# Monogenic Affections of the Gonadotrope Axis in Humans

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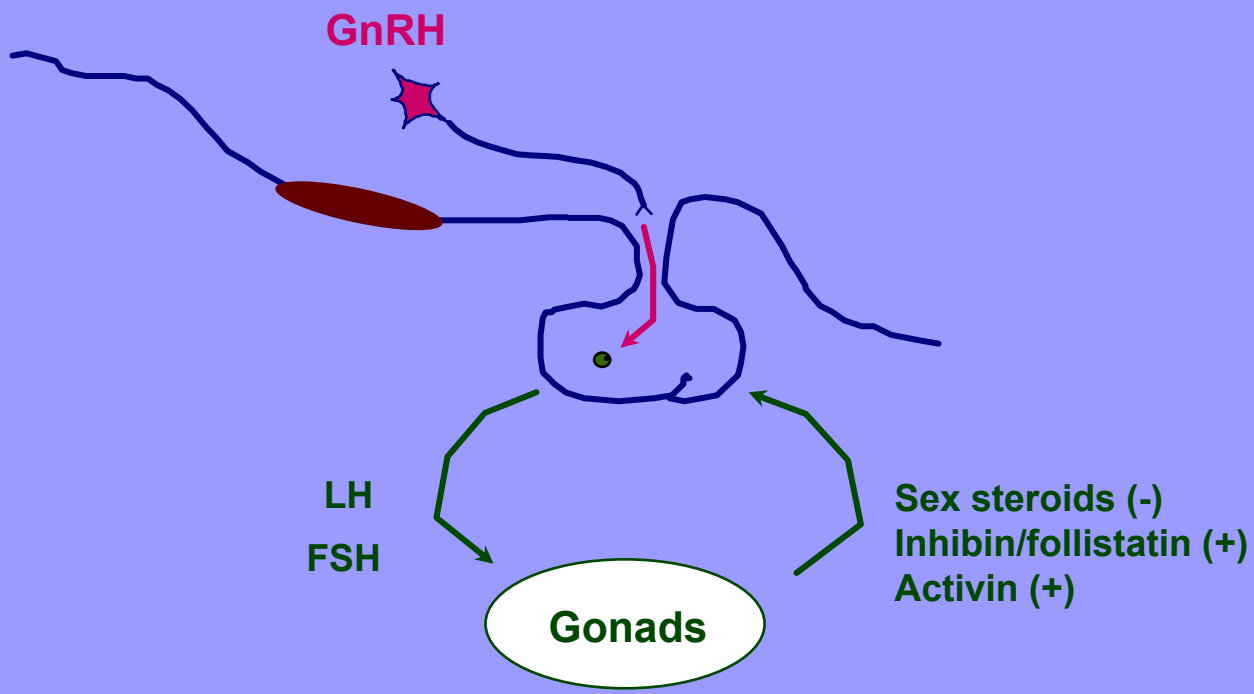
CHUV

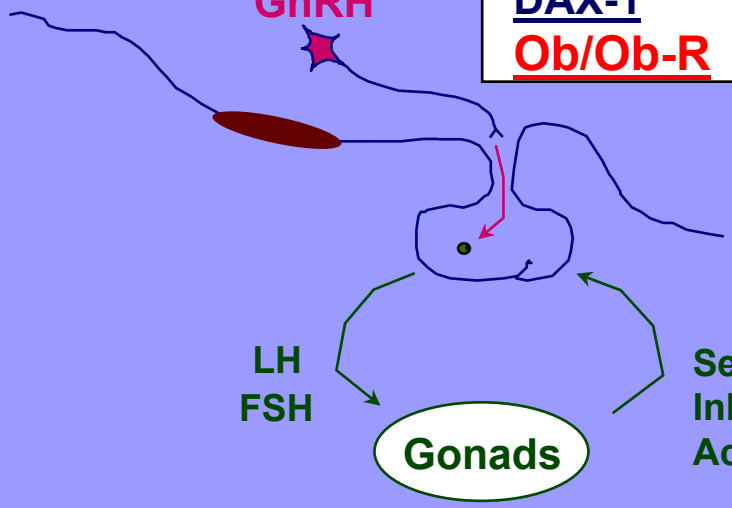
# Hypothalamo-Pituitary-Gonadal Axis

## The Endocrine Angle



# Hypothalamo-Pituitary-Gonadal Axis

## The Molecular Angle




**KAL-1**
**DAX-1**
**Ob/Ob-R**

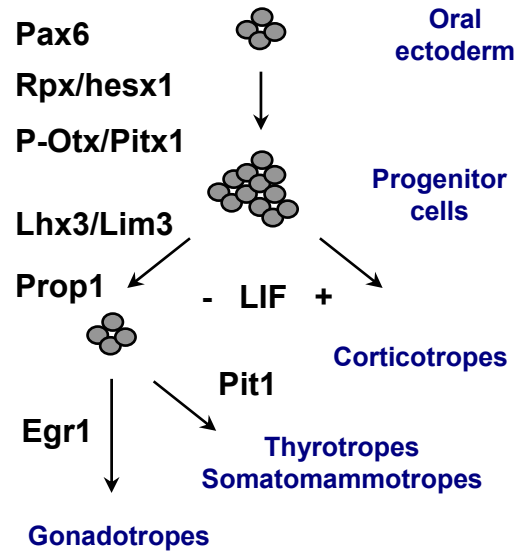
**GnRH-R**
HESX-1
PROP-1
**DAX-1**
**FSH-β**
**LH-β**

**Ovary**
FSH-R
LH-R
FRAXA

SRY
DAX-1
SF-1
WT-1
SOX-9

**Testis**
LH-R
FSH-R
AR
ERα
DAX-1
DAZ
RBM

**Anterior pituitary development**




**Sex chromosome disorders**
- Chromosomal aneuploidies

**Autosomal defects**
- •Mucoviscidose
- •Steinert
- •Kartagener

*Adapted from Achermann and Jameson*
*Mol Endo 13, 1999*

# Case 1

- **1981 work up of hypogonadism, associated with bilateral cryptorchid testes**
  - conserved sense of smell
  - family history negative for infertility
- **LH 0.9 U/L FSH 0.4 U/L T<0.7 nmol/L**
- **Otherwise normal anterior pituitary function**
- **Normal CT of the hypothalamo-pituitary region**

## Isolated GnRH deficiency

# Case 1

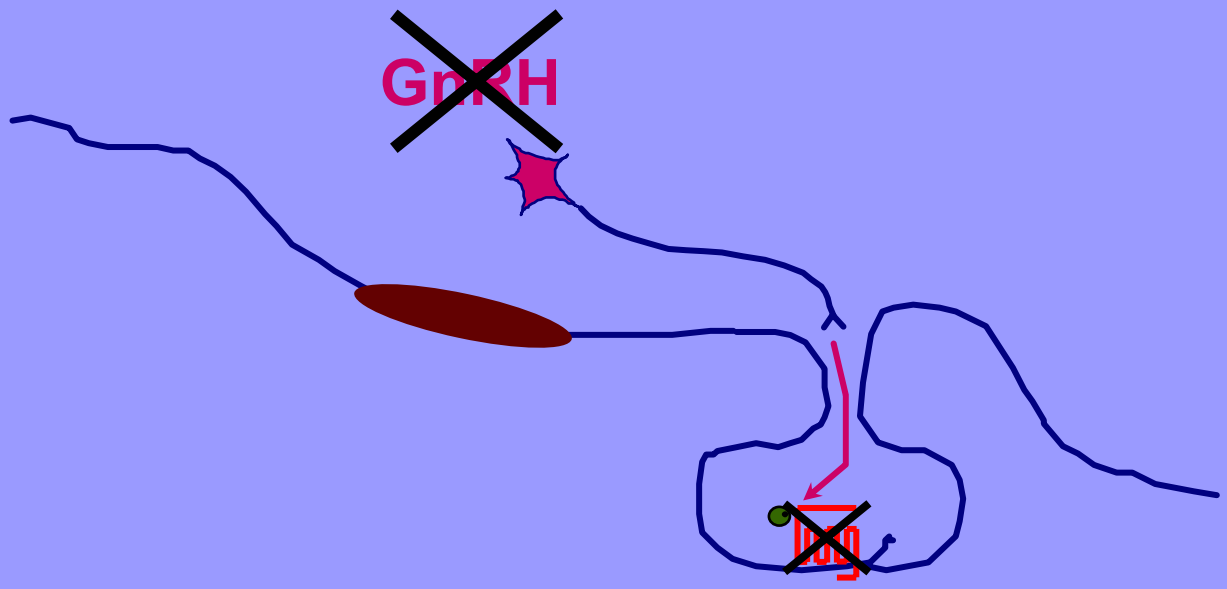
## 1994 desire of fertility

| Date | 10.10 | 23.10 | 9.11 | 20.11 | 1.12 | 8.12 |
|---|---|---|---|---|---|---|
| GnRH (ng/kg) | 60 | 250 | 250 | 250 | 250 | 250 |
| LH | <0.5 | <0.5 | <0.5 | <0.5 | <0.5 | <0.5 |
| FSH | <0.2 | <0.2 | <0.2 | <0.2 | <0.2 | <0.2 |
| T | 3.7 | 2.7 | 1.9 | 1.8 | 1.4 | 0.9 |
| TV R | 8-9 | 8-9 | | | | |
| TV L | 5-6 | 5-6 | | | | |

