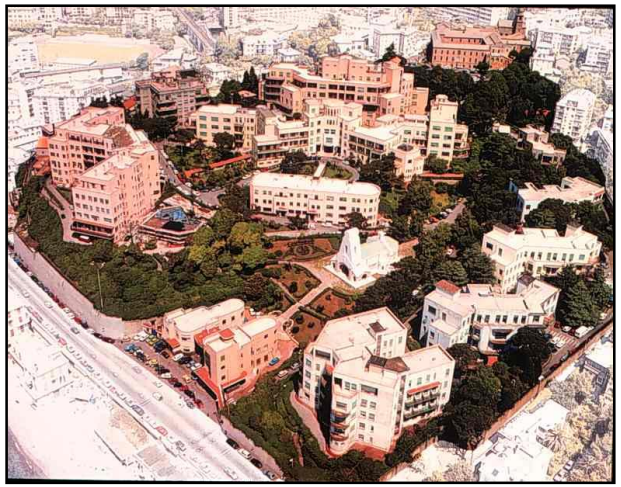
IRCCS Giannina Gaslini
Department of Pediatrics
University of Genova

Il Deficit di Ormone della Crescita Genetica

Mohamad.Maghnie@unige.it
MohamadMaghnie@gaslini.org



Con il Patrocinio di:

SIEDP

Società Italiana di Endocrinologia e Diabetologia Pediatrica

NOVITA' IN ENDOCRINOLOGIA PEDIATRICA

Dalla clinica alla genetica

Cagliari, 14 maggio 2016

Aula Thun

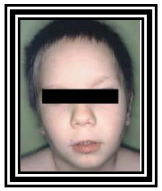
Ospedale Pediatrico Microcitemico "A. Cao"

Outline

- Overview of the GH-IGF Axis: Historical context and molecular considerations
- From Congenital – Genetic– to Idiopathic GH/IGF-I deficiency
- Phenotype
- Genotype

- Genetic Defects Causing GH Insensitivity
  - *GHR*
  - *STAT5B*
  - *IGF1*
  - *IGFALS*
  - *IGF1R ..and beyond*



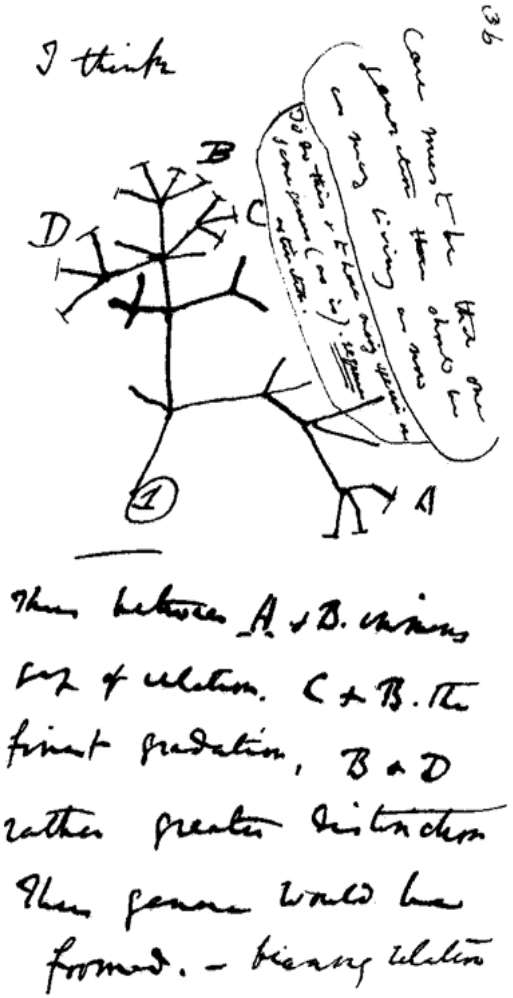



IGF-I Deficiency

IGF-I Insensitivity

- Growth plate matrix and signaling (proteoglycans, TGF …)

Charles Robert Darwin 1809 - 1882



July 1837: 26 years; Transmutation of Species: I think
Above the first evolutionary tree

ON

THE ORIGIN OF SPECIES

BY MEANS OF NATURAL SELECTION,

OR THE

PRESERVATION OF FAVOURED RACES IN THE STRUGGLE FOR LIFE.

By CHARLES DARWIN, M.A.,

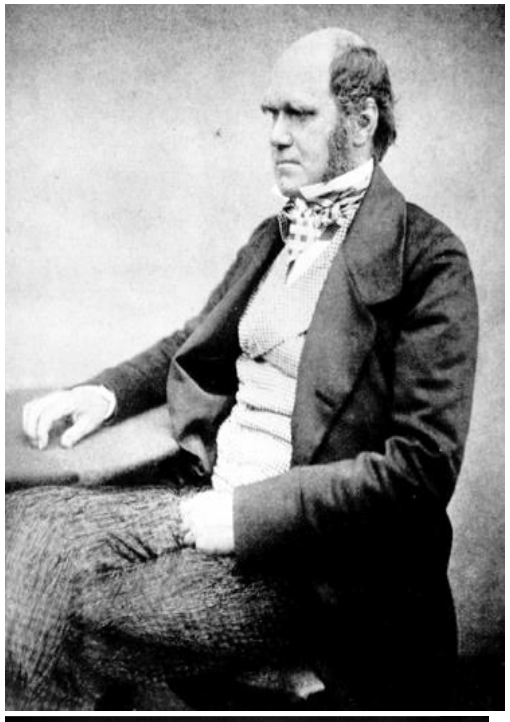
FELLOW OF THE ROYAL, GEOLOGICAL, LINNÆAN, ETC., SOCIETIES;

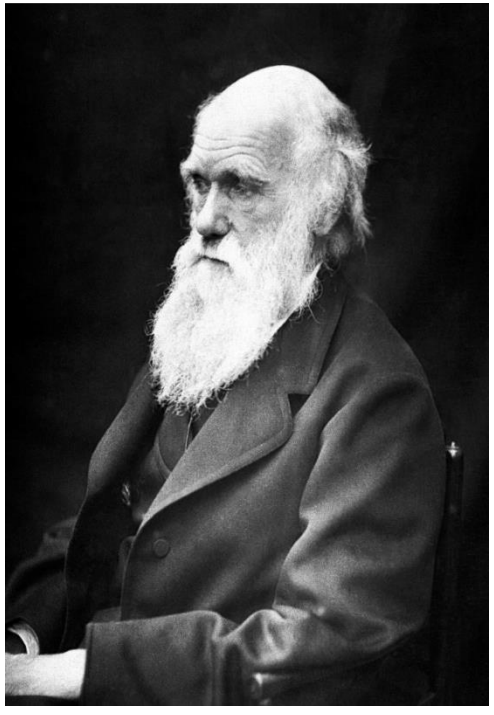
AUTHOR OF 'JOURNAL OF RESEARCHES DURING H. M. S. BEAGLE'S VOYAGE ROUND THE WORLD.'

LONDON:

JOHN MURRAY, ALBEMARLE STREET.

1859.





THE

DESCENT OF MAN,

AND

SELECTION IN RELATION TO SEX.

By CHARLES DARWIN, M.A., F.R.S., &c.

IN TWO VOLUMES.—Vol. I.

WITH ILLUSTRATIONS.

LONDON:

JOHN MURRAY, ALBEMARLE STREET.

1871.

Comparing the human and chimpanzee genomes: Searching for needles in a haystack

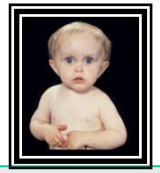
Ajit Varki[1] and Tasha K. Altheide

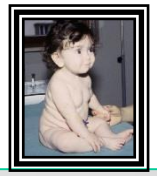
Glycobiology Research and Training Center, Departments of Medicine and Cellular & Molecular Medicine, University of California at San Diego, La Jolla, California 92093, USA

- Humans (Homo sapiens) and chimpanzees (Pan troglodytes) last shared a common ancestor ~5-7 million years ago (Mya) (Chen and Li 2001; Brunet et al. 2002)
- The difference between the two genomes is actually not ~1%, but ~4%—comprising ~35 million single nucleotide differences and ~90 Mb of insertions and deletions
- What makes humans different from their closest evolutionary relatives, and how, why, and when did these changes occur?
- These are fascinating questions, and a major challenge is to explain how genomic differences contributed to this process

CSH PRESS GENOME RESEARCH

Discovery of Rare and Common Variants on a Historical Timeframe





1958 GH treatment For short stature

1977 Sanger Sequencing

1981 GH1 deletion

1985 PCR

1989 GHR Laron S

1992 Mutation POU1F1 Science 1992

1992 FBN1 mutations in Marfan Syndrome

1994 FGFR3 mutations in achondroplasia

1997-1998 SHOX mutations in ISS

1997-1998 Leri-Weill dyschondrogenesis

1997-1998 Langer Dysplasia

2006 Pyrosequencing. Advent of next generation sequencing

2007 First GWAS on height: common variant of HMGA2 associated with height

2008 First large-scale GWAS identifies 54 loci associated with height

2010 GIANT consortium identifies 180 loci associated with height

2011 Short stature associated with increased copy number burden

2014-- 423 loci associated with height

Discovery of the missing heritability?

Rare Variants —— **Monogenic disease**

Common variants —— **Common variation**

Polygenic disease

Genetic influence accounts for 75% - 90% of height variability!
and…all identified loci explained about 20% of the variation of adult height.

Genetic Hypopituitarism

- Incidence rate 4.2 cases per 100,000
- Prevalence rate of 45.5 per 100,000

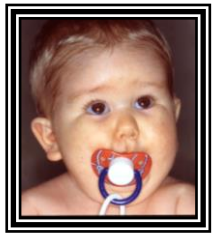
- **GH deficiency**
  - Genes implicated in GH secretion IGHD/CPHD
  - Genes implicated in cellular differentiation IGHD?/CPHD/MPHD
  - Genes implicated in early development (Syndromic GHD/CPHD/MPHD)

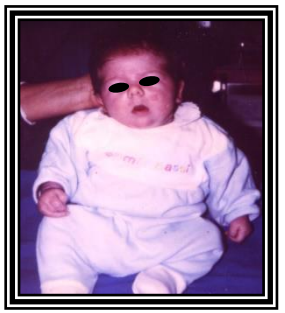
- Sporadic (de novo)
- Familial, AR/ AD/ X-linked
- 5-30% of cases

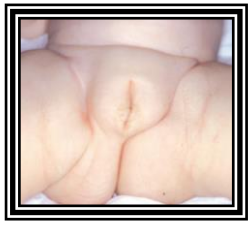
Hypopituitarism-Presentation

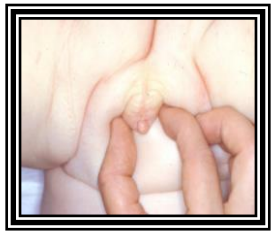
Normal birth length and weight

Neonatal period -infancy (severe GHD-MPHD)



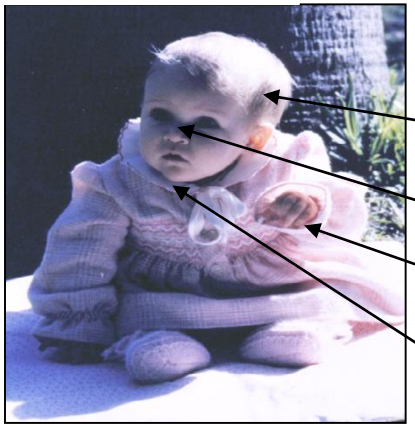






- Hypoglycemia
- Seizures
- Prolonged conjugated jaundice
- Poor feeding
- Hypotonia
- Nystagmus
- Respiratory distress
- Neonatal sepsis
- Micropenis in male newborns (undescended testes)
- Breech deliveries or breech presentation

Hypopituitarism-Presentation



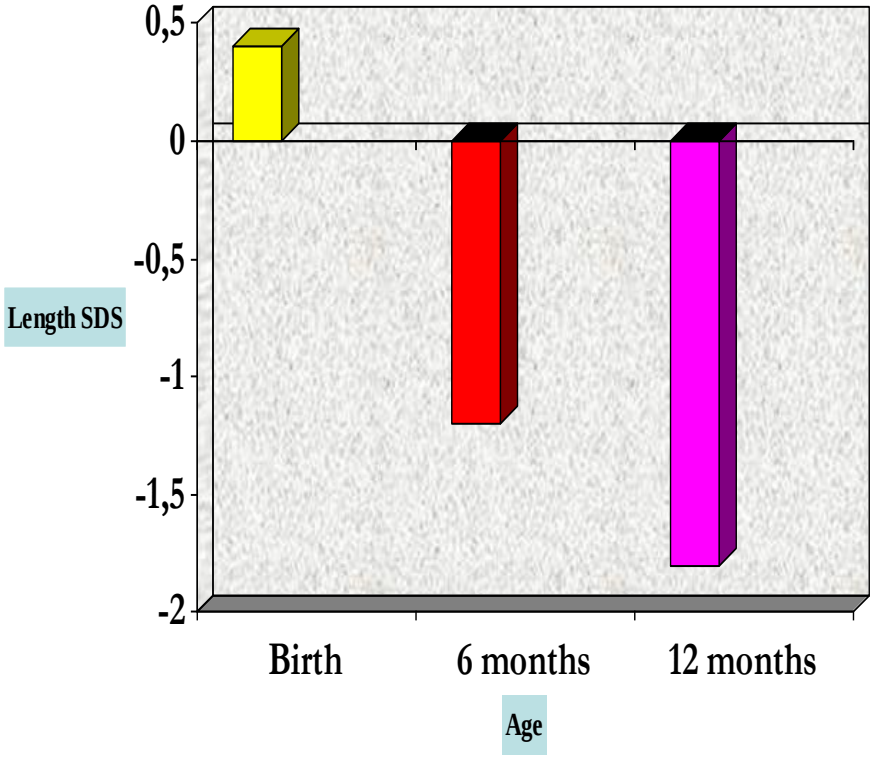
Infancy- childhood

- Immature facies
- Prominent forehead
- Depressed midline development
- Delayed dentition
- Thin and sparse hair
- Low muscle bulk and increased subcutaneous fat
- Slow nail growth
- High-pitched voice
- Micropenis in male newborns

