Acromegaly : irreversible clinical consequences

Wassenaar, M.J.E.

Chapter 10.

IMPACT OF THE EXON 3-DELETED GROWTH HORMONE RECEPTOR POLYMORPHISM ON SPONTANEOUS GROWTH AND THE GROWTH RESPONSE TO RECOMBINANT HUMAN GROWTH HORMONE THERAPY IN GROWTH HORMONE DEFICIENT (GHD) AND NON-GHD CHILDREN WITH SHORT STATURE: A SYSTEMATIC REVIEW AND META-ANALYSIS

MJE Wassenaar[1], OM Dekkers[1,3], A.M. Pereira[1], J.M. Wit[2], J.W. Smit[1], NR Biermasz[1], JA Romijn[1]

Departments of Endocrinology and Metabolism[1], Pediatrics[2], and Clinical Epidemiology[3], Leiden University Medical Center, Leiden, The Netherlands.

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ABSTRACT

Context: The exon-3 deleted growth hormone receptor (GHR_{d3}) polymorphism is associated with an increased growth response to recombinant human GH (rhGH) therapy in some, but not all, studies in growth hormone deficient (GHD) and non-GHD children with short stature.

Objective: To assess the effects of GHR_{d3} on baseline height and the 1st year's growth response to rhGH treatment in pre-pubertal GHD and non-GHD children with short stature.

Design: Systematic review and meta-analysis

Methods: Fifteen studies reporting the effect of GHR_{d3} on growth parameters were included. Principal outcomes were baseline height SDS, and the weighted average of change in growth velocity (delta cm/yr) and height gain (delta height SDS) after 1 year of rhGH.

Results: In GHD, not in non-GHD, baseline height SDS was 0.159 SD higher (95%CI 0.020,0.298) in GHR_{d3} compared with $GHR_{wt\text{-}wt.}$ In GHR_{d3} rhGH therapy resulted in a higher increase in growth velocity (0.521 cm/yr, 95%CI 0.196,1.015) and height gain (0.075 SD; 95%CI 0.007, 0.143) compared with $GHR_{wt\text{-}wt}$. Meta-regression demonstrated a larger difference between GHR_{d3} and $GHR_{wt\text{-}wt}$ in studies using lower rhGH doses and carried out at a higher age, independently of the cause of short stature.

Conclusions: This meta-analysis in prepubertal children with short stature indicates that GHR_{d3} is associated with increased baseline height in GHD, but not in non-GHD. Furthermore, GHR_{d3} stimulates growth velocity by an additional effect of ~0.5 cm during the first year of rhGH treatment and this effect is more pronounced at lower doses of rhGH and higher age.

INTRODUCTION

The aim of treatment of short children with recombinant human growth hormone (rhGH) is to increase growth in childhood and adolescence, and to increase adult height[1]. Treatment with rhGH has been approved for the treatment of children with short stature caused by growth hormone deficiency (GHD), and, more recently, for short children with Turner syndrome, chronic renal failure, Prader-Willi-Labhart syndrome, SHOX-haploinsufficiency, and short children born small for gestational age (SGA). In addition, in the USA rhGH is approved for treatment of children with idiopathic short stature (ISS) (if height standard deviation score (SDS) <-2.25). The first year's growth response, as well as the adult height gain attained as a result of rhGH treatment, is influenced by several factors, including age and height deficit at the start of treatment, the underlying cause of short stature, the severity and duration of GHD, and the dose, injection frequency, and duration of rhGH therapy[2;3]. A possible predictor of adult height gain is the height velocity during the first year of rhGH treatment[4]. Recent reports have suggested that also genetic variations in the GH-IGF-I axis might affect the response to rhGH treatment.

GH acts at the target cell through the growth hormone receptor (GHR)[5]. After binding to the GHR, GH induces activation of the JAK-STAT pathway, ultimately leading to increased expression of IGF-I and other GH dependent genes. One of the genetic factors considered responsible for the variation in response to rhGH treatment is a common polymorphism in the GHR gene, leading to deletion of exon 3 (d3), which encodes a 22-aminoacid residue sequence in the extracellular domain[6;7]. Dos Santos *et al.* described in 2004 that this loss of exon 3 stimulates receptor expression and function, specifically by affecting the binding of rhGH, receptor processing, transport, stability, binding to other ligands, dimerization of GHR monomers, and signal transduction[8].

Since 2004 several studies have addressed the possible influence of the GHR_{d3} polymorphism on the growth response to rhGH treatment in children with diverse clinical conditions, including GHD[9-14], SGA[8;15-19], ISS[8;20;21], and Turner syndrome[22]. However, the results of these studies are remarkably inconsistent, because 6 reports [11;13;19;21;22] confirmed the findings of Dos Santos *et al.* [8], whereas another 8 reports[9;10;12;14;15;17;18;20] could not demonstrate a significant effect of the exon-3 deleted genotype (GHR_{d3}) on the growth response to rhGH. We hypo-

thesized that the discrepancies between these studies might be explained, at least in part, by the relatively small numbers of patients included in these studies, precluding sufficient statistical power.

Theoretically, variations in GH sensitivity due to genetic differences in the GHR can be compensated by reverse changes in endogenous pituitary GH secretion, which might mask the effect of the GHR polymorphism on spontaneous growth. Therefore, we also hypothesized that in patients with GHD this compensatory effect within the GH-IGF-I axis is disturbed. Consequently, one might expect that only in children with short stature due to GHD, but not in children with short stature due to other causes, this GHR_{d3} genotype is associated with a less severe growth failure. However, so far none of the studies in GHD children have been able to demonstrate such an effect on baseline height [4-9], which may be due to confounding factors and insufficient numbers of study subjects.

In view of these contradictory results on the effects of GHR_{d3} on growth parameters in response to rhGH treatment and since the effect on this polymorphism has never been demonstrated on baseline height SDS, we conducted a meta-analysis. The aims of the study were 1) to assess the effect of the GHR_{d3} on baseline height SDS in pre-pubertal GHD and non-GHD children, and 2) to assess the impact of the GHR_{d3} on height gain and change in growth velocity in response to 1 year rhGH treatment.

