Philippe Bouchard
Hôpital Saint Antoine

Conflicts of Interests/ Disclosure

- ***Senior Consultant, The Population Council, New York , New York since 1986***

- ***Consulting, research funding and lecture fees from: Schering Plough (Organon), Merck- Serono ,P & G, Ferring, Grunenthal, Besins Health Int, HRA Pharma,Wyeth, Pfizer, Preglem SA, Pierre Fabre, Repros, Takeda, Pantarhei, Bayer Pharma.***

- ***Consulting: HAS, EMEA, AFSSAPS, WHO***

Pharmacogenetics in Ovarian Stimulation

- Tailoring ovarian stimulation to the individual patient can be challenging because the ovarian response varies substanstially between patients.
- Pharmacogenetics has emerged as a new area of research to improve the balance between desired and undesired action of drugs, based on the the genetic predisposition of the individual patient.

Grebb, Behre and Simoni 2005

Pharmacogenetics in ovarian stimulation

- **Therefore genotyping of patients scheduled for ovarian stimulation could be an attractive tool to individualize FSH/LH dosing according to genetic differences in ovarian sensitivity.**

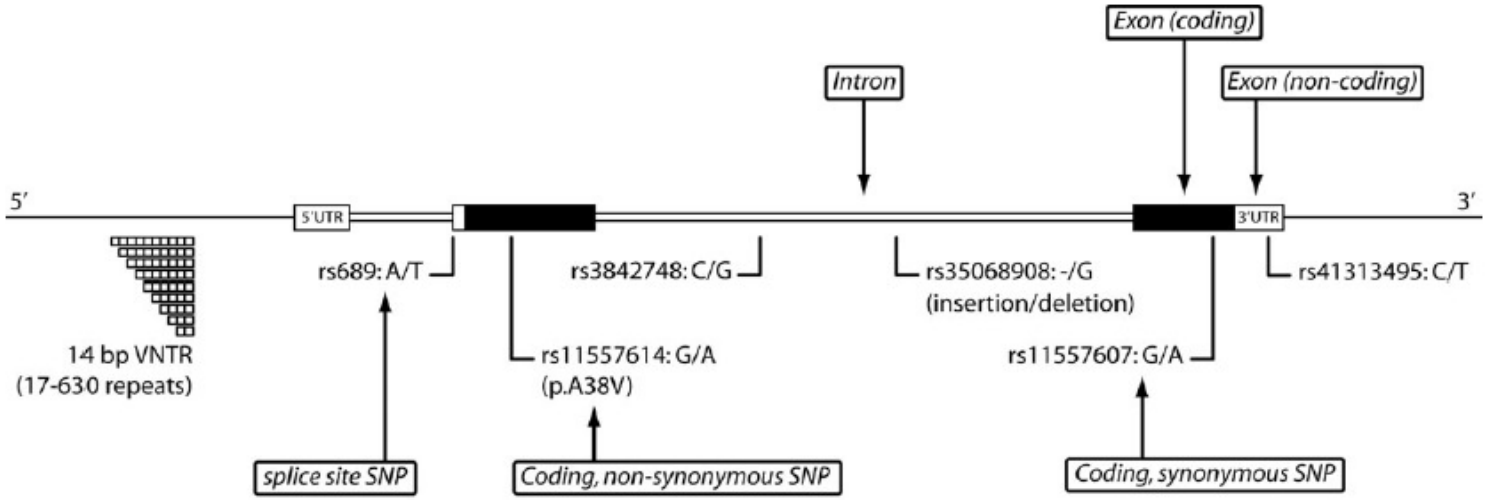
FSH and LH receptor polymorphisms

- Polymorphisms are gene DNA variants that exist in the normal population at a frequency of 1% or more

- Mainly in the form of so-called single nucleotide polymorphisms (SNPs).

Functional Genetic Polymorphisms and Female Reproduction.

Simoni *et al.*



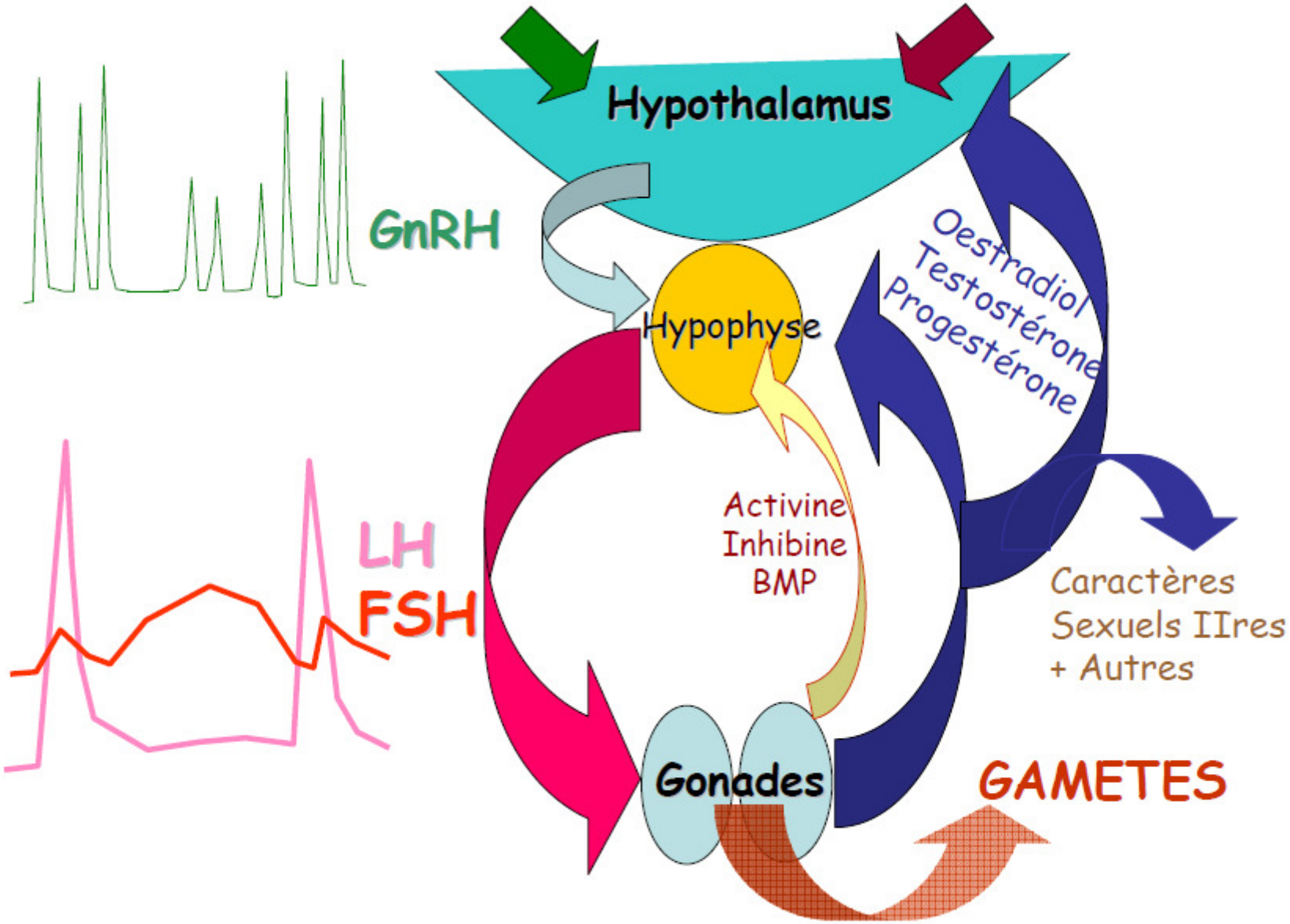
RFLP, restriction fragment length polymorphism.

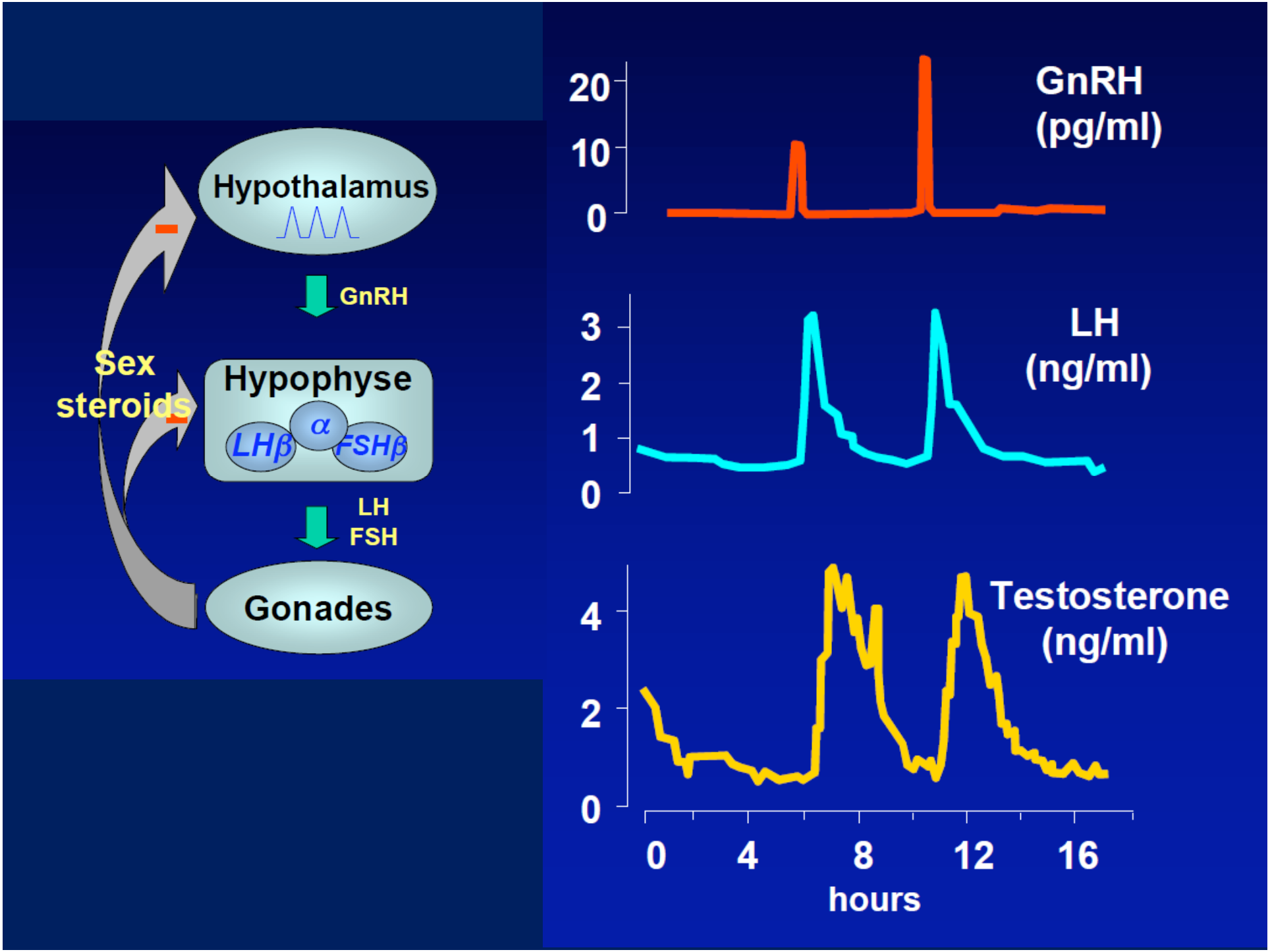
Figure 1: Graphic representation of types of genetic variants, showing insertion/deletion (ins/del) polymorphisms, both coding and non-coding SNPs, and repeat polymorphisms such as tandem repeats or VNTR.
Variants are shown occurring within a gene (in this example the *INS* gene), but can also occur outside of genes. Other types of genetic variations that affect larger regions, such as copy number variations, are not shown. SNP, single-nucleotide polymorphism; VNTR, variable number of tandem repeats.

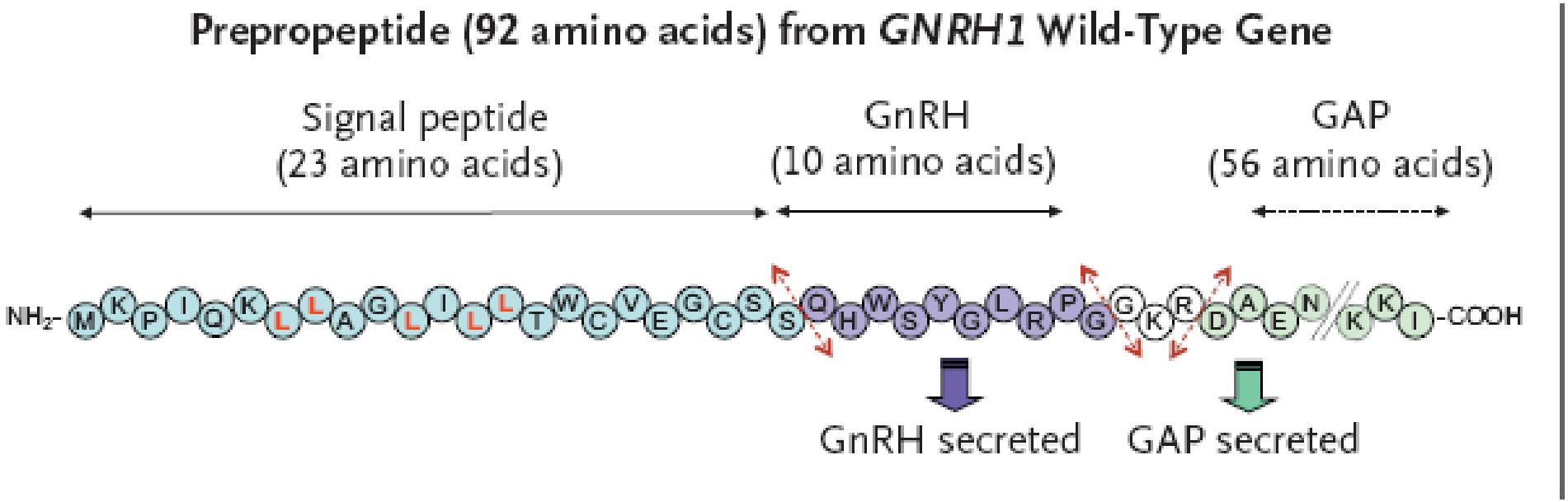
Human Reproduction Update, 14: 459-84,2008

Polymorphic Variants (M Simoni 2008)

- **Variants** can occur within or outside a gene
- **Within the gene**: in exons, introns or regulatory regions. They can be silent or functional.
- **Outside the gene**: they can be used as tags to identify functional regions. They may affect RNA transcription and processing.