Hypopituitarism-Presentation

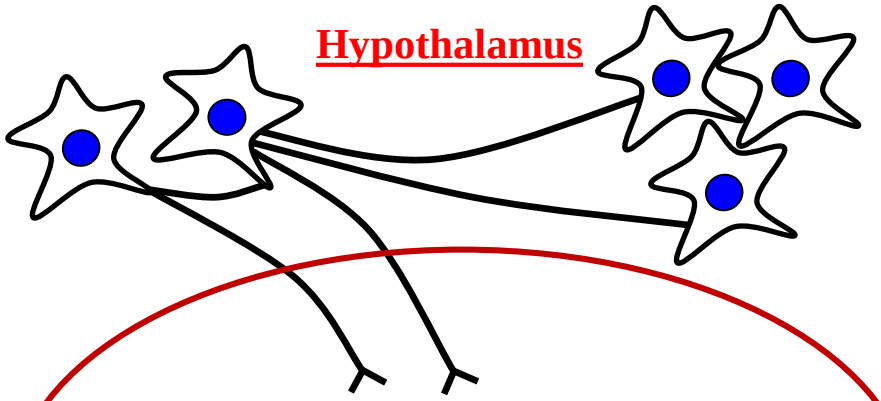
Linear Growth Characteristics of Congenitally GH-Deficient Infants from Birth to One Year

- 46 patients
- Abnormal age-adjusted growth velocity < 7 cm/year in the first 2 yrs
- Abnormal height < 3rd percentile
- GH < 10 ng/ml (2 tests)
- 85% MPHD
- MRI Congenital abnormalities (EPP, ES, SOD, holoprosencephaly)



S Pena-Almazan et al. J Clin Endocrinol Metab, 2001;86:5691-5694

Congenital - Genetic GH Deficiency
Multiple Players at Work



Primary GHD
(secondary IGF-I Deficiency)
Defects in GH production

Pituitary

GHRHR
GH-1
GHSR

Primary IGF-I Deficiency
Defects in GH action/GH Insensitivity

GHR
IGF1
STAT5b
ALS

LIVER

STAT3?

Functional IGF-I Deficiency
(IGF Resistance Insensitivity)
Defects in IGF-I action
Condrocyte abnormalities

SHOX
NPR2/FGFR3
SHP2
IGFRI
SOCS2
ACAN

TARGETS

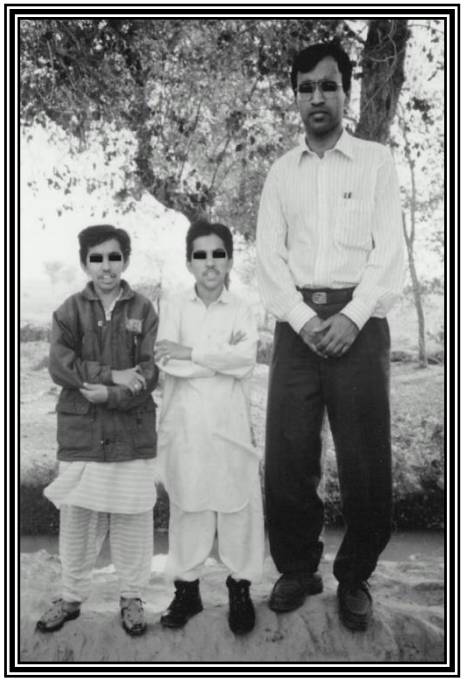
HMGA2
Cell cycle gene
Primordial Dwarfism
TRAIP
XRCC4

Hypopituitarism
Genes implicated in GH secretion (IGHD/CPHD)

Disorder	Mode	Genetic defect	hGH protein	GH antibody	Phenotype
IGHD 1A  *Courtecy L Ghizzoni*	AR	*GH-1 :* Homozygous deletions, mutations	Absent	+/-	Severe dwarfism
IGHD 1B 	AR	*GH-1* :splice site mutations, frameshifts, stop codon *GHRHR :* mutations (missense), deletions	Low	None	Less severe short stature
IGHD II 	AD	*GH-1* Splice site mutations Mutations Exon splice enhancer intron splice enhancer (ISE) IVS-deletions	Low	None	Less severe short stature MPHD
IGHD III	X-linked	Unknown; X-linked agammaglobulinemia? Gain of function in *SOX3?* other genes	Low	None	Short stature± immune defects

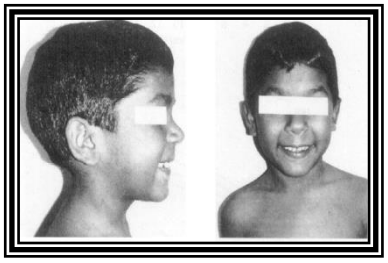
Genetics of GH Deficiency- Dwarfs of Sindh *GHRHR* mutations (Type 1B)

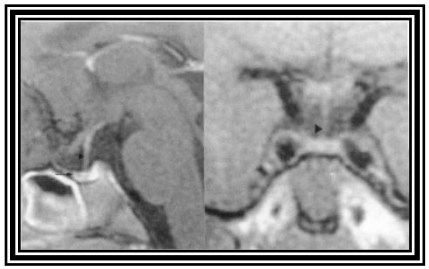
- Patients from Indian subcontinent and Brazilian pedigree: autosomal recessive (Severe growth failure, proportionate dwarfism, minimal facial hypoplasia, no hypoglycemia, no microphallus, pubertal delay
- Low GH, IGF1
- Anterior pituitary hypoplasia ***(Maheshwari HG et al. JCEM 1998;83:4065-74)***



- Absence of frontal bossing
- Small Anterior Pituitary (somatotroph depletion in *little* mouse)
- Normal location of Posterior Pituitary
- Normal Pituitary Stalk

(Netchine et al. JCEM, 1998;3:432-436)





Hypopituitarism
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HORMONE
RESEARCH IN
PÆDIATRICS

Obituary

Horm Res Paediatr 2016;85:219–220
DOI: 10.1159/000444667
Published online: March 12, 2016

Primus-Eugen Mullis, November 1954 – January 2016

Prof. Primus-Eugen Mullis sadly passed away on January 12, 2016. He was an outstanding paediatric endocrinologist who will be greatly missed by his family, colleagues and many friends throughout the world. He was well renowned internationally and was in much demand as a speaker as well as a chairperson and organiser of meetings.

Primus was born on November 10, 1954, in Walenstadt, Switzerland. His mother was of Swedish descent, and his father was Swiss. At the age of 13, Primus was sent to the catholic boarding school of Disentis, Graubünden, Switzerland, where he spent 7 years until his graduation. During these school years, he received a very broad humanistic education; this included studying Latin and ancient Greek with a respective background in history and arts. Primus was a good student and very much liked by his schoolmates for his kind and witty nature. He was also a very sporty young man who even performed in alpine skiing at competition level during his adolescent years. He loved the Swiss mountains and spent a considerable amount of his precious leisure time in nature, either hiking, mountaineering or alpine/cross-country skiing.

After successful graduation from high school, he decided to apply for medical school, which he started at the University of Fribourg at the border to the French-speaking part of Switzerland. He then continued and completed his studies at the University of Bern and also spent some months in Vienna, Austria. He undertook his MD thesis under the guidance of Prof. Ueli Wiesmann, a paediatrician who specialized in paediatric metabolic disorders at the University of Bern and who later became his mentor and supervisor as well as a very close friend. Primus started his initial training in paediatrics at the Children's Hospital Lucerne, in the heart of Switzerland, before he moved to the University Children's Hospital Bern

Prof. Primus-Eugen Mullis

in 1986, which was at that time headed by the late Prof. Ettore Rossi. There he commenced his specialist training in paediatric endocrinology and diabetology with Prof. Klaus Zuppinger. However, it was one of his career highlights when Primus decided to go to London and train with Prof. Charles Brook. He got his first taste of molecular biology in 1988 while working in the laboratory of Prof. David Latchman at University College London. He then returned to Bern and completed his training in paediatric endocrinology and diabetology. He established a research group studying the molecular genetics of growth disorders and was one of the first to identify mutations in *PROP1* in patients with congenital hypopituitarism. Having just returned, he was faced with the death of Prof. Zuppinger, and it was then left to him to carry on and build up a specialized unit for modern paediatric endocrinology and diabetology. The components of this future flagship included a high-quality in- and outpatient service, a routine hormone laboratory and a molecular research laboratory for studies of growth disorders and other genetic disorders of the pituitary gland. In addition, he provided undergraduate teaching at the medical school and training for paediatrics that was both general and specialized. He was appointed Professor and Head of the Division of Paediatric Endocrinology, Diabetology and Metabolism at the University Children's Hospital in Bern in 2000 and ran an active clinical division as well as an active research group working on the molecular basis of growth hormone deficiency, with particular focus on type 2 autosomal dominant growth hormone deficiency.

Additionally, Primus was a founding member of the Department of Clinical Research (DCR), a network connecting small groups of investigators who perform laboratory research within the University Hospital for sharing equipment and expertise. In addition, he was one of the pioneers who formed and led the Graduate School of Bern (GCB) for Cellular and Biomedical Sciences, which is just celebrating its 10th anniversary this year. In this function, he was a dedicated mentor for numerous trainees. Overall, he supervised many doctoral theses of MDs and trained several PhD students and postdoctoral fellows in his research laboratory. In addition, he granted specialized training in paediatric endocrinology and diabetology to more than 10 fellows, who now provide high-quality care all over Switzerland, including a number of academic paediatricians.

Primus published more than 175 peer-reviewed publications and invited reviews and several book chapters and edited the *Developmental Endocrinology* book series. He had been an Associate Editor of *Hormone Research in Paediatrics* since 1996. He was a member of numerous learned societies and served on the councils of the International Growth Hormone Research Society and the Swiss Society for Endocrinology and Diabetology. He also served as Chairman of the Swiss Society for Paediatric Endocrinology and Diabetology. He was a Board Member of the Swiss National Science Foundation between 2000 and 2009, and a Member of the Swiss Academy of Medical Sciences since 2009. He also received a number of awards; those he was most proud of were the Cloetta Prize of the University of Zürich (1998) and the prestigious Research Award of the European Society for Paediatric Endocrinology awarded to him in 2007. Primus was an active member of ESPE and was much respected by his colleagues. He worked hard for the society, and his various roles over the years included his membership of the Programme Organising Committee, the coordination of the Developmental Endocrinology series of meetings, and membership of the steering committee of the ESPE Summer School. In addition to being an outstanding clinician and researcher, he was an excellent teacher and will be remembered by his many students for his teaching prowess.

Primus loved to watch, follow and perform sports. As an endocrinologist, he was therefore predestined to get involved in issues of doping. Thus, he served as an expert to the Swiss Olympic Committee for several years.

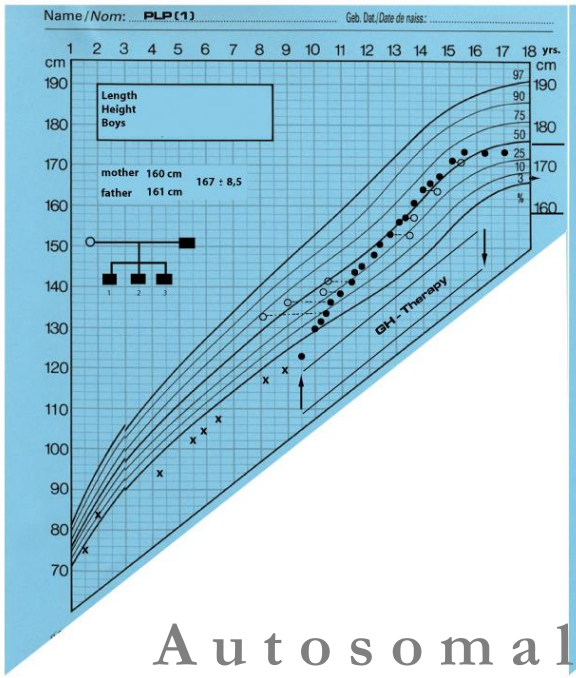
In 2007, Primus went on a 6-month sabbatical leave programme to Great Ormond Street Children's Hospital and the MRC National Institute for Medical Research in London; he was delighted to be in a city for which he had a great fondness and lived with some of his close friends in London during this short time. He pursued a research project on isolated growth hormone deficiency type II together with Prof. Iain Robinson at the MRC National Institute of Medical Research, Mill Hill, London. He returned to Bern refreshed, happy and proud to have received from those institutes a title of Honorary Professor and the status of Honorary Consultant.

