnormal level	0.88
Menarche (n = 50)	Spontaneous start	0.62	$P = 0.0039$	No spontaneous start	0.81
Puberty (n = 50)	Spontaneous start	0.58	$P = 0.0008$	No spontaneous start	0.87
Age (n = 57)	12–16 yr	0.31	NS	Below 12 yr	0.82
	Above 16 yr	0.24			

The factors karyotype, FSH, AMH, spontaneous menarche, and spontaneous start of puberty show statistical significance, but the age factor does not. NS, Not significant.

Borgström et al. J Clin Endocrinol Metab. 94:74-80, 2009

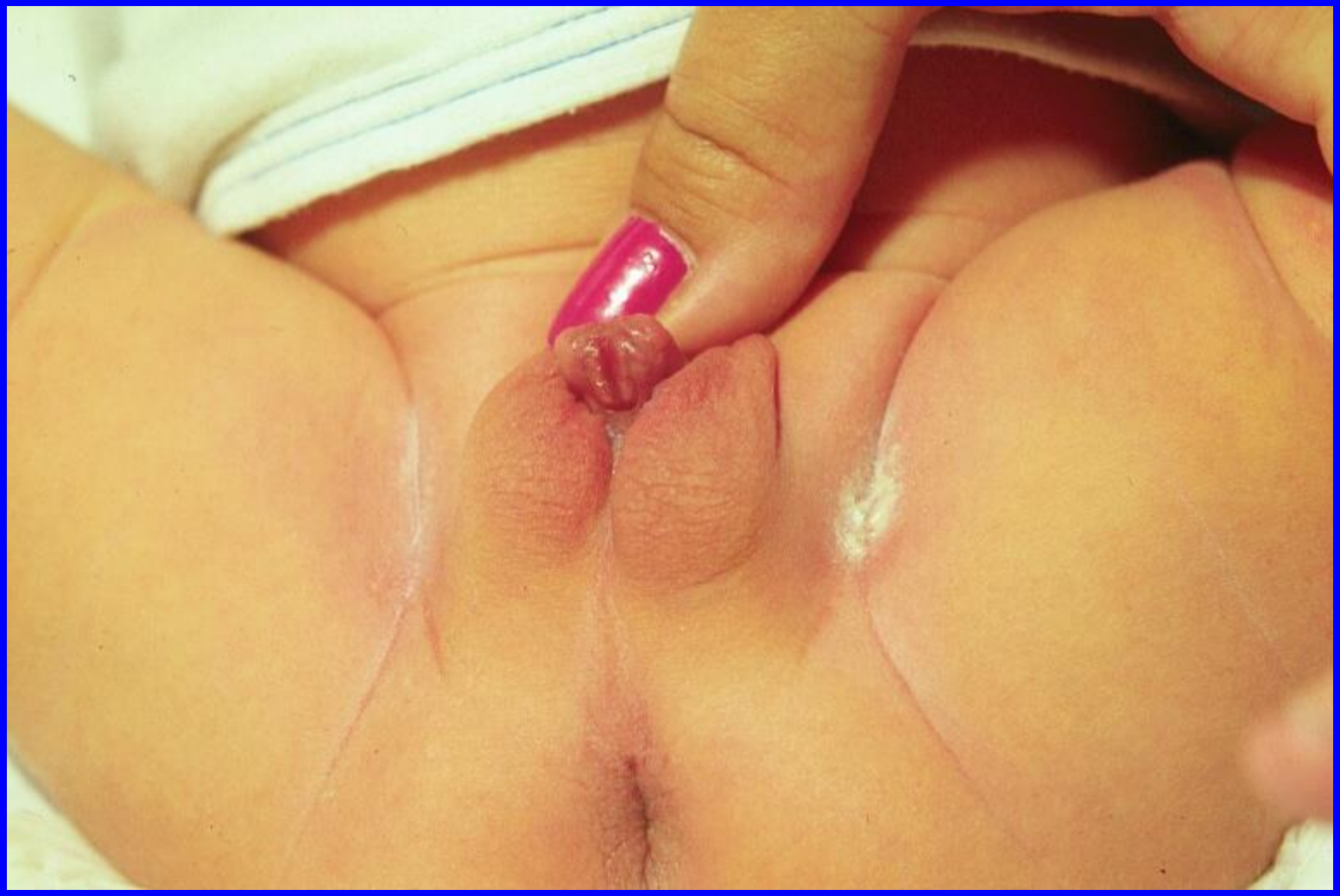
More support of AMH as a predictor of ovarian reserve



Lunding et al. J Clin Endocrinol Metab. 2015 Jul;100(7):E1030-8

Another case lacking normal female karyotype ….

- 2nd child of a 30 year old mother
- 4-year old healthy sister
- Mothers sister has a healthy son
- At delivery the newborn was named Sofía



… 4 hours later

- Examination by pediatrician: Not sure about the gender – needs to be determined after blood sampling
- 2 days later: Karyotype 46XY. Young pediatrician anounces that it is a boy.
- Baby was renamed Joakim.

Intersex team contacted and examines child:

- Hypospadia or virilisation
- Cleaved scotum or enlarged labiae with resistence bilat
- Urethra and 2 cm deep vagina
- Ultrasound with absence of uterus and ovaries

Blood work-up

- 17-OH-progesterone not elevated (CAH)
- DHEAS 1.2 umol/L (normale)
- Androstendion 1.4 nmol/L (normale)
- Testosteron 1.2 nmol/L. After hCG-stimulation 8.0 nmol/L (normale for males)
- Karyotype confirmed 46XY, SRY positive

