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The Effect of the Exon-3-Deleted Growth Hormone Receptor on Pegvisomant-Treated Acromegaly: A Systematic Review and Meta-Analysis

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Key Words

Acromegaly · Pegvisomant · Polymorphism · Growth hormone receptor · Deletion of exon 3 · Meta-analysis

Abstract

Background: The common exon 3 deletion polymorphism of the growth hormone receptor (d3-GHR) is associated with disease severity in acromegaly patients. The GHR antagonist pegvisomant (PEGV) is highly effective in treating severe acromegaly. Response to PEGV treatment seems to be influenced by d3-GHR and appears to be more responsive to PEGV, although available results remain conflicting. ***Objective:*** To assess the influence of d3-GHR on the responsiveness of acromegaly patients to PEGV by compiling the evidence derived from the largest available studies. ***Design:*** A systematic review of the literature identified three published studies and one conference abstract. Acromegaly patients (n = 324, 49.7% d3-GHR carriers) were treated with either PEGV monotherapy or PEGV combined with long-acting somatostatin analogues and/or cabergoline. A meta-analysis of raw data from these studies was performed. ***Results:*** No significant effect of the d3-GHR was observed while bringing insulin-like growth factor I (IGF-I) levels below the upper limit of normal with PEGV, which was defined as the lowest IGF-I level during PEGV treatment (mean difference: –2.3%; 95% CI: –6.5 to 1.8, p = 0.270). The PEGV dose required to achieve the lowest IGF-I levels was also not significantly influenced by individuals carrying d3-GHR (mean difference: 4.1 mg weekly; 95% CI: –5.1 to 13.2, p = 0.385). For both outcomes, separate analysis of PEGV monotherapy and combination treatment gave similar results. ***Conclusion:*** Our findings suggest that the d3-GHR polymorphism has no effect on biochemical disease control in acromegaly, as it is not of added value for either the prediction of PEGV responsiveness or the determination of the required PEGV dose.

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Introduction

Acromegaly is a rare disease characterized by excessive secretion of growth hormone (GH) resulting in a diversified clinical presentation. The disease is almost exclusively caused by a GH-secreting pituitary adenoma [1]. These elevated GH levels subsequently increase insulin-like growth factor I (IGF-I) production, predominantly by the liver, although other tissues also synthesize IGF-I [2]. If untreated, the disease is associated with an increase in morbidity and mortality [2]. Control of disease activity results in mortality rates similar to the general population [3]. Although transsphenoidal surgery remains the first-line treatment in most countries [4], it is often unsuccessful, making additional treatment modalities necessary when GH and IGF-I levels remain elevated. However, primary medical treatment is becoming more and more popular, starting with long-acting somatostatin analogues (LA-SSA), with an average efficacy rate in normalizing GH and IGF-I levels in treatment-naive patients of 44% [5]. A highly effective alternative for patients who are not normalized by LA-SSA monotherapy is the addition of pegvisomant (PEGV) to LA-SSA, or even PEGV monotherapy, provided that the appropriate PEGV dose is used [6–8].

PEGV is a pegylated recombinant GH analogue that acts as a competitive GH receptor (GHR) antagonist in all tissues except the brain, most importantly suppressing GH-dependent production of IGF-I by the liver [9]. The PEGV dose required for normalization of IGF-I levels in acromegaly is variable, depending on disease activity and individual response to the drug [6, 10]. Likewise, a wide interindividual variation in PEGV serum levels is observed despite identical PEGV dosing [11, 12]. These differences in individual responses have been partly attributed to a common polymorphism in the GHR gene characterized by deletion of exon 3. This in-frame deletion causes loss of 22 amino acids from the extracellular domain. In about half of the general population, the polymorphism is homozygous for the full-length GHR (fl/fl-GHR), with the remaining half carrying the exon-3-deleted GHR (d3-GHR) polymorphism; 30–40% being heterozygous and 10–20% homozygous for this deletion [13–15]. A similar distribution of this GHR variant in cohorts of acromegaly patients has been described in the literature [16–20].

