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# Familial Paget's disease of bone: Long-term follow-up of unaffected relatives with and without Sequestosome 1 mutations

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ABSTRACT

*Objective:* Familial Paget's disease of bone is inherited as an autosomal-dominant trait and mutations in the *sequestosome 1* (*SQSTM1*) gene have been reported with variable frequency in patients with familial disease. The natural history, however, of the disease in family members with or without *SQSTM1* mutations is unknown.
*Methods:* To address this question, we investigated members of families with Paget's disease identified and genotyped in 2000 in The Netherlands without clinical, biochemical or radiological signs of Paget's disease. Seventy-five subjects, median age 56 years (range 44–93), with or without *SQSTM1* mutations participated in the present study. Medical history was obtained and clinical examination and laboratory investigations were performed in all. When serum biochemical markers of bone turnover were increased, skeletal scintigraphy with SPECT-CT was performed.
*Results:* After a mean period of 15.9 ± 0.32 (SD) years no subject without *SQSTM1* mutations (either from positive or negative families) developed Paget's disease. Of 14 carriers of *SQSTM1* mutations, Paget's disease of the pelvis was diagnosed in a 74-year old asymptomatic woman.
*Conclusion:* The incidence of new Paget's disease in *SQSTM1 positive subjects* was 7.1% and no mutation-negative subject developed the disease within 16 years of follow-up. Subjects without *SQSTM1* mutations can be reassured whereas mutation carriers should consider screening. Our findings should be confirmed in other populations as currently unknown environmental factors that might be involved in the development of the disease may differ.

## 1. Introduction

Paget's disease of bone (PDB) is a focal disorder of bone remodeling that affects typically the elderly and progresses slowly leading to permanent changes in the shape and size of affected bones [1]. The etiology of the disease is unknown but genetic factors have been implicated in its pathogenesis. In studies in the USA and The Netherlands it has been previously shown that first degree relatives of patients with PDB have a 7- to 10-fold higher risk to develop the disorder than age- and gender-matched controls [2,3] and a positive family history has been reported by up to 40% of patients [4].

In these families PDB is inherited as an autosomal-dominant trait with incomplete penetrance. The importance of genetic factors results from two independent positional cloning studies which linked the chromosome 5q35 locus to Paget's disease of bone and led to the identification of disease-causing mutations in *sequestosome1* (*SQSTM1*) [5,6]. These mutations have been reported with a variable frequency in different populations of patients with familial disease [3,7–11].

In 2000, we studied a large cohort of patients with Paget's disease and we identified and genotyped 18 families with the disease, seven of which (39%) were carriers of *SQSTM1* mutations [3]. Because of the slow progress of the disease and its increasing prevalence with ageing [12], in the present study we investigated unaffected members of these families with or without *SQSTM1* mutations 16 years later with the

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following specific aims: [*1*] to estimate the incidence of PDB in apparently healthy relatives of patients with familial disease and [*2*] to assess whether carriers of *SQSTM1* mutations are at higher risk to develop PDB than these without such mutations.

## 2. Subjects and methods

### 2.1. Subjects

Between October 2016 and July 2017 we contacted the 18 index patients with familial PDB, meaning having 1 or more affected relatives, as described in our previous study [3] and explained the purpose and design of the present study. Following this, we invited their, previously evaluated, unaffected family members with or without *SQSTM1* mutations, to participate in the present study. All identified and consented subjects were visited at the place of their residence, a medical history was taken and all were specifically interviewed for symptoms and signs of PDB as well as use of bisphosphonates; subjects were further clinically examined and blood was taken for laboratory investigations and DNA analysis. Persons without SQSTM1 mutations from mutation positive families were analyzed separately from mutation negative families. Subjects with values of biochemical markers of bone turnover above their respective reference ranges and/or significant increases since the previous study, were invited to attend the Out-Patient Clinic of the Center for Bone Quality where additional investigations, including $^{99}$Tc bone scintigraphy with SPECT-CT scan were performed. The study was approved by the Medical Ethical Review Board of the Leiden University Medical Center and all subjects provided written informed consent.

