ALS deficiency due to a novel and two already described *IGFALS* gene mutations in an unusual non consanguineous family: Two compound heterozygous (father and son) and two heterozygous carriers (brother and mother)

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Introduction

Complete ALS deficiency (ALS-D), caused by inactivating mutations in both *IGFALS* gene alleles, presents severe IGF-I and IGFBP-3 deficiencies associated with moderate growth retardation.

Aim

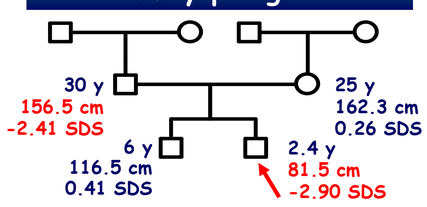
To characterize the molecular defect in a family where the index case and his father presented short stature associated with IGF-I and IGFBP-3 deficiencies.

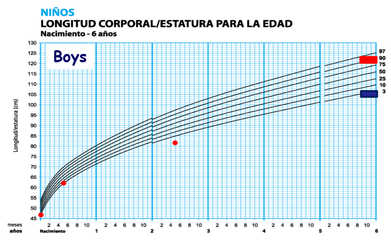
Case report

2.4 year old male boy with short stature

- ✓Height: 81.5 cm (-2.9 SDS)
- ✓Weight: 9.78 kg (-2.7 SDS)
- ✓Born at term (39 w)
  - ✓Birth weight: 2500 g (-2.0 SDS)
  - ✓Birth length: 46.5 cm (-1.7 SDS)
- ✓Non consanguineous family

Family pedigree





Biochemical evaluation

Clinical examination and routine laboratory analysis ruled out non endocrinological causes of short stature.

	Index case (3,7 y)	Reference range
TSH (mIU/ml)	5,03	0,5 - 6,5
FT4 (ng/dl)	1,42	0,8 - 2,2
ATPO (IU/ml)	<10	<20
ACTH (pg/ml)	9	<46
Cortisol (µg/dl)	25,4	6,0 - 21,0
Prolactin (ng/ml)	19,6	2 - 15
LH (mIU/ml)	<0,1	0,05 - 0,30
FSH (mIU/ml)	0,57	0,2 - 2,0
Testosterone (ng/dl)	<10	10 - 32

Arginine-stimulated GH secretion ⟹ Non GH-deficient

	Basal	30 min	45 min	60 min	90 min
GH (ng/ml)	13,3	15,3	4,4	1,6	0,6

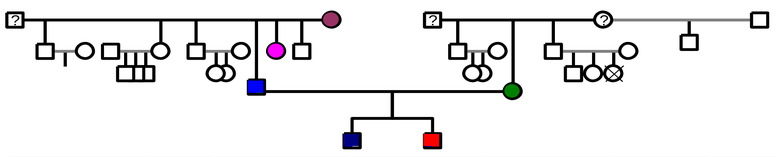
Subject	Age (years)	IGF-I ng/ml (SDS)	IGFBP-3 µg/ml (SDS)	ALS mU/ml
Index case	2.4	< 25	<0.5	<100
	3.2	28 (-2.5)	<0.5	<100
	3.7	27 (-2.6)	<0.5	<100

IGF-I, IGFBP-3 and GH: CLIA (Immulite, Siemmens). ALS: ELISA (Mediagnost)

IGF-I Generation test (3.8 y)

	rhGH 33 µg/kg.d			rhGH 11 µg/kg.d		
	Basal	5th day	8th day	Basal	5th day	8th day
IGF-I (ng/ml)	35	58	52	42	108	103
SDS	-1,97	-0,82	-1,07	-1,56	0,60	0,50
IGFBP-3 (mg/ml)	< 0,5	< 0,5	< 0,5	< 0,5	< 0,5	< 0,5
ALS (mUI/ml)	< 100	< 100	< 100	< 100	< 100	< 100

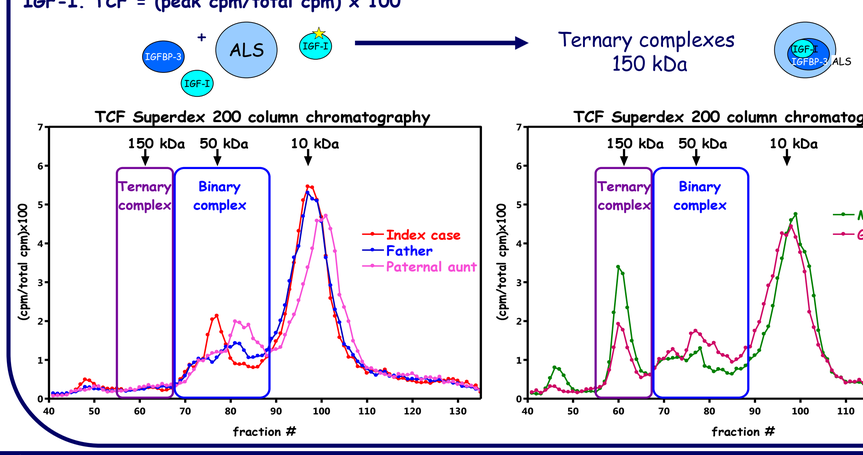
Family auxological and biochemical evaluation



