CLINICAL STUDY

Intrauterine and postnatal growth failure with normal GH/IGF1 axis and insulin-resistant diabetes in a consanguineous kinship

Jaime Guevara-Aguirre^{1,2}, Marco Guevara-Aguirre¹, Vivian Hwa³, Patricio Prócel¹, Jeannette Saavedra¹, Harry Ostrer⁴, Peng Fang³, Ron G Rosenfeld³, Sarah Kerns⁴ and Arlan L Rosenbloom^{1,2}

¹Institute of Endocrinology, Metabolism, and Reproduction (IEMMYR), Avenida Coruña N26-191 (1337) y San Ignacio, Casilla 6337 CCI, Quito, Ecuador; ²Department of Pediatrics, University of Florida College of Medicine, 1701 SW 16th Avenue, Gainesville, Florida 32608, USA; ³Department of Pediatrics, Oregon Health and Science University, Portland, Oregon, USA and ⁴Departments of Pathology and Genetics, Albert Einstein College of Medicine of Yeshiva University, New York, New York, USA

(Correspondence should be addressed to J Guevara-Aguirre at IEMMYR; Email: jguevara@iemmyr-ecuador.org)

Abstract

Objective: To describe the clinical and biochemical features, and perform molecular analysis for candidate abnormalities in a novel familial syndrome of intrauterine growth retardation (IUGR), failure of an adolescent growth spurt with proportional adult short stature, minimal subluxation of the 5th metacarpal–phalangeal joint, and adult-onset insulin-resistant diabetes unrelated to obesity or other manifestations of metabolic syndrome (MS).

Design: Detailed clinical history, auxological, biochemical, radiological, and molecular studies, including DNA analysis and *in vitro* study of the GH/IGF1 pathway.

Materials and methods: Ten affected adults from two generations of five related families were studied in detail, and information obtained about nine other likely affected individuals.

Results: Height Z-scores ranged from -7.3 to -3.8 . Unaffected parents of the older generation and frequency of confirmed and suspected instances of the syndrome in the two generations studied is consistent with autosomal recessive inheritance. Insulin resistance was uniformly present in seven subjects tested who were not taking insulin. Diabetes severity did not correlate with overweight. Subjects did not have other typical manifestations of MS such as substantial hyperlipidemia, osteoporosis, or hypertension. No biochemical abnormality in the GH/IGF1 axis or molecular defect was found.

Conclusions: While the association of IUGR and adult MS, including diabetes, has been well documented, these subjects did not have typical manifestations of MS. Abnormalities in common components that could result in a combination of IUGR, severe postnatal growth, and insulin resistance have been ruled out. A mutation in an unidentified gene may affect intrauterine and postnatal growth, with insulin resistance directly affected or as a result of this growth phenomenon.

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Introduction

Intrauterine growth retardation (IUGR) is followed by catch-up growth postnatally in ~90% of cases (1) and has been associated with increased frequency of obesity and risk for adult metabolic disease, including type 2 diabetes, related to insulin resistance (2). In the absence of syndromic causes of IUGR such as Silver–Russell syndrome (3), which typically involves postnatal growth failure as well, ~50% of instances are due to maternal factors including alcohol consumption, undernutrition, illness, and placental insufficiency, with the remainder being largely unexplained and sporadic (4). Rare hormonal causes include mutations affecting insulin secretion or action, or insulin-like growth factor 1 (IGF1) and its receptor (5).

We have studied ten members in two generations of a consanguineous family having IUGR without

dysmorphism, proportional postnatal growth failure with reported failure of an adolescent growth spurt, and adult insulin resistance. In addition, we have obtained data about a deceased affected relative and statural information about eight individuals likely to be affected on the basis of measured or estimated stature corresponding to that of the affected individuals, but unavailable for detailed examination. This appears to be a new syndrome that is unexplained by current knowledge.

Materials and methods

Ten subjects were studied at the Institute for Endocrinology, Metabolism, and Reproduction (IEMMYR) in Quito, Ecuador. One affected individual was deceased and eight other possibly affected subjects and 32

unaffected relatives had heights and weights measured in their community. Heights were taken at the IEMMYR as the average of three measurements using a wall-mounted calibrated stadiometer. Lower segment was measured as the distance from the top of the symphysis pubis to the floor, and arm span from middle finger tips of outstretched arms. Hand length was taken from distal wrist crease to middle fingertip across the palmar surface and foot length from heel to tip of longest toe along the plantar surface. Height Z-score was calculated from US National Center for Health Statistics data for 20-year-old individuals (6). Other auxological measurements were compared with published reference data (7, 8, 9). Percentage body fat and bone mineral density were determined by dual energy X-ray absorptiometry (DXA; Lunar, Madison, WI, USA). Body fat percentage by DXA was used to define overweight and obesity as a more reliable method than body mass index (BMI) (10). Radiographic studies were done of the long bones, hips, thorax, hands, and feet to rule out skeletal abnormalities.

Detailed medical history was obtained, especially regarding birth length and weight and presence or absence of diabetes. For the deceased subject, stature was estimated from photographs and medical history confirmed by four close relatives. Similarly, detailed familial relationships were explored for a total of six generations.

Five subjects underwent clonidine and insulin-induced hypoglycemia stimulation for GH release, and

three subjects had 7-day GH stimulation of IGF1, IGF binding protein 3 (IGFBP3), and acid labile subunit (ALS). Measurements of GH, GH binding protein (GHBP), IGF1, and IGFBP3 were performed by the commercial laboratory, Esoterix (Calabasas Hills, CA, USA). Seven subjects who had not been treated with insulin had the quantitative insulin sensitivity check index (QUICKI) calculated according to the formula: $1/(\log(\text{fasting insulin in } \mu\text{U/ml}) + \log(\text{fasting glucose in mg/dl}))$. Impaired insulin sensitivity was considered when QUICKI was <0.357 (11).

