ΕΘΝΙΚΟ ΚΑΙ ΚΑΠΟΔΙΣΤΡΙΑΚΟ

ΠΑΝΕΠΙΣΤΗΜΙΟ ΑΘΗΝΩΝ

ΙΑΤΡΙΚΗ ΣΧΟΛΗ

Α’ Παιδιατρική Κλινική Πανεπιστημίου Αθηνών

Νοσοκομείο Παίδων «Η Αγία Σοφία»

Καθ. Γ. Χρούσος

ΔΙΔΑΚΤΟΡΙΚΗ ΔΙΑΤΡΙΒΗ

Κλινική και Μοριακή μελέτη παιδιών με διαταραχές ενήβωσης
(Έλεγχος σχετικών γονιδιακών επιτόπων – γονίδιο GPR54)

ΣΟΦΙΑ Κ. ΛΕΚΑ - ΕΜΙΡΗ

Αθήνα 2014

- **Ημερομηνία αίτησης για ορισμό τριμελούς επιτροπής: 1-12-2008**
- **Ημερομηνία ορισμού τριμελούς επιτροπής: 24-2-2009**
- **Ημερομηνία καθορισμού θέματος διδακτορικής διατριβής: 13-4-2009**

Τριμελής επιτροπή επίβλεψης της Διδακτορικής Διατριβής:

- Αν. Καθηγήτρια Χριστίνα KANAKA-GANTENBEIN (Επιβλέπων)
- Καθηγητής Γεώργιος ΧΡΟΥΣΟΣ
- Επ. Καθηγητής Εμμανουήλ ΖΟΥΜΑΚΗΣ

Επταμελής επιτροπή παρουσίασης της Διδακτορικής Διατριβής:

- Αν. Καθηγήτρια Χριστίνα KANAKA-GANTENBEIN (Επιβλέπων)
- Καθηγητής Γεώργιος ΧΡΟΥΣΟΣ
- Επ. Καθηγητής Εμμανουήλ ΖΟΥΜΑΚΗΣ
- Αν. Καθηγητής Nicolas de ROUX, INSERM U676, Paris
- Αν. Καθηγητής Αναστάσιος ΠΑΠΑΔΗΜΗΤΡΙΟΥ
- Αν. Καθηγητής Νεοκλής ΓΕΩΡΓΟΠΟΥΛΟΣ
- Αν. Καθηγητής Γεώργιος ΜΑΣΤΟΡΑΚΟΣ

NATIONAL UNIVERSITY OF ATHENS

MEDICAL SCHOOL

1st Department of Peadiatrics

The “Aghia Sofia Children’s Hospital”

Prof. G. Chrousos

PhD Thesis

Molecular and clinical investigation of children with disorders of puberty (GPR54 gene and relevant genes)

Sofia K. LEKA - EMIRI

Athens 2014

Scientific committee:

Associate Professor Christina KANAKA-GANTENBEIN (Supervisor)

Professor George CHROUSOS

Assistant Professor Emmanouil ZOUMAKIS

Associate Professor Nicolas de ROUX, INSERM 676, Paris, FRANCE

Associate Professor Anastasios PAPADIMITRIOU

Associate Professor Neoklis GEORGOPOULOS

Associate Professor George MASTORAKOS

Date of presentation: 22-5-2014

Acknowledgements

Professor George CHROUSOS

Chairman of First Department of Pediatrics and Division of Pediatric Endocrinology and Diabetology, "Aghia Sofia" Children's Hospital, University of Athens, Greece

For having accepted me as a young resident in Pediatrics seven years ago and having inspired me in the field of Pediatric Endocrinology

Please find here the expression of my profound respect

Associate Professor Christina KANAKA-GANTENBEIN

Division of Pediatric Endocrinology and Diabetology, First Department of Pediatrics,"Aghia Sofia" Children's Hospital, University of Athens, Greece

For her enthusiastic guidance and trust in my research fields.

For her precious contribution in the finalization of this work.

Please find here the expression of my warmest gratitude

Associate Professor Nicolas De ROUX

Inserm U676 « Equipe Avenir : Génétique et Physiologie de l'Initiation de la puberté », Robert Debré Hospital and University of Paris 7-Denis Diderot, Paris, France

For having accepted me in his laboratory and contributed in unraveling the molecular biological mechanisms of the physiology of puberty

Please find here the expression of my honest reconnaissance

Professor Juliane LEGER

Service d'Endocrinologie et Diabétologie Pédiatrique H. Robert Debré,
University of Paris 7- Denis Diderot, Paris, France

For having accepted me in her department as an ESPE Clinical Fellow and for her kind eagerness that helped me advancing in my research

Please find here the expression of my profound reconnaissance

IOANNIS…

Zacharias and Konstantinos for every one day of my life…

CONTENTS

page

Acknowledgements 5

List of Contents 6

CURRICULUM VITAE 9

HIPPOCRATES OATH 23

Abbreviations 25

I. GENERAL PART

1. NORMAL PUBERTY 26

1.1 Physiology 26

1.1. A. Overview of Kisspeptin signaling 30

1.1. A. 1 Biochemistry 31

1.1. A. 2 Molecular physiology of Kiss1 neurons 34

1.1. A. 3 Comparative physiology 39

1.1. A. 4 Role of kisspeptin in the onset of puberty 47

1.1. A. 4.aThe kisspeptin system is necessary for normal puberty initiation 47

1.1. A. 4.b Changes in the neural Kiss1 system during puberty 49

1.1. A. 4.c Sexual dimorphism of kisspeptin expression 51

1.1. B. Overview of Neurokinin B (NKB) signaling 55

1.1. B. 1 Molecular biology of NKB 55

1.1. B. 2 Anatomic studies of (NKB) and Neurokinin B receptor (NKR3) 56

1.1. B. 3 Estrogen modulation of NKB gene expression 57

1.1. B. 4 Sexual dimorphism of NKB neurons 58

1.1.C. An interconnected network of NKB/kisspeptin/dynorphin neurons 59

1.1.C. 1 Evidence for NKB/kisspeptin/dynorphin neurons in the arcuate nucleus for the modulation of pulsatile GnRH secretion 61

1.1.C. 2 Pharmacological studies 65

1. 2 Clinical manifestations and age references of puberty 67
1. 3 Possible mechanisms of variation in timing of puberty 70
1.3. a Genetic Factors..72
1.3. b Environmental factors..81
1.3. b1 Intrauterine conditions..81
1.3. b2 Nutrition..82
1.3. b3 Stress..75
1.3. b4 Exposure to endocrine-disrupting chemicals (EDCs) 85

2. DISORDERS OF PUBERTAL ONSET 86
2. A. Delayed puberty...86
2. A.1 Age limits 86
2. A.2 Causes and diagnostic work up 86
2. A.3 Consequences...101
2 .B. Precocious puberty 102
2. B.1 Age limits 102
2. B.2 Causes and diagnostic work up 102
2. B.3 Consequences...114

3. SEXUAL DIMORPHISM OF PUBERTAL ONSET DEVIATIONS 115

II. SPECIFIC PART 118
1. RESEARCH PROJECT
TITLE: Molecular and clinical investigation of the GPR54 and TACR3 gene mutations and polymorphisms in Idiopathic Central Precocious Puberty
1. A. Background 118
1. B. Objectives 118
1. C. Patients and Methods..118
1. D. Results...124
1. E. Discussion 125
1. F. Conclusion and perspectives 144

III. REFERENCES 148
IV. ABSTRACT IN GREEK.. 200
V. ABSTRACT IN ENGLISH 201
VI. PUBLICATION OF FINDINGS
XI. APPENDIX

Σοφία Κ. ΛΕΚΑ - ΕΜΙΡΗ

ΠΑΙΔΙΑΤΡΟΣ

Εξειδικευθείσα στην Παιδιατρική Ενδοκρινολογία

Βιογραφικό Σημείωμα

Ημερομηνία Γέννησης: 25/05/1974

Τόπος Γέννησης: Αθήνα

Διεύθυνση Κατοικίας: Χίου 4, Χαλάνδρι, Τ.Κ. 15231

Τηλέφωνα: Οικίας 210 6897387 / 210 6773200 Κινητό 6934227386

FAX: 210 6773200 / 210 6897387

e-mail: sofialekaemiris@gmail.com

1) ΕΠΑΓΓΕΛΜΑΤΙΚΗ ΕΜΠΕΙΡΙΑ

01/2014 - **ΕΠΙΜΕΛΗΤΡΙΑ Β' ΠΑΙΔΙΑΤΡΙΚΗΣ ΕΝΔΟΚΡΙΝΟΛΟΓΙΑΣ**
Τμήμα Παιδιατρικής Ενδοκρινολογίας
Νοσοκομείο Παίδων «Π. & Α. ΚΥΡΙΑΚΟΥ», Αθήνα

12/2008 - 12/2010 **ΜΕΤΕΚΠΑΙΔΕΥΣΗ στην**
ΠΑΙΔΙΑΤΡΙΚΗ ΕΝΔΟΚΡΙΝΟΛΟΓΙΑ και ΔΙΑΒΗΤΟΛΟΓΙΑ
Τμήμα Παιδιατρικής Ενδοκρινολογίας και Διαβητολογίας
(Καθηγητής J-C CAREL) **(ESPE Clinical Fellowship)** και
Εθνικό Ίδρυμα Υγείας και Ιατρικής Έρευνας
(Institut National de la Santé et Recherche Médicale - INSERM)
Μονάδα έρευνας "Παιδικός διαβήτης και Ανάπτυξη"
(Έμμισθη ερευνητής)
(Διευθυντής: Pr. C. LEVY-MARCHAL)
Παιδιατρικό Πανεπιστημιακό Νοσοκομείο «Robert Debré»
Université Paris VII, Παρίσι, ΓΑΛΛΙΑ

6/2008 - 11/2008 **ΜΕΤΕΚΠΑΙΔΕΥΣΗ**
ΠΑΙΔΙΑΤΡΙΚΗ ΕΝΔΟΚΡΙΝΟΛΟΓΙΑ και ΔΙΑΒΗΤΟΛΟΓΙΑ
Τμήμα Παιδιατρικής Ενδοκρινολογίας, Διαβήτη και Μεταβολισμού
Α΄ Παιδιατρική Κλινική Πανεπιστημίου Αθηνών
(Καθηγητής: Γ. ΧΡΟΥΣΟΣ)
Νοσοκομείο Παίδων «Η Αγία Σοφία», Αθήνα

5/2005 - 5/2008 **ΕΙΔΙΚΕΥΟΜΕΝΗ ΙΑΤΡΟΣ ΠΑΙΔΙΑΤΡΙΚΗΣ**
Α΄ Παιδιατρική Κλινική Πανεπιστημίου Αθηνών
(Καθηγητής: Γ. ΧΡΟΥΣΟΣ)
Νοσοκομείο Παίδων «Η Αγία Σοφία», Αθήνα

6/2004 - 5/2005 **ΕΠΙΣΤΗΜΟΝΙΚΗ ΣΥΝΕΡΓΑΤΗΣ**
Τμήμα Παιδιατρικής Ενδοκρινολογίας και Διαβητολογίας
(Διευθυντής: Χ. ΧΑΤΖΗΑΘΑΝΑΣΙΟΥ)
Νοσοκομείο Παίδων «Π. & Α. Κυριακού», Αθήνα

11/2001 - 10/2002 **ΕΙΔΙΚΕΥΟΜΕΝΗ ΙΑΤΡΟΣ ΠΑΙΔΙΑΤΡΙΚΗΣ**
Τμήμα Παιδιατρικής Ενδοκρινολογίας και Διαβητολογίας
(Καθηγητής: P. CZERNICHOW)
Παιδιατρικό Πανεπιστημιακό Νοσοκομείο «Robert Debré»,
Université Paris VII, Παρίσι, ΓΑΛΛΙΑ

5/2001 - 10/2001 **ΙΑΤΡΟΣ (πλήρους έμμισθης απασχόλησης)**
Τμήμα Ενδοκρινολογίας, Διαβητολογίας και Αναπαραγωγής
(Καθηγητής: P. FENICHEL)
Πανεπιστημιακό Νοσοκομείο «L`Archet I»,
Université de Nice, ΓΑΛΛΙΑ.

9/1999 - 12/2000 **Υποχρεωτική Υπηρεσία Υπαίθρου (Αγροτικό),**
Παιδιατρικό Ιατρείο (βοηθός Επιμελητή Α'- Χ. ΣΑΒΒΑΚΗ)
Κέντρο Υγείας Περάματος, Ρέθυμνο, ΚΡΗΤΗ.

2) ΣΠΟΥΔΕΣ - ΔΙΠΛΩΜΑΤΑ

Ιούλιος 2010

Τίτλος Εξειδίκευσης στην

ΠΑΙΔΙΑΤΡΙΚΗ ΕΝΔΟΚΡΙΝΟΛΟΓΙΑ ΚΑΙ ΔΙΑΒΗΤΟΛΟΓΙΑ

Τμήμα Παιδιατρικής Ενδοκρινολογίας και Διαβητολογίας

(Καθ. J-C CAREL),

Παιδιατρικό Πανεπιστημιακό Νοσοκομείο AP-HP,

«Robert Debré», Παρίσι, ΓΑΛΛΙΑ

Φεβρουάριος 2009-

Υποψήφια ΔΙΔΑΚΤΟΡΑΣ

στον τομέα **Παιδιατρικής Ενδοκρινολογίας και Διαβητολογίας**

Α' Παιδιατρική Κλινική Πανεπιστημίου Αθηνών

Νοσοκομείο Παίδων «Η Αγία Σοφία», Αθήνα

Τίτλος Διατριβής: «***Κλινική και Μοριακή μελέτη παιδιών με διαταραχές ενήβωσης (έλεγχος σχετικών γονιδιακών επιτόπων - γονίδιο GPR54)»***

Τριμελής Επιτροπή: Αν. Καθ. Χρ. ΚΑΝΑΚΑ-GANTENBEIN (Επιβλέπουσα)

Επ. Καθ. Εμ. ΖΟΥΜΑΚΗΣ και

Καθ. Γ. ΧΡΟΥΣΟΣ

Ιούλιος 2008

Τίτλος Ιατρικής Ειδικότητας στην ΠΑΙΔΙΑΤΡΙΚΗ (1η Επιτυχούσα)

Α' Παιδιατρική Κλινική Πανεπιστημίου Αθηνών

Νοσοκομείο Παίδων «Η Αγία Σοφία»

(Καθηγητής – Διευθυντής: Γ. ΧΡΟΥΣΟΣ)

Νοσοκομείο Παίδων «Η Αγία Σοφία», Αθήνα

12/2003-1/2006

Μεταπτυχιακό Πρόγραμμα Σπουδών

«Κλινική και Εργαστηριακή Ιατρική – Γενετική» (Άριστα)

Τμήμα Ιατρικής Γενετικής, Ιατρική Σχολή,

Εθνικό και Καποδιστριακό Πανεπιστήμιο ΑΘΗΝΩΝ

Τίτλος: *«Μελέτη με μοριακές τεχνικές του γονιδίου SHOX σε παιδιά με ιδιοπαθή βραχυσωμία»*

Τριμελής Επιτροπή: Σ. ΚΙΤΣΙΟΥ-ΤΖΕΛΗ (Αν. Καθηγήτρια Πανεπιστημίου Αθηνών),

Α. ΚΑΛΠΙΝΗ-ΜΑΥΡΟΥ (Αν. Καθηγήτρια Πανεπιστημίου Αθηνών) και

Ε. ΚΑΝΑΒΑΚΗΣ (Καθηγητής Πανεπιστημίου Αθηνών)

Ιούλιος 1999 **ΠΤΥΧΙΟ ΙΑΤΡΙΚΗΣ (Λίαν Καλώς)**

Τμήμα ΙΑΤΡΙΚΗΣ, Εθνικό και Καποδιστριακό Πανεπιστήμιο ΑΘΗΝΩΝ

Υποτροφία: Ίδρυμα Παπαδάκης (1994-1999)

Ιούνιος 1993 **ΑΠΟΛΥΤΗΡΙΟ Λυκείου (Άριστα) - Πανελλήνιες Εισαγωγικές Εξετάσεις**

Λύκειο Ξυλοκάστρου Νομού Κορινθίας

3) ΕΡΕΥΝΗΤΙΚΗ ΕΜΠΕΙΡΙΑ

(Διεθνή και Εθνικά Ερευνητικά Προγράμματα)

- **2010-2011** Φορέας: **Εθνικό Σύστημα Υγείας και Έρευνας της Γαλλίας (Institut National de la Santé et Recherche Médicale - INSERM)**
  Μονάδα έρευνας στην «Γενετική και Φυσιολογία Έναρξης της Εφηβείας»
  Χρηματοδότηση: **Εθνικό Κέντρο Αποκωδικοποίησης DNA Centre National de Sequencage- GIS- Institut des maladies rares), Παρίσι, Γαλλία**
  Επιστημονικός Υπεύθυνος: Καθηγητής **Nicolas DeROUX**
  Τίτλος: *«Ανάλυση δύο υποψήφιων περιοχών σε οικογενή νευροενδοκρινική νόσο»*

- **2009-2010** Φορέας - Χρηματοδότηση:
  Εθνικό Σύστημα Υγείας και Έρευνας της Γαλλίας (Institut National de la Santé et Recherche Médicale-INSERM)
  Μονάδα έρευνας στην «Ανάπτυξη και τον Παιδιατρικό Διαβήτη»
  Επιστημονικός Υπεύθυνος: Dr **Claire LEVY-MARCHAL**
  Τίτλος:
  1) «Θεραπευτικό πρωτόκολλο με ανθρώπειο ανασυνδυασμένη λεπτίνη (human recombinant Leptin –rmetHuLeptin) σε παιδιά με συγγενή λιποδυστροφικό διαβήτη»
  2) «Μεταβολικές παράμετροι σε παιδιά με ιστορικό ενδομήτριας καθυστέρησης της ανάπτυξης»

- **2008-2009** Φορέας: **Εθνικό και Καποδιστριακό Πανεπιστήμιο Αθηνών**
  Χρηματοδότηση: **Πανελλήνια Ένωση Ενδοκρινολογίας**
  Επιστημονικός Υπεύθυνος: Αν. Καθ. **Χριστίνα KANAKA-GANTENBEIN**
  Τίτλος: *«Κλινική και Μοριακή μελέτη του γονιδίου GPR54 σε παιδιά με ιδιοπαθή κεντρική πρώιμη ή καθυστερημένη ήβη».*

4) <u>ΕΙΔΙΚΗ ΕΚΠΑΙΔΕΥΣΗ - ΣΕΜΙΝΑΡΙΑ</u>

<u>Α) ΣΤΑΤΙΣΤΙΚΗ</u>

- Μάιος **2009** (1 εβδομάδα) Σεμινάριο στην Στατιστική Επεξεργασία Βιο-Ιατρικών δεδομένων

(**<u>SAS statistical logistics),</u> INSERM IR 6,** Ville – Juif, France

<u>Β) ΠΑΙΔΙΑΤΡΙΚΗ</u>

- Φεβρουάριος **2007** (1 εβδομάδα), ***Σεμινάριο Εξειδικευμένης Υποστήριξης της Ζωής Παιδιών και Βρεφών*** **(Advanced Pediatric Life Support - APLS) Hellas,** (Δυευθύνων: Κ. ΒΕΛΑΟΡΑΣ)

<u>Γ) ΠΑΙΔΙΑΤΡΙΚΗ ΕΝΔΟΚΡΙΝΟΛΟΓΙΑ</u>

1. Μάρτιος **2014** (2 ημέρες) **Εντατική Εκπαίδευση στην <u>Παιδιατρική Ενδοκρινολογία και Αναπαραγωγή,</u>** Ελληνική Ενδοκρινολογική Εταιρεία, Μαγγίνειο Αμφιθέατρο, Αρεταίειο Νοσοκ. Αθήνα

2. Μάιος **2012** (1 εβδομαδα) **European Society for Pediatric Endocrinology Science School** and **NICHE conference 2012,** Hersonissos – Heraklion, Crete, May 16-20, 2012, GREECE

3. Μάρτιος **2010** (1 εβδομάδα) **Εντατική Εκπαίδευση στην <u>Παιδιατρική Ενδοκρινολογία,</u>**
   Diplôme Interuniversitaire d'Endocrinologie et Diabétologie Pédiatriques,
   Hôpital d'enfants Armand Trousseau, Paris, FRANCE

4. Ιανουάριος **2010** (2 ημέρες) **Σεμινάριο στην <u>Παιδιατρική Ενδοκρινολογία και Διαβητολογία,</u>**
   Necker - Hôpital des Enfants Malades (Prof. R. RAPPAPORT), Paris, FRANCE

5. Νοέμβριος **2009** (1 εβδομάδα) **International Society for Pediatric and Adolescent Diabetes**
   <u>(ISPAD) Science school for Physicians</u>, Cambridge, UK

6. Σεπτέμβριος **2009** (3 ημέρες) **Lawson & Wilkins Pediatric Endocrinology Society/European Society of Pediatric endocrinology Fellows Workshop on Growth,** New York, USA

7. Μάρτιος **2009** (1 εβδομάδα) **Εντατική Εκπαίδευση στην Παιδιατρική Ενδοκρινολογία,**
Diplôme Interuniversitaire d'Endocrinologie et Diabétologie Pédiatriques,
Hôpital d'enfants Armand Trousseau, Paris, FRANCE

8. Ιανουάριος **2009** (2 ημέρες) **Σεμινάριο στην Παιδιατρική Ενδοκρινολογία και Διαβητολογία,**
Necker - Hôpital des Enfants Malades (Prof. R. RAPPAPORT), Paris, FRANCE

9. Σεπτέμβριος **2008** (1 εβδομάδα), **22nd European Society for Pediatric Endocrinology**
(ESPE) Summer School, Istanbul, Turkey

10. Φεβρουάριος **2008** (2 ημέρες) **Εντατική Εκπαίδευση στην Παιδιατρική Ενδοκρινολογία**
Ελληνική Ενδοκρινολογική Εταιρεία, ΕΚΕΦΕ «Δημόκριτος», Αθήνα

11. Μάιος **2007** (1 μήνας), Visiting observer **Division of Pediatric Endocrinology**
(Professor A. Moran), University of Minnesota Medical School, Minneapolis,
Minnesota, USA

12. Ιανουάριος **2007** (2 ημέρες), **Σεμινάριο στην Παιδιατρική Ενδοκρινολογία και Διαβητολογία,**
Necker - Hôpital des Enfants Malades (Prof. R. RAPPAPORT), Paris, FRANCE

13. Νοέμβριος **2005** (1 εβδομάδα), Visiting Observer **Division of Pediatric Endocrinology**
(Prof. J. Majzoub), Boston Children's Hospital, Harvard Medical School, Boston, USA

14. **2003 – 2013 Ετήσια Προγράμματα Ημερίδων στην Παιδιατρική Ενδοκρινολογία,**
Ελληνική Εταιρεία Παιδικής και Εφηβικής Ενδοκρινολογίας, Αθήνα, ΕΛΛΑΔΑ

15. Μάρτιος **2003** (1 εβδομάδα), **Σεμινάριο στην Παιδιατρική Ενδοκρινολογία και Διαβητολογία**
Universite de Bordeaux, FRANCE

Δ) ΚΥΤΤΑΡΟΓΕΝΕΤΙΚΗ και ΜΟΡΙΑΚΗ ΒΙΟΛΟΓΙΑ

2/2010 **Εθνικό Ίδρυμα Υγείας και Ιατρικής Έρευνας**
(Institut National de la Santé et Recherche Médicale - INSERM)
Hôpital « LE KREMLIN BICETRE »
(Επιστημονικός Υπεύθυνος: Directeur de recherche B. HEMAR)
Θέμα: « Analyse Bioinformatique des séquences moléculaires»

Universite Paris XI, Παρίσι, ΓΑΛΛΙΑ

1/2009 - 3/2009 **Εθνικό Ίδρυμα Υγείας και Ιατρικής Έρευνας**
(Institut National de la Santé et Recherche Médicale - INSERM)
Μονάδα έρευνας **«Γενετική και Φυσιολογία της έναρξης της ήβης»**
(Επιστημονικός Υπεύθυνος: Καθηγητής N. DEROUX)
Παιδιατρικό Πανεπιστημιακό Νοσοκομείο «Robert Debré»,
Université Paris VII, Παρίσι, ΓΑΛΛΙΑ
Θέμα: «Μοριακή ανάλυση δύο υποψήφιων περιοχών σε οικογενή νευροενδοκρινική νόσο»
Υποτροφία: **European Society for Pediatric Endocrinology (ESPE) Visiting Scholarship**

4/2003 έως 5/2005 **Τμήμα Ιατρικής Γενετικής, Πανεπιστημίου Αθηνών**
(Καθηγητής - Διευθυντής Ε. ΚΑΝΑΒΑΚΗΣ),
Χωρέμειο Ερευνητικό Εργαστήριο, Νοσοκομείο Παίδων «Αγία Σοφία», ΑΘΗΝΑ
Θέμα: «Μελέτη με μοριακές τεχνικές του γονιδίου SHOX σε παιδιά με βραχυσωμία»,

1/2001 έως 4/2001 **Εργαστήριο Φυσιολογίας** (Καθηγήτρια D. CRENESSE),
Θέμα: "Ισχαιμία και επαναιμάτωση του ήπατος. Μελέτη της απόπτωσης in vivo και in vitro",
Τμήμα Ιατρικής, Université de Nice, ΓΑΛΛΙΑ ,
Υποτροφία: **Εμπειρίκειο Ίδρυμα**

5) Διεθνείς και Εθνικές Διακρίσεις - Υποτροφίες

6/ 2012 *Μέλος της*

- ***Ελληνικής Εταιρείας Παιδικής και Εφηβικής Ενδοκρινολογίας (ΕΕΠΕΕ)***
- ***Ευρωπαϊκής Εταιρείας Παιδικής Ενδοκρινολογίας (ESPE)***

1/2010 έως 10/2010 **European Society for Pediatric Endocrinology (ESPE)**
Clinical Fellowship
Τμήμα Παιδιατρικής Ενδοκρινολογίας και Διαβητολογίας
(Καθηγητής J-C CAREL)
Παιδιατρικό Πανεπιστημιακό Νοσοκομείο «Robert Debré»
Université Paris VII, Παρίσι, ΓΑΛΛΙΑ

1/2009 έως 3/2009 **European Society for Pediatric Endocrinology (ESPE)**
Visiting Scholarship
Εθνικό Ίδρυμα Υγείας και Ιατρικής Έρευνας
(Institut National de la Santé et Recherche Médicale - INSERM)
Μονάδα έρευνας «Γενετική και Φυσιολογία της έναρξης της ήβης»
(Επιστημονικός Υπεύθυνος: Καθηγητής N. DEROUX)
Παιδιατρικό Πανεπιστημιακό Νοσοκομείο «Robert Debré»,
Université Paris VII, Παρίσι, ΓΑΛΛΙΑ

Ιούλιος 2006 ***Κριτής:*** **Journal of Pediatric Endocrinology and Metabolism**

1/2000 έως 6/2000 **Υποτροφία Εμπειρικείου Ιδρύματος**
(Μεταπτυχιακές Σπουδές στην Γαλλία)

1994 έως 1999 **Υποτροφία Παπαδάκη**
(κατόπιν διαγωνισμού κατά την φοίτηση στην Ιατρική Σχολή Αθηνών)

6) Δημοσιεύσεις - Publications

Περιοδικά - Journals

- Leka-Emiri S, Louizou E, Kambouris M, Chrousos G, De Roux N, Kanaka-Gantenbein C. Absence of GPR54 and TACR3 Mutations in Sporadic Cases of Idiopathic Central Precocious Puberty. Horm Res Paediatr. 2014 Jan 10. [Epub ahead of print]

- Spoulou VI, Tzanakaki G, Lekka S, Chouliaras G, Ladis VA, Theodoridou MC. “Natural and vaccine-induced immunity to Neisseria meningitidis serogroup C in asplenic patients with β-thalassemia”. Vaccine. 2011 Jun 15;29(27):4435-8.

- Leka S, Kousta E, Anyfandakis K, Dolianiti M, Vakaki M, Linos D, Chrousos GP, Papathanassiou A. “Primary Pigmented Nodular Adrenocortical Disease (PPNAD): A case report in a 7-year-old girl” The J Pediatr Endocrinol Metab. 2011;24(3-4):197-202.

- Beltrand J, Lahlou N, Le Carpentier T, Sebag Guy, Leka S, Polak M, Tubiana-Rufi N, Lacombe D, de Kerdanet M, Huet F, Robert J-J, Korpysz A, Chevenne D, Gressens P, Levy-Marchal Cl. “ Resistance to leptin replacement therapy in children with berardinelli-seip congenital lipodystrophy (bscl): an immunological origin” European Journal of Endocrinology 2010 Jun;162(6):1083-91.

- Leka SK, Kitsiou S, Mavrou A, Kanavakis E. “Short stature and dysmorphology associated with SHOX gene defects” HORMONES 5(2): 107-118, 2006

- Leka S. “Arguments and counter arguments concerning HBV vaccination”, ANNALES CLINICAE PAEDIATRICAE UNIVERSITATIS ATHENIENSIS; pp.357-359, Vol 53, Issue 4 Oct-Nov-Dec 2006

- Leka S, Kanaka-Gantenbein Ch, Frysira H. “Tricho-Rhino-Phalangeal syndrome: a case report, differential diagnosis and review of the literature” ANNALES CLINICAE PAEDIATRICAE UNIVERSITATIS ATHENIENSIS; pp.148-154, Vol 52, Issue 2, Apr-May-June 2005

Διεθνή Συνέδρια – International Conferences

1. Léger J, Huijbregts L, Tata B, Jacquier S, Genin E, Leka S, Durr A, Nardelli J, Carel J-C, de Roux N. “Rabconnectin-3α is a synaptic protein that controls pubertal onset and reproduction“9th Joint Meeting of Paediatric Endocrinology, 2013, Milan, Italy September 19-22, Milan, Italy, PL4-4 HORMONE RESEARCH Pediatrics

2. Leka-Emiri S, Louizou E, Kambouris M, Chrousos G, De ROYX N, KANAKA-GANTENBEIN Ch. “Absence of GPR54 (KISS1R) and TACR3 gene mutations in a cohort of girls with idiopathic central precocious puberty (ICPP) of Hellenic origin”, 39th Greek Conference in Endocrinology and Metabolism, Athens, 4-7 April, 2012

3. Leka-Emiri S, Léger J, HH french study group, Carel J-C, de Roux N. “Gender and phenotype differences with respect to genetic defect in 83 patients with Congenital Hypogonadotropic Hypogonadism (HH)” ESPE 2010, The European Society’s for Pediatric Endocrinology 49th Annual Meeting, September 22-25, Prague, Czech Republic, FC13-150, HORMONE RESEARCH Pediatrics 74(suppl 3): 45

4. Leka-Emiri S, Falucar Njuieyon F, Chevenne D, Deghmoun S, Claris O, Levy-Marchal C, Beltrand J. “Fetal growth pattern and post-natal fat mass distribution effects on adiponectin levels”, ESPE 2010, The European Society’s for Pediatric Endocrinology 49th Annual Meeting, September 22-25, Prague, Czech Republic, P2-d1-429, HORMONE RESEARCH; HORMONE RESEARCH Pediatrics 74(suppl 3): 131

5. Leka S, de Roux N, Genin E, Carel J-C, Leger J. “Hypoglycemia during childhood followed by insulin-deficient diabetes mellitus, hypogonadotrophic hypogonadism, central hypothyroidism, demyelinating neuropathy and alopecia: A Woodhouse-Sakati-like syndrome or a new neuroendocrine disease?”, 8th LWPES/ESPE 2009, 9-12 September, New York, USA, PO3-172, HORMONE RESEARCH; 399

6. Leka-Emiri SK, Kanaka-Gantenbein SK, Louizou E, Kambouris M , Chrousos G. “A/G synonymous SNP (dbSNP ID: rs10407968) on the GPR54 gene in children with abnormal puberty onset: A meaningful variant?” ENDO 2009, 91th Annual Meeting of Endocrine Society, Washington DC, 10-13 June, P1-334, pp 25

7. Leka S, Louizou E, Kanaka-Gantenbein Ch, Kambouris M, Chrousos G. “Molecular investigation of the GPR54 gene in children with idiopathic central precocious puberty and delayed puberty”, ESPE 2008, The European Society’s for Pediatric Endocrinology 47th Annual Meeting, September 20-23, Istanbul, Turkey p2-d3-647, HORMONE RESEARCH 2008; 70(suppl 1):193

8. Leka S, Petropoulou Th, Kanaka-Gantenbein Ch. “A rare cause of neonatal diabetes mellitus”, ESPE 2008, The European Society’s for Pediatric Endocrinology 47th Annual Meeting, September 20-23, Istanbul, Turkey, R64, HORMONE RESEARCH 2008; 70(suppl 1); 241

9. Skarpa V, Ioannidis D, Tertipi A, Lekka G, Papachileos P, Leka S, Petrou V, Georgoulas Th, Papastathi E, Vakaki M, Kaimara-Papathanasiou A. “ Effect of treatment with thyroxine on goiter size in euthyroid children with Hashimoto’s thyroiditis”, ESPE 2008, The European Society’s for Pediatric Endocrinology 47th Annual Meeting, September 20-23, Istanbul, Turkey, p2-d2-561, HORMONE RESEARCH 2008; 70(suppl 1):167

10. Leka S, Xaidara A, Platokouki H, Youroukos S. “Neonatal arterial ischaemic stroke and prothrombotic factors: a case report” European Journal Paediatric Neurology, 26-29 September 2007, Kusadasi, Turkey; Vol 11 (suppl 1):66

11. Dimitriou D, Leka S, Tsilifis N, Stamatiou M, Mostrou G, Giannaki M, Theodoridou M. “Measles outbreak-Athens, Greece 2005-2006: Data from the hospitalization of children”, European Academy of Paediatrics, 7-10 October 2006, Barcelona, Spain

12. Leka S, Hadjiathanasiou Ch, Psaromatis I, Skarpa V, Douniadakis D, Papathanasiou A, Anastasakou M, Paraskaki I, Apostolopoulos N. “Pituitary stalk interruption syndrome (PSIS) with ectopic neurohypophysis associated with congenital hearing impairment”, ESPE 2006, 45th Annual Meeting of European Society of Pediatric Endocrinology,30 June – 3 July, Rotterdam, The Netherlands, PO1-630, HORMONE RESEARCH; June 2006 Vol 65 (suppl 4):183

13. Vazeou A, Mitrakou A, Roumanis G, Koumanzeli C, Papathanasiou A, Tournis S, Leka S, Perperidis G, Papadopoulou A, Stamoyannou L, Hadjiathanasiou Ch. “Frequency of impaired glucose tolerance test (IGT) in overweight and obese children in the Greek population”, Obesity Reviews; 1-4 June 2005 Vol 6 (suppl 1):127

14. Leka S, Ballot E, Johanet J, Tubiana-Rufi T, Czernichow P. “Prévalence des anticorps antithyroidiens (Ac AT) dans une population d'enfants diabétiques insulinodépendant

(D.I.D)" a seven year report", 17th Scientific and Medical Meeting on Pediatric Diabetology", "Aide aux jeunes diabetiques" Association, Paris, France, November 2002

Εθνικά Συνέδρια – Greek Conferences

1. Leka-Emiri S, Louizou E, Kambouris M, Chrousos G, De ROYX N, KANAKA-GANTENBEIN Ch. "Absence of GPR54 (KISS1R) and TACR3 gene mutations in a cohort of girls with idiopathic central precocious puberty (ICPP) of Hellenic origin", 39th Greek Conference in Endocrinology and Metabolism, Athens, 4-7 April, 2012

2. Hajdiathanassiou Ch, Konstantopoulos H, Leka S, Evangelopoulou A, Kostakioti E, Fotinou A. "Intrauterine growth retardation with or without GH deficiency: effect of Growth Hormone treatment in glucose homeostasis", 43th Greek Pediatric Congress, June 2005, Kos, Greece; EA047; 34

3. Hadjiathanasiou Ch, Konstantopoulos H, Lekka G, Kostakioti E, Leka S, Evagelopoulou A. "GH treatment effects in children with IUGR do not depend upon GH secretion", 43th Greek Pediatric Congress, June 2005, Kos, Greece; EA 048; 34

4. Kostakioti E, Theodoridis Ch, Petrou V, Leka S, Papathanasiou A, Lekka G, Hadjiathanasiou Ch, Foteinou A. "GH treatment effects on glucose and insulin levels in children with GH deficiency", 43th Greek Pediatric Congress, June 2005, Kos, Greece; AA 073; 115

5. Papathanasiou A, Tertipi A, Fotinou A, Petrou V, Skarpa V, Anastasakou M, Leka S, Hadjiathanasiou Ch. "Growth hormone deficiency in a boy with polyglandular autoimmune syndrome type 2", 32th Greek Congress in Endocrinology and Metabolism, March 2005, Patras, Greece; P78; 117

7) Συνέδρια – Ημερίδες

Συμμετοχή σε συνολικά περίπου **60**

Διεθνή και Εθνικά Συνέδρια **στον τομέα ΕΝΔΟΚΡΙΝΟΛΟΓΙΑΣ (26), ΠΑΙΔΙΑΤΡΙΚΗΣ (21) και ΓΕΝΕΤΙΚΗΣ (8)**

Ενδεικτικά

- **39ο Πανελλήνιο Συνέδριο Ενδοκρινολογίας & Μεταβολισμού, 17-20 Απριλίου, Αθήνα, 2012**
- **49th Annual Meeting of the European Society for Pediatric Endocrinology (ESPE), Prague, 2010**
- **53emes Journées Internationales d' Edocrinologie Clinique H-P KLOTZ, Paris, 2010**
- **8th LWPES/ESPE, New York, September 2009**
- **91th Annual Meeting of Endocrine Society, Washington DC, June 2009**
- **47th Annual Meeting of the European Society for Pediatric Endocrinology (ESPE), Istanbul, Turkey 2008**
- **Πανελλήνιο Συνέδριο Ενδοκρινολογίας και Μεταβολισμού Αθήνα, Μάρτιος 2005, 2008**
- **Ετήσιο Διήμερο Παιδιατρικής Ενημέρωσης «Εφόλης της Ύλης» 2005 - 2008**
- **Ετήσια Θεραπευτική Ενημέρωση της Α' Παιδιατρικής Κλινικής του Πανεπιστημίου Αθηνών 2003 - 2013**
- **Ετήσιο Μετεκπαιδευτικό Σεμινάριο Εργατηρίου Ιατρικής Γενετικής του Πανεπιστημίου Αθηνών 2003 - 2006**
- **Πανελλήνιο Συνέδριο Παιδιατρικής 2004**

8) Ξένες Γλώσσες

ΓΑΛΛΙΚΑ	1988, « Diplôme d'Etudes Françaises », Université de Paris IV Sorbonne
	1996, « Diplôme d'Etudes Supérieures », Γαλλικό Ινστιτούτο Αθηνών
ΑΓΓΛΙΚΑ	1997, «Certificate of Proficiency in English», University of Cambridge

<u>Curriculum Vitae</u>

Sofia LEKA, MD, MSc
Date of birth: 25/05/1974
Chiou 4, 15231 Chalandri, Athens
210 6897387 / 6934227386

Jan 2014-	**Peadiatric Endocrinologist Consultant** Endocrin Division "P.& A. Kyriakou" Children's Hospital, Athens
Feb 2009 -	**PhD Candidate in Pediatric Endocrinology** Medical School, National University of Athens, Greece Supervisor Professor. G. CHROUSOS Thesis's title: "Molecular investigation of the GPR54 gene in children with idiopathic central precocious or delayed puberty" **Hellenic Society of Endocrinology Award 2008**
June 2010	**Diplome Interuniversitaire en Endocrinologie et Diabetologie Pediatrique** Paris VII University, France Supervisor Professor Jean-Claude CAREL and Claire LEVY-MARCHAL Memoire: "Metabolic consequences of IUGR: Adiponectine profiles through the first two years of life" **ESPE Clinical Fellowship Award 2009**
Dec 2008 – Oct 2010	**Research Fellowship in Pediatric Endocrinology** INSERM – Unité de Recherche U690 « Diabete de l'enfant et development » Head : Dr Claire LEVY-MARCHAL and INSERM – Unité de Recherche U676 « Génétique et physiologie de la puberté » Head : Dr Nicolas de ROUX AP-HP Hopital Robert Debré, Paris, FRANCE
May 2005 - May 2008	**Residency in Pediatrics (Highest Honors)** 1st Department of Pediatrics Head: Professor G. CHROUSOS "Aghia Sophia" Children's Hospital National University of Athens, Greece
Jan. 2003 – Apr. 2005	**MSc in Medical Genetics** (**Highest Honors)** Choremio Research Laboratory Department of Medical Genetics Head: Professor E. KANAVAKIS "Aghia Sophia" Children's Hospital National University of Athens, Greece Master's thesis: "Molecular investigation of the SHOX gene in children with idiopathic short stature"
Nov. 2001 – Nov. 2002	**Resident in Pediatric Endocrinology and Diabetology** Department of Pediatric Endocrinology – Diabetology Head: Professor P. CZERNICHOW "Robert Debré" University Children's Hospital, Paris – FRANCE
Jan. 2001 – Oct. 2001	**Resident in Endocrinology - Diabetology** Department of Endocrinology – Diabetology and Medicine of Reproduction Head: Professor P. FENICHEL ARCHET I University Hospital, C.H.U Nice – FRANCE
July 1999	**Medical Degree (High Honors)** Medical School, National University of Athens, Greece.

<u>Ο ΟΡΚΟΣ ΤΟΥ ΙΠΠΟΚΡΑΤΟΥΣ</u>

<u>ΑΡΧΑΙΟ ΚΕΙΜΕΝΟ:</u>

ΟΜΝΥΜΙ ΑΠΟΛΛΩΝΑ ΙΗΤΡΟΝ ΚΑΙ ΑΣΚΛΗΠΙΟΝ ΚΑΙ ΥΓΕΙΑΝ ΚΑΙ ΠΑΝΑΚΕΙΑΝ ΚΑΙ ΘΕΟΥΣ ΠΑΝΤΑΣ ΤΕ ΚΑΙ ΠΑΣΑΣ, ΙΣΤΟΡΑΣ ΠΟΙΟΥΜΕΝΟΣ, ΕΠΙΤΕΛΕΑ ΠΟΙΗΣΕΙΝ ΚΑΤΑ ΔΥΝΑΜΙΝ ΚΑΙ ΚΡΙΣΙΝ ΕΜΗΝ ΟΡΚΟΝ ΤΟΝΔΕ ΚΑΙ ΣΥΓΓΡΑΦΗΝ ΤΗΝΔΕ. ΗΓΗΣΕΣΘΑΙ ΜΕΝ ΤΟΝ ΔΙΔΑΞΑΝΤΑ ΜΕ ΤΗΝ ΤΕΧΝΗΝ ΤΑΥΤΗΝ ΙΣΑ ΓΕΝΕΤΗΣΙΝ ΕΜΟΙΣΙ, ΚΑΙ ΒΙΟΥ ΚΟΙΝΩΣΕΣΘΑΙ ΚΑΙ ΧΡΕΩΝ ΧΡΗΙΖΟΝΤΙ ΜΕΤΑΔΟΣΙΝ ΠΟΙΗΣΕΣΘΑΙ ΚΑΙ ΓΕΝΟΣ ΤΟ ΕΞ ΕΑΥΤΟΥ ΑΔΕΛΦΕΟΙΣ ΙΣΟΝ ΕΠΙΚΡΙΝΕΕΙΝ ΑΡΡΕΣΙ ΚΑΙ ΔΙΔΑΞΕΙΝ ΤΗΝ ΤΕΧΝΗΝ ΤΑΥΤΗΝ, ΗΝ ΧΡΗΙΖΩΣΙ ΜΑΝΘΑΝΕΙΝ, ΑΝΕΥ ΜΙΣΘΟΥ ΚΑΙ ΣΥΓΓΡΑΦΗΣ. ΠΑΡΑΓΓΕΛΙΗΣ ΤΕ ΚΑΙ ΑΚΡΟΗΣΙΟΣ ΚΑΙ ΤΗΣ ΛΟΙΠΗΣ ΑΠΑΣΗΣ ΜΑΘΗΣΙΟΣ ΜΕΤΑΔΟΣΙΝ ΠΟΙΗΣΕΣΘΑΙ ΥΙΟΙΣΙ ΤΕ ΕΜΟΙΣ ΚΑΙ ΤΟΙΣ ΤΟΥ ΕΜΕ ΔΙΔΑΞΑΝΤΟΣ ΚΑΙ ΜΑΘΗΤΑΙΣΙ ΣΥΓΓΕΓΡΑΜΜΕΝΟΙΣΙ ΤΕ ΚΑΙ ΩΡΚΙΣΜΕΝΟΙΣ ΝΟΜΩ ΙΗΤΡΙΚΩ, ΑΛΛΩ ΔΕ ΟΥΔΕΝΙ. ΔΙΑΙΤΗΜΑΣΙ ΤΕ ΧΡΗΣΟΜΑΙ ΕΠ' ΩΦΕΛΕΙΗ, ΚΑΜΝΟΝΤΩΝ ΚΑΤΑ ΔΥΝΑΜΙΝ ΚΑΙ ΚΡΙΣΙΝ ΕΜΗΝ. ΕΠΙ ΔΗΛΗΣΕΙ ΔΕ ΚΑΙ ΑΔΙΚΙΗ ΕΙΡΞΕΙΝ. ΟΥ ΔΩΣΩ ΔΕ ΟΥΔΕ ΦΑΡΜΑΚΟΝ ΟΥΔΕΝΙ ΑΙΤΗΘΕΙΣ ΘΑΝΑΣΙΜΟΝ, ΟΥΔΕ ΥΦΗΓΗΣΟΜΑΙ ΣΥΜΒΟΥΛΙΗΝ ΤΟΙΗΝΔΕ, ΟΜΟΙΩΣ ΔΕ ΟΥΔΕ ΓΥΝΑΙΚΙ ΠΕΣΣΟΝ ΦΘΟΡΙΟΝ ΔΩΣΩ. ΑΓΝΩΣ ΔΕ ΚΑΙ ΟΣΙΩΣ ΔΙΑΤΗΡΗΣΩ ΒΙΟΝ ΤΟΝ ΕΜΟΝ ΚΑΙ ΤΕΧΝΗΝ ΤΗΝ ΕΜΗΝ. ΟΥ ΤΕΜΕΩ ΔΕ ΟΥΔΕ ΜΗΝ ΛΙΘΙΩΝΤΑΣ , ΕΚΧΩΡΗΣΩ ΔΕ ΕΡΓΑΤΗΣΙΝ ΑΔΡΑΣΙΝ ΠΡΗΞΙΟΣ ΤΗΣΔΕ. ΕΣ ΟΙΚΙΑΣ ΔΕ ΟΚΟΣΑΣ ΑΝ ΕΣΙΩ, ΕΣΕΛΕΥΣΟΜΑΙ ΕΠ' ΩΦΕΛΕΙΗ ΚΑΜΝΟΝΤΩΝ, ΕΚΤΟΣ ΕΩΝ ΠΑΣΗΣ ΑΔΙΚΙΗΣ ΕΚΟΥΣΙΗΣ ΚΑΙ ΦΘΟΡΙΗΣ ΤΗΣ ΤΕ ΑΛΛΗΣ ΚΑΙ ΑΦΡΟΔΙΣΙΩΝ ΕΡΓΩΝ ΕΠΙ ΤΕ ΓΥΝΑΙΚΕΙΩΝ ΣΩΜΑΤΩΝ ΚΑΙ ΑΝΔΡΕΙΩΝ, ΕΛΕΥΘΕΡΩΝ ΤΕ ΚΑΙ ΔΟΥΛΩΝ. Α Δ' ΑΝ ΕΝ ΘΕΡΑΠΕΙΗ Η ΙΔΩ Η ΑΚΟΥΣΩ, Η ΚΑΙ ΑΝΕΥ ΘΕΡΑΠΕΙΗΣ ΚΑΤΑ ΒΙΟΝ ΑΝΘΡΩΠΩΝ, Α ΜΗ ΧΡΗ ΠΟΤΕ ΕΚΛΑΛΕΙΣΘΑΙ ΕΞΩ, ΣΙΓΗΣΟΜΑΙ, ΑΡΡΗΤΑ ΗΓΕΥΜΕΝΟΣ ΕΙΝΑΙ ΤΑ ΤΟΙΑΥΤΑ. ΟΡΚΟΝ ΜΕΝ ΟΥΝ ΜΟΙ ΤΟΝΔΕ ΕΠΙΤΕΛΕΑ ΠΟΙΕΟΝΤΙ ΚΑΙ ΜΗ ΣΥΓΧΕΟΝΤΙ ΕΙΗ ΕΠΑΥΡΑΣΘΑΙ ΚΑΙ ΒΙΟΥ ΚΑΙ ΤΕΧΝΗΣ, ΔΟΞΑΖΟΜΕΝΩ ΠΑΡΑ ΠΑΣΙΝ ΑΝΘΡΩΠΟΙΣ ΕΣ ΤΟΝ ΑΙΕΙ ΧΡΟΝΟΝ, ΠΑΡΑΒΑΙΝΟΝΤΙ ΔΕ ΚΑΙ ΕΠΙΟΡΚΟΥΝΤΙ, ΤΑΝΑΝΤΙΑ ΤΟΥΤΕΩΝ.

<u>ΝΕΟΕΛΛΗΝΙΚΗ ΑΠΟΔΟΣΗ:</u>

ΟΡΚΙΖΟΜΑΙ ΕΙΣ ΤΟΝ ΑΠΟΛΛΩΝΑ ΤΟΝ ΙΑΤΡΟ ΚΑΙ ΕΙΣ ΤΟΝ ΑΣΚΛΗΠΙΟ ΚΑΙ ΕΙΣ ΤΗΝ ΥΓΕΙΑ ΚΑΙ ΕΙΣ ΤΗΝ ΠΑΝΑΚΕΙΑ ΚΑΙ ΕΙΣ ΟΛΟΥΣ ΤΟΥΣ ΘΕΟΥΣ ΚΑΙ ΤΙΣ ΘΕΕΣ, ΕΠΙΚΑΛΟΥΜΕΝΟΣ ΑΥΤΟΥΣ ΩΣ ΜΑΡΤΥΡΕΣ, ΟΤΙ ΘΑ ΤΗΡΗΣΩ ΚΑΤΑ ΤΗ ΔΥΝΑΜΗ ΚΑΙ ΤΗΝ ΚΡΙΣΗ ΜΟΥ ΑΥΤΟΝ ΤΟΝ ΟΡΚΟ ΚΑΙ ΑΥΤΟ ΕΔΩ ΤΟ ΣΥΜΒΟΛΑΙΟ ΜΟΥ. ΘΑ ΘΕΩΡΩ ΕΚΕΙΝΟΝ Ο ΟΠΟΙΟΣ ΜΕ ΔΙΔΑΞΕ ΤΗΝ ΤΕΧΝΗ ΑΥΤΗ ΙΣΟ ΠΡΟΣ ΤΟΥΣ ΓΟΝΕΙΣ ΜΟΥ ΚΑΙ ΘΑ ΜΟΙΡΑΣΤΩ ΜΑΖΙ ΤΟΥ ΤΑ ΥΠΑΡΧΟΝΤΑ ΜΟΥ ΚΑΙ ΘΑ ΤΟΝ ΒΟΗΘΩ ΟΤΑΝ ΕΥΡΙΣΚΕΤΑΙ ΣΕ ΟΙΚΟΝΟΜΙΚΗ ΑΝΑΓΚΗ. ΘΑ ΘΕΩΡΩ ΤΟΥΣ ΑΠΟΓΟΝΟΥΣ ΤΟΥ ΩΣ ΑΔΕΛΦΟΥΣ ΜΟΥ ΚΑΙ ΘΑ ΤΟΥΣ ΔΙΔΑΣΚΩ ΤΗΝ ΤΕΧΝΗ ΑΥΤΗ, ΕΑΝ ΕΠΙΘΥΜΟΥΝ ΝΑ ΤΗ ΜΑΘΟΥΝ, ΧΩΡΙΣ ΑΜΟΙΒΗ ΚΑΙ ΣΥΜΒΟΛΑΙΟ. ΘΑ ΜΕΤΑΔΩΣΩ ΜΕ ΠΑΡΑΓΓΕΛΙΕΣ, ΟΔΗΓΙΕΣ ΚΑΙ ΣΥΜΒΟΥΛΕΣ ΟΛΕΣ ΤΙΣ ΠΟΛΟΙΠΕΣ ΓΝΩΣΕΙΣ ΚΑΙ ΕΙΣ ΤΑ ΠΑΙΔΙΑ ΜΟΥ ΚΑΙ ΕΙΣ ΤΑ ΠΑΙΔΙΑ ΤΟΥ ΔΙΔΑΣΚΑΛΟΥ ΜΟΥ ΚΑΙ ΕΙΣ ΤΟΥΣ ΜΑΘΗΤΕΣ ΟΙ ΟΠΟΙΟΙ ΕΧΟΥΝ ΟΡΚΙΣΤΕΙ ΟΤΙ ΘΑ ΤΗΡΟΥΝ ΤΟΥΣ ΙΑΤΡΙΚΟΥΣ ΚΑΝΟΝΕΣ ΚΑΙ ΕΧΟΥΝ ΣΥΜΦΩΝΗΣΕΙ ΕΓΓΡΑΦΩΣ, ΕΙΣ ΟΥΔΕΝΑ ΔΕ ΑΛΛΟΝ. ΘΑ ΘΕΡΑΠΕΥΩ ΤΟΥΣ ΑΣΘΕΝΕΙΣ ΚΑΤΑ ΤΗ ΔΥΝΑΜΗ ΚΑΙ ΤΗΝ ΚΡΙΣΗ ΜΟΥ, ΧΩΡΙΣ ΠΟΤΕ ΕΚΟΥΣΙΩΣ ΝΑ ΤΟΥΣ ΒΛΑΨΩ Η ΝΑ ΤΟΥΣ ΑΔΙΚΗΣΩ. ΔΕ ΘΑ ΧΟΡΗΓΗΣΩ ΘΑΝΑΤΗΦΟΡΟ ΦΑΡΜΑΚΟ ΣΕ ΚΑΝΕΝΑΝ, ΟΥΤΕ ΚΑΙ ΕΑΝ ΜΟΥ ΖΗΤΗΘΕΙ ΚΑΙ ΟΥΤΕ ΘΑ ΔΩΣΩ ΤΕΤΟΙΑ ΣΥΜΒΟΥΛΗ. ΕΠΙΣΗΣ ΔΕ ΘΑ ΔΩΣΩ ΣΕ ΓΥΝΑΙΚΑ ΦΑΡΜΑΚΟ ΓΙΑ ΝΑ ΑΠΟΒΑΛΕΙ. ΘΑ ΔΙΑΤΗΡΗΣΩ ΤΟ ΒΙΟ ΜΟΥ ΚΑΙ ΤΗΝ ΤΕΧΝΗ ΜΟΥ ΚΑΤΑ ΤΡΟΠΟ ΑΓΝΟ ΚΑΙ ΣΥΜΦΩΝΟ ΠΡΟΣ ΤΟΝ ΘΕΙΟ ΝΟΜΟ. ΔΕ ΘΑ ΧΕΙΡΟΥΡΓΗΣΩ ΟΥΤΕ ΤΟΥΣ ΠΑΣΧΟΝΤΑΣ ΑΠΟ ΛΙΘΙΑΣΙΝ, ΑΛΛΑ ΘΑ ΕΚΧΩΡΗΣΩ ΤΗΝ ΠΡΑΞΗ ΑΥΤΗ ΣΤΟΥΣ ΕΙΔΙΚΟΥΣ. ΣΕ ΟΣΕΣ ΔΕ ΟΙΚΙΕΣ ΕΙΣΕΛΘΩ, ΘΑ ΕΙΣΕΛΘΩ ΓΙΑ ΩΦΕΛΕΙΑ ΤΩΝ ΠΑΣΧΟΝΤΩΝ, ΑΠΕΧΟΝΤΑΣ ΑΠΟ ΚΑΘΕ ΕΚΟΥΣΙΑ ΑΔΙΚΙΑ ΚΑΙ ΑΛΛΗ ΖΗΜΙΑ ΚΑΙ ΚΑΘΕ ΓΕΝΕΤΗΣΙΑ ΠΡΑΞΗ ΕΠΙ ΓΥΝΑΙΚΕΙΩΝ ΚΑΙ ΑΝΔΡΙΚΩΝ ΣΩΜΑΤΩΝ, ΕΛΕΥΘΕΡΩΝ Η ΔΟΥΛΩΝ. ΟΣΑ ΔΕ ΔΩ Η ΑΚΟΥΣΩ ΚΑΤΑ ΤΗΝ ΑΣΚΗΣΗ ΤΟΥ ΕΠΑΓΓΕΛΜΑΤΟΣ ΜΟΥ Η ΚΑΙ ΕΚΤΟΣ, ΓΙΑ ΤΗ ΖΩΗ ΤΩΝ ΑΝΘΡΩΠΩΝ, ΤΑ ΟΠΟΙΑ ΔΕΝ ΠΡΕΠΕΙ ΠΟΤΕ ΝΑ ΚΟΙΝΟΠΟΙΗΘΟΥΝ, ΘΑ ΚΡΑΤΗΣΩ ΜΥΣΤΙΚΑ, ΘΕΩΡΩΝΤΑΣ ΟΤΙ ΑΥΤΑ ΕΙΝΑΙ ΑΠΟΡΡΗΤΑ. ΟΣΟ ΛΟΙΠΟΝ ΘΑ ΤΗΡΩ ΤΟΝ ΟΡΚΟ ΜΟΥ ΑΥΤΟΝ ΚΑΙ ΔΕ ΘΑ ΤΟΝ ΠΑΡΑΒΑΙΝΩ, ΕΙΘΕ ΝΑ ΑΠΟΛΑΥΩ ΚΑΙ ΤΗΣ ΖΩΗΣ ΚΑΙ ΤΗΣ ΤΕΧΝΗΣ, ΕΚΤΙΜΩΜΕΝΟΣ ΕΣΑΕΙ ΑΠΟ ΟΛΟΥΣ

ΤΟΥΣ ΑΝΘΡΩΠΟΥΣ. ΕΑΝ ΟΜΩΣ ΤΟΝ ΠΑΡΑΒΩ ΚΑΙ ΓΙΝΩ ΕΠΙΟΡΚΟΣ, ΝΑ ΥΠΟΣΤΩ ΤΑ ΑΝΤΙΘΕΤΑ ΑΠΟ ΑΥΤΑ.

