P1-93

Severe IGF-I deficiency and multi-organ autoimmune disease associated with novel germline *STAT3* mutations.

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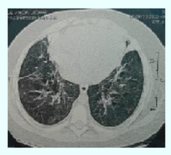
Background

Primary IGF-I deficiency can result from molecular defects in genes encoding for the GH receptor, IGF-I, STAT5b and ALS. Heterozygous, activating mutations in the *STAT3* gene have been recently described in children with severe growth failure associated with a spectrum of early-onset autoimmune disease (1,2).

PATIENT 1



Congenital Hypothyroidism with persistent antibodies
T4

Severe growth retardation with high basal GH and low IGF-I
GH

Pulmonar biopsy (2,7y): Linfhocitic Intertitial Pneumonia

0m 3m 6m 1yr 2.6yr 2.9yr 3.2 yr 3.7 yr 4.2 yr

Oral sirolimus

Oral ciclosporine

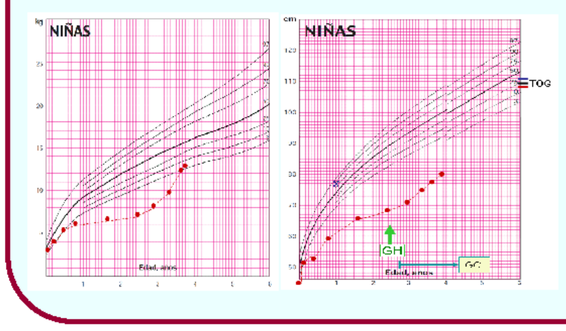
- ✓Recurrent pulmonary diseases
- ✓Oxigen requirement
- ✓Chronic diarrea
- ✓Severe Eczema
- ✓(30 hospitalizations)

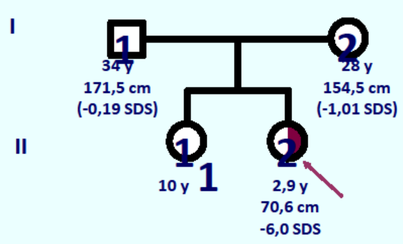
Oral Meprednisone 0,5 mg/kg/d → 1 mg/kg/d → 1.5 mg/kg/d

IV Gammaglobulin 600-800 mg/kg/d every 28 d

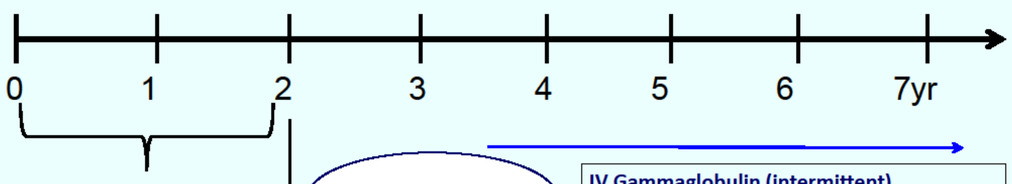
Profilactic TMS

No *STAT5B* mutation





PATIENT 2

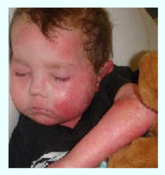


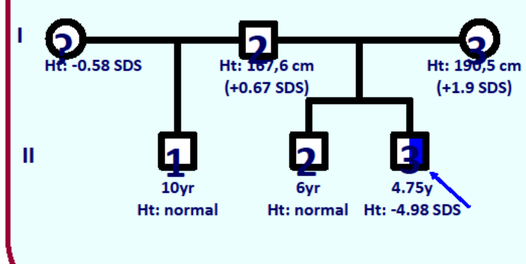
- ✓Pulmonary distress
- ✓Failure to thrive (FTT)
- ✓Chronic diarrhea
- ✓Chronic vomiting
- ✓Abdominal distention
- ✓Severe Eczema
- ✓History of colitis
- ✓Proctitis – intraepthelial lymphocytosis