Cell culture

Primary fibroblast cultures were established from skin biopsies taken from five affected individuals (12). The fibroblasts were maintained in α -MEM (Cellgrow, Mediatech, Herndon, VA, USA) supplemented with 15% fetal bovine serum (Invitrogen Life Technologies, Inc.) at 37 °C in 5% CO₂. Cells were serum starved overnight before treatment with or without IGF1 (10 ng/ml) or insulin (50 ng/ml) for 15 min, and cell lysates were collected for immunoblot analysis.

Genomic DNA and cDNA

Genomic DNA from either whole blood or primary fibroblast cultures was obtained for five subjects (13). Primers and conditions for PCR amplification and

Table 1 List of human cDNA analyzed.

cDNA	Name	Human phenotype associated with defect (OMIM)
Ligand <i>IGF1</i>	Insulin-like growth factor 1	IUGR, severe short stature, intellectual compromise (*147440)
Receptors <i>GHR</i> <i>IGF1R</i> <i>INSR</i>	GH receptor Insulin-like growth factor 1 receptor Insulin receptor	Severe postnatal short stature, GH insensitivity, IGFD IUGR, short stature, resistance to IGF1 (*147370) Rabson–Mendenhall syndrome, NIDDM (*147670)
PI3K–AKT pathway <i>IRS1</i> <i>PIK3CA</i> <i>PI3K</i> <i>PIK3R1</i> <i>PI3K</i> <i>PDPK1</i> <i>AKT1</i> <i>AKT2</i>	Insulin receptor substrate 1 Catalytic subunit (α), p110 α Regulatory subunit 1 (α), p85 α 3-Phosphoinositide dependent protein kinase-1 v-akt murine thymoma viral oncogene homolog 1 v-akt murine thymoma viral oncogene homolog 2	SNPs associated with NIDDM (+ 147545) Tumors, cancers (*171834) ^a Unknown Unknown ^b Insufficiency associated with schizophrenia (*164730) Diabetes mellitus type 2, lipodystrophy, hepatic steatosis (*164731) ^c Hypoglycemia ^d Unknown ^e
<i>PRKACA</i> mTOR–S6K pathway <i>RPS6KB1</i> <i>RPS6KB2</i>	Protein kinase, cAMP-dependent, catalytic, α Ribosomal protein S6 kinase, 70 kDa, polypeptide 1 (also known as S6K1) Ribosomal protein S6 kinase, 70 kDa, polypeptide 2	Unknown ^f Unknown ^g

IUGR, intrauterine growth retardation; IGFD, IGF deficiency; NIDDM, noninsulin-dependent diabetes mellitus. * denotes OMIM (Online Mendelian Inheritance in man)

^aActivating mutations.

^bMouse *Pdpk1a*^{−/−}: reduced islet cells, hyperglycemia (32).

^cLoss of function mutations.

^dActivating mutation (33).

^eMouse *Prkaca*^{−/−}: runted, reduced IGF (34).

^fMouse *Rps6kb1*^{−/−}: IUGR, but had catch-up growth (35, 36).

^gMouse *Rps6kb2*^{−/−}: slightly larger than wild-type (37).

sequencing of genomic GH receptor (*GHR*), *IGF1* receptor (*IGF1R*), and *IGF1* genes have been described in an earlier study (14). Total RNA was extracted from primary fibroblast cells and cDNA was synthesized (13). The primers for PCR amplification and sequencing are shown in Table 1.

Western immunoblot analysis

Preparation of cell lysates and subsequent western immunoblot analyses were performed (13, 14). The antibodies employed in this study were from Cell Signaling Technologies (Beverly, MA, USA), unless otherwise specified: anti-phospho-Tyr-100, anti-phospho-Thr308-Akt rabbit monoclonal IgG, anti-phospho-Ser473-Akt rabbit polyclonal IgG, anti-Akt (pan) rabbit monoclonal IgG, anti-phospho-Ser9-GSK3 β rabbit monoclonal IgG, anti-phospho-Thr389-S6K, anti-PI3K p85, and anti-PI3K p110 α were rabbit polyclonal antibodies. Anti-insulin receptor substrate 1 (IRS1) was from Santa Cruz, Biotechnologies Inc., Santa Cruz, CA, USA. Secondary antibodies (anti-rabbit IgG) were obtained from Amersham-Pharmacia Biotech. Immunoprecipitation was performed as previously described (14). All immunoblot data shown are representative of at least two independent experiments.

The studies were carried out after obtaining informed consent in compliance with the institutional review boards of the IEMYR and Oregon Health and Science University.

Results

Genealogy

The 11 confirmed and eight suspected subjects, from five families, are first, second, or third degree relatives. As is the custom in Spanish culture, offspring carry two last names, from each of the parents' paternal line. All subjects carry a single common name as one of their last names. The members of the older generation do not have affected parents (Fig. 1). Their sibships comprise 42 individuals; therefore, 36% are confirmed or suspected to be affected. Among the offspring of affected individuals in this generation, four of 15 (27%) are confirmed or suspected to be affected (Fig. 1). Unaffected parents of the older generation and frequency of confirmed and suspected instances of the syndrome in the two generations studied is consistent with autosomal recessive inheritance.

