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Genome wide association study identifies variants at *CSF1*, *OPTN* and *TNFRSF11A* as genetic risk factors for Paget's disease of bone

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Abstract

Paget's disease of bone (PDB) is a common disorder with a strong genetic component characterised by focal increases in bone turnover which in some cases is caused by *SQSTM1* mutations. To identify additional susceptibility genes we performed a genome wide association study in 750 PDB cases without *SQSTM1* mutations and 1002 controls and identified three candidate loci for the disease which were replicated in an independent set of 500 cases and 535 controls. The strongest signal was with rs484959 on 1p13 close to the *CSF1* gene ($P = 5.38 \times 10^{-24}$) and significant associations were also observed with rs1561570 on 10p13 within the *OPTN* gene ($P = 6.09 \times 10^{-13}$) and with rs3018362 on 18q21 close to the *TNFRSF11A* gene ($P = 5.27 \times 10^{-13}$). These studies provide new insights into the pathogenesis of PDB and identify *OPTN*, *CSF1* and *TNFRSF11A* as novel candidate genes for disease susceptibility.

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AUTHOR CONTRIBUTIONS OMEA contributed to study design, oversaw the genotyping, performed data management, quality control, statistical and bioinformatics analyses and wrote the first draft of the manuscript. SHR designed the study, obtained funding, co-ordinated the sample collection and phenotyping and revised the manuscript. ALL, TC, RD, WDF, MJH, GI, GCN, JDP, RGS, MDS, JPW contributed toward clinical sample collection and phenotyping. MGD and AT provided genotype data for the stage 1 control samples. MRV and NA assisted in sample preparation and performed DNA sequencing to identify samples with *SQSTM1* mutations. All authors critically reviewed the article for important intellectual content and approved the final manuscript.

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Paget's disease of bone (PDB) is a common disorder of the skeleton that affects up to 2% of Caucasians aged 55 years and above¹. The disease is characterised by focal areas of increased and disorganised bone remodelling which can cause bone pain, bone deformity, pathological fracture, deafness and secondary osteoarthritis². Genetic factors are important in PDB and between 15%-40% of individuals have an affected first degree relative³. Mutations affecting the ubiquitin associated domain of the *SQSTM1* gene have been identified in about 10% of patients with "sporadic" PDB and 40% of patients with a familial PDB^{4,5}. Despite extensive research efforts, and the identification of several susceptibility loci by linkage analysis⁶⁻⁸, the remaining genes that predispose to PDB remain to be identified.

In this study we sought to identify novel genetic variants that predispose to PDB in patients without *SQSTM1* mutations using a genome wide association approach. In the discovery sample (stage 1) we genotyped 750 PDB patients and 1002 controls⁹ using Illumina arrays. In the replication sample (stage 2), we genotyped the most significant SNPs identified from stage 1 in an independent set of 500 cases and 535 controls using the Sequenom MassARRAY iPLEX platform. Details of the subjects used in the discovery and replication stages of the study are provided in the online methods section.

We used multidimensional scaling analysis of identity-by-state (IBS) sharing matrix of all individuals combined with HapMap project samples to assess population ancestry (Supplementary Fig. 1). After application of quality control measures and exclusion of subjects with non-European ancestry, genotypic data in the discovery sample were available for 294,663 SNPs in 692 PDB cases and 1001 controls. Association testing of the discovery stage data was performed using a stratified Cochran-Mantel-Haenszel (CMH) test in which samples were stratified according to their genome-wide IBS sharing similarity. A quantile-quantile plot comparing the observed and expected distribution of $-\log_{10} P$ showed some evidence for inflation of the test statistics given the multiple origin of PDB cases (Genomic inflation factor¹⁰ $\lambda = 1.096$, Supplementary Fig. 2). To exclude the possibility of false positive association due to hidden population substructure, the observed test statistics were corrected using the genomic control method¹⁰. Six SNPs showed genome-wide significant associations with PDB after Bonferroni correction for multiple testing (Fig. 1). Three SNPs were located within a 14kb region of chromosome 1p13.3 (rs10494112, rs499345, and rs484959); one SNP was located on chromosome 10p13 (rs1561570); and two SNPs were located within a 22kb region of chromosome 18q21.33 (rs2957128 and rs3018362; Table 1, Supplementary Table 1 and Fig. 2). We then imputed SNPs located within 1.0Mb of the six SNPs reaching genome wide significance using genotype data from the HapMap project. Association testing of imputed SNPs revealed several variants with genome wide significance close to the six genotyped SNPs identified from stage 1 (Fig. 2). The imputed SNPs showed a slightly stronger association signal than the genotyped markers due to the conservative nature of the stratified CMH test used to analyse genotyped markers compared to the regression methods used to test imputed markers.

