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Growth Hormone Receptor (*GHR*) Gene Polymorphism and Prader-Willi Syndrome

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Abstract

Prader-Willi syndrome (PWS) is a genomic imprinting disorder due to loss of paternally expressed genes in the 15q11-q13 region and characterized by hypotonia, a poor suck, failure to thrive, hypogonadism/hypogenitalism, growth hormone deficiency, learning and behavioral problems and hyperphagia leading to early childhood obesity. Growth hormone acts as a ligand for the growth hormone receptor (GHR) coded by a gene polymorphic for an exon-3 deletion (*d3*) seen in about 50% of Caucasians and associated with an increased response to growth hormone (GH) therapy. We examined 69 individuals with PWS (average age ± SD = 20.1 ± 12.8y). The *GHR* allele distribution in our PWS subjects was similar to reported data in the literature with no gender or PWS genetic subtype differences. A negative correlation was found with age for height standard deviational scores and a positive correlation with age for weight and BMI for non-GH treated PWS subjects. Adjusting for effects of age and gender, individuals with PWS and the *d3/d3* allele showed a significant increase in BMI compared with those having the full length (*fl*) allele. In addition, 12 infants and children with PWS were examined when growth and GH data were available before and during GH treatment. A significant increase in growth rate (1.7 times) was noted in the presence of the *d3* allele (*fl/fl*=0.87cm/month; *fl/d3* or *d3/d3*=1.5 cm/month; $p < 0.05$). The presence of the *d3* allele and its impact on growth and medical care of individuals with PWS while on GH therapy should be further investigated.

Keywords

Growth hormone receptor (GHR); Prader-Willi syndrome; growth hormone treatment; genotype; gene polymorphism

INTRODUCTION

Prader-Willi syndrome (PWS) is characterized by infantile hypotonia; failure to thrive, a poor suck and feeding difficulties; hypogonadism with genital hypoplasia; growth hormone deficiency with short stature and small hands and feet; hyperphagia leading to early childhood obesity; mild mental deficiency and behavioral problems [e.g., Butler et al., 2006; Butler, 2011; Cassidy et al., 2012]. PWS is due to loss of paternally expressed genes from the 15q11-q13 region [e.g., Butler, 1990; Bittel and Butler, 2005], usually from a *de novo* paternally derived deletion [Butler & Palmer, 1983]. When treated with growth hormone (GH), PWS individuals respond favorably in stature, lean body mass and physical activity, but are prone to developing scoliosis requiring braces or surgical intervention [Butler et al., 2006].

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In randomized controlled studies reported in the literature, GH treatment increased the longitudinal growth rate in children with Prader-Willi syndrome [e.g., Oto et al., 2012]. Implementing a controlled diet along with GH therapy and exercise are key in the medical management of individuals afflicted with this condition. For example, Myers et al. [2000] reported that two years of growth hormone treatment in children with PWS was sufficient to reduce fat mass with a sustained increase in lean body mass. Additional long term studies, though limited, suggest that GH treatment for five years may not be sufficient to normalize the lean body mass [Eiholzer et al., 2000], but can help stabilize the body mass index [Lindgren et al., 1999; Tauber et al., 2000].

As the first step in growth hormone action, growth hormone acts as a ligand for the growth hormone receptor (GHR) consisting of an extracellular and cytoplasmic protein domain. GH binding is followed by the activation of the JAK-STAT pathway initiating an increase in expression of insulin-like growth factor I (IGF-I) and other growth hormone genes. The *GHR* gene contains 9 exons with exons 3–7 encoding the extracellular domain [Pantel et al., 2000; Dos Santos et al., 2004]. There are two recognized isoforms of the *GHR* gene in humans due to the presence or absence of exon 3. The exon-3 deletion (*d3*) occurs in about 50% of Caucasians in the general population [Dos Santos et al., 2004; Padidela et al., 2012] and results from exon skipping due to homologous recombination of two retroviral sequences flanking this exon that mimic an alternative splicing event [Pentel et al., 2000]. This exon deletion exhibits increased functional receptor activity by about 30% in transfection studies with an associated growth response to treatment in GH-deficient patients [Dos Santos et al., 2004]. Specifically, non-PWS children with short stature and *d3* allele grew at 1.7 to 2 times faster growth rate when treated with GH.

Prior to growth hormone therapy, PWS individuals from South Korea with at least one d3 *GHR* allele exhibited greater height standard deviational scores than those who did not have this allele [Park et al., 2011]. In addition, a birth cohort studied from the general population in the United Kingdom with two wild type (*fl/fl*) alleles reported to have larger placentas and birth weights with increased intrauterine growth in late gestation [Padidela et al., 2012]. Hence, the aims for our study were to assess for an association between *GHR* allele subtypes and growth parameters before and during GH treatment in individuals with Prader-Willi syndrome.

