P1-596 Functional *in vitro* characterization of two novel germinal *STAT3* mutations associated with short stature, immunodeficiency and autoimmune disease.

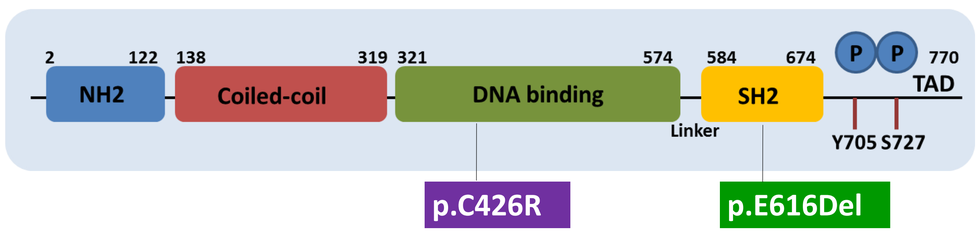
Mariana Gutiérrez[1], Paula Scaglia[1], Ana Keselman[1], Lucía Martucci[1], Liliana Karabatas[1], Sabina Domené[1], Miguel Blanco[2], Nora Sanguinetti[1], Liliana Bezrodnik[3], Daniela Di Giovanni[3], Soledad Caldirola[3], María Esnaola Azcoiti[3], Nana-Hawa Jones[4], Vivian Hwa[4], Santiago Revale[5], Martín Vázquez[5], Héctor Jasper[1], Ashish Kumar[6], Horacio Domené[1].

(1) Centro de Investigaciones Endocrinológicas 'Dr César Bergadá' (CEDIE) CONICET – FEI –División de Endocrinología, Hospital de Niños Ricardo Gutiérrez, Buenos Aires, Argentina. (2) Endocrinología, Hospital Universitario Austral, Buenos Aires, Argentina. (3) Inmunología, Hospital de Niños Ricardo Gutiérrez, Buenos Aires, Argentina. (4) Division of Endocrinology, Cincinnati Center for Growth Disorders, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA. (5) Instituto de Agrobiotecnología de Rosario (INDEAR), CONICET, Rosario, Argentina. (6) Division of BM transplantation and Immunodeficiency, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA.

INTRODUCTION AND OBJECTIVES

We have recently reported (1) the molecular diagnosis of two patients with severe growth failure associated with a spectrum of early-onset autoimmune disease and immunodeficiency, presenting heterozygous *de novo* mutations, c.1847_1849DelAAG (**p.E616Del**) and c.1276T>C (**p.C426R**) in the *STAT3* gene.

We aimed to study the impact of these mutations under basal and GH- or IL-6-stimulated STAT3 activity.