The deletion of exon 3 in GHR is caused by retrovirus-mediated alternative splicing, which results in skipping of coding exons [15]. This alternative splicing pattern is human-specific [15]. Evolutionary conservation of this GHR variant suggests beneficial effects. Transfections experiments by Dos Santos et al. [14] have shown that the lack of exon 3 in the GHR enhances GH signal transduction by approximately 30%. More specifically, the deletion of exon 3 leads to greater stimulation of the intracellular JAK-STAT pathway in response to GH, which results in increased transcription of GH target genes. Following the report of Dos Santos et al. [14], several studies primarily focused on assessing the role of the d3-GHR polymorphism during recombinant GH treatment of GH-deficient and non-GH-deficient prepubertal children with short stature. Carrying one or more d3-GHR alleles was found to be associated with increased baseline height and growth response to GH, according to a meta-analysis by Wassenaar et al. [21]. Thereafter, subsequent studies evaluated the influence of d3-GHR on the severity of acromegaly.

Several studies have addressed the influence of the d3-GHR polymorphism on GH and IGF-I levels. The first study included 44 untreated active acromegaly patients, in whom a higher baseline GH was observed in d3-GHR carriers whereas IGF-I levels were similar across the three genotypes [22]. However, a more recent study in 105 patients with untreated acromegaly could not confirm these findings [18]. The impact of the GHR variant on comorbidities was assessed in 86 acromegaly patients during long-term disease control [23]. The presence of d3-GHR was associated with an increased prevalence of irreversible long-term complications, such as osteoarthritis, dolichocolon, and adenomatous colonic polyps. However, d3-GHR was not associated with other comorbidities such as metabolic syndrome, diabetes mellitus type II, and vertebral and nonvertebral fractures. A recent Turkish study (n = 118) observed no effect of the GHR variant on either clinical features nor comorbidities, but suggested that the polymorphism might play a role in GH/IGF-I level discordance. Posttreatment biochemical characteristics were also assessed by an Italian cohort study, suggesting that more discordant GH/IGF-I levels (high IGF-I and GH ≤2 ng/ml) were observed in d3-GHR carriers, and that this discordance in levels was enhanced after initiation of somatostatin analogue treatment [16].

A previously cited meta-analysis observed an association with increased growth velocity in recombinant human GH-treated GH-deficient children carrying d3-GHR [21]. Subsequently, the question emerged whether d3-GHR influences pharmacodynamics of PEGV in acromegaly as carriers of d3-GHR might need less PEGV to normalize IGF-I levels than patients with the fl/fl-GHR genotype in order to normalize IGF-I levels. PEGV di-

rectly antagonizes the GHR, and, therefore, could have a greater impact on d3-GHRs. Two studies, indeed, reported a lower required PEGV dose during disease control in acromegaly patients with the d3-GHR genotype [16, 24]. However, more recent studies in larger acromegaly cohorts could not confirm these findings [25, 26]. These contradictory reports on the influence of d3-GHR in acromegaly patients regarding PEGV treatment responses and the PEGV doses required to normalize IGF-I levels motivated us to conduct a systematic review of the literature to identify studies examining this question and to perform a meta-analysis. The aim of this study is to address the clinically relevant question: Do clinicians have to take into account d3-GHR genotyping during PEGV dosing?

Materials and Methods

Inclusion Criteria

The two main outcome parameters used by us were (1) lowest IGF-I level expressed as upper limit of normal (ULN) during PEGV treatment and (2) the required PEGV dose to achieve the lowest IGF-I level. Studies reporting these main outcomes in acromegaly cohorts concerning the influence of d3-GHR were included. In these studies, the exon 3-deleted GHR polymorphism has been reported as fl/fl-GHR, d3/fl-GHR, d3/d3-GHR, and/or d3-GHR, in which d3-GHR could be a combination of the d3/fl-GHR and d3/d3-GHR genotype.

Search Strategy

The online literature databases Embase.com, Medline (Ovid-SP), Pubmed Publisher, Web of Science, Google Scholar, and the Cochrane Library were used for this systemic search of studies reporting the influence of d3-GHR on the outcome in response to PEGV treatment in acromegaly patients. This was performed under the guidance of a research librarian. The performed search strategy was: (d3GHR OR d3-GHR OR 'd3-growth hormone receptor' OR 'exon 3' OR d3 OR 'exon 3-deleted' OR 'exon 3 deletion') AND (GHR OR 'GH receptor' OR 'growth hormone receptor' OR GHRs OR 'GH receptors' OR 'growth hormone receptors') AND (Polymorphism OR polymorphisms OR isoform OR isoforms OR genotype OR genotypes OR variant OR variants) AND Acromegaly AND (Pegvisomant OR somavert OR 'growth hormone receptor antagonist' OR 'GH receptor antagonist' OR 'GHR antagonist'). The searches were performed on September 9, 2014. Thereafter, the references of relevant articles were revised for additional studies.