In total, 76 SNPs with P -values of 1×10^{-4} or less were identified in the discovery sample (Supplementary Table 2). From these SNPs we selected those with $P < 1.0 \times 10^{-6}$ and those with $P < 1.0 \times 10^{-5}$ in which another SNP within 50kb attained a P -value of 1.0×10^{-3} or less for further analysis in the replication sample. Following application of quality control measures on the replication dataset, genotype data were obtained in 481 cases and 520 controls for the 16 selected SNPs (Supplementary Table 3). Eight SNPs showed significant association with PDB in the replication stage after correction for multiple testing ($P < 3 \times 10^{-3}$), resulting in the identification of 8 SNPs where P -values attained genome wide significance in the combined dataset (Supplementary Table 3). The distribution of minor alleles and the direction of associations were similar in both the discovery and replication

samples (Table 1 and Supplementary Table 3). Whilst all samples used in the replication stage were of European ancestry, confounding due to population substructure is possible, given the multiple nationalities of the replication cohorts. To address this issue we tested for association only in replication samples of British descent (256 PDB cases and 488 controls) using the CMH test. This yielded results that were qualitatively similar to those obtained from the whole replication cohort (Supplementary Table 4). Linkage disequilibrium (LD) patterns in the associated regions were also similar across the study samples and to those observed in HapMap CEU samples (Supplementary Fig. 3). Furthermore, the distribution of allele frequencies for SNPs showing genome wide significant association was broadly similar across individuals when grouped by origin (Supplementary Table 5) and the replicated hits were not located in genomic regions with known geographic variations in European population^{11,12} indicating that the association reported here is unlikely to be confounded by population substructure.

A significant association with PDB was observed on chromosome 1p13.3 for three SNPs (rs10494112, rs499345, and rs484595) but the strongest signal was with rs484595 (combined $P = 5.38 \times 10^{-24}$; Table 1 and Supplementary Table 1). These SNPs were poorly correlated ($r^2 < 0.36$) with other genotyped SNPs and are located in a 14 kb LD block 87kb upstream of the *CSFI* gene (Fig. 2). Another gene (*EPS8L3*) is located 47kb further upstream but is separated by two recombination hotspots (Fig. 2) making *EPS8L3* a less likely candidate. Stepwise regression analysis adjusting for population clusters and accounting for the genotypic additive effect of the three SNPs showed strong evidence for independent association with rs484595 ($P = 4.7 \times 10^{-10}$) and rs10494112 ($P = 9.28 \times 10^{-3}$) compared to rs499345 ($P = 0.80$; Supplementary Table 6). This is not surprising since rs499345 is correlated with rs10494112 ($r^2 = 0.61$) but rs484595 and rs10494112 are not ($r^2 = 0.21$; Supplementary Fig. 3). Analysis of haplotypes formed by the three SNPs did not show a stronger association than analysis of single-SNP (Supplementary Table 7). The *CSFI* gene encodes macrophage colony stimulating factor which is an extremely strong functional candidate for susceptibility to PDB since it plays a critical role in osteoclast formation and survival^{13,14}. Furthermore, loss of function mutations in rat *Csfl* causes osteopetrosis due to failure of osteoclast differentiation^{14,15}, whereas clinical studies have shown that patients with PDB have increased serum levels of CSF-116,17.