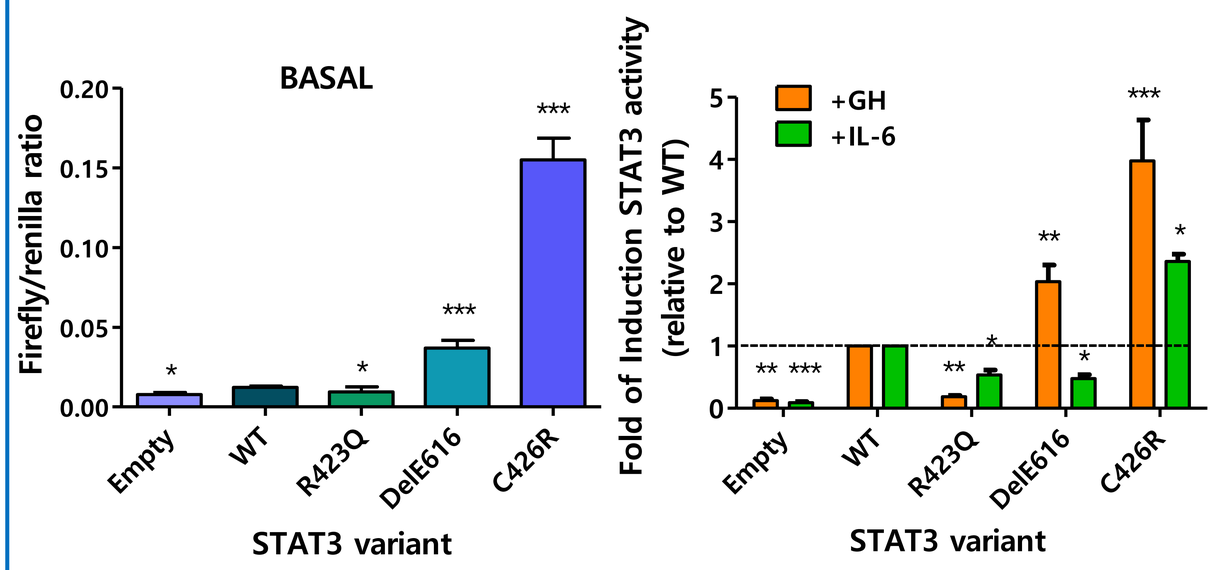


Patient 1 (female) *p.E616Del-STAT3*	Patient 2 (male) *p.C426R-STAT3*
Congenital autoimmune hypothyroidism, descamative eczema, chronic diarrhea, recurrent candidiasis, severe respiratory infections.	IPEX-like syndrome with dermatitis, chronic diarrhea, colitis, and autoimmune hypothyroidism.
↑IgA ↓IgE	
Short stature	
-6,4 SDS (2,4 y)	-5,4 SDS (3,0 y)
Partial GH insensitivity: Normal GH / Undetectable IGF-I	

METHODS

-*STAT3* gene variants were generated by **site-directed mutagenesis.**
-**Variants were transfected** into HEK293T cells transiently expressing hGHR.
-**STAT3-responsive dual Firefly/Renilla Luciferase Cignal reporter system** (Qiagen) was used for evaluating transcriptional activity. R423Q-STAT3 was used as negative control (2).
-IL-6 (20 ng/mL) and GH (200 ng/mL) effects on expression and phosphorylation of STAT3 were assessed by **Western immunoblot.**

TRANSCRIPTIONAL ACTIVITY OF STAT3 VARIANTS
Luciferase assay



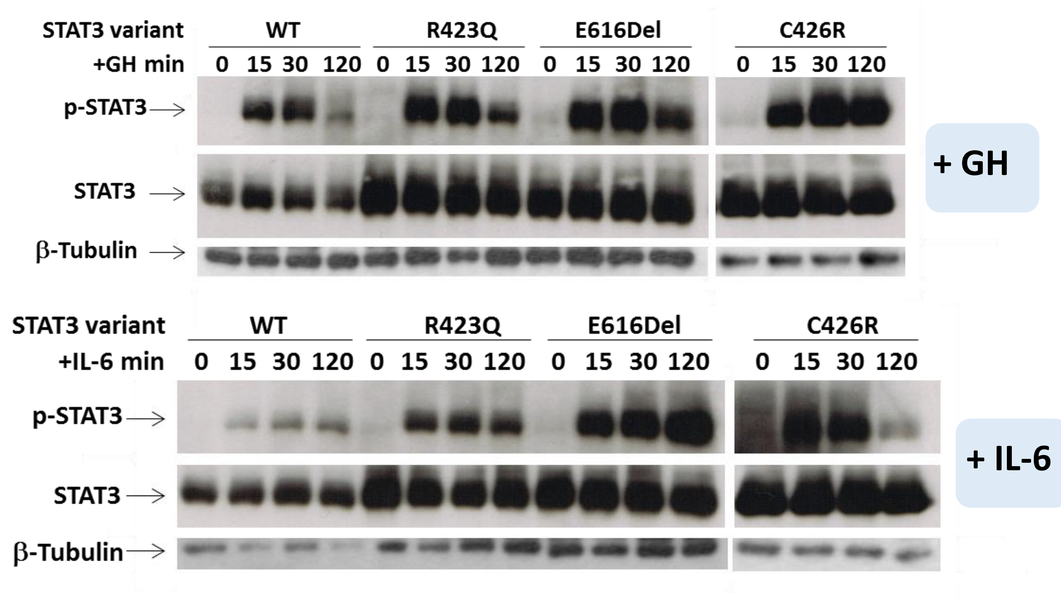
Data represents the mean ratio of firefly/renilla for each construct ± SEM (n=5).

Data are presented as average fold of change relative to WT ± SEM of at least 5 independent experiments.

