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Low Prevalence of Autoantibodies to the Insulin-like Growth Factor I Receptor in Children with Short Stature

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ABSTRACT. Inhibition of IGF-I action by circulating IGF-I receptor autoantibodies is a potential mechanism of IGF-I resistance in growing children. To define the prevalence of IGF-I receptor antibodies in short-statured children, we have examined serum and plasma samples from a well-characterized group of 34 short, prepubertal, growth hormone-sufficient children and three growth hormone-deficient children. IGF-I receptor purified from human placental membranes was radioiodinated by the solid phase radioiodination method. Serum from a patient with severe insulin resistance immunoprecipitated 28.9–44.7% of the ^{125}I-labeled IGF-I receptor. The ranges (mean ± 3 SD) of ^{125}I-labeled IGF-I receptor immunoprecipitated by 1:10 diluted and by undiluted nonimmune human serum were 1.99 ± 0.63% and 4.42 ± 1.32%, respectively. Immunoprecipitation of the ^{125}I-labeled IGF-I receptor by eight samples from six children was > 3 SD above the mean when assayed at a 1:10 dilution. Nevertheless, when assayed undiluted, only one of these samples immunoprecipitated slightly more ^{125}I-labeled IGF-I receptor than nonimmune serum. We conclude from these data that immunoprecipitating autoantibodies to the IGF-I receptor are not commonly present in short-statured children. (*Pediatr Res* 32: 455–459, 1992)

IGF-I is a major mammalian postnatal growth factor that is regulated at least in part by pituitary growth hormone (1). In responsive cells, IGF-I interacts with the IGF-I receptor, a heterotetrameric glycoprotein composed of two disulfide-linked $\alpha\beta$ dimers. The IGF-I receptor is a member of the tyrosine kinase family of growth factor receptors (2) and is structurally homologous to the insulin receptor (3). IGF-I binding to the IGF-I receptor causes the transmembrane activation of the intrinsic tyrosine kinase activity of the IGF-I receptor, which, by mechanisms that are not yet well understood, results in both rapid insulin-like effects on intermediary metabolism and more long-term effects on growth (4).

Autoimmunity to receptors within the endocrine system has been well documented (5). In patients with severe insulin resistance type B, autoantibodies to the insulin receptor have been shown to inhibit insulin action (6). Because of the close homology between the insulin receptor and the IGF-I receptor, Tappy *et al.* (7) examined sera from patients with disorders reported to have an increased prevalence of insulin receptor antibodies to determine if these sera contained autoantibodies to the IGF-I receptor. Eleven of the 141 patient sera tested (7.8%) immunoprecipitated labeled IGF-I receptor. Sera from seven patients contained IgG autoantibodies that did not inhibit IGF-I binding, whereas sera from four patients contained antibodies that bound to the IGF-I receptor at or near the IGF-I binding site and inhibited IGF-I binding. Two of these patients had elevated plasma IGF-I levels, suggesting that they were IGF-I resistant.

Inhibition of IGF-I action by IGF-I receptor autoantibodies would be anticipated to have a significant effect on growth in children. We previously reported (8) the whole body nitrogen kinetics and their relationship to growth in a well-characterized group of 34 short, prepubertal, growth hormone-sufficient children and three growth hormone-deficient children. No mutations were detected in the growth hormone or IGF-I genes of any patients in this study, and the fibroblasts from the 34 non-growth hormone-deficient children responded normally when challenged with recombinant human IGF-I. In this report, we examine serum and plasma from these patients to define the prevalence of immunoprecipitating IGF-I receptor antibodies in short-statured children.

