Growth in Boys with 45,X/46,XY Mosaicism: Effect of Growth Hormone Treatment on Statural Growth

Silvano Bertelloni a Giampiero I. Baroncelli a Francesco Massart a
Benedetta Toschi b

a Pediatric Division, Adolescent Medicine, Department of Obstetrics, Gynecology and Pediatrics, and
b Medical Genetics Laboratory, Clinical Genetics, Azienda Ospedaliero-Universitaria Pisana, Pisa, Italy

Key Words
Growth · Growth hormone · Mixed gonadal dysgenesis · Mosaicism 45,X/46,XY · Short stature · SHOX · Treatment · Y-chromosomal growth gene(s)

Abstract
45,X/46,XY mosaicism is a rare sex chromosome disorder of sex development. Short stature is a main feature of boys with this condition. Different causes likely contribute to growth impairment. Growth hormone (GH) has been administered to treat short stature in boys with 45,X/46,XY mosaicism, but conflicting data are available. Here, spontaneous growth patterns as well as short- and long-term follow-up studies during GH therapy in these patients are reviewed. Short- and mid-term data showed an improvement of the growth pattern in GH-treated boys, mainly when hormonal therapy was started early, while long-term follow-up demonstrated similar adult heights in GH-treated and untreated patients. Individual biological factors (e.g. different chromosome constitution, different mosaicism among various tissues, impaired pubertal growth spurt), non-homogeneous GH doses and different ages at start of therapy may contribute to the variable results. Thus, early GH therapy at pharmacological doses may improve the growth pattern of short boys with 45,X/46,XY mosaicism, but data on adult height are disappointing. Evaluation of larger patient samples treated by homogeneous doses and long-term follow-up studies assessing adult height and safety are needed to reach definitive conclusions on GH therapy in boys with 45,X/46,XY mosaicism.

The term ‘disorders of sex development’ (DSD) defines a group of congenital conditions, in which the development of chromosomal, gonadal or anatomical sex is atypical [Hughes et al., 2006]. Among the various DSD conditions, there are sex chromosome DSD, due to numerical or structural abnormalities of the sex chromosomes [Hughes et al., 2006]. 45,X/46,XY mosaicism is a rare sex chromosome DSD associated with a broad spectrum of clinical phenotypes ranging from normal male appearance to an almost female phenotype, including babies with overt genital ambiguity and stigmata of Turner syndrome; the latter presentation is also defined as mixed gonadal dysgenesis (MGD) [Gantt et al., 1980; Knudtzon and Aarskog, 1987; Telvi et al., 1999; Lindhardt Johansen et al., 2012]. Sex of rearing may be male or female based on the appearance of genitalia at birth [Telvi et al., 1999;
Lindhardt Johansen et al., 2012], but preference for male sex assignment has been documented in recent years in babies with ambiguous genitalia [Kolesinska et al., 2014]. The percentage of 45,X cells in the urogenital ridge, during the critical window of SRY action [Sekido and Lovell-Badge, 2009], likely plays a main role in the abnormal sex determination and differentiation of the gonads in this condition. In fact, the transcripts produced by the SRY gene of the normal 46,XY cell line could be diluted by the 45,X cell line. Therefore, variable levels below the critical threshold necessary for testis formation may determine the varied clinical spectrum of MGD that is usually characterized by a dysgenetic testis plus a contralateral streak gonad [Gantt et al., 1980; Hsu, 1994; Telvi et al., 1999].

45,X/46,XY mosaicism is rare (0.23/1.000 amniocenteses or 1.7/10.000 newborns) [Chang et al., 1990; Huang et al., 2002], but likely underestimated; in fact, short stature, a well-known feature of these children [Gantt et al., 1980; Knudtzon and Aarskog, 1987; Telvi et al., 1999; Lindhardt Johansen et al., 2012], may be the only clinical finding, and affected boys may go unrecognized if signs of MGD are not present and/or karyotyping is not performed [Richter-Unruh et al., 2004; Lee et al., 2006; Jacobsen and Cohen, 2008; Lara Orejas et al., 2008; Efthymiadou et al., 2012].