ΑΓΓΛΙΚΗ ΑΠΟΔΟΣΗ:
HIPPOCRATES OATH

I swear by Apollo Physician and Asclepius and Hygiea and Panacea and all the gods and goddesses, making them my witnesses, that I will fulfil according to my ability and judgment this oath and this covenant:
To hold him who has taught me this art as equal to my parents and to live my life in partnership with him, and if he is in need of money to give him a share of mine, and to regard his offspring as equal to my brothers in male lineage and to teach them this art - if they desire to learn it - without fee and covenant; to give a share of precepts and oral instruction and all the other learning to my sons and to the sons of him who has instructed me and to pupils who have signed the covenant and have taken an oath according to the medical law, but no one else. I will apply dietetic measures for the benefit of the sick according to my ability and judgment; I will keep them from harm and injustice. I will neither give a deadly drug to anybody who asked for it, nor will I make a suggestion to this effect. Similarly I will not give to a woman an abortive remedy. In purity and holiness I will guard my life and my art. I will not use the knife, not even on sufferers from stone, but will withdraw in favour of such men as are engaged in this work. Whatever houses I may visit, I will come for the benefit of the sick, remaining free of all intentional injustice, of all mischief and in particular of sexual relations with both female and male persons, be they free or slaves.
What I may see or hear in the course of the treatment or even outside of the treatment in regard to the life of men, which on no account one must spread abroad, I will keep to myself, holding such things shameful to be spoken about. If I fulfil this oath and do not violate it, may it be granted to me to enjoy life and art, being honoured with fame among all men for all time to come; if I transgress it and swear falsely, may the opposite of all this be my lot.

Abbreviations

ARC = arcuate nucleus

AVPV = anteroventral periventricular nucleus

GRP54 = KISS1R = Kisspeptin-1 receptor

ICPP = idiopathic central precocious puberty

HPG = hypothalamo-pituitary-gonadal axis

ME = median eminence

nIHH = normosmic idiopathic hypogonadotropic hypogonadism

SNP = single nucleotide polymorphism

TAC3 = NKB = neurokinin B

TACR3 = NKB3R = Neurokinin B receptor

GENERAL PART

I. INTRODUCTION

Puberty is the phase of life when secondary sexual characteristics and subsequently reproductive capacity develop and normally takes place between 8 and 13 years in girls and 9 and 14 years in boys. Puberty results from the awakening of a complex neuroendocrine network in which the primary mechanisms are still unclear (Terasawa and Fernandez 2001). A peculiarity of sexual maturation in the human species is the 4- to 5-yr physiological variation in the age at pubertal onset that is observed among normal individuals despite relatively similar life conditions (Tanner 1962). This variability involves genetic factors, as indicated by the studies on heritability of menarcheal age (Kaprio et al. 1995). Other factors such as ethnicity, nutritional conditions, and environment, as well as secular trends have been also shown to influence the physiological range in pubertal onset (Van Wieringen 1978; Eveleth 1978).

A. NORMAL PUBERTY

1. Physiology

The onset of puberty is defined as the activation (or in some species reactivation) of the hypothalamic pituitary gonadal (HPG) axis (Grumbach, 2002; Ojeda and Skinner 2006; Plant and Witchel, 2006). Thus, puberty onset is reflected by increased pulsatile GnRH secretion resulting in pulsatile FSH and LH production, which activates the production of sex steroids by the gonads, thereby stimulating development of secondary sexual characteristics and contributing to the enhanced physical growth (pubertal growth spurt), estrous/menstrual cyclicity, ovulation and gametogenesis (fig. 1).

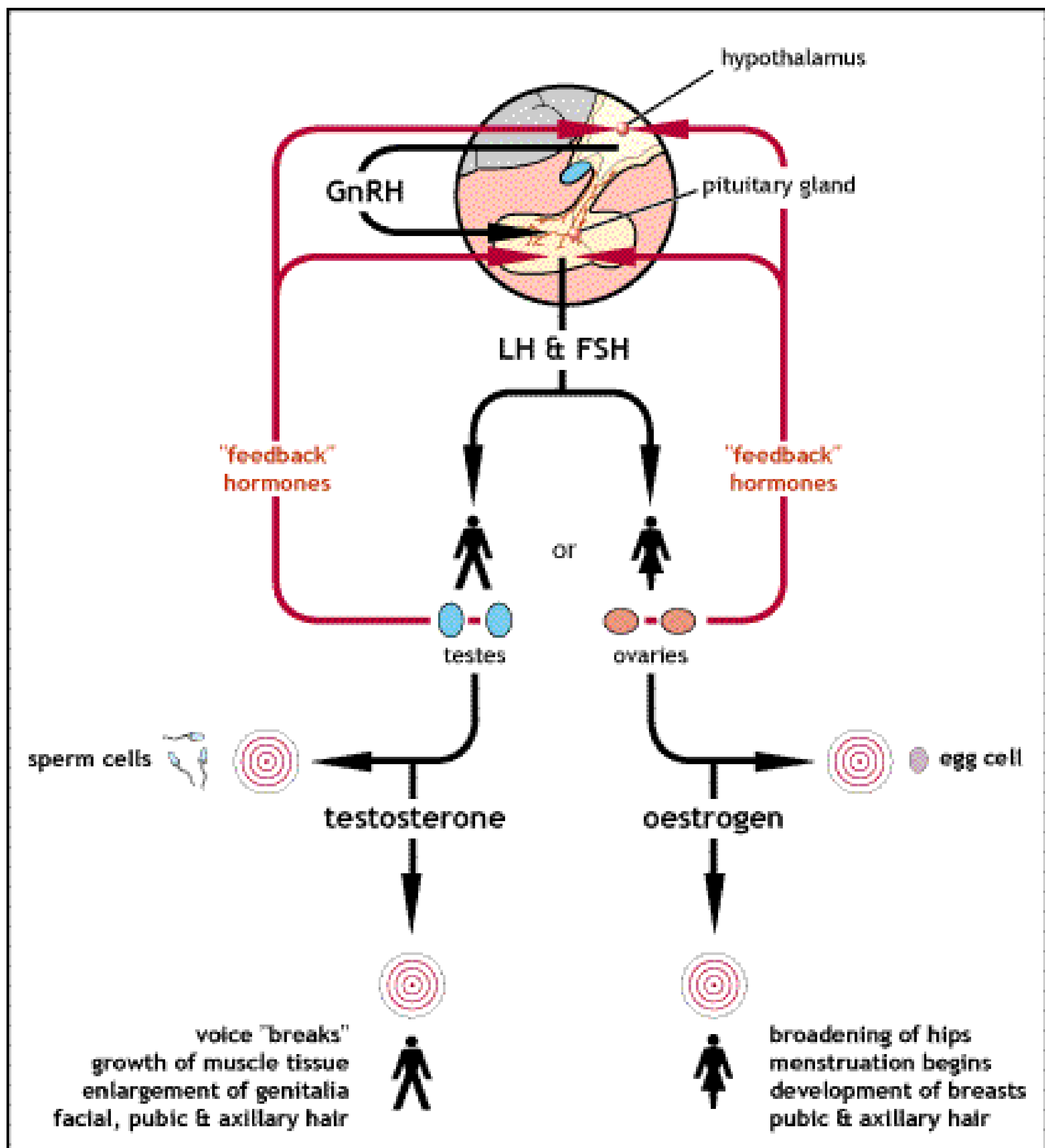


Figure 1. Hormonal changes driving pubertal onset (Smith 2008)

Despite the prevailing dogma that puberty is governed by the enhanced in amplitude and frequency pulsatile GnRH secretion, that is initially restricted at nighttime only and then covering all 24 hours, the specific neural and molecular mechanisms that trigger GnRH secretion in order to initiate puberty remain one of the enigmas of modern science, as does the temporal mechanism that orchestrates when puberty actually occurs (Richter 2006).

GnRH neurons and the pulsatile secretion of LH

The release of GnRH in a pulsatile manner is necessary for pulsatile LH secretion. Pulsatile LH release is indispensable for normal ovarian function (Clarke and Cummins 1982; Knobil 1990). Electrophysiological findings from the arcuate nucleus in monkeys, rats and goats (Knobil 1981; Kimura et al. 1991; Kinsey-Jones et al. 2008; Maeda et al. 1995; Wakabayashi et al. 2010) reveal significant, transient increases in neuronal firing rates (of 2000 spikes/minute approximately) that occur every 20-120 minutes depending mainly upon species and steroid status. These “multiunit volleys” are tied with pulses in serum LH which peaks minutes after the end of each volley. Based on this temporal correlation, it has been proposed that the multiunit volleys come from a group of neurons termed the “GnRH pulse generator”, which signals GnRH neurons to fire (Knobil, 1981). However, this concept remains controversial, with an important divergence of opinions regarding the relationship between the multiunit volleys and the control of GnRH release. Although the anatomical/cellular substrate of the multiunit activity is still unknown, GnRH neurons themselves have been proposed to be the source. In the monkey, GnRH neurons are close to the site of electrical activity (Silverman et al. 1986), and in the rat, unmyelinated GnRH axons passing through the adjacent median eminence are also in close proximity to the multiunit activity (Kimura et al. 1991). In this context, GnRH neurons are probably capable of generating oscillations autonomously (Moenter et al., 2003; Herbison, 2006). Cultures of GnRH-secreting GT1 cells, primary hypothalamic neuronal cultures, and nasal placode explants all exhibit spontaneous oscillations and pulsatile secretion of GnRH (Krsmanovic et al., 1992; Wetsel et al., 1992; Charles and Hales 1995; Terasawa et al., 1999). Despite the fact that GnRH neurons are not confined to a single location or nucleus, they are extensively interconnected providing an anatomical framework for synchronization (Campbell et

al. 2009). Since isolated multi-unit recordings of GnRH neurons in vivo are not possible due to their scattered location, the contribution of GnRH neurons to the multiunit volleys remains to be established.

Additionally, the regulation of pubertal process is proposed to include gonadal steroid-dependent as well as steroid-independent mechanisms. In rodents, negative feedback of reproductive axis by low levels of gonadal steroids is a primary mechanism for inhibitng the axis before puberty (Ebling 2005; Ojeda and Skinner 2006). Through the developmental process approching puberty it has been noticed a decrease in the sensitivity of key neural networks to gonadal steroid negative feedback, allowing with an enhanced activation of the GnRH axis. In rats, this developmental change in gonadal steroid feedback sensitivity normally occurs on the day of vaginal opening wich is a “downstream” event in the pubertal maturation. Therefore it is postulated that puberty onset has already occurred by the time of gonadal steroid sensitivity alteration. As a consequence, additional, non-gonadal components must be involved. This is confirmed in primates. Gonadectomy during the juvenile phase does not lead to elevated gonadotropin release. In agonadal males and females gonadotropin release stays low until just before normal puberty onset and increases importantly at that time. (Plant and Witchel 2006; Herbison 2007). These findings show that there is a steroid-independent regulation of the developing reproductive axis. Puberty onset may consequently reflect not only a removal of inhibitory input onto neurohormonal circuits, but also, an enhancement of stimulatory input, both of which occurs independently of changes in gonadal steroids. In conclusion, GnRH neuronal activation seems to be controlled by a large number of stimulatory, inhibitory and permissive networks (Terasawa and Fernandez 2001). Among these networks Kiss1 neurons along with Kisspeptin/GPR54 complex seem to play a great role in the neuroendocrine control of GnRH pulsatile decretion (Fig. 2).

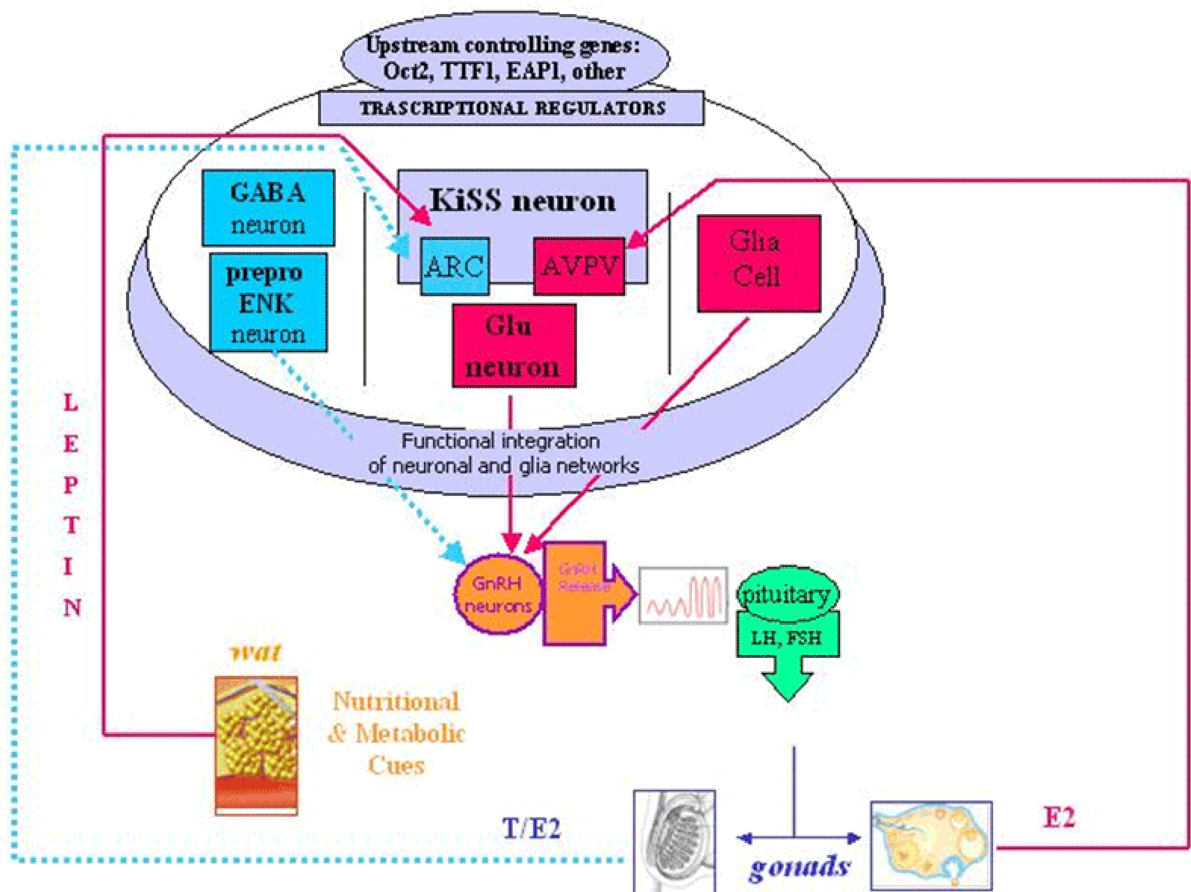


Figure 2

KiSS1 neuron integrates diverse signals to promote GnRH pulsatile release

(www.**endotext**.org)

1. 1. A. Overview of Kisspeptin signaling

Kisspeptin (a Kiss1 gene product) and its receptor (GPR54 or Kiss1r) have emerged as gatekeepers in the regulation of puberty and reproduction. Neurons that express Kiss1/Kisspeptin are present in discrete nuclei in the hypothalamus and in other brain regions in many vertebrates. Their distribution, regulation and function varie widely across species. Kisspeptin neurons: 1) directly innervate and stimulate GnRH neurons, which are the final common pathway that regulates hypothalamic pituitary gonadal (HPG) axis; 2) are dimorphically expressed with respect to cell number and transcriptional activity in certain brain nuclei,; 3) express other co-transmitters,

including dynorphin and neurokinin B and 4) express the estrogen and androgen receptor that direct the action of gonadal steroids in both male and female animals. Kisspeptin signaling in the brain along with triggering and guiding the tempo of sexual maturation during puberty, has been also involved in: 1) mediating the negative feedback action of sex steroids on gonadotropin secretion, 2) generating the preovulatory GnRH/LH surge, 3) controlling seasonal reproduction and 4) restraining reproductive activity during lactation. Finally, kisspeptin signaling may also serve diverse functions such as: 1) the regulation of metastasis in certain cancers, 2) the vascular dynamics, 3) the placental physiology and 4) even higher-order brain functions. Kisspeptin signaling was reviewed in detail by Oakley et al. in 2009 (Oakley et al. 2009) and more recently by Franceschini et al in 2013 (Franceschini et al. 2013) . In this part we will present the most important information on this subject.

1. 1. A.1. Biochemistry

The initial product of the Kiss1 gene is a 145-amino acid peptide, from which a 54-amino acid protein, the kisspeptin-54, is cleaved (West et al. 1998). The sequence of kisspeptin-54 is surrounded by pairs of basic residues, where furin or prohormone convertases are suggested to proteolytically cleave. There are also shorter peptides (kisspeptin-10, -13 and -14). These peptides share a common RF-amidated motif with kisspeptin-54 and collectively, they are termed kisspeptins. Although no obvious cleavage sites have been identified that would result in these shorter peptides it has been suggested that kisspeptin-54 is unstable and may be proteolytically cleaved into shorter products. All four peptides (kisspeptin-10, -13, -14, and 54) exhibit the same affinity and efficacy for the Kiss1r (Kotani et al. 2001). Although all four kisspeptin products are biologically active (Muir et al. 2001), the in vivo relevance of the shorter peptides is as yet unknown.

G protein-coupled receptors (GPCRs) transduce a variety of inputs to activate signaling pathways implicated in diverse functions such as cell growth, proliferation

and migration. The GPCR superfamily is classified into three subdivisions, e.g., rhodopsin-, secretin-, and metabotropic glutamate receptor – like families (Marchese et al. 1999). Typical of the rhodopsin family of GPCRs, Kiss1r has seven transmembrane domains, with three glycosylation sites at the N terminus (Clements et al. 2001). Kiss1r is most similar to the galanin receptor family (~45% homologous), however it does not bind either galanin or galanin like peptide (Lee et al. 1999). Screens for agonists that bind Kiss1r identified several neuropeptides of the RFamide and RWmide family (Clements et al. 2001). The RMRFamide (Phe-Met-Arg-Phe-NH2)-related peptides (RFRPs), of which Kiss1 is a member, constitute a superfamily of neuropeptides that terminate with the sequence Arg-Phe-NH2 and are present in all species (Greenberg and Price 1992; Li et al. 1999). The binding of Kiss1r by Kiss1 peptide results in the activation of G protein-activated phospholipase C (PLCβ), suggesting a Gαq/11-mediated signaling pathway (Kotani et al. 2001; Muir et al. 2001; Stafford et al. 2002; Liu et al. 2008; Constantin et al. 2009) (Fig. 3). PLCβ activation results in the generation of the intracellular second messagers, inositol triphosphate (IP3) and diacylglycerol (DAG); these signaling molecules in turn mediate intracellular Ca^{2+} release and activation of protein kinase C, respectively (Stafford et al. 2002; Constantin et al. 2009).

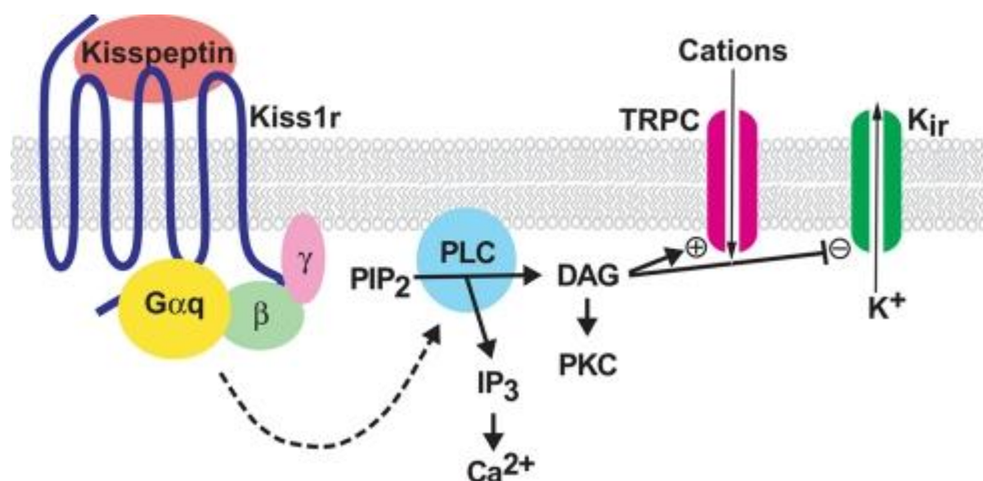


Figure 3

Proposed mechanism of neuronal depolarization by kisspeptin binding to its receptor, Kiss1r. Kisspeptin binding to its GPCR, Kiss1r, activates the G protein, $G\alpha_q$, and PLC to cleave phosphatidylinositol 4,5-bisphosphate (PIP_2) into IP_3 and DAG. DAG activates a signal cascade by activating PKC, whereas IP_3 mobilizes calcium ions (Ca^{2+}), which participate in the cascade by activating other proteins. Membrane depolarization is caused by activation (+) of nonselective TRPC cation channels and inhibition (−) of inwardly rectifying potassium channels (K_{ir}), possibly through involvement of DAG (Oakley et al. 2009).

Kisspeptin is thought to stimulate GnRH secretion by activating transient receptor potential canonical (TRPC)-like channels and inhibiting inwardly rectifying potassium channels (Zhang et al 2008), likely mediated by DAG and/or Ca^{2+}. In addition, Kiss1r has been shown to stimulate arachidonic acid release and ERK1/2 and p38 activation, as well as Rho activation, which causes stress fiber formation (Kotani et al. 2001; Castellano et al. 2006(b)). Endogenous kisspeptin may activate Kiss1r via a ligand transportation mechanism, in which initial binding of a ligand to the membrane is followed by lateral diffusion to the receptor (Lee et al. 2009).
Recently, progress has been made in the development of novel ligands or pharmacologically therapeutic agents, agonists or antagonists, of kisspeptins. Orsini et al. (Orsini et al 2007) have identified a model kisspeptin pharmacophore utilizing a structure-activity relationship approach combining nuclear magnetic resonance, receptor binding and functional assays. The authors demonstrated that the kisspeptin-13 peptide has a relatively stable helix conformation form residues 7 to 13, with three functionally key residues (Phe9, Arg12 and Phe13) that lie on one face of the helix and define its pharmacophore site (Orsini et al. 2007). Through amino acid substitution, Gutierrez-Pascual et al. (Gutierrez-Pascual et al. 2009) have identified alanine at positions 6 and 10 as critical for kisspeptin-10 action at Kiss1r, pointing to potential modifications that could lead to new kisspeptin analogs. The stereochemistry of kisspeptin analog amino acids also appears to be of major

importance; substitution of key residues with the d-isomer significantly decreases peptide agonist activity (Niida et al. 2006). Utilizing a structure-activity relationship approach, Tomita at al. (Tomita et al. 2006; Tomita et al. 2007(a) and (b); Tomita et al. 2008) have identified several pentapeptide kisspeptin analogs as novel Kiss1r agonists. Molecules identified that mimic the key features of the pharmacophore site can act as full agonists, although with reduced potency compared to kisspeptin itself (Orsini et al. 2007).

Several methods have been used to block kisspeptin-Kiss1R signaling. Kinoshita et al. developed a monoclonal anti-rat kisspeptin antibody that, when infused in the preoptic area (POA) completely blocks the proestrus LH surge and inhibits estrous cyclicity (Kinoshita et al. 2005). Roseweir et al. (Roseweir et al. 2009) have developed several kisspeptin antagonists via amino acid substitution of kisspeptin-10 analogs. Based on its structure-activity profile, one potent and specific antagonist ("peptide 234") was selected for use in *ex vivo* and *in vivo* studies. This antagonist inhibits the kisspeptin-induced rise in LH secretion in mice and rats and blocks the postcastration LH secretion in rodents and sheep, suggesting a powerful role of kisspeptin neurons in mediating the negative feedback action of sex steroids on the hypothalamic-pituitary gonadal axis (Roseweir et al. 2009). Furthermore, the antagonist inhibits kisspeptin-10-induced GnRH neuronal firing in the mouse brain and reduces pulsatile GnRH secretion in female pubertal monkeys (Roseweir et al. 2009), underlying the importance of kisspeptin signaling in the control of GnRH secretion.

1.1. A. 2 Molecular physiology of Kiss1 neurons

Kiss1 neurons are regulated by several factors, including steroid hormone feedback, metabolic signals and photoperiodic cues (Fig. 4).

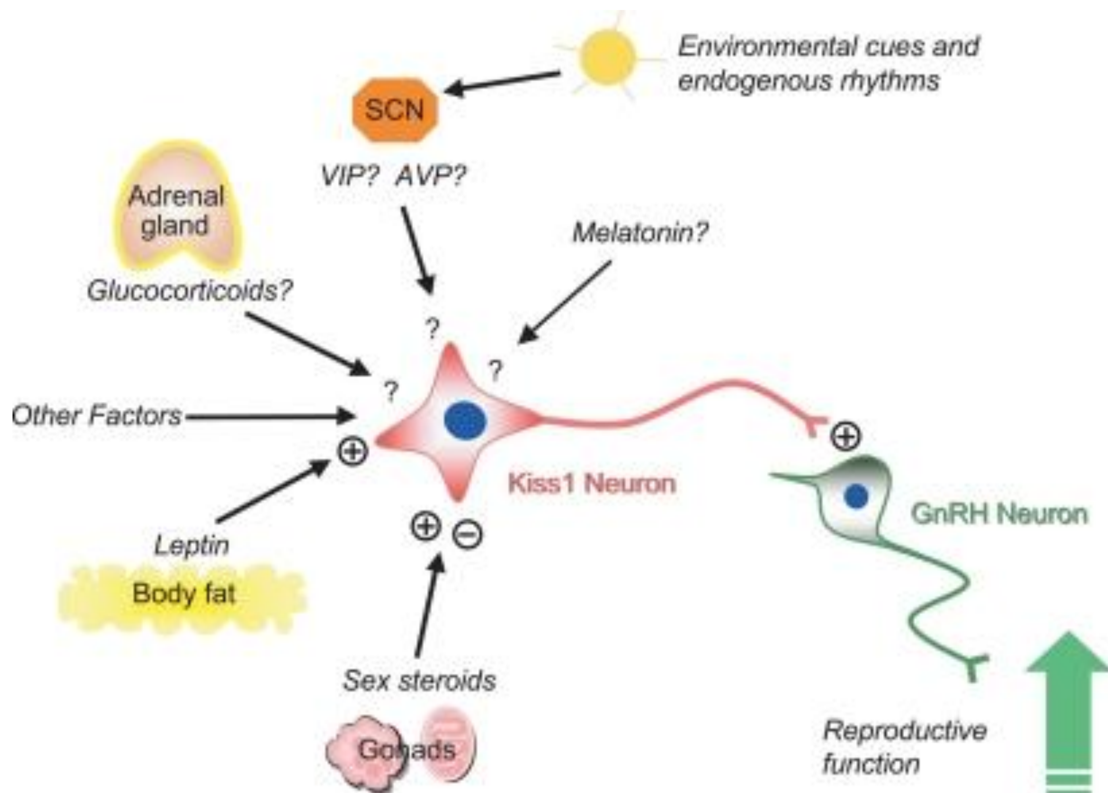


Figure 4

Kisspeptin neurons may act as central processors for relaying signals from the periphery to GnRH neurons. Metabolic and environmental factors regulate reproductive function, which ensures that reproduction proceeds only when metabolic and environmental conditions are favourable. Kisspeptin stimulates GnRH secretion, and *Kiss1* mRNA is both negatively and positively regulated by sex steroids. The expression of Kiss1 may be induced by leptin, whose plasma levels reflect the state of metabolic reserves. Kisspeptin neurons may also receive input from the hypothalamic-pituitary-adrenal axis and from environmental cues such as time of day via the SCN of the hypothalamus and day length via melatonin from the pineal gland. AVP: Arginine vasopressin; VIP: vasoactive intestinal peptide. (Modified from Dungan *et al.*2006).

In accordance with their role as mediators of steroid feedback most Kiss1 neurons express estrogen receptors (ERα) (Adachi et al. 2007; Smith et al. 2006;

Franceschini et al. 2006; Smith et al. 2005 (a) and (b); Clarkson et al. 2008), ERβ (Smith et al. 2006) and progesterone receptors (Pr) (Clarkson et al. 2008; Smith et al. 2007). About 40% of Kiss1-expressing neurons in the ARC of the mouse express the mRNA for the active form of the leptin receptor (Ob-Ob) (Smith et al. 2006), thus providing a potential link between nutrition and reproduction. Numerous studies indicated that Kiss1 neurons are regulated by photoperiod (Martinez-Chavez et al. 2008; Kanda et al. 2008; Revel et al. 2006; Mason et al. 2007; Smith et al. 2008(a); Smith et al. 2007; Gingerich et al. 2009). Goodman et al. (Goodman et al. 2007) describes a subpopulation of ovine kisspeptin neurons in the ARC that co-express dynorphin A and neurokinin B (NKB), and quite likely ERα and PR (Foradori et al 2002; Goubillon et al. 2000). Other investigations have shown a similar co-expression phenomenon in other species, including the rat, mouse and human. For instance, there is extensive colocolisation of NKB and dynorphin in the ARC of the rat (Burke et al. 2006). Rometo et al. (Rometo et al. 2007) observed similar distribution and morphology of NKB- and kisspeptin-containing neurons in the infundibular (ARC) nucleus of postmenopausal women, Furthermore, approximately 50% of the glutamergic neurons in the ARC of sheep express ERα (Pompolo et al. 2003), indicating the presence of a discrete population of kisspeptin/dynorphin/NKB/glutamate estrogen-responsive neurons in the ARC.

Navarro et al. (Navarro et al. 2009) have verified that kisspeptin, NKB and dynorphin are all co-localized in cells of the ARC in the mouse, where all three of these neuropeptides are regulated by estradiol. Moreover, in this species kisspeptin neurons in the ARC also express the cognate receptors for NKB and dynorphin (i.e., NK3 and κ-opiate receptor, respectively), suggesting the existence of autosynaptic contacts among kisspeptin/dynorphin/NKB neurons in this region. In this study by Navarro et al. (Navarro et al. 2009) it has been shown that NKB agonists inhibit LH secretion in ovariectomized mice; moreover, they showed that mice bearing targeted deletions of either dynorphin or κ-opiate receptor gene present a diminished

postcastration rise in LH, implying that NKB and dynorphin signaling play key roles in the regulation of GnRH/LH release. These observations suggest that kisspeptin, dynorphin, NKB (and perhaps glutamate) participate in the regulation of pulsatile GnRH secretion. Indeed, Navarro et al. (Navarro et al. 2009) propose a model on which dynorphin and NKB act autosynaptically on kisspeptin neurons (directly and/or indirectly) to generate discrete pulses of kisspeptin, which in turn drives pulsatile GnRH and LH secretion. This hypothesis is in agreement with the observations of Keen et al. (Keen et al. 2008), that has shown apparent coincidence of pulsatile kisspeptin and GnRH secretion, as measured in the median eminence (ME) of the monkey. It is likely that other peptidergic systems and classical neurotransmitters, such as glutamate and γ-aminobutiric acid, also play important roles in the regulation of kisspeptin activity in the ARC (Clarkson et al. 2006).

In the AVPV, the situation is different from the ARC. Kisspeptin neurons in the AVPV express neither dynorphin nor NKB in any species studied to date. However, in the mouse, kisspeptin neurons in the AVPV appear to co-express tyrosine hydroxylase, suggesting that these cells may be dopaminergic - although this does not appear to be the case in the rat, where few, if any, kisspeptin neurons in the AVPV appear to express tyrosine hydroxylase (Kauffman et al. 2007). The differential expression of various cotransmitters with kisspeptin at different sites (ARC vs. AVPV) evidence that these two populations of “kisspeptin” neurons are phenotypically unique, not only in their molecular fingerprinting, but also in their physiological function. Figure 5 illustrates the main circuits between kiss1 neurons and GnRH neurons.

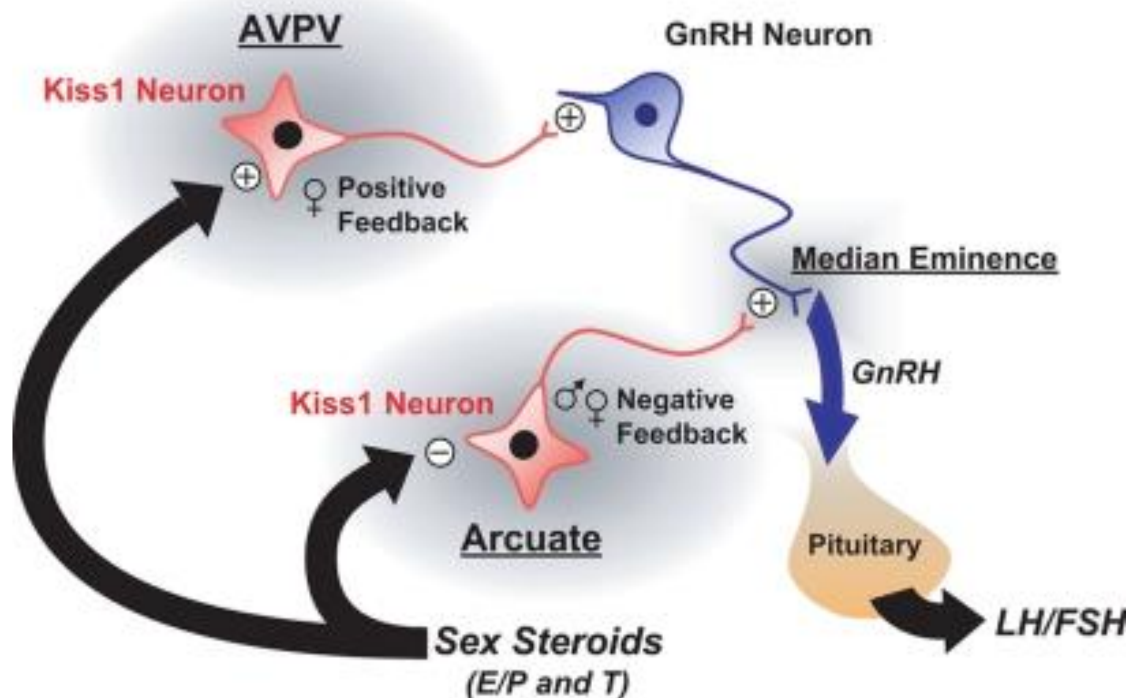


Figure 5

A schematic representation of the initial understanding of Kiss1 signaling in the forebrain of the mouse. Kisspeptin stimulates GnRH secretion by a direct effect on GnRH neurons, most of which express the kisspeptin receptor, Kiss1r. Neurons that express *Kiss1* mRNA reside in the AVPV and the ARC (arcuate). Kiss1 neurons in the ARC appear to be involved in the negative feedback regulation of GnRH/LH by sex steroids. The expression of *Kiss1* mRNA in the arcuate is inhibited by estradiol (E), progesterone (P) and testosterone (T). These same hormones induce *Kiss1* mRNA expression in the AVPV, where Kiss1 neurons are thought to be involved in the positive feedback regulation of GnRH/LH. (Modified with permission from Gottsch *et al.* 2006).

The afferent inputs to Kiss1 neurons (besides possible autosynaptic processes) remain mostly unknon. Kiss1 neurons in the AVPV of the rodent are likely to receive afferent input from the suprachiasmatic nucleus (SCN) because that region projects to the AVPV and is involved in the timing of the LH surge (Watson et al. 1995). Most of the neurotransmitters involved are currently unknown, but they could be arginine vasopressin or vasoactive intestinal peptide because these are the major efferent

projections form the SCN (Watson et al. 1995; Kalsbeek et al. 2006); however, this remains to be clarified.

1. 1. A. 3 Comparative physiology

Some aspects of Kisspeptin anatomy and physiology are highly conserved across species, such as the stimulatory effect of kisspeptin on GnRH. However, other aspects of Kiss1 anatomy and physiology are unique to particular species – such as the mechanisms that govern the preovulatory surge of GnRH/LH.

- *Direct and indirect effects of kisspeptin*

Several studies suggest that kisspeptin signals directly to GnRH neurons (Colledge et al. 2009). First, the majority of GnRH neurons express Kiss1r (Parhar et al. 2004; Han et al. 2005; Messager et al. 2005; Irwig et al. 2004). Second, Kisspeptin-1r fibers are found in close association with GnRH neurons (Kinoshita et al. 2005; Clarkson et al. 2006; Smith et al. 2008; Decourt et al. 2008). Third, kisspeptin can act directly to depolarize and increase firing rates of GnRH neurons in vitro (Liu et al 2008; Zhang et al. 2008; Han et al. 2005; Liu and Herbison 2008; Pielecka-Fortuna et al. 2008; Quaynor et al. 2007; Dumalska et al 2008). Although kisspeptin may act through traditional synaptic mechanisms to stimulate GnRH release, it may also act directly in a nonsynaptic manner, particularly in the median eminence (ME) (Pompolo et al. 2006; Franceschini et al. 2006; Decourt et al.2008; d'Anglemont de Tassigny et al. 2008). Additionally, there is growing evidence to propose that kisspeptin also acts on intermediary neurons, such as GABAergic cells, to regulate GnRH secretion (Pielecka-Fortuna et al. 2008; Zhang et al. 2009).

By acting at the level of the hypothalamus to increase GnRH release, Kisspeptin produces an increase in LH secretion from the pituitary as well. However, some studies suggest that kisspeptin may also act directly at the level of the pituitary..

Richard et al., have reviewed in detail the action of kisspeptin on the pituitary (Richard et al. 2009). Indeed, it appears that both Kiss1 and Kiss1r are present in the pituitary, specifically in gonadotropes, and are differentially regulated by sex steroids (Kinoshita et al. 2005; Richard et al. 2008; Smith et al. 2008; Gutierrez-Pascual et al. 2007). The presence of a functional kisspeptin receptor in the pituitary, combined with the finding that kisspeptin is released in ovine hypophyseal portal blood, assums kisspeptin action at the level of the pituitary to regulate gonadotropin secretion (Smith et al. 2008). This notion is supported by in vitro studies showing a stimulatory increase in gonadotropin release from pituitary fragments or cells treated with kisspeptin (Gutierrez-Pascual et al. 2007; Navarro et al. 2005; Suzuki et al. 2008). Taken together, these findings would suggest a dual action of kisspeptin, at the pituitary and hypothalamus.. However, it remains unclear whether kisspeptin acts as true hypophysiotropic factor to regulate LH secretion. Although kisspeptin can be measured in hypophyseal portal blood, levels are similarly low in both ovariectomized and treated with estrogen to induce LH surge ewes (Smith et al. 2005), suggesting that the action of kisspeptin at the pituitary does not greatly affect the release of LH. Utilizing the hypothalamo-pituitary disconnection sheep model, Smith et al. (Smith et al. 2005) examined the in vivo relevance of kisspeptin at the level of the pituitary. In this model, kisspeptin treatment had no effect on LH secretion, indicating that any effect of kisspeptin on LH secretion occurs upstream of the pituitary. Moreover, a GnRH antagonist blocks the kisspeptin-induced increase in LH, again suggestive of a supra-pituitary action of kisspeptin (Gottsch et al. 2006; Irwig et al. 2004). Thus, whereas some evidence supports a role of kisspeptin action at the pituitary, most findings imply a higher level of action.

- *Continuous vs. pulsatile exposure to kisspeptin*

After an initial stimulation, a continuous exposure of the pituitary to GnRH (or agonists) eventually causes suppression of gonadotropin secretion (Belchetz et al. 1978) through down-regulation and sensitization of the GnRH receptors (McArdle et al. 1987; Wu et al. 1994; Vizcarra et al. 1997; Mason et al. 1994). Because kisspeptin is known to activate the hypothalamic-pituitary-gonadal axis, several groups have examined whether a continuous infusion of kisspeptin would produce the same inhibitory effect on gonadotropin secretion as GnRH. Indeed, continuous delivery of exogenous kisspeptin appears to desensitize Kiss1r, resulting in decrease of LH secretion in agonadal juvenile and adult male monkeys and testicular degeneration in adult male rats (Seminara et al. 2006; Ramaswamy et al. 2007; Thompson et al. 2006). In contrast, repeated peripheral injections of kisspeptin elicit unrestrained LH pulses in male rats and monkeys (Tovar et al. 2006; Plant et al. 2006), suggesting that the efficacy of kisspeptin to drive LH secretion depends on its pulsatile nature, much like for GnRH. Interestingly, sustained (30 or 48h) iv kisspeptin treatment was effective in seasonally acyclic ewes (anestrous season), resulting in ovulation in 80% of animals (compared to 20% controls) (Caraty et al. 2007). However, it is not clear whether this finding reflects a difference in the way sheep respond to continuous exposure to kisspeptin compared with other species or whether the differences reported between studies are due to differences in the dose and mode of kisspeptin administration. Desensitization may have a major impact on the efficacy of kisspeptin analogs and antagonists when used as contraceptives or to treat reproductive disorders.

- *Negative feedback action of sex steroids on Kiss1 gene expression in arcuate nucleus (ARC)*

A classic example of negative feedback in male is that of testosterone acting at the level of the hypothalamus to suppress GnRH and thereby regulating gonadotropin secretion and testicular function. Because GnRH neurons appear to lack both the androgen receptor (AR) and ERα (in either sex), some intermediary neuronal system is believed to indirectly relay the feedback signal from the gonad to the GnRH neurons. Kisspeptin neurons may represent an important element of this negative feedback loop. After castration in mice, rats, hamsters and monkeys, levels of Kiss1 mRNA increase dramatically in the mediobasal hypothalamus, specifically the ARC, In addition, this effect can be reversed with sex steroid replacement (Revel et al. 2006; Irwig et al. 2004;Shibata et al. 2007; Smith et al. 2005 (b), Navarro et al. 2004). A castration-induced increase in Kiss1 expression in the ARC coincides with the increase in GnRH and gonadotropin secretion (Smith et al. 2005(b)) reflecting that they are direct targets for the action of sex steroids. Furthermore, studies of male mice with null mutations in the ERα and hypomorphic alleles of the AR implicate both ERα- and AR-dependent regulation of Kiss1 gene expression in the ARC (Smith et al. 2005(b)). Taken together, these observations provide convincing evidence that Kiss1/Kiss1r signaling (in the ARC) mediates the negative feedback regulation of GnRH secretion – at least in the male.

In the female mammal, during most days of the estrous and menstrual cycle, the negative feedback loop of gonadotropin secretion predominates and a relatively low plasma level of sex steroids restrains GnRH and LH secretion. Kisspeptin neurons appear to play an elemental role in the negative feedback action of estradiol in the female. The expression of Kiss1mRNA in the ARC changes in function of the estrous cycle in the rat, with levels reaching nadirs at or around the time when estradiol levels are highest (Smith et al. 2006). Ovariectomy causes an increase in

hypothalamic expression of Kiss1mRNA in the ARC of rodents, sheep and monkeys (Smith et al. 2008; Rometo et al. 2007; Smith et al. 2005(a); Smith et al. 2007; Navarro et al. 2004; Dungan et al. 2007; Kim et al. 2009). The increase in expression of Kiss1 is reversible upon treatment with estradiol (Smith et al. 2006; Smith et al. 2008; Rometo et al. 2007; Smith et al. 2007; Navarro et al. 2004; Dungan et al. 2007). Finally, female mice bearing targeted deletions of Kiss1r do not show a post castration rise in LH despite exhibiting a dramatic increase in the expression of kiss1mRNA (Dungan et al. 2007). These observations suggest that kisspeptin neurons in the ARC of both the male and female provide tonic drive to GnRH neuronal activity, which is modulated by the negative feedback effects of sex steroids.

- *Circadian signals and positive feedback action of estradiol on Kiss1 gene expression in the anteroventral periventricular nucleus (AVPV)*

Early during proestrous in rodents (or late in the follicular phase of the menstrual cycle in primates), the increasing levels of estradiol in the plasma lead to a surge of GnRH and LH secretion, which induces ovulation. In rodents, this so-called positive feedback effect of estradiol appears to involve estradiol-sensitive neurons in the AVPV, which act directly on GnRH neurons. (Terasawa et al. 1980; Wintermantel et al. 2006). Nearly all kisspeptin expressing cells in the AVPV of the female rodent express ERα (Smith et al. 2005(a)); moreover, the AVPV is a sexually dimorphic nucleus, with sexually differentiated expression of tyrosine hydroxylase (Simerly et al. 1989), Kiss1 (Kauffman et al. 2007), neurotensin (Dungan et al. 2008) and other genes. Kisspeptin neurons in the AVPV of rodents seem to play a central role in relaying the positive feedback effects of estradiol to GnRH neurons. First, treatment with a kisspeptin antiserum to block kisspeptin signaling completely abolishes the LH surge in female rats (Kinoshita et al. 2005; Adachi et al. 2007). Second, in the

mouse, the expression of Kiss1 mRNA in the AVPV is dramatically increased by estradiol (Smith et al. 2005(a); Dungan et al. 2007). Third, in the rat, the expression of Kiss1 mRNA in the AVPV peaks at a time coincident with the GnRH/LH surge, and Kiss1 neurons in the AVPV demonstrate Fos induction at precisely this time (Adachi et al. 2007; Smith et al. 2006). Fourth, a population of rodent ERα- positive neurons has direct synaptic contact with GnRH neurons (Terasawa et al. 1980; Wintermantel et al. 2006), and these neurons are likely to be Kiss1 neurons (Smith et al. 2005 (a). Finally, Clarkson et al. (Clarkson et al. 2008) demonstrated that whereas normal wild-type mice that have been ovariectomized and treated with both estradiol and progesterone show a clear LH surge, mice bearing targeted deletions in Kiss1r seem to lack this capacity. In addition, approximately 50% of GnRH neurons in wild-type mice demonstrated Fos expression coincident with the LH surge, whereas none of the mutant animals showed evidence of Fos expression at this same time (Clarkson et al. 2008). Together, these observations suggest that activation of kisspeptin neurons is a prerequisite for generating the estradiol/progesterone-induced GnRH/LH surge in the female mouse. However, another study added caution to this conclusion. Dungan et al. (Dungan et al. 2007) demonstrated that in another, independently produced line of Kiss1r-knockout mice, ovariectomized knockout females treated with estradiol retain the capacity to elicit a GnRH/LH surge and Fos induction in GnRH neurons, virtually identical to wild-type controls. Although Clarkson et al. and Dungan et al. (Clarkson et al. 2008; Dungan et al. 2007) have provided evidence for having produced a complete knockout of the Kiss1r gene, the method that these groups used to generate a GnRH/LH surge in ovariectomized animals differed. The protocol used to produce a surge in the Dungan et al. (Dungan et al. 2007) study involved sustained treatment with estradiol alone, which produces a diurnal GnRH/LH surge that persists for many days. In the Clarkson et al. study (Clarkson et al. 2008) the protocol involved a combination of estradiol and progesterone to generate a single surge, which produces a single GnRH/LH surge, instead of a daily event.

Consequently, it is possible that these two methods activate different pathways to produce the GnRH/LH surge, which could involve a differential dependency on kisspeptin signaling. Additional observations also argue that kisspeptin signaling is not an absolute prerequisite to sustain some degree of activity of the hypothalamic-pituitary-gonadal axis. In these studies, a subset of animals in another independently derived line of Kiss1r knockout mice and a separate line of Kiss1 knockout mice retain the capacity to show some degree of gonadotropin release and ovarian cyclicity although lacking regularity and evidence of ovulation (Lapatto et al. 2007; Chan et al. 2009). In addition, patients with various mutations in KISS1R show variable reproductive phenotypes (even those with an identical mutation) that, in some cases, indicate a modest level of pulsatile LH secretion (Seminara et al. 2003; Tenenbaum-Rakover et al. 2007; Semple et al. 2005). However, we cannot ignore the possibility that these mutations do not completely disable the KISS1R and thus kisspeptin signaling to GnRH neurons. Taken together, these observations would propose that either some of the various mutations are not fully disabling to the KISS1R and signaling pathway or that GnRH is secreted in low levels independently of trophic activation by kisspeptin. In conclusion, there may exist some degree of GnRH/gonadotropin-dependent reproductive activity to persist in many animal models in which kisspeptin signaling would seem to be completely or partially inactivated whereas in other models, inactivation results in complete reproductive failure.

How might the hypothalamic-pitiutary-gonadal axis retain some activity even when kisspeptin signaling has been completely disabled? First, there may be a compensatory process that occurs throughout development, which drives GnRH release. Second, there may be redundancy in the circuits that drive the GnRH/LH surge, such as the neurotensin pathway in the AVPV (Alexander et al. 1989). Third, the activity of one of the kisspeptin co-transmitters could sustain some level of GnRH activity, such as glutamate or dopamine produced by Kiss1 cells in the AVPV.

Although presumably the negative feedback effect of estradiol and testosterone on GnRH secretion is mediated by kisspeptin/dynorphin/NKB-producing neurons in the ARC of the mammals studied to date, the same principle does not apply in the case of positive feedback. In the rodent, the ability of estradiol to result in a GnRH/LH surge would seem to be mediated by kisspeptin neurons in the AVPV. However in the ewe and primates, there is no homolog of the AVPV. In the ewe, the positive feedback effects of estradiol seem to be mediated by kisspeptin neurons in the rostral region of the mediobasal hypothalamus, as shown by the up-regulation of Kiss1 mRNA in the rostral ARC during the preovulatory period (Estrada et al. 2006). Thus, it appears that there are several "phenotypes" of Kiss1 neurons comprised within the MBH, one involved in negative feedback, and another implicated in positive feedback. In the case of primate species (e.g., monkey and human) it is known that the neuroendocrine mechanisms that drive the preovulatory GnRH/LH surge are also different from the rodent. According to the previous observations, there may exist different site-and species roles for kisspeptin neurons in mediating steroid hormone signaling to GnRH neurons. Figure 6 illustrates the distinct neurophysiology of the Kiss1 neurons.

Figure 6

Simplified scheme of a midsagittal section through the ventral forebrain representing the neuroanatomy of the kisspeptin–GPR54 system. Two anatomically and phenotypically distinct populations of kisspeptin neurons (green) control GnRH (red) secretion: kisspeptin neurons in the POA drive GnRH surges and kisspeptin cells in the ARC modulate the tonic pulsatile release of GnRH. Both populations of kisspeptin neurons interact with GnRH neurons in part directly. A, anterior; P, posterior; D, dorsal; V, ventral; POA, preoptic area; ARC, arcuate nucleus; OC, optic chiasm, VMH, ventromedial hypothalamus; MB, mammillary bodies; APit, anterior pituitary; PPit, posterior pituitary (Franceschini et al. 2013).

1. 1. A.4 Role of the kisspeptin system in the onset of puberty

1.A.4.a The kisspeptin system is necessary for normal puberty initiation

Mice and humans lacking a functional kisspeptin receptor do not progress normally to puberty (de Roux et al. 2003; Seminara et al. 2003). Many species demonstrate a marked increase in Kiss1 and/or Kiss1r expression to concide with the onset of puberty, suggesting that kisspeptin may act as “gatekeeper” for the initiation of puberty (Martinez-Chavez et al. 2008; Filby et al. 2008; Han et al. 2005; Li et al.

2008; Shahab et al. 2005; Navarro et al. 2004; Clarkson et al. 2009). Several reviews have focused on the role of kisspeptin in puberty (Messager et al. 2005; Tena –Sempere 2006; Kuohung et al. 2006; Seminara et al. 2006; Navarro et al. 2007; Kauffman et al. 2007; Smith et al. 2007(b)).

The role of kisspeptin in regulating the neuroendocrine reproductive axis was first brought to light by two clinical studies on subjects that had impaired or absent sexual maturation. In 2003, de Roux et al. and Seminara and colleagues independently reported that puberty was significantly impaired in humans with spontaneous mutations in the Kiss1R (GPR54) gene (de Roux et al. 2003; Seminara et al. 2003). These findings were reported in two separate consanguineous families. Different base pair deletions/substitutions had as common result a non functional Kiss1R protein in either case. An additional study, in 2005, similarly detailed an individual with pubertal impairment in the presence of a disfunctional Kiss1R mutation (Semple et al. 2005).

In addition to the initial findings in humans, the necessity of intact kisspeptin signaling for puberty initiation has since been extended to other mammalian species, mainly mice. Transgenic knockout technology has allowed for the creation of Kiss1RKO mice. Seminara et al. (2003) first showed that Kiss1RKO mice do not progress through puberty, and have severe deficits in adulthood reproductive function. This finding has been extended by other laboratories. In all cases, Kiss1R KO mice are sexually immature. They present low gonadotropin and sex steroid levels, small gonads, absent spermatogenesis, impaired ovulation and absent or impaired estrous cyclicity (Funes et al. 2003; Messager et al. 2005; Dungan et al. 2007; Kauffman et al. 2007c; Lapatto et al. 2007). Additional reports of impaired sexual maturation and reproductive deficiencies in mice lacking a functional Kiss1 gene have followed (d'Anglemont de Tassigny et al. 2007; Lapatto et al. 2007). Of note, targeted deletion of Kiss1R does not affect GnRH neuron migration or synthesis as it has been shown that Kiss1R KO mice have normal hypothalamic distribution and content of GnRH

(Messager et al. 2005). Further evidencing that the GnRH system is functional in these mice some GnRH secretagogues, such as galanin-like peptide, are able to promote GnRH and LH secretion in Kiss1R KO mice (Dungan et al. 2006).

In addition to disfunctional mutations in the Kiss1 system that prevent puberty onset, two clinical studies found that activating mutations of the Kiss1 gene or its receptor were linked to precocious puberty (Teles et al. 2008, Silveira et al. 2010). In these cases, a single base pair substitution in the Kiss1 gene or the Kiss1R gene was reported. In response to kisspeptin binding to the receptor, a prolonged intracellular signaling was noticed, thereby resulting in hyperstimulation of the reproductive axis.