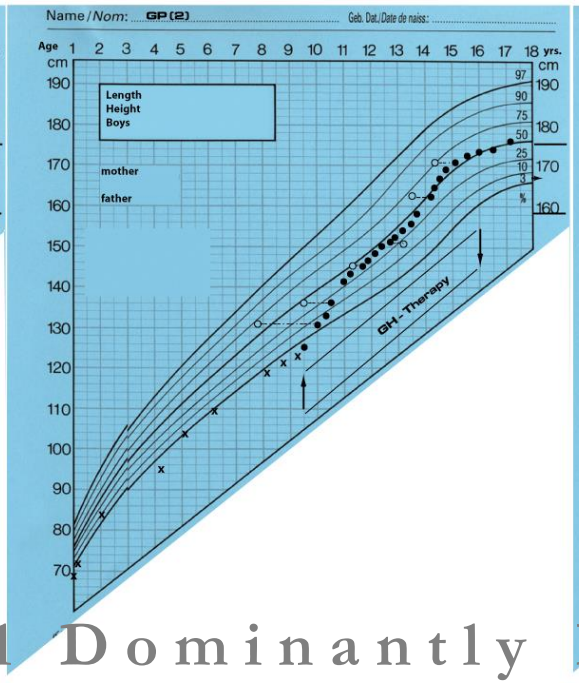
Primus was also a proud father to two children, Daniel and Annina, who are both adults now. Although he was very busy at work, he was always involved with their care and dedicated time to his children. More recently, he and his second wife Pia both enjoyed travelling and being outdoors together. His favorite hobby outside work was trekking in the Swiss mountains, although the highest peaks he had climbed were the Kilimanjaro in Tanzania, Africa, and Mt. Blanc in France. A major skiing accident left him severely incapacitated for a while in 2004. However, with his typical courage, he valiantly fought back to good health.

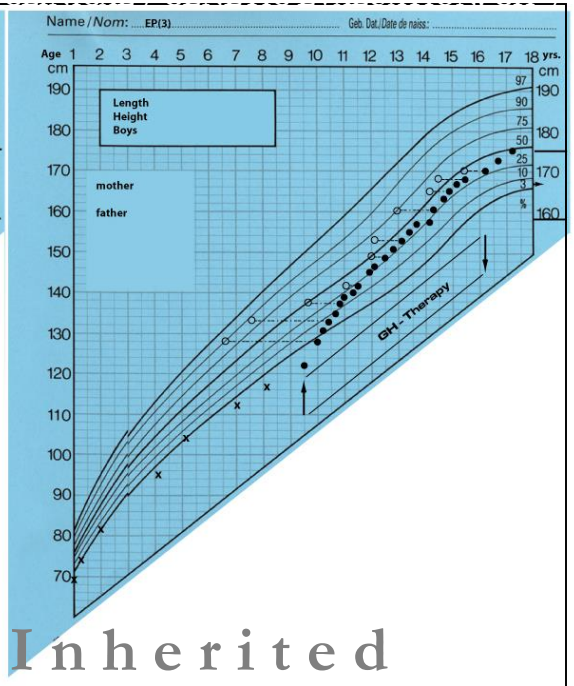
When Primus was challenged with a potentially life-threatening diagnosis in 2014, he decided to keep this a secret shared by only his family and close friends. He carried on with his beloved profession and research as much as he could and was determined to conquer the disease. Sadly, he finally passed away on January 12, 2016, far too young and with so much that he could still have offered to the world.

Friends and colleagues will remember Primus for his intelligence, scientific rigour, warmth, kindness, wit, charm and generosity, as well as for his energy and enthusiasm. He had a great fun-loving streak and was a real prankster at times – all of us will have our favourite story to tell about him! Primus will be greatly missed by the paediatric endocrinology world, his family and colleagues, and his many friends and collaborators throughout the world.

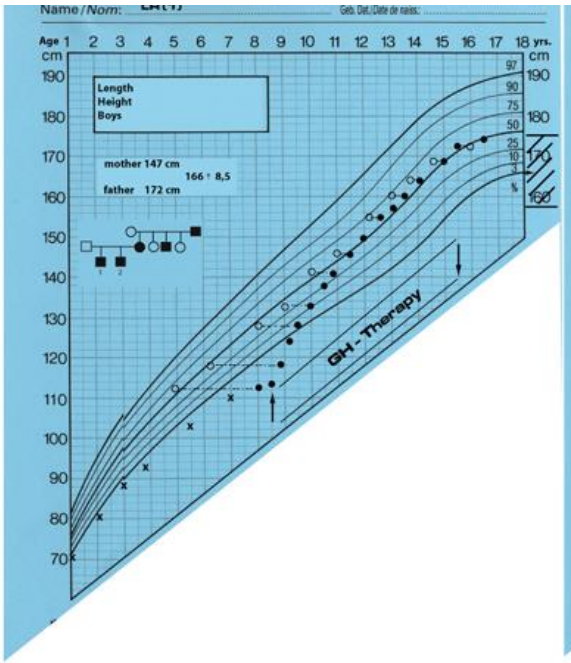
Christa E. Flück, Bern
Mehul Dattani, London
Annina Mullis, Bern

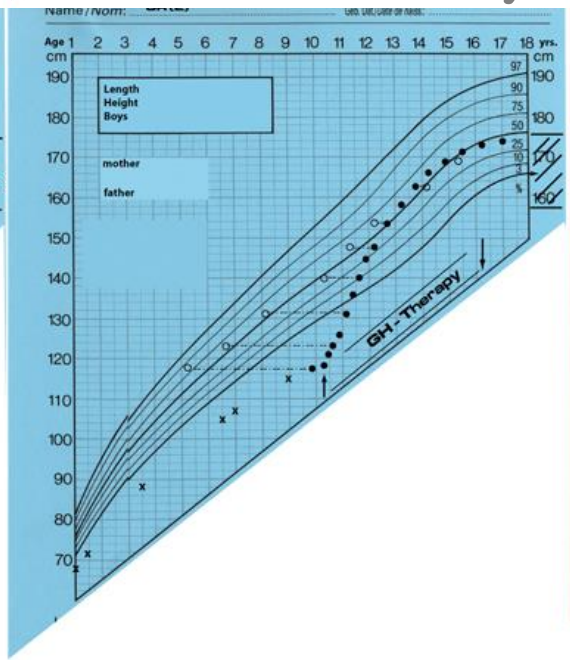


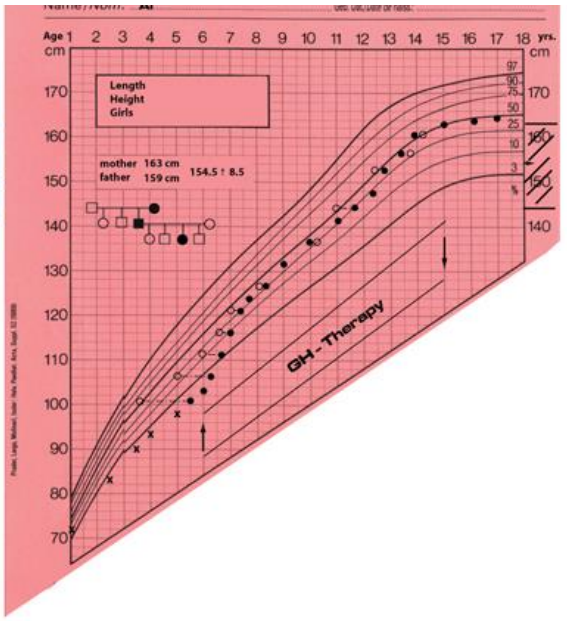




Autosomal Dominantly Inherited

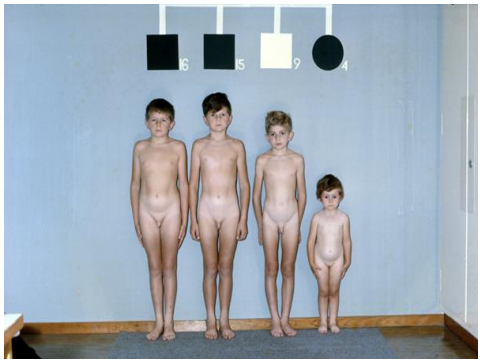


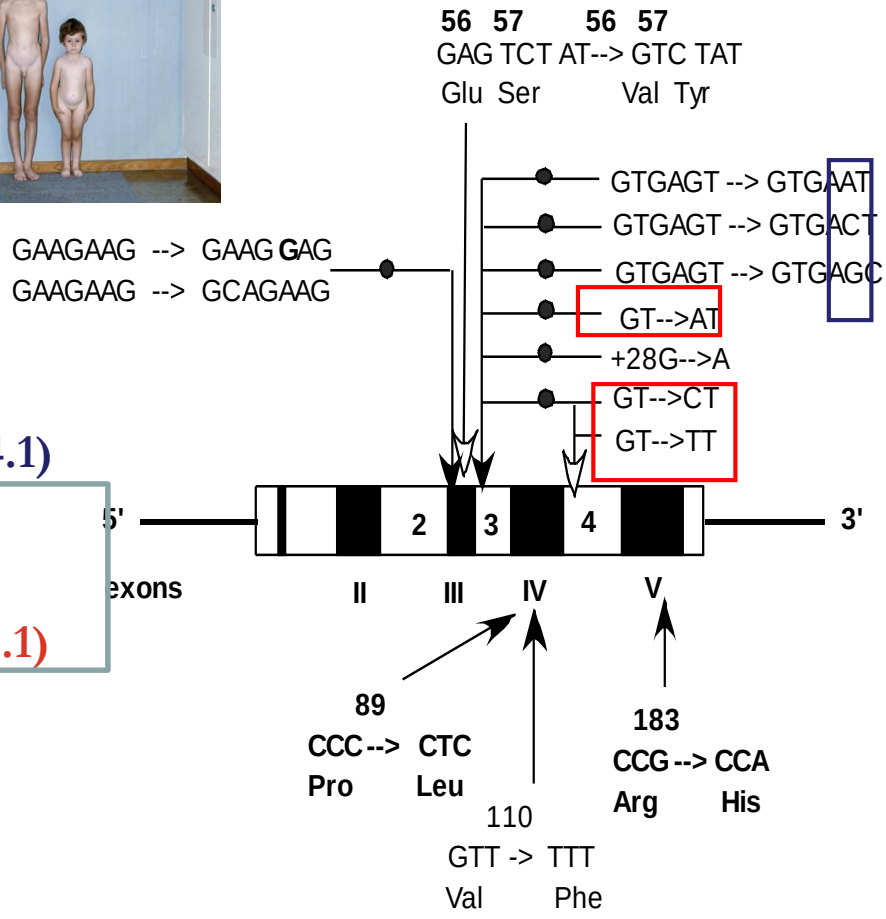




Genetics of GH Deficiency - IGHD Type II
IGHD II: an evolving pituitary deficit?

- 89 subjects with IGHD belonging to 32 families
- were studied on clinical basis, GH-1 gene was sequenced
- 69 subjects belonging to 27 families
- splice site, missense mutation





- **5' IVS-3 +1 / 2 bp**
  - **Age median (range): 3.6 years (1.3 - 5.4)**
  - **Height median (range): -3.2 SDS (2.6 - 4.1)**
- **5' IVS-3 +5 / 6 bp**
  - **Age median (range): 4.2 years (2.1 - 6.4)**
  - **Height median (range): -3.1 SDS (2.5 - 3.1)**
- missenses mutations
  - Age median (range): 7.6 years (1.9 - 9.1)
  - Height median (range): -2.8 SDS (2.2 - 3.7)

Genetics of GH Deficiency - IGHD Type II
IGHD II: an evolving pituitary deficit?

- **5' IVS-3 +1 / 2 bp**
  - **treated rhGH: n: 21; 4 ACTH, 3 TSH insufficient (20%)**
  - **untreated : n: 24; 3 ACTH, 2 TSH insufficient (17%)**
    - **Age median (range): 44 years (21 - 71).**
  - **intrafamilial variability**

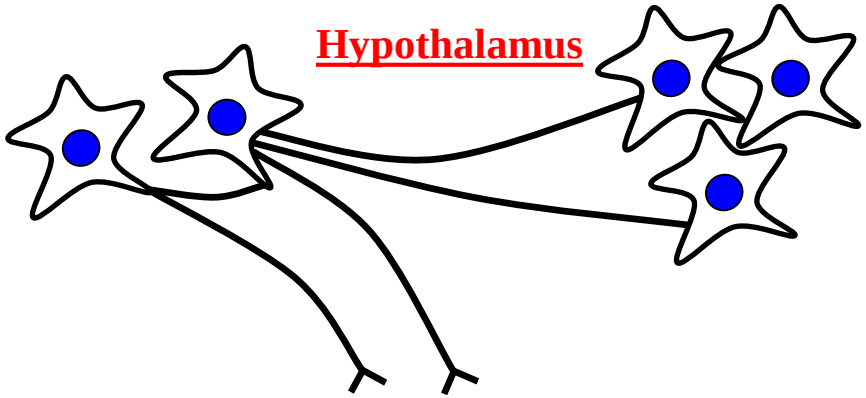
- **5' IVS-3 +5 / 6 bp**
  - **No abnormalities found: totally 19 index patients and 21 untreated subjects studied**

- missense mutations
  - P89L:
    - treated rhGH n:12, 8 ACTH and/or TSH insufficient
    - Untreated: n: 5, 4 ACTH and/or TSH deficient
  - R183H: no abnormalities found; n:6

Genetics of GH Deficiency - IGHD Type II
IGHD II: an evolving pituitary deficit?

