Insulin-like growth factors in embryonic and fetal growth and skeletal development (Review)

GEORGIOS D. AGROGIANNIS¹, STAVROS SIFAKIS², EFSTRATIOS S. PATSOURIS¹ and ANASTASIA E. KONSTANTINIDOU¹

1st Department of Pathology, School of Medicine, University of Athens, Athens; 2Department of Obstetrics and Gynecology, University Hospital of Heraklion, Crete, Greece

Received February 17, 2014; Accepted April 16, 2014

DOI: 10.3892/mmr.2014.2258

Correspondence to: Dr Georgios D. Agrogiannis, 1st Department of Pathology, School of Medicine, University of Athens, 75 Mikras Asias Street, Athens GR-11527, Greece
E-mail: agrojohn@med.uoa.gr

Key words: insulin-like growth factors, insulin-like growth factor-I, insulin-like growth factor-II, fetal growth, skeletal development

Abstract. The insulin-like growth factors (IGF)-I and -II have a predominant role in fetal growth and development. IGFs are involved in the proliferation, differentiation and apoptosis of fetal cells in vitro and the IGF serum concentration has been shown to be closely correlated with fetal growth and length. IGF transcripts and peptides have been detected in almost every fetal tissue from as early in development as pre-implantation to the final maturation stage. Furthermore, IGFs have been demonstrated to be involved in limb morphogenesis. However, although ablation of Igf genes in mice resulted in growth retardation and delay in skeletal maturation, no impact on outgrowth and patterning of embryonic limbs was observed. Additionally, various molecular defects in the Igf1 and Igf1r genes in humans have been associated with severe intrauterine growth retardation and impaired skeletal maturation, but not with truncated limbs or severe skeletal dysplasia. The conflicting data between in vitro and in vivo observations with regard to bone morphogenesis suggests that IGFs may not be the sole trophic factors involved in fetal skeletal growth and that redundant mechanisms may exist in chondro- and osteogenesis. Further investigation is required in order to elucidate the functions of IGFs in skeletal development.

Contents

1. Introduction
2. Role of IGFs in fetal growth
3. Expression levels of IGF genes and proteins in fetal serum and tissues
4. IGF-I in limb morphogenesis
5. IGF-I genetic disorders in humans
6. Conclusion

1. Introduction

The components of the insulin-like growth factor (IGF) system, include IGFs (IGF-I and IGF-II), type 1 and type 2 IGF receptors, a family of six secreted IGF-binding proteins (IGFBPs) and IGFBP proteases (1). The IGFs are single-chain mitogenic polypeptides, structurally similar to proinsulin, that function in an autocrine/paracrine manner and also as classical hormones. The two IGF receptors are structurally and functionally unrelated. IGF ligand signaling is mediated by IGF-1R, which is a transmembrane glycoprotein with tyrosine kinase activity (2). IGF-2R is a single-chain protein without kinase activity (3). IGF-1R binds IGF-I with up to 20-fold higher affinity than for IGF-II, while IGF-2R strongly binds IGF-II, but barely recognizes IGF-I (2,3). In biological fluids, IGFs are usually bound by members of the secreted IGFBP family, of which the exact role remains unknown. IGFBPs are considered to mediate IGF-independent actions via their own receptors (1). The Igf2 and Igf2r genes are imprinted, expressed in a monoallelic manner depending on parental legacy. In the murine embryo, only the paternal Igf2 allele is expressed, while only the maternal Igf2r allele is expressed (4). However, subsequent to birth, Igf2 expression becomes biallelic in certain tissues, for example, in the liver (5). The present review focuses on the role of IGF-I in fetal growth and development, paying particular attention to skeletal development.