Data Review and Data Collection

Data selection was independently assessed by the investigators S.E. Franck and S.J.C.M.M. Neggers. Besides our own cohort, two articles and one conference abstract met our selection criteria. The study of Bianchi et al. [16] from 2009 (n = 19) was excluded from this meta-analysis as the vast majority of the patients was also included in a larger cohort (n = 127) published by the same author group in 2012 [25]. We contacted the principal investigators of these three research groups, in order to collect raw data from these acromegaly cohorts. We were able to obtain from all three selected articles the variables needed to perform the meta-analysis: genotype coded as fl/fl, d3/fl or d3/d3, sex, age at diagnosis, PEGV monotherapy versus PEGV combined with long-acting LA-SSA and/or cabergoline (CAB) (combination treatment), lowest IGF-I levels during PEGV treatment and required PEGV dose to achieve this lowest IGF-I level. In total 135 patients were treated with PEGV monotherapy and 189 with combination treatment. Medical ethics committees from each hospital approved the protocol, and a written informed consent was obtained from all patients. The paragraph on 'Included Study Characteristics' in the Results section describes this approach in more detail.

Statistical Analysis

Raw data from each cohort were used to calculate betas (βs) and standard errors (SEs) by linear regression analysis. βs and SEs were calculated per cohort and medication group (PEGV monotherapy, combination treatment and total cohort: monotherapy + combination treatment). The variable lowest IGF-I level during PEGV treatment was corrected for sex, age and required PEGV dose to achieve the lowest IGF-I level. This latter variable itself was corrected for sex and age, when used for meta-analysis individually. Hardy-Weinberg equilibrium (HWE) was analyzed with the χ^2 test via the observed and expected genotype frequencies.

Statistical analyses were performed with SPSS version 20 (SPSS software, Chicago, Ill., USA) and GraphPad Prism version 6 for Windows (GraphPad Software, San Diego, Calif., USA). Potential effects of the genotype GHR deletion of exon 3 were calculated by an inverse variance meta-analysis in R, version 3.2.1 [27]. Fixed effects meta-analyses were performed as implemented in the R package 'rmeta' [28]. As we tested two independent medical treatment variants (PEGV monotherapy and combination treatment) as well as the total cohort, we set the significance threshold of our one-sided p values at 0.025 in order to correct for multiple testing (Bonferroni correction).

Results

Literature Search

We identified 511 potentially relevant studies by a literature search in Embase, Medline, Pubmed, Web of Science, Google Scholar, and the Cochrane Library (fig. 1). Of these studies, 497 were found not to meet the inclusion criteria on the basis of title and abstract. Fourteen papers were relevant for more detailed examination, of which 9 were excluded for different reasons: 4 papers presented a description/citation of an included study in our meta-analysis in a book, 3 papers presented an abstract of an included study in our meta-analysis, and 2 studies did not report original data. One original study [16] was excluded as the majority of the patients were included in a larger more recent study, which was already part of this meta-analysis [25]. Finally, we included 3 published original studies and 1 conference abstract describing one relevant

