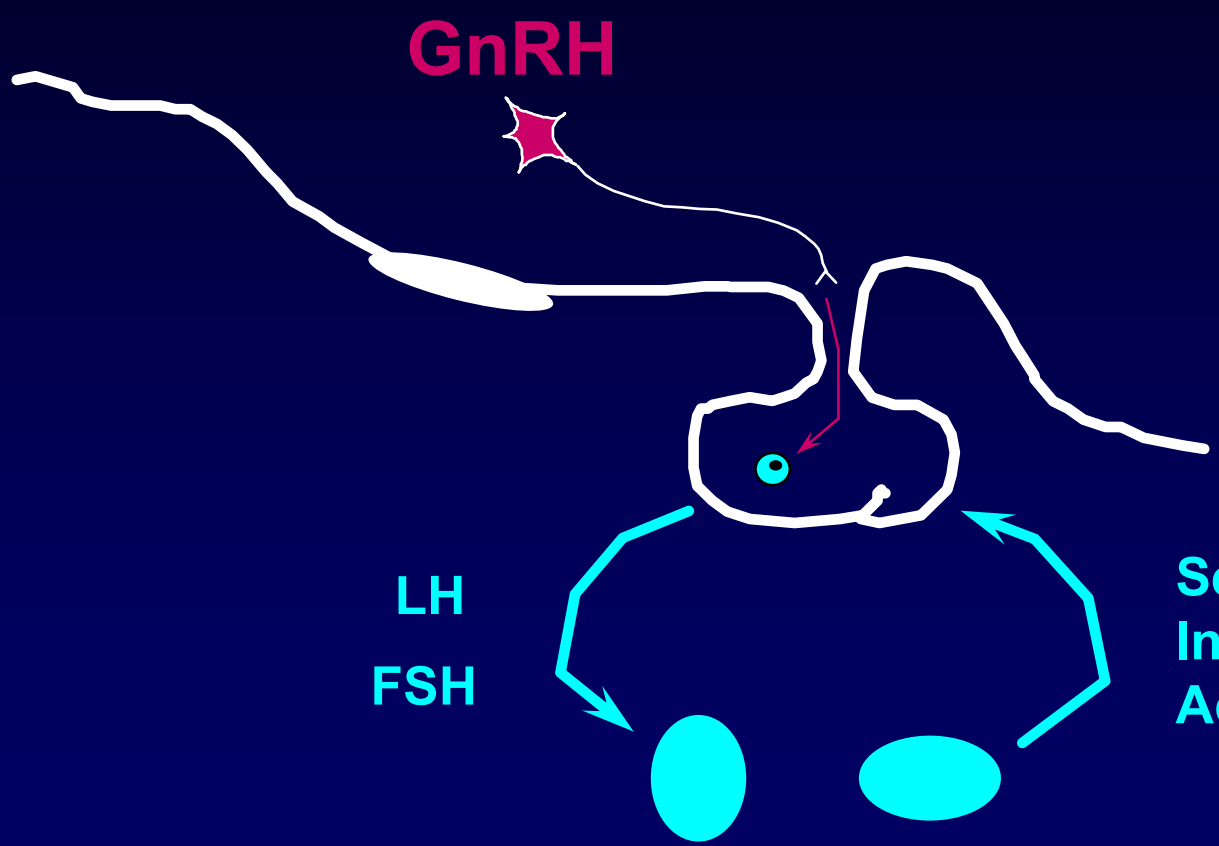
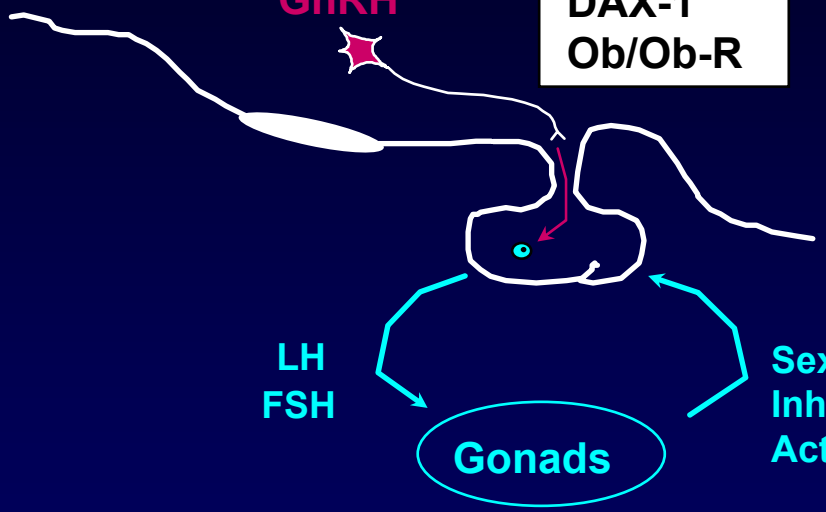
Genetics of Hypogonadism

François Pralong
Division of Endocrinology
Lausanne University Hospital

The Gonadotrope Axis



Single Gene Mutations and Hypogonadism



KAL
DAX-1
Ob/Ob-R

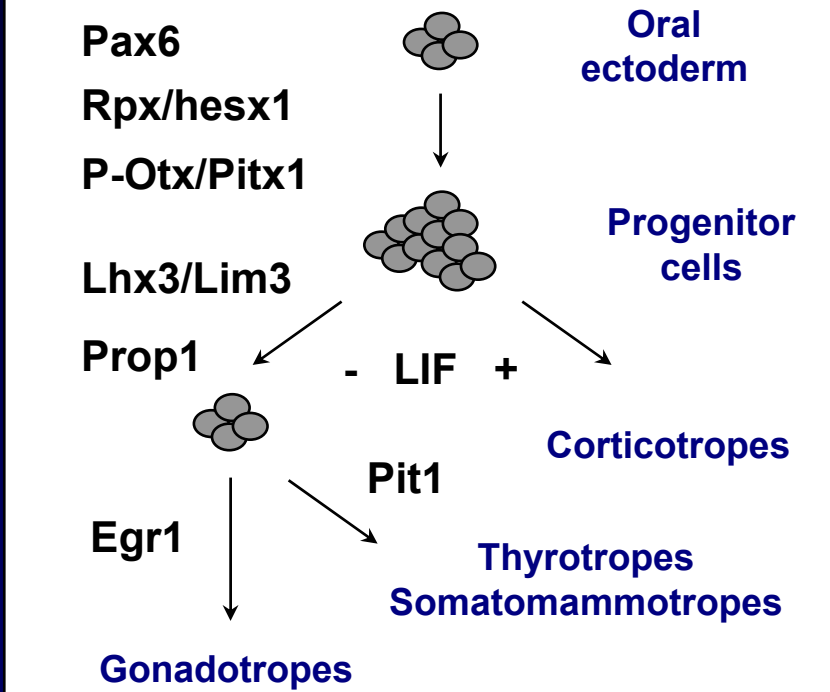
GnRH-R
HESX-1
PROP-1
DAX-1
FSH-β
LH-β

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



Achermann and Jameson, Mol Endo 13, 1999

Case Presentation
Mr C.G. 1962

- **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 hypogonadotropic hypogonadism

