Pegvisomant in combination with long-acting somatostatin analogues in acromegaly: the role of the GH receptor deletion of exon 3

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Abstract

Background: Doses of the GH receptor (GHR) antagonist pegvisomant (PEGV) that normalize insulin-like growth factor 1 (IGF1) levels vary widely among acromegaly patients. Predictors for PEGV response are baseline IGF1 levels, sex, body weight and previous radiotherapy. A GHR polymorphism lacking exon 3 (d3-GHR) is frequent in the general population. The influence of d3-GHR on PEGV responsiveness in acromegaly is unclear.

Objective: To assess the influence of d3-GHR on IGF1 levels and PEGV responsiveness in acromegaly patients using combined PEGV and long-acting somatostatin receptor ligand (LA-SRIF) treatment.

Design: Data were collected at the Rotterdam Pituitary Centre between 2004 and 2013. Patients with elevated IGF1 levels (>1.2 upper limit of normal; n = 112) and over 6 months of high-dose LA-SRIF treatment were co-treated with PEGV. GHR genotype was assessed using genomic DNA in 104 patients.

Results: D3-GHR was observed in 51 (49.0%) of the patients (7.7% homozygous, 41.3% heterozygous) and was in Hardy–Weinberg equilibrium (P = 0.859). Baseline characteristics were similar in d3-GHR and full-length (fl)-GHR genotypes. During PEGV/LA-SRIF treatment IGF1 levels were not different between d3-carriers and non-carriers. Similarly, no difference in PEGV dose required to normalize IGF1 (P = 0.337) or PEGV serum levels (P = 0.433) was observed between the two groups. However, adenoma size decreased significantly (>20% of largest diameter) in 25.6% of the fl-GHR genotype but only in 7.5% of d3-carriers (P = 0.034, OR: 4.6 (CI: 1.1–18.9)).

Conclusions: GHR genotype does not predict the IGF1 normalizing dose of PEGV in acromegaly patients using combination PEGV/LA-SRIF treatment. However, fewer d3-carriers showed significant reductions in adenoma size.

Introduction

Disease activity and phenotype is diverse among patients with acromegaly. Comorbidities such as hypertension, cardiomyopathy, type 2 diabetes, sleep apnea and osteoarthritis are influenced by the severity and the duration of growth hormone (GH) hypersecretion (1). Pegvisomant (PEGV) is a competitive GH receptor (GHR) antagonist that is used in the treatment of acromegaly (2). The required dose of PEGV to achieve disease control as assessed by the normalization of insulin-like growth factor 1 (IGF1) levels differs significantly between individual patients (2). Baseline IGF1 appears to be a predictor for the required dose (3). Other factors known to influence the required dose are sex, body weight and previous radiotherapy (4). However, GHR polymorphisms seem to have an influence as well (5, 6).
A polymorphism of the GHR that lacks exon 3 (d3-GHR) during splicing is common in the general population. About half of the population is homozygous for the full-length GHR (fl-GHR), 30–40% is heterozygous for d3-GHR and 10–20% is homozygous for this deletion (7, 8, 9). It has been reported that the d3-GHR polymorphism shows a comparable distribution between different cohorts of acromegalic patients (6, 10, 11, 12). Multiple studies show that the fl-GHR and d3-GHR have comparable binding properties, and that internalization of fl-GHR is as effective as d3-GHR (13, 14, 15). However, Dos Santos et al. (8) showed in transfection experiments that the lack of exon 3 results in an enhanced signal transduction by the STAT-5-dependent pathway, which increases the expression of IGF1 and other GH-dependent genes. Scientific attention on d3-GHR was in the beginning focused on the outcome of recombinant GH replacement therapy in GH-deficient children and later in adults, in which the studies often show different conclusions (16, 17, 18). Thereafter research was focused on the severity of acromegaly regarding the GHR-genotype.

Previous research showed that d3-GHR carriers with acromegaly have a more severe clinical and biochemical phenotype; however, inconsistent results have also been reported. For example, Wassenaar et al. (19) reported an increased prevalence of osteoarthritis, dolichocolon and adenomatous colonic polyps in d3-GHR carriers with acromegaly, but no difference in cardiovascular risk and bone mineral density. Mercado et al. (11) observed that diabetes mellitus was more prevalent in patients with the d3-GHR genotype apart, whereas several other phenotypic features were independent of GHR genotype. The authors also observed a significantly higher serum IGF1 concentration after treatment (surgery, radiotherapy and/or pharmacological therapy) in d3-GHR carriers (11). Cinar et al. (20) reported that d3-GHR genotype did not have an effect on clinical features nor on comorbidities in acromegaly patients.