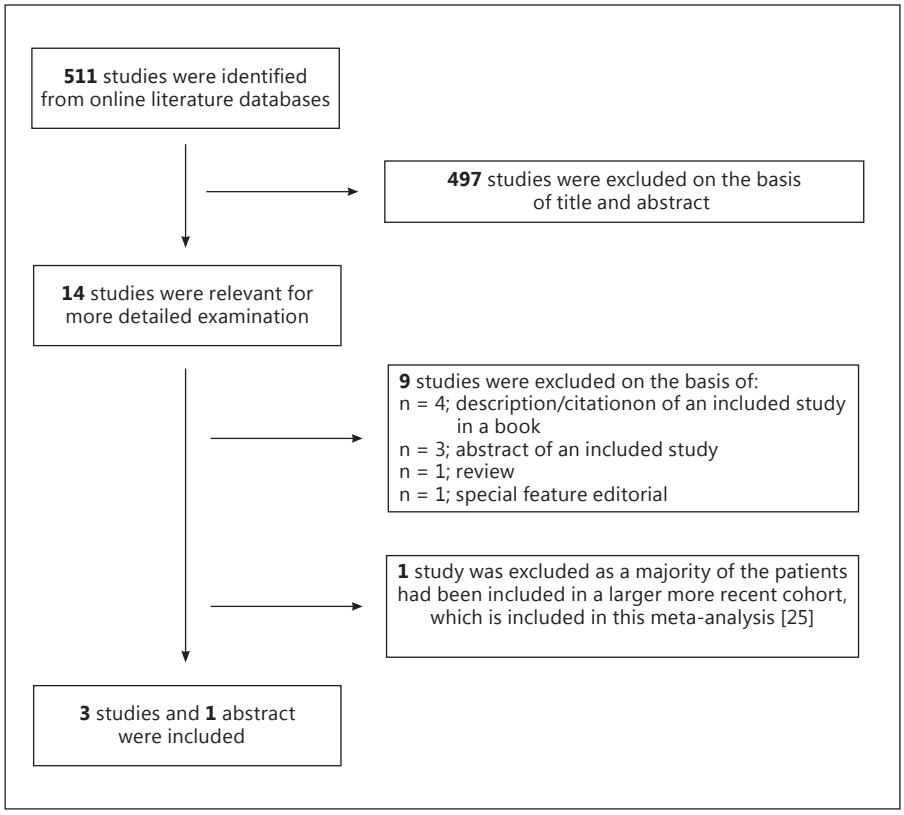

Fig. 1. Flow diagram of the study selection and exclusion stages.

acromegaly cohort [24–26, 29]. All 4 selected papers included data of both requested outcomes: (1) lowest IGF-I level expressed as ULN during PEGV treatment and (2) the required PEGV dose to achieve the lowest IGF-I level.

Included Study Characteristics

Characteristics of the 4 included studies are summarized in table 1. The reports were published between 2010 and 2015, and the range of the number of included patients varied between 44 and 127. All reports included only acromegaly patients with elevated IGF-I levels after at least 6 months of the maximum tolerated dose of LA-SSA. Kamenicky et al. [29] included 53 patients, of which 1 patient was excluded because LA-SSA pretreatment was not administered, and GHR genotype was not available in 3 patients, resulting in 49 acromegaly patients relevant for analysis.

In total, 135 patients were treated with PEGV monotherapy and 189 with combination treatment (177 PEGV + LA-SSA, 8 PEGV + CAB, 4 PEGV + LA-SSA + CAB). Treatment modalities were different per study. Bernabeu et al. [24] (n = 44) and Kamenicky et al. [29] (n = 49) predominantly included patients treated with PEGV monotherapy (Bernabeu: 29 PEGV alone; 7 PEGV + LA-SSA; 7 PEGV + CAB; 1 PEGV + LA-SSA + CAB, Kamenicky: 42 PEGV alone; 3 PEGV + LA-SSA; 1 PEGV + CAB; 3 PEGV + LA-SSA + CAB). Filopanti et al. [25] (n = 127) included an almost equal number of patients treated with PEGV monotherapy and combination treatment (64 PEGV alone, 63 PEGV + LA-SSA). Franck et al. [26] (n = 104) included only patients treated with LA-SSA in combination with PEGV.

The frequency distribution of the d3-GHR genotype showed some variation between studies and ranged between 46.5% in the cohort of Filopanti et al. [25] and 59.1% in the cohort of Bernabeu et al. [24] shown in table 1. The total sample of this meta-analysis included the following genotypes: fl/fl: 163 (50.3%); d3/fl: 122 (37.7%); d3/d3: 39 (12.0%). With the exception of Franck et al. [26] (p = 0.859), none of the included cohorts had genotype frequencies following HWE proportions. When all cohorts (n = 324) were pooled together, the genotype distribution was not in accordance with HWE (p = 0.034).

In 1 of the 4 studies, an effect of d3-GHR was observed on the duration of successful PEGV treatment, as carriers required less time to reach IGF-I normalization [24].

Table 1. Characteristics of included cohorts

First author [Ref.], year	n	Males, %	Age, years	GHR genotype, %				Effect of d3-GHR
				fl/fl	d3/fl	d3/d3		
Bernabeu [24], 2010	44	40.9	46 [36–56]	40.9	56.8	2.3	quantitative PCR	
Normalization of IGF-I during PEGV								no effect
Months to normalization of IGF-I								effect: ↓ months
Required PEGV dose								effect: ↓ PEGV dose
Kamenicky [29], 2011	49	57.1	36 [26–48]	49.0	26.5	24.5	multiplex PCR	
Required PEGV dose								no effect
Filopanti [25], 2012	127	57.5	42 [32–50]	53.5	32.3	14.2	multiplex PCR	
IGF-I SDS during PEGV								no effect
Required PEGV dose								no effect
Franck [26], 2015	104	58.7	45 [36–56]	51.0	41.3	7.7	quantitative PCR	
IGF-I ULN during PEGV								no effect
Required PEGV dose								no effect