**Primary failure of pulsatile GnRH therapy**

# Isolated GnRH Deficiency

## Functional GnRH deficiency



# Case 1

## Rx HCG (500 IU 3x/week) and HMG (75 IU 3x/week)

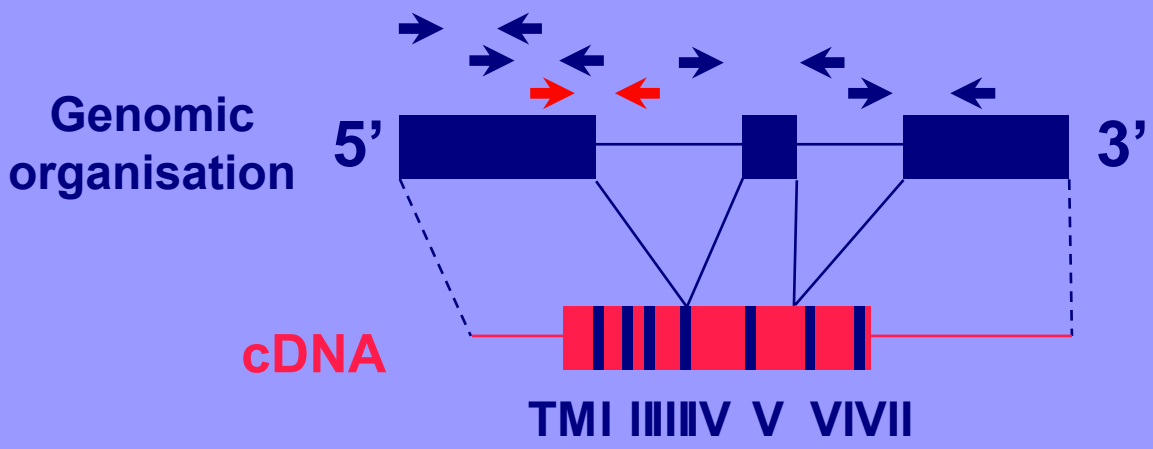
| Date | 13.1.96 | 23.2 | 19.7 | 28.10 | 6.3.97 |
|---|---|---|---|---|---|
| T | 11.8 | 16.8 | 9 | 27 | 24 |
| TV R | 8-10 | 10-12 | 12 | 12 | 15 |
| TV L | 6-8 | 8-10 | 8-10 | 10 | 14-15 |

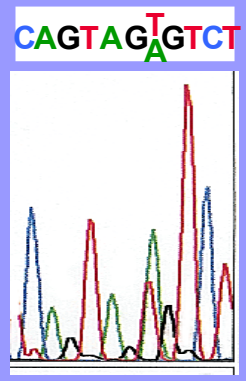
**Spermogram:**

28.10.96: $1x10^3$ sperm cells/mL

20.02.97: $9x10^6$ sperm cells/mL

# Sequencing Strategy

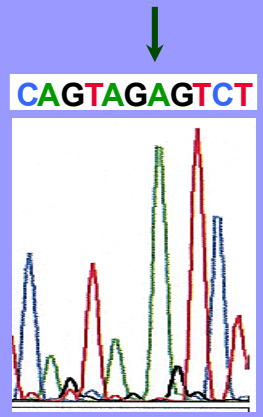


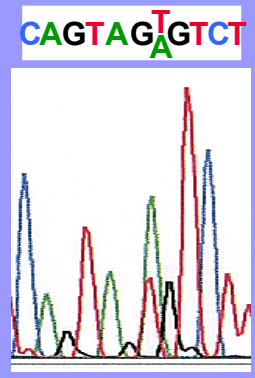

Wild type

*Mutant*

Ser Ser Val
AG*A*
*Arg*

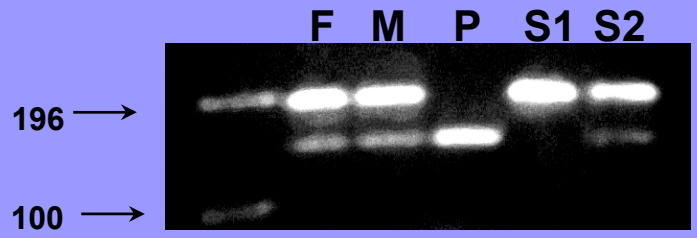



Ser *Arg* Val




Ser Ser Val
AG*A*
*Arg*

# The S168R mutation introduces a HinfI restriction enzyme site

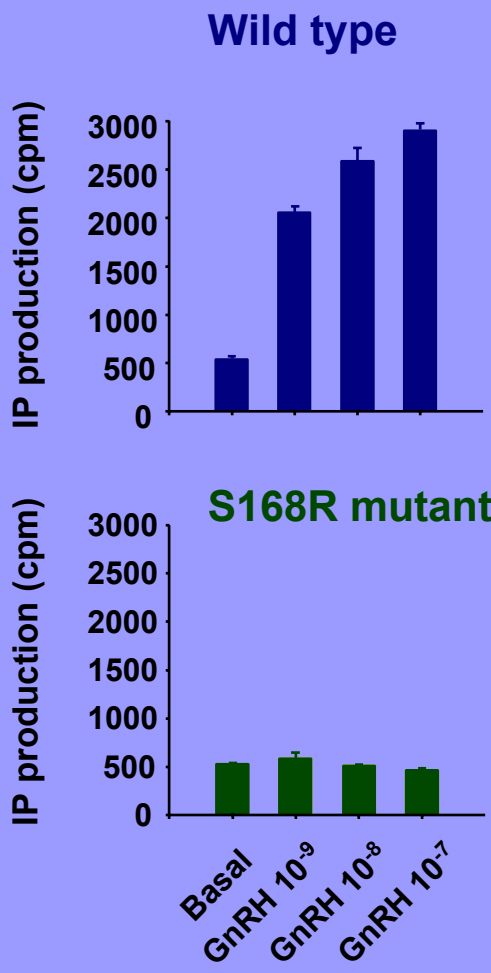


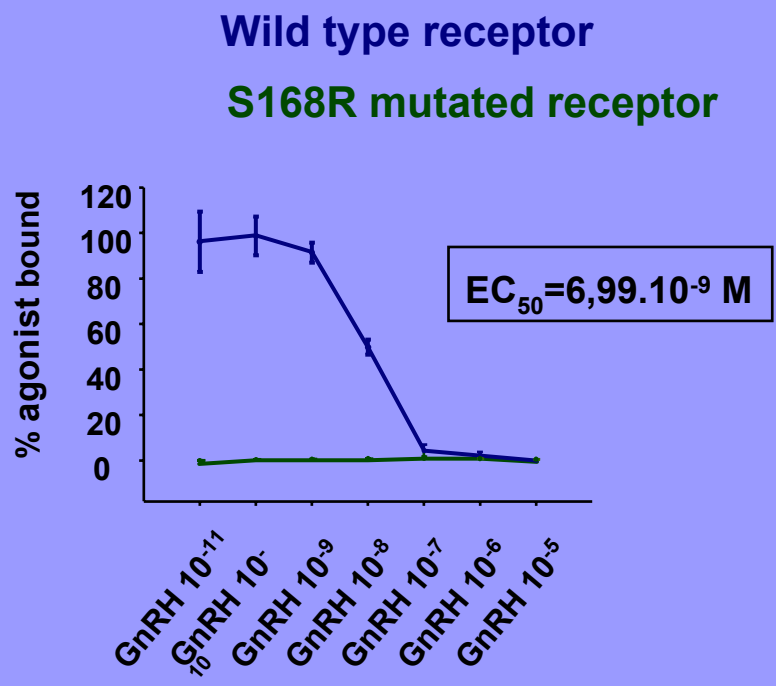

Both parents (F and M) are heterozygote for this mutation

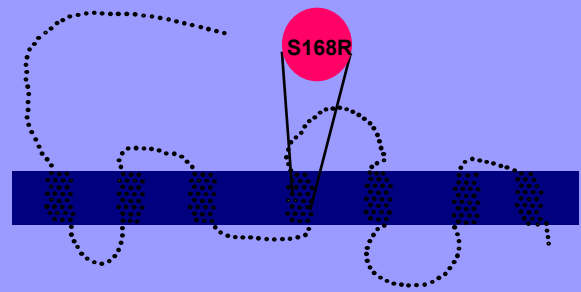
The proband (P) is homozygote for this mutation

The two unaffected sisters are either homozygote for the wild type sequence (S1) or heterozygote for this familial mutation (S2)

