# P1-D1-140 Severe short stature and GH insensitivity due to a *de novo* heterozygous STAT5B missense mutation

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## Objective

To characterise clinical and biochemical features of two severely growth retarded twins, to identify the genetic cause of their GH insensitivity and to define molecular properties of the affected proteins and pathways.

## Background

GH insensitivity (GHI) is caused by disturbances of GH receptor function or inability to transduce the hormone signal. Affected children are severely growth retarded and may also present immune complications when the transducer STAT5B is defective. Only autosomal-recessive STAT5B mutations have been described to date.

## Index Patient

- second child of unrelated parents
- 36. gestational week; birth weight, 2500 g (-1.9 SDS); birth length, 45 cm (-1.9 SDS)
- proportionate short stature; no dysmorphic signs (Fig.1)
- onset of puberty at approximately 15 yrs
- recombinant human rhIGF1 treatment commenced at 14.8 yrs (0.12 mg/kg bds); moderate response after 2.6 yrs [Δ(height) 1.0 SDS; Table 1, Fig.1]
- biochemical evaluation indicative for GHI (Table 1)
- clinical and biochemical characteristics of the monozygotic twin very similar to the index patient
- healthy parents; father, 174 cm (-0.9 SDS); mother, 160 cm (-1.2 SDS)
- siblings (brother and half-sister): normal

**Table 1.** Auxological and biochemical characteristics of the patient

| | before rhIGF1 tx (14.5 yrs) | during rhIGF1 tx (17.1 yrs) |
|---|---|---|
| height , cm (SDS) | 131.5 (-5.3) | 149.0 (-4.3) |
| weight , kg (SDS) | 28.0 (-4.5) | 42.0 (-3.5) |
| bone age, yrs | 9.6 | 13.5 |
| GH, basal, µg/l | n.d. | 8.1 |
| GH, stimulated, µg/l | 16.2 | n.d. |
| GHBP, pmol/l | n.d. | 404 (369-1876) |
| IGF1, µg/l | 56 (76-499) | 102.7 (-5.1 SDS) |
| IGFBP3, mg/l | n.d. | 2.3 (-1.7 SDS) |
| ALS, pmol/l | n.d. | 418 (986-1678) |
| Prolactin, mU/l | n.d. | 290.6 (86-324) |

- recurrent eczema, but otherwise healthy
- elevated IgE levels (340 kU/l , normal <114)
- immunological phenotyping otherwise unremarkable
- no chronic pulmonary disease, no lung fibrosis or lymphocytic interstitial pneumonia

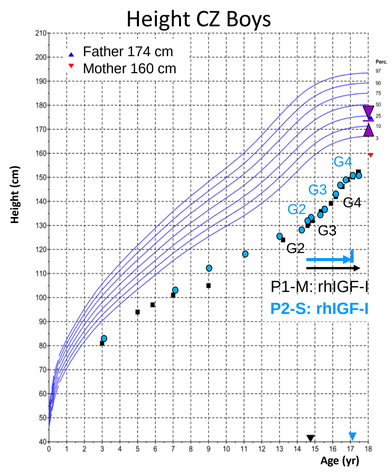


**Fig. 1:** Growth chart (CZ standards 2001) and photograph of the patients; black symbols index patient; blue symbols, twin brother.

## Genetic evaluation

- molecular genetic analyses of *GHR* and *STAT5B* revealed an heterozygous A to C transversion within exon 5 of *STAT5B* (CAG to CCG; c.530A>C; Fig.2)
- twin brother but not parents bear the same mutation (*de novo* mutation)
- analysis of *GH1*, *IGF1*, *IGFALS* and *IGF1R* genes did not show any further potentially pathogenic aberration
- mutant *STAT5B* allele expressed on mRNA level (Fig.2)

