GH receptor isoforms and skeletal fragility in acromegaly

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Abstract

Objective: Acromegaly is associated with an increased prevalence of vertebral fractures (VFs) in close relationship with GH hypersecretion. Two isoforms of the GH receptor (GHR) have been identified; the two isoforms differ or not by the expression of the protein fragment encoded by exon 3 of the *GHR* gene. Deletion of the exon 3 may influence the functional properties of the GHR and affect fracture risk in acromegalic patients.

Design: A cross-sectional study was designed to investigate the association between the d3-GHR isoform and the prevalence of VFs in patients with acromegaly.

Methods: In this study, 109 acromegalic patients were included (M/F, 48/61): 73 with controlled/cured acromegaly and 36 with active disease. *GHR* genotype was assessed in each patient. All patients were evaluated for VFs and bone mineral density at lumbar spine and hip. Serum IGF1 levels and bone metabolism markers were measured. A multivariate analysis was performed to establish risk factors for VFs in our population.

Results: d3-GHR carriers showed an increased prevalence of VFs when compared with patients expressing full-length *GHR* (35/55 vs 12/54; $P < 0.001$). The association between *GHR* deletion and VFs was demonstrated both in patients with active disease and in those with controlled/cured disease. Out of 35 patients who were prospectively evaluated, 13 (37.1%) developed incident VFs. The incidence of VFs was significantly higher in patients for whom the *GHR* gene has been deleted when compared with those harboring the *fl* gene ($P = 0.04$). In multivariate analysis, male sex (odds ratio (OR), 3.250; $P = 0.041$), IGF1 levels (OR, 1.183; $P = 0.031$), length of active diseases (OR, 1.038; $P = 0.001$), and d3-GHR genotype (OR, 3.060; $P = 0.015$) were all confirmed as risk factors of VFs in our population.

Conclusions: This study suggests for the first time that exon 3 deletion of *GHR* may predispose patients with active and controlled acromegaly to a higher risk of VFs.

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Introduction

Growth hormone (GH) and insulin-like growth factor 1 (IGF1) are important regulators of the bone remodeling process. Although GH may act directly on skeletal cells, most of its effects are mediated by IGF1 that exerts its action as a systemic hormone synthesized in the liver under GH stimulation or as a local growth factor synthesized in peripheral tissues (1). Circulating GH binds to a single-chain transmembrane glycoprotein

receptor (GH receptor (GHR)). The GHR in its final form consists of an extracellular, a transmembrane, and an intracellular domain, whereas the *GHR* gene consists of nine exons encoding the receptor protein and several additional untranslated exons (2). To date, two isoforms of human GHR have been identified: a full-length (fl-GHR) isoform retaining the protein fragment encoded by exon 3 and an exon 3-deficient (d3-GHR) isoform excluding this

fragment (3). Exon 3 encodes a segment in the extracellular domain of the receptor; although both the isoforms retain the capacity of binding GH with a high affinity, there may well be significantly functional differences between the two isoforms (4, 5). In fact, GH-deficient patients expressing d3-GHR isoform are known to be more responsive to GH replacement therapy than fl-GHR patients (5, 6, 7). Acromegalic patients with exon 3 being deleted in the *GHR* gene consistently express higher serum IGF1 levels even after treatment with somatostatin analogs or pegvisomant (8, 9, 10, 11). Moreover, exon 3 deletion in acromegalic patients has been associated with a higher incidence of long-term complications of the disease, such as diabetes, osteoarthritis, and colon polyps (12, 13).

Over the recent past years, an expanding body of knowledge is supporting the idea that patients with acromegaly suffer from skeletal fragility and are at an increased risk for vertebral fractures (VFs). Fracture risk correlates with the activity of the disease and the presence of concomitant additional risk factors, such as hypogonadism (14, 15, 16). Nonetheless, it is still largely unclear as to whether *GHR* polymorphisms are implicated in this phenomenon and have an impact on skeletal fragility in acromegaly. Recently, Wassenaar *et al.* (13) did not find any significant association between the deletion of *GHR* exon 3 and VFs. However, the authors restricted their analysis to patients with longstanding controlled acromegaly. In our opinion, active acromegaly may well provide a better model to study the potential impact of *GHR* polymorphisms on skeletal fragility. Therefore, the aim of our study is to investigate the effects of exon 3 deletion of *GHR* on bone metabolism, bone mineral density (BMD), and incidence of VFs in patients with active and controlled/cured acromegaly.