Case Presentation
Mr C.G. 1962

HCG: 2000 IU 3x/week, replaced by HCG/HMG (1.82):

Date	3.8.81	7.8.81	1.9.81	6.11.81	7.12.81	13.1.82	2.4.82
T (nmol/L)	<0.7	1.4	<2.0	1.9	23.2	36.3	19.4
TV R (mL)	-	-	3	4	4	4	4
TV L (mL)	-	-	-	3	3	3	3

Case Presentation
Mr C.G. 1962

1994 patient wants 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

Case Presentation
Mr C.G. 1962

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:

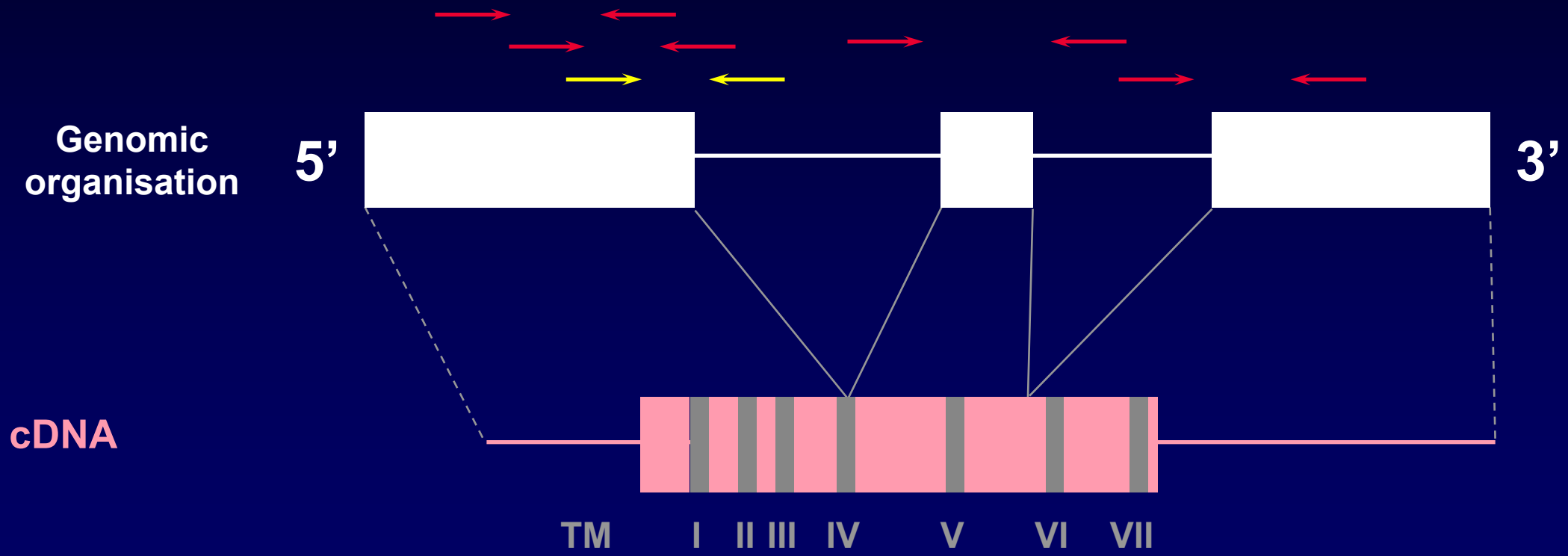
28.10.96: 1×10^{3} sperm cells/mL