Animal studies have mimicked this effect by experimentally treating prepubertal rodents or monkeys with exogenous kisspeptin. In these animals, kisspeptin treatment was found to initiate various aspects of precocious puberty (such as enhanced LH secretion or early vaginal opening) (Navaro et al, 2004a,b; Shahab et al. 2005)

1. 1. A. 4. b Changes in the neural Kiss1 system during puberty

Although it was determined in 2004 that exogenous kisspeptin treatments can induce indices of pubertal onset, it was not until 2008 that endogenous secretion of kisspeptin was found to increase during puberty. Working with hypothalamic explants from female monkeys, Keen et al. (2008) showed that neural kisspeptin secretion, as measured with radioimmunoassay, was elevated in pubertal compared to prepubertal juvenile monkeys. In addition, kisspeptin secretion was shown to be pulsatile and to pulse in synchrony with GnRH pulses. Thus, Keen et al. proposed that increased kisspeptin pulsatility results in increased GnRH pulsatility in the pubertal monkey. However, the neuroanatomical source of the pubertal kisspeptin secretion was not determined. In other wrds, it is not known whether the kisspeptin pulses were coming from the ARC Kiss1 neurons, preoptic neurons, or both (or neither). Takase et al.

(2009) have addressed this issue in developing female rats. Kiss1 mRNA levels were found to be higher in adulthood than in prepubertal animals in both the ARC and AVPV/PeN, as was Kisspeptin protein immunoreactivity. This finding in female rats contradicted that in male mice in which the ARC Kiss1 system did not appear to change with puberty (Han et al. 2005). To determine if sex steroids influence the increase in Kiss1 levels in rats, Takase et al. (2009) treated separate cohorts of female rats with constant low E2 levels at each age and measured Kiss1 mRNA levels several days later. As in intact animals, Kiss1 was found to be higher in both ARC and AVPV/PeN in adults than in prepubertal females, even when gonadal steroid levels were equal between age groups. Concequently, at least in females rats, Kiss1/kisspeptin increases with puberty in both the ARC and AVPV/PeN, and this increase is independent of changes in circulating gonadal steroids. Whether this is the case for male rats, or males and females of other species remains to be determined.

In mice, the number of kisspeptin-containing fibers that oppose GnRH neurons was reported to increase at puberty (Clarkson and Herbison, 2006). This suggests that pubertal maturation may also include the completion of developmental circuit coupling Kiss1 and GnRH neurons. However, higher gonadal steroids in adulthood would result in elevated kisspeptin synthesis in the AVPV/PeN, which could cause more kisspeptin immunoreactivity to be present. Thus, it is possible that the degree of innervation of GnRH neurons by kisspeptin neurons is not different before and after puberty, but that the technical ability to visualize these kisspeptin axonal fibers is enhanced in adulthood when more kisspeptin is being produced.

In addition to Kiss1, changes in the kisspeptin receptor, Kiss1R, may also be implicated in pubertal maturation. Low doses of Kisspeptin treatment are less effective in stimulating GnRH neuronal firing activity and gonadotropin secretion in juvenile than adult rodents, suggesting that kisspeptin has a reduced ability to activate the GnRH system before puberty (Han et al. 2005; Castellano et al.

2006a,b). In support of this, in rats of both sexes and female monkeys, but not male mice, hypothalamic Kiss1R expression is lower in juveniles than in adulthood (Navarro et al. 2004 a, b; Han et al. 2005; Shahab et al. 2005). However, in most cases, Kiss1R was only measured well before or well after puberty, not during the pubertal transition. Navarro et al. (2004a, b) measured Kiss1R levels on day 1 during the actual pubertal period and reported that total hypothalamic Kiss1R expression was higher on that first day than in both juvenile and adult rats. It is worth to say that, the elevated Kissi1R levels occurred earlier in female than male rats, corresponding with earlier puberty onset in the former. Consequently, increases in hypothalamic Kiss1R may constitute a critical aspect of the pubertal process, though more work on this subject is needed. In addition, it is possible that the ability of the Kiss1R protein to signal within GnRH neurons changes with puberty, which could be independent of changes in Kiss1R mRNA levels.

1.1.A.4.c Sexual dimorphism of kisspeptin expression

Kisspeptin expression, kisspeptin neurons, and/or serum kisspeptin have been shown to be sexually dimorphic in many species, including humans. Expression of the kisspeptin receptor has also been reported to be sexually dimorphic in rats, Rhesus monkeys and teleost fish cobia (Navarro et al. 2004 a; Shahab et al. 2005 Mohamed et al. 2007). This dimorphism has been associated to the onset of puberty and fertility in some species (Wray and Gainer 1987; Kauffman et al. 2007a,b; Homma et al. 2009; Kauffman et al. 2009; Bakker et al. 2010; Hrabovszky et al. 2010; Jayasena et al. 2011; Pita et al. 2011a). Prenatal exposure to sex steroids may be responsible for part of the sexual dimorphism in kisspeptin and lack of kisspeptin dimorphism can result in irreversible abnormalities of the sexual bexavior in some species (Kauffman et al. 2007 a,b; Gonzalez-Martinez et al. 2008). Moreover, circulating kisspeptin has been reported to be sexually dimorphic in humans. Women

having significantly more elevated kisppeptin when compared to men (Wray and Gainer 1987; Kauffman et al. 2007a,b; Hrabovszky et al. 2010; Pita et al. 2011 a,b). Additionally, in other species, such as in the adult sheep, the ARC is sexually differentiated, with ewes expressing higher numbers of Kiss1 neurons than rams. (Estrada et al. 2006; Caraty et al. 1998). In the rodent, the AVPV is sexually differentiated, being larger and with more Kiss1 neurons in the female than the male (Clarkson and Herbison 2006; Kauffaman et al. 2007; Adachi et al. 2007; Simerly 1989; Dungan et al. 2008). Because the AVPV is thought to play a critical role in relaying the positive feedback effects of estradiol to GnRH neurons (Smith et al. 2006; Dungan et al. 2007), it is not surprising that the male rodent is incapable of generating a GnRH/LH surge. Kiss1 expression of the adult rodent is organized perinatally, when neonatally androgenized females display a male-like pattern of Kiss1 expression on the AVPV in adulthood and lack the capacity to generate a GnRH/LH surge (Kauffman et al. 2007; Navarro et al. 2009). On the opposite, neonatally castrated males show a feminized pattern of Kiss1 expression in the AVPV (Homma et al. 2009). Kiss1 expression in the ARC of the adult rodent is not sexually differentiated and thus not apparently dependent upon the perinatal sex steroid milieu (Kauffman et al. 2007). However, this generalization does not apply to the prepubertal animal, where it appears that Kiss1 and NKB in the ARC are sexually differentiated. Prepubertal male, compared to female, rodents show a reduced rise in Kiss1/NKB expression in the ARC after gonadectomy and this phenomenon is associated with a more restrained postcastration rise in LH. This sexually differentiated response to castration in the prepubertal animal does not occur in the adult (Kauffman 2008). In other words, sex differences in the tempo of sexual maturation (females being earlier than males) may reflect a differential sensitivity to the steroid milieu in the prepubertal animal.

Kauffman et al. 2009 suggested that earlier puberty onset in females compared to males may reflect sex differences in the timing of the gating of peripubertal Kiss1

circuits, such that there is greater (or longer lasting) inhibition of Kiss1 gene in prepubertal males as opposed to prepubertal females. They found that, in prepubertal female mice, but not in male mice, Kisss1 levels and LH secretion in the ARC increased dramatically 2 and 4 days after gonadectomy. Moreover, adult mice of both sexes exhibited increases in both LH levels and ARC Kiss1 expression 4 days following gonadectomy. In other words sex differences in the steroid-independent regulation of ARC Kiss1 neurons and LH secretion are manifested only during peripubertal development. These findings show that the regulation of reproductive status during prepubertal development is sexually dimorphic, with males, but not females, exhibiting gonadal steroid-independent suppression of Kiss1 and LH levels. This Kiss1 sex difference may relate to known sex differences in pubertal maturation in mammals, including humans (boys usually mature later than girls) though additional data are needed to support this idea.

Recent studies have investigated the expression and /or secretion of kisspeptin in adults as well as in pubertal children. The results of these studies show sexually dimorphic differences in the expression of kisspeptin in humans as well as in serum levels. In one study, sexually dimorphic diefferences were found in the distribution and number of immunolabeled kisspeptin in hypothalamic areas in humans. Females presented heavily labeled kisspeptin in the infundibulus, whereas very few, if any, were present in males. Brain samples were obtained from healthy subjects who died of sudden death. Results were confirmed with an additional antibody from a distinct source, the analysis was blinded and the age of subjects did not influence the results (Hrabovszky et al. 2010). The homogeneity of the data in the female group was reassuring against the potential variability of unknown sex steroid levels at the time of death among subjects.

Although the source of circulating kisspeptin has not been established, experimental studies show that intravenously injected kisspeptins can significantly stimulate GnRH/gonadotropin/ steroid secretion in rats (Matsui et al. 2004; Pheng et al. 2009),

mice (Mikkelsen et al. 2009), Rhesus monkeys (Ramaswamy et al. 2007) and humans (Dhillo et al. 2005; George et al. 2011). Additionally, systemic injections of physiologically relevant concentrations of kissspeptin synchronize LH surge in cycling ewes and induces ovulation in non-cycling ewes on the anestrous season (Jayasena et al. 2010). Similarly, kisspeptin injected peripherally to women is capable of inducing desensitization of the LH response (Jayasene et al. 2009) as well as of bypassing the suppression of LH in patients affected from hypothalamic amenorrhea (Caraty et al. 2007). These observations demonstrate that circulating kisspeptins are physiologically relevant and likely to play a role in the regulation of the HPG axis in many species, including humans.

Serum kisspeptin in adult, sexually mature women was significantly elevated as opposed to adult men of similar age in two studies of different populations from distinct ethnic backgrounds (Pita et al. 2011 a,b). This reinforces the sexually dimorphic character of kisspeptin secretion.

In healthy children, serum kisspeptin is reported to positively correlate with rises in LH and testosterone during all stages of puberty in boys (Bano et al. 2009). Similarly, serum kisspeptin in pubertal girls is reported to positively correlate to bone age, peak/basal LH, and LH/FSH ratios (Rhie et al. 2011). Additionally, healthy pubertal girls from an unrelated population were reported to have significantly elevated serum kisspeptin when compared to Tanner stage -matched healthy boys, who were, in average, one year older (Pita et al. 2011a). These observations show that kisspeptin is not only a faithful indicator but an important mediator as well of onset and progression of puberty in healthy children.

Consequently, Kisspeptin signaling is essential in initiating puberty onset. However, although prepubertal kisspeptin treatments stimulate GnRH secretion before it is normally activated, it remains unclear if these treatments alter the developmental mechanisms that normally time and trigger puberty. Indeed, it is not known if kisspeptin signaling represents a key element of the puberty-triggering mechanism,

including, but not limited to, the pubertal ”clock”, or simply a downstream effector of the puberty machinery elsewhere in the brain.

1. 1. B. Overview of Neurokinin B (NKB) signaling

Several studies implicate NKB and its receptor NK3R as essential components of the human reproductive axis. Over the last two decades it was shown that a group of neurons in the hypothalamic infundibular/arcuate nucleus form an important component of this regulatory circuit. These neurons are steroid-responsive and co-express NKB, kisspeptin, dynorphin, NK3R and estrogen receptor a (ERa) in a variety of mammalian species. Important studies in the human have indicated that these neurons function in the hypothalamic circuity regulating estrogen negative feedback on gonadotropin-releasing hormone (GnRH) secretion. Loss of function mutations in the genes encoding either neurokinin B (NKB) or its receptor, (NK3R), result in hypogonadotropic hypogonadism, characterized by low circulating levels of LH and gonadal steroids and an absence of pubertal development . A detailed review on neurokinin B signaling was provided by Rance et al. in 2010 (Rance et al. 2010) and more recently by Navarro et al. in 2013 (Navarro et al. 2013). In this part we will share critical information on this subject.

1.1. B.1. Molecular biology of NKB

Neurokinin B (NKB) is a member of the tachykinin family of peptides. Tachykinins are characterized by a common C-terminal amino-acid sequence (Phe-X-Gly-Leu-Met-NH2) and include substance P, neurokinin A and NKB, as well as neuropeptide K, neuropeptide γ, and hemokin-1. NKB is the only tachykinin synthesized from the preprotachykinin-B gene (Almeida et al., 2004; Bonner et al., 1987; Helke et al., 1990; Kotani et al., 1986; Page et al., 2001) which is currently designated as TAC3 in humans, Tac3 in non-humans primates, cattle and dogs and Tac2 in rodents. The

TAC3 precursor mRNA contains 7 exons, 5 of which are translated to form the preprotachykinin B peptide. This prepropeptide undergoes enzymatic cleavage to form proneurokinin B, then NKB. The primary amino acid sequence of the final active peptide is encoded by exon 5 (Bonner et al., 1987; Kotani et al., 1986; Page et al., 2000). TAC3 precursor mRNA variants have been described, but the NKB peptide is widely conserved across vertebrates (Page et al., 2009). NKB preferentially binds to NK3R, encoded by the TACR3 gene. Three tachykinin receptors have been identified, although the existence of additional receptors has been proposed (Grant et al., 2002; Pennefather et al., 2004). The three receptors (NK1R, NK2R and NK3R) belong to the rhodopsin-like family of G-protein coupled receptors and share considerable structural homology (Almeida et al., 2004; Takahashi et al., 1992). After NKB is bound to its receptor, NK3R activation increases intracellular Ca2+ concentration through inositol phospholipids hydrolysis. Alternatively, NK3R activation can increase intracellular cAMP levels through adenylate cyclase activation (Satake and Kawada 2006). A feature of G-protein related receptors, however, is that down stream cascades of the receptor can be either excitatory or inhibitory depending on which intracellular proteins are expressed (Kenakin et al., 1995). Other G-protein coupled receptors, such as the serotonin receptor, can associate with more than one intracellular cascade (Berg et al., 1998). Thus the cellular responses arising from NK3R signaling are likely complex.

1.1. B. 2 Anatomic studies of NKB and NK3R in the hypothalamus and basal forebrain

The location of neurons expressing NKB mRNA has been mapped in detail in the human hypothalamus and basal forebrain (Chawala et al., 1997). NKB mRNA-expressing neurons are present predominantly in the infundibular nucleus and the anterior hypothalamic area. Magnocellular neurons in the septal region, diagonal

band of Broca and nucleus basilis of Meynert also express NKB mRNA. The bed nucleus of the stria terminalis and the amygdala are also prominent sites of NKB mRNA-containing cell bodies and scattered neurons are localized in the adjacent neocortex (Chawala et al., 1997). A major population of NKB neurons resides in the arcuate nucleus of the rat. Small numbers of NKB neurons are also scattered throughout the anterior, lateral and dorsomedial hypothalamus and preoptic regions (Krajewski et al., 2010). NKB neurons have also been described in the arcuate nucleus of the monkey (Abel et al.,1999; Ramaswamy et al., 2010), sheep (Foradori et al., 2006; Goodman et al, 2007), goat (Wakabayashi et al., 2010) and mouse (Duarte et al., 2006; Navarro et al., 2009). Many of the regions receiving arcuate NKB projections in the rat also express NK3R (Ding et al.,1996; Krajewski et al., 2005). Moreover, NK3R neurons are present in the zona incerta and the lateral hypothalamic area and perifornical region. Magnocellular neurons of the paraventricular nucleus, suppraoptic nucleus and accessory magnocellular nucleus are intensely labeled with NK3R antibodies. There are also magnocellular NK3R neurons in the septal nuclei, diagonal band and nucleus basalis (Krajewski et al., 2005).

1.1.B.3 Estrogen modulation of NKB gene expression

Early studies demonstrated that neurons in the rat arcuate nucleus concentrate radioactive estradiol after intraperitoneal injections (Pfaff and Keiner 1973). Later on, two isoforms of estrogen receptor were identified, ERα and ERβ. Studies using knockout mice have shown that ERα is critical for estrogen negative feedback on LH release (Dorling et al., 2003; Hewitt and Korach et al., 2002). However, GnRH neurons have only been shown to express ERβ, suggesting that estrogen negative feedback is mediated via a separate set of neurons (Hrabovsky et al., 2001). NKB and ERα co-expression has been localized in the arcuate nucleus of humans (Rance

and Young et al., 1991), sheeps (Goubillon et al., 2000) and rats (Burke et al., 2006), indicating that estrogens could act directly on arcuate NKB neurons. Additionaly, progesterone receptors have been identified on these neurons in the ewe (Foradori et al., 2002). The transcription of NKB could be directly altered by estrogen receptors, as sequences corresponding to the estrogen response element (ERE) and the imperfect palindromic ERE have been reported upstream of the TAC3 gene transcription start site (Page et al. 2001). Experiments in multiple species have documented sex steroid modulation of the gene encoding NKB in the arcuate nucleus. NKB gene expression changes during the estrous cycle of the rat (Rance and Bruce 1994). Gonadectomy increases NKB gene expression in arcuate nucleus of female rats (Race and Bruce 1994), mice (Kauffman et al., 2009; Navarro et al., 2009) and monkeys (Gelid et al., 2010). Orchidectomy increases NKB gene expression in the arcuate nucleus of male rats (Danzer et al., 1999) and mice (Kauffman et al., 2009). Moreover, estrogen treatment of gonadectomized animals suppresses NKB gene expression in rats, of both genders (Rance and Bruce 1994; Danzer et al., 1999), monkeys (Abel et al., 1999), sheep (Pillon et al., 2003) and mice (Dellovade and Merchenthaler 2004; Navarro et al., 2009). Finally, the suppression of NKB gene expression by estradiol does not occur in ERa knockout mice.(Dellovade and Merchenthaler 2004).

1.1.B.4 Sexual dimorphism of NKB neurons

Although gonadectomy and steroid replacement modify NKB gene expression in both sexes (Danzer et al., 1999; Rance and Bruce 1994), gender differences exist in the number and morphology of NKB neurons. Prenatal testosterone treatment of ewes results in a male-type pattern, indicating an organizational effect of androgens early in development. Increased numbers of NKB neurons have been identified in the

arcuate nucleus of ewes compared to rams (Cheng et al., 2010; Goubillon et al., 2000). In the rat male, there are dense projections of NKB axons around blood vessels in the median eminence, as opposed to a more diffuse distribution in the female. This morphology is altered by treatment with gonadal steroids even after the postnatal period (Ciofi et al., 2006; Ciofi et al., 2007). Finally, the response of NKB neurons to gonadectomy before puberty is gender dimorphic. Prepubertal female mice exhibit increased NKB (and Kisspeptin) gene expression in the arcuate nucleus in response to gonadectomy, while this response is delayed until after puberty in male mice (Kauffman et al., 2009).

1. 1. C. An interconnected network of arcuate NKB/kisspeptin/dynorphin neurons

First showed in the ewe (Goodman et al., 2007), kisspeptin (or its mRNA) is co-expressed with NKB in the actuate nucleus in the rat (Kirigiti et al., 2009), mouse (Navarro et al., 2009) and goat (Wakabayashi et al., 2010). Additionally, NKB and kisspeptin mRNAs are co- expressed in the infundibular (arcuate) nucleus of the human (Rance and Young 1991; Rometo et al., 2007) and monkey (Abel et al., 1999; Rometo et al., 2007). This fact is further supported by the recent immunohistochemical demonstration of kisspeptin and NKB co-localization in the human (Hrabovszky et al., 2010) and monkey (Ramaswamy et al., 2010) infundibular nucleus. Dynorphin is an endogenous opioid peptide involved in progesterone negative feedback on GnRH release in the luteal phase of the ewe (Goodman et al., 2004). Dynorphin is co-expressed with NKB in the arcuate nucleus of the rat (Burke et al., 2006; Ciofi et al., 2006), ewe (Foradori et al., 2006), mouse (Navarro et al., 2009) and goat (Wakabayashi et al., 2010). Coexistence of multiple peptides in a single neuron is a common finding in the central and peripheral nervous system.

Typically, two or more peptides are stored together in large dense core vesicles and may be differentially released depending on their relative synthesis (Salio et al., 2006). Through a series of studies, a bilateral network of NKB neurons within the arcuate nucleus in the female rat has been described that project to GnRH axons in the median eminence as well as in the contralateral arcuate nucleus (Burke et al., 2006; Krajewski et al., 2005; Krajewski et al., and 2010). There is also evidence of a similar network of arcuate NKB/kisspeptin/dynorphin neurons projecting to the median eminence of the monkey (Ramaswamy et al., 2008; Ramaswamy et al., 2010), mouse (Navarro et al., 2009), sheep (Foradori et al., 2006; Lehman et al., 2010) and goat (Wakabayashi et al., 2010).

Within the arcuate nucleus there is a dense network of NKB/dynorphin axons and apposition of these axons on NKB/dynorphin cell bodies and dendrites that is indicative of communication between these neurons (Burke et al., 2006). An important feature of the arcuate nucleus is the presence of NK3R on NKB/kisspeptin/dynorphin neurons (Burke et al., 2006). This feature has been shown in the rat (Burke et al., 2006), mouse (Navarro et al., 2009) and ewe (Amstalden et al., 2009). Bilateral connections among arcuate NKB/Kisspeptin/dynorphin/ ERα neurons via NK3R may provide an anatomic framework for coordinated activity of this important neuronal network. From the arcuate nucleus, NKB fibers project to both the internal and external zones of the median eminence, including the lateral palisade zone, a site with dense GnRH terminals (Krajewski et al., 2005; Krajewski et al., 2010). Using electron microscopy, Ciofi et al. showed direct apposition between NKB fibers and GnRH axons in the median eminence without synaptic specializations (Ciofi et al., 2006). NKB would be expected to be released by large dense core vesicle exocytosis, a mode of neurotransmission that does not require the presence of classic synapses (Salio et al., 2006).

1. 1. C. 1 Evidence for NKB/kisspeptin/dynorphin neurons in the arcuate nucleus for the modulation of pulsatile GnRH secretion

Even if GnRH neurons generate pulsatile activity, this would not exclude the possibility of an external oscillator in the arcuate nucleus that relays information from sex-steroids to influence pulsatile GnRH secretion (a GnRH pulse modulator). Modulation of GnRH neurons via external sources might also need to be pulsatile or at least coordinated, to ensure synchronized GnRH secretion. There are currently several lines of evidence that arcuate NKB/kisspeptin/dynorphin neurons could modulate pulsatile GnRH secretion, and be the source of the multiunit volleys of electrical activity that are timed with pulses of serum LH (Rance et al. 2010):

1. NKB/kisspeptin/dynorphin neurons are located in the arcuate nucleus where the multiunit volleys of activity are present in monkeys (Knobil, 1981), rats (Kimura et al., 1991; Kinsey-Jones et al., 2008) and goats (Maeda et al., 1995; Okhura et al., 2009; Wakabayashi et al., 2010).
2. Destruction of the arcuate nucleus in the rhesus monkey (with relative preservation of more lateral GnRH neurons) abolishes spontaneous and estrogen-induced LH secretion (Plant et al., 1978; Plant and Ramaswamy, 2009).
3. NKB/kisspeptin/dynorphin neurons in the arcuate nucleus of the rat form bilateral interconnected network that could provide an anatomical framework for the coordination and synchronization of activity (Burke et al., 2006; Krajewski et al., 2010).
4. NKB/kisspeptin/dynorphin neurons in the arcuate nucleus project to GnRH terminals in the median eminence in the rat (Krajewski et al., 2005; Krajewski et al., 2010), monkey (Ramaswami et al. 2008) and sheep (Lehman et al., 2010), an ideal location for final modulation of GnRH output (Moenter et al., 2003).

5. The expression of NKB and kisspeptin mRNAs in arcuate neurons and the frequency of multiunit activity and LH pulses (Mori et al., 1991; Nishihara et al., 1999; Wakabayashi et al., 2010) are modulated in a similar direction by ovariectomy and steroid replacement.
6. There is strong evidence that progesterone inhibition of LH pulse frequency in the luteal phase of the ewe is mediated by NKB/kisspeptin/dynorphin. Neurons in the arcuate nucleus (Goodman et al., 2004; Lehman et al., 2010).
7. In monkeys, the duration of each multiunit volley increases over a 4-6 week period after ovariectomy (O'Byrne et al., 1993) consistent with a time-course of cellular remodeling in the form of neuronal hypertrophy (Rance et al., 1990; Rance and Young, 1991). On the other hand, estradiol treatment of ovariectomized monkeys reduces the duration of multiunit activity within hours (O'Byrne et al., 1993), consistent with the time course that estradiol suppresses arcuate NKB mRNA in the ewe (Pillon et al., 2003).
8. Kisspeptin is released in a pulsatile manner into stalk-median eminence of female rhesus monkeys and these pulses occur with the majority of GnRH pulses (Keen et al. 2008). Based on morphological descriptions, the kisspeptin released in the stalk-median eminence of monkeys is likely secreted from NKB/kisspeptin-co-expressing neurons in the arcuate nucleus (Ramaswamy et al., 2008; Ramaswamy et al., 2010; Rometo et al., 2007; Sandoval-Guzman et al., 2004) and could have direct effects on GnRH axons at the level of the median eminence (d'Anglemont de Tassigny et al., 2008).
9. The frequency of multiunit volleys and pulsatile LH secretion are altered by infusions of NKB or dynorphin into the arcuate nucleus of the goat (Wakabayashi et al., 2010), consistent with the identification of NK3R (Amstalden et al., 2009; Burke et al., 2006; Navarro et al., 2009) and kappa opioid receptor mRNA (Navarro et al., 2009) on arcuate NKB/kisspeptin/dynorphin neurons.

10. Patients with hypogonadotropic hypogonadism due to TAC3 and TACR3 mutations exhibit low levels of serum LH but normal, or nearly normal, levels of FSH (Topaloglu et al., 2009; Young et al., 2010). This profile of gonadotropin secretion can be simulated by changing the pattern of pulsatile GnRH secretion (Wildt et al., 1981; Wise et al., 1979; Young et al., 2010).
11. In contrast to the normal frequency and low amplitude LH pulses detected in patients with kisspeptin receptor gene mutations (Tenenbaum-Rakover et al., 2007), no LH pulses were reported in untreated patients with TAC3 and TACR3 mutations (Gianetti et al. 2010; Young et al., 2010). After gonadal steroid treatment of patients with TAC3 and TACR3 mutations, despite some recovery of function, LH pulse frequency is slow or irregular (Gianetti et al., 2010). Thus, it was hypothesized that loss of functional NK3R signaling on arcuate NKB/kisspeptin/dynorphin neurons leads to alterations in the frequency of LH pulses due to dysfunctional coordination of the arcuate nucleus network. Figure 7 recapitulates the role of *Kiss1/Dyn/NKB* neurons in the generation of the GnRH pulses.

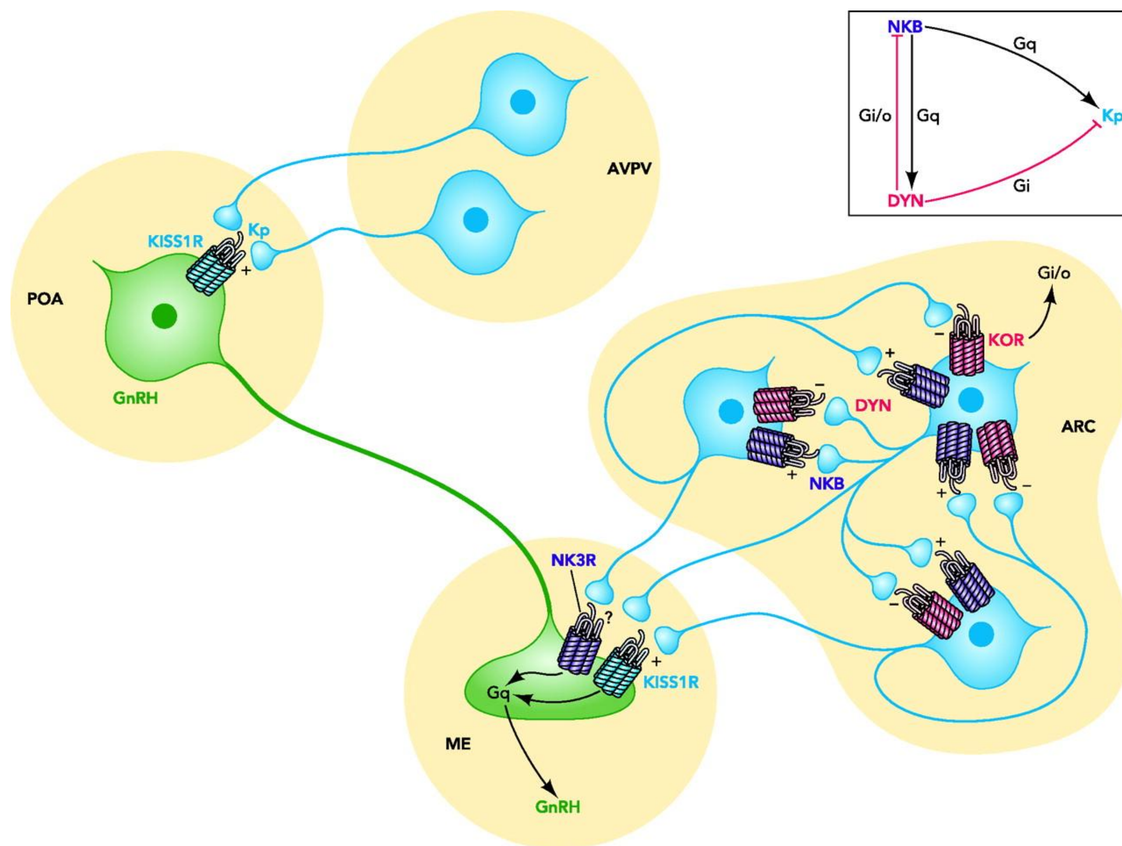


Figure 7

According to this model, *Kiss1/Dyn/NKB* neurons receive autosynaptic input from NKB and Dyn and target GnRH fibers in the ME, which are responsive to kisspeptin and NKB (through Kiss1r and NK3, respectively). When E_2 levels decline, *Kiss1/Dyn/NKB* neurons become spontaneously active. This activity would be amplified by positive autosynaptic feedback through NKB/NK3 signaling, which would also propagate by collaterals to trigger synchronized firing in the ensemble of *Kiss1/DYN/NKB* neurons in the Arc. DYN which would be released along with NKB, would act on *Kiss1/DYN/NKB* neurons (or interneurons that express KOR) with a small phase lag to clamp further discharge from *Kiss1/Dyn/NKB* neurons and thus extinguish their activity. In the absence of additional activity, Dyn release would cease, and eventually the inhibitory effect of Dyn would wane, causing the *Kiss1/Dyn/NKB* neurons to reactivate and initiate another cycle of regenerative activity, followed by inhibition. Each time *Kiss1/Dyn/NKB* neurons would undergo a burst of activity, a “pulse” of kisspeptin, Dyn, and NKB would be released in the ME, where kisspeptin and NKB would act directly on GnRH fibers or terminals. It is unlikely that Dyn acts directly on GnRH neurons, because GnRH neurons apparently do not express KOR. Kisspeptin would evoke prolonged activation of GnRH fibers or terminals, which, if unchecked, would last for hours. Thus, some mechanism must subsequently inactivate GnRH neurons so that a discrete pulse of GnRH can be delivered into the portal circulation. It is postulated that NKB acts via NK3 on GnRH fibers or terminals to accomplish this task (Franceschini et al. 2013).

1.1. C. 2 Pharmacological studies of the effects of NK3R agonists on LH secretion

As reviewed in detail by Rance et al. in 2010 (Rance et al. 2010) pharmacological administration of NKB or NKR3 agonists has resulted in disparate effects on LH secretion, depending on the animal model and gonadal status. Initial studies showed that intraventricular injections of senktide, a potent and selective NK3R agonist, importantly decreased serum LH in ovariectomized rats treated with very low levels of estradiol (Sandoval-Guzman and Rance, 2004). Inhibitory effects on LH secretion have also been noticed after intraventricular injection of senktide in ovariectomized mice (Navarro et al., 2009) and direct injection of NKB into the arcuate nucleus of ovariectomized goats (Wakabayashi et al., 2010). On the other hand, senktide stimulated LH secretion in the ewe during the follicular phases but not during the luteal phase (Billings et al., 2010). Moreover, stimulation of LH secretion was found in prepubertal rhesus monkeys after intravenous infusion of either NKB or senktide (Ramaswamy et al., 2010). NKB also modulates the effects of kisspeptin on LH release. For example, intraventricular co-administration of NKB and kisspeptin amplified kisspeptin's stimulatory effects in male rodents while co-administration of NKB and kisspeptin to mouse hypothalamic explants inhibited kisspeptin's positive effect on GnRH secretion (Corander et al., 2010). Thus, the effects of pharmacological administration of NKB or senktide are complex and contradictory. This complexity contrasts with kisspeptin, which consistently stimulates LH secretion in a wide variety of mammalian species and experimental settings (Dhillo et al., 2006; Han et al., 2005; Plant et al., 2006; Thompson et al., 2004). While administration of NK3R agonists clearly alters LH secretion, the interpretation of these studies is complicated by many aspects. First, senktide is a potent and selective NK3R agonist, but NKB binds to other tachykinin receptors (Laufer et al., 1986; Pennefather et al., 2004). Second, there may be species differences in the efficacy of pharmacological

agents (Leffler et al., 2009) and in the location of NK3R (Amstalden et al., 2009; Krajewski et al., 2005). Third, the steroid environment could alter the basal activity of the neurons, relative levels of gene expression and the number or responsiveness of receptors (Kelly et al., 2003). Finally, endogenous neuropeptides are released in specific spatial and temporal patterns, while intraventricular infusion of a pharmacological agent could simultaneously interact with NK3R at numerous sites at nonphysiologic concentrations. For NK3R agonists in particular, there could be direct effects on GnRH neurons, as well as indirect effects via the arcuate nucleus that could modulate the pattern of GnRH secretion and thereby alter the responsiveness of the anterior pituitary gland.

In summary, there is strong evidence for proposing that arcuate (infundibular) NKB/kisspeptin/dynorphin neurons are part of the neural network influencing the pulsatile secretion of GnRH and contribute to the multiunit activity known as the "GnRH pulse generator". This hypothesis does not exclude GnRH neurons in generating pulses or a role of other neurons within the arcuate nucleus or elsewhere. It must be emphasized that there is currently no definitive demonstration of the source of multiunit activity and thus, these concepts are speculative. It is exciting, however, to have substantial clues on a cellular identity of the multiunit activity, and these data are currently being used to develop models of how GnRH pulses are generated (Lehman et al., 2010; Navarro et al., 2009; Okamura et al., 2010; Wakabayashi et al. 2010). Of note, if this theory is correct, then NK3R signaling on arcuate NKB/kisspeptin/dynorphin neurons may represent an integral part of network coordination (Amstalden et al., 2009; Burke et al., 2006; Navarro et al., 2009; Wakabayashi et al., 2010). Since pulsatile GnRH secretion is essential for normal reproductive function, dysfunctional coordination of the NKB/kisspeptin/dynorphin network may explain the etiology of hypogonadotropic hypogonadism and of idiopathic central precocious puberty.

1.2 Clinical manifestations and age references of normal puberty

Although the concerns about sexual precocity and changes in timing of puberty appear to be much greater in girls than in boys, these issues must be addressed in both sexes in a comprehensive and comparative perspective.

The first sign of the onset of puberty in females is thelarche, otherwise breast development. Menarche, the occurrence of first menstruation is the last characteristic in the development of puberty. Thelarche is defined as Tanner B2 stage that normally occurs between 8 and 13 years of age (Tanner 1962). The Tanner stages provide semi-quantitative information with less accuracy than using the menarcheal age to access the timing of pubertal development. Menarche, however, is a relatively late marker of female puberty and might provide information different from breast budding because the former is the end point of a complex sequence of maturational events, whereas the later results more simply from the onset of estrogenic action reflecting hypothalamic-pituitary-ovarian axis stimulation. In addition, there are possible confounding factors that can explain that the ages at the onset of breast development and at menarche are not strictly correlated (de Ridder et al. 1992). Because food and/or energy availability influence sexual maturation (Van Wieringen 1978; Warren 1983) and are unequally distributed around the world, the age limits for puberty should be discussed separately in well-off and underprivileged conditions. Data published during the last 20yr on the age at onset of breast development and menarche in different European countries, are represented in Figure 8.

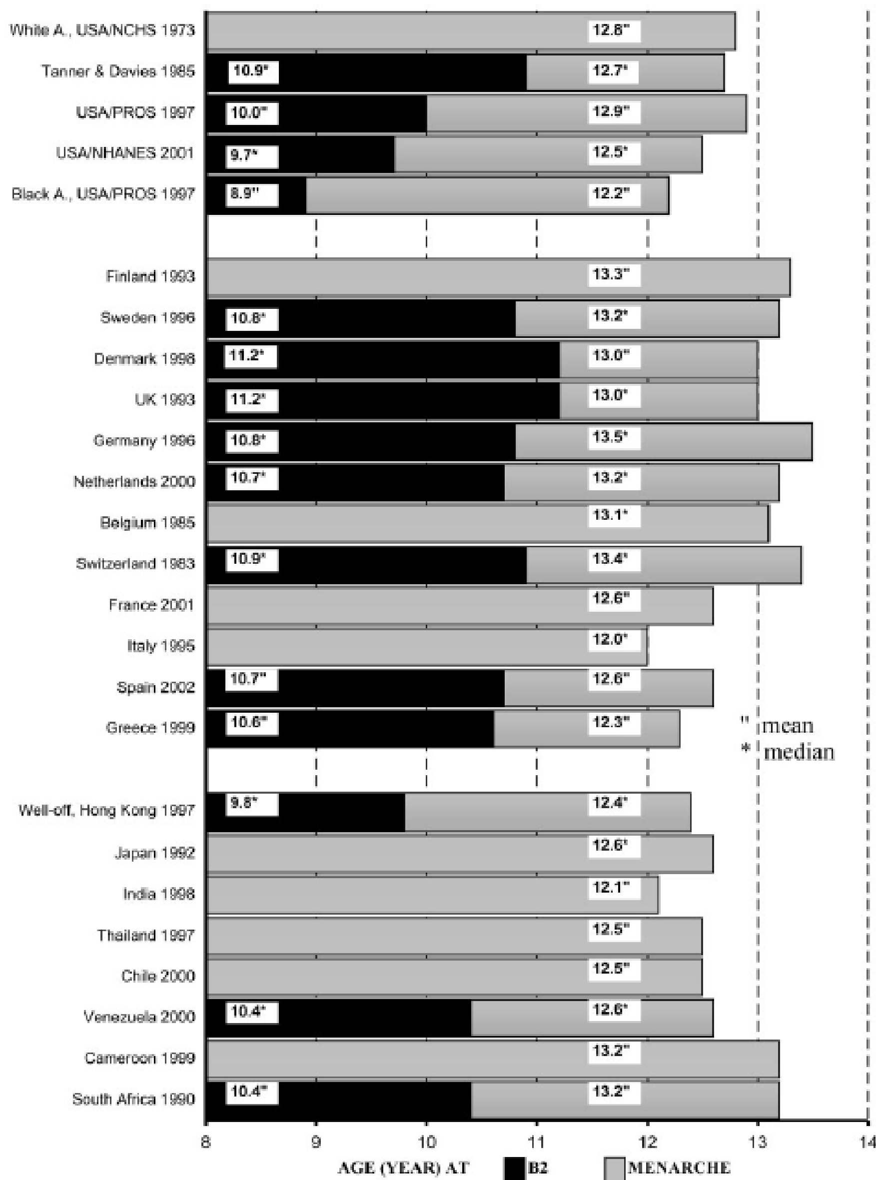


Figure 8

Average (mean or median) ages at onset of breast development (B2) or menarche in different well-off populations around the world (Parent et al. 2003).

In boys, the first sign of pubertal development is an increase in testicular volume above 3 ml, consistent with Tanner G2 stage that normally ranges from 9 to 14 years

and can be assessed only by thorough evaluation at physical examination (Tanner 1962; Marshall and Tanner 1970). However, most data from all around the world were obtained based on visual inspection without palpation of testicular volume or assessment of testicular size. For instance, in the pioneering work of Marchall and Tanner (Marshall and Tanner 1970) which provided age references for male pubertal development in 1970, the mean age for G2 stage was found to be 11.6 yr in UK. Similar data have been reported for the US (11.5 yr) in 1985, Sweden (11.6 yr) in 1996, The Netherlands (11.5 yr) in 2001, Switzerland (11.2 yr) in 1983, Spain (12.3 yr)in 2002 (Tanner and Davies 1985; Lindgren 1996; Mul et al. 2001; Largo and Prader 1983). In a longitudinal study of 78 boys, Roche et al. (Roche et al. 1995) reported a mean age of 11.3 yr at G2, but the validity of these data may be limited because of self-assessment. In a more recent study analyzing the data collected and evaluated biannually in 1112 Turkish school children aged from 8 to 18 years showed that mean age at onset of puberty was 11.6 +/- 1.2 years (Bundak et al. 2007). In a recent Greek study (Papadimitriou et al. 2008) the median age at onset of breast development (B2) was at 10 years (9,2; 10,6) for girls whereas the median (95% CI]) age for boys at G2, defined as testicular volume (TV) ≥ 4 mL, was 11.3 (10.9-11.6) years (Papadimitriou et al. 2011). On the other hand in urban Chinese boys (Ma et al. 2011) the median age of onset of puberty defined as the age at attainment of testicular volume of 4mL or greater was 10.55 (95% CI 10.27-10.79) years. In a Danish study (Sørensen K, 2010) onset of puberty, defined as age at attainment of testicular volume above 3 ml, occurred significantly earlier in 2006-2008 [11.66 yr (11.49-11.82); mean (95% confidence interval)] than in 1991-1993 [11.92 yr (11.76-12.08); P = 0.025]. Thus, it is difficult to draw conclusions without additional data from a prospective study with assessment of testicular volume or size.

1. 3 Possible mechanisms of Variation in Timing of Puberty

Puberty is determined by an increase in the secretion of the pituitary gonadotropins, LH and FSH, which are dependent on the frequency and amplitude of pulsatile GnRH neurosecretion from the hypothalamus (Grumbuch and Styne 1998; Terasawa and Fernandez 2001).The timing of puberty can be influenced by signals involving neurotransmitters and neuropeptides that originate in the hypothalamus. Additionally, signals linked to the environment such as intrauterine conditions, nutrition, endocrine disruptors might impinge on the hypothalamic signaling network directly or through peripheral signals (fig. 9).

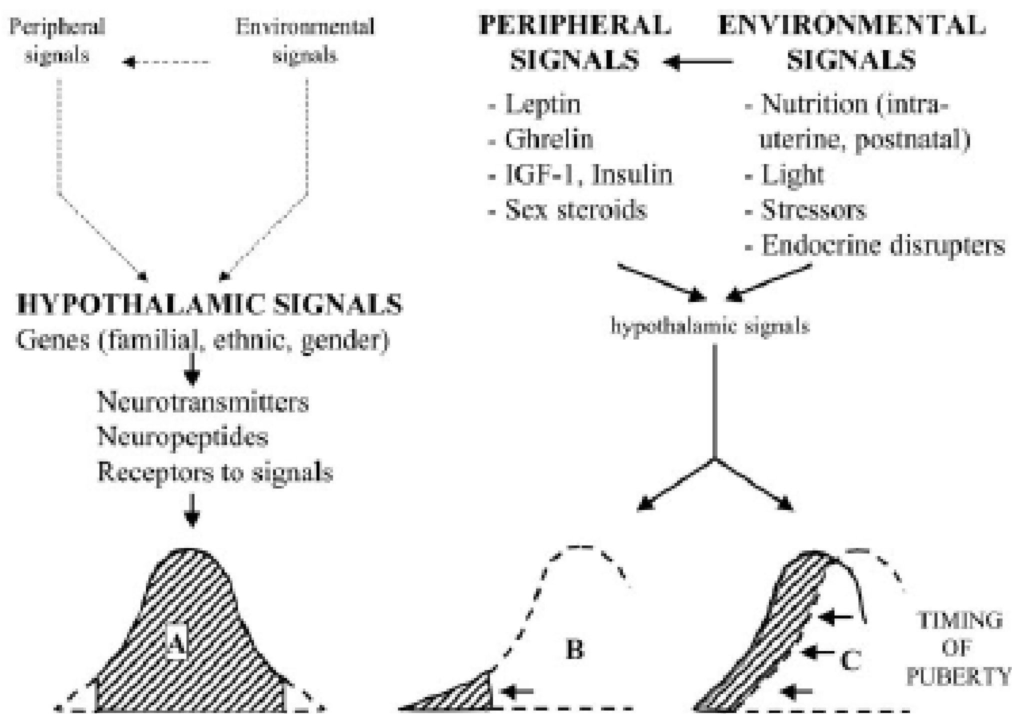


Figure 9

Schematic illustration of the relative influences of hypothalamic, peripheral and environmental signaling on the physiological variability in timing of puberty (A) or the occurrence of a subset with sexual precocity (B) or the shift of the whole study population toward early pubertal timing (C) (Parent et al. 2003).

As summarized schematically in figure 10, the individual variability, which involves familial, ethnic and gender patterns, is likely to depend on the genetic control of the

expression of signals or signal receptors in the hypothalamus. This process is only slightly influenced by peripheral and environmental signals, which play an essential permissive role in those conditions. In specific situations, however, these peripheral and environmental signals may play a crucial role in the occurrence of either abnormal precocious or delayed puberty.

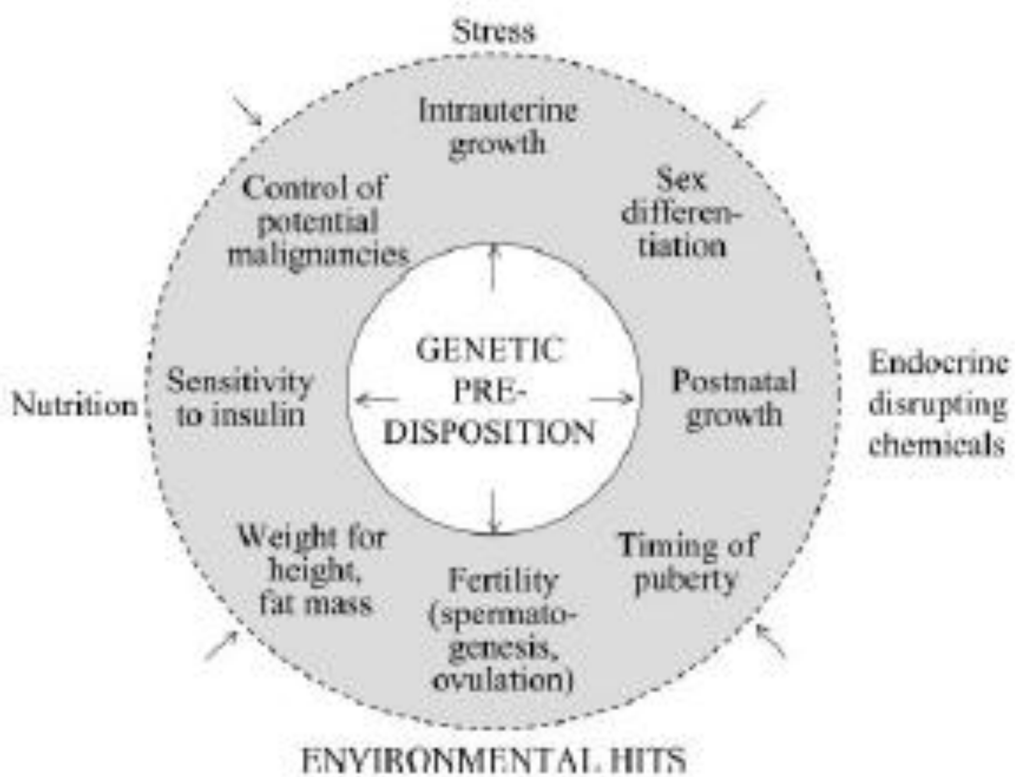


Figure 10

Integration of timing of puberty within a spectrum of processes that are influenced by both genetic and environmental factors (Parent et al. 2003)

1.3.a Genetic Factors

Puberty is an important developmental stage. The timing of puberty varies greatly in the general population and is influenced by genetic factors (Parent et al. 2003; Palmert and Hirschhorn 2003; Demerath et al. 2004; Euling et al. 2008). Early observations (Zacharias and Wurtman 1969; Fischbein 1977) derived from monozygotic twin correlation studies indicated that 70-80% of the variance in pubertal timing can be explained by or genetic factors. Kaprio et al (Kaprio et al. 1995) used a bivariance twin ANOVA in age and BMI and concluded that 74% of the variance involved genetic effects and 26% environmental effects. In a review article, Palmert and Boepple (Palmert and Boepple 2001) suggested that the genetic control of the variance in pubertal timing was likely to be a complex polygenic trait.

The high correlation of the onset of puberty seen within racial/ethnic groups, within families and between monozygotic as opposed to dizygotic twins all provide evidence for genetic regulation of pubertal onset. These data suggest that 50-80% of the variation in pubertal onset is determined by genetic factors (Parent et al. 2003; Palmert and Hirschhorn 2003; Towne et al. 2005; van den Berg and Boomsma 2007).

“Mistakes of nature” have contributed to the unraveling of the networks implicated in the normal initiation of puberty. Genes that have been proved to be involved in the normosmic Idiopathic Hypogonadotropic Hypogonadism (nIHH), in the X-linked or autosomal forms of Kallmann syndrome (KS), in the obesity and Hypogonadotrophic Hypogonadism (HH), in precocious puberty and in the abnormal Hypothalamic Pituitary Gonadal (HPG) axis development have been identified (GNRH1, GNRHR, GPR54, FGFR1, FGF8, PROK2, PROKR2, TAC3, TACR3, KAL1, PROK2, PROKR2, CHD7, LEP, LEPR, PC1, DAX1, SF-1, HESX-1, LHX3 and PROP-1). All of these genes were found to play critical roles in the development and regulation of the HPG

axis (Herbison 2007; Bianco and Kaiser 2009). Recently, the first two loci associated with population variation in the timing of puberty were identified at 6q21, in or near LIN28, and at 9q31.2 (Perry et al. 2009). Human genetics has been a powerful contributor to the discovery of molecular elements critically involved in the embryonic migration of GnRH neurons as well as in the secretion, regulation and action of hypothalamic GnRH. At this point we will review main genes proved to be implicated in the GnRH-mediated pathway initiating puberty.

- *GNRH1 and GNRHR*

Gonadotropin-releasing hormone (GnRH) is the master hormonal regulator of the reproductive endocrine system, largely controlling the pulsatile secretion of luteinizing (LH) and follicle stimulating hormone (FSH) from pituitary. These gonadotropins then lead to steroidogenesis and gametogenesis from the gonads, culminating in secondary sexual characteristics development and fertility.

The human GnRH is a decapeptide produced by a small number of GnRH neurons in the preoptic area. The GnRH neurons originate in the olfactory placode and undergo a remarkable axophilic migration along the scaffold of olfactory, vomeronasal and terminal nerves into the forebrain. Ultimately, the GnRH neurons dissociate from their olfactory guiding fibers to reach the preoptic area, where their axons extend into the median eminence. These complex developmental events are tightly regulated by specific spatiotemporal expression patterns of adhesion molecules, growth factors, and attractants and repellents (Gonzalez-Martinez et al. 2004).

The human GnRH receptor (GNRHR) gene, located at 4q13.2-3, encodes a 328 amino acid G protein-coupled receptor with seven transmembrane domains and an extracellular amino terminus, but no intracellular carboxy terminus. Ligand binding results in activation of phospholipase C, with increased inositol triphosphate (IP3) production and intracellular calcium mobilization (Kakar et al. 1992).

- *Kisspeptin (KISS1) and and KISS1 receptor (GPR54 or KISS1R)*

Kisspeptin and its receptor (KISS1R or GPR54) have emerged as the gatekeepers of puberty onset and the reproductive function, as already described (Han et al. 2005, 2007). The human KISS1R gene, located at 19p13.3, encodes a 398-amino acid heptahelical G-protein-coupled receptor with homology to the galanin receptor family

(Lee et al. 1999). Kisspeptin binding to the KISS1R results in coupling to the Gaq/11 pathway, with activation of phospholipase C, leading to IP3 production and intracellular calcium mobilization (Ohtaki et al. 2001; Kotani et al. 2001; Muir et al. 2001; Castano et al. 2008). In the central nervous system, Kisspeptin expression is highest in the arcuate (ARC) and anteroventral periventricular (AVPV) nuclei, sends projections to the media preoptic area, where there is an abundance of GnRH cell bodies, whereas KISS1R is expressed in the surface of GnRH neurons (Smith and Clarke 2007). Physiological and pharmacological studies conducted in humans and animal models showed that Kisspeptin is the most potent known stimulator of GnRH-dependent LH secretion (Gottsch et al. 2004a). Low doses of Kisspeptin, administrated to rodents, primates or humans, either centrally or peripherally, are capable to trigger a robust GnRH-dependent gonadotropin release (Gottsch et al. 2004b; Matsui et al. 2004; Thompson et al. 2004; Dhillo et al. 2005; Messager et al. 2005; Navarro et al. 2005; Shahab et al. 2005).

- *TAC3 (Neurokinin B) and TACR3 (Neurokinin B Receptor)*

Neurokinin B, as already described, is a member of the substance-P-related tachykinin family highly expressed in the hypothalamus arcuate nucleus, where it co-localizes with estrogen receptor-α. In rodents, neurokinin B and GnRH axons are in close anatomical apposition within the median eminence, and neurokinin B receptors have been identified on GnRH-expressing neurons, suggesting a role at the level of the hypothalamic GnRH release (Krajewski et al. 2005). In addition, both neurokinin B and its receptor are co-expressed in neurons that express Kisspeptin and dynorphin further emphasizing the role of kisspeptin in the regulation of pubertal timing (Goodman et al. 2007).

- *KAL1*

This gene encodes an extracellular 680-amino-acid protein called anosmin-1, which contains a cystein-rich region, a whey acidic protein domain, four fibronectin-like type III repeats that are homologous to cell adhesion molecules, and several predicted heparin sulfate binding regions (Franco et al. 1991). During development, anosmin-1 is expressed in basement membranes of developing olfactory bulb, retina and kidney (Duke et al. 1995)). Anosmin-1 is involved in the control of different cell functions, including cell adhesion, neuro/axonal elongation and fasciculation, epithelial morphogenesis as well as in the migratory activity of GnRH neurons (Soussi-Yanicostas et al. 2002; Bulow et al. 2002).

- *Fibroblast growth factor 1 receptor (FGFR1) and FGF8*

In the recent years it has been hypothesized that FGF signaling is critical for the proper formation and maintenance of a functional GnRH system and that it can be modulated by anosmin-1 (Gonzalez-Martinez et al. 2004). The FGFR1 gene, located at 8p11.1, has been considered a potential causative gene for Kallmann syndrome, following characterization of patients with contiguous syndromes (Vermeulen et al. 2001; Dode et al. 2003). The FGFR1 is a member of the fibroblast growth factor receptor family containing three extracellular Ig-like domains, one acid box domain, one transmembrane domain, and two intracellular tyrosine-kinase domains. It is expressed in multiple embryonic tissues and organs such as skeletal tissue, inner ear, and rostral forebrain (Pirvola et al. 2002; Rice et al. 2003). FGFR1 signaling is achieved by receptor conformational changes upon ligand binding, resulting in dimerization and subsequent activation by autophosphorylation of the tyrosine-kinase

intracellular domains. Heparin sulfate proteoglycan (HSPG) binding is essential for the dimerization and activation of the FGF-FGFR complex (McKeehan and Kan et al. 1994; Ibrahimi et al. 2004). Several lines of evidence hypothesized that FGF signaling is critical for the proper formation and maintenance of a functional GnRH system, and it can be modulated by anosmin-1 (Gonzalez-Martinez et al. 2004).

Additionally, the fibroblast growth factor 8 (FGF8) was considered as a key ligand of the FGFR1 in the ontogenesis of GnRH neurons. It was initially suspected by the observation that FGFR1 mutated receptors have dramatically reduced affinity for FGF8 (Pitteloud et al. 2007). Subsequently, experimental results of Chung et al. (2008) clearly indicated that the heterozygous state of FGF8 hypomorphic mice alone is sufficient to cause significant disruption of GnRH neuronal development.