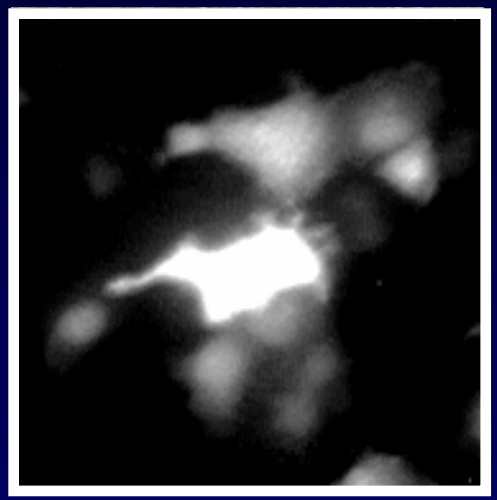
# The S168R Mutation Is a Complete Loss-Of-Function Mutation



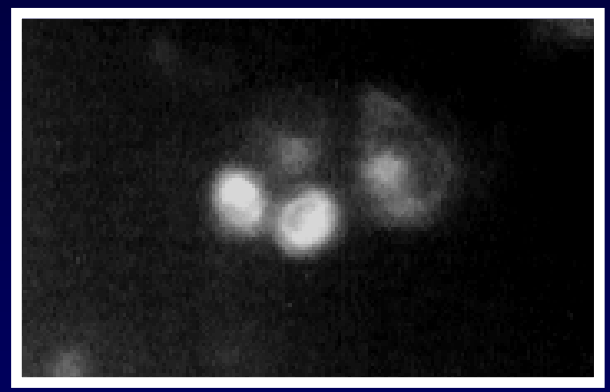



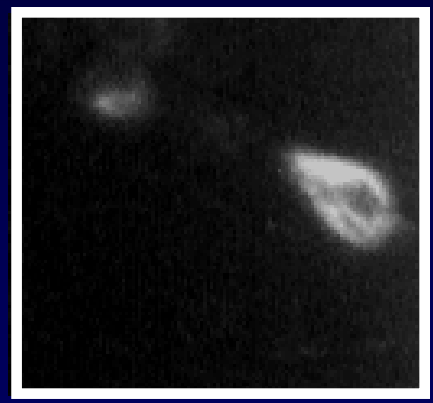
# Intracellular Localization of the S168R Mutated GnRH-R



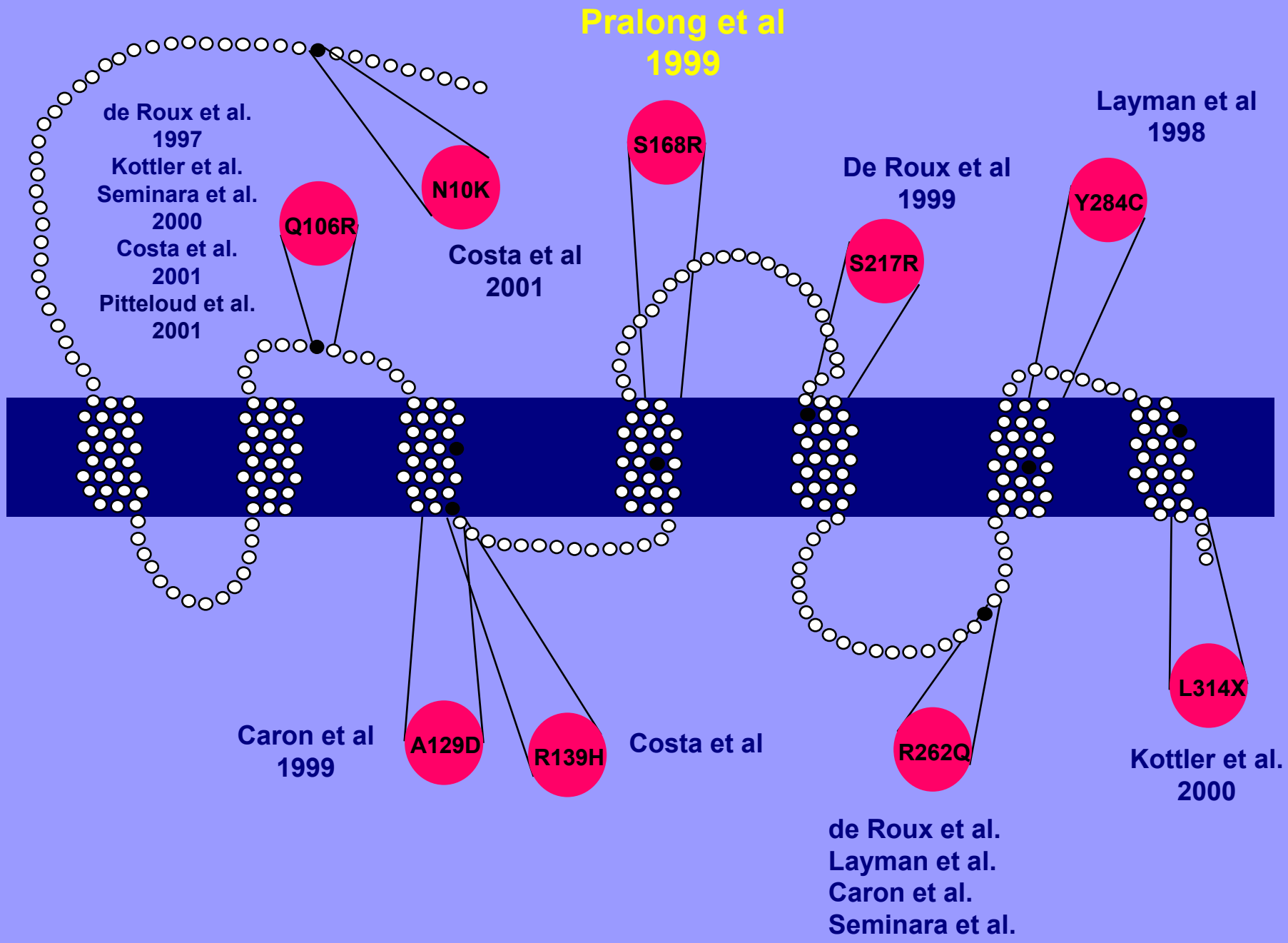
GFP



Wild type-GFP

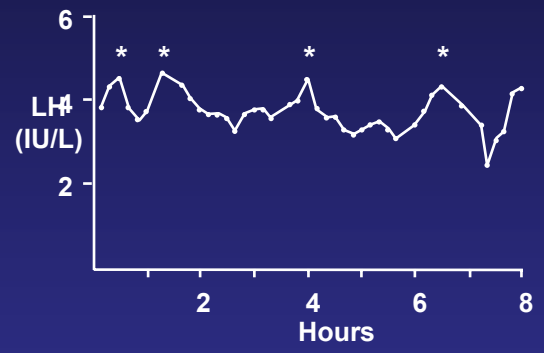


S168R-GFP


Pralong et al
1999
de Roux et al.
1997
Kottler et al.
Seminara et al.
2000
Costa et al.
2001
Pitteloud et al.
2001
N10K
Q106R
Costa et al
2001
S168R
De Roux et al
1999
S217R
Layman et al
1998
Y284C
L314X
Caron et al
1999
A129D
R139H
Costa et al
R262Q
Kottler et al.
2000
de Roux et al.
Layman et al.
Caron et al.
Seminara et al.

## Phenotype of compound heterozygote patients :

- Male : scrotal testis (8 mL each)
- LH : 4.0 IU/L, FSH : 5.9 IU/L
- Puberty at age 16 years
- Basal LH secretion displays blunted pulsatility:

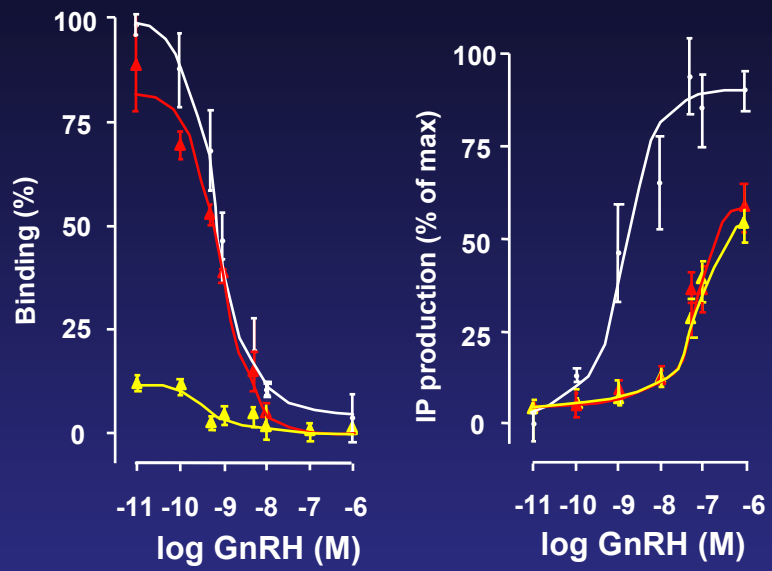



Normal response to GnRH (100 μg iv) :
Peak LH=24 IU/L, peak FSH=8.9 IU/L

- Female : primary amenorrhea and infertility
- Telarche at age 14 years
- LH : 5.0 IU/L, FSH : 5.2 IU/L, E2 128 pmol/L