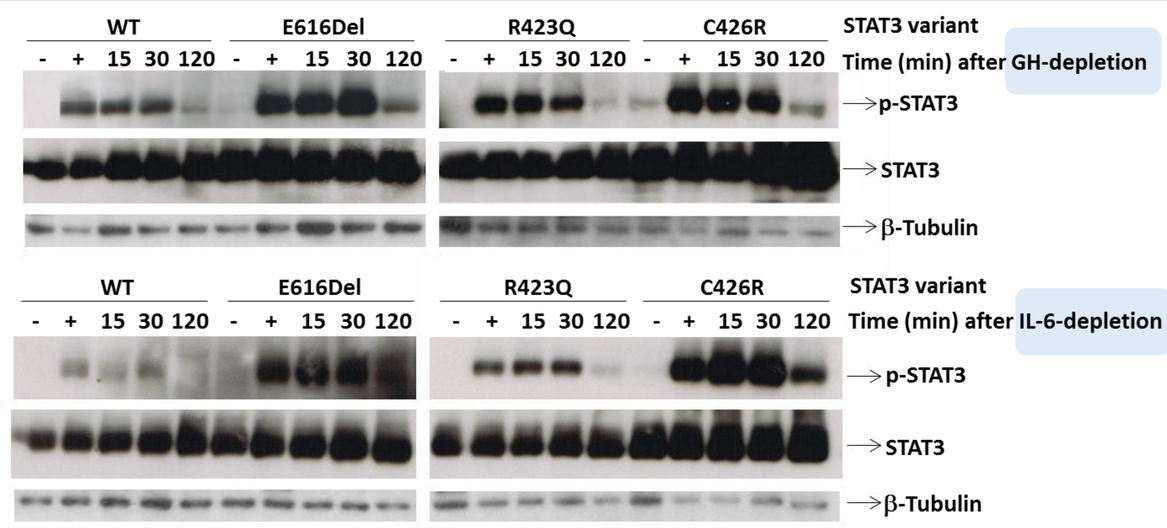
***P < 0.05, **P < 0.01, ***P < 0.001, t test.**

- **Both studied mutants display constitutive transcriptional activity.**
- **Transcriptional activity in response to IL-6 is only enhanced by C426R-STAT3 variant.**
- **GH induces STAT3-mediated transcriptional activity for both activating variants.**

ANALYSIS OF Y705-pSTAT3 IN TRANSIENTLY TRANSFECTED CELLS
Western Immunoblot



Stimuli depletion

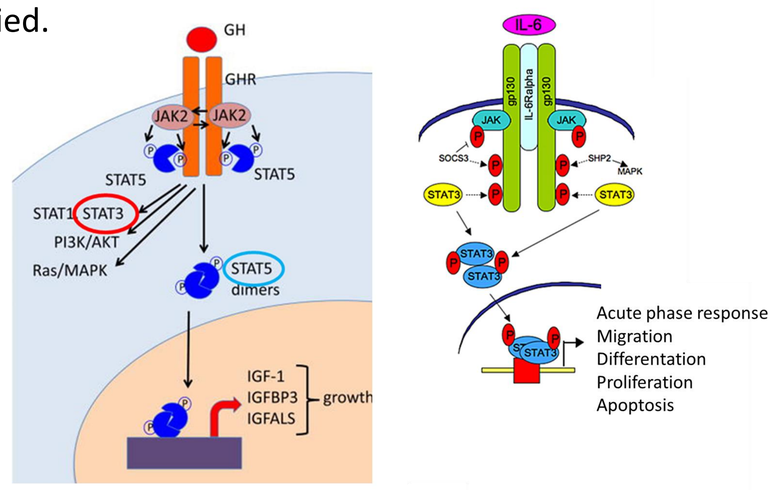


p.E616Del- and p.C426R-STAT3 variants:

- are **NOT constitutively phosphorylated.**
- are **phosphorylated in response** to both GH and IL-6 stimuli.
- show **different dephosphorylation kinetics** under both treatments.

CONCLUSIONS

- ***E616Del-* and *C426R-STAT3* are GAIN-OF-FUNCTION** mutations displaying constitutive transcriptional activity in the absence of stimuli, despite the observation that they are **NOT constitutively phosphorylated.**
- These findings suggest that gain-of-function STAT3 variants may exert their transcriptional activity through different mechanisms depending upon the type of mutation and the affected protein domain.
- How these STAT3 mutants affect STAT5b in the GH-signaling pathway remains to be studied.



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REFERENCES

1. Scaglia PA *et al*. Severe IGF-I deficiency and multi-organ autoimmune disease associated with novel germline STAT3 mutations. ESPE 2015, P1-93. **2.** Holland SM *et al*. STAT3 mutations in the hyper-IgE syndrome. N Eng J Med 2007, 357:1608-19.

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