

# The growth hormone (GH) and insulin-like growth factor (IGF) system in girls and women with Turner syndrome

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**Abstract.** Growth hormone (GH), its receptor and signaling cascade and the insulin-like growth factor (IGF) system are important regulators of growth and metabolic homeostasis. The GH–IGF axis in girls and women with Turner syndrome has been studied by many groups. A variety of distinct abnormalities as to synthesis and secretion of GH and IGFs as well as perturbed signaling and impaired biologic responses to GH and/or IGFs have been reported. This chapter summarizes the literature and gives a synopsis of current knowledge as to findings related to the GH and IGF system in girls and women with Turner syndrome. © 2006 Elsevier B.V. All rights reserved.

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## 1. Introduction

Growth hormone (GH; somatotropin) and a family of peptide hormones referred to as insulin-like growth factors (IGFs; somatomedins) are the most important stimulants of skeletal and somatic growth. They are also key modulators of energy homeostasis and metabolic control in many tissues. GH is secreted by somatotroph cells in the lateral parts of the anterior pituitary gland. Growth hormone releasing hormone (GHRH) stimulates while somatostatin (SRIF) inhibits GH synthesis and GH secretion. In the circulation GH is complexed to a binding protein (GHBP) corresponding to the extracellular portion of the GH membrane receptor. The GH receptor is a monomeric transmembrane protein that lacks

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tyrosine kinase activity and signals through the JAK–STAT pathway [1]. Suppressors of cytokine signaling (SOCS) typically limit cytokine receptor such as GH receptor signaling via the JAK–STAT pathway. Considerable evidence demonstrates that SOCS2 limits growth hormone (GH) action on body and organ growth. Biochemical evidence that SOCS2 binds to the type I insulin-like growth factor receptor (IGF-IR) supports the novel possibility that SOCS2 also limits IGF-I action [1–6].

The IGFs are important mediators of cell proliferation and longitudinal bone growth. IGF-I and IGF-II share very similar chemical structure and complement the metabolic effects of insulin. IGF-I is GH dependent and mediates many biologic effects of GH. IGF-II is less GH dependent and is thought to play a role during embryonic and fetal development. The IGFs are pleiotropic factors which are also involved in regulating metabolic homeostasis and modulation of apoptosis and tumor transformation. Most importantly, IGF-I is considered to be crucial for both pre- and postnatal growth in the human. Growth during human fetal life represents the most rapid phase of human growth [7]. Fetal growth depends upon substrate and oxygen supply, vascularization of placental and fetal tissues, and a complex endocrine modulation of cellular proliferation, tissue expansion, inhibition of apoptosis and tissue remodeling. IGF-I and IGF-II have both been implicated to be important regulators of human fetal growth. IGF-I is produced by the fetal liver [1,2,8,9]. This production is growth hormone-independent but is directly stimulated by insulin and fetal glucose uptake [6]. IGF-I has been shown to be a key stimulus of placental substrate uptake. IGF-I inhibits fetal placental catabolism and reduces placental lactate production. Targeted disruption of the mouse IGF-II gene leads to a 40% reduction of fetal but normal postnatal growth. Disruption of the IGF-I gene leads to both pre- and post-natal growth failures. Mice with deletion of the IGF-I receptor gene have the most severe phenotype with birth weights of only 45% of normal. Mice with an IGF-I receptor knock-out genotype usually die shortly after birth. Muscular hypotrophy leads to respiratory insufficiency in these animals pointing to the key role of the IGF-I receptor for the development and expansion of skeletal muscle [10,11].

The IGFs circulate bound to so-called IGF binding proteins, IGFBPs. There are six IGFBPs, IGFBP-1, to -6. In addition, a number of IGFBP-like proteins have also been described, the function of which is less known when it comes to their putative role as IGF modulators [1,2]. The IGFs signal through binding to the insulin receptor family: the insulin receptor (IR) binds insulin with the highest affinity and IGF-I and -II with lower affinity. The IGF-II/mannose-6-phosphate receptor is a monomer which has a small cytoplasmic domain lacking tyrosine kinase activity. In addition to IGF-II it binds lysosomal enzymes bearing the mannose-6-phosphate residues. This receptor targets lysosomal enzymes to lysosomes [1]. The insulin receptor related receptor (IRR) is mainly expressed in neuronal cells and interestingly its expression in human tumor cells relates to disease-free survival in children with neuroblastomas. No ligand has been found for the IRR [3].