2. Role of IGFs in fetal growth

In initial IGF studies, the predominant roles of IGF-I and -II in fetal growth were elucidated by abundant but largely indirect evidence. IGFs were shown to act as proliferation and differentiation factors in cultured fetal cells (6-8) and preimplantation embryos (9), and were demonstrated to be secreted by cultured fetal cells and explants in vitro (10-11).
Direct evidence of the importance of IGFs and IGF receptors in the regulation of embryonic and early postnatal growth was provided by a series of studies using gene knockout, analyzing the phenotypes manifested by mutations, alone or in combination (4). Igf2(-/-) nullizygotes and heterozygous mice carrying a paternally derived mutated Igf2 gene were phenotypically indistinguishable (12). The mice were viable dwarfs with a birth weight 60% that of normal. Ablation of the Igf1 gene (Igf1 nullizygotes) resulted in a similar reduction of fetal growth (13) contradicting the prevailing hypothesis that IGF-II was the predominant mediator of fetal growth. Furthermore, the growth deficiency of the Igf1 mutants became evident at mouse embryonic day 13.5, when the size of the mutant embryos was ~90% that of normal size, subsequent to which the Igf1(-/-) embryos continued to grow at a slower rate, thus the mice were ~60% of normal size at the end of gestation (14). Surviving mutants continued to grow postnatally at a retarded rate, resulting in gaining only 30% of normal body weight as adults (14). This is in contrast to the normal birth weights observed in mice with GH-deficiency or GH-resistance (15,16) and suggests that in prenatal mice, IGF-1 is secreted independently of GH. Nevertheless, evidence indicates that GH acts as a local growth, differentiation and cell survival factor in the embryo, independent of IGF-1 (17). IGF1r nullizygotes exhibited an even greater reduction in birthweight (45% of normal) and died immediately following birth (13). The proposed underlying mechanism for growth retardation of Igf1 knock-out mice is that IGF-I and -II are not mitogenic per se. Deletion of Igf1 is suggested to lead to elongation of cell cycle time, resulting in fewer proliferation events during the same period and the generation of fewer cells than those required for the completion of embryonic development. In addition, evidence provided by Wajnkopf et al (18) and Bhakta et al (19) show that the influence of IGF-1 on fetal growth is dose-related.

3. Expression levels of IGF genes and proteins in fetal serum and tissues

The two IGFs have been detected in the fetal plasma early in gestation in the majority of animal species investigated thus far (20-22), with plasma concentrations of IGF-II found to be several fold higher than those of IGF-I (20,22). Notably, high IGF-II concentrations in fetal serum were demonstrated to decline within days following birth (20,23), while serum concentrations of IGF-I appeared to be low in the fetus and rise to decline within days following birth (20,23), while serum concentrations of IGF-I were low in the fetus and rise to decline within days following birth (20,23). Notably, high IGF-II concentrations in fetal serum were demonstrated to decline within days following birth (20,23), while serum concentrations of IGF-I appeared to be low in the fetus and rise to decline within days following birth (20,23). Notably, high IGF-II concentrations in fetal serum were demonstrated to decline within days following birth (20,23), while serum concentrations of IGF-I appeared to be low in the fetus and rise to decline within days following birth (20,23).

In accordance with the findings regarding plasma concentrations of IGF-II, the majority of studies reported higher abundance of Igf2 mRNA in fetal tissues compared with adult tissues (25). This raised the suggestion that IGF-II is the IGF that mediates growth and differentiation in developing fetal tissues. However, while IGF-II was revealed to be more abundant than IGF-I within the conceptus (serum and tissues), IGF-I was most closely associated with fetal growth in the majority of species. Thus, the plasma concentration of IGF-I, but not IGF-II, was found to correlate positively with fetal size and length, as well as birth and placental weight in humans (26-29). Alterations in the plasma or serum concentrations of IGF-I and IGFBP-1 and -3 have been identified in pregnancies complicated by preeclampsia and intrauterine growth restriction, where placental function is inadequate and fetal growth reduced (30-33). In such complicated pregnancies, the placent al expression levels of IGF-I and IGFBP-1 are also decreased (34,35).

Since serum concentrations may not reflect the production of peptides in specific tissues, several studies have attempted to detect the expression levels of Igf genes and/or peptides in vivo. Using reverse transcription-polymerase chain reaction (RT-PCR), transcripts of Igf1 and Igf2 receptor genes were detected in the fetal tissues of various species between the earliest stage of pre-implantation and the final phase of tissue maturation (36-39), while sensitive hybridization methods have shown that Igf gene expression was present in almost all human and rodent fetal tissues (40), including the liver, pancreas and osteochondral tissue.

