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Genetic Basis of Short Stature:

Defects in Growth Hormone Signaling and Beyond

Vivian Hwa, PhD
Division of Endocrinology
Basic Research Director
The Cincinnati Center of Growth Disorders
Cincinnati Children's Hospital Medical Center
Cincinnati, OH, USA

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Disclosure

Consultant, Pfizer

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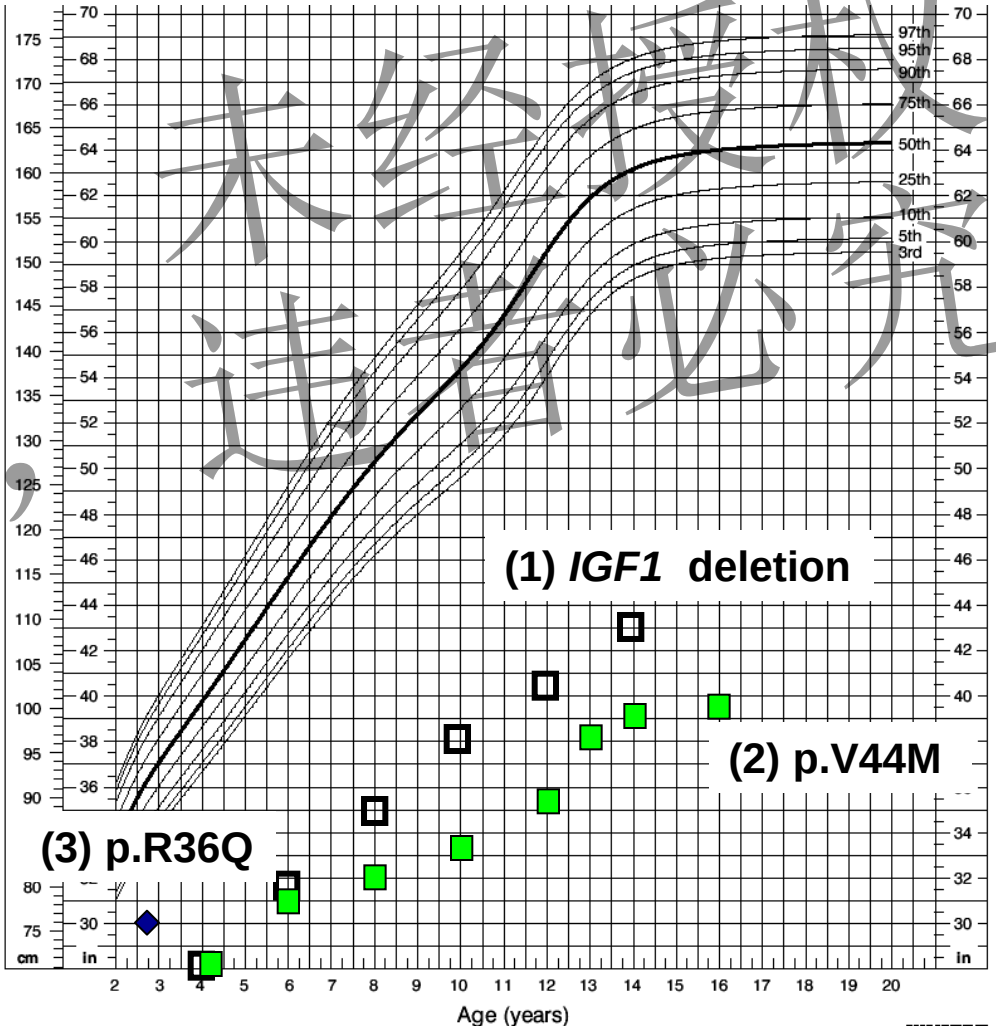
INSULIN-LIKE GROWTH FACTOR-I (IGF-I): CRITICAL FOR NORMAL HUMAN PRE- & POST-NATAL GROWTH

Homozygous *IGF1* Mutations

(1) Woods KA, NEJM, 1996
(2) Walenkamp MJ, JCEM, 2005
(3) Netchine I, JCEM, 2009

- IUGR (birth wt: <-2.5 SDS)
- severe post-natal growth failure (Ht: < -4.9 SDS)
- Microcephaly
- Intellectual impairment
- sensorineural deafness

(1 and 2 only)

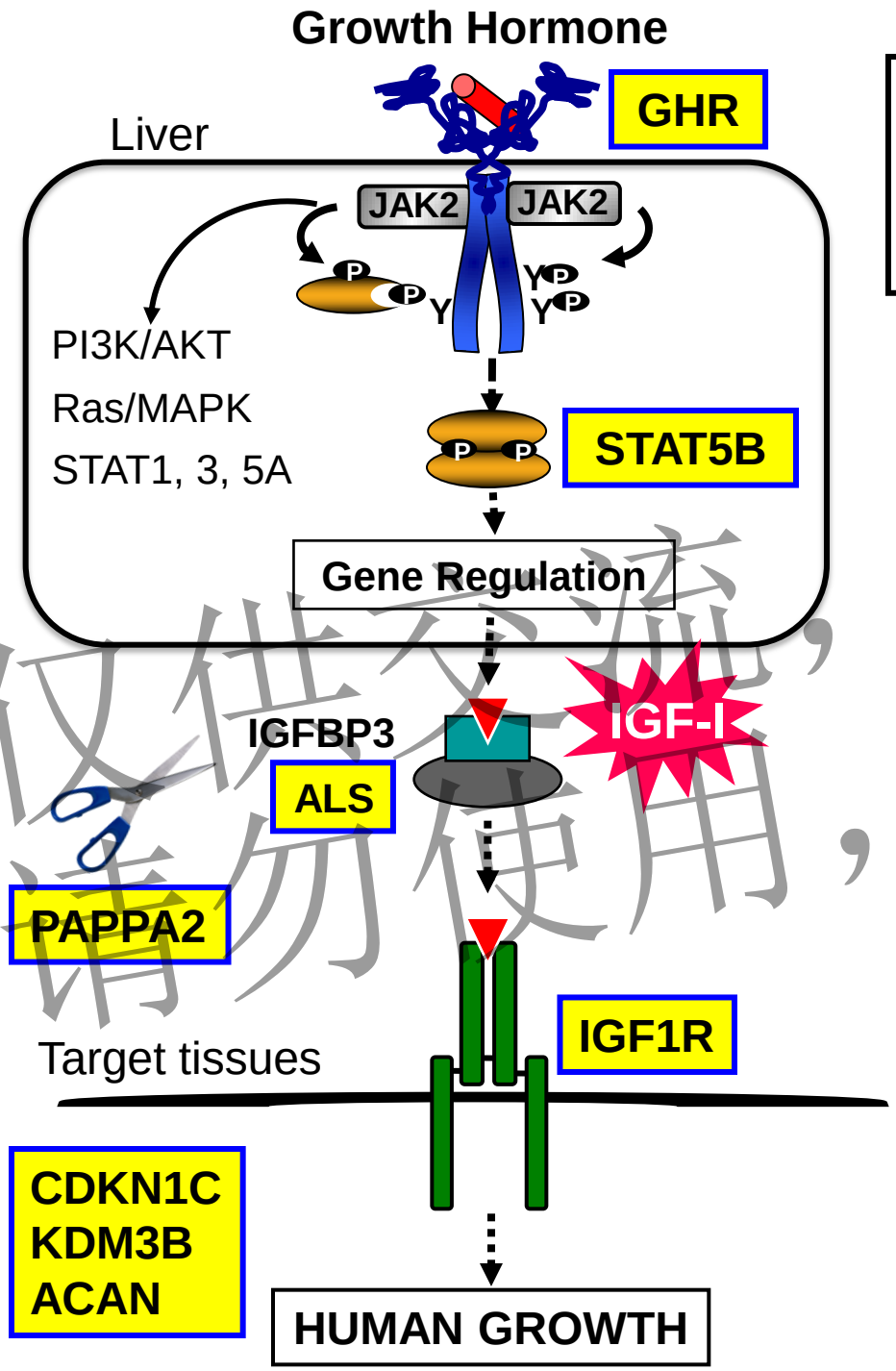


***In Utero*:**

IGF-I production appears to be dependent on insulin and nutrition.

Post-natal growth:

IGF-I production is GH-dependent.



GH-IGF-I Pathway

(Growth hormone-Insulin-like growth factor-I)

Genetic defects

↓

GH Insensitivity (GHI) Syndrome

↓

IGF-I Deficiency

or

IGF-I Resistance

↓

Spectrum of growth failure

-2.0 to -10 SDS below normal

Genetic Basis for Growth Hormone Insensitivity (GHI)

I. Defects disrupting IGF-I production

II. Defects disrupting IGF-I actions

III. Defects disrupting fundamental cellular functions

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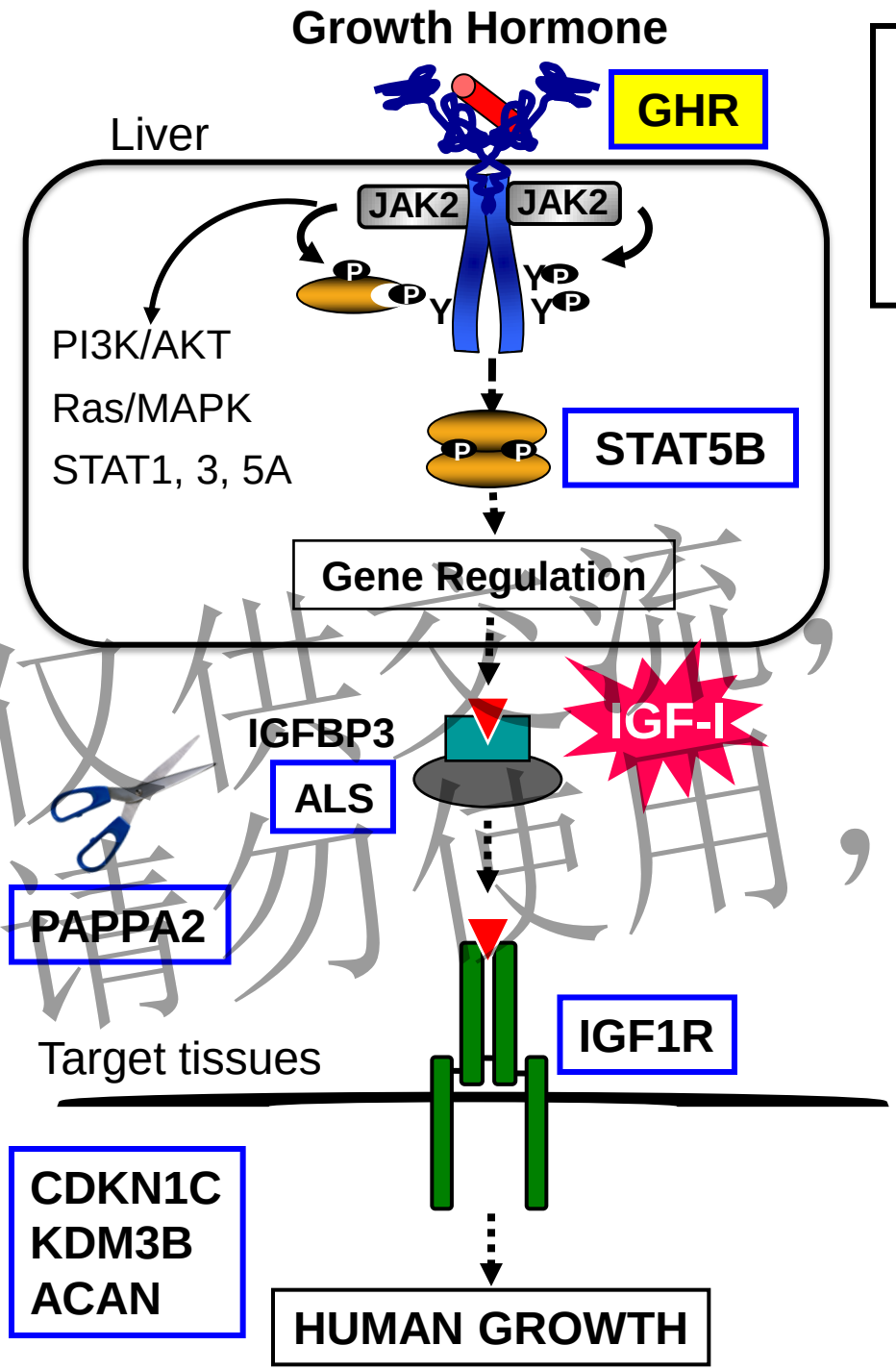
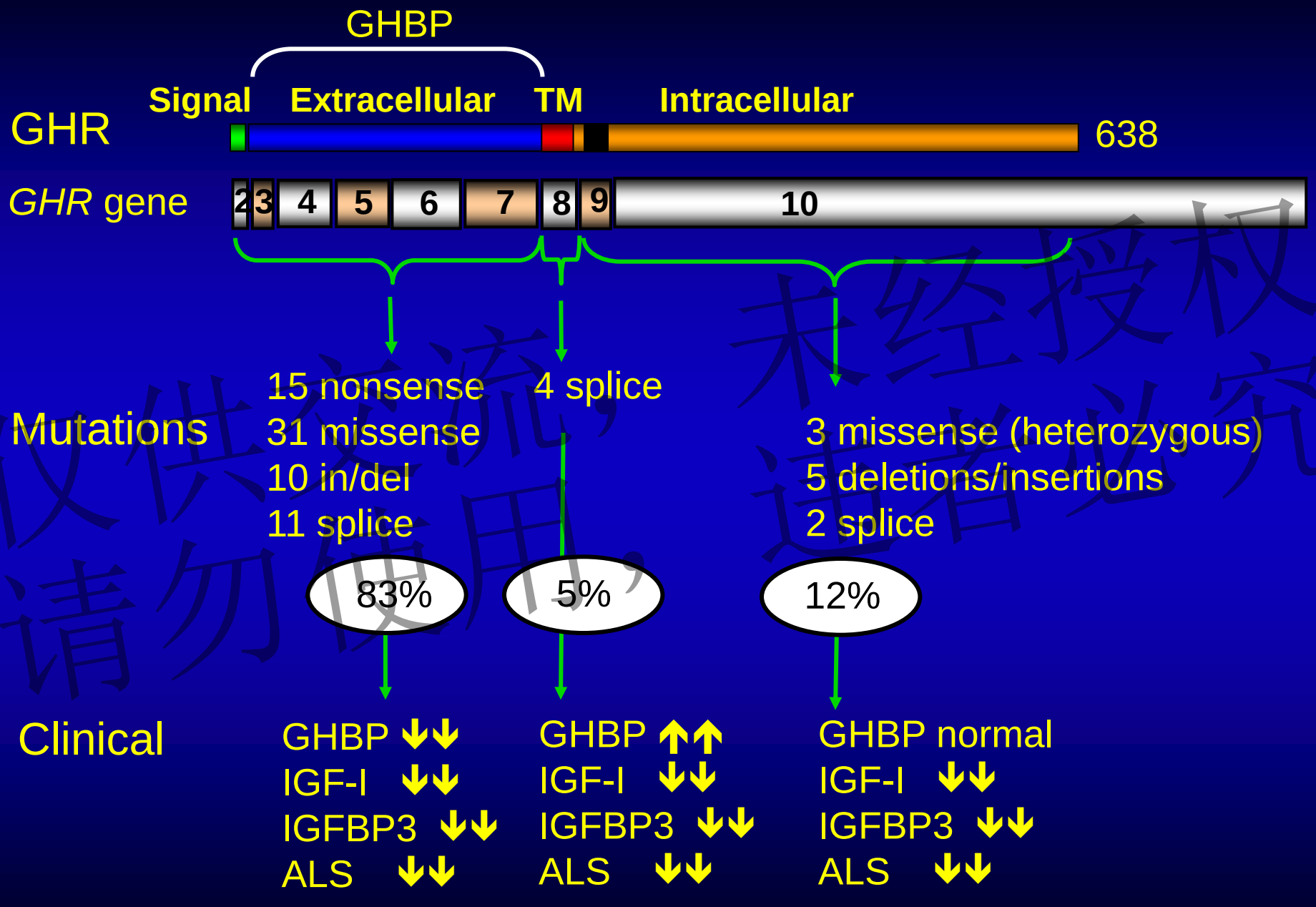


