P2-480

In vitro functional characterization of *IGFALS* gene variants found in ALS deficient or idiopathic short stature children

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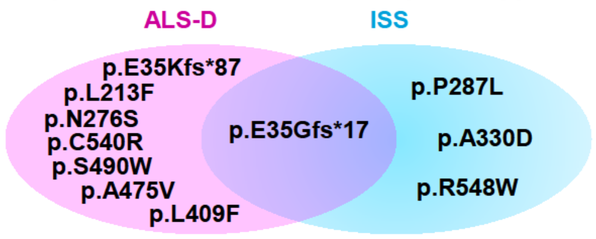
Background

ALS deficient (ALS-D) patients present severe IGF-I and IGFBP-3 deficiencies and variable degree of growth retardation. Heterozygous carriers for *IGFALS* variants, ALS-D relatives or a subset of idiopathic short stature (ISS) children, have levels of IGF-I, IGFBP-3 and ALS intermediate between ALS-D and wildtype (WT) subjects. This supports that *IGFALS* gene variants may affect ALS synthesis, secretion and/or function and could be responsible for the observed phenotype.

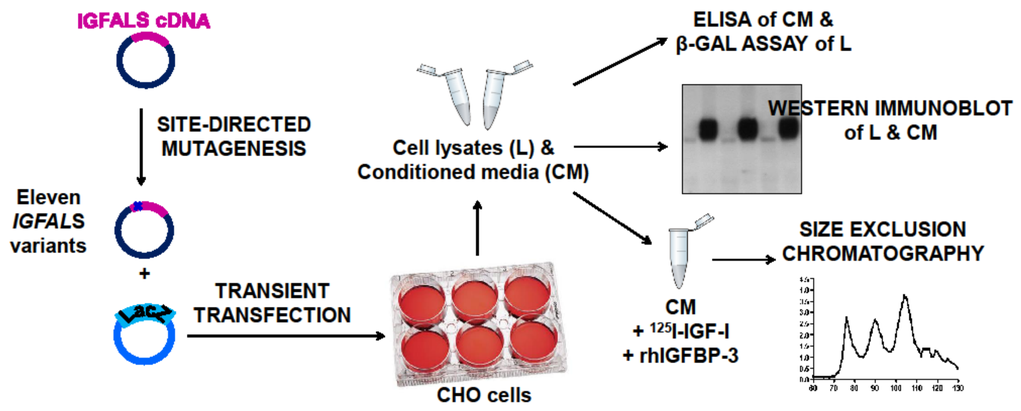


Objective

We aim to study the impact of eleven *IGFALS* gene variants identified in ALS-D or ISS children (p.E35Kfs*87, p.E35Gfs*17, p.L213F, p.N276S, p.P287L, p.A330D, p.L409F, p.A475V, p.S490W, p.C540R, and p.R548W) on ALS protein synthesis and secretion and functional capacity for ternary complex formation *in vitro* (Iv-TCF).

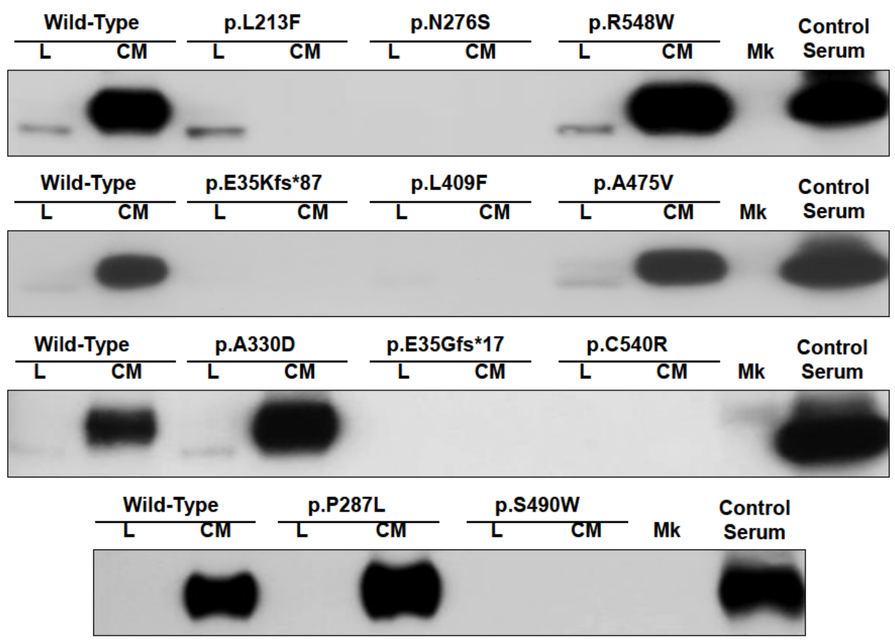
Methods

***IGFALS* gene variants were introduced by site-directed mutagenesis into a plasmid containing the human *IGFALS* cDNA. CHO cells were transiently transfected with WT-*IGFALS* or each of the variants. Cell lysates (L) and conditioned media (CM) were analyzed by Western immunoblot. For each secreted ALS variant, new experiments were carried out and L and CM were collected at 12, 24, 36 and 48 hs after transfection and immunoblotted for ALS. Besides, ALS levels in CM were measured by ELISA at 24 hs post-transfection and corrected by β-galactosidase activity and total protein in L. Iv-TCF was performed with equal amounts of WT-ALS and secreted ALS variants by Superdex 200 size exclusion chromatography.**



