TSH RECEPTOR GENE (*TSHR*) VARIANTS IN PEDIATRIC PATIENTS WITH NON AUTOIMMUNE HIPERTHYROTROPINEMIA

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BACKGROUND

TSH resistance is defined as reduced sensitivity to TSH, associated with molecular defects hampering the adequate transmission of TSH stimulatory signal into thyroid cells. Non autoimmune yperthyrotropinemia (NAH) is a state of mild TSH resistance characterized biochemically by elevated TSH associated to normal thyroid hormones serum levels, in the absence of anti-thyroid antibodies.

Several studies in different populations have reported the presence of heterozygous *TSHR* gene variants associated to this condition. The prevalence of *THSR* gene variants varies in these reports and should be considered as a potential cause of subclinical hypothyroidism.

AIM

To study the frequency of *TSHR* gene variants in a pediatric population with NAH.

SUBJECTS

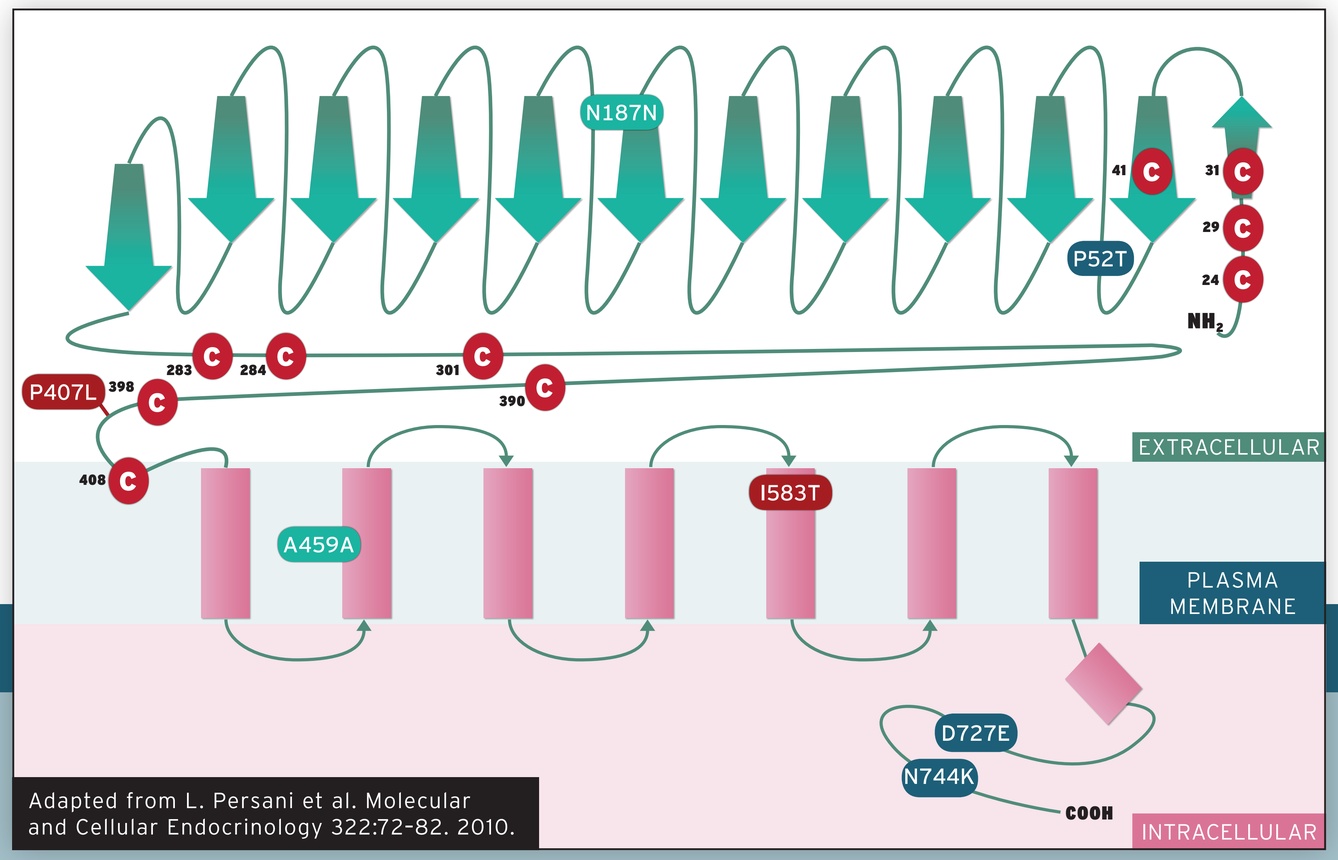
- 35 non obese unrelated children with NAH with
  - at least two TSH measurements > 5 mIU/L (ECLIA)
  - normal total (T_4) and free thyroxine (FT_4) (ECLIA)
  - negative TPO and ATG-antibodies (ICMA, Immulite)