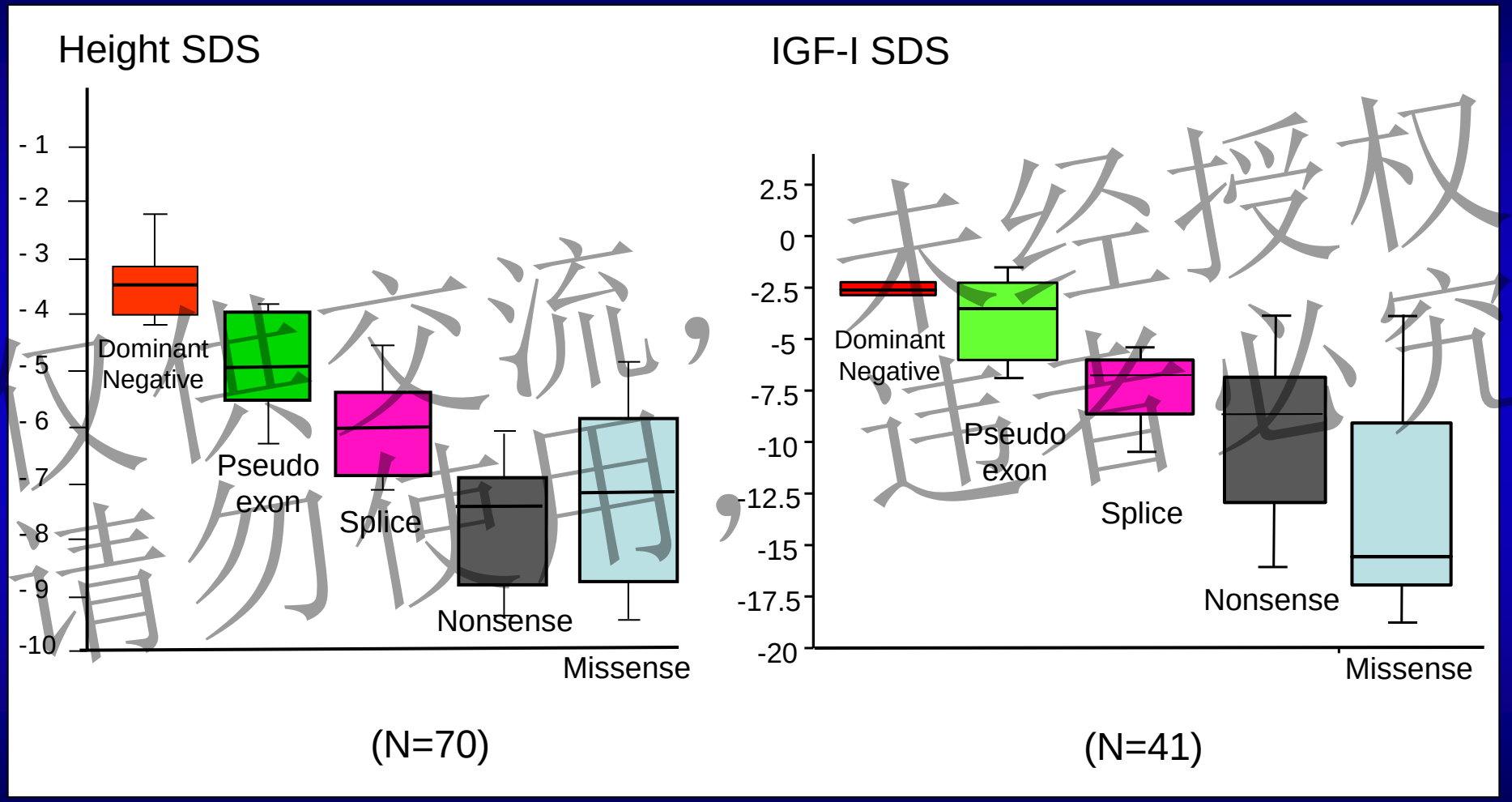
Growth Hormone Receptor (*GHR*) Defects: GHI and IGF Deficiency

***GHR:* GHBP+/-**
>300 cases
>80 mutations

- Autosomal Recessive
- Loss of function mutations
- Spectrum: from “Classical” to atypical GHI

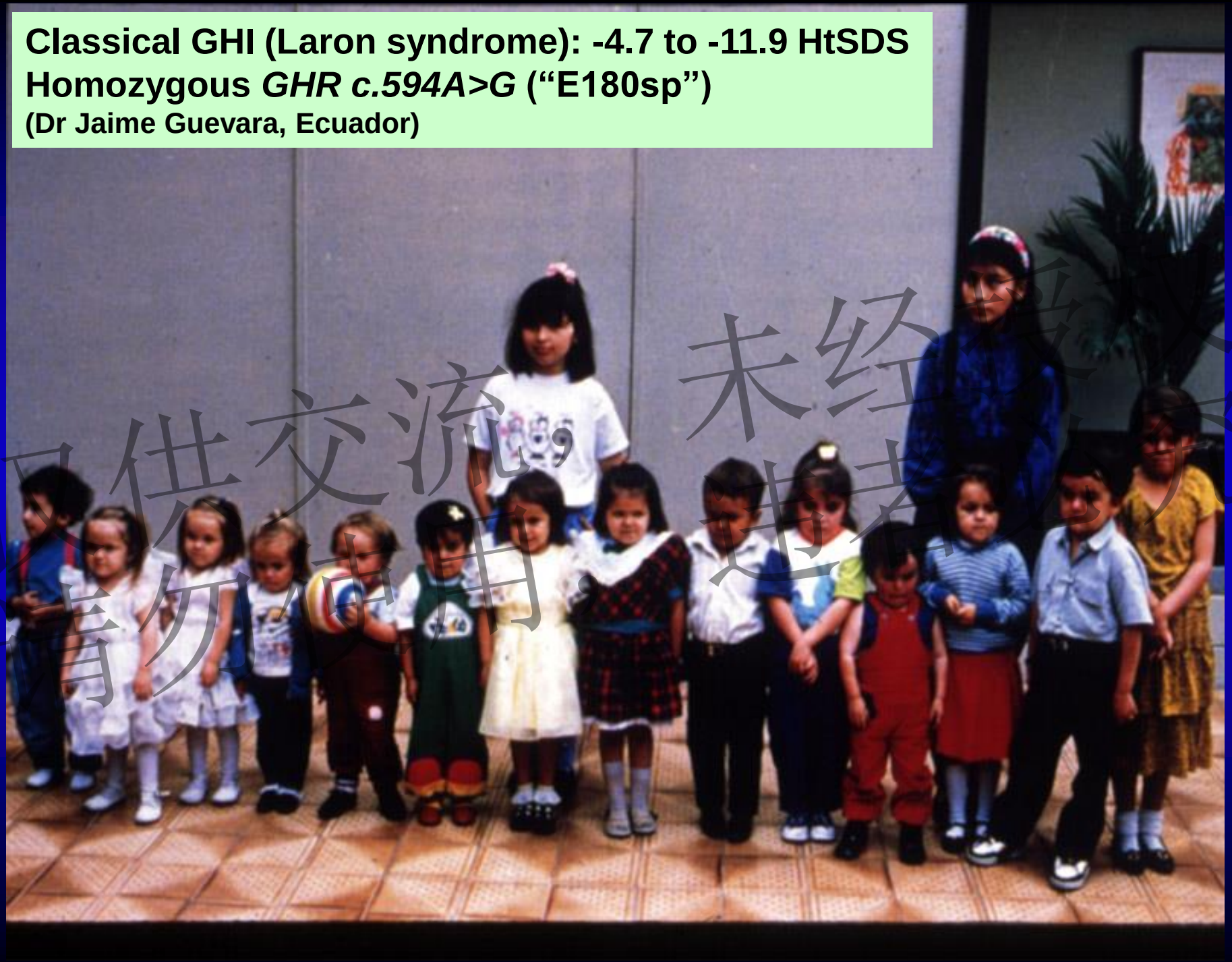

GHBP
Signal
Extracellular
TM
Intracellular
GHR
638
GHR gene
2
3
4
5
6
7
8
9
10
Mutations
15 nonsense
31 missense
10 in/del
11 splice
4 splice
3 missense (heterozygous)
5 deletions/insertions
2 splice
83%
5%
12%
Clinical
GHBP ↓↓
IGF-I ↓↓
IGFBP3 ↓↓
ALS ↓↓
GHBP ↑↑
IGF-I ↓↓
IGFBP3 ↓↓
ALS ↓↓
GHBP normal
IGF-I ↓↓
IGFBP3 ↓↓
ALS ↓↓

Impact of *GHR* mutations on Height and IGF-I Production

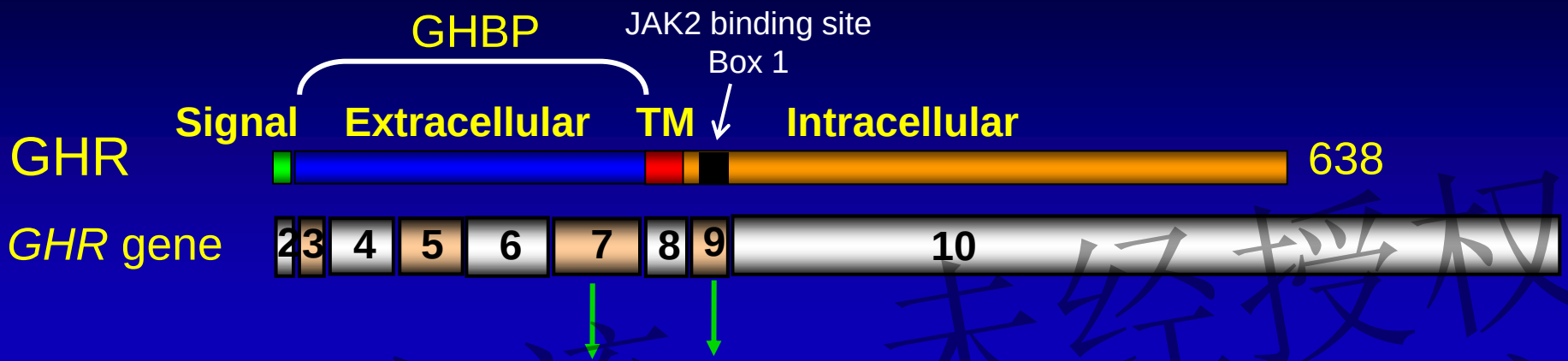


David *et al*, Endocr Rev, 2011. 32: 472

Classical GHI (Laron syndrome): -4.7 to -11.9 HtSDS
Homozygous *GHR c.594A>G* (“E180sp”)
(Dr Jaime Guevara, Ecuador)



Case: Atypical GHI - Compound Heterozygous *GHR*



Male: 4 yr
Height SDS: -4.17
Normal birth weight

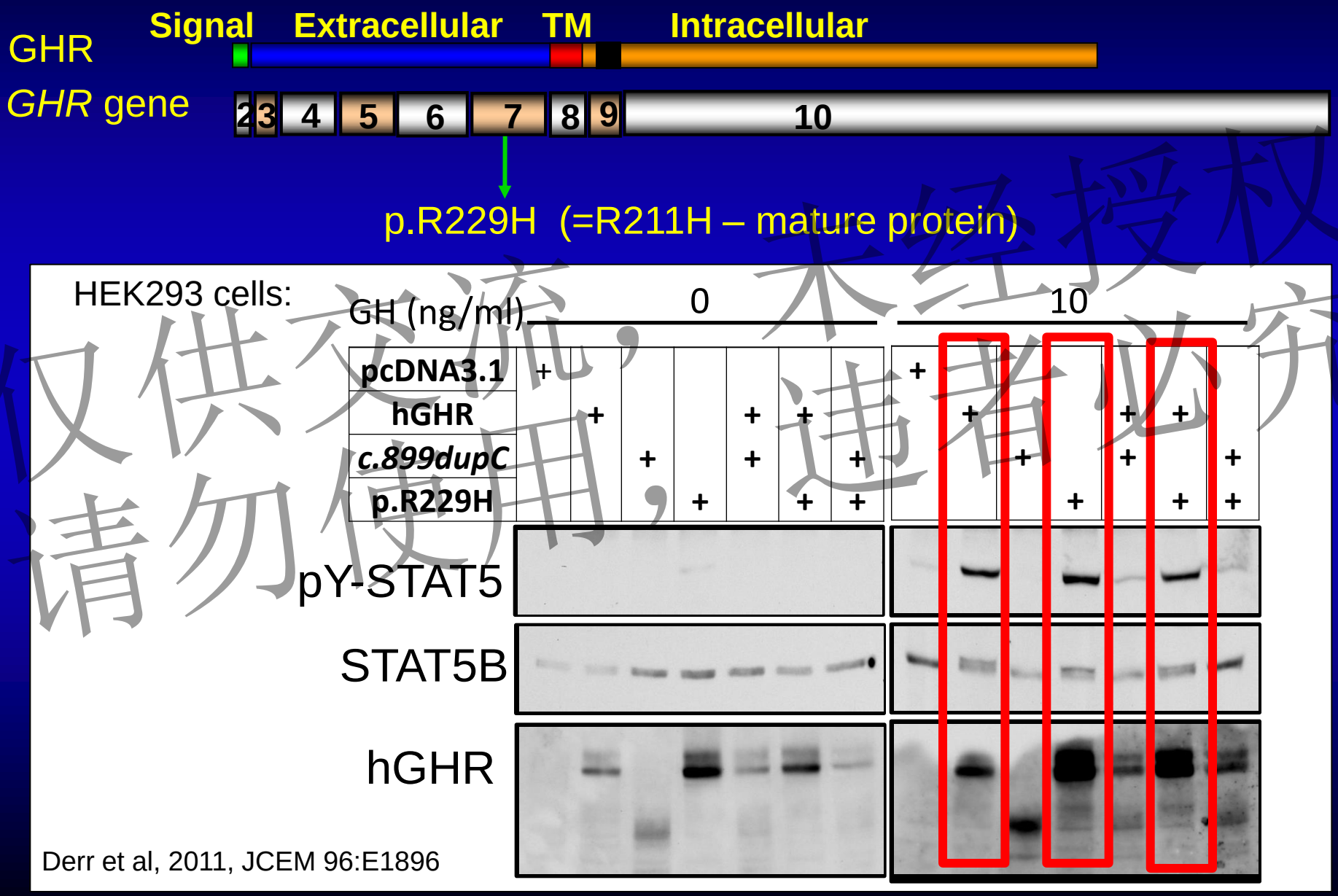
Father, HtSDS: 0.48
Mother, HtSDS: -1.38
Consanguinity: no
Two siblings: normal Ht

Biochemistries

GH, ng/ml: 8 (normal)
GHBP, pmol/L: 1379 (267-1638)
IGF-I, ng/ml: 16 (54-178)
IGFBP-3, mg/L: 0.7 (0.8-3.0)
ALS, mg/L: 2.6 (1.9-10)

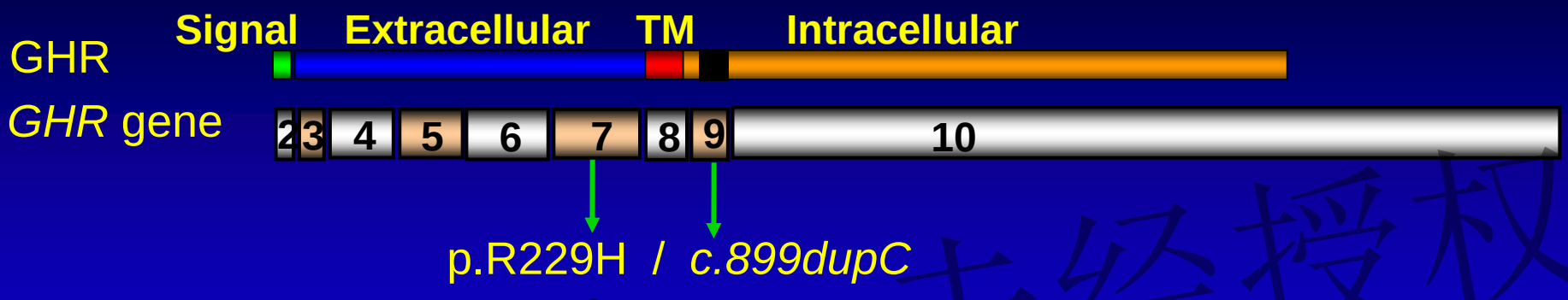
Aisenberg et al, 2010, Horm Res Paediatr 74:406

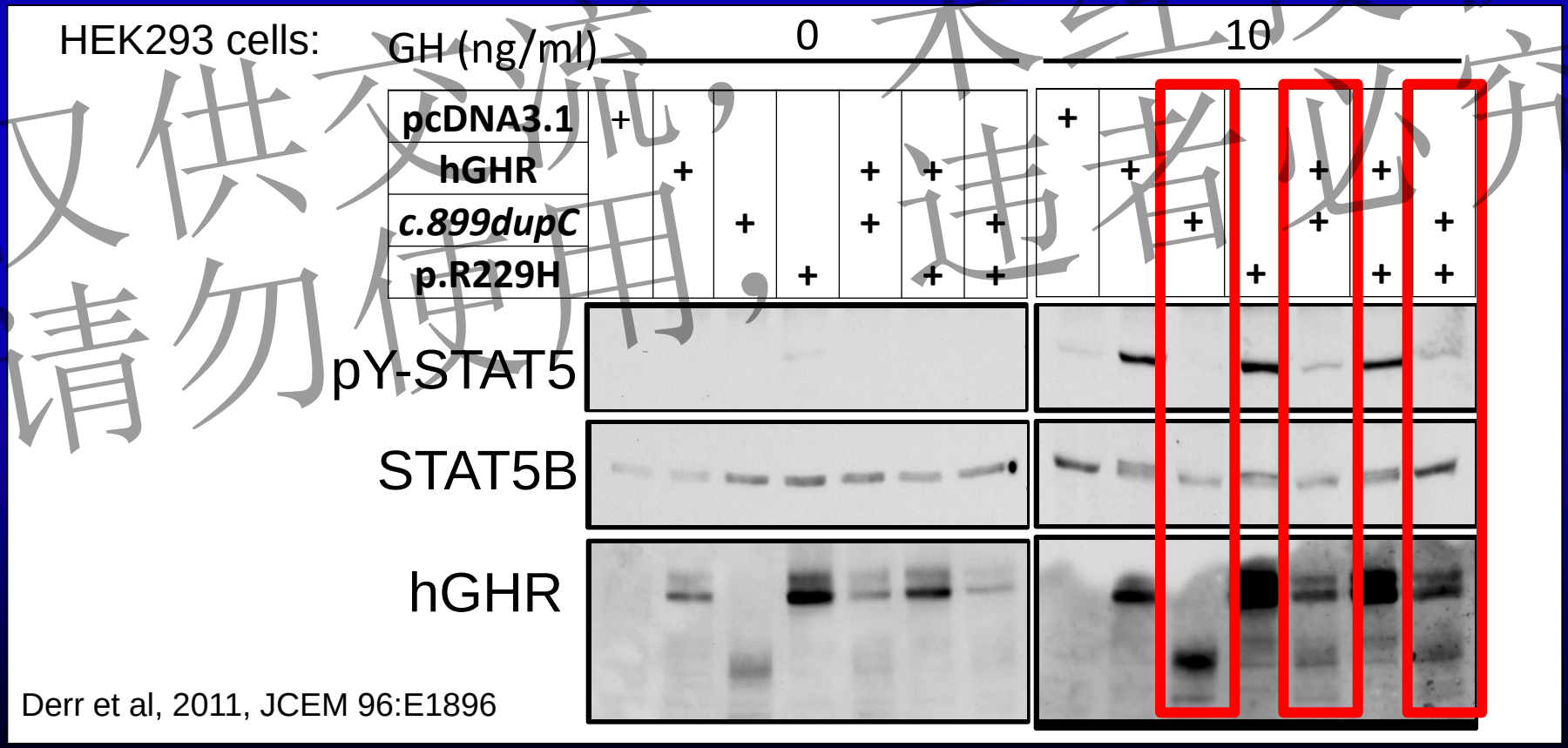
Heterozygous *GHR* p.R229H: Functionally Benign