MATERIALS AND METHODS

Participants

The study sample was comprised of 69 participants who were genetically confirmed with PWS and consisted of 30 males and 39 females with a mean age + SD = 20.1y ± 12.8y and age range of 2 mo to 50 yrs. Forty-one (58%) of the study participants had the typical 15q11-q13 deletion and the remaining 28 PWS subjects (42%) had maternal disomy 15 (UPD) or an imprinting center defect determined by established cytogenetic and molecular genetic techniques [Butler et al., 2008; Henkhaus et al., 2012]. Ninety percent of our PWS study subjects were Caucasian, 4% were African-American, 4% were Hispanic and 2% were Asian. Fifty-nine percent (N= 41) of our PWS study subjects were naïve to GH treatment while the remaining 41 percent (N=28) were undergoing GH treatment at the time of study or had been treated in the past. The study was conducted under the authority of the University of Kansas Medical Center Office of Research Compliance who reviewed the study protocol and monitored study activities to ensure that appropriate steps were taken to protect the rights and welfare of humans participating as research subjects.

Growth Hormone Receptor *(GHR)* Genotyping Method

The *GHR* exon 3 deletion was identified using PCR-based technology with primers (G1, G2, and G3) described in GenBank, accession number AF155912. The *GHR*-exon 3 genotyping test was performed, to identify GHRfl and GHRd3 alleles, using a multiplex PCR procedure with primers G1 (5'-TGTGCTGGTCTGTTGGTCTG-3'), G2 (5'-AGTCGTTCCTGGGACAGAGA-3'), and G3 (5'-CCTGGATTAACACTTTGCAGACTC-3'). The following PCR cycle parameters were used as reported previously by Dos Santos et al. [2004]: the initial step of denaturation for five minutes at 94°C, then followed by 35 cycles with each cycle having a duration of 30 sec at 94°C, 30 sec at 60°C; and for 1 min 30 sec at 72°C, the extension period followed for 7 min at 72°C. Electrophoresis of the PCR fragments was performed with 1% agarose, and the gel stained with ethidium bromide. Primers for G1 and G3 generate a PCR fragment of 935 bp indicating the wild type full length (fl) allele while primers G1 and G2 generate a PCR fragment of 532 bp when the exon 3 deletion is present representing the d3 allele (Fig 1).

Statistical Analysis

Measurements of length (height) to the nearest 0.1 cm, weight to the nearest 0.1 kg, and head circumference to the nearest 0.1 cm were extracted from patient medical records and growth charts for all subjects along with growth hormone dosage and plasma IGF-I levels and reference ranges. Standard deviational scores were generated for the growth parameters from growth curves relative to normally developing children. Descriptive statistics are presented as means and standard deviations. One Way Analysis of Variance adjusted for age and gender was used to compare means by *GHR* subtype and correlation coefficients determined. Linear equations were used to determine the rate of change in growth parameters for each subject and the mean rate of change was compared by *GHR* subtype. We also examined the rate of height change during GH treatment in relationship to *GHR* allele subtype in a subset of 12 infants and children selected from our PWS cohort with available growth and GH treatment data.

RESULTS

Non-GH Treatment

The distribution of *GHR* alleles (*fl/fl*, N=36 or 52%; *fl/d3*, N=25 or 36%; *d3/d3*, N=8 or 12%) in our 69 PWS patients was similar to reported data in Caucasian control participants. There were no gender or PWS genetic subtype (e.g., 15q11-q13 deletion, maternal disomy 15) differences identified in the distribution of *GHR* alleles or in the individual growth parameters studied. The association between *GHR* allele subtypes and growth parameters (height, weight, head circumference) obtained prior to GH treatment as well as body mass index (BMI) was examined in the PWS individuals. Prior to GH treatment, we found a negative correlation ($p<0.05$) with age for height standard deviational scores (SDS) and a positive correlation ($p<0.05$) with age for weight and BMI regardless of *GHR* allele subtype. Adjusting for effects of age and gender, we found that individuals with PWS carrying the *d3* allele showed a significantly increased mean BMI compared with those having the full length allele ($F=3.9$; $p<0.02$). However no differences were found in standard deviational scores for height, weight or head circumference by *GHR* subtype prior to GH treatment (Figs 2 and 3).

Height SDS as a function of age in the absence of GH treatment was compared to *GHR* allele subtype and shown in Figure 4. Linear regression analysis was significant ($F= 4.1$, $p<0.05$) and revealed a main effect of age ($F=19.6$; $p<0.0001$) as well as age by allele subtype interaction ($F=3.8$; $p<0.05$). Growth rate for the *d3/d3* subtype showed a faster growth rate prior to 18 years of age and a slower growth rate over 20 years of age. The

overall rate of decline in SDS for age was faster for the *d3/d3* allele subtype in comparison with the *fl/fl* and *fl/d3* subtypes.

GH Treatment

The rate of height change during GH treatment was examined in relationship to *GHR* allele subtype in a subset of 12 infants and children from our PWS cohort (Table I). The *GHR* allele subtype for this group included eight patients with *fl/fl* (four males and four females), three with *fl/d3* (two males and one female) and one infant female with the *GHR d3/d3* allele subtype. Age at pre-GH treatment measurements for height and weight ranged from 1 month to 19 months while measurements during the GH treatment phase ranged from 6 months to 7 years of age at the time when GH dosage and plasma IGF-I levels were within therapeutic range for age. Individuals with the *fl/fl* subtype had a height increase of 0.87 cm/month or 0.03 SDS/month while individuals possessing the *d3* allele (i.e., *fl/d3* or *d3/d3*) had a significantly faster rate of height increase of 1.5 cm/month or 0.16 SDS/month (t-test; $p<0.05$) during GH treatment compared with −.11 SDS/month for *fl/fl* and 0.08 SDS/month for those with the *d3* allele during the pre-GH treatment period. Figure 5 illustrates a representative example of a PWS female infant (Patient 5, Table I) with the *GHR fl/fl* allele subtype and maternal disomy 15. Figure 6 illustrates a representative example of a PWS female infant (Patient 9, Table I) with the *GHR fl/d3* allele subtype and the 15q11-q13 deletion.

