FUNCTIONAL CHARACTERIZATION OF THREE NOVEL MUTATIONS IN THE IGF1R GENE

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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.Arg1255Ser, de novo p.Asn395Tyr and p.Try865Cys (ENDO 2013, OR20-2).

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

Cases reports

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<th>Molecular Studies</th>
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<td>Fibroblast cell primary culture</td>
<td>Functional Studies (in vitro assays)</td>
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<td>Fibroblasts 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°C in a 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.</td>
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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-3H] thymidine (1 mCi/ml) was added for 4 h prior to basal and conditions to 12, 16, 20 h of IGF-1 treatment. A significant increase of [3H] 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 vs 16 h and 24 h.

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. Postnatral growth failure
  3. Normal karyotype
  4. Microcephaly

Discussion

We characterized three novel heterozygous mutations, de novo p.Arg1255Ser (P1), de novo p.Asn395Tyr(P2), and familial p.Try865Cys (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 IGFR1 and cause the phenotype of pre- and postnatal 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.