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ABSTRACT

Paget's disease of bone (PDB) has a strong genetic component. Here, we investigated possible associations between genetic variants that predispose to PDB and disease severity. Allelic variants identified as predictors of PDB from genome-wide association studies were analyzed in 1940 PDB patients from the United Kingdom, Italy, Western Australia, and Spain. A cumulative risk allele score was constructed by adding the variants together and relating this to markers of disease severity, alone and in combination with *SQSTM1* mutations. In *SQSTM1*-negative patients, risk allele scores in the highest tertile were associated with a 27% increase in disease extent compared with the lowest tertile ($p < 0.00001$) with intermediate values in the middle tertile (20% increase; $p = 0.0007$). The effects were similar for disease severity score, which was 15% ($p = 0.01$) and 25% ($p < 0.00001$) higher in the middle and upper tertiles, respectively. Risk allele score remained a significant predictor of extent and severity when *SQSTM*-positive individuals were included, with an effect size approximately one-third of that observed with *SQSTM1* mutations. A genetic risk score was developed by combining information from both markers, which identified subgroups of individuals with low, medium, and high levels of severity with a specificity of 70% and sensitivity of 55%. Risk allele scores and *SQSTM1* mutations both predict extent and severity of PDB. It is possible that with further refinement, genetic profiling may be of clinical value in identifying individuals at high risk of severe disease who might benefit from enhanced surveillance and early intervention. © 2013 American Society for Bone and Mineral Research

Introduction

Paget's disease of bone (PDB) is a common skeletal disorder characterized by focal abnormalities of bone remodeling that disrupt bone architecture, leading to the development of various complications such as bone pain, deformity, secondary osteoarthritis, nerve compression syndromes, and pathological fractures.[1] The severity of PDB is highly variable however; some patients have severe disease that negatively impacts on quality of life,[2, 3] whereas others are completely asymptomatic.[1]

Paget's disease has a strong genetic component. The single most important susceptibility gene is *SQSTM1*, which harbors mutations in up to 40% of patients with a family history of PDB and in 5% to 10% of patients with no family history.[4, 5] These mutations are thought to play a causal role in the disease because they have been found to reproduce the disease phenotype in one experimental model[6] and to increase osteoclastogenesis significantly in another.[7] Mutations of *SQSTM1* also segregate with the disease in families and are seldom found in unaffected controls.[4, 8-14] Recent genome-wide association studies have identified additional susceptibility loci for PDB near the *CSF1* gene on chromosome 1p13; the *NUP205* gene on 7q33; the *TM7SF4* gene on chromosome 8q22; the *OPTN* gene on chromosome 10p13; the *RIN3* gene on 14q32; the *PML* gene on 15q24; and near the *TNFRSF11A* gene on 18q21.[15, 16] When combined, these alleles have a strong effect on susceptibility to PDB, accounting for about 13% of the heritability,[15] but it is currently unknown if they influence disease severity.

The aim of the present study was to determine if risk alleles at the above loci influence extent, severity, or complications of PDB either alone or in combination with *SQSTM1* mutations.

Materials and Methods

Patients

The primary analysis was conducted in participants of the Paget's Disease, Randomised Trial of Intensive versus Symptomatic Management (PRISM) study (ISRCTN12989577), which was a randomized comparative trial of two treatment strategies for PDB.[17] In brief, the PRISM trial recruited 1324 patients with PDB attending secondary-care referral centers in the United Kingdom. They were randomized to receive either "symptomatic" therapy in which treatment was administered only in patients who had bone pain or "intensive" bisphosphonate therapy in which the aim of treatment was to reduce and maintain serum alkaline phosphatase levels within the reference range by use of bisphosphonate therapy. The bisphosphonate of choice in the "intensive" group was risedronate because the trial was initiated in 2001 before the licensing of zoledronic acid for the treatment of PDB. The present report is based on a subgroup of 770 study participants who consented to provide a blood sample for genetic analysis. For replication, four separate clinic-based cohorts of PDB patients were used. The GenePage cohort comprised 384 unrelated Italian patients with Paget's disease recruited from 13 Italian centers as previously described;[9] the Naples/Siena cohort comprised a nonoverlapping cohort of 363 unrelated Italian patients with Paget's disease recruited from northern, central, and southern Italy as described;[18] the Salamanca cohort comprised 191 unrelated patients attending a specialist clinic for Paget's disease in Salamanca, Spain;[19] and the Western Australian cohort comprised 232 unrelated patients attending a specialist clinic in Perth.[20]

Assessment of disease extent

In all cohorts, disease extent was assessed on the basis of radionuclide bone scan by counting the number of affected sites with radiographic and/or scintigraphic evidence of PDB.