### 2.2. Laboratory investigations

Serum 25-hydroxy vitamin D (25-OHD), alkaline phosphatase activity (ALP) and procollagen 1 amino-terminal propeptide (P1NP) were measured by fully automated techniques on a Roche Cobus 8000 modulator system (Roche Diagnostics, Almere, The Netherlands). Upper limits of references ranges for ALP: women 98 IU/l, men 115 IU/l and for P1NP: pre-menopausal women and men 59 ng/ml, post-menopausal women 76 ng/ml.

### 2.3. DNA analysis

Samples from all subjects of mutation positive or negative families previously identified as *SQSTM1* negative were re-tested. Patient samples were screened for genetic variation leading to loss of function of the SQSTM1 UBA domain, known to be involved in PDB pathogenesis. To this end, we performed PCR amplification followed by enzymatic clean-up and Sanger sequencing. Primers for exons 7 and 8 were designed using Primer3Plus based on the SQSTM1 template sequence (accession code: NM_003900). The amplicons were amplified using the GoTaq® G2 DNA Polymerase (Promega Corporation, Madison, WI, USA). Amplification of the PCR product was verified by agarose gel electrophoresis. Unincorporated dNTPs and primer oligonucleotides were removed from the PCR reactions using Exonuclease I (New England Biolabs, Inc., Ipswich, MA, USA) and Calf Intestinal Alkaline Phosphatase (Roche Applied Science, Hoffmann-La Roche AG, Basel, Switzerland). Sanger sequencing was performed using the ABI Prism BigDye terminator cycle sequencing ready reaction kit (Applied Biosystems, Foster City, CA, USA) using the same primers used for PCR amplification. Using the BigDye Xterminator purification kit we removed unincorporated BigDye terminators, prior to sequence determination using the ABI 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

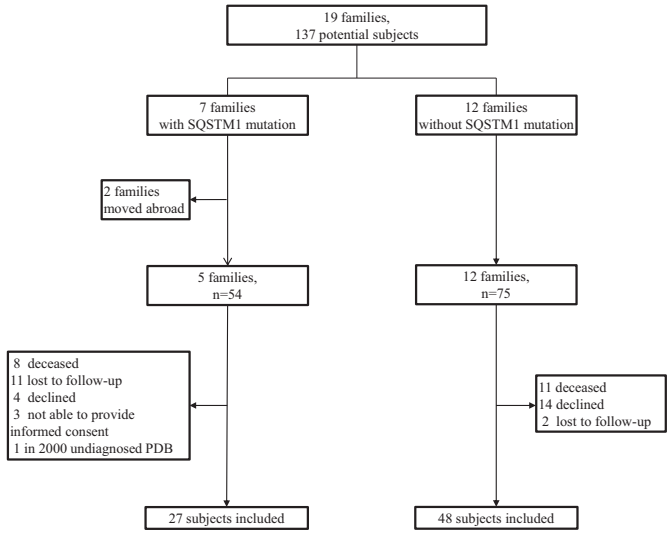


**Fig. 1.** Flow-chart of the inclusion of subjects with familial Paget's disease of the bone. SQSTM1 = sequestosome 1, PDB = Paget's disease of the bone.

### 2.4. Statistics

Data are expressed as median ± SE unless otherwise stated. Descriptive statistics were used for the presentation of the characteristics of studied subjects. Comparisons between subjects with and without a mutation were done by means of independent samples *t*-test. All analyses were done using IBM SPSS Statistics 23.

## 3. Results

A total of 137 subjects met the inclusion criteria. Of those, 19 were deceased and 3 were mentally or physically unable to participate in the study (4 were mutation positive), see also Fig. 1. According to information from family members and available medical records none of these subjects had been diagnosed with PDB. All 8 alive, previously reported, family members of index patients with the S399P and M404 T mutations of *SQSTM1* had immigrated to Suriname and Indonesia, respectively, and 3 additional subjects had left the country. Of the remaining subjects, 13 were lost to follow-up and another 18 declined participation; all were mutation negative and the latter subjects did not report a suspected diagnosis of PDB during the follow-up period.