Potential causes of poor masculinization

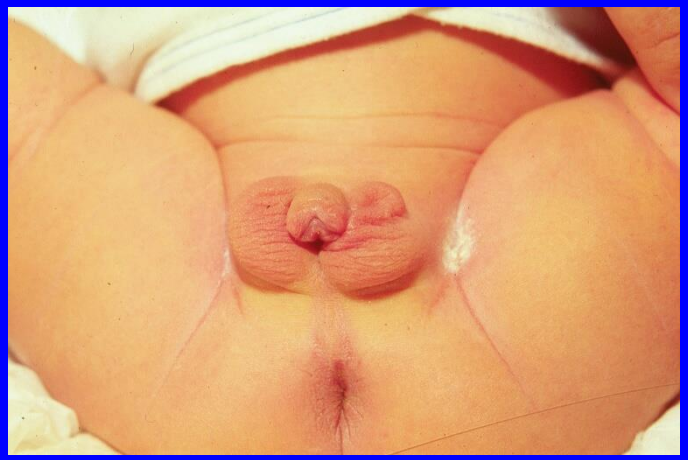
Poor androgen produktion or sensitivity

- LH receptor defect (Leydigcell aplasia)
- 3β-HSD- , 17-keto-oxido-reductase- or, 5α-reductase deficiency
- Androgen insensitivity

Genetics

- Androgen receptor gene: DNA-binding region mutation (Glu653Lys)
- Mother carrier of mutation
- Diagnosis PAIS (partial androgen insensitivity)
- 5α-reductase gene normal