DISCUSSION

Our study showed that the distribution of *GHR* alleles in individuals with PWS was not different from the expected frequency reported in non-PWS controls from the general population. In addition, our study of growth parameters in PWS subjects confirmed the well-known pattern of growth in this obesity-related syndrome with decreased height but increased weight and BMI with advancing age relative to normally developing children without PWS and in the absence of GH therapy [Myers et al., 2000; Burman et al., 2001; Butler et al., 2006; Carrel et al., 2010]. Examination of the influence of *GHR* allele subtypes in PWS found an association between the *GHR* subtype and the rate of change of height SDS in the presence and absence of exogenous GH treatment. Without GH treatment, subjects homozygous for the *d3* allele showed an accelerated growth rate in infancy and childhood compared with both heterozygous (*fl/d3*) subjects and homozygous (*fl/fl*) subjects suggesting that the *d3* allele influences height without GH treatment supporting increased sensitivity to GH and with more responsiveness to GH secretions even if below normal range with GH deficiency being common in PWS. This observation was also reported by Park et al. [2011] in patients with PWS from South Korea in which the *d3* allele was associated with increased height and IGF-I levels before GH therapy and thus the *d3* allele influences height through GH secretion sensitivity. If endogenous GH levels decrease with age in infants and children with PWS, then the rate of growth (e.g., length) would significantly decrease relative to a normal growth rate particularly in those the *fl/fl* allele.

Growth rates for PWS subjects with the *d3/d3* subtype decreased sharply after puberty if GH treatment is not administered. When treated with growth hormone, all PWS individuals respond favorably in stature, with decreased fat, increased muscle mass and physical activity. Heterozygous (*fl/d3*) individuals and homozygous (*d3/d3*) individuals showed a higher sensitivity to GH treatment than homozygous *fl/fl* subjects as indicated by greater acceleration in growth rate in PWS subjects with the *fl/d3* or *d3/d3* alleles. PWS infants with the *fl/fl GHR* allele subtype in our study were responsive to GH treatment but did not appear to be as sensitive as those possessing the *d3* allele. We estimated that the presence of the *d3* allele increased height change by 1.7 times in comparison to the response of the full length

allele as similarly seen in the literature in GH treated non-PWS children [Dos Santos et al., 2004].

In summary, the *d3* allele was associated with significantly increased BMI in our cohort of PWS study participants prior to GH treatment but not for height or weight. The presence of the *d3* allele was associated with an increased rate of height change compared to the response of the full length (*fl*) allele as similarly seen in GH treated non-PWS children. The sensitivity to GH treatment and relationship to *GHR* allele subtype could influence management decisions about GH dosage by following IGF-I levels more closely (e.g., 3 month intervals) and for monitoring for scoliosis. The presence of the *d3* allele and its impact on management decisions and caring for individuals with PWS while on GH therapy should be addressed in future studies with a larger sample of PWS subjects for investigation and for an extended period of observational time.

Acknowledgments

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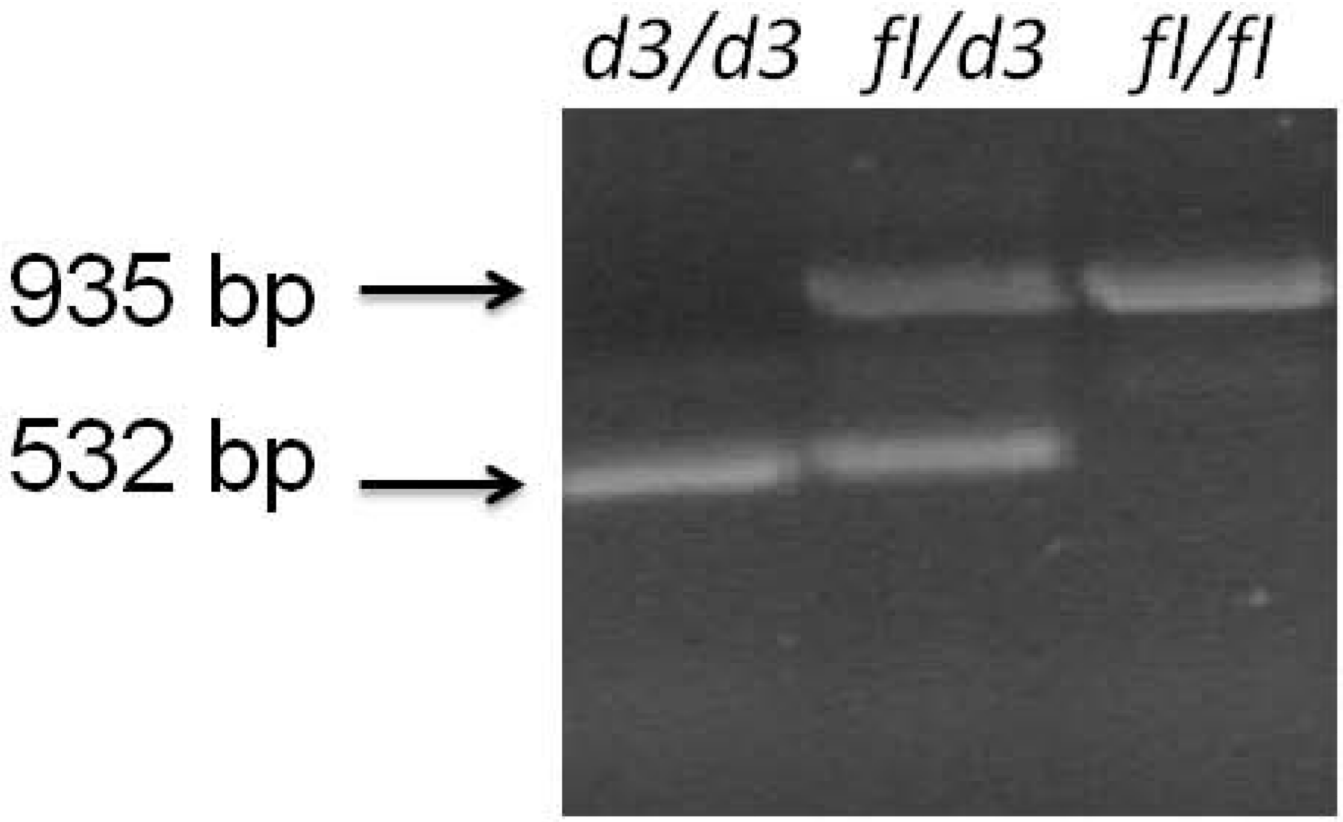

