Prevalence of Asymptomatic Vertebral Fractures in Late-Onset Pompe Disease

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Context: Bone fragility and low bone mass have been reported in small case series of patients with Pompe disease with severely reduced muscle strength or immobilization.

Objective: Our objective was to determine the prevalence of morphometric vertebral fractures and to evaluate bone mass in adults with late-onset Pompe disease.

Design: We conducted a multicenter cross-sectional observational study from August 2012 to December 2013.

Study Setting: All subjects were outpatients referred to University Referral Centers.

Patients: Patients included 22 late-onset Pompe disease patients with progressive proximal myopathy and minimal respiratory involvement without other diseases affecting bone mass.

Main Outcome Measure: The prevalence of morphometric vertebral fractures was systematically assessed by semiquantitative analysis of lateral spine x-rays (T4–L5).

Results: A high prevalence of morphometric vertebral fractures was found. At least 1 vertebral fracture was present in 17 of 22 patients (77%). All vertebral fractures were asymptomatic. Bone mineral density was normal in 36.5% of the patients, whereas 36.5% were osteopenic and 27% were osteoporotic in at least 1 site. Fracture prevalence was independent of muscular and respiratory functional parameters and of genotype.

Conclusions: Our data show for the first time that asymptomatic and atraumatic vertebral fractures occur frequently in late-onset Pompe disease patients without a significant impairment of bone mass. Screening for asymptomatic vertebral fractures should be routinely performed in Pompe disease irrespective of the disease severity. Fracture risk should be confirmed in longitudinal studies. **(*J Clin Endocrinol Metab* 100: 401–406, 2015)**

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Abbreviations: BMD, bone mineral density; CTX, C-telopeptide of type I collagen; CV, coefficient of variation; 25(OH)D, 25-hydroxyvitamin D; DXA, dual-energy x-ray absorptiometry; ERT, enzyme replacement therapy; FVC, forced vital capacity; GAA, acid α-glucosidase; GSGC, gait, stairs, Gower, chair; LOPD, late-onset Pompe disease; 6MWT, 6-minute walking test; PLS, potentially less severe mutation; SDI, spine deformity index; SQ, semiquantitative; VS, very severe mutation.

Pompe disease is a rare, inherited glycogen storage disease caused by a large variety of mutations of the acid α-glucosidase (*GAA*) gene leading to partial or complete deficiency of the lysosomal enzyme acid α-glucosidase (1). Severity and age of onset of the disease are related to the level of residual enzymatic activity, and among the clinical presentations, 2 main variants can be singled out: a more severe infantile form and a late-onset form (late-onset Pompe disease [LOPD]) characterized by a slowly progressive proximal myopathy with respiratory involvement. The introduction of enzyme replacement therapy (ERT) with recombinant human GAA has modified the course of the disease, increasing the survival in the infantile-onset and reducing the disease progression in LOPD (2, 3). Recently, bone fractures were reported in infantile-onset Pompe patients (4) and moderate to severe reduction of bone mineral density (BMD) was described in LOPD (5, 6). The concern for fragility fracture in Pompe disease, as a potential long-term complication, is growing particularly in late-onset patients treated with ERT with better outcome and improvement of clinical performance. Until now, relatively small series of patients with fractures were described, and the real prevalence of fragility fractures in this disease has not yet been defined (5, 7). We systematically explored BMD and the prevalence of vertebral and nonvertebral fractures in a selected group of LOPD patients without risk factors for osteoporosis to try to establish whether fragility and low bone mass may be considered a new aspect of this disease.