Subjects and methods

Subjects

A cross-sectional study was designed and a total of 109 patients consecutively treated at our Institution (Pituitary Unit of Endocrinology Department, Gemelli Hospital, Rome) were enrolled. All patients were diagnosed with acromegaly or had a previous diagnosis of acromegaly. Exclusion criteria from the study were: i) diagnosis of active neoplastic disease; ii) ongoing treatment with anti-osteoporotic drugs with the exception of calcium and vitamin D supplements; iii) treatment with drugs causing osteoporosis with the exception of glucocorticoid

replacement therapy in hypopituitary patients; iv) clear history of moderate or high-energy VFs; v) history of surgical intervention on the spine; and vi) prolonged immobilization (more than 6 weeks). Out of the 109 enrolled patients, 84 had been already evaluated in previously published studies from our research group (14, 15, 16). Both surgically treated and medically treated patients were included.

Active acromegaly was defined as the failure of suppression of GH plasma levels ≤ 1 ng/ml after a 75 g oral glucose load and fasting plasma IGF1 levels above the normal ranges for sex and age (17). Patients undergoing somatostatin analog treatment were evaluated by measurement of serum random GH and IGF1, and those undergoing pegvisomant were evaluated by serum IGF1 alone, whereas patients treated by neurosurgery alone were evaluated also by serum GH after a 75 g oral glucose load, according to the current guidelines (18). Acromegaly was defined as controlled if the IGF1 values were within the reference ranges for age and, in patients undergoing treatment with somatostatin analogs and after neurosurgery, random GH was below 1.0 ng/ml. When the 75 g oral glucose load was performed, the GH values of 0.4 ng/ml or below were considered as expression of cured disease. Disease activity was defined as 'cured' if, after a previous treatment for acromegaly, patients had normal biochemical parameters at follow-up with no ongoing medical therapy. Patients' gonadal status was also assessed: hypogonadism was defined as absence of or irregular menstrual cycles in women and low total testosterone associated with symptoms and signs of sexual dysfunction in men. Hypogonadic patients undergoing replacement therapy with sex steroids were assumed to be eugonadal only if treatment was started at least 12 months before study entry. All patients signed an informed consent form after full explanation of the purpose and nature of all procedures used. The study was also approved by our Institutional Ethical Committee.

Biochemical measurements

All biochemical measurements were performed within 6 months of lumbar and femoral neck dual energy X-ray absorptiometry (DXA) scan and vertebral X-ray assessment. Blood samples were collected in fasting conditions. GH and IGF1 levels were measured using the Immulite 2000 immunoassay system (Siemens, Diagnostic Product Corp., Los Angeles, CA, USA). The inter-assay coefficient of variation was 5.5–6.2% for GH assay, and 6.4–11.5% for IGF1 assay; detection lower limits were 0.01 and 0.2 μ g/l

respectively. IGF1 levels were reported as absolute concentrations and SDS relative to normal age-adjusted adult values (normal range from -2 to $+2$ SDS). The formula used for SDS calculation is, as reported previously, $((IGF1_p - IGF1_{adj-value}) / IGF1_{s.d.})$, where $IGF1_p$ is the absolute level of IGF1 of the patient, and $IGF1_{adj-value}$ and $IGF1_{s.d.}$ are the average and the s.d. of the age-matched IGF1 levels in healthy patients respectively (19). Serum 25-hydroxy vitamin D was measured using a chemiluminescent assay (Liaison Instrument, DiaSorin Inc, Stillwater, MN, USA), reference intervals for the study were <10 ng/ml for severe deficiency, 10 – 30 ng/ml for mild to moderate deficiency, and 31 – 100 ng/ml for normal status. Serum parathyroid hormone levels were also measured using an immunoenzymatic method (reference interval, 10 – 65 pg/ml), osteocalcin by the ECLIA method (reference interval, 15 – 45 ng/ml), and β -crosslaps (β -CTX) by a chemiluminescent immunoassay (reference interval, 0.2 – 1.0 ng/ml).