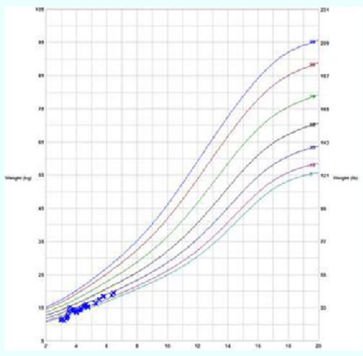
No *FOXP3* mutation

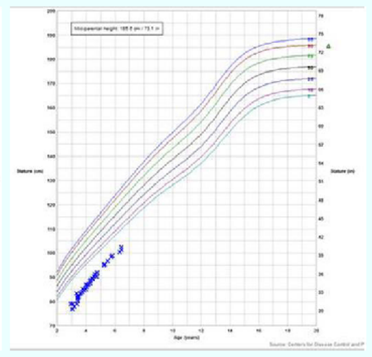
IV Gammaglobulin (intermittent)
Prednisolone (3.4 y onwards)
Oral Tacrolimus (4y onwards)

Endoscopy:
esophagus – normal
stomach – normal
duodenum - mild lymphoid nodularity
Hypothyroidism diagnosed
T4 started - poor compliance



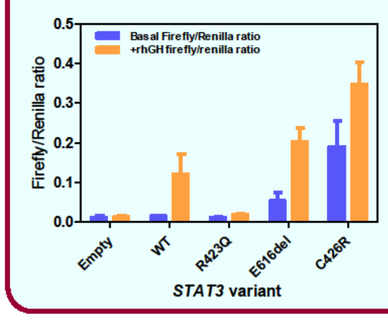


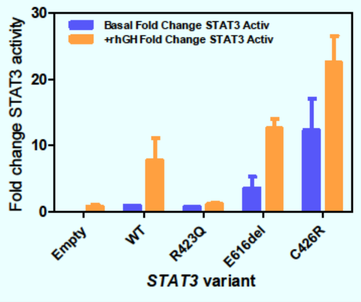




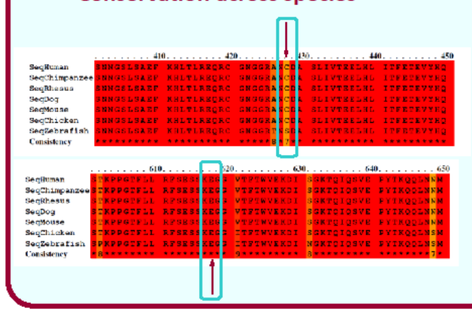
	Patient 1 (female)	Patient 2 (male)
Chronological Age (years) Height (SDS) Weight (SDS)	2,4 -6.4 -3,4	3,0 -5,36 -2,71
Gestational Age (weeks) Birth weigth (g) Birth lenght (cm/SDS)	38 3155 44 (- 3.3)	38 3586 50.8 (-0.75)
Clinical features	congenital hypothyroidism, descamative eczema, chronic diarrhea, recurrent candidiasis, severe respiratory infections	History of IPEX-like syndrome with dermatitis, chronic diarrhea, colitis, and autoimmune hypothyroidism
Immunological evaluation		
IgG / IgA / IgM / IgE (mg/dl) CD3 / CD4 / CD8 / CD19 (%) FOXP3 / Treg CD127 / Th17	637/389↑/103/<5↓ 56/34/19/27 N/N/low	760/211↑/154/<1↓ 82↑/35/45↑/12 (L) N/nd/nd
Endocrine evaluation at diagnosis		
GH (ng/ml)	20	
IGF-I (ng/ml) basal post IGF-GT (rhGH for 7d)	<12 20	< 25
IGF-BP3 (µg/ml) basal post IGF-GT (rhGH for 7 d)	1,0 2,2	0,5
Prolactin (ng/ml)	30,6	----
TSH (mIU/ml)/FT4 (ng/dl) TPO-Ab / TG-Ab (IU/ml)	238 / 0.4 83 / 48	364 / 0,2 >1000 / 165
rhGH treatment (period, dose) IGF-I (ng/ml)/IGFBP-3 (µg/ml)	10 mo (0,43mg/kg.wk) 240 / 4,4	No
Molecular studies		
Candidate gene sequencing	*STAT5B*: no mutation	*FOXP3*: no mutation
WES: Heterozygous *de novo* *STAT3* variants	c.1847_1849delAAG (p.Glu616 del) SH2 domain	c.1276T>C (p.Cys426Arg) DNA binding domain