Patients and Methods

Diagnosis of LOPD patients included in this study was based on decreased GAA activity in muscle tissue and was confirmed by mutational analysis. All patients were ambulant without aid and did not need respiratory support. Muscle strength was assessed by Medical Research Council (MRC) sum score, ie, the sum of the MRC score of 6 muscles (3 at the upper and 3 at the lower limbs) on both sides, each muscle graded from 0 to 5 (8). The functional exercise capacity was assessed by the 6-minute walking test (6MWT) and by timing and grading 4 functional performances, that is, walking for 10 m (gait), climbing 4 steps on a stair, Gower's maneuver, and rising from a chair (gait, stairs, Gower, chair [GSGC] score) (9). Spirometric evaluations were performed and forced vital capacity (FVC) was expressed in percent decrease compared with controls. Exclusion criteria comprised systemic bone diseases (such as primary hyperparathyroidism, hyperthyroidism, and idiopathic hypercalciuria) or other diseases affecting bone mass such as celiac disease or rheumatologic diseases and any drug affecting bone mass such anticonvulsants, glucocorticoid therapies, thyroid hormones, estrogen replacement therapies, and bisphosphonates to list a few. Body mass index was calculated for all the patients. Serum calcium (Roche/Hitachi coefficient of variation [CV] <10%; Roche Diagnostics), serum PTH (Diasorin Liaison N-tact PTH, CV <10%), serum 25-hydroxyvitamin D (25(OH)D; total CV <15%; DiaSorin), and C-telopeptide of type I collagen (CTX; Elecsys B-CrossLaps/serum assay, CV <20%; Roche Diagnostics) were analyzed as biochemical parameters of bone metabolism. BMD was measured by dual-energy x-ray absorptiometry (DXA) (Hologic Discovery) at lumbar spine L1–L4 (LS; in vivo precision 1.0%) and femoral neck (in vivo precision, 1.8%). For adult patients, the results are expressed as T-score and for the patient aged 16 years as Z-score. World Health Organization criteria consider a T-score below −2.5 SD at the spine or hip diagnostic of osteoporosis and a value below −1 SD and above −2.5 SD diagnostic of osteopenia (10). For Z-score, a value <-2 SD is considered as osteoporosis in children and adolescents (11). Fractured vertebrae were excluded from the DXA scan. All skeletal fractures for low-energy trauma were included in this study. The prevalence of morphometric vertebral fractures (both symptomatic and asymptomatic) were evaluated by conventional spinal radiographs in lateral (T4–L5) obtained in all patients with standardized technique. Two expert readers (F.B. and M.B.), who were blinded to clinical patient data, independently reviewed the radiographs and discussed questionable cases to agree on a diagnosis. The interrater reliability between the 2 readers was good (κ = 0.82). Vertebral fractures were diagnosed on visual inspection using a semiquantitative (SQ) visual assessment (12). According to this technique, fractures were defined as reductions of more than 20% in anterior, middle, or posterior vertebral height. Each vertebra is visually assessed as intact (SQ grade 0) or as having approximately mild (20%–25% compression), moderate (25%–40% compression), or severe (>40% compression) deformity (SQ grades 1, 2, and 3, respectively). Subsequently for each patient, the spine deformity index (SDI) was calculated by summing the SQ grade for each of the 14 vertebrae from T4 to L5 (13). Nonvertebral fractures were included only if radiologic reports or surgical procedures were available. Fractures of toes, facial bones, and fingers and all those caused by high-energy trauma were excluded.

This study is supported by the School of Medicine of Verona, which required a formal approval by the Ethical Board of the School of Medicine of Verona as coordinator center. Informed consent was obtained from all patients, and the personal data were encoded.

Statistical analysis

The demographic and clinical characteristics are reported as median and interquartile range, due to the departure from the normal distribution of the continuous parameters. The Kolmogorov-Smirnov test was used to compare the medians. The independence of categorical variables was tested by χ^2 test. Continuous variables were categorized into below and above median. For 25(OH)D, the patients were categorized to have normal, deficient, or insufficient serum levels. Linear regression was used to explore relationships between continuous variables. A logistic regression model was used to identify independent determinants of the fractures. $P < .05$ was considered statistically significant at the 95% confidence levels. The statistical analyses were done with Statgraphics Centurion (version XV).

Results

Twenty-two LOPD patients (13 males and 9 females) were enrolled in this study. The median age was 40.5 years

(range 16–68 years; interquartile range, 15). Females were all premenopausal with normal menses. All patients walked without assistance and were not ventilated. The most frequent gene mutation was IVS1-13T>G on allele 1; 12 (57%) patients had the very severe mutation (VS) and 9 patients (43%) the potentially less severe mutation (PLS) according to Kroos et al (14). For one patient, the mutation was not available. Nineteen patients were on treatment with ERT, and the duration of therapy ranged between 1 month and 5 years. The genotypes and demographic and clinical characteristics of the patients as well as muscular functional and respiratory parameters are summarized in Table 1. No patient had known risk factors for osteoporosis (low body mass index, delayed puberty, amenorrhea or significant menstrual menses abnormalities, menopause, cigarette smoking, excessive alcohol intake, <100 mg/d calcium intake, or family history of hip fracture) and were naive for bisphosphonates or other drugs for bone fragility. BMD T-score at the lumbar spine, femoral neck, and total hip were −0.55 (−1.4/0.7), −1.2 (−2.2/−0.3), and −1.1 (−2.3/−0.4), respectively. BMD was normal in 36.5% of the population, whereas 36.5% of the patients were osteopenic and 27% were osteoporotic. The semiquantitative morphometric analysis of the spine radiograms showed at least 1 vertebral fracture in 17 of 22 patients (77%). Eight of them had 2 or more verte-

