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Genome-wide association identifies three new susceptibility loci for Paget's disease of bone

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Paget's disease of bone (PDB) is a common disorder characterized by focal abnormalities of bone remodeling. We previously identified variants at the *CSF1*, *OPTN* and *TNFRSF11A* loci as risk factors for PDB by genome-wide association study[1]. Here we extended this study, identified three new loci and confirmed their association with PDB in 2,215 affected individuals (cases) and 4,370 controls from seven independent populations. The new associations were with rs5742915 within *PML* on 15q24 (odds ratio (OR) = 1.34, $P = 1.6 \times 10^{-14}$), rs10498635 within *RIN3* on 14q32 (OR = 1.44, $P = 2.55 \times 10^{-11}$) and rs4294134 within *NUP205* on 7q33 (OR = 1.45, $P = 8.45 \times 10^{-10}$). Our data also confirmed the association of *TM7SF4* (rs2458413, OR = 1.40, $P = 7.38 \times 10^{-17}$) with PDB. These seven loci explained ~13% of the familial risk of PDB. These studies provide new insights into the genetic architecture and pathophysiology of PDB.

PDB is a common skeletal disorder with a strong genetic component that affects up to 2% of individuals of European ancestry aged 55 years and above[2,3]. Mutations of *SQSTM1* are known to cause a high-penetrance form of PDB which is clinically severe[4] and occurs in about 40% of individuals with a family history of the disorder[5,6]. We recently identified additional susceptibility alleles for PDB at the *CSF1*, *OPTN* and *TNFRSF11A* loci by a genome-wide association study (GWAS) involving 692 cases with PDB and 1,001 controls and a replication cohort of 481 cases and 520 controls[1]. In order to identify additional susceptibility loci for the disease, we performed an extended GWAS involving a total of 749 cases with PDB of British descent in whom *SQSTM1* mutations had been excluded and 2,930 British controls derived from the 1958 Birth Cohort[7] with replication in a further 1,474 cases and 1,671 controls from six independent populations.

After applying quality control measures and excluding samples of non-European ancestry, the extended cohort (henceforth referred to as the GWAS stage) comprised 741 cases and 2,699 controls with genotype information for 290,115 SNPs, providing a fourfold increase in power to detect loci of moderate effect size (OR ≥ 1.4) compared with our previous study[1]. To increase SNP coverage, we performed genome-wide SNP imputation for the GWAS stage samples using phased haplotype data from the HapMap project as a reference. The results of the association testing of genotyped and imputed SNPs (a total of 2,487,078 SNPs) from the GWAS stage are shown in **Figure 1**. A locus on chromosome 8q22.3 showed genome-wide evidence of association with PDB ($P < 5.0 \times 10^{-8}$), in addition to the previously identified genome-wide significant loci on 1p13.3, 10p13 and 18q21.33 (ref. 1).

In the second stage of this study, we analyzed the highest ranking SNPs observed in the GWAS stage ($P \leq 5 \times 10^{-5}$) for replication after excluding those in linkage disequilibrium (LD; $r^2 > 0.8$ or

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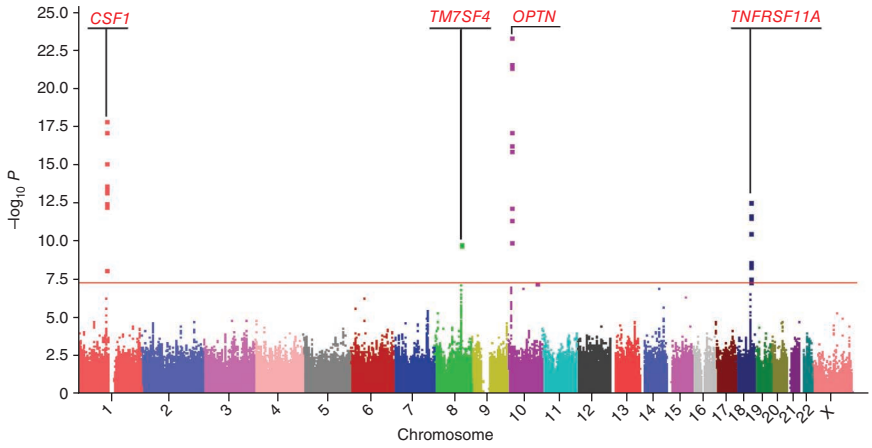

Figure 1 Loci for susceptibility to PDB detected by GWAS. Manhattan plot of association test results of GWAS stage data showing the chromosomal position of 2,487,078 genotyped or imputed SNPs plotted against genomic-control–adjusted $-\log_{10} P$. The red horizontal line represents the threshold for genome-wide significance ($P < 5 \times 10^{-8}$).