One SQSTM1 mutation carrier had calcaneal tracer uptake and in retrospect this was present on the scintigraphy in 2000, at an age of 42 years. Therefore Paget's was already present in the previous study and we excluded the patient for the present analyses and moved the subject to the index-cases. Therefore, 75 subjects were included in the present study. Importantly, all available carriers of the P329L and G425R mutations participated.

Seventy-five (44 women) subjects, median age 56 years (range 44–93) participated in the study 15.9 ± 0.32 (SD) years (range 15.0–16.5 years) after the initial investigation. Of these, 64 were siblings or offsprings of patients with PDB and 11 were second degree relatives (nephews or nieces). In one member of a family in whom no *SQSTM1* mutations were identified in our original study, upon retesting a, previously unreported, mutation was detected; this was a Ala381Thr substitution Details of all studied subjects are summarized in Fig. 2. Of the 14 SQSTM1 positive individuals, 2 were siblings of index cases and 12 were offspring. None of these subjects reported symptoms or signs of PDB or and serum 25-OHD concentrations were > 20 nmol/l in all but 3 subjects. Mean serum 25-OHD ± SD concentrations were not different between subjects with and without *SQSTM1*mutations [51.0 (9.2) vs 54.0 (3.0) nmol/l, respectively, $p = 0.66$].

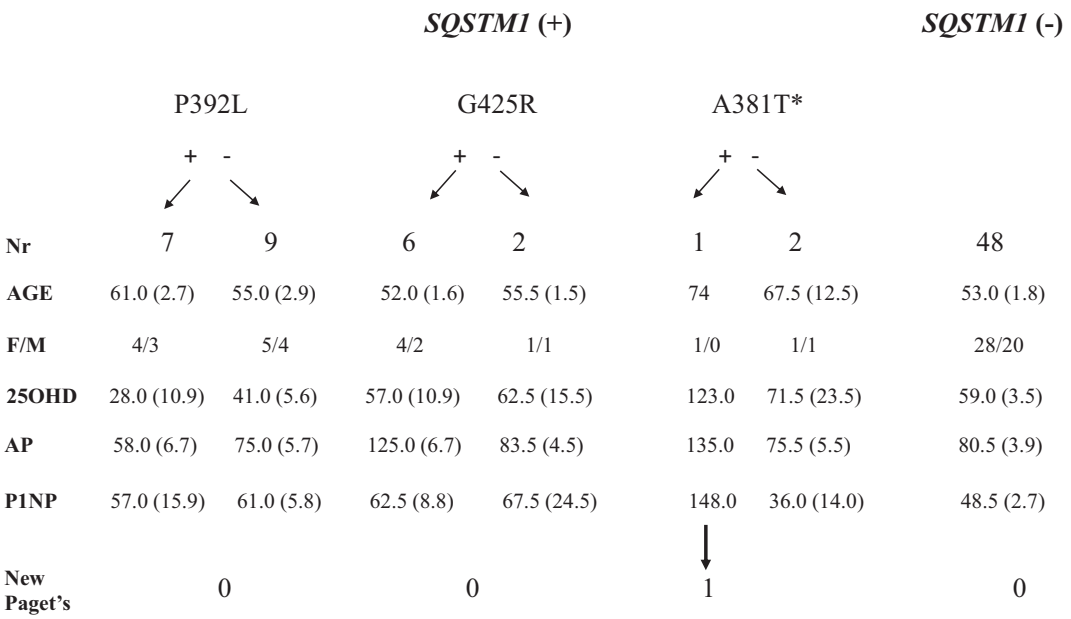


**Fig. 2.** Clinical and biochemical characteristics of members of families with and without Sequestosome 1 (SQSTM1) mutations and no Paget's disease of bone at baseline.
F/M = Female/Male, 25-OHD = 25-hydroxyvitamin D (nmol/l), AP = Alkaline Phosphatase (IU/l), P1NP = procollagen 1 amino-terminal propeptide (ng/ml). Median ± SE are shown.