Results I

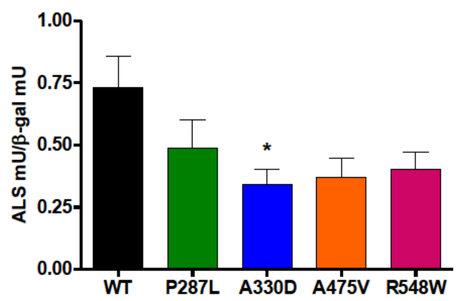
ALS determined by Western immunoblot of cell lysates and conditioned media from CHO cells transfected with WT-*IGFALS* or each variant



Results II

ALS in conditioned media at 24 hs post-trasfection

n ≥ 5 for each variant
ANOVA test (*P*=0.0372)
Tukey's Test (* p<0.05)



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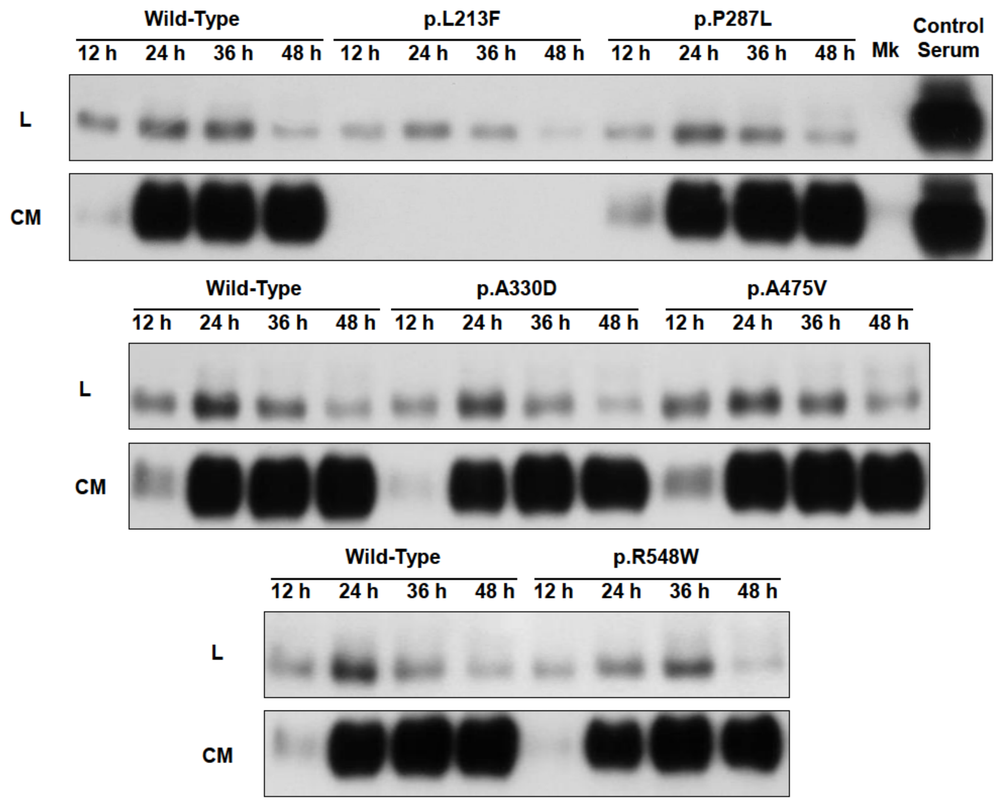
The authors have nothing to disclose.