  (Data retrieved from the medical records)
  - 18/35 small for gestational age (SGA)
  - The protocol was approved by the Institutional Review Board and all the patients involved and their parents gave their informed consent to participate

Children born SGA were younger and shorter at consultation and had significantly lower TSH and higher FT_4 than AGA children.

	TOTAL	SGA	AGA	P
N	35 18 Girls	18 8 Girls	17 10 Girls	NS
Age (years)	6.0 (1.0 - 18.0)	5.3 (1.5 - 18.0)	7.0 (1.0 - 18.0)	P < 0.01
GA (weeks)	38.0 (31.0 - 40.0)	38.5 (31.0 - 40.0)	39.5 (36.0 - 40.0)	NS
Birth weight (g)	3000 (680 - 4500)	2250 (680 - 3200)	2990 (2600 - 4500)	NS
Height (SDS)	-1.59 (-4.8 to 2.5)	-2.2 (-1.2 to -4.8)	-0.37 (-2.3 to 2.5)	P < 0.01
BMI (SDS)	-0.09 (-3.1 to 1.9)	-0.7 (-2.9 to 1.0)	-0.1 (-3.1 to 1.9)	NS
TSH (mIU/l)	8.8 (5.7 - 14.0)	7.9 (5.7 - 11.4)	9.2 (6.0 - 14.0)	P < 0.05
T_4 (mg/dl)	9.0 (6.4 - 11.8)	9.7 (6.9 - 11.8)	8.8 (6.4 - 11.5)	NS
FT_4 (ng/dl)	1.27 (1.00 - 1.65)	1.34 (1.00 - 1.65)	1.20 (1.00 - 1.42)	P < 0.05

Results are expressed as Median (range). Mann Whitney test was used to compare demographic variables and hormone levels between two groups of patients (SGA and AGA).



Adapted from L. Persani et al. Molecular and Cellular Endocrinology 322:72-82. 2010.

METHODS

- The whole coding sequence of *TSHR* gene (exons 1 to 10) and intronic flanking regions were amplified by PCR from genomic DNA and automatically sequenced.
- Different softwares were used for in silico prediction of gene variants effects:
  - PolyPhen 2 (http://genetics.bwh.harvard.edu/pph2/)
  - Mutation Taster (http://www.mutationtaster.org/)
  - SIFT (http://sift.jcvi.org/www/SIFT_enst_submit.html)
  - MutPred (http://mutpred.mutdb.org/)
  - SNAP (https://www.rostlab.org/services/snap/submit)
- Chi-square test was used for allelic frequencies comparisons.
- Population frequency databases:
  - NCBI dbSNP (http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?locusId=7253): 1000Genome project, phase 1 genotype data from 1094 worldwide individuals, released in the May 2011 dataset.
  - Exome Variant Server, NHLBI GO Exome Sequencing Project (ESP), Seattle, WA (URL: http://evs.gs.washington.edu/EVS/) [june, 2014].