Measurement of BMD and quantitative morphometrical assessment of VFs

BMD of femoral neck and lumbar spine was measured by DXA (Lunar Prodigy 8743, GE Medical System, Madison WI, USA). Measurements were made at the time of the spinal X-ray. BMD is expressed as absolute values and Z-scores using a standard Caucasian European reference population. Fractured vertebrae, defined as described below, were excluded from the lumbar BMD analysis. A quantitative morphometrical assessment of VFs in the T4–L4 region was performed using a dedicated morphometrical software (Spine-X Analyzer, ICAM Diagnostics, Milan, Italy) (20). Briefly, using a translucent digitizer and a cursor, six points were marked on the borders of each vertebral body. Anterior (Ha), middle (Hm), and posterior (Hp) vertebral heights were measured and height ratios were calculated for each vertebra from T4 to L4; fractures were defined as mild, moderate, or severe based on a height ratio decrease of 20 – 25 , 25 – 40 , and more than 40% respectively (21). All VFs identified by the morphometrical scan were independently and blindly reviewed by two experienced physicians (M Mormando and LA Nasto). Moreover, a spinal deformity index (SDI) was calculated for each patient. Each VF was assigned a score according to the fracture severity (one for mild, two for moderate, and three for severe); SDI was defined as the sum of all scores in a single patient (22).

After 3 years of follow-up, 35 patients without pre-existing VFs were evaluated for incident VFs. Incident VFs were defined as a decrease of 20% or more and at least

4 mm in length in any of the three vertebral heights (Ha, Hm, or Hp) compared with baseline radiograph (16).

Genetic testing

DNA was extracted from 100 – 200 μ l peripheral blood using the Illustra blood genomicPrep Mini Spin Kit (GE Healthcare, Little Chalfont, UK) for the detection of the *GHR* gene polymorphism. Polymorphisms were studied through standard PCR amplification by Eppendorf Master Mix ($2.5\times$) (Eppendorf, Hamburg, Germany). The following primers were used for amplification, $5'$ -TGTGCTGGTCTGTTGGTCTG- $3'$, $5'$ -AGTCGGTTCC-TGGGACGAGA- $3'$, and $5'$ -CCTGGATTAACACTTTGCA-GACTC- $3'$ (23). The reaction was carried out with one cycle at 94 $^{\circ}$ C for 5 min (denaturing), 35 cycles at 94 $^{\circ}$ C for 30 s (denaturing), 60 $^{\circ}$ C for 30 s (annealing), 72 $^{\circ}$ C for 60 s (extension), and one cycle at 72 $^{\circ}$ C for 7 min (last extension). The amplification products were separated by electrophoresis on 2% agarose gel and visualized by ethidium bromide labeling. The full-length allele (*fl-GHR*) is represented by a 935 -bp fragment and the exon 3-deficient allele (*d3-GHR*) by a 532 -bp fragment.

Statistical analysis

Data are expressed as mean $\pm$ s.d., unless otherwise stated. Continuous variables were tested using the *t*-test, whereas frequencies were compared using the χ^2 -test with the Fisher correction, when appropriate. Correlation between variables was sought using Pearson's correlation coefficient. A multivariate logistic regression model was used for statistical analysis of risk factors for occurrence of VFs. Statistical significance was assumed when $P \leq 0.05$. Data were analyzed using the SPSS Software, version 17.

Results

A total of 48 men and 61 women were included in the study (mean age 47 years, ± 19 – 79). Of them, 58 patients had controlled disease (nine of them received medical therapy as first-line treatment, whereas 49 were treated with somatostatin analogs or pegvisomant after unsuccessful neurosurgery) and 15 patients were cured by neurosurgery. The remaining 36 patients had active disease; of them, 31 were on medical therapy after unsuccessful neurosurgery and radiotherapy and five were waiting for surgery or refused it at the time of this study. Of the total number of patients, 61 patients were eugonadal (35 men and 26 women), 20 patients were

hypogonadal (13 men and seven women), and the remaining 28 women were post-menopausal and were assigned to the hypogonadic group as well.