A second locus showing significant association with PDB was situated on chromosome 10p13. Three SNPs (rs1561570, rs825411 and rs2095388) all located within a 30kb region were analysed in both stages of the study and the strongest signal was observed for rs1561570 (combined $P = 6.09 \times 10^{-13}$; Table 1 and Supplementary Table 3). These three SNPs are poorly correlated with other genotyped SNPs ($r^2 < 0.37$). The rs825411 is not in LD with rs1561570 ($r^2 = 0.04$, $D' = 0.21$; Supplementary Fig. 3) but attained borderline genome wide significance ($P = 7.82 \times 10^{-8}$) and appeared to have an independent association with PDB as revealed by regression analysis accounting for the genotypic additive effect of rs1561570 ($P = 2.23 \times 10^{-9}$) and rs825411 ($P = 5.15 \times 10^{-6}$; Supplementary Table 6). Haplotype analysis showed a stronger association signal for alleles formed by rs1561570 and rs825411 combined, compared to single-SNP analysis with the risk haplotype "TC" showing the strongest association ($P = 2.50 \times 10^{-17}$; OR = 1.67; Supplementary Table 7). The third SNP (rs2095388) showed no significant association with PDB in the combined analysis ($P = 3.08 \times 10^{-6}$; Supplementary Table 3) and had no independent effect after accounting for rs1561570 and rs825411 ($P = 0.14$; Supplementary Table 6). The 10p13 locus is marked by two recombination hotspots and contains only one known gene (*OPTN*; Fig. 2). It is interesting to note that this region of chromosome 10p13 has been previously linked to familial PDB but the causal gene has not been identified⁶. It is therefore possible that the risk haplotype could be tagging rare allele(s) within *OPTN* that markedly increase susceptibility to PDB. In this regard there have been other reports where

GWAS studies have identified common variants associated with diseases within regions previously mapped by linkage analysis. Examples include amyotrophic lateral sclerosis¹⁸ and Crohn's disease¹⁹. Alternatively the risk haplotype could be tagging another common susceptibility variant within the gene but further studies will be required to investigate these possibilities. The *OPTN* gene, which encodes Optineurin is a novel candidate gene for PDB. Mutations in *OPTN* have been linked to glaucoma²⁰, but until now, *OPTN* has not been implicated in the regulation of bone metabolism. Optineurin is a ubiquitously expressed cytoplasmic protein²¹ which contains a ubiquitin binding domain, similar to that present in NEMO. Optineurin negatively regulates TNF- α induced NF κ B activation by interacting with ubiquitylated RIP2²². Furthermore, a putative NF κ B binding site has been identified in *OPTN* promoter²³ and studies have shown that Optineurin interacts with myosin VI suggesting a role in vesicular trafficking between the Golgi apparatus and plasma membrane²⁴. This is of interest since mutations affecting the VCP protein which is also involved in vesicular trafficking cause the syndrome of inclusion body myopathy with early-onset Paget's disease and frontotemporal dementia (IBMPFD)²⁵. Taken together these data indicate that optineurin may well play a hitherto unrecognised role in the regulation of bone metabolism through its effects on NF κ B signaling and/or vesicular trafficking, but further studies will be required to investigate this issue.

The third region showing a significant association with PDB was located on chromosome 18q21.33 close to the *TNFRSF11A* gene which encodes receptor activator of NF κ B (RANK). Four SNPs within a 300kb region reached genome wide significance in the combined analysis (rs663354, rs2980996, rs2957128, and rs3018362). Regression analysis accounting for the genotypic additive effect of the four SNPs showed that only rs2957128 ($P = 0.047$) and rs3018362 ($P = 0.022$) had independent effects (Supplementary Table 6). Analysis of haplotypes formed by alleles of rs2957128 and rs3018362 showed that a risk haplotype "AA" was consistently over-represented in PDB cases as compared with controls in the combined sample of cases and controls ($P = 8.71 \times 10^{-14}$; OR = 1.55; Supplementary Table 7). These two SNPs are moderately correlated ($r^2 = 0.55$) and are located in adjacent LD blocks about 5kb downstream of *TNFRSF11A* (Fig.2c).