Previous studies regarding the distribution of IGFs in the bones of piglets and mice, revealed localization within the growth plate (41,42). Igf1 and Igf2 mRNA was expressed throughout all zones, albeit Igf1 less extensively. Immunohistochemical techniques also revealed the expression of IGFs within the resting zone, the hypertrophic zone and the proliferative zone of the growth plate (41) (Fig. 1). Additionally, with the use of RT-PCR, IGFs were also detected within the perichondrium and metaphyseal bone in rats (43).

4. IGF-I in limb morphogenesis

During mammalian embryogenesis, growth factors are important not only in cellular proliferation and differentiation but also in morphogenesis. The developing limb constitutes an attractive model of tissue morphogenesis. At the end of week 4 of gestation, the developing limb buds become visible as outpocketings from the ventrolateral body wall. Initially, the limb buds consist of a mesenchymal core derived from the lateral plate mesoderm that forms the bones and connective tissues of the limb, covered by a layer of ectoderm. The ectoderm at the distal border of the limb thickens and forms the apical ectodermal ridge (AER) (44). This ridge exerts an inductive influence on the adjacent mesenchyme, causing the mesenchyme to remain as a population of undifferentiated, rapidly proliferating cells, the progressing zone. As the limbs grow, cells farther from the influence of the AER begin to differentiate into cartilage and muscle. In this manner, development of limb proceeds proximodistally (44). Fingers and toes are formed when cell death in the AER separates the ridge into five parts. The zone of polarizing activity (ZPA) is an additional signaling region at the posterior margin of the limb mesenchyme that controls the antero-posterior patterning of the limb (45).

While the external shape is being established, the mesenchyme in the buds begins to condense and differentiate into chondrocytes. By week 6 of development, the first hyaline cartilage models, foreshadowing the bones of the extremities, are formed by these chondrocytes. Ossification of the bones of the extremities, endochondral ossification, begins by the end of the embryonic period. Primary ossification centers are present in all long bones of the limb by week 12 of development (44).

Several studies have demonstrated the predominant role of the IGFs in limb development. IGF-I has been demonstrated
to stimulate proliferation of dissociated limb mesenchymal cells (46), isolated human fetal chondocytes (6), and explanted limb buds of rat and chicken embryos in vitro (47,48). Other studies, using in situ hybridization and immunohistochemistry, have demonstrated that IGF-I and its receptor (IGF-1R) are expressed in vivo by the sub ridge mesodermal cells of the developing rat and chicken limb buds (49-52), while in mouse embryos IGF-I has also been detected in the progress zone (53), suggesting that IGF-I may be involved in promoting the proliferation and outgrowth of the limb mesoderm in response to the AER or ZPA regions. Furthermore, several studies have revealed Igf1 transcripts in the condensing central core of mouse and chicken limbs (52,53), which implicates IGF-I in the regulation of chondrogenic differentiation. However, other studies did not detect Igf1 transcripts, and reported only Igf2 and Igf1r transcripts in the undifferentiated mesenchymal condensations and differentiated chondrocyte precursors in murine fetus chondrogenesis (54), verifying the results of in vitro experiments in limb organ cultures (47,48,52). Igf1 transcripts have been reported to be present in the osteoblast, osteo- and chondroclasts and nascent matrix of the long bones of developing chicken and mouse limbs, a location consistent with a potential role for IGF-I in endochondral bone formation (49). Notably, during the outgrowth and patterning of the limbs, IGF-I has been identified in mesoderm regions that undergo programmed cell death, including the interdigital zone in mouse and chicken embryos (52,53,55). Therefore, IGF-I is implicated in all activities (proliferation, differentiation and apoptosis) essential for proper limb morphogenesis.