20.02.97: 9×10^{6} sperm cells/mL

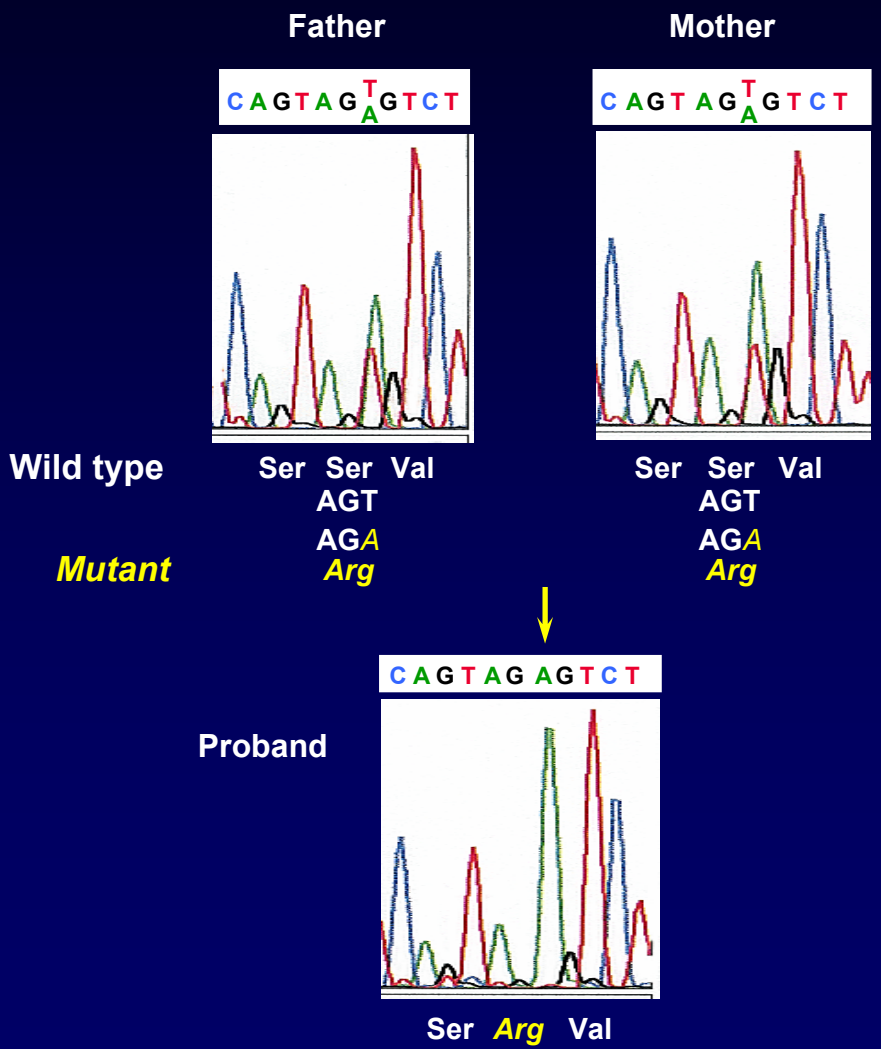
Hypothesis

- The origin of this patient's hypogonadism is at the pituitary level, rather than the hypothalamic level
- Presence of an inactivating mutation of the GnRH receptor gene

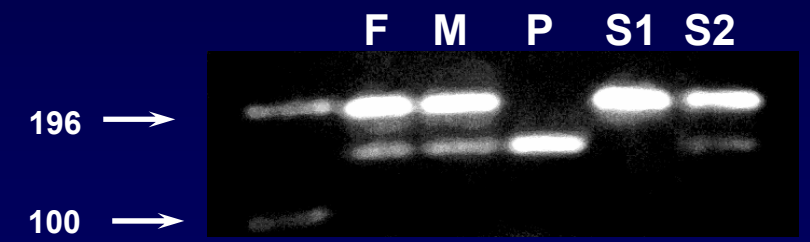
Sequencing Strategy

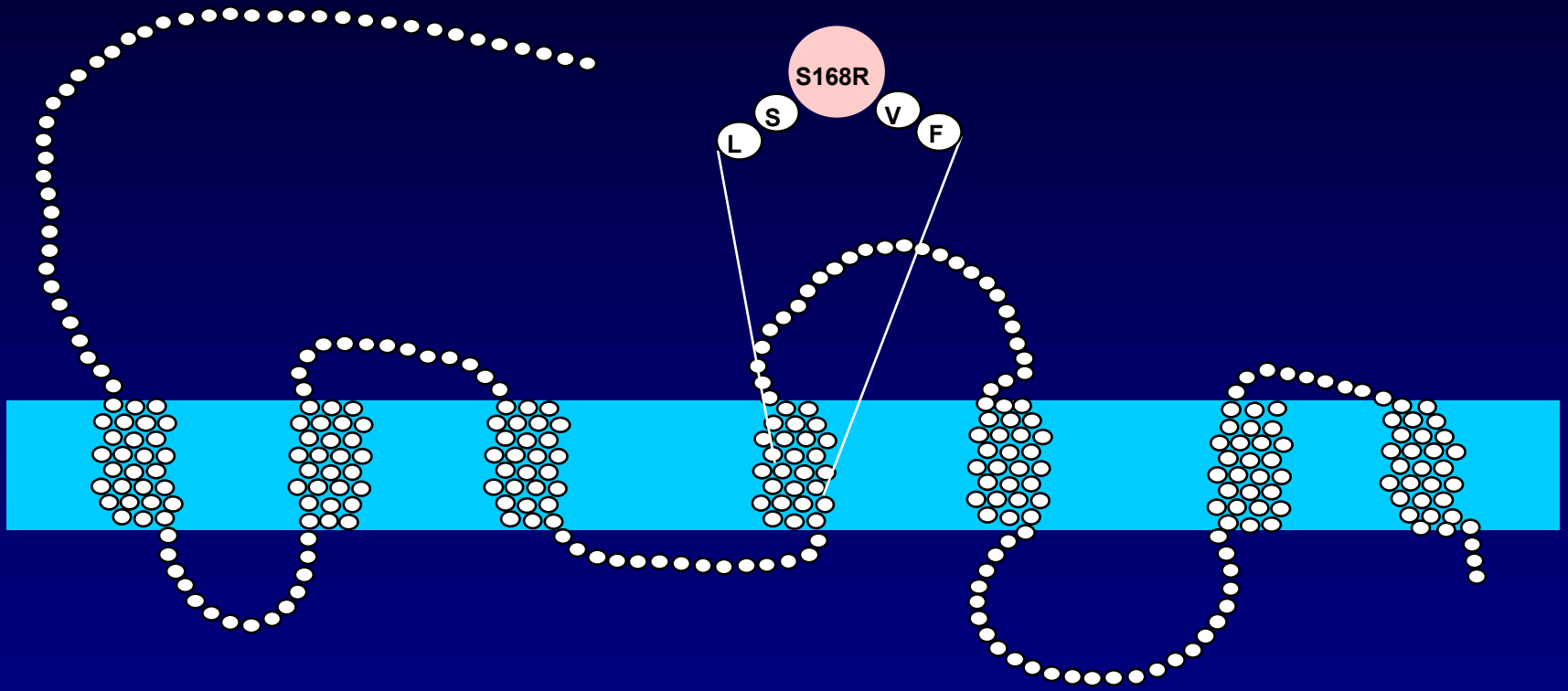


A T to A Point Mutation at Position 504 Results in a Change from Serine to Arginine at Residue 168 of the GnRH-R