A meta-analysis on GH-deficiency concluded that the presence of d3-GHR increases the response to recombinant GH treatment in GH-deficient children (21). This pharmaco-genetic phenomenon could be important for PEGV treatment in acromegaly. In theory, carriers of d3-GHR might need less PEGV than non-carriers to reach a comparable decrease in IGF1 levels. Indeed, two studies reported that the required PEGV dose for normalization of IGF1 levels was significantly lower in acromegaly patients with a d3-GHR genotype (5, 6). A later study, however, did not observe a better response of d3-GHR carriers during monotherapy PEGV nor during combination treatment with PEGV and somatostatin analogues (10). These inconsistent findings indicate that larger cohorts of acromegaly patients are needed to investigate whether the response to PEGV differs between d3-GHR and fl-GHR genotypes. We therefore examined whether there were differences in the clinical and biochemical responses during PEGV treatment between both genotypes in our cohort of 104 patients using somatostatin analogues combined with PEGV.

**Table 1** Patient characteristics of fl-GHR and d3-GHR genotypes.

<table>
<thead>
<tr>
<th></th>
<th>fl-GHR</th>
<th>All d3-carriers</th>
<th>P valuea</th>
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<tbody>
<tr>
<td>n (%)</td>
<td>53 (51.0)</td>
<td>51 (49.0)</td>
<td>0.432</td>
</tr>
<tr>
<td>Males (%)</td>
<td>54.7</td>
<td>62.7</td>
<td>0.995</td>
</tr>
<tr>
<td>Age at diagnosis (years)</td>
<td>43.0 (35.4–52.7)</td>
<td>46.8 (36.8–56.2)</td>
<td>0.614</td>
</tr>
<tr>
<td>Time between diagnosis and start of PEGV (years)</td>
<td>1.4 (0.9–3.5)</td>
<td>1.6 (0.9–3.5)</td>
<td>0.099</td>
</tr>
<tr>
<td>Age at start PEGV (years)</td>
<td>46.8 (37.2–59.2)</td>
<td>48.9 (40.1–59.2)</td>
<td>0.309</td>
</tr>
<tr>
<td>Years of PEGV treatment</td>
<td>4.2 (2.0–7.4)</td>
<td>5.2 (3.2–7.2)</td>
<td>0.181</td>
</tr>
<tr>
<td>Tumor volume (Macro %)</td>
<td>79.2</td>
<td>84.3</td>
<td>0.614</td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
<td>30.2</td>
<td>43.1</td>
<td>0.222</td>
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<tr>
<td>Previous therapy (%)</td>
<td>43.4</td>
<td>41.2</td>
<td>0.845</td>
</tr>
<tr>
<td>Surgery</td>
<td>41.2</td>
<td>42.3</td>
<td>0.751</td>
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<tr>
<td>Radiotherapy</td>
<td>17.6</td>
<td>12.5</td>
<td>0.614</td>
</tr>
<tr>
<td>GH at start of PEGV (µg/l)</td>
<td>4.5 (2.5–10.2)</td>
<td>3.5 (2.2–7.3)</td>
<td>0.206</td>
</tr>
<tr>
<td>IGF1 at start of PEGV (nmol/l)</td>
<td>68.8 (46.1–94.0)</td>
<td>62.6 (48.9–78.3)</td>
<td>0.558</td>
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<tr>
<td>IGF1 × ULN at start of PEGV</td>
<td>1.77 (1.52–2.31)</td>
<td>2.03 (1.47–2.72)</td>
<td>0.521</td>
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</tbody>
</table>

Expressed as median (interquartile range). ULN, upper limit of normal; PEGV, pegvisomant; IGF1, insulin-like growth factor 1.