MATERIALS AND METHODS

Eligibility criteria

Two principal measures of outcome were used: 1) baseline height SDS in GHD and non-GHD children and 2) height gain and/or growth velocity in response to 1 year of rhGH treatment. Studies reporting one of these outcomes in prepubertal children and stratified according to the exon 3-deleted GHR polymorphism, i.e. $GHR_{wt\text{-}wt,}$ $GHR_{wt\text{-}d3,}$ $GHR_{d3\text{-}d3,}$ and/or GHR_{d3} (GHR_{d3} is the combination of $GHR_{wt\text{-}d3}$ and $GHR_{d3\text{-}d3}$ genotypes) were eligible for inclusion in the present study. A large proportion of studies reported outcomes for $GHR_{wt\text{-}d3}$ and $GHR_{d3\text{-}d3}$ separately. For the purpose of the present meta-analysis, we re-calculated the weighted mean of the outcome parameter of interest (baseline height and first year's growth velocity and height gain) as well as the combined standard error of the $GHR_{wt\text{-}d3}$ and $GHR_{d3\text{-}d3}$ group. Studies reporting

incomplete data or studies providing the effects of the separate genotypes on growth only in a figure could not be included in this meta-analysis.

Search strategy

We searched Medline, Embase, Web of Science, and the Cochrane Library for studies reporting the effect of d3GHR on growth parameters in response to rhGH treatment. Searches were performed using the following search strategy: d3GHR OR d3-GHR OR d3-growth hormone (GH) receptor OR (("Exon 3-deleted" OR "exon 3 deletion") AND Growth Hormone Receptor) OR (("exon 3" OR d3) AND (growth hormone receptor OR GHR OR gh receptor OR gh receptors OR growth hormone receptors) AND (polymorphism OR polymorphisms OR genotype OR genotypes OR isoform OR isoforms)). Searches were performed on the 4th of December 2008. In addition, the references of relevant articles were checked for additional articles. Abstracts of meetings and unpublished results were not included in the analysis.

Data review and data analysis

Data extraction and eligibility was assessed by two independent investigators (M.W. and N.B.). Inconsistencies in data extraction were resolved by consensus. We compared the effect of rhGH treatment and baseline height between subjects with at least one copy of the exon-3 deleted GHR, i.e. GHR_{d3}, and subjects without exon-3 deleted GHR, i.e. $GHR_{wt\text{-}wt}$. $GHR_{wt\text{-}wt}$ was used as the reference group. For studies assessing the effect of rhGH, the weighted mean difference in height gain (delta SDS) and growth velocity (cm/year) in the first year of treatment was calculated. The effect of the GHR_{d3} on baseline height, expressed as SDS, was assessed separately in GHD and non-GHD. A meta-analysis was performed for baseline height, height gain and growth velocity by using both a fixed effects model and a random effects model. To assess to which extent the effect measured by the set of single studies was hetero- or homogeneous, the I^2 index was assessed. The test seeks to determine whether there are genuine differences underlying the results of the studies (heterogeneity), or whether the variation in findings is compatible with chance alone (homogeneity), 0% is very homogeneous, 100% very heterogeneous. In addition, a meta-regression was performed to assess the effects of age, dose, and diagnosis on the effect of the different d3GHR genotypes on height gain and growth velocity. Statistical analyses were done in Comprehensive Meta-Analysis (version 2·0, Biostat, Englewood, New Jersey, USA) and in STATA for Windows (version 10.1, StataCorp, Texas, USA).

RESULTS

Literature search

We identified 136 studies by search in Medline, Embase, Web of Science, and the Cochrane Library (*Figure 1*).

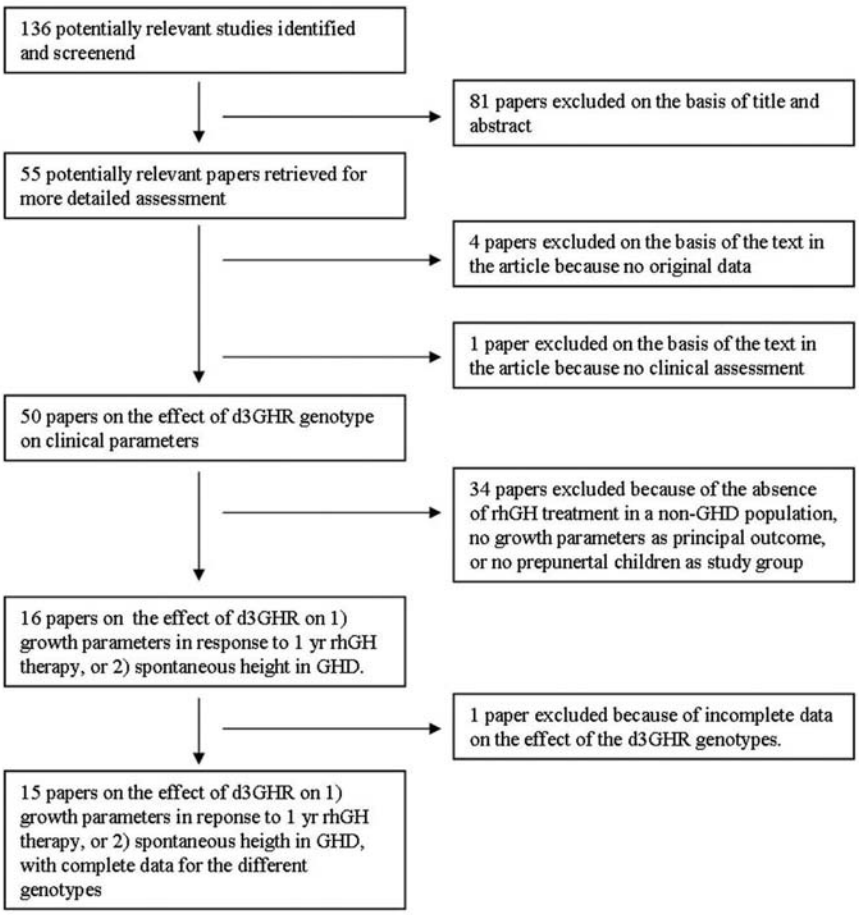


Figure 1. Summary of study assessment and exclusion stages.