Figure 1.
Representative examples of growth hormone receptor gene (*GHR*) alleles using polymerase chain reaction to identify the wild type *fl/fl*; heterozygous *fl/d3* and homozygous exon-3 deletion (*d3/d3)* alleles in Prader-Willi syndrome.

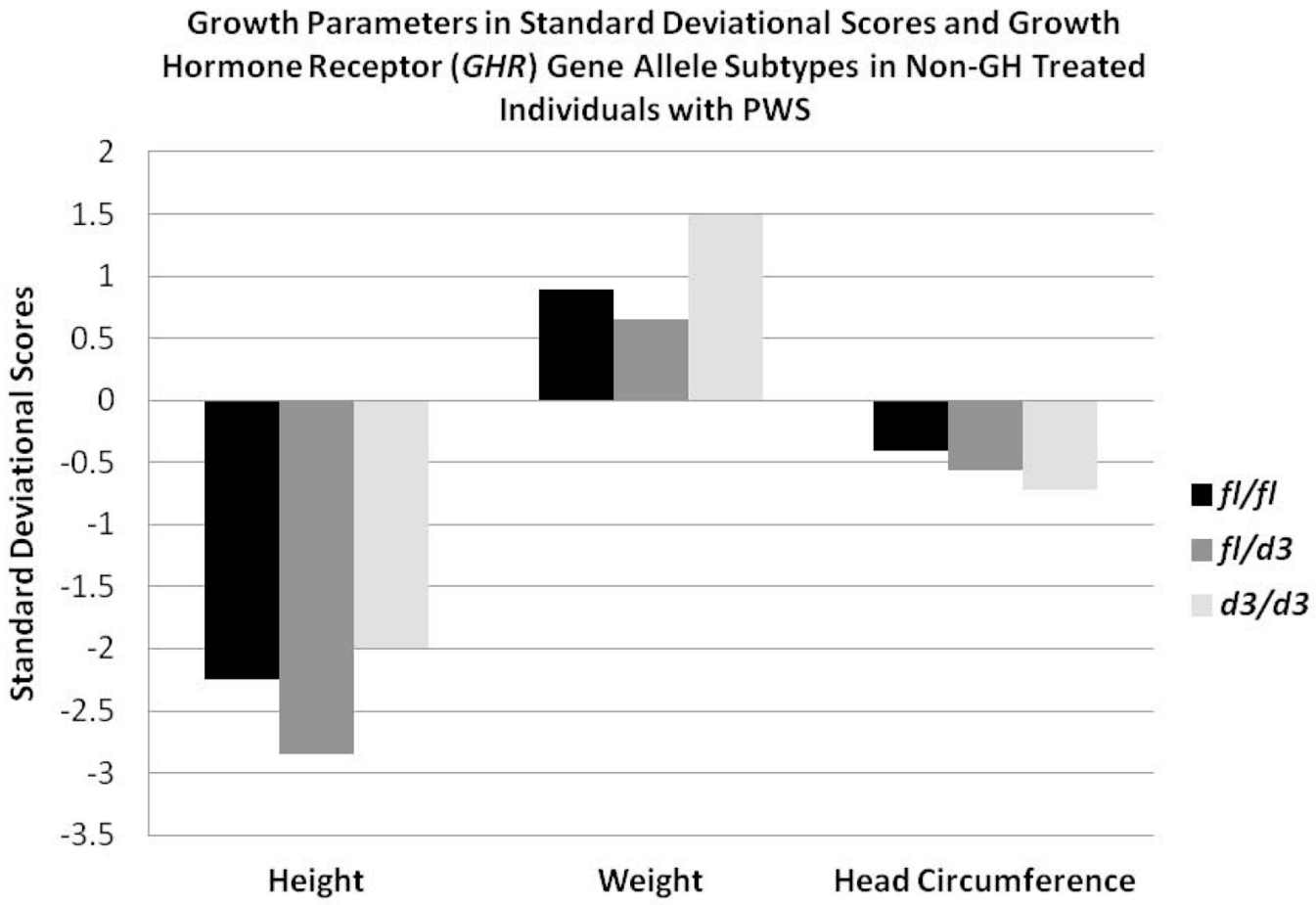

Figure 2.

