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

Contents

1. How it started
2. Discovery of the gene defect (Yu Sun)
3. What was previously known of IGSF1
4. Human phenotype (10 mutations in 11 families, 26 patients)
5. Mouse phenotype
6. Conclusions

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)
  -GH, PRL, IGF-I, CRH and GnRH test nl.
  -Start L-T4 treatment.
  -*TRHR* 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

4

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)

5

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 0.6 SDS). BMI 24.4 (+1.5 SDS). Retesting: 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. T↑ from 14.5 y

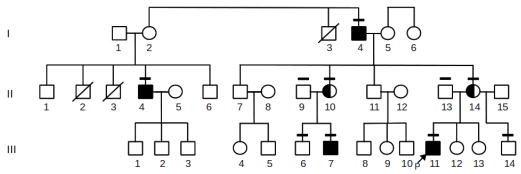
6

Maternal grandfather A-I.4

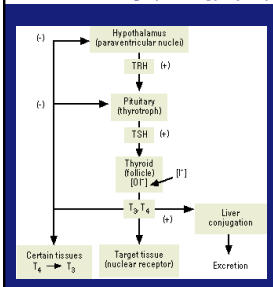
- Growth:
  -normal stature, overweight (BMI 33), GH: normal
- Thyroid/prolactin:
  -FT4 9 pmol/L, TSH 0.9 mU/L, PRL normal
- Adrenal:
  -possibly central hypocortisolism. Low DHEAS
- Gonads:
  -late puberty, large testis (removed after torsion), contralateral testis atrophic, postoperative primary hypogonadism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

7

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?

9

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

10

AMC

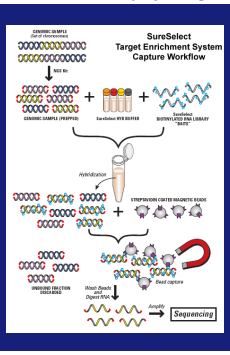
5 ½ families with clinical picture of "X-linked" central hypothyroidism:

- TE (+RH) - nephews
- CS (1984) + YS (1989) - nephews
- AJ + MH (1990) + (1991) - nephews
- PK (1999)+ SK (2003) - nephews
- DL (1991) + SR (2002) - (half-)brothers
- JS (1995) + KS (2000) - brothers

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

11

2. Discovery of the gene defect: Exome sequencing

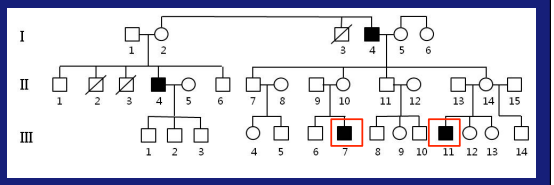


- Mutation detection in monogenic diseases
- Drawback
  - In principle only exonic regions
  - Variable capture efficiency
  - Difficulties in analysis (comparison)

12

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



13

Standard variant calling and filtering

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

	III-7	III-11	in common
#indels	735	770	510
#SNVs	2278	2366	1922
#SNVs not in 1000 Genomes Project			591
#SNVs hapmap freq <1%			471
#SNVs in region			129

SNV = single nucleotide variant

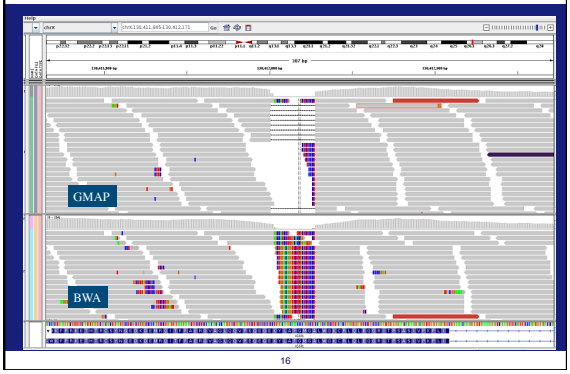
14

Searching for candidate gene

129 SNVs in region

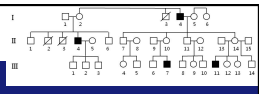
	All	Not in dbSNP
nonsense	0	0
missense	10	2
silent	1	0
Splice-3	0	0
Splice-5	0	0
3-utr	11	3
5-utr	12	1
intron	63	20
intergenic	20	3
Near-gene-3	9	1
Near-gene-5	7	2