Table 1. Demographic and Clinical Characteristics, Genotypes, and Vertebral Fractures of LOPD Patients

Patient	Sex	Age, y	Genotype[a]	Phenotype	6MWT, m	GSGC Score	MRC Sum Score	FVC, %	ERT Duration, mo	Vertebral Fractures (Grade)	SDI
1	F	46	IVS1-13T>G/c.2331 + 1GA	Prox muscle weakness	468	10/27	52/60	88	54	T11 (1)	1
2	F	42	IVS1-13T>G/c.1836CG	Prox muscle weakness	438	12/27	52/60	80	57	T7 (1), T11 (1)	2
3	F	40	IVS1-13T>G/525 delT	Prox muscle weakness	440	8/27	53/60	74	52	None	0
4	M	58	IVS1-13T>G/c.1655TC	Prox muscle weakness	324	8/27	54/60	93	1	None	0
5	M	50	IVS1-13T>G/525 delT	Prox muscle weakness	504	14/27	53/60	100	7	T8 (2), T11 (2)	4
6	F	43	IVS1-13T>G/2237G>A	Prox muscle weakness	540	14/27	56/60	114	0	T8 (2), T11 (2)	4
7	M	36	IVS1-13T>G/525 delT	Prox muscle weakness	612	6/27	58/60	100	0	T6 (1)	1
8	M	60	Not available	Prox muscle weakness	n.v.	27/27	42/60	71	5	T6 (1)	1
9	M	52	IVS1-13T>G/2237G>A	Prox muscle weakness	452	4/27	58/60	98	39	T9 (1)	1
10	M	44	IVS1-13T>G/525 delT	Prox muscle weakness	144	18/27	52/60	63	41	T10 (3), L1 (3)	6
11	F	42	IVS1-13T>G/2237G>A	Prox muscle weakness	296	24/27	35/60	91	56	T7 (1)	1
12	M	38	IVS1-13T>G/c.1124G>T	Prox muscle weakness	620	8/27	54/60	73	36	T12 (1)	1
13	F	50	IVS1-13T>G/c.2237G>A	Prox muscle weakness	450	12/27	58/60	96	36	None	0
14	M	33	IVS1-13T>G/c.1927G>A	Prox muscle weakness	460	14/27	53/60	32	39	T12 (2)	2
15	M	41	IVS1-13T>G/c.1802C>T	Prox muscle weakness	425	9/27	58/60	73	48	T8 (1), T9 (1), T10 (2), T12 (1)	5
16	F	38	IVS1-13T>G/c.1802C>G	Prox muscle weakness	395	19/27	46/60	68	48	T8 (1)	1
17	M	68	IVS1-13T>G/del exon18	Prox muscle weakness	437	10/27	54/60	81	60	T11 (1), T12 (1)	2
18	F	16	IVS1-13T>G/c.1927G>A	Asymptomatic increased sCK	625	5/27	60/60	94	17	None	0
19	M	31	IVS1-13T>G/525 delT	Asymptomatic increased sCK	593	4/27	60/60	98	57	T7 (1), T8 (1), T9 (1), T10 (1)	4
20	F	33	IVS1-13T>G/1927G>A	Prox muscle weakness	66	22/27	42/60	68	57	T10 (1), T11 (1)	2
21	M	34	IVS1-13T>G/2104C>T	Prox muscle weakness	528	6/27	47/60	73	0	T6 (1)	1
22	M	20	IVS1-13T>G/c.546 + 1G>T	Asymptomatic increased sCK	550	4/27	60/60	85	42	None	0

Abbreviations: F, female; M, male; Prox, proximal; sCK: serum creatine kinase.

[a] Patients 1, 3, 5, 6, 7, 9, 10, 11, 13, 17, 19, and 22 are categorized as VS; patients 2, 4, 12, 14, 15, 16, 18, 20, and 21 are categorized as PLS according to Kroos and colleagues (14).