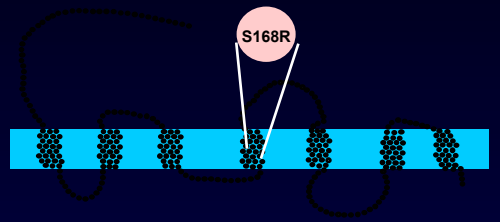


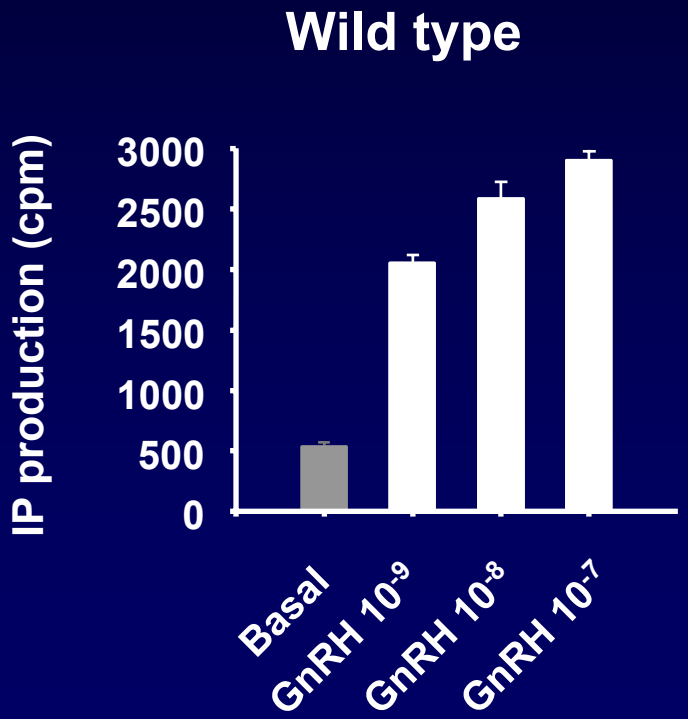
The T to A Mutation Introduces a HinfI Restriction Enzyme Site

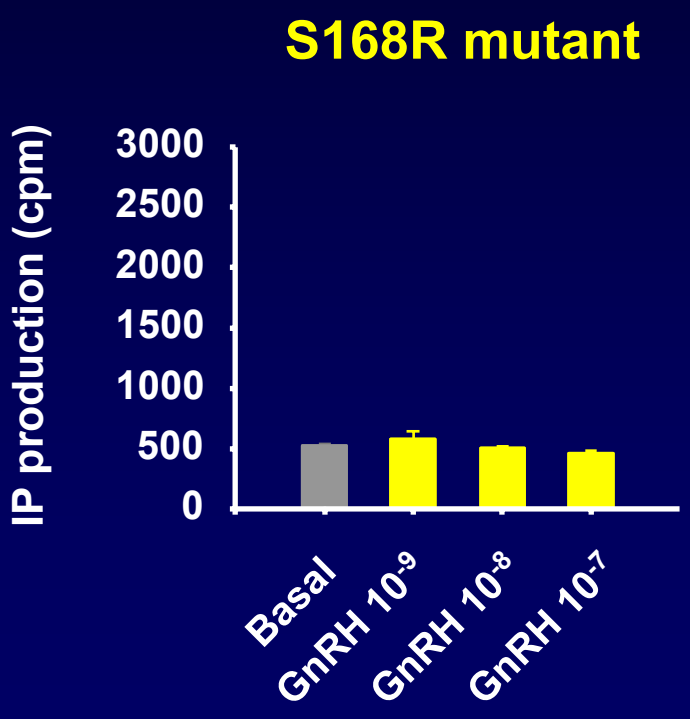



S168R
L
S
V
F

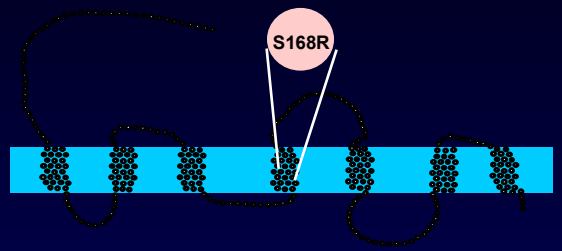
The S168R Mutation Is a Complete Loss-Of-Function Mutation