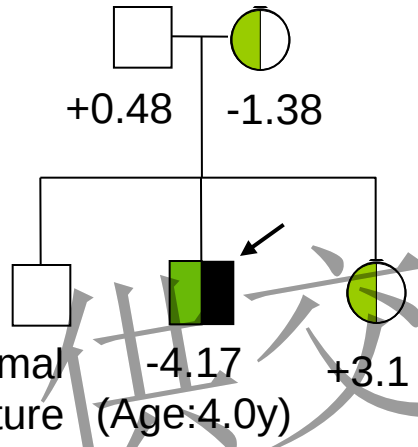
Derr et al, 2011, JCEM 96:E1896

Nonclassical GHI due to Dominant-Negative *GHR c.899dupC*

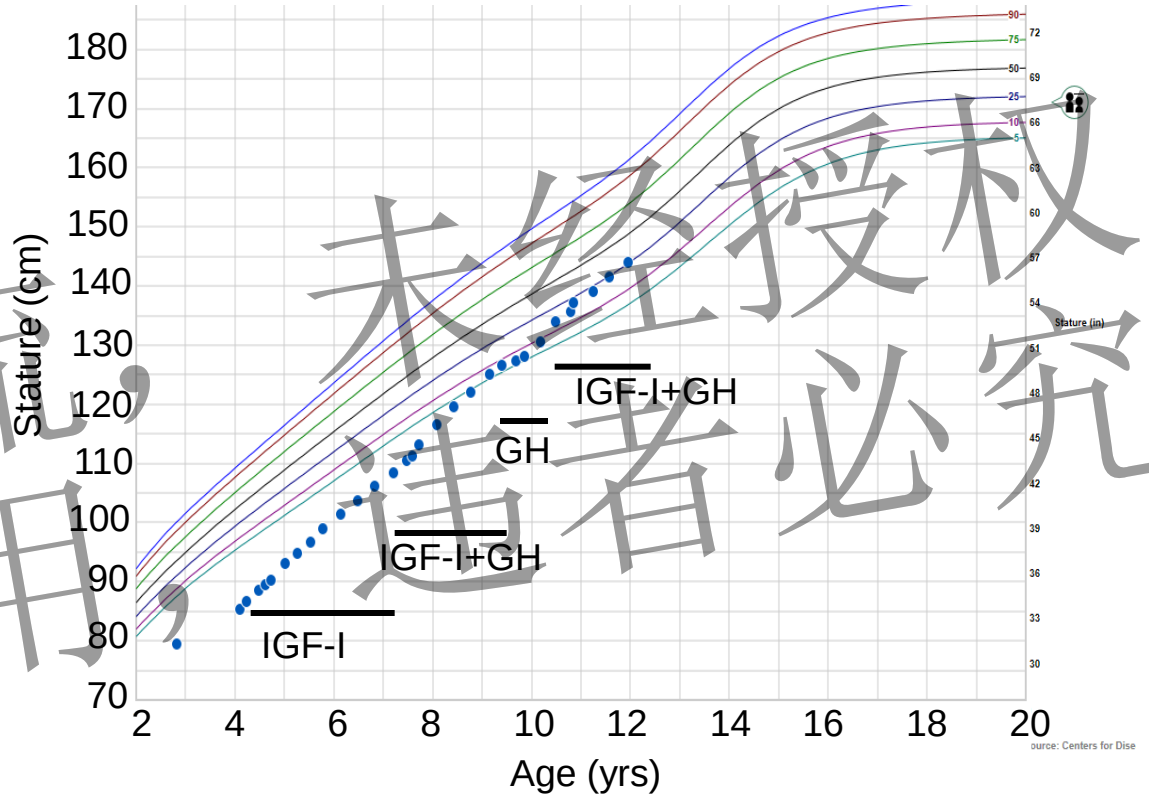




Combination rhGH+rhIGF-I Therapy Improved Growth Velocity of Patient carrying *de novo* Dominant-negative *GHR c.899dupC*



Derr *et al*, JCEM 96:E1896, 2011



J. Aisenberg, M.D., unpublished

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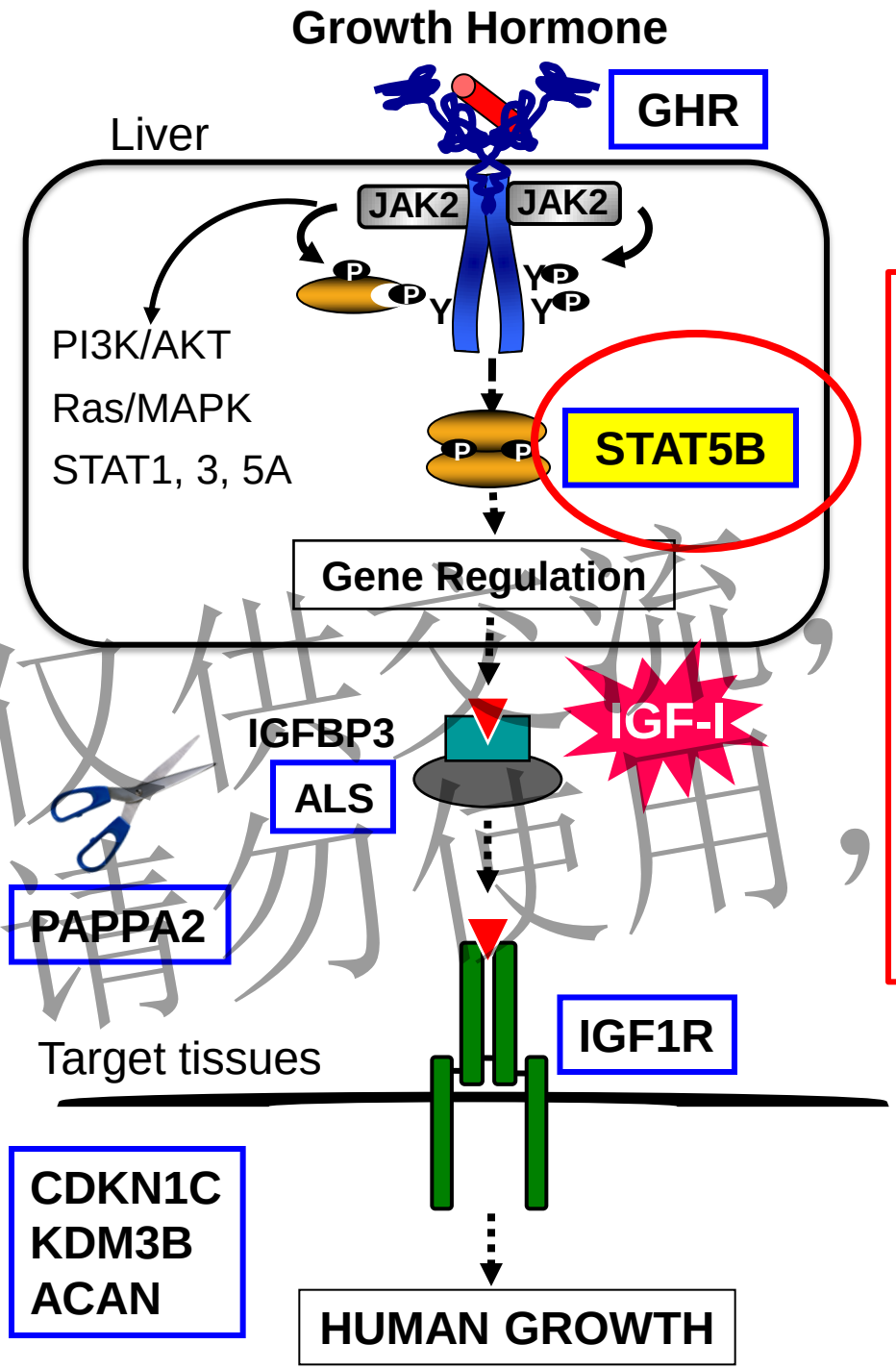
GHR MUTATIONS: LESSONS LEARNT

- Most common molecular cause of GHI, IGF deficiency, and severe short stature
- Severity of disruption to GHR expression and function correlate to severity of IGF-I deficiency and growth failure
- Majority of mutations: autosomal recessive, located in the extracellular domain
- Seven proven dominant-negative *GHR* mutations: located in the intracellular domain; less severe growth impairment, therapeutic options available.
- Prevalence of dominant-negative *GHR* defects may be underestimated in endocrine clinics.
- Functional studies of *GHR* defects: essential to prove causality and improve understanding of GH responsiveness



GH-IGF-I Pathway

STAT5B Mutations:

- Loss of function mutations
- Autosomal Recessive
- Patients:
  - Severe growth failure
  - GH Insensitivity
  - IGF-I deficiency
  - Immune deficiency
  - Pulmonary fibrosis ➔ 3 death

SIGNAL TRANSDUCERS AND ACTIVATORS OF TRANSCRIPTION (STAT)

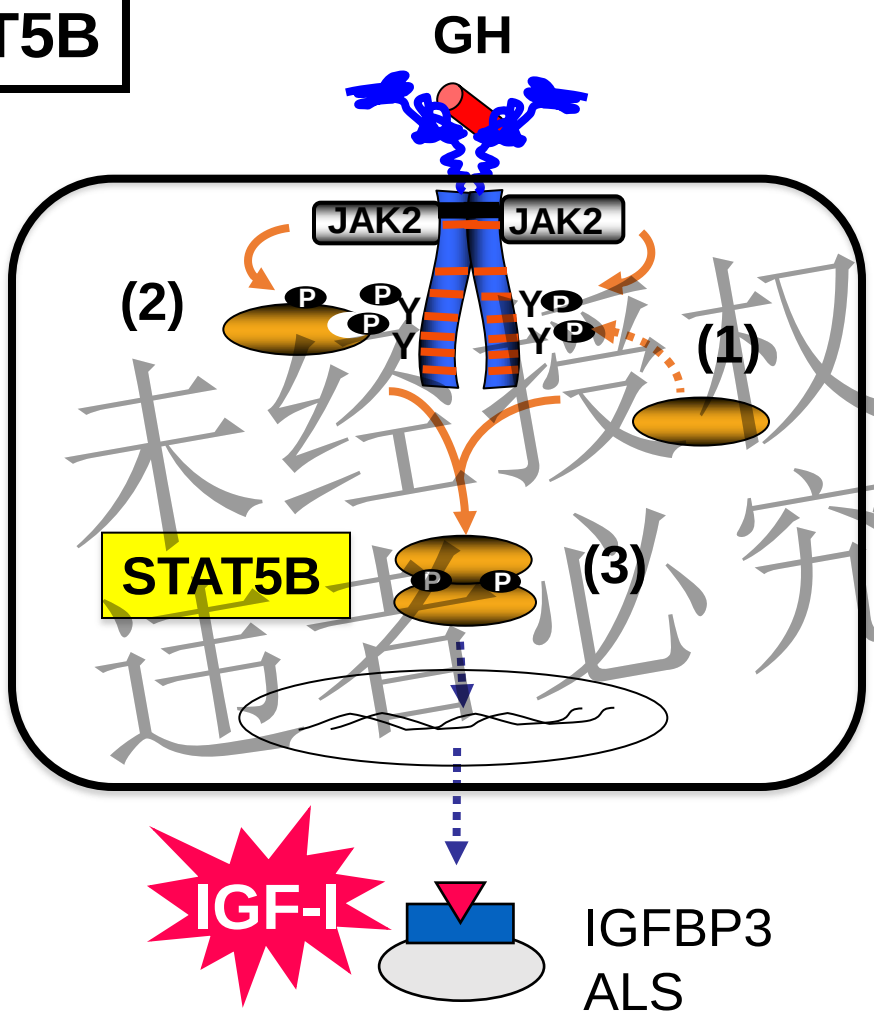
- 7 human STATs (STAT1, -2, -3, -4, -5A, -5B, -6).
- STAT5A and STAT5B share ~96% amino acid identity.
- Activated by multiple growth factors and cytokines
- GH activates STAT1, 3, 5A and 5B.

GH-induced Activation of STAT5B

(1) STAT5B is recruited and docks to 3 of the 7 phosphorylated tyrosines on activated human GHR

(2) Recruited STAT5B: tyrosine phosphorylated (Y699) by JAK2

(3) Phosphorylated STAT5B homo-dimerize, translocate to the nucleus, bind DNA and transcriptionally regulates genes



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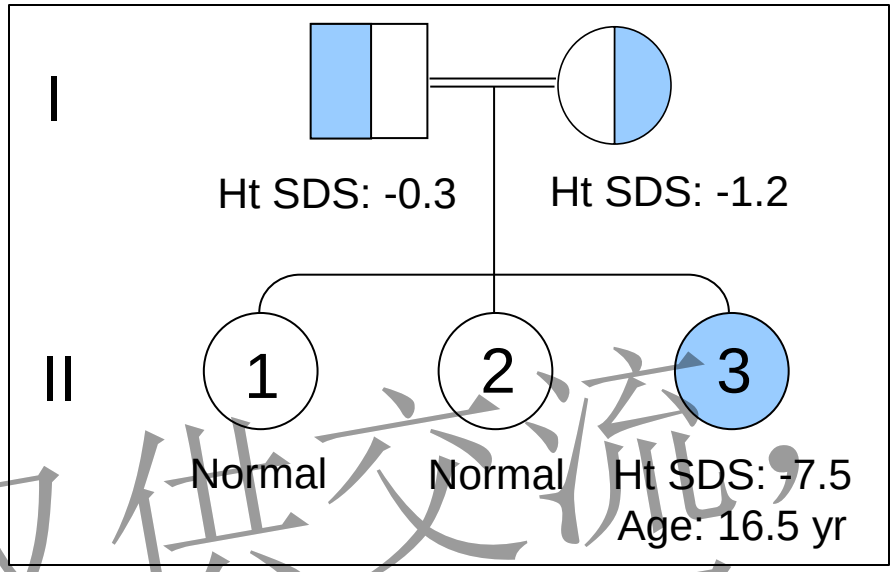
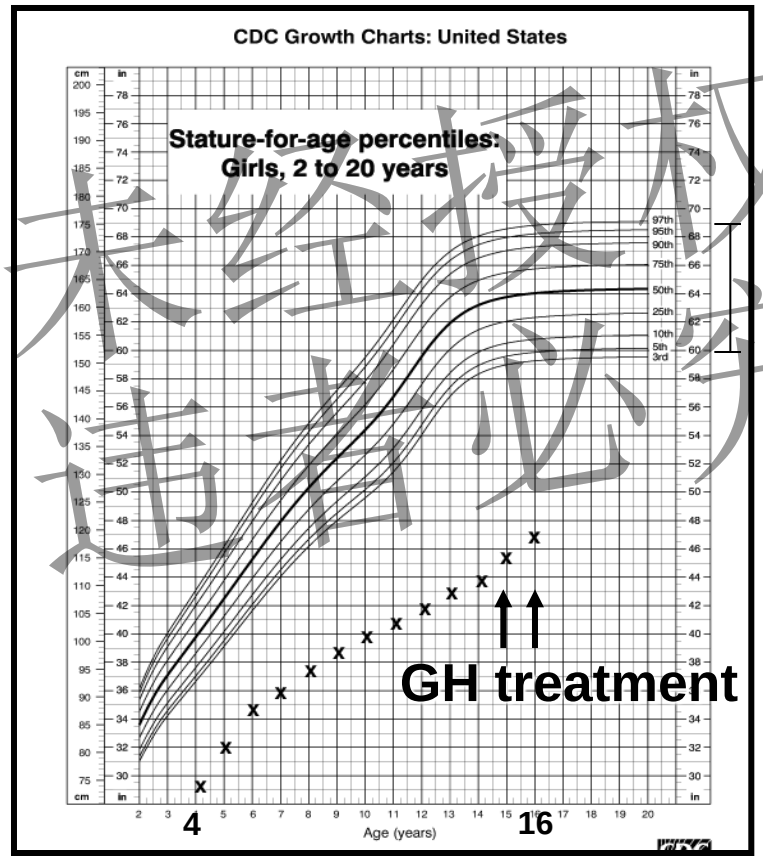
First *STAT5B* Mutation: Homozygous *STAT5B* p.Ala630Pro



Phenotypic features
Normal serum GH
IGF-I, IGFBP-3, ALS deficiencies
Poor response to GH therapy