Although short stature in boys with 45,X/46,XY mosaicism is likely not due to growth hormone (GH) deficiency, GH treatment was used, showing some benefit on the growth pattern in short-term follow-up studies [Richter-Unruh et al., 2004; Lee et al., 2006; Jacobsen and Cohen, 2008; Lara Orejas et al., 2008; Efthymiadou et al., 2012]. However, long-term results of this treatment seem to be disappointing [Tosson et al., 2010; Martinerie et al., 2012].
In this paper, the spontaneous growth pattern of boys with 45,X/46,XY mosaicism and the effects of GH treatment are briefly reviewed.

**Spontaneous Growth Pattern in Boys with 45,X/46,XY Mosaicism**

Short stature is a well-reported feature of boys with 45,X/46,XY mosaicism [Gantt et al., 1980; Knudtson and Aarskog, 1987; Telvi et al., 1999; Richter-Unruh et al., 2004; Lee et al., 2006; Jacobsen and Cohen, 2008; Tosson et al., 2010; Lindhardt Johansen et al., 2012; Martinerie et al., 2012]. Impaired intrauterine linear growth may be present at birth [de Andrade et al., 2010; Lindhardt Johansen et al., 2012; Martinerie et al., 2012; Bertelloni et al., 2015], but usually these babies show normal or increased birth length [Jacobsen and Cohen, 2008; Tosson et al., 2010; Lindhardt Johansen et al., 2012; Martinerie et al., 2012]. Then, growth deceleration occurs in infancy, and short stature is often present during childhood when height is usually below the 50th centile [Tosson et al., 2010; Lindhardt Johansen et al., 2012; Martinerie et al., 2012]. Pubertal growth spurt is severely affected, being about 4 standard deviations (SD) below the normal mean of male growth spurt (12.3 vs. 28.0 cm) [Tanner and Whitehouse, 1976; Martinerie et al., 2012].

In some individuals, growth impairment may be the only clinical feature of otherwise normal phenotypic males [Richter-Unruh et al., 2004; Lee et al., 2006; Jacobsen and Cohen, 2008; Lara Orejas et al., 2008], suggesting that karyotyping should be assured to all boys with unexplained short stature [Jacobsen and Cohen, 2008; Lara Orejas et al., 2008], as recommended in girls [Bondy, 2007]. At least 50 metaphases should be evaluated to exclude low frequency mosaicism in peripheral blood cells [Gantt et al., 1980; de Andrade et al., 2010].

Individual adult heights (AH) of GH-untreated adult males (n = 22) from recent literature data are shown in table 1 and summarized in figure 1. While some males reached an AH near to or above the normal mean and their mid-parental height (MPH) [Tho et al., 2007], the majority had severe short stature (table 1). In fact, the mean AH of late adolescents/young adult men with 45,X/46,XY mosaicism is more than 2.5 SD below the normal mean (fig. 1). In addition, AH was significantly below MPH when the latter parameter was reported (161.0 ± 9.9 and 178.2 ± 7.3 cm, respectively; n = 9, p = 0.007) (table 1). In the study of Martinerie et al. [2012], AH was independent of the absence or presence of intrauterine growth retardation and of spontaneous or induced puberty. Thus, adult short stature seems to be the consequence of low linear growth during prepubertal years and severely affected pubertal growth spurt.

An ascertainment bias is likely present since boys with 45,X/46,XY mosaicism, normally developed genitalia and not grossly impaired growth pattern are usually undiagnosed. In addition, long-term follow-up of babies with normal phenotype individuated by prenatal diagnosis is largely unknown.