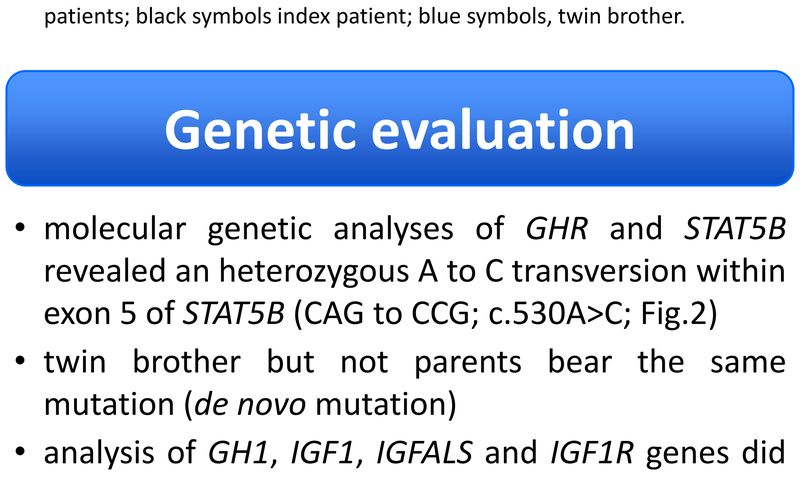


**Fig. 2:** Analysis of genomic DNA and reversely transcribed mRNA (whole blood) of the brothers and their parents (mother not shown)

- mutation predicted to result in a p.Gln177Pro amino acid substitution
- disrupts α-helix 1 within coiled-coil domain (CCD) presumably affecting secondary structure (Fig.3)

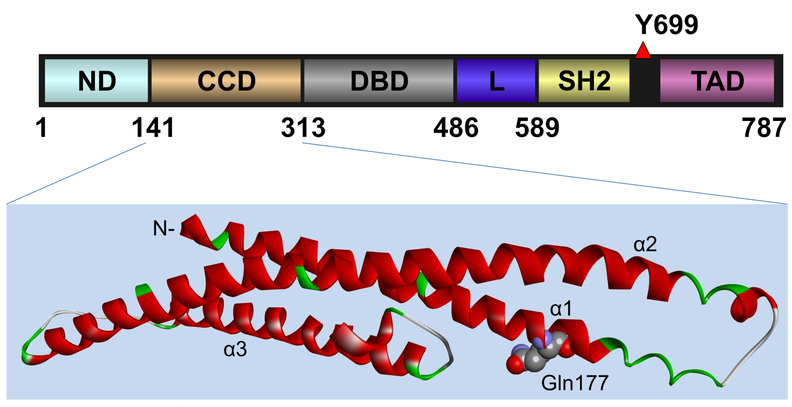


**Fig. 3:** Scheme of the STAT5B protein, domain organisation and location of the Gln177 residue within α-helix 1 [pdb: 1Y1U, STAT5A; D. Neculai et al., J Biol Chem, 2005, 280: 40782–40787]

## Summary

We describe a novel heterozygous p.Gln177Pro STAT5B mutation with potential dominant-negative properties conferring clinical manifestations comparable to reported STAT5B deficient patients but with less severe co-morbidities.

## Normal STAT5B activation

- reconstitution studies and immunoblotting in hGHR transfected HEK293 cells (Fig.4)
- p.Gln177Pro expression and phosphorylation comparable to STAT5B wild-type in response to GH

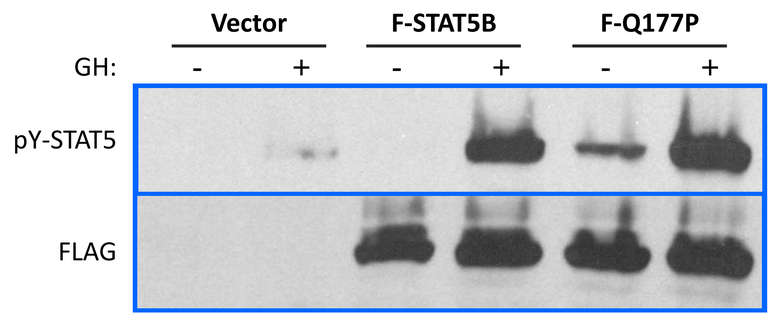


**Fig. 4:** Immunoblots of HEK293 cells expressing proteins shown above the X-ray scans using antibodies indicated on the left