- PAIS most often diagnosed at birth due to ambiguous genitalia

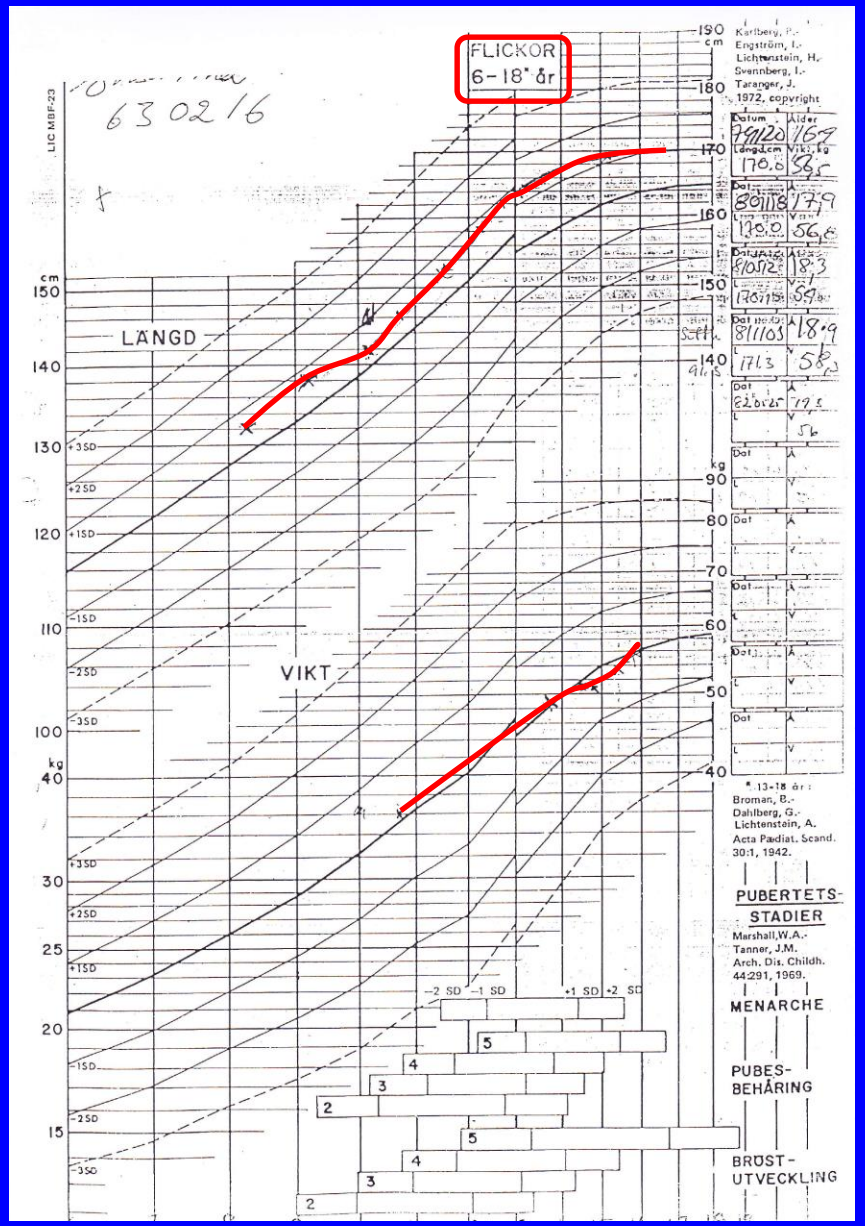


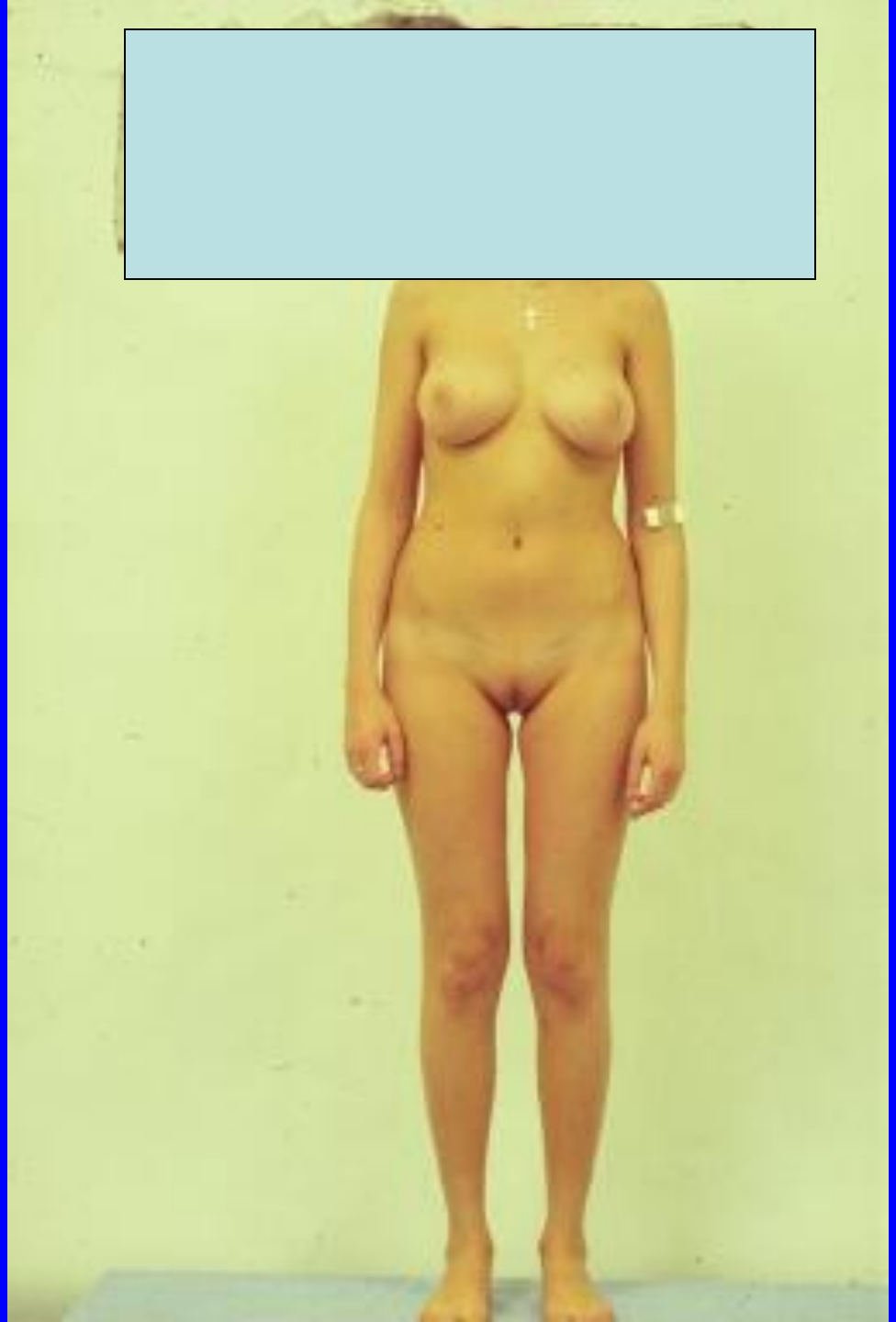
Androgen insensitivity - another case

- ✓ Normal breast development
- ✓ Near normal female pubertal growth
