FUNCTIONAL CHARACTERIZATION OF THREE NOVEL MUTATIONS IN THE *IGF1R* GENE

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Authors have nothing to disclose

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Introduction

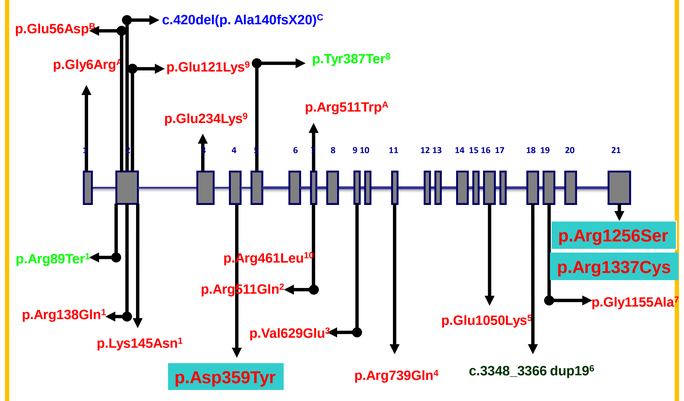
Components of the IGF system are ubiquitously expressed throughout pre- and postnatal life regulating the development of most, if not all, tissues and organs.

Since 2003, several *IGF1R* gene mutations have been associated with varying degrees of intrauterine and postnatal growth retardation and microcephaly due to IGF1 insensitivity in humans.

We have previously reported three novel variants in the *IGF1R* gene: *de novo* p.Arg1256Ser, *de novo* p.Asn359Tyr and p.Tyr865Cys (ENDO 2013, OR20-2)

Aim: To characterize the functional effects of the novel *IGF1R* gene allelic variants.

Gene and Mutations



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nonsense * homozygous
missense
duplication
small deletion

Study Population

- Of 74 SGA patients without catch-up growth, we selected 28 unrelated Argentinean children suspected of having IGF-1 insensitivity according to the following criteria:

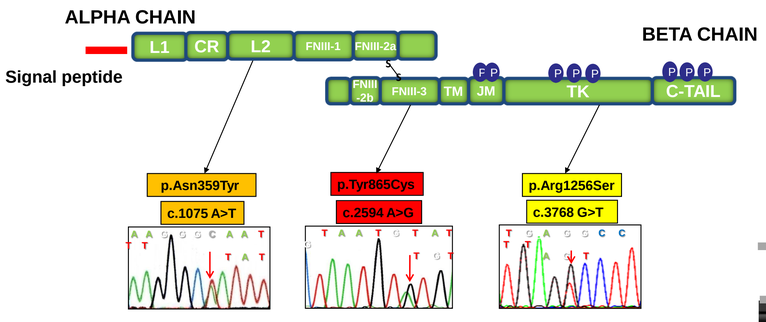
1. Being born SGA
2. Postnatal growth failure
3. Normal karyotype
4. Microcephaly

Cases reports

AT BIRTH	P1	P2	P3	P3 Mother
SEX	male	male	female	female
Gestational Age wk	37	38	38.5	at term
Weight Kg (SDS)	1.9 (-2.98)	2.37 (-2.05)	2.23 (-2.31)	not available
Lenght cm (SDS)	42 (-4.77)	48 (-1.44)	43 (-3.9)	not available
Head Circumference (HC) cm (SDS)	not available	not available	31 (-2.6)	not available
ON ADMISSION				
Chronological Age yr	1.49	2.8	1.9	35
Bone Age yr	1.4	1.49	1.5	
Length / Height cm (SDS)	74 (-2.17)	81.2 (-3.19)	77.7 (-2.25)	161 (0)
Weight Kg (SDS)	7.75 (-3.33)	10.5 (-2.56)	7.91 (-2.97)	
HC cm (SDS)	42.5(-4)	46.2 (-3)*	43 (-2.89)	52.2 (-2.1)
MENTAL DEVELOPMENT	Mild delay	Normal	Normal	
ADDITIONAL FINDINGS	Clinodactyly Triangular face Thin lips Long filtrum Forehead	Forehead Broad nasal bridge		
KARYOTYPE	46 XY	46 XY	46 XX	
LABORATORY FINDINGS				
IGF1 ng/ml (SDS)	231 (+2.94)	58 (+0.54)	211 (+2.43)	
IGFBP3 mg/l (SDS)	5.2 (+3.37)	2.6 (-0.20)	4.7 (+2.76)	
Basal serum GH ng/ml	8	0.27	1.18	
Maximun GH peak ng/ml #		9.6		
OGTT	normal	normal	normal	
HOMA	0.4	0.18	0.89	