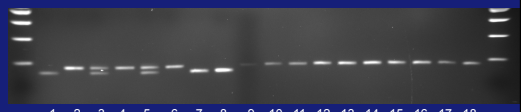
Alignment with GMAP/GSNAP: 27bp deletion in IGSF1



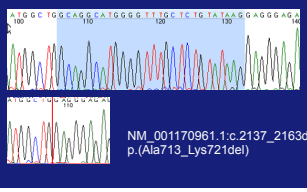
16

Confirmation





1	I:4
2	II:9
3	II:10 (m)
4	II:13
5	II:14 (m)
6	III:6
7	III:7
8	III:11
9	III:12
12-18	controls

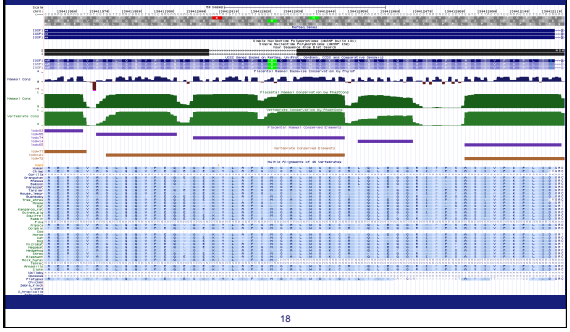


NM_001170961.1:c.2137_2163del
p.(Ala713_Lys721del)

17

IGSF1

- Conserved region



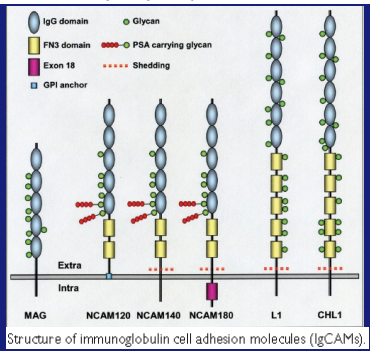
18

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.

19

Other members of the family



Structure of immunoglobulin cell adhesion molecules (IgCAMs).

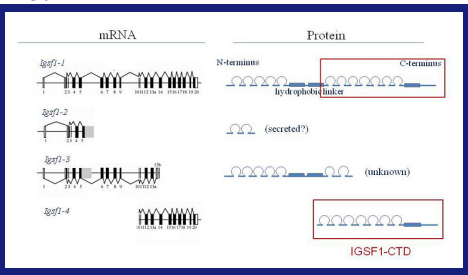
Irintchev, The Neuroscientist 2011 20

IGSF1

- 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.