- Clinical phenotype depends on *GH-1* gene alteration
- Splice site mutations within 5'IVS-3 +1 /+ 2bp are more severe than 5'IVS-3 +5 /+ 6bp
- Variability in onset, severity, and progression is evident between mutation genotypes and may also occur within families with the same mutation
- That other hormone deficits can develop in IGHD II patients, underscores the clinical importance of maintaining vigilance for development of other hormonal deficiencies over the years

Congenital - Genetic GH Deficiency
Multiple Players at Work



Hypothalamus

HESX1
SOX2
SOX3
LHX3
LHX4
PROP1
POU1F1
PITX
GLI3
GLI2
OTX2
BRAF
ARNT2
IGSF1

Gene overlap KS
FGF8 - FGFR1
PROKR2- PROK2
CDH7- WDR11

TCF7L1 – RNPC3

New Genes ?
IFT72 (ciliopathy)
GPR161
CDON (SHH)

Primary GHD
(secondary IGF-I Deficiency)
Defects in GH production

Pituitary

GHRHR
GHSR
GH-1

PIT-1 antibodies
MicroRNAs

Primary IGF-I Deficiency
Defects in GH action/GH Insensitivity

Functional IGF-I Deficiency
(IGF Resistance Insensitivity)
Defects in IGF-I action
Condrocyte abnormalities

GHR
IGF1
STAT5b
ALS

LIVER

STAT3?

TARGETS

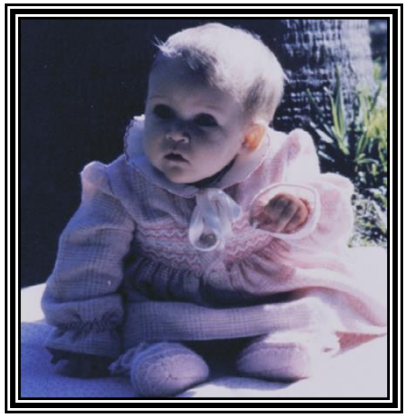
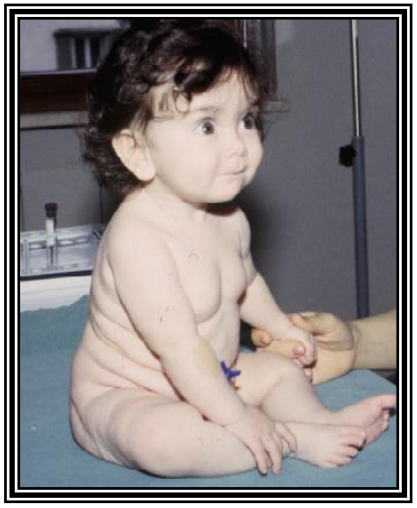
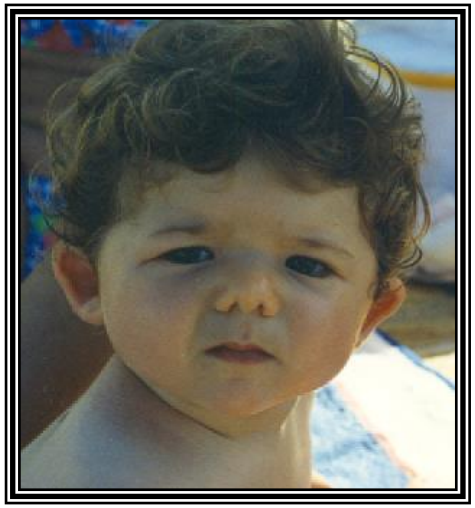
SHOX
NPR2/FGFR3
SHP2
IGFRI
SOCS2
ACAN

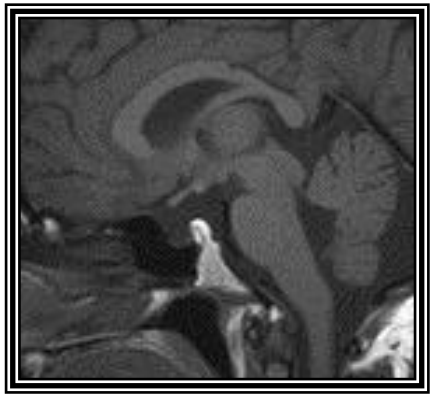
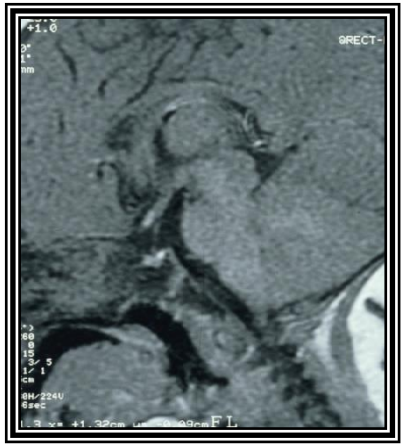
HMGA2
Cell cycle gene
Primordial Dwarfism
TRAIP
XRCC4

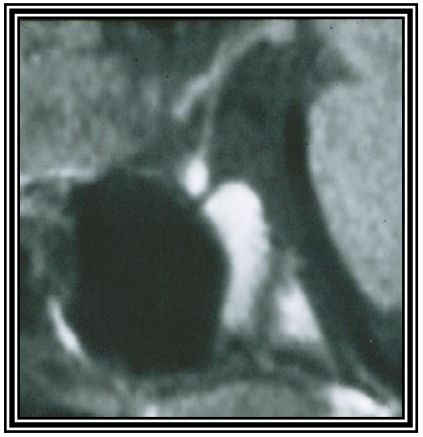
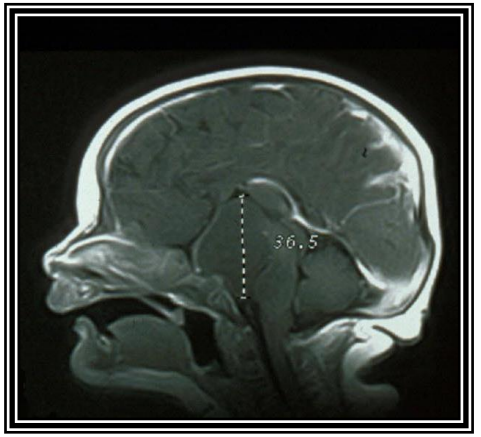
Management of Congenital GH Deficiency

- Who should be screened?
- How should they be screened?
- When should they be be screened?

Congenital–Genetic–Idiopathic Hypopituitarism Phenotype/Genotype Relevance

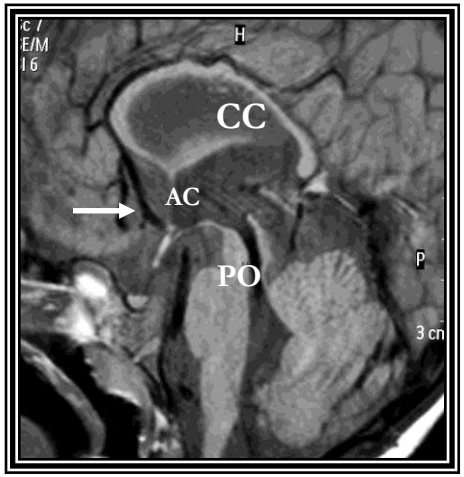



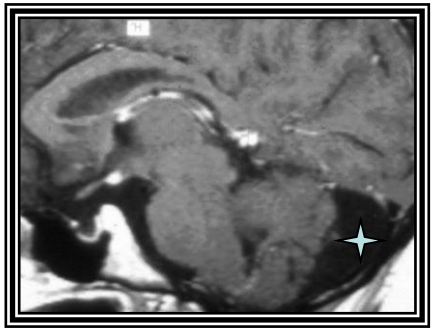



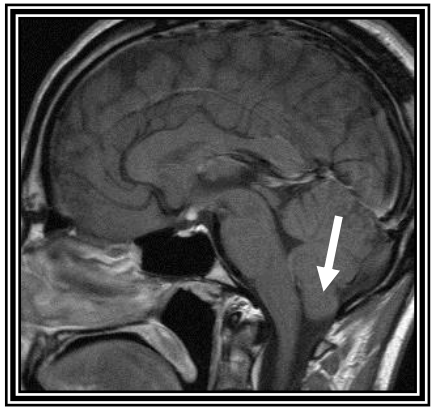
Structural & Pituitary Abnormalities in Congenital GH Deficiency

CNS Malformations

Extra Pituitary

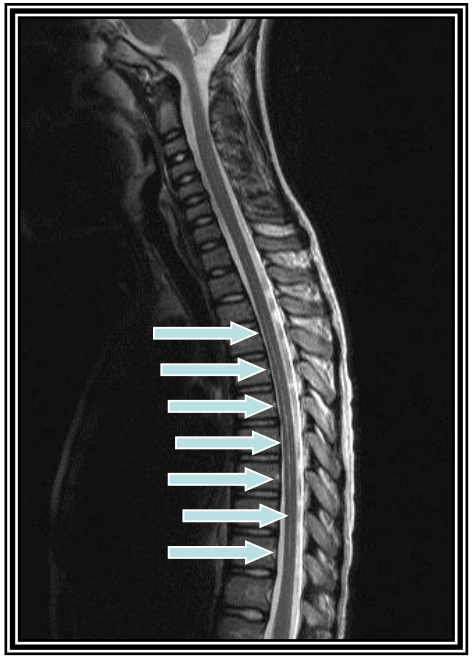






- Arnold-Chiari type I
- Tentorial anomaly
- Septum pellucidum agenesis
- Septo optic dysplasia
- Cortical dysplasia
- Absence of internal carotid artery

- Arnold-Chiari type II
- Vermis dysplasia
- Periventricular heterotopia
- Corpus callosum dysgenesis
- Basilar impression
- Arachnoid Cyst
- Syringomielia



Idiopathic - Genetic GH Deficiency
Genes implicated in cellular differentiation

Congenital hypothyroidism in a young man with growth hormone, thyrotropin, and prolactin deficiencies

A growth-retarded, mentally deficient, young man is described with diminished secretory response of growth hormone, thyrotropin, and prolactin to the pharmacologic stimuli of insulin, arginine, chlorpromazine, and thyrotropin-releasing hormone. Gonadotropin and ACTH functions were normal both basally and upon pharmacologic stimulation. Additionally, the patient was unresponsive to exogenous thyrotropin injections. These data suggest that the hypothyroidism in this patient was due to combined thyroid dysgenesis and pituitary insufficiency, i.e., primary and secondary hypothyroidism.

Alan D. Rogol, M.D., Ph.D.,* and C. Ronald Kahn, M.D., *Bethesda, Md.*

HYPOTHYROIDISM may be subdivided according to the level at which the hypothalamic-pituitary-thyroid axis is altered: in primary hypothyroidism there is a disturbance of the thyroid gland, and in secondary hypothyroidism there is a disturbance of production or secretion of TSH by the pituitary. Secondary hypothyroidism itself can be subdivided into pituitary TSH deficiency, in which the pituitary fails to respond to thyrotropin-releasing hormone, and hypothalamic hypothyroidism in which there is a presumed disturbance of production or secretion of TRH. These usually may be distinguished by measurements of plasma TSH, response of the pituitary to exogenous TRH, and response of the thyroid to exogenous TSH.[1]

The present report is of a patient who presented with athyreotic cretinism with severe growth retardation. Growth response to therapy with thyroid hormone was poor, although bone age rapidly matured. On re-evaluation 15 years later the patient was found to have, in addition to thyroid dysgenesis, deficiencies of TSH, prolactin, and growth hormone. Thus this patient presents both primary and secondary hypothyroidism. Furthermore, in contrast to previous studies[2-4] of patients with GH- and TSH-deficient dwarfism, the lack of response to *TRH in this patient suggests a pituitary rather than a* hypothalamic origin of the TSH deficiency.

From the Diabetes and Clinical Endocrinology Branches, National Institute of Arthritis, Metabolism, and Digestive Diseases, National Institutes of Health.
**Reprint address: University of Virginia School of Medicine, Department of Pediatrics, Charlottesville, Va. 22901.*

Abbreviations used

TSH:	thyroid-stimulating hormone
TRH:	thyrotropin-releasing hormone
GH:	growth hormone
hPRL:	human pituitary prolactin
MUU:	mouse uterine units
HGH:	human growth hormone
TEBG:	testosterone-estrogen binding globulin
LH-RH:	luteinizing hormone-releasing hormone

CASE REPORT

Patient W. T. R.,* a 3,100 gm. full-term infant, was born May 16, 1945; pregnancy, labor, and delivery were uncomplicated. The mother was a 26-year-old, gravida 3, para 2 woman.