*afl/fl genotype vs d3 genotype (fl/d3 and d3/d3).*
Methods

Patients

Data of acromegaly patients were collected at our Rotterdam Pituitary Centre between 2004 and 2013. Inclusion criteria were i) elevated serum IGF1 levels (>1.2×upper limit of normal (ULN)) after at least 6 months on the highest dose of LA-SRIFs (Sandostatin LAR 30 mg or Lanreotide Autogel 120 mg every 28 days) and ii) genomic DNA could be obtained (n=104). After the initial start with monotherapy of LA-SRIF, co-treatment with PEGV was added by weekly injections. For starting doses of PEGV and the protocol of PEGV dose titration to achieve normal IGF1 levels, see Neggers et al. (22). All patients gave informed consent. The study was approved by the local Institutional review board.

Hormone assays

Serum levels of IGF1 and GH were measured with the Immulite 2000 assay (DPC Biermann GmbH/Siemens, Fernwald, Germany), a solid-phase, enzyme-labeled chemiluminescent immunometric assay, with an intra-assay variability of 2–5%, and an intra-assay variability of 3–7%. The IGF1 age-adjusted reference ranges were used in accordance with earlier reports (23). PEGV serum levels were assessed in Aarhus, as described previously (24).

Assessments of side effects included serum concentrations of alanine aminotransferase (ALT), aspartate aminotransaminase (AST), alkaline phosphatase, γ-glutamyltranspeptidase and total bilirubin. Magnetic resonance imaging (MRI) was used to assess changes in pituitary tumor volume at least every 2 years. Changes in tumor size were assessed by a single radiologist who was blinded for patient characteristics and treatment regimens. A ‘significant decrease’ was defined as a reduction of more than 20% of the largest diameter of the tumor during combination treatment compared with the largest diameter of the last MRI before the addition of PEGV.

Q-PCR of GHR deletion of exon 3

The exon-3 deleted GHR polymorphism (d3-GHR) could be assessed in 104 patients. Genomic DNA was extracted from peripheral blood leukocytes by standard procedures. Analysis of the d3-GHR polymorphism was carried out using quantitative PCR (Q-PCR) as previously described (5). Briefly, primer/probe sets binding to exons 3 and 10 of Table 2

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Clinical and biochemical response during treatment of fl-GHR and d3-GHR genotypes.</th>
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<tbody>
<tr>
<td></td>
<td>All patients</td>
</tr>
<tr>
<td>n (%)</td>
<td>104</td>
</tr>
<tr>
<td>Normalization of IGF1 &lt;1.0×ULN (mol/l)</td>
<td>97.1</td>
</tr>
<tr>
<td>Lowest IGF1 during treatment (mol/l)</td>
<td>18.5 (14.5–23.5)</td>
</tr>
<tr>
<td>Lowest IGF1 × ULN during treatment</td>
<td>0.57 (0.43–0.75)</td>
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<tr>
<td>Decrease IGF1 (%)</td>
<td>69.5 (59.6–78.3)</td>
</tr>
<tr>
<td>PEGV dose (mg weekly)</td>
<td>80.0 (60.0–120.0)</td>
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<tr>
<td>PEGV dose (mg/kg weekly)</td>
<td>0.90 (0.66–1.28)</td>
</tr>
<tr>
<td>PEGV serum level (µg/l)</td>
<td>4625 (2975–11 962)</td>
</tr>
<tr>
<td>Ratio PEGV (serum/dose)</td>
<td>63.0 (38.5–11962)</td>
</tr>
<tr>
<td>Transient elevated transaminases (%)</td>
<td>16.3</td>
</tr>
<tr>
<td>Decrease of tumor size during treatment (%)</td>
<td>16.5</td>
</tr>
</tbody>
</table>