RESULTS

TSHR SEQUENCING: frequent SNPSs (coding)

Several known and frequent polymorphic variants were found in the coding region.

dbSNP database reference	Variant	Exon	MAF in our cohort	dbSNP 1000Genome MAF	Exome Variant Server MAF	P (vs dbSNP) (vs EVS)
rs2234919	p.Pro52Thr (c.154C>A)	1	0.043 (3/70)	0.034	0.047 (606/13006)	NS NS
rs2075179	p.Asn187Asn (c.561T>C)	7	0.143 (10/70)	0.287	0.270 (3508/13006)	0.01 vs dbSNP 0.02 vs EVS
rs113951800	p.Ala459Ala (c.1377G>A)	10	0.014 (1/70)	0.008	0.017 (224/13006)	NS NS
rs1991517	p.Asp727Glu (c.2181C>G)	10	0.157 (11/70)	0.109	0.077 (997/13006)	NS 0.02 vs EVS
rs61743974	p.Asn744Lys (c.2232C>G)	10	0.014 (1/70)	0.011	0.019 (242/13006)	NS NS

In our cohort:
- N187N MAF was significantly lower compared to both population databases (dbSNP and EVS).
- D727E MAF was significantly higher compared to EVS database.

*MAF: Minor Allele Frequency

TSHR SEQUENCING: uncommon variants

In two patients, two uncommon heterozygous missense variants were found in exon 10.

Variant	dbSNP database reference	MAF in our cohort	dbSNP 1000Genome MAF	Exome Variant Server MAF	Additional Data
p.Ile583Thr (c.1748T>C)	-	0.0143 (1/70)	NA	NA	Described in one patient with NAH(1)
p.Pro407Leu (c.1220C>T)	rs367815744	0.0143 (1/70)	NA	0.00008 (1/13006)	Novel

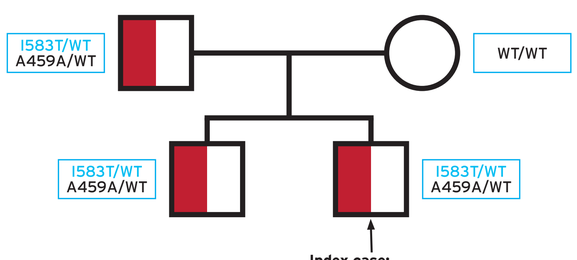
TSHR SEQUENCING: uncommon variants

Both variants were predicted as pathogenic by different bioinformatic tools.

Gene variant	Polyphen 2 prediction (score)	Mutation Taster prediction (probability)	SIFT (score)	MutPred (Probability of deleterious mutation)	Exome Variant Server MAF	*In vitro*
p.Ile583Thr (c.1748T>C)	Probably damaging (0.909)	Disease causing (0.999996)	Damaging (0)	0.566	Neutral (0/53%)	Less responsive to TSH in vitro.(1)
p.Pro407Leu (c.1220C>T)	Probably damaging (0.999)	Disease causing (0.999999)	Damaging (0)	0.720	Non-neutral (1/63%)	NA

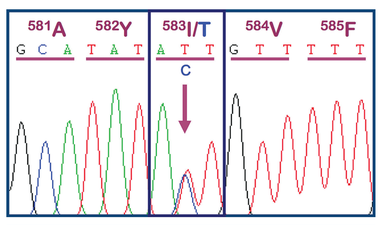
(1) Calebiro D. et al. JCEM 97: E156-E160, 2012.

PATIENT 1: p.Ile583Thr

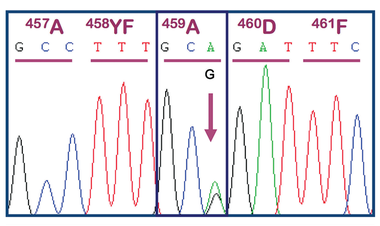


Brother:
- 12.1 y
- HeightSDS: 2.0
- BMISDS: -0.54
- G4 VP4 Ts 20/20

Index case:
7.6 y:
Healthy, prepubertal
Height -0.82 SDS, BMI 1.61 SDS.
Elevated TSH found in a routine pediatric examination.
Followed up till 11.2 y: Height SDS 0.76
BMISDS 1.42 SDS, G4 TS 12/15.