$D' > 0.95$) with the highest ranking SNP from each region. We genotyped a total of 27 SNPs in the replication cohorts, which consisted of 1,474 *SQSTM1*-negative cases with PDB from six different geographic regions and 1,671 unaffected controls from the same regions that were matched with the cases by gender, as described in the Online Methods section and **Supplementary Table 1**. We performed a meta-analysis of data from the GWAS stage and the individual replication cohorts, and the results are summarized in **Supplementary Table 2**. This strengthened the association with PDB for the *CSF1*, *OPTN* and *TNFRSF11A* loci that we identified in our previous study[1] and confirmed the association with the 8q22.3 locus that was suggestively associated with PDB in our previous GWAS[1] and which was confirmed to be associated with PDB in a small study of Belgian and Dutch subjects[8]. Furthermore, we identified three additional genome-wide significant loci on 7q33, 14q32.12 and 15q24.1 in the combined dataset ($P < 5 \times 10^{-8}$; **Table 1** and **Fig. 2**).

The strongest signal on 8q22.3 was with rs2458413 (combined $P = 7.38 \times 10^{-17}$, OR = 1.4). There was no significant heterogeneity between the study groups (**Table 1**, **Fig. 3** and **Supplementary Table 3**), and the direction of association was similar in all cohorts. The associated region spans ~220 kb, but the SNPs with the highest association signal appear to cluster within an 18-kb LD block spanning the entirety of *TM7SF4*, the transmembrane 7 superfamily member 4 gene (**Fig. 2** and **Supplementary Fig. 1**). This gene encodes the dendritic-cell–specific transmembrane protein (DC-STAMP)[9], which is a strong functional candidate gene for PDB because it is required for the fusion of osteoclast precursors to form mature osteoclasts[10]. Previous studies have shown that RANKL-induced DC-STAMP expression is essential for osteoclast formation[11], and a recent study showed that the connective tissue growth factor CCN2 stimulates osteoclast fusion through interaction with DC-STAMP[12]. Because osteoclasts from individuals with PDB are larger in size and contain more nuclei than normal osteoclasts, it seems likely that the genetic variants that predispose to PDB do so by enhancing *TM7SF4* expression or by causing gain of function at the protein level, but further studies will be required to investigate these possibilities.

The first new locus for PDB susceptibility was on 7q33, which is tagged by rs4294134 (combined $P = 8.45 \times 10^{-10}$, OR = 1.45). The direction of association was similar in all study cohorts, and analysis of the combined dataset showed no evidence for heterogeneity between study groups (**Table 1**, **Fig. 3** and **Supplementary Table 3**). The associated region spans ~350 kb (**Fig. 2**), but the strongest signal was with rs4294134, located within the twenty-second intron of *NUP205*. This gene encodes nucleoporin 205 kDa, which is one of the main components of the nuclear pore complex involved in the regulation of transport between the cytoplasm and nucleus[13]. All SNPs with $P < 1 \times 10^{-5}$ in the 350-kb associated region were in moderate to strong LD with rs4294134 ($r^2 \geq 0.5$, $D' \geq 0.95$), with the exception of two SNPs (rs3110788 and rs3110794) that were poorly correlated with rs4294134 ($r^2 \leq 0.21$, $D' \geq 0.95$; **Fig. 2**). Conditional analysis in the GWAS stage indicated that the association signal appeared to be driven by rs4294134 ($P = 8.8 \times 10^{-3}$) after adjusting for rs3110788 ($P = 0.31$) and rs3110794 ($P = 0.10$). None of the genes located in this region are known to affect bone metabolism, and further studies will be required to identify the functional variant(s) responsible for association with PDB.