Expressed as median (interquartile range). ULN, upper limit of normal; PEGV, pegvisomant; IGF1, insulin-like growth factor 1.
*fl/fl genotype vs d3 genotype (fl/d3 and d3/d3).
**Dichotomous variable based on the lowest IGF1 during treatment period.
†Difference between IGF1 before the start of PEGV and lowest IGF1 during combination treatment.
*Required PEGV dose to achieve normalization of the IGF1 level.
*More than 20% volume reduction.
*Patients with an empty sella were not included in the analyses, as decrease and increase are not applicable.
*Tested by logistic regression, correction for radiotherapy did not influence the significance.
*Tested by Fisher’s exact test.
the human GHR gene were added to the DNA samples. GHR exon 10 served as an internal positive control. Q-PCR was performed in triplicate 384-wells plates with 20 ng genomic DNA in a volume of 5 μl using 1× Taqman Universal PCR Master Mix (Life Technologies), 3 μM GHR exon 3 primers/probe and 9 μM GHR exon 10 primers/probe. Amplification was performed using a real-time TaqMan 7900 HT instrument (Applied Biosystems) with the following cycle conditions: 50 °C for 2 min, 95 °C for 10 min followed by 40 cycles of 95 °C for 15 s, and 60 °C for 1 min. Differences in cycle threshold (ΔCt) between exon 3 and exon 10 amplicons were used to determine the exon 3 copy number for each sample. A ΔCt value of 1 indicates two exon 3 copies (genotype fl/fl), a ΔCt value of 2 indicates one exon 3 copy (genotype fl/d3) and no signal for exon 3 in the presence of a normal exon 10 signal indicates an absent exon 3 (genotype d3/d3). To validate the genotyping accuracy, 25 samples were randomly selected to determine the GHR exon 3 polymorphism for a second time using a multiplex PCR assay (7). No discrepancies were found between the genotypes obtained by either method.

Statistical analysis

Data are expressed as medians (interquartile range (IQR)) unless otherwise specified. Nominal variables were analyzed using the χ² test. Differences between two or more independent subgroups were analyzed using the Mann–Whitney U test or the Kruskal–Wallis ANOVA respectively. Hardy–Weinberg equilibrium was analyzed with the χ² test via the observed and expected genotype frequencies. Results of correlation analyses are expressed as Spearman’s rank correlation coefficient. P values <0.05 (two-tailed) are considered statistically significant. Statistical analyses were performed with SPSS version 20 and GraphPad Prism version 6 for Windows (SPSS software, Chicago, IL, USA and GraphPad Software, San Diego, CA, USA).

Results

Patient characteristics

Characteristics of the 104 patients are presented in Table 1. The median age of all patients at time of diagnosis was 45.4 years and the majority of the patients were male. The median time between the diagnosis and the start of PEGV was 1.4 years. The median duration of PEGV treatment was 5 years. The majority of the patients had a macro adenoma. Diabetes mellitus was present in one-third of subjects. Previous therapies were surgery (42.3%) and radiotherapy (12.5%). Before the start of PEGV in combination with LA-SRIFs, the median absolute IGF1 level was 63.8 nmol/l or 1.81 (expressed as times the ULN of IGF1). The median GH level at the start of PEGV was 4.0 μg/l. For 18 patients, GH levels were missing at the start of PEGV.

Figure 1
Decrease of IGF1 during PEGV treatment. Expressed as median. Decrease of IGF1 level: before PEGV and the lowest IGF1 during PEGV treatment. *fl/fl genotype vs d3 genotype (fl/d3 and d3/d3).

Figure 2
Required PEGV dose for normalization of IGF1. Expressed as median (interquartile range). Required PEGV dose to achieve normalization of IGF1 level. *fl/fl genotype vs d3 genotype (fl/d3 and d3/d3).
Patient characteristics and GHR genotype

The d3-GHR polymorphism assessed in 104 (92.9%) of the 112 patients. The eight patients from whom genomic DNA was not retrieved did not exhibit phenotypical features different from the genotyped patients (data not shown).

d3-GHR was observed in 51 (49.0%) of the patients, of which 7.7% were homozygous and 41.3% were heterozygous. This distribution of the d3-GHR genotype followed the Hardy–Weinberg equilibrium ($P = 0.859$).

Patient characteristics for both groups are depicted in Table 1. No statistically significant differences were present at baseline between the GHR genotypes regarding age at diagnosis and at start of PEGV, years of treatment, sex, tumor volume assessed as macro vs micro adenomas, presence of diabetes mellitus, kind of previous therapy, and GH and IGF1 levels before the start of PEGV.

Clinical and biochemical response during treatment

IGF1 levels ▶ Normalization of IGF1 corrected for age, defined as a dichotomous variable based on the lowest IGF1 during treatment, was observed in almost all fl-GHR patients and d3-GHR carriers and was not significantly different between the groups ($P = 0.587$, Table 2). The lowest median absolute IGF1 level during treatment (18.0 nmol/l) in the fl-GHR genotype was not significantly different from that in the d3-GHR genotype (18.4 nmol/l, $P = 0.592$). Furthermore, IGF1×ULN and a decrease of IGF1 during treatment were not different between d3-carriers and non-carriers ($P = 0.780$ and 0.728 respectively, Fig. 1).