The *TNFRSF11A* gene product RANK plays a critical role in osteoclast differentiation and function. Mice with targeted disruption of *TNFRSF11A* exhibit severe osteopetrosis due to complete absence of osteoclasts²⁶ and loss of function mutations in *TNFRSF11A* cause osteoclast-poor osteopetrosis in humans²⁷. Mutations affecting the signal peptide region of RANK cause the PDB-like syndromes of Familial Expansile Osteolysis, early onset familial PDB and expansile skeletal hyperphosphatasia²⁸⁻³⁰. Mutations of *TNFRSF11A* have not so far been identified in patients with classical PDB^{28,31}, although this region of chromosome 18q22 has been linked to PDB in some families³². It is also of interest to note that rs3018362 and rs884205 located downstream of *TNFRSF11A* have recently been associated with bone mineral density (BMD) and fracture³³⁻³⁵. The allele of rs3018362 that was associated with PDB was also associated with reduced BMD raising the possibility that this allele may be associated with increased bone turnover but further studies will be required to confirm this. The rs884205 was not directly genotyped in our study but it is moderately correlated with both rs3018362 ($r^2 = 0.53$) and rs2957128 ($r^2 = 0.52$). Imputation analysis showed evidence for association of rs884205 with PDB (Imputed P value = 5.93×10^{-11}) confirming the importance of *TNFRSF11A* in the genetic regulation of bone metabolism.

The three loci on chromosomes 1p13, 10p13 and 18q21 identified in this study appear to have an independent role, since we found no evidence to suggest that the associated SNPs within these loci interacted to regulate susceptibility to PDB ($P > 0.33$ for all inter-locus pairwise interactions; Supplementary Table 8). These data are consistent with a multiplicative model for association with PDB. The cumulative population attributable risk for the SNPs

showing independent association with PDB was 70% indicating substantial contribution of the identified loci to PDB development. Additionally the risk of PDB increased with increasing number of risk allele scores ($OR_{\text{per-riskallele}} = 1.34$, 95% CI = 1.29 – 1.40, $P = 5.81 \times 10^{-45}$) with individuals carrying 10 or more risk alleles having six fold increase in PDB risk compared to those with median number of risk alleles (Table 2).

It is likely that other genomic regions might also contribute to PDB since the present study was powered only to detect variants with a moderate effect size (risk allele OR > 1.6). A quantile-quantile plot showing the distribution of P values after removal of all genome wide significant SNPs and correlated markers showed excess in the number of SNPs with small P values compared to what is expected by chance (Supplementary Fig. 2). For example, we observed 19 SNPs with $P < 1 \times 10^{-5}$ compared to 3 expected SNPs suggesting that other risk variants with modest effect remain to be identified. Among these are variants located on chromosome 3p24, 8q22, 10q24, and 14q32 which didn't reach genome wide significance but could be considered suggestively associated with PDB (combined $P < 1 \times 10^{-5}$; Supplementary Table 3). Of particular interest is the 14q32 locus containing *RIN3* (Ras interaction/interference protein 3) gene which is involved in vesicular trafficking³⁶ and could be important in osteoclast function.

In summary we have demonstrated that common genetic variants at loci close to the *CSF1*, *OPTN*, and *TNFRSF11A* genes are independently associated with PDB. Further studies are now warranted to explore the mechanisms responsible for these associations.