The second new susceptibility locus was located on 14q32.12 and was tagged by rs10498635. This SNP showed borderline evidence of association with PDB in our previous study ($P = 9.69 \times 10^{-8}$)[1] but reached genome-wide significance in the present study (combined $P = 2.55 \times 10^{-11}$, OR = 1.44). Association testing showed no evidence for heterogeneity between the study groups (**Table 1**, **Fig. 3** and **Supplementary Table 3**). The 62-kb associated region is bounded by two recombination hotspots and contains *RIN3* (**Fig. 2**), which encodes the Ras and Rab interactor 3, a protein that plays a role in vesicular trafficking through

Table 1 Summary of the seven loci showing genome-wide significant association with Paget's disease of bone

			GWAS Stage		Replication		Combined overall effect				
					Fixed effect		Fixed effect		Heterogeneity		
Chr.	SNP	RA	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	P_{het}	I^2	Closest gene
1	rs10494112	G	5.83×10^{-17}	1.75 (1.54–1.99)	4.93×10^{-19}	1.69 (1.50–1.89)	7.06×10^{-35}	1.72 (1.57–1.87)	0.97	00.0	*CSF1, EPS8LS*
7	**rs4294134**	**G**	$\mathbf{1.20 \times 10^{-5}}$	**1.50 (1.25–1.79)**	$\mathbf{2.29 \times 10^{-5}}$	**1.42 (1.20–1.66)**	$\mathbf{8.45 \times 10^{-10}}$	**1.45 (1.29–1.63)**	**0.83**	**00.0**	**NUP205**
8	rs2458413	A	7.85×10^{-11}	1.51 (1.34–1.71)	1.09×10^{-7}	1.32 (1.19–1.46)	7.38×10^{-17}	1.40 (1.29–1.51)	0.10	44.3	*TM7SF4*
10	rs1561570[a]	T	9.56×10^{-18}	1.71 (1.51–1.93)	2.09×10^{-21}	1.64 (1.48–1.81)	4.37×10^{-38}	1.67 (1.54–1.80)	0.01	65.7	*OPTN*
14	**rs10498635**	**C**	$\mathbf{1.51 \times 10^{-5}}$	**1.45 (1.23–1.71)**	$\mathbf{5.64 \times 10^{-7}}$	**1.42 (1.29–1.63)**	$\mathbf{2.55 \times 10^{-11}}$	**1.44 (1.29–1.60)**	**0.62**	**00.0**	**RIN3**
15	**rs5742915**	**C**	$\mathbf{1.40 \times 10^{-7}}$	**1.38 (1.22–1.54)**	$\mathbf{3.99 \times 10^{-8}}$	**1.32 (1.20–1.46)**	$\mathbf{1.60 \times 10^{-14}}$	**1.34 (1.25–1.45)**	**0.56**	**00.0**	**PML**
18	rs3018362	A	1.87×10^{-11}	1.50 (1.34–1.69)	1.27×10^{-10}	1.40 (1.26–1.55)	7.98×10^{-21}	1.45 (1.34–1.56)	0.46	00.0	*TNFRSF11A*

RA, risk allele; OR, odds ratio for the risk allele; CI, confidence interval; I^2, heterogeneity statistics; P_{het}, *P* value for heterogeneity. Newly identified loci are shown in bold. [a]rs1561570 showed significant heterogeneity but random-effect results were genome-wide significant ($P = 4.34 \times 10^{-12}$, OR = 1.68).