Hypothalamus
GnRH
Hypophyse
Oestradiol
Testostérone
Progestérone
Activine
Inhibine
BMP
LH
FSH
Caractères
Sexuels IIres
+ Autres
Gonades
GAMETES


Hypothalamus
GnRH
Hypophyse
α
LHβ
FSHβ
LH
FSH
Gonades
Sex steroids
20
10
0
GnRH (pg/ml)
3
2
1
0
LH (ng/ml)
4
2
0
Testosterone (ng/ml)
0
4
8
12
16
hours

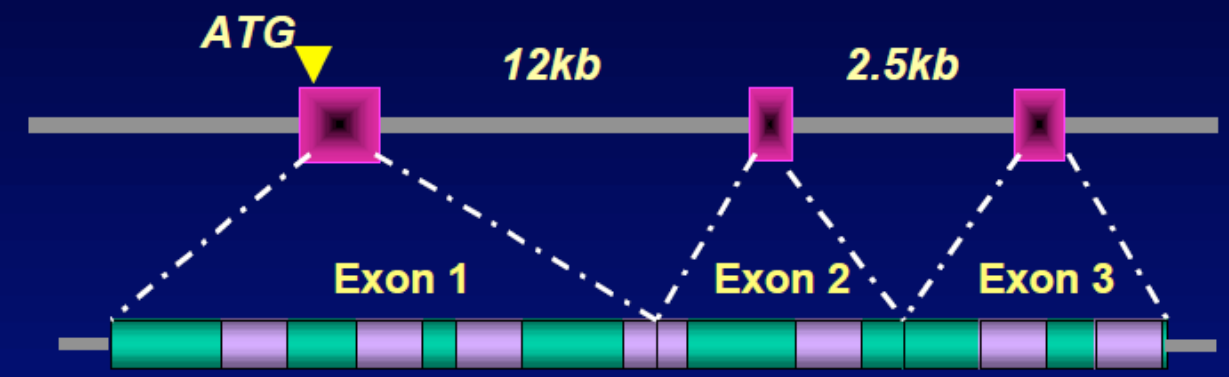


Bouligand J et al NEJM, 2009

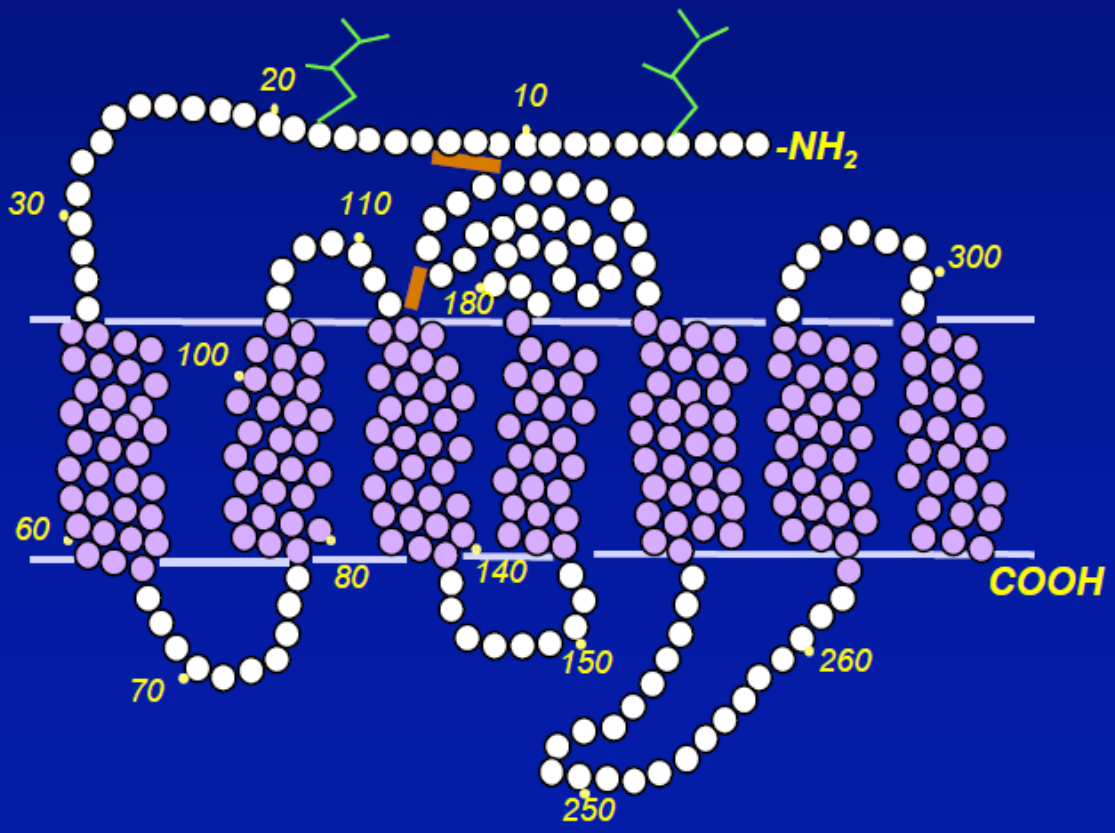
Polymorphisms: GnRH1

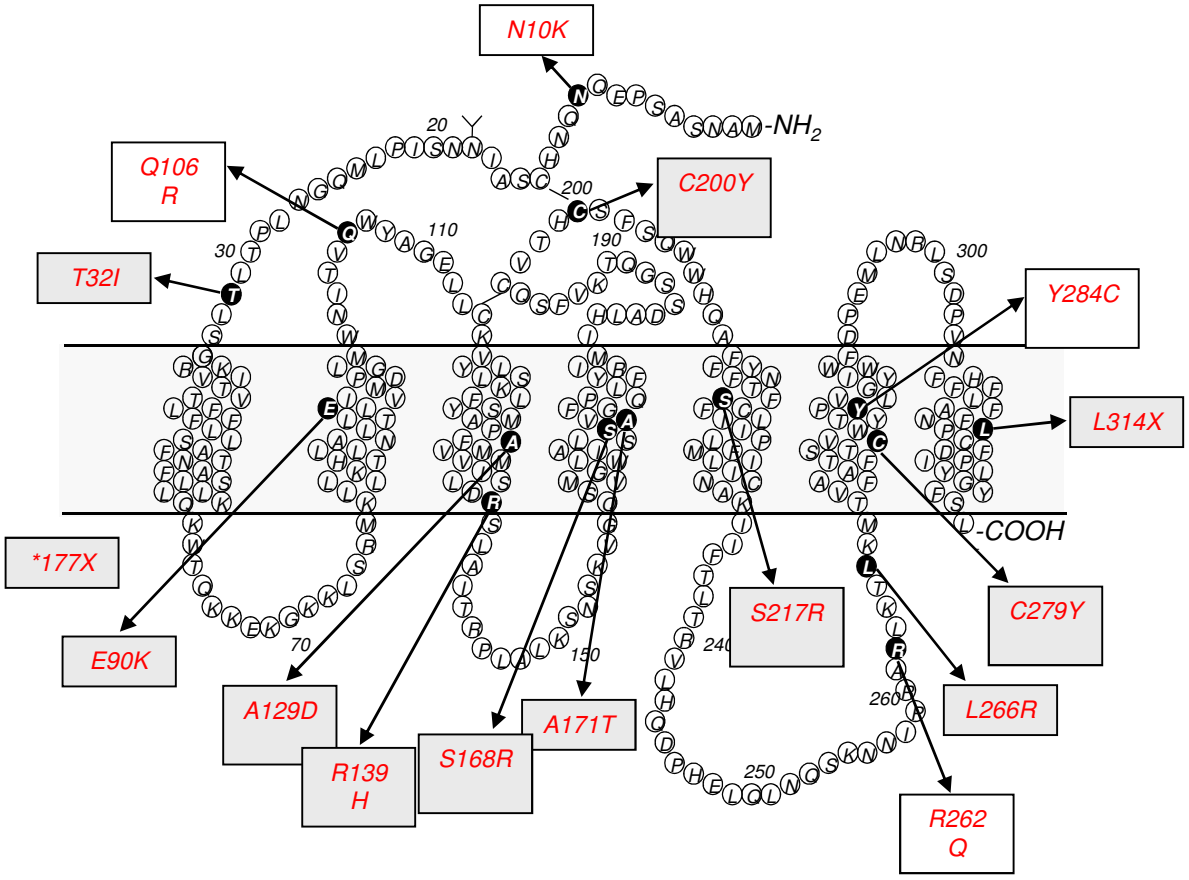
- A polymorphism in the first exon of
- GnRH 1has been described, constituting an amino acid variation at codon 16 (Trp16Ser).

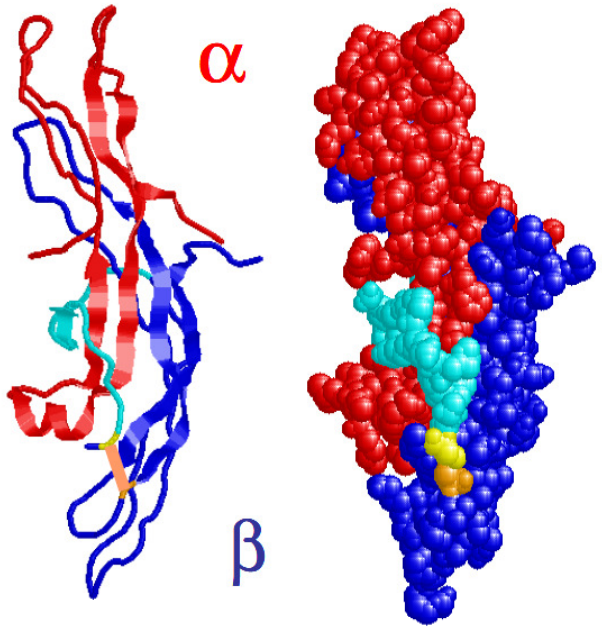
- Results are contradictory in terms of Estrogen exposure.



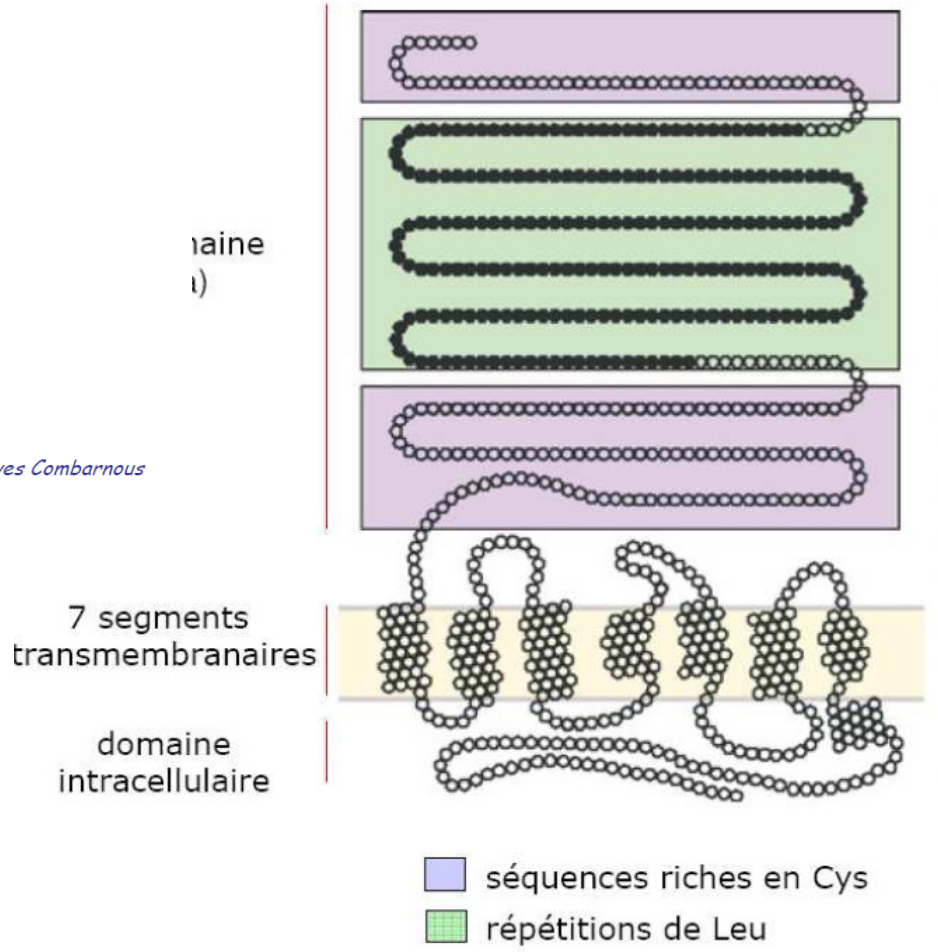
Transmembrane Helices = TMH



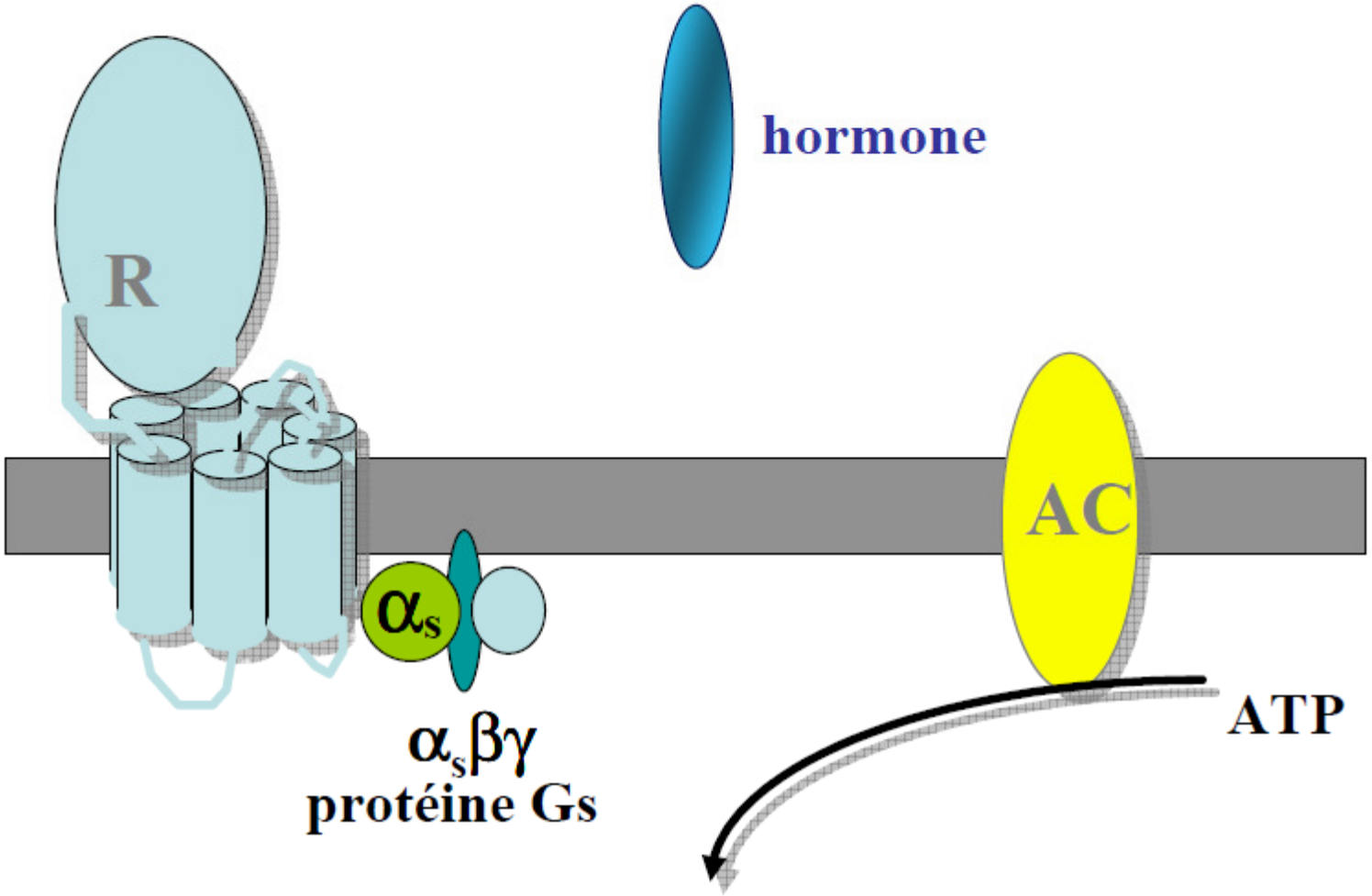

N10K
Q106
R
C200Y
T32I
Y284C
L314X
*177X
E90K
A129D
R139
H
S168R
A171T
S217R
C279Y
L266R
R262
Q
-NH2
-COOH
20
30
110
190
200
300
70
150
240
250
260