Clinical assessments

Within the PRISM study, health-related quality of life was assessed by the SF-36 questionnaire.[21] Deformity was assessed by the attending physicians who were asked to assess whether the patient had clinical evidence of bone deformity using a three-point scale as follows: 0 = no deformity; 1 = mild or moderate deformity; 2 = severe deformity. The presence of bone pain was recorded, and physicians were asked to assess if they thought the pain was caused by PDB. Information was collected on previous fractures and whether they had occurred in affected bone; on orthopedic surgical procedures; on the use of a hearing aid for deafness; on age at diagnosis of PDB; and family history of PDB. Information was recorded on whether the patient had previously received bisphosphonate treatment and the number of treatment courses given.

Within most of the other cohorts, information was also available on the presence or absence of bone deformity; previous fractures through Pagetic bone; use of a hearing aid in patients with skull involvement; orthopedic surgery for PDB; age at diagnosis; family history of PDB; and previous courses of bisphosphonates for PDB. In the Perth cohort, however, information on bone deformity, orthopedic surgery for PDB, and use of a hearing aid in association with skull involvement had not been collected.

We employed a composite disease severity score taking several clinical features and complications of the disease into account as previously described.[22] In brief, one point was assigned for each bone affected and additional points were assigned for the following: previous fractures through Pagetic bone (0 = no; 1 = yes); previous orthopedic surgery for PDB (0 = no; 1 = yes); history of osteosarcoma (0 = no; 1 = yes); bone deformity (0 = no deformity; 1 = mild or moderate deformity; 2 = severe deformity, for each bone affected); use of a hearing aid if the patient had PDB of the skull bones (excluding mandible and maxilla) (0 = no; 1 = yes); bisphosphonate treatment in the previous 12 months (0 = no; 1 = yes); bisphosphonate treatment >12 months ago (0 = no; 1 = yes); and age at diagnosis (1 = ≥70 years; 2 = 60–69 years; 3 = 40–59 years; 4 = < 40 years).

Genotyping

Genomic DNA was extracted from peripheral blood using standard procedures. Mutations screening of *SQSTM1* was conducted on PCR-amplified DNA fragments focusing on exons 7 and 8 and the intron-exon boundaries because all previously reported mutations occur in these regions.[23] The PCR methodology was essentially as described previously.[8] For exon 7, we used the following primers: forward-TTAAAGTCACGCTGGGAACCTGCT; reverse-AGGGCAGGATGCTCTAAAGGG. For exon 8, we used the following primers: forward-TCTGGGCAGGCTCGGACACT; reverse-CCCTAAATGGCTTCTTGCACCC. The PCR products were sequenced using the same primers in both forward and reverse directions. The traces were analyzed by Chromas-pro software and compared with the reference sequence (NC_000005.8, GI:51511721, NCBI Entrez Gene, http://www-ncbi-nlm-nih-gov.offcampus.dam.unito.it/). The prevalence and type of *SQSTM1* mutations observed in the PRISM study are shown in Supplemental Table S1. Genotyping for the other SNP that attained genome-wide significance for association with PDB (rs10494112, rs4294134, rs2458413, rs1561570, rs10498635, rs5742915, rs3018362) was performed either using the Illumina HumanHapDuo300 array; the Sequenom MassARRAY iPLEX platform as previously described;[15] or TaqMan SNP genotyping assays. A number of randomly selected samples were genotyped on the different genotyping platforms (*n* = 96 on Illumina and Sequenom; *n* = 12 on all three platforms), and the cross-platform genotype concordance rate was 100%.

Ethics

The PRISM study was approved by the multicenter ethics committee in the UK. Collection of DNA samples and clinical data were approved by local ethics committees of the other participating centers. All patients gave written informed consent to being included in the study.

Statistical analysis

For each individual in each cohort, we assigned a score of 0, 1, or 2 to genotypes at the rs10494112, rs4294134, rs2458413, rs1561570, rs10498635, rs5742915, and rs3018362 loci, depending on whether subjects carried the wild-type allele or were heterozygous or homozygous for the allele that was associated with PDB. We then adjusted the score for each locus depending on the strength of association with PDB derived from the odds ratio for association with PDB as described previously.[15] The adjustment factor for each allele carried was 1.72 for rs10494112; 1.45 for rs4294134; 1.40 for rs2458413; 1.67 for rs1561570; 1.44 for rs10498635; 1.34 for rs5742915; and 1.45 for rs3018362. We then added the scores across all seven loci to create a cumulative allelic risk score and divided subjects from each cohort into tertiles based on the cumulative risk score. For *SQSTM1* mutations, a score of 0 or 1 was assigned depending on whether a mutation was present. We also devised a genetic risk score in which patients were classified into six groups based on their risk allele score and *SQSTM1* mutation status, ranging from those in the lowest tertile of risk allele score who were *SQSTM1* mutation negative to those in the top tertile for risk allele score who were *SQSTM1* positive. Analysis of variance (ANOVA) and general linear model ANOVA were used to evaluate differences between the genotype groups for continuous variables, and the chi-square test was used for categorical variables. Data were synthesized across cohorts by meta-analysis using Review Manager software. The Mantel-Haenszel method was used to calculate odds ratios and confidence intervals for categorical variables, and the inverse variance method was used to calculate standardized mean differences for continuous variables.