**Causes of Short Stature in Males with 45,X/46,XY Mosaicism**

GH deficiency has been rarely reported in boys with 45,X/46,XY mosaicism [Efthymiadou et al., 2012]. Thus, classic GH deficiency likely does not occur in this disorder [Richter-Unruh et al., 2004], as confirmed by the normal GH response to provocative stimuli [Bertelloni et al., 2015]. Although appropriate IGF1 values for delayed bone age have been reported [Jacobsen and Cohen, 2008], low-normal or slightly reduced IGF1 concentrations have been documented in other studies [Richter-Unruh et al., 2004; Tosson et al., 2010; Lindhardt Johansen et al., 2012], suggesting some degree of GH resistance [Bertelloni et al., 2015]. This hypothesis seems to be supported by the finding that serum IGF1 levels remained in the normal range during GH treatment at pharmacolog-
ical doses [Richter-Unruh et al., 2004; Bertelloni et al., 2015].

As in girls with Turner syndrome [Binder, 2011], SHOX gene haploinsufficiency in the 45,X cell line likely plays a role in the growth impairment and may explain the observed growth improvement during GH administration [Richter-Unruh et al., 2004; Tosson et al., 2010; Martinerie et al., 2012; Bertelloni et al., 2015]. In fact, both girls with Turner syndrome and children with non-chromosomal SHOX haploinsufficiency benefit from GH treatment [Blum et al., 2009; Binder, 2011; Iughetti et al., 2012]. However, untreated boys with 45,X/46,XY mosaicism reach a mean AH (table 1) higher than untreated or GH-treated patients with Turner syndrome [Martinerie et al., 2012], suggesting that other factors are operative.

Loss of the Y chromosome in the 45,X cell line and consequent loss of possible Y chromosomal growth gene(s) could contribute to growth impairment [Kirsch et al., 2000; Dati et al., 2011]. In this regard, it should be kept in mind that the peripheral blood cells’ karyotype does not predict the chromosome constitution of other body tissues (e.g. growth plate) [Tosson et al., 2010], and phenotypic features are independent of the percentage of 45,X cells in the lymphocyte karyotype [Telvi et al., 1999; Martinerie et al., 2012].

Prenatal growth impairment is a recognized factor affecting postnatal linear growth [Clayton et al., 2007; Boguszewski et al., 2011], but no difference has been found in boys with 45,X/46,XY mosaicism and intrauterine growth retardations in comparison to those with normal prenatal growth pattern [Martinerie et al., 2012].

Prepubertal (mainly during the so-called minipuberty) and pubertal impairment of gonadal steroid secretion may contribute to growth deceleration in childhood and to impaired pubertal growth spurt [Tosson et al., 2010], but androgen substitutive therapy did not improve the growth pattern [Martinerie et al., 2012].

Finally, some authors suggested that children with non-Turner SHOX haploinsufficiency can have a rapid progression of pubertal bone maturation due to a deleterious effect of gonadal steroids, leading to premature growth plate fusion, compromised pubertal growth and loss of height potential [Fukami et al., 2004; Scalco et al., 2010]. However, this phase of growth failure has not been confirmed [Ross et al., 2001]; recently no difference in the progress of pubertal stages from that of the reference population has been reported, suggesting that both timing and tempo of puberty is normal in children with SHOX haploinsufficiency and adequate gonadal function [Blum et al., 2013]. Thus, the net effect of 45,X-related SHOX haploinsufficiency on pubertal growth impairment in males with 45,X/46,XY mosaicism should be highlighted.

In synthesis, the causes of growth impairment in children with 45,X/46,XY mosaicism are not completely known, but likely different factors act together.

Effects of GH Treatment in Boys with 45,X/46,XY Mosaicism

The response to GH treatment in boys with 45,X/46,XY mosaicism and short stature has been evaluated in a limited number of studies, enrolling few patients or single case reports (table 2). Often therapy was started at a late chronological age, and some authors did not provide any outcome data. Different GH doses were used, ranging from physiological to pharmacological ones [Richter-Unruh et al., 2004; Lee et al., 2006; Jacobsen and Cohen, 2008; Lara Orejas et al., 2008; Tosson et al., 2010; Efthymiadou et al., 2012; Lindhardt Johansen et al., 2012; Martinerie et al., 2012; Bertelloni et al., 2015; Risso et al., 2015].