21

Igsf1 in the mouse

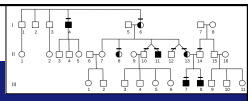


Normal reproductive function in KO mice

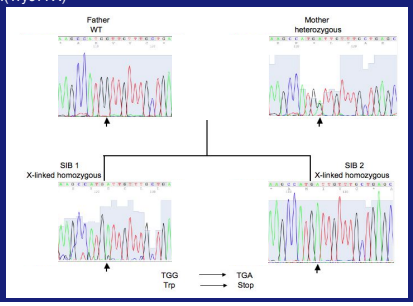
Courtesy D. Bernard

22

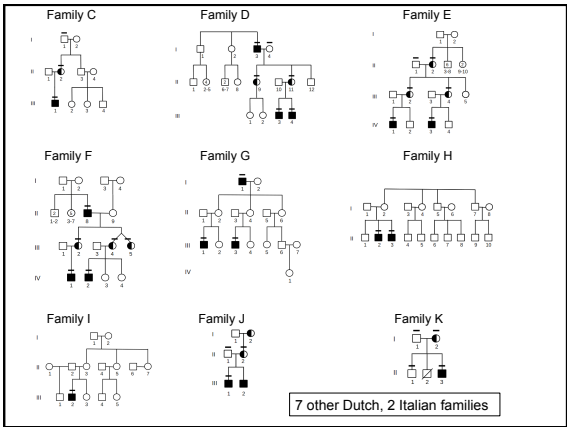
4. Human phenotype: Family UK



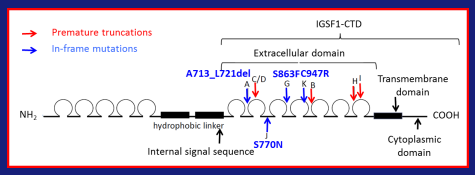
NM_001170961.1:c.2931G>A
p.(Try977X)



23



IGSF1 mutations predicted to result in loss of function

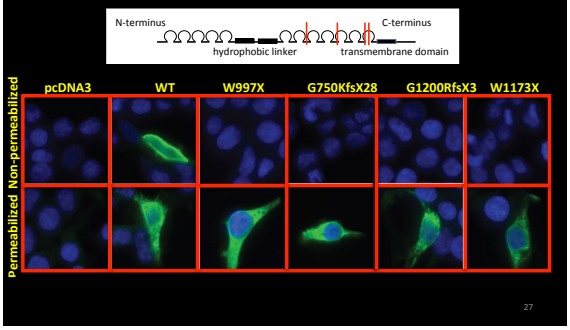


Whole gene deletions:
Family E 126kb deletion arr Xq26.1q26.2(130.386.267-130.512.002)x0 (hg19)
Family F 328kb deletion arr Xq26.1q26.2(130.310.905-130.639.353)x0 (hg19)

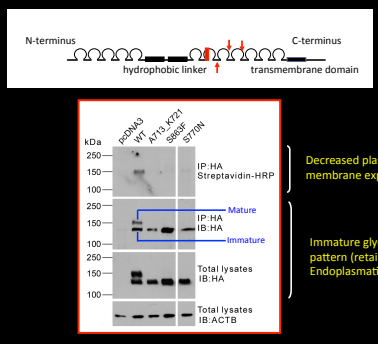
The variants

- 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



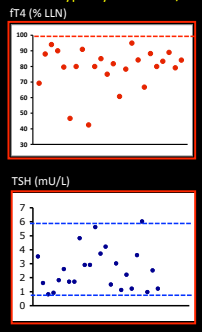
IGSF1 in-frame mutants are inefficiently trafficked to the plasma membrane



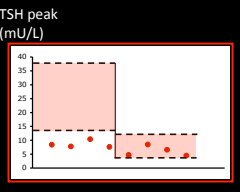
28

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

Central Hypothyroidism 26/26 cases



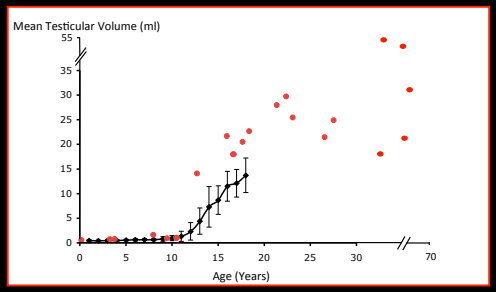
TRH Tests



Prolactin deficiency 18/26 cases

29

IGSF1 Loss-of Function Causes Adult Macroorchidism



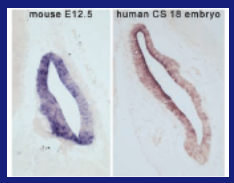
Pubertal development is disharmonious: testosterone low for testis size

Ultrasound references from Goede et al Horm Res Paediatr 2011; 76:56-64

30

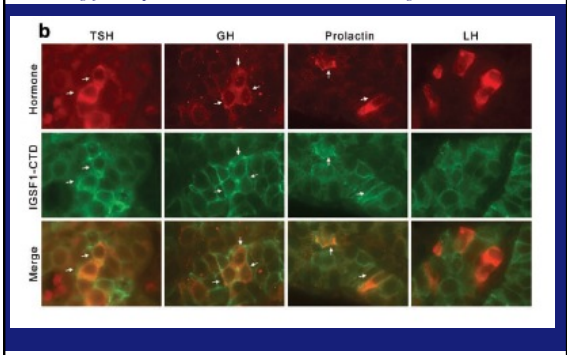
5. Mouse phenotype

- 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)