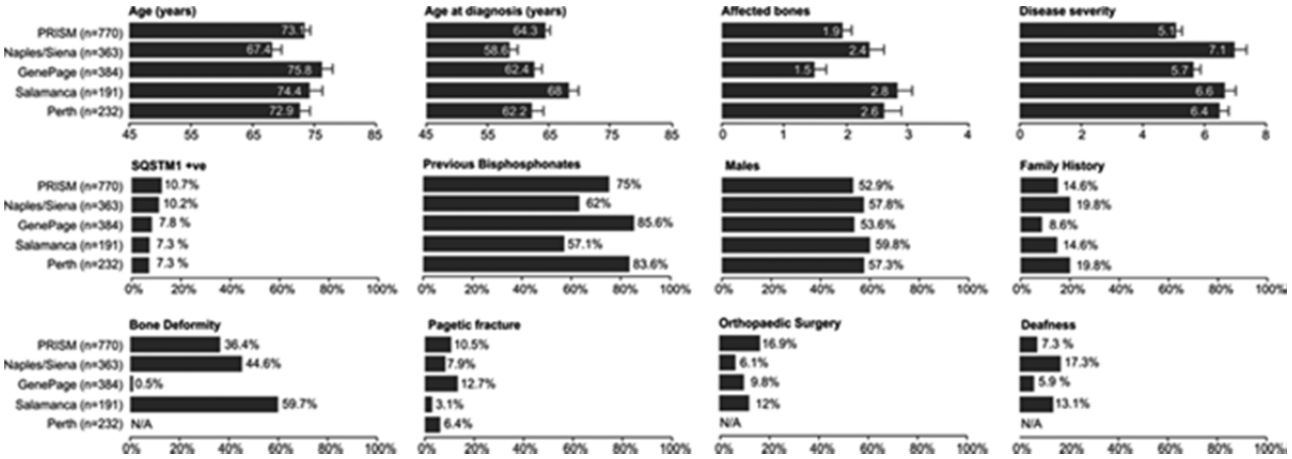
Results

Characteristics of study cohorts

The characteristics of the cohorts included in the study are summarized in Fig. 1. The average age at recruitment ranged from 67 to 75 years, and the average age at diagnosis from 58 to 68 years. There was a

predominance of male subjects in each cohort ranging from 53% to 60%, and between 8.6% and 19.8% of patients reported a family history of the disease. The frequency of complications varied between cohorts. Fractures through Pagetic bone ranged from 3.1% in Salamanca to 12.7% in the GenePage cohort, whereas the prevalence of bone deformity ranged from 0.5% in the GenePage cohort to 59.7% in the Salamanca cohort. The frequency of deafness with skull involvement ranged from 5.9% in the GenePage cohort to 17.3% in the Naples/Siena cohort, and the frequency with which orthopedic surgery had been required for PDB ranged from 6.1% in the Naples/Siena cohort to 16.9% in the PRISM cohort. The frequency with which patients had previously been given bisphosphonate therapy for Paget's disease ranged from 57.1% in the Salamanca cohort to 85.6% in the GenePage cohort.

Figure 1.



Characteristics of included studies. Values in the figures are means for continuous variables and percentages for categorical variables. Error bars in the bar charts are standard error of the mean.

Association between PDB susceptibility alleles and severity in the PRISM study

The relation between PDB susceptibility alleles, disease extent, and complications in 688 subjects from the PRISM cohort who tested negative for *SQSTM1* mutations are shown in Table 1. There was a significant association between risk allele score and number of affected bones with evidence of an allele dose effect ($p = 0.04$). A similar trend was observed for disease severity score, but the differences were not significant. Family history of PDB was significantly associated with risk allele score in this subgroup of patients ($p = 0.01$) as was the number of previous courses of bisphosphonates received for PDB ($p = 0.04$). There was no significant association between allele risk score and sex, age, age at diagnosis of PDB, quality-of-life measures, the presence of deafness owing to PDB (reflected by use of a hearing aid and skull involvement), fractures through Pagetic bone, orthopedic surgery, or bone deformity.