Yves Combarnous

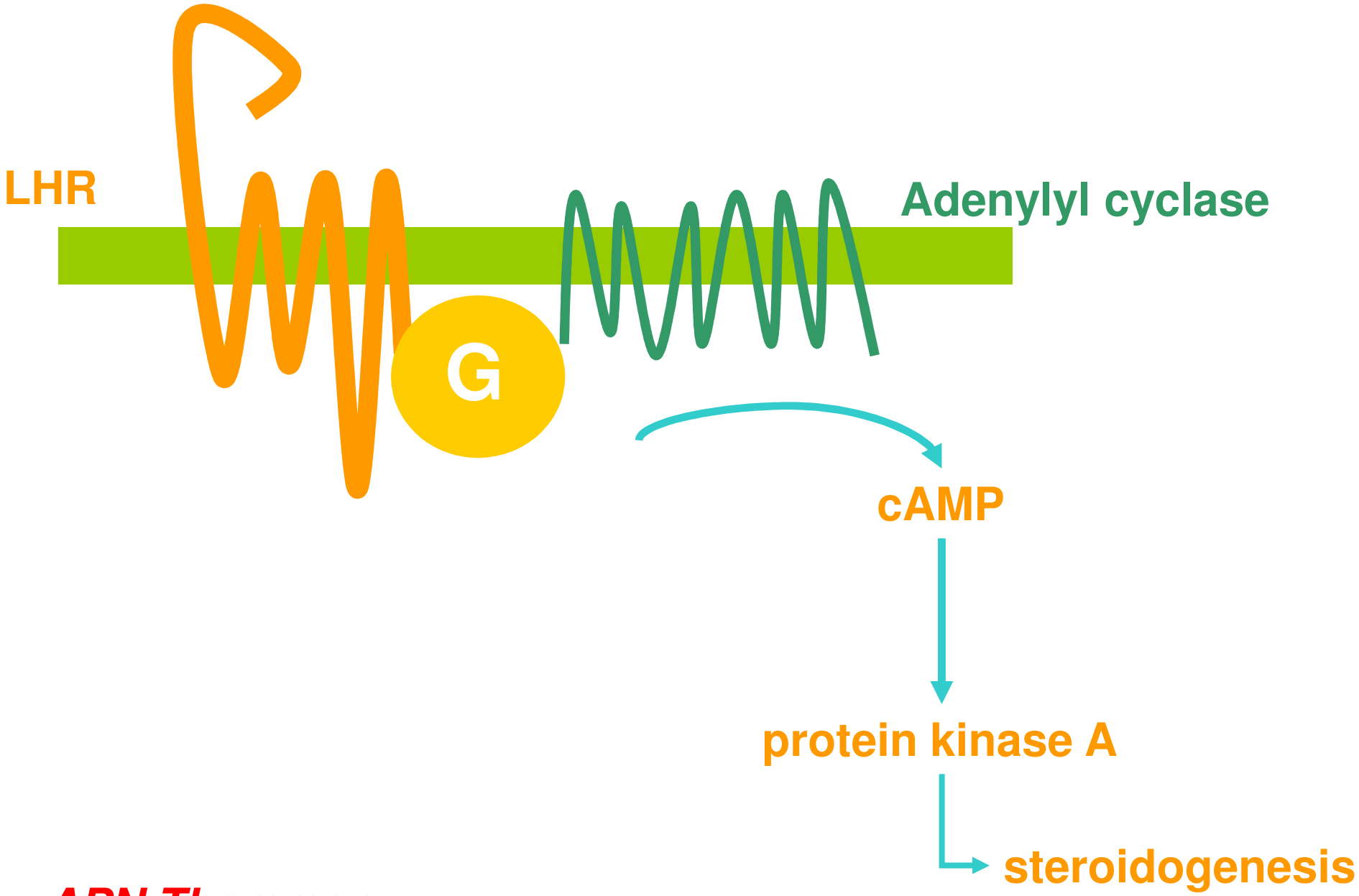


Vassart *et al.* (2004), *TRENDS in Biochemical Sciences.*, **29**:119-126



Yves COMBARNOUS

	mâle	femelle
FSH 	Cellules de Sertoli	Cellules de granulosa
LH 	Cellules de Leydig	Cellules de granulosa Cellules de thèque Cellules lutéales

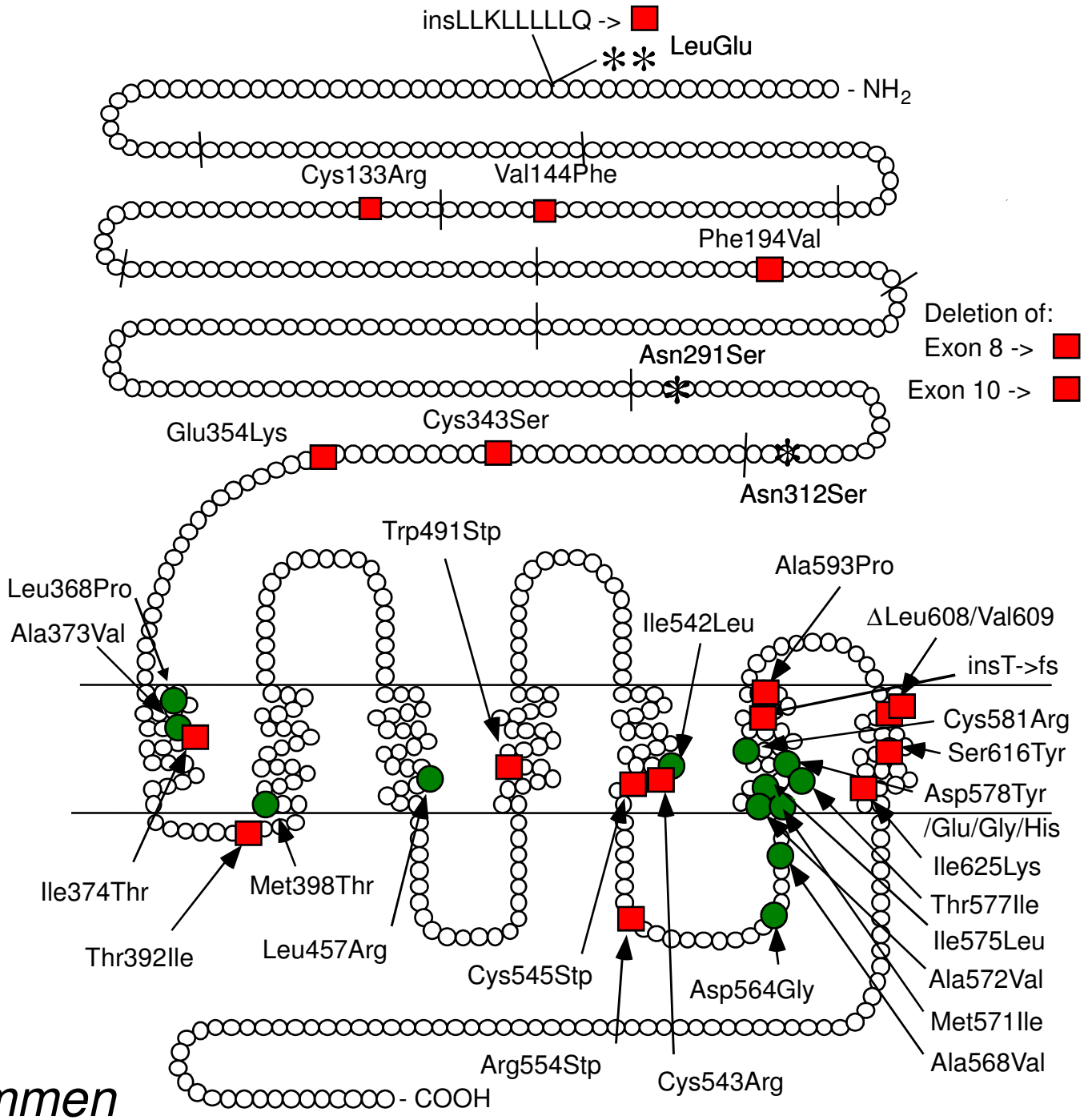

LHR
Adenylyl cyclase
G
cAMP
protein kinase A
steroidogenesis

APN Themmen

LH receptor gene mutations

- Activating mutations
- Inactivating mutations
- More subtle: polymorphisms

APN Themmen


insLLKLLLLLQ ->
LeuGlu
- NH2
Cys133Arg
Val144Phe
Phe194Val
Deletion of:
Exon 8 ->
Exon 10 ->
Asn291Ser
Cys343Ser
Glu354Lys
Asn312Ser
Trp491Stp
Ala593Pro
Leu368Pro
Ala373Val
Ile542Leu
ΔLeu608/Val609
insT->fs
Cys581Arg
Ser616Tyr
Asp578Tyr
/Glu/Gly/His
Ile625Lys
Thr577Ile
Ile575Leu
Ala572Val
Met571Ile
Ala568Val
Ile374Thr
Met398Thr
Thr392Ile
Leu457Arg
Cys545Stp
Asp564Gly
Arg554Stp
Cys543Arg
- COOH

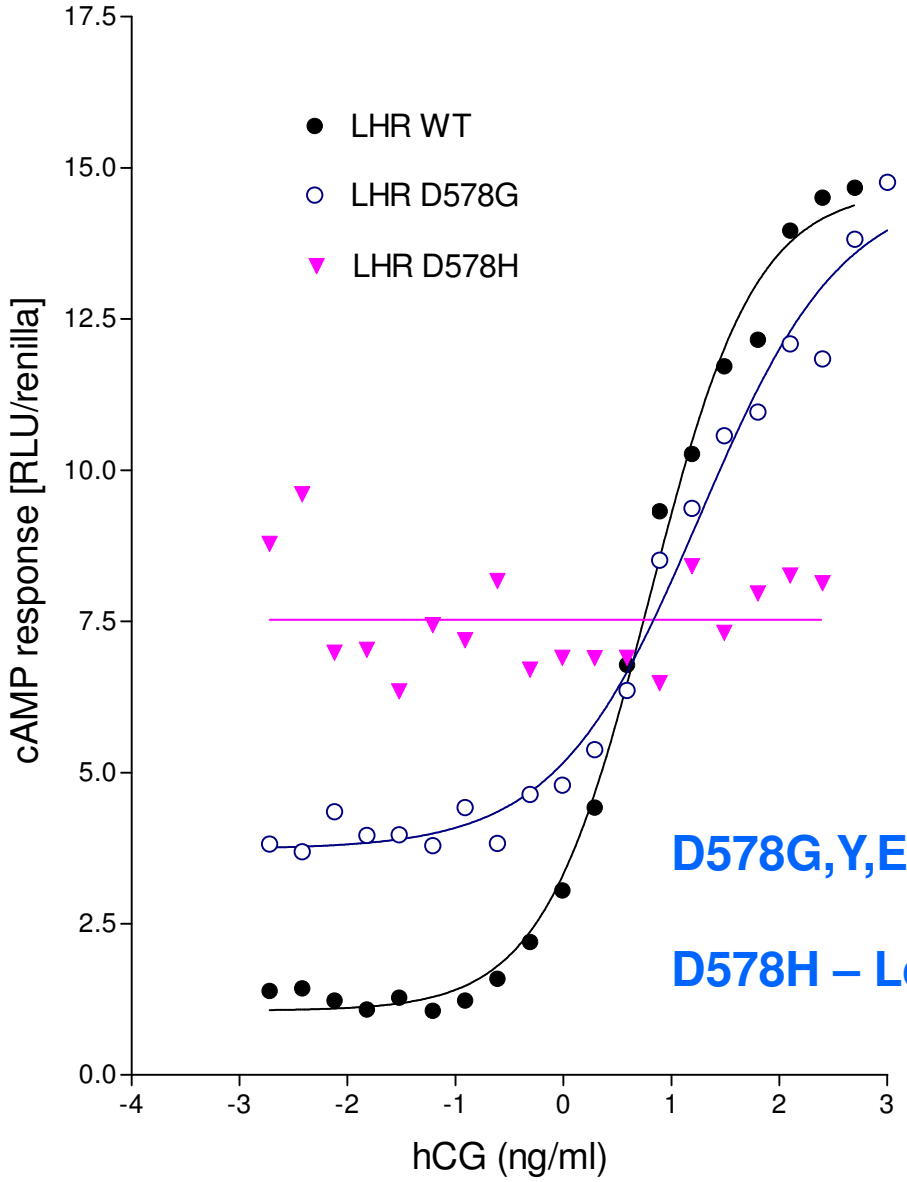
APN Themmen

Familial male-limited precocious puberty

- onset before the age of four
- high testosterone levels
- low LH levels
- autosomal dominant inheritance
- only boys are affected

Hypothesis - activated LH receptor

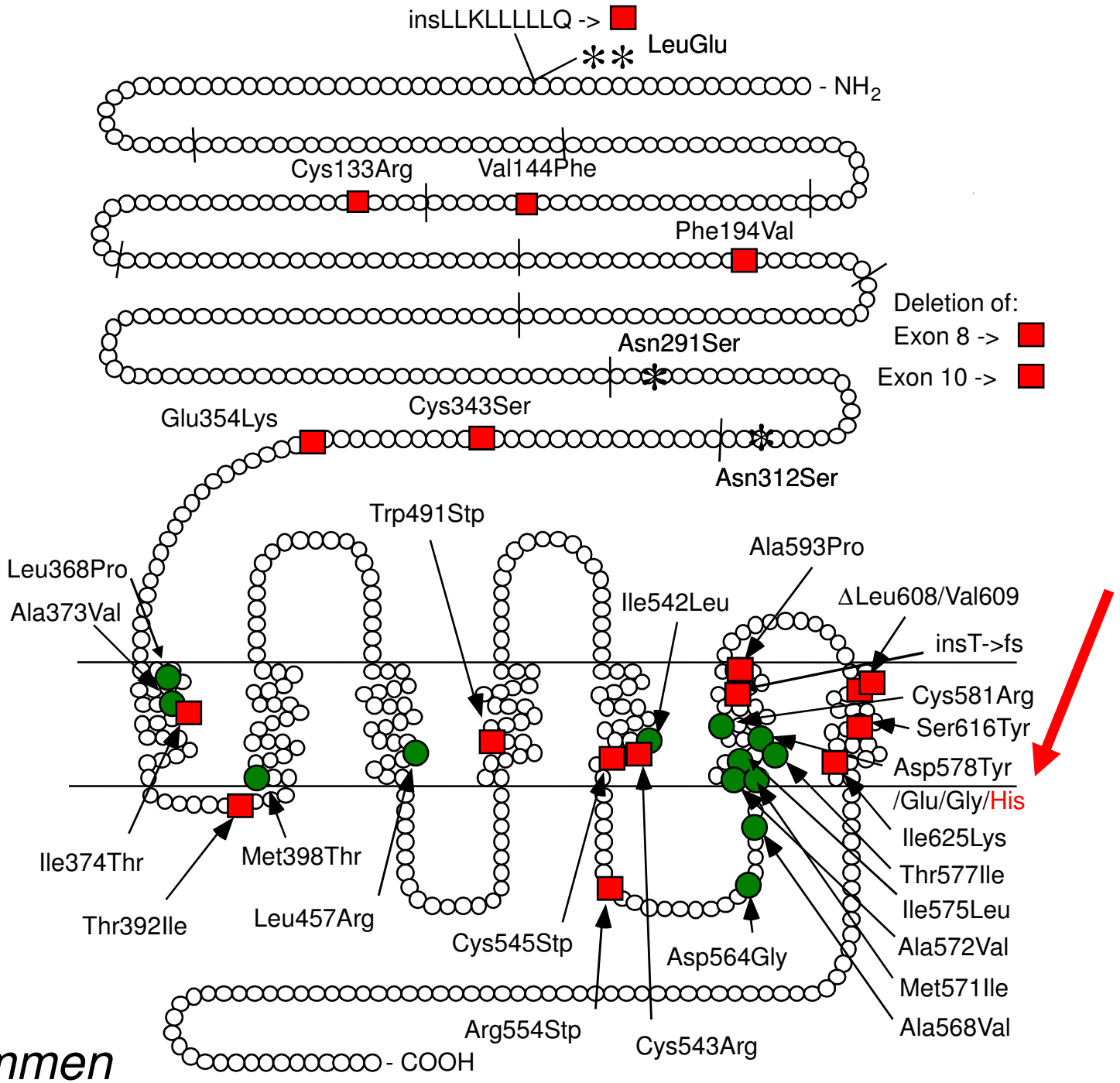
hLHR mutants



D578G,Y,E – precocious puberty

D578H – Leydig cell adenoma

APN Themmen


insLLKLLLLLQ ->
LeuGlu
- NH2
Cys133Arg
Val144Phe
Phe194Val
Deletion of:
Exon 8 ->
Exon 10 ->
Asn291Ser
Glu354Lys
Cys343Ser
Asn312Ser
Trp491Stp
Leu368Pro
Ala373Val
Ala593Pro
Ile542Leu
ΔLeu608/Val609
insT->fs
Cys581Arg
Ser616Tyr
Asp578Tyr
/Glu/Gly/His
Ile625Lys
Thr577Ile
Ile575Leu
Ala572Val
Met571Ile
Ala568Val
Ile374Thr
Met398Thr
Thr392Ile
Leu457Arg
Cys545Stp
Asp564Gly
Arg554Stp
Cys543Arg
- COOH

