A novel cause of congenital central hypothyroidism: From two cousins to a novel X-linked endocrine syndrome

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On behalf of the international IGSF1 consortium

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1. How it started

- Index case TE, d.o.b. 29-10-1994
- BW +2.3 SDS, BL +1.1 SDS, BHC +3.5 SDS (40.5 w)
  Jaundice for 2 weeks, hydrocephalus.

- Screening for congenital hypothyroidism (age 9 and 13 d):
  - T4 -2.4 and -3.3 SDS
  - TSH 5 mU/l
1. How it started

- Hospital, AMC:
  - FT4 8.3 pmol/L, TSH 3.5 mU/L
  - TRH test: TSH max 10.4 mU/L (low)
  - Start L-T4 treatment.
  - TRH and TSHB genes nl.
- MRI: external hydrocephalus. VP drain at 1.0 yr.
- At age 1.8 y: transfer of care to LUMC
- Height SDS → -1.3
- 11.0-15.2 y testes 4->16 mls, but low T until 15.2 yrs → T treatment; BMI +2.5 SDS. PRL borderline

His cousin

- RH, dob 8-2-1991
- BW +2.2 SDS, BL +1.6 SDS (43 w)
- At 7.2 y referred to LUMC
  - growth deviation
  - height SDS -1.4,
  - BMI +2.2,
  - delayed bone age (3.1 y)

His cousin

- Lab. investigation:
  - FT4 8.8 pmol/L, TSH 1.6 mU/L
  - TRH test: TSH max 4.3 mU/L, PRL low, IGF-I -1.3 SDS.
  - Start L-thyroxine
- Lab. investigation 2:
  - GH max 16.9 and 13.1 mU/L, IGF-I -1.0 SDS
- 8.8 y: start GH treatment: excellent catch-up, adult height
  191.6 cm (+1.3 SDS) (Target height 0.8 SDS). BMI 24.4 (+1.5 SDS). Relesting: GH max normal.
- 9.9-14.2 y testes 4->16 mls, while T still low. GnRH test: LH
  0.7-13.4, FSH 6.5-13.4 U/L. T1 from 14.5 y
Maternal grandfather A-I.4

- Growth:
  - normal stature, overweight (BMI 33), GH normal
- Thyroid/prolactin:
  - FT4 9 pmol/L, TSH 0.9 mU/L, PRL normal
- Adrenal:
  - possibly central hypocortisolism. Low DHEAS
- Gonads:
  - late puberty, large testis (removed after torsion), contralateral testis atrophic, postoperative primary hypogonadism
- CNS/behavior:
  - normal (retired general practitioner)

Family A

(Patho)physiology of thyroid hormone secretion

Causes of hypothyroidism
- Hypothalamus/pituitary: TRHR and TSHB genes defect
- Central (sec./tert.) hypothyroidism
- Thyroid: agenesis, hypoplasia, enzyme defects
- Primary hypothyroidism

CH screening in Netherlands: T4+TSH+TBG approach
- If low T4 = normal TSH + low T4/TBG ratio: central hypo?
Conclusion family A

Novel X-linked syndrome of:
• Pituitary TSH deficiency
• Variable prolactin deficiency
• Macroorchidism and delayed puberty
• Partial GH deficiency?
• Large birth size, overweight

Plan: exome sequencing of the X-chromosome

AMC

5 1/2 families with clinical picture of “X-linked” central hypothyroidism:
• TE (+RH) - nephews
• Pk (1999) + SK (2003) - nephews
• DL (1991) + SR (2002) - (half-)brothers

2003/2004: linkage studies - not successful
2010, plan: exome sequencing of the X-chromosome

2. Discovery of the gene defect: Exome sequencing

• Mutation detection in monogenic diseases
• Drawback
  • In principle only exonic regions
  • Variable capture efficiency
  • Difficulties in analysis (comparison)
Exome sequencing Family A (Yu Sun)

- SNP array to detect candidate region (III.11 vs 14)
- Exome sequencing III-7 and III-11
- Sureselect Agilent, X-exome capture
- GAII Illumina, 51nt paired end run

Standard variant calling and filtering

- BWA + samtools: SNVs and short indels
- SeattleSeq Annotation
- 1000 Genomes Project
- Allele frequency Hapmap

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SNV = single nucleotide variant

Searching for candidate gene

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Alignment with GMAP/GSNAP: 27bp deletion in IGSF1

Confirmation

IGSF1

- Conserved region
3. What was known of IGSF1

- Immunoglobulin superfamily member 1
- Membrane glycoprotein
- 3 isoforms (REFSEQ)
- Function
  - Unclear
  - Possibly a co-receptor in inhibin signaling, but not a high-affinity inhibin receptor.
  - Antagonizes activin A signaling in the presence or absence of inhibin B (by similarity).
  - Necessary to mediate a specific antagonistic effect of inhibin B on activin-stimulated transcription.

Other members of the family

- Expression
  - Highly expressed in pituitary, hypothalamus, pancreas, testis and fetal liver.
  - Moderately expressed in heart, prostate and small intestine.
  - Expressed at very low levels in thymus, ovary, colon, fetal lung and fetal kidney.
Igsf1 in the mouse

Normal reproductive function in KO mice

4. Human phenotype: Family UK

NM_001170961.1:c.2931G>A
p.(Try977X)

Family C                              Family D                                            Family E
Family F                               Family G                                            Family H
Family I                               Family J                                           Family K

7 other Dutch, 2 Italian families
IGSF1 mutations predicted to result in loss of function

Whole gene deletions:
Family E: 126kb deletion
arr Xq26.1q26.2(130.310.905-130.639.353)x0 (hg19)

Family F: 328kb deletion
arr Xq26.1q26.2(130.310.905-130.639.353)x0 (hg19)

The variants

- Not present in database
  - Local inhouse database, dbSNP, 1000 Genomes Project, HGMD, LOVD
- Cosegregate with phenotype
- Might affect function of the protein

IGSF1 truncation mutants are not trafficked to the plasma membrane
IGSF1 in-frame mutants are inefficiently trafficked to the plasma membrane

Decreased plasma membrane expression

Immature glycocalyx pattern (preserved in Endoplasmic Reticulum)

IGSF1 Loss-of-Function Causes Central Hypothyroidism and Prolactin Deficiency

Central Hypothyroidism 26/26 cases

TRH Tests

TSH peak (mU/L)

Infant Child Adult

Prolactin deficiency 18/26 cases

IGSF1 Loss-of-Function Causes Adult Macroorchidism

Pubertal development is disharmonious: testosterone low for testis size

Ultrasound references from Goede et al Virurn Res Pediatr 2011; 76:56-64
5. Mouse phenotype

- Expression of IGSF1/IGSF1 mRNA in murine embryonic day 12.5
- Also expressed in human embryo Carnegie stage 18 Rathke’s pouch (in situ hybridization)

Igsf1 is expressed in TSH, GH and PRL secreting cells.

TSH and T3 levels are decreased in male Igsf1<sup>Δex1</sup> mice

- Decreased serum TSH
- Decreased serum T3
TRH signalling may be impaired in male Igsf1Δex1 mice

Central Hypothyroidism due to IGSF1 Loss-of-Function
Conclusions

- IGSF1 mutations cause a novel, X-linked syndrome of central hypothyroidism, testicular enlargement and variable prolactin deficiency.
- Central hypothyroidism (and PRL deficiency?) caused by defects in TRHR expression and TRH signalling.
- Croorchidism: mechanism unresolved.
- Detection important because of theoretical reasons that hypothyroidism should be treated.

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