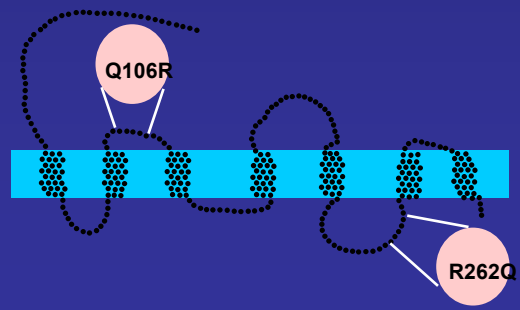
## Conclusion : incomplete hypogonadotropic hypogonadism

## Functional characterization of these two mutations :




Q106R mutation

R262Q mutation

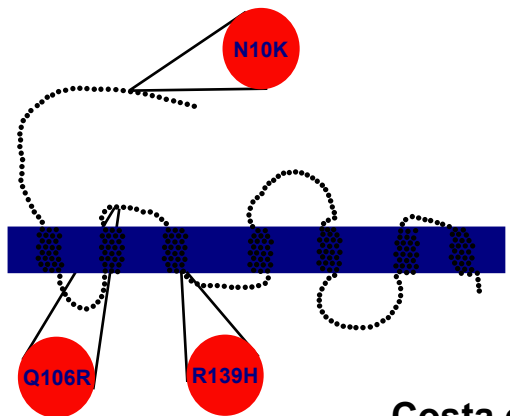



de Roux et al.
*NEJM 337*, 1997

# Mutations in the GnRH-R Are More Frequent than Previously Thought

**Screening of 17 HH patient, in 14 unrelated families: three mutations identified**

- **N10K:** novel compound heterozygote mutation (with Q106R) affected patients have partial HH
- **R139H:** novel homozygote mutation affected patient has complete HH




Costa et al
In press

# Conclusions

- **There is a wide range of phenotypic expression of loss-of-function mutations of the GnRH-R, characterized by a variable degree of resistance to GnRH**
- **The incidence of GnRH-R mutations in complete or partial HH is probably around 15%**

# Menstrual cycles: Fatness as a Determinant of Minimum Weight for Height Necessary for Their Maintenance or Onset

***Weight loss causes loss of menstrual function, and weight gain restores menstrual cycles.....***
***.....The data suggest that a minimum level of stored, easily mobilized energy is necessary for ovulation and menstrual cycles in the human female.***

***Frisch and McArthur, Science 185, 1974***

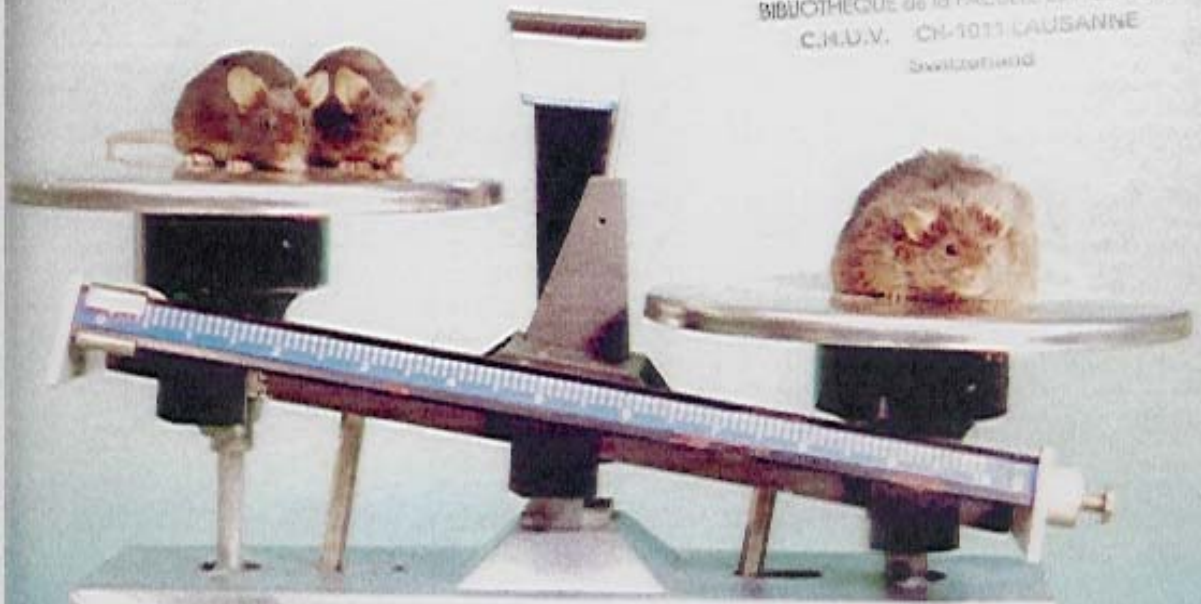
## The Critical Fatness Hypothesis

# nature

INTERNATIONAL WEEKLY JOURNAL OF SCIENCE

Volume 372 No. 6505 1 December 1994 £4.00 FF44 DM13 Lire13000



## Mouse weighed down by genetics

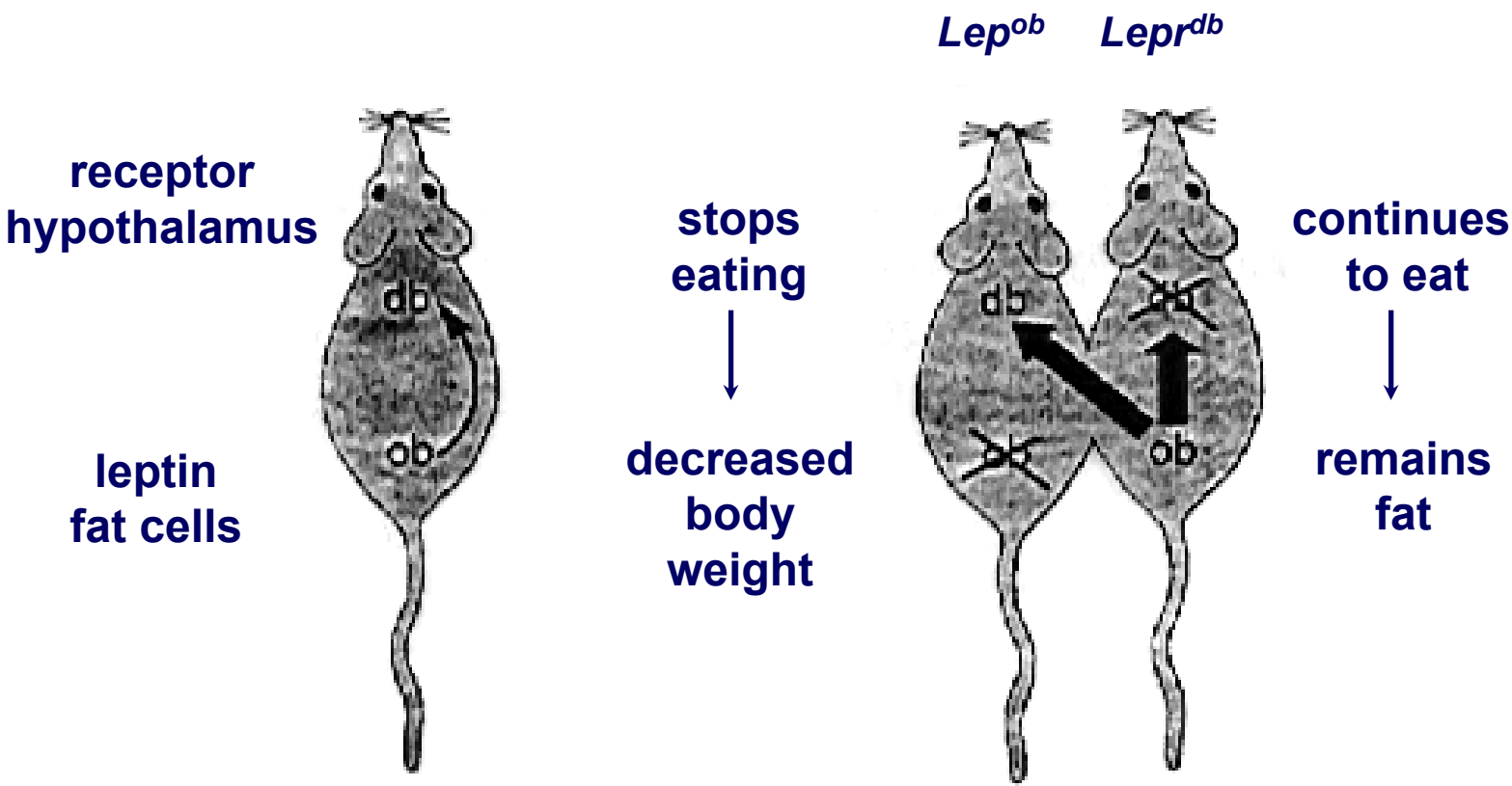
# The *Lep*$^{Ob}$ Mouse

- **Genetically obese**
- **Multiple metabolic and endocrine abnormalities**
    - ***hyperglycemia and insulin resistance***
    - ***defects in thyrotrope and corticotrope axes***