We excluded 81 papers on the basis of title and abstract. Four of the 55 potentially relevant papers retrieved for more detailed assessment, were excluded from further analysis, because these studies did not report original data and one more could be excluded because the

effect of d3GHR on clinical parameters was not assessed. Additionally, 34 of the 50 papers with data on the effect of d3GHR on clinical parameters were excluded because of the absence of rhGH treatment in GHD or non-GHD populations, of reporting on growth parameters, or of pre-pubertal children as study group. The study from Pilotta *et al.*[23] did not provide precise data on genotypic differences and was therefore excluded from the present analysis. Consequently, a total of 15 studies were included in this meta-analysis (*Figure 1*).

These 15 studies provided data on the effect of rhGH therapy on height gain (n=1)[10], growth velocity (n=4)[8;12;13;21], or both (n=9) [9;11;14;15;17-20;22]. From 5 studies (one publication reported on 2 study populations[10]) [9-11;13] in GHD and from 11 studies (two publications reported on 2 different study populations [6;20] in non-GHD [13-19] data on baseline height could be extracted.

Study characteristics

Details of the 15 included studies are summarized in *Table 1*. Studies on the effect of d3GHR on baseline height or the growth response to rhGH were published between 2004 and 2008, and the number of included patients ranged from 28 to 240. Dos Santos *et al.*[8], Binder *et al.* [22] and de Graaff *et al.* [10] reported on 2 different study populations in the same paper, and for the purpose of the present analysis these populations were described and analyzed separately. Dos Santos *et al.* studied 2 separate populations of both SSA and ISS pre-pubertal children [8]. Binder *et al.* studied SGA and ISS children separately in the same paper[22], and De Graaff *et al.* [10] studied isolated GHD and multiple GHD pre-pubertal children in the same paper.

Indications for rhGH treatment were GHD (isolated GHD or in combination with multiple endocrine deficiencies) [9-14], small for gestational age (SGA) [8;15;17-19;22], idiopathic short stature (ISS) [8;20;21], and Turner syndrome [16;22]. The dosages of rhGH applied in the 15 included studies ranged from 26 μg/kg/day to 66 μg/kg/day and mean age ranged from 7 to 9 years. The genotypic distribution showed some inter-study variation, but in all included studies the distribution was according to the Hardy Weinberg Equilibrium (HWE). The distribution remained in HWE when all 1680 patients of the 15 studies were analyzed together. The genotypes included in this meta-analysis were: wt-wt=851 (51%), wt-d3=631 (37%), and d3-d3= 198 (12%).

Table 1. Overview of publications on the pharmacogenetic effects of rhGH in relation to d3GHR genotype on 1) baseline height in GHD and 2) growth velocity and/or height SDS gain in response to 1 year of rhGH treatment, arranged according to clinical condition

First author	N	Male	Age	GHR genotye (%)			Outcome	Significant effect of d3GHR polymorphism vs. wildtype		
		(%)	(yrs)	wt-wt	wt-d3	d3-d3		Effect of WT-D3	Effect of D3-D3	Effect of D3
GH deficiency in children										
Blum, 2006 (9)	107	68	7.0	59 (55%)	45 (42%)	3 (3%)	1. baseline height	NR	NR	No effect
							2. growth velocity and height gain	NR	NR	No effect
Jorge, 2006 (11)	58	62	8.9	28 (48%)	23 (40%)	7 (12%)	1. baseline height	NR	NR	↑ growth velocity
							2. growth velocity and height gain	NR	NR	No effect
Wan, 2007 (14)	154	70	7.8	79 (51%)	55 (36%)	20 (13%)	growth velocity and height gain	No effect	No effect	NR
De Graaff I, 2008 (10)	40	NG	5.5	17 (43%)	19 (47%)	4 (9%)	1. baseline height	NR	NR	No effect
							2. height gain	NR	NR	No effect
De Graaff II, 2008 (10)	45	NG	4.8	19 (43%)	21 (47%)	5 (9%)	1. baseline height	NR	NR	No effect
							2. height gain	NR	NR	No effect
Marchisotti, 2008 (12)	28	57	11.2	14 (50%)	11 (39%)	3 (11%)	1. growth velocity	NR	NR	No effect
Räz, 2008 (13)	181	54	6.6	90 (50%)	71 (39%)	20 (11%)	1. baseline height	No effect	↑ growth velocity	↑ growth velocity
							2. growth velocity	No effect	No effect	No effect
Small for gestational age										
Dos Santos I , 2004 (8)	76	61	6.6	36 (47%)	24 (32%)	16 (21%)	growth velocity	NR	NR	↑ growth velocity
Binder I, 2006 (22)	60	63	7.1	29 (48%)	23 (38%)	8 (14%)	growth velocity and height gain	↑ growth velocity	No effect	↑ growth velocity
Carrascosa, 2006 (17)	68	49	± 7.1	30 (44%)	32 (47%)	6 (9%)	growth velocity and height gain	No effect	No effect	NR
Tauber, 2007 (19)	240	58	6.6	144 (60%)	65 (27%)	31 (13%)	growth velocity and height gain	No effect	No effect	↑ growth velocity
Audi, 2008 (15)	219	50	9.1	99 (45%)	96 (44%)	24 (11%)	growth velocity and height gain	No effect	No effect	NR
Carrascosa, 2008 (18)	49	72	7.7	23 (47%)	21 (43%)	5 (10%)	growth velocity and height gain	No effect	No effect	NR
Idiopathic short stature										
Dos Santos II, 2004 (8)	96	64	7.7	50 (52%)	38 (40%)	8 (8%)	growth velocity	NR	NR	↑ growth velocity
Carrascosa, 2008 (20)	106	55	7.8	46 (43%)	42 (40%)	18 (17%)	growth velocity and height gain	No effect	No effect	NR
Ko, 2008 (21)	52	51	8.0	37 (71%)	13 (25%)	2 (4%)	growth velocity	NR	NR	↑ growth velocity
Turner syndrome										
Binder II, 2006 (22)	53	0	9	27 (51%)	15 (28%)	11 (21%)	growth velocity and height gain	No effect	↑ growth velocity compared with wt-wt and wt-d3	↑ growth velocity
Binder, 2008 (16)	48	0	9.1 - 16.0	24 (50%)	17 (36%)	7 (14%)	growth velocity	No effect	↑ period of high growth velocity	NR