APN Themmen

Leydig Cell Hypoplasia

46,XY individuals

phenotypic males

micropenis
hypospadias

phenotypic females

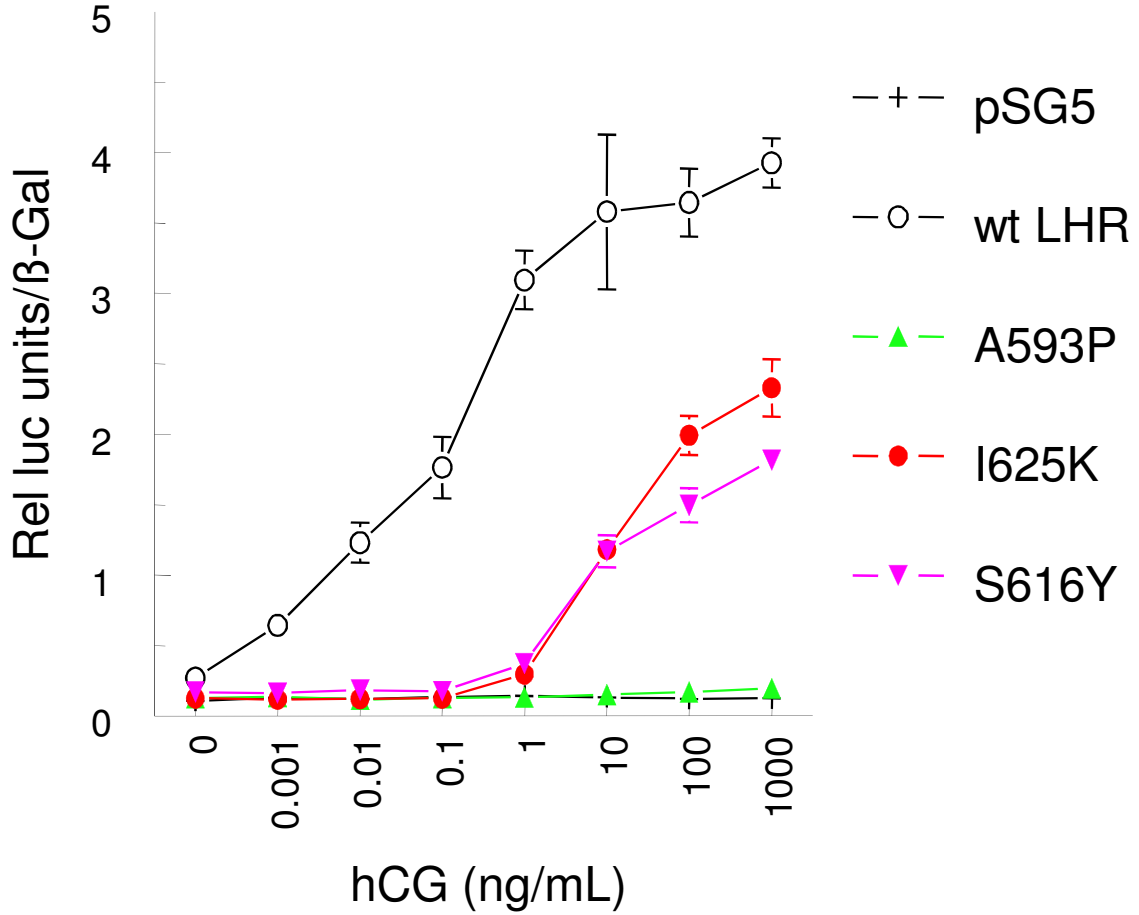
blind ending vagina

lack of breast development

- very low testosterone levels; high LH levels
- FSH levels within the normal range
- testes with very few Leydig cells

Hypothesis - inactive LH receptor

46 XY DSD:
inactivating LH receptor mutants

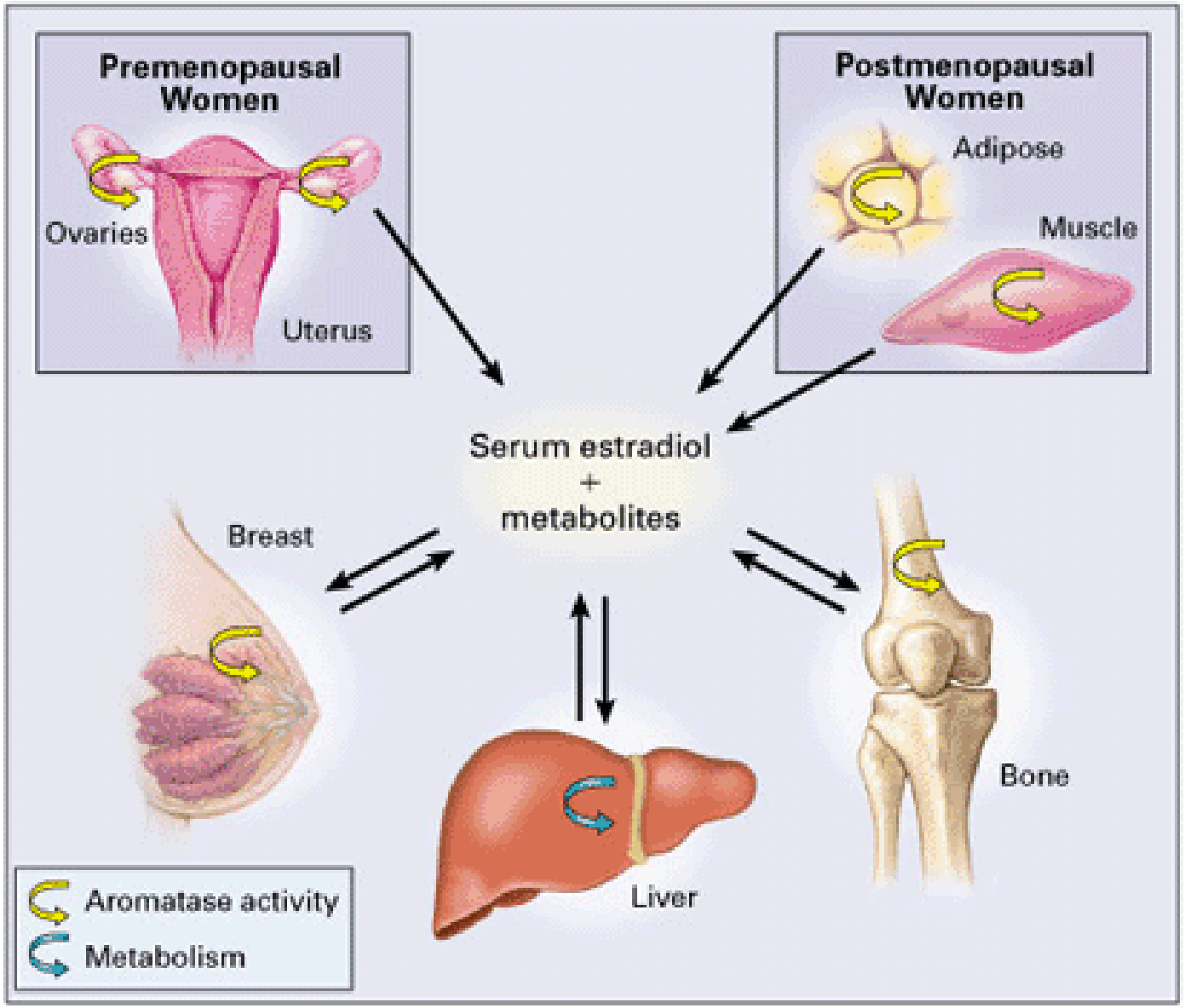


APN Themmen

LH receptor

- LH receptor mutations - disasters
- pseudohermaphroditism <-> precocious puberty
- **What about polymorphisms**

Production and action of estrogens



Estrogen and the risk of breast cancer. M Clemons and P Goss. *NEJM* 2001; 344(4): 276-285.

LHR polymorphisms

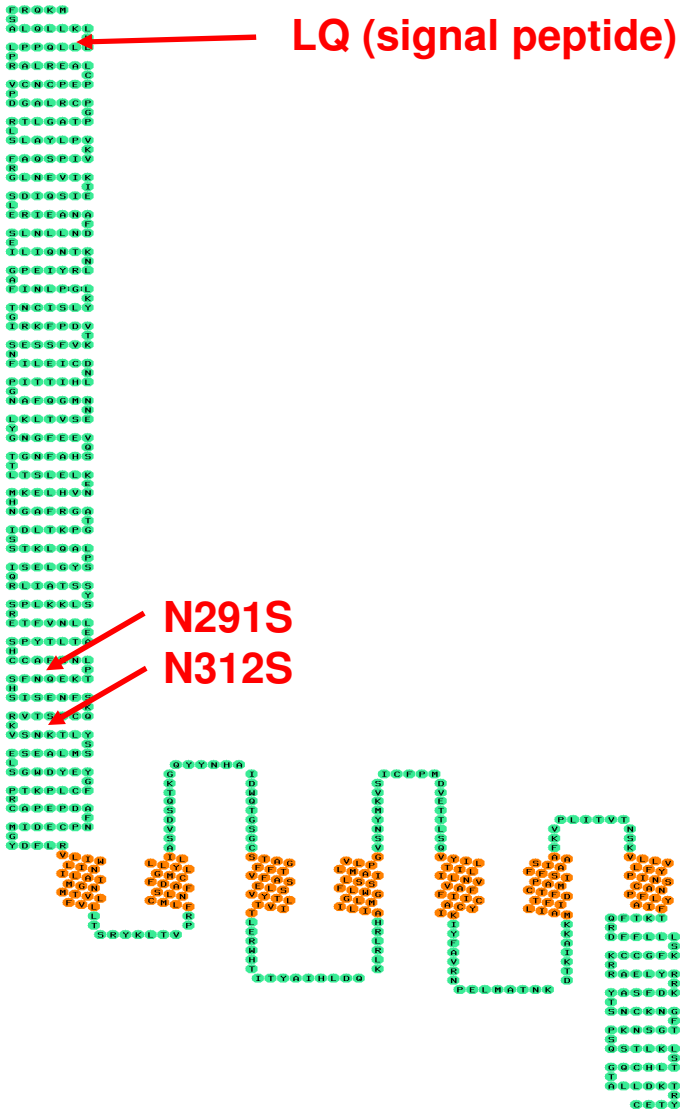
- 282 SNPshave been identified
- Most are located in large introns, which account for more than 95% of the LHR gene
- The most frequent polymorphisms is the absence or presence of a 2 aminoacid insertion at positon 18 in exon 1,
- And 2 variable aminoacids in postions 291 and 312: 291 NS, and 312 SN in exon 10.

LH receptor polymorphisms

- **LQ (Leu-Gln) insertion at pos 18 in signal peptide**
- N291S - glycosylation site
- N312S - near glycosylation site

- others silent (no AA change)

Human LH receptor



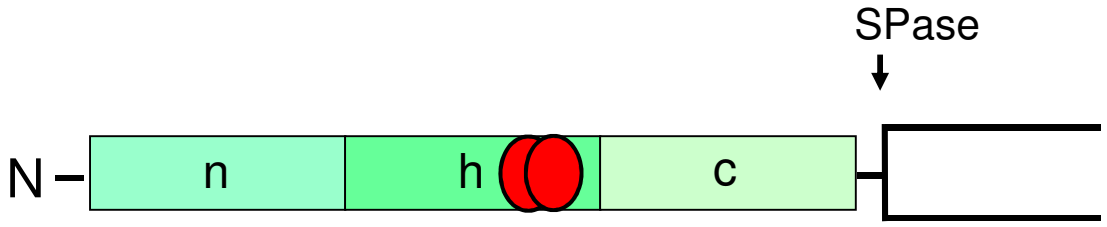
APN Themmen

LQ variant

insLQ-LHR polymorphism

exon 1- codon 18

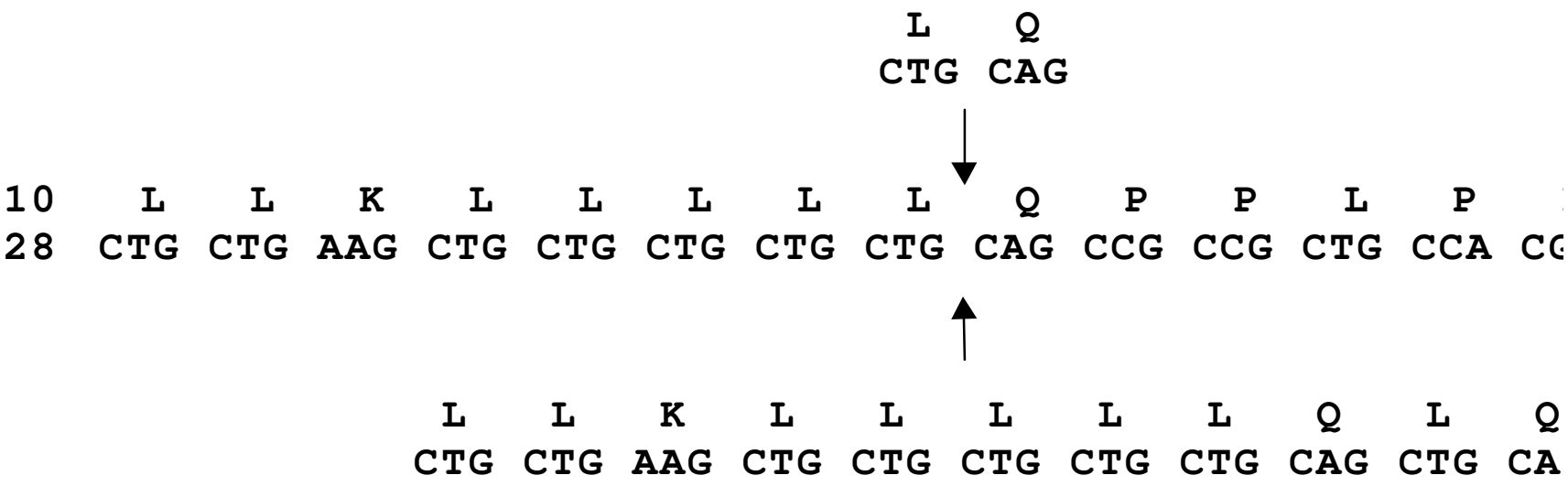
h-region of signal sequence



Polymorphisms: LHR

- An insertion of 2 aa L(leucine) & Q (glutamine) in the signal peptide of LHR (18insLQ) was shown in vitro to result in a increased LH receptor activity (Piersma, 2006).
- In BC this pm is associated with a shorter disease free interval (Powell 203 and Piersma 2006).