↓

"Classical" GHI syndrome
GHR = wild-type
Prolactin – above normal



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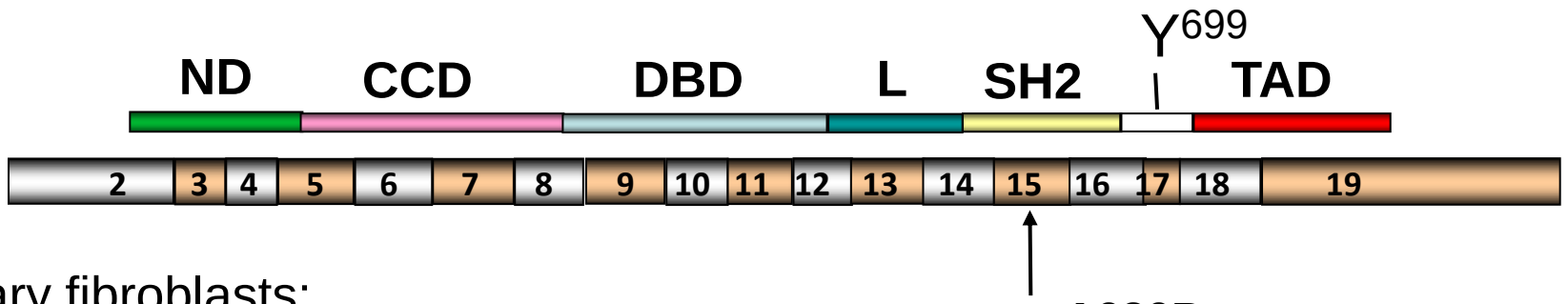
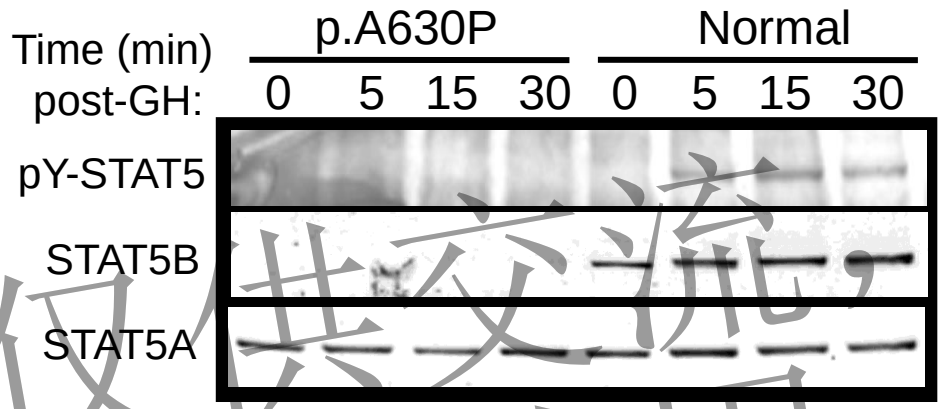
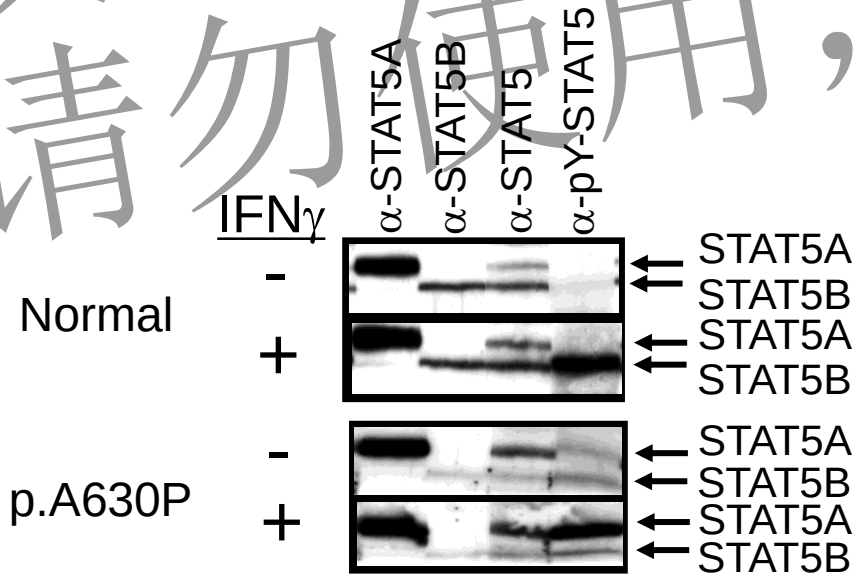
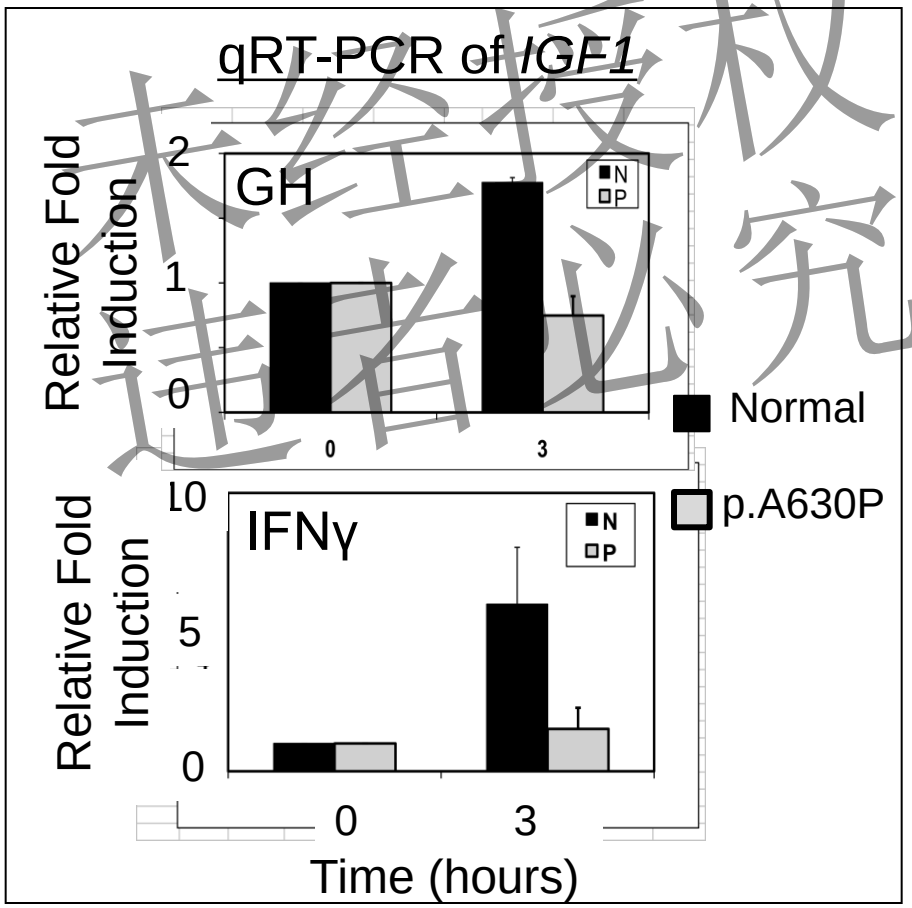
Kofoed *et al*, 2003, *New Engl J Med* 349:1139



Primary fibroblasts:

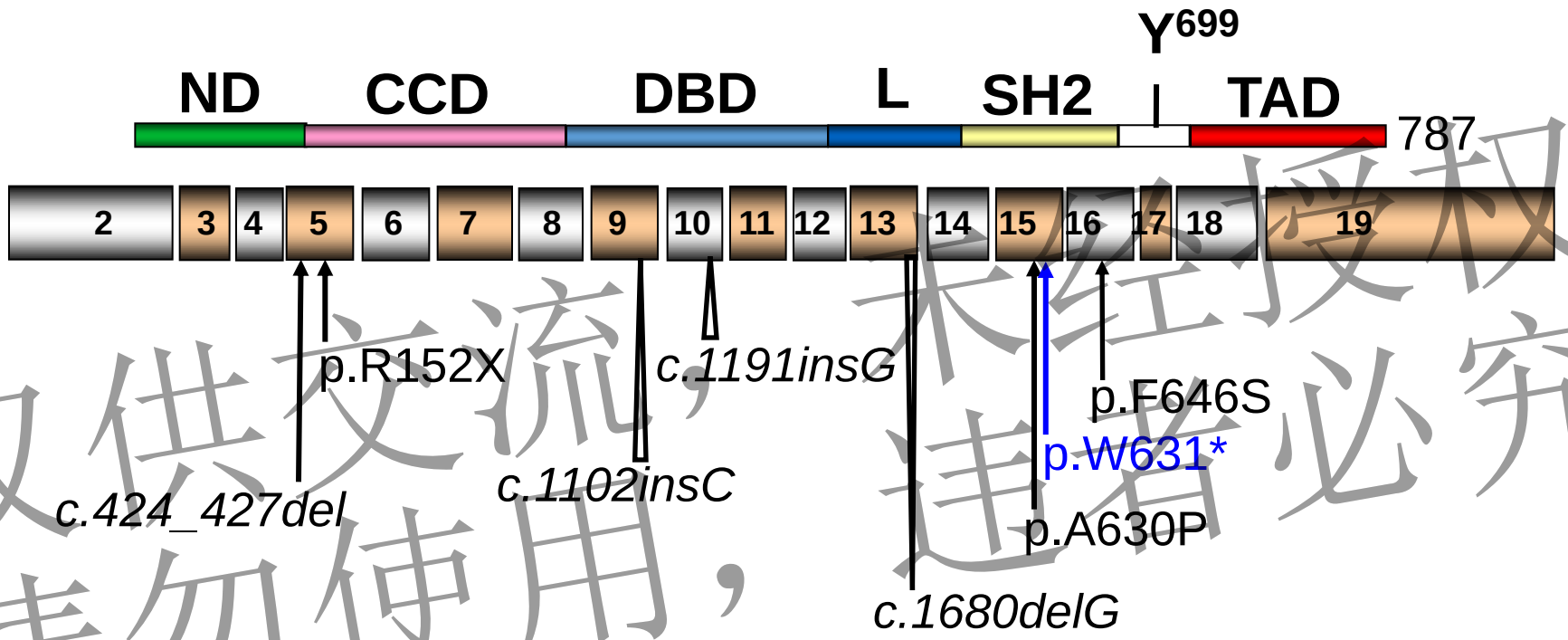






Kofoed *et al*, 2003, *New Engl J Med* 349:1139
Hwa *et al*, 2004, *J Biol Chem* 279: 2728

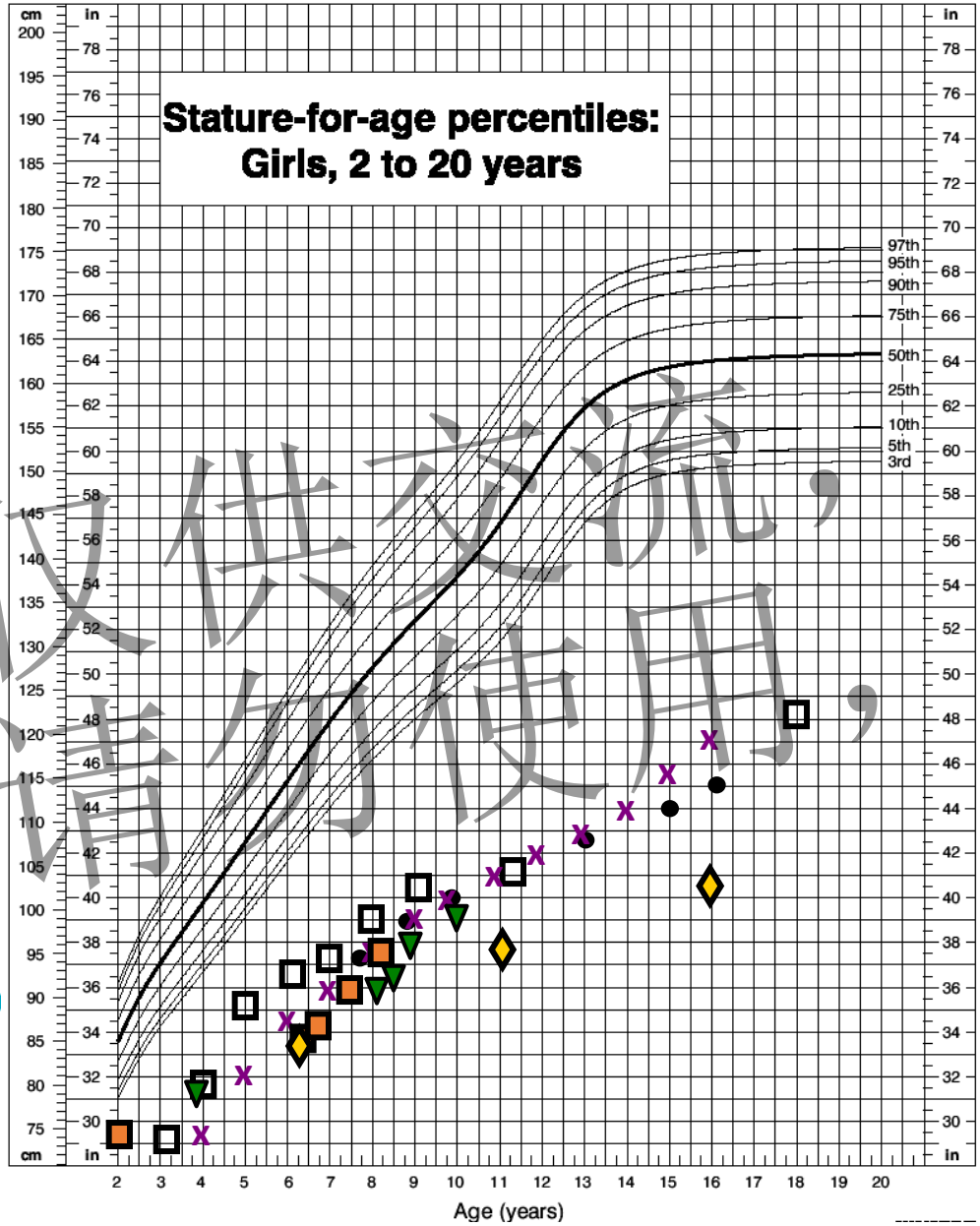
PATHOPHYSIOLOGICAL *STAT5B* MUTATIONS: HOMOZYGOUS, INACTIVATING



Resembles patients with *GHR* defects

but can also present with severe immune deficiencies

SEVERE POST-NATAL GROWTH FAILURE



Height SDS: -5 to -11

***STAT5B* Mutations**

- x **p.A630P**[1]
- ∘ ***c.1191insG***[2]
- ◊ **p.R142X**[3]
- ▽ ***c.1680delG*** **(Sib1**[4]**)**
- □ ***c.1680delG*** **(Sib2**[4]**)**
- □ **p.F646S**[5]

(1) Kofoed EM *et a*l, 2003. *N Engl J Med* 349:1139
(2) Hwa V *et al*, 2005. *J Clin Endocrinol Metab* 90:4260
(3) Bernasconi et al, 2006. *Pediatrics* 118:e1584
(4) Hwa V *et al*, 2007. *Horm Res* 68:218
(5) Scaglia, et al, 2012, *J clin Endocrinol Metab* 97:E830

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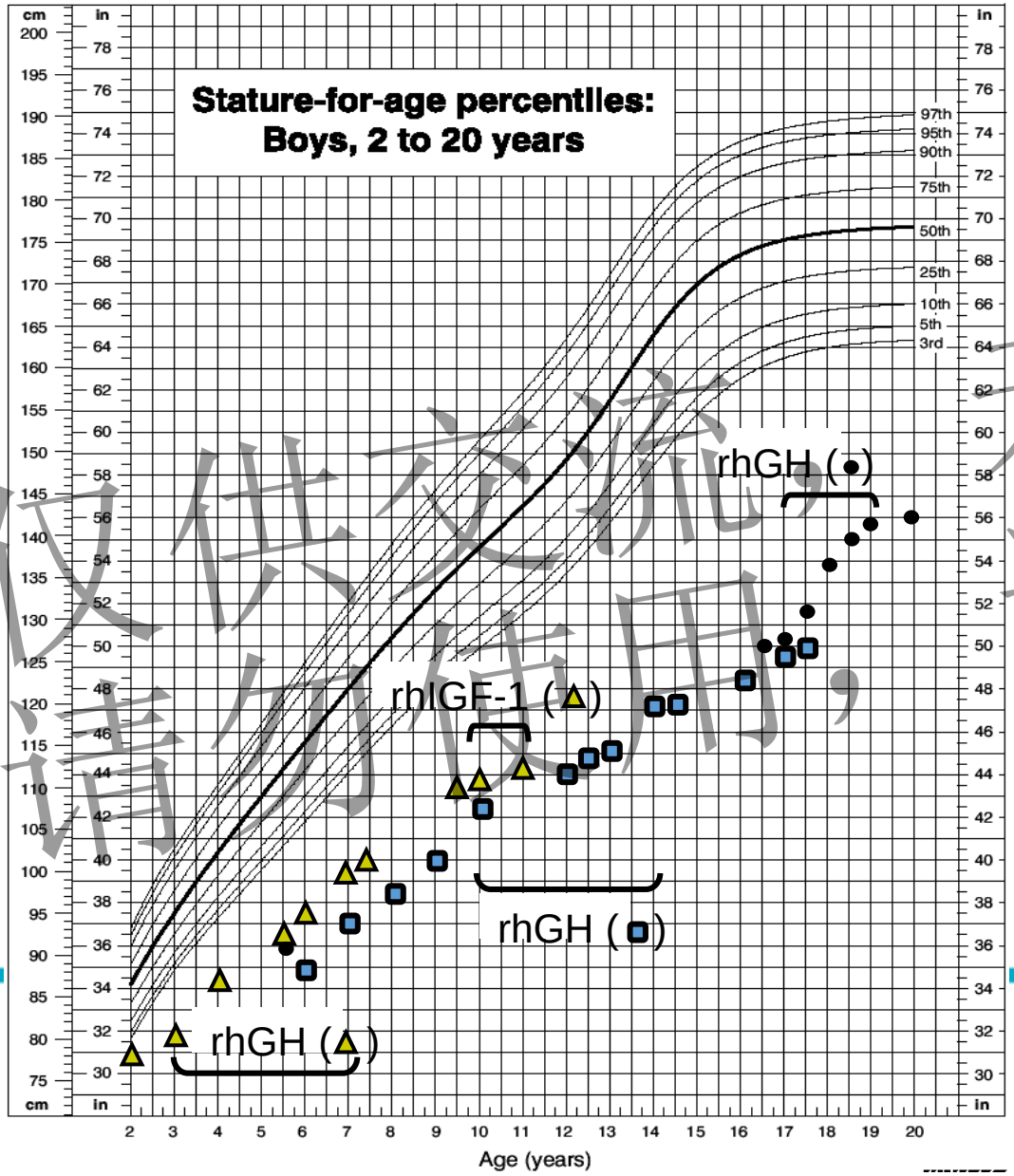
SEVERE POST-NATAL GROWTH FAILURE



Height SDS: -5 to -5.9

- ● **STAT5B:*c.1102insC*[1]**
- ■ **STAT5B:*c.424_427del* (1)[2]**
- ▲ **STAT5B:*c.424_427del* (2)[2]**

(1) Vidarsdottir S *et a*l, 2006. *J Clin Endocrinol Metab* 91:3482
(2) Pugliese-Pires PN *et al*, 2010 Eur J Endocrinol 163:349