Legend Table 1. Effect means: p<0.05, no effect: p>0.05. NR: not reported, yrs: years, GHR: growth hormone receptor, rhGH: recombinant human growth hormone (treatment). D3 = $GHR_{wt\text{-}d3}$ and $GHR_{d3\text{-}d3}$ *vs* $GHR_{wt\text{-}wt}$. The use of 2 different study groups in 1 paper is referred to as either I or II of those authors.

Meta-analysis

Baseline height in GHD pre-pubertal children (Figure 2A)

The effect of d3GHR on baseline height (SDS) in GHD children without previous rhGH treatment was assessed in five studies [9-11;13]. In 4 of these 5 studies baseline height (SDS) was higher in the GHR_{d3} than in the $GHR_{wt\text{-}wt}$ groups, which reached significance in only one study[11]. The mean difference in baseline height (SDS) between GHR_{d3} and $GHR_{wt\text{-}wt}$ in the combined data from these 5 studies was -0.159 (95% CI: -0.298, -0.020), which reflects a small positive effect in GHR_{d3} when compared with $GHR_{wt\text{-}wt}$ in the absence of rhGH treatment (I^2 0%). This was significant, even when with for age (p=0.04).

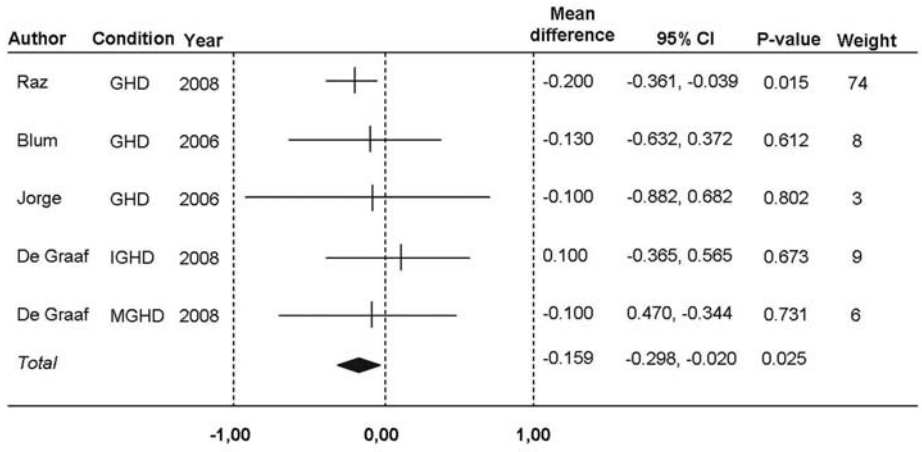


Figure 2A. Meta analysis of the effect of $GHR_{wt\text{-}wt}$ *vs.* GHR_{d3} genotypes on baseline height (SDS) in children with short stature caused by GHD. 'Total' represents fixed effects. A negative score points towards lower baseline height in $GHR_{wt\text{-}wt}$ subjects. De Graaff *et al.* studied isolated GHD (IGHD) and multiple GHD (MGHD) in the same paper[10]. CI: confidence interval. GHD: growth hormone deficiency, SGA: small for gestational age, ISS: idiopathic short stature.

Baseline height in non-GHD pre-pubertal children (Figure 2B)

In non-GHD children baseline height (SDS) was cross-sectionally assessed in 9 of 15 studies, and 2 of these 9 articles reported on 2 study populations[6;20]. None of these studies demonstrated a significant difference in baseline height (SDS) between GHR_{d3} and $GHR_{wt\text{-}wt}$. The mean difference in baseline height between GHR_{d3} and $GHR_{wt\text{-}wt}$ was -0.05 SDS (95% CI: -0.180,

0.080), which indicates the absence of a significant difference between GHR_{d3} and $GHR_{wt\text{-}wt}$ children (I^2 0%).