## Normal STAT5B dimerisation

- reconstitution experiments in HEK293 cells, co-immunoprecipitation and immunoblotting (Fig.5)
- p.Gln177Pro retains the capability to dimerise with the wild-type STAT5B in response to GH

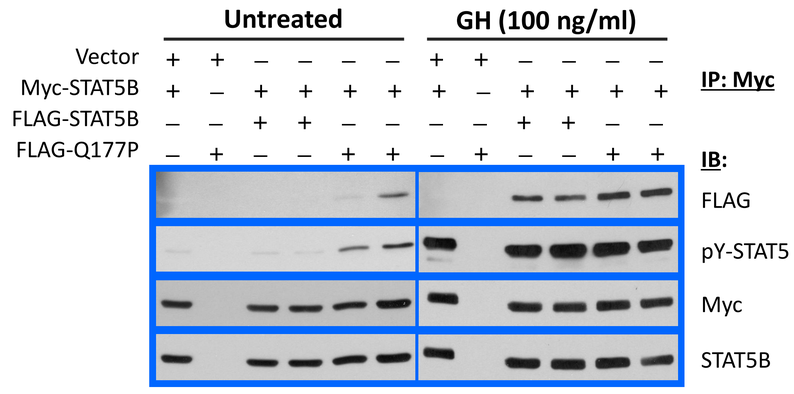


**Fig. 5:** Immunoblots using antibodies indicated on the right after co-transfection (plasmids shown above the X-ray scans) and anti-Myc-STAT5B (WT) immunoprecipitation.

## Abnormal STAT5B trafficking

- reconstitution experiments in HEK293 cells, immunofluorescence and deconvolution microscopy
- p.Gln177Pro does not translocate into the nucleus, neither when transfected alone (data not shown) nor when associated with wild-type STAT5B (Fig. 6)
- wild-type STAT5B is prevented from nuclear translocation when associated with the mutant

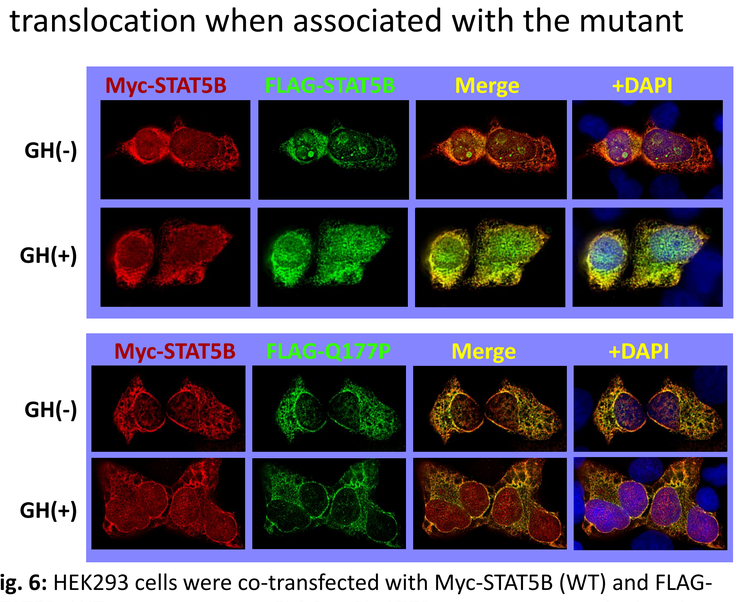


**Fig. 6:** HEK293 cells were co-transfected with Myc-STAT5B (WT) and FLAG-STAT5B (WT; upper panel) or FLAG-p.Glu177Pro (lower panel). Antibodies: monoclonal anti-FLAG, secondary goat-anti-mouse-FITC; polyclonal anti-Myc, secondary goat-anti-rabbit-ALEXA555.

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