We found 54 patients (49.5%) to be homozygous for the *fl*-GHR isoform and 55 patients (50.5%) to be carriers of at least one allele of the *d3*-GHR isoform. A total of 18 patients were homozygous for the *d3*-GHR isoform. Patients were assigned to two groups: i) homozygous carriers of the WT allele (*flfl*-GHR, referred as *fl* patients hereafter) and ii) homozygous or heterozygous carriers of the exon 3-deficient allele (*fld3*-GHR and *d3d3*-GHR, referred as *d3* patients hereafter). We decided to apply a dominant model because we did not observe any significant difference in terms of VFs between carriers of one *d3* allele and carriers of two *d3* alleles; we did not observe any gene-dosage effect on fractures. The two groups did not differ in sex, lumbar BMD values, serum IGF1 levels, and bone metabolism parameters (PTH, vitamin D, osteocalcin, and β -CTx). BMD and Z-score of the lumbar spine were not different between *fl* patients and *d3* patients even after adjusting values for gender and BMI (Table 1). A significantly positive linear correlation between IGF1-SDS levels and β -CTx was observed both in *d3* patients (Pearson's R^2 , 0.552; $P=0.001$) and in *fl* patients (Pearson's R^2 , 0.194; $P=0.022$), whereas only a positive trend was observed between IGF1-SDS and osteocalcin in *d3* patients (Pearson's R^2 , 0.0433;

$P=0.407$). No correlation was found between IGF1-SDS and osteocalcin levels in *fl* patients (Pearson's R^2 , 0.060; $P=0.192$; Fig. 1).

Of the total number of patients, 47 (43.1%) had at least one VF, with a total of 53 mild, 13 moderate, and eight severe fractures. The average SDI of fractured patients was 2.19 (± 1.48). Fractured patients did not differ from non-fractured patients with regard to age (48.49 vs 45.32 years, $P=0.213$), lumbar Z-score (-0.57 vs -0.10 , $P=0.100$), femoral Z-score (0.153 vs 0.280, $P=0.527$), and duration of total disease (91.43 vs 72.17 months, $P=0.151$). Patients with fractures were more frequently male (27/47 males, 57.4% vs 21/62 females, 33.8%) ($P=0.012$). Moreover, fractured patients showed significantly higher IGF1 levels (SDS 3.45 vs 2.10; $P=0.022$), and had longer duration of active disease (52.43 vs 23.31 months; $P<0.001$) than non-fractured patients. PTH and vitamin D levels did not differ significantly between the two groups. Osteocalcin levels did not differ significantly either ($P=0.769$), whereas β -CTx levels were higher in fractured patients although not significantly so (0.61 vs 0.45 ng/ml; $P=0.071$) (Table 2).

Prevalence of VFs was significantly higher in *d3* carriers than *fl* patients (63.6 vs 22.2%; $P<0.001$) and this association was confirmed in patients with controlled/cured and active disease (Fig. 2). The mean SDI was significantly higher in fractured *d3* carriers (1.24 ± 1.44)

Table 1 Baseline characteristics of the two populations of the study (*flfl*-GHR carriers vs *fld3/d3d3*-GHR carriers). Values are expressed as mean $\pm$ s.d. unless otherwise stated.

Variables	<i>flfl</i> (n=54)	<i>fld3-d3d3</i> (n=55)	P value
Age	46.52 (± 14.21)	46.85 (± 12.06)	0.894
Sex (M/F)	54 (23/31)	55 (25/30)	0.457
Length of disease			
Total (months)	83.87 (± 68.63)	77.44 (± 69.37)	0.631
Active (months)	38.79 (± 41.40)	33.86 (± 36.52)	0.531
Disease status	20 active, 28 controlled, and six healed	16 active, 29 controlled, and ten healed	0.484
Lumbar BMD	1.165 (± 0.168)	1.113 (± 0.163)	0.146
Lumbar Z-score	-1.132 (± 1.328)	-0.468 (± 1.287)	0.240
Femoral neck BMD	1.036 (± 0.212)	0.980 (± 0.143)	0.141
Femoral neck Z-score	0.288 (± 0.948)	0.154 (± 0.893)	0.503
IGF1 (SDS)	3.13 (± 3.35)	2.65 (± 2.97)	0.382
PTH (pg/ml)	51.33 (± 26.92)	54.51 (± 39.10)	0.631
Vitamin D (ng/ml)	15.80 (± 11.03)	15.66 (± 11.07)	0.951
Osteocalcin (ng/ml)	22.48 (± 12.50)	27.67 (± 25.09)	0.322
Crosslaps (ng/ml)	0.57 (± 0.41)	0.62 (± 0.41)	0.616
No. of fractured patients (%)	12/54 (22%)	35/55 (63%)	<0.001
No. of fractures	19 (nine mild, four moderate, and six severe)	55 (44 mild, nine moderate, and two severe)	
Fractures/patient	0.35 (± 0.70)	1.00 (± 1.00)	<0.001
SDI	0.65 (± 1.43)	1.24 (± 1.44)	0.035

SDI, spinal deformity index.