Histograms of growth parameters (height, weight, head circumference) in standard deviational scores and *GHR* allele subtypes (*fl/fl, fl/d3, d3/d3*) in non-growth hormone (GH) treated individuals with Prader-Willi syndrome (PWS).

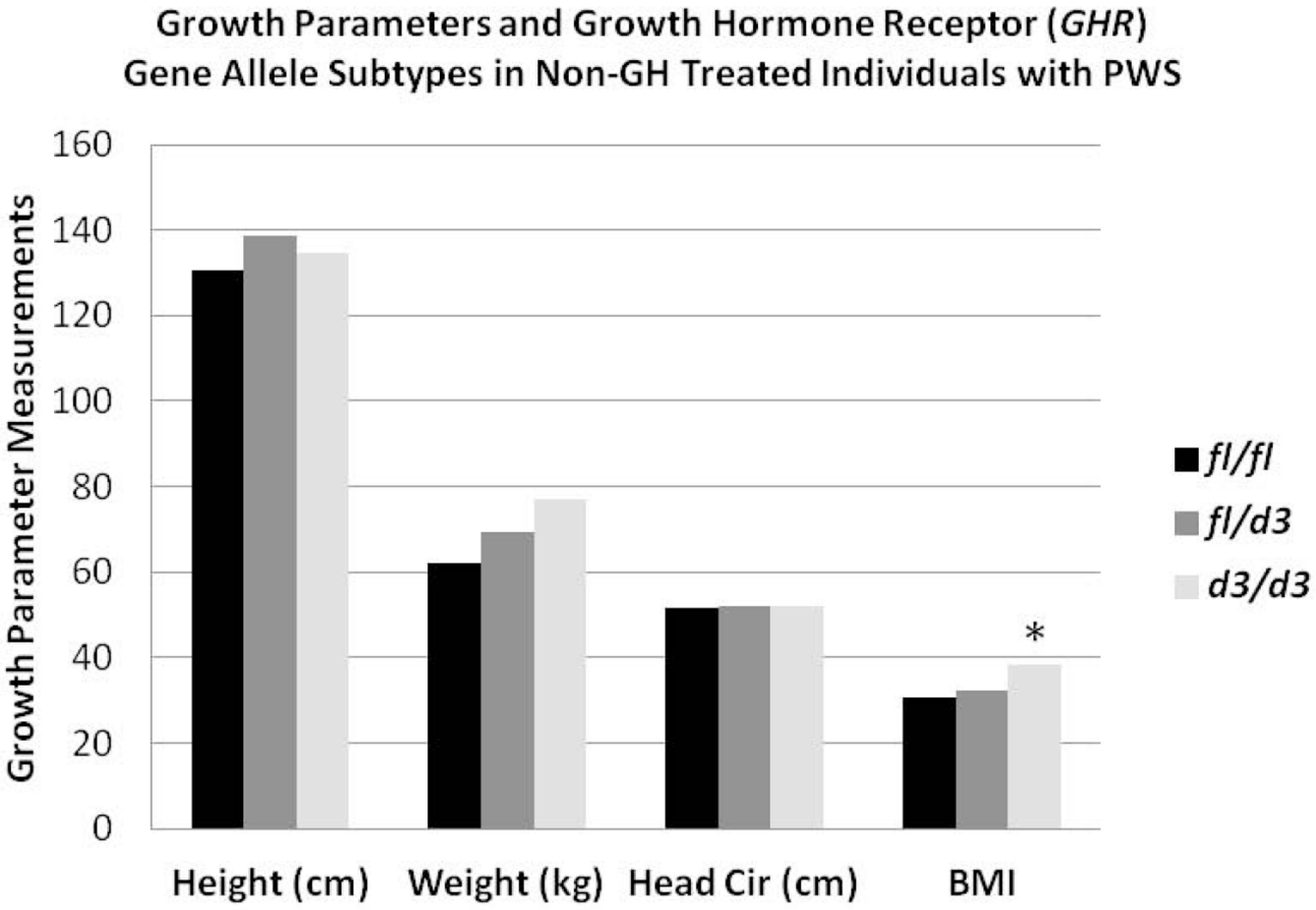

Figure 3.

Histograms of growth parameters (height, weight, head circumference) and body mass index (BMI) and *GHR* allele subtypes (*fl/fl, fl/d3, d3/d3*) in non-growth hormone (GH) treated individuals with Prader-Willi syndrome (PWS). A significant difference ($F=3.7$; $p<0.05$) was found for the *d3/d3* allele (*) compared with the other *GHR* allele subtypes for BMI.