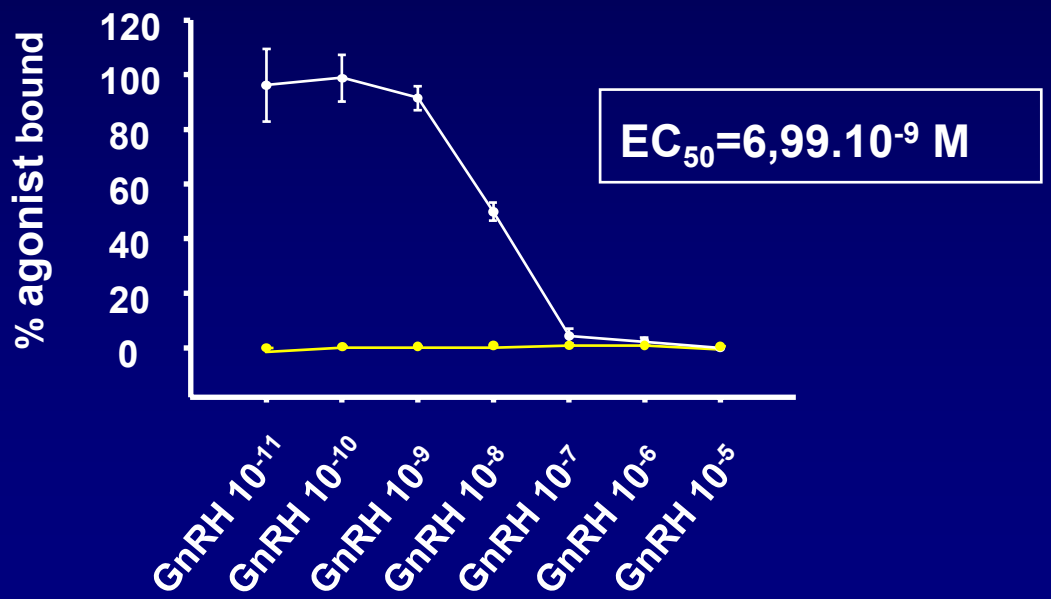


The S168R Mutation Abolishes Ligand Binding

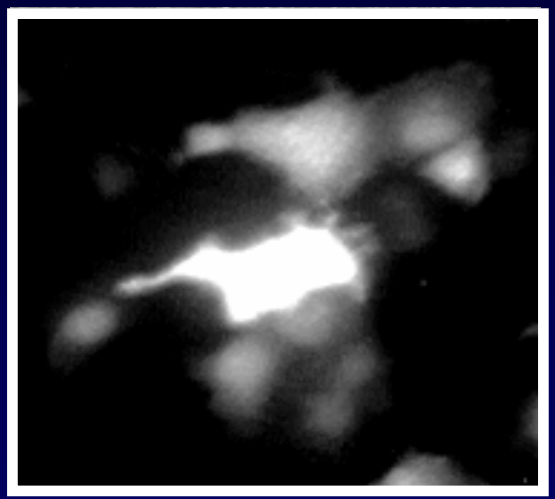


Wild type receptor

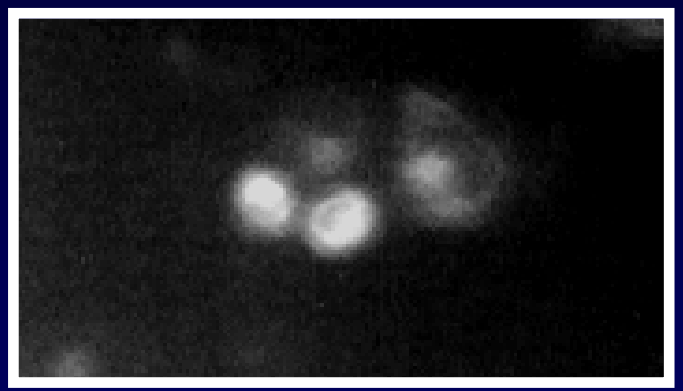
S168R mutated receptor



Intracellular Localization of the S168R Mutated GnRH-R



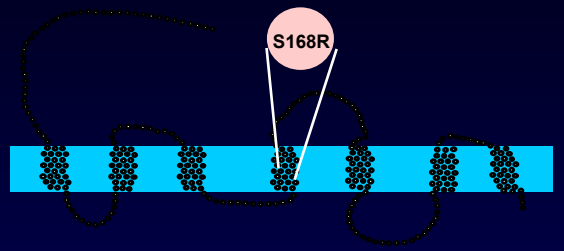
GFP

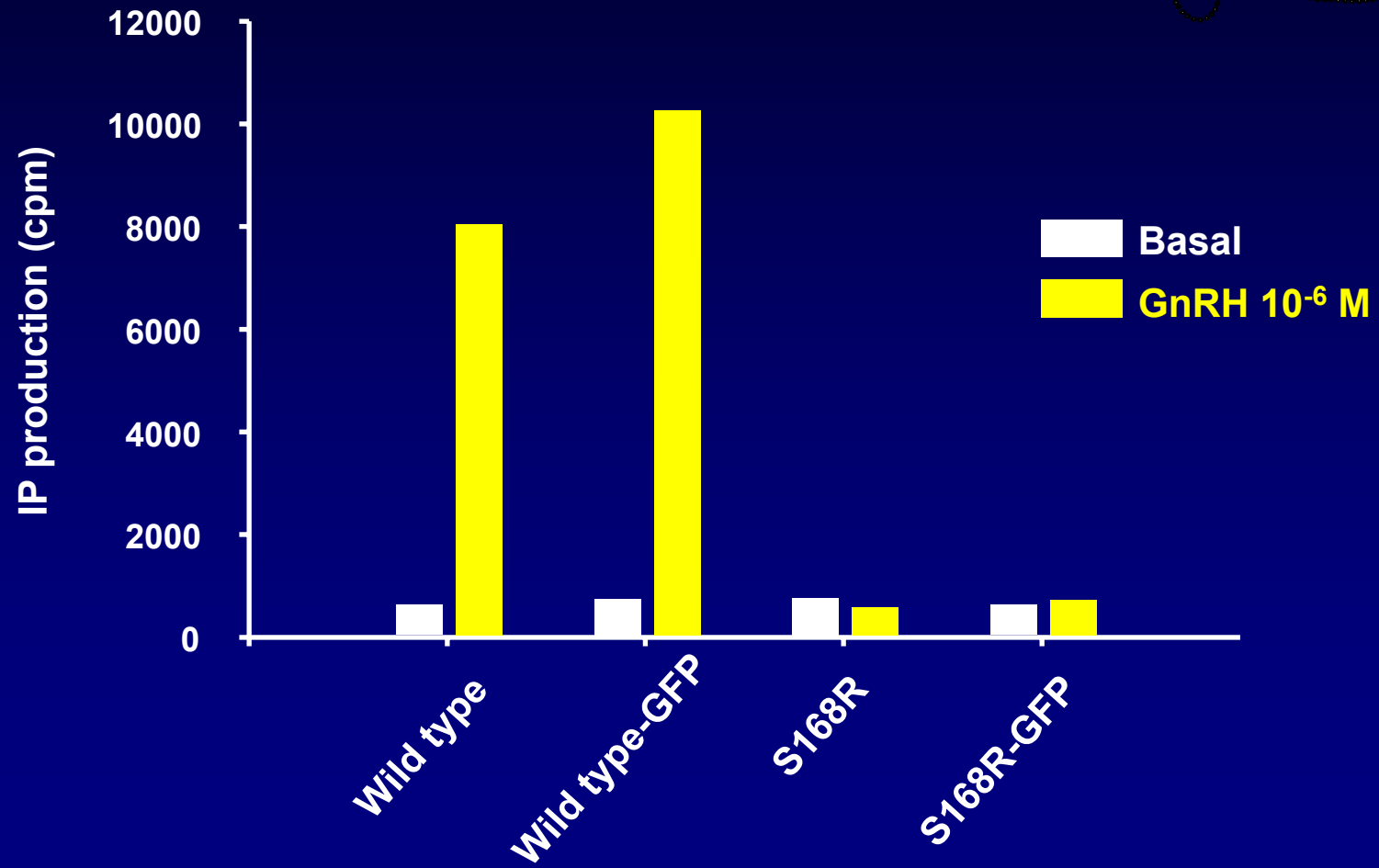


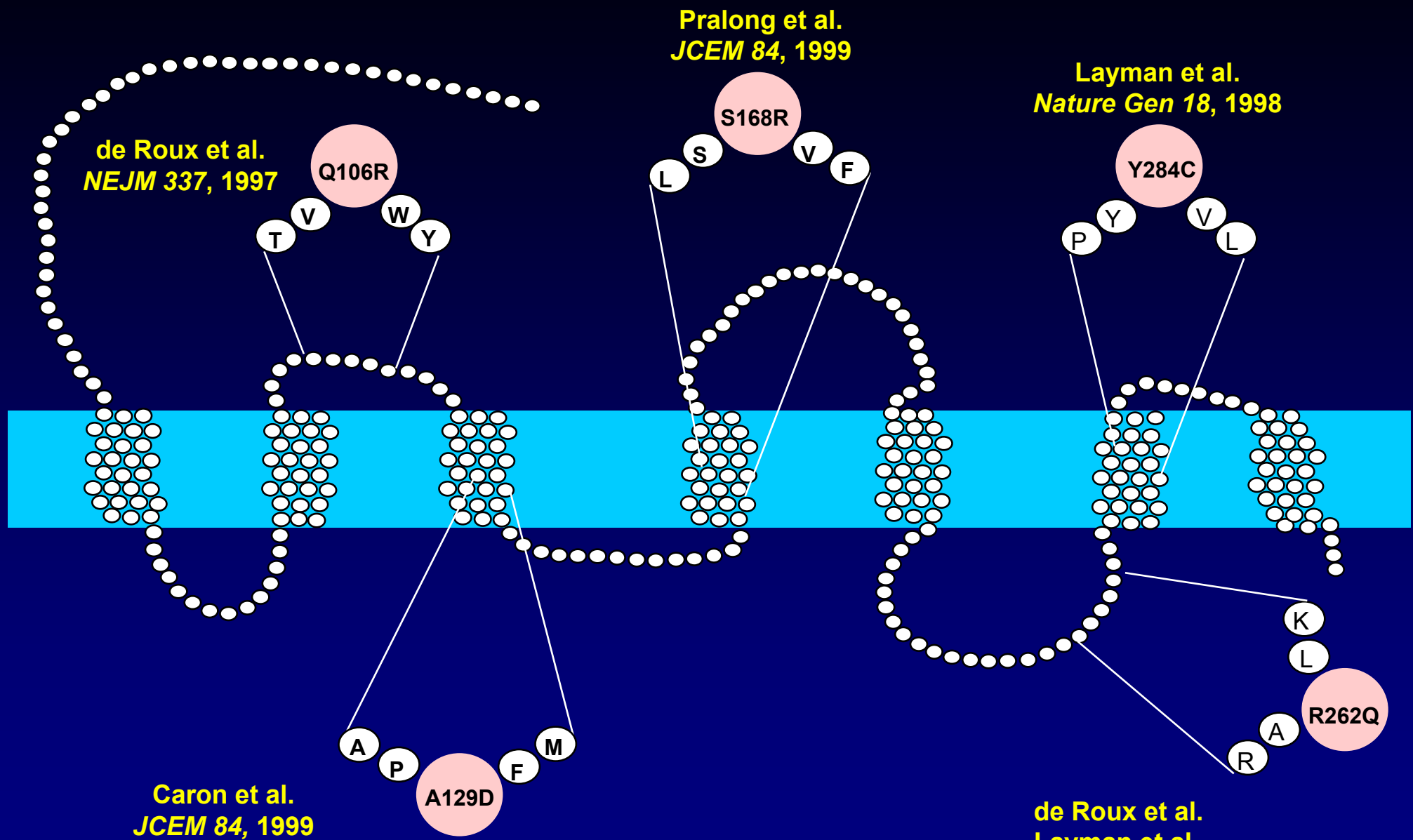
Wild type-GFP

S168R-GFP

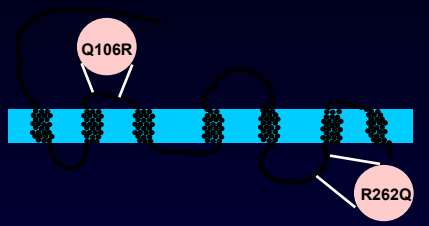
The GnRH Receptor-GFP Fusion Protein Is Functionally Active