- *Leptin and Leptin receptor (LEPR)*

While there is no doubt that leptin plays a role in the onset of puberty, current evidence is not enough to define the precise nature of this effect. Some argue that the initiation of puberty in humans (Frish and Revelle 1970) and rodents (Kennedy and Mitra 1963) would require a critical fat mass, and that the resulting increase in fat produced leptin would be the signal to initiate puberty once this critical mass is achieved (Barash et al. 1996; Chehab et al. 1996). The fact that injection of leptin accelerates puberty in normal female mice would support the requirement of a critical fat mass for the onset of puberty (Ahima et al. 1997; Chehab et al. 1997).

- *Prokineticin 2 (PROK2) and PROK Receptor (PROKR2)*

Prokineticins are secreted bioactive proteins that regulate several biological processes, including olfactory bulb morphogenesis and reproduction. Masumoto et al. in 2006 (Masumoto et al. 2006) have shown that the activation of the prokineticin

receptor-2 (PROKR2), a G protein-coupled receptor, is essential for the normal development of the olfactory bulbs and sexual maturation.

- *Nasal embryonic LHRH factor (NELF)*

Nasal embryonic LHRH factor (NELF) has been hypothesized to participate in the migration of GnRH and olfactory neurons into the forebrain. However, the biological functions of NELF, which has no homology to any human protein, remain largely elusive. Although mRNA expression did not differ, NELF protein expression has been described to be greater in migratory than postmigratory GnRH neurons. Pituitary Nelf mRNA expression was also observed and increased 3-fold after exogenous GnRH administration. NELF has been shown to display predominant nuclear localization in GnRH neurons and NELF knockdown impaired GnRH neuronal migration of NLT cells in vitro. These findings and the identification of two putative zinc fingers suggest that NELF being a transcription factor could be involved in the onset of puberty (Xu et al. 2010).

- *CHD7*

Clues to the candidate genes for GnRH deficiency in humans could also be elaborated by other syndromes that combine hypogonadism and olfactory abnormalities. CHARGE syndrome is a developmental disorder consisting of eye coloboma, heart defects, choanal atresia, growth retardation, genitourinary anomalies and ear abnormalities (Pagon et al. 1981). However, no single feature is universally present or sufficient for the diagnosis of CHARGE syndrome. Other frequently occurring features include semicircular canal agenesis, hearing

impairment and characteristic face and hand dysmorphia. In 2007, new diagnostic criteria have been proposed (Sanlaville and Verloes 2007). This syndrome has as an estimated birth incidence of 1/8500 -12000 and is caused by CHD7 mutations (Vissers et al. 2004). CHD7 encodes the chromodomain helicase DNA-binding protein 7, belonging to a family which shares a unique combination of functional domains consisting of two N-terminal chromodomains, followed by SWI2/SNF2-like ATPase/-helicase domain and aDNA-binding domain. CHD protein complexes may affect chromatin structure and gene expression and thereby play important roles in regulating embryonic development (Higgs et al. 2007). CHD7 is expressed in the disease-associated organs of CHARGE syndrome, but also in affected tissues in Kallmann syndrome, including the olfactory epithelium and pituitary in mice, the olfactory placode in mouse embryo, as well as the olfactory nerve and bulb, hypothalamus and pituitary in humans (Kim et al. 2008; Jongmans et al. 2009). The CHD7 pattern of expression is consistent with involvement in the development of the olfactory pathway and the GnRH neurons.

- *LIN28B*

LIN28B is the human homolog of a C.elegans gene with a role in timing larvae to adult maturation, which suggests that LIN28B could play a role in human sexual maturation. This is further supported by genome-wide association studies indicating that polymorphisms in or near the LIN28B gene could be significant sources of variation in the age of menarche in girls (He et al. 2009; Ong et al. 2009; Perry et al. 2009).

- *GABRA1*

Gamma-Aminobutyric acid (GABA) is a dominant inhibitory neurotransmitter involved in the modulation of brain electric activity and puberty onset in primates. GABA

inhibitory effects on GnRH neurons are mainly mediated by GABA-A receptor alpha1-subunit. GABRA1 is reported to be essential for the effects of the gamma amino butyric acid type A (GABAA) receptors on GnRH neurons (Lee et al. 2010). Early studies showed that a GABAA receptor antagonist (bicuculine) accelerated puberty in monkeys (Keen et al. 1999). Recently it was demonstrated that this effect was mediated by kisspeptin as indicated by robust increases in kisspeptin secretion in response to bicuculine (Kurian et al. 2012). Additionally, the effect of bicuculine on GnRH neurons was prevented by pre-treatment with anti-kisspeptin serum (Terasawa et al. 2010; Kurian et al. 2012). However, selective reduction of GABAA receptors in GnRH neurons in mice did not lead to visible pubertal abnormalities (Lee et al. 2010), suggesting that deficiencies in this receptor would be compensated for in rodents.

- *NPYR*

The NPYR gene encodes the receptor for neuropeptide Y (NPY), which antagonizes GABA effects on GnRH neurons. This antagonism was shown to play a role in pubertal development in monkeys and rodents (Terasawa and Fernandez 2001). Additionally, hypothalamic NPY-producing neurons were shown to co-express Kiss1r and respond to kisspeptin in mouse cells and sheep hypothalamic explants. These observations raised the possibility that mutations in the NPYR gene could play a role in the etiology of ICPP (Backholer et al. 2010; Kim et al. 2010).

- *Makorin RING –finger protein 3 (MKRN3)*

Very recently MKRN3 protein was proved to have an inhibitory effect on GnRH release (Abreu et al. 2013). The authors showed that Mkrn3 mRNA, which is abundant in the ARC nucleus in mice, was highest on postnatal days 10 and 12, began to decline on day 15, and reached a nadir by days 18 to 22, at which time

Mkrn3 expression was 10 to 20% of the levels detected at 10 days, precisely consonant with the onset of puberty, and remained low after puberty. The timing of the decline in Mkrn3 expression correlated with the ages at which arcuate Kiss1 and Tac2 expression have been shown to increase, heralding the onset of puberty.

1.3.b Environmental factors

1.3.b1 Intrauterine conditions

Early studies proposed that the intrauterine milieu might influence physiological events occurring throughout life (Barker et al. 1993). Evidence of central precocious puberty associated with intra-uterine growth restriction (IUGR) has been provided in some patients with Russel-Silver syndrome (Silver 1964) or with maternal uniparental isodisomy of chromosome 14 (Tomkins et al. 1996; Fokstuen et al. 1999). In the UK, the age at menarche was 0.2 yr earlier in girls with birth weight below 2.85 Kg as opposed to those weighing more than 3.75 Kg (Cooper et al. 1996). In Spain, among girls with early puberty (B2 between 8 and 9 yr), menarcheal age was 1yr earlier in girls with a birth weight below 2.7 Kg (Ibanez et al. 2000). However, in France, IUGR was found to be associated with a pubertal delay averaging 0.8 yr in girls and 2.1 yr in boys (Lienhardt et al. 2002). A sexual dimorphism in the relationship between birth weight and timing of puberty was observed in a limited group of 35 girls who demonstrated pubertal age positively and significantly correlated with birth weight tertiles whereas a trend toward a negative correlation was seen in 34 boys (Delemarre-van de Waal et al. 2002). This female predisposition to early onset of puberty in IUGR (Marks and Bergeson 1977, Delemarre-van de Waal et al. 2002) is in agreement with the gender dimorphism seen in other health conditions. This suggests that factors linked with IUGR may be superimposed to a more general mechanism rendering females prone to develop sexual precocity.

1.3.b 2. Nutrition

Among the factors linked with the living standards nutrition is likely to play a key role in the downward secular trend in timing of puberty and the differences between underprivileged and privileged settings. A direct relationship between body weight and the age at onset of puberty was suggested by Frisch and Revelle (Frisch and Revelle 1970; Frisch and Revelle 1971) based on comparison between early and late maturers. Frish et al. (Frisch and Revelle 1973) concluded that a critical amount of body fat was needed for the onset of puberty. The Frish and Revelle hypothesis has triggered a number of studies that confirmed (Wattigney et al. 1999; Stark et al. 1989; Moisan et al. 1990; Merzenich et al. 1993) a significant relationship between menarcheal age and fat mass estimated through BMI, the sum of skinfold thickness or dual energy x-ray absorptiometry. On the other hand it has been shown that girls with early menarche are more likely to be obese than those with late menarche (Stark et al. 1989). In comparison with non obese girls, the average menarcheal age of obese girls was 9 months earlier in Japan (Murata and Hibi 1992) and 0.9 yr earlier in Thailand (Jaruratanasirikul et al. 1997). However, it is debatable whether the Frisch and Revelle hypothesis could be relevant when only the physiological variations in body fatness are considered and thus the mechanisms involved in these pathological conditions may be different from those in normal subjects. Importantly, there were studies suggesting that childhood might be a critical period for weight to influence the timing of puberty. Kaprio et al. (Kaprio et al. 1995) suggested that the association between relative body weight and menarcheal age was mainly due to correlated genetic effects. On the other hand, Cooper et al. (Cooper et al. 1996) showed that menarcheal age was inversely related to weight at 7 yr. In this same direction, Wang et al. (Wang 2002) demonstrated that early sexual maturation was associated with an increased prevalence of fatness in girls. Such sexual dimorphism

could involve genetic and/ or endocrine factors. As a consequence, it is tempting to conclude that the link between nutritional status and physiological variations in the onset of puberty can be significant but not particularly strong, suggesting that the relationship is indirect or partial or superseded by other factors.

Fat-produced leptin is regarded as the main mediator of nutritional signals to reproduction. Leptin deficient ob/ob mice have arrested puberty and infertility (Swedloff et al. 1976; Batt et al. 1982) and humans with congenital leptin deficiency present hypogonadotropic hypogonadism and early onset obesity. Negative energy balance with suppression of serum leptin is also associated with loss of body fat due to extreme exercise routines or eating disorders (Licinio et al. 2004; Welt et al. 2004). These abnormalities are at least partially rescued with leptin supplementation (Farooqi et al. 2002; Gibson et al. 2004; Kiess et al. 1998). Although it is clear that leptin plays a role in the timing of puberty, current evidence is not enough to define the precise mechanism of this effect. Kisspeptin is a recognized target of leptin (Smith et al. 2006a; Bakholer et al. 2010) and suggested to be the main mediator of pubertal effects of leptin (Roa et al. 2008; Kalamatianos et al. 2008). In this context, the hypotheses to explain accelerated induction of puberty in foreign adopted children after migration have incriminated the transition from an underprivileged to a privileged high energy intake environement, through leptin mediated awaking of the hypothalamic-pituitary-gonadal axis (Proos et a. 1991; Bourguignon et al. 1992).

Evidence suggests that the activity of Kiss1 neurons is influenced by body weight nutrition, metabolism and hormonal signals (Fernandez-Fernandez et al. 2006; Forbes et al. 2009; Wu et al. 2009). More precisely, a significant fraction of Kiss1 neurons in the ARC express the leptin receptor, Ob-Ob (Smith et al. 2006). Additionally, Kiss1mRNA is significantly reduced in obese ob/ob mice compared with wild-type controls (Smith et al. 2006). In other models in which the leptin receptor is dysfunctional as a result of a mutation, such as obese, diabetic Zucker rat (fa/fa), reproduction is also impaired. Treatment with exogenous kisspeptin can induce an

acute release of LH in these animals, suggesting that the kisspeptin signaling might be responsible for their dysfunction (Navarro et al. 2004). In rats with streptozotocin-induced diabetes, hypothalamic levels of Kiss1 mRNA as well as pituitary gonadotropin levels are decreased. The hypogonadotropic state associated with streptozotocin-induced diabetes can be rescued by kisspeptin administration, suggesting that reduced kisspeptin signaling may explain the reproductive failure that often accompanies diabetes (Castellano et al. 2006(b); Castellano et al. 2009). In states of undernutrition (or fasting) which reduce Kiss1 expression as well as gonadotropin secretion, exogenous kisspeptin administration can establish reproductive function (Navarro et al. 2004; Roa et al. 2008; Castellano et al. 2005). Collectively, these findings point to a potentially important role of Kiss1 neurons in regulation of reproduction by metabolic factors.

1.3. b.3 Stress

Different stress situations, such as adverse physical or psychological conditions and acute or chronic illnesses, are known to supress the hypothalamic-pitiutary-gonadal axis activation (Van den Berghe et al. 1998; Chrousos 1992). Intensive physical training and sport competition, such as in elite gymnasts, may lead to combined physical, psychological and nutritional stresses that are associated with delayed puberty and late menarche (Theintz et al. 1989; Georgopoulos et al. 1999). In war conditions, which involve nutritional deprivation and psychological/emotional insult such as occurred in Bosnia and Croatia, a delay in menarcheal age was observed (Tahirović 1998; Prebeg and Bralic 2000). In these situations, it is difficult to separate the participation of each stress factor. The relative difference in impact of the components of a stressful situation is further suggested by the heterogeneity of the

neuroendocrine response to various acute stressors and by the fact of different sensitivity of each individual to a specific stressor (Pacak and Palkoviits 2001). Among the neuronal circuits involved, signaling through CRH and interleukin-1 (IL-1) may be particularly important (Rivest and Rivier 1995). In this context, it is possible that, in foreign migrating children, withdrawal from a stressful environment contributes to potentiation of maturation, although some stress may result from the adoption and migration process as well. This hypothesis might be consistent with the observation of precocious pubertal development in conditions of stressful rearing and insecure attachment to parents (Belsky et al. 1991; Wierson et al. 1993).

1.3.b.4 Exposure to endocrine-disrupting chemicals (EDCs)

EDCs are environmental substances that have been introduced by man and that may influence the endocrine system in a harmful manner (Marshall 1993; Toppari et al. 1996). Krstevska-Konstantinova and co-workers (Krstevska-Konstantinova et al. 2001) have hypothesized that moving to Belgium could result in a change in exposure to EDCs leading to sexual precocity. The screening for eight organochlorine pesticides in serum of foreign migrating patients with precocious puberty in comparison with Belgian native patients has revealed the presence of *p,p'*-DDE [1,1-dichloro-2,2-bis (4-chlorophenyl) ethane] with a half life of several decades. DDT has been banned in the US and Western European countries since the late 1960s (Partsch and Sippell 2001; Key and Reeves 1994), but is still used extensively in developing countries. DDT and some isomers behave as estrogen agonists (Clark et al. 1998; Kelce et al. 1995) or may promote the hypothalamic-pituitary-gonadal axis maturation. On the other hand migration may interrupt exposure of foreign children to some EDCs. As a consequence, it is unknown whether central precocious

puberty could result indirectly from withdrawal of the negative feedback effects of the sex steroids or their environmental analogs and/or directly from accelerated hypothalamic maturation caused by sex steroids. The biopotency of EDCs on the reproductive system in general is further supported by data showing that spermatogenesis was delayed or advanced after neonatal administration of high or low doses of diethylstilbestrol respectively (Atanassova et al. 2000). Another study demonstrated that increased lead levels were associated with delayed pubic hair development and menarche but not with breast development in a mixed cohort of American girls (Wu et al. 2003). In conclusion, EDCs may act at different hypothalamic-pituitary-gonadal or extragonadal levels to influence pubertal timing.

2. DISORDERS OF PUBERTAL ONSET

2. A. Delayed puberty

2. A.1 Age limits

Delayed puberty (DP) is defined by the absence of testicular development in boys beyond 14 years old (or a testicular volume lower than 4 ml) and by the absence of breast development in girls beyond 13 years old. DP occurs in approximatively 3% of cases.

2. A.2 Causes and diagnostic work up

Causes and diagnostic work up of delayed puberty are detailed in figures 11 and 12.

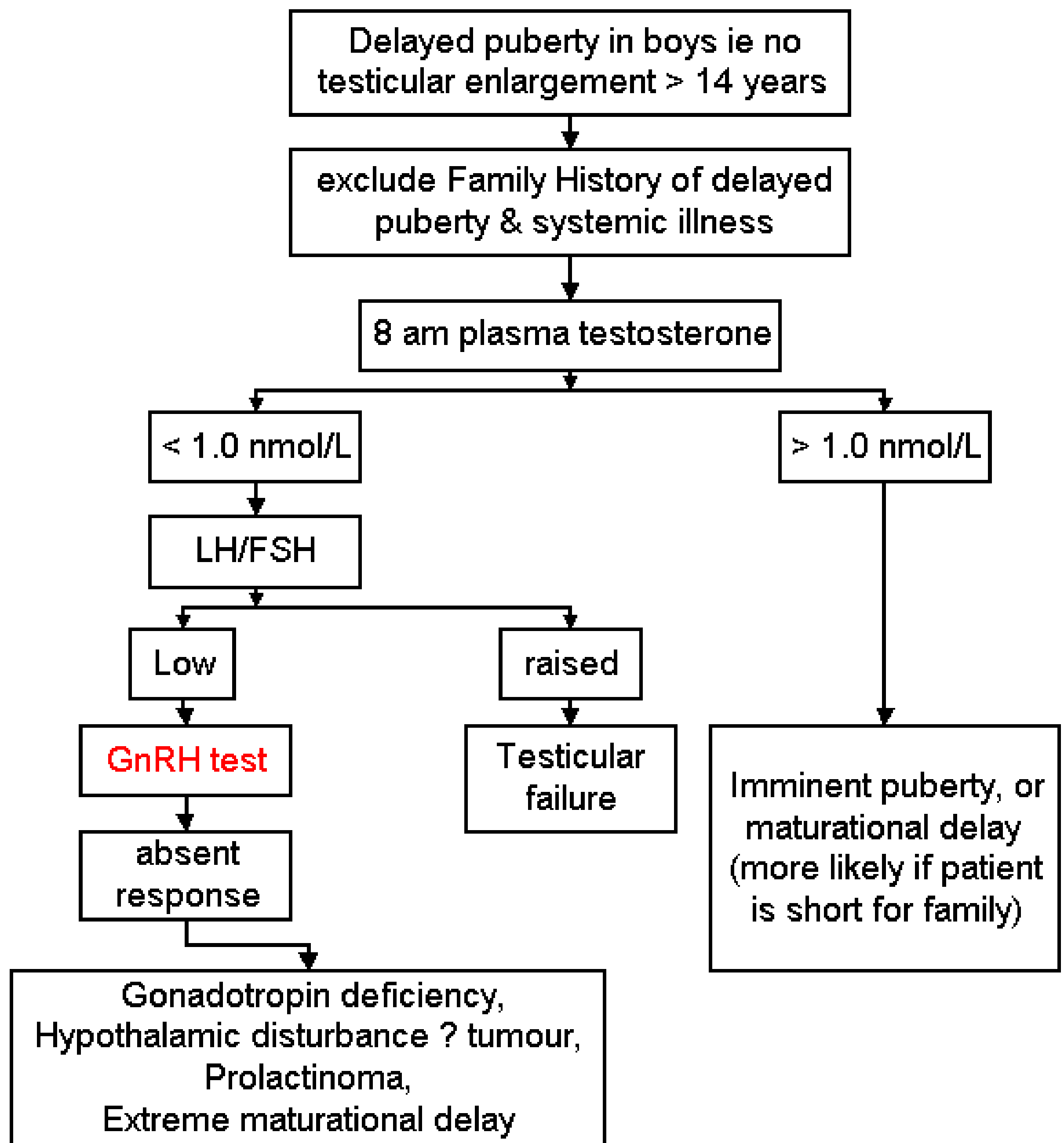


Figure 11 Diagnostic work-up of delayed puberty in boys (www.pathology.leedsth.nhs.uk)

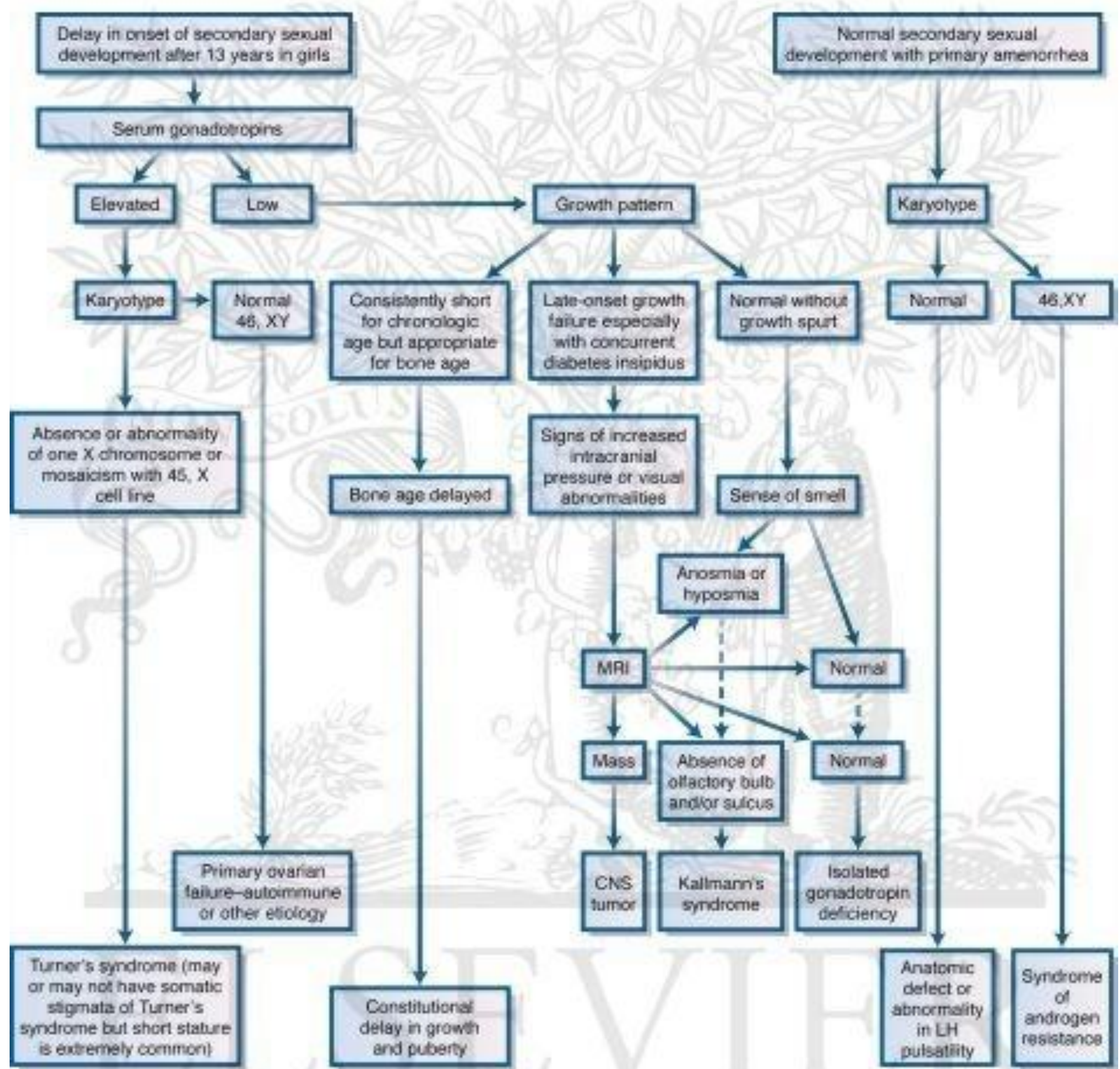


Figure 12. Diagnostic work-up of delayed puberty in girls (elsevierimages.com)

Congenital hypogonadotropic hypogonadism (CHH) is one od the causes of pubertal failure in both boys and girls. CHH is usually due to defective secretion of LH and FSH, leading to abnormal testicular/ovarian function during the physiological activation of the gonadotropic axis. The prevalence of CHH has been estimated at 1/4000 to 1/10000 in males and is reported to be 2 to 5 times less frequent in

females than males (Tanner 1962; Kaprio et al. 1995). Isolated forms are most frequently discovered during adolescence or early adulthood because of incomplete or absent pubertal development. Genetic alterations affecting GnRH secretion (mutations in GNRH1, KISS1 and KISS1R, TAC3 and TACR3) or GnRH action (GNRHR) account for mainly familial cases. Patients with isolated CHH can usually be distinguished from adolescents with constitutional delay of growth and puberty because they often have normal height for chronological age (de Ridder et al. 1992), while the latter tend to be short (Van Dop et al. 1987). CHH is less often diagnosed in boys before the normal age of puberty, usually based on unilateral or bilateral cryptorchidism and/or micropenis during the neonatal period. These cases can be documented by hormonal investigations if done before the age of 6 months, the only period before puberty during which testosterone and gonadotropin deficiency can be documented due to the mini-puberty during the first 3-6 months of life (Tanner and Whitehouse 1976; Sklar et al. 1980; Palmert et al. 2001; Hergenroeder et al. 1999). Patients with more complex forms are usually diagnosed by pediatricians or pediatric endocrinologists during early infancy or childhood, due to multiple anterior pituitary hormone deficiencies (Van Wieringen 1978; Eveleth 1978), leading to growth failure, hypoglycemia, adrenal insufficiency, or in other forms in combination with adrenal failure (Herman-Giddens et al. 1997), obesity (NHANES III 1997; Lee 2001; Grumbach 1998; Bridges 1994; Proos 1991) or neurological disorders that appear before the normal age of puberty and are usually predominant. For example, Kallmann's syndrome may be suspected in a prepubertal patient with anosmia or mirror movements (Krstevska-Konstantinova et al. 2001) especially when there is already a positive family history. At this point, we will review the main gene defects associated with GnRH-dependent hypogonadotropic hypogonadism, including normosmic idiopathic hypogonadotrophic hypogonadism (nIHH) and Kallman Syndrome (KS) (Fig.13).

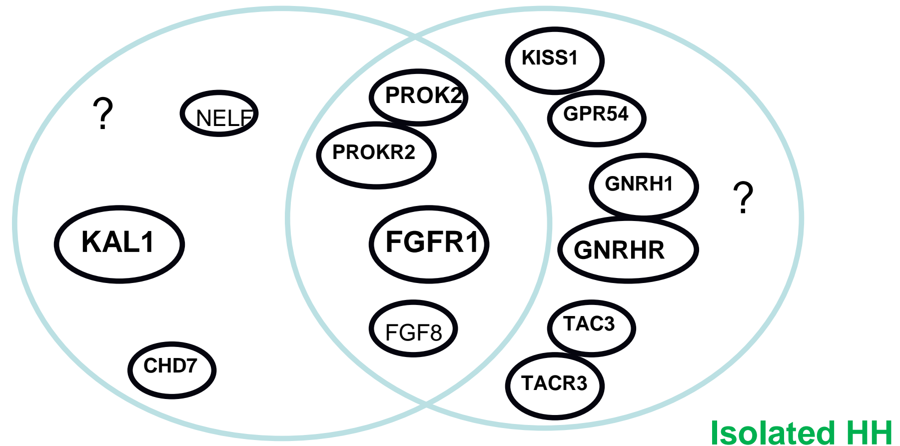


Figure 13. Genes implicated in GnRH-dependent hypogonadotropic hypogonadism

- **Normosmic idiopathic hypogonadotrophic hypogonadism (nIHH) or Isolated hypogonadotropic hypogonadism**

Congenital nIHH is characterized by partial or complete lack of pubertal development after the age of 13 years in girls and 14 years in boys, secondary to deficient GnRH-induced gonadotropin secretion. The diagnosis is based on the presence of low levels of sex steroids associated with low or inappropriately normal LH and FSH serum levels, with no anatomical lesion in the hypothalamic-pituitary tract and no other pituitary hormone deficiencies (Seminara, Oliveira et al. 2000). nIHH is a rare and genetically heterogeneous disease, which occurs most commonly in the sporadic form or, less frequently, inherited as an autosomic recessive trait (Quinton et al. 2001). Its prevalence has been estimated at 1/4000 to 1/10000 in males and it is reported to be between 2 to 5 times less frequent in females (Seminara et al. 1998; Quinton et al. 2001) but it is probably underestimated in this gender, as in women breast development can be highly variable, it is often present and sometimes almost normal, and the disease is revealed by primary amenorrhea in over 90% of cases.

In the last few years, a number of neuropeptides and their receptors involved in the control of different stages of GnRH function were implicated in the pathogenesis of nIHH, especially in familial cases.

- *GnRH1 and GnRHR*

Mutations in the GNRHR gene were first described by de Roux et al. (1997). To date, approximately twenty different homozygous or compound heterozygous point mutations in the GNRHR have been reported in patients with sporadic or familial forms of nIHH, in an autosomal recessive mode of inheritance (de Roux et al. 1997; Layman et al. 1998; Kottler et al. 1999; Seminara et al. 2000b; Pralong et al. 1999; Costa et al. 2001; Soderlund et al. 2001; Silveira, Stewart et al. 2002; Meysing et al. 2004). Large-scale screening revealed that GNRHR mutations account for 3.5-16% of sporadic cases of nIHH and up to 40% of familial cases of nIHH (Beranova et al. 2001), representing the most frequent genetic cause of the disease.

The mechanisms responsible for the inactivation of the mutant GnRH receptors include defects in the synthesis, trafficking to the cell membrane and/or in internalization, recycling, or degradation of receptors, impaired ligand binding and/or ligand –induced signal transduction, leading to various degrees of LH and FSH deficiency (Conn and Janovick, 2009). The phenotype of nIHH patients with GNRHR mutations varies from partial to complete hypogonadism (de Roux et al. 1997; Costa et al. 2001; Silveira, Stewart et al. 2002; Pitteloud et al. 2001).

Considering the fundamental role of GnRH in human reproduction, the gene that encodes GnRH is an obvious candidate for explaining nIHH. Bouligand et al. (2009) reported a homozygous GNRH1 frameshift mutation (c.18-19insA) in the amino-terminal region of the signal peptide-containing protein precursor of GnRH in a teenage brother and sister out of 145 patients screened with sporadic nIHH. The

severity of gonadotropin deficiency was demonstrated by very low levels of plasma gonadotropins and sex steroids in both siblings. Their unaffected parents and sister were heterozygous and had a normal phenotype, indicating an autosomal recessive mode of transmission. In another study (Chan et al. 2009), the DNA screening of 310 patients with nIHH revealed a different homozygous frameshift mutation (c.87delA) in one boy with micropenis, bilateral cryptorchidism and absent puberty at 15 years of age, suggestive of severe congenital HH. Heterozygous variants which were not seen in the normal controls were identified in four patients with IHH in this study. Therefore, the large number of patients with nIHH without GNRH1 defects in these two recent studies indicated that the GNRH1 mutations represent a very rare cause of nIHH. Loss-of-function mutations in genes encoding ligands arise less commonly than in genes encoding receptors (Chanc et al. 2009). One possibility to explain this difference is related with the size of the peptide. Indeed, GNRH1 represents a smaller "target" for mutation and the infertility caused by genetic mutations results in the rapid disappearing of these inherited defects in future generations.

- *Kisspeptin and KISS1 receptor (KISS1R or GPR54)*

In 2003, two independent groups identified a new candidate region in the short arm of chromosome 19 using linkage analysis on two large consanguineous families with several members affected with nIHH (de Roux et al. 2003; Seminara et al. 2003). After screening several candidate genes, homozygous inactivating mutations in the GPR54 gene were identified in the affected patients, establishing a new and unsuspected cause of nIHH. Segregation analysis showed that heterozygous carriers of GPR54 mutations had normal pubertal development, confirming the autosomic recessive mode of inheritance. A recent report estimated that the prevalence of KISS1R mutations in humans encompasses approximately 5% of normosmic idiopathic hypogonadotropic hypogonadism (IHH) cases and about 2% of total IHH cases although this prevalence is expected to be higher in familial cases (Bianco and

Kaiser 2009)..After the initial description a few different GPR54 loss-of-function mutations have been described. So far, GPR54 abnormalities were identified in three out of 180 sporadic cases (1,6%) and five out of 24 familial cases (20,8%) (de Roux et al. 2003; Seminara et al. 2003; Semple et al. 2005; Tenenbaum-Rakover et al. 2007; Lanfranco et al. 2005, Nimri et al. 2011).

Patients carrying GPR54 mutations present with impaired pubertal development with no other associated conditions. Reproductive phenotype varies from partial to severe hypogonadism. However, the majority of patients had at least partial LH and FSH responses to GnRH stimulation. De Roux et al. (2003) were the first to describe a consanguineous French family with eight children, five of whom had HH. The index case was a 20-year-old man referred for delayed puberty. He had small intra-scrotal testes (4ml), a penis of 7cm, sparse pubic hair and a bone age of 15 years With no other associated abnormalities. His 16-year-old sister had partial breast development and a single episode of uterine bleeding. She had low plasma gonadotropin levels with a “blunted” response in the GnRH test and low sex steroid levels. Sequencing revealed a homozygous deletion of 155 bp spanning the splice acceptor site of the intron 4 - exon 5 junction and part of exon 5. Seminara et al. (2003) described a male patient who presented at the age of 18 years with delayed puberty (hypotrophic intrascrotal testes measuring 1.2ml, sparse pubic hair) and had a GPR54 compound heterozygous mutation (Arg331X and X399Arg). Although he had low basal testosterone and gonadotropin levels, response to GnRH stimulation test was normal and frequent gonadotropin sampling showed the presence of low amplitude, endogenous LH pulses. On the other hand, Semple et al. (2005) described another compound heterozygous mutation (C223R and R297L) in a patient of mixed Turkish-Cypriot and Afro-Caribbean ancestry who was diagnosed at 2 months of age with micropenis, bilateral cryptorchidism and undetectable levels of gonadotropins. Micropenis and/or cryptorchidism were described in three cases, suggesting a role

for kisspeptin/Kiss1R in the gonadotropin induced androgen secretion in the last trimester of gestation. Additionally, Tenenbaum-Rakover et al. (2007) identified five patients with HH belonging to two unrelated consanguineous Arab-Muslim families from Syria and Israel. All affected subjects were homozygous for a GPR54 missense mutation (L102P). The index case of the first family presented partial pubertal development (Tanner stage B4) and primary amenorrhea. In the second family the propositus had micropenis and cryptorchidism at birth but hormonal studies revealed persistent with a normal frequency but a very low amplitude LH pulsatile secretion. Finally, Nimri et al. (2011) described a new homozygous mutation (c.T815C) in GPR54 leading to a phenylalanine substitution by serine (p.F272S). Functional analysis showed an almost complete inhibition of kisspeptin-induced GPR54 signaling and a dramatic decrease of the mutated receptor expression at the cell surface. The males exhibited the same clinical features from infancy to adulthood, characterized by cryptorchidism, a relatively short penis, and no spontaneous pubertal development. The female patient presented at 18 yr with absence of secondary sexual characteristics and primary amenorrhea. Repeated stimulation tests demonstrated complete gonadotropin deficiency throughout follow-up. It is interesting to state that male and female patients carrying GPR54 mutations have been successfully treated, either with exogenous gonadotropin or long –term pulsatile GnRH infusion and achieved fertility and normal pregnancy outcomes, suggesting that the Kisspeptin/GPR54 system is operative above the GnRH receptor level, most likely stimulating GnRH secretion. This was already demonstrated in animal models, but without discernible direct effect on the pituitary or gonads. (Bo-Abbas et al. 2003; Seminara et al. 2003; Lanfranco et al. 2005; Pallais et al. 2006; Tenenbaum-Rakover et al. 2007; Colledge 2009).. Further studies in animal models showed that kisspeptins can stimulate GnRH secretion in the hypothalamus: Kisspeptin-10 was shown to elicit GnRH release by rat hypothalamic explants (Plant and Ramaswami 2009; Colledge et al. 2009; Roa et al. 2008), while intracerebral

kisspeptin injection to sheep induced GnRH release into the cerebrospinal fluid. An additional interesting finding in men with HH and GPR54 mutations is the relatively high frequency of patients with cryptorchidism and micropenis that are considered to reflect gonadotropin deficiency during the antenatal period (Grumbach et al. 2005). More recently, Topaloglu et al. reported the first inactivating mutation in KISS1 gene in a large consanguineous family that results in failure of pubertal progression, indicating that functional kisspeptin is important for puberty and reproduction in humans (Topaloglu et al. 2012). These data indicate that the activation of Kisspeptin/GPR54 signaling is not only necessary to trigger puberty but is also involved in all phases of physiological activation of the hypothalamic-pitiutary-gonadal axis. However, whether the KISS1/GPR54 system is the initial trigger of puberty or whether it acts as a downstream effector of other yet to be identified factors it remains unclear.

- *Neurokinin B (TAC3) and Neurokinin B recetor (NKR3 or TACR3)*

By means of genome-wide analysis of nine inbred Turkish families with IHH Topaloglu et al. (2009) were able to identify that the neurokinin B pathway has a critical role in the human reproductive axis. Homozygous loss-of-function missense mutations in TAC3 and TACR3, encoding neurokinin B and its receptor, respectively, were identified in four unrelated consanguineous families; while pedigree analysis also demonstrated that heterozygous carriers were unaffected, indicating autosomal recessive HH. The TACR3 mutations (G93D and P353S) demonstrated evidence of impaired receptor signaling in HEK293 cells. Likely, a marked reduced activity of the synthetic peptide containing the M90T variant of the neurokinin B was demonstrated using the same expression system. All patients with TAC3 or TACR3 gene mutations exhibited a severe gonadotropin deficiency. Micropenis was reported in all affected males, suggesting failure of normal intrauterine and perinatal activation of the

reproductive axis. Additionally, a novel missense mutation (H148L) in the first extracellular loop of the neurokinin B receptor was reported in three siblings with nIHH (Guran et al. 2009). The signaling activity of the mutant receptor in response to neurokinin B or its analog was severely impaired in a heterologous expression system. This study further strengthens the link between the neurokinin B signaling defects and the pathogenesis of nIHH. More recently, Young et al. (2010) identified three unrelated patients with the same homozygous substitution in the TAC3 intron splice acceptor site (c.209-1G>C) and three siblings with a homozygous mutation in the TACR3 intron 2 splice acceptor site (c.738-1G>A) both of which invalidate neurokinin B and its receptor respectively. Like Topaloglou et al. (2009) they observed dissociation between very low LH levels and normal or near-normal FSH levels that responded excessively to GnRH challenge. Finally, the extensive analysis of the TAC3 and TACR3 in a large cohort of 345 patients with nIHH revealed that mutations in this system appear to have an attenuated effect over time, as a significant proportion of patients carrying mutations exhibited reversal of the hypogonadotropism (Gianetii et al. 2010). These findings demonstrate the hypothalamic origin of the gonadotropin deficiency showing that neurokinin B and NK3R both play a crucial role in human GnRH release.

- **Kallmann syndrome (KS)**

Most cases with KS are also diagnosed at the time of puberty because of the lack of sexual development, identified by small testes in males or the lack of breast development and primary amenorrhea in females. The prevalence of Kallmann syndrome has been roughly estimated to occur in 1/10.000 males and in 1/50.000 females (Pawlowitzki et al. 1987). Although Kallmann syndrome is most commonly sporadic, X-linked, autosomal dominant and autosomal recessive inheritance has

been described (Waldstreicher et al. 1996; Georgopoulos et al. 1997). In Kallmann syndrome, the hypogonadism is due to GnRH deficiency (Naftolin et al. 1971), probably by defective migration of GnRH-synthesizing neurons. Anosmia is related to hypoplasia or aplasia of the olfactory bulbs, even though normal olfactory bulbs images have been reported (Quinton et al. 1996; Sato et al. 2004). Genetic disruption of factors putatively involved in the GnRH migration has been described in patients with KS. Of these, the Kallmann 1 (KAL1) and the fibroblast growth factor receptor 1 (FGFR1) genes were the major loci identified in the etiology of KS.

- *KAL1*

Various types of KAL1 abnormalities have been described that are distributed throughout the entire gene, including missense, nonsense and splice site mutations, intragenic deletions and submicroscopic chromosomal deletions (Tsai and Gill 2006; Trarbach et al. 2007). These KAL1 abnormalities have been identified in approximately 8-11% of sporadic and 14-50% of familial cases of X-linked Kallmann syndrome (Franco et al. 1991; Legouis et al. 1991; Hardelin et al. 1993; Tsai and Gill 2006; Trarbach et al. 2007). Patients with KAL1 mutations most usually present severe and highly penetrant reproductive phenotypes (Salenave et al. 2008; Pitteloud et al. 2002). In the X-linked Kallmann syndrome most patients have micropenis and bilaterally undescended testes at birth, reflecting severe congenital GnRH and gonadotropin insufficiency (Salenave et al. 2008). The non-reproductive, non-olfactory abnormalities frequently associated with X-linked Kallmann syndrome are cleft lip and/or palate, tooth agenesis, high arched palate, sensorineural hearing impairment, abnormal eye movements, congenital ptosis, abnormal visual spatial attention, short metacarpals, unilateral renal agenesis, agenesis of the corpus callosum, involuntary upper limb mirror movements (bimanual synkinesis) (White et al. 1983; Schwankhaus et al. 1989; Zenteno et al. 1999; Mayston et al. 1997; Quinton et al. 2001; Dode and Hardelin 2009).

- *FGFR1 and FGF8*

The FGFR1/anosmin1 connection is supported by the shared clinical findings of patients carrying KAL1 and FGFR1 mutations. Dode et al. (2003) were the first investigators reporting the association of loss-of-function mutations in FGFR1 with an autosomal dominant form of Kallmann syndrome. Since then, FGFR1 gene was extensively studied and mutations were identified in approximately 10-17% of Kallmann syndrome affected individuals. Some non-reproductive phenotypes, including cleft lip or palate, dental agenesis and, less often, bimanual synkinesis were also reported (Tsai and Gill 2006; Trarbach et al. 2007). FGFR1 mutations have been associated with marked phenotypic variability both within and among families and apparent incomplete penetrance. Most reported FGFR1 mutations are small insertion/deletion or point mutations. Only a heterozygous FGFR1 deletion was reported in a female patient with IHH (Trarbach et al. 2009).

The fibroblast growth factor 8 (FGF8) is considered as a key ligand of the FGFR1 in the ontogenesis of GnRH neurons. It was initially suspected by the observation that FGFR1 mutated receptors have dramatically reduced affinity for FGF8 (Pitteloud et al. 2007). Falardeau et al. (2008) screened a large cohort of patients with IHH and identified six FGF8 missense mutations that reduce the receptor biological activity in vitro. The affected patients presented a different degree of GnRH deficiency, including the rare adult onset form of hypogonadotrophic hypogonadism and a variability of olfactory phenotypes.

- *PROK2 and PROKR2*

Mutations in PROKR2 and its ligand were first described by Dode et al. (2006), who studied a cohort of 192 patients with Kallmann syndrome. One frameshift and nine

missense mutations on PROKR2 and four PROK2 defects were identified in these patients. However most of these mutations were found in heterozygous state and some in apparently unaffected individuals, while no functional study was provided, raising questions regarding their pathogenic role. Later, PROK2 and PROKR2 mutations were found in patients with GNRH deficiency with or without olfactory abnormalities (Pitteloud, Zhang et al. 2007; Cole et al. 2008; Abreu et al. 2008). In the study performed by Cole et al. (2008), mutations in PROKR2 or PROK2 genes were observed in 2 and 5% of Kallmann syndrome, respectively, and in 2% of nIHH probands. Genotype/phenotype correlations showed considerable variability even within families (Dode et al. 2006; Cole et al. 2008; Abreu et al. 2008). Reproductive phenotypes of patients with heterozygous or homozygous PROK2/PROKR2 mutations ranged from complete to partial IHH, including complete reversal of GnRH deficiency. Additional non-reproductive phenotypes such as fibrous dysplasia, severe obesity, sleep disorder, synkinesis and epilepsy have been described in these patients. Abreu et al. (2008) described a homozygous nonsense PROKR2 mutation, in an anosmic boy with micropenis, bilateral cryptorchidism and high arched palate. Many mutations were identified in the heterozygous state but segregation analysis and in vitro functional studies suggest that the presence of monoallelic PROKR2 mutations is not sufficient to produce hypogonadotrophic hypogonadism (Abreu et al. 2008; Monnier et al. 2009). Different defective mechanisms of the PROKR2 missense mutations were demonstrated in vitro. However, when wild-type and mutant receptors were co-expressed, none of the mutant receptors affected cell surface-targeting or signaling activity of the wild-type receptor, arguing against a dominant negative effect of the PROKR2 hetorozygous mutations in vivo (Monnier et al. 2009). In other words, patients heterozygous for PROK2 and PROKR2 are expected to carry additional in other, as yet unknown genes, arguing in favor of a digenic or oligogenic mode of inheritance of the disease.

- *CHD7*

Given that most if not all CHARGE patients have both olfactory aplasia or hypoplasia and IHH Kim et al. (2008) hypothesized that CHD7 mutations could be involved in the pathogenesis of Kallmann syndrome. The authors performed a mutation screening of the CHD7 gene in 197 patients with IHH with and without olfactory abnormalities. Seven heterozygous mutations were identified in three sporadic Kallmann syndrome and four sporadic nIHH patients. Therefore, it was proposed that these conditions could be considered mild allelic variants of CHARGE syndrome. On the other hand, another study of Jongamans et al. (2009) identified CHD7 mutations only in Kallmann syndrome patients who had additional phenotypic features of CHARGE syndrome. This study suggested that the patients diagnosed with IHH and anosmia should be screened for clinical features consistent with CHARGE syndrome and only in the presence of deafness, dysmorphic ears and /or hypoplasia or aplasia of the semicircular canals CHD7 sequencing should be recommended.

- *Digenic (oligogenic) defects*

The distinction among the different abnormalities of pubertal development may not be absolute. It is worth mentioning that mutations in FGFR1 can cause both KS and nIHH (Pitteloud et al. 2006) and a homozygous mutation in PROK2 has been reported to cause both KS and HH within a single family (Pitteloud et al. 2007a). A more comprehensive study of PROK2 and PROKR2 in HH and KS patients found mutations in both genes distributed in both groups of patients (Cole et al. 2008)

IHH has been classically considered a monogenic disorder with Mendelian inheritance pattern. However, in light of the report by Pitteloud et al. (2007a), where two IHH families were described, one with Kallmann syndrome with FGFR1 and NELF mutations and another with nIHH with GNRHR and FGFR1 mutations, a possible digenic model causing the IHH phenotype was proposed. Co-existence of

mutations in PROKR2 and KAL1 genes, PROKR2 and PROK2 and FGFR1 and FGF8 has also been described in cases of Kallmann syndrome (Hardelin et al. 1992; Miura et al. 2004; Dode et al. 2006; Cole et al. 2008; Canto et al. 2009). Defects in different genes seem to act synergistically to modify the severity of the GnRH deficiency, partially explaining the wide phenotypic variability observed within and across families with nIHH and Kallmann syndrome.

2. A.3 Consequences

All patients with CHH have normal statural growth during childhood and, despite the absence of the pubertal growth spurt, statural retardation is very rarely a presenting symptom (Tanner and Davies 1985). On the contrary, the absence of long-bone epiphyseal closure explains these patients' frequent eunuchoid habitus and relative tallness. Retarded bone maturation, osteopenia and osteoporosis are frequent when gonadotropin deficiency is diagnosed in adulthood (Tanner and Davies 1985; Ducros and Warren 1983). Additionally, two different polymorphisms of the estrogen receptor α gene, which were found previously to be associated with reduced breast cancer risk, were also associated with a relative delay in menarcheal age in Greek adolescent girls (Stavrou et al. 2002).

2.B. Precocious puberty

2.B.1 Age limits

In accordance with the above mentioned average ages at onset of pubertal development and assuming a Gaussian distribution in the normal population, abnormally precocious puberty has been defined in Europe as less than 8yr for the B2 stage in girls and less than 9yr for the G2 stage in boys (Bridges et al. 1994; Lebrethon and Bourguignon 2000; Klein 1999). These age limits, which are below the 3rd centile, have been used for several decades and are still currently used. The definition of appropriate age limits is important to restrict diagnostic investigations and possible therapeutic intervention to children with abnormal precocious pubertal development.

2.B.2 Causes and diagnostic work up

Main causes of precocious puberty in girls and boys are illustrated in table 1. Sexual precocity is classified as central, gonadotropin-dependent, when it results from early hypothalamic-pituitary-gonadal maturation (Lee et al. 2001). Central precocious puberty (CPP) mimics the physiological pubertal development and results from premature activation of hypothalamic GnRH-secreting neurons. Children present with pubertal basal or stimulated gonadotropins and sex steroids levels, acceleration in linear growth and progressive bone age advancement (>2SD) resulting in premature epiphyseal closure and short stature in untreated children (Palmert and Boepple 2001; Partsch et al. 2002; Carel and Leger 2008). CPP represents four fifths of the total number of patients with precocious puberty and is much more frequent in girls than in boys (Klein 1999; Pescovitz et al. 1986).

Precocious Puberty in girls	**Precocious Puberty in boys**
Incomplete variants	***Gonadotropin independent***
Premature thelarche Premature adrenarche Premature menarche	**Exogenous** Testosterone cream
Complete variants	**Endogenous**
Gonadotropin independent Estrogenic ovarian cyst Estrogenic ovarian tumor Estrogenic adrenal tumor McCune Albright syndrome Primary hypothyrodism	**Testicular source** Testicular tumor McCune Albright Syndrome Testotoxicosis HCG producing tumor **Adrenal source** CAH (21OH deficinecy or 11OH deficiency) Adrenal tumor
Gonadotropin dependent **Idiopathic** **Organic** **Brain tumor** Hypothalamic hamartoma Glioma **Congenital anomaly** Hydrocephalus Arachnoid cyst Cerebral dysgenesis **Central Nervous System insult** Infection Trauma Surgery Radiation **Genetic** Activating KISS1 or KISS1R mutation Rare variants in TAC3 or TACR3 genes Loss of function mutations in MKRN3 gene	

Table 1 Causes of precocious puberty

As shown in Fig. 14, the female to male ratio found among patients with central precocious puberty in the studies published between 1961 and 1990 was relatively similar, around 3:1 to 4:1 (Pescovitz et al. 1986, Thamdrup 1961; Wilkins 1965;

Sigurjonsdottir and Hayles 1968; Kaplan and Grumbach 1990; Blanco-Garcia et al.1983; Caragorri et al. 1982; Lee et al. 1989).

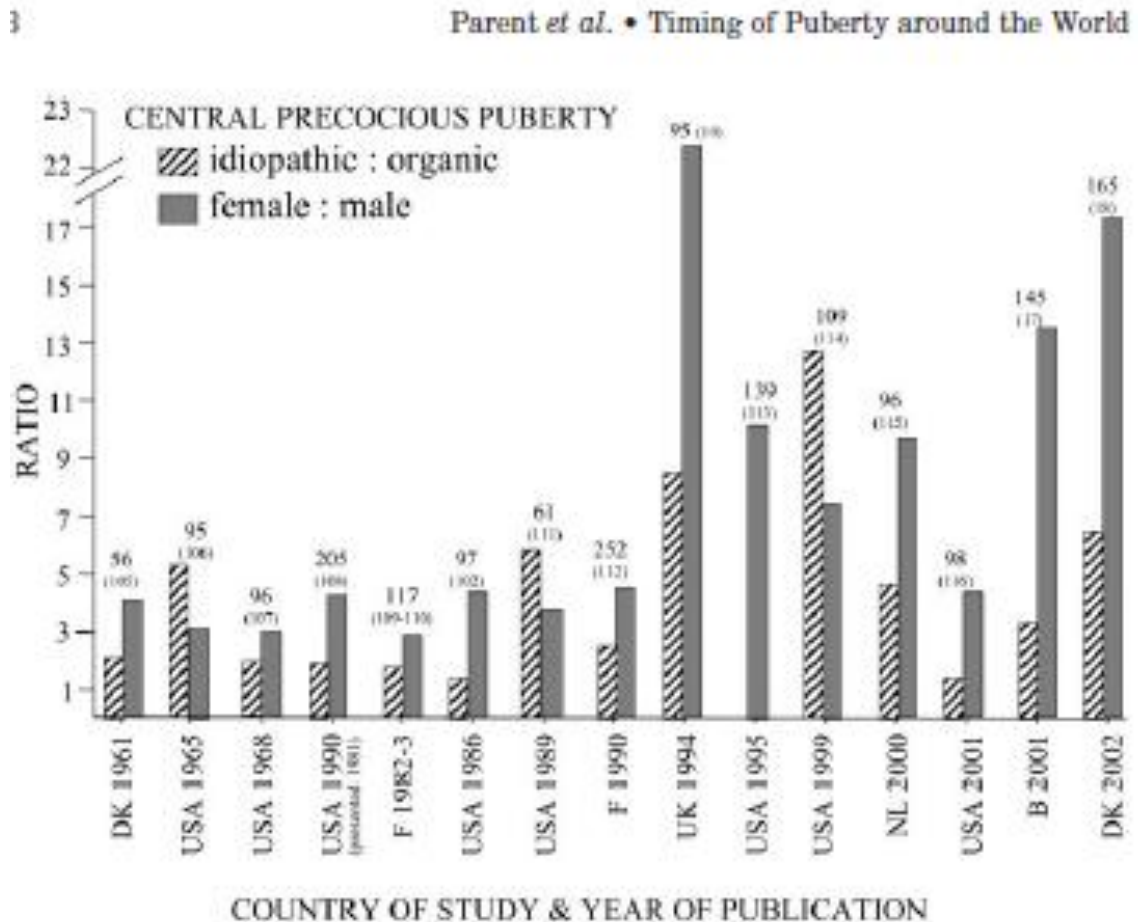


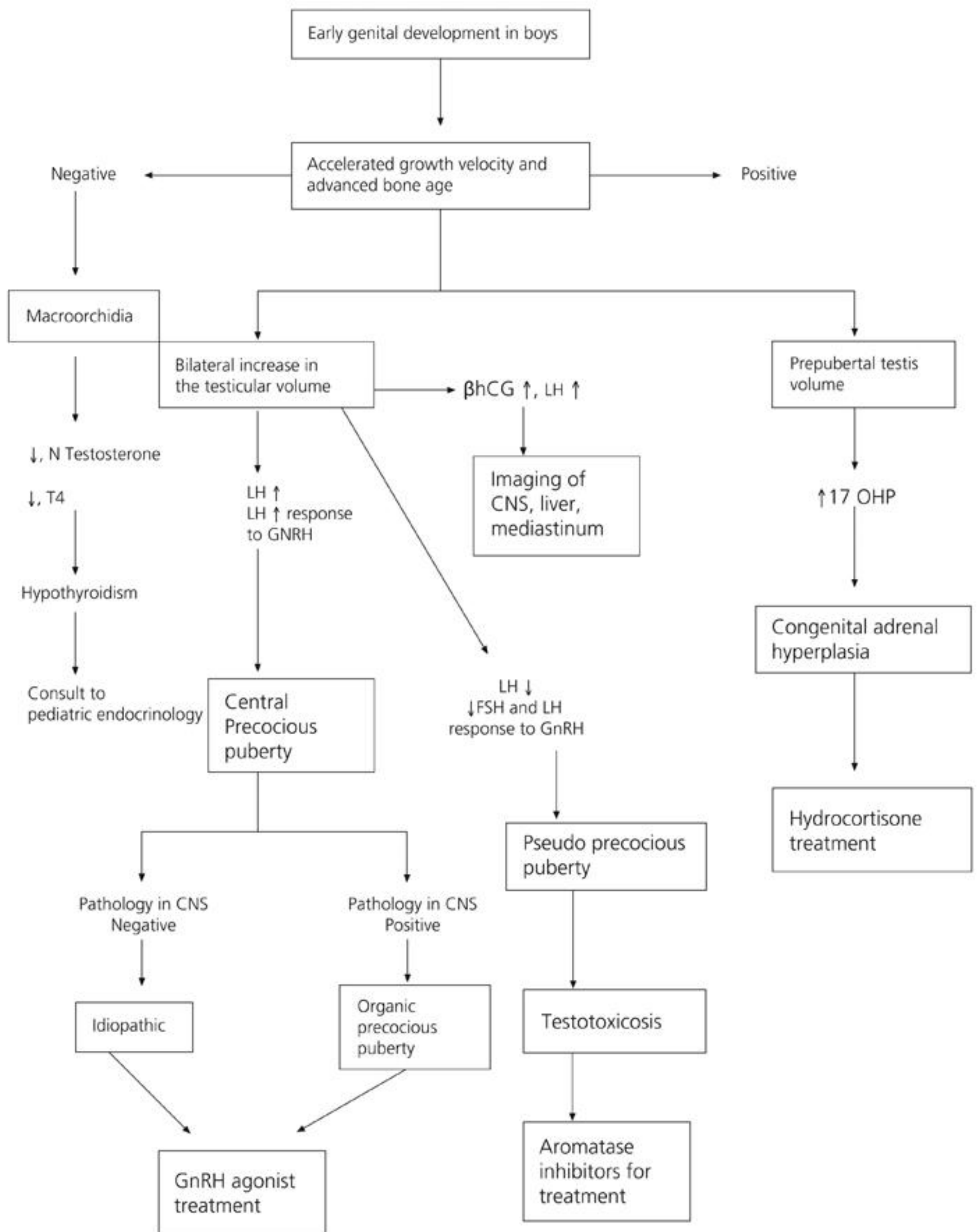
Figure 14

Gender and etiologic distribution of patients with central precocious puberty in the series published between 1961 and 2001 in relation to the year of publication and the country of study. The number of patients and reference (in parenthesis) are indicated at the top of each bar (Parent et al. 2003).