Notably, no knock-outs of any IGF-axis member to date have been reported to result in defects in limb initiation, outgrowth or patterning. Thus, although Igf1(-/-) and Igf2(-/-) mutants exhibited growth impairment, only marginal ossification retardation occurred, and this did not exceed one embryonic day (12,13). However, postnatal comparisons of wild-type and surviving Igf1(-/-) mutants revealed the rate of long bone ossification to be greatly reduced in the mutants (14). Ablation of the Igf1r gene resulted in a greater delay in the appearance of the ossification centers in facial and cranial bones (lag of 2 embryonic days), and ossification of the interparietal bone exhibited an even longer delay (~4 days) (13). In support of these findings, the intravascular infusion of recombinant IGF-I in late gestation of fetal sheep resulted in no change in the lengths of the fetus and long bones. However, a rise in skeletal maturation was observed, as assessed by the acceleration of the appearance of epiphysial centers and the increase in cross-sectional areas of the bones (56). Furthermore, overexpression of IGF-I in mice resulted in disproportionate overgrowth of certain organs but no increase in the length of long bones (57). These observations indicate the existence of redundant mechanisms for the developmental processes of limb morphogenesis, including chondro- and osteogenesis, and/or compensatory actions of IGF-axis members. In this regard, Dealy and Kosher (58) observed that insulin mimics the effects of IGF-I in promoting AER induction and limb outgrowth in vitro, and Messiano et al (59) demonstrated that hypophysectomized lamb fetuses with normal plasma concentrations of IGF-I and IGF-II exhibited delayed osseous maturation, which was restored by thyroxine administration. These observations suggest that IGFs are not the sole trophic factors involved in fetal skeletal development. More recent studies, using novel methods to visualize and quantify differences in the structure and mineral density of fetal bones in Igf1(-/-) knock-out mice compared with bones in wild-type mice, report hypomineralization and differences in bone microstructure, possibly representing impaired remodeling activity in the absence of IGF-I (60,61).

5. IGF-I genetic disorders in humans

In the last few years, reports of patients with genetic defects in various components of the IGF-axis have broadened knowledge regarding the role of IGFs in intra-uterine growth and development. The first human case concerning a patient with a homozygous partial deletion (exons 4 and 5) of the Igf1 gene was described by Woods et al (62). This mutation was
manifested by severe intrauterine growth retardation [birth weight -3.9 standard deviations (SD), birth length -5.4 SD and microcephalia] and dysmorphic features (micrognathia and bilateral clinodactyly), in addition to postnatal growth failure, including delayed bone age and severe osteopenia. Although the growth impairment of the Igf1 null patient was relatively more severe than that of the Igf1 knock-out mice (13), the overall phenotypic features were similar.

Since then, a variety of molecular defects in the Igf1 gene have been reported, including homozygosity for a missense mutation (18), another missense mutation with a milder phenotype (63) and a nucleotide substitution (polymorphism) (64), which was later found to also occur in healthy controls (65). In 2010, van Duyvenvoorde et al (66), described a case of heterozygosity for a frameshift mutation characterized by short stature and microcephaly. Later, using molecular methods, the same group concluded that the short stature of the patients cannot be attributed exclusively to the Igf1 gene defect but to the combination of the Igf1 gene with other factors, including placental IGF-I insufficiency and other genetic factors (67).

In any case, severe pre- and postnatal growth impairment, microcephaly, dysmorphic features, retarded skeletal maturation, deaf-mutism and mental retardation, though variable in density, appear to be common characteristics of patients with Igf1 gene molecular defects.

In humans, the potential lethal effect from total loss of IGF-1R may explain why only heterozygous mutations in the Igf1r gene have been reported to date. Thus, several cases of either heterozygosity for Igf1r gene mutations (68-75) or Igf1r gene haploinsufficiency (loss of the distal long arm of chromosome 15q26) have been reported (76-79). All patients exhibit a similar phenotype to patients with mutations in the Igf1 gene. However, compared with patients exhibiting heterozygosity for Igf1r mutations (Igf1r haploinsufficiency), patients with loss of the Igf1r gene tend to have more prominent phenotypic abnormalities, with greater dysmorphic features, an increased delay in motor development and impaired psychosocial skills. The extent to which these features reflect the loss of contiguous genes on chromosome 15 is uncertain. The heterogeneity in the clinical phenotypes of patients with molecular defects in the Igf1 or Igf1r genes may suggest variability in the degree of functional loss of the IGF-I/IGF-1R interaction and/or the involvement of other genetic or environmental factors.