PEGV-dose and PEGV serum level ▶ The median required PEGV dose to achieve the lowest IGF1 level was 80.0 mg weekly (60.0–110.0) for non-carriers and 80.0 mg weekly (60.0–140.0) for the d3-GHR genotype. No significant difference between the two groups was found for the PEGV dose ($P = 0.337$), as presented in Fig. 2. Correlation analysis showed a positive correlation between the decrease in IGF1 level and the PEGV dose ($\rho = 0.211, P = 0.030$), Fig. 3. The median required PEGV dose between men and women was not significantly different ($P = 0.650$). Weight correlated positively with the PEGV dose ($\rho = 0.265, P = 0.007$). The PEGV dose in weekly mg per kg was not significantly different between fl-GHR and d3-GHR ($P = 0.655$).

The PEGV serum levels ($n=83$, genotyped cohort), measured during the end of the inclusion period of this study, were also not significantly different between the two genotypes ($P = 0.433$), as shown in Fig. 4. Correlation analysis showed a positive correlation between the decrease of IGF1 and the PEGV serum level ($\rho = 0.225, P = 0.041$, Fig. 5). The median PEGV
serum levels were not different between men and women ($n=86$, $P=0.870$). Weight was not correlated to the PEGV serum level ($n=79$, $r=0.027$, $P=0.814$). The ratio of PEGV serum levels over the PEGV dose was not significantly different between the d3-GHR carriers and non-carriers ($n=83$, $P=0.293$).

Samples of 29 patients (25.9%) were not available for the assessment of PEGV concentrations. In 7.8% of the samples, a PEGV serum level of 0 $\mu$g/l was measured during PEGV treatment and was left out of the analysis.

**Tumor shrinkage and liver function tests**

A significant reduction of the adenoma size was observed in 25.6% of the fl-GHR genotype and in 7.5% of the d3-carriers, which is a significant difference between the groups ($P=0.034$, OR: 4.6 (CI: 1.1–18.9) tested by logistic regression). When tested by Fisher’s exact test, $P$ value was 0.036. Of the d3-carriers, 17.6% had transiently elevated transaminases (TET), the most common side effect of the combination treatment with PEGV and LA-SRIF, compared to 15.1% of patients with the fl-GHR genotype ($P=0.725$).

**Discussion**

The results of our study did not show a significant difference in patient characteristics or treatment response to PEGV between fl-GHR and d3-GHR genotypes in patients with acromegaly. Carriage of d3-GHR did not affect the PEGV dose nor the PEGV serum levels during treatment. However, a significantly larger reduction in tumor volume during treatment was observed in patients with the fl-GHR genotype compared to d3-carriers ($P=0.034$).

Transfection studies have shown that the lack of exon 3 in the GHR enhances GH signal transduction (8) and there are clinical data to suggest that this polymorphism confers a better response to GH replacement therapy and also impacts on patients with acromegaly (5, 6, 25, 26). The group of acromegaly patients in our study is suitable to further analyze the clinical relevance of the d3-GHR genotype for two reasons. First, acromegaly patients using LA-SRIFs and PEGV have a more severe disease activity, as LA-SRIF monotherapy was not effective enough to normalize IGF1. Secondly, d3-GHR carriers, having a higher GHR signal transduction, are considered to respond better to PEGV compared to patients with the fl-GHR. Therefore, the d3-GHR carriers can be hypothesized to need a lower dose of PEGV to achieve disease control. Indeed, studies during PEGV monotherapy by Bianchi et al. (6) ($n=19$) and Bernabeu et al. (5) ($n=44$) revealed that the required PEGV dose to normalize IGF1 levels was significantly lower in acromegaly patients with d3-GHR genotype. However, Filopanti et al. (10) studied two groups of acromegaly patients using monotherapy PEGV ($n=64$) and LA-SRIFs in combination with PEGV ($n=63$) and could not confirm the superior treatment response of d3-GHR carriers in either group. It could be argued that the sample sizes of these two cohorts in the last negative study were too small to observe an effect. However, in our current study, with a reasonable sample size, an effect of the d3-GHR was also not observed.