The IGF-IR is closely related to the insulin receptor with a homology of 80–95% in the tyrosine kinase domain [1,3,4,11]. IGF-I binding to the IGF-IR causes the transmembrane activation of the tyrosine kinase activity of the IGF-IR [5]. In contrast to the insulin receptor, an impairment of the closely related IGF-IR has only been suggested on rare occasions under clinical circumstances (for review see [4]). In humans, the IGF-I receptor gene is located on the distal long arm of chromosome 15 (15q26.3). The receptor is synthesized as a large

Table 1

IGF-I receptor mutations or loss of fragments of chromosome 15 lead to intrauterine growth retardation, postnatal growth deficits, and in addition occasionally craniofacial and skeletal abnormalities and mild to moderate mental retardation [4]

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- a. Compound heterozygosity for point mutations in exon 2 of the IGF-I receptor gene
  - b. Nonsense mutation (Arg59stop) of the IGF-I receptor gene
  - c. Deletions of the long arm of chromosome 15
  - d. Hemizyosity for *IGF-IR*
  - e. Ring chromosome 15, with loss of sequences of the long arm
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precursor protein that undergoes extensive post-translational modifications including cleavage and glycosylation [3,11]. The mature and functional IGF-I receptor is a heterotetramer, consisting of two alpha- and two beta-subunits. The alpha subunits form the extracellular domain for ligand binding. The IGF-I receptor binds IGF-I with high and IGF-II and insulin with lower affinity. In contrast, the insulin receptor is activated by low concentrations of insulin but higher doses of IGFs are required for activation of the IR [3]. The beta-subunits of the receptors contain intracellular tyrosine kinase domains and are responsible for transphosphorylation of the receptors. Phosphorylation of the IGF-I receptor leads to interaction with a number of signaling molecules, phosphorylation of insulin-receptor substrates (IRS), activation of PI3-kinase (phosphatidylinositol-3) and MAP kinase [3,11]. We have recently described a family who were found to be heterozygous for the point mutation CGA → TGA (Arg59stop), in exon 2 of the IGF-I receptor gene. Sequencing of all exons encoding the IGF-I receptor showed no additional mutation, thus a compound heterozygous mutation on both alleles was excluded. Suggesting autosomal dominant inheritance, the grandfather (I/I) presenting short stature would be a candidate to carry the mutation, unfortunately he was lost for follow up. The mutation creates a novel *DdeI* site, resulting in two additional fragments of 97 and 155 bp to the 252 bp PCR product [4,12,13]. This finding is further proof for the concept that GH and IGF-I and their signaling pathways are crucial for longitudinal growth in the human (Table 1).

The GH–IGF axis in girls and women with Turner syndrome has been studied by many groups. A variety of distinct abnormalities as to synthesis and secretion of GH and IGFs as well as perturbed signaling and impaired biologic responses to GH and/or IGFs have been reported. This chapter summarizes the literature and gives a synopsis of current knowledge as to findings related to the GH and IGF system in girls and women with Turner syndrome.

## 2. GH and GH secretion in girls and women with Turner syndrome

GH secretion upon clonidine stimulation and/or 1–29 GHRH stimulation showed a variable and somewhat reduced response in girls with TS in one study. However, GH measurements were not useful in indicating GH therapy or predicting the response to GH therapy [14]. This lack of correlation between GH secretory capacity and response to GH therapy was also confirmed in a study in 41 girls with TS. In this study 22% of the girls undergoing GH stimulation tests had a subnormal (<11 ng/ml) and 7% had a frank pathological (<7 ng/ml) GH test, the majority of the TS patients showing normal GH secretion upon provocative testing [14]. In a cohort of 81 girls with TS, Pirazzoli et al.