6. Conclusion

The two IGFs and the main IGF receptor IGF-1R are indisputably important in embryonic and fetal growth and development, as indicated by in vitro findings, in vivo experiments with knockout mice and case reports of patients with molecular defects in the IGF-axis members. Although IGF-II is more abundantly expressed in the serum and tissues of the conceptus than IGF-I, IGF-I appears to be more closely associated with fetal growth in the majority of species. IGF-I is generally considered to affect fetal growth in a dose-related manner, independently of GH. However, controversy remains surrounding the data from in vitro and in vivo observations, and the exact role of IGFs, as pertains to the prenatal development of the skeleton, remains uncertain. Further investigation is required in fetuses with impaired skeletal development, in the context of fetal growth restriction or skeletal dysplasia, in order to elucidate the role of the IGFs in fetal growth and skeletal development.

References

1. Jones HI and Clemmons DR: Insulin-like growth factors and their binding proteins: biological actions. Endocr Rev 16: 3-34, 1995.
2. LeRoith D, Werner H, Beiter-Johnson and Roberts CT Jr: Molecular and cellular aspects of the insulin-like growth factor I receptor. Endocr Rev 16: 143-163, 1995.
3. Kornfeld S: Structure and function of the mannose-6-phosphate/insulin-like growth factor II receptors. Annu Rev Biochem 61: 307-330, 1992.
4. Esfjatadi A: Genetics of mouse growth. Int J Dev Biol 42: 955-976, 1998.
5. Davies SM: Developmental regulation of genomic imprinting of the Igf2 gene in human liver. Cancer Res 54: 2560-2562, 1994.
6. Vetter U, Zapf J, Heit W, et al: Human fetal and adult chondrocytes. Effect of insulin-like growth factors I and II, insulin, and growth hormone on clonal growth. J Clin Invest 77: 1903-1908, 1986.
7. Bhaumick B and Bala RM: Differential effects of insulin-like growth factors I and II on growth, differentiation and glucose-regulation in differentiating chondrocyte cells in culture. Acta Endocrinol (Copenh) 125: 201-211, 1991.
8. Lorenzo M, Valverde AM, Ternel T and Benito M: IGF-I is a mitogen involved in differentiation-related gene expression in fetal rat brown adipocytes. J Cell Biol 123: 1567-1575, 1993.
9. Harvey MB and Kaye PL: Insulin-like growth factor I-stimulates growth of mouse preimplantation embryos in vitro. Mol Reprod Dev 31: 195-199, 1992.
10. Canalies E, McCarthy T and Centrella M: Isolation and characterization of insulin-like growth factor I (somatomedin-C) from cultures of fetal rat calvariae. Endocrinology 122: 22-27, 1988.
11. D’Ercole AJ, Applewhite GT and Underwood LE: Evidence that somatomedin is synthesized by multiple tissues in the fetus. Dev Biol 75: 315-328, 1980.
12. DeChiara TM, Esfjatadi A and Robertson J: Growth deficiency phenotype in heterozygous mice carrying an insulin-like growth factor I gene disrupted by targeting. Nature 345: 78-80, 1990.
13. Liu J-P, Baker J, Perkins AS, Roberson EJ and Esfjatadi A: Mice carrying null mutations of the genes encoding insulin-like growth factor I (Igf1) and type 1 IGF receptor (Igf1r). Cell 75: 99-72, 1993.
14. Baker J, Liu J-P, Robertson EJ and Esfjatadi A: Role of insulin-like growth factors in embryonic and postnatal growth. Cell 75: 73-82, 1993.
15. Wajnrajch MP, Gertner JM, Harbisson MD, Chua SC Jr and Leibel RL: Nonsense mutation in the human growth hormone-releasing hormone receptor causes growth failure analogous to the little (lit) mouse. Nat Genet 12: 88-90, 1996.
16. Savage MO, Blum WF, Ranke MB, et al: Clinical features and endocrine status in patients with growth hormone insensitivity (Laron syndrome). J Clin Endocrinol Metab 77: 1465-1471, 1993.
17. Sanders EJ and Harvey S: Growth hormone as an early embryonic growth and differentiation factor. Anat Embryol (Berl) 209: 1-9, 2004.
18. Wallenkamp MJ, Karpenien M, Pereira AM, et al: Homozygous and heterozygous expression of a novel insulin-like growth factor-I mutation. J Clin Endocrinol Metab 90: 2855-2864, 2005.
19. Bhakta KY, Marlin SJ, Shen JJ and Fernandes CJ: Terminal deletion of chromosome 15q26.1: case report and brief literature review. J Perinatol 25: 429-432, 2005.
20. Gluckman PD and Butler JH: Parturition-related changes in insulin-like growth factors-I and -II in the perinatal lamb. J Endocrinol 199: 223-232, 1983.
21. Holland MD, Hossner KL, Williams SE, Wallace CR, Niswender GD and Odde KG: Serum concentrations of insulin-like growth factors and placental lactogen during gestation in cattle. Domest Anim Endocrinol 14: 231-239, 1997.
22. Lee CY, Chung CS and Simmen FA: Ontogeny of the porcine insulin-like growth factor system. Mol Cell Endocrinol 93: 71-80, 1993.
23. Moses AC, Nissley SP, Short PA, et al: Increased levels of multiplication-stimulating activity, an insulin-like growth factor, in fetal rat serum. Proc Natl Acad Sci USA 77: 3649-3653, 1980.
IGF expression
Growth
Evaluation of fetal
The skeletal structure of
36.
35.
34.
33.
32.
31.
30.
27.
26.
25.
24.
23.
22.
21.
20.
19.
18.
17.
16.
15.
14.
13.
12.
11.
10.
9.
8.
7.
6.
5.
4.
3.
2.
1.