Short-term growth improvement has been reported in some papers. Lindhardt Johansen et al. [2012] treated 7 children (3 males) with GH (dose not shown); these children had a median 1-year height increase of 0.5 SD (range 0.1–1.2 SD), but individual data are not reported. The 3 short-term-treated boys by Richter-Unruh et al. [2004] improved their height by 0.9 SD (GH 0.35 mg/kg/week), 1.2 SD (GH 0.35 mg/kg/week) and 0.56 SD (GH 0.196–0.33 mg/kg/week), respectively. In the boy described by Jacobsen and Cohen [2008], growth velocity increased from 4.36 cm/year to 10.2 cm/year during an 8-month period of GH administration (0.35 mg/kg/week). Lee et al. [2006] treated 2 boys and reported a height increase of 20 cm/43 months (5.6 cm/year) and 6.5 cm/8 months (9.7 cm/year). We treated 1 short boy with 45,X/46,XY MGD (height –2.5 SD) with a pharmacological dose of GH (0.33 mg/kg/week) from the age of 4.6 years; he showed a progressive improvement of his stature during the follow-up [Bertelloni et al., 2015]. His height completely normalized after 6.5 years of treatment, when he reached his MPH (–0.3 vs. –0.4 SD) before the onset of puberty [Bertelloni et al., 2015]. Albeit only additional follow-up up to AH will permit definitive conclusions, our data suggest that early GH administration, long duration of therapy and high GH doses progressively modified according to the patient’s growth response and IGFI serum levels may determine a better outcome, normalizing the growth pattern before the onset of puberty, as reported in Turner syndrome [van Pareren et al., 2003].
Table 2. Adult height of GH-treated males with 45,X/46,XY mosaicism

<table>
<thead>
<tr>
<th>Reference</th>
<th>Country</th>
<th>MPH</th>
<th>AH, cm (SD)(^{a})</th>
<th>Phenotype</th>
<th>Age at GH start, years (duration of treatment, years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tosson et al. [2010](^{b})</td>
<td>USA</td>
<td>186.7 cm</td>
<td>161.1 (–2.2)</td>
<td>hypospadias, UG(L)(^{c})</td>
<td>9.2 (–)(^{d})</td>
</tr>
<tr>
<td></td>
<td></td>
<td>177.7 cm</td>
<td>166.7 (–1.4)</td>
<td>UG(L)</td>
<td>11.5 (–)(^{e})</td>
</tr>
<tr>
<td></td>
<td></td>
<td>178.2 cm</td>
<td>164.7 (–1.7)</td>
<td>hypospadias</td>
<td>7.7 (–)(^{f})</td>
</tr>
<tr>
<td>Richter-Unruh et al. [2004]</td>
<td>Germany</td>
<td>–0.74 SD</td>
<td>161.2 (–1.3)(^{c})</td>
<td>normal</td>
<td>10.5 (4.8)(^{f})</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.22 SD</td>
<td>154.8 (–2.2)(^{c})</td>
<td>normal</td>
<td>14.5 (0.9)(^{g})</td>
</tr>
<tr>
<td>Martinerie et al. [2012](^{h})</td>
<td>France</td>
<td>–</td>
<td>150.2 (–4.0)</td>
<td>hypospadias, UG</td>
<td>–0.74 SD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>–</td>
<td>151.5 (–3.0)(^{c})</td>
<td>hypospadias, UG(R)</td>
<td>–0.74 SD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>–</td>
<td>152.4 (–3.7)</td>
<td>hypospadias, UG(L)</td>
<td>–0.74 SD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>–</td>
<td>161.5 (–2.2)</td>
<td>–</td>
<td>–0.74 SD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>–</td>
<td>155.3 (–3.2)</td>
<td>hypospadias, UG(L)</td>
<td>–0.74 SD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>–</td>
<td>139.0 (–3.5)(^{c})</td>
<td>hypospadias, UG(L)</td>
<td>–0.74 SD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>–</td>
<td>150.0 (–4.0)</td>
<td>hypospadias, UG(L)</td>
<td>–0.74 SD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>–</td>
<td>157.5 (–3.0)</td>
<td>hypospadias, UG(L)</td>
<td>–0.74 SD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>–</td>
<td>160.7 (–2.4)</td>
<td>hypospadias, UG(L)</td>
<td>–0.74 SD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>–</td>
<td>168.5 (–1.0)</td>
<td>hypospadias, UG(R)</td>
<td>–0.74 SD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>–</td>
<td>156.0 (–1.5)(^{c})</td>
<td>hypospadias</td>
<td>–0.74 SD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>–</td>
<td>162.0 (–1.5)</td>
<td>hypospadias, UG(L)</td>
<td>–0.74 SD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>–</td>
<td>150.0 (–1.2)(^{c})</td>
<td>hypospadias, UG(L)</td>
<td>–0.74 SD</td>
</tr>
<tr>
<td>Risso et al. [2015]</td>
<td>Italy</td>
<td>–</td>
<td>166.2 (–1.8)</td>
<td>hypospadias, UG(B)</td>
<td>9.7 (5.5)(^{a})</td>
</tr>
</tbody>
</table>