31

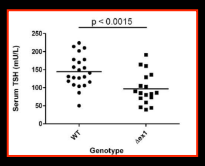
Igsf1 is expressed in TSH, GH and PRL secreting cells



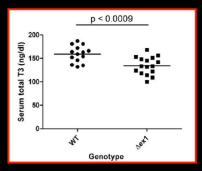
32

TSH and T3 levels are decreased in male Igsf1$^{\Delta ex1}$ mice

Decreased Serum TSH



Decreased Serum T3

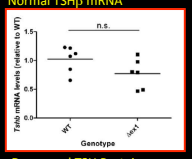


33

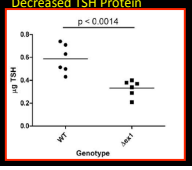
TRH signalling may be impaired in male Igsf1$^{\Delta ex1}$ mice

Pituitary

Normal TSHβ mRNA

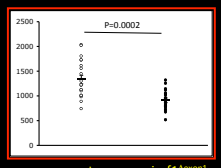


Decreased TSH Protein



Impaired TSH response to TRH

Peak TSH (mU/L)



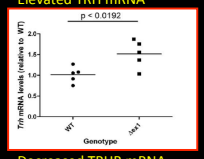
wt igsf1$^{\Delta exon1}$

34

TRH signalling may be impaired in male Igsf1$^{\Delta ex1}$ mice

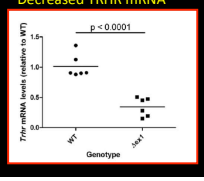
Hypothalamus

Elevated TRH mRNA



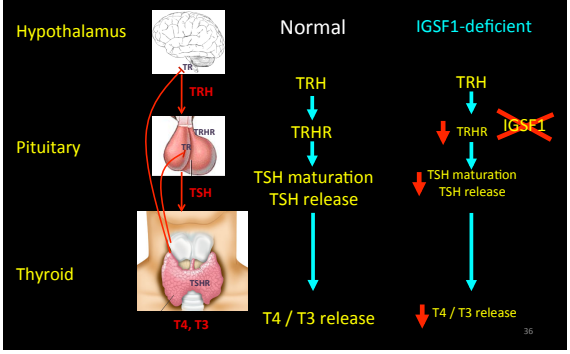
Pituitary

Decreased TRHR mRNA



35

Central Hypothyroidism due to IGSF1 Loss-of-Function



36

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

37

Acknowledgements

Leiden
Yu Sun
Jet Stokvis Wilma Oostdijk
Nienke Biermasz, Natasha Appelman
Eleonora Corssmit, Guido Hovens
Alberto Pereira
Sarina Kant
Claudia Ruivenkamp
Cathy Bosch
Annemieke Aartsma-Rus
Jeroen Laros
Emmelien Aten
Marjolein Kriek
Bert Bakker
Johan den Dunnen
Martijn Breuning
Jan Maarten Wit
LGTC, LDGA

Canada: Montreal
Daniel Bernard, Beata Bak, Michael Wade

UK: London, Cambridge
Mehul Dattani, Paul le Tissier, Neda Mousavy, Juan P Martinez-Barbera
Krishna Chatterjee, Nadia Schoenmakers, Emma Cambridge, Jackeline White, Peter Voshol

Amsterdam (AMC)
Paul van Trotsenburg, Raoul Hennekam, Tom Vulsma, Marlies Kempers

Rotterdam
Anita Hokken-Koelega, Daria Gorbenko

Italy: Milan
Luca Persani, Paolo Beck-Peccoz, Irene Campi, Marco Bonomi

Australia: Perth
Hongdong Zhu, Tim Davis

38