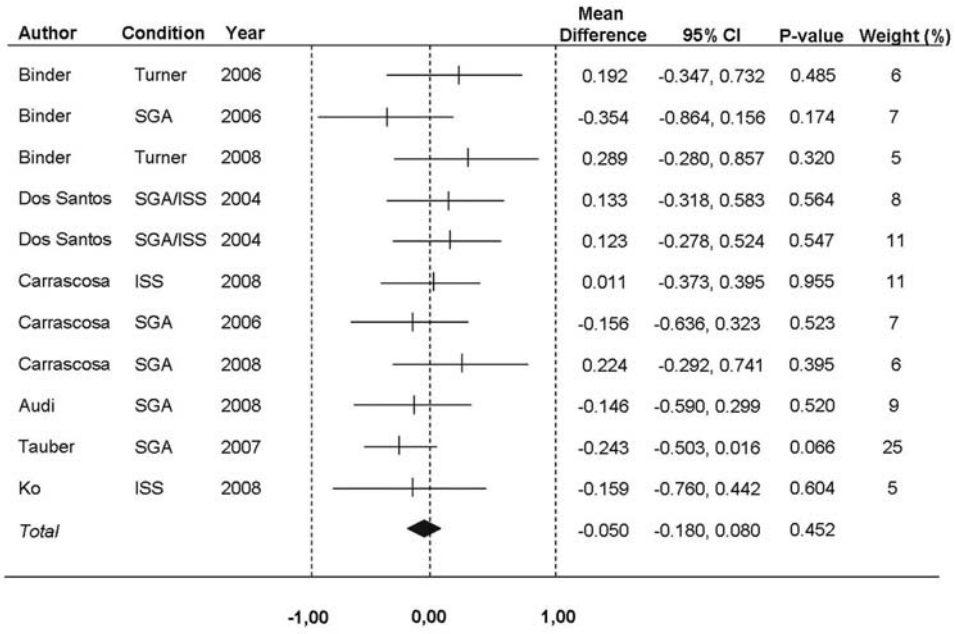


Figure 2B. Meta analysis of the effect of $GHR_{wt\text{-}wt}$ *vs.* GHR_{d3} genotypes on baseline height (SDS) in children with non-GHD short stature. 'Total' represents fixed effects. A negative score points towards lower baseline height in $GHR_{wt\text{-}wt}$ subjects. Binder *et al.* studied Turner syndrome and SGA, in the same paper[22]. Dos Santos *et al.* studied two populations of SGA/ISS in the same paper[8]. CI: confidence interval. GHD: growth hormone deficiency, SGA: small for gestational age, ISS: idiopathic short stature.

Height gain in response to 1 year rhGH treatment (Figure 3)

Height gain was measured as change in height SDS after 1 year of rhGH treatment. As demonstrated in *Figure 3*, for 8 of the 11 studies assessing the effect of height gain in GHD and non-GHD small children, the mean difference in height gain SDS after one year of rhGH treatment was lower for $GHR_{wt\text{-}wt}$ when compared with GHR_{d3} (mean difference -0.075, 95% C.I. - 0.143, -,007), which reflects 0.075 SD more increase in height in GHR_{d3} when compared with $GHR_{wt\text{-}wt}$ after 1 year of rhGH treatment. This pharmacogenetic effect was not statistically significant in the majority of the original studies. The I^2 for this comparison was 46%, indicating moderate heterogeneity [24].

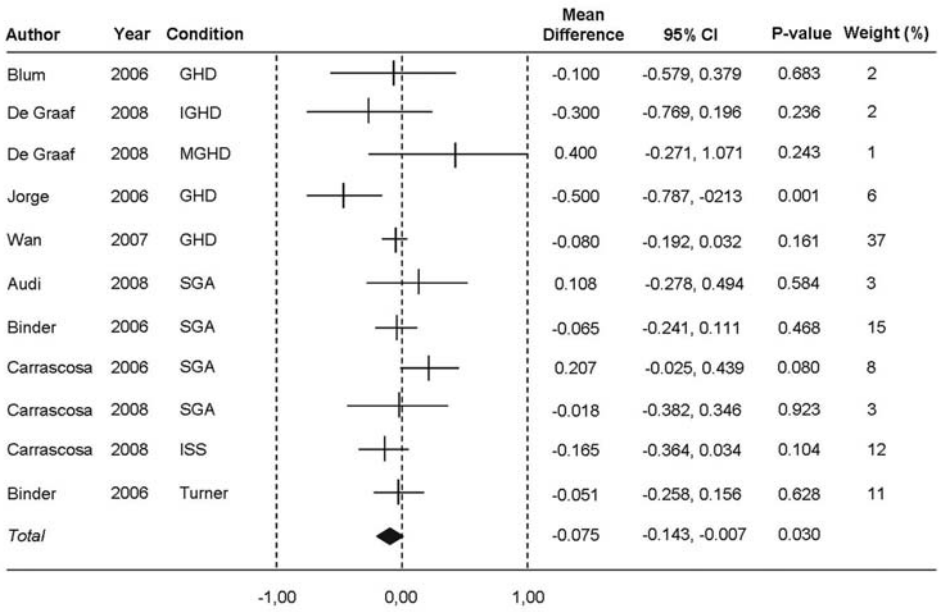


Figure 3. Meta-analysis of the effect of GHR_{wt-wt} *vs.* GHR_{d3} genotypes on height gain (SD) in children with short stature after 1 year of rhGH treatment. 'Total' represents fixed effects. A negative score points towards less height gain in GHR_{wt-wt} subjects. Binder *et al.* studied Turner syndrome and SGA, in the same paper[22]. De Graaff *et al.* studied isolated GHD (IGHD) and multiple GHD (MGHD) in the same paper[10]. GHD: growth hormone deficiency, SGA: small for gestational age, ISS: idiopathic short stature. CI: confidence interval.

First year's growth velocity in response to rhGH treatment (Figure 4)

For 16 studies the effects of d3GHR polymorphism on the first year's growth velocity, measured in cm/year, during rhGH treatment could be compared between GHR_{wt} and GHR_{d3} children. In 6 of these 16 studies there was a significant difference in growth velocity during rhGH treatment between GHR_{d3} and GHR_{wt-wt}, favoring $GHR_{d3.}$ After meta-analysis, mean growth velocity was lower for GHR_{wt-wt} compared with GHR_{d3} (mean difference -0.521 cm/yr, 95% CI: -0.709,-0.333), which reflects an additional increase in height of 0.521 cm in GHR_{d3} compared with GHR_{wt-wt} after 1 year of rhGH treatment. The I^2 was 67%, but the use of a random effects model did not influence the point estimate nor the statistical significance.