Birth and infancy history

All confirmed affected subjects were born of uneventful pregnancies at term, with birth lengths varying from 25 to 40 cm (-14 to -5.6 SDS), and all were said to have very low birth weights. Similar information was

Figure 1 Abbreviated pedigrees of five related families. Solid symbols indicate confirmed of intrauterine growth retardation, short stature, and diabetes or insulin resistance. Half solid symbols indicate individuals likely to be affected based on measured or estimated stature but not yet fully evaluated. Males are represented by squares and females by circles, numbers of individuals of the gender are indicated within the symbol, and deceased individuals by a diagonal line. Numbers below the symbols refer to the designation in Table 2.

obtained for the deceased individual, #11 (Fig. 1, Table 2). Subject #8 was so small, at 25–30 cm and <1000 g, that the parents were told she would not live, but she survived with intense maternal care and breastfeeding for her first 18 months. Subject #2 had knee contractures and required surgical intervention at 7 and 8 years of age before she was able to walk. None of the normal statured relatives had IUGR.

Clinical phenotype

Considering all 19 confirmed or suspected affected individuals, there were 11 females and eight males, ranging in age from 20 to 69 years. Demographic and auxological data are presented in Table 2. Height Z-scores varied from -7.3 to -3.8 . The 20 unaffected relatives <70 years of age had height Z-scores of -2.3 to $+0.4$. Upper to lower segment ratio (U/L) Z-scores, with the exception of the woman with leg deformities, ranged from -0.3 to $+3.0$, and were within 2 S.D.s of normal in 7/10 subjects. This contrasts with the observations made in 35 Ecuadorian adults with short stature due to *GHR* deficiency (GHRD), 75% of whom had U/L Z-scores $> +2.5$ (15). Subject #2 had a markedly abnormal U/L Z-score of $+6$ as the result of her lower extremity deformities; if her lower segment were proportional to her upper segment, her height would be 141 cm, -3.8 Z-score, and therefore still abnormal. Her arm span of 133 cm and hand and foot length, the shortest among the subjects, were further consistent with the syndrome. All subjects reported an absence of an adolescent growth spurt, but they underwent normal sexual maturation. Arm span was normal for height in all subjects. Head

Table 2 (A) Demographic and auxological data of subjects #1–#10 studied in detail and (B) height measurements/estimations of subjects #11–#19. U/I is the ratio of upper segment to lower segment. Z-scores for U/I and arm span-standing height (AS-SH) were estimated from sex-specific data for fully mature adolescents (8). Head circumference Z-scores are from international sex-specific reference data for 18-year-olds (9), and hand (both sexes) and foot (sex-specific) Z-scores were estimated from percentiles for 18-year-olds (7). Bone mineral density (BMD) Z-score for age and gender was determined by dual energy X-ray absorptiometry.

									Z-score						
No.	M/F	Age (years)	Height (cm)	U/I	AS-SH	Head (cm)	Hand (cm)	Foot (cm)	Height	U/I	AS-SH	Head	Hand	Foot	BMD
(A) Detailed data of subjects 1–10															
1	F	32	135	1.05	+3	52.5	15	18.5	−4.8	+1.5	+0.9	−1.9	−2.7	−4.4	−0.1
2	F	37	133	1.32	0	52.3	14	17.6	−5.2	+6.0	−0.1	−2.0	−3.4	−5.1	−0.4
3	F	43	132	1.00	+4.5	55	15.1	19.3	−5.4	+0.4	+1.2	0.0	−2.6	−3.8	+2.3
4	M	47	141	1.10	+1.8	54	16.8	20.8	−5.6	+2.5	−0.1	−1.5	−1.4	−4.2	−1.0
5	F	49	126	1.03	−1.5	54.2	14	18.2	−6.2	+1.0	−0.5	−0.6	−3.4	−4.6	+1.1
6	F	57	132	0.98	+1.5	53.3	14.5	17.9	−5.4	0.0	+0.5	−1.2	−3.1	−4.9	+0.9
7	M	59	132	1.14	+2.5	56	15	18.2	−7.0	+3.0	+0.5	0.0	−2.7	−6.24	+1.7
8	F	59	127	0.99	0	52	13.8	18.8	−6.2	+0.2	−0.1	−2.1	−3.5	−4.2	+1.6
9	M	61	140	1.03	+7	55	15.8	19.8	−5.8	+1.5	+1.5	−0.7	−2.1	−5.0	−0.5
10	M	69	138	0.91	+4	54.5	16.8	21.5	−6.0	−0.3	+0.8	−1.2	−1.4	−3.6	0.0
(B) Data of subjects 11–19															
11	F	54 ^a	130						−6.4 ^b						
12	F	60	139						−4.1 ^c						
13	F	39	141						−3.8 ^c						
14	M	60	130						−7.3 ^b						
15	M	53	140						−5.6 ^b						
16	M	68	138						−5.9 ^c						
17	M	27	145						−4.9 ^c						
18	F	34	134						−5.2 ^c						
19	F	20	141						−3.8 ^c						

^aAge deceased from kidney failure.

^bStatural data estimated from photographs and confirmed by at least three relatives.

^cStatural data confirmed by investigators.

circumferences were more normal than stature, varying from −3.5 to −1.4 Z-score. Hand length Z-scores ranged from −1.4 to −3.5, less abnormal than stature. Foot length Z-scores corresponded to the range in statural Z-scores (−6.0 to −3.6). Radiographs showed minimal subluxation of the 5th metacarpal–phalangeal joints bilaterally in all ten subjects (Fig. 2). Deformity was not noted on physical examination.

Diabetes, body composition, and insulin sensitivity

Diabetes developed between 26 and 37 years of age in six of the ten subjects studied at the IEMYR and by report in three of the other nine at about age 30 (#11–#13, Table 2B). As part of the present study, one subject (#7, Table 2A) was diagnosed at 57 years of age and another (#4) at age 47 was found to have impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) based on American Diabetes Association criteria (16).

