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

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**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.

**METHODS**

- STAT3 gene variants were generated by site-directed mutagenesis.
- Variants were transected 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.

**CONCLUSIONS**

- E616Del- and C426R-STAT3 variants 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.

**REFERENCES**


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