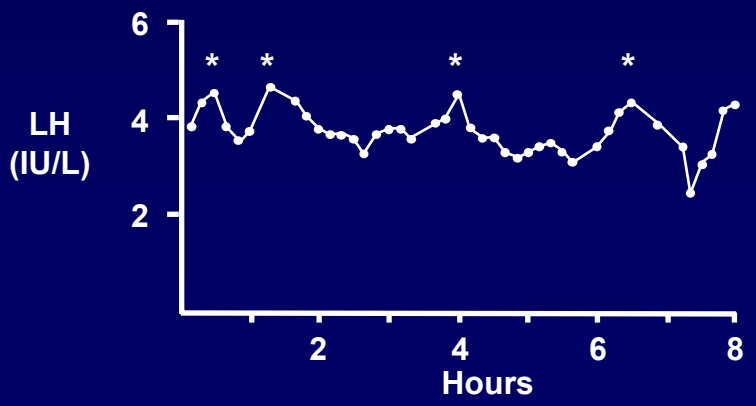

de Roux et al.
NEJM 337, 1997
Q106R
T
V
W
Y
Pralong et al.
JCEM 84, 1999
S168R
L
S
V
F
Layman et al.
Nature Gen 18, 1998
Y284C
P
Y
V
L
A
P
A129D
F
M
Caron et al.
JCEM 84, 1999
K
L
R262Q
A
R
de Roux et al.
Layman et al.
Caron et al.

Phenotype of compound heterozygote patients



de Roux et al.
NEJM 337, 1997

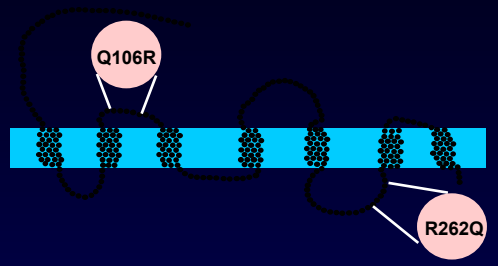
- Male : scrotal testis (8 mL)
- LH : 4.0 IU/L, FSH : 5.9 IU/L
- Puberty at age 16 years
- Basal LH secretion displays blunted pulsatility:



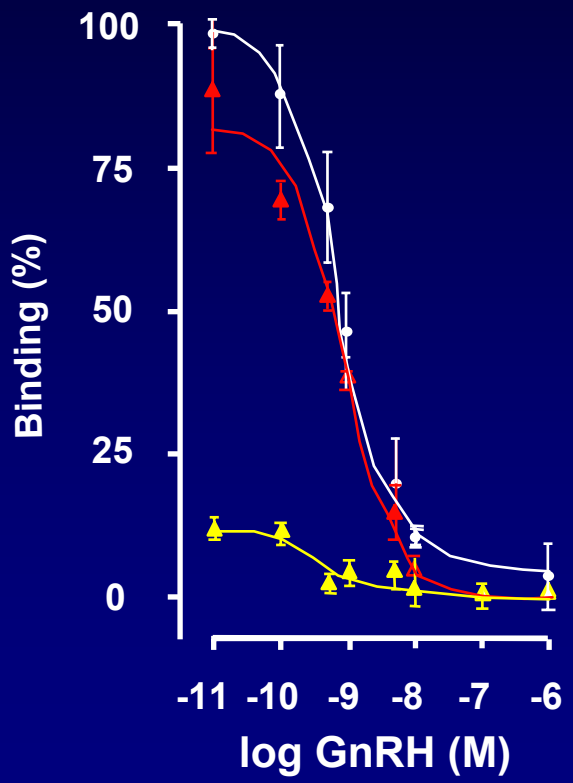
- Normal response to GnRH (100 μg)
- Female : primary amenorrhea and infertility
- Telarche at age 14 years
- LH : 5.0 IU/L, FSH : 5.2 IU/L, E2 128 pmol/L

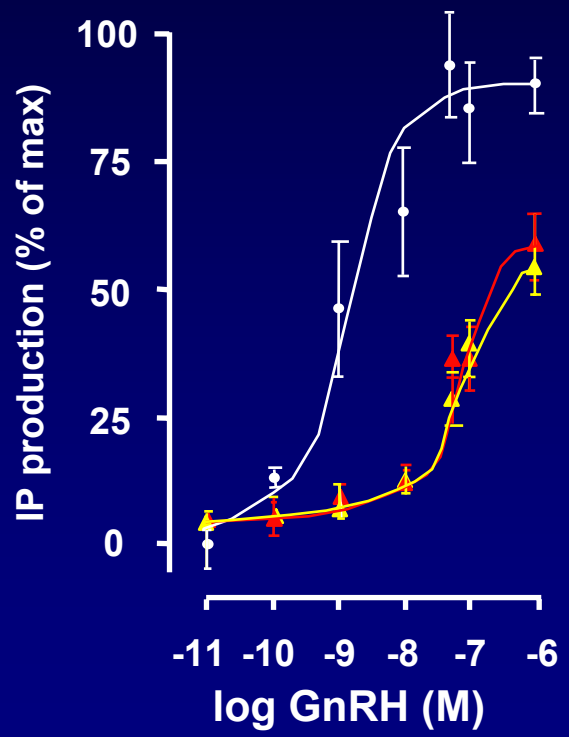
Conclusion : incomplete hypogonadotropic hypogonadism

Functional characterization of compound heterozygote mutations



de Roux et al.
NEJM 337, 1997





Q106R mutation

R262Q mutation

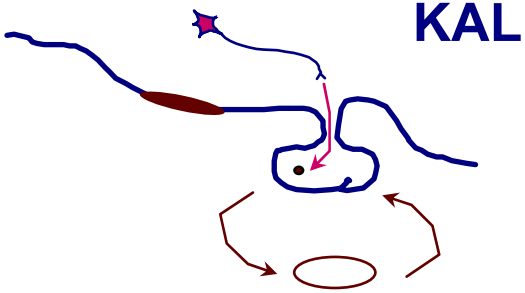
Conclusions

- 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
- This phenotypic heterogeneity seems to correlate well with the degree of functional impairement of the receptor

Conclusions

Genotype/phenotype correlations performed on these patients provide invaluable information about structure-function relationships of the GnRH receptor

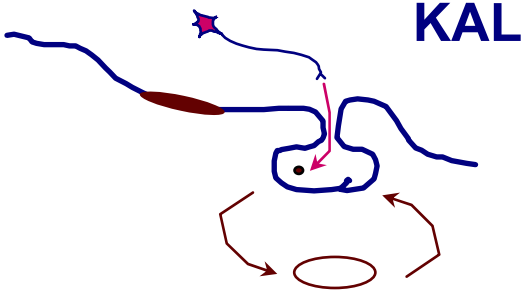
Kalmann's Syndrome



HH associated with anosmia

- X-linked syndrome
- due to mutations of KAL-1 (member of the superfamily of Neural Cell Adhesion Molecules)

Kalmann's Syndrome



The Candidate Gene for the X-Linked Kallmann Syndrome Encodes a Protein Related to Adhesion Molecules

Legouis et al., Cell 67, 1991

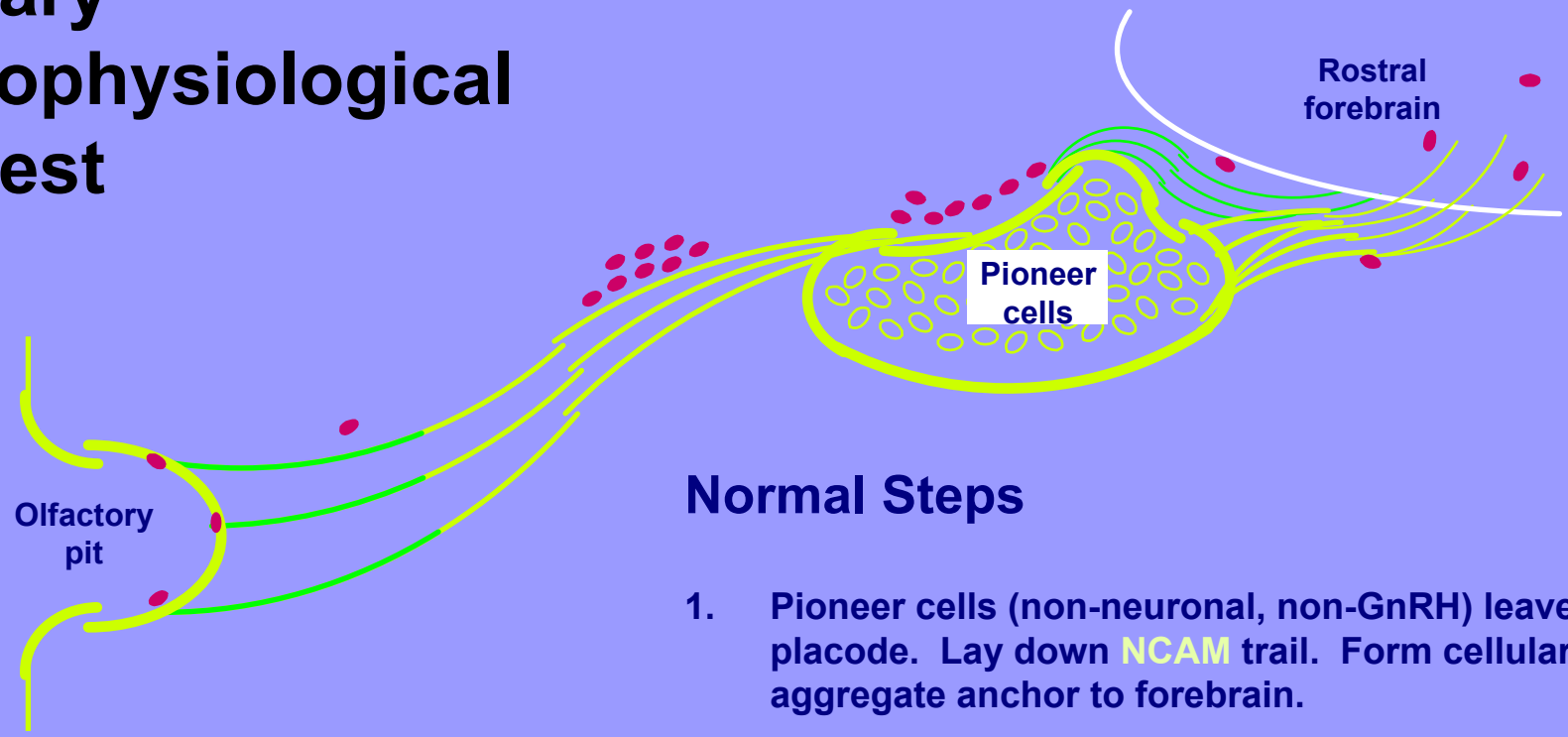
A Gene Deleted in Kallmann's Syndrome Shares Homology with Neural Cell Adhesion and Axonal Path-Finding Molecules