Polymorphism

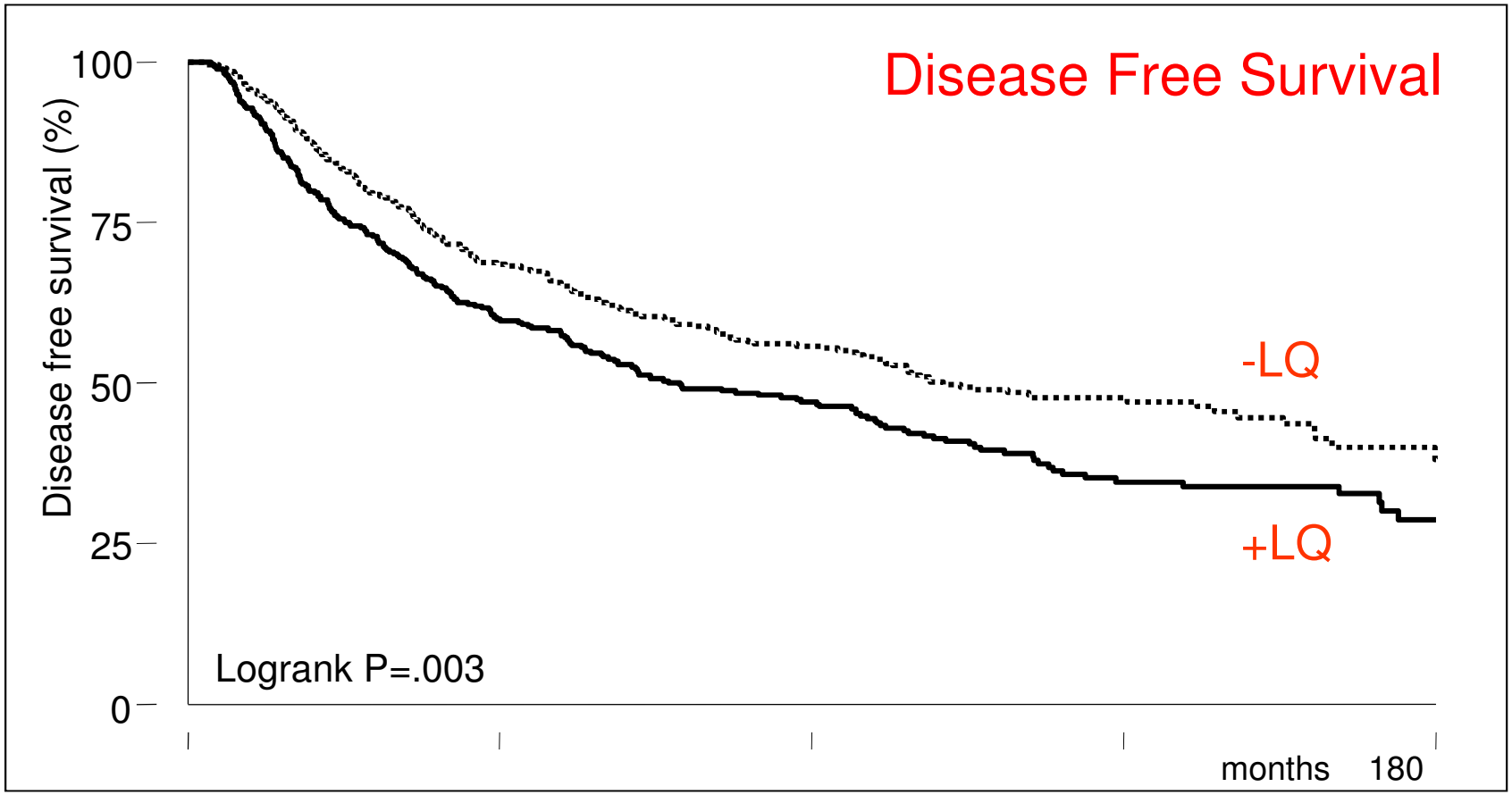


Mutational insert (inactivating)

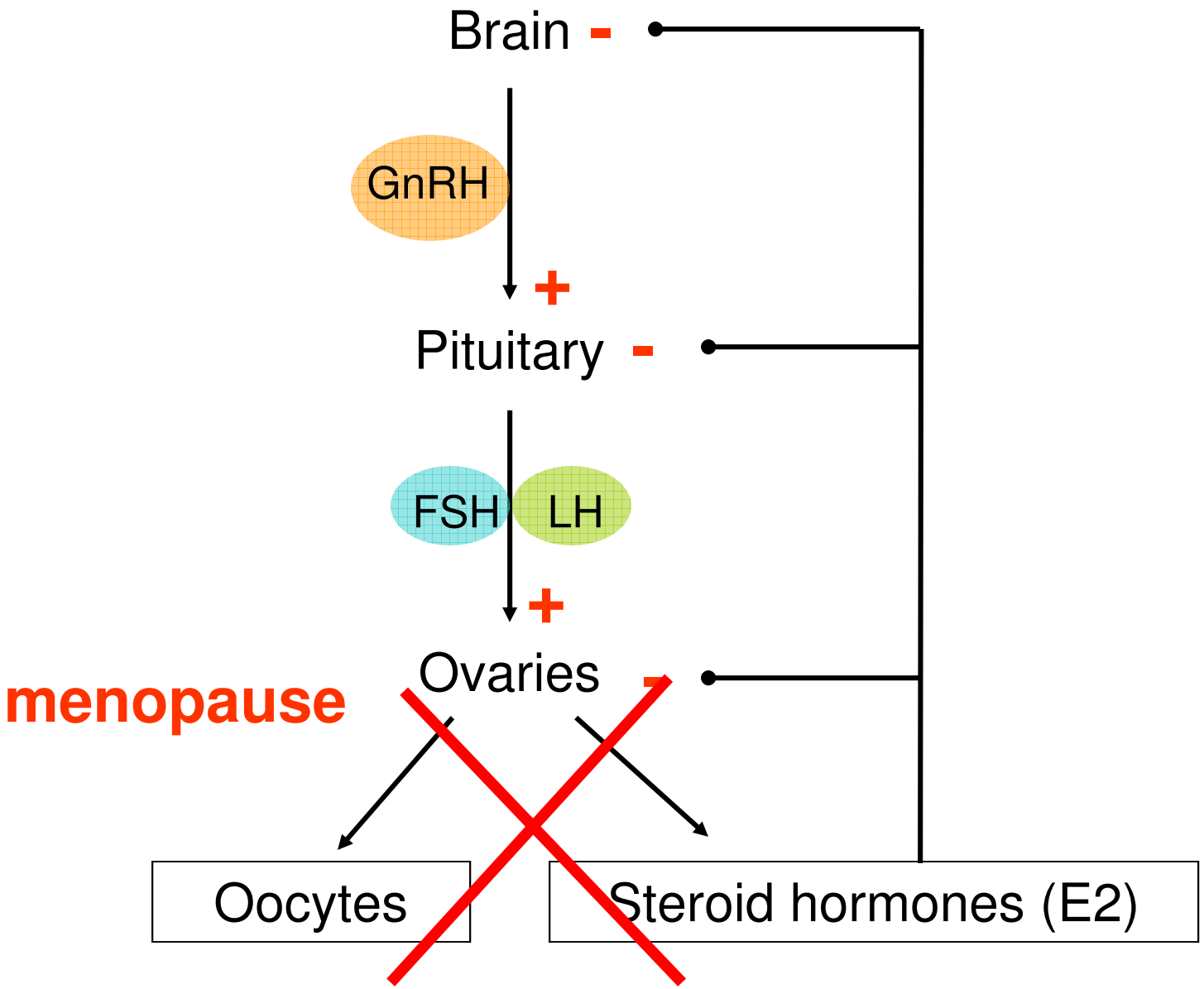
APN Themmen

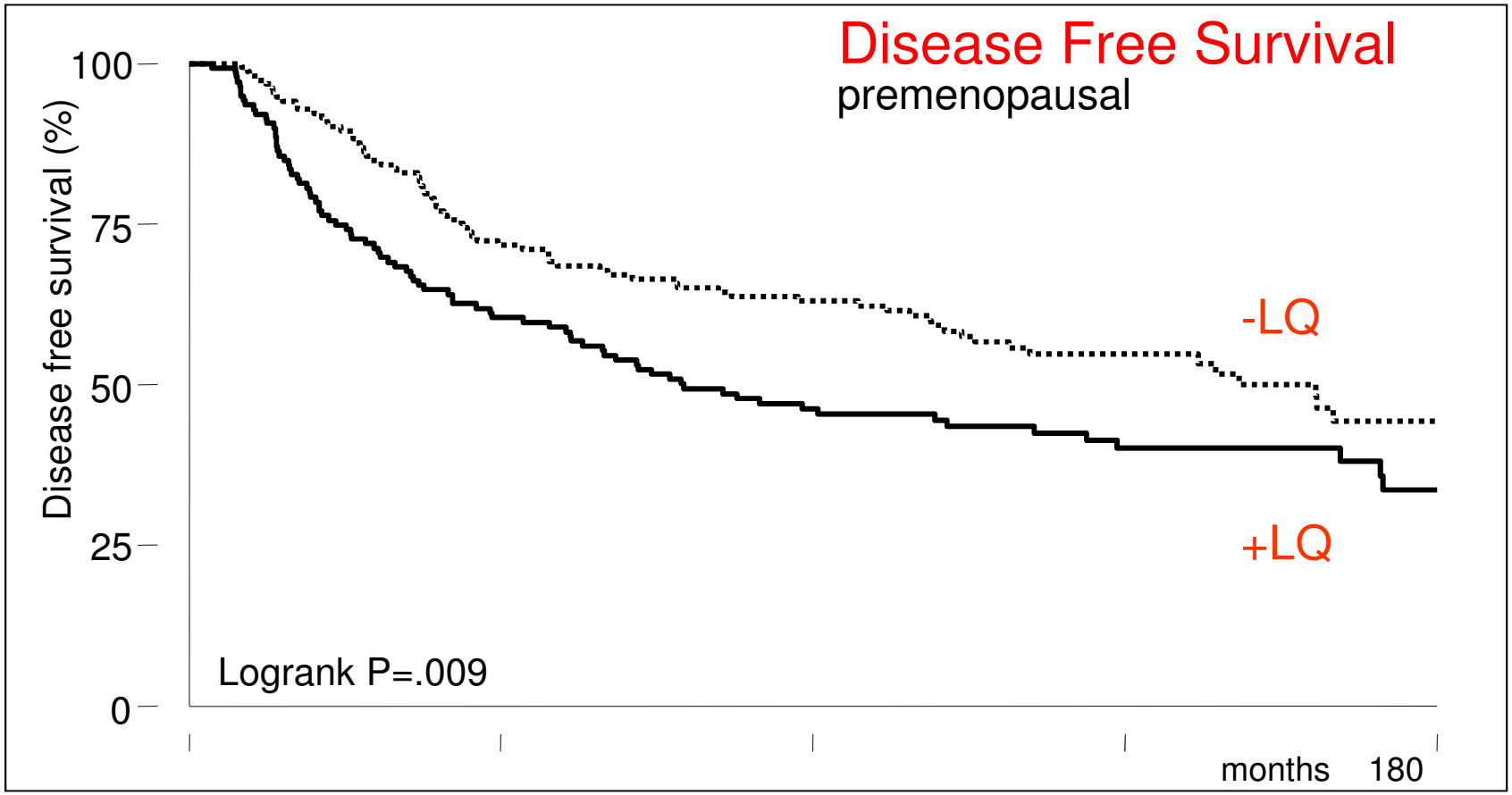
LH receptor LQ variant and breast cancer

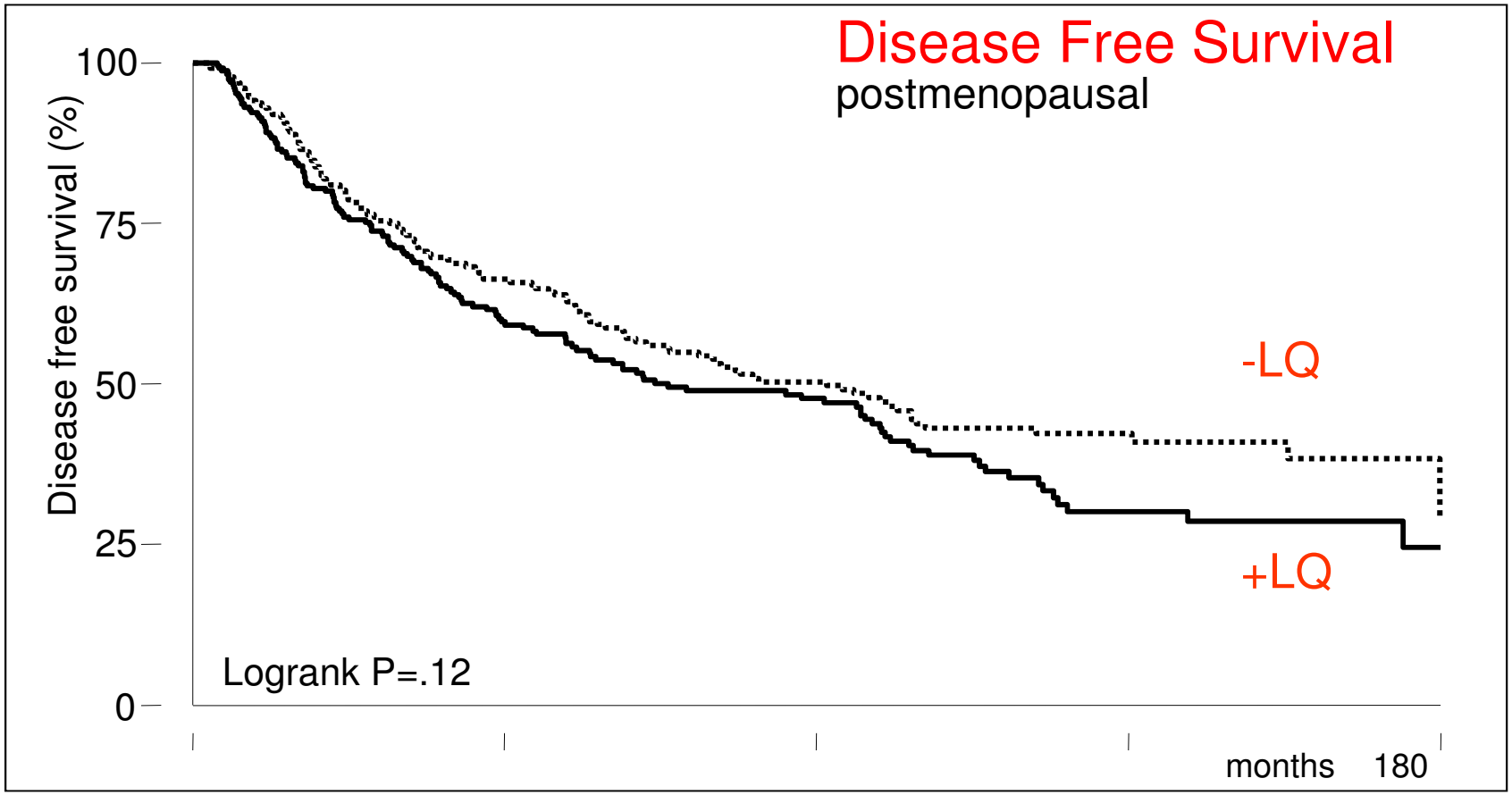
- 751 breast cancer cases
- median 130 months follow-up
- LQ not a risk allele
- disease free survival