**Isolated GnRH Deficiency**

# The Leptin-Leptin Receptor System

## Parabiosis experiments




*Coleman et al., Diabetologia 14, 1978*

# The Leptin Gene

**A positional cloning approach in the *Lep*$^{ob}$ mouse allows to identify the locus of the gene encoding for the *ob* protein**

**Encodes for a 167 amino acids, with a cytokine-like tertiary structure**

**C to T missense mutation in *Lep*$^{ob}$ mice results in an Arg105X mutation in the *ob* protein**

**Levels of *ob* gene expression are markedly increased in WAT of *Lep*$^{ob}$ mice, suggesting that the truncated protein is biologically inactive**

**Zhang *et al.*, Nature 372, 1994**

# Expression Cloning Of The Leptin Receptor: OB-R

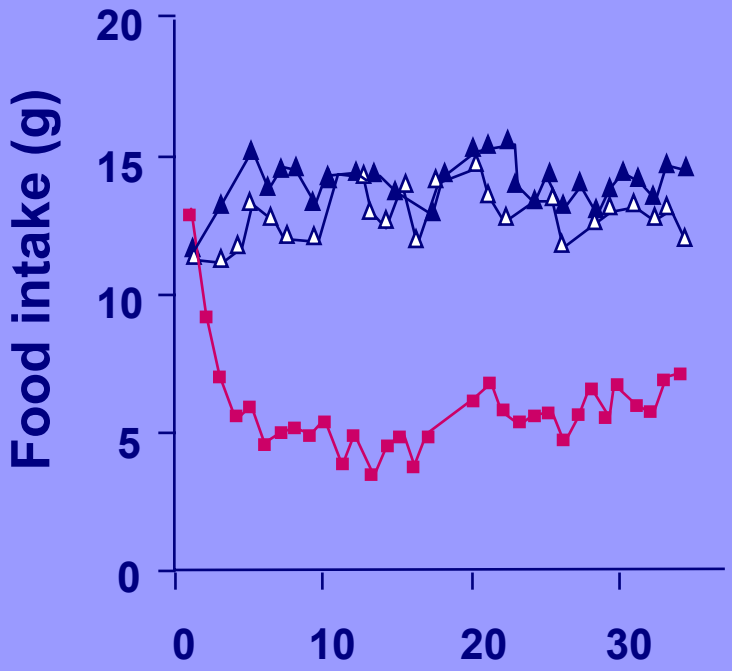
**Screening of a wide variety of mammalian cell lines and tissues for leptin binding, using 125I-leptin and AP-OB fusion proteins**

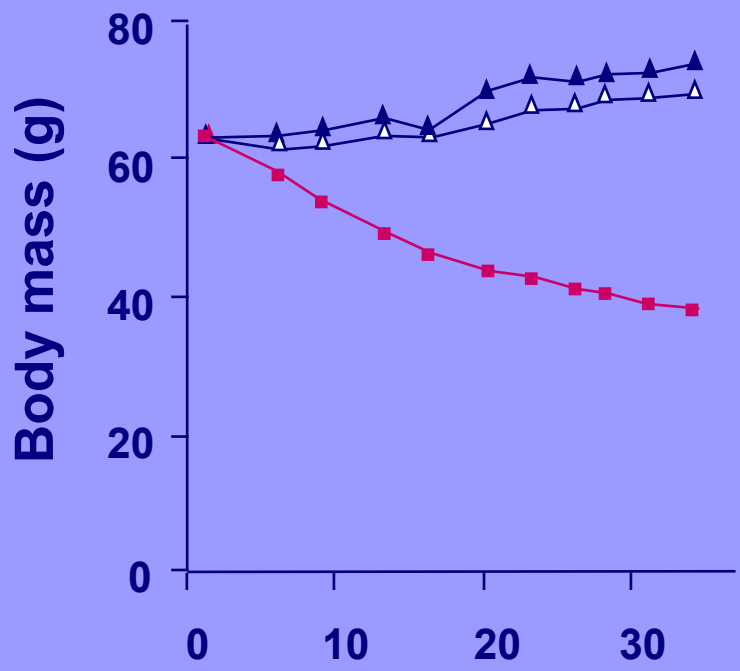
**Leptin binding identified in mouse choroid plexus**

**Member of the cytokine receptor superfamily (single membrane-spanning receptor)**

***Tartaglia et al., Cell 83, 1995***

# Leptin Decreases Food Intake in *Lep$^{ob}$* Mice




*Halaas at al., Science 269, 1995*

**Correction of the sterility defect in homozygous obese female mice by treatment with the human recombinant leptin**

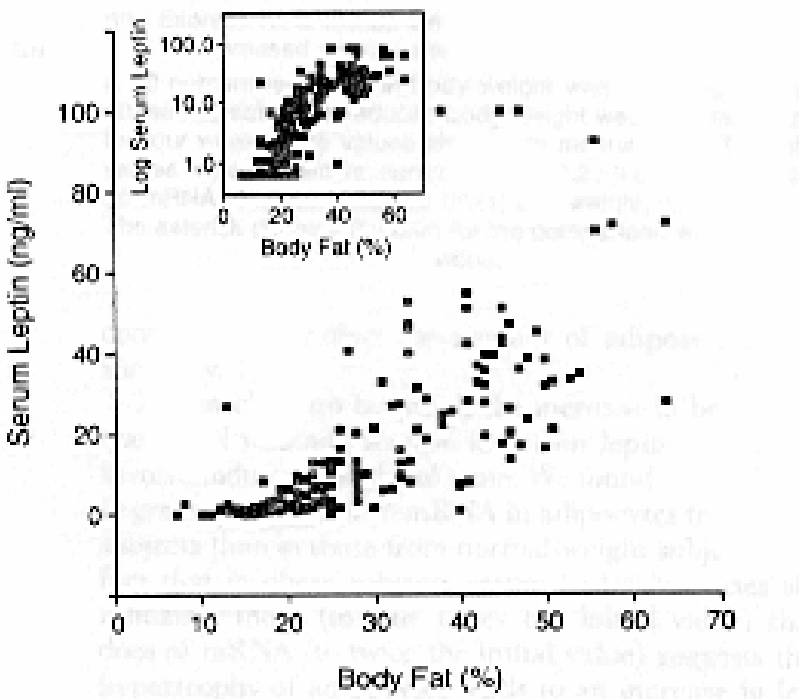
***Chehab et al., Nat Genet 1996***

**Leptin treatment rescues the sterility of genetically obese ob/ob males**

***Mounzih et al., Endocrinology 138, 1997***

# Correlations in Human Reproductive Physiology

# Is Human Obesity Caused By Leptin Deficiency ?

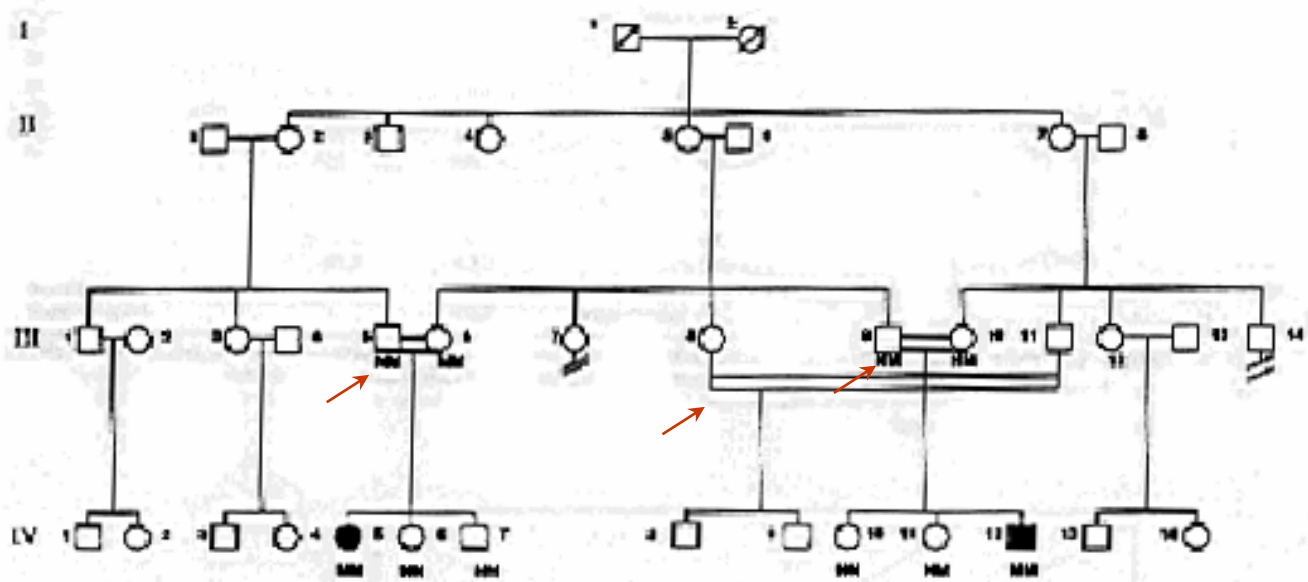



**Large, population-based screenings show that circulating leptin levels are appropriately high in the humans**

Considine *et al.*, NEJM 334, 1996

# Leptin Gene Mutation In Humans

**Study of two first degree cousins, members of a highly consanguineous family, presenting with marked, early onset hyperphagia**