At age 32, subject #1 had an oral glucose tolerance test (OGTT) with a fasting level of 4.8 mmol/l, and a level of 7.5 mmol/l at 2 h with normal HbA1c (Table 3). Subject #2 had acanthosis nigricans of the neck, axilla, linea alba, and inner thighs. Subject #3 had maintained a healthy lifestyle to avoid developing diabetes; her older sister (#5) and brother (#7) were affected. Subject #4

had maintained a healthy lifestyle with regular exercise in a job requiring 5–6 h of walking a day and did not have symptoms of diabetes. His fasting blood glucose level was 6.22 mmol/l (IFG) with a 2-h level of 9.78 mmol/l (IGT). Subject #5 had diabetes for 23 years, since age 26,

Figure 2 Radiograph of left hand of a 47-year-old affected male (#4) showing minimal fifth metacarpal–phalangeal subluxation.

Table 3 Metabolic data. Body mass index (BMI) calculated as the ratio of weight to height in meters squared. Percent body fat was determined by dual energy X-ray absorptiometry. Fasting serum glucose was measured in mmol/l and insulin in pmol/l for those individuals not receiving insulin therapy. Quantitative insulin sensitivity check index (QUICKI) was calculated for those not taking insulin, according to the formula: $1/(\log(\text{fasting insulin in } \mu\text{U/ml}) + \log(\text{fasting glucose in mg/dl}))$; impaired insulin sensitivity was considered when QUICKI was <0.357 (4) in subjects not requiring insulin (10). No subjects were receiving medication for hyperlipidemia.

Subject no.	DM (years)	Weight (kg)	BMI	Fat (%)	Fasting serum glucose (mmol/l)	Insulin (pmol/l)	QUICKI	HbA1c (%)	DM therapy	Cholesterol (nmol/l)	HDL (nmol/l)	TG (nmol/l)	LDL (nmol/l)
1	0	43	23.3	42.4 ^O	5.4	91.1	0.324	5.4	0	4.74	1.45	1.28	2.72
2	7	43.4	24.5	36.5 ^{OW}	4	—	—	11.4	Insulin	6.76*	1.37	2.38*	4.30
3	0	40	23	34.3 ^N	5.5	56.0	0.344	6.4	Metformin	6.03*	1.32	1.20	4.17
4	IFG/IGT	48.3	24	31.6 ^O	6.2	144.2	0.298	5.8	Diet/exercise	7.10*	1.22	2.90*	4.01
5	23	34.2	21.5	35.3 ^{OW}	7.6	—	—	—	Insulin	6.11*	1.58	1.59	3.81
6	20	38.7	22	38.9 ^{OW}	8.4	71.8	0.331	7.2	Intermittent OHA	3.57	1.30	1.01	1.81
7	31	33.2	19	17.3 ^N	7.5	—	—	7.8	Insulin/metformin	5.96*	1.22	1.07	4.25
8	<1	40.5	25	39.9 ^{OW}	7.4	66.7	0.324	6.8	Oral agents	5.59*	1.32	1.18	3.73
9	25	43.3	22	24.3 ^N	9.1	53.8	0.325	7.0	Metformin/glibenclamide	4.92	1.14	0.80	3.39
10	35	38	20	21.6 ^N	9.5	45.9	0.329	7.3	Diet/exercise	5.57*	1.17	1.20	3.83

^N, normal; ^O, obese; ^{OW}, overweight; DM, Diabetes mellitus; OHA, oral hypoglycemic agents; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; C, cholesterol (normal <5.18 mmol/l); HDL, high-density lipoprotein-C (normal $0.907\text{--}1.81$ mmol/l); TG, triglycerides (normal <1.62 mmol/l); LDL, low-density lipoprotein-C (normal $2.59\text{--}4.92$ mmol/l); DM, diabetes mellitus. Normal cholesterol <5.18 mmol/l. Abnormal values and impaired fasting glucose/impaired glucose tolerance are indicated by (*).

initially treated with oral agents. Pregnancy at age 27 resulted in insulin dependence which persisted with poor control and blindness due to retinopathy. Diabetes was diagnosed in subject #6 when she was 37 years of age and had been present for 20 years, controlled with oral agents taken irregularly. Subject #7 had diabetes for 31 years, since age 28, initially diagnosed on routine examination with a fasting glucose concentration of 8.1 mmol/l and confirmed by OGTT. Diet and exercise were adequate for control for ~ 10 years and oral agents for another 20 years until recent institution of a small dose of long-acting insulin together with metformin. Diabetes was diagnosed in subject #8 by OGTT during the present study with a 2-h glucose level of 12.5 mmol/l. Her diabetes was well controlled with low-dose metformin. Subject #9 had diabetes for 25 years, with onset at age 36. For the first 15 years, control was with diet and exercise, followed by various oral hypoglycemic agents which he continued to take at the time of study. Subject #10 had diabetes for 36 years, since age 33, controlled with diet and exercise.

Insulin sensitivity analyses of the seven subjects who did not have manifest diabetes or require insulin for management of their diabetes are summarized in Table 3. All of them demonstrated impaired insulin sensitivity by QUICKI. While seven of the ten test subjects had elevated serum total cholesterol concentrations, and two of these seven individuals also had elevated triglycerides, all ten subjects had normal high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) serum levels (Table 3).