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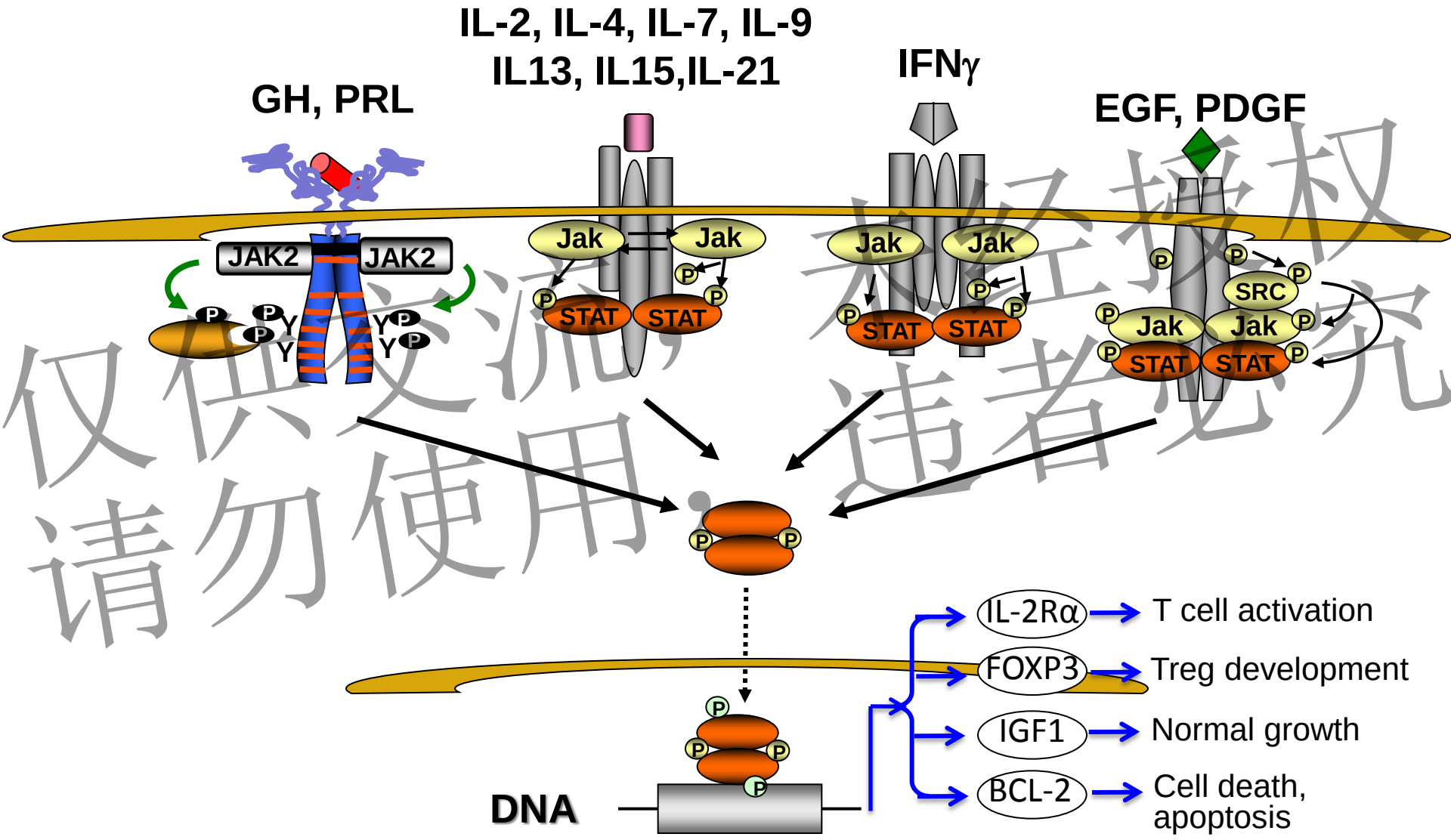
STAT5B ACTIVATION: CYTOKINES, GROWTH FACTORS



STAT5B Deficiency: Immune & Pulmonary Dysfunction

(1) Shared symptoms in 8 of 10 patients:

- Severe eczema
- Chronic pulmonary disease, from as young as 1 yr
- Confirmed lung fibrosis and/or LIP (2 yr – 10 yr)

(2) Additional symptoms:

- Hemorrhagic *varicella* (5 cases)
- Thrombocytopenic purpura (2 case)
- Autoimmune thyroiditis (2 cases)
- Sickle cell anemia (1 case)
- Juvenile idiopathic arthritis (1 case)

STAT5B Deficiency: Immune Dysfunction

Immunological Evaluations:

- Hypergammaglobulinemia: elevated IgG, **IgE**
- NK cells (CD16+56+): below normal
- T-cell lymphopenia: reduced CD4+ and CD8+

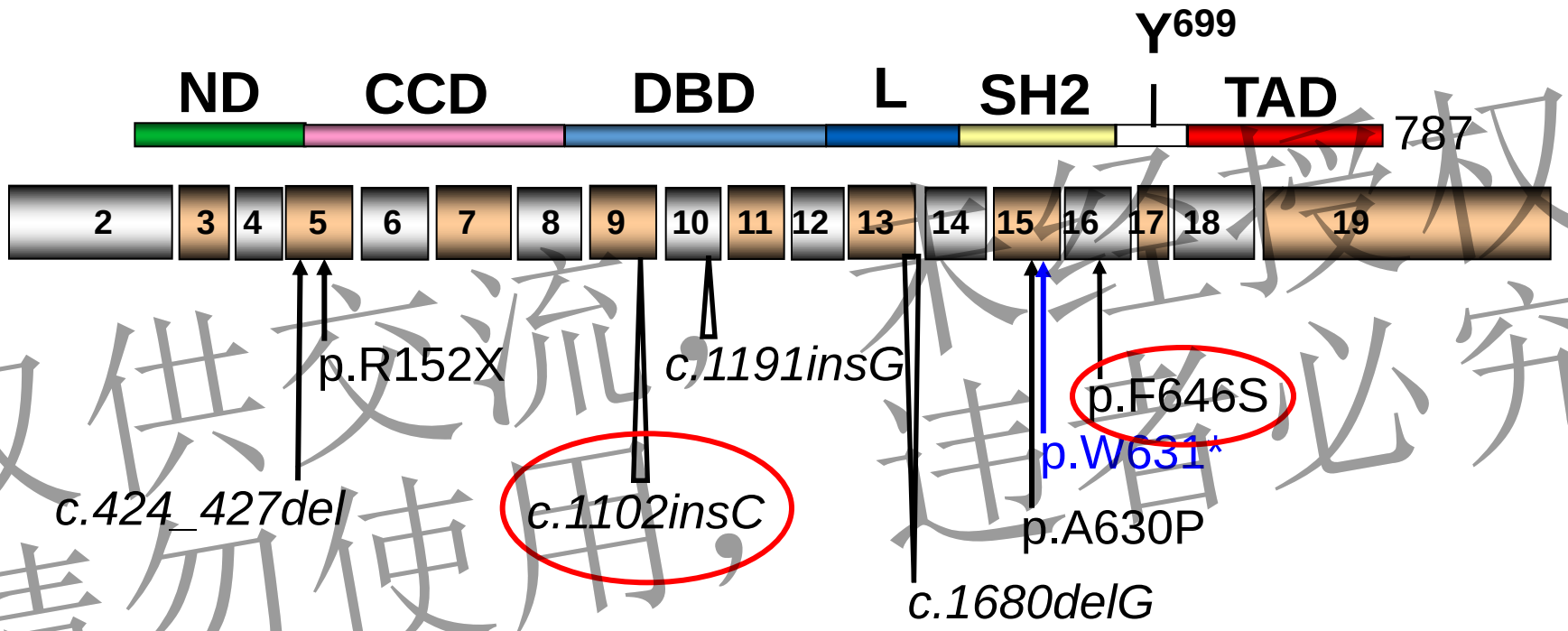
CD4+ T cells: poorly responsive to IL-2

Treg ($CD4\ CD25^{hi}$): reduced; deficient in FOXP3

Treg function: significantly impaired

Cohen *et al*, 2006 *J Immunol* 177:2770

Two Homozygous STAT5B Mutations Associated with Lack of Severe Immune Dysfunctions and Pulmonary Disease



Implication: severe growth failure associated with STAT5B deficiency is NOT secondary to immune complications

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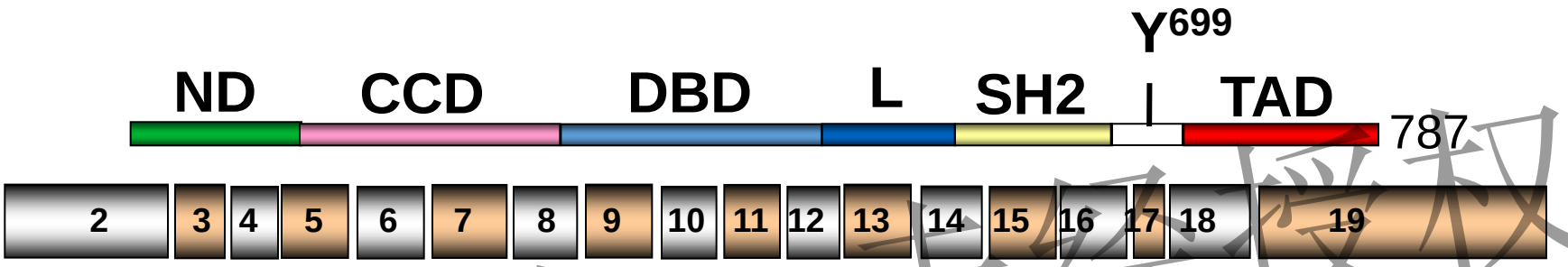
STAT5B Deficiency: Summary 1

- *STAT5B* mutations: autosomal recessive.
- STAT5B is critical for GH-induced regulation of IGF-I: defects associated with severe GHI and growth failure
- *STAT5B* mutations: novel primary immune deficiency
  - 3 patients: succumbed, died of pulmonary fibrosis
  - 1 patient: lung transplantation (~6yrs) – deceased
- Growth functions of STAT5B can be delineated from immune functions
- STAT5A cannot compensate for loss of STAT5B in humans (unlike *Stat5b*$^{-/-}$ knock-out mouse models)

Heterozygous STAT5B Variants Associated with Short Stature but Lack of Severe Immune Problems



p.Q177P
HtSDS: -5.3
Eczema
Elevated IgE
(Dr David Neumann, Czech Republic)

p.Q474R
HtSDS: <-2.5
Eczema
Elevated IgE
(Dr David Schwartz, MO)

p.A478V HtSDS: -2.9
Eczema
Elevated IgE
(Dr Evelein Gevers, England)

J. Klammt, D. Neumann, S. Andrew et al, unpublished

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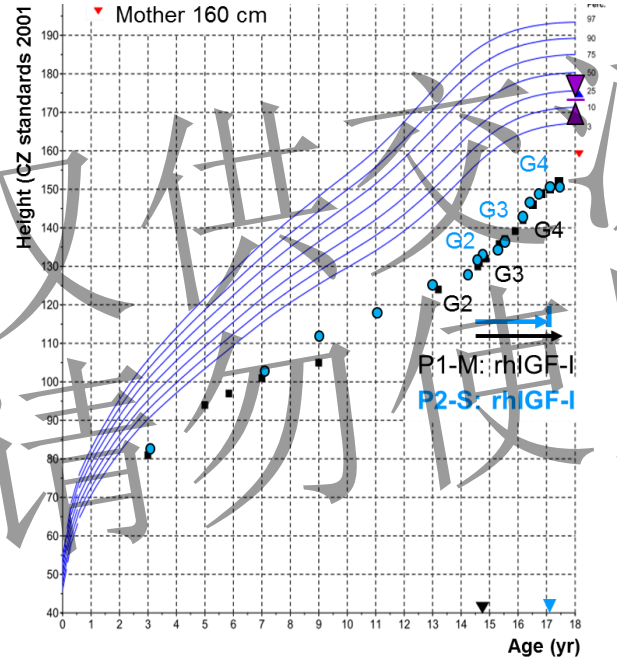
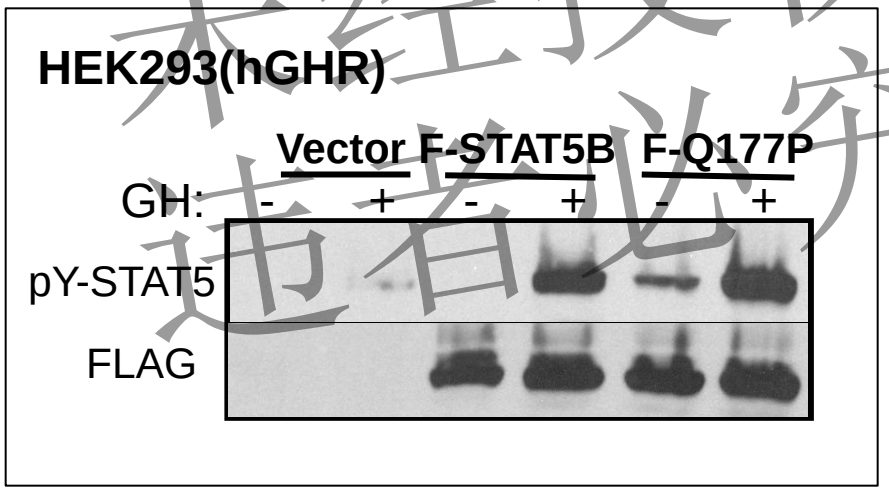
Dominant-Negative STAT5B p.Q177P: Defect in Nuclear Localization

Identical twin boys
Final Height SDS: -4.3
Lack immune deficiency



Functional Analysis



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J. Klammt, D. Neumann, S. Andrew et al, unpublished

Dominant-Negative STAT5B p.Q177P: Defect in Nuclear Localization

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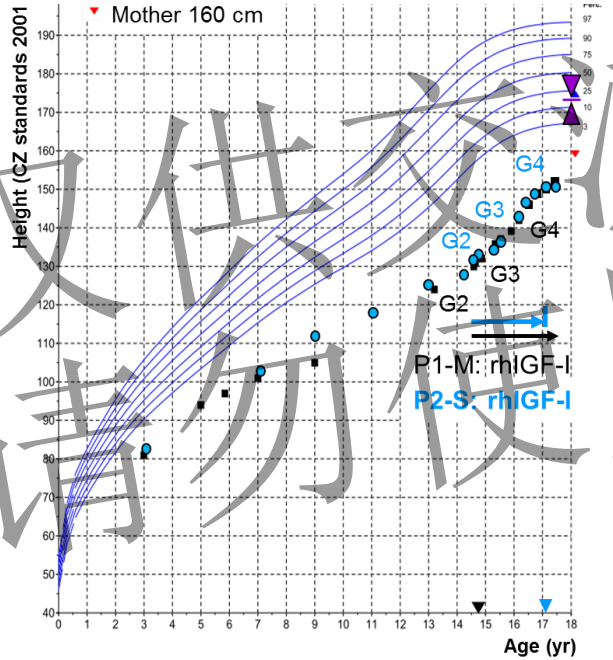
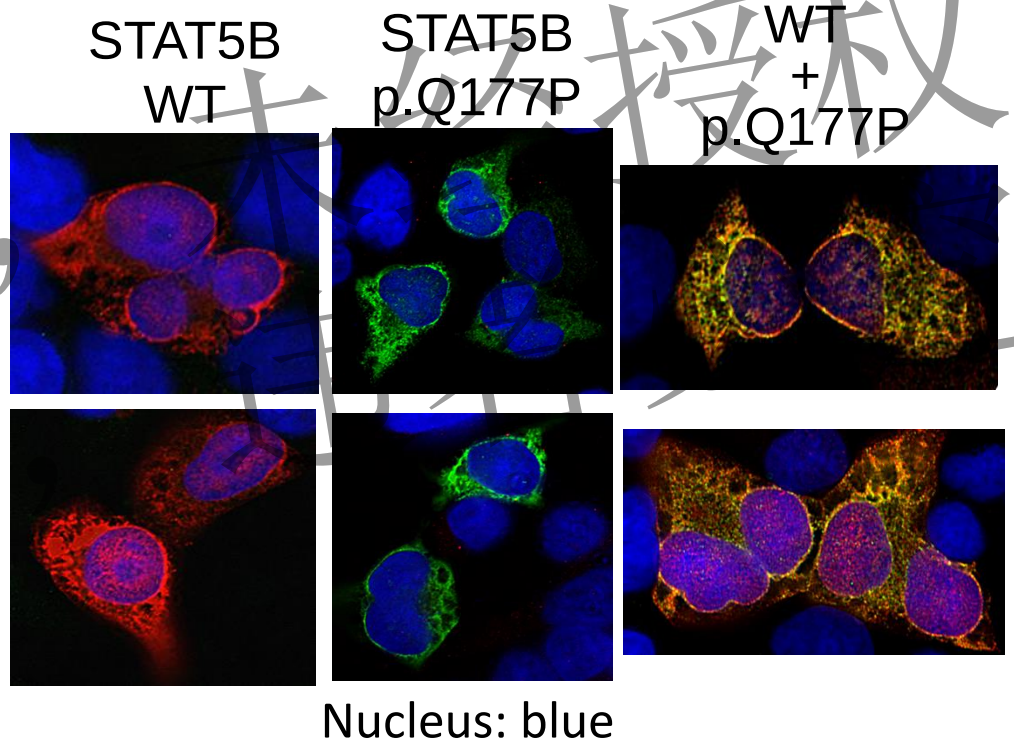


Functional Analysis



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J. Klammt, D. Neumann, S. Andrew et al, unpublished

LESSONS LEARNT:

Expanding the Spectrum of STAT5B Deficiency

- Autosomal recessive to dominant-negative mutations
- Severe growth failure (-5 to -12 SDS) to less severe (-2.8 to -5 SDS)
- Severe (high mortality) to minimal immune dysfunctions
- ~25% of normal, functional, STAT5B: sufficient for normal immunity but insufficient to maintain normal human growth
- STAT5B deficiency: prevalence may be underestimated in endocrine, immunology, pulmonary clinics

Genetic Basis for Growth Hormone Insensitivity (GHI)