### 3.1. Biochemical markers of bone turnover

Bone turnover markers (either ALP, P1NP or both) were elevated in 8 of 14 SQSTM1 positive subjects and all of them underwent bone scintigraphy. In addition, 2 of the 13 SQSTM1 negative subjects from mutation positive families and 12/48 subjects from mutation negative families, underwent bone scintigraphy because of increased and/or higher bone markers than during the initial study. Elevated serum ALP values ranged between 101 and 151 and those of P1NP between 60 ng/ml and 148 ng/ml.

In a 74 year-old asymptomatic woman, carrier of the newly identified A381T *SQSTM1* mutation and sibling of an index patient with a diagnosis of poly-ostotic PDB at the age of 36, PDB of the right pelvis was diagnosed.; Her serum ALP activity was 135 IU/l compared with a baseline value of 73 IU/l, 15.5 years ago and serum P1NP value was 148 ng/ml. In a 58-year-old man, carrier of the G425R mutation, a nephew of the index patient, who was diagnosed at age 46 with poly-ostotic PDB, with slightly increased serum P1NP values (63 ng/ml) but normal serum ALP activity (103 IU/l), a previously suspected PDB of the calcaneus was confirmed. Interestingly, although serum ALP was higher than his baseline value (86 IU/l) this was still within the normal range probably due to the limited extent of the disease [13,14]. While in all mutation positive subjects bone scans were performed, independently of the magnitude of increase in BTM, in 8 male subjects, members of mutation negative families, with marginally elevated serum P1NP values (62–68 ng/ml) no scintigraphy was performed. These 8 subjects had normal serum ALP activity values that were not different from their baseline values 16 years ago and their serum 25-OHD concentrations were > 20 nmol/l[median 40.0 (4.3) nmol/l.

## 4. Discussion

Our study with the longest reported systematic follow-up of unaffected members of families with PDB demonstrates that the overall incidence of familial disease in our Dutch cohort is 1.3% despite the increased risk of these subjects. However, none of 61 subjects without *SQSTM1* mutations, either from mutation positive or negative families developed the disease within the 16-year follow-up period. The only new case of PDB was a carrier of a *SQSTM1* mutation accounting for an incidence of 7.1% and a prevalence of 13.3% among mutation carriers. Another, previously reported, longitudinal study in New Zealand identified 2 patients with Paget's disease among 28 carriers of *SQSTM1* mutation after a mean follow-up period of 5.1 (3.7–6.3) years [15]; an incidence of 7.7% that is similar to that in the Dutch cohort. The rate of new PDB in the New Zealand subjects was 15 per 1000 pt-yrs and in our study this was 4.5 per 1000 pt-yrs. While direct comparison of these rates is inappropriate due to the very small numbers of patients, the 3-fold difference in follow-up time, and, perhaps, also the different environmental factors, they both indicate that family members, of patients with familial PDB, despite being carriers of *SQSTM1* mutations, are at relatively low risk of developing the disease within the time-frame of the studies. These results have implications for the management of first degree relatives of patients with familial PDB and may contribute to the understanding of the suggested but currently unidentified interactions between genetic and environmental factors in the pathogenesis of the disease.

PDB is currently efficaciously treated with a single infusion of zoledronate 5 mg that induces long-term biochemical and clinical remissions [16,17]. Treating asymptomatic patients is currently advised for consideration [18]. Therefore, a strategy for early identification of the disease in those at higher risk, such as individuals with a SQSTM1 mutation, is desirable. With a single ALP measurement PDB might be missed in individuals with limited disease, as in the case of the subject with calcaneal disease. However, this subject had serial measurements of ALP where ALP rose from 86 U/l to 103 during follow up. In addition, he had an increased P1NP and therefore a scintigraphy was made.