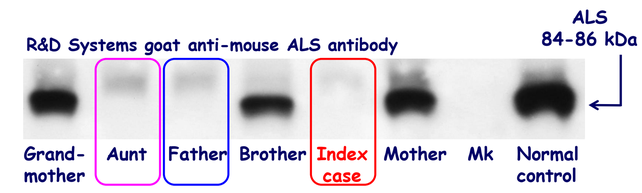
Subject	Age (y)	Height cm (SDS)	IGF-I ng/ml (SDS)	IGFBP-3 µg/ml (SDS)	ALS (mU/ml)	TCF (%)
Index case	3,2	88,6 (-2,30)	28 (-2,50)	<0,5	<100	1,6
Father	30,4	156,5 (-2,40)	25 (-7,94)	<0,5	<100	2,7
Mother	25,8	162,3 (0,26)	128 (-1,52)	2,6 (-2,7)	800	17,9
Brother	6,0	116,5 (0,41)	126 (0,67)	2,0 (-3,0)	700	15,7
Aunt	24,8	153,0 (-1,26)	27 (-7,15)	<0,5	<100	2,5
Grandmother	56,5	156,4 (-0,70)	133 (0,11)	2,7 (-2,0)	1044	10,4

In vitro ternary complex formation (TCF)

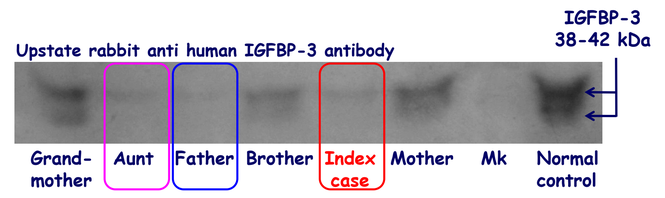
Size exclusion chromatography (Superdex column, GE Healthcare) after incubating patients' sera with ^{125}I-IGF-I. TCF = (peak cpm/total cpm) x 100



ALS Western immunoblot

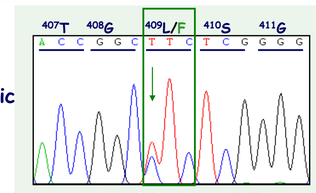


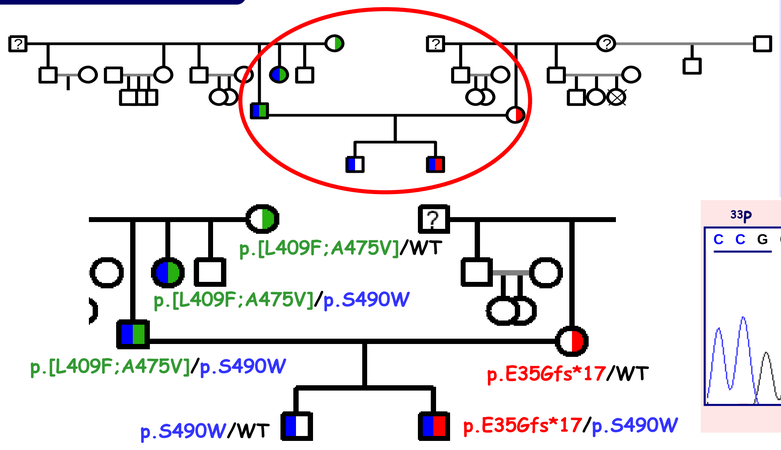
IGFBP-3 Western immunoblot

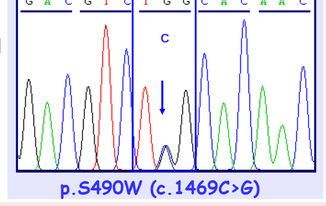


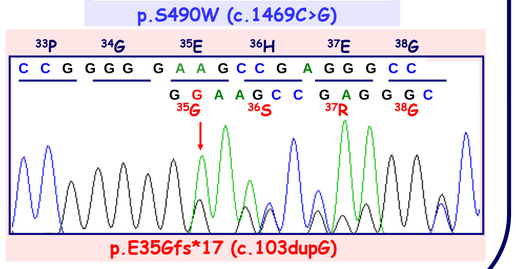
IGFALS gene sequencing

- ✓Written consent for genetic studies was obtained from the parents.
- ✓Genomic DNA was extracted by CTAB method from peripheral blood leukocytes
- ✓The whole *IGFALS* gene was PCR amplified and automatically sequenced (Macrogen, Seoul, Korea).