Parker EA, Hegde A, Buckley M, Barnes KM, Baron J and
the PTHrP/Ihh pathway. J Bone Miner Res 26: 1437-46, 2011.

Wang Y, Cheng Z, Elalieh HZ, et al: IGFIR signaling in chondrocytes modulates growth plate development by interacting with the PTHR/P/Ip/hippathway. J Bone Miner Res 26: 1437-46, 2011.

E363-E367, 2010.

Short stature associated with a novel heterozygous mutation in the upstream core polyadenylation signal of IGF1 gene is associated with short stature. J Pediatr 157: 331-335, 2010.

Netchine I, Azzi S, Housang M, et al: Altered expression of insulin-like growth factor I- and insulin-like growth factor binding proteins 2 and 5 in the mouse mutant Hypoactinity (Hd) correlates with sites of apoptotic activity. Anat Embryol (Berl) 202: 1-11, 2000.

Wang E, Wang J, Chin E, Zhou J and Bondy CA: Cellular patterns of insulin-like growth factor system gene expression in murine chondrogenesis and osteogenesis. Endocrinology 136: 2741-2751, 1995.

Allan GJ, Flint DJ, Darling SM, Geh J and Patel K: Altered expression of insulin-like growth factor 1 and insulin-like growth factor binding proteins 2 and 5 in the mouse mutant Hypoactinity (Hd) correlates with sites of apoptotic activity. Anat Embryol (Berl) 202: 1-11, 2000.

Lok F, Owens JA, Mundy L, Robinson JS and Owens PC: Insulin-like growth factor I promotes growth selectively in fetal sheep in late gestation. Am J Physiol 270: R1148-R1155, 1996.

Mathews LS, Hammer RE, Behringer RR, et al: Growth enhancement of transgenic mice expressing human insulin-like growth factor I. Endocrinology 125: 287-293, 1988.