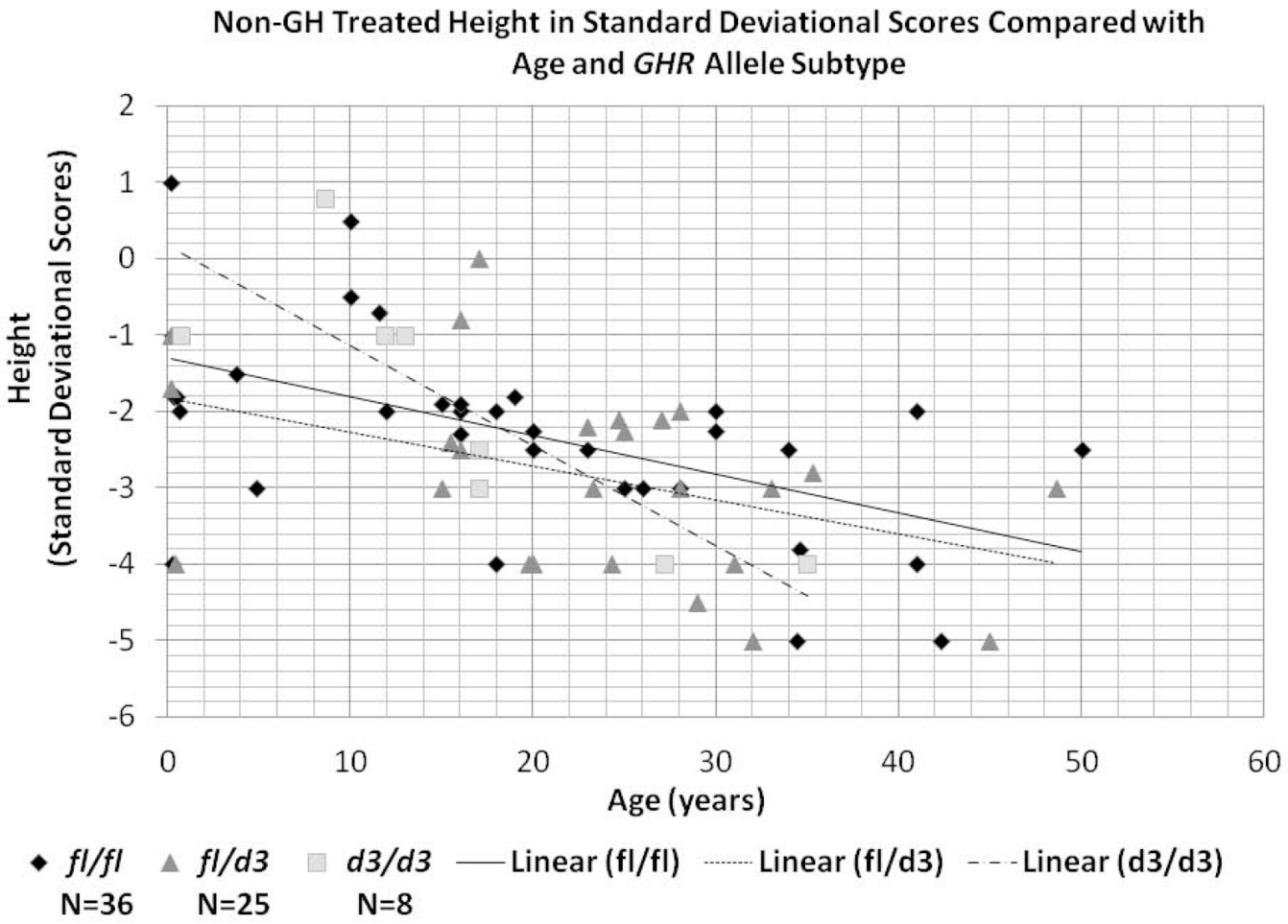

Figure 4.
Scatterplot of non-growth hormone (GH) treatment data for height in standard deviational scores compared with age (in years) and *GHR* allele subtypes for all individuals studied with Prader-Willi syndrome.