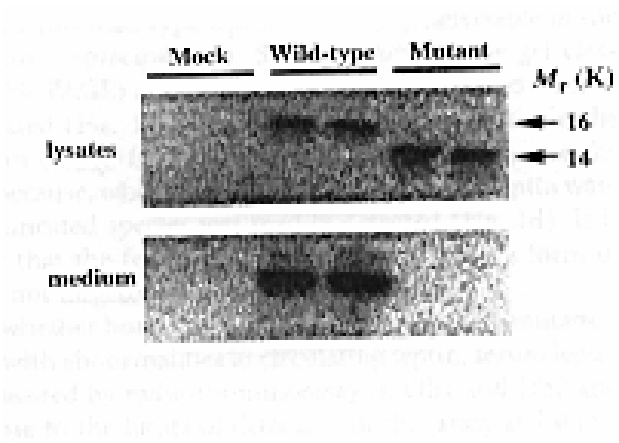
**Montague *et al.*, Nature 387, 1997**

# Identification Of A Leptin Gene Point Mutation In Humans

## Single G deletion at codon 133

- disruption of reading frame
- 14 aberrant aa after Gly 132
- premature stop codon

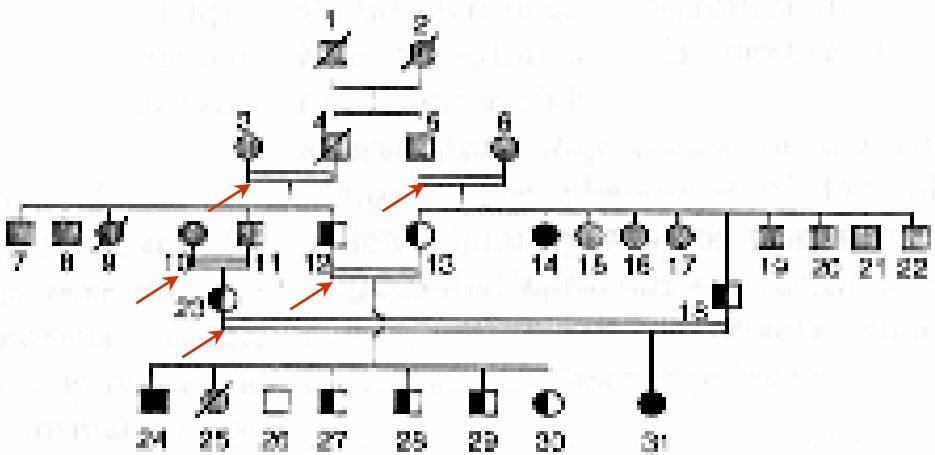
## Impaired secretion of mutant protein




Montague *et al.*, Nature 387, 1997

# A Novel Leptin Gene Point Mutation In Humans

**Study of a markedly obese (BMI=55.8 kg/m$^2$) patient with very low serum leptin concentrations (0.9 ng/mL)**

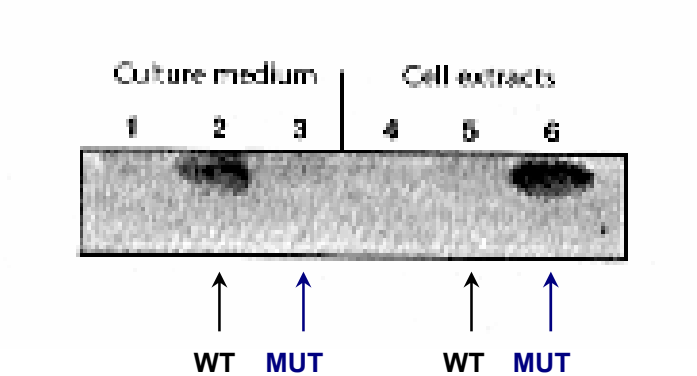


**Highly consanguineous family**

Strobel *et al.*, Nature Genetics 18, 1998

# A Novel Leptin Gene Point Mutation In Humans

## C105T substitution, resulting in Arg to Trp replacement



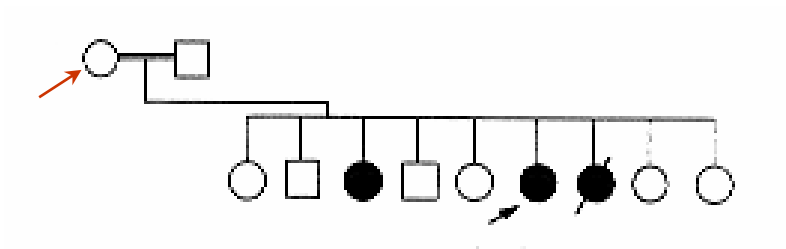

**Impaired secretion of the mutant protein**

Strobel *et al.*, Nature Genetics 18, 1998

# A Mutation In The Human OB-R Gene Causes Obesity

**Study of a family with strong prevalence of morbid obesity occurring early in life**

**Affected patients with markedly elevated leptin levels**



**Clément *et al.*, Nature 392, 1998**

# Phenotype of Leptin/Leptin Receptor Deficiency in Humans

- Early-onset morbid obesity
- Hyperphagia
- Impaired GH and TSH secretion (Ob-R)

