

Currently reported patients:

Case 1: A boy of 6.7 years of Mayan origin with a height z-score of -2.91 SD, delayed bone age (5.5 years) and homozygous *IGFALS* 1308_1316 dup9 mutation in the 17th LRR domain. GH treatment began at the age of 8.5 years and was discontinued 1 year later due to development of nonalcoholic steatotic hepatitis. The patient's transaminase levels continued to climb when he was off treatment, however they subsequently returned to normal. GH therapy was tried again from age 10 years for another 2 years. Despite increasing doses of GH, he failed to improve his growth velocity or normalize his IGF-I and IGF binding protein (IGFBP)-3 levels. During this time, at the chronological age of 10.5 years, he initiated spontaneous puberty and was started on LH-RH analogue therapy to preserve growth potential while on GH. At age 12 years, he was switched from GH to IGF-I therapy.

Case 2: A girl aged 4.1 years of Eastern European Jewish/Icelandic-Western European ethnic origin with a height z-score of -2.14 SD, bone age consistent to her chronologic age, and compound heterozygous *IGFALS* C60S/L244F missense mutations in the 1st and 9th LRR domains, respectively. She started GH treatment at age 4.4 years, increasing her height z-score in 13 months to -1.67 SD; IGF-I and IGFBP-3 levels nonetheless remained abnormally low, and ALS was undetectable.

Patients reported in the amendment:

Case 1: An Indian/Pakistani boy aged 15.2 years with a height z-score of -3.17 SD, delayed bone age (11 years), sexual infantilism and homozygous *IGFALS* L134Q missense mutations in the 4th LRR domain. His parents, both heterozygous carriers, had normal heights (-0.09 and -1.35 SDS).

Case 2: An Ashkenazi Jewish boy aged 12.7 years with a height z-score of -2.87 SD, bone age of 11.5 years, sexual infantilism and compound heterozygous *IGFALS* P73L/L241P missense mutations in the 1st and 8th-9th LRR domains, respectively. His parents, both heterozygous carriers of one of the mutations, had normal heights (-1.68 and $+0.85$ SDS).

ALS protein, a member of the LRR superfamily of proteins involved in protein-protein interactions, contains 20 LRR domains that form a donut shape with a closed structure. The LRRs contain β -strands that form sheets inside the donut, and α -helices that flank the structure's outer circumference. This paper highlights the ethnic

and genetic heterogeneity of *IGFALS* mutations that are pathogenic in causing PIGFD and modest short stature that responds poorly to GH therapy. Although GH can induce IGF-I and IGFBP-3 production, without ALS, circulating levels of the growth factor are not sustained. This is a nice in vivo illustration of the importance of the ternary complex in prolonging the circulating half-life, and hence activity, of IGF-I.

Fofanova-Gambetti OV, Hwa V, Kirsch S, et al. Three novel *IGFALS* gene mutations resulting in total ALS and severe circulating IGF-I/IGFBP-3 deficiency in children of different ethnic origins. *Horm Res.* 2009;71:100-110.

Editor's Comment: *Genotyping of the parents of Girl #2 in this paper was not available. The authors hypothesized that her mutations must be in the compound heterozygous state because her ALS protein was undetectable; had her mutations occurred in cis, then her wild-type allele would be expected to produce wild-type ALS that should have been detected, as was the case for the carrier parent of the Turkish boy with a homozygous missense mutation.² Another possibility is that the double mutations in cis so altered the ALS protein product that it functioned as a dominant negative, tying up the wild-type ALS in the ER or Golgi and preventing its secretion. This second hypothesis would require that one of the parents similarly carry the dominant negative in cis mutations, have undetectable ALS, and be affected. The father's height z-score was $+0.30$ SD while the mother's was -2.13 SD. Since one of the main teaching points of this paper is that ALS mutations cause PIGFD with only modest short stature, perhaps the mother is affected like her daughter?*

Adda Grimberg, MD

References

1. Domene HM, Bengolea SV, Martinez AS, et al. Deficiency of the circulating insulin-like growth factor system associated with inactivation of the acid labile subunit gene. *N Engl J Med.* 2004;350:570-577.
2. Hwa V, Haeusler G, Pratt K, et al. Total absence of functional acid labile subunit, resulting in severe insulin-like growth factor deficiency and moderate growth failure. *J Clin Endocrinol Metab.* 2006;91:1826-1831.
3. Domene HM, Scaglia PA, Lteif A, et al. Phenotypic effects of null and haploinsufficiency of acid-labile subunit in a family with two novel *IGFALS* gene mutations. *J Clin Endocrinol Metab.* 2007;92:4444-4450.

Acute Vascular Effects of GH Appear to be Independent of Both Local and Systemic IGF-I Production

Growth hormone (GH) has been shown to regulate vascular tone and reactivity in humans, but it is unclear whether this action is a result of a direct stimulatory effect of GH or if it is dependent on systemic and local insulin-like growth factor (IGF)-I production. In this study, Li et al

evaluated the mechanisms underlying the acute vascular effects of GH. Ten healthy lean young volunteers (20 to 27 years of age; 7 male and 3 females) were studied after an overnight fast. GH was infused for 6 hours at 0.06 mcg/kg/minute and a biopsy of the vastus lateralis muscle was

obtained in 7 of these subjects before and after infusion for analysis of IGF-I mRNA and Akt phosphorylation. Blood was obtained serially every 10 minutes during the infusion for GH, IGF-I, insulin and glucose assessments. GH infusion increased plasma GH and forearm blood flow by 66% ($p < 0.001$), but did not change plasma IGF-I concentrations, muscle IGF-I mRNA expression, or muscle Akt phosphorylation—therefore suggesting a lack of IGF-I action in muscle. Additionally, human aortic endothelial cells (HAECs) were incubated with GH (30 ng/mL) in vitro for 3 or 6 hours. GH did not alter endothelial nitric oxide synthase (eNOS) protein content, but induced a time-dependent increase of the phosphorylation of eNOS. This study demonstrated that GH exerts an acute vascular effect, independent of both systemic and local IGF-I production and that this effect probably occurs via direct action on GH receptors and eNOS in the vascular endothelium.