Description of patient characteristics of the included studies and the effect of d3-GHR on IGF-I during PEGV treatment and required PEGV dose. Effect indicates $p \leq 0.05$; no effect indicates $p \geq 0.05$; the required PEGV dose is provided to normalize IGF-I levels. Age was noted at diagnosis and expressed as median [interquartile range]. GHR = Growth hormone receptor; fl = full-length; d3 = deletion of exon 3; n = number of patients; IGF-I = insulin-like growth factor I; SDS = standard deviation score; ULN = upper limit of normal; PEGV = pegvisomant; PCR = polymerase chain reaction.

However, no difference was observed in genotype distribution between normalized and nonnormalized patients regarding IGF-I levels during PEGV treatment. Meanwhile, the required PEGV dose per body weight was approximately 20% lower in d3-GHR carriers [24]. The other 3 studies did not report an influence of the genotype on PEGV-treated acromegaly patients regarding either IGF-I levels or the required PEGV dose.

Meta-Analysis

Lowest IGF-I Level during PEGV Treatment

The effects of d3-GHR on the lowest IGF-I level during PEGV treatment could be assessed in all 4 cohorts using the raw data sets obtained from the principal investigators and the individual effects of the studies are summarized in table 2. The mean difference in lowest IGF-I level (ULN) between d3-GHR carriers and fl/fl-GHR in the total cohort was –2.3% (95% CI: –6.5 to 1.8), which reflects a small negative effect in d3-GHR carriers when compared with fl/fl-GHR in combined data from the 4 studies. However, this effect was not significant ($p = 0.270$, heterogeneity $p = 0.535$, fig. 2). Similar results were observed when the total cohort was subdivided into patients using either PEGV monotherapy or combination treatment and analyses were performed separately (PEGV

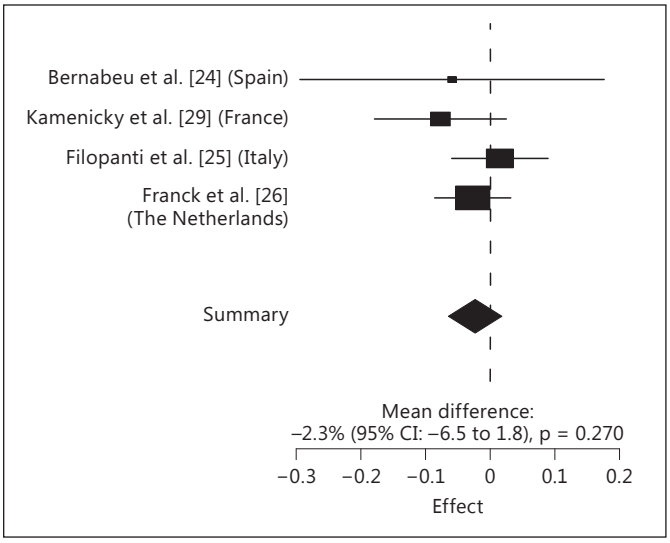

Fig. 2. Forest plot of meta-analysis: effect of d3-GHR on IGF-I during PEGV. The summary effect of d3-GHR genotype with respect to fl/fl-GHR on the lowest IGF-I level ULN during PEGV treatment in patients treated with PEGV alone or in combination with LA-SSA and/or CAB (n = 43) has a mean difference of –2.3% relative to the ULN of IGF-I (95% CI: –6.5 to 1.8, p = 0.270). GHR = Growth hormone receptor; fl = full-length; d3 = deletion of exon 3; IGF-I = insulin-like growth factor I; ULN = upper limit of normal; PEGV = pegvisomant; LA-SSA = long-acting somatostatin analogues; CAB = cabergoline; CI = confidence interval.