Table 1. Susceptibility Alleles and Markers of Disease Severity in PRISM Study Participants Without *SQSTM1* Mutations

Variable	Tertile 1 (*n* = 228)	Tertile 2 (*n* = 230)	Tertile 3 (*n* = 230)	*p* Value
Age (years)	73.3 ± 7.8	73.7 ± 8.1	72.7 ± 7.8	0.47
Male	121 (53.1%)	131 (57.0%)	117 (50.9%)	0.39
Age (years) at diagnosis	65.2 ± 10.4	65.7 ± 10.4	64.0 ± 10.5	0.19
Family history of PDB	18 (7.9%)	23 (10.0%)	38 (16.5%)	0.01
Patients with bone deformity	84 (36.8%)	75 (32.6%)	90 (39.1%)	0.33

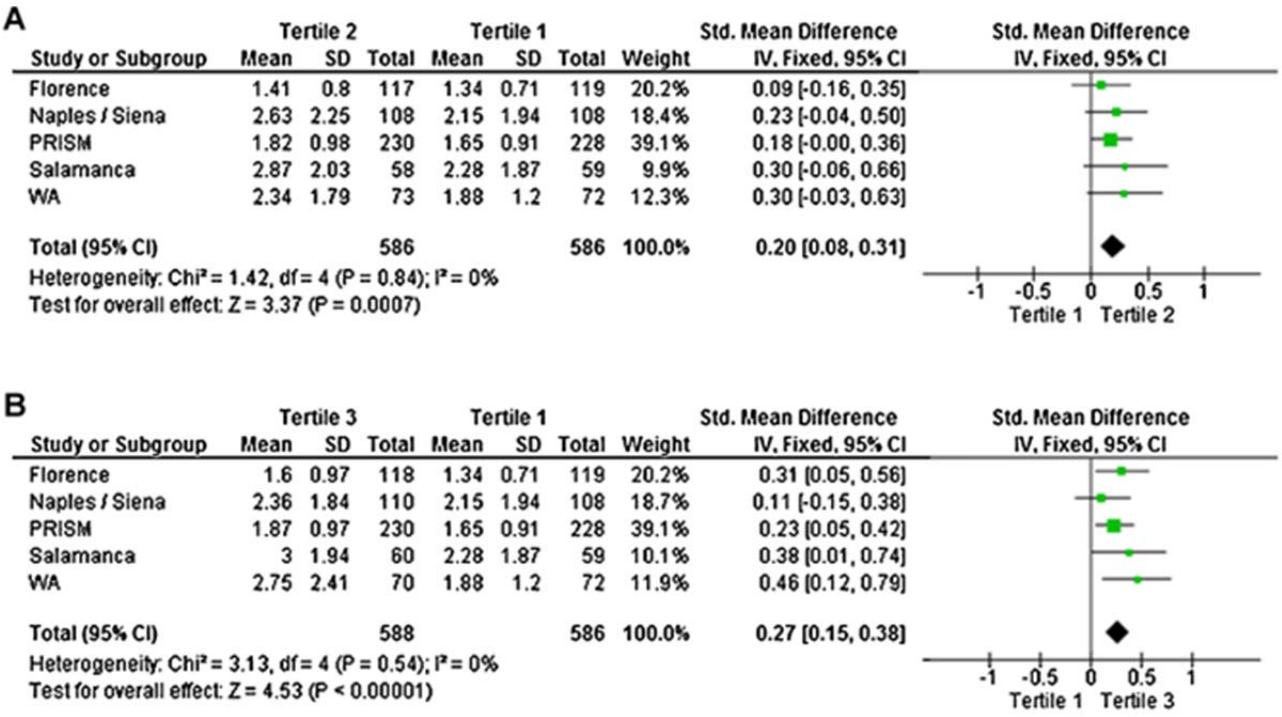
1. Values are mean ± SD or number (%). The *p* values refer to the differences between the genotype groups assessed by ANOVA or chi-square test. The ALP values have been standardized to the upper limit of the reference range, which was set at 1.0.

Variable	Tertile 1 (*n* = 228)	Tertile 2 (*n* = 230)	Tertile 3 (*n* = 230)	*p* Value
Fracture in Pagetic bone	17 (7.5%)	29 (12.6%)	25 (10.9%)	0.18
Orthopedic surgery for PDB	36 (15.8%)	38 (16.5%)	37 (16.1%)	0.97
Deafness and skull PDB	17 (7.5%)	17 (7.4%)	14 (6.1%)	0.80
No. of affected bones	1.66 ± 0.92	1.82 ± 0.98	1.87 ± 0.98	0.04
Disease severity score	4.82 ± 2.18	4. 87 ± 2.34	5.19 ± 2.22	0.17
No. of previous courses of bisphosphonate				
0	69 (30.3%)	68 (29.6%)	41 (17.7%)	
1	87 (38.2%)	91 (39.6%)	98 (42.6%)	
2	47 (20.6%)	48 (20.9%)	57 (24.8%)	0.04
3 or more	25 (10.9%)	23 (10.0%)	34 (14.7%)	
Pain and quality of life				
SF36 bodily pain	40.6 ± 10.6	40.5 ± 10.6	40.7 ± 11.1	0.98
SF36 physical summary	37.7 ± 11.0	37.0 ± 11.6	37.1 ± 11.8	0.77
SF36 mental summary	49.4 ± 10.7	49.5 ± 11.9	49.7 ± 11.5	0.94
Alkaline phosphatase	1.18 ± 0.85	1.37 ± 1.25	1.21 ± 0.91	0.10