Li G, del Rincon P, Jahn LA, et al. Growth hormone exerts acute vascular effects independently of systemic or muscle insulin-like growth factor I. *J Clin Endocrinol Metab.* 2008;93:1379-1385.

Editor's Comment: *Endothelial dysfunction appears to explain much of the increased cardiovascular risk of GH deficiency. GH seems to play an important role in the regulation of peripheral vascular resistance and vascular reactivity; these effects appear to be mediated*

by the activation of the NO pathway. GH deficiency is associated with decreased systemic NO formation and decreased forearm release of nitrite and cyclic GMP during acetylcholine stimulation, as well as a decreased peak hyperemic response to ischemia, which reverts to normal during GH replacement. Significant endothelial dysfunction—as determined by an impaired endothelium-dependent brachial artery dilatory response to occlusion ischemia and by abnormalities of several biochemical markers of endothelial cell activation—has been reported in adolescents and adults with GH deficiency.^{1,2} It is not clear whether these effects are a result of a direct effect of GH on the vascular endothelium or whether they are dependent on systemic and local IGF-I production. This study seems to indicate that the acute vasodilatory effect of GH is exerted independent of IGF-I, very possibly through GH receptor mediated eNOS activation.

Roberto Lanes, MD

References

1. Lanes R, Marcano H, Villaroel O, et al. Circulating levels of high-sensitivity C-reactive protein and soluble markers of vascular endothelial cell activation in growth hormone-deficient adolescents. *Horm Res.* 2008;70:230-235.
2. Elhadd TA, Abdu TA, Oxtoby J, et al. Biochemical and biophysical markers of endothelial dysfunction in adults with hypopituitarism and severe GH deficiency. *J Clin Endocrinol Metab.* 2001;86:4223-4232.

Nedd4 Controls Animal Growth by Regulating IGF-I Signaling

Nedd4 (Neural precursor cell expressed developmentally down regulated 4 - OMIM 602278, chromosome 15q) is a cytoplasmic ubiquitin ligase that regulates protein movement and structure thereby its function or directs a protein into ubiquitin-proteasomal degradative pathway. Cao et al demonstrated that Nedd4 is essential for transduction of intracellular signals initiated by insulin and insulin-like growth factor (IGF)-I and the localization of the insulin receptor (IR, OMIM 147670, chromosome 19p13.2) and the IGF-I receptor (IGF1R, OMIM 147370, chromosome 15q25-q26) to the cell plasma membrane. Nedd4 does not bind to IR or IGF1R directly, but links to an adaptor protein, Grb10 (Growth factor receptor-bound protein10, OMIM 601523, chromosome 7p12-p11.2), which in turn is bound by IR and IGF1R. Grb10 inhibits movement of these receptors to their localization sites in the plasma membrane and thereby impairs function of IR and IGF1R. This effect is opposed by the binding of Nedd4 to Grb10. Cao and colleagues generated Nedd4 knockout (KO) mice. Nedd4^{-/-} mice died during gestation or shortly after birth due to immature lung development and aeration (Figure); their linear growth and weight were severely impaired by embryonic day 12.5. Heterozygous Nedd4^{+/-} mice were also small at birth and through post-natal age 3 months (the end of the study period). In vitro, the proliferation of Nedd4^{-/-} fibroblasts was impaired relative to that of

wild-type fibroblasts due to decreased progression through the cell cycle at phases G₀ and G₁. IGF-I and insulin mediated intracellular signaling was substantially reduced in Nedd4^{-/-} and Nedd4^{+/-} fibroblasts and could be restored by expression of Nedd4 in these cells. However, in Nedd4^{-/-} fibroblasts, the expression and translation of IR and IGF1R were normal, but the receptors did not reach the cell surface, an abnormality that could also be reversed by expression of Nedd4 in these cells. Further studies demonstrated that the amount of Grb10 was increased in Nedd4^{-/-} fibroblasts and that “knockdown” of Grb10 by small interfering RNA (siRNA) restored insulin and IGF-I signaling in Nedd4^{-/-} fibroblasts. The investigators concluded that Nedd4 positively regulates IGF-I and insulin signaling by enhancing the movement of their receptors to the cell surface. Nedd4 does so by dis-inhibiting the inhibitory effect of Grb10 on this process—perhaps by controlling the rate of degradation of Grb10 itself through the ubiquitin-proteasomal system.

Cao XR, Lill NL, Boase N, et al. Nedd4 controls animal growth by regulating IGF-1 signaling. *Sci Signal.* 2008;1:ra5. [DOI:10.1126/scisignal.1160940]

Editor's Comment: *This study has identified another intracellular signal transduction site (Nedd4-Grb10) to examine when a patient with severe growth retardation due*