\(^{a}\)Mean: 156.8 ± 7.4 cm. \(^{b}\)SD calculated from the authors’ raw data according to US Clinical Growth Charts (http://www.cdc.gov/growthcharts/clinical_charts.htm). \(^{c}\)Near-final height. \(^{d}\)Dose unreported. \(^{e}\)Dose 0.6 mg/kg/week. \(^{f}\)Dose 0.046 mg/kg/day. \(^{g}\)Dose 0.04 mg/kg/day. \(^{h}\)Mean dose for treated boys: 0.3 mg/kg/day (range 0.025–0.035). (B) = Bilateral; (L) = left; (R) = right; UG = undescended gonad.

Individual AHs of boys with 45,X/46,XY mosaicism treated with GH are reported in table 2. As a group, treatment was started at relatively late chronological age (mean 10.9 ± 3.0 years; n = 15) [Richter-Unruh et al., 2004; Tosson et al., 2010; Martinerie et al., 2012; Risso et al., 2015].

Among the 6 boys described by Richter-Unruh et al. [2004], 2 patients reached near-AH.

One boy was treated for ~5 years, and his height increased from –2.68 SD at beginning of therapy (age of 10.5 years) to –1.34 SD at 15.3 years. The other boy was treated by the age of 14.5 years for 10.8 months, and his height did not improve (table 2). In both patients, near-AH was below their MPH (table 2) [Richter-Unruh et al., 2004]. Martinerie et al. [2012] described the height outcome of 13 boys treated with GH for a mean period of 6.5 years; no significant difference in AH was found in comparison with that of an untreated group (157.0 ± 2.3 vs. 156.6 ± 4.2 cm); only 5 patients were early treated (between 4 and 7 years of age), and 4 boys of this subgroup reached AH [Martinerie et al., 2012]. No significant difference between the early treated boys and those treated after the chronological age of 10 years (n = 8) (157.0 ± 5.2 and 155.9 ± 6.5 cm, respectively) was found, but GH was administered at relatively low doses (0.17–0.24 mg/kg/week) in comparison with other studies [Richter-Unruh et al., 2004; Jacobson and Cohen, 2008; Tosson et al., 2010; Lindhardt Johansen et al., 2012; Bertelloni et al., 2015], and the GH dose was not adjusted according to IGF1 levels [Martinerie et al., 2012]. Among the boys described by Tosson et al. [2010], 3 GH-treated patients reached AH (table 2); they remained about 17 cm below their MPH (164.2 vs. 180.9 cm). In this study, GH treatment was started between 7.7 years and 11.5 years, but total therapy periods were unavailable, and GH dose was reported only for 1 boy (table 2) [Tosson et al., 2010]. AH of treated boys was similar to that of the 3 untreated children (159.1 ± 8.7 and 162.5 ± 3.8 cm, respectively) [Tosson et al., 2010]. Published data of GH-treated males with 45,X/46,XY mosaicism are summarized in figure 1. Mean AH of GH-treated patients is more than 2 SD below the normal mean and not significantly different to that of untreated subjects (fig. 1).
Thus, some short- to mid-term results suggested some efficacy of GH treatment in improving the growth pattern in boys with 45,X/46,XY mosaicism, while data at AH are disappointing till now.