Slow development was first noted at 8 months when he was unable to hold his head against gravity. Evaluation at 1 6/12 years revealed an enlarged "hydrocephalic-appearing" head and marked delay in growth and development. Bilateral subdural aspirations yielded only clear fluid. At 1 11/12 years, a nondepressed *linear fracture of the parietal bone* was noted. Urinalysis, glucose tolerance test, intravenous pyelogram, STS, nonprotein nitrogen, serum alkaline phosphatase, calcium, and phosphorus concentrations were within normal limits. The serum concentration of cholesterol was 187 mg/dl.

*Patient T.R. in reference 5.

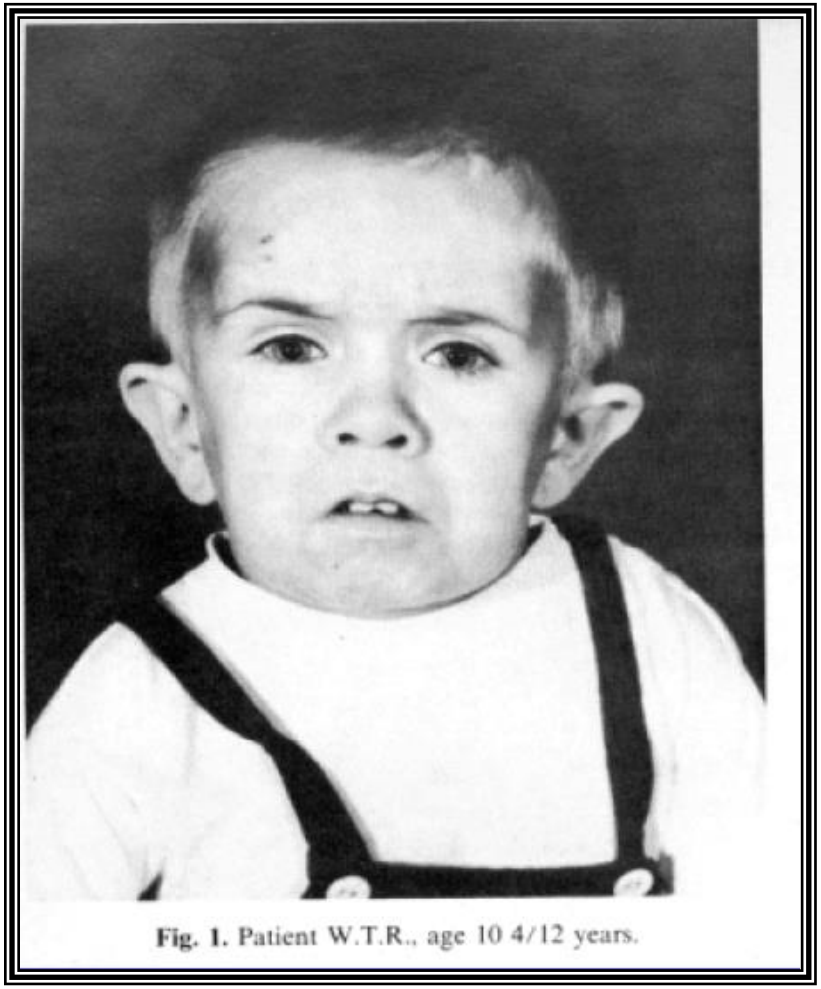


Fig. 1. Patient W.T.R., age 10 4/12 years.

Congenital/Genetic GH Deficiency
Genes implicated in cellular differentiation

Science. 1992 Aug 21;257(5073):1118-21.

Mutation of the POU-specific domain of Pit-1 and hypopituitarism without pituitary hypoplasia.

Pfäffle RW, DiMattia GE, Parks JS, Brown MR, Wit JM, Jansen M, Van der Nat H, Van den Brande JL, Rosenfeld MG, Ingraham HA.

Department of Pediatrics, Emory University School of Medicine, Atlanta, GA 30322.

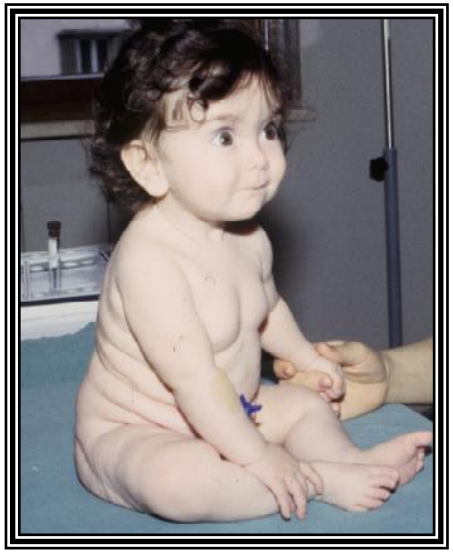
Abstract

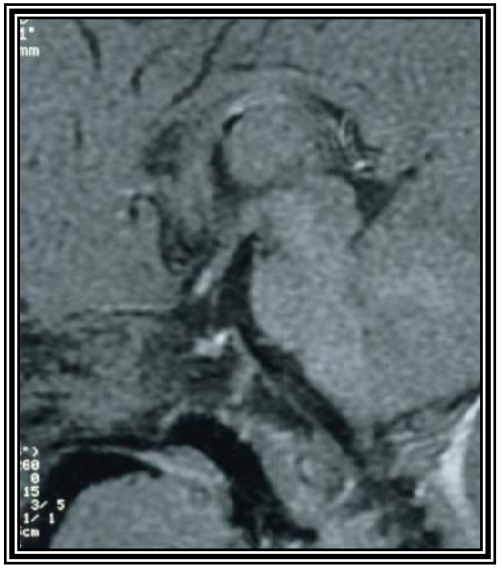
- A point mutation in the POU-specific portion of the human gene that encodes the tissue-specific POU-domain transcription factor, Pit-1, results in hypopituitarism, with deficiencies of growth hormone, prolactin, and thyroid-stimulating hormone. In two unrelated Dutch families, a mutation in Pit-1 that altered an alanine in the first putative alpha helix of the POU-specific domain to proline was observed. This mutation generated a protein capable of binding to DNA response elements but unable to effectively activate its known target genes, growth hormone and prolactin. The phenotype of the affected individuals suggests that the mutant Pit-1 protein is competent to initiate other programs of gene activation required for normal proliferation of somatotrope, lactotrope, and thyrotrope cell types. Thus, a mutation in the POU-specific domain of Pit-1 has a selective effect on a subset of Pit-1 target genes.

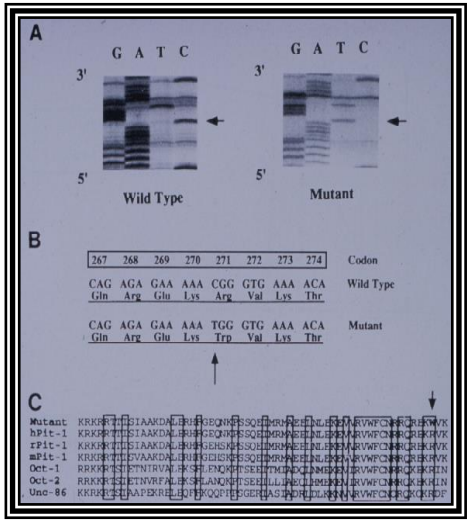
Congenital- Idiopathic-Genetic GH Deficiency
Genes implicated in cellular differentiation
"In situ Posterior Pituitary"

- Birth weight 4 kg
- Birth length 49.5 cm
- Nonconsanguineous parents
- Neonatal hyperbilirubinemia
- Failure to thrive
- Mild hypotonia
- Pale skin
- Prominent frontal bone
- Depressed nasal bridge
- Central hypothyroidism
- Undetectable GH
- Undetectable PRL (inability to breast feed in affected mothers)

- small pituitary MR imaging







- MRI at the age of 2 months
- Pituitary height 2.5 mm (controls 2.6-5 mm)

R271 Mutation

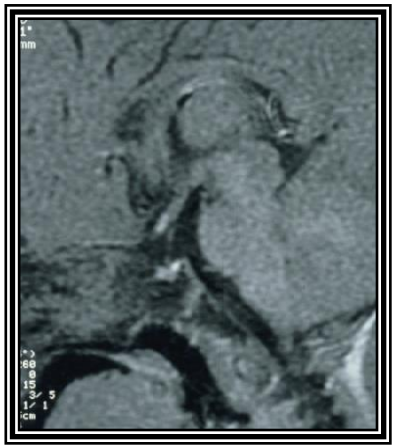
J Clin Endocrinol Metab 1995;80:679-684

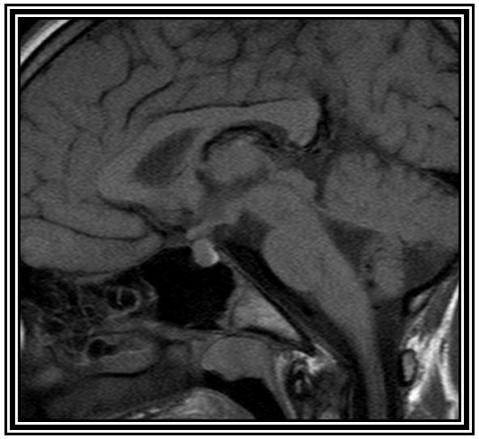
Genetic GH Deficiency

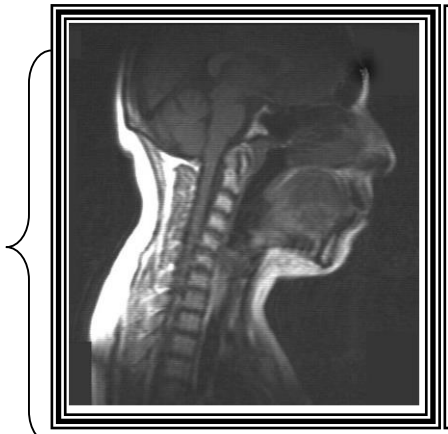
Genes implicated in cellular differentiation

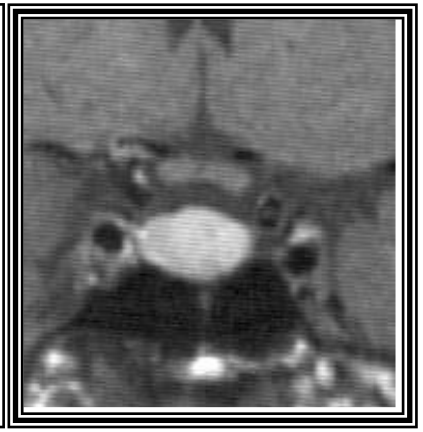
"In situ Posterior Pituitary"

Gene Inheritance	Hormone Deficiencies					Pituitary Phenotypes	Associated Abnormalities
POU1F1 AR/AD	GH	PRL	TSH			Small / Normal	No
PROP1 /AR	GH	PRL	TSH	LH/FSH	± ACTH	All sizes	No
LHX3/AR	GH	PRL	TSH	LH/FSH	±ACTH	AP hypoplasia / large, Cystic Chiari I	Stubby neck Limited rotation, skeletal, Scoliosis Deafness, narrow acoustic nerve
SOX 2/AR	±GH			LH/FSH		AP hypoplasia	Microphtalmia, anophtalmia, optic nerve hypoplasia, SOD, H hamartoma, micropenis, neurosensorial deafness, GI tract defects