Table 2. Effect of d3-GHR on the lowest IGF-I level ULN during PEGV treatment

First author [Ref.], year	β of ULN	SE of β	p value	Heterogeneity p value
PEGV monotherapy				
Bernabeu [24], 2010	0.017	0.147	0.907	
Kamenicky [29], 2011	–0.052	0.060	0.393	
Filopanti [25], 2012	0.026	0.046	0.576	
Meta-analysis PEGV monotherapy	–0.002	0.035	0.961	0.582
Combination treatment				
Bernabeu [24], 2010	–0.193	0.309	0.547	
Kamenicky [29], 2011	–0.185	0.193	0.439	
Filopanti [25], 2012	0.025	0.062	0.684	
Franck [26], 2015	–0.027	0.030	0.371	
Meta-analysis combination treatment	–0.022	0.027	0.417	0.654
Total cohort				
Bernabeu [24], 2010	–0.059	0.120	0.626	
Kamenicky [29], 2011	–0.077	0.052	0.145	
Filopanti [25], 2012	0.015	0.038	0.702	
Franck [26], 2015	–0.027	0.030	0.371	
Meta-analysis total cohort	–0.023	0.021	0.270	0.535

Meta-analyses of the effect of d3-GHR versus fl-GHR on the lowest IGF-I level ULN during PEGV treatment in patients treated with PEGV alone, in combination with LA-SSA and/or CAB and in the total cohort (PEGV monotherapy + combination treatment). βs and SEs are corrected for sex, age at diagnosis and required PEGV dose. Effect indicates $p \leq 0.025$; no effect indicates $p \geq 0.025$. GHR = Growth hormone receptor; fl = full-length; d3 = deletion of exon 3; IGF-I = insulin-like growth factor I; SE = standard error; ULN = upper limit of normal; PEGV = pegvisomant; β = beta; SE = standard error.

monotherapy; mean difference: –0.2%, 95% CI: –0.071 to 0.068, p = 0.961 and combination treatment; mean difference: –2.2%, 95% CI: –0.074 to 0.031, p = 0.417; heterogeneity both not significant).

Required PEGV Dose to Achieve the Lowest IGF-I Levels

The effect of d3-GHR on the required PEGV dose needed for the lowest IGF-I level during treatment and the individual effects of the studies are summarized in table 3. The mean difference in required PEGV dose was 4.1 mg weekly (95% CI: –5.1 to 13.2), which suggests a small positive effect in d3-GHR carriers when compared with fl/fl-GHR in the total cohort from the 4 studies; however, this effect was not significant (p = 0.385, heterogeneity p = 0.535, fig. 3). Similar results were observed when the total cohort was subdivided in patients using PEGV monotherapy or in combination with LA-SSA and analyses were performed separately (PEGV monotherapy; mean difference: 7.9 mg weekly, 95% CI: –4.5 to 20.3, p = 0.210 and combination treatment; mean difference: 1.4 mg weekly, 95% CI: –11.5 to 14.2, p = 0.837; heterogeneity both not significant). In a multivariate linear regression model, the required PEGV dose was not different between the three genotypes with adjustment for sex, age and the different cohorts included in this meta-analysis (β = –0.5, SE = 5.2, p = 0.923). When the total cohort was subdivided in monotherapy PEGV or combination treatment with LA-SSA and separately analyzed for the required PEGV dose, the linear multivariate regression model showed similar results.

Discussion

The aim of this meta-analysis was to answer the question whether clinicians should take into account d3-GHR genotyping during PEGV prescription, as previous studies of its function are contradictory. Our results indicate that d3-GHR does not influence the pharmacodynamics of PEGV in acromegaly, at least not clinically relevant. No effect was observed concerning the response of PEGV

Table 3. Effect of d3-GHR on the required PEGV dose

First author [Ref.], year	β of ULN	SE of β	p value	Heterogeneity p value
PEGV monotherapy				
Bernabeu [24], 2010	21.880	15.523	0.171	
Kamenicky [29], 2011	–22.323	19.305	0.255	
Filopanti [25], 2012	9.213	7.424	0.219	
Meta-analysis PEGV monotherapy	7.930	6.327	0.210	0.193
Combination treatment				
Bernabeu [24], 2010	–73.166	26.187	0.017	
Kamenicky [29], 2011	–39.367	106.970	0.737	
Filopanti [25], 2012	3.061	10.123	0.763	
Franck [26], 2015	9.440	9.200	0.312	
Meta-analysis combination treatment	1.350	6.577	0.837	0.029
Total cohort				
Bernabeu [24], 2010	–10.624	15.288	0.419	
Kamenicky [29], 2011	–19.448	18.215	0.291	
Filopanti [25], 2012	6.696	6.127	0.277	
Franck [26], 2015	9.440	9.200	0.312	
Meta-analysis total cohort	4.060	4.675	0.385	0.374