found that mean nocturnal GH concentration was significantly lower than that measured in 27 prepubertal normal girls and even equaled the low levels measured in 29 prepubertal GH-deficient children [15]. However, mean GH concentrations as measured in 24-h profiles is similar in girls with Turner syndrome to that of prepubertal normally growing children. The number of GH peaks is 7–8 over the 24-h period in girls with Turner syndrome as well as in normal control girls. The mean GH secretion in untreated girls with TS does not change between 6 and 18 years. Since GH secretion expressed either as area under the curve or as the maximal GH level over the 24-h period increases during puberty in healthy, normally growing children and adolescents, there is an increasing disparity in adolescence, with GH secretion remaining low in untreated TS patients [16–18]. However, the mean GH concentrations in estrogen-treated girls with Turner syndrome are significantly higher than those of untreated girls with TS. Treated girls showed similar GH secretory capacity as healthy girls during puberty. Ishikawa et al. showed that serum levels of 20-kDa human growth hormone parallel those of 22-kDa human GH both in normal children, GH-deficient children and in girls with TS. Thus, the percentage of 20-kDa GH in the circulation is constant, regardless of age, gender, puberty, height SD score, body mass index and GH secretion status [19]. In conclusion, GH secretion in TS is similar to that of normally growing short children but shows no change with age. Periodicity of GH secretion is no different in various groups of girls with TS, normally growing healthy children and GH-sufficient short children. Estrogen treatment of girls with TS will lead to progression of puberty and enhancement of GH secretion similar to the one seen in healthy girls during pubertal development [20]. It is important to note that endogenous GH secretion does not correlate with growth in patients with TS as found by the Italian Study Group for Turner syndrome [21]. In contrast, and importantly, in a recent paper by Binder et al. the d3-GH receptor polymorphism has been shown to be associated with increased responsiveness to GH in Turner syndrome and small-for-gestational-age children. This finding – once confirmed in larger cohorts – could explain the differences in response to GH therapy in girls with TS and be important for guidance and dosing of growth promoting therapies [22]. In addition, GH binding protein levels have been measured in a large cohort of 6447 subjects and actually were highest in patients with TS. GHBP levels were GH independent and not predictive of responses to GH therapy, although low GHBP levels might have indicated GH receptor abnormalities and partial GH insensitivity [9].

However, in one study of 27 adult women with TS and 24 age-matched healthy controls, the integrated 24-h GH concentration was reduced in women with TS. Multiple regression analysis revealed that fat-free mass and maximal oxygen uptake accounted for 60% of the variance in the 24-h GH secretory capacity. After adjustment of these two variables, any difference between GH concentration between Turner patients and controls disappeared [20]. Importantly, 17 $\beta$ -estradiol replacement therapy was associated with normalizing GH secretion in the TS women [23]. Thus, it seems clear that there is no intrinsic underlying defect in GH secretion in girls and women with TS [24].

### **3. IGF and IGFBP serum concentrations in girls and women with Turner syndrome**

Reference ranges for IGF-I, -II and IGFBP-1, -2 and -3 serum concentrations have been established for healthy children, adolescence and adults over the life span. In most studies

using such reference values, IGF-I and IGFBP-3 levels are lower in groups of girls with TS than in normally growing, healthy controls [23,24]. In addition to the decreased total IGF-I levels, free IGF-I serum concentrations are also lower in patients with TS [23]. Interestingly, Western ligand blotting of sera from girls with TS revealed an increased IGFBP-3 proteolysis in TS. This finding could explain why in some studies using particular assays low IGFBP-3 levels have been reported while in others normal IGFBP-3 levels in TS patients have been shown. In the latter studies presumably assays which in addition to intact IGFBP-3 also measure proteolysed IGFBP-3 have been employed. Finally, Cianfarani et al. assessed fasting serum levels of IGFBP-1 to test the hypothesis of whether or not IGFBP-1 could play a role in the pathogenesis of growth failure and metabolic derangements in TS. IGFBP-1 serum levels in TS girls were within the normal range and were inversely related to bone age, body weight and body mass index. Thus, IGFBP-1 levels were confirmed to be regulated by nutritional factors (and insulin), but did not add to the pathogenesis of growth failure in TS [25].