- ✓ Lack/sparse pubic hair

- ✓ Acute inguinal hernia: ovo-testis structure: Extirpated at 11 and 15 years of age
- ✓ FSH 28.9 IU/l, LH 81.6 IU/l, Testosterone 113.8 nmol/l at peak HtV- AIS

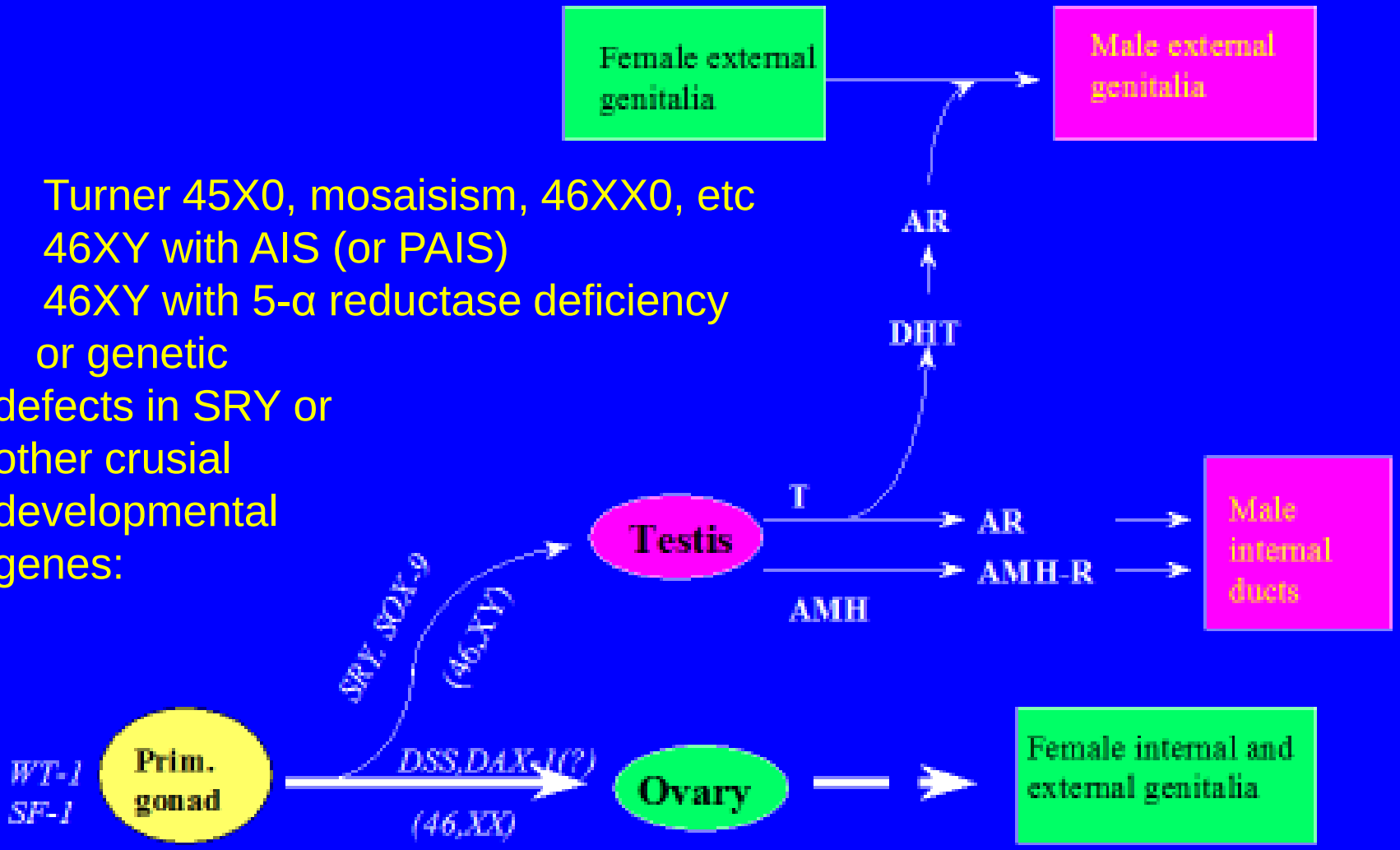
- ✓ DHT w some hairgrowth
- ✓ Estradiol gel