Meta-analysis of PDB susceptibility alleles in relation to disease severity

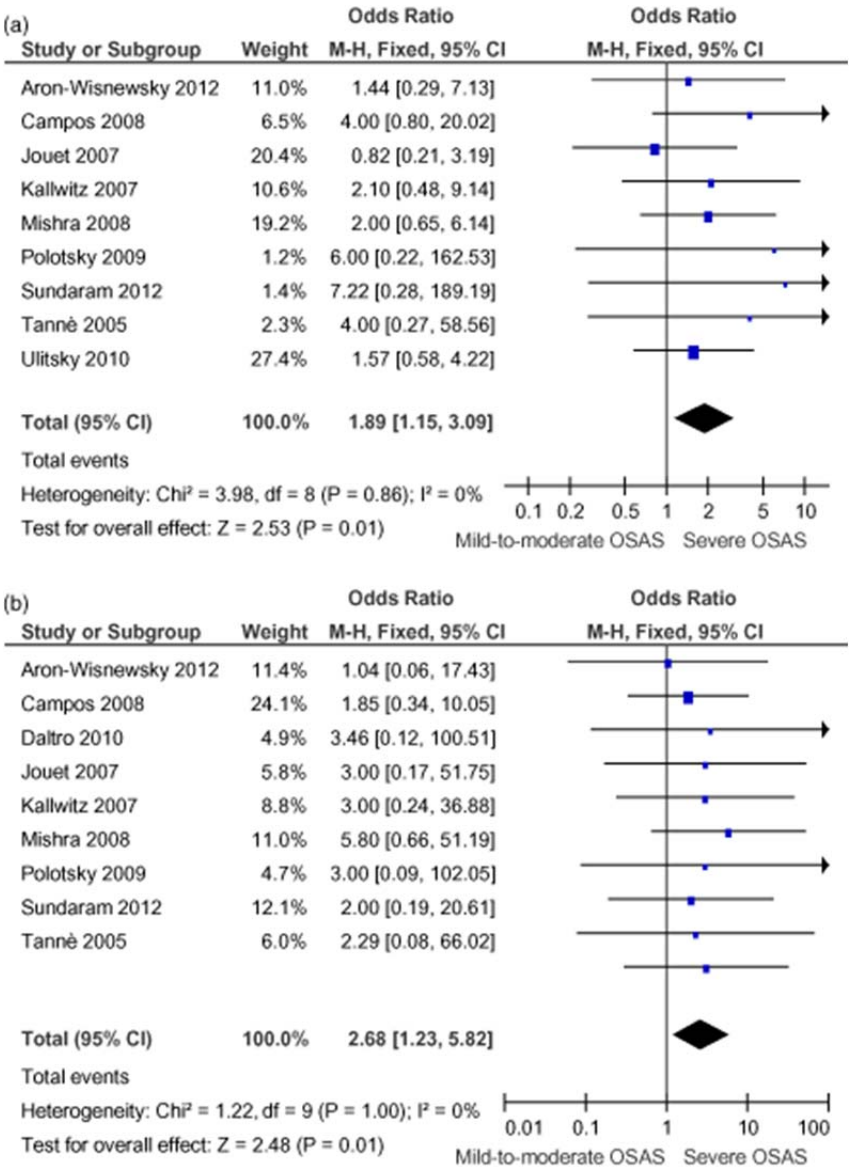
To determine if risk alleles were associated with disease severity in other populations, we employed meta-analysis to examine the relation between risk allele score and markers of disease severity in *SQSTM1*-negative subjects from all centers. This showed a highly significant association between risk allele score and both disease extent and severity with evidence of an allele dose effect. The number of affected bones was significantly greater in tertile 2 compared with tertile 1 (standardized mean difference 0.20 [0.08–0.31], $p = 0.0007$) and greater still in tertile 3 compared with tertile 1 (standardized mean difference 0.27 [0.15–0.38], $p = 0.00001$) (Fig. 2). A similar effect was noted for disease severity score, which was greater in tertile 2 than in tertile 1 (0.15 [0.03–0.26], $p = 0.01$) and greater still in tertile 3 compared with tertile 1 (0.25 [0.13–0.36], $p < 0.0001$) (Fig. 3). There was no significant association between fractures through Pagetic bone, use of a hearing aid and Paget's disease of the skull, orthopaedic surgery for Paget's disease, or bone deformity and risk allele score (Supplementary Figures S1–S4). However, the number of previous courses of bisphosphonates received was significantly greater for those within tertile 3 as opposed to tertile 1 (0.22 [0.10–0.33], $p = 0.0002$) (Supplemental Fig. S5). Analysis of individual loci for PDB susceptibility in relation to disease extent and severity in the whole cohort of *SQSTM1*-negative subjects revealed weakly positive associations for some markers (Supplemental Table S1). These included rs104941, rs2458413, rs1561570, and rs3018362 with the number of affected bones ($p = 0.048$, $p = 0.012$, $p = 0.030$, and $p = 0.043$, respectively) and rs2458413 for disease severity ($p = 0.041$).