Franco et al., Nature 353, 1991

KAL-1

Schwanzel-Fukuda

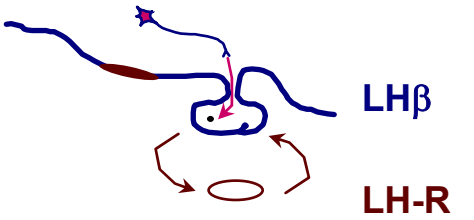
primary pathophysiological interest



Normal Steps

1. Pioneer cells (non-neuronal, non-GnRH) leave placode. Lay down NCAM trail. Form cellular aggregate anchor to forebrain.
2. Vomeronasal and terminal nerves follow trail.
3. GnRH cells follow nerves and trail.
4. Polysialated NCAM helps acceleration from placode and aggregate.

LH Deficiency - Males



LHβ

One single case described

Bio-inactive LH

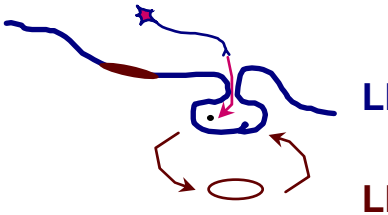
- **Phenotype:**
  - normal male
  - delayed puberty
  - response to hCG: normal virilization, **but not fertility**
- **Male heterozygotes: 3/4 infertile**

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

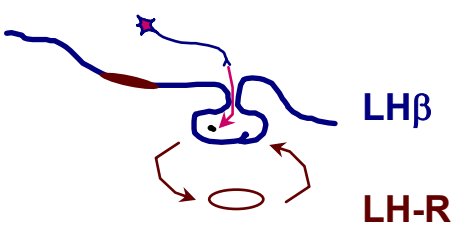
LH-R

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

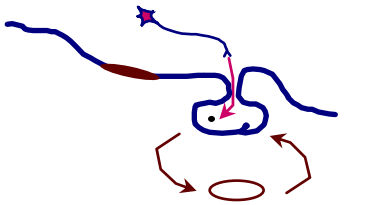
Role of the LH/LH-R System

- **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



FSH Deficiency - Males



FSHβ

Two cases described

- **Phenotype:**
  1) delayed puberty, low testosterone and absent spermatogenesis

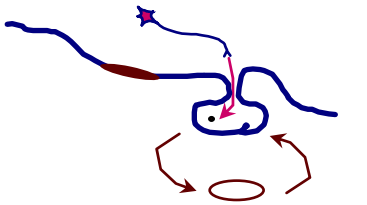
  2) normal puberty and virilization, spermatogenic arest

FSH-R

Finnish study

- normal virilization
- decreased testicular volume
- variable suppression of spermatogenesis

FSH Deficiency - Females



FSHβ

Three cases described

- **Phenotype:**
  - delayed puberty
  - primary amenorrhea
  - normal response to FSH with achivement of fertility

FSH-R

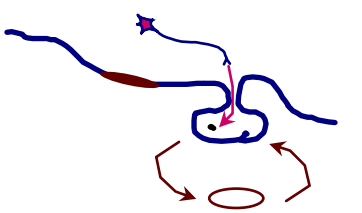
Finnish study

- **Phenotype:**
  - primary amenorrhea
  - ovarian dysgenesis with normal karyotype

Role of the FSH/FSH-R System

- **Important for estrogen production, follicular maturation and fertility**

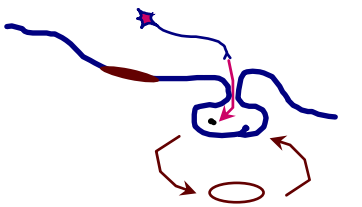
- **Role of FSH in spermatogenesis remains unclear:**
  - **variable spermatogenesis in FSH-R mutations**
  - **absent spermatogenesis in FSHβ mutations**



FSHβ

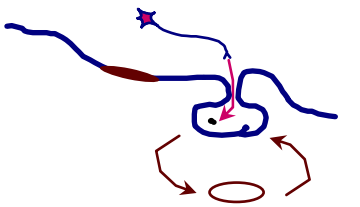
FSH–R

Conclusions



The study of inactivating mutations of several genes throughout the gonadotrope axis has provided invaluable insights into the physiology of reproduction in humans

Conclusions



These mutations offer a model of single-gene diseases, allowing genotype/phenotype correlations to be drawn and structure-function relationships to be inferred

Acknowledgments

Division of Endocrinology Lausanne

Einar Castillo
Luc Portmann
Fulgencio Gomez
Rolf Gaillard

Division of Pediatric Endocrinology, Geneva

Michel Aubert

Institute of Pharmacology and Toxicology, Lausanne

Liliane Abuin
Susanna Cotecchia