CPP can be organic or idiopathic (ICPP). The idiopathic to organic ratio has been reported to be around 2:1, indicating that two thirds of the patients had idiopathic forms and one third had identified neurogenic organic causes. Data published from four European countries as well as from the US (Bridges et al. 1994; Krstevska-Konstantinova et al. 2001; Neely 1995; Palmert et al. 1999; Mil et al. 2000) showed that the proportion of female patients and of idiopathic forms has clearly increased.

Among patients with central precocious puberty the proportion of idiopathic forms varies between 58 and 96% (Partsch and Sippel 2001). This proportion is greater in girls than in boys, who show a higher prevalence of recognizable organic causes (Grumbach and Styne 1998; Lebrethon and Bourguignon 2000). Finally, some partial forms of sexual precocity, such as isolated premature thelarche, can secondarily evolve into central precocious puberty (Lebrethon and Bourguignon 2000, 2001).

In peripheral, gonadotropin-independent sexual precocity, the underlying cause is increased secretion of adrenal or gonadal sex steroids or exposure to exogenous sex steroids without activation of the HPG axis (Bridges et al. 1994; Pescovitz et al. 1986). Peripheral sexual precocity represents one third to one fifth of the total number of patients with precocious puberty and involves both sexes. Some forms of peripheral sexual precocity predominate in boys, such as chorionic gonadotropin-secreting tumors and familial testotoxicosis, the latter caused by activating mutations of the LH receptor. Other causes, such as constitutive Gsα activity which accounts for the McCuneAlbright syndrome, predominate in girls (Bridges et al. 1994; Lebrethon and Bourguignon 2001). In peripheral precocious puberty the development of secondary sexual characteristics is due to the effect of the sex steroids without activation of the hypothalamic-pituitary-gonadal axis that is actually found suppressed. However, some cases of peripheral precocious puberty, such as untreated forms of congenital adrenal hyperplasia in males, may be complicated by the secondary activation of the hypothalamic-pituitary gonadal axis and therefore by a gonadotropin-dependent central precocious puberty. Figures 15 and 16 demonstrate the detailed diagnostic flow chart of precocious puberty in boys and girls.



FSH: follicle stimulating hormone, LH: luteinizing hormone, CNS: central nervous system; GnRH: gonadotropin releasing hormone, hCG: human chorionic gonadotropin, 17OHP: 17 hydroxyprogesterone

Figures 15 Diagnostic work up of precocious puberty in boys (www.biomedsearch.com)

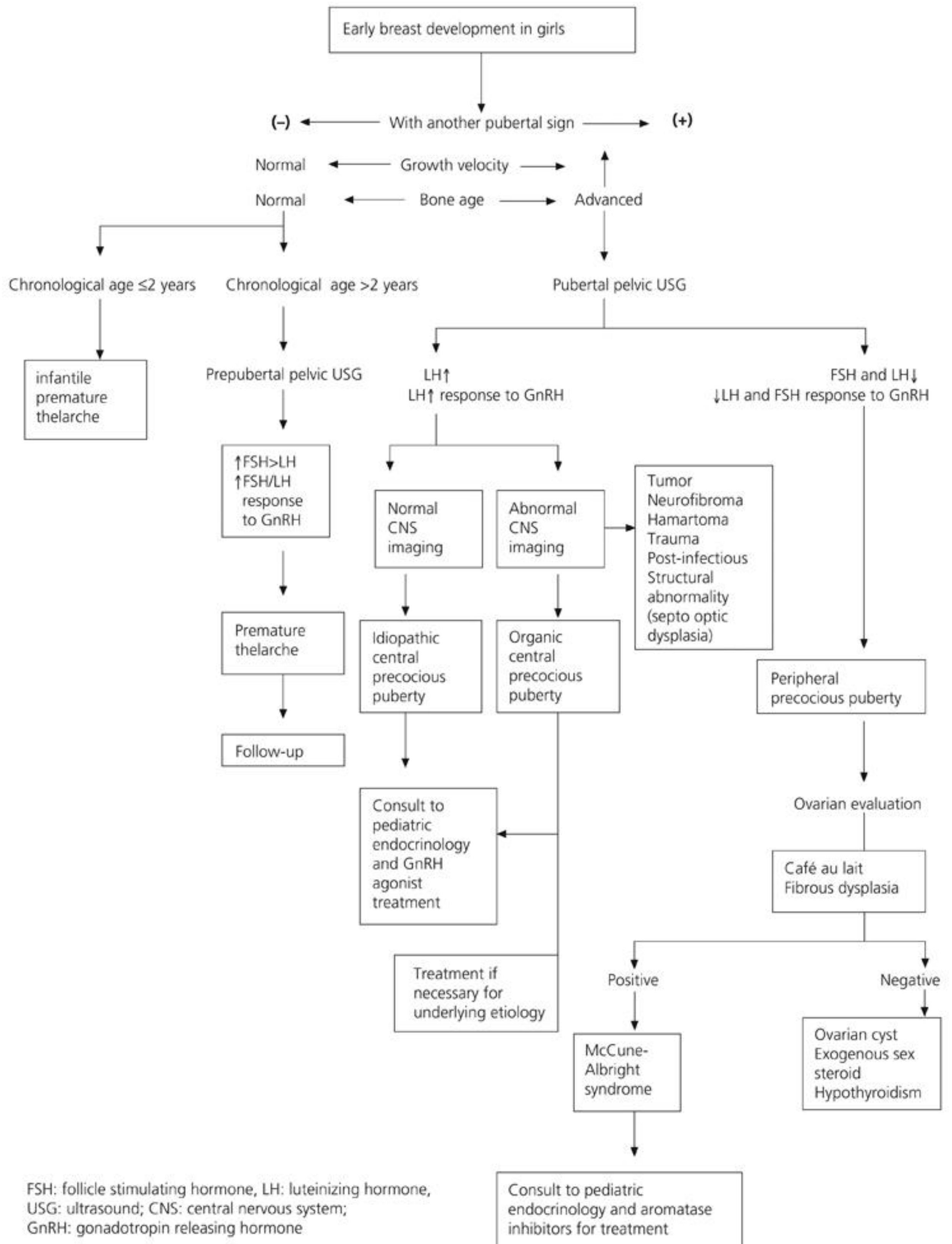


Figure 16 Diagnostic work-up of precocious puberty in girls (www.biomedsearch.com)

Idiopathic central precocious puberty (ICPP)

Over 90% of the girls and up to 25% of the boys with CPP have an idiopathic form (Palmert and Boepple 2001; Partsch et al. 2002; Kakarla and Bradshaw 2003). Up to 27.5% prevalence of familial cases has been reported among 156 patients with idiopathic CPP, suggesting a role for genetic factors in the pathogenesis of this condition (de Vries et al. 2004). At this point we will review pathophysiology and gene defects associated to idiopathic central precocious puberty (ICPP).

- *GABRA1*

Initial research and sequencing of the GABRA1 gene in a cohort of thirty-one girls from 28 unrelated families with ICPP-mean age at onset of pubertal signs 4.7 (2.1) years did not lead to mutations' detection (Brito et al. 2006). In that study seven different GABRA1 polymorphisms, including two exonic (156T>C and 1323G>A) and five intronic [IVS2-712(GT)n, IVS3+12A>T, IVS8+45T>G, IVS9+76A>G, and IVS10+15G>A] were found in patients and controls.

- *NPYR1*

Later on, sequencing of the NPYR1 detected only a synonymous SNP (K374T) in the heterozygous state in a girl with familial ICPP-out of trhirty three patients tested, and this polymorphism was present at a higher rate in the control population (28%). Moreover, in vitro assays failed to show altered activity for this mutant (Freitas et al. 2007).

- *Kisspeptin and KISS1R*

The Kisspeptin system has been recently implicated in the pathogenesis of CPP in humans. A cohort of 67 Brazilian children with idiopathic CPP, including 13 familial cases, was screened for KISS1R mutations (Teles et al. 2008; Silveira et al. 2008). A

heterozygous activating mutation of the KISS1R gene (R386P) was described in an adopted girl with CPP (Teles et al. 2008). She had slow progressive thelarche from birth, suggesting an early, persistent, and slightly increased estrogen secretion. Accelerated growth, skeletal maturation and progression of breast development were noticed at 7 years of age. She had pubertal estradiol levels and GnRH- stimulated LH levels were borderline for pubertal activation. Interestingly, in vitro studies demonstrated that the R386P mutation, located in the carboxy-terminal tail of the KISS1R, led to prolonged activation of intracellular signaling pathways in response to Kisspeptin, resulting in significantly higher inositol phosphate accumulation for as long as 18h. These findings suggested a significant reduction in the rate of desensitization of the mutant KISSI1R. This mechanism would result in an increased, prolonged cellular response and hence the release of an increased-amplitude pulse of GnRH in response to Kisspeptin stimulation. Ligand binding affinity and intracellular signaling capacity of the mutant KISS1R under basal conditions were not altered, indicating that the R386P is a non-constitutively activating mutation. Indeed a constitutive activation of KISS1R might be expected to disrupt pulsatile GnRH release and result in delayed puberty, since continuous GnRH secretion leads to receptor desensitization.

In addition to the KISS1R mutation, two KISS1 missense mutations (P74S and H90D) were identified in two unrelated Brazilian children out of 83 patients with ICPP tested (Silveira et al. 2010). The P74S mutation was identified in the heterozygous state in a boy who had sporadic CPP at 1 year of age. His mother and maternal grandmother, who had history of normal pubertal development, also carried the P74S mutation in the heterozygous state, suggesting incomplete sex-dependent penetrance. The H90D mutation was identified in the homozygous state in a girl with familial CPP, who developed puberty at 6 years of age. Her mother, who had menarche at 10 years of age, was heterozygous for this variant. Both KISS1

mutations were absent in 400 control alleles and are located in the amino-terminal region of the KISS1 gene, which may be involved in protein stabilization and protection against proteolytic digestion. In vitro studies revealed that the capacity of the P74S and H90D mutants to stimulate inositol phosphate (IP) production was similar to the wild type GPR54. However, after pre-incubation of wild-type and mutant GPR54 in human serum, the capacity to stimulate signal transduction was significantly greater for P74S and H90D compared to the wild-type, indicating that both variants were associated with higher Kisspeptin resistance to degradation as opposed to the wild type. These findings suggest a role for these mutations in the CPP. Additionally, while no mutations in KISS1 were found in 101 Korean girls with ICPP - mean age at first breast budding was 7.09 (1.14) years (Ko et al. 2010), eight polymorphisms were identified. Although two of them were novel, those polymorphisms could not lead to amino acid changes. p.P110T was detected less frequently in CPP patients than in the controls (P = 0.022). Moreover, the CPP patients with p.P110T evidenced lower peak FSH values under GnRH stimulation than those without p.P110T (P = 0.002) and therefore p.P110T, appeared to be a meaningful polymorphism. Additionally, very recent data demonstrated that polymorphisms in the 3'UTR of KISS1 are associated to CPP and interfere with the conformation of the DNA sequence and potentially the RNA sequence of KISS1 further verifying a role of the KISS1/KISS1R in the abnormal initiation of puberty [Huijbregts et al. 2012]. Finally, two mutations identified in Chinese patients with ICPP in genome wide association studies (His196Pro-KISS1R and Pro110Thr-kisspeptin) stil await confirmation of this association (Luan et al. 2002; 2007). On the other hand no mutations in KISS1 or KISS1R genes were found in a cohort of thirty girls with ICPP -mean age at onset of puberty was 7.5 (6.5 ;7.9) years and mean bone age advancement was 1.4 (-0.1; 2.8) years (Tommiska et al. 2011). Finally, very recently, no rare variants were detected in KISS1 or KISS1R out of twenty-eight girls with ICPP. Age at diagnosis was 5.72±2.59, with a mean bone age

advancement of 1.4 years (-0.1 to 2.8) and height at onset of therapy in SD score was 0.90±1.48 for age. Luteinizing hormone-releasing hormone test was performed in all subjects, and all of them had a pubertal response (LH 20.35±32.37 mIU/mL; FSH 23.32±15.72 mIU/mL) (Krstevska-Konstantinova et al. 2014).

- *LIN28B*

As already exposed, LIN28B has also been implicated in the pathophysiology of onset of puberty. However on the aformentioned study of Tommiska et al. (Tommiska et al. 2011) no mutations were detected. Later on, out of 178 Brazilian children with CPP (171 girls with mean pubertal onset at age 5.4, 16.8% familial cases) one mutation in LIN28B was identified in the heterozygous state (p.H199R) in a girll who developed CPP at 5.2 years. Nonetheless, functional assays performed did not detect significant changes in the activity of the mutant (Sileveira-Neto et al. 2012). More precisely, when ectopically expressed in cells the mutant protein was capable of binding pre-let-7 miRNA and inhibiting let-7 expression to the same extent as wild-type Lin28B protein; thus, the significance of LIN28B for human pubertal maturation remains unknown.

- *Leptin and Leptin receptor (LEPR)*

Three SNPs in the leptin (nt-2548) or leptin receptor (223 A/G, 109 A/G) genes were assessed for their association with CPP in a population of 249 female patients as opposed to 219 healthy controls. Allele frequencies in SNPs were compared with anthropometric measures and circulating hormone concentrations (estradiol, FSH, LH and leptin). SNPs at LEPR223 and LEPR109 were significantly associated with higher levels of LH in girls with CPP, but none of the genotypes at these SNPs were significantly associated with CPP (Su et al. 2012).

- *TAC3/TACR3 complex*

As detailed above, TAC3/TACR3 complex could also be implicated in ICPP. In 2012, Tusset et al. published a new variant of TAC3 gene (c.187G>C) in the heterozygous state, in a Brazilian girl out of 114 patients (107girls/7boys) with CPP. The affected girl had pubertal onset at 7 years of age. She had advanced bone age (11 years) and breast development pubertal stage Tanner 3. Hormonal evaluation revealed pubertal basal LH level (IFMA) of 1.2U/L, LH after acute GnRH stimulation of 17.9 U/L and pubertal basal estrogen level (IFMA) of 35.2pg/ml. This variant was absent in the control population of 150 Brazilian individuals. *In silico* analyses suggested that this variant does not alter the splicing sites and its pathogenicity was not supported using the Polyphen-2 tool. No mutation in TACR3 was found in their population with ICPP [Tusset et al. 2012].

- *Estrogen reaceptor a (ERα)*

Estrogen is the final key factor that triggers the onset of puberty. The raised sensitivity of ERa, which may be caused by an ERα gene mutation or polymorphism, has been implicated in the etiology of precocious puberty. Two novel polymorphisms, p.G145S and p.R55H were idenitfied in a population of 204 Korean female patients with CPP as compared to 102 healthy female controls. The subgroup with p.G145S showed significantly higher level of peak LH levels than the subgroup without this SNP (Lee et al. 2013). However, no solid conclusion could be made from this study and further studies are needed to validate the function of these SNPs.

- *Thyroid transcription factor 1 (TTF1) and enhanced at puberty (EAP1) genes*

Thyroid transcription factor 1 (TTF1) and enhanced at puberty (EAP1) are transcription factors that modulate GnRH expression but also were connected, via gene networks, to genes implicated in the control of menarche. Several studies indicated that EAP1 is crucial for the ARC to maintain menstrual cyclicity in higher

primates. Using the lentiviruses delivery system it was determined that decreasing the production of EAP1 would alter the onset of female puberty and adult reproductive cyclicity (Garcia-Rundaz et al., 2007; Hager et al., 2007). It was then observed that Eap1 siRNA-producing lentiviral particles injected bilaterally into the preoptic area (POA) of juvenile 23-day-old rats resulted in delayed puberty, disrupted estrous cyclicity, reduced plasma LH, FSH and estradiol levels, and delayed growth of ovarian follicles. Additional experiments were conducted to determine if EAP1 is also important for the hypothalamic control of menstrual cyclicity in nonhuman primates and found that knocking down EAP1 expression in the arcuate nuclei (ARC) of the hypothalamus abolished menstrual cyclicity (Dissen et al., 2009). In this context, 86 cases with central precocious puberty were analysed for TTF1 or EAP1 mutations or polymorphisms (Cukier et al. 2013). Direct sequencing of the TTF1 did not reveal any mutation or polymorphism, whereas two EAP1 synonymous variants were identified in two sisters but without resulting in alteration in their transcriptional activity.

- *MKRN3*

In 2013, Abreu et al. (Abreu et al. 2013) showed that loss of function mutations in the imprinted gene MKRN3, located in 15q11-q13, predict to cause CPP. After whole – exome sequencing performed in 40 members of 15 families with history of CPP, 32 patients (27 females/5 males) with CCP and 8 with normal puberty (5 females/3 males) they identified four novel heterozygous mutations in MKRN3. All fifteen affected persons (8 females / 7 males) inherited the mutations from their fathers, the median age at the onset of puberty was 5.75 (5-6.6) years for girls and 8.1 (5.9-8.5) for boys.

In 2014, two additional studies (Settas et al. 2014; Macedo et al. 2014) further confirmed the implication of MKRN3 in precocious puberty. A novel heterozygous

missense variant in the MKRN3 gene (p.C340G) was detected in two affected siblings, a girl with CPP, her brother with early puberty and their unaffected father. In silico analysis predicted the mutation possibly damaging in all five software packages used and structural alignment of the ab initio native and mutant MKRN3 models predicts that this variant leads to significant structural perturbations in the 3-dimensional structure of the C3HC4 (Settas et al. 2014). Finally, in the study of Macedo et al. (Macedo et al. 2014) five novel heterozygous mutations in MKRN3 gene were found in eight unrelated girls out of 215 children with CPP (213 sporadic, 207 girls). Four were frameshift mutations predictive to encode truncated proteins and one was a missence mutation, which was suggested to be deleterious by in silico analysis. All patients with MKRN3 mutations had classical features of CPP with a median age of onset of puberty at 6 years and the mutation was inherited on the paternal allele.

2.B.3 Consequences

Several authors reported that early menarche was associated with an increased risk of obesity, type 2 diabetes and cardiovascular disease in adulthood (Sherman et al. 1981; Van Lenthe et al. 1996; Elks et al. 2010). Additionally, several studies pointed that the timing of puberty could be linked to the risk of breast cancer later in life. In pairs of monozygotic twins discordant for breast cancer an earlier menarche did not predict an increased risk of the disease, whereas in twins concordant for breast cancer, an earlier menarche predicted an earlier occurrence or diagnosis of breast cancer suggesting that the same genes could be involved in determining the timing of menarche and the risk of breast cancer (Hamilton and Mack 2003). These data indicate that, in the heritable or familial forms of breast cancer, genetic susceptibility can cause unusual early sensitivity to sex hormones or unusual early load in sex

hormones. Additionally, precocious puberty has also been associated with increased incidence of conduct and behavior disorders during adolescence (Golub et al. 2008).

3. Sexual dimorphism of pubertal onset deviations

In almost all mammals studied, puberty onset is significantly different between the sexes, usually earlier in females. The onset of puberty in girls occurs 1-2 years earlier than in boys; menarche happens even earlier than reproductive maturity in boys (Iuliano-Burns et al. 2009). The prevelence of pubertal disorders in humans is also sexually dimorphic: the incidence of precocious puberty being disproportianally higher in girls when compared to boys (de Vries et al. 2004). The ratio for ICPP is estimated to be 15-20 females for each male with the disorder (Teles et al. 2011). On the other hand, incidence of IHH is 5-fold elevated in males when compared to females (Seminara et al. 1998; Sykiotis et al. 2010) and males quite often exhibit more severe symptoms than females carrying the same mutation. It is noteworthy that the majority of mutations studied to date were identified in patients with IHH, a disorder far less frequent than ICPP. Prevalence of premature puberty has been predicted to be 0,2% in one population (Teilmann et al. 2005), whereas incidence of IHH is estimated to be 1-10 cases per 100.000 births (or 0.001-0.01%) (Seminara et al.1998). Another important difference between ICPP and IHH is the mode of inheritance. The pedigree of families with history of IHH suggests an autosomal recessive mode of inheritance (Bianco and Kaiser 2009). In other words, only individuals carrying the associated mutation in the homozygous (or compound heterozygous) state present the IHH phenotype, whereas heterozygous parents and siblings have no obvious reproductive abnormalities (de Roux et al. 2003; Seminara et al. 2003; Bhagavath et al. 2006; Topaloglu et al. 2006; Bedecarrats and Kaiser 2007; Nimri et al. 2011). On the other hand, the pedigree of families with a history of ICPP indicates an autosomal dominant mode of transmission (de Vries et al. 2004).

The later is further supported by recent reports of human mutations with confirmed association with ICPP, which were identified in the heterozygous state in affected children (Teles et al. 2008; Silveira et al. 2010; Abreu et al. 2013; Settas et al. 2014; Macedo et al. 2014)

The mechanisms underlying this key sex difference in sexual maturation, as well as the adaptive significance of early or late puberty in one sex versus the other, remain to be investigate, but the underlying mechanisms could implicate sexually dimorphic signaling pathways with a role on GnRH release.

In all species, activation of GnRH neurons remains a consistent pubertal hallmark, and it was shown that any species differences occur “up stream” of GnRH secretion at afferent regulatory signals. However, GnRH neurons were not found to be sexually dimorphic in experimental animals such as rats or guinea pig (Clarkson and Herbison 2006; Cheng et al. 2010). This would be compatible with the involvement of KISS1/NKB signaling, which has been shown to be sexually dimorphic in the hypothalamus of mice (Wray and gainer 1987; Kauffman et al. 2007b; Kauffman et al. 2009), rats (Kauffaman et al. 2007a; Ciofi et al. 2006, 2007), and sheep (Schanzel-Fukunda et al. 1981) (fig. 17).

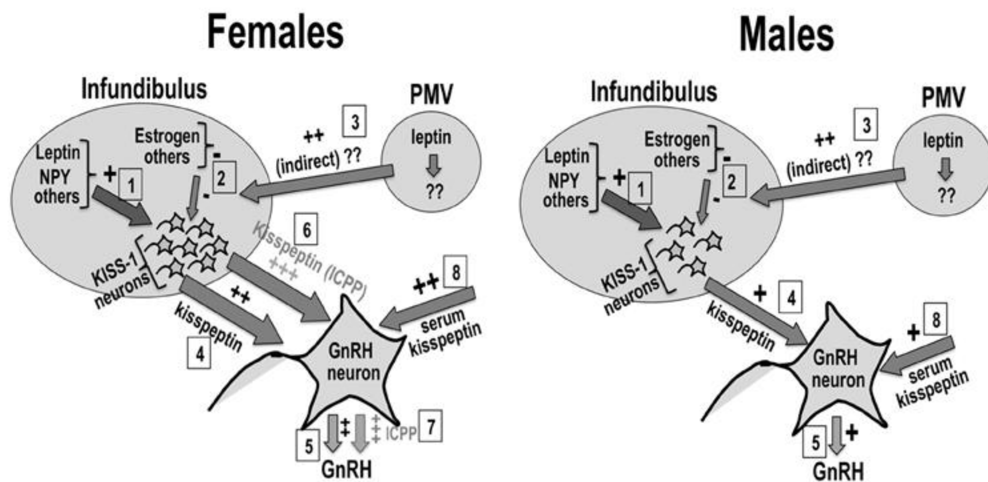


Figure 17

Kisspeptin, sexual dimorphism, and puberty: Kisspeptin neurons (KISS-1) in the infundibular nucleus would be regulated by positive (1) and negative (2) inputs from nutritional, hormonal and environmental sources. Possible leptin input on kisspeptin neurons is shown in (1) and (3). Kisspeptin from KISS-1 neurons (4) or the circulation (8) stimulate GnRH neurons to secrete GnRH (5). The number of KISS-1 neurons in the infundibulus is reported to be elevated in women (left panel) when compared to men (right panel). This sexual difference in kisspeptin is proposed to contribute to earlier onset of puberty in healthy girls when compared to boys (6), as well as to the higher incidence of idiopathic CPP in girls (7) (Bianco 2012).

II. SPECIFIC PART

1. RESEARCH PROJECT

TITLE: Molecular and clinical investigation of the GPR54 and TACR3 gene mutations and polymorphisms in Idiopathic Central Precocious Puberty

I. A. Background

In the last few years, GPR54 (KISS1R) and TACR3 (NK3R) loss of function mutations have been reported to be associated with absence of pubertal initiation, namely with idiopathic hypogonadotropic hypogonadism (IHH) [Cerrato and Seminara 2007; de Roux et al. 2003; Seminara et al. 2003; Semple et al. 2005; Tenenbaum-Rakover et al. 2007; Topaloglu et al. 2009; Gianetti et al. 2010; Nimri et al. 2011]. At the time of the initiation of the current study, only one publication so far reported GPR54 (KISS1R) gain of function mutations to be associated with Idiopathic Central Precocious Puberty ([Teles et al. 2008) while no publication existed so far, exploring the putative role of TACR3 mutations in the pathogenesis of precocious puberty.

1. B. Objectives

The aim of the current study was to investigate the presence of GPR54 (KISS1R) and TACR3 (NK3R) gene mutations and polymorphisms in a population of girls of Greek origin with ICPP and evaluate their possible role in phenotypic variations.

1. C. Patients and Methods

Subjects

Thirty-eight girls were enrolled in this study based on the diagnosis of ICPP that was settled by a paediatric endocrinologist according to the combination of clinical and laboratory data: breast development before the age of 8 years, accelerated progression from one Tanner stage to the next in a period of ≤ 6 months, advanced

bone age by at least 1yr, predicted adult height [Bayley and Pinneau 1952] below target height, uterine length > 34mm, FSH, LH and E2 levels in the pubertal range and LH peak after GnRH testing in the pubertal range. Additional elements for ICPP were: no evidence of hypothalamic-pituitary anatomical lesion on imaging and otherwise normal pituitary function as documented by normal basal levels of prolactin, thyroid stimulating hormone, IGF1 and cortisol. At initial evaluation, the median (25th; 75th) and mean (SD) age were 8.5 (7.5; 9.5) and 8.2 (1.3) years respectively. The median bone age (25th; 75th) was 10.75 (10; 11) years. The median difference (25th; 75th) between target and predicted average final height was 7.2 (3.1; 13.2) cm, the median basal LH levels (25th; 75th) were 1.5 (0.3; 3.9) IU/lt, the median peak LH levels after GnRH stimulating test were 13.6 (6.5; 22.5) IU/lt, the median E2 levels (25th; 75th) were 26.2 (17; 48.5) and the median uterine length (25th; 75th) was 44mm (35; 51.8) (detailed data available on table 2).

The girls have been followed in the Division of Paediatric Endocrinology, Diabetes and Metabolism of the First Department of Paediatrics, National University of Athens, in "Aghia Sofia" Children's Hospital, Athens, Greece. Medical history and clinical examination data (weight and height, pubertal Tanner staging) as well as hormonal assessment (basal FSH, LH, E2 values and GnRH Test) and imaging studies (bone age, pelvic U/S, MRI of the hypothalamo-pituitary area) have also been registered from their medical files.

Case no.	Age (ys)	Puberty Tanner stage	Bone Age (ys)	Height (cm)	Target Height (cm)	FSH (IU/L)	LH (IU/L)	Peak LH (IU/L)	E2 (pg/ml)	U/S Uterus length (mm)	LHRH analog therapy
1	8.5	3	10	125.8	149,3	3	0.1	6.6	na	na	Yes
2	7.1	3	9	143	168,5	6.4	0.7	3.7	20	60	No
3	7.9	5	10.5	124.6	na	7.5	1.4	9.2	31	50	Yes
4	9.3	4	11	130.0	151,0	12.6	6.5	22.2	49	60	Yes
5	8.3	4	10	138.8	153,0	14.6	6.1	30.3	16	43	No
6	8.5	5	12	145.0	155,5	3.4	0.8	20.3	63	83	No
7	7.8	3	10	133.5	152,0	3.8	0.3	8.8	15	na	Yes
8	9.5	3	10.5	131.3	162,0	3.6	0.7	12.5	25	47	Yes
9	6	2	9	113.0	na	3.6	0.4	12.8	4	38	Yes
10	9.8	4	12.5	139.0	159,0	7.1	14.2	88.3	na	46	Yes
11	6.1	4	11	136.0	163,5	5.7	4.6	51.7	26	60	Yes
12	9.5	5	12.5	145.0	160,3	2	0.3	8.2	na	70	Yes
13	4.5	4	6	102.5	162,5	4.9	1.6	70.8	50	36	Yes
14	8	3	10.5	130.3	161,5	7.5	5	43.5	na	37	Yes
15	8.8	4	13	142	155,3	2.9	1.7	15.5	na	27	Yes
16	9.8	3	10.5	140.8	164,5	2.3	2.1	13.6	27	na	Yes
17	9	4	11	142.2	164,0	7.5	2.9	13.6	na	35	Yes
18	9.7	5	12	154.5	170,0	2.6	0.1	3.9	na	64	No
19	7.7	3	10	141.0	162,0	4.7	2	6.2	na	na	Yes
20	6.8	3	10	123.8	159,9	3.3	1.8	35.2	10	20	Yes
21	7.8	3	11	133.0	162,5	2.2	0.2	5.5	na	na	Yes
22	8.7	4	11	141.2	159,5	4.9	2.1	na	35	57	Yes
23	9.5	4	10.5	143	159,5	3.1	1.6	na	20	46	Yes
24	8	2	11	134.0	167,8	1.8	0.1	3	na	34.2	No
25	7.2	3	8.5	124.2	163,0	2.1	0.1	2.9	26	35.8	No
26	7.6	2	10.5	136.0	na	4.9	0.4	16.6	10	45	Yes
27	8.5	3	10	139.0	165,8	10.5	6.1	22.8	47	34	No
28	6.5	3	9	128.0	162,5	2.2	0.2	16	8	35	Yes
29	9.5	3	12	139.4	165,2	4.8	1.5	40.6	na	35	Yes
30	9.7	4	12	145.5	169,0	2.2	0.1	12.7	60	49	Yes
31	9	5	11	138.0	164,8	5.4	1.2	na	58	na	Yes
32	8	3	11	132.5	na	3.4	1.1	20	na	na	Yes
33	7	2	8	124.5	156,0	7.3	16	11.4	20	na	Yes
34	5.5	3	7.5	114.8	161,8	0.7	0.1	4.3	26	21	Yes
35	9.5	4	11	138.5	160,0	3.9	4	na	na	43	Yes
36	8.5	4	11	131.6	155,0	3.1	0.2	6.3	45	45	Yes
37	9	4	11	143.9	162,1	6.1	4.3	14.6	na	40	Yes
38	9.5	4	11	146.5	167,0	4.4	3.8	na	83	48	Yes

Table 2: Clinical and laboratory data for each case with ICPP at referral, na:not available

Ethical Issues

The study has been approved by the Institutional Ethic Committee of the "Aghia Sophia" Children's Hospital and blood has been withdrawn from the children for genetic analysis only after written informed consent has been obtained from their parents or guardians.

Methods

GPR54 (KISS1R) *and TACR3 gene analysis*

Genomic DNA was extracted from peripheral blood lymphocytes of the patients according to classical methods. The entire coding region as well as the exon - intron boundaries of GPR54 (KISS1R) (exons 1 through 5) and TACR3 (NK3R) (exons 1 through 5) genes were amplified by the polymerase chain reaction (PCR) method with a GeneAmp PCR System 9700 (Applied Biosystems). The nucleotide sequences were determined by fluorescent dye chemistry sequencing with an ABI PRISM3000 DNA Analyser and analyzed with Sequencing Analysis software (Applied Biosystems). Primers for GPR54 (KISS1R) and TACR3 (NK3R) were designed as described in previous reports [de Roux et al. 2003; Topaloglu et al. 2009] (detailed data available on tables 3 and 4). By referencing the assembled sequence in the Ensemble genome database, the presence of mutations or single nucleotide polymorphisms (SNP) was checked.

.

EXONS	Primers	Annealing Temperature/cycles number	PCR product	DNA polymerase
Exon 1	forward GGGCGGCCGGGAGGAGGA reverse CCGGGACGGCAGCAGGTG	62^0C /35 cycles	315bp	Platinum Taq (Invitrogen, Greece)
Exon 2	forward GCCCAGCGCCCGCGCATC reverse GTCCCCAAGTGCGCCCTCTC	66^0C /35 cycles	195bp	Platinum Taq (Invitrogen, Greece)
Exon 3	forward CAGGCTCCCAACCGCGCAG reverse CGTGTCCGCCTTCTCCCGTG	61^0C /35 cycles	200bp	AmpliTaq Gold (Applied Biosystems, Greece)
Exon 4	forward, CTTCATCCTGGCTTGTGGCAC reverse CTTGCTGTCCTCCCACCCAC	62^0C /35 cycles	290bp	Platinum Taq (Invitrogen, Greece)
Exon 5	forward GCCTTTCGTCTAACCACCTTC reverse GGAGCCGCTCGGATTCCCAC	65^0C/35 cycles	525bp	Phusion Master Mix (Finnzymes, Finland)

Table 3: Primers and annealing temperatures for GPR54's exons PCR amplification

PCR reactions were carried out in 25 μl total volume with 20-100 ng of purified DNA, 1.5mM MgCl2, 0.4 μM of primers, 0.2 mM dNTPs, 5% DMSO and 0.1μl U of DNA polymerase

EXONS	Primers	Annealing Temperature/cycles number	PCR product
Exon 1 A	forward CCAGCAGGGATTGCAGTATC reverse GCCAGGATGATCCAGATGAC	57^{0}C /35 cycles	330bp
Exon 1 B	forward CCAACCTCACCAACCAGTTTC reverse ACTCGAGGGCTACAAATGGG	57^{0}C /35 cycles	499bp
Exon 2	forward GCCATGATTACCATTCTACGC reverse CAACTTATTGACCACACACAAATC	57^{0}C /35 cycles	535bp
Exon 3	forward CAACTGGCAGCATTTGAAAC reverse GATTACAGTATGTGGACAGCAGC	57^{0}C /35 cycles	529bp
Exon 4	forward CTGTCCGTATATTGCTTCACC reverse AAAGCCTGTGCCTCTCTCAG	57^{0}C /35 cycles	496bp
Exon 5	forward TGTGACATAAATTCTAAGAGTCTGGC reverse CCTTTCTCAATTTGACCATAGC	57^{0}C /35 cycles	603bp

Table 4: Primers and annealing temperatures for TACR3's exons PCR amplification. PCR reactions were carried out in 25 µl total volume with 20-100 ng of purified DNA, 1.5mM MgCl2, 0.2 µM of primers, 0.2 mM dNTPs and 0.1/µl U of Red Taq DNA polymerase (Eurogentec, Brussels)

1. D. Results

No mutation in *GPR54* that might explain the occurrence of central precocious puberty in our subjects was detected. An A/G coding sequence SNP in *GPR54* (rs10407968) (fig. 18) was identified in the homozygous state in 2 out of the 38 patients with ICPP corresponding to a frequency of 6% for the minor allele G in this population. This is a synonymous SNP located in exon 1 of *GPR54*. Global Minor Allele Frequency (MAF) of the minor allele of rs10407968 in Caucasian populations is 15.7%. Since there is no intron before exon 1, this identified SNP cannot modify pre-mRNA splicing and lead to an abnormal mRNA processing. Moreover, at the protein level, this change does not result in the substitution of an amino-acid, indicating that the GPR54 (KISS1R) protein encoded would not differ from the wild–type protein.

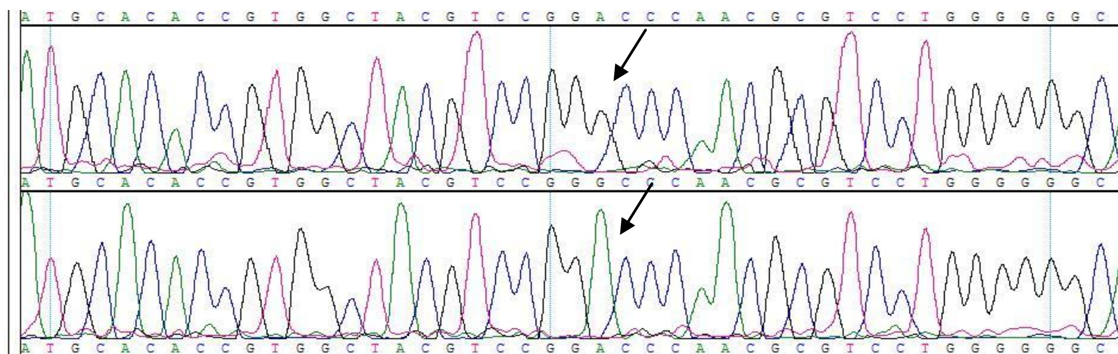


Figure 18 The A/G coding sequence SNP identified in *GPR54* (rs10407968)

The sequence analysis for TACR3 gene revealed neither a mutation nor a polymorphism in this gene in our cohort of girls with ICPP.

1. E Discussion

In this study we have screened for GPR54 (KISS1R) and TACR3 (NK3R) gene mutations and polymorphisms a population of 38 girls with ICPP but found no mutation of these genes in this cohort.

It has been shown that Kisspeptin/GPR54 complex is an established positive regulator of GnRH secretion (Oakley et al. 2009). Kisspeptin consistently stimulates LH secretion in a wide variety of mammalian species and experimental settings (Dhillo 2008). Given the central role of the kisspeptin-KISS1R signaling complex in the pubertal activation of GnRH neurons and the reproductive axis (Seminara et al. 2003), a defective kisspeptin system appears to be an obvious candidate in the pathogenesis of sexual precocity. Until the initiation of the current study only one publication so far reported GPR54 (KISS1R) gain of function mutations to be associated with Idiopathic Central Precocious Puberty (Teles et al. 2008). In that study of 53 unrelated children with ICPP, an autosomal dominant missense mutation, Arg386Pro, in KISS1R (GPR54), leading to prolonged activation of intracellular pathways in response to kisspeptin, has been suggested to cause central precocious puberty in an adopted girl whose premature breast development with slow progression had been observed since birth. Biologic family history was not available. At 8 years breast development was Tanner stage 4, pubic hair was Tanner stage 2 and bone age was 11 years. Her basal FSH levels were 2.6IU/lt, and after GnRH stimulation test 5.9IU/lt, her basal LH were <0.6IU/lt and after GnRH stimulation test 6.4IU/lt and her estradiol levels were 22 pg/ml. To the best of our knowledge, no other ICPP cases with activating KISS1R mutations have been reported.

In our study, patients presented with advanced ICPP on the basis of early breast development (at least B2) at median age (25th; 75th) of 8.5 years (7.5; 9.5) given that the latest Greek report for median age at onset of breast development (B2) was at 10 years (9,2; 10,6) (Papadimitriou et al. 2008). The patients were selected based on their clinical and hormonal data according to their medical files available in the

Division of Endocrinology, Diabetes and Metabolism of the First Department of Pediatrics of the University of Athens. However, the retrospective nature of patients' data collection has inherent limitations and cannot guarantee the ideal selection of patients included in the genetic study, since some patients may have been cases of early puberty and not clear cut cases of precocious puberty.

In the current study we have identified a synonymous SNP (rs 10407968) residing in exon 1 of the GPR54 gene in two patients with ICPP, out of 38 screened. Until now few variations of the GPR54 (KISS1R) gene have been reported:http://www.ensembl.org. GPR54 (KISS1R) receptor's polymorphisms have been poorly studied so far. The A/G coding sequence SNP on the GPR54 (KISS1R) gene (dbSNP ID: rs10407968) was previously identified by others (Tenenbaum-Rakover et al. 2007; Lanfranco et al. 2005) in a population of male patients with IHH but no association with this disorder was found. Moreover, this polymorphism has not been recorded in a large population of Chinese girls (24 girls randomly selected from 272 girls with idiopathic central precocious puberty vs 288 unrelated normal girls) that were screened for GPR54 (KISS1R)'s SNPs through direct sequencing (Luan et al. 2007). The discordance in these data between Chinese and Caucasian populations cannot be explained by a different frequency of rs10407968 polymorphism in the Chinese general population, since it is reported to be 13,3% for G allele and therefore it is not represented significantly lower than the 15.7% representing the frequency of this allele in the Caucasian population (Chi-Square test, p:0.3). However, a further limitation of our study is the limited size of enrolled cases and the absence of control group.

Recently, Silveira et al. (Silveira et al. 2010) studied 83 children (77 girls) with ICPP for mutations in KISS1, and reported two missence variants, c.369C>T (p.Pro74Ser) and c.417C>G (p.His90Asp), in three unrelated children. These variants were not detected in 200 controls, but only Pro74Ser differed from the wild type in the *in vitro* studies: the variant appeared to result in higher kisspeptin resistance to degradation.

However, the proband's mother and maternal grand-mother were also carriers of the variant, but had menarche at appropriate ages. On the other hand, no mutations in KISS1 gene were found in 101 Korean girls with ICPP (Ko et al. 2010). Moreover, very recent data demonstrated that polymorphisms in the 3'UTR of KISS1 are associated with CPP and interfere with the conformation of the DNA sequence and potentially the RNA sequence of KISS1 further verifying a role of the KISS1/KISS1R in the abnormal initiation of puberty (Huijbregts et al. 2012). In accordance to the aforementioned findings, no mutations were found in either KISS1R or KISS1 in 30 girls with ICPP (Tommiska et al. 2011).

Our findings of absence of any mutation in the GPR54 gene are in agreement with those reported previously, suggesting that defects in the kisspeptin system are a rare cause for ICPP.

Moreover, several lines of evidence sugest that neurokin B might have a role as regulator of GnRH secretion (Goodman et al. 2007). Neurokinin B/NKB3R signaling pathway shares many similarities with the Kisspeptin/GPR54 pathway. Navarro et al. [Navarro et al. 2009] proposed a model whereby NKB acts autosynaptically on Kisspeptin neurons in the arcuate nucleus to synchronize and shape the pulsatile secretion of Kisspeptin and drive the release of GnRH in the median eminence. Kisspeptin and NKB (along with Dyn and ERa) signaling pathways may represent the so far called "GnRH pulse generator system". Imbalance of this functional network may result in alterations in the timing of puberty. Therefore, it has been also reasonable to hypothesize that gain-of-function mutations in TACR3 (or NKB3R) might be identified in children with ICPP.

In 2012, a new heterozygous variant (p.A63P) in proneurokinin B was identified in a Brazilian girl out of a population of 114 patients (107girls) with ICPP (Tusset et al. 2012). Comparative analysis of the amino acid sequence of neurokinin B showed that the alanine in position 63 is a conserved residue among primates. Nonetheless, this variant was not predicted to alter the splicing site, and the functional effects of

this amino acid substitution were controversial using different *in silico* algorithms. In other words, this new variant does not seem to have a direct causative role in the precocious puberty. Accordingly, we were not able to identify any mutation or polymorphism in TACR3 gene in this cohort of 38 girls with ICPP, suggesting that TACR3 mutations can rarely, if ever, be the underlying genetic abnormality leading to the premature initiation of puberty.

Taken the above studies and our findings together it appears that no isolated pathway is responsible for the neuroendocrine control of puberty and that the initiation of puberty could involve several inhibitory and stimulatory pathways. It is noteworthy that, a hypothetical transcriptional model that control mammalian puberty via "activators" and "repressors" regulating genes involved in advancing the pubertal process has been proposed [Ojeda et al. 2010].

In the last decade a wealth of evidence has accumulated demonstrating the contribution of different, yet partially overlapping, sub-systems to the neuroendocrine mechanism controlling the pubertal onset in mammals.The final event required for puberty to occur is a sustained increase in pulsatile gonadotropin-releasing hormone (GnRH) release. In rodents, GnRH neurons reside in the preoptic area; in primates they are mostly located in the medial basal hypothalamus,(Ojeda and Skinner, 2006; Plant and Witchel, 2006). The pubertal changes in GnRH output are, in turn determined by modifications in transsynaptic (Kordon et al., 1994; Ojeda and Terasawa, 2002) and glial (Ojeda et al., 2003; Ojeda and Terasawa, 2002) inputs to the GnRH neurons. Studies in monkeys and rodents have shown that the transynaptic changes involve an increase in excitatory inputs and a reduction in inhibitory influences (Ojeda and Terasawa, 2002; Plant and Witchel, 2006; Terasawa and Fernandez, 2001) The glial component of the system is predominantly facilitatory, and consists of growth factors and small diffusible molecules that directly or indirectly stimulate GnRH release (Ojeda et al., 2003; Ojeda and Skinner 2006).

Individual components of each of these regulatory systems continue to be identified (fig.19).

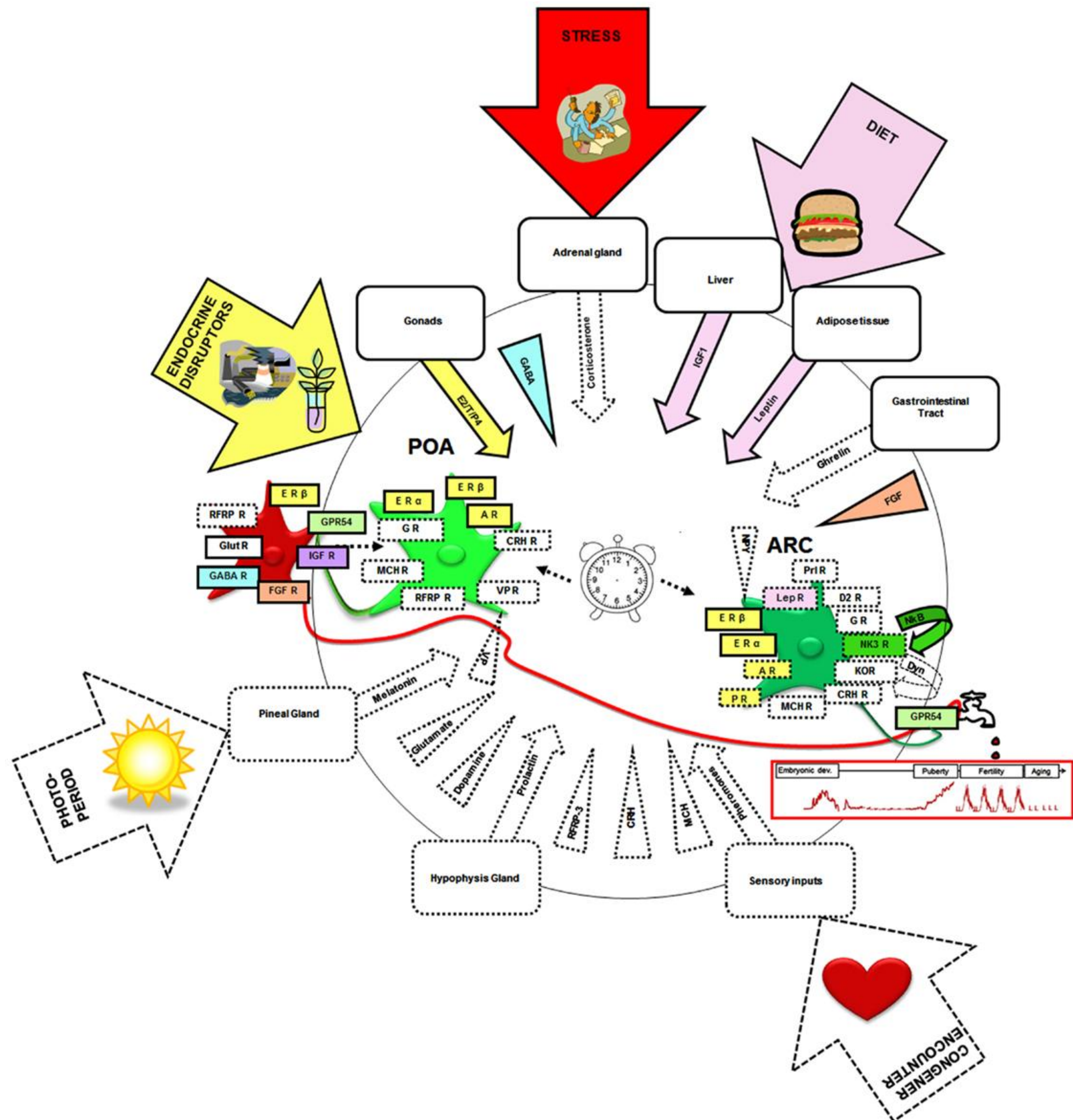


Figure 19 Neural, hormonal, and environmental factors regulating the kisspeptin–GPR54 system. Scheme summarizes the different factors that have been shown to regulate the kisspeptin–GPR54 system only during adulthood (squared by dashed lines) or also during development (squared by full lines and colored). Hormonal factors are codified by an arrow and central factors by a triangle. Molecular factors have been included whose receptors have been found on some kisspeptin neurons, factors found within fibers in close apposition to kisspeptin neurons, factors eliciting c-fos expression, or an electrophysiological response within kisspeptin neurons or changing *Kiss1* or *GPR54* mRNA levels, kisspeptin or GPR54

immunoreactivities, or the number of Kiss1/kisspeptin expressing cells when exogenously administered. Of note this synthetic scheme combines data from mice, rats, sheep, and monkeys and therefore occults potential species differences that may exist in these regulations. It is hypothesized that the developmental pattern of GnRH release (red graph below the tap) is shaped by interactions of these different neural and hormonal factors with an intrinsic differentiation program of the system (central clock). The developing kisspeptin–GPR54 system is particularly vulnerable to some environmental factors like endocrine disruptors, diet and stress which can alter GnRH secretion and reproductive function on the long-term. POA, preoptic area; ARC, arcuate nucleus; E2, estradiol; T, testosterone; P4, progesterone; ER, estrogen receptor; AR, androgen receptor; PR, progestin receptor; IGF, insulin-like growth factor; IGF-R, insulin-like growth factor receptor; FGF, fibroblast growth factor; FGF-R, fibroblast growth factor receptor; GABA-R, GABA receptor; RFRP3, RF-amides related peptide-3; RFRP3-R, RFRP3 receptor; LepR, leptin receptor; Prl-R, Prolactin receptor; NKB, neurokinin B; NK3R, NKB receptor; Glut-R, glutamate receptor; VP, vasopressin; VP-R, vasopressin receptor; MCH, melanocortin; MCH-R, MCH receptor; Dyn, dynorphin; KOR, kappa-opioid receptor (Dyn-receptor); GR, Glucocorticoid receptor; CRH, corticotrophin-releasing hormone; CRH R, corticotrophin-releasing hormone receptor; D2-R, dopamine-receptor (Franceschini et al. 2013).

.

As already exposed, in addition to glutamatergic neurons (Brann, 1995; Ojeda and Skinner 2006; Plant and Witchel, 2006), which are widely distributed through the basal forebrain, the hypothalamus contains neurons that use the peptide kisspeptin to stimulate GnRH secretion (Kauffman et al., 2007; Oaklay et al., 2009). These neurons also produce neurokinin B (Navarro et al., 2009), a neuropeptide that has been shown to stimulate GnRH release and dynorphin, an opioid peptide that inhibits GnRH release (Kinoshita et al., 1982; Navarro et al., 2009; Schulz et al., 1981). In rodents, one group of kisspeptin neurons is located in the periventricular region of the anteroventral periventricular nucleus (AVPV) and the other in the periventricular region of the arcuate nucleus (ARC) (Clakson et al., 2009b). Thus, the excitatory transynaptic regulation of GnRH secretion is provided by neurons that use glutamate, kisspeptin (Dungan et al., 2006; Ojeda and Skinner, 2006) and apparently, neurokinin B (Topaloglu et al., 2008).

With regard to the inhibitory transynaptic network controlling GnRH release, it clearly appears that GABAergic and opioergic neurons are major players (Terasawa and Fernandez 2001). However, growing evidence suggests that a product of the RFamide-related peptide gene (RFRP), which is the mammalian ortholog of the peptide gonadotropin-inhibiting hormone (GnIH) in birds (Ebling and Luckman, 2008) is a physiological inhibitor of GnRH neurons (Ducret et al., 2009; Gibson et al., 2008; Tsutsui et al., 2010). These observations propose that RFRP-containig neurons provide an additional, and perhaps important, transsynaptic mode of inhibitory systems. The RFamide-component seems to be the simplest, because it is composed of one or two peptides (RFRP1 and RFRP3) and a single receptor termed GPR147 (Hinuma et al., 2000; Tsutsui et al., 2010), which appears to be expressed in GnRH neurons (Ducret et al., 2009). The GABA system presents a higher level of complexity. GABA can inhibit GnRH secretion indirectly, via effects exerted on neurons connected to the GnRH neuronal network (Ojeda and Skinner 2006; Terasawa and Fernandez, 2001), but can also stimulate GnRH neurons directly through activation of GABA receptors (DeFazio et al., 2001; Moenter and DeFazio, 2005). The opioid inhibitory system is even more complex. Opioergic neurons imply different peptides and several receptors to inhibit GnRH release (Kordon et al., 1994). Similarly to GABAergic inputs, opiatergic inhibition may be exerted directly (Dudas and Merchenthaler, 2006) or indirectly on GnRH neurons or on neurons involved in the stimulatory control of the GnRH neuronal network, such as kisspeptin neurons (Navarro et al., 2009). In addition to transsynaptic inputs, the pubertal activation of GnRH secretion also requires information from glial cells (Ojeda et al., 2000; Ojeda and Terasawa, 2002). Astrocytes and ependymoglial cells lining the ventral surface of the third ventricle produce cell to cell signaling molecules that stimulate GnRH release (Lomniczi and Ojeda, 2009). Glial cells contribute to the pubertal activation of GnRH secretion via two mechanisms. One of them involves growth factors of at least four different families. Transforming growth factor-beta

(TGFβ), of the TGF superfamily, is recognized by cell-membrane receptors endowed with serine-threonine kinase activity that are located on GnRH neurons (Prevot et al., 2000). Upon binding, TGFβ enhances GnRH gene expression and GnRH secretion (Mahesh et al., 2006; Prevot et al, 2002). Growth factors of the other three families, include the epidermal growth factor (EGF) family, basic fibroplast gorwth factor (bFGF), and insulin-like growth factor 1 (IGF-1). These factors are recognized by receptors with tyrosine kinase activity. Some of these receptors (FGFR, IGF-1R) are expressed in GnRH neurons, but erbB receptors (which recognize EGF and EGF-like peptides) are mostrly expressed on glial cells. Genetic disruption of erbB receptors delays female sexual development due, at least in part, to impaired erbB ligand-induced glial prostaglandin E2 (PGE2) secretion (Lomniczi and Ojeda, 2009). While growth factors of glial origin set in motion glia-to-neurons signaling pathways, at least one neuron-to-glia regulatory pathway initiated by glutamatergic neurons has been shown to facilitate astrocytic signaling mediated by erbB receptors (Dziedzic et al., 2003). The second mechanism of glia-to-GnRH neuron communication involves plastic rearrangement in cell adhesiveness. The adhesion of glial cells to GnRH neurons appears to require at least three different cell-to-cell communications systems. One is thought to be provided by the sialylated form of the neural cell adhesion molecule NCAM (PSA-NCAM) (Parkash and Kaur, 2005; Perera et al., 1993). PSA-NCAM is abundant in brain regions that present a high degree of postnatal plasticity (Gascon et al., 2007), such as the medial basal hypothalamus-median eminence (ME) region (Perera et al., 1993). PSA-NCAM is abundant in GnRH nerve terminals and glial cells of the ME (Parkash and Kaur, 2005), suggesting an involvement in glia-GnRH nerve terminal adhesiveness. The addtional adhesive systems are the Synaptic Cell Adhesion Molecule 1 (SynCAM1) (Ojeda et al., 2008) and the Receptor-like Protein Tyrosine Phosphatase-β (RPTPβ) (Parent et al., 2007). Because in all three cases the participating proteins contain intracellular domains with signalling capabilities, it is likely that the interaction of glial cells with

GnRH neurons may not only involve secreted bioactive molecules, but also the activation of cell-to-cell signalling mechanisms (Lomniczi and Ojeda, 2009).