MATERIALS AND METHODS

Materials. Iodine-125 was purchased from Du Pont/New England Nuclear Research Products (Boston, MA) (17.4 Ci/mg I) or from Amersham Corp. (Arlington Heights, IL) (14.5 Ci/mg I). Enzymobead Radioiodination Reagent and Bio-Gel P-2 (200–400 mesh) were produced by Bio-Rad Laboratories (Richmond, CA). Sephacryl S-200 Superfine was obtained from Pharmacia LKB Biotechnology (Piscataway, NJ). Triton X-100, BSA, leupeptin, phenylmethylsulfonyl fluoride, and aprotinin were purchased from Sigma Chemical Co. (St. Louis, MO). Serum from a patient with severe insulin resistance who had high titers of antibodies to the insulin receptor was a gift of Dr. James R. Gavin III (University of Oklahoma Health Sciences Center, Oklahoma City, OK). The MAb to the IGF-I receptor, αIR-3 (0.5 mg/mL), was generously provided by Dr. Steven Jacobs (The Wellcome Research Laboratories, Research Triangle Park, NC). Pansorbin (fixed protein A-bearing *Staphylococcus aureus* cells, 10% wt/vol, binding capacity of 2.0–2.4 mg human IgG/mL) was produced by Behring Diagnostics (San Diego, CA). These cells have been reported to bind IgG1, IgG2, IgG4, IgA2, and some IgM in human serum (9).

Subjects. Thirty-seven prepubertal children between the ages of 4.2 and 13.8 y and > 2 SD below the mean height for age were recruited from the Washington University Pediatric Endocrinology Clinic. Their clinical characteristics have been previously summarized (8). Thirty patients had normal levels of serum IGF-I, and seven patients had serum IGF-I levels > 2 SD below

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the mean for their age or pubertal status and gender. Informed consent had been obtained from all families according to procedures approved by the Washington University Human Studies Committee.

Purification of IGF-I receptor from human placental membranes. The high-affinity IGF-I receptor was purified from normal full-term human placentas using wheat germ agglutinin-Sepharose chromatography, insulin affinity chromatography, and IGF-I affinity chromatography, as previously described (10).

Radioiodination of purified IGF-I receptor. Purified IGF-I receptor was concentrated four times in a Centricon 30 microconcentrator (Amicon Corp., Danvers, MA) and then dialyzed against PBS at 4°C on an MF-Millipore membrane filter (0.05 μm). Radioiodination of the receptor (0.5–2.1 pmol) was performed with Enzymobead Radioiodination Reagent, according to the manufacturer's instructions. As shown in Figure 1, the reaction mixture, containing the labeled IGF-I receptor, and a 50-μL wash of the reaction vial were applied to a Bio-Gel P-2 column (1 × 24 cm) equilibrated in PBS with 0.1% Triton X-100 to separate bound from free ^{125}I. Fractions (0.5 mL) were collected at a flow rate of 9 mL/h, and radioactivity was monitored by counting 1-μL aliquots in a Micromedic Automatic Gamma Counter (Micromedic Systems, Inc., Horsham, PA). Void volume fractions from the Bio-Gel P-2 column were pooled and applied to a Sephacryl S-200 column (1.5 × 46 cm) equilibrated in PBS with 0.1% Triton X-100. Fractions (1.0 mL) were collected at a flow rate of 18 mL/h, and radioactivity was monitored by counting 5-μL aliquots. Peak fractions containing the ^{125}I-labeled IGF-I receptor were pooled and stored at −20°C until use. The ^{125}I-labeled IGF-I receptor had a sp act of 1.3–8.5 μCi/pmol and was used within 2 wk of its preparation.

SDS-PAGE and autoradiography. SDS-PAGE was performed according to the method of Laemmli (11) with 7.5% or 3–10% gradient acrylamide resolving gels. Electrophoresis sample buffer was added to samples at a final concentration of 1% SDS or 2% SDS with 5% β-mercaptoethanol, and samples were heated at 100°C for 2 min or 5 min, respectively. Molecular weight standards (Bio-Rad) included myosin (200 000), β-galactosidase (116 250), phosphorylase b (97 400), BSA (66 200), and ovalbumin (42 700). Prestained molecular weight standards (Bio-Rad) included all of the above except phosphorylase b. Gels were either fixed in 50% methanol/10% acetic acid for 1 h or fixed, stained, and destained as previously described (12). The gels were then dried and exposed to Kodak XAR-5 x-ray film in the presence of a Du Pont Cronex Lightning-Plus intensifying screen at −70°C. Autoradiographs were scanned with an LKB Ultroscan XL laser densitometer.

Immunoprecipitation of IGF-I receptor. Before use, Pansorbin was washed twice with PBS with 1 mg/mL BSA (washing buffer). ^{125}I-labeled IGF-I receptor was then precleared by incubation for 30 min at 4°C with an equal volume of 10% Pansorbin in washing buffer with 0.1% Triton X-100 and protease inhibitors (1 μg/mL leupeptin, 0.2 mM phenylmethlysulfonyl fluoride, and 20 μg/mL aprotinin) and centrifugation at 15 500 × *g*. The supernatant was removed and used directly in the immunoprecipitation assay. In eight experiments, 1.8–4.8% of the ^{125}I label was removed from the ^{125}I-labeled IGF-I receptor by preclearance with 10% Pansorbin.