ONLINE METHODS

Study subjects

The genome wide association study was conducted in a discovery sample of 750 patients of predominantly British descent with clinical and radiological evidence of PDB in whom mutations of the *SQSTM1* gene had been excluded by DNA sequencing. These comprised subjects who had participated in the PRISM study (n=597) a randomised trial of two different treatment strategies for PDB³⁸; clinic-based patients from the UK with sporadic PDB (n=55); and patients with a family history of PDB derived from the UK (n=20), Australia (n=66), New Zealand (n=8) and Italy (n=4). Details of the 1002 control subjects have previously been described⁹, but in brief, they comprised healthy subjects of Scottish descent with no clinical evidence of PDB. For the replication study we conducted genotyping in an additional 500 PDB patients without *SQSTM1* mutations diagnosed according to standard techniques. These comprised subjects with sporadic PDB who had been recruited from hospital clinics in the UK (n=226), Italy (n=20) and Spain (n=200); patients with sporadic PDB who had participated in the PRISM study (n=43) and subjects with a positive family history of PDB who had been recruited from hospital clinics in Australia (n=10), and the UK (n=1). The 535 replication controls comprised subjects from the UK who had been referred for investigation of osteoporosis but who had been found to have normal bone density on examination by dual energy x-ray absorptiometry (n=248); spouses of participants of the PRISM study who were not known to be affected by PDB (n=252) and clinic based controls from Spain (n=35). All study participants were of European descent and they provided informed consent. The discovery sample had 96 % power to detect disease associated allele with MAF = 0.2 and genotype relative risk of 1.6 assuming a multiplicative model and a disease with population prevalence of 2%.

Stage 1 genotyping and quality control

Genotyping of PDB cases was performed at the genetics core of the Wellcome Trust Clinical Research Facility (Edinburgh, UK) using Illumina HumanHap300 BeadChip

version 2 duo. Genotyping of the controls had been previously performed by Illumina Inc. (San Diego) using HumanHap300 v1 and HumanHap240S arrays⁹. Genotypes for cases and controls were called using BeadStudio v3.2 (Illumina, Inc.) by following the manufacturer's recommended protocol. Genotype data for control subjects were provided after applying the quality control (QC) measures described previously⁹. For the cases, we used a no-call threshold of 0.15 in BeadStudio and quality control metrics such as cluster separation, AB T mean and AB R mean to exclude badly performing SNPs. Samples with a call rate of less than 90% were excluded ($n = 30$). The data were then subjected to further QC measures using PLINK39 to exclude SNPs with a call rate of less than 95%, those with Hardy-Weinberg equilibrium (HWE) P -values of less than 1.0×10^{-4} in controls, and those with a minor allele frequency of less than 1%. This left a total of 294,663 SNPs common to cases and controls with at least 95% call rates in each set. Samples with excess heterozygosity (1 case), non-European ancestry (21 cases and 1 control), and related subjects (6 cases) were excluded before analysis leaving a final total of 692 cases and 1001 controls with an average (\pm SD) genotype call rate of 99.63 ± 1.0 . The genotype cluster plots for all SNPs showing association with PDB at $P < 1.0 \times 10^{-4}$ were visually inspected in BeadStudio. Population ancestry was determined using multidimensional scaling analysis of identity-by-state (IBS) distances matrix of all individuals after combining genotype data from the HapMap project (release 22) samples of European (CEU), Asian (CHB and JPT), and African (YRI) ancestry. For this analysis we first removed SNPs in areas of extended linkage disequilibrium (LD) (Chr2: 134.0 -138.0, Chr6: 25.0 - 34.0, Chr8: 8.0 - 12.0, Chr11: 45.0 - 57.0)⁴⁰, and those with $r^2 > 0.2$ within a 150 SNP window. SNPs with call rate $< 99\%$, MAF $< 5\%$, and HWE $P < 1.0 \times 10^{-4}$ in cases or controls were also excluded leaving a total of 63,528 SNPs. The genome wide average IBS distances matrix for all pair of individuals was then calculated based on the 63,528 SNPs using PLINK and then used for multidimensional scaling analysis. Supplementary Fig 1 is a plot of the first two component of the multidimensional scaling analysis showing three clusters corresponding to CEU, CHB +JPT, and YRI samples with the majority of cases and controls located within the European CEU cluster. We identified 21 cases and 1 control as outliers from the CEU cluster and these were excluded from further analysis. Based on genome wide IBS distance, we identified 5 identical pairs (IBS distance $> 99\%$) and 1 related pair (IBS distance $> 85\%$) of samples from the cases cohort; the sample with the lowest call rate was excluded from each pair before further analysis.