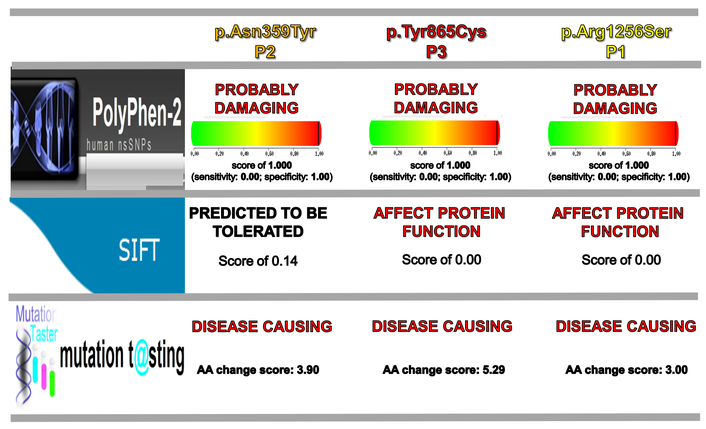
* at 3.2 yr chronological age # after Arginine stimulation

Molecular Studies



Species	Patient 2	Patient 3	Patient 1
Human	QMLQGCTIFKGNLLINIRRG	EPENPNGLILMYEIKYGSQSQ	MRMCWQYNPKMRPSFL
Mutated	QMLQGCTIFKGYLLINIR RG	EPENPNGLILMCEIKYGSQSQ	MRMCWQYNPKMSPSFL
Chimp	QMLQGCTIFKGNLLINIRRG	EPENPNGLILMYEIKYGSQSQ	MRMCWQYNPKMRPSFL
Rhesus	QMLQGCTIFKGNLLINIRRG	EPENPNGLILMYEIKYGSQSQ	MRMCWQYNPKMRPSFL
Mouse	QMLQGCTILKGNLLINIRRG	EPENPNGLILMYEIKYGSQSQ	MRMCWQYNPKMRPS
Chicken	QMLQGCTILKGNLLINIRRG	EPTNPNGLILMYEIKYGQHGE	MRMCWQYNPKMRPS
Xenopus	NLQLNIRKG	EPKRPNGLILMYEIEYKQ QGE	MRMCWQYNPKMRPSFLEI
Zebrafish	VIKG NLQINIRRG	EP LHPNGL ILMYEIKYRLGTEA	CWQYNPKMRPSFLEI
Fugu	T VIDGNLDINIRHG	EP IT PNG LILMYEVKFRPGNE	MRMCWQYNPKMRPSFLEI

***In silico* tools were applied to predict the functional effects of the novel variants.**



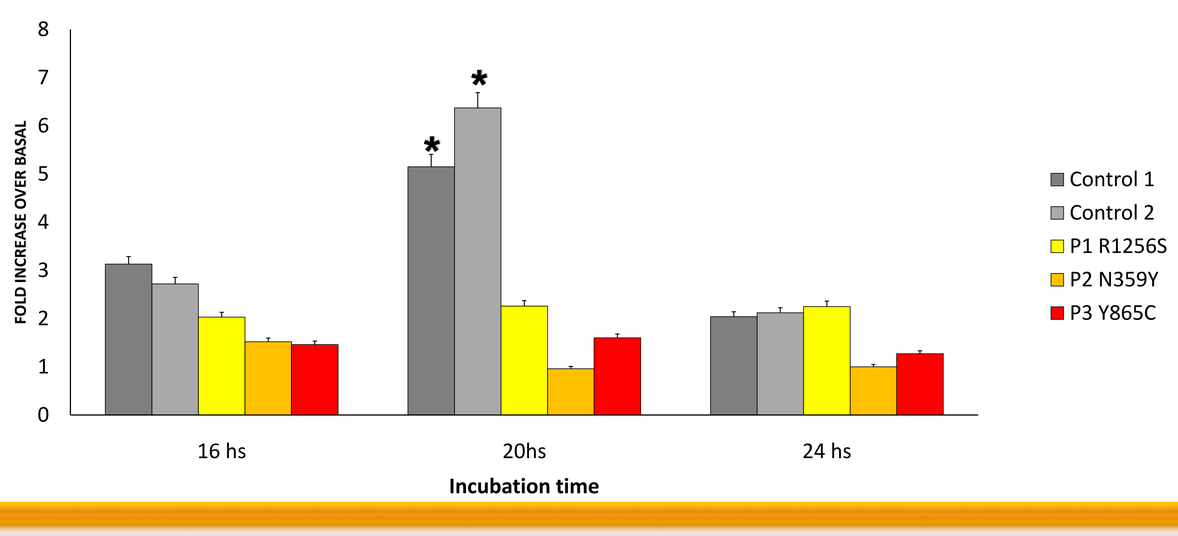
Functional Studies (*in vitro* assays)