Karyotypes that may come to diagnosis in older girls/young women:

- ✓ Turner 45X0, mosaisism, 46XX0, etc
- ✓ 46XY with AIS (or PAIS)
- ✓ 46XY with 5-α reductase deficiency
- ✓ or genetic defects in SRY or other crusial developmental genes:



Abnormal gonadal function – inadequate estrogen production:

- ✓ LH/FSH receptor defects

- ✓ Gonadal dysgenesis (46 XY/46 XO)
- ✓ Aromatase deficiency in 46XX (ambiguous genitalia, hypergonadotropic hypogonadism, lack secondary sexual characteristics, exhibit progressive virilization)

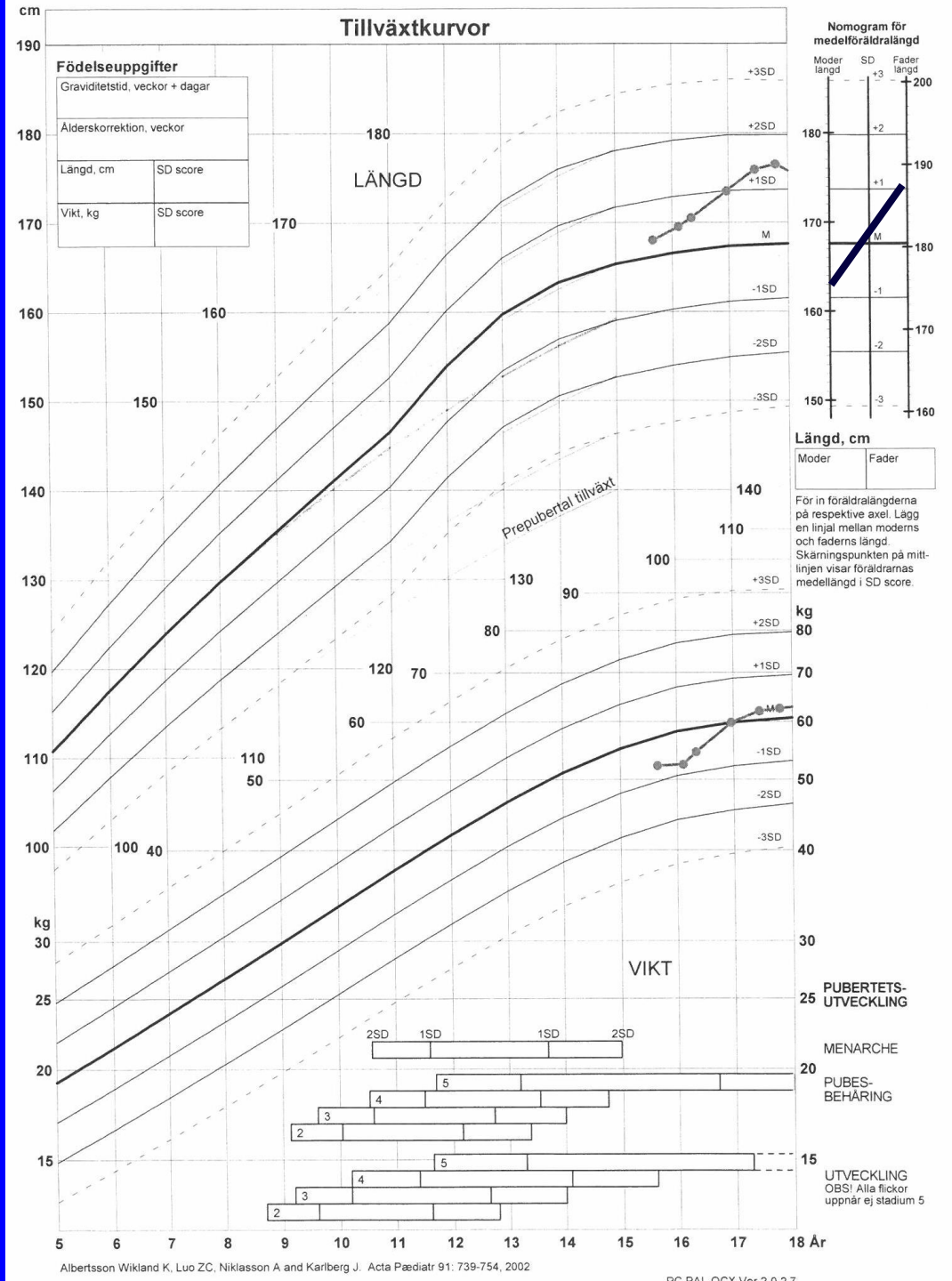
Case – Julia (not really her name)

- ✓ Lack of pubertal development (B1) and primary amenorrhea at age 15 years old
- ✓ Mother menarche at 15 years of age
- ✓ Midparental height + 0.5 SDS

- ✓ FSH 88 IU/L / LH 16 IU/L / Estradiol < 45 pmol/L
- ✓ Bone age delayed > 3 years
- ✓ Ultrasound (vaginal in full anestesia performed by gynecologist): Vagina, small uterus, but ovaries cannot be visualized

- ✓ Laparoskopi at Karolinska
- ✓ Ovarian tissue 46 XX
- ✓ Suspected FSH receptor defect – genetic diagnosis not known

- ✓ Pubertal induction by estradiol gel
- ✓ Gestapuran added 2 years later 10 days in 1 month cycles



Abnormal gonadal function (cont.)