Stage 2 genotyping and quality control

Genotyping of replication samples was performed by Sequenom (Hamburg, Germany) using the MassARRAY iPLEX platform. DNA from cases and controls were distributed into 384 well plates so that each plate had the same number of cases and controls to minimize genotyping bias due variations between runs. We included 100 samples from stage 1 as a quality control measure. The concordance rate between Illumina and Sequenom platforms was $> 99.9\%$. Replication samples with call rate $< 95\%$ were excluded (19 cases and 15 controls) leaving a total of 481 cases and 520 controls with an average genotype call rate of 99.61%. The call rate of all genotyped SNPs was $> 95\%$.

Imputation

Genotypes were imputed for untyped variants located within 2.0 Mb of SNPs identified in stage 1 with genome wide significant association with PDB using MACH41. The HapMap European (CEU) genotype data from release 22 were used as a reference. To minimize end effects, only data from the middle 2 Mb of each 4 Mb imputed segment was used for further analysis. We used 200 rounds of Markov chain iterations to estimate allele dosage and best guess genotypes in stage 1 data. Imputation quality was assessed by estimating the correlation (r^2) between imputed and true genotypes. SNPs with $r^2 < 0.3$ were excluded

before further analysis. Analysis of imputed data was performed using logistic regression implemented in mach2dat42 in which the imputed allelic dosage was used to account for uncertainty in imputed genotypes.

Statistical analysis

Statistical analyses were performed using PLINK (Version 1.07) 39. In stage 1, genotyped SNPs were tested for association with PDB using a stratified Cochran-Mantel-Haenszel (CMH) test. Samples were stratified based on their genome-wide IBS similarity so that individuals assigned to one cluster were not genetically different ($P > 0.001$ from pair wise population concordance test). The quantile-quantile plot and genomic control λ were used to assess overdispersion of the test statistics and were calculated using the statistical package R version 2.7.2 based on the 90% least significant SNPs as described previously¹⁰. Stepwise logistic regression was used to test for independent effects of SNP where the allelic dosage of the conditioning SNP was entered as a covariate in the regression model along with the population clusters identified by IBS sharing analysis described above to adjust for population substructure. Haplotype analysis was performed by logistic regression looking at the presence or absence of the test haplotype and including the population clusters as a covariate in the model. Haplotypes were phased using the E-M algorithm implemented in PLINK and only haplotypes with a frequency of $\geq 1\%$ were analyzed. The cut off point for genome wide significance was set as $P < 1.7 \times 10^{-7}$ ($0.05/294,663$) for stage 1, and $P < 3 \times 10^{-3}$ ($0.05/16$) for the replication stage. For the combined analysis we set the threshold for significance as $P < 5 \times 10^{-8}$ as recently proposed⁴³. The replication and combined data sets were analysed as described above except that the replication dataset was considered as a separate cluster when population clusters were used in a stratified CMH test or as a covariate in logistic regression models. The population attributable risk (PAR) for markers showing association with PDB was calculated according to the following formula: $PAR = p(OR-1) / [p(OR-1)+1]$; 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: Cumulative PAR = $1 - (\pi_{j \rightarrow n} (1 - PAR_j))$; where n is the number of variants and PAR_j is the individual PAR for the i th SNP.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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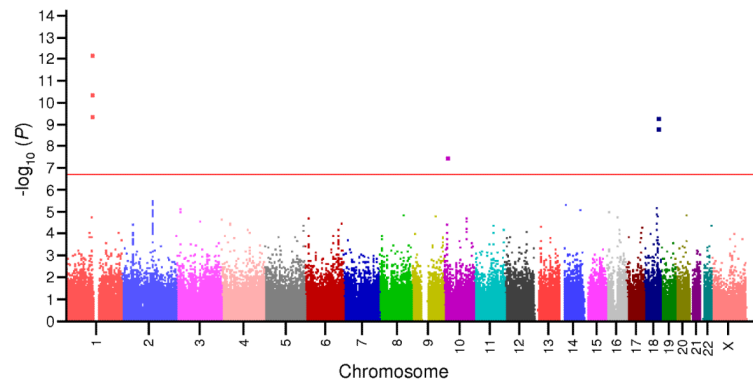