Percentage body fat determined by DXA (Table 3) met the gender- and age-specific criteria for obesity in one man and one woman and for overweight in four women (10). This contrasts with the BMI data which identified no individuals as obese ($\text{BMI} > 30 \text{ kg/m}^2$) and only one at the borderline for overweight ($\text{BMI} 25 \text{ kg/m}^2$) (6). Bone mineral density Z-scores adjusted for age and gender ranged from -1.0 to $+2.3$, with the majority of the subjects having above-average scores (Table 2A). Thus, there was no evidence of osteoporosis as a late complication of IUGR (2). Blood pressures (data not shown) were normal ($<130/70$ mmHg) in all but one subject who had a pressure of 140/78 mmHg.

Among the 32 unaffected relatives aged 26 to 91 years, only one was known to have diabetes – a 91-year-old male who developed his diabetes at 70 years of age, which was controlled with diet and exercise.

Social adaptation

All subjects did average or better work in school; three of the four males who were studied at the IEMYR were very good students. All ten individuals in Table 2A were married. Eight of the subjects had children. One man and one woman decided not to have children because they did not want to run the risk of passing on the condition.

GH/IGF1 axis

All subjects had normal GHBP, IGF1, IGFBP3, and ALS serum concentrations, and all but one responded normally to GH stimulation (Table 4). IGF1 concentrations increased 2.5-, 3-, and 4.5-fold, in response to GH administration, in the three subjects thus tested, while IGFBP3 concentrations increased 50% or more and ALS levels increased >100, 50, and 10%.

Genetic and cellular evaluations

The severe short stature, with normal serum IGF1 levels that increased with administration of rhGH, was suggestive of resistance to IGF1. Molecular defects of the *Igf1r* gene are associated with IGF1 resistance (5). Furthermore, in rodents, pancreas-specific ablation of the *IGF1R* gene led to diabetes (17, 18). Therefore, the *IGF1R* gene and cDNA of our subjects were sequenced and *IGF1R* was found to be wild-type as was the insulin receptor (*INSR*) gene (Table 1). The *IGF1* and *GHR* genes were also normal.

Because the *IGF1R* and *INSR* genes were wild-type (Table 1), the possibility of defects downstream of these two highly homologous receptors was considered. For sequencing, we targeted genes encoding for signal molecules known to be activated by both systems and associated with short stature or potential diabetes phenotypes for sequencing. As shown in Table 1, all cDNAs analyzed were wild-type.

Although all components analyzed were normal at the cDNA level, it is possible that one or more of the components was not normally expressed at the protein level or was incapable of being normally activated. IGF1-induced signaling, specifically activation of PI3K/AKT, was evaluated in fibroblasts from one of the subjects and compared with normal, control fibroblasts. PI3K/AKT signaling, exemplified by phosphorylation of IRS1 (tyrosines) and AKT (serine and threonine), appeared to be normally activated by IGF1

treatment (Fig. 3A). Insulin, furthermore, similarly activated AKT (Fig. 3B), indicating that at least the pathway to PI3K/AKT appeared to be normal.

Discussion

We have described a previously unrecognized familial association of IUGR, postnatal growth failure with absence of an adolescent growth spurt despite normal sexual maturation, minimal subluxation of the 5th metacarpal–phalangeal joint, and insulin resistance with frequent development of generally non-insulin-requiring diabetes unrelated to obesity. As is typical of type 2 diabetes, the need for insulin therapy occurred in some individuals, indicating inadequate β cell response to the increasing demand with peripheral resistance. Both the resistance and the β cell failure could theoretically result from defects in combined insulin and IGF1 action.

The relationship of IUGR and later development of components of the metabolic syndrome (MS), including obesity, diabetes, hypertension, dyslipidemia, and osteoporosis, has been thought to be an effect of programming the fetus for insulin resistance (2, 19). The relationship between IUGR and adult development of glucose intolerance and the MS has been documented in numerous populations and ethnicities (20).

The most prevalent explanation for the association of decreased fetal growth and adult metabolic disease is the thrifty phenotype hypothesis (19). Fetal under-nutrition is postulated to lead to selective distribution of nutrients to essential survival functions, such as brain growth, with metabolic changes that increase postnatal survival in a similarly undernourished environment. These adaptations then become detrimental in the presence of adequate or greater nutrition. This scenario has been substantiated in survivors of the Dutch famine at the end of World War II who were *in utero* at the time (21).

Table 4 Peak serum GH response to clonidine and insulin-induced hypoglycemia, GH binding protein (GHBP) concentrations (normal range 686–2019 pmol/l), baseline insulin-like growth factor 1 (IGF1), IGF binding protein 3 (IGFBP3), and acid labile subunit (ALS, normal range 7.0–16 mg/l) in five subjects and response to 7-day GH stimulation in three subjects. Age- and gender-specific norms for IGF1 and age-specific norms for IGFBP3 are given in parentheses.

	Subjects				
	#1	#6	#8	#9	#10
GH/clonidine (μ g/l)	5.0	1.6	0.6	2.3	10.1
GH/hypoglycemia (μ g/l)	14.9	10.1	11.8	1.0	13.3
GHBP (pmol/l)	1385	2218	2087	1519	1421
Basal IGF1 (μ g/l)	146 (87–368)	105 (53–287)	110 (53–287)	131 (60–220)	78 (60–220)
Peak IGF1 (μ g/l)	–	–	500	401	295
Basal IGFBP3 (mg/l)	3.3 (2.0–4.2)	3.4 (1.9–3.6)	3.2 (1.9–3.6)	2.3 (1.9–3.6)	1.9 (1.9–3.6)
Peak IGFBP3 (mg/l)	–	–	4.6	3.9	3.5
Basal ALS (mg/l)	15	18	21	10	10
Peak ALS (mg/l)	–	–	23	23	15

Figure 3 The IGF1- and insulin-induced PI3K signaling pathway is normal in the affected proband. Primary fibroblasts were treated with IGF1 or insulin as described in the section Materials and methods, and cell lysates analyzed. C, normal fibroblasts; P, fibroblasts established from proband. (A) IRS1 is normally activated by IGF1. From 130 μ g of total cell lysates, IRS1 was first immunoprecipitated with anti-IRS1 antibody and immunoblot analyzed by probing with a general anti-phospho-tyrosine (p-Y-100) antibody. The immunoblot was subsequently stripped and re-probed with anti-IRS1. (B) Immunoblot analysis of cell lysates for expression of PI3K components p85 and p110 α . (C) Immunoblot analysis of cell lysates for phosphorylation of AKT at Thr308 (p-T-AKT) and at Ser473 (p-S-AKT). (D) Phosphorylation of AKT in cells treated with insulin compared with cells treated with IGF1.