Dealy CN and Kosher RA: Studies on insulin-like growth factor-I and insulin in chick limb morphogenesis. Dev Dyn 202: 67-79, 1995.

Mesiario S, Dunn MS, Baxter RC, Hintz RL, Browne CA and Thorburn GD: Effect of hypophysectomy with and without thyroxine replacement on growth and circulating concentrations of insulin-like growth factors I and II in the fetal lamb. Endocrinology 120: 1821-1830, 1987.

Bikle D, Majumdar S, Laib A, et al: The skeletal structure of insulin-like growth factor I-deficient mice. J Bone Miner Res 16: 2330-2339, 2001.

Burghardt AJ, Wang Y, Elalieh H, et al: Evaluation of fetal bone structure and mineralization in IGF-I deficient mice using synchrotron radiation microtomography and Fourier transform infrared spectroscopy. Bone 40: 160-168, 2007.

Majumdar S, Barter D, Clark AJL and Savage MO: Insulin-like growth factor I gene deletion causing intrauterine growth retardation and distal limb malformations: underlying mechanisms and clinical associations. Clin Genet 60: 165-172, 2001.

Kaplowitz PB, D’Erocle AJ and Underwood LE: Stimulation of embryonic mouse limb bud mesenchymal cell growth by peptide growth factors. J Cell Physiol 112: 353-359, 1982.

Bhattacharjee B and Bala RM: Receptors for insulin-like growth factor I and II in developing embryonic mouse limb bud. Bioch Biophys Acta 927: 117-128, 1987.

Geduspan JS and Solursh M: Effects of the mesonephros and insulin-like growth factor I on chondrogenesis of limb explants. Endocrinology 125: 1560-1566, 1989.

Sifakis S, Basel D, Ianakiev P, Kilpatrick M and Tsipouras P: Distal limb malformations: underlying mechanisms and clinical associations. Clin Genet 60: 165-172, 2001.

Kaplowitz PB, D’Erocle AJ and Underwood LE: Stimulation of embryonic mouse limb bud mesenchymal cell growth by peptide growth factors. J Cell Physiol 112: 353-359, 1982.

Bhattacharjee B and Bala RM: Receptors for insulin-like growth factor I and II in developing embryonic mouse limb bud. Bioch Biophys Acta 927: 117-128, 1987.

Geduspan JS and Solursh M: Effects of the mesonephros and insulin-like growth factor I on chondrogenesis of limb explants. Endocrinology 125: 1560-1566, 1989.

Sifakis S, Basel D, Ianakiev P, Kilpatrick M and Tsipouras P: Distal limb malformations: underlying mechanisms and clinical associations. Clin Genet 60: 165-172, 2001.

Kaplowitz PB, D’Erocle AJ and Underwood LE: Stimulation of embryonic mouse limb bud mesenchymal cell growth by peptide growth factors. J Cell Physiol 112: 353-359, 1982.

Bhattacharjee B and Bala RM: Receptors for insulin-like growth factor I and II in developing embryonic mouse limb bud. Bioch Biophys Acta 927: 117-128, 1987.

Geduspan JS and Solursh M: Effects of the mesonephros and insulin-like growth factor I on chondrogenesis of limb explants. Endocrinology 125: 1560-1566, 1989.

Sifakis S, Basel D, Ianakiev P, Kilpatrick M and Tsipouras P: Distal limb malformations: underlying mechanisms and clinical associations. Clin Genet 60: 165-172, 2001.

Kaplowitz PB, D’Erocle AJ and Underwood LE: Stimulation of embryonic mouse limb bud mesenchymal cell growth by peptide growth factors. J Cell Physiol 112: 353-359, 1982.

Bhattacharjee B and Bala RM: Receptors for insulin-like growth factor I and II in developing embryonic mouse limb bud. Bioch Biophys Acta 927: 117-128, 1987.

Geduspan JS and Solursh M: Effects of the mesonephros and insulin-like growth factor I on chondrogenesis of limb explants. Endocrinology 125: 1560-1566, 1989.