Figure 1. Loci for susceptibility to PDB detected by genome wide association study
Manhattan plot of association test results from the discovery cohort showing chromosomal positions of the 294,633 SNPs passing quality control plotted against genomic-control adjusted $-\log_{10} P$. Association with Paget's disease was tested using stratified Cochran-Mantel-Haenszel test. The red horizontal line indicates the threshold for genome wide significance ($P < 1.7 \times 10^{-7}$).

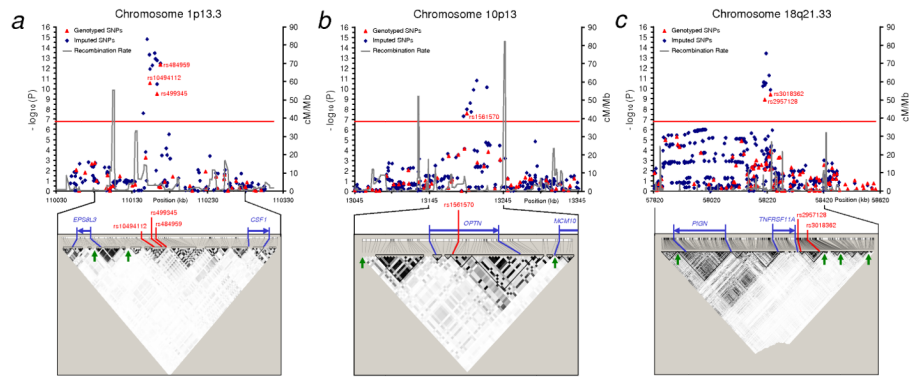


Figure 2. Details of loci associated with Paget's disease

Association and linkage disequilibrium (LD) plots of regions showing genome wide significant association with Paget's disease located on (a) chr 1p13, (b) chr 10p13 and (c) 18q21. The chromosomal position (based on NCBI human genome Build 36) of SNPs is plotted against genomic-control adjusted $-\log_{10} P$. Genotyped SNPs are shown as red triangles and imputed SNPs as blue diamonds. The estimated recombination rates (cM/Mb) from HapMap CEU release 22 are shown as grey lines and the red horizontal line indicates genome wide significance threshold ($P < 1.7 \times 10^{-7}$). Genotyped SNPs were tested using stratified Cochran-Mantel-Haenszel test and imputed SNPs were tested using regression analysis based on imputed allelic dosage and adjusting for population clusters. SNPs reaching genome wide significance are shown with red text. LD plots for the indicated regions are based on HapMap CEU release 22 showing LD blocks depicted for alleles with MAF > 0.05 using the r^2 colouring scheme of Haploview37. The blue arrows indicate known genes in the region and possible recombination hotspots (> 20 cM/Mb) are shown as green arrows on the LD plots.

Table 1

SNPs showing genome-wide significant association with Paget's disease of bone.