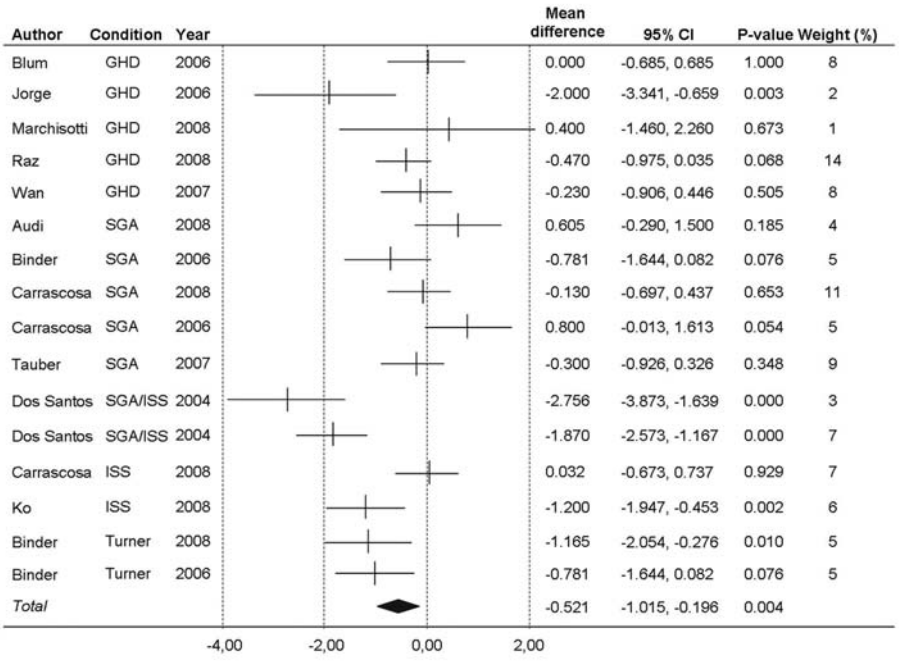
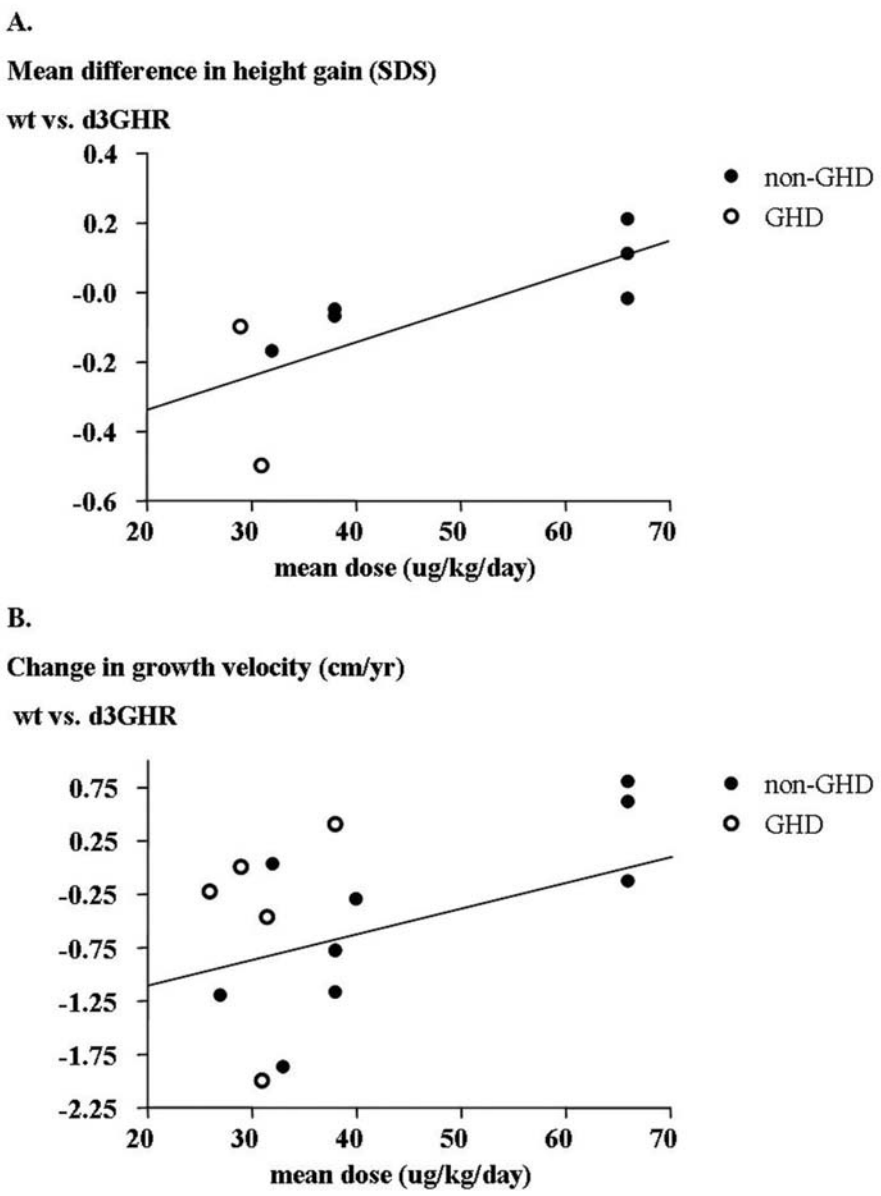


Figure 4. Meta-analysis of the effect of $GHR_{wt\text{-}wt}$ *vs.* GHR_{d3} genotypes on growth velocity (cm/yr) in children with short stature after 1 year rhGH treatment. 'Total' represents fixed effects. A negative score points towards lower growth velocity in $GHR_{wt\text{-}wt}$ subjects. Binder *et al.* studied Turner syndrome and SGA, in the same paper[22]. De Graaff *et al.* studied isolated GHD (IGHD) and multiple GHD (MGHD) in the same paper [10]. Dos Santos *et al.* studied two populations of SGA/ISS in the same paper[8]. CI: confidence interval. GHD: growth hormone deficiency, SGA: small for gestational age, ISS: idiopathic short stature.