There are noteworthy differences between these well-documented observations and the present population. Firstly, it is not possible to invoke an environmental explanation for selective undernutrition (e.g. famine, maternal illness) of the subjects, who have younger and older siblings who are unaffected. The apparent autosomal recessive pattern of inheritance also would be inconsistent with an environmental or maternal explanation for fetal underdevelopment. The subjects

in the present study had postnatal growth failure, did not have hypertension or uniform or severe dyslipidemia, and did not have osteoporosis. While they were not, as a group, obese as defined by percentage body fat on DXA study, four of the six women and one of the four men met the criteria for obesity or overweight. However, there did not appear to be any correlation between degree of obesity by this definition and severity of diabetes; the single man thus defined as obese only had IFG and IGT with a normal HbA1c, controlled by lifestyle, while the three men who did not meet the DXA criteria for overweight had symptomatic diabetes for 10–15 years by the time they were his age. Similarly, the 32-year-old woman obese as per DXA criteria was the only subject with normal glucose metabolism despite insulin resistance. The three insulin-requiring individuals included only one who was overweight by DXA criteria.

An alternative explanation for the association of diminished fetal growth and adult insulin-resistant diabetes would be the presence of gene defects causing both low birth weight and insulin resistance with susceptibility to diabetes in adulthood, which has been termed the fetal insulin hypothesis (22). Monogenic diseases that affect glucose sensing (glucokinase deficiency), insulin secretion (transient neonatal diabetes), or insulin sensitivity (Donohue and Rabson–Mendenhall syndromes) can impair fetal growth. While mutations in the gene encoding glucokinase can result in low birth weight and mild diabetes, the diabetes is of young onset, postnatal growth is normal, and the condition is dominantly transmitted (23). Donohue syndrome is characterized by facial dysmorphism with severe postnatal failure to thrive and death in infancy (24). Newborns with Rabson–Mendenhall syndrome have fasting hypoglycemia and develop severe insulin-dependent diabetes in childhood; they have premature dysplastic dentition, coarse facial features, and pineal hyperplasia (25). A single individual with a syndrome of severe IUGR with modest postnatal growth failure and adult statural deficit (–3 SDS), early puberty, diabetes onset at age 23, and failure of lactation had a paternally derived translocation breakpoint disrupting regulation of the *IGF2* gene; her daughter carrying this balanced translocation was unaffected (26). The subjects in this study had more severe short stature, normal age of onset of puberty, the familial occurrence was quite different, and the females had no problem with nursing their offspring. Although the subjects in this report do not correspond to any of these disorders, a genetic cause of insulin resistance that also influences postnatal growth is a credible explanation for their condition. An example is suggested by the observation in a large cohort that birth size was influenced by common genetic variation in expression of the insulin gene regulated by the variable number of tandem repeats (VNTR) locus, and that this effect was most pronounced in those individuals who did not have postnatal catch-up growth (27).

The uniform presence of insulin resistance in this syndrome with little or no obesity is in marked contrast to our experience with GHRD, in which severe obesity was generally accompanied by remarkable insulin sensitivity. The obese GHRD subjects had normal triglyceride levels as a reflection of insulin sensitivity and absence of diabetes (28, 29).

The components of the GH/IGF1 pathway were found to be intact, with GH, IGF1, and IGF1R expression and functions apparently normal. IUGR as a result of disturbance in this pathway only occurs with defects of *IGF1* and *IGF1R* genes (30). The *IGF1* mutations also result in severe mental retardation, deafness, micrognathia, and severe postnatal growth failure. With heterozygosity for *IGF1R* mutation, there is more modest postnatal growth failure than with *IGF1* mutation or in the present syndrome, and no or mild retardation; there is also microcephaly with *IGF1* mutation and less severe reduction in head circumference with mutation of *IGF1R* (5). Bilateral minimal metacarpal-phalangeal subluxation of the fifth finger is an unusual and distinctive feature. We are unaware of any dysmorphic syndrome with this abnormality, which has only been described as the result of trauma or rheumatoid arthritis (31).

The genetic basis of the syndrome in these subjects remains elusive despite an extensive analysis of known components downstream of the IGF1R and insulin receptor signaling systems. Our preliminary analysis of serine (Ser9) phosphorylation of GS3K β and threonine (Thr398) phosphorylation of S6K, as consequences of activated AKT and the mTOR pathways, suggests normal signaling (data not shown). Hence, common components that could result in a combination of IUGR, severe postnatal growth, and insulin resistance have been ruled out. It is likely that an as yet unidentified component or mutation is affecting intrauterine and postnatal growth, with the insulin resistance directly affected or as a by-product of this growth phenomenon. One possibility would be a tissue-specific mutation affecting insulin secretion, as has been suggested by studies on mice with specific knockout of β cell IGF1Rs (17, 18).

Declaration of interest

R G Rosenfeld has received payments for consulting or lectures from Tercica Inc. This potential conflict of interest has been received and managed by OHSU.