The issue of IGF and IGFBP levels as measures of poor growth and/or of biochemical GH deficit is hampered by the fact that there are cases with non-detectable levels of circulating IGF-I yet normal height and growth velocity, or with non-detectable levels of GH yet normal growth and IGF-I levels. The interpretation and clinical utility of IGF measurements are currently being widely discussed [26]. Many authors propose a role for IGF-I measurements in optimizing GH dosing [27]. Since upon GH treatment in TS patients supraphysiological levels of IGF-I are reached when optimal growth is induced, these questions are of particular relevance for TS patients. Accordingly, the development of easy to use and convenient laboratory methods such as IGF-I and IGFBP-3 measurements on filter paper blood spots are of clinical importance in monitoring IGF levels during GH treatment [28]. However, no clear correlation between IGF-I and IGFBP-3 levels in growth response to GH treatment was demonstrable in a large cohort of GH treated patients [29]. Therefore IGF and IGFBP measurements during growth promoting therapies might be for safety reasons only and not clinically useful in regards to aiding dose adjustments [29].

#### **4. IGF signaling and GH and IGF mediated responses in girls and women with Turner syndrome**

Turner syndrome is associated with increased insulin resistance and adiposity, which might be associated with type 2 diabetes in later life. Salgin et al. therefore used hyperinsulinemic euglycemic clamp techniques to investigate insulin sensitivity and putative causal factors in sixteen adult women with TS. In a multiple regression analysis the Turner karyotype was significantly related to insulin sensitivity independent of any differences in fat-free mass and percent whole-body fat mass [30]. Similarly, IGF-I resistance has been suggested to be present in TS patients. In fact, clinical data show that high levels of IGF-I might be required to overcome such 'IGF-I resistance' in patients with TS [31]. Decreased sensitivity to IGF-I was demonstrated at the molecular level in one study: lymphocytes derived from peripheral blood of TS patients did not respond as well to GH and/or IGF-I in regards to LDL degradation, mixed lymphocyte reaction, of IL-2 secretion after blastoid transformation as did lymphocytes from normal healthy donors [32]. Furthermore, Barreca et al. using an in vitro fibroblast culture model demonstrated that

Table 2

Synopsis of GH secretion, IGF-I and IGFBP serum levels in girls and women with Turner syndrome

GH secretion	Low/ <b>normal</b>
GH stimulation tests	Frequently normal
GHBP	High/ <b>normal</b>
IGF-I serum concentration	Low/ <b>normal</b>
IGFBP-1	Normal
IGFBP-3	Low/ <b>normal</b>
IGFBP-3 proteolysis	Enhanced

fibroblasts isolated from TS patients released into cell culture medium significantly lower amounts of IGF-I and IGF-II than fibroblasts from normal controls [33]. Since the interassay variability of such cell culture models is considerably large, the conclusions derived from these studies may be limited. No specific cellular defect which might indicate and/or cause a relative IGF-I insensitivity and hence growth failure in TS has been found so far (Table 2).

## 5. Discussion

Insulin-like growth factors are involved in many physiological processes, including classical endocrine functions, like growth hormone dependent longitudinal growth during childhood and adolescence, or paracrine and autocrine functions, affecting embryonal proliferation, differentiation, and regression of various organs and tissues [2]. The IGF-I receptor mediates most effects on proliferation and inhibition of apoptosis, induced by both IGF-I and IGF-II [3]. Hence, many groups have focussed their attention on the GH–IGF system to search for putative abnormalities in TS patients that might explain the growth failure and growth deficit in these patients. Both perinatal growth retardation and postnatal lack to catch up growth despite sufficient IGF-I levels have been identified in TS patients. It seems reasonable to conclude that there may be an intrinsic end organ unresponsiveness to GH–IGF promoting effects in cartilage and/or bone in TS patients. It seems clear to date that there is no distinct abnormality of the GH nor IGF system in TS patients (Table 2). However, secondary derangements of the GH–IGF axis may enhance the growth deficit in TS patients particularly so during puberty.

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