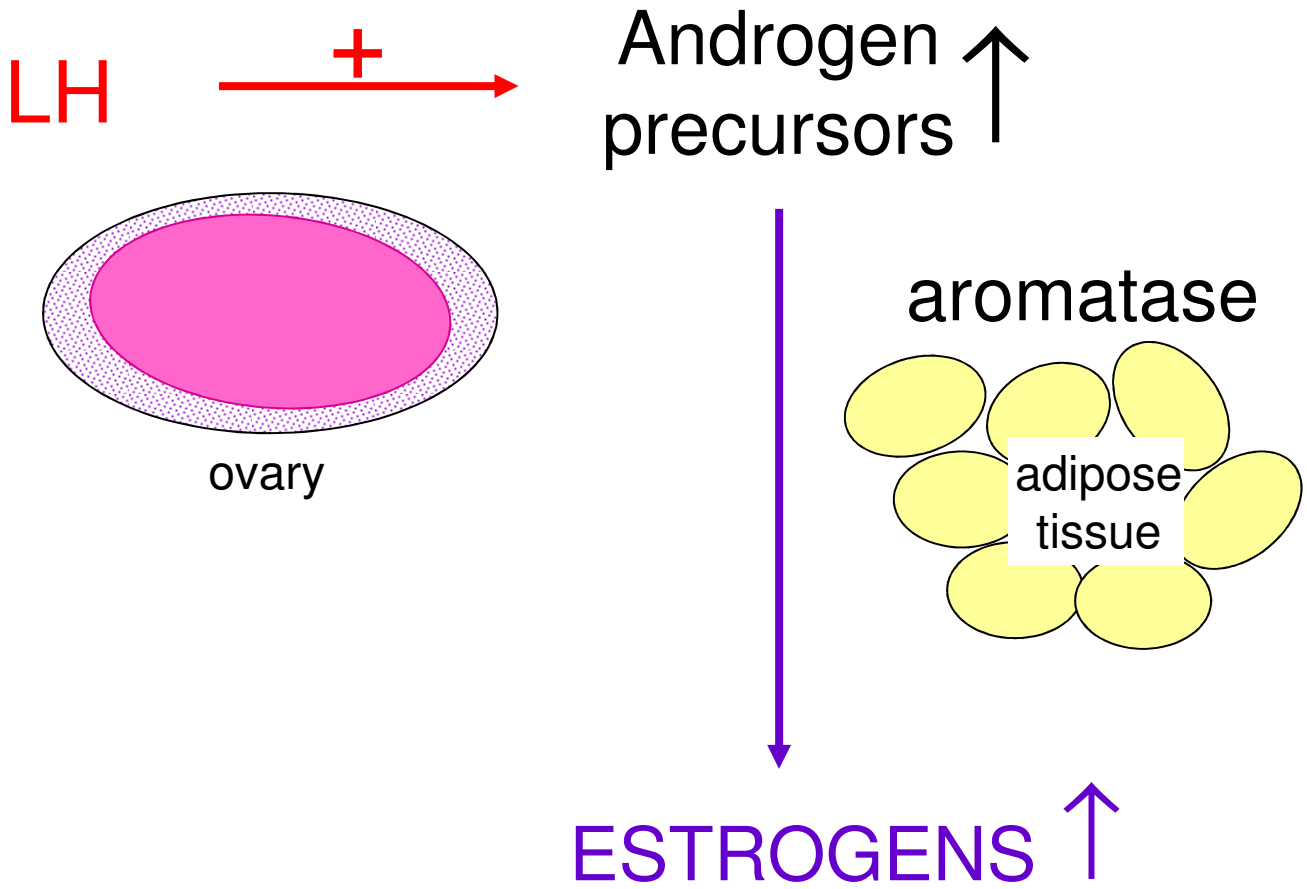

Disease Free Survival
Disease free survival (%)
100
75
50
25
0
-LQ
+LQ
Logrank P=.003
months
180

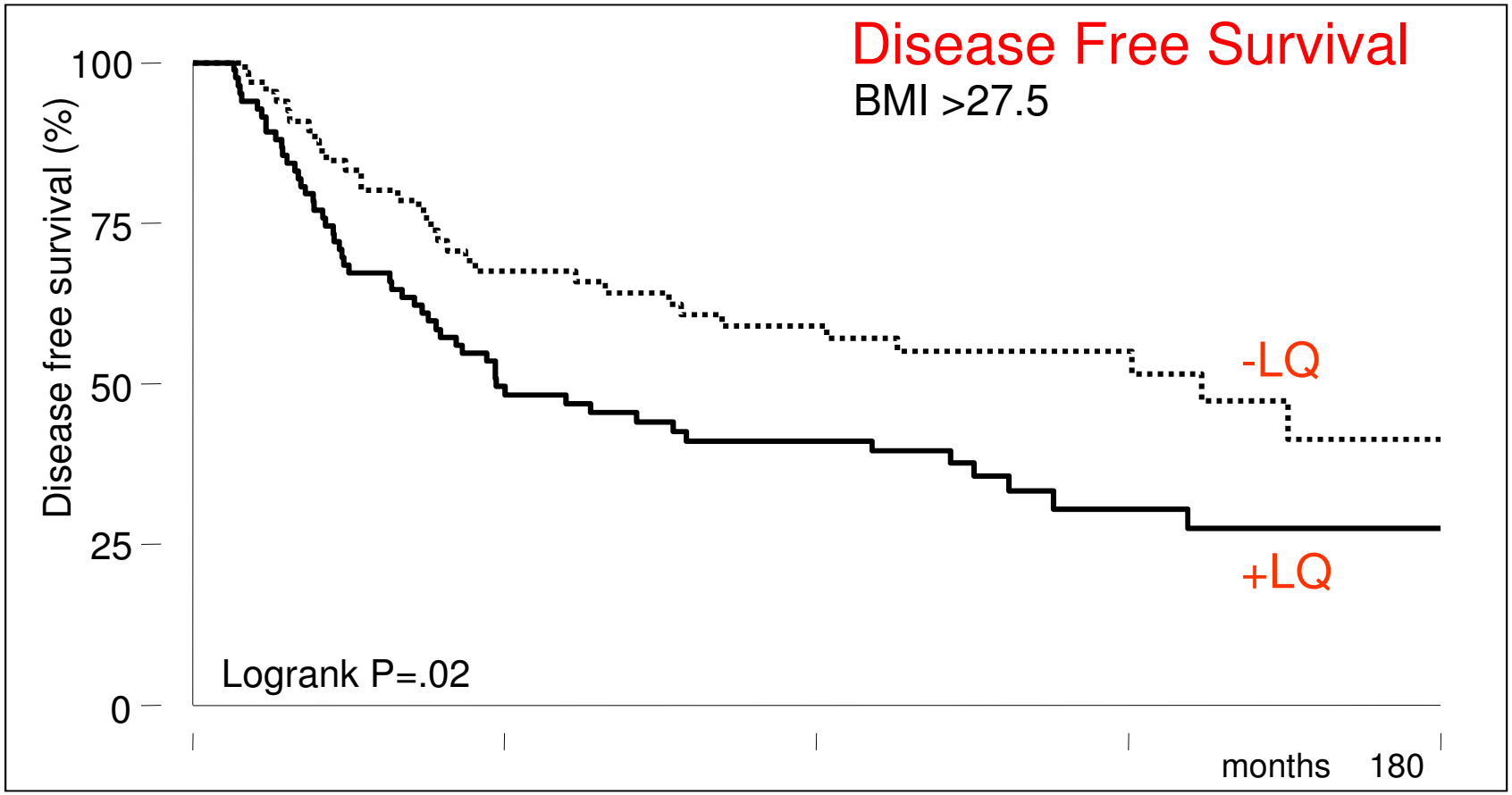
APN Themmen

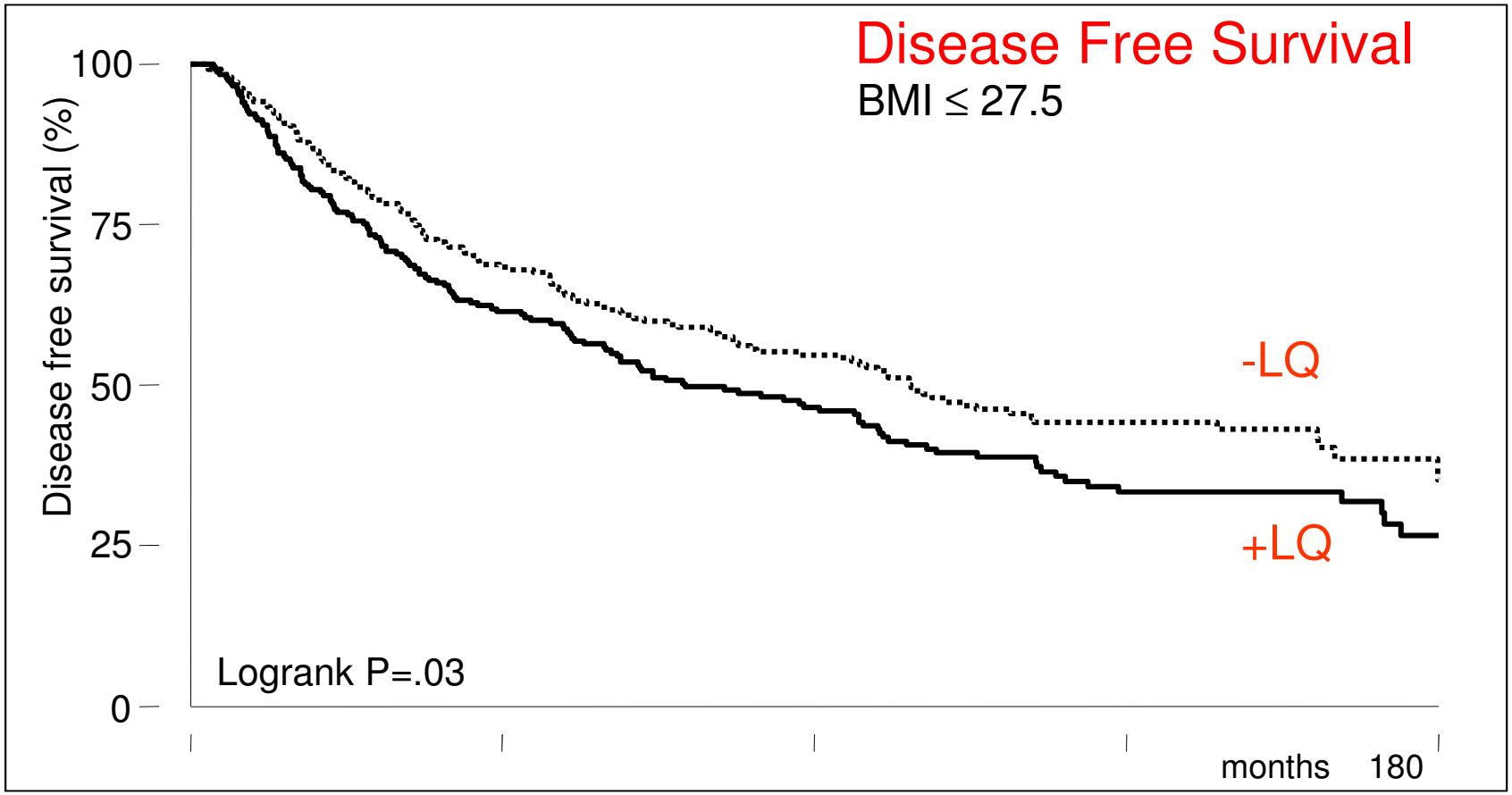

Brain -
GnRH
+
Pituitary -
FSH
LH
+
Ovaries -
menopause
Oocytes
Steroid hormones (E2)


Disease Free Survival
premenopausal
Disease free survival (%)
100
75
50
25
0
-LQ
+LQ
Logrank P=.009
months
180


Disease Free Survival
postmenopausal
Disease free survival (%)
100
75
50
25
0
-LQ
+LQ
Logrank P=.12
months
180


LH
+
Androgen
precursors
ovary
aromatase
adipose
tissue
ESTROGENS


Disease Free Survival
BMI >27.5
Disease free survival (%)
100
75
50
25
0
-LQ
+LQ
Logrank P=.02
months
180


Disease Free Survival
BMI ≤ 27.5
Disease free survival (%)
100
75
50
25
0
-LQ
+LQ
Logrank P=.03
months
180

Summary

Association

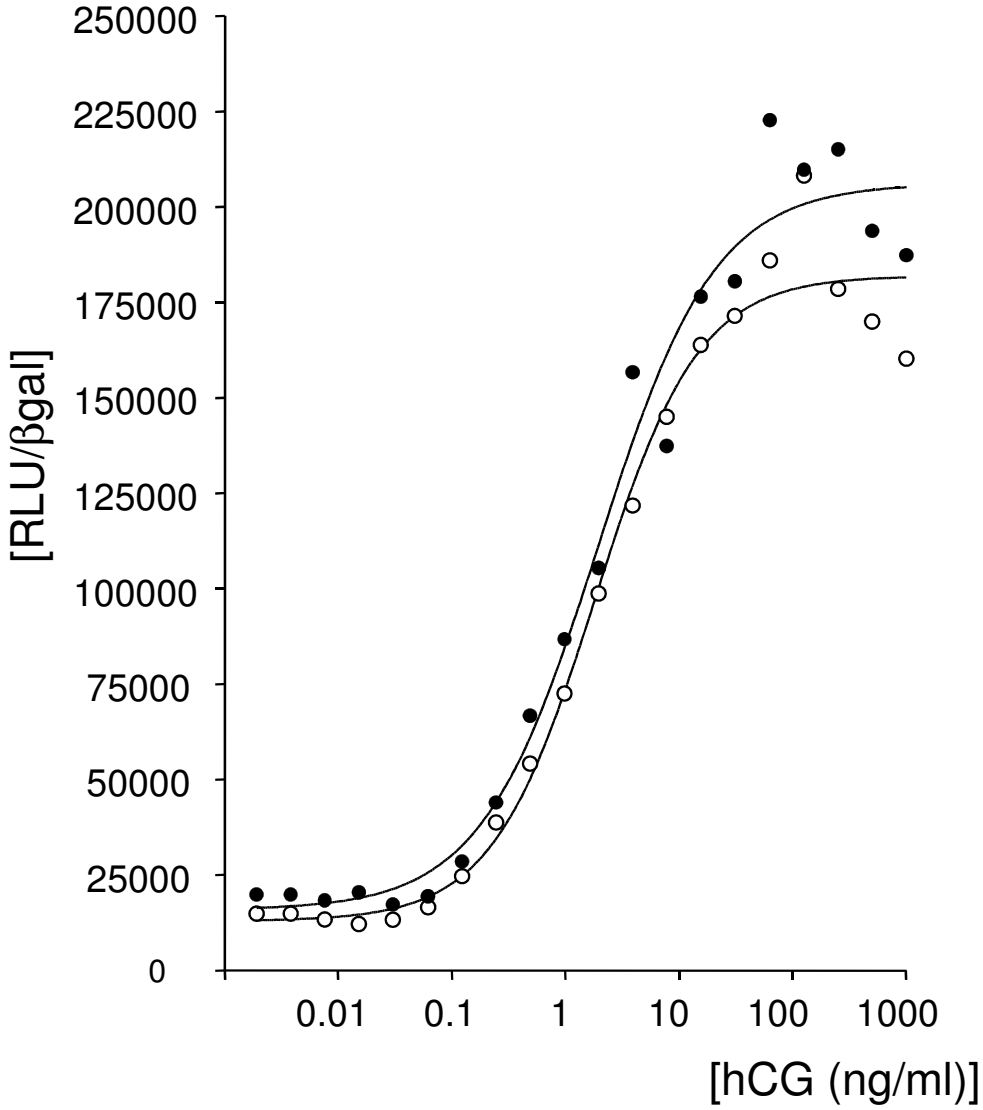
worse disease free survival

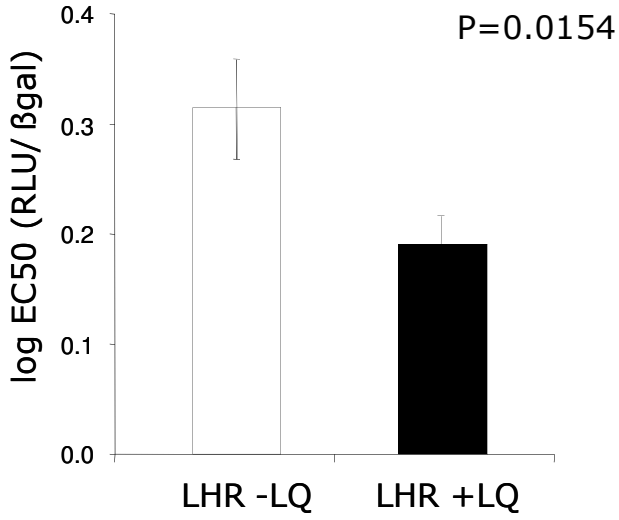
effect strong in premenopause

effect strong in obese women

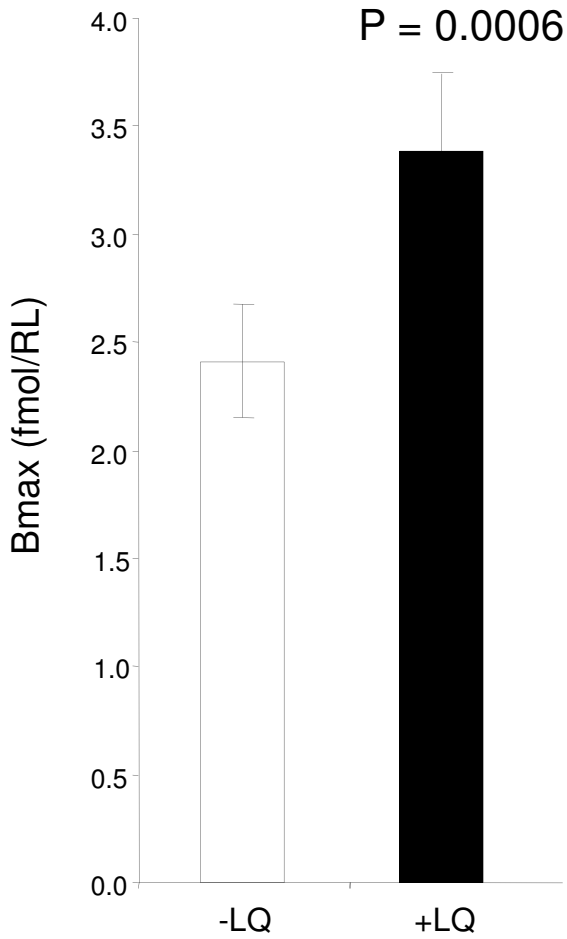
Effect of insLQ on LH receptor function?