- ✓ Adrenal hormone excess (secundary amenorrhea)
  - ✓ Premature adrenache – PCOS
  - ✓ CAH has pubertal start normal or slightly ealier
  - ✓ Doping
- ✓ Gonadotoxic therapy
  - ✓ Total body irradiation or directed toward gonads
  - ✓ Cytotoxix drugs
- ✓ Autoimmune Polyendocrine Syndromes

A 10-year follow up of reproductive function in women treated for childhood cancer

Table 4 Ovarian reserve in survivors included in 2010 according to different treatment groups.

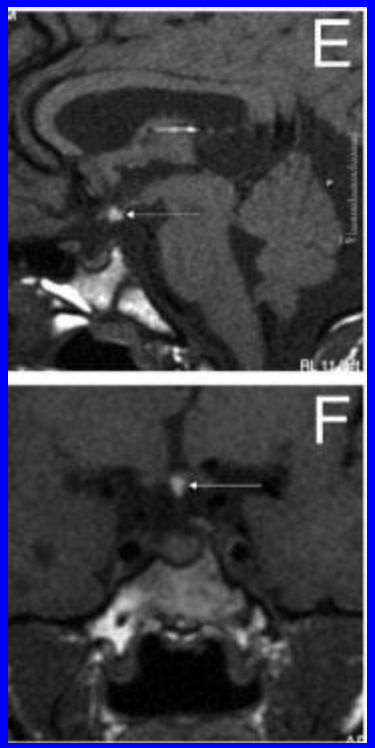
	Group 1: minimal gonadotoxic treatment (n = 36)	*Group 2: potentially gonadotoxic treatment (n = 26)*	*Group 3: gonadotoxic treatment (n = 9)*	*P-value*
Age at inclusion (years)	35.4 (27.5–45.2)	34.9 (27.8–53.6)	33.3 (29.6–42.4)	NS
AMH (pmol/l)	20.0 (<3–66.0)	5.8 (<3–71.0)	<3 (<3–4.7)	<0.001
AFC (2–10 mm)	15 (0–40)	9 (0–34)	2 (0–7)	0.003

Values are median (range). Kruskal–Wallis test.
Group 1 = non-alkylating chemotherapy and ovaries not in radiation field in seven cases also treated with radiotherapy; cytotoxic agents asparaginase, cytaribine, dactinomycin, daunorubicin, doxorubicin, 6-mercaptopurine, methotrexate, prednisone, vincristine.
Group 2 = chemotherapy including alkylating agents and ovaries not in radiation field in four cases also treated with radiotherapy; cytotoxic agents camustine, chlorambucil, cisplatin, cyclophosphamide, dacarbazine, ifosfamide, mustagene, procarbazine, asparaginase, bleomycin, cytaribine, dactinomycin, doxorubicin, 6-mercaptopurine, methotrexate, prednisone, thioguanine, vepeside, vinblastine, vincristine, VM-26.
Group 3 = chemotherapy including alkylating agents and radiotherapy with ovaries in radiation field (pelvic irradiation and total body irradiation); cytotoxic agents asparaginase, busulfan, cisplatin, cyclophosphamide, cytaribine, dacarbazine, dactinomycin, doxorubicin, dounorubicin, 6-mercaptopurine, methotrexate, prednisone, thioguanine, vincristine, VM-26.
AFC = antral follicle count (both ovaries); AMH = anti-Müllerian hormone.

Nielsen et al. Reprod Biomed Online. 2013 Aug;27(2):192-200.

Abnormal pituitary function:

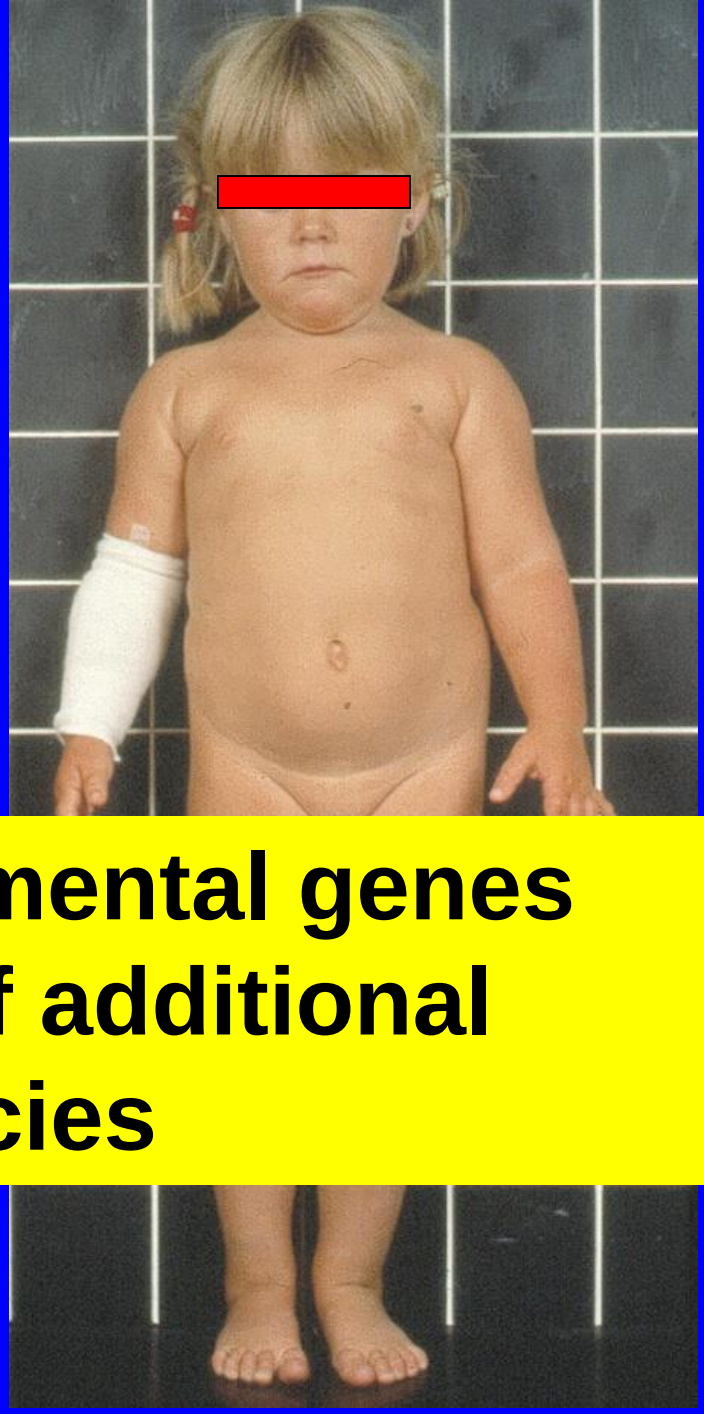
- ✓ Gonadal axis deficiency or multiple pituitary hormone deficiencies
  - ✓ Survivors of pediatric brain tumours and their treatment - irradiation
  - ✓ Kallman – absence of GnRH neuron (and olfactorius) migration
  - ✓ Midline defects - SOD
- ✓ Syndromes affecting the pituitary-gonadal axis
  - ✓ Prader Willi
  - ✓ Noonan
- ✓ Other pituitary hormone deficiencies or excess states
  - ✓ GH deficiency or GH insensitivity syndrome
  - ✓ Hypothyroidism
  - ✓ Cushing's syndrome