Table 2. Comparison of BMD, Bone Metabolism, and Muscular and Respiratory Function in LOPD Stratified by Vertebral Fractures, ERT, and Genotype Severity[a]

	n	BMD Lumbar Spine T-Score SD	BMD Femoral Neck T-Score SD	BMD Total Hip T-Score SD	CTX, ng/mL	25(OH)D, ng/mL	PTH, pg/mL	6MWT m	GSGC Score	MRC Sum Score	FVC %
Fx	17	−0.9 (2.4)	−1.6 (2.5)	−1.4 (2.0)	0.35 (0.16)	15.0 (12.5)	39.2 (41.5)	456.0 (124.0)	12 (10)	53.0 (9.0)	80.0 (27.0)
No fx	5	0.1 (0.7)	−0.8 (1.6)	−1.1 (1.7)	0.29 (0.30)	14.4 (4.8)	45.9 (32.5)	450.0 (110.0)	8 (3)	58.0 (6.0)	93 (9.0)
ERT	18	−0.8 (2.1)	−1.8 (2.1)	−1.8 (2.0)	0.36 (0.31)	15.8 (13.5)	40.7 (47.0)	450.0 (79.0)	11 (10)	53.0 (6.0)	80.5 (23.0)
No ERT	4	0.4 (1.6)	−0.4 (1.9)	−1.0 (1.9)	0.21 (0.18)	14.2 (9.5)	24.0 (21.8)	534.0 (150.0)	7 (5)	55.0 (6.5)	96.5 (24.0)
VS	12	−1.1 (1.7)	−1.4 (1.9)	−1.0 (2.4)	0.30 (0.25)	13.6 (13.8)	56.5 (50.2)	460.0 (106.5)	10 (9)	55.0 (5.5)	93.5 (16.0)
PLS	9	0.6 (1.8)	−0.4 (2.3)	−0.9 (2.0)	0.35 (0.16)	16.6 (8.9)	26.0 (12.9)	438.0 (133.0)	9 (6)	53.0 (7.0)	73.0 (12.0)[b]

Abbreviation: Fx, vertebral fractures.

[a] Data are expressed as median (interquartile range).

[b] $P = .043$.

bral fractures. A slightly higher prevalence of fractured subjects was observed among males (11 of 13, 85%) than in females (6 of 9, 67%). In all patients but 1, the vertebral fractures were located in the thoracic tract of the spine. Twelve patients had mild (grade 1) vertebral fractures, 3 patients had moderate (grade 2) vertebral fractures, 1 patient had severe (grade 3) vertebral fractures, and 1 patient had 3 mild (grade 1) and 1 moderate (grade 2) vertebral fracture. The median SDI was 1.0 (0–2). The site and the number of vertebral fractures and the SDI are reported in Table 1. Only 6 of 17 patients with prevalent vertebral fracture had a T-score value, at least in a skeletal site, at or below −2.5 SD, but in 7 of them, BMD was normal in all the measured skeletal sites. In the other 4 fractured patients, T-score ranged between −1 and −2.4 SD. In particular, in 65% of fractured patients, vertebral fractures occurred with a normal or slightly decreased lumbar spine BMD (T-score greater than −2.5 SD). In all patients, vertebral fractures were asymptomatic, and none reported a significant trauma. No low-energy nonvertebral fractures were recorded. The 25(OH)D levels were low in almost all patients (14.7 [10.8–23.3] ng/mL); in particular, 4 of 22 had insufficiency (below 10 ng/mL), 16 of 22 had deficiency (between 30 and 10 ng/mL), and only 2 had normal vitamin D levels (>30 ng/mL). Serum CTX levels (0.33 [0.28–0.58] ng/mL), available from 20 patients, were high, in accordance with Italian normal range matched for age (15), in 40% of the patients, ie, in 40% of fractured (6 of 15) and in 40% of patients without vertebral fractures (2 of 5). No significant differences were found for BMD, SDI, 25(OH)D, CTX, and PTH levels or physical (MRC, 6MWT, and GSGC) and respiratory (FVC) parameters when we compared fractured with nonfractured patients, ERT with non-ERT patients, and VS with PLS patients (Table 2). There was no statistical relationship of BMD T-score, CTX, and 25(OH)D with ERT duration, MRC, 6MWT, GSGC, or FVC (Supplemental Data). The prevalence of fracture (yes/no) and SDI (Supplemental Data) was independent of 25(OH)D levels (Figure 1), ERT (yes/no) (Figure 2), and genotype (VS and PLS) (Figure 3), and when fracture prevalence (yes/no) was tested by logistic regression with all continuous and categorical parameters, no independent factor was found (Supplemental Data).