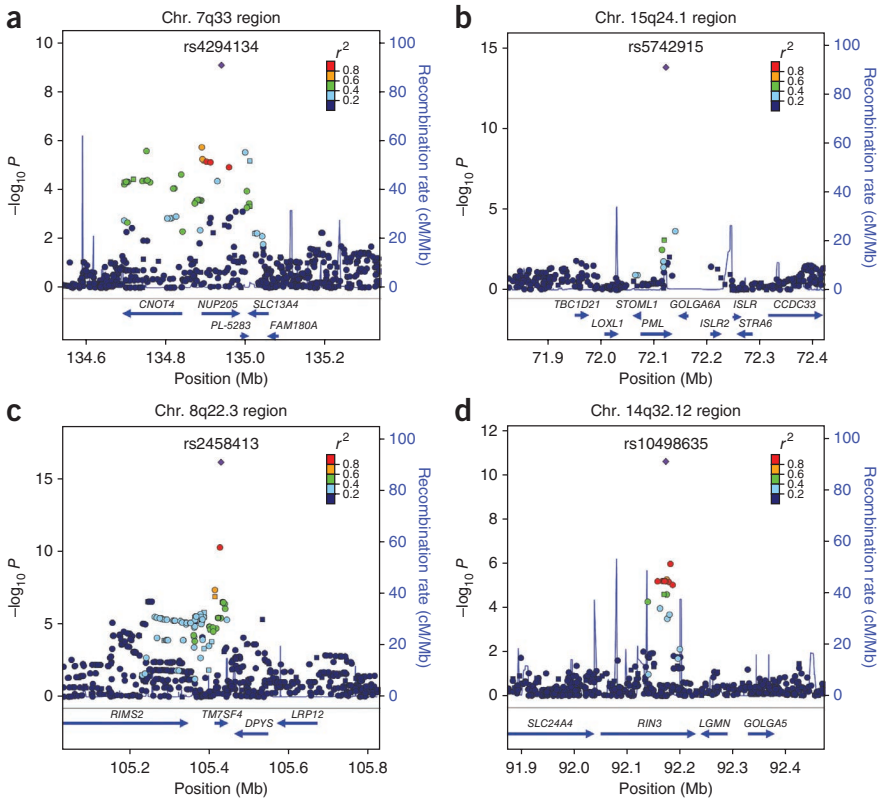

Figure 2 Regional association plots of loci showing genome-wide significant association with PDB. (**a**–**d**) Details of loci on chromosome 7q33 (**a**), 15q24.1 (**b**), 8q22.3 (**c**) and 14q32.12 (**d**) showing the chromosomal position (based on NCBI human genome build 36) of SNPs in each region plotted against $-\log_{10}$ *P* values. Genotyped (squares) and imputed (circles) SNPs are color coded according to the extent of LD with the SNP showing the highest association signal (represented as purple diamonds) from each region in the combined analysis. The estimated recombination rates (cM/Mb) from HapMap CEU release 22 are shown as light blue lines, and the blue arrows represent known genes in each region. We defined the associated regions based on LD with the highest association signal ($r^2 > 0.2$) within a window of 500 kb.

interaction with small GTPases such as Ras and Rab[14,15]. The function of *RIN3* in bone metabolism is currently unknown, but it could play a role in bone resorption in view of the importance that small GTPases play in vesicular trafficking and in osteoclast function[16,17]. It is of interest to note that mutations affecting VCP, a protein also involved in vesicular trafficking, cause the syndrome of inclusion body myopathy with early onset Paget's disease and frontotemporal dementia[18].

The third new susceptibility locus was located on 15q24.1, and the strongest association was with rs5742915 (combined $P = 1.60 \times 10^{-14}$; OR = 1.34; **Table 1**, **Fig. 3** and **Supplementary Table 3**). The associated region is bounded by two recombination hotspots and spans ~200 kb, but we observed a gap spanning ~40 kb in this region with no SNP coverage in the Illumina arrays or the HapMap European CEU population. The associated SNPs were clustered within *PML*, the promyelocytic leukemia gene (**Fig. 2**), and we observed the strongest signal for rs5742915, which results in a phenylalanine to leucine amino acid change at codon 645 (p.Phe645Leu) of PML. The function of *PML* in bone metabolism is unclear, but it is known to be involved in TGF-β signaling[19]. Accordingly, researchers from a previous study showed that cells from *Pml* knockout mice were resistant to TGF-β–dependent growth arrest and apoptosis and had impaired induction of TGF-β target genes[19]. Because TGF-β is known to play a role in the regulation of bone remodeling, it is possible that the association between PDB and *PML* could be mediated by an effect on TGF-β signaling, but further research will be required to investigate this possibility. *GOLGA6A* is also located in the associated region and encodes a protein that belongs to golgin, a family of coiled-coil proteins associated with the Golgi apparatus and which play a role in membrane fusion and as structural supports for the Golgi cisternae. This gene is located in the 40-kb gap region that contains a large low-copy repeat sequence. Although *GOLGA6A* has no known role in bone metabolism, mutations in other members of the golgin family have been shown to cause lethal skeletal dysplasia[20] and a severe form of osteoporosis[21].