Figure 2.



Risk allele score and number of affected bones. Meta-analysis of association between risk alleles and number of affected bones in *SQSTM1*-mutation-negative individuals using a fixed effects model, comparing tertile 1 with tertile 2 (*A*) and tertile 1 with tertile 3 (*B*).

Figure 3.



Risk allele score and disease severity score. Meta-analysis of association between risk alleles and disease severity score in *SQSTM1*-mutation-negative individuals using a fixed effects model, comparing tertile 1 with tertile 2 (*A*) and tertile 1 with tertile 3 (*B*).

Interaction between *SQSTM1* mutations, risk alleles, and disease severity

Because we and others have previously reported that *SQSTM1* mutations influence severity of PDB,[8, 12, 22] we looked for evidence of an additive effect of risk allele score and *SQSTM1* mutations in predicting disease severity. We analyzed the relationship between risk allele score, *SQSTM1* mutations, number of affected bones, and overall severity using a general linear model ANOVA, entering age, sex, and study center into the model for the whole study population. The results are summarized in Table 2. For number of affected bones, male gender ($p < 0.0001$), risk allele category ($p < 0.0001$), *SQSTM1* mutations ($p < 0.0001$), and study center ($p < 0.0001$) were all significant predictors. For disease severity score, significant predictors were male gender ($p < 0.0001$), family history of Paget's ($p = 0.001$), *SQSTM1* mutations ($p < 0.0001$), risk allele category ($p < 0.0001$), center ($p = 0.002$ to 0.0001), and age ($p = 0.01$, beta coefficient 0.005). The effect size of *SQSTM1* mutations on number of affected bones and overall disease severity as reflected by the beta coefficients was about three times larger than that of the risk allele score.

Table 2. Predictors of Disease Severity and Extent in the Whole Study Population

	No. of affected bones			Disease severity score		
	Mean ± SD	**Beta**	***p* Value**	**Mean ± SD**	**Beta**	***p* Value**
Gender						
Female	2.67 ± 0.07	−0.137	<0.0001	6.88 ± 0.12	−0.199	<0.0001
Male	2.95 ± 0.07	—		7.28 ± 0.12	—	
Family history						
No	2.72 ± 0.06	−0.092	0.055	6.80 ± 0.11	−0.277	0.001
Yes	2.90 ± 0.09	—		7.36 ± 0.15	—	
SQSTM1 + ve						
No	2.15 ± 0.05	−0.659	<0.0001	6.20 ± 0.09	−0.883	<0.0001
Yes	3.47 ± 0.11	—		7.97 ± 0.18	—	
Risk allele category						
Tertile 1	2.61 ± 0.08	−0.200	<0.0001	6.80 ± 0.13	−0.282	<0.0001
Tertile 2	2.91 ± 0.08	0.105	0.025	7.16 ± 0.13	0.080	0.303
Tertile 3	2.90 ± 0.08	—		7.28 ± 0.13	—	
Center						
Florence	2.10 ± 0.09	−0.709	<0.0001	6.72 ± 0.16	−0.365	0.002
PRISM	2.42 ± 0.07	−0.386	<0.0001	5.98 ± 0.12	−1.105	<0.0001
Salamanca	3.38 ± 0.12	0.566	<0.0001	7.57 ± 0.19	0.490	0.001
Naples/Siena	3.00 ± 0.09	0.188	0.009	7.82 ± 0.16	0.740	<0.0001
Western Australia	3.15 ± 0.10	—		7.32 ± 0.18	—	

1. The data shown are least square means (SD) from a general linear model analysis of predictors of disease extent in the whole study population. The beta-coefficients (Beta) refer to the effect size of the indicated variable on the response variable (no. of affected bones or disease severity score). The *p* values refer to differences between subgroups in each category as compared with the reference subgroup, which is indicated by the (—) symbol.