The neuroendocrine control of puberty seems to involve many genes with different functions

So, can the initiation of puberty be attributed to the activation of a single gene? Superficially the answer to this question may be affirmative if one considers that rare single gene mutations, such as those affecting GNRHR (Bedecarrats and Kaiser, 2007), GPR54 (de Roux et al., 2003; Seminara et al., 2003; Teles et al. 2008) KiSS1 (Lapatto et al., 2007; Topaloglu et al., 2010; Silveira et al. 2010) TAC3 and TACR3 (Topaloglu et al., 2008) result in failure or acceleration of pubertal onset. However, none of these genes seems to be in a commanding position to synchronize neuronal and glial networks involved in the initiation of puberty. Contrary to earlier studies, recent evidence suggests that KiSS1 may not be a gene that sets in motion the pubertal process. Instead, KiSS1 – expressing neurons more likely are activated in response to developmental changes in estradiol production (Clarkson et al., 2009a). These observations along with the recent finding that common genetic variation in a set of genes thought to play an essential role in puberty does not affect the pubertal timing in humans (Gajdos et al., 2008), have made clear that no isolated pathway is responsible for the neuroendocrine control of pubertal onset (Eaves et al., 2004; Krewson et al., 2004; Ojeda et al., 2006; Seminara and Crowley 2001). Moreover, this notion is supported by recent genome-wide association studies that, as already exposed, have found an association of a sequence variation in LIN28B with early menarche (He et al., 2009; Ong et al., 2009; Perry et al., 2009; Sulem et al., 2009). However, to the best of our knowledge, only one sequence variant (rs11800887) was found in the coding region of LIN28B, that was detected in a patient out of 30 girls with ICPP (Tommiska et al. 2011), but it was also present in 1 of the 132 controls

who did not deviate from the other controls with respect to weight, height or Tanner stage as well as gonadotropins or sex steroids levels. Thus, this missence change is not likely to be causative for ICPP and is not of clinical value in the early detection of girls at risk of ICPP.

Further studies found a significant association of sequence polymorphisms in at least 10 other genes with age at menarche (Perry et al., 2009; Sulem et al., 2009). Very recently, it was reported that – in addition to the observations in humans – mice overexpressing Lin28α have delayed puberty (Zhu et al., 2010). However, it is currently unknown if Lin28α overexpression delays puberty due to a general effect on body metabolism or via a mechanism involving an alteration in GnRH secretion. Several other studies identified and characterized other elements of the regulatory system controlling the onset of female puberty, such as novel molecules required for glutamate release (Choi et al., 2008; Ha et al., 2008), homeostatic maintenance of GnRH neuron excitability (Garcia-Rudaz et al., 2008), unidirectional glia-to-GnRH neuron signaling (Lomniczi et al., 2006), and glia-GnRH neuron adhesive communication (Parent et al., 2007; Sandau et al., 2009). Transcriptional regulators of the pubertal onset were also identified, such as the POU-domain gene *Oct2* (Ojeda et al., 1999), the homeodomain gene *Ttf1/Nkx2.1* (Mastronardi et al., 2006), and a novel gene termed *Eap1* (Enhanced At Puberty 1) (Rampazzo et al., 2000; Heger et al., 2007). Based on a variety of experimental studies, including the use of anti-sense oligodeoxynucleotides (Ojeda et al., 1999), CreloxP-mediated, neuron-specific conditional gene deletion (Mastronardi et al., 2006) and siRNA-mediated region-specific knock-down of gene expression (Heger et al., 2007) it was suggested that these genes may play a central role in the hierarchical arrangement of networks controlling the pubertal process (Ojeda et al., 2006). In most of these studies GnRH secretion had been affected by manipulating the expression of these genes selectively in the hypothalamus and the endpoints were manifestations of puberty, such as vaginal opening and age at first ovulation. In other words there exist strong

evidence that puberty is controlled by regulatory gene networks composed of multiple functional modules operating with overlaps of partially redundant pathways as opposed to being in a strict hierarchy (Ojeda et al., 2006).

Genes controlling puberty are organized in functional networks

In accordance with the above reports it may be concluded that there are genes required for the acquisition of reproductive function, such as GPR54, KISS1 and TAC3, and genes that contribute to define the correct timing of the pubertal onset, such as those encoding molecules involved in glia-neuron communication, transsynaptic information, glia-GnRH neuron adhesiveness, and intracellular signaling. Others, such as OCT2, TTF1/NKX2.1, EAP1, and LIN28B appear to be involved in the transcriptional control of puberty. As such they are supposed to control the expression of subordinate genes that are needed for the neuron-to-neuron and glia-to neuron regulation of GnRH secretion at puberty (Ojeda et al., 2010b). Using DNA arrays evidence was provided that an additional gene network exists that contributes to the hypothalamic control of puberty. Genes composing this network have diverse cellular functions but share the common feature of being first identified being involved in tumor suppression/tumor formation (Roth et al., 2007). Consequently this network was termed “TSG” (for Tumor Suppressor Gene) network. Quantitative PCR studies verified the array results (Parent et al., 2008; Roth et al., 2007) and *in silico* analysis of transcription factor recognition sites present in gene promoters indicated that kiss1, Gpr54 and Syncam1, are subordinate genes of the network. Importantly, before the discovery of kisspeptin being involved in the control of puberty, the Kiss1 gene was known as a tumor metastases suppressor (Ohtaki et al., 2001; Steeg et al., 2003).

Cis-regulatory analysis of shared TSG binding sites predicted the existence of five central hubs (*Cdp/Cutl1, Maf, p53, Yy1 and Usf2*) controlling the TSG network at the

transcriptional level (Roth et al., 2007). This analysis involved: 1) the computer-assisted inspection of the 5'flanking region of the genes of interest for predicted transcription factor binding sites using databases such as TransFac (Matys et al., 2003; Wingender etal., 1996) and P-MATCH (http://www.gene-regulation.com/pub/programs.html), 2) the establishment of directional links among genes encoding transcription factors to all genes containing a binding site for those transcription factors, and 3) the visualization of these interactions using the on-line tool CytoScape (http//www.expasy.org). Several hubs were identified and found to be connected to both subordinate genes encoding proteins required for intracellular signaling and cell-to-cell communication and to other presumptive non-TSG upper-echelon genes (*Oct2, Ttf1 and Eap1*) involved in the transcriptional regulation of the pubertal process. Immunohistochemistry and *in situ* hybridization analysis demonstrated that these three genes, as well as other subordinate nodes of the network, are expressed in neuronal and/or glial subsets involved in the control of GnRH secretion, including GnRH neurons themselves (Heger et al. 2007; Mastronardi et al., 2006; Ojeda et al., 2008; Roth et al., 2007). However, the current model of this TSG network is imperfect and needs to be fully validated by functional studies. Such studies are necessary to not only verify *in silico* predictions, but also to provide a more accurate and comprehensive architecture of the network. Also in need of definition is the identity of the neuronal and glial subsets expressing each putative central hub, and the possibility that individual central hubs have different, cell-specific, puberty-related developmental patterns of expression. It is likely that the TSG network is just one of several sub-networks involved in the control of puberty. As indicated above, *Oct2, Ttf1 and Eap1* may form part of another, functionally connected network, because they do not behave as TSGs. LIN28b, on the other hand, may be a hub of the TSG network, because of its role in puberty and its well-established contribution to cancer biology (Viswanathan et al., 2009; Ojeda et al. 2010).

Based on both *in silico* models and experimental data, it was suggested that there may exist a controlling system in which transcriptional regulators are shared by different neuronal and glial subsets, with sets of subordinate genes specifically expressed in particular cellular subsets. It is tempting to speculate that these transcriptional control systems extend to genes encoding enzymes involved in processing precursors of puberty-related peptides, such as kisspeptin, dynorphin, RFRP1/3, etc. Likewise, little is kown about the transcriptional machinery operating in hypothalamic astrocytes to control the production of growth factors (e.g. TGFa, neurogulins) and small molecules (e.g., prostangladin E2, glutamate, ATP) involved in stimulating GnRH release. Of all of these factors, only TGFa expression has been shown to be controlled by a gene *(Oct2)* involved in the transcriptional regulation of puberty (Ojeda et al. 2010).

Gain-of-function methods were used to assess the involvement of transcription factors in the control of the pubertal process. (Davidson et al., 2002; Ideker et al., 2001). Such approaches have been employed to define the involvement of astroglial cells (Ma et al., 1994; Prevot et al., 2003; Rage et al., 1997; Sandau et al., 2009) and specific neuronal subsets (Bilger et al., 2001; Heger et al., 2003) in the hypothalamic control of puberty. Loss-of-function approaches were used to identify three upstream transcriptional regulators of the pubertal process (Heger et al., 2007; Mastronardi et al., 2006; Ojeda et al., 1999), and four subordinate genes involved in neuron-to-neuron communication (Choi et al., 2008; Garcia-Rudaz et al., 2008; Ha et al., 2008; Sandau et al., 2009).

The transcriptional repression of puberty

Growing evidence suggests that puberty may not occur earlier because it is held in check by a developmental program involving transcriptional/posttranscriptional repression of genes that are stimulatory to the pubertal process. For instance, as

already exposed, LIN28B encodes RNA-binding proteins that control gene expression via posttranscriptional regulation (Moss et al., 1997). Nucleotide polymorphisms near the LIN28B gene in chromosome 6(q21) are associated with earlier puberty and shorter stature in girls (Ong et al., 2009; Perry et al., 2009; Sulem et al., 2009). Increasing Lin28b expression in C.Elegans (Moss et al., 1997) and Lina in mice (Zhu et al., 2010) leads to developmental delay. Lin28b exerts its effect by preventing the early expression of genes, which should normally be activated at a subsequent phase of development (Ambros and Horvitz, 1984; Moss et al., 1997). Mammalian LIN 28A and B control cellular function by blocking the maturation of *let-7* miRNA precursors into mature miRNAs. An excess of LIN28B has been shown to reduce production of this miRNA family leading to depression of *let-7* miRNA target genes (Viswanathan et al., 2009). Considering that Lin28a overexpression in mice results in delayed puberty (Zhu et al., 2010), both *Lin28 and let-7 miRNAs* can be considered as repressive components of the TSG network controlling the onset of puberty.

Other potential mechanisms of transcriptional/ posttranscriptional repression that may be involved in controlling the onset of puberty could exist. For instance, it was observed that expression of a family of genes encoding Zinc-finger (ZNF)-containing proteins changes in the hypothalamus of castrated male nonhuman primate at the expected time of puberty (Matagne et al., 2009). The expression profiles of these genes were inversely correlated to the prepubertal changes in LH output.. ZNF genes encode proteins that function as transcriptional repressors (Urrutia, 2003; Vogel et al., 2006). Additionally, two families of transcriptional repressors were expressed in the pre- and peripubertal female rat hypothalamus (Lomniczi et al., 2010). Interestingly, expression of genes belonging to one of these families decreases at puberty, while that of members from the other family increases. Family 1 consists of genes of the POZ-ZF (poxvirus and Zinc finger) family of transcriptional regulators, also konown as BTB (broad complex, Tramtrack, bric –à - bric) (Kelly and Daniel,

2006). It has been observed that expression of a subset of these genes, known as the POK (POZ and Kruppel) subfamily, increases in the hypothalamus of female rats at puberty, suggesting that POK proteins may be repressing downstream repressors of the pubertal process. Family 2 consists of genes of the Polycomb group (PcG). The PcG silencing complex is considered as a major regulator of genomic programs, because it acts at different stages of development to define which set of genes are active and which are quiescent (Kohler and Villar, 2008; Schwrtz and Pirrotta, 2007; Simon and Kingston, 2009). Expression of key members of this complex decreases in the hypothalamus at puberty, suggesting that PcG proteins may provide a repressive influence on the initiation of puberty. Like POK genes, genes of the PcG silencing complex can be considered as TSGs, because they have tumor suppressive nodes (Classen et al., 2009; Kelly and Daniel, 2006; Martinez et al., 2009). Based on these considerations, the concept of a TSG network controlling puberty can be refined by postulating that the network's core is composed of both trans-activational and repressive nodes. While the former move the process along by facilitating the sequential activation of key stimulatory events, the latter may impose a repressive, and likely encompassing, tone to the system, so that premature reproductive maturation is prevented. Obviously, these preliminary observations will need to be thoroughly tested before they can be universally employed to support the idea that transcriptional repression is an integral component of the developmental program controlling mammalian puberty at the hypothalamic level (Ojeda et al. 2010).

The epigenetic control of puberty

As reported, pubertal timing is influenced by complex interactions among genetic, nutritional, environmental, and socioeconomic factors (Palmert and Boepple 2001). It appears obvious that alternative mechanisms of control of puberty onset must exist. It is believed that a powerful biological regulatory system that meets these requirements is epigenetics (Herman and Bylin, 2003; Wolffe and Matzke, 1999). Chemical modification of DNA or chromatin-associated proteins, particularly histones, has a major influence on chromatin structure and gene expression. DNA can be modified by methylation of cytocine residues in CpG dinucleotides (Bjornsson et al., 2004; Jaenish and Bird, 2003). N-terminal tails of histone proteins are subject to a wide range of different modifications, including acetylation, methylation, phosphorylation and ubiquitylation (Jenuwein and Allis 20001; Kouzarides 2007). Epigenetic mechanisms can not only provide gene-specific gate keeper functions (Garcia-Bassets et al., 2007), but are also endowed with an unsuspected degree of plasticity able to transiently change gene expression within hours (Miller and Sweatt 2007) and even minutes (Kangaspeska et al., 2008; Metivier et al., 2008). Even more remarkably, epigenetic regulation of certain genes (Metivier et al., 2008), such as the gene encoding ERα (Kangaspeska et al., 2008), is unexpectedly cyclic, exhibiting a periodicity that results in a rapid, tight and dynamic control of gene expression. It has been demonstrated that epigenetic information is also essential for a variety of neural functions, including estrogen-induced gene expression (Perillo et al., 2008; Subramanian et al., 2008), glial-neuronal interactions (Shen et al., 2008), circadian rhythms (Nakahata et al., 2008) and sexual differentiation of the brain (McCarthy et al., 2009). Lomniczi et al. (2010) suggested that an epigenetic mechanism of transcriptional repression operating in the hypothalamus plays a significant role in the timing of the initiation of female puberty. Recent results suggest that the PcG group

of transcriptional silencers is a major contributor to this repressive mechanism. It was observed that hypothalamic expression of core components of the PcG complex decreases at puberty, and that this change is associated with acquisition of epigenetic silencing marks (DNA methylation, repressive histones) and loss of activating histone marks from their promoter regions. Using the *Kiss1* gene as a prototype, it was then found that PcG proteins interact with the 5' flanking region of this gene, and that the pubertal increase in *Kiss1* expression is accompanied by the acquisition of epigenetic modifications associated with gene activation, i.e. DNA demethylation, recruitment of activating histones and loss of repressive histones. More precisely,in pre-pubertal period, Kiss1 expression has been described to be inhibited by the Eed and Cbx7 (components of PcG complexes). At pubertal onset, the Eed and Cbx7 promoters become methylated, causing a decrease in their expression, allowing the Kiss1 gene to be turned on by other epigenetic modifications, and thereby initiate puberty (Lomniczi et al., 2013).

Moreover, very recently, Abreu et al. (Abreu et al. 2013) reported four loss of function mutations in 12 patients with familial CPP (6 girls) in the imprinted gene MKRN3. MKRN3 is a maternally imprinted gene (only the paternal allele is expressed) and is associated with protein ubiquitination, in which an ubiquitin moiety is attached to a protein, thus tagging it for movement to the proteasome, where it is degraded. Ubiquitination can also be an indicator for signal transduction, cell-cycle regulation, differentiation, morphogenesis and other nonproteolytic fates. The precise mechanism by which the deletion of MKRN3 leads to the early reactivation of pulsatile GnRH secretion remains to be elucidated. However, the widespread species conservation of the makorin protein family, in which MKRN3 protein belongs, suggests that it plays one or more vital roles in cells, with high levels of expression in the developing nervous system (Gray et al. 2000). Recently, increased levels of mkrn3 mRNA at young ages in the arcuate nucleus of male and female mice were found, with a striking reduction in levels immediately before puberty and low levels in

adulthood. The arcuate nucleus is considered to play a key role in puberty control in mice (Navarro et al. 2009), and the pattern of Mkrn3 mRNA expression correlates with an inhibitory effect on the initiation of puberty in these animals. These data are in agreement with the identification of a loss of function mutation in patients with CPP, corroborating the view that the mutation has an inhibitory effect on the secretion of GnRH. Thus the initiation of puberty is thought to result from a decrease in factors that inhibit the release of GnRH combined with an increase in stimulatory factors (fig. 20). These results support the notion that epigenetic mechanisms are integral components of the neuroendocrine process controlling puberty.

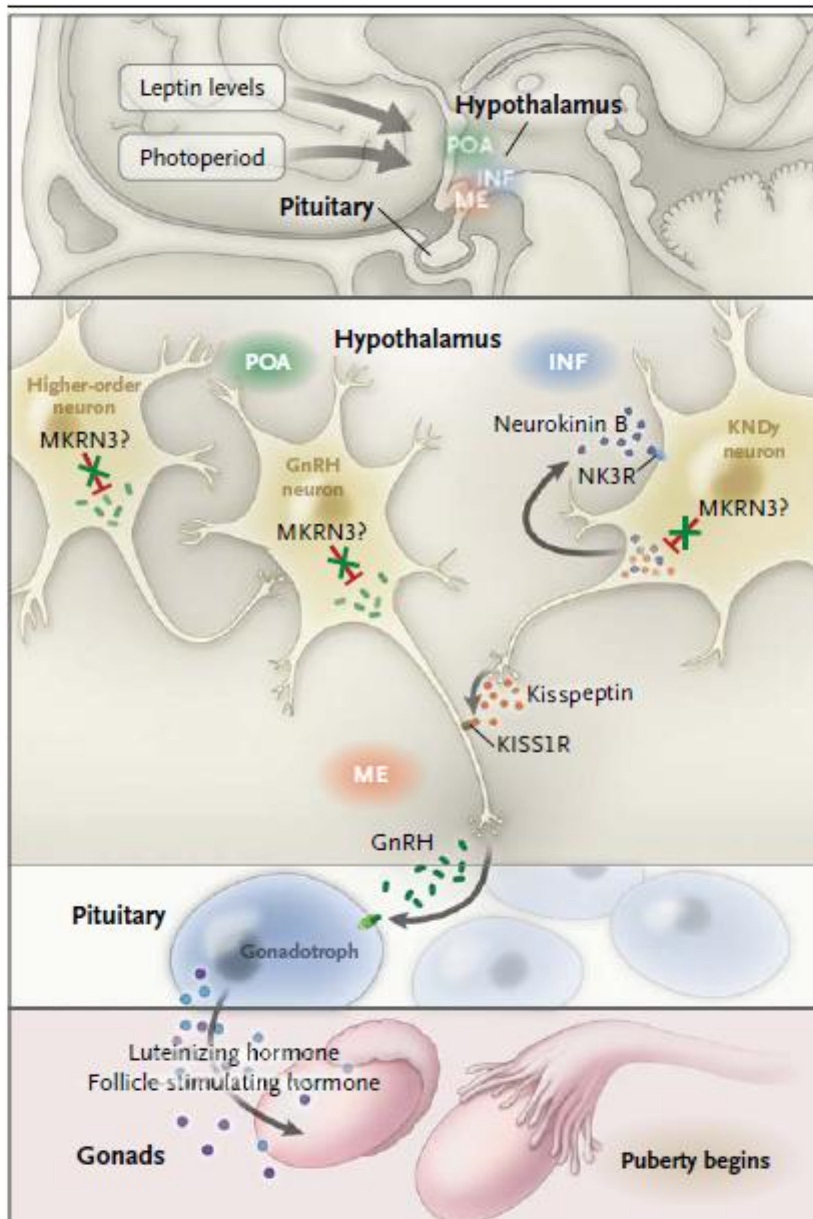


Figure 20 **Timing of Puberty**

A pivotal event in the onset of puberty in mammals is the resumption of pulsatile release of GnRH from neurons of the hypothalamus. Known influences on the timing of the onset of puberty I mammals include the photoperiod, leptin levels, and the increased expression of neurokinin B, kisspeptin, and their receptors (NK3R and KISS1R, respectively). Abreu et al. (Abreu et al. 2013) implicate MKRN3, a protein that is believed to mediate ubiquitination, in puberty onset. In contrast with kisspeptin and neurokinin B, which stimulate the commencement of puberty, MKRN3 seems to inhibit puberty: Abreu et al. show that mutations in MKRN3 predicted to cause loss of function of the protein, cause central precocious puberty. KNDy denotes kisspeptin-neurokinin B-dynorphin, INF infudibular nucleus, ME medin eminence, and POA preoptic area (Hughes 2013).

1. F Conclusions and perspectives

In conclusion, mutations in coding sequences of *GPR54* or *TAC3R* do not seem to be a frequent cause of ICPP. Large-scale international collaborative studies comprising more candidate genes implicated in the initiation of puberty are certainly necessary to delineate in different ethnic groups the impact of specific mutations in the abnormal initiation of puberty.

GnRH decapeptide structure was elucidated in 1971. However, debate continues on the relative importance of neuronal circuits controlling GnRH neuronal secretion. This is due to the complexity of a system regulated by multiple inputs. Under various physiological and pathological conditions, hormonal and metabolic signals either regulate GnRH neurons directly or indirectly. Neuronal inputs to GnRH cells mediate important metabolic-, stress-, sex steroid-, lactational-, and circadian signals to the reproductive axis, among other effects. Based on several studies, it is likely that this critical function is carried out by an interconnected network of sex-steroid sensitive neurons in the infundibular/arcuate nucleus that coexpress NKB/kisspeptin/dynorphin and project to GnRH axons in the median eminence. Connections among the NKB/kisspeptin/dynorphin neurons within the arcuate nucleus provide an anatomic framework to explain how these neurons could be coordinated bilaterally to relay feedback information from the ovaries to modulate pulsatile GnRH release. Thus it is expected that a more complex network of neuronal circuits interplays to exert their regulation on GnRH release under specific physiologic conditions. reasoning this context, we do believe that the tandem Kisspeptin/NKB is essential for the tonic episodic release of GnRH while yet there are significant open questions in this model that await to be addressed in the upcoming years. Given the rarity of KISS1R/TACR3 mutations in patients with ICPP as opposed to patients with IHH, roles of kisspeptins in the timing of puberty could be challenged. Are they triggers, amplifiers, or

dispensable regulators? What are the interactions and hierarchy of Kiss1 neurons with respect to other central regulators of the HPG axis: glutamate, GABA, RFRPs? What are the exact roles and mode of action of KNDy neurons in the central control of GnRH secretion? In addition, it will be important to further define the essential regulatory regions of Kiss1 gene and identify critical transcription factors that interact with Kiss1, in order to better elucidate the complex mechanisms involved in its regulation (Semaan et al. 2013). Moreover, it would be of great interest to decode what the genuine role of dynorpin in this model is and what interneurons it is acting on. It will also be very important to determine the role of NKB signaling in the early activation of the reproductive axis in the neonatal period. In addition, it is known that GnRH neurons exhibit a prolonged period of activation after a single kisspeptin pulse (Han et al. 2005), therefore, there must be a factor that actively block kisspeptin's action on GnRH neurons after every pulse, whose nature is to be discovered.

The above observations support the notion that the onset of puberty depends on the contribution of more than one gene. There are many genes and many pathways that contribute to the process, but as seen in embryonic developmental processes, puberty also appears to be controlled at the transcriptional/post transcriptional level by discrete groups of genes. "Activators' might move the process along by promoting key developmental events; "repressors' might prevent the untimely activation of activating genes (Fig. 21).

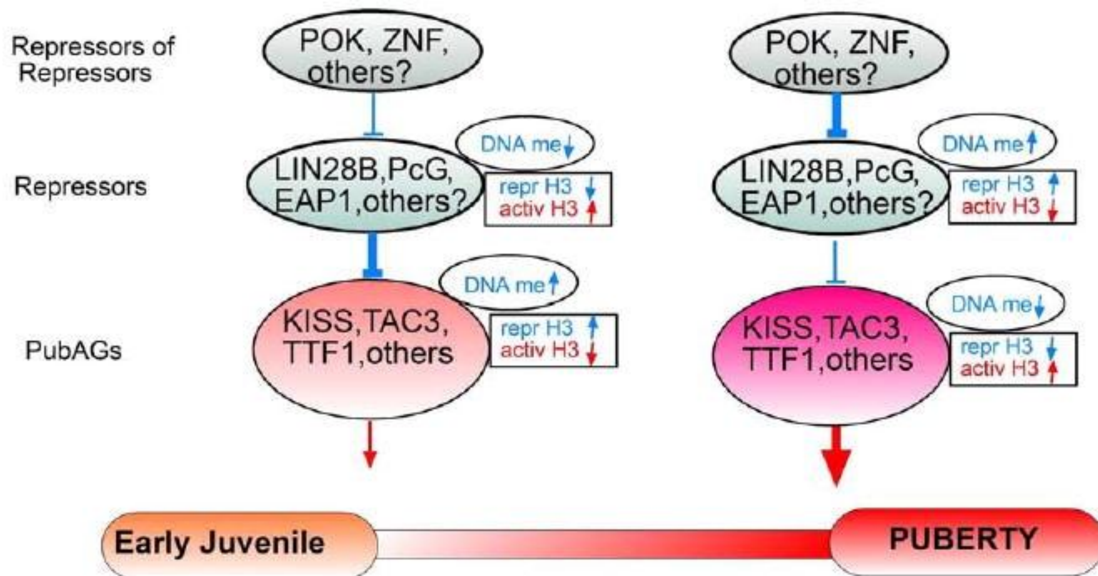


Figure 21 General organization of a hypothetical transcriptional complex controlling the activational arm of female puberty

According to this model the highest level of control is exerted by *repressors of repressors* (*e.g., POK, ZNF* genes); because these genes repress other repressors (e.g. *PcG* genes), their influence would be expected to be low during juvenile development, increasing at puberty. In contrast, the repressive influence of a second, less "central" level of control formed by "repressors" (e.g. *PcG* genes) would be higher during juvenile development than at puberty, because these genes function to prevent the premature activation of puberty activating genes (PubAGs). As a result of these changes in repressive tone, PubAG expression (e.g, *Kiss1, Tac3, TTF1*), would increase at and/or preceding the onset of puberty. Because *Eap1* predominantly represses gene expression, it may be considered as a second tier repressor, but more information is needed before an accurate assignment can be made. An additional level of regulation is provided by epigenetic mechanisms. These mechanisms involve opposite changes in DNA methylation (DNA me) and association of modified histones to PcG and PubAG promoters (repr H3 = histones associated with gene repression; activ H3 = histones associated with gene activation). Not considered in this model is the possibility that *repressors of repressors* also modulate the activity of neuronal populations involved in the inhibitory control of puberty; should this be the case, an increased repressive influence of these factors at puberty would remove transsynaptic inhibitory influences on GnRH neurons, allowing stimulatory inputs to operate at full force. Lastly, this model does not consider "further upstream" mechanisms of control that may govern the expression of repressors of repressors, and that are likely to impose an even more encompassing regulation of the transcriptional cascade controlling puberty onset (Ojeda and Lomniczi 2010).

As detailed in the above figure, it is speculated that both inhibitory and stimulatory pathways of transcriptional regulation operate within neuronal and glial cell populations involved in controlling the onset of puberty and that an encompassing level of regulation is provided by two layers of gene repression, acting in concert with epigenetic mechanisms (Ojeda and Lomniczi 2010).

One of these layers, formed by transcriptional/posttranscriptional 'repressors" (e.g. PcG genes, Lin28b, etc) may function to prevent the premature activation of puberty-inducing genes (such as Kiss1, Tac3, Ttf1, Eap1, Oct2, etc); the other layer, located more centrally in the network (e.g., POK, ZNF genes, and possibly others), may modulate in a temporally dynamic manner the expression of repressors. As such, these more centrally located genes can be considered as 'repressors of repressors". Both layers may also directly control key subordinate genes of the network (such as those operating within kisspeptin, GABAergic, glutamatergic, opioid and RFRP neurons) not by variations in DNA sequence, but by epigenetic changes in gene expression (Lomniczi et al., 2010; Ojeda and Lomniczi 2010). Puberty onset seems to integrate diverse genetic and environmental signals. The control of gene expression via epigenetic mechanisms enables the integration of both intrinsically programmed and environmental factors, thus allowing for the ability to adapt to a changing environment by altering the activity of genes (Bernstein et al. 2007; Rando et al. 2012). Much research is obviously needed to verify or challenge the validity of these concepts.

III. REFERENCES

- Abel TW, Voytko ML, Rance NE. The effects of hormone replacement therapy on hypothalamic neuropeptide gene expression in a primate model of menopause. J Clin Endocrinol Metab. 1999 84:2111-8.
- Abel TW, Rance NE. Stereologic study of the hypothalamic infundibular nucleus in young and older women. J Comp Neurol. 2000 424:679-88.
- Abreu AP, Dauber A, Macedo DB, et al.: Central precocious puberty caused by mutations in the imprinted gene MKRN3. N Engl J Med. 2013 368:2467-75.
- Abreu AP, Trarbach EB, de Castro M, Frade Costa EM, Versiani B, Matias Baptista MT, Garmes HM, Mendonca BB, Latronico AC: Loss-of-function mutations in the genes encoding prokineticin-2 or prokineticin receptor-2 cause autosomal recessive kallmann syndrome. J Clin Endocrinol Metab 2008 93:4113-4118.
- Adachi S, Yamada S, Takatsu Y, Matsui H, Kinoshita M, Takase K, Sugiura H, Ohtaki T, Matsumoto H, Uenoyama Y, Tsukamura H, Inoue K & Maeda K. Involvement of anteroventral periventricular metastin/kisspeptin neurons in estrogen positive feedback action on luteinizing hormone release in female rats. J Reprod Dev 2007 53 367-378.
- Alexander MJ, Dobner PR, Miller MA, Bullock BP, Dorsa DM, Leeman SE. Estrogen induces neurotensin/neuromedin N messenger ribonucleic acid in a preoptic nucleus essential for the preovulatory surge of luteinizing hormone in the rat. Endocrinology. 1989 125:2111-7.
- Almeida TA, Rojo J, Nieto PM, Pinto FM, Hernandez M, Martín JD, Candenas ML. Tachykinins and tachykinin receptors: structure and activity relationships. Curr Med Chem. 2004 11:2045-81.
- Ambros V, Horvitz HR. Heterochronic mutants of the nematode Caenorhabditis elegans. Science. 1984 226:409-16.
- Amstalden M, Coolen LM, Hemmerle AM, Billings HJ, Connors JM, Goodman RL, Lehman MN. Neurokinin 3 receptor immunoreactivity in the septal region, preoptic area and hypothalamus of the female sheep: colocalisation in neurokinin B cells of the arcuate nucleus but not in gonadotrophin-releasing hormone neurones. J Neuroendocrinol. 2010 22:1-12.
- Atanassova N, McKinnell C, Turner KJ, Walker M, Fisher JS, Morley M, Millar MR, Groome NP, Sharpe RM. Comparative effects of neonatal exposure of male rats to potent and weak (environmental) estrogens on spermatogenesis

at puberty and the relationship to adult testis size and fertility: evidence for stimulatory effects of low estrogen levels. Endocrinology 2000 141:3898-907.

- Backholer K, Smith J.T, Rao A, Pereira A, Iqbal J, Ogawa S, et al. Kisspeptin cells in the ewe brain respond to leptin and communicate with neuropeptide Y and proopiomelanocortin cells. Endocrinology 2010 151:2233-2243
- Bakker J, De Mees C, Douhard Q, Balthazart J, Gabant P, Szpirer J, et al. Alpha-fetoprotein protects the developng female mouse brain from masculinization and defeminization by estrogens. Nat Neurosci. 2006 9:220-226
- Bakker J, Pierman S, Gonzalez-Martinez D. Effects of aromatase mutation (ArKO) on the sexual diferentiation of kisspeptin neuronal numbers and their activation by same versus opposite sex urinary pheromones. Horm Behav. 2010 57:390-395
- Ballabio A, Bardoni B, Carrozzo R, Andria G, Bick D, Campbell L, Hamel B, Ferguson-Smith MA, Gimelli G, Fraccaro M, et al.: Contiguous gene syndromes due to deletions in the distal short arm of the human x chromosome. Proc Natl Acad Sci U S A 1989 86:10001-10005.
- Bano R, Wahab F, Riaz T, Jabeen s, Irfan S, Arslan M, et al. Pripheral metastatin (kisspeptin 54) levels changes duting progression of puberty in boys in The 91th Endocrine Society Meeting (Washington, DC), 287, P3
- Barker DJ, Gluckman PD, Godfrey KM, Harding JE, Owens JA, Robinson JS. Fetal nutrition and cardiovascular disease in adult life. Lancet 1993 341:938-41.
- Batt RA, Everard DM, Gillies G, Wilkinson M, Wilson CA, Yeo TA. Investigation into the hypogonadism of the obese mouse (genotype ob/ob). J Reprod Fertil. 1982 64:363-71.
- Bédécarrats GY, Kaiser UB. Mutations in the human gonadotropin-releasing hormone receptor: insights into receptor biology and function. Semin Reprod Med. 2007 25:368-78.
- Belchetz PE, Plant TM, Nakai Y, Keogh EJ & Knobil E. Hypophysial responses to continuous and intermittent delivery of hypopthalamic gonadotropin-releasing hormone. Science 1978 202 631-633.
- Belsky J, Steinberg L, Draper P. Childhood experience, interpersonal development, and reproductive strategy: and evolutionary theory of socialization. Child Dev 1991 62:647-70.

- Berg KA, Maayani S, Goldfarb J, Scaramellini C, Leff P, Clarke WP. Effector pathway-dependent relative efficacy at serotonin type 2A and 2C receptors: evidence for agonist-directed trafficking of receptor stimulus. Mol Pharmacol. 1998 54:94-104.
- Bernstein BE, Meissner A, Lander ES. The mammalian epigenome. Cell. 2007 128:669-81.
- Bhagavath B, Pdolsky R,H, Ozata M, Bolu E, Bick D.P, Kulharya A, et al. Clinical and molecular characteriation of a large sample of patients with hypogonadotropic hypogonadism. Fertil. Steril 2006 85 706-713.
- Bianco SD, Kaiser UB: The genetic and molecular basis of idiopathic hypogonadotropic hypogonadism. Nat Rev Endocrinol 2009 5:569-576.
- Bilger M, Heger S, Brann DW, Paredes A, Ojeda SR. A conditional tetracycline-regulated increase in Gamma amino butyric acid production near luteinizing hormone-releasing hormone nerve terminals disrupts estrous cyclicity in the rat. Endocrinology. 2001 142:2102-14.
- Billings H, Connors JM, Altman SN, Hileman SM, Holaskova I, Lehman MN, et al. Neurokinin B acts via the neurokinin-3 receptor in the retrochiasmatic area to stimulate luteinizing hormone secretion in sheep. Endocrinology 2010 151: 3836-3846.
- Bjornsson HT, Fallin MD, Feinberg AP. An integrated epigenetic and genetic approach to common human disease. Trends Genet. 2004 20:350-8.
- Bonner TI, Affolter HU, Young AC, Young WS III. A cDNA encoding the precursor of the rat neuropeptide, neurokinin B. Brain Res. 1987 388:243-9.
- Bo-Abbas Y, Acierno JS, Jr., Shagoury JK, Crowley WF, Jr., Seminara SB: Autosomal recessive idiopathic hypogonadotropic hypogonadism: Genetic analysis excludes mutations in the gonadotropin-releasing hormone (gnrh) and gnrh receptor genes. J Clin Endocrinol Metab 2003 88:2730-2737.
- Bouligand J, Ghervan C, Tello JA, Brailly-Tabard S, Salenave S, Chanson P, Lombes M, Millar RP, Guiochon-Mantel A, Young J: Isolated familial hypogonadotropic hypogonadism and a gnrh1 mutation. N Engl J Med 2009;360:2742-2748.
- Bourguignon JP, Gérard A, Alvarez Gonzalez ML, Fawe L, Franchimont P. Effects of changes in nutritional conditions on timing of puberty: clinical evidence from adopted children and experimental studies in the male rat. Horm Res. 1992 38 Suppl 1:97-105.

- Brann DW. Glutamate: a major excitatory transmitter in neuroendocrine regulation. Neuroendocrinology. 1995 61:213-25.
- Bridges NA, Christopher JA, Hindmarsh PC, Brook CG. Sexual precocity: sex incidence and aetiology. Arch Dis Child 1994 70:116-8.
- Brito VN, Mendonca BB, guilhoto LM, Freitas KC, Arnhold IJ, Latronico AC: Allelic variants of the gamma-aminobutyric acid-A receptor alpha1-subunit gene (GABRA1) are not associated with idiopathic gonadotropin-dependent precocious puberty in girls with and without electroencephalographic abnormalities. J Clin Endocrinol Metab 2006 91 2432-2436
- Bulow HE, Berry KL, Topper LH, Peles E, Hobert O: Heparan sulfate proteoglycan-dependent induction of axon branching and axon misrouting by the kallmann syndrome gene kal-1. Proc Natl Acad Sci U S A 2002 99:6346-6351.
- Bundak R, Darendeliler F, Gunoz H, Bas F, Saka N, Neyzi O. Analysis of puberty and pubertal growth in healthy boys. Eur J Pediatr. 2007 166:595-600.
- Burke MC, Letts PA, Krajewski SJ, Rance NE. Coexpression of dynorphin and neurokinin B immunoreactivity in the rat hypothalamus: Morphologic evidence of interrelated function within the arcuate nucleus. J Comp Neurol. 2006 498:712-26.
- Campbell RE, Gaidamaka G, Han SK, Herbison AE. Dendro-dendritic bundling and shared synapses between gonadotropin-releasing hormone neurons. Proc Natl Acad Sci U S A. 2009 106:10835-40.
- Canto P, Munguia P, Soderlund D, Castro JJ, Mendez JP: Genetic analysis in patients with kallmann syndrome: Coexistence of mutations in prokineticin receptor 2 and kal1. J Androl 2009 30:41-45.
- Caraty A, Fabre-Nys C, Delaleu B, Locatelli A, Bruneau G, Karsch FJ, Herbison A. Evidence that the mediobasal hypothalamus is the primary site of action of estradiol in inducing the preovulatory gonadotropin releasing hormone surge in the ewe. Endocrinology. 1998 139:1752-60.
- Caraty A, Smith JT, Lomet D, Ben Saïd S, Morrissey A, Cognie J, Doughton B, Baril G, Briant C, Clarke IJ. Kisspeptin synchronizes preovulatory surges in cyclical ewes and causes ovulation in seasonally acyclic ewes. Endocrinology. 2007 148:5258-67.
- Carel JC, Leger J: Clinical practice. Precocious puberty. N Engl J Med 2008 358:2366-2377.

- Castano JP, Martinez-Fuentes AJ, Gutierrez-Pascual E, Vaudry H, Tena-Sempere M, Malagon MM: Intracellular signaling pathways activated by kisspeptins through gpr54: Do multiple signals underlie function diversity? Peptides 2009 30:10-15.
- Castellano JM, Navarro VM, Fernandez-Fernandez R, Castano JP, Malagon MM, Aguilar E, Dieguez C, Magni P, Pinilla L & Tena-Sempere M. Ontogeny and mechanisms of action for the stimulatory effect of kisspeptin on gonadotropin-releasing hormone system of the rat. Mol Cell Endocrinol 2006 (a) 257-258 75-83.
- Castellano JM, Navarro VM, Fernández-Fernández R, Nogueiras R, Tovar S, Roa J, Vazquez MJ, Vigo E, Casanueva FF, Aguilar E, Pinilla L, Dieguez C, Tena-Sempere M. Changes in hypothalamic KiSS-1 system and restoration of pubertal activation of the reproductive axis by kisspeptin in undernutrition. Endocrinology. 2005 146:3917-25.
- Castellano JM, Navarro VM, Fernández-Fernández R, Roa J, Vigo E, Pineda R, Dieguez C, Aguilar E, Pinilla L, Tena-Sempere M. Expression of hypothalamic KiSS-1 system and rescue of defective gonadotropic responses by kisspeptin in streptozotocin-induced diabetic male rats. Diabetes. 2006(b) 55:2602-10.
- Castellano JM, Navarro VM, Roa J, Pineda R, Sánchez-Garrido MA, García-Galiano D, Vigo E, Dieguez C, Aguilar E, Pinilla L, Tena-Sempere M. Alterations in hypothalamic KiSS-1 system in experimental diabetes: early changes and functional consequences. Endocrinology. 2009 150:784-94.
- Chan YM, de Guillebon A, Lang-Muritano M, Plummer L, Cerrato F, Tsiaras S, Gaspert A, Lavoie HB, Wu CH, Crowley WF, Jr., Amory JK, Pitteloud N, Seminara SB: Gnrh1 mutations in patients with idiopathic hypogonadotropic hypogonadism. Proc Natl Acad Sci U S A 2009 106:11703-11708.
- Chan YM, Broder-Fingert S, Seminara SB. Reproductive functions of kisspeptin and Gpr54 across the life cycle of mice and men. Peptides. 2009 30:42-8.
- Charles AC, Hales TG. Mechanisms of spontaneous calcium oscillations and action potentials in immortalized hypothalamic (GT1-7) neurons. J Neurophysiol. 1995 73:56-64.
- Chawla MK, Gutierrez GM, Young WS 3rd, McMullen NT, Rance NE. Localization of neurons expressing substance P and neurokinin B gene

transcripts in the human hypothalamus and basal forebrain. Comp Neurol. 1997 384:429-42.

- Cheng G, Coolen LM, Padmanabhan V, Goodman RL, Lehman MN. The kisspeptin/neurokinin B/dynorphin (KNDy) cell population of the arcuate nucleus: sex differences and effects of prenatal testosterone in sheep. Endocrinology. 2010 151:301-11.
- Choi J, Ha CM, Choi EJ, Jeong CS, Park JW, Baik JH, Park JY, Costa ME, Ojeda SR, Lee BJ. Kinesin superfamily-associated protein 3 is preferentially expressed in glutamatergic neurons and contributes to the excitatory control of female puberty. Endocrinology. 2008 149:6146-56.
- Chrousos GP, Gold PW. The concepts of stress and stress system disorders. Overview of physical and behavioral homeostasis. JAMA. 1992 267:1244-52.
- Chung WC, Moyle SS, Tsai PS: Fibroblast growth factor 8 signaling through fibroblast growth factor receptor 1 is required for the emergence of gonadotropin-releasing hormone neurons. Endocrinology 2008 149:4997-5003.
- Ciofi P, Leroy D, Tramu G. Sexual dimorphism in the organization of the rat hypothalamic infundibular area. Neuroscience. 2006 141:1731-45.
- Ciofi P, Lapirot OC, Tramu G. An androgen-dependent sexual dimorphism visible at puberty in the rat hypothalamus. Neuroscience. 2007 146:630-42.
- Clark EJ, Norris DO, Jones RE. Interactions of gonadal steroids and pesticides (DDT, DDE) on gonaduct growth in larval tiger salamanders, Ambystoma tigrinum. Gen Comp Endocrinol 1998 109:94-105.
- Clarkson J, Boon WC, Simpson ER, Herbison AE. Postnatal development of an estradiol-kisspeptin positive feedback mechanism implicated in puberty onset. Endocrinology. 2009 150:3214-20.
- Clarkson J, d'Anglemont de Tassigny X, Colledge WH, Caraty A, Herbison AE. Distribution of kisspeptin neurones in the adult female mouse brain. J Neuroendocrinol. 2009 21:673-82.
- Clarkson J, d'Anglemont de Tassigny X, Moreno AS, Colledge WH & Herbison AE. Kisspeptin-GPR54 signaling is essential for preovulatory gonadotropin-releasing hormone neuron activation and the luteinizing hormone surge. J Neurosci 2008 28 8691-8697.
- Clarkson J & Herbison AE. Postnatal development of kisspeptin neurons in mouse hypothalamus; sexual dimorphism and projections to gonadotropin-releasing hormone neurons. Endocrinology 2006 147 5817-5825.

- Clarkson J & Herbison AE. Development of GABA and glutamate signaling at the GnRH neuron in relation to puberty. Mol Cell Endocrinol 2006 254-255 32-38.
- Classen AK, Bunker BD, Harvey KF, Vaccari T, Bilder D. A tumor suppressor activity of Drosophila Polycomb genes mediated by JAK-STAT signaling. Nat Genet. 2009 41:1150-5.
- Clements MK, McDonald TP, Wang R, Xie G, O'Dowd BF, George SR, Austin CP & Liu Q. FMRFamide-related neuropeptides are agonists of the orphan G-protein-coupled receptor GPR54. Biochem Biophys Res Commun 2001 284 1189-1193.
- Cole LW, Sidis Y, Zhang C, Quinton R, Plummer L, Pignatelli D, Hughes VA, Dwyer AA, Raivio T, Hayes FJ, Seminara SB, Huot C, Alos N, Speiser P, Takeshita A, Van Vliet G, Pearce S, Crowley WF, Jr., Zhou QY, Pitteloud N: Mutations in prokineticin 2 and prokineticin receptor 2 genes in human gonadotrophin-releasing hormone deficiency: Molecular genetics and clinical spectrum. J Clin Endocrinol Metab 2008 93:3551-3559.
- Colledge WH. Kisspeptins and GnRH neuronal signalling. Trends Endocrinol Metab 2009 20 115-121.
- Conn PM, Janovick JA: Trafficking and quality control of the gonadotropin releasing hormone receptor in health and disease. Mol Cell Endocrinol 2009 299:137-145.
- Constantin S, Caligioni CS, Stojilkovic S & Wray S. Kisspeptin-10 facilitates a plasma membrane-driven calcium oscillator in gonadotropin-releasing hormone-1 neurons. Endocrinology 2009 150 1400-1412.
- Cooper C, Kuh D, Egger P, Wadsworth M, Barker D. Childhood growth and age at menarche. Br J Obstet Gynaecol. 1996 103:814-7.
- Costa EM, Bedecarrats GY, Mendonca BB, Arnhold IJ, Kaiser UB, Latronico AC: Two novel mutations in the gonadotropin-releasing hormone receptor gene in brazilian patients with hypogonadotropic hypogonadism and normal olfaction. J Clin Endocrinol Metab 2001 86:2680-2686.
- Cukier P, Wright H, Rulfs T, Silveira LF, Teles MG, Mendonca BB, Arnhold IJ, Heger S, Latronico AC, Ojeda SR, Brito VN. Molecular and gene network analysis of thyroid transcription factor 1 (TTF1) and enhanced at puberty (EAP1) genes in patients with GnRH-dependent pubertal disorders. Horm Res Paediatr. 2013 80:257-66.

- d'Anglemont de Tassigny X, Fagg LA, Carlton MB, Colledge WH. Kisspeptin can stimulate gonadotropin-releasing hormone (GnRH) release by a direct action at GnRH nerve terminals. Endocrinology. 2008 149:3926-32.
- Danzer SC, Price RO, McMullen NT, Rance NE. Sex steroid modulation of neurokinin B gene expression in the arcuate nucleus of adult male rats. Brain Res Mol Brain Res. 1999 66:200-4.
- Davidson EH, Rast JP, Oliveri P, Ransick A, Calestani C, Yuh CH, Minokawa T, Amore G, Hinman V, Arenas-Mena C, Otim O, Brown CT, Livi CB, Lee PY, Revilla R, Rust AG, Pan Z, Schilstra MJ, Clarke PJ, Arnone MI, Rowen L, Cameron RA, McClay DR, Hood L, Bolouri H. A genomic regulatory network for development. Science. 2002 295:1669-78.
- Decourt C, Tillet Y, Caraty A, Franceschini I & Briant C. Kisspeptin immunoreactive neurons in the equine hypothalamus Interactions with GnRH neuronal system. J Chem Neuroanat 2008 36 131-137.
- DeFazio RA, Heger S, Ojeda SR, Moenter SM. Activation of A-type gamma-aminobutyric acid receptors excites gonadotropin-releasing hormone neurons. Mol Endocrinol. 2002 16:2872-91.
- Delemarre-van de Waal HA. Regulation of puberty. Best Ptract Res Clin Endocrinol Matab. 2002 16 1-12
- Delemarre-van de Waal HA, van Coeverden SC, Engelbregt MT. Factors affecting onset of puberty. Horm Res 2002 57 Suppl 2:15-8.
- Dellovade TL, Merchenthaler I. Estrogen regulation of neurokinin B gene expression in the mouse arcuate nucleus is mediated by estrogen receptor alpha. Endocrinology. 2004 145:736-42.
- de Ridder CM, Thijssen JH, Bruning PF, Van den Brande JL, Zonderland ML, Erich WB. Body fat mass, body fat distribution, and pubertal development: a longitudinal study of physical and hormonal sexual maturation of girls. J Clin Endocrinol Metab 1992 75:442-6.
- de Roux N, Genin E, Carel JC, Matsuda F, Chaussain JL, Milgrom E: Hypogonadotropic hypogonadism due to loss of function of the kiss1-derived peptide receptor gpr54. Proc Natl Acad Sci U S A 2003 100:10972-10976.
- de Roux N, Young J, Misrahi M, Genet R, Chanson P, Schaison G, Milgrom E: A family with hypogonadotropic hypogonadism and mutations in the gonadotropin-releasing hormone receptor. N Engl J Med 1997 337:1597-1602.

- de Vries L, Kauschansky A, Shohat M, Phillip M: Familial central precocious puberty suggests autosomal dominant inheritance. J Clin Endocrinol Metab 2004 89:1794-1800.
- Demerath EW, Towne B, Chumlea WC, Sun SS, Czerwinski SA, Remsberg KE, Siervogel RM: Recent decline in age at menarche: The fels longitudinal study. Am J Hum Biol 2004 16:453-457.
- Dhillo WS, Chaudhri OB, Patterson M, Thompson EL, Murphy KG, Badman MK, McGowan BM, Amber V, Patel S, Ghatei MA, Bloom SR: Kisspeptin-54 stimulates the hypothalamic-pituitary gonadal axis in human males. J Clin Endocrinol Metab 2005 90:6609-6615.
- Ding YQ, Shigemoto R, Takada M, Ohishi H, Nakanishi S, Mizuno N. Localization of the neuromedin K receptor (NK3) in the central nervous system of the rat. J Comp Neurol. 1996 364:290-310.
- Dissen GA, Lomniczi A, Neff TL, Hobbs TR, Kohama SG, Kroenke CD, Galimi F, Ojeda SR. In vivo manipulation of gene expression in non-human primates using lentiviral vectors as delivery vehicles. Methods. 2009 49:70-7.
- Dode C, Hardelin JP: Kallmann syndrome. Eur J Hum Genet 2009 17:139-146.
- Dode C, Levilliers J, Dupont JM, De Paepe A, Le Du N, Soussi-Yanicostas N, Coimbra RS, Delmaghani S, Compain-Nouaille S, Baverel F, Pecheux C, Le Tessier D, Cruaud C, Delpech M, Speleman F, Vermeulen S, Amalfitano A, Bachelot Y, Bouchard P, Cabrol S, Carel JC, Delemarre-van de Waal H, Goulet-Salmon B, Kottler ML, Richard O, Sanchez-Franco F, Saura R, Young J, Petit C, Hardelin JP: Loss-of-function mutations in fgfr1 cause autosomal dominant kallmann syndrome. Nat Genet 2003 33:463-465.
- Dode C, Teixeira L, Levilliers J, Fouveaut C, Bouchard P, Kottler ML, Lespinasse J, Lienhardt-Roussie A, Mathieu M, Moerman A, Morgan G, Murat A, Toublanc JE, Wolczynski S, Delpech M, Petit C, Young J, Hardelin JP: Kallmann syndrome: Mutations in the genes encoding prokineticin-2 and prokineticin receptor-2. PLoS Genet 2006 2:e175.
- Dorling AA, Todman MG, Korach KS, Herbison AE. Critical role for estrogen receptor alpha in negative feedback regulation of gonadotropin-releasing hormone mRNA expression in the female mouse. Neuroendocrinology. 2003 78:204-9.
- Duarte CR, Schütz B, Zimmer A. Incongruent pattern of neurokinin B expression in rat and mouse brains. Cell Tissue Res. 2006 323:43-51.

- Ducret E, Anderson GM, Herbison AE. RFamide-related peptide-3, a mammalian gonadotropin-inhibitory hormone ortholog, regulates gonadotropin-releasing hormone neuron firing in the mouse. Endocrinology. 2009 150:2799-804.
- Ducros A, Pasquet P. Evolution of age at menarche in France. Biom Hum. 1978 13:35-43.
- Dudas B, Merchenthaler I. Three-dimensional representation of the neurotransmitter systems of the human hypothalamus: inputs of the gonadotrophin hormone-releasing hormone neuronal system. J Neuroendocrinol. 2006 18:79-95.
- Duke VM, Winyard PJ, Thorogood P, Soothill P, Bouloux PM, Woolf AS: Kal, a gene mutated in kallmann's syndrome, is expressed in the first trimester of human development. Mol Cell Endocrinol 1995 110:73-79.
- Dumalska I, Wu M, Morozova E, Liu R, van den Pol A & Alreja M. Excitatory effects of the puberty-initiating peptide kisspeptin and group I metabotropic glutamate receptor agonists differentiate two distinct subpopulations of gonadotropin-releasing hormone neurons. J Neurosci 2008 28 8003-8013.
- Dungan HM, Clifton DK, Steiner RA. Minireview: kisspeptin neurons as central processors in the regulation of gonadotropin-releasing hormone secretion. Endocrinology. 2006 147:1154-8.
- Dungan HM, Gottsch ML, Zeng H, Gragerov A, Bergmann JE, Vassilatis DK, Clifton DK, Steiner RA. The role of kisspeptin-GPR54 signaling in the tonic regulation and surge release of gonadotropin-releasing hormone/luteinizing hormone. J Neurosci. 2007 27:12088-95.
- Dungan HM, Naderi R, Clifton DK, Steiner RA Role of neurotensin in the positive feedback control of GnrH/LH secretion in the mouse. Society for Neuroscience Meeting, Washington DC, November 2008. 381.5/RR30
- Ebling FJ, Luckman SM. RFAmide-related peptide: another sexy peptide? Endocrinology. 2008 149:899-901.
- Eaves L, Silberg J, Foley D, Bulik C, Maes H, Erkanli A, Angold A, Costello EJ, Worthman C. Genetic and environmental influences on the relative timing of pubertal change. Twin Res. 2004 7:471-81.
- Eghlidi DH, Haley GE, Noriega NC, Kohama SG, Urbanski HFInfluence of age and 17beta-estradiol on kisspeptin, neurokinin B, and prodynorphin gene expression in the arcuate-median eminence of female rhesus macaques. Endocrinology. 2010 151:3783-94.