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References

- 1 Leger J, Limoni C, Collin D & Czernichow P. Prediction factors in the determination of final height in subjects born small for gestational age. *Pediatric Research* 1998 **43** 808–812. (doi:10.1203/00006450-199806000-00015)
- 2 Holt RIG. Fetal programming of the growth hormone–insulin-like growth factor axis. *Trends in Endocrinology and Metabolism* 2002 **13** 392–397. (doi:10.1016/S1043-2760(02)00697-5)
- 3 Preece M. The genetics of the Silver–Russell syndrome. *Reviews in Endocrine and Metabolic Disorders* 2002 **3** 369–379. (doi:10.1023/A:1020961909991)
- 4 Wollman HA. Intrauterine growth restriction: definition and etiology. *Hormone Research* 1998 **49** 1–6. (doi:10.1159/000053079)
- 5 Rosenbloom AL & Guevara-Aguirre J. Growth hormone resistance. *eMedicine from WebMD* 2010. <http://emedicine.medscape.com/article/922902-overview>.
- 6 National Center for Health Statistics & Hamil PVV. NCHS growth curves for children birth–18 years: United States. Vital and health statistics, series 11, no. 165. Washington DC: Government Printing Office; DHEW publication no. (PHS) 78–1650, 1977.
- 7 Hall J, Froster-Iskenius JG & Allanson JE. *Handbook of Normal Physical Measurements*. New York: Oxford University Press, 1989.
- 8 Turan S, Bereket A, Omar A, Berber M, Ozen A & Bekiroglu N. Upper segment/lower segment ratio and armspan–height difference in healthy Turkish children. *Acta Paediatrica* 2005 **94** 407–413. (doi:10.1111/j.1651-2227.2005.tb01909.x)
- 9 Nellhaus G. Head circumference from birth to eighteen years. Practical composite international and interracial graphs. *Pediatrics* 1968 **41** 106–114.
- 10 Kennedy AP, Shea JL & Sun G. Comparison of the classification of obesity by BMI vs dual energy X-ray absorptiometry in the Newfoundland population. *Obesity* 2009 **17** 2004–2009. (doi:10.1038/oby.2009.101)
- 11 Hrebíček J, Janout V, Malincíková J, Horáková D & Cízek L. Detection of insulin resistance by simple quantitative insulin sensitivity check index QUICKI for epidemiological assessment and prevention. *Journal of Clinical Endocrinology and Metabolism* 2002 **87** 144–147. (doi:10.1210/jc.87.1.144)
- 12 Kofoed EM, Hwa V, Little B, Woods KA, Buckway CK, Tsubaki J, Pratt KL, Bezrodnik L, Jasper H, Tepper A, Heinrich JJ & Rosenfeld RG. Growth hormone insensitivity associated with a STAT5b mutation. *New England Journal of Medicine* 2003 **349** 1139–1147. (doi:10.1056/NEJMoa022926)
- 13 Fang P, Riedl S, Amselem S, Pratt KL, Little BM, Haeusler G, Hwa V, Frisch H & Rosenfeld RG. Primary growth hormone (GH) insensitivity and insulin-like growth factor deficiency caused by novel compound heterozygous mutations of the GH receptor gene: genetic and functional studies of simple and compound heterozygous states. *Journal of Clinical Endocrinology and Metabolism* 2007 **92** 2223–2231. (doi:10.1210/jc.2006-2624)
- 14 Fang P, Schwartz ID, Johnson BD, Derr MA, Roberts CT Jr, Hwa V & Rosenfeld RG. Familial short stature caused by haploinsufficiency of the insulin-like growth factor I receptor due to nonsense-mediated messenger ribonucleic acid decay. *Journal of Clinical Endocrinology and Metabolism* 2009 **94** 1740–1747. (doi:10.1210/jc.2008-1903)
- 15 Guevara-Aguirre J, Rosenbloom AL, Fielder PJ, Diamond FB Jr & Rosenfeld RG. Growth hormone receptor deficiency in Ecuador: clinical and biochemical phenotype in two populations. *Journal of Clinical Endocrinology and Metabolism* 1993 **76** 417–423. (doi:10.1210/jc.76.2.417)
- 16 American Diabetes Association. Diagnosis and classification of diabetes. *Diabetes Care* 2003 **26** 3160–3167. (doi:10.2337/diacare.26.11.3160)
- 17 Xuan S, Kitamura T, Nakae J, Politi K, Kido Y, Fisher PE, Morroni M, Cinti S, White ME, Herrera PL, Accili D &