STAT3 Luciferase assay

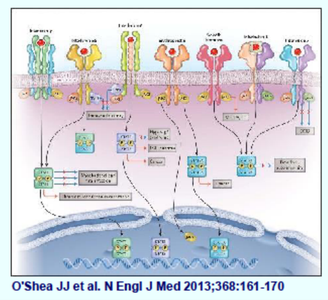




STAT3 sequence alignment: Conservation across species



Disorders Associated with Mutations of JAKs and STATs.



O'Shea JJ et al. N Engl J Med 2013;368:161-170

Patient 1
(WES performed in index case and unaffected father, mother and sister)

42639 variants (13110 genes)
Pass filter
31615 variants (11270 genes)
Consequence filter
10706 variants (5961 genes)
Population freq <10%
1245 variants (660 genes)

De novo model: 159 variants (117 genes) → Gene list filter (250 genes) → 3 variants (3 genes) *FCGR3A, PMS2 STAT3* → Manual curation

AR model: 441 variants (97 genes) → 4 variants (2 genes) *CASP8 PMS2*

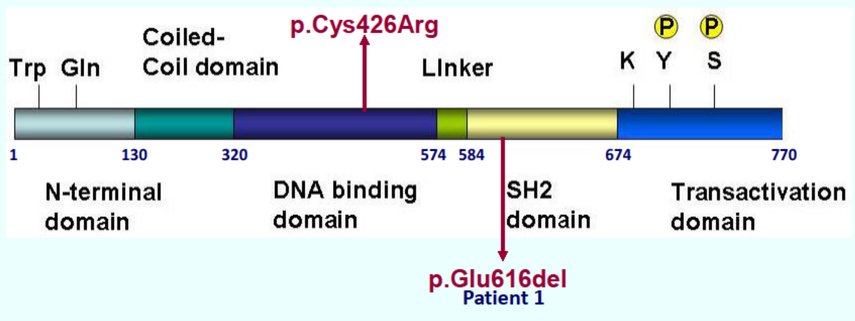
STAT3 de novo heterozygous variant : c.1847_1849delAAG (p.Glu616 del)

Patient 2
(WES performed on index case, unaffected father and mother)

907,411 variants (19,841 genes)
Common Variant filter <3%
592,152 variants (17379 genes)
Inheritance filter
8976 variants (2438 genes)
Biological Filter (Lymphocyte Activation)
170 variants (50 genes)
Other filters: Eczema, colitis Autoimmune thyroiditis AD autoinflammatory disorders
30 variants (14 genes)
Candidate

STAT3 de novo heterozygous variant: c.1276T>C (p.Cys426Arg)

Patient 2



Methods

- ✓Candidate gene study by Sanger sequencing (*STAT5B / FOXP3*)
- ✓Whole Exome Sequencing (WES): Illumina HiSeq 1500.
- ✓Functional studies: In HEK293 cells transfected with hGHR expression vector, transcriptional activity of WT and C426R E616del STAT3 mutants was assessed via a STAT3-responsive dual Firefly/Renilla Luciferase Cignal reporter system (Qiagen). The activity was measured before and 30 minutes after rhGH (200ng/ml). Previously reported LOF R423Q-STAT3 mutant was used as negative control (3).

References

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Conclusions

- ✓Although the gene-candidate approach has been useful to identify the genetic defect of several immune dysregulation and autoimmune diseases (STAT5B, FOXP3, CD25, ITCH) only the application of WES techniques has been successful to characterize novel genetic defects.
- ✓Activating *STAT3* mutations represent a novel monogenic defect presenting multi-organ autoimmune disease associated with severe growth retardation as the result of marked IGF-I deficiency. In contrast to STAT5b deficiency, patients carrying activating *STAT3* mutations appear to preserve partial GH responsiveness.

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