Functional studies LQ variant: EC 50





Functional studies LQ variant: B max



Conclusions insLQ-LHR:

Breast cancer patients

- worse disease free survival
- interaction ovary and peripheral aromatase

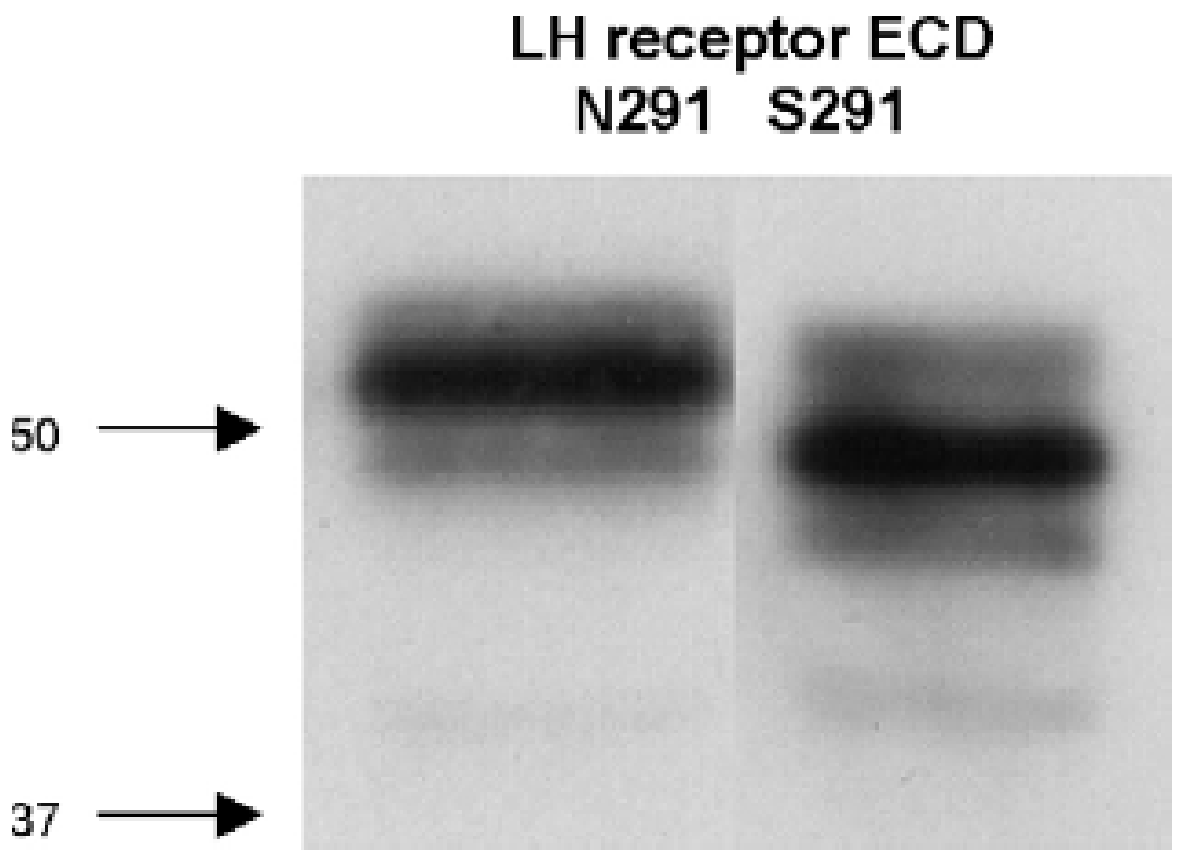
In vitro function

- more sensitive
- higher cell surface expression
- more effective translocation into ER membrane

Polymorphisms: LHR

- In addition, Exon 10 of LHR contains 2 coding SNPs that cause a change in amino acids: Asn 291Ser and Ser312Asn).
- Asn312 has been associated with increased rik of BC (piersma 2007) suggesting a more active LHR;
- Similarly, a lower frequency of the Asn312 allele has been described in infertile men with impaired spermatogenesis (Simoni 2008).

LHR polymorphisms

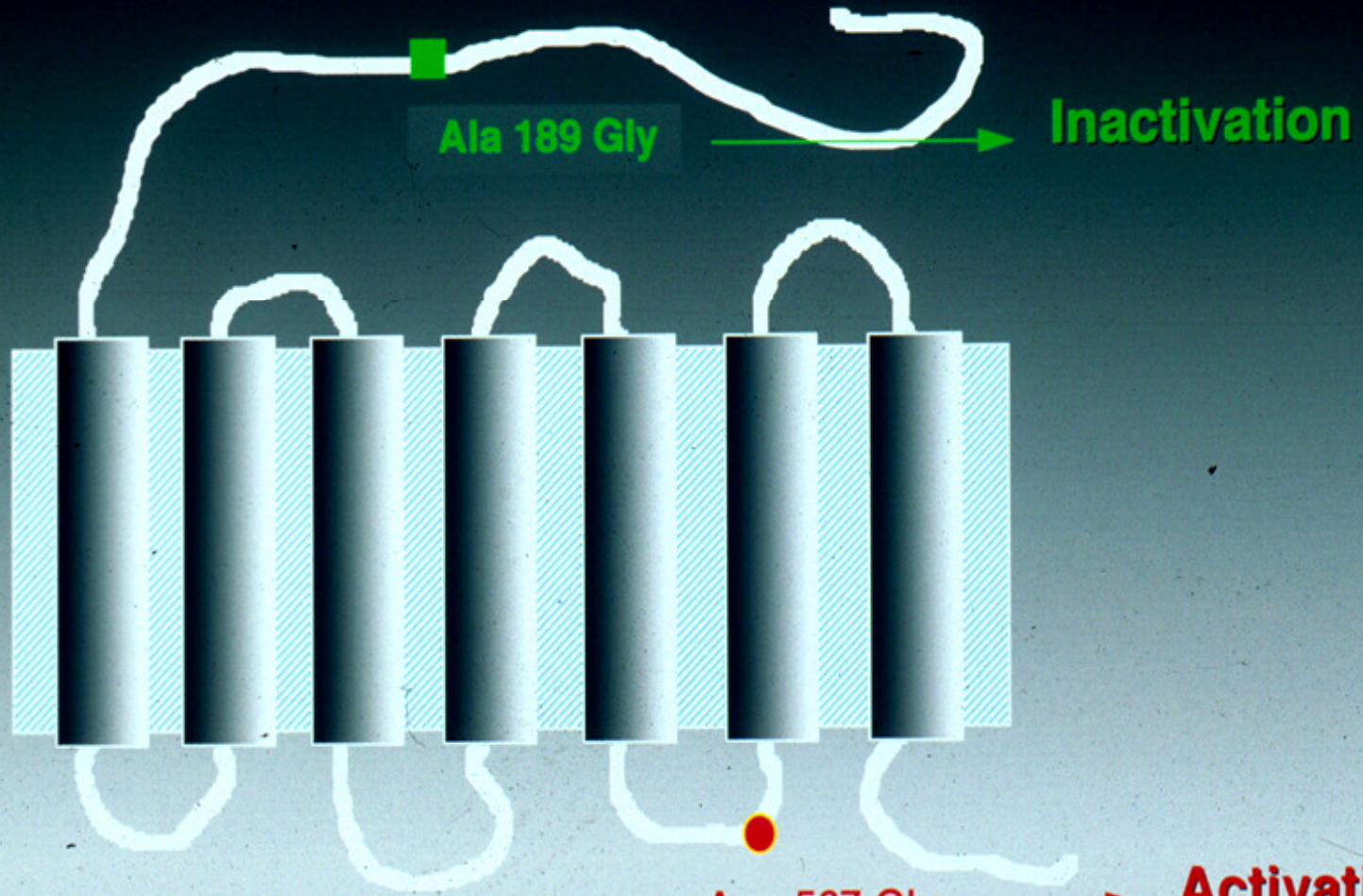


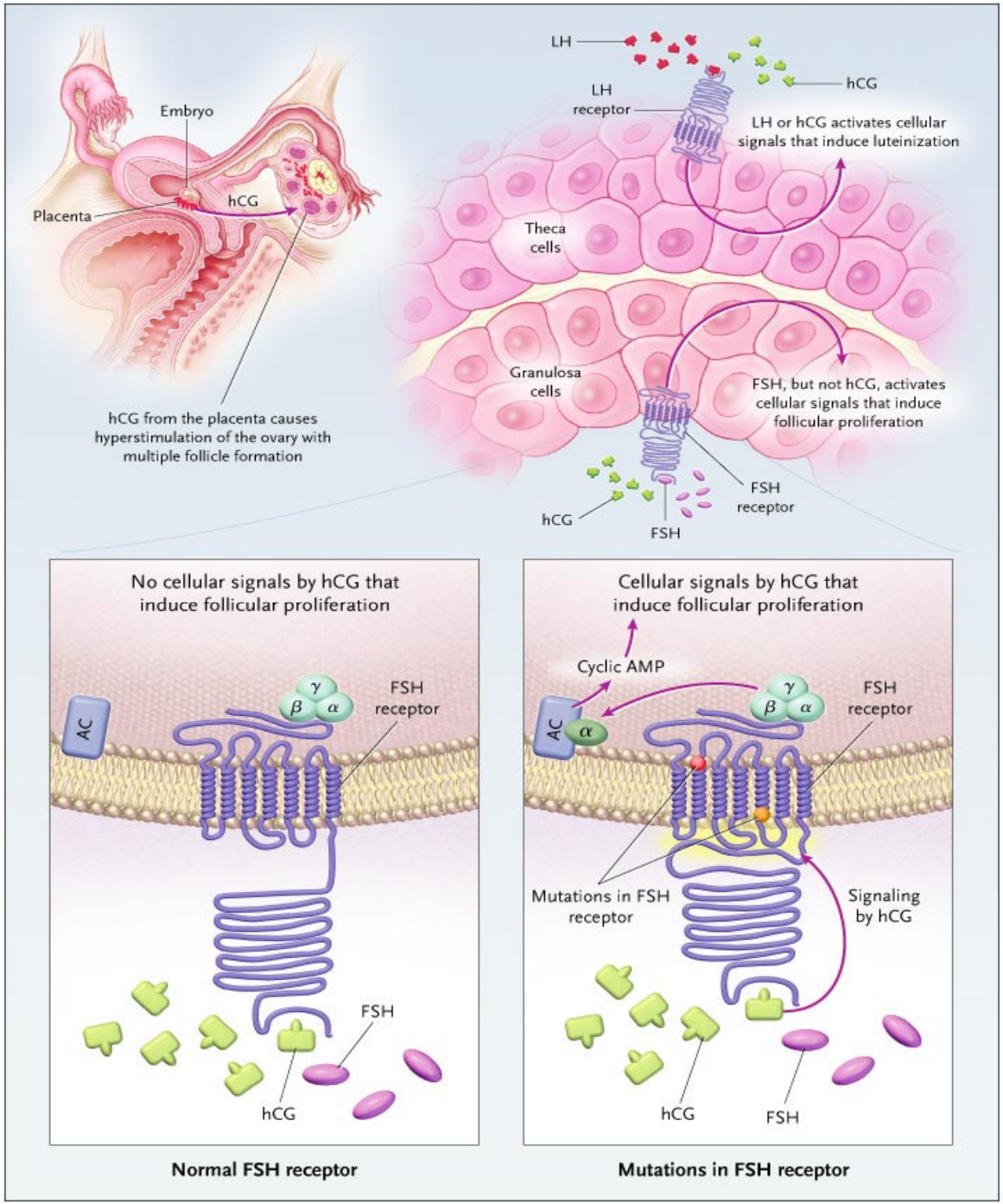
LH receptor

- Central to sex differentiation
- Activating vs inactivating mutations
- Polymorphic variants and disease

Récepteurs pour les hormones gonadotropes

. Localisation des mutations dans le récepteur FSH.

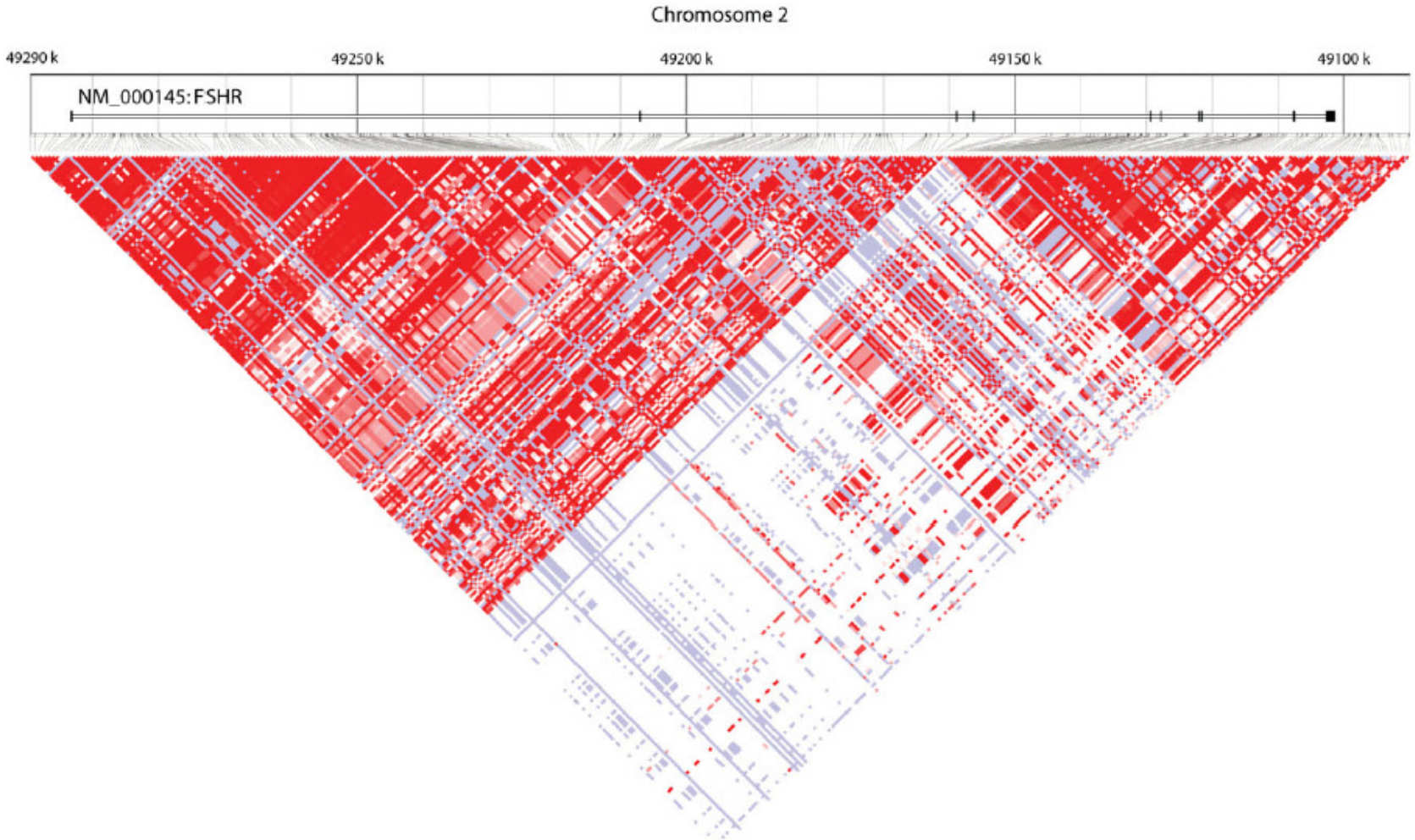




UB Kaiser
NEJM
2003

FSHR polymorphisms

- The FSHR harbours more than 900 SNPs arranged in two major linkage disequilibrium blocks.