- Elks CE, Perry JR, Sulem P, et al. Thirty new loci for age at menarche identified by a meta-analysis of genome-wide association studies. Nat Genet 2010 42: 1077-85
- Estrada KM, Clay CM, Pompolo S, Smith JT, Clarke IJ. Elevated KiSS-1 expression in the arcuate nucleus prior to the cyclic preovulatory gonadotrophin-releasing hormone/lutenising hormone surge in the ewe suggests a stimulatory role for kisspeptin in oestrogen-positive feedback. J Neuroendocrinol. 2006 18:806-9.
- Eveleth PB 1978 Population differences in growth: environmental and genetic factors. In Falkner F, Tanner JM, eds. Human Growth. New York: Plenum Press; 373-394
- Euling SY, Herman-Giddens ME, Lee PA, Selevan SG, Juul A, Sorensen TI, Dunkel L, Himes JH, Teilmann G, Swan SH: Examination of us puberty-timing data from 1940 to 1994 for secular trends: Panel findings. Pediatrics 2008 121 Suppl 3:S172-191.
- Falardeau J, Chung WC, Beenken A, Raivio T, Plummer L, Sidis Y, Jacobson-Dickman EE, Eliseenkova AV, Ma J, Dwyer A, Quinton R, Na S, Hall JE, Huot C, Alois N, Pearce SH, Cole LW, Hughes V, Mohammadi M, Tsai P, Pitteloud N: Decreased fgf8 signaling causes deficiency of gonadotropin-releasing hormone in humans and mice. J Clin Invest 2008 118:2822-2831.
- Farooqi IS, Matarese G, Lord GM, Keogh JM, Lawrence E, Agwu C, Sanna V, Jebb SA, Perna F, Fontana S, Lechler RI, DePaoli AM, O'Rahilly S. Beneficial effects of leptin on obesity, T cell hyporesponsiveness, and neuroendocrine/metabolic dysfunction of human congenital leptin deficiency. J Clin Invest. 2002 110:1093-103.
- Fernandez-Fernandez R, Martini AC, Navarro VM, Castellano JM, Dieguez C, Aguilar E, Pinilla L, Tena-Sempere M. Novel signals for the integration of energy balance and reproduction. Mol Cell Endocrinol. 2006 254-255:127-32.
- Filby AL, van Aerle R, Duitman J & Tyler CR. The kisspeptin/gonadotropin-releasing hormone pathway and molecular signaling of puberty in fish. Biol Reprod 2008 78 278-289.
- Fischbein S. Intra-pair similarity in physical growth of monozygotic and of dizygotic twins during puberty. Ann Hum Biol 1977 4:417-30.

- Fokstuen S, Ginsburg C, Zachmann M, Schinzel A. Maternal uniparental disomy 14 as a cause of intrauterine growth retardation and early onset of puberty. J Pediatr 1999 134:689-95.
- Foradori CD, Coolen LM, Fitzgerald ME, Skinner DC, Goodman RL, Lehman MN. Colocalization of progesterone receptors in parvicellular dynorphin neurons of the ovine preoptic area and hypothalamus. Endocrinology. 2002 143:4366-74.
- Foradori CD, Goodman RL, Adams VL, Valent M, Lehman MN. Progesterone increases dynorphin a concentrations in cerebrospinal fluid and preprodynorphin messenger ribonucleic Acid levels in a subset of dynorphin neurons in the sheep. Endocrinology. 2005 146:1835-42.
- Foradori CD, Amstalden M, Goodman RL, Lehman MN. Colocalisation of dynorphin and neurokinin B immunoreactivity in the arcuate nucleus and median eminence of the sheep. J Neuroendocrinol. 2006 18:534-41.
- Forbes S, Li XF, Kinsey-Jones J, O'Byrne K. Effects of ghrelin on Kisspeptin mRNA expression in the hypothalamic medial preoptic area and pulsatile luteinising hormone secretion in the female rat. Neurosci Lett. 2009 460:143-7.
- Franceschini I, Desroziers E. Development and Aging of the Kisspeptin-GPR54 System in the Mammalian Brain: What are the Impacts on Female Reproductive Function? Front Endocrinol (Lausanne). 2013 28:4-22.
- Franceschini I, Lomet D, Cateau M, Delsol G, Tillet Y & Caraty A. Kisspeptin immunoreactive cells of the ovine preoptic area and arcuate nucleus co-express estrogen receptor alpha. Neurosci Lett 2006 401 225-230.
- Franco B, Guioli S, Pragliola A, Incerti B, Bardoni B, Tonlorenzi R, Carrozzo R, Maestrini E, Pieretti M, Taillon-Miller P, Brown CJ, Willard HF, Lawrence C, Graziella Persico M, Camerino G, Ballabio A: A gene deleted in kallmann's syndrome shares homology with neural cell adhesion and axonal path-finding molecules. Nature 1991 353:529-536.
- Francou B, Bouligand J, Voican A, Amazil L, Trabado S, Fagart J et al. Normosmic congenital hypogonadotropic hypogonadism due to TAC3/TACR3 mutations: characterization of neuroendocrine phenotypes and novel mutations. Plos ONE 6:e25614
- Freitas KC, Ryan G, Brito VN, Tao YX, Costa EM, Mendonca BB, Segaloff D, Latronico AC: Molecular analysis of the neuropeptide y1 receptor gene in

human idiopathic gonadotropin-dependent precocious puberty and isolated hypogonadotropic hypogonadism. Fertil Steril 2007 87:627-634.

- Frisch RE, Revelle R. Height and weight at menarche and a hypothesis of critical body weights and adolescent events. Science 1970 169:397-9.
- Frisch RE, Revelle R. Height and weight at menarche and a hypothesis of menarche. Arch Dis Child 1971 46:695-701.
- Frisch RE, Revelle R, Cook S. Components of weight at menarche and the initiation of the adolescent growth spurt in girls: estimated total water, llean body weight and fat. Hum Biol 1973 45:469-83.
- Fukami M, Muruyama T, Dateki S, Sato N, Yoshimura Y, Ogata T. Hypothalamic dysfunction in a female with isolated hypogonadotropic hypogonadism and compound heterozygous TACR3 mutations and clinical manifestation in her heterozygous mother. Horm Res Paeditr 73 477-481
- Gajdos ZK, Butler JL, Henderson KD, He C, Supelak PJ, Egyud M, Price A, Reich D, Clayton PE, Le Marchand L, Hunter DJ, Henderson BE, Palmert MR, Hirschhorn JN. Association studies of common variants in 10 hypogonadotropic hypogonadism genes with age at menarche. J Clin Endocrinol Metab. 2008 93:4290-8.
- Garagorri JM, Chaussain JL, Job JC. Precocious puberty in girls. Study of 98 cases]. Arch Fr Pediatr 1982 39:605-11.
- Garcia-Bassets I, Kwon YS, Telese F, Prefontaine GG, Hutt KR, Cheng CS, Ju BG, Ohgi KA, Wang J, Escoubet-Lozach L, Rose DW, Glass CK, Fu XD, Rosenfeld MG. Histone methylation-dependent mechanisms impose ligand dependency for gene activation by nuclear receptors. Cell. 2007 128:505-18.
- Garcia-Rudaz C, Deng V, Matagne V, Ronnekleiv OK, Bosch M, Han V, Percy AK, Ojeda SR. FXYD1, a modulator of Na,K-ATPase activity, facilitates female sexual development by maintaining gonadotrophin-releasing hormone neuronal excitability. J Neuroendocrinol. 2009 21:108-22.
- Gascon E, Vutskits L, Kiss JZ. Polysialic acid-neural cell adhesion molecule in brain plasticity: from synapses to integration of new neurons. Brain Res Rev. 2007 56:101-18.
- George JT, Veldhuis JD, Roseweir AK, Newton CL, Faccenda E, Millar RP, Anderson RA. Kisspeptin-10 is a potent stimulator of LH and increases pulse frequency in men. J Clin Endocrinol Metab. 2011 96:E1228-36.

- Georgopoulos N, Markou K, Theodoropoulou A, Paraskevopoulou P, Varaki L, Kazantzi Z, Leglise M, Vagenakis AG. Growth and pubertal development in elite female rhythmic gymnasts. J Clin Endocrinol Metab 1999 84:4525-30.
- Georgopoulos NA, Pralong FP, Seidman CE, Seidman JG, Crowley WF, Jr., Vallejo M: Genetic heterogeneity evidenced by low incidence of kal-1 gene mutations in sporadic cases of gonadotropin-releasing hormone deficiency. J Clin Endocrinol Metab 1997 82:213-217.
- Gianetti E, Tusset C, Noel SD, Au MG, Dwyer AA, Hughes VA, Abreu AP, Carroll J, Trarbach E, Silveira LF, Costa EM, de Mendonca BB, de Castro M, Lofrano A, Hall JE, Bolu E, Ozata M, Quinton R, Amory JK, Stewart SE, Arlt W, Cole TR, Crowley WF, Kaiser UB, Latronico AC, Seminara SB: Tac3/tacr3 mutations reveal preferential activation of gonadotropin-releasing hormone release by neurokinin b in neonatal life followed by reversal in adulthood. J Clin Endocrinol Metab 2010 95:2857-2867.
- Gibson WT, Farooqi IS, Moreau M, DePaoli AM, Lawrence E, O'Rahilly S, Trussell RA. Congenital leptin deficiency due to homozygosity for the Delta133G mutation: report of another case and evaluation of response to four years of leptin therapy. J Clin Endocrinol Metab. 2004 89:4821-6.
- Gibson EM, Humber SA, Jain S, Williams WP 3rd, Zhao S, Bentley GE, Tsutsui K, Kriegsfeld LJ. Alterations in RFamide-related peptide expression are coordinated with the preovulatory luteinizing hormone surge. Endocrinology. 2008 149:4958-69.
- Gingerich S, Wang X, Lee PK, Dhillon SS, Chalmers JA, Koletar MM & Belsham DD. The generation of an array of clonal, immortalized cell models from the rat hypothalamus: analysis of melatonin effects on kisspeptin and gonadotropin-inhibitory hormone neurons. Neuroscience 2009 162 1134-1140.
- Glidewell-Kenney C, Hurley LA, Pfaff L, Weiss J, Levine JE, Jameson JL. Nonclassical estrogen receptor alpha signaling mediates negative feedback in the female mouse reproductive axis. Proc Natl Acad Sci U S A. 2007 104:8173-7.
- Golub MS, Collman GW, Foster PM, et al. Public health implications of altered puberty timing. Pediatrics 2008 121: Suppl 3: S218-S230
- González-Martínez D, De Mees C, Douhard Q, Szpirer C, Bakker J. Absence of gonadotropin-releasing hormone 1 and Kiss1 activation in alpha-fetoprotein

knockout mice: prenatal estrogens defeminize the potential to show preovulatory luteinizing hormone surges. Endocrinology. 2008 149:2333-40.

- Gonzalez-Martinez D, Hu Y, Bouloux PM: Ontogeny of gnrh and olfactory neuronal systems in man: Novel insights from the investigation of inherited forms of kallmann's syndrome. Front Neuroendocrinol 2004 25:108-130.
- Goodman RL, Coolen LM, Anderson GM, Hardy SL, Valent M, Connors JM, Fitzgerald ME, Lehman MN. Evidence that dynorphin plays a major role in mediating progesterone negative feedback on gonadotropin-releasing hormone neurons in sheep. Endocrinology. 2004 145:2959-67.
- Goodman RL, Lehman MN, Smith JT, Coolen LM, de Oliveira CV, Jafarzadehshirazi MR, Pereira A, Iqbal J, Caraty A, Ciofi P, Clarke IJ: Kisspeptin neurons in the arcuate nucleus of the ewe express both dynorphin a and neurokinin b. Endocrinology 2007 148:5752-5760.
- Gottsch ML, Clifton DK, Steiner RA: Galanin-like peptide as a link in the integration of metabolism and reproduction. Trends Endocrinol Metab 2004 15:215-221.
- Gottsch ML, Cunningham MJ, Smith JT, Popa SM, Acohido BV, Crowley WF, Seminara S, Clifton DK, Steiner RA: A role for kisspeptins in the regulation of gonadotropin secretion in the mouse. Endocrinology 2004 145:4073-4077.
- Gottsch ML, Navarro VM, Zhao Z, Glidewell-Kenney C, Weiss J, Jameson JL, Clifton DK, Levine JE, Steiner RA. Regulation of Kiss1 and dynorphin gene expression in the murine brain by classical and nonclassical estrogen receptor pathways. J Neurosci. 2009 29:9390-5.
- Goubillon ML, Forsdike RA, Robinson JE, Ciofi P, Caraty A, Herbison AE. Identification of neurokinin B-expressing neurons as a highly estrogen-receptive, sexually dimorphic cell group in the ovine arcuate nucleus. Endocrinology. 2000 141:4218-25.
- Grant AD, Akhtar R, Gerard NP, Brain SD. Neurokinin B induces oedema formation in mouse lung via tachykinin receptor-independent mechanisms. J Physiol. 2002 543:1007-14.
- Gray TA, Hernandez L, Carey AH, et al. The ancient source of a distinct gene family encoding proteins featuring RING and C(3)H zinc-finger motifs with abundant expression in developing brain and nervous system. Genomics 2000 66:76-86
- Greenberg MJ & Price DA. Relationships among the FMRFamide-like peptides. Prog Brain Res 1992 92 25-37.

- Grumbach MM: The neuroendocrinology of human puberty revisited. Horm Res 2002 57 Suppl 2:2-14.
- Grumbach MM: A window of opportunity: The diagnosis of gonadotropin deficiency in the male infant. J Clin Endocrinol Metab 2005 90:3122-3127.
- Grumbach MM, Styne DM 1998 Puberty: ontogeny, neuroendocrinology, physiology, and disorders. In: Wilson JD, Foster DW, Kronenberg HM, Larsen PR, eds. Williams' textbook of endocrinology. 9th ad. Phyladelphia: W.B. Saunders Ca; 1509-1625
- Guran T, Tolhurst G, Bereket A, Rocha N, Porter K, Turan S, Gribble FM, Kotan LD, Akcay T, Atay Z, Canan H, Serin A, O'Rahilly S, Reimann F, Semple RK, Topaloglu AK: Hypogonadotropic hypogonadism due to a novel missense mutation in the first extracellular loop of the neurokinin b receptor. J Clin Endocrinol Metab 2009 94:3633-3639.
- Gutierrez-Pascual E, Leprince J, Martinez-Fuentes AJ, Segalas-Milazzo I, Pineda R, Roa J, Duran-Prado M, Guilhaudis L, Desperrois E, Lebreton A, Pinilla L, Tonon MC, Malagon MM, Vaudry H, Tena-Sempere M & Castano JP. In vivo and in vitro structure-activity relationships and structural conformation of Kisspeptin-10-related peptides. Mol Pharmacol 2009 76 :58-67.
- Gutierrez-Pascual E, Martinez-Fuentes AJ, Pinilla L, Tena-Sempere M, Malagon MM & Castano JP. Direct pituitary effects of kisspeptin: activation of gonadotrophs and somatotrophs and stimulation of luteinising hormone and growth hormone secretion. J Neuroendocrinol 2007 19:521-530.
- Hamilton AS, Mack TM. Puberty and genetic susceptibility to breast cancer in a case-control study in twins. N Engl J Med 2003 348:2313-22.
- Ha CM, Choi J, Choi EJ, Costa ME, Lee BJ, Ojeda SR. NELL2, a neuron-specific EGF-like protein, is selectively expressed in glutamatergic neurons and contributes to the glutamatergic control of GnRH neurons at puberty. Neuroendocrinology. 2008 88:199-211.
- Han SK, Gottsch ML, Lee KJ, Popa SM, Smith JT, Jakawich SK, Clifton DK, Steiner RA, Herbison AE: Activation of gonadotropin-releasing hormone neurons by kisspeptin as a neuroendocrine switch for the onset of puberty. J Neurosci 2005 25:11349-11356.
- Hardelin JP, Levilliers J, del Castillo I, Cohen-Salmon M, Legouis R, Blanchard S, Compain S, Bouloux P, Kirk J, Moraine C, et al.: X

chromosome-linked kallmann syndrome: Stop mutations validate the candidate gene. Proc Natl Acad Sci U S A 1992 89:8190-8194.

- He C, Kraft P, Chen C, Buring JE, Paré G, Hankinson SE, Chanock SJ, Ridker PM, Hunter DJ, Chasman DI. Genome-wide association studies identify loci associated with age at menarche and age at natural menopause. Nat Genet. 2009 41:724-8.
- Heger S, Mastronardi C, Dissen GA, Lomniczi A, Cabrera R, Roth CL, Jung H, Galimi F, Sippell W, Ojeda SR. Enhanced at puberty 1 (EAP1) is a new transcriptional regulator of the female neuroendocrine reproductive axis. J Clin Invest. 2007 117:2145-54.
- Heger S, Seney M, Bless E, Schwarting GA, Bilger M, Mungenast A, Ojeda SR, Tobet SA. Overexpression of glutamic acid decarboxylase-67 (GAD-67) in gonadotropin-releasing hormone neurons disrupts migratory fate and female reproductive function in mice. Endocrinology. 2003 144:2566-79.
- Helke CJ, Krause JE, Mantyh PW, Couture R, Bannon MJ. Diversity in mammalian tachykinin peptidergic neurons: multiple peptides, receptors, and regulatory mechanisms. FASEB J. 1990 4:1606-15.
- Herbison, AE. Physiology of the gonadotropin-releasing hormone neuronal network. In: Neil, JD.; Plant, TM.; Pfaff, DW.; Challis,JG.; de Kretser, DM.; Richards, JS.; Wassarman, PM., editors. In Knobil and Neill's Physiology of reproduction. Vol. Vol. II; Elsevier; 2006. p. 1415-1482.ed.^eds.
- Hergenroeder AC, Hill RB, Wong WW, Sangi-Haghpeykar H, Taylor W. Validity of self-assessment of pubertal maturation in African American and European American adolescents. J Adolesc Health 1999 24:201-5.
- Herman JG, Baylin SB. Gene silencing in cancer in association with promoter hypermethylation. N Engl J Med. 2003 349:2042-54.
- Herman-Giddens ME, Slora EJ, Wasserman RC, Bourdony CJ, Bhapkar MV, Koch GG, Hasemeier CM. Secondary sexual characteristics and menses in young girls seen in office practice: a study from the Pediatric Research in Office Settings network. Pediatrics 1997 99:505-12.
- Herbison AE: Genetics of puberty. Horm Res 2007 68 Suppl 5:75-79.
- Hewitt SC, Korach KS. Estrogen receptors: structure, mechanisms and function. Rev Endocr Metab Disord. 2002 3:193-200.
- Hinuma S, Shintani Y, Fukusumi S, Iijima N, Matsumoto Y, Hosoya M, Fujii R, Watanabe T, Kikuchi K, Terao Y, Yano T, Yamamoto T, Kawamata Y, Habata Y, Asada M, Kitada C, Kurokawa T, Onda H, Nishimura O, Tanaka M, Ibata

Y, Fujino M. New neuropeptides containing carboxy-terminal RFamide and their receptor in mammals. Nat Cell Biol. 2000 2:703-8.

- Higgs DR, Vernimmen D, Hughes J, Gibbons R: Using genomics to study how chromatin influences gene expression. Annu Rev Genomics Hum Genet 2007 8:299-325.
- Homma T, Sakakibara M, Yamada S, Kinoshita M, Iwata K, Tomikawa J, Kanazawa T, Matsui H, Takatsu Y, Ohtaki T, Matsumoto H, Uenoyama Y, Maeda K, Tsukamura H. Significance of neonatal testicular sex steroids to defeminize anteroventral periventricular kisspeptin neurons and the GnRH/LH surge system in male rats. Biol Reprod. 2009 81:1216-25.
- Hrabovszky E, Ciofi P, Vida B, Horvath MC, Keller E, Caraty A, Bloom SR, Ghatei MA, Dhillo WS, Liposits Z, Kallo I. The kisspeptin system of the human hypothalamus: sexual dimorphism and relationship with gonadotropin-releasing hormone and neurokinin B neurons. Eur J Neurosci. 2010 31:1984-98.
- Hughes IA. Releasing the brake on puberty. N Engl J Med. 2013 368:2513-5.
- Huijbregts L, Roze C, Bonafe G, Houang M, Le Bouc Y, Carel JC, Leger J, Alberti P, de Roux N. DNA polymorphisms of the KiSS1 3' Untranslated region interfere with the folding of a G-rich sequence into G-quadruplex. Mol Cell Endocrinol 2012 351:239-248
- Ibrahimi OA, Zhang F, Hrstka SC, Mohammadi M, Linhardt RJ: Kinetic model for fgf, fgfr, and proteoglycan signal transduction complex assembly. Biochemistry 2004 43:4724-4730.
- Ideker T, Thorsson V, Ranish JA, Christmas R, Buhler J, Eng JK, Bumgarner R, Goodlett DR, Aebersold R, Hood L. Integrated genomic and proteomic analyses of a systematically perturbed metabolic network. Science. 2001 292:929-34.
- Irwig MS, Fraley GS, Smith JT, Acohido BV, Popa SM, Cunningham MJ, Gottsch ML, Clifton DK & Steiner RA. Kisspeptin activation of gonadotropin releasing hormone neurons and regulation of KiSS-1 mRNA in the male rat. Neuroendocrinology 2004 80:264-272.
- Jaenisch R, Bird A. Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. Nat Genet. 2003 33 Suppl:245-54.

- Jaruratanasirikul S, Mo-suwan L, Lebel L. Growth pattern and age at menarche of obese girls in a transitional society. J Pediatr Endocrinol Metab 1997 10:487-90.
- Jenuwein T, Allis CD. Translating the histone code. Science. 2001 293:1074-80.
- Jongmans MC, van Ravenswaaij-Arts CM, Pitteloud N, Ogata T, Sato N, Claahsen-van der Grinten HL, van der Donk K, Seminara S, Bergman JE, Brunner HG, Crowley WF, Jr., Hoefsloot LH: Chd7 mutations in patients initially diagnosed with kallmann syndrome--the clinical overlap with charge syndrome. Clin Genet 2009 75:65-71.
- Ibáñez L, Ferrer A, Marcos MV, Hierro FR, de Zegher F. Early puberty: rapid progression and reduced final height in girls with low birth weight. Pediatrics. 2000 106:E72.
- Iuliano-Burns S, Hopper J, Seeman E. The age of puberty determines sexual dimorphism in bone structure: a male/female co-twin control study. J Clin Endocrinol Metab. 2009 94:1638-43.
- Jayasena CN, Nijher GM, Abbara A, Murphy KG, Lim A, Patel D, Mehta A, Todd C, Donaldson M, Trew GH, Ghatei MA, Bloom SR, Dhillo WS. Twice-weekly administration of kisspeptin-54 for 8 weeks stimulates release of reproductive hormones in women with hypothalamic amenorrhea. Clin Pharmacol Ther. 2010 88:840-7.
- Jayasena CN, Nijher GM, Chaudhri OB, Murphy KG, Ranger A, Lim A, Patel D, Mehta A, Todd C, Ramachandran R, Salem V, Stamp GW, Donaldson M, Ghatei MA, Bloom SR, Dhillo WS. Subcutaneous injection of kisspeptin-54 acutely stimulates gonadotropin secretion in women with hypothalamic amenorrhea, but chronic administration causes tachyphylaxis. J Clin Endocrinol Metab. 2009 94:4315-23.
- Jayasena CN, Nijher GM, Comninos AN, Abbara A, Januszewki A, Vaal ML, Sriskandarajah L, Murphy KG, Farzad Z, Ghatei MA, Bloom SR, Dhillo WS. The effects of kisspeptin-10 on reproductive hormone release show sexual dimorphism in humans. J Clin Endocrinol Metab. 2011 96:E1963-72.
- Kakarla N, Bradshaw KD: Disorders of pubertal development: Precocious puberty. Semin Reprod Med 2003 21:339-351.
- Kalamatianos T, Grimshaw SE, Poorun R, Hahn JD, Coen CW. Fasting reduces KiSS-1 expression in the anteroventral periventricular nucleus (AVPV): effects of fasting on the expression of KiSS-1 and neuropeptide Y in

the AVPV or arcuate nucleus of female rats. J Neuroendocrinol. 2008 20:1089-97.

- Kalsbeek A, Palm IF, La Fleur SE, Scheer FA, Perreau-Lenz S, Ruiter M, Kreier F, Cailotto C & Buijs RM. SCN outputs and the hypothalamic balance of life. J Biol Rhythms 2006 21:458-469.
- Kanda S, Akazome Y, Matsunaga T, Yamamoto N, Yamada S, Tsukamura H, Maeda K & Oka Y. Identification of KiSS-1 product kisspeptin and steroid-sensitive sexually dimorphic kisspeptin neurons in medaka (oryzias latipes). Endocrinology 2008 149:2467-2476.
- Kangaspeska S, Stride B, Métivier R, Polycarpou-Schwarz M, Ibberson D, Carmouche RP, Benes V, Gannon F, Reid G. Transient cyclical methylation of promoter DNA. Nature. 2008 452:112-5.
- Kaplan SL, Grumbach MM 1990 Pathogenesis of sexual precocity. In: Grumbach M, Sizonenko PC, Aubert ML, eds. Control of the onset of puberty. Baltimore: Williams & Wilkins; 620-662
- Kaprio J, Rimpelä A, Winter T, Viken RJ, Rimpelä M, Rose RJ. Common genetic influences on BMI and age at menarche. Hum Biol 1995 67:739-53.
- Kauffman AS. Sex differences in the Kiss1 system of rodents as the basis for generating the GnRH/Lh surge and guiding the tempo of pubertal development. Proc first World conference on Kisspeptin Signaling in the Brain, Cordova, Spain, 2008; p27
- Kauffman AS, Clifton DK, Steiner RA. Emerging ideas about kisspeptin-GPR54 signaling in the neuroendocrine regulation of reproduction. Trends Neurosci. 2007 30:504-11.
- Kauffman AS, Gottsch ML, Roa J, Byquist AC, Crown A, Clifton DK, Hoffman GE, Steiner RA & Tena-Sempere M. Sexual differentiation of Kiss1 gene expression in the brain of the rat. Endocrinology 2007a 148 1774-1783.
- Kauffman AS, Navarro VM, Kim J, Clifton DK, Steiner RA. Sex differences in the regulation of Kiss1/NKB neurons in juvenile mice: implications for the timing of puberty. Am J Physiol Endocrinol Metab. 2009 297:E1212-21.
- Kauffman AS, Park JH, McPhie-Lalmansingh AA, Gottsch ML, Bodo C, Hohmann JG, Pavlova MN, Rohde AD, Clifton DK, Steiner RA, Rissman EF. The kisspeptin receptor GPR54 is required for sexual differentiation of the brain and behavior. J Neurosci. 2007 27:8826-35.

- Kelce WR, Stone CR, Laws SC, Gray LE, Kemppainen JA, Wilson EM. Persistent DDT metabolite p,p'-DDE is a potent androgen receptor antagonist. Nature. 1995 375:581-5.
- Keen KL, Burich AJ, Mitsushima D, Kasuya E, Terasawa E. Effects of pulsatile infusion of the GABA(A) receptor blocker bicuculline on the onset of puberty in female rhesus monkeys. Endocrinology. 1999 140:5257-66.
- Keen KL, Wegner FH, Bloom SR, Ghatei MA, Terasawa E. An increase in kisspeptin-54 release occurs with the pubertal increase in luteinizing hormone-releasing hormone-1 release in the stalk-median eminence of female rhesus monkeys in vivo. Endocrinology. 2008 149:4151-7.
- Kelly KF, Daniel JM. POZ for effect--POZ-ZF transcription factors in cancer and development. Trends Cell Biol. 2006 16:578-87.
- Kenakin T. Agonist-receptor efficacy. II. Agonist trafficking of receptor signals. Trends Pharmacol Sci. 1995 16:232-8.
- Key T, Reeves G. Organochlorines in the environment and breast cancer. BMJ 1994 308:1520-1.
- Kiess W, Blum WF, Aubert ML. Leptin, puberty and reproductive function: lessons from animal studies and observations in humans. Eur J Endocrinol. 1998 138:26-9.
- Kim GL, Dhillon SS, Belsham DD. Kisspeptin directly regulates neuropeptide Y synthesis and secretion via the ERK1/2 and p38 mitogen-activated protein kinase signaling pathways in NPY-secreting hypothalamic neurons. Endocrinology. 2010 151:5038-47.
- Kim W, Jessen HM, Auger AP, Terasawa E. Postmenopausal increase in KiSS-1, GPR54, and luteinizing hormone releasing hormone (LHRH-1) mRNA in the basal hypothalamus of female rhesus monkeys. Peptides. 2009 30:103-10.
- Kim HG, Kurth I, Lan F, Meliciani I, Wenzel W, Eom SH, Kang GB, Rosenberger G, Tekin M, Ozata M, Bick DP, Sherins RJ, Walker SL, Shi Y, Gusella JF, Layman LC: Mutations in chd7, encoding a chromatin-remodeling protein, cause idiopathic hypogonadotropic hypogonadism and kallmann syndrome. Am J Hum Genet 2008 83:511-519.
- Kimura F, Nishihara M, Hiruma H, Funabashi T. Naloxone increases the frequency of the electrical activity of luteinizing hormone-releasing hormone pulse generator in long-term ovariectomized rats. Neuroendocrinology. 1991 53:97-102.

- Kinoshita F, Nakai Y, Katakami H, Imura H. Suppressive effect of dynorphin-(1-13) on luteinizing hormone release in conscious castrated rats. Life Sci. 1982 30:1915-9.
- Kinoshita M, Tsukamura H, Adachi S, Matsui H, Uenoyama Y, Iwata K, Yamada S, Inoue K, Ohtaki T, Matsumoto H & Maeda K. Involvement of central metastin in the regulation of preovulatory luteinizing hormone surge and estrous cyclicity in female rats. Endocrinology 2005 146:4431-4436.
- Kinsey-Jones JS, Li XF, Luckman SM, O'Byrne KT. Effects of kisspeptin-10 on the electrophysiological manifestation of gonadotropin-releasing hormone pulse generator activity in the female rat. Endocrinology. 2008 149:1004-8.
- Kirigiti MA, True C, Ciofi O, Grove KL, Smith MS. Kisspeptin and NKB fiber distribution in the adult female rat: relation to GnRH cell bodies and fibers/terminals in the median eminence. Endocrine Soc. Abstr. 2009: P3-P223
- Klein KO. Precocious puberty: who has it? Who should be treated? J Clin Endocrinol Metab 1999 84:411-4.
- Knobil E. Patterns of hypophysiotropic signals and gonadotropin secretion in the rhesus monkey. Biol Reprod. 1981 24:44-9.
- Ko JM, Lee HS, Hwang JS. KISS1 gene analysis in Korean girls with central precocious puberty: a polymorphism, p.P110T, suggested to exert a protective effect. Endocrine Journal 2010 57:701-709
- Köhler C, Villar CB. Programming of gene expression by Polycomb group proteins. Trends Cell Biol. 2008 18:236-43.
- Kordon C, Drouva SV, Martinez de la Escalera, Weiner RI. Role of classi and peptide neuromediators in the neuroendocrine regulation of luteinizing hormona and prolactin. In: knobil, E.; Neil, JD.; editors. The Physiology of Reproduction. 2. Vol.1. Raven Press; New York: 1994. p. 1621-1681
- Kotani M, Detheux M, Vandenbogaerde A, Communi D, Vanderwinden JM, Le Poul E, Brezillon S, Tyldesley R, Suarez-Huerta N, Vandeput F, Blanpain C, Schiffmann SN, Vassart G & Parmentier M. The metastasis suppressor gene KiSS-1 encodes kisspeptins, the natural ligands of the orphan G protein-coupled receptor GPR54. J Biol Chem 2001 276:34631-34636.
- Kotani H, Hoshimaru M, Nawa H, Nakanishi S. Structure and gene organization of bovine neuromedin K precursor. Proc Natl Acad Sci U S A. 1986 83:7074-8.

- Kouzarides T. Chromatin modifications and their function. Cell. 2007 128:693-705.
- Kottler ML, Counis R, Bouchard P: Mutations of the gnrh receptor gene: A new cause of autosomal-recessive hypogonadotropic hypogonadism. Arch Med Res 1999 30:481-485.
- Krajewski SJ, Anderson MJ, Iles-Shih L, Chen KJ, Urbanski HF, Rance NE: Morphologic evidence that neurokinin b modulates gonadotropin-releasing hormone secretion via neurokinin 3 receptors in the rat median eminence. J Comp Neurol 2005 489:372-386.
- Krajewski SJ, Burke MC, Anderson MJ, McMullen NT, Rance NE. Forebrain projections of arcuate neurokinin B neurons demonstrated by anterograde tract-tracing and monosodium glutamate lesions in the rat. Neuroscience. 2010 166:680-97.
- Krewson TD, Supelak PJ, Hill AE, Singer JB, Lander ES, Nadeau JH, Palmert MR. Chromosomes 6 and 13 harbor genes that regulate pubertal timing in mouse chromosome substitution strains. Endocrinology. 2004 145:4447-51.
- Krsmanović LZ, Stojilković SS, Merelli F, Dufour SM, Virmani MA, Catt KJ. Calcium signaling and episodic secretion of gonadotropin-releasing hormone in hypothalamic neurons. Proc Natl Acad Sci U S A. 1992 89:8462-6.
- Krstevska-Konstantinova M, Charlier C, Craen M, Du Caju M, Heinrichs C, de Beaufort C, Plomteux G, Bourguignon JP. Sexual precocity after immigration from developing countries to Belgium: evidence of previous exposure to organochlorine pesticides. Hum Reprod 2001 16:1020-6.
- Krstevska-Konstantinova M, Jovanovska J, Tasic VB, Montenegro LR, Beneduzzi D, Silveira LF, Gucev ZS. Mutational analysis of KISS1 and KISS1R in idiopathic central precocious puberty. J Pediatr Endocrinol Metab. 2014 27:199-201.
- Kuohung W, Kaiser UB. GPR54 and KiSS-1: role in the regulation of puberty and reproduction. Rev Endocr Metab Disord. 2006 7:257-63.
- Kurian JR, Keen KL, Guerriero KA, Terasawa E. Tonic control of kisspeptin release in prepubertal monkeys: implications to the mechanism of puberty onset. Endocrinology. 2012 153:3331-6.
- Lanfranco F, Gromoll J, von Eckardstein S, Herding EM, Nieschlag E, Simoni M: Role of sequence variations of the gnrh receptor and g protein-coupled receptor 54 gene in male idiopathic hypogonadotropic hypogonadism. Eur J Endocrinol 2005 153:845-852.

- Lapatto R, Pallais JC, Zhang D, Chan YM, Mahan A, Cerrato F, Le WW, Hoffman GE & Seminara SB. Kiss1-/- mice exhibit more variable hypogonadism than Gpr54-/- mice. Endocrinology 2007 148:4927-4936.
- Largo RH, Prader A. Pubertal development in Swiss girls. Helv Paediatr Acta. 1983 38:229-43.
- Layman LC, Cohen DP, Jin M, Xie J, Li Z, Reindollar RH, Bolbolan S, Bick DP, Sherins RR, Duck LW, Musgrove LC, Sellers JC, Neill JD: Mutations in gonadotropin-releasing hormone receptor gene cause hypogonadotropic hypogonadism. Nat Genet 1998 18:14-15.
- Lebrethon MC, Bourguignon JP. Management of central isosexual precocity: diagnosis, treatment, outcome. Curr Opin Pediatr 2000 12:394-9.
- Lee PA, Guo SS, Kulin HE. Age of puberty: data from the United States of America. APMIS 2001 109:81-8.
- Lee JY, Moon JS, Eu YJ, Lee CW, Yang ST, Lee SK, Jung HH, Kim HH, Rhim H, Seong JY & Kim JI. Molecular interaction between kisspeptin decapeptide analogs and a lipid membrane. Arch Biochem Biophys 2009 485: 109-114.
- Lee DK, Nguyen T, O'Neill GP, Cheng R, Liu Y, Howard AD, Coulombe N, Tan CP, Tang-Nguyen AT, George SR & O'Dowd BF. Discovery of a receptor related to the galanin receptors. FEBS Lett 1999 446:103-107.
- Lee HS, Park HK, Kim KH, Ko JH, Kim YJ, Yi KH, Hwang JS. Estrogen receptor α gene analysis in girls with central precocious puberty. J Pediatr Endocrinol Metab. 2013 26:645-9.
- Lee PA, Page JG. Effects of leuprolide in the treatment of central precocious puberty. J Pediatr 1989 114:321-4.
- Lee K, Porteous R, Campbell RE, Lüscher B, Herbison AE. Knockdown of GABA(A) receptor signaling in GnRH neurons has minimal effects upon fertility. Endocrinology. 2010 151:4428-36.
- Legouis R, Hardelin JP, Levilliers J, Claverie JM, Compain S, Wunderle V, Millasseau P, Le Paslier D, Cohen D, Caterina D, et al.: The candidate gene for the x-linked kallmann syndrome encodes a protein related to adhesion molecules. Cell 1991 67:423-435.
- Lehman MN, Coolen LM, Goodman RL. Minireview: kisspeptin/neurokinin B/dynorphin (KNDy) cells of the arcuate nucleus: a central node in the control of gonadotropin-releasing hormone secretion. Endocrinology. 2010 151:3479-89.

- Li C, Kim K & Nelson LS. FMRFamide-related neuropeptide gene family in Caenorhabditis elegans. Brain Res 1999 848:26-34.
- Li S, Ren J, Yang G, Guo Y & Huang L. Characterization of the porcine Kisspeptins receptor gene and evaluation as candidate for timing of puberty in sows. J Anim Breed Genet 2008 125:219-227.
- Licinio J, Caglayan S, Ozata M, Yildiz BO, de Miranda PB, O'Kirwan F, Whitby R, Liang L, Cohen P, Bhasin S, Krauss RM, Veldhuis JD, Wagner AJ, DePaoli AM, McCann SM, Wong ML. Phenotypic effects of leptin replacement on morbid obesity, diabetes mellitus, hypogonadism, and behavior in leptin-deficient adults. Proc Natl Acad Sci U S A. 2004 101:4531-6.
- Lienhardt A, Carel JC, Preux PM, Coutant R, Chaussain JL. Amplitude of pubertal growth in short stature children with intrauterine growth retardation. Horm Res 2002 57 Suppl 2:88-94.
- Lindgren G. Pubertal stages 1980 of Stockholm schoolchildren. Acta Paediatr. 1996 85:1365-7.
- Liu X & Herbison AE. Small-conductance calcium-activated potassium channels control excitability and firing dynamics in gonadotropin-releasing hormone (GnRH) neurons. Endocrinology 2008 149:3598-3604.
- Liu X, Lee K & Herbison AE. Kisspeptin excites gonadotropin-releasing hormone neurons through a phospholipase C/calcium-dependent pathway regulating multiple ion channels. Endocrinology 2008 149:4605-4614.
- Lomniczi A, Cornea A, Costa ME, Ojeda SR. Hypothalamic tumor necrosis factor-alpha converting enzyme mediates excitatory amino acid-dependent neuron-to-glia signaling in the neuroendocrine brain. J Neurosci. 2006 26:51-62.
- Lomniczi A, Loche A, Ojeda SR. Epigenetic regulation of female puberty. 4oth Ann Mtg Society for neuroscience; San Diego, CA. November 13-17, 2010
- Lomniczi A, Ojeda SR. A role for glial cells of the neuroendocrine brain in the central control of female sexual development. In: Parpura, V, Haydon P, editors. Astrocytes in (Patho)Physiology of the Nervous System. Springer; NY: 2009. p. 487-511
- Lomniczi A, Loche A, Castellano JM, Ronnekleiv OK, Bosch M, Kaidar G, Knoll JG, Wright H, Pfeifer GP, Ojeda SR. Epigenetic control of female puberty. Nat Neurosci. 2013 16:281-9.

- Luan X, Yu H, Wei X, Zhou Y, Wang W, Li P, Gan X, Wei D, Xiao J. GPR54 polymorphisms in Chinese girls with central precocious puberty. Neuroendocrinology. 2007 86:77-83.
- Luan X, Zhou Y, Wang W, Yu H, Li P, Gan X, Wei D, Xiao J. Association study of the polymorphisms in the KISS1 gene with central precocious puberty in Chinese girls. Eur J Endocrinol. 2007 157:113-8.
- Ma HM, Chen SK, Chen RM, Zhu C, Xiong F, Li T, Wang W, Liu GL, Luo XP, Liu L, Du ML; Pubertal Study Group of the Society of Pediatric Endocrinology and Genetic Disease, Chinese Medical Association. Pubertal development timing in urban Chinese boys. nt J Androl 2011 34:e435-45.
- Ma YJ, Dissen GA, Merlino G, Coquelin A, Ojeda SR. Overexpression of a human transforming growth factor-alpha (TGF alpha) transgene reveals a dual antagonistic role of TGF alpha in female sexual development. Endocrinology. 1994 135:1392-400.
- Macedo DB, Abreu AP, Reis AC, Montenegro LR, Dauber A, Beneduzzi D, Cukier P, Silveira LF, Teles MG, Carroll RS, Guerra Junior G, Guaragna Filho G, Gucev Z, Arnhold IJ, de Castro M, Moreira AC, Martinelli CE Jr, Hirschhorn JN, Mendonca BB, Brito VN, Antonini SR, Kaiser UB, Latronico AC. Central Precocious Puberty that appears to be sporadic caused by Paternally inherited mutations in the imprinted GENE makorin ring finger 3. J Clin Endocrinol Metab. 2014 Mar 14:jc20133126. [Epub ahead of print]
- Maeda K, Tsukamura H, Ohkura S, Kawakami S, Nagabukuro H, Yokoyama A. The LHRH pulse generator: a mediobasal hypothalamic location. Neurosci Biobehav Rev. 1995 19:427-37.
- Mahesh VB, Dhandapani KM, Brann DW. Role of astrocytes in reproduction and neuroprotection. Mol Cell Endocrinol. 2006 246:1-9.
- Marchese A, George SR, Kolakowski LF, Jr., Lynch KR & O'Dowd BF. Novel GPCRs and their endogenous ligands: expanding the boundaries of physiology and pharmacology. Trends Pharmacol Sci 1999 2: 370-375.
- Marks LJ, Bergeson PS. The Silver-Russel syndrome. Am J Dis Child. 1977 131:447-51.
- Marshall E. Epidemiology. Search for a killer: focus shifts from fat to hormones. Science. 1993 259:618-21.
- Marshall WA, Tanner JM. Variations in the pattern of pubertal changes in boys. Arch Dis Child 1970 46:13-23.

- Martinez-Chavez CC, Minghetti M & Migaud H. GPR54 and rGnRH I gene expression during the onset of puberty in Nile tilapia. Gen Comp Endocrinol 2008 156:224-233.
- Martinez AM, Schuettengruber B, Sakr S, Janic A, Gonzalez C, Cavalli G. Polyhomeotic has a tumor suppressor activity mediated by repression of Notch signaling. Nat Genet. 2009 41:1076-82.
- Mason DR, Arora KK, Mertz LM, Catt KJ. Homologous down-regulation of gonadotropin-releasing hormone receptor sites and messenger ribonucleic acid transcripts in alpha T3-1 cells. Endocrinology. 1994 135:1165-70.
- Mason AO, Greives TJ, Scotti MA, Levine J, Frommeyer S, Ketterson ED, Demas GE & Kriegsfeld LJ. Suppression of kisspeptin expression and gonadotropic axis sensitivity following exposure to inhibitory day lengths in female Siberian hamsters. Horm Behav 2007 52:492-498.
- Mastronardi C, Smiley GG, Raber J, Kusakabe T, Kawaguchi A, Matagne V, Dietzel A, Heger S, Mungenast AE, Cabrera R, Kimura S, Ojeda SR. Deletion of the Ttf1 gene in differentiated neurons disrupts female reproduction without impairing basal ganglia function. J Neurosci. 2006 26:13167-79.
- Masumoto KH, Nagano M, Takashima N, Hayasaka N, Hiyama H, Matsumoto S, Inouye ST, Shigeyoshi Y: Distinct localization of prokineticin 2 and prokineticin receptor 2 mrnas in the rat suprachiasmatic nucleus. Eur J Neurosci 2006 23:2959-2970.
- Matagne V, Ramaswamy S, Lomniczi A, Plant TM, Ojeda SR. Program No 703.9, 2009 Neuroscience Meeting Planner. Chicago, IL: Society for Neuroscience; 2009. Hypothalamic expression of a gene cluster encoding transcriptional repressors and maping to chromosome 19 is developmentally regulated and linked to sexual maturation in the rhesus monkey. Online
- Matsui H, Takatsu Y, Kumano S, Matsumoto H, Ohtaki T: Peripheral administration of metastin induces marked gonadotropin release and ovulation in the rat. Biochem Biophys Res Commun 2004 320:383-388.
- Matys V, Fricke E, Geffers R, Gössling E, Haubrock M, Hehl R, Hornischer K, Karas D, Kel AE, Kel-Margoulis OV, Kloos DU, Land S, Lewicki-Potapov B, Michael H, Münch R, Reuter I, Rotert S, Saxel H, Scheer M, Thiele S, Wingender E. TRANSFAC: transcriptional regulation, from patterns to profiles. Nucleic Acids Res. 2003 31:374-8.
- McArdle CA, Gorospe WC, Huckle WR & Conn PM. Homologous down-regulation of gonadotropin-releasing hormone receptors and desensitization

of gonadotropes: lack of dependence on protein kinase C. Mol Endocrinol 1987 1:420-429.

- McCarthy MM, Auger AP, Bale TL, De Vries GJ, Dunn GA, Forger NG, Murray EK, Nugent BM, Schwarz JM, Wilson ME. The epigenetics of sex differences in the brain. J Neurosci. 2009 29:12815-23.
- McKeehan WL, Kan M: Heparan sulfate fibroblast growth factor receptor complex: Structure-function relationships. Mol Reprod Dev 1994 39:69-81; discusison 81-62.
- Merzenich H, Boeing H, Wahrendorf J. Dietary fat and sports activity as determinants for age at menarche. Am J Epidemiol. 1993 138:217-24.
- Messager S. Kisspeptin and its receptor: new gatekeepers of puberty. J Neuroendocrinol. 2005 17:687-8.
- Messager S, Chatzidaki EE, Ma D, Hendrick AG, Zahn D, Dixon J, Thresher RR, Malinge I, Lomet D, Carlton MB, Colledge WH, Caraty A, Aparicio SA: Kisspeptin directly stimulates gonadotropin-releasing hormone release via g protein-coupled receptor 54. Proc Natl Acad Sci U S A 2005 102:1761-1766.
- Métivier R, Gallais R, Tiffoche C, Le Péron C, Jurkowska RZ, Carmouche RP, Ibberson D, Barath P, Demay F, Reid G, Benes V, Jeltsch A, Gannon F, Salbert G. Cyclical DNA methylation of a transcriptionally active promoter. Nature. 2008 452:45-50. Erratum in: Nature. 2010 463(7279):384.
- Meysing AU, Kanasaki H, Bedecarrats GY, Acierno JS, Jr., Conn PM, Martin KA, Seminara SB, Hall JE, Crowley WF, Jr., Kaiser UB: Gnrhr mutations in a woman with idiopathic hypogonadotropic hypogonadism highlight the differential sensitivity of luteinizing hormone and follicle-stimulating hormone to gonadotropin-releasing hormone. J Clin Endocrinol Metab 2004 89:3189-3198.
- Mikkelsen JD, Bentsen AH, Ansel L, Simonneaux V, Juul A. Comparison of the effects of peripherally administered kisspeptins. Regul Pept. 2009 152:95-100.
- Miller CA, Sweatt JD. Covalent modification of DNA regulates memory formation. Neuron. 2007 Mar 15;53(6):857-69. Erratum in: Neuron. 2008 59:1051.
- Miura K, Acierno JS, Jr., Seminara SB: Characterization of the human nasal embryonic lhrh factor gene, nelf, and a mutation screening among 65 patients with idiopathic hypogonadotropic hypogonadism (ihh). J Hum Genet 2004 49:265-268.

- Moenter SM, DeFazio RA. Endogenous gamma-aminobutyric acid can excite gonadotropin-releasing hormone neurons. Endocrinology. 2005 146:5374-9.
- Moenter SM, DeFazio AR, Pitts GR, Nunemaker CS. Mechanisms underlying episodic gonadotropin-releasing hormone secretion. Front Neuroendocrinol. 2003 24:79-93.
- Mohamed JS, Benninghoff AD, Holt GJ, Khan IA. Developmental expression of the G protein-coupled receptor 54 and three GnRH mRNAs in the teleost fish cobia. J Mol Endocrinol. 2007 38:235-44.
- Moisan J, Meyer F, Gingras S. A nested case-control study of the correlates of early menarche. Am J Epidemiol. 1990 132:953-61.
- Monnier C, Dode C, Fabre L, Teixeira L, Labesse G, Pin JP, Hardelin JP, Rondard P: Prokr2 missense mutations associated with kallmann syndrome impair receptor signalling activity. Hum Mol Genet 2009 18:75-81.
- Mori Y, Nishihara M, Tanaka T, Shimizu T, Yamaguchi M, Takeuchi Y, Hoshino K. Chronic recording of electrophysiological manifestation of the hypothalamic gonadotropin-releasing hormone pulse generator activity in the goat. Neuroendocrinology. 1991 53:392-5.
- Moss EG, Lee RC, Ambros V. The cold shock domain protein LIN-28 controls developmental timing in C. elegans and is regulated by the lin-4 RNA. Cell. 1997 88:637-46.
- Muir AI, Chamberlain L, Elshourbagy NA, Michalovich D, Moore DJ, Calamari A, Szekeres PG, Sarau HM, Chambers JK, Murdock P, Steplewski K, Shabon U, Miller JE, Middleton SE, Darker JG, Larminie CG, Wilson S, Bergsma DJ, Emson P, Faull R, Philpott KL, Harrison DC: Axor12, a novel human g protein-coupled receptor, activated by the peptide kiss-1. J Biol Chem 2001 276:28969-28975.
- Mul D, Fredriks AM, van Buuren S, Oostdijk W, Verloove-Vanhorick SP, Wit JM. Pubertal development in The Netherlands 1965-1997. Pediatr Res. 2001 50:479-86.
- Mul D, Oostdijk W, Otten BJ, Rouwé C, Jansen M, Delemarre-van de Waal HA, Waelkens JJ, Drop SL Final height after gonadotrophin releasing hormone agonist treatment for central precocious puberty: the Dutch experience. J Pediatr Endocrinol Metab. 2000 13:765-72.
- Murata M. Nutrition and the secular trend of growth. Horm Res. 1992 38 Suppl 1:89-96.

- Naftolin F, Harris GW, Bobrow M: Effect of purified luteinizing hormone releasing factor on normal and hypogonadotrophic anosmic men. Nature 1971 232:496-497.
- Nakahata Y, Kaluzova M, Grimaldi B, Sahar S, Hirayama J, Chen D, Guarente LP, Sassone-Corsi P. The NAD+-dependent deacetylase SIRT1 modulates CLOCK-mediated chromatin remodeling and circadian control. Cell. 2008 134:329-40.
- Navarro VM, Castellano JM, Fernández-Fernández R, Barreiro ML, Roa J, Sanchez-Criado JE, Aguilar E, Dieguez C, Pinilla L, Tena-Sempere M. Developmental and hormonally regulated messenger ribonucleic acid expression of KiSS-1 and its putative receptor, GPR54, in rat hypothalamus and potent luteinizing hormone-releasing activity of KiSS-1 peptide. Endocrinology. 2004a 145:4565-74.
- Navarro VM, Castellano JM, Fernandez-Fernandez R, Tovar S, Roa J, Mayen A, Nogueiras R, Vazquez MJ, Barreiro ML, Magni P, Aguilar E, Dieguez C, Pinilla L, Tena-Sempere M: Characterization of the potent luteinizing hormone-releasing activity of kiss-1 peptide, the natural ligand of gpr54. Endocrinology 2005 146:156-163.
- Navarro VM, Castellano JM, Fernandez-Fernandez R, Tovar S, Roa J, Mayen A, Barreiro ML, Casanueva FF, Aguilar E, Dieguez C, Pinilla L & Tena-Sempere M. Effects of KiSS-1 peptide, the natural ligand of GPR54, on follicle-stimulating hormone secretion in the rat. Endocrinology 2005 146: 1689-1697.
- Navarro VM, Fernandez-Fernandez R, Castellano JM, Roa J, Mayen A, Barreiro ML, Gaytan F, Aguilar E, Pinilla L, Dieguez C, Tena-Sempere M: Advanced vaginal opening and precocious activation of the reproductive axis by kiss-1 peptide, the endogenous ligand of gpr54. J Physiol 2004b 561:379-386.
- Navarro VM, Castellano JM, Garcia-Galiano D, Tena-Sempere M: Neuroendocrine factors in the initiation of puberty: The emergent role of kisspeptin. Rev Endocr Metab Disord 2007 8:11-20.
- Navarro VM, Gottsch ML, Chavkin C, Okamura H, Clifton DK, Steiner RA. Regulation of gonadotropin-releasing hormone secretion by kisspeptin/dynorphin/neurokinin B neurons in the arcuate nucleus of the mouse. J Neurosci. 2009 29:11859-66.

- Navarro VM, Sánchez-Garrido MA, Castellano JM, Roa J, García-Galiano D, Pineda R, Aguilar E, Pinilla L, Tena-Sempere M. Persistent impairment of hypothalamic KiSS-1 system after exposures to estrogenic compounds at critical periods of brain sex differentiation. Endocrinology. 2009 150:2359-67.
- Navarro VM, Gottsch ML, Wu M, García-Galiano D, Hobbs SJ, Bosch MA, Pinilla L, Clifton DK, Dearth A, Ronnekleiv OK, Braun RE, Palmiter RD, Tena-Sempere M, Alreja M, Steiner RA. Regulation of NKB pathways and their roles in the control of Kiss1 neurons in the arcuate nucleus of the male mouse. Endocrinology. 2011 152:4265-75.
- Navarro VM. Interactions between kisspeptins and neurokinin B. Adv Exp Med Biol. 2013 784:325-347
- Neely EK 1995 A multicenter trial of depot leuprolide for central precocious puberty. In: Plant TM, Lee PA, The neurobiology of puberty. Bristol, UK: J Endocrinol Ltd; 313-318
- Niida A, Wang Z, Tomita K, Oishi S, Tamamura H, Otaka A, Navenot JM, Broach JR, Peiper SC & Fujii N. Design and synthesis of downsized metastin (45-54) analogs with maintenance of high GPR54 agonistic activity. Bioorg Med Chem Lett 2006 16:134-137.
- Nimri R, Lebenthal Y, Lazar L, Chevrier L, Phillip M, Bar M, Hernandez-Mora E, de Roux N, Gat-Yablonski G. A novel loss-of-function mutation in GPR54/KISS1R leads to hypogonadotropic hypogonadism in a highly consanguineous family. J Clin Endocrinol Metab. 2011 96:536-45.
- Nishihara M, Takeuchi Y, Tanaka T, Mori Y. Electrophysiological correlates of pulsatile and surge gonadotrophin secretion. Rev Reprod. 1999 4:110-6.
- NHANESIII 1997 NHANES III Reference manuals and reports (CD-ROM). Analytic and reporting guidelines: the Third National Health and Nutrition Examination Survey (1988-94). Hyattsville,MD: National Center for Health Statistics, Centers for Disease Control and Prevention.
- O'Byrne KT, Chen MD, Nishihara M, Williams CL, Thalabard JC, Hotchkiss J, Knobil E. Ovarian control of gonadotropin hormone-releasing hormone pulse generator activity in the rhesus monkey: duration of the associated hypothalamic signal. Neuroendocrinology. 1993 57:588-92.
- Oakley AE, Clifton DK, Steiner RA. Kisspeptin signaling in the brain.Endocr Rev. 2009 30:713-43.
- Ohtaki T, Shintani Y, Honda S, Matsumoto H, Hori A, Kanehashi K, Terao Y, Kumano S, Takatsu Y, Masuda Y, Ishibashi Y, Watanabe T, Asada M,

Yamada T, Suenaga M, Kitada C, Usuki S, Kurokawa T, Onda H, Nishimura O, Fujino M: Metastasis suppressor gene kiss-1 encodes peptide ligand of a g-protein-coupled receptor. Nature 2001 411:613-617.