Fibroblast cell primary culture

Fibroblast cultures were established from skin biopsies obtained from the abdomen zone from patients affected by the variations (P1, P2 and P3) and two control subjects (C1 and C2). Cell cultures were maintained in Dulbecco's modified Eagle medium and F12 (DMEM/F12, SIGMA, Buenos Aires, Argentina) containing 10 or 20% fetal bovine serum (FBS), at 37 °Cina humidified atmosphere with 5% CO2. Fibroblasts were subcultured for 3 passages and then stored in liquid N until the time of performing the assay. Finally, all fibroblasts were subcultured once more to collect the necessary cell number for the study. All studies were performed at passage 4. Fibroblasts were stimulated with different concentrations of IGF-1, and the highest response was observed at 50 ng/ml.

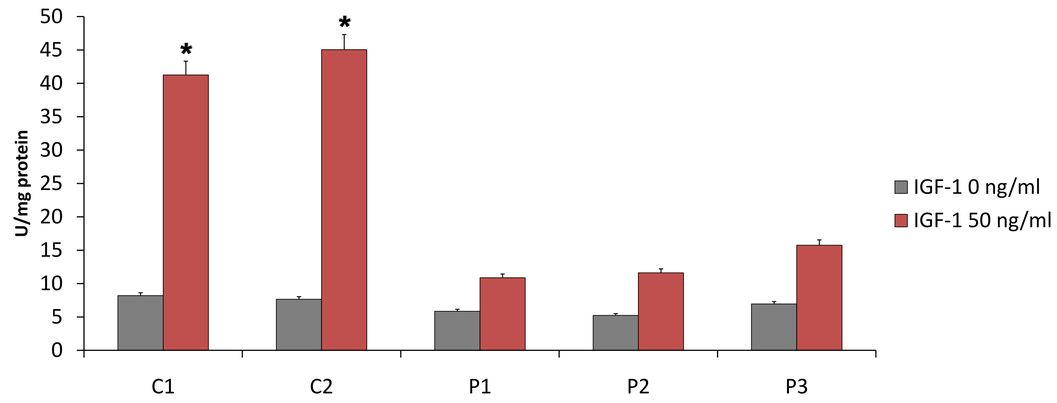
IGF1-dependent DNA synthesis assay (3[methyl-H] Thymidine incorporation)

Fibroblasts were stimulated with 50 ng/ml of IGF-1 for 16, 20, and 24 h. [Methyl- 3 H] thymidine (1 mCi/ml) was added for 4 h prior to basal conditions and to 12, 16, 20 h of IGF-1 treatment.
A significant increase of ^{3}[H] thymidine incorporation was observed after 20 hours of IGF-1 treatment in C1 and C2 ($p<0.05$ by ANOVA and Student's t Test). No significant increase was observed in P1, P2, and P3.
Results are expressed as fold increase over basal X ± SD.
*P < 0.05 20 h vs 16 and 24 h.



AKT phosphorylation stimulation by IGF-1 (Phospho-Akt (Ser473) STAR ELISA Kit (Millipore)

A total of 80000 cells/wells were incubated in 24-well plates and stimulated with 50 ng/ml of IGF-1. The fibroblasts were lysed and the protein concentration was measured by Bradford assay.
AKT phosphorylation was measured with phospho-Akt (Ser473) STAR ELISA Kit (Merk Millipore, Tecnolab, Buenos Aires, Argentina). The entire assay was performed in duplication. Results are
expressed as units per mg of protein.
Akt phophorylation was significantly stimulated in the control subjects by IGF-1 ($P < 005$ by ANOVA and Bonferroni tests). No significant stimulation in the PI3K/Akt pathway was observed in P1, P2 and P3.
*P < 005 vs basal conditions.



Discussion

We characterized three novel heterozygous mutations, *de novo* p.Arg1256Ser (P1), *de novo* p.Asn359Tyr(P2), and familial p.Tyr865Cys (P3) in the *IGF1R* gene that inhibit cell proliferation induced by IGF-1 and affect IGFR signal transduction in patients' fibroblast cultures. These findings strongly suggest that these mutations lead to failure of the IGF1R and cause the phenotype of pre- and postanatal growth retardation and microcephaly.

Using this approach, we found 3/28 affected patients with mutations in the IGF1R (estimated frequency 10.7%), which reinforces the importance of measuring head circumference in the evaluation of SGA and short-statured patients.