Precleared receptor (~25 000 cpm/45 μL) was incubated overnight at 4°C with 5 μL diluted or undiluted patient serum or plasma, nonimmune human serum, or serum from a patient with severe insulin resistance. Sample dilutions used are described in the legends to Figures 2 and 3 and were prepared in PBS. To precipitate immune complexes, samples were incubated with 75 μL of a 20–40% suspension of Pansorbin in washing buffer for 30 min at 4°C. Because different Pansorbin preparations were used in our experiments, each lot was tested to determine the percentage of Pansorbin required to maximally precipitate receptor-antibody complexes using undiluted immune serum. After centrifugation at 15 500 × *g*, the supernatants were removed and the pellets were washed three times in washing buffer. The supernatants, washes, and pellets were then counted to determine the percentage of ^{125}I-labeled IGF-I receptor immunoprecipitated.

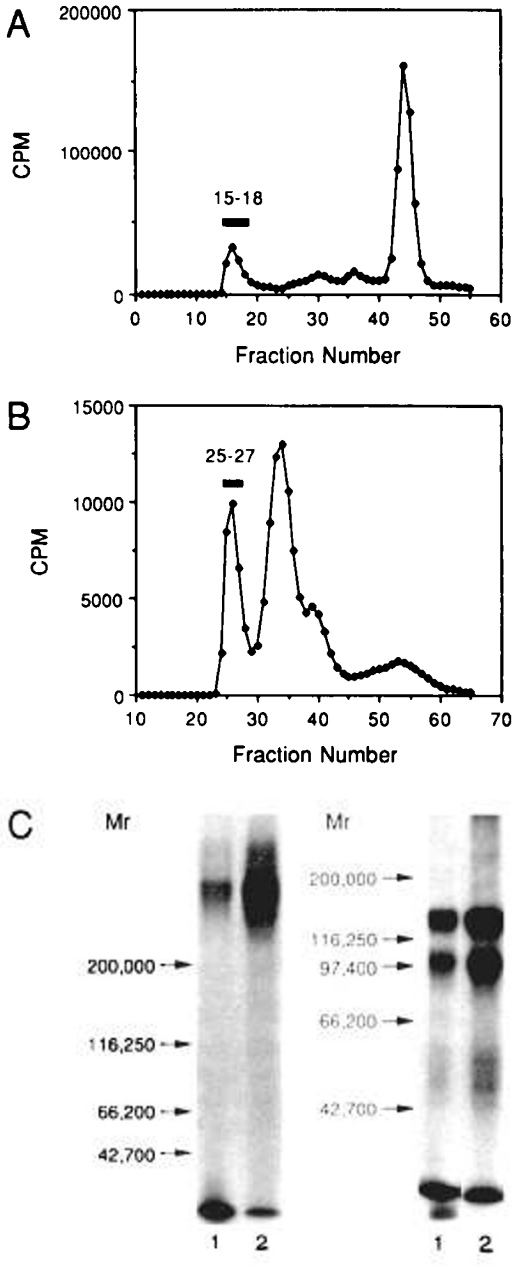

Fig. 1. Column chromatography profiles and SDS-PAGE analysis of the ^{125}I-labeled IGF-I receptor. Purified IGF-I receptor was concentrated, dialyzed, and radioiodinated as described in Materials and Methods. *A*, elution profile from the Bio-Gel P-2 gel filtration column. Void volume fractions (*15–18*) from the Bio-Gel P-2 column were pooled and applied to a Sephacryl S-200 column. *B*, elution profile from the Sephacryl S-200 gel filtration column. Fractions 25–27 containing the ^{125}I-labeled IGF-I receptor were pooled and used in the immunoprecipitation studies. *C*, autoradiographs of the ^{125}I-labeled IGF-I receptor analyzed by SDS-PAGE. *Left lanes*, samples analyzed under nonreducing conditions using a 3–10% gradient acrylamide resolving gel. The migration of prestained molecular weight standards is indicated. *Right lanes*, samples analyzed under reducing conditions using a 7.5% acrylamide resolving gel. The migration of molecular weight standards is indicated. *Lanes 1*, 5-μL aliquot of pooled fractions 15–18 from the Bio-Gel P-2 column. *Lanes 2*, 5-μL aliquot of pooled fractions 25–27 from the Sephacryl S-200 column.