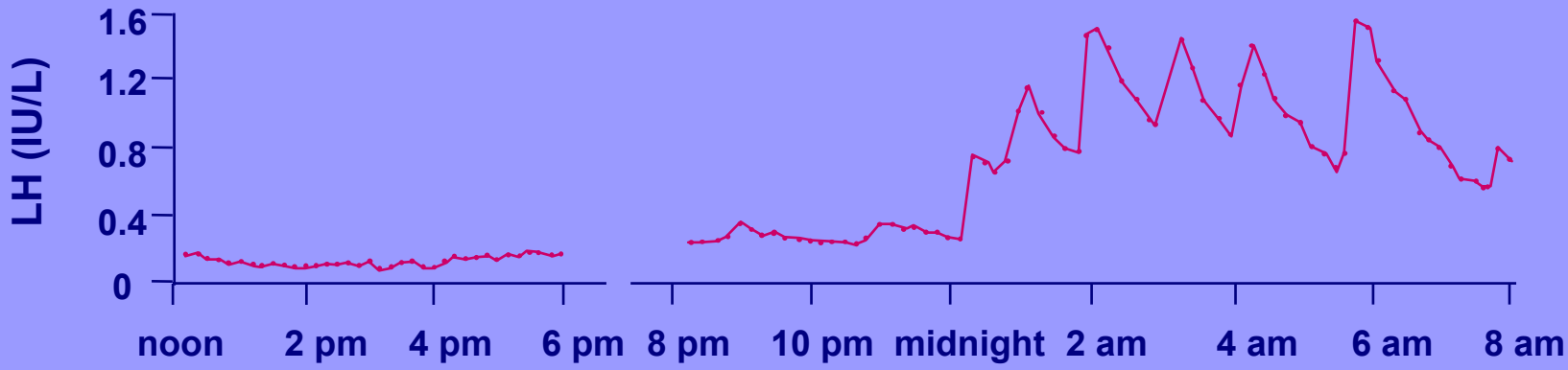
- Delayed puberty

- Autosomal recessive inheritance

# Summary Of The Phenotype Of Human Leptin-Leptin receptor Mutations

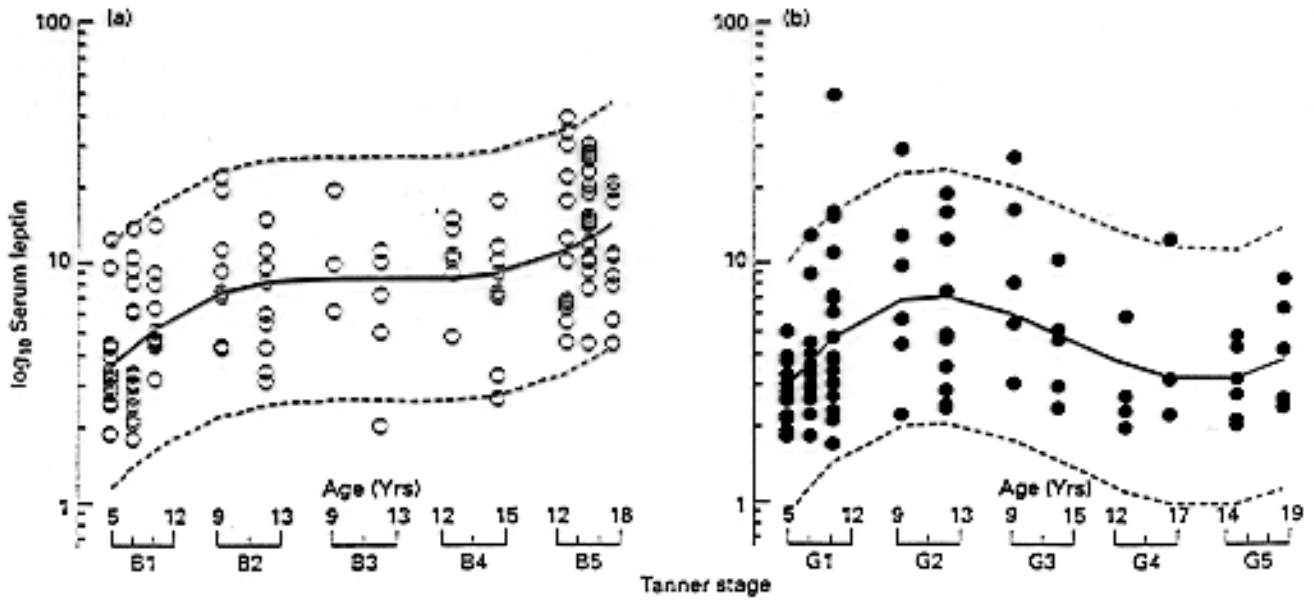
| | OB 1 | OB 2 | OB 3 | OB 4 | OB-R 1 | OB-R 2 |
|---|---|---|---|---|---|---|
| | Montague *et al* | Montague *et al* | Strobel *et al* | Strobel *et al* | Clément *et al* | Clément *et al* |
| **Age at diag** | **8 y** | **2 y** | **34 y** | **22 y** | **19 y** | **19 y** |
| **Sex** | **F** | **M** | **F** | **M** | **F** | **F** |
| **Mutation** | G deletion at codon 133 (frameshift) | G deletion at codon 133 (frameshift) | R105W | R105W | G to A in splice donor site, exon 16 | G to A in splice donor site, exon 16 |
| **Clinical feat** | **Pre-pubertal** | **Pre-pubertal** | **Primary amenorrhoea** | **Delayed puberty impuberism** | **Primary amenorrhoea** | **Primary amenorrhoea** |
| **LH (IU/L)** | **<0.2** | **<0.2** | **NA** | **4.4** | **<0.2** | **<0.8** |
| **FSH (IU/L)** | **0.8** | **0.2** | **NA** | **9.0** | **<0.1** | **1.2** |
| **E2 (pmol/L)** | **<20** | **-** | **NA** | **-** | **17** | **13** |
| **T (nmol/L)** | **-** | **<0.2** | **-** | **5** | **-** | **-** |