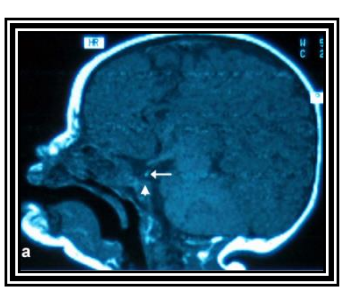


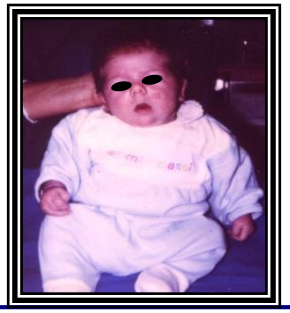


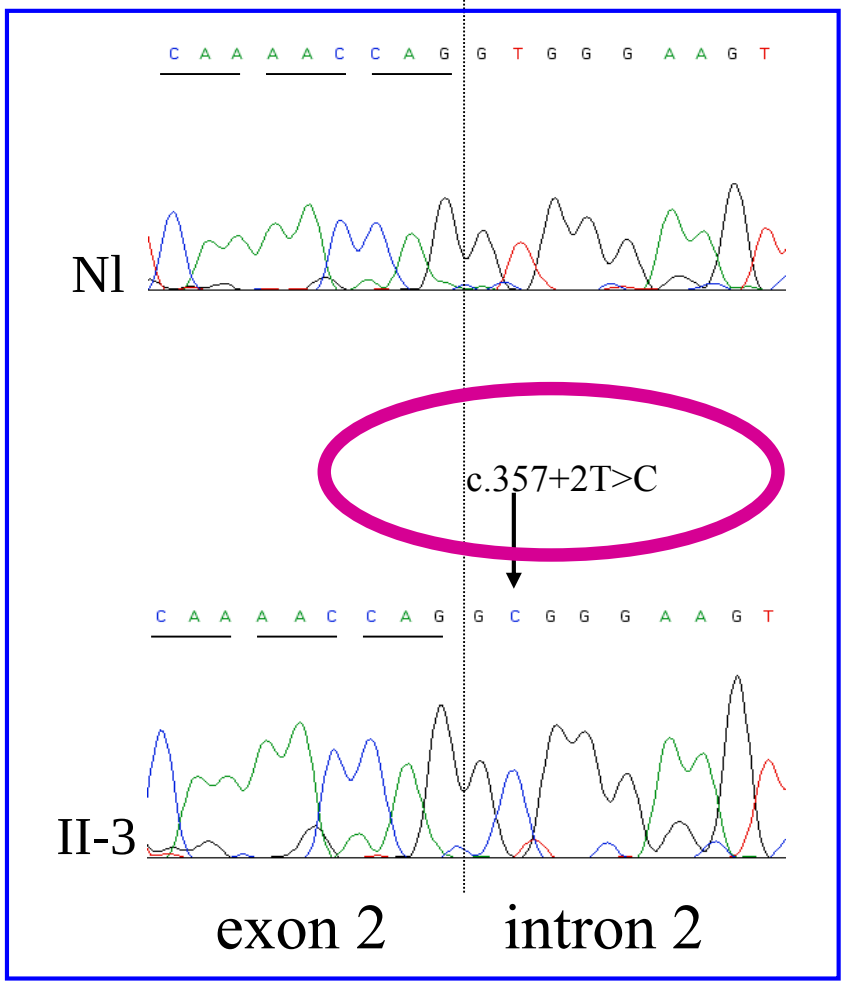
Developmental/Differentiation Defects

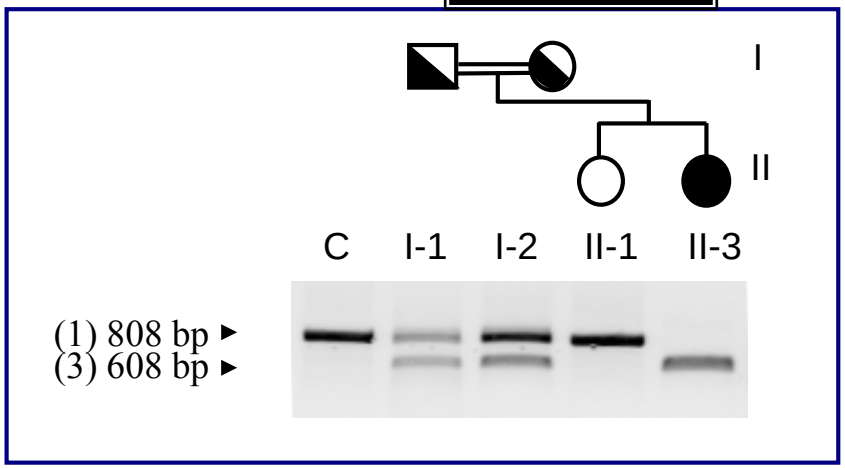
Gene Inheritance	Hormone Deficiencies					Pituitary Phenotypes	Associated Abnormalities
POU1F1 AR/AD	GH	PRL	TSH			Small / Normal	No
PROP1 /AR	GH	PRL	TSH	LH/FSH	± ACTH	All sizes	No
HESX1 AR/AD/v.p.	GH	± PRL	±TSH	± LH/FSH	± ACTH	Normal / Aplasia / AP hypo-EPP / AP hypo	SOD SOD variants
LHX3/AR	GH	PRL	TSH	LH/FSH		AP hypoplasia / large	Stubby neck Limited rotation
LHX4/AD	GH		TSH	LH/FSH (?)	ACTH	AP hypoplasia / EPP	Chiari- I
GLI2/AD/ v.p.	GH	?	TSH	?	ACTH	**AP hypoplasia / AP Aplasia**	Holoprosencephaly-like
SOX3/X- linked R	GH	PRL	TSH	LH/FSH	ACTH	AP hypoplasia / EPP	Mental retardation?
SOX2	±		±		±	AP hypoplasia / EPP/ **Normal AP**	Microphtalmia/anophtal mia, ONH, CNS Micropenis
OTX2	GH		TSH	FSH/LH	ACTH	Normal AP hypoplasia / EPP	Bil. Anophtalmia Chiari-I

Molecular studies: Case 2, S.S.
Sequencing of the HESX1 gene (4 exons and flanking intronic regions) and RT-PCR on RNA from EBV-transformed lymphocytes

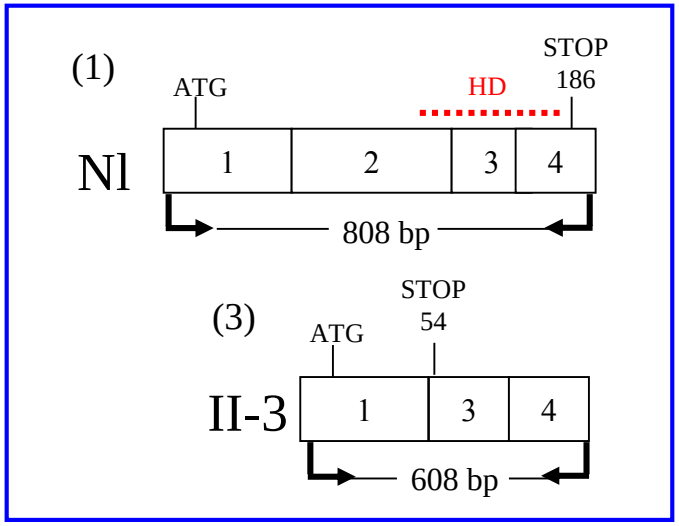








HESX1
AP Aplasia
No EP
No ONH



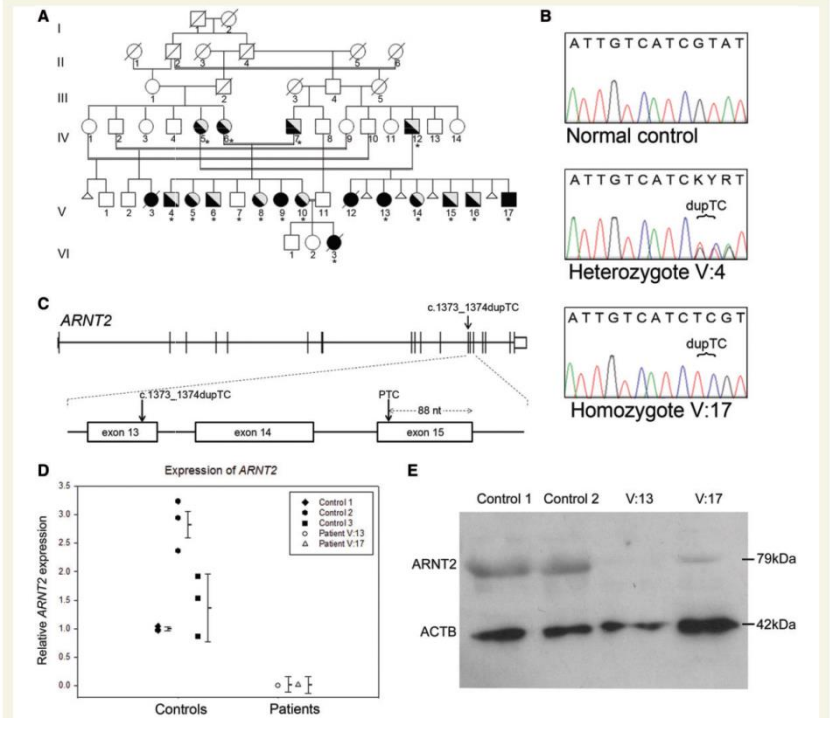
Detection of a "splice"

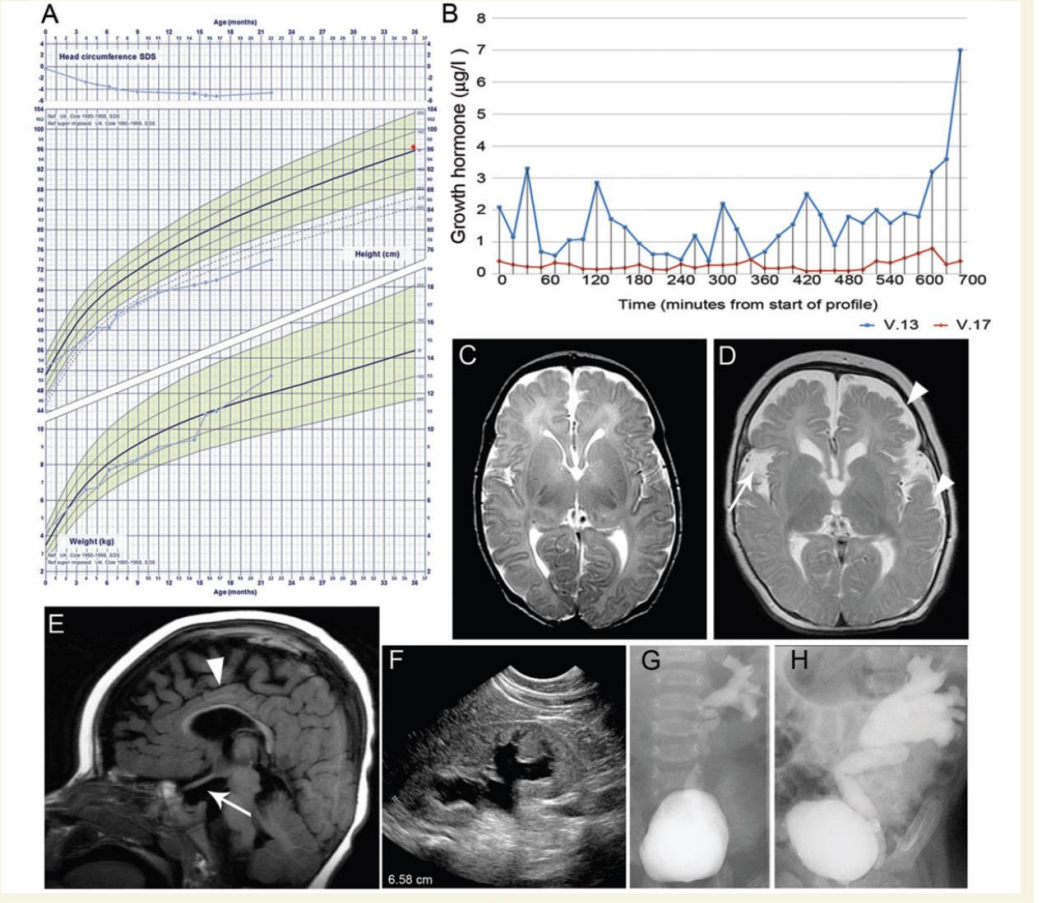
J Clin Endocrinol Metab. 2006 ; 91:4528-36.

doi:10.1093/brain/awt218 Brain 2013: 136; 3096–3105 | 3096

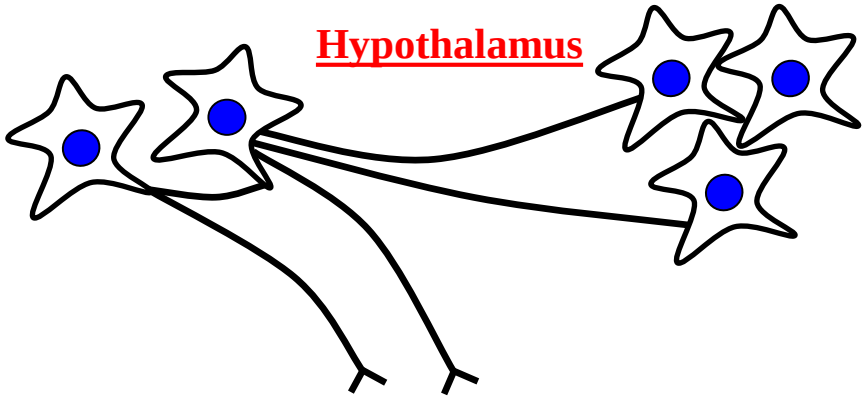
BRAIN
A JOURNAL OF NEUROLOGY

ARNT2 mutation causes hypopituitarism, post-natal microcephaly, visual and renal anomalies





Congenital - Genetic GH Deficiency
Multiple Players at Work



Hypothalamus

HESX1
SOX2
SOX3
LHX3
LHX4
PROP1
POU1F1
PITX
GLI3
GLI2
OTX2
BRAF
ARNT2
IGSF1

Gene overlap KS
FGF8 - FGFR1
PROKR2- PROK2
CHD7- WDR11

TCF7L1 – RNPC3

New Genes ?
IFT72 (ciliopathy)
GPR161
CDON (SHH)

Primary GHD
(secondary IGF-I Deficiency)
Defects in GH production

Pituitary

GHRHR
GHSR
GH-1

PIT-1 antibodies
MicroRNAs

Primary IGF-I Deficiency
Defects in GH action/GH Insensitivity

Functional IGF-I Deficiency
(IGF Resistance Insensitivity)
Defects in IGF-I action
Condrocyte abnormalities

GHR
IGF1
STAT5b
ALS

LIVER

STAT3?