For screening purposes we would advise to use serial measurements of ALP with large intervals and to consider P1NP as this BTM has been shown to be useful in PDB [19]. Literature is conflicting regarding age of development of PDB in offspring [10,15]. In the present study the earliest observed case of PDB was at the age of 42 in the case of the subject with the calcanean Pagets'. Therefore we would advise to consider screening above the age of 40.

An on-going 5-yr, placebo-controlled clinical trial investigates the effect of a single zoledronate infusion in preventing the development of PDB in unaffected carriers of *SQSTM1* mutations (http://www.

controlledtrials.com/ISRCTN11616770). Combining the New Zealand with our own data, we expect that ~7% of *SQSTM1* carriers will develop monostotic, asymptomatic disease with time. This is a very low incidence of mild disease that may be related to the reported declining prevalence and decreasing severity of PDB [20–23] and does not, in our opinion, justify preventive treatment of all mutation positive subjects even if this treatment could successfully prevent the disease. This suggestion should, however, await the final results of the clinical trial.

An intriguing finding of our study was the increased levels of serum ALP activity, that did not change with time, in carriers of the G425R mutation without any scintigraphic evidence of PDB. We noted this is our original study [3] and we hypothesized that in subjects with *SQSTM1* mutations, raised serum ALP activity without evidence of PDB may represent an early stage of the natural history of the disease that will develop with time. The present study failed to confirm this hypothesis over a 16-year period and we still have no explanation for this finding.

The strength of our study is the very long follow-up and the inclusion of a substantial number of members of families with PDB without *SQSTM1* mutations for whom no data about potential clinical consequences are currently available. Limitations include inability to contact members of the two families with the S399P and M404 T mutations and the inclusion of a relatively small number ($n = 18$) of older subjects. In the initial study the mean age at diagnosis was 56.5 (SE 1.65) years. Other studies have shown that mean age of a Paget diagnosis in SQSTM1 positive offspring was similar [10] or 10 years older [15] than age at parental diagnosis. Although the mean age of the subjects in the present study is comparable to age at diagnosis of the index patients, some of the subjects were screened at younger age than the parental diagnosis and therefore it might be that the offspring was not old enough to develop PDB, being a limitation of the present study. Furthermore, 1 subject was on Alendronate in osteoporosis dose (70 mg/week) with fully suppressed bone turnover markers and 1 subject had stopped using Risedronate at least 6 months before the study, what might have influenced results as well.

All alive members with the P329L and G425R mutations were identified and investigated. In addition, search for evidence of PDB in most of those who did not participate in the study did not reveal any diagnosed cases. Whether the results of our current study are applicable to all populations is currently unknown given the uncertainty about the contribution of environmental factors in the pathogenesis of the disease [23]. Another limitation of the study is the fact that scintigraphy was performed after confirmed elevated levels of bone turnover, whereas in limited disease radiological changes can be visible with BTM within normal range [12–14]. However all these studies have been performed in relationship with Alkaline Phosphatase and imaging. P1NP however has now been shown to be a more sensitive marker of bone activity [19] and therefore it is not likely that if P1NP levels are within the normal range, any relevant PDB might be missed.

In conclusion, long-term follow up of unaffected members of families with PDB revealed a low incidence of the disease only in carriers of *SQSTM1* mutations allowing the planning of a management strategy for familial PDB.

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## Author roles

JP: data collection, data interpretation, data analysis, writing manuscript, approving of the final version of the manuscript.

RR: mutation analysis, data interpretation, writing manuscript, approving of the final version of the manuscript.

EH: data collection, approving of the final version of the manuscript.

EE: approving of the final version of the manuscript.

FS: writing manuscript, approving of the final version of the manuscript.

EB: mutation analysis, approving of the final version of the manuscript.

WH: data interpretation, revising manuscript, approving of the final version of the manuscript.

SP: data analysis, data interpretation, writing manuscript, revising manuscript, approving of the final version of the manuscript.

NA: study design, data analysis, data interpretation, writing manuscript, approving of the final version of the manuscript.

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## Declaration of competing interest

The authors have nothing to disclose.

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