Meta-analyses of the effect of d3-GHR versus fl-GHR on the required PEGV dose needed to achieve normalization of IGF-I levels in patients treated with PEGV alone, in combination with LA-SSA and/or CAB and in the total cohort (PEGV monotherapy + combination treatment). βs and SEs are corrected for sex and age. Effect indicates $p \leq 0.025$; no effect indicates $p \geq 0.025$. GHR = Growth hormone receptor; fl = full-length; d3 = deletion of exon 3; IGF-I = insulin-like growth factor I; SE = standard error; ULN = upper limit of normal; PEGV = pegvisomant; β = beta; SE = standard error.

treatment as the mean change in IGF-I levels was not significantly different between the GHR genotypes. Furthermore, the required PEGV dose to achieve normalization of IGF-I levels did not differ between carriers of the d3-GHR and the fl/fl-GHR genotypes.

The first two published studies reporting the effect of d3-GHR genotype on PEGV pharmacodynamics in acromegaly observed beneficial effects regarding d3-GHR carriers. An Italian study (n = 19) observed lower IGF-I levels in d3-GHR carriers compared to those with the fl/fl-GHR genotype after 3 and 6 months of PEGV [16]. However, after 12 months of PEGV treatment, this difference was lost, although the final PEGV dose was significantly lower in d3-GHR carriers compared to the fl/fl-GHR genotype. Similar results were observed in a Spanish cohort (n = 44); no difference was observed in genotype distribution between IGF-I controlled and noncontrolled patients, but the required PEGV dose per kilogram of weight to normalize IGF-I levels was 20% lower (p = 0.033) in patients with the d3-GHR polymorphism compared with the fl/fl-GHR genotype [24]. The previous 19 Italian patients were later included in a larger cohort (n = 127) in which the same research group reported that they could not confirm their previous results [25]. Similarly, we did not observe a difference between the two genotypes regarding IGF-I levels during PEGV treatment. Concerning PEGV dosing, we only observed a significant difference in a subset of the patients undergoing combination treatment (n = 15, table 3). This effect was not observed in patients receiving PEGV monotherapy (n = 29) or in the total cohort (monotherapy and combination treatment tested together). More recent studies, reporting larger cohorts (n = 49, n = 127, n = 104), were also unable to confirm the beneficial effects on PEGV pharmacodynamics in acromegaly patients carrying the d3-GHR polymorphism [25, 26, 29]. This phenomenon could be due to the presence of a publication bias in the beginning of this publication series about the effect of d3-GHR in acromegaly, the so-called 'winner's curse'.

This meta-analysis included 324 acromegalic patients; the d3-GHR polymorphism was observed in 161 (49.7%) of the patients, of which 122 (37.7%) were heterozygous

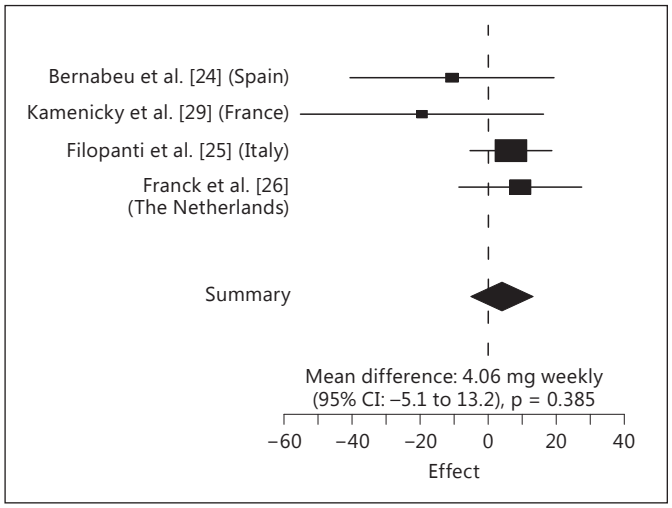

Fig. 3. Forest plot of meta-analysis: effect of d3-GHR on the required PEGV dose. The summary effect of d3-GHR genotype with respect to fl/fl-GHR on the required PEGV dose needed to achieve normalization of IGF-I levels in patients treated with PEGV alone or in combination with LA-SSA and/or CAB (n = 43) has a mean difference of 4.06 mg weekly (95% CI: −5.1 to 13.2, p = 0.385). GHR = Growth hormone receptor; fl = full-length; d3 = deletion of exon 3; IGF-I = insulin-like growth factor I; ULN = upper limit of normal; PEGV = pegvisomant; LA-SSA = long-acting somatostatin analogues; CAB = cabergoline; CI = confidence interval.