Figure 1

(A) Linear correlation between IGF1 (SDS) levels and osteocalcin levels. (B) Linear correlation between IGF1 (SDS) levels and β -crosslaps levels.

than in fractured *fl* carriers (0.65 ± 1.43) ($P=0.035$). In 35 patients prospectively evaluated, 13 (37.1%) developed incident VFs. The incidence of VFs was significantly higher in patients for whom the *GHR* gene has been

deleted when compared with those harboring the *fl* gene (52.6 vs 18.8%; χ^2 , 4.3; $P=0.04$).

Multivariate analysis

Variables associated with VFs were entered in a multivariate logistic regression model in order to assess the fracture risk associated with each variable. GHR isoform, IGF1 levels, length of active disease, and male sex were all significantly associated with an increased risk of VFs. Odds ratio (OR) of having a VF in patients who were carriers of one or two d3 alleles was 3.060 ($P=0.015$). Each 1 + IGF1-SDS level was associated with a 1.183 OR ($P=0.031$), whereas length of active disease each month had 1.038 OR ($P=0.001$). Male sex was associated with an increased risk of VFs with an OR of 3.250 ($P=0.041$; Table 3).

Discussion

Data from our study demonstrate for the first time, to the best of our knowledge, that the deletion of the exon 3 of GHR (*d3-GHR*) in at least one allele is associated with a higher prevalence of VFs in acromegalic patients. This is the first report examining the effects of GHR isoforms on bone fragility and risk of VFs in a heterogeneous population of patients (active acromegaly, controlled/cured disease).

The *d3-GHR* polymorphism enhances GHR signal transduction by altering the structure of the extracellular domain of the receptor. The increased intracellular signal transduction may enhance sensitivity to GH as demonstrated by studies conducted on GH-deficient children

Table 2 Baseline characteristics of the two populations of the study (fractured patients vs patients without fractures).

Variables	Fractured (n=47)	Non-fractured (n=62)	P value
Age	48.49 (± 14.48)	45.32 (± 11.91)	0.213
Sex (M/F)	27/20	21/41	0.012
Lumbar BMD	1.112 (± 0.165)	1.158 (± 0.167)	0.210
Lumbar Z-score	-0.57 (± 1.374)	-0.10 (± 1.230)	0.100
Femoral BMD	1.007 (± 0.170)	1.008 (± 0.193)	0.980
Femoral Z-score	0.153 (± 0.801)	0.280 (± 1.017)	0.527
IGF1-SDS	3.45 (± 3.52)	2.10 (± 2.29)	0.022
Length of disease			
Total (months)	91.43 (± 77.38)	72.17 (± 60.49)	0.151
Active (months)	52.43 (± 47.46)	23.31 (± 23.67)	<0.001
PTH (pg/ml)	57.20 (± 46.63)	49.66 (± 17.72)	0.256
Vitamin D (ng/ml)	14.97 (± 11.39)	16.33 (± 10.74)	0.540
Osteocalcin (ng/ml)	20.61 (± 8.96)	19.82 (± 8.96)	0.769
Crosslaps (ng/ml)	0.61 (± 0.36)	0.45 (± 0.21)	0.071
Gonadal status	24 ipogon/23 no ipogon	24 ipogon/38 no ipogon	0.137
GHR polymorphism	12 fl/fl/35 d3	42 fl/fl/20 d3	<0.001

ipogon, hypogonadic.