I. Defects disrupting IGF-I production

II. Defects disrupting IGF-I actions

III. Defects disrupting fundamental cellular functions

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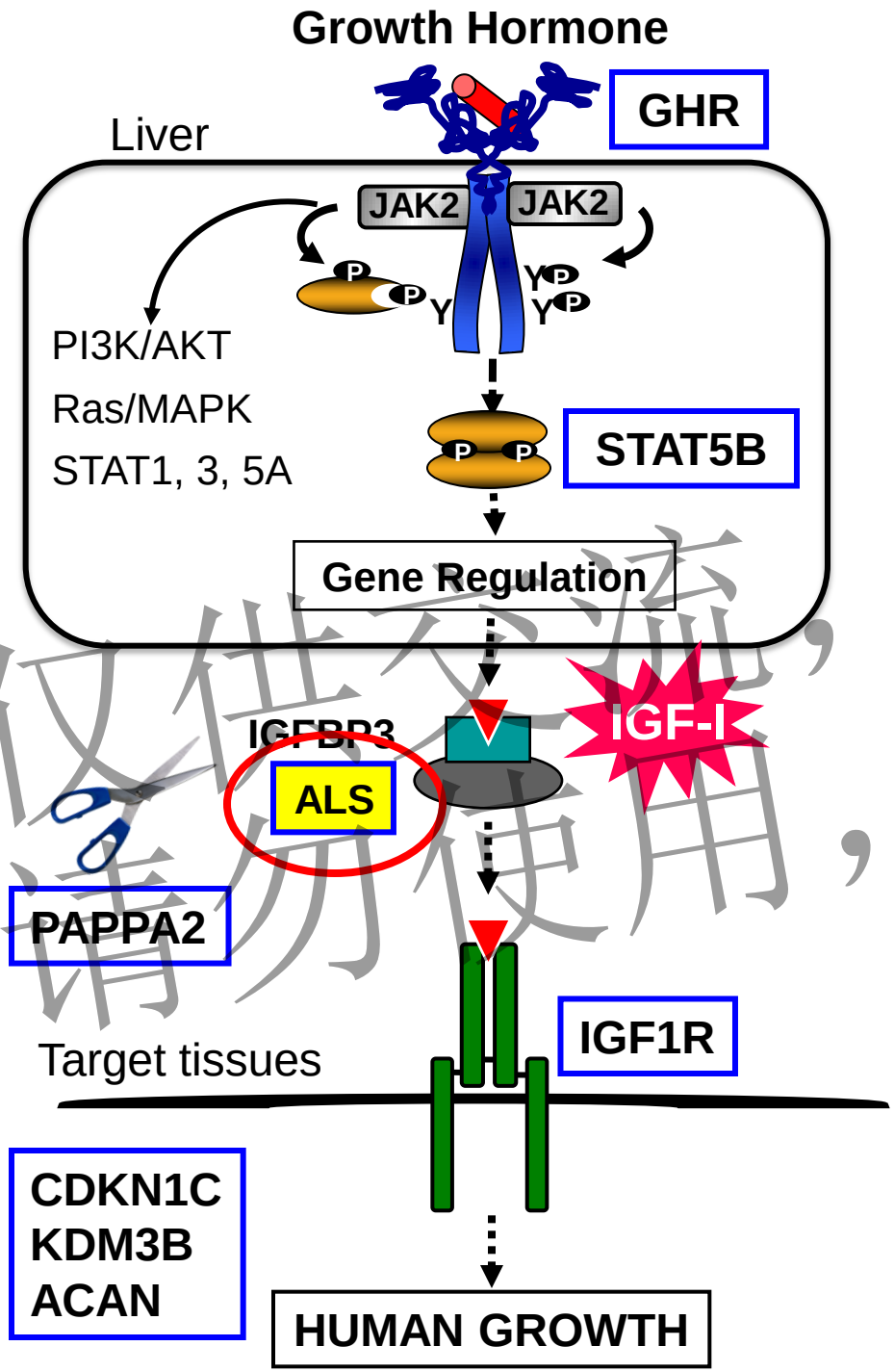

***IGFALS* Deficiency**
Autosomal Recessive
> 16 mutations

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Disruption of IGF-I transport

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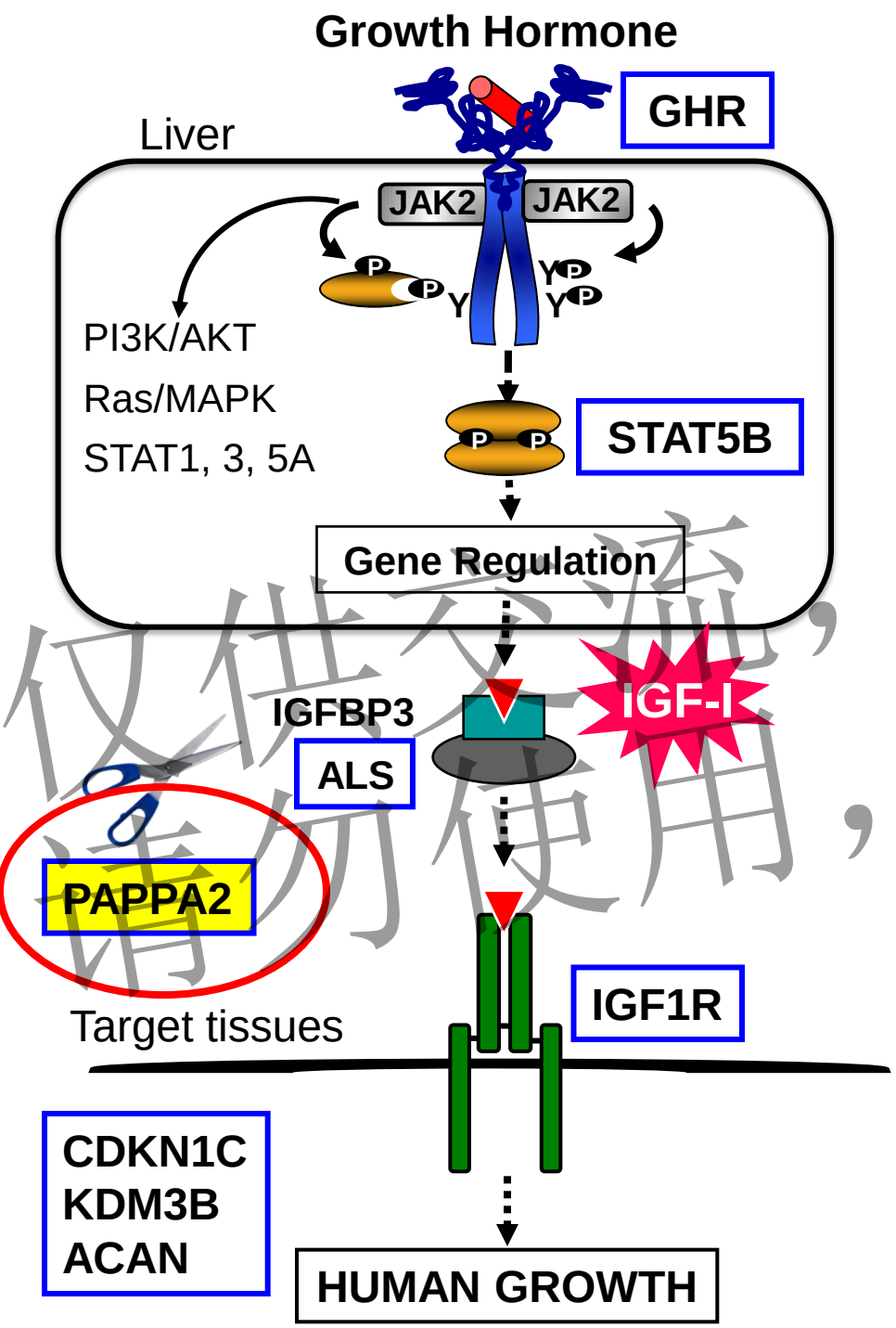
Severe deficits in detectable circulating IGF-I and IGFBP-3

↓

Mild post-natal growth failure (-2 to -4 HtSDS)



***PAPPA2* Deficiency**

↓

Decrease in free IGF-I bioavailability

↓

Elevated total IGF-I, IGFBP-3 and IGFBP5

↓

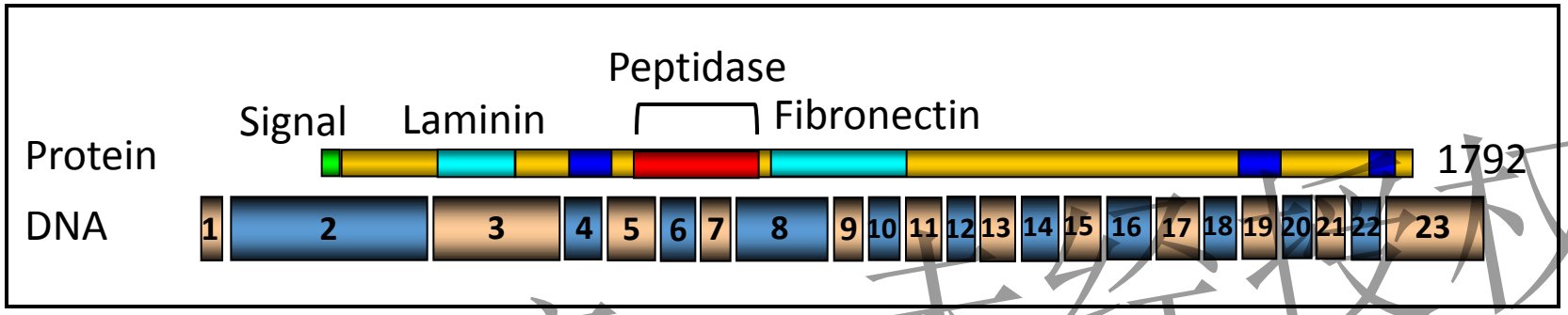
Mild post-natal growth failure (-1.0* to -3.8 HtSDS)

Dauber *et al*, EMBO Mol Med 2016

(*1 - 2 SD below mid-parental target height)

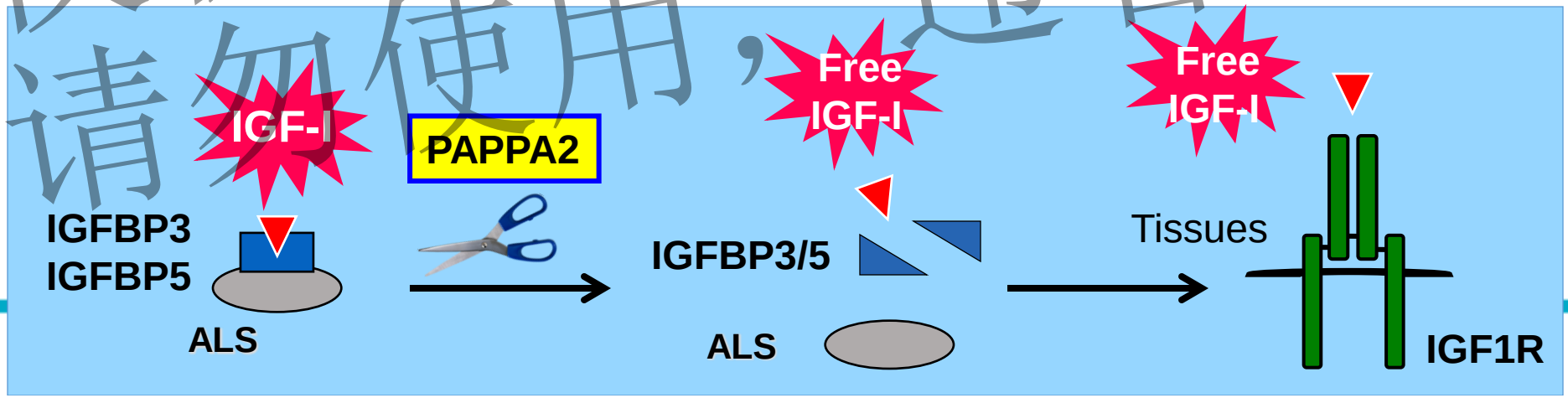
PAPPA2: Pregnancy-Associated Plasma Protein A2

Chromosome 1q23-q.25



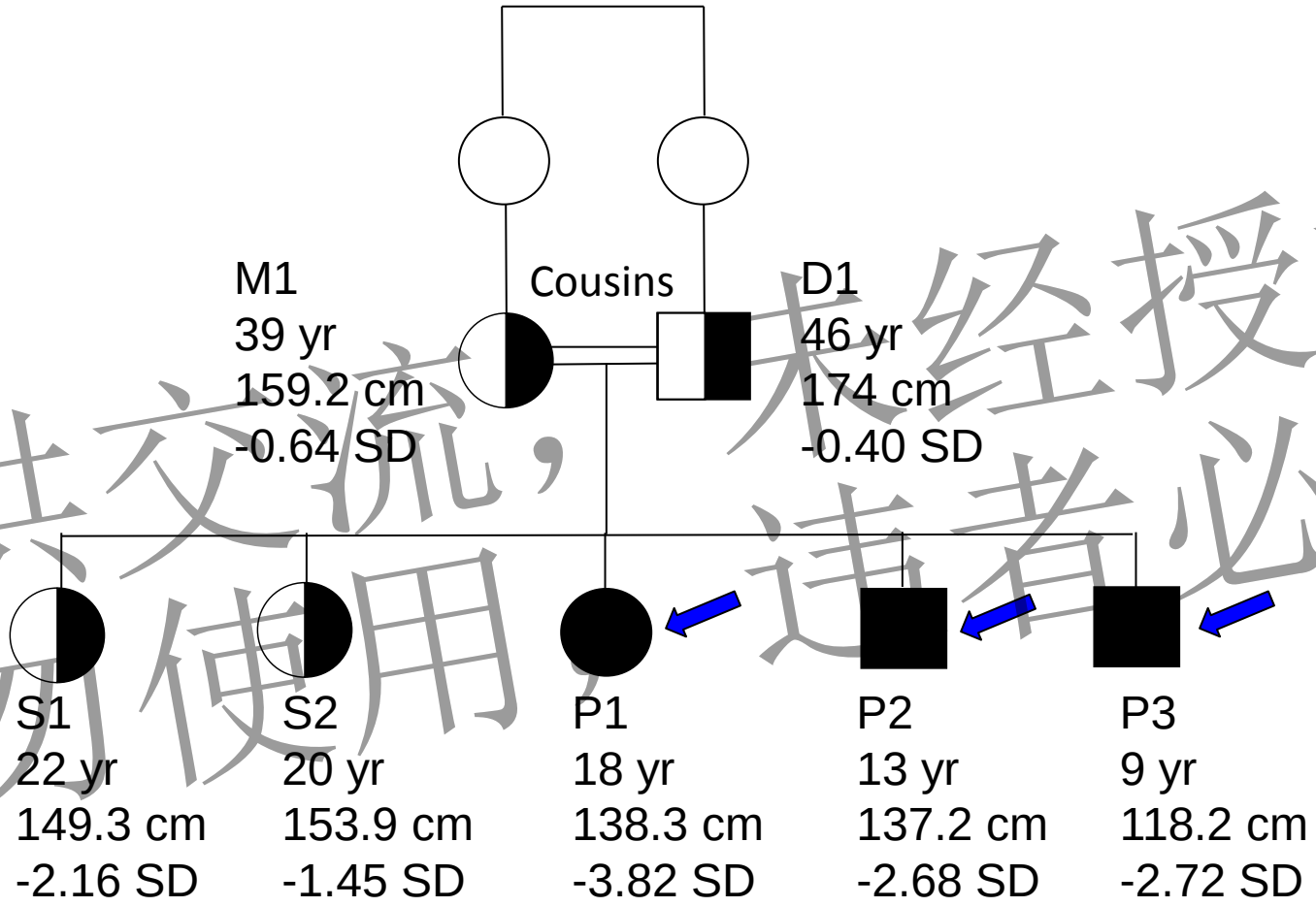
PAPPA2: a circulating protease of the pappalysin protein family