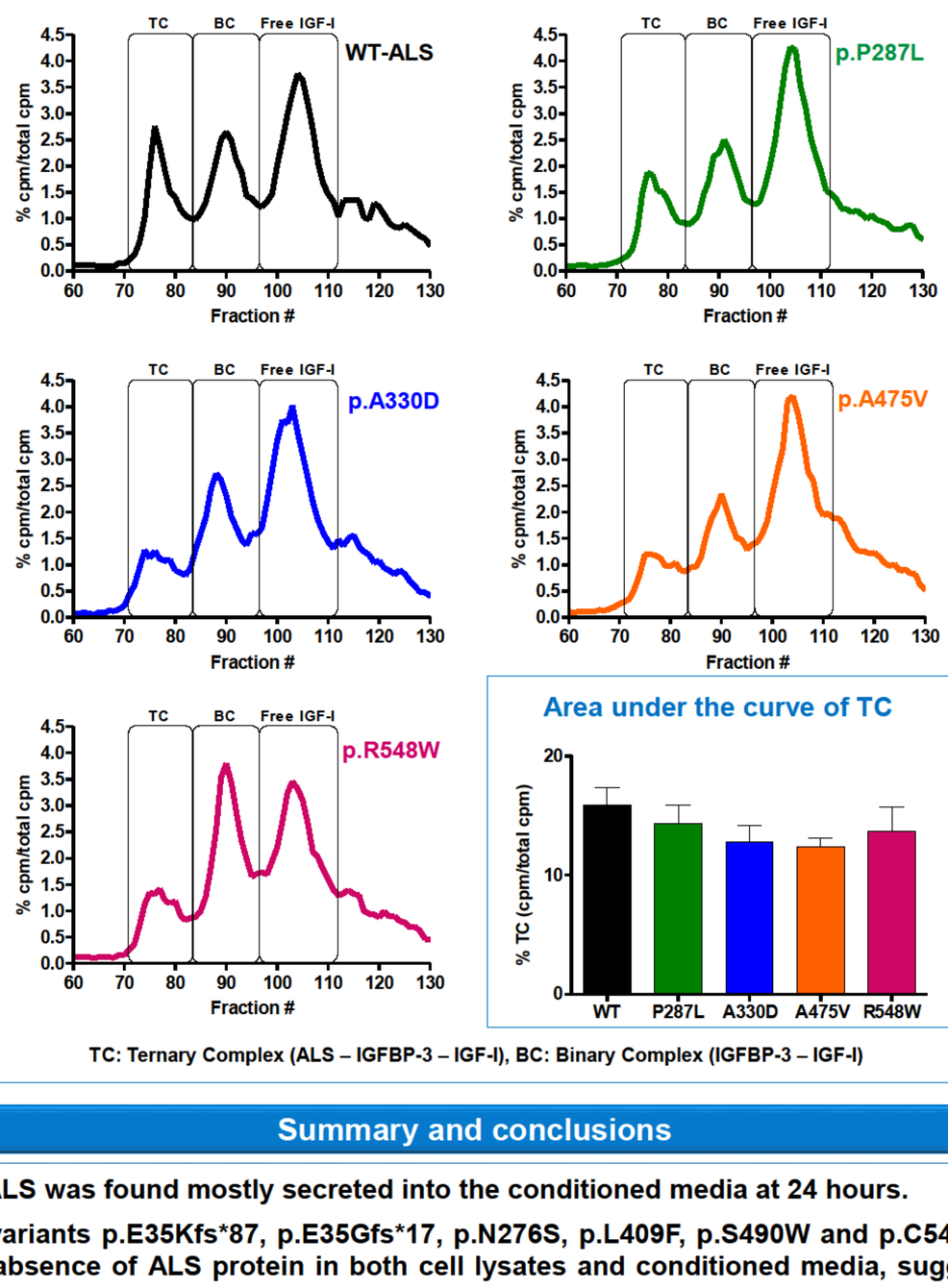
Results III

Temporal study of WT-ALS and ALS variants in L and CM by Western immunoblot



Results IV

Iv-TCF of ALS-containing conditioned media incubated with rhIGFBP-3 and [125I]IGF-I



TC: Ternary Complex (ALS – IGFBP-3 – IGF-I), BC: Binary Complex (IGFBP-3 – IGF-I)

Summary and conclusions

- **WT-ALS was found mostly secreted into the conditioned media at 24 hours.**
- **For variants p.E35Kfs*87, p.E35Gfs*17, p.N276S, p.L409F, p.S490W and p.C540R, we found absence of ALS protein in both cell lysates and conditioned media, suggesting that these variants impair the biosynthesis and/or stability of the protein.**
- **p.L213F-ALS was present in cell lysates but absent in conditioned media, suggesting an impairment on its secretion pathway.**
- **p.P287L-ALS, p.A330D-ALS, p.A475V-ALS and p.R548W-ALS were present in cell lysates and conditioned media and all retain their ability to form TC *in vitro*.**
- **Secreted p.A330D-ALS levels were significantly reduced 24 hs after transfection comparing to WT-ALS.**
- **All but one of the variants found in homozygosis or compound heterozygosis in ALS-D patients were not secreted.**
- **Two variants (p.E35Gfs*17 and p.A330D) found in heterozygosis in ISS patients might have a role in the pathogenesis of their short stature.**

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Growth

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