Figure 2

Both in active acromegalic and controlled disease, carriers of at least one *d3* allele had a significantly increased prevalence of VFs ($P=0.0001$ and $P=0.001$). A, *d3* genotype was associated to increased prevalence of VFs ($P<0.001$); B, Both in active acromegaly and controlled disease, carriers of at least one *d3* allele had a significantly increased prevalence of VFs ($P=0.001$ and $P=0.002$).

treated with recombinant human GH. Patients expressing *d3*-GHR have an increased growth velocity and eventually they reach a higher final height (6, 7). On the other hand, *d3*-GHR polymorphism confers higher susceptibility to GH stimulation in acromegalic patients and may negatively affect phenotypic presentation and long-term complications of the disease. Nonetheless, the effect of *d3*-GHR polymorphism on GH/IGF1 levels is controversial. Mercado *et al.* (24) reported a significant correlation between IGF1 and GH levels only in *d3*-GHR carriers. Bianchi *et al.* (9) demonstrated that basal GH vs IGF1 relationship was concordant in *d3* carriers, but it became discordant (GH normal and IGF1 increased) after neurosurgery or somatostatin analog treatment, thus suggesting that small differences in GHR isoforms sensitivity exist and are possible although these differences could not be immediately evident in active acromegaly because of the elevated levels of circulating GH. At the time of our study,

there were no differences observed in terms of IGF1 levels between *fl*-GHR and *d3*-GHR carriers, although we did not measure GH levels because of the confounding effect of some therapies (pegvisomant) on this assessment.

In this study, we focused our attention on bone phenotype of acromegalic patients and particularly on the prevalence of VFs. With regard to the impact of *GHR* isoforms on complications of acromegaly, Montefusco *et al.* (25) found that patients carrying at least one *d3* allele had a lower BMI, normal glucose tolerance, and lower insulin levels 120 min after an oral glucose load than patients homozygous for *fl*-GHR; these data suggest that *GHR* polymorphism may well affect the weight and metabolic phenotype. Wassenaar *et al.* (13) investigated the impact of *d3* deletion of *GHR* on BMD, osteoarthritis, and (non) VFs. The authors also studied the prevalence of other comorbidities such as adenomatous polyps, dolichocolon, and the association between anthropometric parameters, cardiovascular risk factors, and GHR isoforms. In a selected population of patients with long-term remission of acromegaly, Wassenaar *et al.* demonstrated that *d3* carriers had an increased prevalence of osteoarthritis, adenomatous polyps, and dolichocolon but anthropometric parameters, cardiovascular risk factors, BMD, and (non) VFs did not differ between *fl* homozygous patients and *d3* patients.

In our population, comprising patients with active and controlled/cured acromegaly, we confirmed some of the previous results concerning BMD not significantly correlated with fractures (14, 15). However, in our cohort, a significant difference in terms of prevalence of VFs was observed between *fl* homozygous patients and *d3* carriers. In fact, patients carrying at least one *d3* allele had a significantly increased prevalence of VFs (Fig. 2). Another original observation of our study was concerned with bone metabolism markers and their association with fractures. Previous studies demonstrated that active acromegaly was associated with increased calcium and phosphate serum levels, increased calciuria and markers of bone formation, and bone resorption (26, 27, 28, 29, 30, 31). Bonadonna *et al.* (14) found that fractured acromegalic women had

Table 3 Results from a logistic regression analysis of independent variables correlating with fracture risk.

Independent variables	OR (95% CI)	P value
Sex (male)	3.250 (1.051–10.048)	0.041
<i>d3</i> genotype	3.060 (0.700–13.381)	0.015
IGF1–SDS (per SDS)	1.183 (0.951–1.472)	0.031
Length of active disease (per month)	1.038 (1.016–1.060)	0.001

higher serum bone alkaline phosphatase and urinary deoxypyridinoline and lower serum levels of vitamin D compared with non-fractured patients, but the study population was too small (36 women) to draw any real final conclusion. In our population, β -CTx (a bone catabolism marker) correlated with a higher prevalence of VFs and, although not significant ($P=0.07$), this assessment may be helpful to estimate bone fragility in acromegalic patients. In addition, we found a positive linear correlation between IGF1 levels (increased in active disease) and β -CTx in both *f1* and *d3* patients, although the correlation was stronger for *d3* patients (Fig. 1). Taking the stronger linear correlation between IGF1-SDS and β -CTx in *d3* carriers into consideration, we can hypothesize that in these patients when acromegaly is active, the increased sensitivity to GH may increase bone remodeling and consequently plasma levels of bone catabolism markers.

Our study confirmed previous cross-sectional observations in which acromegalic patients developed VFs independent of normal BMD and there were no significant differences in terms of BMD values between fractured and non-fractured patients (14, 15, 32). For this reason, X-ray assessment is of paramount importance in establishing the real fracture risk in patients affected by acromegaly (33, 34) as well as in all patients affected by other forms of secondary osteoporosis (35). In accordance with previous studies (14, 15), fracture rate in our population was 43% and the biochemical control of acromegaly appeared effective in reducing the risk of fractures.