Sifakis S, Basel D, Ianakiev P, Kilpatrick M and Tsipouras P: Distal limb malformations: underlying mechanisms and clinical associations. Clin Genet 60: 165-172, 2001.

Kaplowitz PB, D’Erocle AJ and Underwood LE: Stimulation of embryonic mouse limb bud mesenchymal cell growth by peptide growth factors. J Cell Physiol 112: 353-359, 1982.
67. van Duyvenvoorde HA, van Doorn J, Koenig J, et al: The severe short stature in two siblings with a heterozygous IGF1 mutation is not caused by a dominant negative effect of the putative truncated protein. Growth Hormone IGF Res 21: 44-50, 2011.

68. Abuzzahab MJ, Schneider A, Goddard A, et al: IGF-I receptor mutations resulting in intrauterine and postnatal growth retardation. N Engl J Med 349: 2211-2222, 2003.

69. Kawashima Y, Kanzaki S, Yang F, et al: Mutation at cleavage site of insulin-like growth factor receptor in a short-stature child born with intrauterine growth retardation. J Clin Endocrinol Metab 90: 4679-4687, 2005.

70. Raile K, Klammt J, Schneider A, et al: Clinical and functional characteristics of the human Arg59Ter insulin-like growth factor I receptor (IGF1R) mutation: implication for a gene dosage effect of the human IGF1R. J Clin Endocrinol Metab 91: 2264-2271, 2006.

71. Walenkamp MJ, van der Kamp HJ, Pereira AM, et al: A variable degree of intrauterine and postnatal growth retardation in a family with a missense mutation in the insulin-like growth factor I receptor. J Clin Endocrinol Metab 91: 3062-3070, 2006.

72. Inagaki K, Tulipakov A, Rubtsov P, et al: A familial insulin-like growth factor I receptor mutant leads to short stature: clinical and biochemical characterization. J Clin Endocrinol Metab 92: 1542-1548, 2007.

73. Kruis T, Klammt J, Galli-Tsinopoulou A, et al: Heterozygous mutation within a kinase-conserved motif of the insulin-like growth factor I receptor causes intrauterine and postnatal growth retardation. J Clin Endocrinol Metab 95: 1137-1142, 2010.

74. Wallborn T, Wüller S, Klammt J, et al: A heterozygous mutation of the insulin-like growth factor-I receptor causes retention of the nascent protein in the endoplasmic reticulum and results in intrauterine and postnatal growth retardation. J Clin Endocrinol Metab 95: 2316-2324, 2010.

75. Choi JH, Kang M, Kim GH, et al: Clinical and functional characteristics of a novel heterozygous mutation of the IGF1R gene and IGF1R haploinsufficiency due to terminal 15q26.2->qter deletion in patients with intrauterine growth retardation and postnatal catch-up growth failure. J Clin Endocrinol Metab 96: E130-E134, 2011.

76. Roback EW, Barakat AJ, Dev VG, Mbikay M, Chrétien M and Butler MG: An infant with deletion of the distal long arm of chromosome 15 (q26.1 - qter) and loss of insulin-like growth factor I receptor gene. Am J Med Genet 38: 74-79, 1991.

77. Walenkamp MJ, de Muinck Keizer-Schrama SM, de Mos M, et al: Successful long-term growth hormone therapy in a girl with haploinsufficiency in the insulin-like growth factor-I receptor due to a terminal 15q26.2->qter deletion detected by multiplex ligation probe amplification. J Clin Endocrinol Metab 93: 2421-2425, 2008.

78. Fang P, Schwartz ID, Johnson BD, et al: Familial short stature caused by haploinsufficiency of the insulin-like growth factor I receptor due to nonsense-mediated messenger ribonucleic acid decay. J Clin Endocrinol Metab 94: 1740-1747, 2009.

79. Ester WA, van Duyvenvoorde HA, de Wit CC, et al: Two short children born small for gestational age with insulin-like growth factor I receptor haploinsufficiency illustrate the heterogeneity of its phenotype. J Clin Endocrinol Metab 94: 4717-4727, 2009.