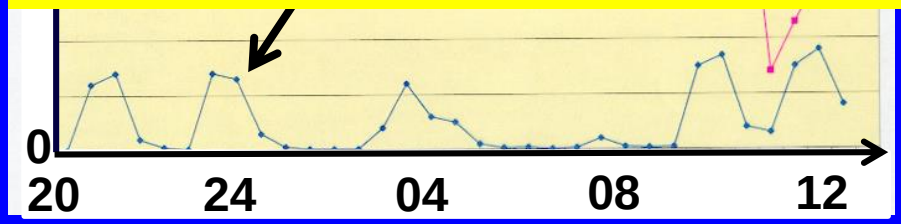
GHD

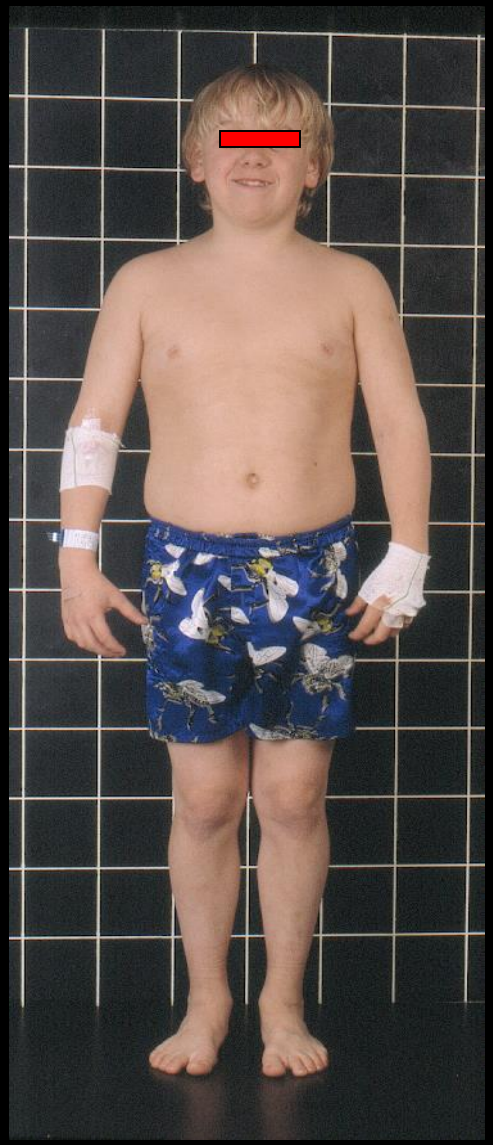
Organic or Idiopathic

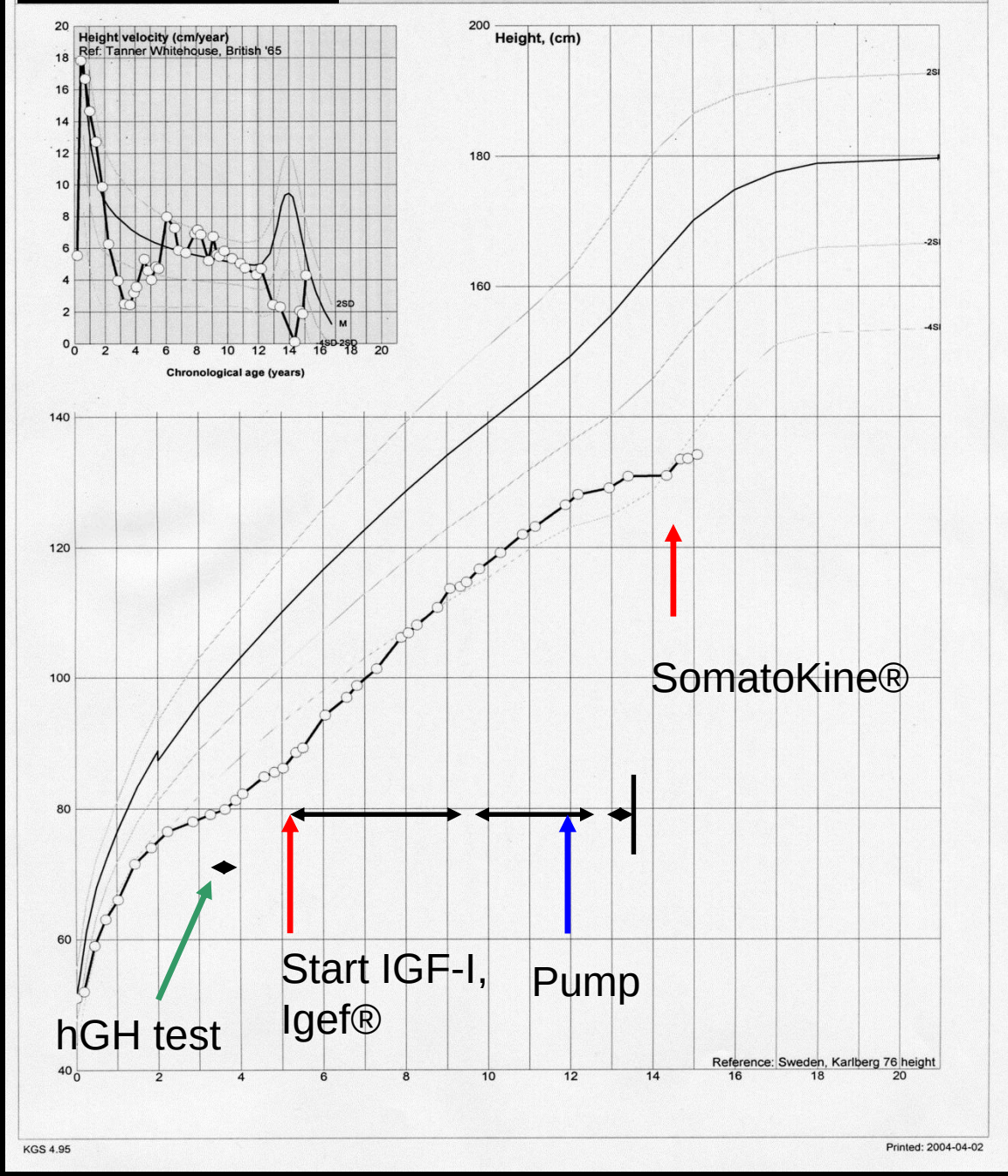
Genetic causes: HESX1, LHX3, LHX4, PROP1, POU1F1, SOX3, SOX2, GH1, GHRH-R