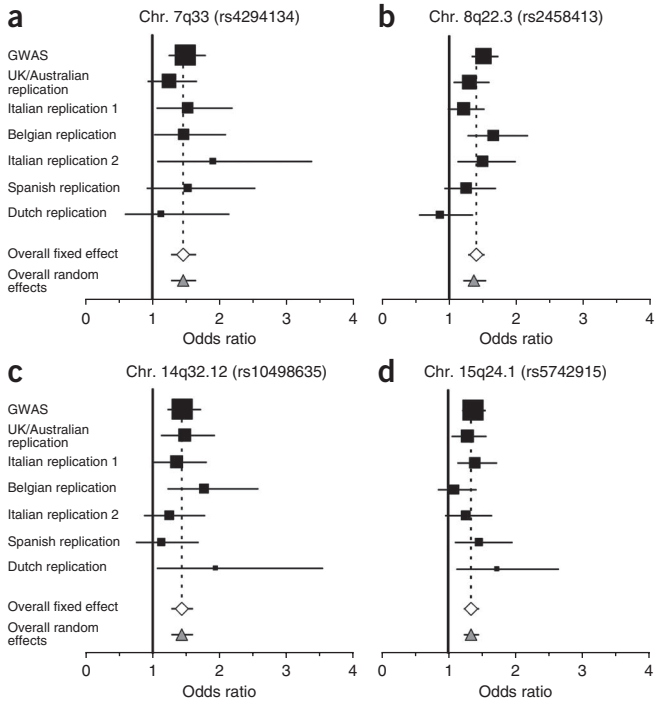

Figure 3 Forest plots showing association in the different datasets for SNPs at 7q33, 8q22.3, 14q32.12 and 15q24.1. (**a**–**d**) Forest plots of overall effect size for SNPs associated with PDB risk from the identified loci on 7q33 (rs4294134) (**a**), 8q22.3 (rs2458413) (**b**), 14q32.12 (rs10498635) (**c**) and 15q24.1 (rs5742915) (**d**). We estimated the overall effect size using meta-analysis of the GWAS sample and the six replication samples. The black squares represent the effect estimates for the individual cohorts, and the horizontal lines represent the 95% CIs of the estimates. The sizes of the squares are proportionate to the weights of the estimates. The diamonds and triangles represent the overall estimate under fixed-effect and random-effect models, respectively. The dotted vertical lines represent the overall fixed effect estimates.

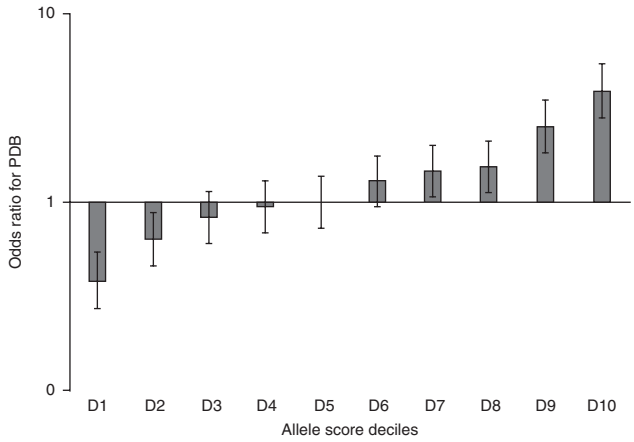

Figure 4 Cumulative contribution of genome-wide significant loci to the risk of PDB. Risk allele scores defined by the seven loci associated with PDB risk are plotted against the OR for PDB. We weighted risk alleles according to their estimated effect size and divided weighted risk allele scores into ten equal parts (deciles) using data from the replication cohorts. We calculated the OR for PDB risk for each decile in reference to the fifth decile (D5). Vertical bars represent 95% CIs.

We were also able to replicate our previously reported association between variants at the *CSF1*, *OPTN* and *TNFRSF11A* loci and PDB in the present study[1]. The results of our meta-analysis of the combined dataset for these loci are shown in **Table 1** and **Supplementary Figure 2**, which provide conclusive evidence for association of variants at *CSF1* ($P = 7.06 \times 10^{-35}$), *OPTN* ($P = 4.37 \times 10^{-38}$) and *TNFRSF11A* ($P = 7.98 \times 10^{-21}$) with PDB. We observed evidence of heterogeneity between the study groups for rs1561570 ($I^2 = 65.7\%$, $P_{het} = 0.01$) at *OPTN*, but this was because of differences in effect size rather than the direction of effect, and the association remained genome-wide significant after accounting for heterogeneity ($P = 4.34 \times 10^{-12}$, OR = 1.68). The heterogeneity was caused by the larger effect size observed in the Dutch cohort (**Supplementary Fig. 2**) and possibly because of the small sample size of this cohort. These observations provide highly robust evidence for association between these loci and PDB and extend those recently reported[8] in the Dutch and Belgian populations, which were also included in the present study.