Regulates release of IGF-I by cleaving IGFBP3 and IGFBP5



Pappa2$^{-/-}$ knock-out mouse exhibit post-natal growth failure

Case: Growth Failure Associated with Elevated IGF-1



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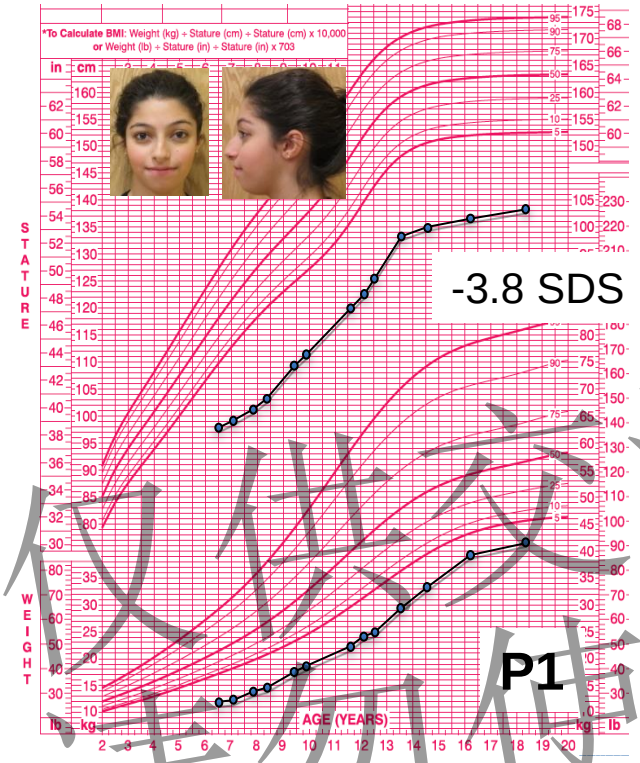
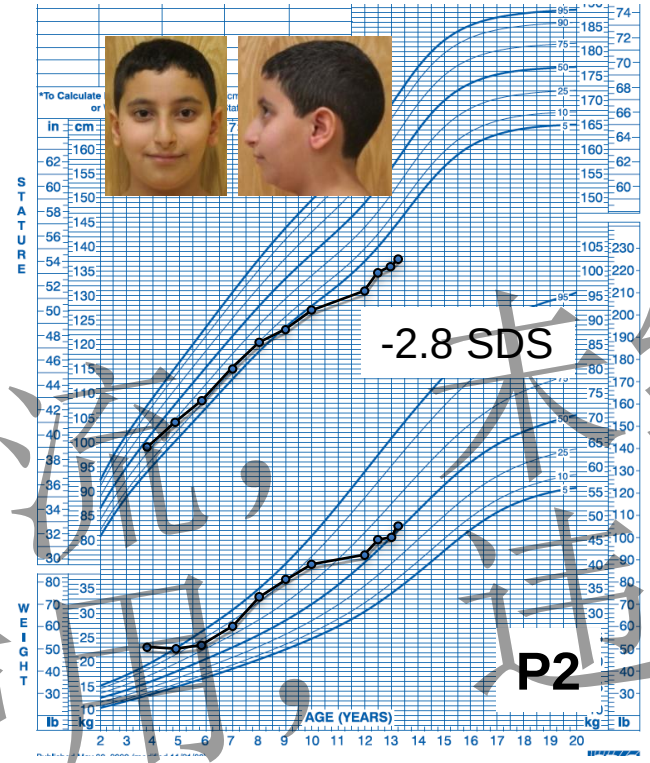
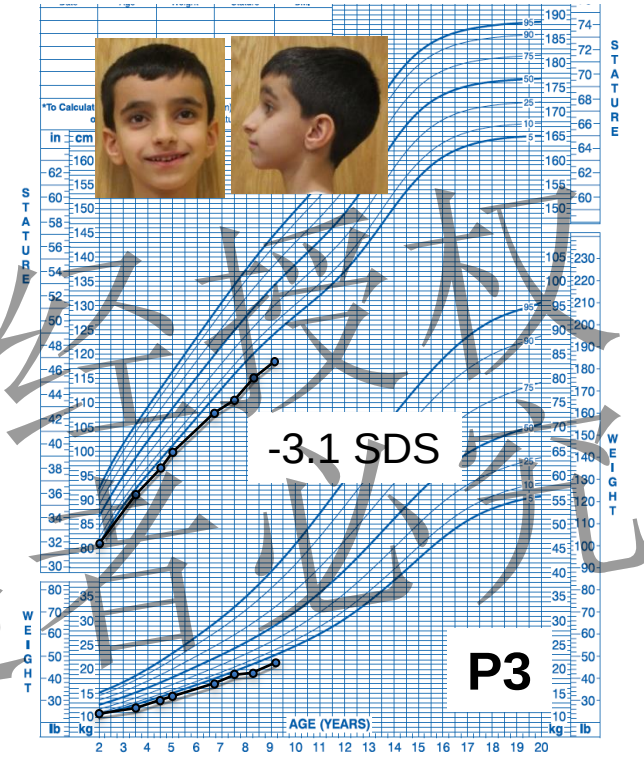
Dauber A *et al*, EMBO Mol Med 2016

Case: Growth Failure Associated with Elevated IGF-1







Total IGF-I: **above** normal range
Free IGF-I: **below** normal range
IGFBP-3: **above** normal range
IGFBP-5: **above** normal range
ALS: normal range

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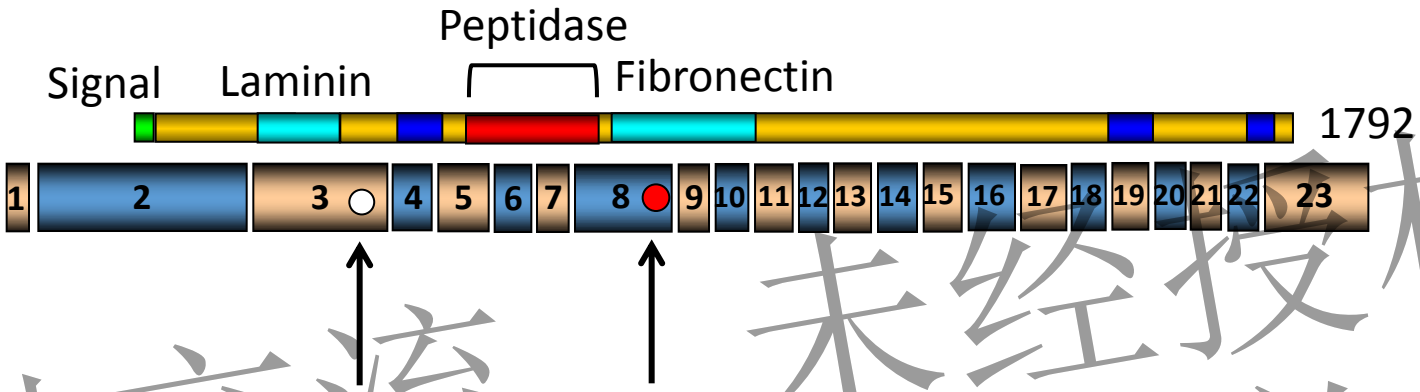
Dauber A *et al*, EMBO Mol Med 2016

Whole Exome Sequence (WES) Analysis: Homozygous Missense *PAPPA2* Mutation



c.1927_1928insAT
p.Asp643fs25*
(frameshift mutation)

Serum PAPPA2: undetectable

c.3098C>T
p.Ala1033Val

Serum PAPPA2: detected, normal range

Could not cleave IGFBP3 or 5

Elevated total IGF-I, IGFBP3 and IGFBP5
Free IGF-I: **below** normal

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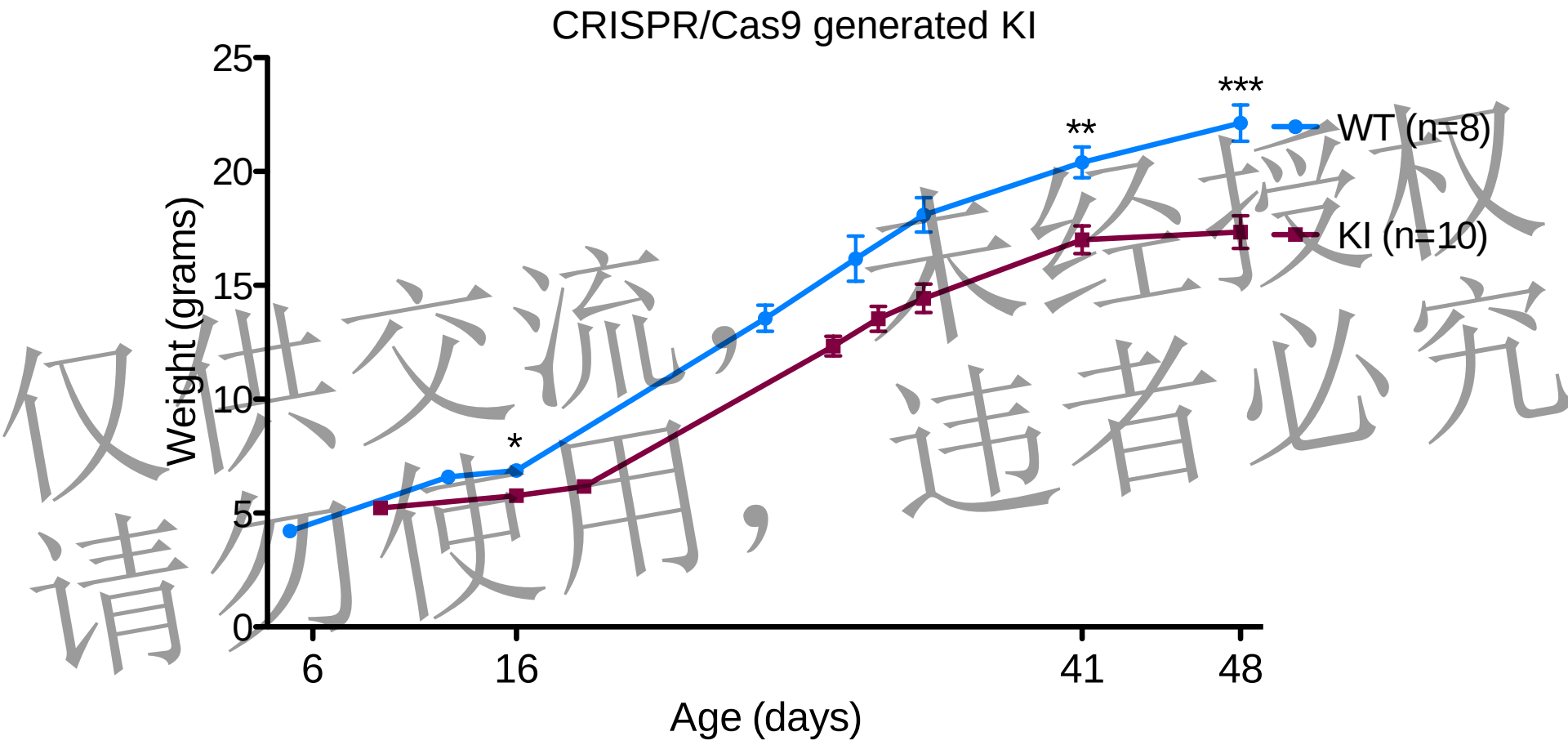
Dauber A *et al*, EMBO Mol Med 2016

Pappa2 p.Ala1034Val Knock-In (KI) Mice Mouse Model: Progressive Post-natal Growth Failure



Melissa Andrew, Lihong Liao, unpublished

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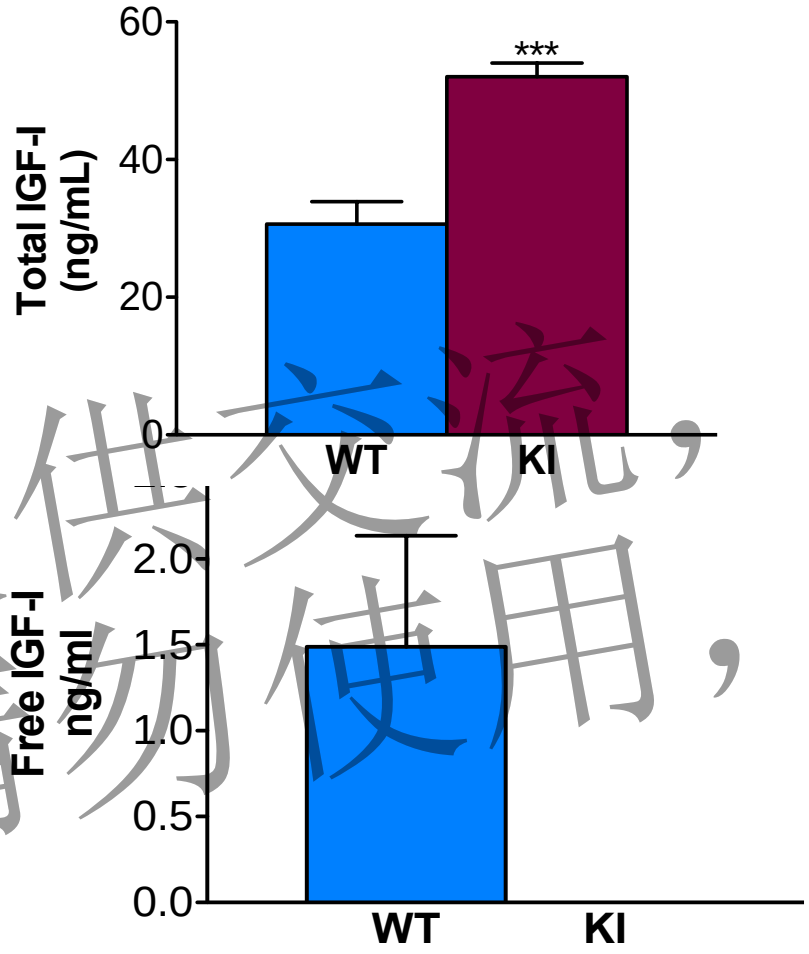
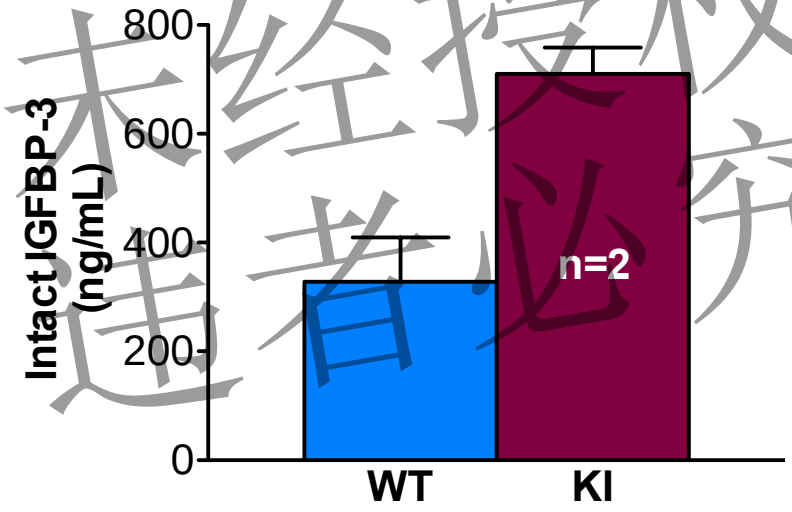
KI Mouse Model: Total IGF-I, Free IGF-1, IGFBP-3 Recapitulates Human Clinical Biochemistries





Melissa Andrew, Lihong Liao, unpublished

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PAPPA2 Deficiency: a New Molecular Cause of GHI

- 2 autosomal recessive, loss-of-function, mutations identified in 5 individuals in 2 unrelated families
- Key clinical presentations:
  - Mild, progressive, growth failure (as low as -3.8 HtSDS)
  - Markedly elevated total serum IGF-I, IGFBP-3 and -5
  - Free IGF-I: below normal
  - Other clinical manifestations are variable: SGA, mild microcephaly, thin long bones.
- Current treatment option: rhIGF-I – efficacy, TBD.

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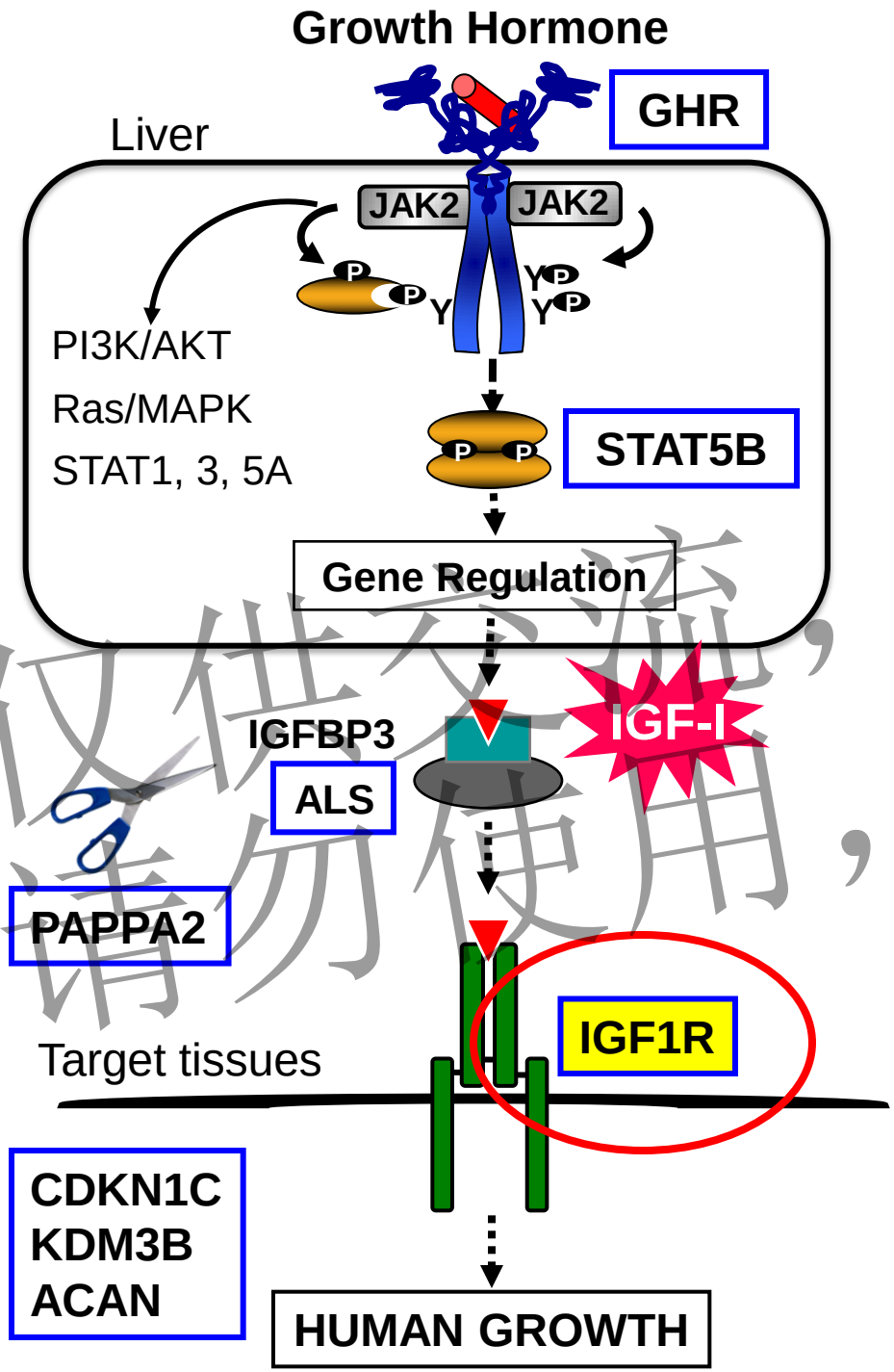
Dauber A *et al*, EMBO Mol Med 2016