and 39 (12.0%) were homozygous. As previously mentioned, half of the general population is homozygous for the fl/fl-GHR; 30–40% is heterozygous for d3-GHR, and 10–20% is homozygous for this deletion [13–15]. A similar distribution of the d3-GHR polymorphism is reported in several acromegaly cohorts [16–20], including the total cohort in this meta-analysis, although the distribution was not in accordance with HWE. When HWE was tested in the individual cohorts, only the Dutch cohort was in HWE. An explanation for this deviation from HWE in the smaller cohorts may indicate a possible random genetic drift related to the small sample size. The smaller French cohort [18] (n = 49) has an increased prevalence of the d3/d3 genotype and a lower prevalence of the d3/fl genotype. When 3 d3/3 patients are shifted to the d3/fl genotype, the distribution is in HWE. Interestingly, when only these 3 French patients are shifted, the total cohort of 324 patients is also in accordance with the HWE. These 3 patients from a smaller cohort that could explain the disbalance in the HWE distribution are not expected to affect the results of this meta-analysis. In the larger cohort of Filopanti et al. [25], the deviation from HWE could be explained by a genotyping error; however, this seems to be unlikely as these data were obtained following the same methods and by the same laboratory analysts that found consistent distributions of the d3-GHR genotype following HWE in healthy individuals and several series of acromegaly patients [20], which was also additionally monitored and confirmed by an independent staff.

Since acromegaly is a rare disease and patients treated with PEGV are limited, we could only include a relatively small number of large studies in this meta-analysis. To date, however, this is the largest data set available for this specific group of acromegaly patients. Furthermore, GHR genotyping was done locally in several laboratories using multiplex and quantitative polymerase chain reaction techniques. Similarly, different assays were used to measure IGF-I levels, and, therefore, it was chosen to express the IGF-I level as the upper limit of normal. In addition, a comparison between fl/fl-GHR and d3-GHR regarding baseline GH and IGF-I levels in untreated acromegaly patients is missing, which would have given a more complete overview. Especially, as there are data reporting an association between d3-GHR and discordant GH and IGF-I levels (high IGF-I vs. normal GH) [22, 30], however conflicting data regarding this topic are also reported [31]. Despite these limitations, we were able to obtain all the available raw data from the studies derived from our systematic literature search on d3-GHR and PEGV treatment. Moreover, data were all collected using similar definitions, as response to PEGV treatment was objectified by the lowest IGF-I level during the PEGV treatment period, as was the associated required PEGV dose linked to these IGF-I levels. Furthermore, PEGV monotherapy and combination therapies showed similar results for both the response to PEGV treatment and the required PEGV dose. If d3-GHR carriers were to exhibit greater biological activity of GH, a dose effect for PEGV per haplotype is also to be expected. In this meta-analysis, we aimed to evaluate a dose effect regarding the required PEGV dose in a pairwise comparison between the three genotypes. No difference in PEGV dose was observed between the three genotypes either in the PEGV monotherapy or in the combination treatment group, making it highly unlikely that there is a clinically relevant effect of d3-GHR on the pharmacodynamics of PEGV in acromegaly. This outcome leaves room for discussion about the effect of the d3-GHR polymorphism in healthy individuals. d3-GHR carriers present in the general population should either maintain normal GH activity despite less circulating GH, and, therefore, have similar GH end-targets such as metabolic state, body composition and final height or exhibit an increase in these GH end-targets. In this respect, it

is interesting to note that genome-wide association studies on final height [32, 33] and metabolic state [34] did not report any association with the GHR locus in healthy individuals, corroborating our findings. In summary, we believe that our study demonstrates that the presence of the d3-GHR genotype in acromegaly patients has no impact on clinical practice. Moreover, we are convinced that this meta-analysis provides us a final conclusion regarding the d3-GHR polymorphism and its lack of effect on PEGV response and dosing in acromegaly.

Conclusion

In our meta-analysis of a combined group of 324 acromegaly patients obtained from 4 separate study cohorts, the presence of 1 or 2 copies of the d3-GHR polymorphism had no significant effect on the lowest IGF-I levels during PEGV treatment nor on the required PEGV dose to achieve these levels. Similarly, no difference between subgroups of subjects that used PEGV either as monotherapy or in combination with LA-SSA was observed. Our results indicate that there is no evidence supporting a role for the d3-GHR polymorphism in either predicting responses to PEGV therapy or determining PEGV dosing during treatment of acromegaly.

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