| Chr | SNP | Position | MA | Discovery | | | Replication | | | Combined | | | |
|-----|------------|-------------|----|-----------|----------|------------------------|--------------------|---------|----------|------------------------|--------------------|------------------------|--------------------|
| | | | | MAF PDB | MAF Cont | P^a | OR (95%CI) | MAF PDB | MAF Cont | P^a | OR (95%CI) | P^a | OR (95%CI) |
| 1 | rs10494112 | 110,154,000 | G | 0.312 | 0.200 | 9.14×10^{-12} | 1.80 (1.53 – 2.12) | 0.277 | 0.210 | 4.34×10^{-4} | 1.45 (1.18 – 1.77) | 1.67×10^{-14} | 1.65 (1.45 – 1.88) |
| 1 | rs499345 | 110,163,205 | A | 0.396 | 0.284 | 1.13×10^{-10} | 1.68 (1.44 – 1.95) | 0.400 | 0.293 | 5.15×10^{-7} | 1.61 (1.33 – 1.94) | 3.02×10^{-16} | 1.65 (1.47 – 1.85) |
| 1 | rs484959 | 110,167,606 | A | 0.355 | 0.489 | 1.15×10^{-13} | 0.56 (0.49 – 0.65) | 0.340 | 0.491 | 7.25×10^{-12} | 0.53 (0.45 – 0.64) | 5.38×10^{-24} | 0.55 (0.49 – 0.62) |
| 10 | rs1561570 | 13,195,732 | C | 0.346 | 0.451 | 1.11×10^{-8} | 0.64 (0.56 – 0.74) | 0.366 | 0.463 | 1.19×10^{-5} | 0.67 (0.56 – 0.80) | 6.09×10^{-13} | 0.65 (0.58 – 0.73) |
| 18 | rs2957128 | 58,211,715 | A | 0.499 | 0.384 | 4.21×10^{-10} | 1.61 (1.40 – 1.86) | 0.463 | 0.408 | 1.35×10^{-2} | 1.25 (1.05 – 1.49) | 1.86×10^{-11} | 1.46 (1.30 – 1.63) |
| 18 | rs3018362 | 58,233,073 | A | 0.458 | 0.341 | 1.35×10^{-10} | 1.64 (1.42 – 1.89) | 0.415 | 0.343 | 9.81×10^{-4} | 1.36 (1.13 – 1.62) | 5.27×10^{-13} | 1.52 (1.36 – 1.70) |

^a Genomic-control adjusted P values from association testing using stratified Cochran-Mantel-Haenszel test. MA, minor allele; MAF, minor allele frequency; PDB, Paget's disease of bone subjects; Cont, Control subjects; OR, odds ratio for the minor allele and CI is confidence interval.

Table 2

The cumulative contribution of identified variants to PDB risk.

| Risk Allele Score ^a | Allele score frequency (%) | | OR ^b (95% CI) | P |
|--------------------------------|----------------------------|----------|--------------------------|------------------------|
| | Cases | Controls | | |
| 0,1 | 0.94 | 3.02 | 0.41 (0.21 - 0.82) | 9.00×10^{-3} |
| 2 | 1.71 | 6.57 | 0.34 (0.21 - 0.58) | 2.81×10^{-5} |
| 3 | 6.99 | 12.49 | 0.74 (0.54 - 1.02) | 0.07 |
| 4 | 11.59 | 18.67 | 0.83 (0.63 - 1.09) | 0.17 |
| 5 | 15.17 | 20.18 | 1.00 (0.77 - 1.30) | 1.00 |
| 6 | 18.33 | 16.90 | 1.44 (1.11 - 1.87) | 5.00×10^{-3} |
| 7 | 19.10 | 11.18 | 2.27 (1.73 - 2.98) | 2.25×10^{-9} |
| 8 | 13.73 | 6.51 | 2.81 (2.05 - 3.83) | 4.42×10^{-11} |
| 9 | 6.39 | 3.16 | 2.69 (1.79 - 4.05) | 1.07×10^{-6} |
| 10 | 4.43 | 0.99 | 5.98 (3.27 - 10.93) | 1.60×10^{-10} |
| 11,12 | 1.62 | 0.33 | 6.55 (2.40 - 17.85) | 3.06×10^{-5} |

^aRisk allele score of the 6 SNPs showing independent association with PDB in the combined dataset (chr1:rs10494112, rs484959; chr10:rs1561570, rs825411; chr18:rs2957128, rs3018362). Allele scores were normally distributed in cases and controls. Individuals carrying low frequency scores (0,1 and 11,12) were combined together.

^bOdds ratios (OR) are relative to the median number of risk alleles in the controls (5 risk alleles).