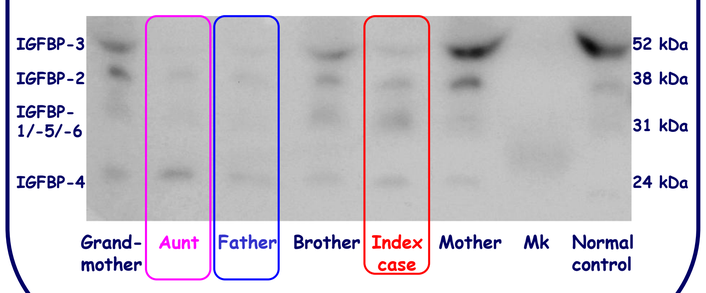








Western ligand blot (^{125}I-IGF-I)



In silico analysis

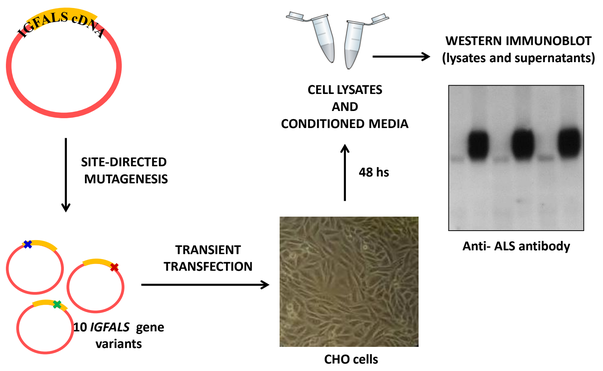
In silico bioinformatic tools were used to predict the effect of each variant on protein function.

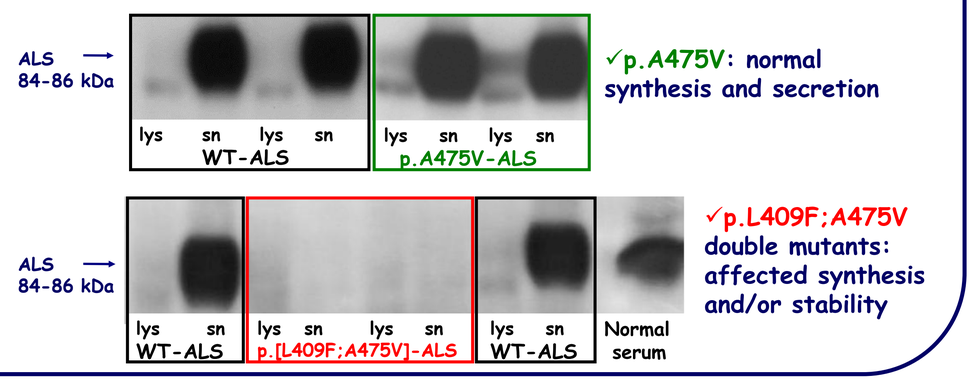
Gene variant	Polyphen 2 prediction (score)	Mutation Taster prediction (probability)	SIFT (score)	MutPred (probability of deleterious mutation)	SNAP prediction (reliability)
p.E35Gfs*17 (c.103dupG)	NA	Disease causing (1)	NA	NA	NA
p.L409F (c.1225C>T)	Probably damaging (0,999)	Disease causing (0,99999)	Damaging (0,02)	0,484	Non-neutral (2)
p.A475V (c.1424C>T)	Benign (0,011)	Polymorphism (0,99999)	Tolerated (0,78)	0,503	Neutral (2)
p.S490W (c.1469C>G)	Probably damaging (0,999)	Polymorphism (0,99946)	Damaging (0,01)	0,667	Non-neutral (4)

The p.S490W, p.L409F and p.E35Gfs*17 variants were predicted to be pathogenic while p.A475V was predicted as benign by *in silico* bioinformatic tools.

In vitro characterization of ALS variants

Three of these *IGFALS* gene variants were expressed *in vitro* in CHO cells. ALS expression was evaluated by WIB.





Summary

Four different *IGFALS* variants were identified in a non consanguineous family.

- ✓p.S490W is novel, while p.L409F, p.A475V and p.E35Gfs*17 have been previously reported in ALS-D patients.
- ✓The index case and his father were both compound heterozygous, sharing the p.S490W variant.
- ✓*In vitro* expression of these variants in CHO cells showed that p.L409F and p.E35Gfs*17 mutants result in affected protein synthesis and/or stability, while p.A475V is normally synthesized and secreted.
- ✓Even if 4 out of 5 bioinformatic tools predict the p.S490W variant as damaging, it remains to be expressed *in vitro* in order to demonstrate its pathogenicity.

Conclusions

- ✓Functional evaluation of these variants by *in vitro* cell culture expression demonstrates that p.E35Gfs*17 and p.L409F are loss-of-function mutations.
- ✓The finding of *IGFALS* gene variants in non consanguineous families suggests that these genetic variants are present in the population and are not under a strong negative selection pressure.
- ✓This is the first report showing fertility is preserved in an adult ALS-D patient.