Meta-regression

We performed a meta-regression analysis to assess whether the differences in response to rhGH between genotypes in growth velocity and height gain were dependent on the dose of rhGH, age, or cause of short stature (*i.e.* GHD versus non-GHD). Random effects meta-regression showed a significant interaction between rhGH dose and the difference in height gain between $GHR_{wt\text{-}wt}$ and GHR_{d3} (p=0.02) (*Figure 5A*) and suggested a trend for the interaction between rhGH dose and the difference in growth velocity between $GHR_{wt\text{-}wt}$ and GHR_{d3} (p=0.06) (*Figure 5B*) and This points towards smaller differences in outcome with increasing doses of growth hormone.

Figure 5AB



There was also an interaction between genotype and age for the treatment effect of rhGH: the differences in height gain between $GHR_{wt\text{-}wt}$ and GHR_{d3} (p=0.02) (*Figure 5C*) and growth velocity (p=0.02) (*Figure 5D*) were more pronounced in studies with a higher mean age. Meta-regression demonstrated that age and dose were both significant predictors for the ef-

fect of the d3GHR genotypes (p=0.005 and p=0.002, respectively), irrespective of the cause of short stature (*i.e.* GHD *versus* non-GHD children). The cause of short stature did not significantly affect the difference between genotypes (p=0.069), although the trend observed

Figure 5CD

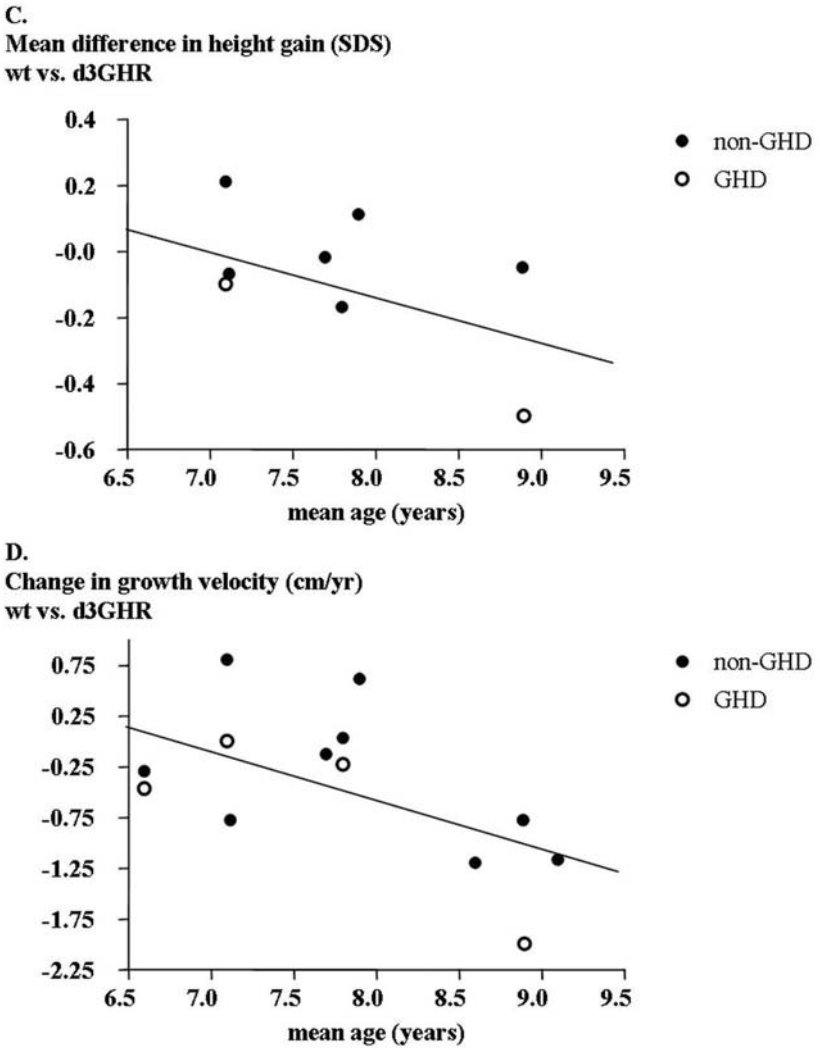


Figure 5 A-D. Association between the dose of rhGH / age and the difference between GHR_{wt-wt} and GHR_{d3} genotypes in change in growth velocity and height gain during 1 year of rhGH treatment. Regression coefficient (R) and 95% confidence interval (CI): Fig 5A: R 1.89, 95% CI -0.12, 3.17; Fig 5B: R 1.48, 95% CI 0.07, 0.88, Fig 5C: R -3.55, 95% CI -1.15, -7.25; Fig 5D:R -1.71, 95% CI -0.64, -2.99. GHD: growth hormone deficient.

in the interaction between genotype and diagnosis pointed towards a smaller effect in non-GHD children. When GHD and non-GHD patients were analyzed separately, this difference in height gain between $GHR_{wt\text{-}wt}$ and GHR_{d3} in non-GHD patients with short stature was not significant anymore (p=0.18), whereas it was significant in the GHD patients (p=0.01).

DISCUSSION

In the present meta-analysis, we show that the growth response during the first year of rhGH treatment, both expressed as height SDS gain and as growth velocity, is significantly increased in pre-pubertal short children with the GHR_{d3} genotype in comparison with the $GHR_{wt\text{-}wt}$ genotype. Moreover, this meta-analysis demonstrates an association between the stimulatory effect of the GHR_{d3} genotype and baseline height in children with GHD. Both findings are in line with the differences in activity between both GH receptor variants *in vitro*[8]. In addition, a lower rhGH dose and a higher age at onset of rhGH treatment were associated with a larger difference in growth response between $GHR_{wt\text{-}wt}$ and GHR_{d3} genotypes.