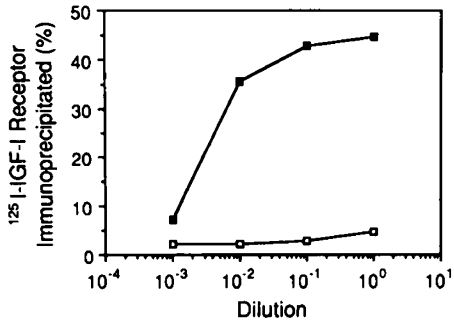

Fig. 2. Immunoprecipitation of IGF-I receptor by immune and nonimmune human serum. Forty-five μL (25 075 cpm) of precleared ^{125}I-labeled IGF-I receptor in washing buffer with 0.1% Triton X-100 and protease inhibitors were incubated overnight at 4°C with 5 μL of serum from a patient with severe insulin resistance (■) or with 5 μL of nonimmune human serum (□) at the dilutions indicated. Immune complexes were precipitated by the addition of 75 μL of 20% Pansorbin in washing buffer, as described in Materials and Methods. Results are expressed as cpm immunoprecipitated/total cpm recovered × 100%.

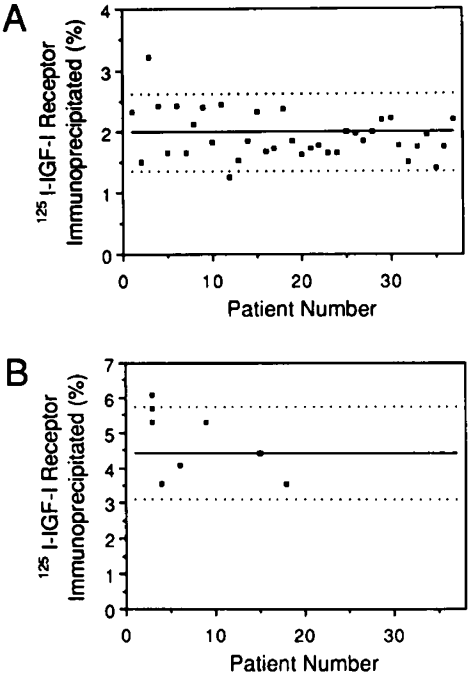

Fig. 3. Immunoprecipitation of IGF-I receptor by serum or plasma from short-statured children. Immunoprecipitation of the ^{125}I-labeled IGF-I receptor was performed as described in Materials and Methods. Results are expressed as cpm immunoprecipitated/total cpm recovered × 100%. The *solid* and *dotted lines* indicate the percentage (mean ± 3 SD) of ^{125}I-labeled IGF-I receptor immunoprecipitated by 1:10 diluted or by undiluted nonimmune human serum. *A*, immunoprecipitation of the ^{125}I-labeled IGF-I receptor by a 1:10 dilution of patient serum or plasma. Data are plotted as the mean percentage of ^{125}I-labeled IGF-I receptor immunoprecipitated by three samples obtained at different times from each patient, except for patient no. 29, from whom only two samples were obtained. *B*, immunoprecipitation of the ^{125}I-labeled IGF-I receptor by selected undiluted patient serum or plasma. Patient samples in which ^{125}I-labeled IGF-I receptor immunoprecipitation was > 3 SD above the mean when assayed at a 1:10 dilution were tested undiluted. Data are plotted as the mean percentage of ^{125}I-labeled IGF-I receptor immunoprecipitated by each sample in one to three assays.

Immunoprecipitation of the precleared IGF-I receptor by αIR-3 was performed as previously described (13), except that washing buffer with 0.1% Triton X-100 and protease inhibitors was used in the incubation.