Because both risk allele score and *SQSTM1* were independent predictors of disease extent and severity, we combined information from both markers to create a genetic risk score, dividing patients into three groups: group 1 (*SQSTM1* negative and tertile 1 of risk allele score); group 2 (*SQSTM1* negative and tertiles 2 and 3 of risk allele score); and group 3 (*SQSTM1* positive and tertiles 1 to 3 of risk allele score). Analysis of various markers of disease severity in the whole study population showed a highly significant and stepwise increase in disease extent, severity, and number of previous bisphosphonates given for Paget's in relation to the genetic risk score (Table 3). When we defined “severe disease” as a disease severity score of 7 or greater (representing the top 20% of the whole population), there was a stepwise increase in severity according to genetic risk score. In terms of specificity and sensitivity, the highest category of genetic risk score had 70% specificity and 55% sensitivity for predicting severe disease.

Table 3. Combined Genetic Risk Score and Markers of Severity in the Whole Study Population

Variable	Low risk (*n* = 570)	Medium risk (*n* = 1190)	High risk (*n* = 180)	*p* Value
No. of affected bones	1.74 ± 1.19	2.04 ± 1.48	3.20 ± 2.48	<0.0001
Disease severity score	5.44 ± 2.15	5.86 ± 2.59	7.58 ± 3.67	<0.0001
Severe disease (score >7.0)	146 (25.6%)	378 (31.7%)	99 (55.0%)	<0.0001
No. of previous bisphosphonates	1.12 ± 1.04	1.23 ± 1.00	1.50 ± 1.11	<0.0001
Bone deformity	154 (30.8%)	346 (33.1%)	58 (35.6%)	0.46
Deafness owing to Paget's	49 (9.8%)	95 (9.1%)	23 (14.1%)	0.13
Fracture in Pagetic bone	45 (7.9%)	113 (9.5%)	22 (12.2%)	0.20
Orthopedic surgery	57 (11.4%)	133 (12.7%)	23 (14.1%)	0.59

1. Values are mean ± SD or number (%). The *p* values refer to the differences between the genotype groups assessed by ANOVA or chi-square test. Those in the low-risk group were *SQSTM1* negative and tertile 1 of the risk allele score; the medium risk group *SQSTM1* negative and tertiles 2 to 3 of the risk allele score; and the high-risk group *SQSTM1* positive and tertiles 1 to 3 of the risk allele score.

Role of *SQSTM1* mutations and risk alleles as predictors of treatment response

In view of the fact that the genetic risk score was a strong predictor of disease extent and severity, we wanted to determine if this also influenced treatment response. This analysis was restricted to the PRISM study, where we had prospective data on treatment response. The results are summarized in Table 4, which showed that although the genetic risk score strongly predicted disease extent and severity at the baseline visit, it was not significantly associated with the response to bisphosphonate treatment in terms of change in ALP activity or change in quality-of-life measures as assessed by the SF36 score or with the total dose of various bisphosphonates received during the study. This suggests that patients at increased genetic risk of severe disease do not have an impaired response to bisphosphonate therapy.

Table 4. Combined Genetic Risk Score and Response to Treatment in the PRISM Study

Variable	Low risk (*n* = 220)	Medium risk (*n* = 468)	High risk (*n* = 82)	*p* Value
Symptomatic treatment	105 (47.7%)	229 (48.9%)	43 (52.4%)	0.76
No. of affected bones at baseline	1.66 ± 0.92	1.83 ± 0.97	2.48 ± 1.22	<0.0001
Disease severity score at baseline	4.85 ± 2.27	4.99 ± 2.29	6.22 ± 2.63	<0.0001
Received bisphosphonate during study	134 (60.9%)	289 (61.7%)	50 (62.2%)	0.97
Total dose risedronate (g)	2.18 ± 3.24	2.00 ± 3.34	2.17 ± 3.64	0.79
Total dose etidronate (g)	0.29 ± 3.12	1.19 ± 8.72	2.98 ± 21.5	0.10
Total dose pamidronate (mg)	45.0 ± 156	66.9 ± 202.8	39.7 ± 107.5	0.21
Total dose tiludronate (g)	4.94 ± 20.0	6.51 ± 23.5	7.56 ± 28.2	0.60
Change in ALP	−0.34 ± 0.90	−0.32 ± 0.97	−0.39 ± 1.11	0.39
Change in SF36 pain score	−0.53 ± 8.81	0.12 ± 9.7	−0.88 ± 11.2	0.59
Change in SF36 physical summary	−4.27 ± 9.0	−4.62 ± 9.7	−4.98 ± 10.6	0.86
Change in SF36 mental summary	−1.56 ± 10.1	−2.41 ± 10.8	−3.74 ± 12.1	0.39

1. Values are mean ± SD or number (%). The *p* values refer to the differences between the genotype groups assessed by ANOVA or chi-square test. The ALP values have been standardized to the upper limit of the reference range, which was set at 1.0.