The studies included in the present meta-analysis differ with respect to the conditions causing short stature in children, the presence/severity of GHD, age (although all children were pre-pubertal with mean ages between 7 and 9 years), and the duration and/or dose of rhGH treatment. Nonetheless, the pharmacogenetic effects on the growth response to rhGH were remarkably constant across the various studies and the various indications for rhGH therapy. In the majority of studies on height SDS gain or first year's growth velocity, the effect of rhGH was larger in GHR_{d3} compared with $GHR_{wt\text{-}wt}$. Moreover, in 4 out of 5 included studies in GHD children baseline height (SDS) was higher in GHR_{d3} compared with $GHR_{wt\text{-}wt}$. The problem of statistical power in the individual studies is illustrated by the fact that the pharmacogenetic effects are largely consistent among the different studies, even though these effects were small and not always statistically significant. Unfortunately, the number of subjects in the studies included in the present meta-analysis was too small to reliably sub-analyze data for GHD and non-GHD children. In the meta-regression, the cause of short stature (GHD *versus* non-GHD) did not appear to be a significant predictor for first year's height gain and growth velocity in the total group. On the other hand, the difference in height gain between genotypes lost its statistical significance in a separate analysis of non-GHD children. Therefore, larger

studies are needed to enable firm conclusions on the (magnitude of the) effect of the GHR_{d3} on height gain in non-GHD children and to assess whether this effect is larger in GHD than in non-GHD children. In addition, we chose to analyze the pharmacogenetic effects in homozygous and heterozygous d3 deletions patients together, since the number of studies that reported separate results for GHR_{wt-d3} and GHR_{d3-d3} was too small. In analogy with the *in vitro* data by Dos Santos *et al.* which indicate increased bioactivity in GHR_{d3-d3} compared with GHR_{wt-d3}, additional studies are needed to establish whether there are comparable pharmacogenetic differences with respect to the growth response to rhGH between patients with the GHR_{wt-d3} and the GHR_{d3-d3} genotype[8].

The observation of a larger difference in first year's height gain and growth velocity between GHR_{wt-wt} and GHR_{d3} in patients treated with a lower rhGH dose in our meta-regression is in line with theoretical considerations that differences of any predictor will be uncovered more easily when a relatively low dose is given. With a high dose it would be more difficult to detect the subtle effects of variation in responsiveness. Our observation is in contrast to the suggestion that was raised in the recent review by Keni *et al*. that the GHR_{d3} genotype has a larger impact at higher doses. The discrepancy between our results and the analysis of Keni *et al.* may be caused by methodological differences, because Keni *et al.* did not perform a meta-analysis of the data and included only some of the studies included in our study [25]. The interaction between the pharmacogenetic effect with age can be explained by the relatively high GH responsiveness at a young age, which may overrule the subtle difference in responsiveness between the two genotypes. Since age and GH dose are independent predictors of the growth response to GH[4], it is uncertain what the relative effect of the GHR variant is in a prediction model including multiple parameters. We believe, however, that the small size of the effect estimated in the univariate analysis makes it unlikely that the assessment of GHR polymorphism will be a clinically useful predictor. In addition, in a large study in children with severe GHD no effect of the GHR polymorphism on height gain and final height was demonstrated[13]. Future studies should confirm this finding.

Interestingly, in 2 studies differences in growth parameters associated with the d3GHR genotypes were not reflected by different increases in IGF-I levels[16;22]. The opposite discrepancy was reported by Marchisotti *et al.,* who were unable to demonstrate an effect of the GHR_{d3} genotype on the growth response to rhGH in pre-pubertal GHD children, but did demonstrate an effect on IGF-I levels[12]. Therefore, one may speculate that IGF-I independent

effects of rhGH dose at the epiphyseal growth plates could also be involved in the pharmacogenetics of rhGH[26]. Alternatively, serum IGF-I levels may not be a good marker of the total IGF-I mediated effects at the epiphyseal growth plates, as autocrine and paracrine effects of locally secreted IGF-I (which are also influenced by rhGH therapy) are not reflected by circulating IGF-I concentrations[26].

The GHR_{d3} genotype is not expected to be associated with physiological variations in human growth or to be a primary cause of short-stature in humans, since potential variations in GH sensitivity due to genotypic differences in GHR activity can be compensated by alterations in endogenous pituitary GH secretion, which might mask the effect of the GHR polymorphism on spontaneous growth. In addition, the distribution of the d3GHR genotypes is in accordance to Hardy Weinberg equilibrium in all clinical conditions studied. Nonetheless, we hypothesized that in patients with GHD this compensatory effect within the GH-IGF-I axis does not function properly and, therefore, the GHR_{d3} genotype may be associated in GHD patients with increased baseline height. Individual studies in pre-pubertal GHD children did not report a stimulatory effect of this polymorphism on baseline height, but in this meta-analysis we were able to demonstrate a small effect, even when adjusted for age. As expected, this genotypic effect was not demonstrated on baseline height in non-GHD children. This finding is supported by observations of Lettre *et al*. who were unable to detect a role for common genetic variation in eight candidate genes of the GH/IGF-I axis in stature variation in the general population[27].

A possible limitation of this meta-analysis is the relatively small number of large studies on the effect of the d3GHR polymorphism on baseline or stimulated growth. In addition, in the various studies there were considerable differences in the degree of GH deficiency, which also may have influenced our findings. Despite the fact that both positive and negative results on the stimulatory effect on growth of the d3GHR polymorphism have been published, we can not exclude the presence of a publication bias in this field. However, this meta-analysis of 15 studies including a total of 1680 patient from the presently available peer-reviewed literature is useful to put available results into perspective.

In conclusion, this meta-analysis shows that the d3GHR genotype is significantly associated with a ~0.5cm/year higher growth response to 1 year of rhGH therapy in prepubertal children with short stature due to GHD and other causes (non-GHD). This pharmacogenetic effect is one of the many factors contributing to the growth response to rhGH therapy and

might be considered to be included in future prediction models[25]. These pharmacogenetic effects of GHR_{d3} on the growth response to rhGH treatment were stronger with lower rhGH dose and higher age. In addition, a d3GHR dependent effect was demonstrated on baseline height in GHD, but not in non-GHD children. Additional studies are required to establish to which extent these pharmacogenetic effects of the GHR_{d3} genotype on rhGH treatment translate into ultimate adult height gain.

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