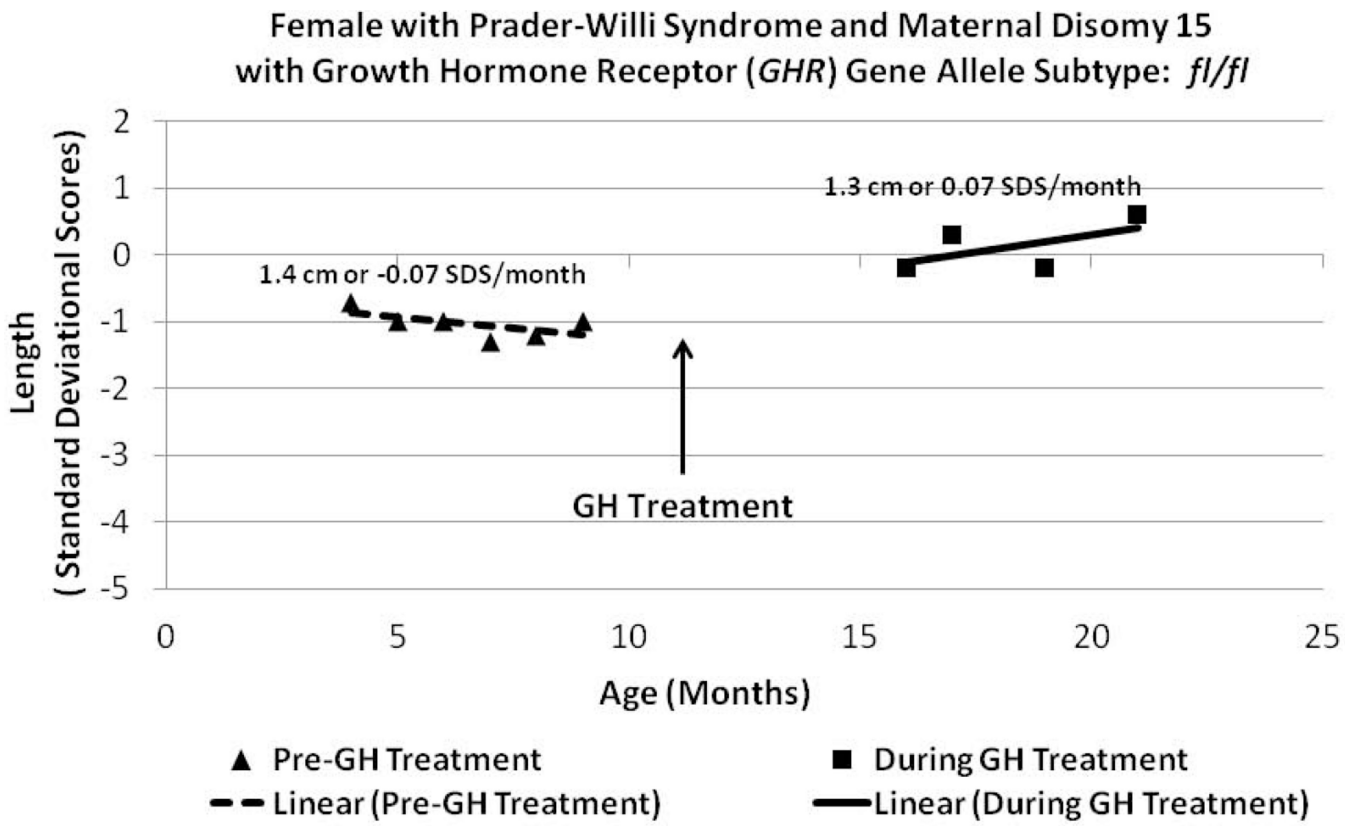

Figure 5.
Length data plotted from a Prader-Willi syndrome (PWS) female with maternal disomy 15 (Patient 5, Table I) before and during GH treatment within the therapeutic range monitored by plasma IGF-I levels and having the *GHR fl/fl* allele subtype.