We also found an increased prevalence of VFs in men, which has not been previously reported in other studies (14, 15, 26, 32). In other previous cross-sectional studies, acromegalic men and only post-menopausal women were analyzed separately. Conversely, two recent studies have demonstrated a higher prevalence of VFs in acromegalic males (36, 37), and also in the recently published prospective study (16), the percentage of incident fractures was slightly, but not significantly, higher in males (45%) than in females (36%). The authors explained this finding by assuming a bone protective effect of estrogens in pre-menopausal women included in this study. Alternatively, an increased bone tissue sensitivity to GH stimulation in males may be hypothesized as already demonstrated for other clinical end-points of GH (38). However, this effect appeared to be independent of the polymorphisms of *GHR*.

Although the lack of sex steroids was traditionally considered as a risk factor for bone loss at lumbar spine in patients with acromegaly (39), data on association between VFs and hypogonadism have been inconclusive (15, 32, 40, 41), probably because the negative effects of

hypogonadism seem to be variable related to the activity of acromegaly (16). In fact, hypogonadism was shown to be associated with the high risk of VFs in patients with controlled/cured disease but not in those with active disease (16). Consistent with these findings, in this study performed on a mixed population of acromegaly patients (i.e. with active and controlled/cured disease), we did not find a significant association between hypogonadism and VFs.

The real novelty of this work is the strong correlation found between *d3*-GHR isoform and high prevalence of VFs in patients with acromegaly. Interestingly, we also observed a significant correlation between *d3*-GHR isoform and incident VFs in a small subgroup of acromegalic patients followed up for 3 years. Notably, all these patients did not have a high prevalence of VFs at baseline and this feature provides further evidence that deletion of *GHR* might have been involved in the pathogenesis of skeletal fragility in acromegaly regardless of coexistent risk factors for fractures (16). Very few contrasting data are currently available on the relationship between GHR polymorphisms and osteoporosis in healthy patients. A progressive decrease in GH secretion has been hypothesized; however, recent experimental evidences have suggested that, besides the age-dependent decline of GH and IGF1 serum levels, dysregulation of GH and IGF1 action may be due to an impairment of the post-receptor signaling of GHR (42). In rodents, complete inhibition of GH action by disrupting or knocking out of the *GHR* gene (which resulted in GH resistance, elevated GH levels, and markedly reduced IGF1 levels) led to reduced muscle mass and BMD (43). In line with these observations, some authors reported that subjects receiving GH replacement therapy showed no significant changes in BMD (44, 45), probably because of other mechanisms being involved in elderly subjects affected by pituitary disease (i.e. impaired post-receptor GH signaling). The effects of *GHR* exon 3 isoform expression on osteoporosis has been studied in healthy patients previously. Kenth *et al.* (46) studied the possible influence of *GHR* exon 3 isoform on final adult height, quantitative ultrasound (QUS) of the heel, and BMD (spine and femoral neck) in a healthy population of 368 white women; there was no correlation between *GHR* exon 3 genotype and final height, QUS, and BMD. In acromegalic patients, Waassenar *et al.* (13) did not find a significant difference in terms of BMD and prevalence of VFs between carriers of exon 3 deletion and patients carrying the WT allele. In our population, on the other hand, which consists also of patients affected by active acromegaly, a stronger impact of GHR polymorphism on the risk of fracture due to GH excess can be expected.

In conclusion, our study demonstrates for the first time that another important risk factor for vertebral fragility fractures in acromegalic patients does exist, i.e. the presence of d3 deletion of *GHR* gene. Genetic testing may represent an additional tool for stratification of risk of VFs in acromegalic patients and help in devising an ordered plan and adequate management in each patient.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

L De Marinis designed this study and M Mormando conducted the study. M Mormando and A Giampietro collected data. L A Nasto, A Bianchi, and G Mazziotti conducted data analysis and interpretation. M Mormando drafted the manuscript. L A Nasto, A Giustina, and E Pola revised the manuscript content. A Pontecorvi, E Pola, and L De Marinis approved the final version of the manuscript. M Mormando and L De Marinis were responsible for the integrity of the data.

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