RESULTS

Radioiodination of purified IGF-I receptor. Previous experiments in our laboratory had suggested that radioiodination of the purified IGF-I receptor under some conditions caused reduction of intersubunit disulfide bonds. The solid phase radioiodination method, however, resulted in the generation of ^{125}I-labeled IGF-I receptor that remained intact for use in immunoprecipitation studies. Figure 1 shows the column chromatography profiles and SDS-PAGE analysis of labeled IGF-I receptor after radioiodination using the Enzymobead Radioiodination Reagent. The ^{125}I-labeled IGF-I receptor elutes in the void volume of the Bio-Gel P-2 column and is effectively separated from free ^{125}I (*panel A*). Human serum albumin is the major contaminant in our purified receptor preparations (10) and is effectively removed by gel filtration on the Sephacryl S-200 column (*panel B*). SDS-PAGE and autoradiography were performed to demonstrate that the labeled IGF-I receptor was intact after radioiodination and to verify its purity (*panel C*). Under nonreducing conditions (*left lanes*), a single broad band with an apparent M_r > 300 000 is resolved, indicating that the IGF-I receptor remains in its heterotetrameric form after radioiodination. Under reducing conditions (*right lanes*), the α- and β-subunits of the IGF-I receptor with apparent M_r = 131 600 and 100 400, respectively, and a less prominent band with an apparent M_r = 44 000, which represents the β′-subunit (13), are resolved. Densitometric scans of the autoradiographs of ^{125}I-labeled IGF-I receptor after Sephacryl S-200 column chromatography (*lanes 2*) demonstrated that the amount of label associated with the IGF-I receptor under nonreducing and reducing conditions was 74.8% and 73.7%, respectively.

Immunoprecipitation of IGF-I receptor. Serum from a patient with severe insulin resistance significantly blocked IGF-I binding to renal basolateral membranes (personal communication, Gavin III JR) and was used as a positive control in our studies. Maximal immunoprecipitation of the ^{125}I-labeled IGF-I receptor by this serum was achieved at a 1:10 dilution, as demonstrated in Figure 2. In five experiments, maximal immunoprecipitation of the IGF-I receptor by undiluted immune serum ranged from 28.9 to 44.7%. Precipitation of the IGF-I receptor by undiluted nonimmune human serum ranged from 3.86 to 4.82%. Immunoprecipitation of the IGF-I receptor was also performed using αIR-3, a mouse MAb specific to the human IGF-I receptor (14). Maximal immunoprecipitation of the ^{125}I-labeled IGF-I receptor by this antibody (5 μL undiluted) was 55%. The comparable maximal immunoprecipitation achieved with serum from a patient with severe insulin resistance and with αIR-3 indicates that the immune serum contains antibodies that cross-react with the ^{125}I-labeled IGF-I receptor.

Immunoprecipitation of IGF-I receptor by serum or plasma from children with short stature. Serum and plasma from 37 prepubertal children between the ages of 4.2 and 13.8 y and > 2 SD below the mean height for age were tested for their ability to immunoprecipitate purified ^{125}I-labeled IGF-I receptor. Samples had been collected before the treatment protocol described previously (8). Figure 3*A* shows the mean percentage of ^{125}I-labeled IGF-I receptor immunoprecipitated by 1:10 dilutions of serum or plasma samples from each patient. Three samples obtained at different times from each patient were examined, except for patient no. 29, from whom only two samples were obtained. The range of ^{125}I-labeled IGF-I receptor immunoprecipitated by a 1:10 dilution of nonimmune human serum was 1.99 ± 0.63% (mean ± 3 SD, n = 6), as indicated by the *solid* and *dotted lines*. Three samples from patient no. 3 immunoprecipitated 3.12%, 2.71%, and 3.82% of the ^{125}I-labeled IGF-I receptor. In addition,

immunoprecipitation of ^{125}I-labeled IGF-I receptor by a single sample from patients no. 4, 6, 9, 15, and 18 was > 3 SD above the mean. Five of these six patients had normal levels of serum IGF-I. These samples were further tested undiluted, as shown in Figure 3*B*. The range of ^{125}I-labeled IGF-I receptor immunoprecipitated by undiluted nonimmune human serum was 4.42 ± 1.32% (mean ± 3 SD, $n = 3$), again as indicated by the *solid* and *dotted lines*. Immunoprecipitation of ^{125}I-labeled IGF-I receptor by seven of the eight samples from six patients was within 3 SD of the mean. One of three samples from patient no. 3 immunoprecipitated slightly more ^{125}I-labeled IGF-I receptor than nonimmune human serum. We conclude from these data that among this patient group of short-statured children no significant circulating immunoprecipitating antibodies to the IGF-I receptor are present.