We next wanted to determine if the identified loci on 15q24.1, 7q33 and 14q32.12 interacted with each other or with the previously identified loci on 1p13.3, 8q22.3, 10p13 and 18q21.33 to affect the risk of PDB. Pairwise interaction analysis showed weak evidence for interaction of 7q33 (rs4294134) with 8q22.3 (rs2458413, $P = 0.03$) and 10p13 (rs1561570, $P = 0.02$). However, these interactions were not significant after adjusting for multiple testing, and none of the other loci showed evidence for interaction ($P > 0.05$), suggesting a multiplicative model of association with PDB risk. In order to estimate the effect size of the identified loci on the development of PDB, we calculated the proportion of familial risk explained by the genome-wide significant loci in the replication sample assuming a sibling relative risk for PDB of 7.0 (ref. 22). This showed that the proportion of familial risk explained was ~13%, which is much greater than that observed for other common bone diseases, such as osteoporosis[23]. We also estimated the cumulative population attributable risk of these loci in the replication cohort and found it to be 86%, and we found that the risk of PDB increased with the increasing number of risk allele scores defined by the seven loci (per risk allele OR = 1.44, 95% CI 1.38–1.51, $P = 5.4 \times 10^{-57}$). When we weighted allele scores according to their estimated effect size, we found that subjects in the top 10% of the allele score distribution (D10; $n = 315$) had a 10.1-fold (95% CI 7.0–14.6, $P = 2.4 \times 10^{-39}$) increase in risk of developing PDB compared to those in the bottom 10% of the distribution (D1; $n = 315$) from the replication dataset (**Fig. 4**). Although these data suggest that a large part of the genetic risk of PDB in individuals without *SQSTM1* mutations is accounted for by these loci, we acknowledge that the functional variants need to be identified before we can precisely estimate the contribution that these loci make to the risk of developing PDB. To assess the functional effect of the identified SNPs on gene expression, we tested the association between the top PDB-associated SNPs (or those in LD with these SNPs; $D' \geq 0.8$) from each of the seven loci and *cis*-allelic expression of genes located in the associated regions using publicly available expression quantitative trait loci (eQTL) data. This showed highly significant associations for transcripts of *TM7SF4* (rs2458415, expression $P = 1.22 \times 10^{-18}$) and *OPTN* (rs1561570, expression $P = 6.61 \times 10^{-62}$) in peripheral blood monocytes[24], suggesting that the association with PDB risk for these loci could be mediated by influencing gene expression levels.

In addition to the loci mentioned above, we identified additional variants that showed suggestive evidence for association with PDB. For example, a locus on chromosome Xq24 showed borderline evidence for association with PDB (rs5910578 within *SLC25A43*, combined $P = 1.26 \times 10^{-7}$, OR = 1.34), as did another locus on chromosome 6p22.3 (rs1341239 near *PRL*, combined $P = 3.83 \times 10^{-6}$, OR = 1.20; **Supplementary Table 2**). Given that we observed six genotyped variants with $P < 1 \times 10^{-5}$ in the GWAS stage after removal of confirmed SNPs and associated variants when only three are expected by chance (**Supplementary Fig. 3**), it is likely that some of the associations observed are true, but our study was not sufficiently powered to detect them at a genome-wide significant level.

This study has been successful in identifying seven loci that contribute substantially to the risk of developing PDB. The identified loci have relatively large effect sizes compared to other common diseases of the musculoskeletal system such as osteoporosis and rheumatoid arthritis. This indicates that susceptibility to PDB is probably mediated by inheritance of a relatively small number of genes with large effect sizes as opposed to a large number of genes with small effect sizes, as seen in other complex diseases. Many of the susceptibility variants are within or close to genes that are known to play important roles in regulating osteoclast differentiation and function, whereas other variants are within genes not previously implicated in the regulation of bone metabolism. Although further work will be required to identify functional variants, the present study has provided new insights into the genetic architecture of PDB and has identified several genes that were not previously suspected to play a role in bone metabolism. Finally, the large effect size of the variants identified means that it may be possible in the future to identify people at risk of developing PDB by genetic profiling.

METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/naturegenetics/.

URLs. Wellcome Trust Case Control Consortium, http://www.wtccc.org.uk/.

Note: Supplementary information is available on the Nature Genetics website.