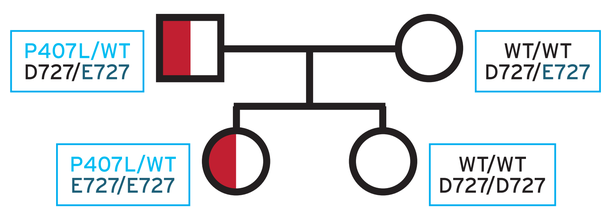
p.I583T (c.1748T>C)



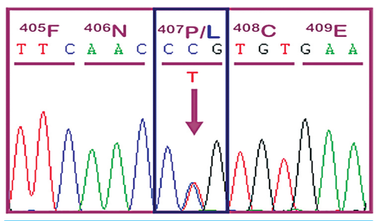
p.A459A (c.1377G>A)

	Index Case	Brother	Father	Mother	Reference range
TSH (mIU/l)	10.0 / 9.2	8.5	2.5	2.3	0 - 5
T4 (mg/dl)	7.2 / 6.6	7.1	6.3	7.1	6.0 - 14.0
FT4 (ng/dl)	1.27 / 1.14	1.20	0.94	1.06	0.8 - 2.0
T3 (ng/dl)	133 / 160	155	115	-	80 - 220
Tg (ng/ml)	14.5	14.5	5.6	14.8	6 - 40
TPO-Ab/ TG-Ab	Negative	Negative	Negative	Negative	<35/<35
Thyroid US	Normal	Normal	Normal	NA	
Molecular findings p.I583T p.A459A	p.I583T/WT p.A459A/WT	p.I583T/WT p.A459A/WT	p.I583T/WT p.A459A/WT	WT/WT WT/WT	

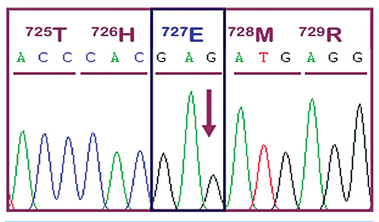
PATIENT 2: p.Pro407Leu



Index case
- Elevated TSH : casual finding at 12 y of age. No goiter. Normal puberty (T III).
- Assumed as hypothyroid, she received LT4 treatment till 18.6 y of age.
- Normal thyroid profile during treatment. When reevaluated, persistent NAH.
- Last visit (21.1 y): Healthy, normal height, overweight (BMI 26), no goiter.



p.P407L (c.1220C>T)



P407L/WT E727/E727

	Index Case	Sister	Father	Mother	Reference range
TSH (mIU/l)	8.8 / 13.5	7.1 / 10.6	4.9	2.5	0 - 5
T4 (mg/dl)	7.3	8.9	8.4	9.7	6.0 - 14.0
FT4 (ng/dl)	1.01	1.24	1.26	1.19	0.8 - 2.0
T3 (ng/dl)	136	129	131	114	80 - 220
Tg (ng/ml)	7.6	4.9	4.6	2.8	6 - 40
TPO-Ab/TG-Ab/Trab	Negative	Negative	Negative	Negative	
Thyroid US	Normal	Normal	Normal	Normal	
Molecular findings p.407L p.D727E	p.P407L/WT p.D727E/D727E	p.I583T/WT WT/WT	p.P407L/WT p.D727E/WT	WT/WT p.D727E/WT	

She was also homozygous for p.D727E.(c.2181C>G)

A coexisting defect should be ruled out given the finding of low levels of TG in all the members of the family and the hyperthyrotropinemia in the sister.

SUMMARY & CONCLUSIONS

In a relatively small cohort of pediatric patients with NAH we were able to find two potential pathogenic *TSHR* gene variants in 2/35 of the patients (6%), both of them present in AGA children (2/17, 11.7%)

p.Ile583Thr

- Already reported in one NAH patient.
- Expressed *in vitro* and demonstrated to be less responsive to TSH stimulation ($G_{q/11}$-dependent signaling pathway).

p.Pro407Leu

- *In vitro* expression of this novel variant is required to establish its role in thyroid pathogenesis.
- Expression studies should also include the double mutant (p.[P407L;D727E]).
- The finding of low levels of thyroglobulin in all the members of the affected family requires to rule out a coexisting *TG* gene defect.

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Authors have nothing to disclose.