- Efstratiadis A. Defective insulin secretion in pancreatic beta cells lacking type 1 IGF receptor. *Journal of Clinical Investigation* 2002 **110** 1011–1019.
- 18 Kulkarni RN, Holzenberger M, Shih DQ, Ozcan U, Stoffel M, Magnuson MA & Kahn CR. Beta-cell-specific deletion of the Igf1 receptor leads to hyperinsulinemia and glucose intolerance but does not alter beta-cell mass. *Nature Genetics* 2002 **31** 111–115.
 - 19 Hales CN & Barker DJP. Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. *Diabetologia* 1992 **35** 595–601. (doi:10.1007/BF00400248)
 - 20 Ozanne SE & Hales CN. Early programming of glucose–insulin metabolism. *Trends in Endocrinology and Metabolism* 2002 **13** 368–373. (doi:10.1016/S1043-2760(02)00666-5)
 - 21 Ravelli AC, van der Meulen JH, Michels RP, Osmond C, Barker DJ, Hales CN & Bleker OP. Glucose tolerance in adults after prenatal exposure to famine. *Lancet* 1998 **351** 173–177. (doi:10.1016/S0140-6736(97)07244-9)
 - 22 Hattersley AT & Tooke JE. The fetal insulin hypothesis: an alternative explanation of the association of low birth weight with diabetes and vascular disease. *Lancet* 1999 **353** 1789–1792. (doi:10.1016/S0140-6736(98)07546-1)
 - 23 Ravelli A, Beards F, Ballantyne E, Appleton M, Harvey R & Ellard S. Mutations in the glucokinase gene of the fetus result in reduced birth weight. *Nature Genetics* 1998 **19** 268–270. (doi:10.1038/953)
 - 24 Cantan A, Ziruolo MG & Tacconi ML. A rare polydysmorphic syndrome: leprechaunism – review of forty-nine cases reported in the literature. *Annales de Génétique* 1987 **30** 221–227.
 - 25 West RJ, Lloyd JK & Turner WML. Familial insulin-resistant diabetes, multiple somatic anomalies, and pineal hyperplasia. *Archives of Disease in Childhood* 1975 **50** 703–708. (doi:10.1136/adc.50.9.703)
 - 26 Murphy R, Baptista J, Holly J, Umpleby AM, Ellard S, Harries LW, Crolla J, Cundy T & Hattersley AT. Severe intrauterine growth retardation and atypical diabetes associated with a translocation breakpoint disrupting regulation of the insulin-like growth factor 2 gene. *Journal of Clinical Endocrinology and Metabolism* 2008 **93** 4373–4380. (doi:10.1210/jc.2008-0819)
 - 27 Dunger DB, Ong KK, Huxtable SJ, Sherriff A, Woods KA, Ahmed ML, Golding J, Pembrey ME, Ring S, Bennett ST & Todd JA. Association of the INS VNTR with size at birth. ALSPAC study team. Avon longitudinal study of pregnancy and childhood. *Nature Genetics* 1998 **19** 98–100. (doi:10.1038/ng0598-98)
 - 28 Rosenbloom AL, Martinez V, Kranzier JH, Bachrach LK, Rosenfeld RG & Guevara-Aguirre J. Natural history of growth hormone receptor deficiency. *Acta Paediatrica* 1999 **88** 153–156. (doi:10.1111/j.1651-2227.1999.tb14374.x)
 - 29 Guevara-Aguirre J, Balasubramanian P, Guevara-Aguirre M, Wei M, Madia F, Cheng CW, Hwang D, Martin-Montalvo A, Saavedra J, Ingles S, de Cabo R, Cohen P & Longo VD. Growth hormone receptor deficiency is associated with a major reduction in pro-aging signaling, cancer, and diabetes in humans. *Science Translational Medicine* 2011 **3** 1–9. (doi:10.1126/scitranslmed.3001845)
 - 30 Guevara-Aguirre J. Insulin-like growth factor I – an important intrauterine growth factor. *New England Journal of Medicine* 1996 **335** 1389–1391. Erratum in: *New England Journal of Medicine*, 303–336, 1997. (doi:10.1056/NEJM199610313351810)
 - 31 Apfelberg DB, Maser MR, Lash H, Kaye RL, Britton MC & Bobrove A. Rheumatoid hand deformities: pathophysiology and treatment. *Western Journal of Medicine* 1978 **129** 267–272.
 - 32 Hashimoto N, Kido Y, Uchida T, Asahara S, Shigeyama Y, Matsuda T, Takeda A, Tsuchihashi D, Nishizawa A, Ogawa W, Fujimoto Y, Okamura H, Arden KC, Herrera PL, Noda T & Kasuga M. Ablation of PDK1 in pancreatic beta cells induces diabetes as a result of loss of beta cell mass. *Nature Genetics* 2006 **38** 589–593. (doi:10.1038/ng1774)
 - 33 Hussain K, Challis B, Rocha N, Payne F, Minic M, Thompson A, Daly A, Scott C, Harris J, Smillie BJL, Savage DB, Ramaswani U, De Lonlay P, O'Rahilly S, Barroso I & Semple RK. An activating mutation of AKT2 and human hypoglycemia. *Science* 2011 **334** 474. (doi:10.1126/science.1210878)
 - 34 Skalhogg BS, Huang Y, Su T, Idzerda RL, McKnight GS & Burton KA. Mutation of the C-alpha subunit of PKA leads to growth retardation and sperm dysfunction. *Molecular Endocrinology* 2002 **16** 630–639. (doi:10.1210/me.16.3.630)
 - 35 Shima H, Pende M, Chen Y, Fumagalli S, Thomas G & Kozma SC. Disruption of the p70(s6k)/p85(s6k) gene reveals a small mouse phenotype and a new functional S6 kinase. *EMBO Journal* 1998 **17** 6649–6659. (doi:10.1093/emboj/17.22.6649)
 - 36 Pende M, Kozma SC, Jaquet M, Oorschot V, Burcelin R, Le Marchand-Brustel Y, Klumperman J, Thorens B & Thomas G. Hypoinsulinaemia, glucose intolerance and diminished beta-cell size in S6K1-deficient mice. *Nature* 2000 **408** 994–997. (doi:10.1038/35050135)
 - 37 Pende M, Um SH, Mieulet V, Sticker M, Goss VL, Mestan J, Mueller M, Fumagalli S, Kozma SC & Thomas G. S6K1^{-/-}/S6K2^{-/-} mice exhibit perinatal lethality and rapamycin-sensitive 5-prime-terminal oligopyrimidine mRNA translation and reveal a mitogen-activated protein kinase-dependent S6 kinase pathway. *Molecular and Cellular Biology* 2004 **24** 3112–3124. (doi:10.1128/MCB.24.8.3112-3124.2004)

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