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AUTHOR CONTRIBUTIONS

O.M.E.A. contributed to the study design and funding, oversaw the genotyping, performed data management, quality control, statistical and bioinformatics analyses, and wrote the first draft of the manuscript. S.H.R. designed the study, obtained funding, coordinated the sample collection and phenotyping, and revised the manuscript. K.G., M.L.B., T.C., P.Y.J.C., R.D., J.-P. D., A.F., W.D.F., L.G., F.G., M.J.H., W.V.H, G.I., G.C.N., R.N., S.P., J.d.P.M., T.R., S.L.R, D.R., R.G.-S., M.d.S., L.C.W. and J.P.W. contributed toward clinical sample collection and phenotyping. M.R.V., N.A., S.E.W., R.G.-S., P.Y.J.C. and F.G. contributed to sample preparation and carried out DNA sequencing to identify samples with *SQSTM1* mutations. All authors critically reviewed the article for important intellectual content and approved the final manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare competing financial interests: details accompany the full-text HTML version of the paper at http://www.nature.com/naturegenetics/.

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ONLINE METHODS

GWAS stage study subjects. This study describes an extension to our previously reported GWAS of PDB, in which we used genotype data from 692 cases with PDB from our previously described study[1] and extended the case group by genotyping an additional 57 cases with PDB. The additional cases were selected from recently recruited subjects in the PRISM study[25], which was a randomized trial of two different treatment strategies for cases with PDB from the UK. We also increased the size of the control group by using genotype data from 2,930 subjects from the British 1958 Birth Cohort genotyped by the Wellcome Trust Case Control Consortium[7]. This control group is a better match to our cases with PDB than the previous controls, which were recruited from Scotland[1] because, like the PRISM participants, they were recruited from all over the UK. The extended samples size used in this study provided 90% power to detect disease-associated alleles with minor allele frequency of 0.2 and genotype relative risk of 1.4, assuming a multiplicative model and a disease with population prevalence of 2%. This represents a substantial increase in power compared to our previous study[1], in which we had 20% power to detect alleles with genotyped relative risk of 1.4.

GWAS-stage genotyping and quality control. Genotyping and quality control for the 692 cases with PDB were performed using Illumina HumanHap300-Duo arrays as described previously[1]. The additional 57 cases with PDB were genotyped using Illumina Human660W Quad version 1 arrays, and quality control measures were applied as previously described[1]. Briefly, SNPs with call rate <95% were excluded, and samples with call rate <90% ($n = 1$), excess heterozygosity ($n = 1$), and non-European ancestry ($n = 6$; **Supplementary Fig. 4**) were removed before analysis. The genotyping of the British 1958 Birth Cohort was previously performed by the Wellcome Trust Case Control Consortium using the Illumina Human 1.2M Duo custom array (see URLs)[7]. For the control group, SNPs with call rate <95% were excluded, and we removed 231 samples because they failed at least one of the following quality control criteria: low call rate, non-European ancestry, gender mismatch or cryptic relatedness. Population ancestry was determined using multidimensional scaling analysis of identity-by-state distances matrix as previously described[1]. After quality control, we analyzed 741 cases with PDB and 2,699 controls with genotype data for 290,115 SNPs, which were common to the three different genotyping arrays. To ensure consistent genotyping between different platforms, a subset of samples were genotyped using at least two different platforms, and the cross-platform genotype concordance rate was >99.7% (**Supplementary Table 4**). Additionally, the genotype cluster plots for all SNPs showing association with PDB at $P < 1.0 \times 10^{-4}$ were visually inspected in cases and controls, and only high quality genotype data were included in the analysis. Furthermore, genotype call rate for the top associated SNPs was consistent between cases and controls (**Supplementary Table 5**).

Replication samples. The replication study groups were derived from clinic-based individuals with PDB and gender-matched controls selected from the same region. Individuals with *SQSTM1* mutations were excluded, and all study participants provided informed consent. The first replication cohort comprised 175 individuals with PDB from the UK, 8 cases with PDB from Sydney, Australia and 215 cases with PDB from Western Australia. These individuals were of British descent and were matched with 485 unaffected British controls. The second replication cohort (Italian replication cohort 1) comprised 354 cases with PDB and 390 unaffected controls enrolled from various referral centers in Italy who took part in the GenPage project[26]. The third replication cohort (Italian replication cohort 2) comprised 205 Italian cases with PDB and 238 unaffected controls enrolled from referral centers in northern, central and southern Italy as previously described[27]. The fourth replication cohort comprised 246 individuals with sporadic PDB recruited from various referrals centers in Belgium, and these individuals were matched with 263 controls with no clinical evidence of PDB as previously described[8]. The fifth replication cohort comprised 85 individuals with PDB and 93 controls recruited from various centers in The Netherlands, as previously described[8,28]. The sixth replication comprised 186 cases with sporadic PDB recruited from the Salamanca region in the Castilla-Leon region of Spain and 202 unaffected controls from the same region.