Pituitary developmental genes predicts risks of additional deficiencies

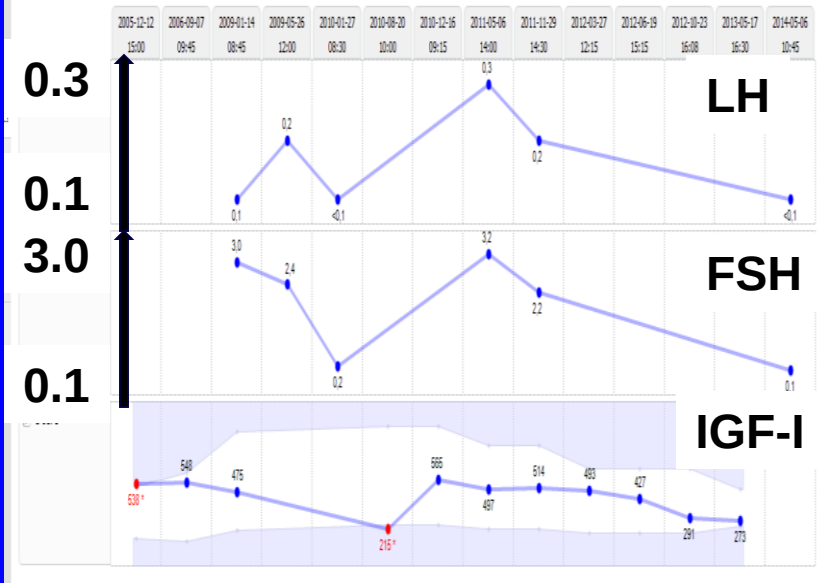


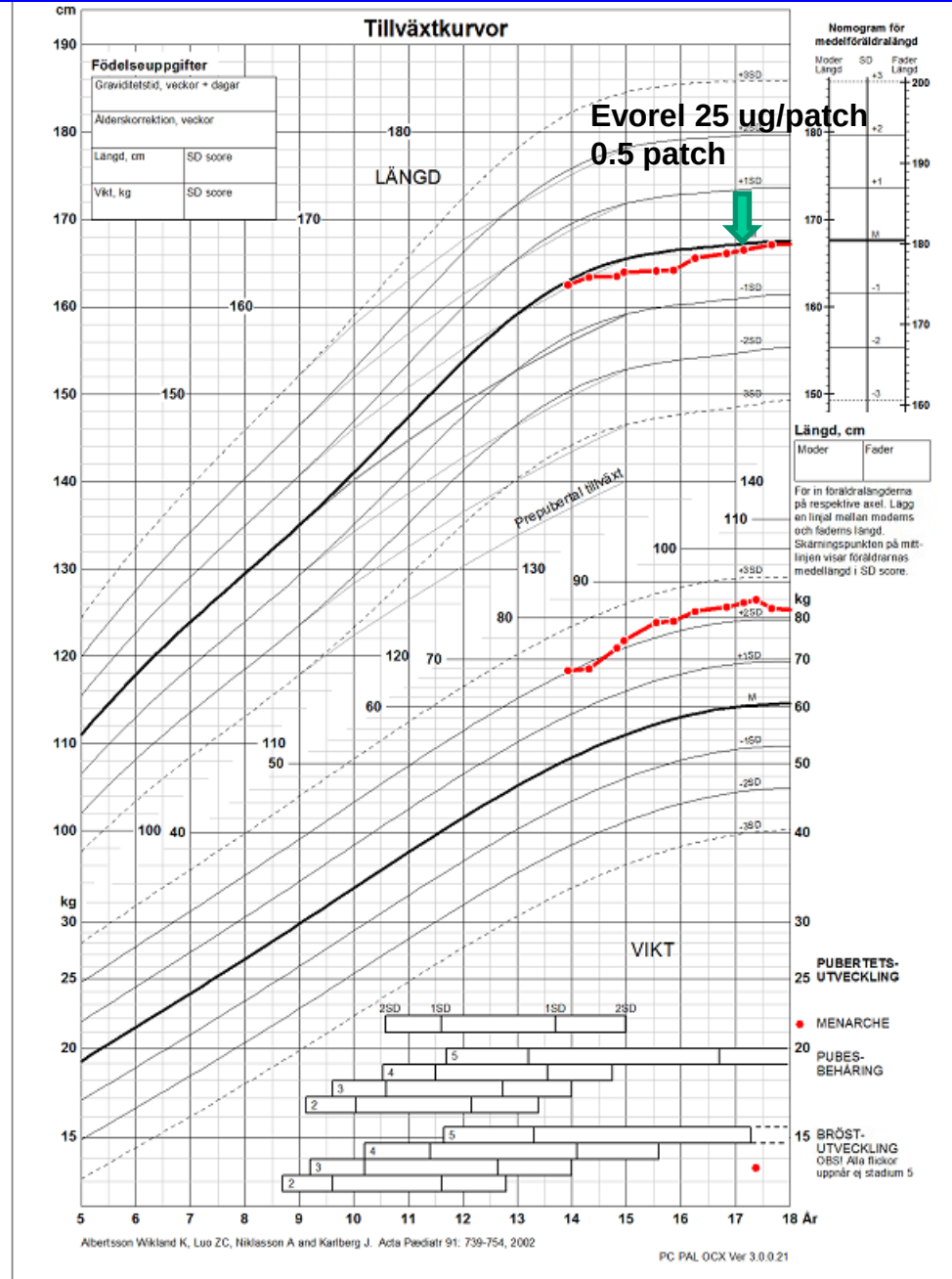


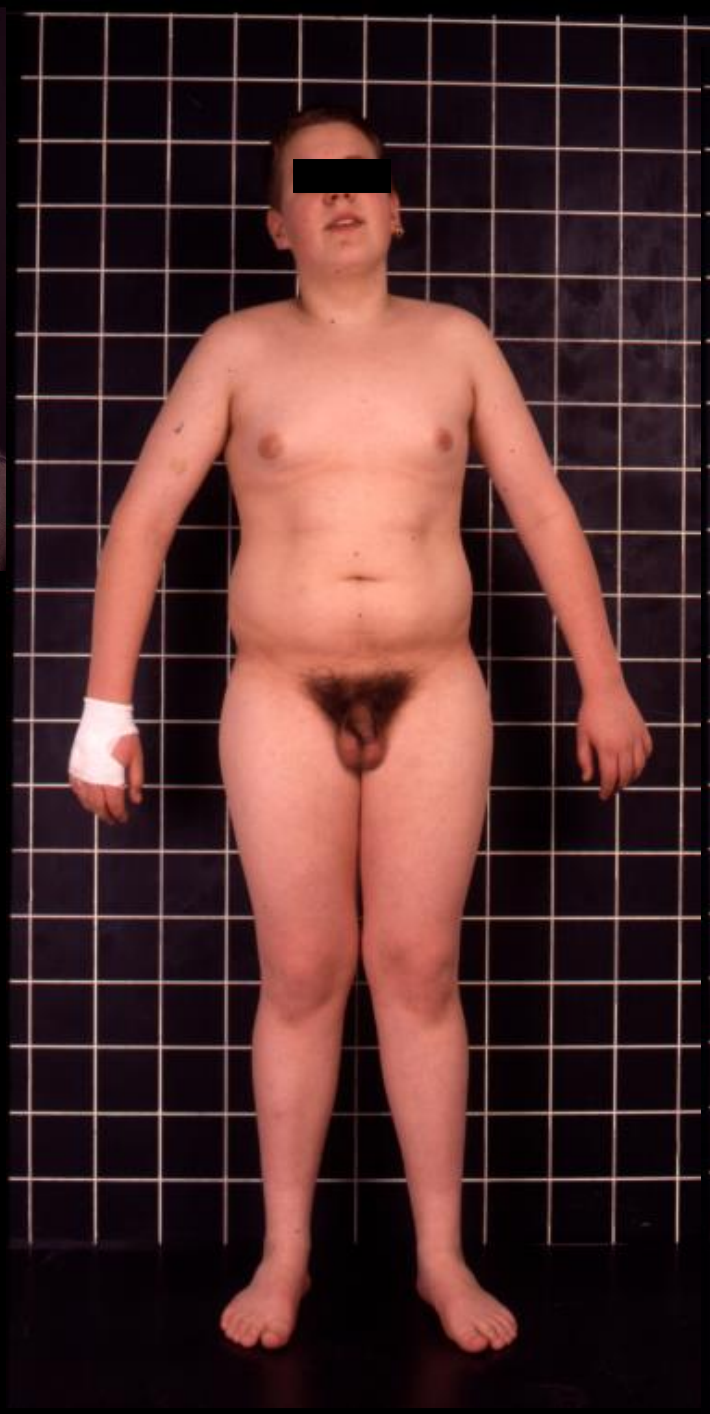

Height velocity (cm/year)
Ref: Tanner Whitehouse, British '65
Chronological age (years)
Height, (cm)
SomatoKine®
hGH test
Start IGF-I,
Igef®
Pump
Reference: Sweden, Karlberg 76 height
KGS 4.95
Printed: 2004-04-02

Prader Willi

- ✓ GH therapy
- ✓ Spontaneous pubertal start
- ✓ Primary amenorrhea at 17 years of age
- ✓ Pubertal induction by estradiol gel (too late)







Summary

- ✓ Age at menarche is affected by nutritional and genetic (epigenetic) factors with little secular trend
- ✓ CDGP and nutritional deficits or stressers associated with chronic disease or lifestyle are common causes of primary amenorrhea in the pediatric population
- ✓ An increasing number of karyotypic and genetic abnormalities associated with amenorrhea are diagnosed early and treated by pediatricians
- ✓ Fertility counseling and treatment should involve OBGY
- ✓ An increasing number of children treated for cancer may have pituitary and/or gonadal damage that causes primary amenorrhea

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Martin Ritzen
Katarina Algovik

Thanks for you attention