***IGF1R* Defects**
(>21 mutations reported)
(15q26.3 deletions)

↓

Resistance to IGF-I

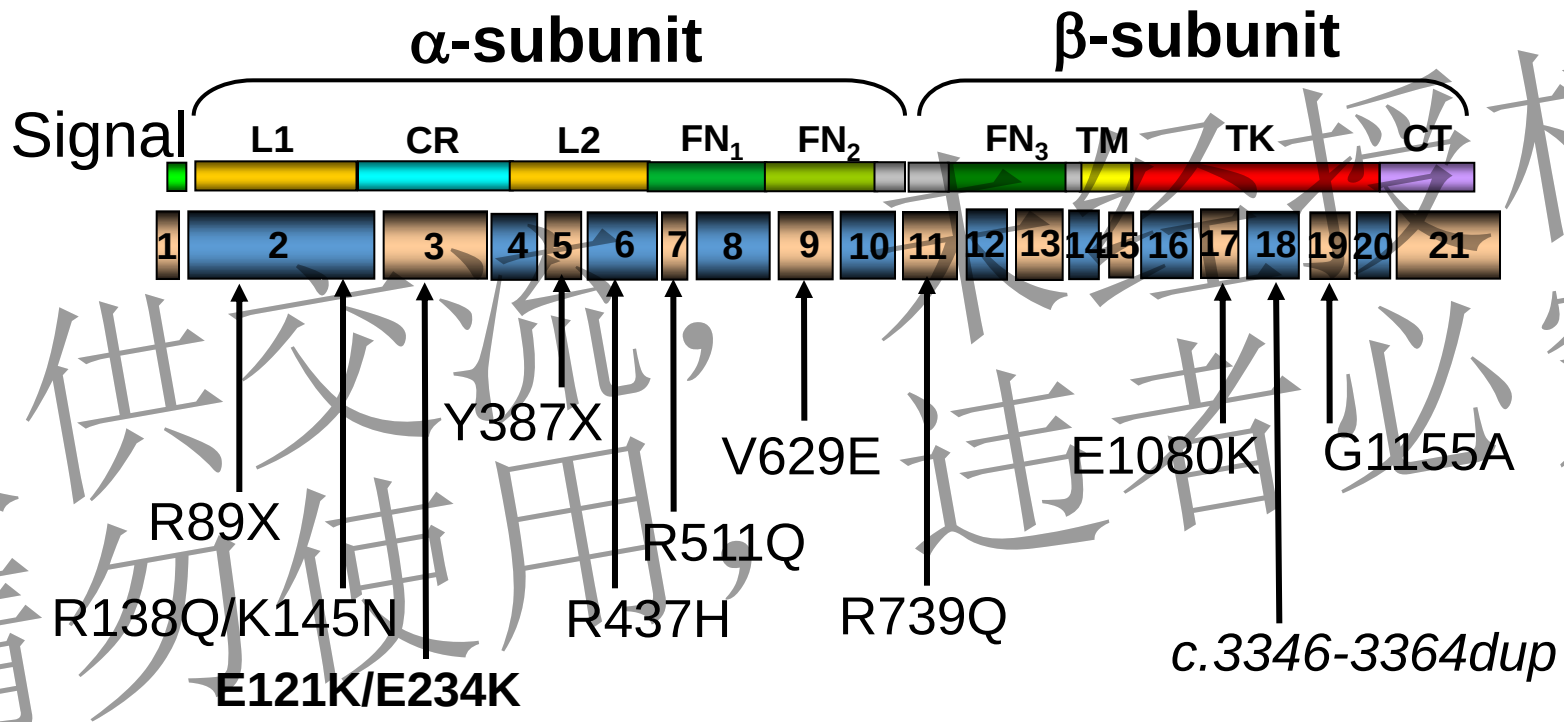
↓

Elevated total IGF-I
Normal IGFBP-3 and ALS

↓

***In utero* and post-natal growth failure**
(-2 to -6 HtSDS)

Inactivating, Heterozygous *IGF1R* Mutations



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Clinical Presentations of IGF1R Insufficiency

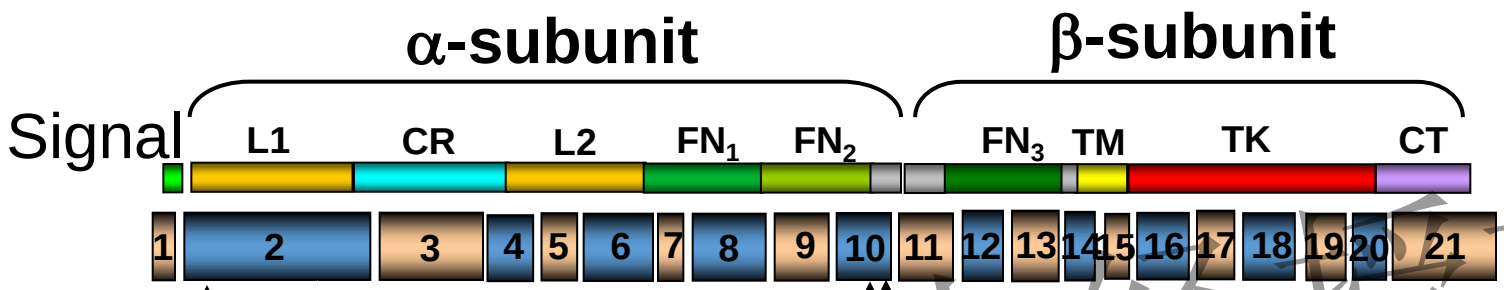
(1) Shared Characteristics:

- Pre-natal growth retardation – SGA
- Post-natal growth failure: -1.5 to -5.9 SDS less severe than patients with *GHR*, *STAT5B* or *IGF1* mutations
- Normal to elevated IGF-I levels, consistent with IGF-I resistance

(2) Variable Characteristics:

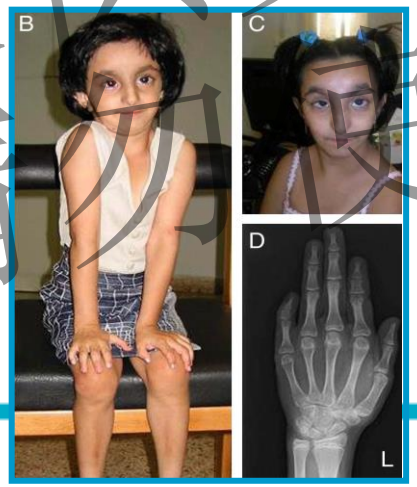
- Microcephaly
- Intellectual impairment
- Moderate insulin resistance (3 cases)

Inactivating, Homozygous *IGF1R* Mutations



p.R40L

HtSDS: -4.4



Gannagé-Yared et al, Eur J Endocrinol. 2012. 168:K1

c.2132_2143del

(in-frame deletion)

I.Maystadt, S.Andrew, unpublished

HtSDS

-6.2 -4.5

c.2201G>T

(splicing defect)

Prontera et al, Hum Mutat, 2015

Length SD: -3.2

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Lessons from Rare Homozygous *IGF1R* Mutations

- Clinical phenotype more severe than IGF1R insufficiency:
  - intrauterine growth retardation (IUGR)
  - severe post-natal growth failure
  - microcephaly, dysmorphic features, intellectual disability
- Markedly elevated total serum IGF-I
- Insulin insensitivity, Type 2 diabetes
- Associated with sub-set of neonatal cardiac malformation
- Functionally: mutant IGF1R has detectable residual activities

Genetic Basis for Growth Hormone Insensitivity (GHI)

I. Defects disrupting IGF-I production

II. Defects disrupting IGF-I actions

III. Defects disrupting fundamental cellular functions

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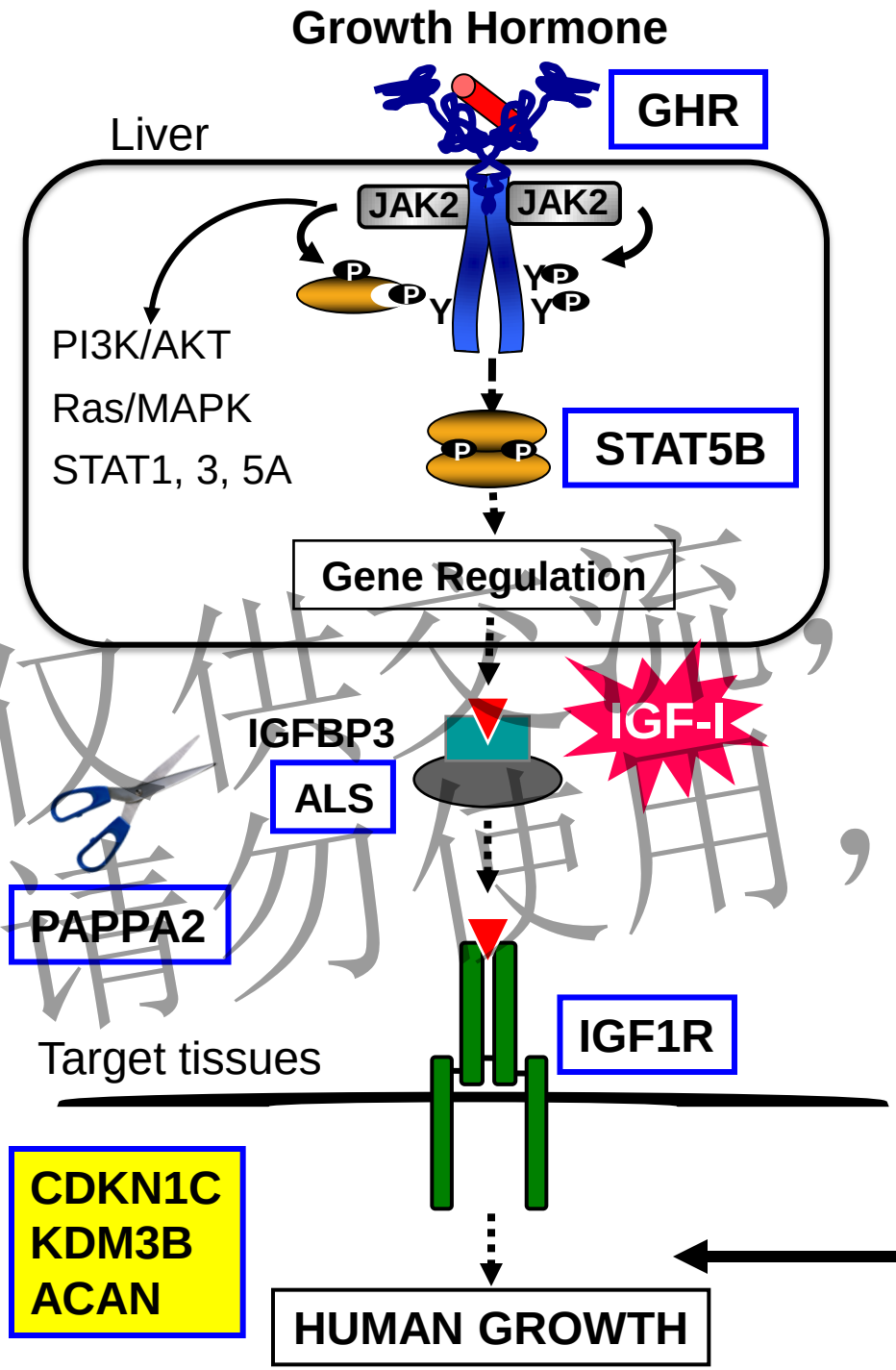


Growth Failure Associated with GHI and Normal to Elevated IGF-I

WES Analyses: Identified Defects in Genes Involved in Global, Fundamental Cellular Functions

Centrosomal Defects:
- ***NIN*** **(Ninein)**
- ***PCNT*** **(pericentrin)**

Cell Cycle Defects:
- ***CDKN1C*** **(p57): imprinted**

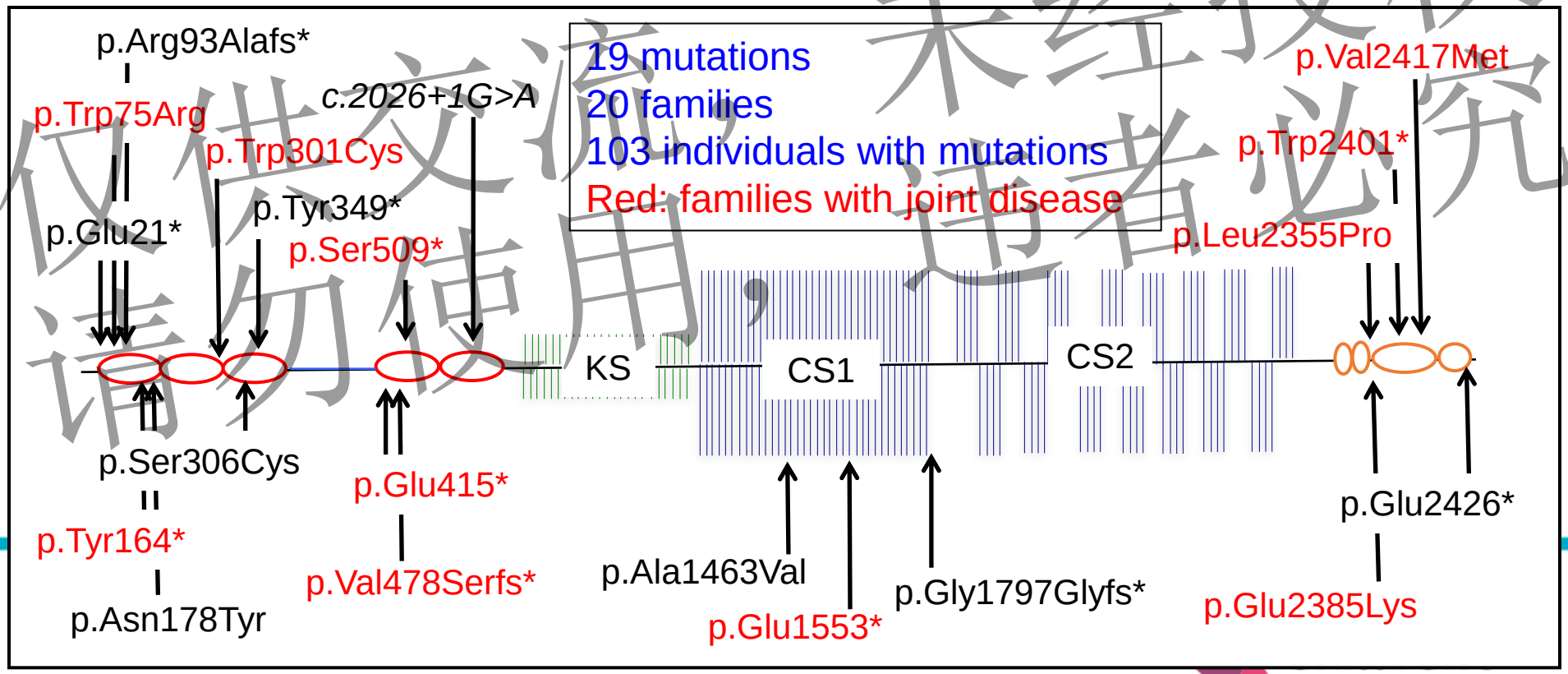
DNA Repair Defects:
- ***ERCC6***
- ***XRCC4***

Extracellular (matrix) Defects:
- ***ACAN***

Aggrecan (*ACAN*) Mutations: a Cause of Autosomal Dominant Short Stature

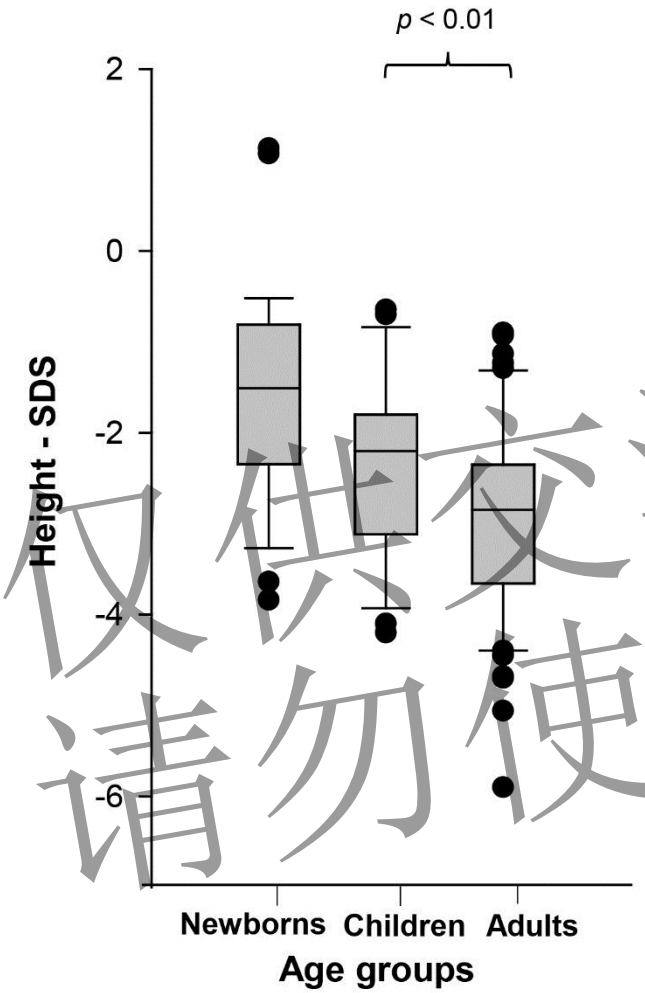
Chromosome 15q26.1

Aggrecan: large chondroitin sulfated (CS) proteoglycan, a major structural component of cartilage, e.g. growth plate, intervertebral discs.



Gkourogianni A, ... Dauber A. J Clin Endocrinol Metab 2016

Clinical Presentations of ACAN Insufficiency



- Advanced BA (1 – 4 yrs) is common
- Mild proportionate short stature
- No obvious indications of chondrodysplasia
- Common co-morbidities: early onset osteoarthritis and degenerative disc disease
- Normal endocrine evaluations
- Modest growth response to therapeutic GH
- Poor genotype-phenotype correlations: functional studies necessary

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SUMMARY

- Defects along the GH-IGF-I axis that affect:
  - production of IGF-I (e.g. *GHR*, *STAT5B*, *IGF1*)
  - action of IGF-I (e.g. *IGFALS, PAPPA2*, *IGF1R*)

  result in GHI and growth failure, associated co-morbidities.
- Defects beyond the GH-IGF-I axis: may account for ~75-80% of GHI cases with unknown causes of short stature
- Expansion of genetic approaches (linkage, WES, GWAS, epigenetic): help resolve the molecular basis of short stature
- Functional evaluation of identified candidate variants/genes (e.g. *in vitro*, *in vivo* models, iPS, etc):
  - essential to prove causality
  - provide insights into structure/function of affected protein and improve our understanding of growth physiology

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