Replication sample genotyping and quality control. Genotyping of replication samples was performed by Sequenom using the MassARRAY iPLEX platform. To minimize genotyping bias caused by variations between runs, DNA from cases and controls from the six different replication cohorts were distributed into 384-well plates so that each plate had the same number of cases and controls. We included 4,000 known genotypes as a quality control measure, and the concordance rate between the genotype calls was >99.8%. We removed 64 samples because of low call rate (<90%), and the call rate for all genotyped SNPs was >95%.

Imputation. Genome-wide genotype imputation for autosomal SNPs was performed using MACH[29], and the HapMap European (CEU) phased haplotype data from release 22 were used as a reference. We excluded SNPs with poor imputation quality based on the estimated correlation between imputed and true genotypes ($r^2 < 0.3$). Additionally, a subset (2%) of known genotypes were masked during imputation, and then imputed genotypes were compared with true genotypes, and the average per allele imputation error rate was 2.9%. Imputed SNPs were tested for association using ProbABEL software[30] implementing a logistic regression model in which the allelic dosage of the imputed SNP was used to adjust for uncertainty in imputed genotypes.

Statistical analysis. Statistical analyses were performed using PLINK (Version 1.07)[31] and R (v2.11.1). In the GWAS stage, genotyped SNPs were tested for association with PDB using a standard allelic (1-degree-of-freedom) χ^2 statistic. We also performed association testing using regression models in which we adjusted for gender and population clusters (as determined by multidimensional scaling analysis), but the results were essentially identical to those obtained from the standard allelic test reported here (data not shown). The genomic inflation factor λ_{GC} was calculated based on the 90% least significant SNPs as described previously[32]. The observed test statistic values were corrected using the genomic control method ($\lambda_{GC} = 1.05$; **Supplementary Fig. 3**). Logistic regression was used to test for the independent effects of SNPs where the allelic dosage of the conditioning SNP was entered as a covariate in the regression model. To assess if the reported associations were confounded by age, age of onset or recruitment center, we performed a regression analysis using case-only data from the GWAS stage to test if any of these factors were associated with the top hits using linear regression models. The results of this analysis showed no evidence to suggest that the reported association is confounded by age, age of onset or recruitment center ($P > 0.10$). The cut-off point for genome-wide significance was set as $P < 5 \times 10^{-8}$, as recently proposed[33]. Association testing of replication data was performed in each replication cohort using a standard (1-degree-of-freedom) χ^2 statistic. To assess combined genetic effects, we performed a meta-analysis of all studies using the inverse-variance method assuming a fixed-effect model. We also tested the random-effects model using the DerSimonian-Laird method[34], and between-study heterogeneity was assessed using the Cochran's Q and I^2 metrics. Heterogeneity was considered significant if $P_{het} < 0.05$. The population attributable risk (PAR) for markers showing association with PDB was calculated according to the following formula:

$$\text{PAR} = p(\text{OR} - 1)/(p(\text{OR} - 1) + 1)$$

where p is the frequency of the risk allele in controls, and OR is the risk allele odds ratio. The cumulative PAR was calculated as follows:

$$\text{cumulative PAR} = 1 - (\Pi_{1 \to n}\,(1 - \text{PAR}_i))$$

where n is the number of variants, and PAR_i is the individual PAR for the *i*th SNP. The proportion of familial risk attributable to the identified loci was calculated as previously described[35] assuming a multiplicative model of association and a sibling relative risk $\lambda_s = 7.0$, as estimated from previous epidemiological studies[22]. Regional association plots were generated using the locuszoom tool[36].

eQTL analysis. SNPs showing genome-wide significant association with PDB (or those in strong LD; $D' \geq 0.8$) were tested for association with *cis*-allelic expression of gene transcripts located in the associated regions using publicly available eQTL data[24,37–40]. Only *cis*-acting allelic associations located within 250 kb of either 5′ or 3′ end of the associated gene with expression $P < 1 \times 10^{-5}$ were considered. To avoid false detection, we excluded expression data if the gene probe contained a polymorphic SNP or was located in a highly repetitive sequence.

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