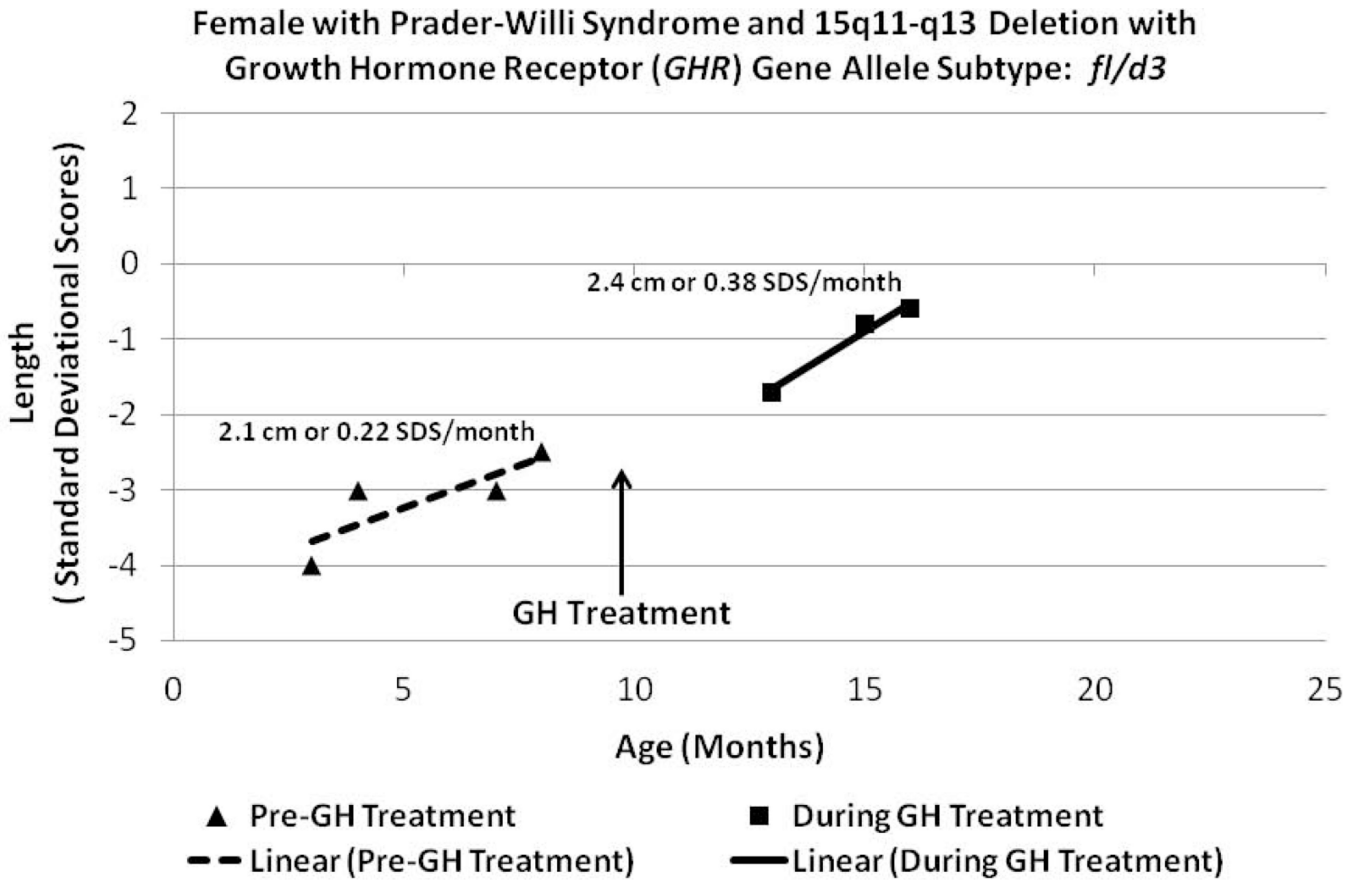

Figure 6.

Length data plotted from a Prader-Willi syndrome (PWS) female with the 15q11-q13 deletion (Patient 9, Table I) before and during GH treatment within the therapeutic range monitored by plasma IGF-I levels and having the *GHR fl/d3* allele subtype.

Table 1

Clinical Summary of 12 Infants with Prader-Willi Syndrome before and during Growth Hormone Therapy

Subject	Sex	Race	Genetic Subtype		Pregnancy/Birth	Birth Measures		History of G-tube	Scoliosis (>10°)	Age GHT Initiated (months)	Average Height (SDS)/ Interval Studied (months)		Other
			PWS	GHR		Weight [kg (SDS)]	Length [cm (SDS)]				Pre-GHT	GHT	
1	F	C	15q11-q13 type I deletion	fl/fl	34 week gestation, C-section, polyhydramnios, pre-eclampsia, dizygotic twin birth	1.8 kg (−1.3)	45 cm (−0.7)	Yes	No	9.6	−1.9/3.0	−1.3/2.0	
2	F	A/C	15q11-q13 type II deletion	fl/fl	34 week gestation, PROM, emergency C-section	1.9 kg (−1.3)	49 cm (0.7)	No	No	3.6	−1.3/1.0	−1/27.0	
3	M	C	15q11-q13 type II deletion	fl/fl	38 week gestation, C-section	2.3 kg (−1.9)	49 cm (−0.7)	No	No	20.4	−0.8/7.0	0.9/9.0	
4	F	C	15q11-q14 atypical deletion	fl/fl	Term	2.7 kg (−1.6)	51 cm (0.3)	Yes	No	6.0	0.5/3.5	0.4/25	Coarctation of aorta, PDA, ear tags
5	F	C	UPD	fl/fl	Term	2.7 kg (−1.6)	48 cm (−1.3)	Yes	No	10.8	−1.0/5.0	0.1/5.0	Hip subluxation
6	M	C/AA	UPD	fl/fl	31 week gestation, maternal hypertension, abnormal placenta, oligohydramnios	0.9 kg (−1.9)	35 cm (−1.9)	Yes	No	6.0	−6.7/2.5	−1.6/12.0	Adrenal insufficiency, pulmonary hypertension, hypospadias, inguinal hernia
7	M	C	UPD	fl/fl	38 week gestation	2.5 kg (−1.3)	50 cm (0)	No	No	12.0	−2.2/8.0	0.2/ 81.0	
8	M	C	UPD	fl/fl	Term, polyhydramnios, gestational diabetes	4.2 kg (1.3)	55 cm (1.9)	Yes	No	7.2	0.7/4.5	0.5/22	NF1
9	F	C	15q11-q13 type I deletion	fl/d3	35 week gestation	1.9 kg (−1.6)	46 cm (−0.7)	Yes	No	6.0	−3.1/3.0	−1.0/3.0	
10	M	C	15q11-q13 type II deletion	fl/d3	Term	2.3 kg (−2.3)	48 cm (−1.3)	Yes	No	0.6	NA	−0.8/8.0	
11	M	C	UPD	fl/d3	37 week gestation	2.0 kg (−2.3)	46 cm (−1.3)	No	No	7.2	−3.6/2.5	−0.2/62.0	Multiple bone fractures
12	F	C	15q11-q13 type II deletion	d3/d3	38 week gestation, C-section	2.4 kg (−1.6)	48 cm (−0.7)	Yes	No	9.6	−0.9/5.0	0.1/11.0	Horseshoe kidney, hip dysplasia

M= Male; F= Female; AA= African-American; A= Asian; C= Caucasian; PWS= Prader-Willi syndrome; GHR= Growth hormone receptor gene; UPD= Uniparental maternal disomy 15; PROM= Premature rupture of membranes; SDS= Standard deviational score; GHT= Growth hormone therapy; NF1= Neurofibromatosis 1; PDA= Patent ductus arteriosus; NA= Not applicable