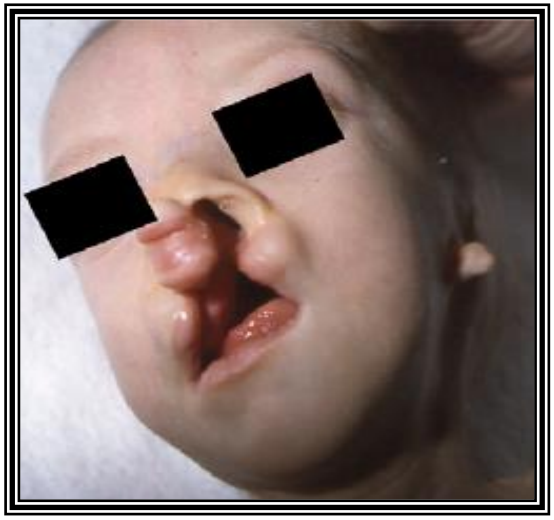
TARGETS

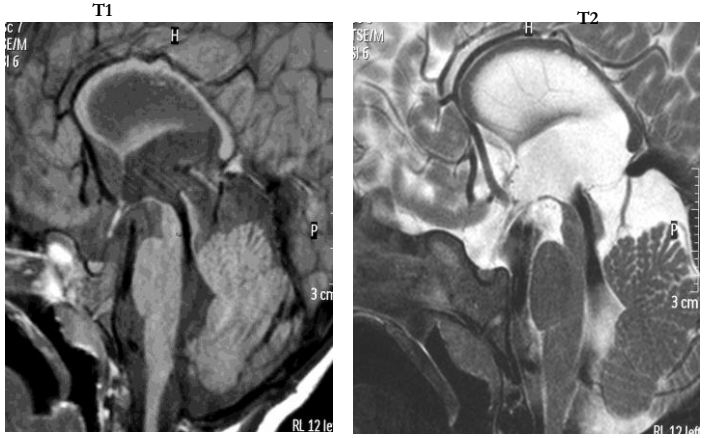
SHOX
NPR2/FGFR3
SHP2
IGFRI
SOCS2
ACAN

HMGA2
Cell cycle gene
Primordial Dwarfism
TRAIP
XRCC4

FGFR1 Mutations
Gene Overlap

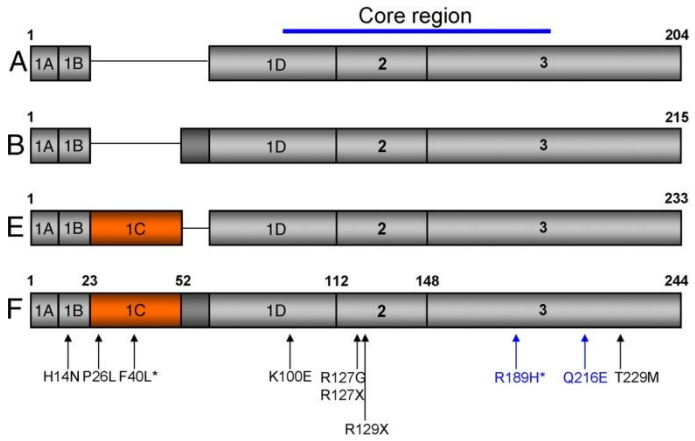
- HH variably associated with anosmia and midline defects (cleft palate, dental agenesis,..)
- First report: ***Dode et al, Nature Genet 2003***
- Incidence: about 10% of KS

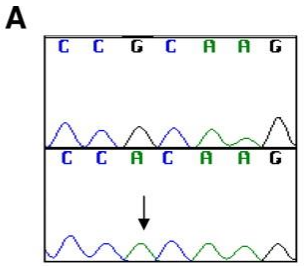




Two novel mutations associated with recessive HPE and SOD. A and B, A total of 421 patients with hypopit phenotypes ranging from HPE to SOD were screened for mutations in FGF8 by direct equencing.

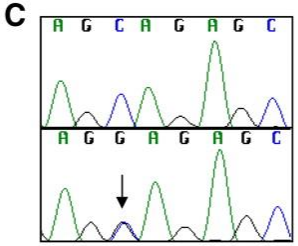
Structure of FGF8 isoforms.





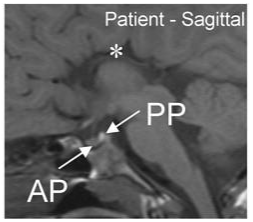
B

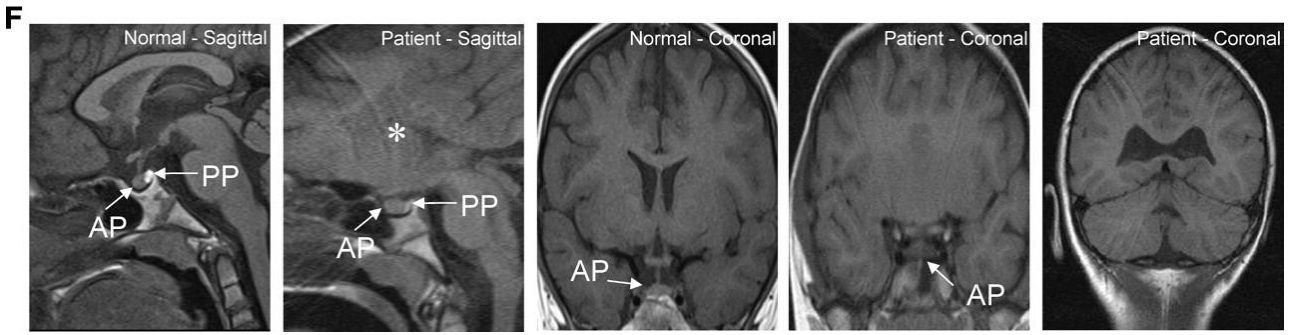
Human:	R--K--G--R--P--R--K--G--S--K--T
Chimpanzee:	R--K--G--R--P--R--K--G--F--K--T
Mouse:	R--K--G--R--P--R--K--G--S--K--T
Rat:	R--K--G--R--P--R--K--G--S--K--T
Dog:	R--K--G--R--P--R--K--G--S--K--T
Guinea Pig:	R--K--G--R--P--R--K--G--S--K--T
Dolphin:	R--K--G--R--P--R--K--G--S--F--T
Cat:	R--K--G--R--P--R--K--G--S--K--T
Wallaby:	R--K--G--R--P--R--K--G--S--K--T
Chicken:	R--K--G--R--P--R--K--G--S--K--T
Lizard:	R--K--G--R--P--R--K--G--S--K--T
Xenopus:	R--R--G--R--P--R--K--G--S--K--T
Zebrafish:	R--K--G--R--P--R--K--G--S--K--T

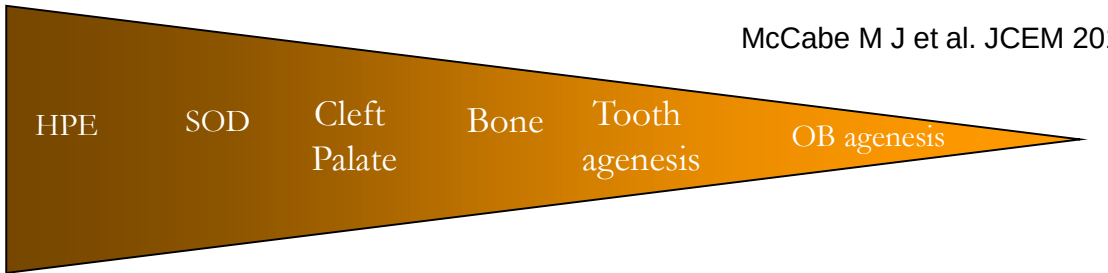


D

Human:	H--H--T--T--E--Q--S--L--R--F--E
Chimpanzee:	H--H--T--T--E--Q--S--L--R--F--E
Mouse:	H--H--T--T--E--Q--S--L--R--F--E
Rat:	H--H--T--T--E--Q--S--L--R--F--E
Dog:	H--H--T--T--E--Q--S--L--R--F--E
Guinea Pig:	H--H--T--T--E--Q--S--L--R--F--E
Dolphin:	H--H--T--T--E--Q--S--L--R--F--E
Cat:	H--H--T--T--E--Q--S--L--R--F--E
Wallaby:	H--H--T--T--E--Q--S--L--R--F--E
Chicken:	H--Q--T--T--E--P--H--R--R--F--E
Lizard:	H--Q--T--T--E--P--H--R--R--F--E
Xenopus:	H--H--T--T--E--P--H--K--R--F--E





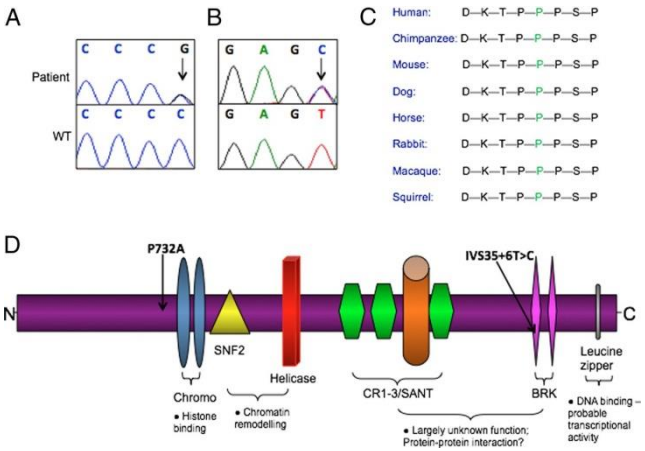


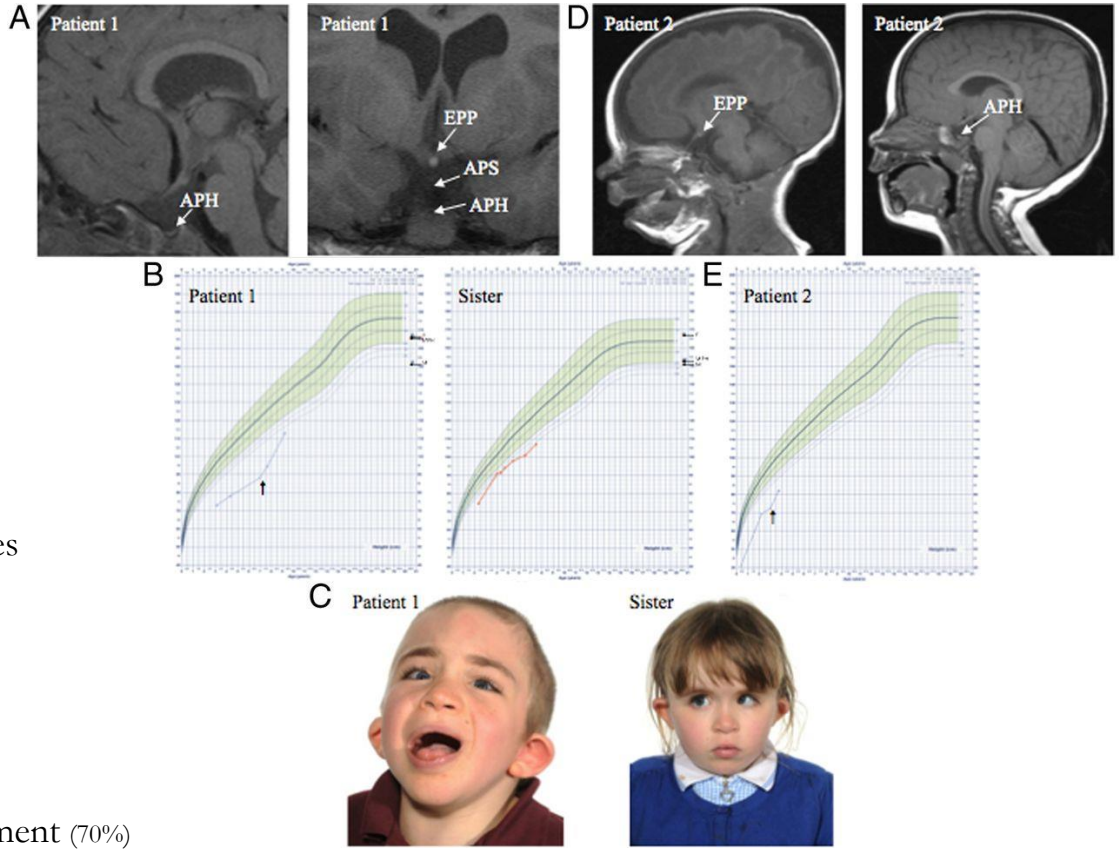
McCabe M J et al. JCEM 2011;96:E1709-E1718

The Journal of Clinical Endocrinology & Metabolism

A CHD7 mutation associated with CHARGE syndrome and hypopituitarism.
MRI in patient 1 revealed APH, an absent pituitary stalk (APS) with an undescended/EPP at the tuber cinereum and a thin corpus callosum.