DISCUSSION

IGF-I resistance has been described in only a few children with growth failure. Bierich *et al.* (15) reported that fibroblasts from a short child with growth failure bound 50% less ^{125}I-labeled IGF-I than normal fibroblasts, although the structure and function of the IGF-I receptor in this child's fibroblasts were not directly examined. Heath-Monnig *et al.* (16) reported that fibroblasts from a child with short stature were significantly less responsive to stimulation by IGF-I in α-aminoisobutyric acid uptake assays. We have found that the resistance to IGF-I action in this patient's fibroblasts is caused by an abnormal production and/or cell association of IGF binding proteins (17). Potential mechanisms of IGF-I resistance include not only abnormalities in IGF-I receptor structure/function and abnormalities in the modulation of IGF-I action by IGF binding proteins but also inhibition of IGF-I action by circulating autoantibodies to IGF-I, to the IGF-I receptor, or to one or more IGF binding proteins.

Autoantibodies to the homologous insulin receptor are not widespread. Insulin receptor antibodies were first identified in the plasma of patients with severe insulin resistance type B associated with acanthosis nigricans (6). Although some antibodies from these patients inhibit insulin binding, other antibodies mimic insulin action *in vitro* and *in vivo*. Recently, insulin receptor autoantibodies have also been reported in several patients who developed severe fasting hypoglycemia without a previous history of insulin resistance (18). The balance between agonistic and antagonistic antibodies, the titer of antibodies, and/or the chronicity of exposure may be important determinants in the clinical response to insulin receptor antoantibodies (18). Although insulin receptor autoantibodies account for only a small number of patients with diabetes and/or hypoglycemia in the general population, studies of these antibodies have provided important insights into insulin action.

We undertook the present study to define the prevalence of autoantibodies to the IGF-I receptor in growing children. Kasuga *et al.* (19) reported that sera from five of seven patients with severe insulin resistance and acanthosis nigricans immunoprecipitated both placental insulin and IGF-I receptors. Autoantibodies to the IGF-I receptor were also found in sera from 11 patients with a variety of disorders reported to have an increased prevalence of insulin receptor antibodies (7). In this study, two sera from patients with severe insulin resistance type B were found to immunoprecipitate the labeled IGF-I receptor and to inhibit IGF-I binding. Maximal immunoprecipitation of ^{125}I-labeled IGF-I receptor by these sera using a second antibody procedure was ~14% and ~40%, which is similar to the immunoprecipitation achieved with the immune serum used as a positive control in our study. Immunoprecipitation of ^{125}I-labeled IGF-I receptor by sera from eight of the remaining nine patients was only slightly greater than 3 SD above the mean when compared to immunoprecipitation by sera from control subjects. This study did not report any clinical effects related to IGF-I receptor antibodies in two patients (one of 31 with rheumatic disorders and one of seven with polycystic ovary syndrome) with modestly elevated IGF-I levels and did not include growing children, in whom inhibition of IGF-I action by IGF-I receptor autoantibodies would be anticipated to have significant clinical effects. We therefore examined serum and plasma samples from a well-characterized group of children with short stature. Because of potential interference by IGF binding proteins in screening sera with an assay to detect antibodies that block IGF-I binding, an immunoprecipitation assay method was used. It is unlikely that serum antibodies that block IGF-I binding but do not immunoprecipitate well would be missed in our assay, however, because Pansorbin (fixed protein A-bearing *S. aureus* cells) rather than a second antibody procedure was used. The equivalence titrations required for optimal precipitation of antigen-antibody complexes in the second antibody procedure can be dispensed with merely by providing protein A sites in excess of the antiserum IgG sites, as we have done, resulting in superior recovery (20). A single serum sample from patient no. 3 immunoprecipitated slightly more ^{125}I-labeled IGF-I receptor than nonimmune human serum, but immunoprecipitation of ^{125}I-labeled IGF-I receptor by the other two of three samples from this patient was within 3 SD of the mean. This result therefore was probably not significant and was not investigated further. Although a small number of children were studied, our report documents for the first time that immunoprecipitating autoantibodies to the IGF-I receptor are not commonly present in short-statured children.

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