Chromosome 2
49290 k
49250 k
49200 k
49150 k
49100 k
NM_000145:FSHR

Polymorphisms: FSHR (p.N680S)

- Simoni et al 1999
- Perz Mayorga 2000
- Sudo 2002
- De Castro 2003, 2004
- Laven 2003
- Behre 2005
- Falconer 2005
- Jun 2006
- Loutradis 2006
- De Koning 2006, Yang 2006

Human Reproduction Update, Vol.14, No.5 pp. 459–484, 2008
Advance Access publication July 4, 2008
doi:10.1093/humupd/dmn024

Functional genetic polymorphisms and female reproductive disorders: Part I: polycystic ovary syndrome and ovarian response

M. Simoni[1,5], C.B. Tempfer[2], B. Destenaves[3] and B.C.J.M. Fauser[4]

Human Reproduction Update, Vol.15, No.1 pp. 97–118, 2009
Advanced Access publication on September 19, 2008 doi:10.1093/humupd/dmn040

human reproduction update

Functional genetic polymorphisms and female reproductive disorders: Part II—endometriosis

C.B. Tempfer[1,5], M. Simoni[2], B. Destenaves[3], and B.C.J.M. Fauser[4]

Human Reproduction, Vol.24, No.8 pp. 2014–2022, 2009
Advanced Access publication on April 29, 2009 doi:10.1093/humrep/dep113

human reproduction ORIGINAL ARTICLE *Reproductive genetics*

Genetic polymorphisms of GnRH and gonadotrophic hormone receptors affect the phenotype of polycystic ovary syndrome

O. Valkenburg[1,5], A.G. Uitterlinden[2,3], D. Piersma[2], A. Hofman[3], A.P.N. Themmen[2], F.H. de Jong[2], B.C.J.M. Fauser[4], and J.S.E. Laven[1]

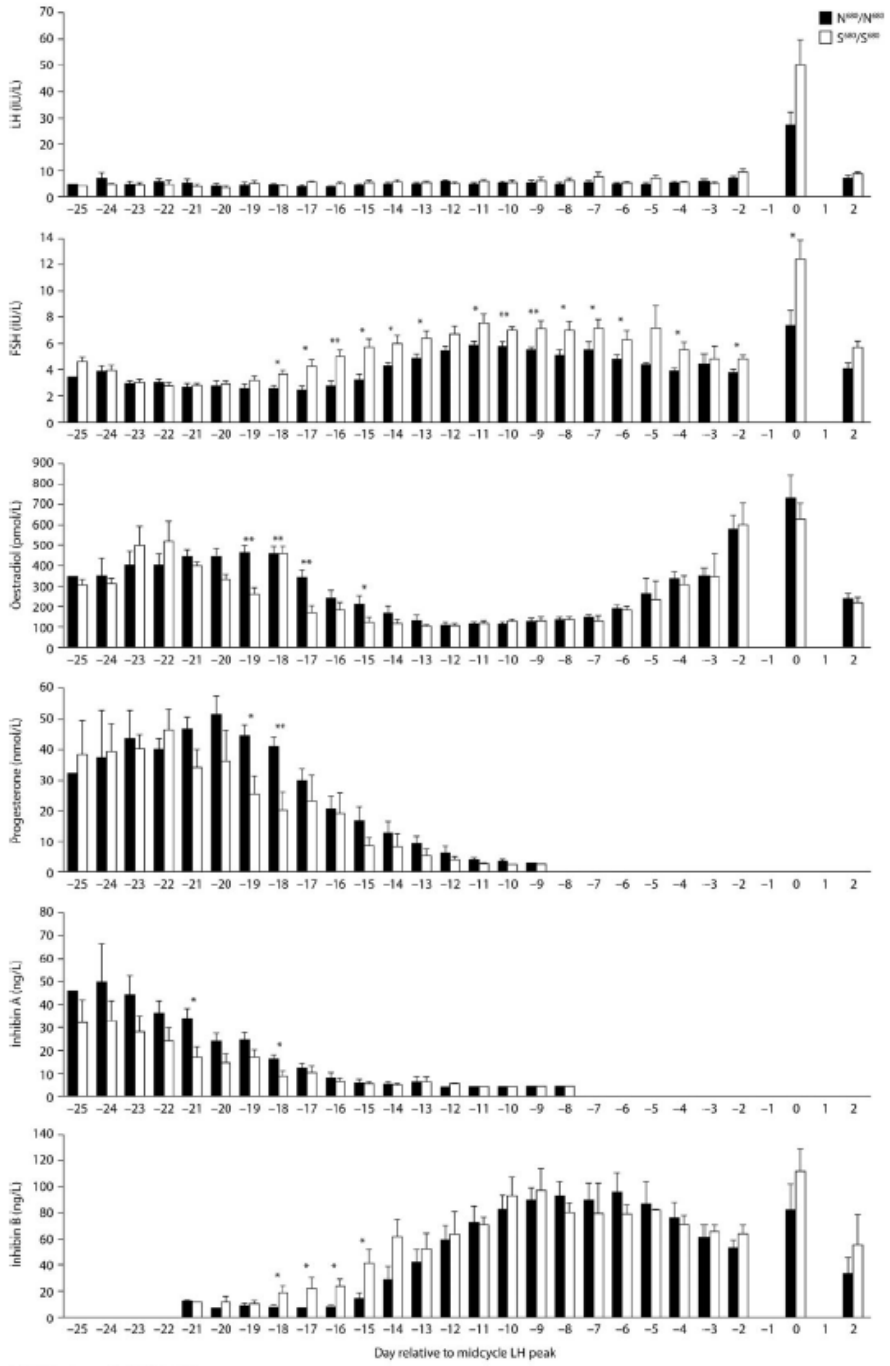
Polymorphisms: FSHR

- A well known combination of two polymorphisms has been described in exon 10 of the FSHR gene
- As coding SNPs at codon position 307 and 680 (Simoni 1999).
- The minor allele at position 680 (Ser 680) is associated with significantly higher levels of FSH, and altered ovarian response to FSH: **N680S** or **N/N vs S/S** (Perez Mayorga 2000)

Polymorphisms: FSHR Ser/Asn 680

- Higher frequency of Ser 680 has been reported in anovulatory subjects (Laven 2003).

- However, although the FSHR is less responsive, this polymorphism does not modify the response to exogenous FSH.



H, luteinizing hormone. *P < 0.05; **P < 0.005.

N680S

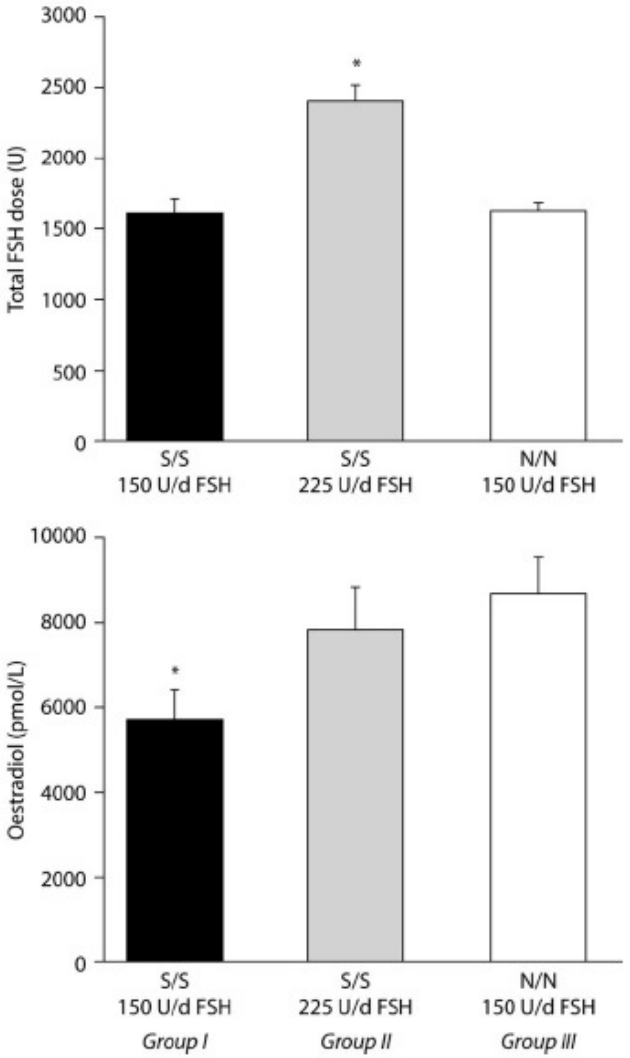


Figure 5: Serum levels of oestradiol before ovulation induction were significantly lower in women with the Ser/Ser allele variant (group I, n=24) compared to the Asn/Asn allele variant (group III, n=44) of the FSH receptor (lower panel: *significant difference between group I and III). This difference in ovarian response could be overcome by increasing the daily FSH dose from 150U/day to 225U/day (upper panel:*significant higher total FSH dose) in women with the Ser/Ser allele variant (group II, n=25); lower panel: no significant difference between group II and III.

Table VI. Comparison of the study designs and outcomes of two conflicting studies investigating the relation between the FSHR polymorphism at amino acid position 680 (Asn/Ser) and pregnancy rate.

	Jun *et al.* (2006)	Klinkert *et al.* (2006)
Number of patients	263	105
Status regarding ICSI patients	Not excluded	Excluded
Frequency of unexplained infertility (%)	35.6	25.0
Genotype associated with significantly higher FSH levels	Ser/Ser	No difference
Genotype associated with significantly higher clinical pregnancy rate	Asn/Asn	Ser/Ser

ICSI, intracytoplasmic sperm injection.

Human Reproduction, Vol.24, No.8 pp. 2014–2022, 2009
Advanced Access publication on April 29, 2009 doi:10.1093/humrep/dep113

human reproduction

ORIGINAL ARTICLE *Reproductive genetics*

Genetic polymorphisms of GnRH and gonadotrophic hormone receptors affect the phenotype of polycystic ovary syndrome

O. Valkenburg[1,5], A.G. Uitterlinden[2,3], D. Piersma[2], A. Hofman[3], A.P.N. Themmen[2], F.H. de Jong[2], B.C.J.M. Fauser[4], and J.S.E. Laven[1]

Polymorphisms: PCOS

Table I Primers and probe sequences used in the study of polymorphisms in PCOS women and controls

Gene variant	Rs number	PCR primers		Taqman probes	
GNRH1					
Ser16Trp	rs6185	Fw	AATTCAAAAACTCCTAGCTGGCCTTA	VIC	CACGCACCAAGTCA
		Rv	CATAGGACCAGTGCTGGCT	FAM	ACGCACGAAGTCA
FSHR					
Ala307Thr	rs6165	Fw	GCAACAAATCTATTTTAAGGCAAGAAGTTGA	VIC	TGACCCCTAGTCTGAGTC
		Rv	TGTCTTCTGCCAGAGAGGATCT	FAM	ACCCCTAGCCTGAGTC
Asn680Ser	rs6166	Assay on demand (Applied Biosystems, C_2676874_10)			
LHR					
Asn291Ser	rs12470652	Fw	CTGAAGTCCAAAAGCTCAAATGCT	VIC	CAGACAGAATTTTTC
		Rv	TGTGCTTTCACATTGTTTGGAAAAGT	FAM	CAGACAGAGTTTTTC
Ser312Asn	rs2293275	Fw	TTTTCCAAACAATGTGAAAGCACAGT	VIC	TTACAGTGTTTTGTTATTCACTT
		Rv	GATACGACTTCTGAGTTTCCTTGCA	FAM	CAGTGTTTTGTTACTCACTT

FSHR: FSH receptor, LHR: LH receptor.

Valkenburg et al 2009

Polymorphisms: GnRH1 in PCOS

- The GnRH1 Trp16 Ser polymorphism was associated with milder forms of PCOS.
- However this polymorphism has no effect on FSH/LH levels
- This argues against a direct effect of this polymorphism on the function of GnRH.

Valkenburg et al 2009

Polymorphisms: PCOS: The most striking association was observed with FSHR

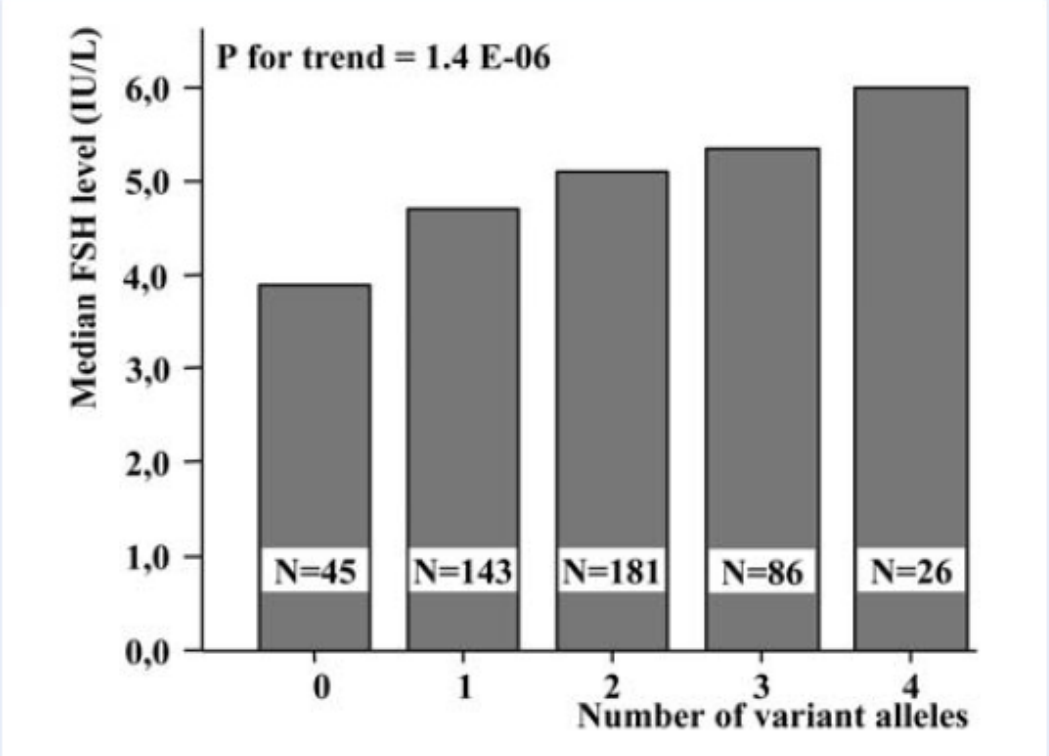


Figure 1 Median FSH levels in women with PCOS, stratified according to number of allelic variants (FSH receptor Ser^{680} and LH receptor Asn^{312}).

Polymorphisms: Ser 680 in PCOS

- This variant did not constitue a risk allelle for PCOS,
- And no association was found with the number of antral follicules or with AMH levels.
- FSH levels were increased in carriers
- but also LH levels.

Polymorphisms: PCOS

- The contributions of these polymorphisms to the phenotype of PCOS is small and may only be relevant in conjunction with other genetic variants that contribute to minor phenotypical variation (Simoni 2008).

Gene Polymorphisms

- In the coming years, the focus of research will be on the association studies of gene polymorphism with endpoints of disease in cohort of sex differentiation and infertility patients and other patients with aberrations of hormonal homeostasis.

Acknowledgements

- APN Themmen, Erasmus University