CHD7 - DNA-binding protein-7 (8q12.1, 7q21.11)





Diagnostic Criteria Pagon (1981) 4 among 6 anomalies

C - Coloboma of the eye (80%), CNS anomalies

H - Heart defects (85%)

A - Atresia of the choanae (57%)

R - Retardation of growth (80%) and/or development (70%)

G - Genital and/or urinary defects

E - Ear anomalies (90-100%) and/or deafness (62%)

Gregory L C et al. JCEM 2013;98:E737-E743

THE JOURNAL OF CLINICAL ENDOCRINOLOGY & METABOLISM

Management of Congenital GH Deficiency

Who should be screened?

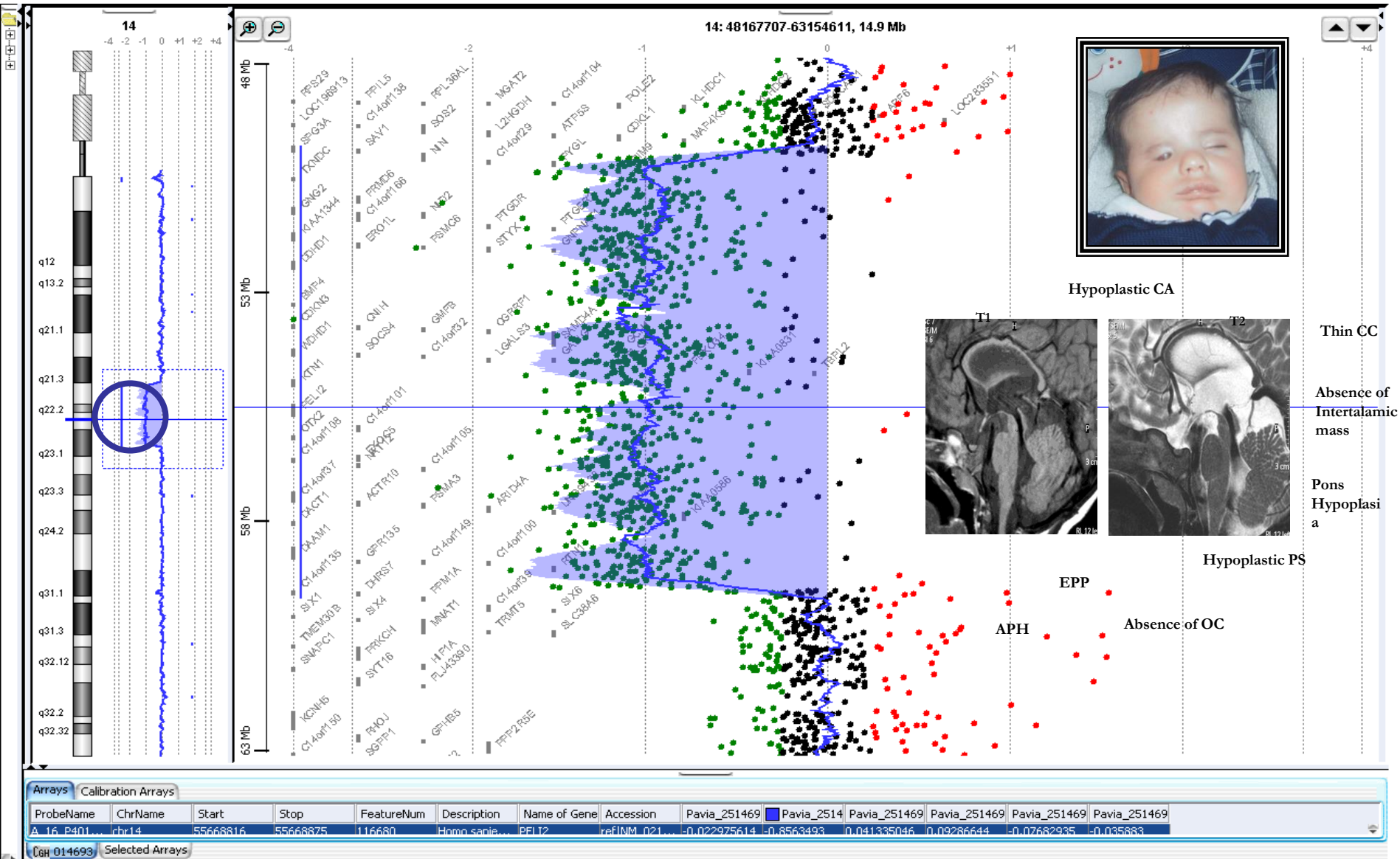
- Familial forms
- Consanguinity
- Neonatal-Early-onset severe hypopituitarism
- Clinical phenotype (Cranio-facial, eyes, ears, deafness, midline defects…)
- Anosmia, heart, kidney,….
- Isolated GHD with severe clinical and endocrine phenotype
- CPHD/MPHD patients
- MRI phenotype with the classic «Triad» of EPP, pituitary stalk agenesis and pituitary hypoplasia/aplasia with CNS abnormalities

How should they be screened?

- Caryotyping
- CGH-Array
- Sanger sequencing
- Next generation sequencing
- Whole genome sequensing

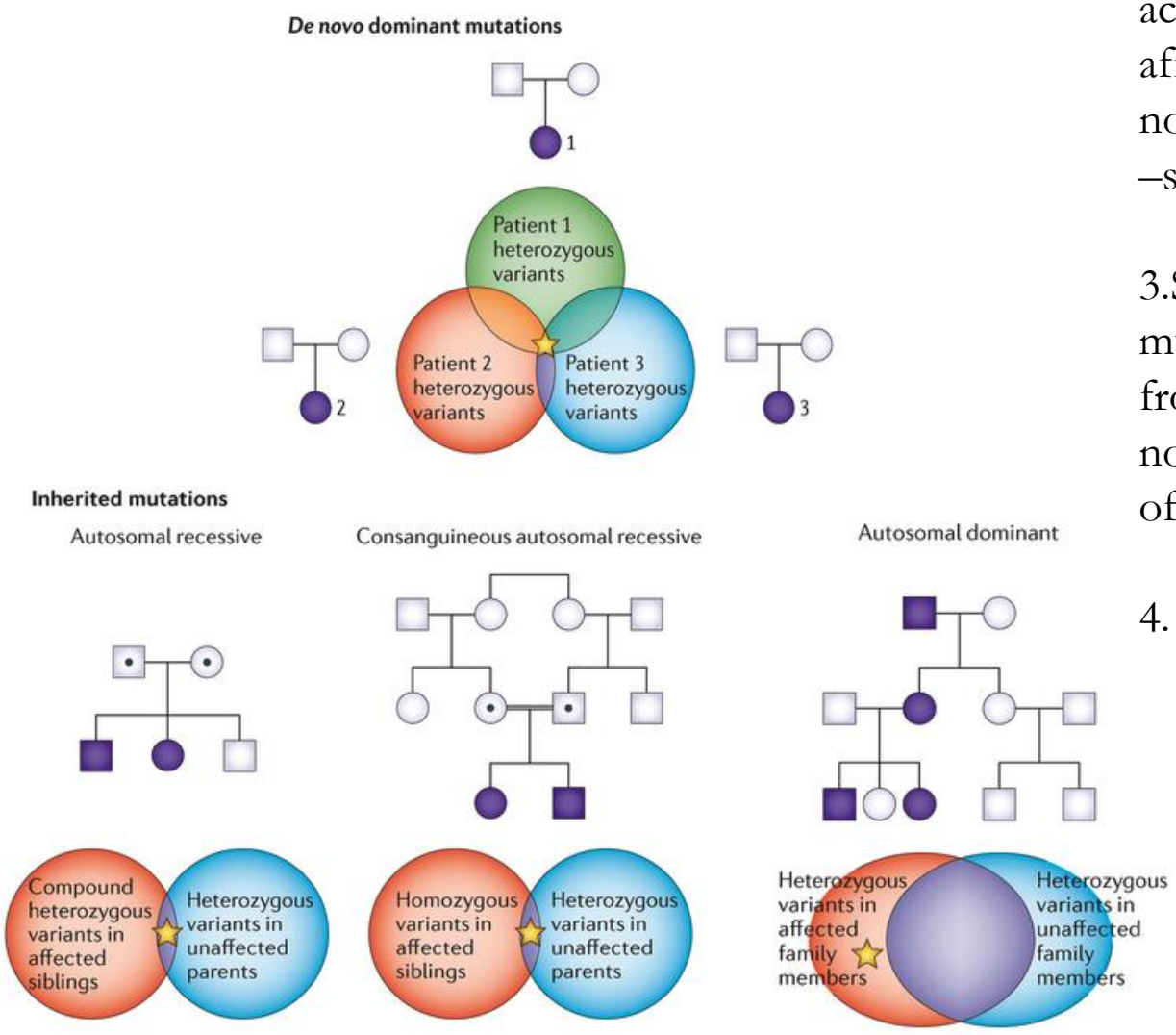
Developmental/Migration Defect

Micro-array CGH: [del(14)(q22.1q23.1)]



Exome sequencing technical approach for Mendelian gene discovery

Bamshad, M. J. et al. Exome sequencing as a tool for Mendelian disease gene discovery. Nature reviews. Genetics 12, 745–55 (2011)

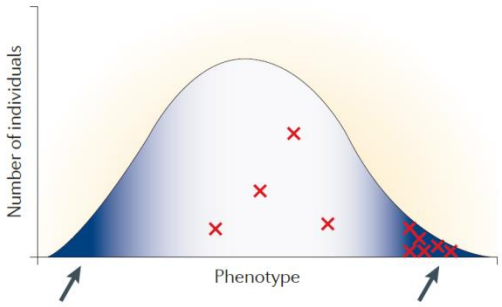


1. Sequencing parent-child trios for identifying new mutations

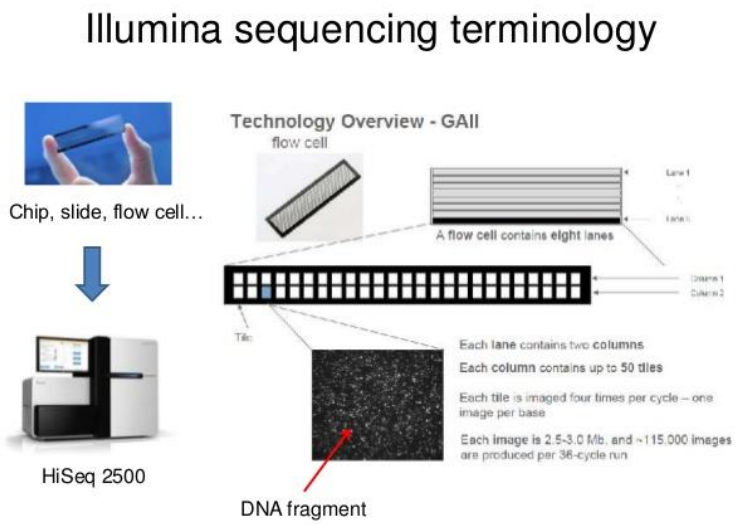
2.Sequencing and filtering accross multiple unrelated, affected individuals to identify novel variants in the same gene –shared- by the individuals

3.Sequencing and filtering among multiple affected individuals from within a pedigree with a novel variant in a shared region of the genome

4. Rare variants in one extreme



Exome sequencing technical approach for Mendelian gene discovery



- da luglio 2016 l'analisi molecolare su piattaforma MiSeq (Illumina mediante next generation sequencing e verranno analizzati in totale 31 geni candidati; il pannello comprende i geni

- 1 IGF1 2 GHR 3 IGFALS(ALS) 4 SOCS1, 5 IGF1, 6 SHOX, 7 COMP, 8 SOX9, FGFR3, 10 HMGA2, 11 GHRHR, 12 GH1, 13 SHH, 14 GLI2, 15 GLI3, 16 FGF8, 17 CDH7, 18 HESX1, 19 OTX2, 20 POU1F1, 21 SOX2, 22 LHX3, 23 LHX4, 24 FGFR1, 25 PROKR2, 26 ARNT2, 27 GPR161, 28 XRCC4, 29 PAPPA2, 30 PCNT, 31 STAT5B

- Con tale screening, si garantisce una copertura tra l'80 e il 90% della regione target dei geni HMGA2, IGFALS, SHH, SHOX, SOX9, SAT5B, FGF8 e una copertura superiore al 95% dei restanti geni, con una profondità di analisi di almeno 20X.

Management of Congenital GH Deficiency

- Who should be screened?
  - All patients with congenital hypopituitarism should have a genetic evaluation
  - Those with a family history and/or severe short stature (IGHD and early–onset presentation)
  - Those with CPHD /MPHD
- How should be screened?
  - Sanger for specific phenotypes (POU1F1, PROP1 …)
  - NGS /Gene panel
- When should they be be screened?
  - Genetic screening should occur after the clinical diagnosis of hypopituitarism for better counselling and prognosis

Take Home Message

- Mutations/variations in genes implicated in pituitary development are rare
- Pleiotropic phenotypes
- Evolving phenotypes
- Variable inheritance
- Variably penetrant

  -Cranio-facial
  - Eyes abnormalities
  - Deafness
  - Skeletal
  - Pituitary
  - Extra pituitary (midline, midbrain, olfactory bulbs …)
  - Role of other genes
  - Role of environmental factors
- Careful interpretation required at any changes
- Care with genetic counselling
- Re-evaluation with time

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Maura Mazzoni e staff

Patients and Families