In our cohort of acromegaly patients, the d3-GHR polymorphism was observed in 49% of the patients and followed the Hardy–Weinberg equilibrium. This demonstrates the absence of a selection benefit in our acromegaly cohort using combination therapy. Acromegaly cohorts in the studies of Bernabeu et al. and Filopanti et al. did not follow the Hardy–Weinberg equilibrium. The latter study suggested an association between d3GHR genotype and a more severe phenotype of acromegaly patients.

In our study, we measured PEGV serum levels to investigate whether a discrepancy might exist between the PEGV dose and the serum levels of PEGV between the genotypes. For both PEGV serum levels and PEGV doses,
we could not detect a significant difference between the two genotypes. PEGV serum levels of 0 µg/l (n=5) measured during PEGV treatment were left out of the analysis. These undetectable PEGV serum levels could be explained by a false negative error of the assay, non-compliance of the patient in taking the drug or the absence of PEGV due to the half-life of the drug (T1/2el = 74–172 h (27)). Although the half-life of the drug is probably increased during combination treatment, since PEGV serum levels increase by 20% (24, 28), in our opinion it is the loss of PEGV from the circulation that is the most likely explanation for the undetectable PEGV serum levels. The majority of patients with undetectable PEGV serum levels were using a low PEGV dose, between 10 and 60 mg once weekly.

Apart from differences in sample sizes, the discrepancies between our data and those previously published could be due to an IGF1-independent pathway. Binder et al. (25, 26) published two studies on the use of recombinant human GH (rhGH) in children short for gestational age with remarkable outcomes. They observed a higher growth velocity in d3-GHR carriers during rhGH therapy, although this was not reflected by increases in IGF1 levels, and may allow cautious speculation about an IGF1 independent pathway. If this perspective held true, our study and previous research may not have been suitable to assess the influence of d3-GHR genotype on IGF1 levels during PEGV treatment in acromegaly patients. We could not find a significant difference in baseline characteristics, clinical or biochemical response between fl-GHR and d3-GHR carriers. However, we did observe a difference between the decrease in adenoma volume during treatment between patients with fl-GHR genotype and the d3-carriers. An explanation for the higher decrease in the fl-GHR group is not readily available as PEGV dose and PEGV serum levels were not significantly different between carriers and non-carriers. Moreover, Veldhuis et al. (29) observed that PEGV does not cross the human blood-brain barrier. However, many areas of the hypothalamus and the pituitary lack a blood-brain barrier (30, 31). A direct effect of PEGV on the difference between fl-GHR and d3-GHR genotype in tumor shrinkage is thus difficult to estimate. LA-SRIFs are more likely to have influence on the different rates of tumor shrinkage between the two genotypes. A meta-analysis by Giustina et al. (32) reported that LA-SRIFs induced relevant tumor shrinkage in more than half of the acromegaly patients studied. Predictors of tumor shrinkage by LA-SRIFs are age, decrease of GH and IGF1 levels, treatment-naive patients and duration of LA-SRIF-treatment (32, 33, 34). Age at diagnosis, age at start of PEGV, decrease of IGF1 levels during treatment and previous therapy (such as neurosurgery and radiotherapy) were not significantly different in our study between fl-GHR and d3-GHR genotype. The other studies about d3-GHR and PEGV treatment (Bianchi, Bernabeu and Filopanti) did not describe tumor shrinkage (5, 6, 10). Bernabeu et al. did report two cases of tumor increase during PEGV monotherapy, but genotypes were not specified. Filopanti et al. reported four cases of tumor increase in the PEGV monotherapy group, of which one patient had the fl-GHR genotype and three patients were carriers of d3-GHR. In a single-center study in Mexico City (11), the authors observed more severe disease activity with lower efficacy of treatment (RTx, surgery and/or pharmacological therapy) in acromegaly subjects with the d3-GHR genotype. Therefore, it might be expected that carriers of the d3-GHR have less tumor regression, since biochemical response and tumor regression seem to be linked.

Conclusion

The clinical data in our study do not support a role for GHR genotype in the treatment response to PEGV combined with long-acting somatostatin analogues in patients with acromegaly. Our observation that the reduction in pituitary tumor volume during combination therapy was smaller in d3-carriers was unexpected and merits further attention.

Declaration of interest

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