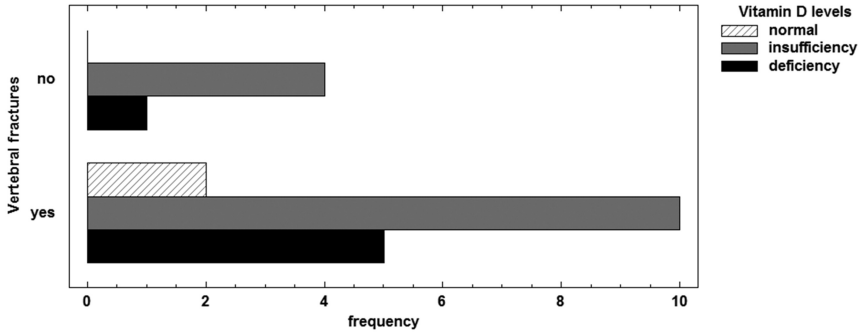

Figure 1. Distribution of fractured/nonfractured patients by vitamin levels ($\chi^2 = 0.986$, $P = .61$).

Discussion

We observed in a relatively large series of LOPD patients with preserved motility and pulmonary function a high prevalence of osteoporosis/osteopenia and a remarkably high prevalence of asymptomatic and atraumatic vertebral fractures. It is noteworthy that the prevalence of vertebral fracture is quite high also in patients with normal or very slightly reduced BMD. An increased incidence of low BMD in adults and children affected by Pompe disease and the occurrence of fractures in children with the infantile form were recently reported (4, 6, 16). However, in these studies, most of the patients were nonambulatory and needed respiratory support so that the prevalence of low BMD found was ascribed to significant residual motor impairment and absence of weight bearing (5–7). In about half of our LOPD patients, we

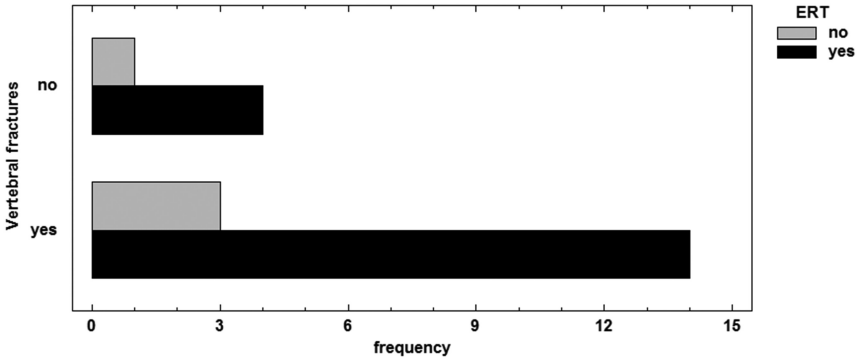

Figure 2. Distribution of fractured/nonfractured patients by ERT (χ^2 = 0.014, P = .90).

confirmed the low BMD reported by Papadimas et al (5–7). The prevalence of osteoporosis (27%) and osteopenia (36.5%) in our population of LOPD patients exceeds the one reported in literature for age-matched controls (0.5% and 15%, respectively) and reaches the prevalence found in celiac disease, rheumatoid arthritis, inflammatory bowel diseases, and anorexia nervosa (17). As previously reported (6), we noted in our patients a trend toward a lower BMD at the hip than at the spine. Based on these data, Pompe disease should be included among the causes of secondary osteoporosis in young adults (17). In our cohort of LOPD patients, the unexpected prevalence of vertebral fracture was quite high also in subjects with normal or very slightly reduced BMD. This is the first report of a systematic analysis for morphometric vertebral fractures in Pompe disease. Most of our patients (77%) had at least 1 vertebral fracture, and about half of them had multiple vertebral fractures. None had fractures in other skeletal sites, eg, long bones. The prevalence of fragility fractures in Pompe disease is not well defined. Case et al (4) reported 19 fractures in 14 children with Pompe disease on ERT. All had fragility fractures of long bones ascribed to a significant residual motor impairment and absence of weight bearing. Bone fractures were not reported in 46 patients with Pompe disease (36 adults and 10 children) with low BMD or in 8 adult patients with moderately reduced BMD (5, 6).