Discussion

Genetic factors play an important role in regulating susceptibility to PDB, but less is known about the genetic determinants of disease severity. We previously reported that in the PRISM study, *SQSTM1* mutations were associated with more severe and extensive disease and a higher incidence of certain complications.[22] Positive associations between *SQSTM1* mutations and disease extent have been

observed in other cohorts,[8, 12, 18] and we recently reported that the valine to alanine variant at codon 192 of the receptor activator of nuclear factor κB protein (RANK) was associated with increased disease severity in Italian patients with PDB, with particularly strong effects in patients who also carried *SQSTM1* mutations.[24]

The results of the present study confirm and extend these observations in showing that the risk alleles that we previously found to predispose to PDB by genome-wide association study[15, 16] are also associated with disease extent and severity in several populations. When we analyzed the association between PDB susceptibility alleles in *SQSTM1*-negative patients from the PRISM study, we found an allele dose effect of risk allele score on disease extent and a trend for association with increased disease severity. The positive association between risk allele score and disease extent was confirmed by meta-analysis of this data combined with that derived from *SQSTM1*-negative patients from Italy, Spain, and Western Australia. Here, we found a strong association between risk allele score and both disease extent and severity with evidence of an allele dose effect for both outcomes. In addition, we found that subjects in the highest tertile of risk allele score had received a greater number of courses of bisphosphonate therapy for PDB than those in the lowest category, which is in keeping with that observed in the PRISM study cohort.

Further analysis that included *SQSTM1*-negative and *SQSTM1*-positive patients from all these cohorts revealed that risk allele score and *SQSTM1* mutations acted in an additive manner to predict disease extent and severity, although the effect size of *SQSTM1* mutations was about three times as great as that of the risk allele score. When we combined information from *SQSTM1* mutations and risk allele score, we were able to define three distinct subgroups of patients with markedly differing disease extent and severity. Importantly, however, we found no difference in response of ALP or quality-of-life measures in response to therapy during the PRISM study according to the combined genetic risk score. This indicates that patients at high genetic risk of severe PDB show no evidence of resistance to the therapeutic effects of bisphosphonates, raising the possibility that complications associated with increased disease severity could be preventable.

Although the results presented here show that genetic testing for *SQSTM1* mutations and other susceptibility alleles can define subgroups of patients with different levels of disease severity, further research will now be required to define how this information should best be used in clinical practice. The predictive value of the genetic markers tested here was modest with a sensitivity of 70% and specificity of 55% for predicting severe disease. This illustrates that the factors that influence disease severity and extent of PDB are incompletely understood. It is likely that identification of the causal genetic variants in the loci identified by GWAS will improve the performance of the genetic risk analysis outlined here. There is strong evidence that environmental factors also influence the severity and extent of PDB,[25, 26] and it could be that environmental factors could have contributed to the development of severe disease in patients with a low genetic risk score. The identity of these risk factors remains poorly understood, but possibilities include infectious agents, nutritional factors, an urban as opposed to a rural lifestyle, and a more sedentary lifestyle with reduced mechanical loading of the skeleton and fewer skeletal injuries.[27] Further work will clearly be required to determine how information from genetic markers and environmental risk factors can be combined to improve the identification of patients with mild and severe disease.

Previous studies have shown that potent bisphosphonates such as zoledronic acid and risedronate are highly effective at suppressing bone turnover in established PDB[28, 29] and that zoledronic acid gives prolonged suppression of ALP levels for up to 6.5 years after a single infusion.[30] Because these drugs are generally safe and highly effective, there would be little clinical need for genetic markers to target treatment in patients already diagnosed with PDB. In clinical practice, however, it is common to encounter patients who have already developed complications or irreversible skeletal damage by the time they first present.[31] It is, therefore, possible that with further refinement and independent validation, genetic profiling as described here could be of value in identifying subjects at increased risk of developing PDB (such as those with a positive family history) and in prioritizing these subjects for enhanced surveillance and possibly even prophylactic bisphosphonate therapy. It is important to emphasize, however, that use of

prophylactic bisphosphonate therapy in the absence of symptoms would need to be carefully evaluated to ensure that the benefits of treatment outweigh the potential risks. In this regard, it is relevant to point out that a trial is now in progress, called the ZiPP study (Zoledronate in the Prevention of Paget's disease; ISRCTN11616770), which aims to explore the risks and benefits of prophylactic zoledronic acid therapy versus placebo in patients with *SQSTM1* mutations who have not yet been diagnosed with PDB. It would be of great interest to evaluate the effect of these new markers within this study to determine if they affect penetrance, extent, and severity of the disease in *SQSTM1* mutation carriers.

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