# Correction of Hypogonadotropic Hypogonadism by Leptin Treatment in Human Leptin Deficiency



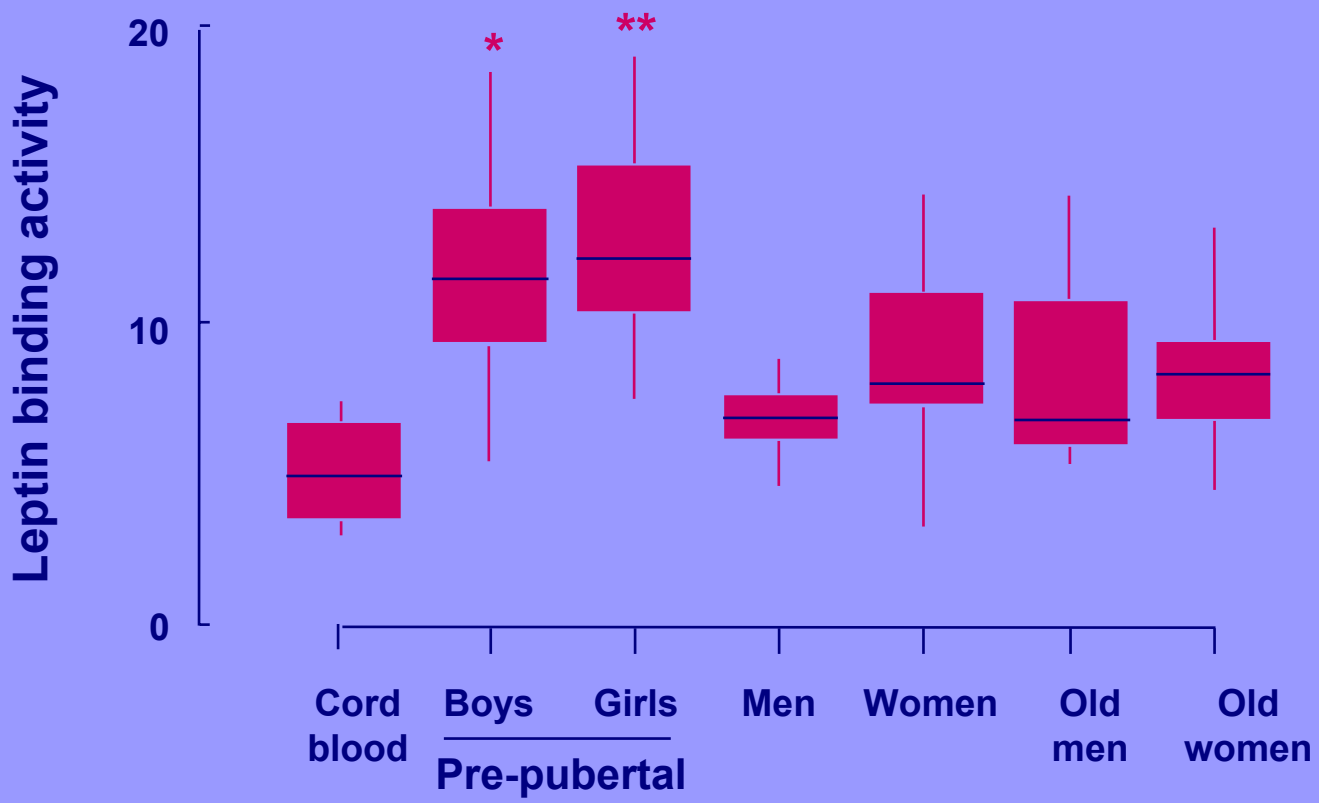

*Farooqi et al., NEJM 341, 1999*

# Rising Serum Leptin Levels Before Puberty




*Clayton et al., Clin Endocrinol 46, 1997*

# Elevated Serum Leptin Binding Protein Levels Before Puberty

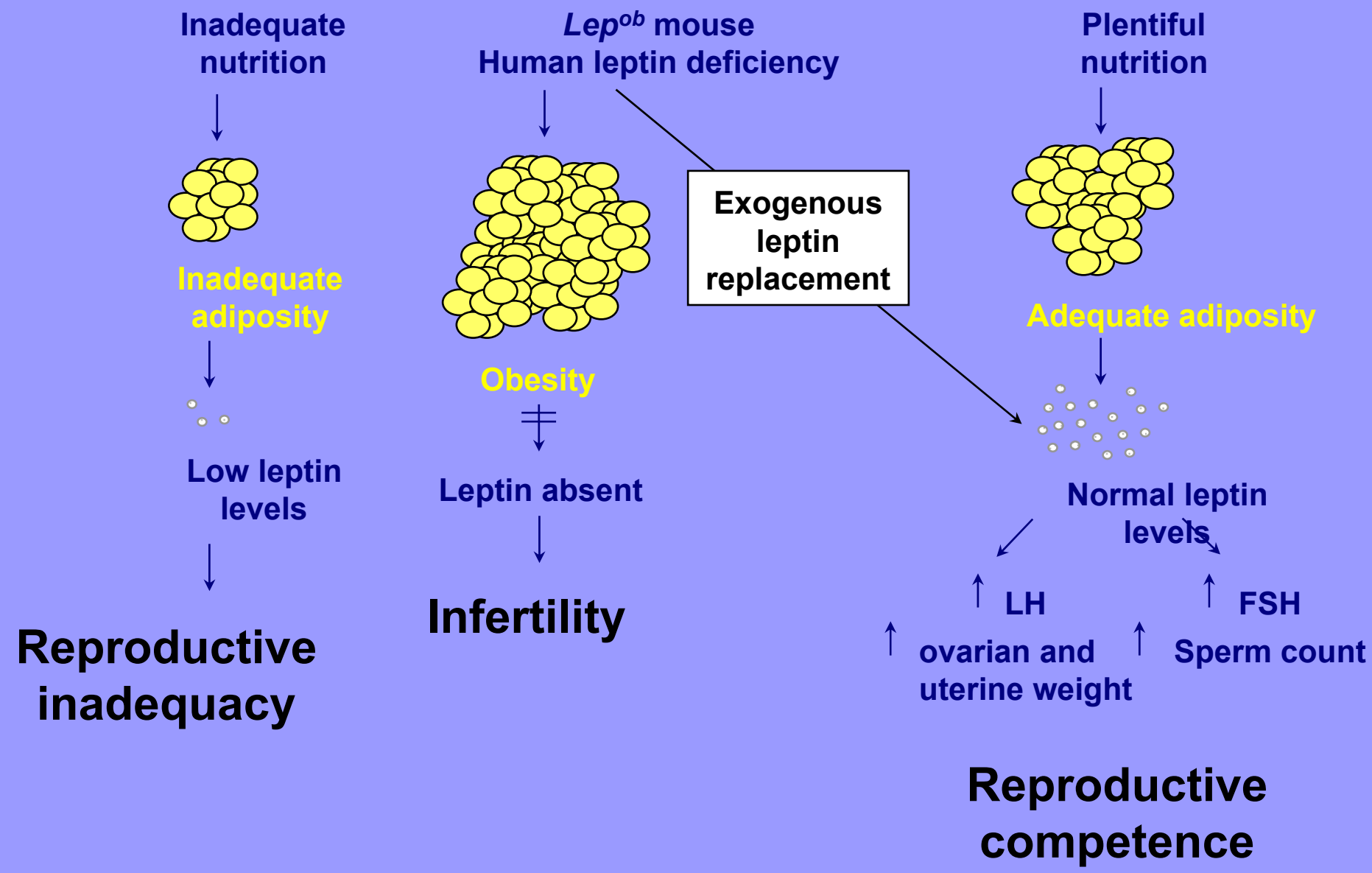



*Quinton et al., JCEM 84, 1999*

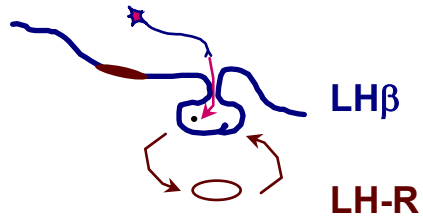
# Leptin Meets the Criteria for a Blood-borne Metabolic Signal Timing Puberty

- **The circulating leptin levels are different in the sexually immature and mature individuals**
- **When administered, leptin leads to a change in the hypothalamic secretion of GnRH**

# The Critical Fatness Hypothesis Revisited



# LH Deficiency - Males




## LHβ

**One single case described**

**Bio-inactive LH**

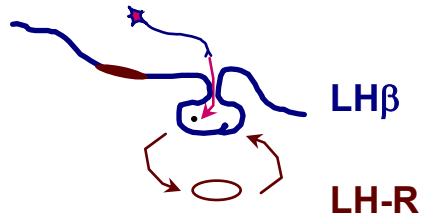
- **Phenotype:**
  - normal male
  - delayed puberty
  - response to hCG: normal virilization, but not fertility
- **Male heterozygotes: 3/4 infertile**

## LH-R

**Broad spectrum of phenotypic expression of inactivating mutations**

- pseudohermaphroditism and complete azoospermia
- micropenis, delayed puberty and arrest of spermatogenesis

# LH Deficiency - Females




**No LH-β mutation yet described in a female patient**

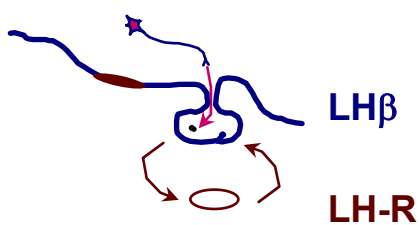
**LH-R**

- normal external genitalia
- normal pubertal development
- primary amenorrhea
- **no pre-ovulatory follicles**

# Role of the LH/LH-R System

- **Important for normal male development**
- **LH-R plays a role in spermatogenesis as well as ovulation**

**LH-R is a candidate gene for male as well as female infertility**



# Case presentation

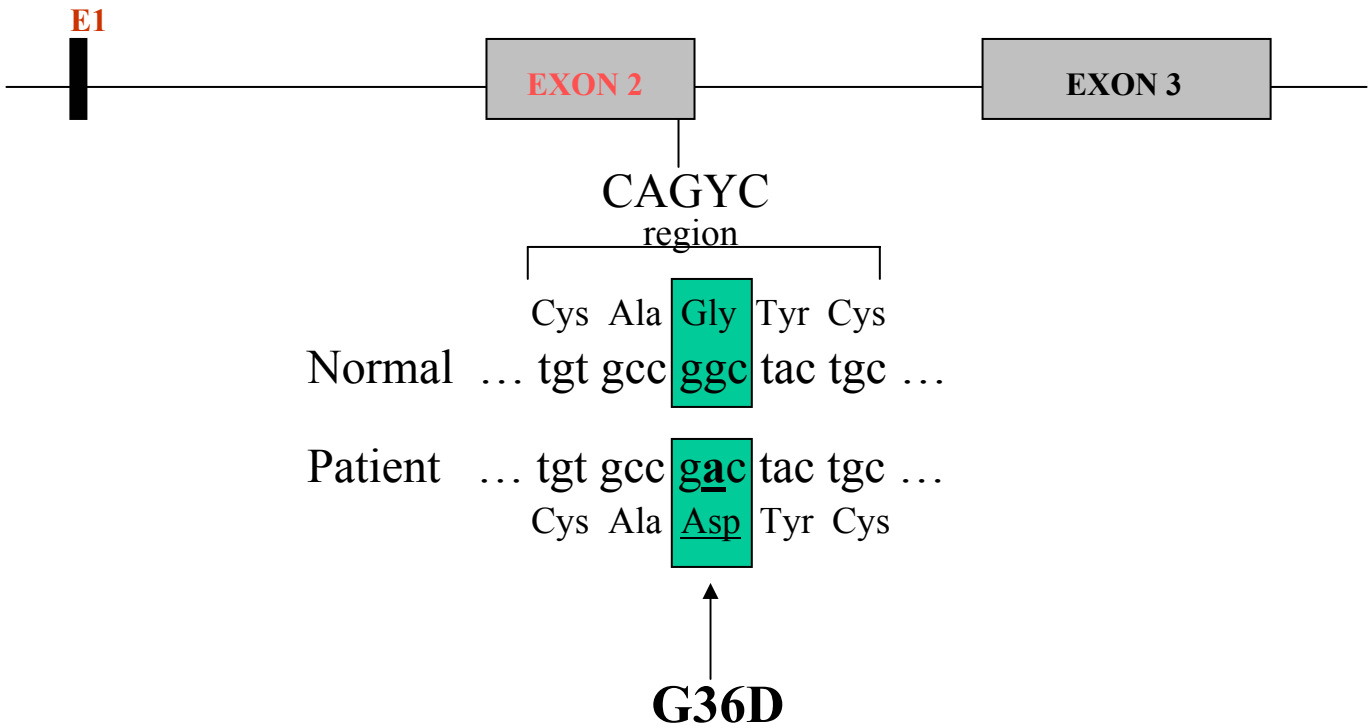
- **30 yo male, evaluated for delayed puberty**
  - conserved sense of smell
  - family history negative for infertility (reliable?)
- **Micropenis, TV: L=R=8 mL**
- **LH: undetectable  FSH=23 mIU/L  T=0.3 μg/L**
- **αSU<0.1 mIU/L  βHCG<2 IU/L**
- **Otherwise normal anterior pituitary function**
- **Normal MRI of the hypothalamo-pituitary region**

## Isolated LH deficiency

# Case presentation

- **<u>Testicular biopsy:</u> immature Leydig and Sertoli cells, intact seminal tubules, rare spermatogenic cells**
- **GnRH test:**
  - **LH undetectable  FSH=48mIU/L**
- **im testosterone Rx:**
  - **normalization of FSH levels**
  - **azoospermic ejaculation**

# LH Beta gene


E1
EXON 2
EXON 3
CAGYC
region
Cys Ala Gly Tyr Cys
Normal … tgt gcc ggc tac tgc …
Patient … tgt gcc gac tac tgc …
Cys Ala Asp Tyr Cys
G36D

# CONCLUSIONS

- **This inactivating point mutation of LHβ is a novel cause of human hypogonadotropic hypogonadism**
- **Demonstrates the role of T in FSH feedback regulation**
- **Confirms the role of LH in human spermatogenesis**
- **Confirms the importance of maternal βHCG in testicular genesis**

- **Inactivating mutations recently described throughout the gonadotrope axis provide a model for single-gene diseases in humans**

- **The careful phenotyping of affected patients provides insight into the physiology and pathophysiology of reproduction and associated disorders**

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