Khan et al (7) recently described several long-bone fragility fractures in 1 young adult patient and a significant reduction of BMD in 3 other patients. To date, only 2 vertebral fractures have been reported in Pompe disease (4, 18) and that induced Case et al to suggest screening for morphometric vertebral fractures (4). The pathogenesis of bone health impairment in Pompe disease is unknown. Osteopenia and osteoporosis were reported in other lysosomal storage diseases such as Fabry and Gaucher disease, where an imbalance between osteoblast and osteoclast activity was identified (19–21). Low BMD was also described in Duchenne muscular dystrophy and spinal muscular atrophy (22, 23) and was attributed to the reduced muscle strength, inactivity, or immobilization (5–7). To verify whether genotype could influence bone fragility in LOPD, we compared 2 homogeneous subgroups of LOPD patients based on the second allele mutations (VS and PLS, according with Kroos et al [14]). LOPD VS patients showed a slight trend to have lower BMD and 25(OH)D levels than LOPD PLS, but the genotype does not correlate with the prevalence and the severity of the vertebral fractures. The classification of GAA mutations in VS and PLS is related to muscular and respiratory impairment (24), and the possible impact in bone metabolism is unknown. Because the first allele mutation was found in almost all our patients, the impact of this mutation on bone health or fractures cannot be explored. We did not find any relationship between parameters of muscular activity (MRC, 6MWT, and GSGC) or respiratory function (FVC) with BMD T-score or parameters of bone metabolism, but our data should be confirmed in a larger series of patients. Furthermore, no differences between muscle function and respiratory parameters and fractured or nonfractured LOPD patients were found. The fracture prevalence was not significantly associated with any functional phenotype or with vitamin D levels (the only 2 patients with normal levels of 25(OH)D were fractured). Overall, these data suggest that in LOPD, the bone health impairment, and particularly the fracture risk, is likely to be independent from muscular and respiratory phenotype or vitamin D levels. A potential effect of ERT on bone mass in Pompe disease has been suggested, but data are poor and corrupted by bisphosphonate use (7). It could be mediated by an improvement of motility and muscle performance because a direct effect on bone metabolism has not yet been

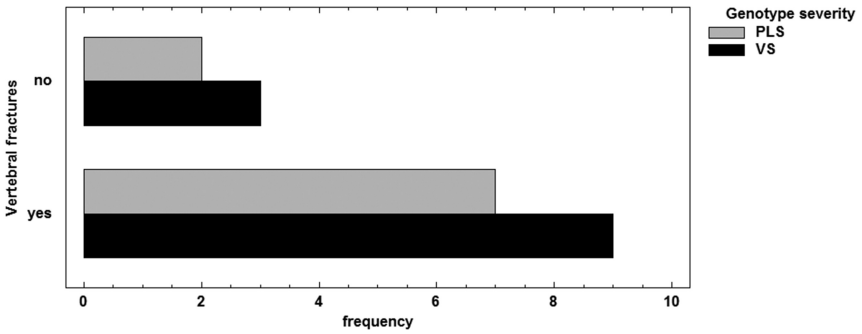

Figure 3. Distribution of fractured/nonfractured patients by genotype severity (χ^2 = 0.022, P = .88).

proven. Most of our patients have been on ERT treatment since 1 to 5 years before the study. In our LOPD population, we did not find any influence of ERT and its duration on BMD and the prevalence and severity of vertebral fracture. To our knowledge, the relationships between phenotype/genotype and the pathogenesis of bone health in Pompe disease is completely unexplored and the physiopathology of fragility is currently merely speculative. The fracture risk could be an intrinsic feature of the disease and potentially linked to the first mutation (IVS1-13T>G), which is common to almost all our patients.

In summary, we report here for the first time a high prevalence of asymptomatic vertebral fractures in a cohort of late-onset Pompe disease patients. The impressive high risk for vertebral fracture seems to be independent of the clinical phenotype and genotype and from other known fracture risk factors. Our data suggest that screening for asymptomatic vertebral fractures should be routinely performed in Pompe disease patients independent of disease severity or pain. Our data are not conclusive, and more studies should be promoted to understand the pathogenesis of bone alterations in Pompe disease.

Acknowledgments

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P.T. and F.B. designed the study. F.B., F.Z., M.S., and P.T. conducted the study. P.T., M.M., V.L., C.A., C.S., M.F., S.C., and A.T. collected data. M.B. and S.P. analyzed data. F.B. and P.T. interpreted data. F.B. and P.T. drafted the manuscript. F.B., P.T., and M.B. revised the manuscript content. F.B., P.T., F.Z., M.M., V.L., C.A., C.S., M.F., S.C., A.T., M.S., M.B., and S.P. approved the final version of the manuscript. PT takes responsibility for the integrity of the data analysis.

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