- Ojeda SR, Dubay C, Lomniczi A et al.: Gene networks and the neuroendocrine regulation of puberty. Mol Cell Endocrinol 2010a 3:243-11
- Ojeda SR, Hill J, Hill DF, Costa ME, Tapia V, Cornea A, Ma YJ. The Oct-2 POU domain gene in the neuroendocrine brain: a transcriptional regulator of mammalian puberty. Endocrinology. 1999 140:3774-89.
- Ojeda SR, Lomniczi A. The Epigenetics of Mammalian Puberty. 14th International Congress of Endocrinology; Kyoto, Japan. March 26-30, 2010b
- Ojeda SR, Lomniczi A, Mastronardi C, Heger S, Roth C, Parent AS, Matagne V, Mungenast AE. Minireview: the neuroendocrine regulation of puberty: is the time ripe for a systems biology approach? Endocrinology. 2006 147:1166-74.
- Ojeda SR, Lomniczi A, Sandau US. Glial-gonadotrophin hormone (GnRH) neurone interactions in the median eminence and the control of GnRH secretion. J Neuroendocrinol. 2008 20:732-42.
- Ojeda, SR.; MA, YJ.;Dziedzic, B.; Prevot, V. Astrocyte-neuron signalling and the onset of female puberty. In: Bourguignon, J-P.; Plant, TM., editors. The Onset of Puberty in Perspective. Elsevier Science B. V; Amsterdam: 2000.p.41-57
- Ojeda SR, Prevot V, Heger S, Lomniczi A, Dziedzic B, Mungenast A. Glia-to-neuron signaling and the neuroendocrine control of female puberty. Ann Med. 2003 35:244-55.
- Ojeda, SR, Skinner, MK. Puberty in the rat. In: Neil, JD., editor. The Physiology of reproduction. 3. Academic Press/Elsevier; San Diego: 2006. p. 2061-2126
- Ojeda, SR, Terasawa, E. Neuroendocrine regulation of puberty. In: Pfaff, D.; Arnild, A.; Etgen, A.; Fahabach, S.; Moss, R.; Rubin, R., editors. Hormones, Brain and Behavior. Vol.4. Elsevier; New York: 2002.p. 589-659.
- Ohkura S, Takase K, Matsuyama S, Mogi K, Ichimaru T, Wakabayashi Y, Uenoyama Y, Mori Y, Steiner RA, Tsukamura H, Maeda KI, Okamura H. Gonadotrophin-releasing hormone pulse generator activity in the hypothalamus of the goat. J Neuroendocrinol. 2009 21:813-21.
- Ong KK, Elks CE, Li S, Zhao JH, Luan J, Andersen LB, Bingham SA, Brage S, Smith GD, Ekelund U, Gillson CJ, Glaser B, Golding J, Hardy R, Khaw KT,

Kuh D, Luben R, Marcus M, McGeehin MA, Ness AR, Northstone K, Ring SM, Rubin C, Sims MA, Song K, Strachan DP, Vollenweider P, Waeber G, Waterworth DM, Wong A, Deloukas P, Barroso I, Mooser V, Loos RJ, Wareham NJ. Genetic variation in LIN28B is associated with the timing of puberty. Nat Genet. 2009 41:729-33.

- Orsini MJ, Klein MA, Beavers MP, Connolly PJ, Middleton SA & Mayo KH. Metastin (KiSS-1) mimetics identified from peptide structure-activity relationship-derived pharmacophores and directed small molecule database screening. J Med Chem 2007 50:462-471.
- Pacák K, Palkovits M. Stressor specificity of central neuroendocrine responses: implications for stress-related disorders. Endocr Rev. 2001 22:502-48.
- Page NM, Woods RJ, Gardiner SM, Lomthaisong K, Gladwell RT, Butlin DJ, Manyonda IT, Lowry PJ. Excessive placental secretion of neurokinin B during the third trimester causes pre-eclampsia. Nature. 2000 405:797-800.
- Page NM, Woods RJ, Lowry PJ. A regulatory role for neurokinin B in placental physiology and pre-eclampsia. Regul Pept. 2001 98:97-104.
- Pagon RA, Graham JM, Jr., Zonana J, Yong SL: Coloboma, congenital heart disease, and choanal atresia with multiple anomalies: Charge association. J Pediatr 1981 99:223-227.
- Pallais JC, Bo-Abbas Y, Pitteloud N, Crowley WF, Jr., Seminara SB: Neuroendocrine, gonadal, placental, and obstetric phenotypes in patients with ihh and mutations in the g-protein coupled receptor, gpr54. Mol Cell Endocrinol 2006 254-255:70-77.
- Palmert MR, Boepple PA: Variation in the timing of puberty: Clinical spectrum and genetic investigation. J Clin Endocrinol Metab 2001 86:2364-2368.
- Palmert MR, Hayden DL, Mansfield MJ, Crigler JF Jr, Crowley WF Jr, Chandler DW, Boepple PA. The longitudinal study of adrenal maturation during gonadal suppression: evidence that adrenarche is a gradual process. J Clin Endocrinol Metab. 2001 86:4536-42.
- Palmert MR, Hirschhorn JN: Genetic approaches to stature, pubertal timing, and other complex traits. Mol Genet Metab 2003 80:1-10.
- Palmert MR, Mansfield MJ, Crowley WF Jr, Crigler JF Jr, Crawford JD, Boepple PA. Is obesity an outcome of gonadotropin-releasing hormone agonist administration? Analysis of growth and body composition in 110

patients with central precocious puberty. J Clin Endocrinol Metab. 1999 84:4480-8.

- Papadimitriou A, Gousia E, Pitaouli E, Tapaki G, Philippidis P. Age at menarche in Greek girls. Ann Hum Biol. 1999 26:175-7.
- Papadimitriou A, Pantsiotou S, Douros K, Papadimitriou DT, Nicolaidou P, Fretzayas A. Timing of pubertal onset in girls: evidence for non-Gaussian distribution. J Clin Endocrinol Metab. 2008 93:4422-5. Epub 2008 Aug 26.
- Papadimitriou A, Douros K, Kleanthous K, Papadimitriou DT, Attilakos A, Fretzayas A. Pubertal maturation of contemporary Greek boys: no evidence of a secular trend. J Adolesc Health. 2011 49:434-6.
- Parent AS, Matagne V, Westphal M, Heger S, Ojeda S, Jung H. Gene expression profiling of hypothalamic hamartomas: a search for genes associated with central precocious puberty. Horm Res. 2008 69:114-23.
- Parent AS, Mungenast AE, Lomniczi A, Sandau US, Peles E, Bosch MA, Rønnekleiv OK, Ojeda SR. A contactin-receptor-like protein tyrosine phosphatase beta complex mediates adhesive communication between astroglial cells and gonadotrophin-releasing hormone neurones. J Neuroendocrinol. 2007 19:847-59.
- Parent AS, Teilmann G, Juul A, Skakkebaek NE, Toppari J, Bourguignon JP: The timing of normal puberty and the age limits of sexual precocity: Variations around the world, secular trends, and changes after migration. Endocr Rev 2003 24:668-693.
- Parhar IS, Ogawa S & Sakuma Y. Laser-captured single digoxigenin-labeled neurons of gonadotropin-releasing hormone types reveal a novel G protein-coupled receptor (Gpr54) during maturation in cichlid fish. Endocrinology 2004 145:3613-3618.
- Parkash J, Kaur G. Neuronal-glial plasticity in gonadotropin-releasing hormone release in adult female rats: role of the polysialylated form of the neural cell adhesion molecule. J Endocrinol. 2005 186:397-409.
- Partsch CJ, Heger S, Sippell WG: Management and outcome of central precocious puberty. Clin Endocrinol (Oxf) 2002 56:129-148.
- Pawlowitzki IH, Diekstall P, Schadel A, Miny P: Estimating frequency of kallmann syndrome among hypogonadic and among anosmic patients. Am J Med Genet 1987 26:473-479.

- Pennefather JN, Lecci A, Candenas ML, Patak E, Pinto FM, Maggi CA. Tachykinins and tachykinin receptors: a growing family. Life Sci. 2004 74:1445-63.
- Perera AD, Lagenaur CF, Plant TM. Postnatal expression of polysialic acid-neural cell adhesion molecule in the hypothalamus of the male rhesus monkey (Macaca mulatta). Endocrinology. 1993 133:2729-35.
- Perillo B, Ombra MN, Bertoni A, Cuozzo C, Sacchetti S, Sasso A, Chiariotti L, Malorni A, Abbondanza C, Avvedimento EV. DNA oxidation as triggered by H3K9me2 demethylation drives estrogen-induced gene expression. Science. 2008 319:202-6.
- Perry JR, Stolk L, Franceschini N, Lunetta KL, Zhai G, McArdle PF, Smith AV, Aspelund T, Bandinelli S, Boerwinkle E, Cherkas L, Eiriksdottir G, Estrada K, Ferrucci L, Folsom AR, Garcia M, Gudnason V, Hofman A, Karasik D, Kiel DP, Launer LJ, van Meurs J, Nalls MA, Rivadeneira F, Shuldiner AR, Singleton A, Soranzo N, Tanaka T, Visser JA, Weedon MN, Wilson SG, Zhuang V, Streeten EA, Harris TB, Murray A, Spector TD, Demerath EW, Uitterlinden AG, Murabito JM: Meta-analysis of genome-wide association data identifies two loci influencing age at menarche. Nat Genet 2009 41:648-650.
- Pescovitz OH, Comite F, Hench K, Barnes K, McNemar A, Foster C, Kenigsberg D, Loriaux DL, Cutler GB Jr. The NIH experience with precocious puberty: diagnostic subgroups and response to short-term luteinizing hormone releasing hormone analogue therapy. J Pediatr. 1986 108:47-54.
- Pheng V, Uenoyama Y, Homma T, Inamoto Y, Takase K, Yoshizawa-Kumagaye K, Isaka S, Watanabe TX, Ohkura S, Tomikawa J, Maeda K, Tsukamura H. Potencies of centrally- or peripherally-injected full-length kisspeptin or its C-terminal decapeptide on LH release in intact male rats. J Reprod Dev. 2009 55:378-82.
- Pfaff D, Keiner M. Atlas of estradiol-concentrating cells in the central nervous system of the female rat. J Comp Neurol. 1973 151:121-58.
- Pielecka-Fortuna J, Chu Z & Moenter SM. Kisspeptin acts directly and indirectly to increase gonadotropin-releasing hormone neuron activity and its effects are modulated by estradiol. Endocrinology 2008 149 :1979-1986.
- Pillon D, Caraty A, Fabre-Nys C, Bruneau G. Short-term effect of oestradiol on neurokinin B mRNA expression in the infundibular nucleus of ewes. J Neuroendocrinol. 2003 15:749-53.

- Pirvola U, Ylikoski J, Trokovic R, Hebert JM, McConnell SK, Partanen J: Fgfr1 is required for the development of the auditory sensory epithelium. Neuron 2002 35:671-680.
- Pita J, Barrios V, Gavela-Pérez T, Martos-Moreno GÁ, Muñoz-Calvo MT, Pozo J, Rovira A, Argente J, Soriano-Guillén L. Circulating kisspeptin levels exhibit sexual dimorphism in adults, are increased in obese prepubertal girls and do not suffer modifications in girls with idiopathic central precocious puberty. Peptides. 2011 32:1781-6.
- Pita J, Rado-Peralta S, Gavela-Pérez T, Aragón I, Barrios V, Rovira A, Argente J, Soriano-Guillén L. Plasma kisspeptin levels are elevated in cord blood and present sexual dimorphism in the adult population: relation with leptin, gonadotropins and anthropometrical data. Peptides. 2011 32:983-8.
- Pitteloud N, Boepple PA, DeCruz S, Valkenburgh SB, Crowley WF, Jr., Hayes FJ: The fertile eunuch variant of idiopathic hypogonadotropic hypogonadism: Spontaneous reversal associated with a homozygous mutation in the gonadotropin-releasing hormone receptor. J Clin Endocrinol Metab 2001 86:2470-2475.
- Pitteloud N, Hayes FJ, Boepple PA, DeCruz S, Seminara SB, MacLaughlin DT, Crowley WF, Jr.: The role of prior pubertal development, biochemical markers of testicular maturation, and genetics in elucidating the phenotypic heterogeneity of idiopathic hypogonadotropic hypogonadism. J Clin Endocrinol Metab 2002 87:152-160.
- Pitteloud N, Meysing A, Quinton R, Acierno JS, Jr., Dwyer AA, Plummer L, Fliers E, Boepple P, Hayes F, Seminara S, Hughes VA, Ma J, Bouloux P, Mohammadi M, Crowley WF, Jr.: Mutations in fibroblast growth factor receptor 1 cause kallmann syndrome with a wide spectrum of reproductive phenotypes. Mol Cell Endocrinol 2006 254-255:60-69.
- Pitteloud N, Quinton R, Pearce S, Raivio T, Acierno J, Dwyer A, Plummer L, Hughes V, Seminara S, Cheng YZ, Li WP, Maccoll G, Eliseenkova AV, Olsen SK, Ibrahimi OA, Hayes FJ, Boepple P, Hall JE, Bouloux P, Mohammadi M, Crowley W: Digenic mutations account for variable phenotypes in idiopathic hypogonadotropic hypogonadism. J Clin Invest 2007 117:457-463.
- Pitteloud N, Zhang C, Pignatelli D, Li JD, Raivio T, Cole LW, Plummer L, Jacobson-Dickman EE, Mellon PL, Zhou QY, Crowley WF, Jr.: Loss-of-function mutation in the prokineticin 2 gene causes kallmann syndrome and

normosmic idiopathic hypogonadotropic hypogonadism. Proc Natl Acad Sci U S A 2007 104:17447-17452.

- Plant TM, Gay VL, Marshall GR, Arslan M. Puberty in monkeys is triggered by chemical stimulation of the hypothalamus. Proc Natl Acad Sci U S A. 1989 86:2506-10.
- Plant TM, Krey LC, Moossy J, McCormack JT, Hess DL, Knobil E. The arcuate nucleus and the control of gonadotropin and prolactin secretion in the female rhesus monkey (Macaca mulatta). Endocrinology. 1978 102:52-62.
- Plant TM, Ramaswamy S: Kisspeptin and the regulation of the hypothalamic-pituitary-gonadal axis in the rhesus monkey (macaca mulatta). Peptides 2009 30:67-75.
- Plant TM, Ramaswamy S, Dipietro MJ: Repetitive activation of hypothalamic g protein-coupled receptor 54 with intravenous pulses of kisspeptin in the juvenile monkey (macaca mulatta) elicits a sustained train of gonadotropin-releasing hormone discharges. Endocrinology 2006 147:1007-1013.
- Plant, TM.; Witchel, SF. Puberty in nonhuman primates and humans. In: Neil, JD., editor. The Physiology of Reproduction. 3. Academic Press/Elsevier; San Diego: 2006.p. 2177-2230.
- Pompolo S, Pereira A, Estrada KM & Clarke IJ. Colocalization of kisspeptin and gonadotropin-releasing hormone in the ovine brain. Endocrinology 2006 147:804-810.
- Pompolo S, Pereira A, Scott CJ, Fujiyma F & Clarke IJ. Evidence for estrogenic regulation of gonadotropin-releasing hormone neurons by glutamatergic neurons in the ewe brain: An immunohistochemical study using an antibody against vesicular glutamate transporter-2. J Comp Neurol 2003 465:136-144.
- Pralong FP, Gomez F, Castillo E, Cotecchia S, Abuin L, Aubert ML, Portmann L, Gaillard RC: Complete hypogonadotropic hypogonadism associated with a novel inactivating mutation of the gonadotropin-releasing hormone receptor. J Clin Endocrinol Metab 1999 84:3811-3816.
- Prebeg Z, Bralic I. Changes in menarcheal age in girls exposed to war conditions. Am J Hum Biol. 2000 12:503-508.
- Prevot V, Rio C, Cho GJ, Lomniczi A, Heger S, Neville CM, Rosenthal NA, Ojeda SR, Corfas G. Normal female sexual development requires neuregulin-erbB receptor signaling in hypothalamic astrocytes. J Neurosci. 2003 23:230-9.

- Proos LA, Hofvander Y, Tuvemo T. Menarcheal age and growth pattern of Indian girls adopted in Sweden. I. Menarcheal age. Acta Paediatr Scand. 1991 80:852-8.
- Quaynor S, Hu L, Leung PK, Feng H, Mores N, Krsmanovic LZ & Catt KJ. Expression of a functional g protein-coupled receptor 54-kisspeptin autoregulatory system in hypothalamic gonadotropin-releasing hormone neurons. Mol Endocrinol 2007 21:3062-3070.
- Quinton R, Duke VM, de Zoysa PA, Platts AD, Valentine A, Kendall B, Pickman S, Kirk JM, Besser GM, Jacobs HS, Bouloux PM: The neuroradiology of kallmann's syndrome: A genotypic and phenotypic analysis. J Clin Endocrinol Metab 1996 81:3010-3017.
- Quinton R, Duke VM, Robertson A, Kirk JM, Matfin G, de Zoysa PA, Azcona C, MacColl GS, Jacobs HS, Conway GS, Besser M, Stanhope RG, Bouloux PM: Idiopathic gonadotrophin deficiency: Genetic questions addressed through phenotypic characterization. Clin Endocrinol (Oxf) 2001 55:163-174.
- Rage F, Hill DF, Sena-Esteves M, Breakefield XO, Coffey RJ, Costa ME, McCann SM, Ojeda SR. Targeting transforming growth factor alpha expression to discrete loci of the neuroendocrine brain induces female sexual precocity. Proc Natl Acad Sci U S A. 1997 94:2735-40.
- Ramaswamy S, Guerriero KA, Gibbs RB, Plant TM. Structural interactions between kisspeptin and GnRH neurons in the mediobasal hypothalamus of the male rhesus monkey (Macaca mulatta) as revealed by double immunofluorescence and confocal microscopy. Endocrinology. 2008 149:4387-95.
- Ramaswamy S, Seminara SB, Ali B, Ciofi P, Amin NA, Plant TM. Neurokinin B stimulates GnRH release in the male monkey (Macaca mulatta) and is colocalized with kisspeptin in the arcuate nucleus. Endocrinology. 2010 151:4494-503.
- Ramaswamy S, Seminara SB, Pohl CR, DiPietro MJ, Crowley WF Jr, Plant TM. Effect of continuous intravenous administration of human metastin 45-54 on the neuroendocrine activity of the hypothalamic-pituitary-testicular axis in the adult male rhesus monkey (Macaca mulatta). Endocrinology. 2007 148:3364-70.
- Rampazzo A, Pivotto F, Occhi G, Tiso N, Bortoluzzi S, Rowen L, Hood L, Nava A, Danieli GA. Characterization of C14orf4, a novel intronless human

gene containing a polyglutamine repeat, mapped to the ARVD1 critical region. Biochem Biophys Res Commun. 2000 278:766-74.

- Rance NE, Young WS 3rd. Hypertrophy and increased gene expression of neurons containing neurokinin-B and substance-P messenger ribonucleic acids in the hypothalami of postmenopausal women. Endocrinology. 1991 128:2239-47.
- Rando OJ. Combinatorial complexity in chromatin structure and function: revisiting the histone code. Curr Opin Genet Dev. 2012 22:148-55.
- Revel FG, Saboureau M, Masson-Pevet M, Pevet P, Mikkelsen JD & Simonneaux V. Kisspeptin mediates the photoperiodic control of reproduction in hamsters. Curr Biol 2006 16:1730-1735.
- Rhie YJ, Lee KH, Eun SH, Choi BM, Chae HW, Kwon AR, Lee WJ, Kim JH, Kim HS. Serum kisspeptin levels in Korean girls with central precocious puberty. J Korean Med Sci. 2011 26:927-31.
- Rice DP, Rice R, Thesleff I: Fgfr mrna isoforms in craniofacial bone development. Bone 2003 33:14-27.
- Richard N, Corvaisier S, Camacho E & Kottler ML. KiSS-1 and GPR54 at the pituitary level: overview and recent insights. Peptides 2009 30:123-129.
- Richard N, Galmiche G, Corvaisier S, Caraty A & Kottler ML. KiSS-1 and GPR54 genes are co-expressed in rat gonadotrophs and differentially regulated in vivo by oestradiol and gonadotrophin-releasing hormone. J Neuroendocrinol 2008 20:381-393.
- Rivest S, Rivier C. The role of corticotropin-releasing factor and interleukin-1 in the regulation of neurons controlling reproductive functions. Endocr Rev. 1995 16:177-99.
- Roa J, Aguilar E, Dieguez C, Pinilla L, Tena-Sempere M: New frontiers in kisspeptin/gpr54 physiology as fundamental gatekeepers of reproductive function. Front Neuroendocrinol 2008 29:48-69.
- Roa J, Vigo E, García-Galiano D, Castellano JM, Navarro VM, Pineda R, Diéguez C, Aguilar E, Pinilla L, Tena-Sempere M. Desensitization of gonadotropin responses to kisspeptin in the female rat: analyses of LH and FSH secretion at different developmental and metabolic states. Am J Physiol Endocrinol Metab. 2008 294:E1088-96.
- Roche AF, Wellens R, Attie KM, Siervogel RM. The timing of sexual maturation in a group of US white youths. J Pediatr Endocrinol Metab. 1995 8:11-8.

- Rometo AM, Krajewski SJ, Voytko ML, Rance NE. Hypertrophy and increased kisspeptin gene expression in the hypothalamic infundibular nucleus of postmenopausal women and ovariectomized monkeys. J Clin Endocrinol Metab. 2007 92:2744-50.
- Roseweir AK, Kauffman AS, Smith JT, Guerriero KA, Morgan K, Pielecka-Fortuna J, Pineda R, Gottsch ML, Tena-Sempere M, Moenter SM, Terasawa E, Clarke IJ, Steiner RA & Millar RP. Discovery of potent kisspeptin antagonists delineate physiological mechanisms of gonadotropin regulation. J Neurosci 2009 29:3920-3929.
- Roth CL, Mastronardi C, Lomniczi A, Wright H, Cabrera R, Mungenast AE, Heger S, Jung H, Dubay C, Ojeda SR. Expression of a tumor-related gene network increases in the mammalian hypothalamus at the time of female puberty. Endocrinology. 2007 148:5147-61.
- Salenave S, Chanson P, Bry H, Pugeat M, Cabrol S, Carel JC, Murat A, Lecomte P, Brailly S, Hardelin JP, Dode C, Young J: Kallmann's syndrome: A comparison of the reproductive phenotypes in men carrying kal1 and fgfr1/kal2 mutations. J Clin Endocrinol Metab 2008 93:758-763.
- Salio C, Lossi L, Ferrini F, Merighi A. Neuropeptides as synaptic transmitters. Cell Tissue Res. 2006 326:583-98.
- Sandoval-Guzmán T, Stalcup ST, Krajewski SJ, Voytko ML, Rance NE. Effects of ovariectomy on the neuroendocrine axes regulating reproduction and energy balance in young cynomolgus macaques. J Neuroendocrinol. 2004 16:146-53.
- Sanlaville D, Verloes A: Charge syndrome: An update. Eur J Hum Genet 2007 15:389-399.
- Satake H, Kawada T. Overview of the primary structure, tissue-distribution, and functions of tachykinins and their receptors. Curr Drug Targets. 2006 7:963-74.
- Sato N, Katsumata N, Kagami M, Hasegawa T, Hori N, Kawakita S, Minowada S, Shimotsuka A, Shishiba Y, Yokozawa M, Yasuda T, Nagasaki K, Hasegawa D, Hasegawa Y, Tachibana K, Naiki Y, Horikawa R, Tanaka T, Ogata T: Clinical assessment and mutation analysis of kallmann syndrome 1 (kal1) and fibroblast growth factor receptor 1 (fgfr1, or kal2) in five families and 18 sporadic patients. J Clin Endocrinol Metab 2004 89:1079-1088.

- Schulz R, Wilhelm A, Pirke KM, Gramsch C, Herz A. Beta-endorphin and dynorphin control serum luteinizing hormone level in immature female rats. Nature. 1981 Dec 294:757-9
- Schwankhaus JD, Currie J, Jaffe MJ, Rose SR, Sherins RJ: Neurologic findings in men with isolated hypogonadotropic hypogonadism. Neurology 1989 39:223-226.
- Schwanzel-Fukuda M, Robinson JA, Silverman AJ. The fetal development of the luteinizing hormone-releasing hormone (LHRH) neuronal systems of the guinea pig brain. Brain Res Bull. 1981 7:293-315
- Schwartz YB, Pirrotta V. Polycomb silencing mechanisms and the management of genomic programmes. Nat Rev Genet. 2007 8:9-22.
- Semaan SJ, Kauffman AS. Emerging concepts on the epigenetic and transcriptional regulation of the Kiss1 gene. Int J Dev Neurosci. 2013 31:452-62.
- Seminara SB. Mechanisms of Disease: the first kiss-a crucial role for kisspeptin-1 and its receptor, G-protein-coupled receptor 54, in puberty and reproduction. Nat Clin Pract Endocrinol Metab. 2006 2:328-34.
- Seminara SB, Beranova M, Oliveira LM, Martin KA, Crowley WF, Jr., Hall JE: Successful use of pulsatile gonadotropin-releasing hormone (gnrh) for ovulation induction and pregnancy in a patient with gnrh receptor mutations. J Clin Endocrinol Metab 2000 85:556-562.
- Seminara SB, Crowley WF Jr. Perspective: the importance of genetic defects in humans in elucidating the complexities of the hypothalamic-pituitary-gonadal axis. Endocrinology. 2001 142:2173-7.
- Seminara SB, Dipietro MJ, Ramaswamy S, Crowley WF, Jr., Plant TM: Continuous human metastin 45-54 infusion desensitizes g protein-coupled receptor 54-induced gonadotropin-releasing hormone release monitored indirectly in the juvenile male rhesus monkey (macaca mulatta): A finding with therapeutic implications. Endocrinology 2006 147:2122-2126.
- Seminara SB, Hayes FJ, Crowley WF, Jr.: Gonadotropin-releasing hormone deficiency in the human (idiopathic hypogonadotropic hypogonadism and kallmann's syndrome): Pathophysiological and genetic considerations. Endocr Rev 1998 19:521-539.
- Seminara SB, Messager S, Chatzidaki EE, Thresher RR, Acierno JS, Jr., Shagoury JK, Bo-Abbas Y, Kuohung W, Schwinof KM, Hendrick AG, Zahn D, Dixon J, Kaiser UB, Slaugenhaupt SA, Gusella JF, O'Rahilly S, Carlton MB,

Crowley WF, Jr., Aparicio SA, Colledge WH: The gpr54 gene as a regulator of puberty. N Engl J Med 2003 349:1614-1627.

- Seminara SB, Oliveira LM, Beranova M, Hayes FJ, Crowley WF, Jr.: Genetics of hypogonadotropic hypogonadism. J Endocrinol Invest 2000 23:560-565.
- Semple RK, Achermann JC, Ellery J, Farooqi IS, Karet FE, Stanhope RG, O'Rahilly S, Aparicio SA: Two novel missense mutations in g protein-coupled receptor 54 in a patient with hypogonadotropic hypogonadism. J Clin Endocrinol Metab 2005 90:1849-1855.
- Settas N, Dacou-Voutetakis C, Karantza M, Kanaka-Gantenbein C, Chrousos GP, Voutetakis A. Central Precocious Puberty in a Girl and Early Puberty in Her Brother Caused by a Novel Mutation in the MKRN3 Gene. J Clin Endocrinol Metab. 2014 99:E647-51.
- Shahab M, Mastronardi C, Seminara SB, Crowley WF, Ojeda SR, Plant TM: Increased hypothalamic gpr54 signaling: A potential mechanism for initiation of puberty in primates. Proc Natl Acad Sci U S A 2005 102:2129-2134.
- Shen S, Sandoval J, Swiss VA, Li J, Dupree J, Franklin RJ, Casaccia-Bonnefil P. Age-dependent epigenetic control of differentiation inhibitors is critical for remyelination efficiency. Nat Neurosci. 2008 11:1024-34.
- Sherman B, Wallace R, Bean J, Schlabaugh L. Relationship of body weight to menarcheal and menopausal age: implications for breast cancer risk. J Clin Endocrinol Metab. 1981 52:488-93.
- Shibata M, Friedman RL, Ramaswamy S & Plant TM. Evidence that down regulation of hypothalamic KiSS-1 expression is involved in the negative feedback action of testosterone to regulate luteinising hormone secretion in the adult male rhesus monkey (Macaca mulatta). J Neuroendocrinol 2007 19 432-438.
- Sigurjonsdottir TJ, Hayles AB. Precocious puberty. A report of 96 cases. Am J Dis Child. 1968 115:309-21.
- Silveira LG, Noel SD, Silveira-Neto AP, Abreu AP, Brito VN, Santos MG, Bianco SD, Kuohung W, Xu S, Gryngarten M, Escobar ME, Arnhold IJ, Mendonca BB, Kaiser UB, Latronico AC. Mutations of the KISS1 gene in disorders of puberty. J Clin Endocrinol Metab. 2010 95:2276-80.
- Silveira LF, Stewart PM, Thomas M, Clark DA, Bouloux PM, MacColl GS: Novel homozygous splice acceptor site gnrh receptor (gnrhr) mutation: Human gnrhr "Knockout". J Clin Endocrinol Metab 2002;87:2973-2977.

- SILVER HK. ASYMMETRY, SHORT STATURE, AND VARIATIONS IN SEXUAL DEVELOPMENT. A SYNDROME OF CONGENITAL MALFORMATIONS Am J Dis Child. 1964 107:495-515.
- Silverman AJ, Wilson R, Kesner JS, Knobil E. Hypothalamic localization of multiunit electrical activity associated with pulsatile LH release in the rhesus monkey. Neuroendocrinology. 1986 44:168-71.
- Simerly RB. Hormonal control of the development and regulation of tyrosine hydroxylase expression within a sexually dimorphic population of dopaminergic cells in the hypothalamus. Brain Res Mol Brain Res. 1989 6:297-310.
- Simon JA, Kingston RE. Mechanisms of polycomb gene silencing: knowns and unknowns.Smith JT, Acohido BV, Clifton DK & Steiner RA. KiSS-1 neurones are direct targets for leptin in the ob/ob mouse. J Neuroendocrinol 2006 18:298-303.
- Sklar CA, Kaplan SL, Grumbach MM. Evidence for dissociation between adrenarche and gonadarche: studies in patients with idiopathic precocious puberty, gonadal dysgenesis, isolated gonadotropin deficiency, and constitutionally delayed growth and adolescence. Clin Endocrinol Metab. 1980 51:548-56.
- Smith JT, Popa SM, Clifton DK, Hoffman GE, Steiner RA. Kiss1 neurons in the forebrain as central processors for generating the preovulatory luteinizing hormone surge. J Neurosci. 2006 26:6687-94.
- Smith JT, Clarke IJ: Kisspeptin expression in the brain: Catalyst for the initiation of puberty. Rev Endocr Metab Disord 2007 8:1-9.
- Smith JT, Clay CM, Caraty A & Clarke IJ. KiSS-1 messenger ribonucleic acid expression in the hypothalamus of the ewe is regulated by sex steroids and season. Endocrinology 2007(a) 148: 1150-1157.
- Smith JT, Coolen LM, Kriegsfeld LJ, Sari IP, Jaafarzadehshirazi MR, Maltby M, Bateman K, Goodman RL, Tilbrook AJ, Ubuka T, Bentley GE, Clarke IJ & Lehman MN. Variation in kisspeptin and RFamide-related peptide (RFRP) expression and terminal connections to gonadotropin-releasing hormone neurons in the brain: a novel medium for seasonal breeding in the sheep. Endocrinology 2008(a) 149:5770-5782.
- Smith JT, Cunningham MJ, Rissman EF, Clifton DK & Steiner RA. Regulation of Kiss1 gene expression in the brain of the female mouse. Endocrinology 2005(a) 146:3686-3692.

- Smith JT, Dungan HM, Stoll EA, Gottsch ML, Braun RE, Eacker SM, Clifton DK & Steiner RA. Differential regulation of KiSS-1 mRNA expression by sex steroids in the brain of the male mouse. Endocrinology 2005(b) 146:2976-2984.
- Smith JT, Popa SM, Clifton DK, Hoffman GE & Steiner RA. Kiss1 neurons in the forebrain as central processors for generating the preovulatory luteinizing hormone surge. J Neurosci 2006 26:6687-6694.
- Smith JT, Rao A, Pereira A, Caraty A, Millar RP & Clarke IJ. Kisspeptin is present in ovine hypophysial portal blood but does not increase during the preovulatory luteinizing hormone surge: evidence that gonadotropes are not direct targets of kisspeptin in vivo. Endocrinology 2008(b) 149:1951-1959.
- Soderlund D, Canto P, de la Chesnaye E, Ulloa-Aguirre A, Mendez JP: A novel homozygous mutation in the second transmembrane domain of the gonadotrophin releasing hormone receptor gene. Clin Endocrinol (Oxf) 2001 54:493-498.
- Sørensen K, Aksglaede L, Petersen JH, Juul A. Recent changes in pubertal timing in healthy Danish boys: associations with body mass index. J Clin Endocrinol Metab. 2010 95:263-70.
- Soussi-Yanicostas N, de Castro F, Julliard AK, Perfettini I, Chedotal A, Petit C: Anosmin-1, defective in the x-linked form of kallmann syndrome, promotes axonal branch formation from olfactory bulb output neurons. Cell 2002 109:217-228.
- Stafford LJ, Xia C, Ma W, Cai Y & Liu M. Identification and characterization of mouse metastasis-suppressor KiSS1 and its G-protein-coupled receptor. Cancer Res 2002 62:5399-5404.
- Stark O, Peckham CS, Moynihan C. Weight and age at menarche. Arch Dis Child. 1989 64:383-7.
- Stavrou I, Zois C, Ioannidis JP, Tsatsoulis A. Association of polymorphisms of the oestrogen receptor alpha gene with the age of menarche. Hum Reprod. 2002 17:1101-5.
- Steeg PS, Ouatas T, Halverson D, Palmieri D, Salerno M. Metastasis suppressor genes: basic biology and potential clinical use. Clin Breast Cancer. 2003 4:51-62.
- Steiner RA, Navarro VM. Tacking toward reconciliation on Tacr3/TACR3 mutations. Endocrinology. 2012 153:1578-81

- Subramanian K, Jia D, Kapoor-Vazirani P, Powell DR, Collins RE, Sharma D, Peng J, Cheng X, Vertino PM. Regulation of estrogen receptor alpha by the SET7 lysine methyltransferase. Mol Cell. 2008 30:336-47.
- Su PH, Yang SF, Yu JS, Chen SJ, Chen JY. Study of leptin levels and gene polymorphisms in patients with central precocious puberty. Pediatr Res. 2012 71:361-7.
- Sulem P, Gudbjartsson DF, Rafnar T, Holm H, Olafsdottir EJ, Olafsdottir GH, Jonsson T, Alexandersen P, Feenstra B, Boyd HA, Aben KK, Verbeek AL, Roeleveld N, Jonasdottir A, Styrkarsdottir U, Steinthorsdottir V, Karason A, Stacey SN, Gudmundsson J, Jakobsdottir M, Thorleifsson G, Hardarson G, Gulcher J, Kong A, Kiemeney LA, Melbye M, Christiansen C, Tryggvadottir L, Thorsteinsdottir U, Stefansson K. Genome-wide association study identifies sequence variants on 6q21 associated with age at menarche. Nat Genet. 2009 41:734-8.
- Suzuki S, Kadokawa H & Hashizume T. Direct kisspeptin-10 stimulation on luteinizing hormone secretion from bovine and porcine anterior pituitary cells. Anim Reprod Sci 2008 103:360-365.
- Sykiotis GP, Hoang XH, Avbelj M, Hayes FJ, Thambundit A, Dwyer A, Au M, Plummer L, Crowley WF Jr, Pitteloud N. Congenital idiopathic hypogonadotropic hypogonadism: evidence of defects in the hypothalamus, pituitary, and testes. J Clin Endocrinol Metab. 2010 95:3019-27.
- Swerdloff RS, Batt RA, Bray GA. Reproductive hormonal function in the genetically obese (ob/ob) mouse. Endocrinology. 1976 98:1359-64.
- Tahirović HF. Menarchal age and the stress of war: an example from Bosnia. Eur J Pediatr. 1998 157:978-80.
- Takahashi K, Tanaka A, Hara M, Nakanishi S. The primary structure and gene organization of human substance P and neuromedin K receptors. Eur J Biochem. 1992 204:1025-33.
- Tanner JM 1962 Growth at adolescence. 2nd ed. Oxford, UK: Blackwell
- Tanner JM, Davies PS. Clinical longitudinal standards for height and height velocity for North American children. J Pediatr. 1985 107:317-29.
- Tanner JM, Whitehouse RH. Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. Arch Dis Child. 1976 51:170-9.
- Teilmann G, Pedersen CB, Jensen TK, Skakkebaek NE, Juul A. Prevalence and incidence of precocious pubertal development in Denmark: an

epidemiologic study based on national registries. Pediatrics. 2005 116:1323-8.

- Teles MG, Bianco SD, Brito VN, Trarbach EB, Kuohung W, Xu S, Seminara SB, Mendonca BB, Kaiser UB, Latronico AC: A gpr54-activating mutation in a patient with central precocious puberty. N Engl J Med 2008 358:709-715.
- Teles MG, Silveira LF, Tusset C, Latronico AC. New genetic factors implicated in human GnRH-dependent precocious puberty: the role of kisspeptin system. Mol Cell Endocrinol. 2011 346:84-90.
- Tenenbaum-Rakover Y, Commenges-Ducos M, Iovane A, Aumas C, Admoni O, de Roux N: Neuroendocrine phenotype analysis in five patients with isolated hypogonadotropic hypogonadism due to a l102p inactivating mutation of gpr54. J Clin Endocrinol Metab 2007 92:1137-1144.
- Tena-Sempere M. The roles of kisspeptins and G protein-coupled receptor-54 in pubertal development. Curr Opin Pediatr. 2006 18:442-7.
- Terasawa E, Fernandez DL: Neurobiological mechanisms of the onset of puberty in primates. Endocr Rev 2001 22:111-151.
- Terasawa E, Keen KL, Mogi K, Claude P. Pulsatile release of luteinizing hormone-releasing hormone (LHRH) in cultured LHRH neurons derived from the embryonic olfactory placode of the rhesus monkey. Endocrinology. 1999 140:1432-41.
- Terasawa E, Kurian JR, Guerriero KA, Kenealy BP, Hutz ED, Keen KL. Recent discoveries on the control of gonadotrophin-releasing hormone neurones in nonhuman primates. J Neuroendocrinol. 2010 22:630-8.
- Terasawa E, Wiegand SJ, Bridson WE. A role for medial preoptic nucleus on afternoon of proestrus in female rats. Am J Physiol. 1980 238:E533-9.
- Thamdrup E 1961 Precocious sexual development: a clinical study of 100 patients. Sprinfield, IL: Charles C Thomas
- Theintz GE, Howald H, Allemann Y, Sizonenko PC. Growth and pubertal development of young female gymnasts and swimmers: a correlation with parental data. Int J Sports Med. 1989 10:87-91.
- Thompson EL, Murphy KG, Patterson M, Bewick GA, Stamp GW, Curtis AE, Cooke JH, Jethwa PH, Todd JF, Ghatei MA, Bloom SR. Chronic subcutaneous administration of kisspeptin-54 causes testicular degeneration in adult male rats. Am J Physiol Endocrinol Metab. 2006 291:E1074-82.
- Thompson EL, Patterson M, Murphy KG, Smith KL, Dhillo WS, Todd JF, Ghatei MA, Bloom SR: Central and peripheral administration of kisspeptin-10

stimulates the hypothalamic-pituitary-gonadal axis. J Neuroendocrinol 2004 16:850-858.

- Tomita K, Narumi T, Niida A, Oishi S, Ohno H & Fujii N. Fmoc-based solid-phase synthesis of GPR54-agonistic pentapeptide derivatives containing alkene- and fluoroalkene-dipeptide isosteres. Biopolymers 2007(a) 88:272-278.
- Tomita K, Niida A, Oishi S, Ohno H, Cluzeau J, Navenot JM, Wang ZX, Peiper SC & Fujii N. Structure-activity relationship study on small peptidic GPR54 agonists. Bioorg Med Chem 2006 14:7595-7603.
- Tomita K, Oishi S, Cluzeau J, Ohno H, Navenot JM, Wang ZX, Peiper SC, Akamatsu M, Fujii N. SAR and QSAR studies on the N-terminally acylated pentapeptide agonists for GPR54. J Med Chem. 2007(b) 50:3222-8.
- Tomita K, Oishi S, Ohno H & Fujii N. Structure-activity relationship study and NMR analysis of fluorobenzoyl pentapeptide GPR54 agonists. Biopolymers 2008 90:503-511.
- Tomkins DJ, Roux AF, Waye J, Freeman VC, Cox DW, Whelan DT. Maternal uniparental isodisomy of human chromosome 14 associated with a paternal t(13q14q) and precocious puberty. Eur J Hum Genet. 1996 4:153-9.
- Tommiska J, Sørensen K, Aksglaede L et al.: LIN28B, LIN28A, KISS1, and KISS1R in idiopathic central precocious puberty. BMC Res Notes 2011 4:363
- Topaloglu AK, Lu ZL, Farooqi IS, Mungan NO, Yuksel B, O'Rahilly S, Millar RP. Molecular genetic analysis of normosmic hypogonadotropic hypogonadism in a Turkish population: identification and detailed functional characterization of a novel mutation in the gonadotropin-releasing hormone receptor gene. Neuroendocrinology. 2006 84:301-8.
- Topaloglu AK, Reimann F, Guclu M, Yalin AS, Kotan LD, Porter KM, Serin A, Mungan NO, Cook JR, Ozbek MN, Imamoglu S, Akalin NS, Yuksel B, O'Rahilly S, Semple RK. Tac3 and tacr3 mutations in familial hypogonadotropic hypogonadism reveal a key role for neurokinin b in the central control of reproduction. Nat Genet 2009 41:354-358.
- Toppari J, Larsen JC, Christiansen P, Giwercman A, Grandjean P, Guillette LJ Jr, Jégou B, Jensen TK, Jouannet P, Keiding N, Leffers H, McLachlan JA, Meyer O, Müller J, Rajpert-De Meyts E, Scheike T, Sharpe R, Sumpter J, Skakkebaek NE. Male reproductive health and environmental xenoestrogens. Environ Health Perspect. 1996 104 Suppl 4:741-803.

- Tovar S, Vázquez MJ, Navarro VM, Fernández-Fernández R, Castellano JM, Vigo E, Roa J, Casanueva FF, Aguilar E, Pinilla L, Dieguez C, Tena-Sempere M. Effects of single or repeated intravenous administration of kisspeptin upon dynamic LH secretion in conscious male rats. Endocrinology. 2006 147:2696-704.
- Towne B, Czerwinski SA, Demerath EW, Blangero J, Roche AF, Siervogel RM: Heritability of age at menarche in girls from the fels longitudinal study. Am J Phys Anthropol 2005 128:210-219.
- Trarbach EB, Silveira LG, Latronico AC: Genetic insights into human isolated gonadotropin deficiency. Pituitary 2007 10:381-391.
- Tsai PS, Gill JC: Mechanisms of disease: Insights into x-linked and autosomal-dominant kallmann syndrome. Nat Clin Pract Endocrinol Metab 2006 2:160-171.
- Tsutsui K, Bentley GE, Kriegsfeld LJ, Osugi T, Seong JY, Vaudry H. Discovery and evolutionary history of gonadotrophin-inhibitory hormone and kisspeptin: new key neuropeptides controlling reproduction. J Neuroendocrinol. 2010 22:716-27.
- Tusset C, Noel SD, Trarbach EB, Silveira LF, Jorge AA, Brito VN, Cukier P, Seminara SB, Mendonça BB, Kaiser UB, Latronico AC. Mutational analysis of TAC3 and TACR3 genes in patients with idiopathic central pubertal disorders. Arq Bras Endocrinol Metabol. 2012 56:646-52.
- Urrutia R. KRAB-containing zinc-finger repressor proteins. Genome Biol. 2003 4:231.
- van den Berg SM, Boomsma DI: The familial clustering of age at menarche in extended twin families. Behav Genet 2007 37:661-667.
- Van den Berghe G, de Zegher F, Bouillon R. Clinical review 95: Acute and prolonged critical illness as different neuroendocrine paradigms. J Clin Endocrinol Metab. 1998 83:1827-34.
- Van Dop C, Burstein S, Conte FA, Grumbach MM. Isolated gonadotropin deficiency in boys: clinical characteristics and growth. J Pediatr. 1987 111:684-92.
- van Lenthe FJ, Kemper CG, van Mechelen W. Rapid maturation in adolescence results in greater obesity in adulthood: the Amsterdam Growth and Health Study. Am J Clin Nutr. 1996 64:18-24.
- Van Wieringen JC 1978 Secular growth changes. In: Falkner F, Tanner JM, eds. Human growth. New York: Plenum Press: 445-473

- Vermeulen S, Messiaen L, Scheir P, De Bie S, Speleman F, De Paepe A: Kallmann syndrome in a patient with congenital spherocytosis and an interstitial 8p11.2 deletion. Am J Med Genet 2002 108:315-318.
- Vissers LE, van Ravenswaaij CM, Admiraal R, Hurst JA, de Vries BB, Janssen IM, van der Vliet WA, Huys EH, de Jong PJ, Hamel BC, Schoenmakers EF, Brunner HG, Veltman JA, van Kessel AG: Mutations in a new member of the chromodomain gene family cause charge syndrome. Nat Genet 2004 36:955-957.
- Viswanathan SR, Powers JT, Einhorn W, Hoshida Y, Ng TL, Toffanin S, O'Sullivan M, Lu J, Phillips LA, Lockhart VL, Shah SP, Tanwar PS, Mermel CH, Beroukhim R, Azam M, Teixeira J, Meyerson M, Hughes TP, Llovet JM, Radich J, Mullighan CG, Golub TR, Sorensen PH, Daley GQ. Lin28 promotes transformation and is associated with advanced human malignancies. Nat Genet. 2009 41:843-8.
- Vizcarra JA, Wettemann RP, Braden TD, Turzillo AM, Nett TM. Effect of gonadotropin-releasing hormone (GnRH) pulse frequency on serum and pituitary concentrations of luteinizing hormone and follicle-stimulating hormone, GnRH receptors, and messenger ribonucleic acid for gonadotropin subunits in cows. Endocrinology. 1997 138:594-601.
- Vogel MJ, Guelen L, de Wit E, Peric-Hupkes D, Lodén M, Talhout W, Feenstra M, Abbas B, Classen AK, van Steensel B. Human heterochromatin proteins form large domains containing KRAB-ZNF genes. Genome Res. 2006 16:1493-504.
- Wakabayashi Y, Nakada T, Murata K, Ohkura S, Mogi K, Navarro VM, Clifton DK, Mori Y, Tsukamura H, Maeda K, Steiner RA, Okamura H. Neurokinin B and dynorphin A in kisspeptin neurons of the arcuate nucleus participate in generation of periodic oscillation of neural activity driving pulsatile gonadotropin-releasing hormone secretion in the goat. J Neurosci. 2010 30:3124-32.
- Waldstreicher J, Seminara SB, Jameson JL, Geyer A, Nachtigall LB, Boepple PA, Holmes LB, Crowley WF, Jr.: The genetic and clinical heterogeneity of gonadotropin-releasing hormone deficiency in the human. J Clin Endocrinol Metab 1996 81:4388-4395.
- Wang Y. Is obesity associated with early sexual maturation? A comparison of the association in American boys versus girls. Pediatrics. 2002 110:903-10.

- Warren MP. Effects of undernutrition on reproductive function in the human. Endocr Rev. 1983 4:363-77.
- Watson RE, Jr., Langub MC, Jr., Engle MG & Maley BE. Estrogen-receptive neurons in the anteroventral periventricular nucleus are synaptic targets of the suprachiasmatic nucleus and peri-suprachiasmatic region. Brain Res 1995 689:254-264.
- Wattigney WA, Srinivasan SR, Chen W, Greenlund KJ, Berenson GS. Secular trend of earlier onset of menarche with increasing obesity in black and white girls: the Bogalusa Heart Study. Ethn Dis. 1999 9:181-9.
- Welt CK, Chan JL, Bullen J, Murphy R, Smith P, DePaoli AM, Karalis A, Mantzoros CS. Recombinant human leptin in women with hypothalamic amenorrhea. N Engl J Med. 2004 351:987-97.
- West A, Vojta PJ, Welch DR & Weissman BE. Chromosome localization and genomic structure of the KiSS-1 metastasis suppressor gene (KISS1). Genomics 1998 54 145-148.
- Wetsel WC, Valença MM, Merchenthaler I, Liposits Z, López FJ, Weiner RI, Mellon PL, Negro-Vilar A. Intrinsic pulsatile secretory activity of immortalized luteinizing hormone-releasing hormone-secreting neurons. Proc Natl Acad Sci U S A. 1992 89:4149-53.
- White BJ, Rogol AD, Brown KS, Lieblich JM, Rosen SW: The syndrome of anosmia with hypogonadotropic hypogonadism: A genetic study of 18 new families and a review. Am J Med Genet 1983 15:417-435.
- Wierson M, Long PJ, Forehand RL. Toward a new understanding of early menarche: the role of environmental stress in pubertal timing. Adolescence. 1993 28:913-24.
- Wilkins L 1965 The diagnosis and treatment of endocrine disorders in childhood and adolescence. Springfield, IL: Charles C Thomas
- Wingender E, Dietze P, Karas H, Knüppel R. TRANSFAC: a database on transcription factors and their DNA binding sites. Nucleic Acids Res. 1996 24:238-41.
- Wintermantel TM, Campbell RE, Porteous R, Bock D, Gröne HJ, Todman MG, Korach KS, Greiner E, Pérez CA, Schütz G, Herbison AE. Definition of estrogen receptor pathway critical for estrogen positive feedback to gonadotropin-releasing hormone neurons and fertility. Neuron. 2006 52:271-80.

- Wolffe AP, Matzke MA. Epigenetics: regulation through repression. Science. 1999 286:481-6.
- Wray S, Gainer H. Effect of neonatal gonadectomy on the postnatal development of LHRH cell subtypes in male and female rats. Neuroendocrinology. 1987 45:413-9.
- Wu T, Buck GM, Mendola P. Blood lead levels and sexual maturation in U.S. girls: the Third National Health and Nutrition Examination Survey, 1988-1994. Environ Health Perspect. 2003 111:737-41.
- Wu M, Dumalska I, Morozova E, van den Pol A, Alreja M. Melanin-concentrating hormone directly inhibits GnRH neurons and blocks kisspeptin activation, linking energy balance to reproduction. Proc Natl Acad Sci U S A. 2009 106:17217-22.
- Wu JC, Sealfon SC & Miller WL. Gonadal hormones and gonadotropin-releasing hormone (GnRH) alter messenger ribonucleic acid levels for GnRH receptors in sheep. Endocrinology 1994 134 1846-1850.
- Xu N, Bhagavath B, Kim HG, Halvorson L, Podolsky RS, Chorich LP, Prasad P, Xiong WC, Cameron RS, Layman LC. NELF is a nuclear protein involved in hypothalamic GnRH neuronal migration. Mol Cell Endocrinol. 2010 319:47-55.
- Young J, Bouligand J, Francou B, Raffin-Sanson ML, Gaillez S, Jeanpierre M, Grynberg M, Kamenicky P, Chanson P, Brailly-Tabard S, Guiochon-Mantel A: Tac3 and tacr3 defects cause hypothalamic congenital hypogonadotropic hypogonadism in humans. J Clin Endocrinol Metab 2010 95:2287-2295.
- Zacharias L, Wurtman RJ. Age at menarche. Genetic and environmentalinfluences. N Engl J Med. 1969 280:868-75.
- Zenteno JC, Mendez JP, Maya-Nunez G, Ulloa-Aguirre A, Kofman-Alfaro S: Renal abnormalities in patients with kallmann syndrome. BJU Int 1999 83:383-386.
- Zhang C, Bosch MA, Ronnekleiv OK & Kelly MJ. Gamma-aminobutyric acid B receptor mediated inhibition of gonadotropin-releasing hormone neurons is suppressed by kisspeptin-G protein-coupled receptor 54 signaling. Endocrinology 2009 150:2388-2394.
- Zhang C, Roepke TA, Kelly MJ & Ronnekleiv OK. Kisspeptin depolarizes gonadotropin-releasing hormone neurons through activation of TRPC-like cationic channels. J Neurosci 2008 28:4423-4434.

- Zhu H, Shah S, Shyh-Chang N, Shinoda G, Einhorn WS, Viswanathan SR, Takeuchi A, Grasemann C, Rinn JL, Lopez MF, Hirschhorn JN, Palmert MR, Daley GQ. Lin28a transgenic mice manifest size and puberty phenotypes identified in human genetic association studies. Nat Genet. 2010 42:626-30.

IV. ABSTRACT IN GREEK

Η έναρξη της εφηβείας είναι ένα σύνθετο και κρίσιμο φαινόμενο στην εξέλιξη κάθε είδους και οι μηχανισμοί που εμπλέκονται σε αυτήν παραμένουν εν πολλοίς άγνωστοι. Πρόσφατα τα νευροπεπτίδια Kisspeptin και Neurokinin B (NKB) έχουν προταθεί ότι παίζουν κεντρικό ρόλο στην ενεργοποίηση των GnRH νευρώνων κατά την έναρξη της ήβης. Μελέτες σε ανθρώπους και πειραματόζωα έχουν δείξει ότι μεταλλάξεις στα γονίδια που κωδικοποιούν αυτά τα νευροπεπτίδια ή τους υποδοχείς τους (GPR54 και TACR3 αντίστοιχα) οδηγούν σε διαταραχές ενήβωσης Ενδιαφέρον παρουσιάζει το γεγονός ότι η πλειοψηφεία των μεταλάξεων που έχουν μελετηθεί έως σήμερα έχουν ανιχνευτεί σε ασθενείς με Ιδιοπαθή Υπογοναδοτροφικό Υπογοναδισμό, μια νόσο πολύ πιο σπάνια από την Ιδιοπαθή Κεντρική Πρώιμη Ήβη (ΙΚΠΗ) (επίπτωση 0.001-0.01% και 0.2% αντίστοιχα). Σκοπός της παρούσας μελέτης ήταν να μελετηθεί η επίπτωση μεταλλάξεων και πολυμορφισμών των γονιδίων GPR54 και TACR3 στην παθογένεια της ιδιοπαθούς κετρικής πρώιμης ήβης (ΙΚΠΗ).
Σε αυτή τη μελέτη 33 θήλεα άτομα με διάγνωση ΙΚΠΗ και τακτική παρακολούθηση από παιδίατρο ενδοκρινολόγο στη Μονάδα Ενδοκρινολογίας, Μεταβολισμού και Διαβήτη της Α' Πανεπιστημιακής Κλινικής, Νοσοκομείου Παίδων «Η Αγία Σοφία» μελετήθηκαν αναδρομικά. Το γενομικό DNA των ασθενών πολλαπλασιάστηκε με PCR και έγινε μοριακή ανάλυση των γονιδίων GPR54 και TACR3 με direct sequencing. Η βάση δεδομένων Ensemble genome database χρησιμοποιήθηκε για των έλεγχο τυχών μεταλλάξεων και πολυμορφισμών.
Δεν διαπιστώθηκαν μεταλλάξεις στα γονίδια GPR54 και TACR3. Η σιωπηλή παραλλαγή (SNP) A/G coding sequence στη θέση 857526 στο γονίδιο GPR54 (dbSNP ID: rs10407968) βρέθηκε σε 2 ασθενείς με ΙΚΠΗ.
Συμπερασματικά μεταλλάξεις στα γονίδια GPR54 και TACR3 μάλλον δεν αποτελούν συχνά αίτια στην παθογένεια της ΙΚΠΗ.

V. ABSTRACT IN ENGLISH

Kisspeptin (KISS1)/GPR54 (KISSR) signaling complex and Neurokinin B (NKB)/ NKB receptor (TACR3) signaling have been proposed as an integral part of the network coordinating GnRH release. GPR54 (KISS1R) and TACR3 gene mutations have been described in cases of idiopathic hypogonadotrophic hypogonadism, while limited data exist on gain of function mutation in GPR54 (KISS1R) gene causing idiopathic central precocious puberty (ICPP). No data on TACR3 mutations in ICPP have been described so far. Aim of this study was to elucidate the possible impact of GPR54 (KISS1R) and TACR3 mutations in ICPP. PCR-amplified genomic DNA of 38 girls with ICPP was analysed for GPR54 and TACR3 genes mutations. No GPR54 or TACR3 mutations were found. The A/G coding sequence SNP on the GPR54 gene (dbSNP ID: rs10407968) was found in 2 patients with ICPP.

Conclusion: Our data indicate that GPR54 and TACR3 gene mutations are not a frequent cause of ICPP. The identified A/G synonymous SNP (dbSNP ID: rs10407968) located in exon 1 of the gene is not probable to have a pathogenic role in exon splicing and therefore in the premature initiation of puberty.