Gonadal Cancer Risk and GH Treatment

In individuals with 45,X/46,XY mosaicism, the presence of a specific region on the Y chromosome (gonadoblastoma locus on Y chromosome) increases the risk for development of gonadal malignant germ cell tumors (13% in the case of mild undervirilization; 52% in the case of overt ambiguous phenotype) in the environment of dysgenetic gonads [Cools et al., 2011; Kaprova-Pleskacova et al., 2013]. In addition, a recent systematic review showed that the overall standardized incidence ratio for cancer was significantly increased in subjects treated with GH (2.74; 95% CI 1.18–5.41), raising some concern on the long-term safety of GH treatment [Deodati et al., 2014]. However, the authors concluded that several confounders and biases may affect their analysis [Deodati et al., 2014], and no increased risk for testicular cancer has been found at now [Cianfarani, pers. commun.].

Unfortunately, a prospective evaluation of testicular cancer risk in GH-treated males with 45,X/46,XY mosaicism is missing, and any conclusion on this aspect should be careful at this time. At least periodic clinical and sonographic evaluation of gonads, if not removed, seems to be advisable.

Looking to the Future

Several individual biological factors (e.g. abnormal chromosome constitution, different mosaicism among the various tissues, low birth weight, impaired pubertal growth, variable GH sensitivity, etc.) and treatment-related issues (e.g. different ages at start of GH treatment, no homogeneous GH doses, GH doses not adjusted to IGF1 levels) may explain short stature and the variable results of GH therapy in 45,X/46,XY mosaicism. The following points should be taken into consideration to improve growth management of these boys:

- The causes of growth impairment should be better defined, since they will provide the basis for a more appropriate therapeutic approach.
- Karyotyping should be considered in all male children with unexplained short stature in order to individuate the boys with this mosaicism, but without phenotypic features of MGD.
- GH treatment should be started as soon as possible after diagnosis if short stature is present, or as soon as the growth curve decelerates in order to optimize the growth pattern before the onset of puberty.
- Supraphysiological GH doses are likely needed, as in Turner syndrome and SHOX haploinsufficiency [van Pareren et al., 2003; Bondy, 2007; Binder, 2011; Iughetti et al., 2012], but specific trials addressing dose/growth response relationship need to be developed.
- Strategies should be developed to optimize growth during the pubertal period [e.g. increased doses of GH, starting age and doses of androgen substitutive therapy (if needed), adjunctive therapy with GnRH analogs or aromatase inhibitors], since severely impaired pubertal growth spurt seems to be a greater contribution to adult short stature, but experiences addressing this item are not available.
- Surveillance studies to better define safety of GH treatment, mainly regarding the risk of testicular cancer, should be done.

Finally, a larger number of patients should be assessed in multicenter prospective trials with long follow-up till the attainment of AH to reach more clear criteria for growth promoting therapy(ies) in this DSD and to provide better indications for practice.

Acknowledgement

This paper was developed under the auspices of the Italian Society for Pediatric Research.
Growth in 45,X/46,XY Boys: Effect of GH

Sex Dev 2015;9:183–189
DOI: 10.1159/000441342

189