Supplementary Information

Genetic Diagnostics in Routine Osteological Assessment of Adult Low Bone Mass Disorders

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Supplementary Figure 1. Statistical analysis of phenotype data in relation to DCV status.

Supplementary Table 1. Genes used for CADD score enrichment analysis

Supplementary Table 2. List of GWAS hits used for calculation of genetic risk score (GRS)

Supplementary Table 3. List of class IV and V disease causing variants (DCVs) in EOOP patients.

Supplementary Table 4. List of class III variants of unknown significance (VUS) in EOOP patients.

Supplementary Table 5. Biochemical assessment of EOOP study population at baseline.

Supplementary Table 6. CADD-score enrichment analysis for variants in high BMD genes
Oheim et al. Genetic Diagnostics in Routine Osteological Assessment of Adult Low Bone Mass Disorders

Supplementary Figure 1. Statistical analysis of phenotype data in relation to DCV status. For EOOP individuals subdivided into the cohorts DCV and No DCV no differences were observed for (A) age, (B) BMD DXA Z-score lumbar spine, (C) BMD DXA Z-score hip. (D) Grip strength measurements available for 68 individuals showed significantly lower values for DCV carriers.
### Supplementary Table 1. Genes used for CADD score enrichment analysis

| Gene List | Low BMD | High BMD |
|-----------|---------|----------|
| **ADAMTS2, ALPL, ATP6V0A2, ATP7A, BANF1, BMP2, CASR, COL1A1, COL1A2, CREB3L1, CRTAP, DMP1, ENPP1, FGF23, FGFR1, FKBP10, GBA, GORAB, IFITM5, LEPRE1, LMNA, LRP4, LRP5, LRP6, MAFB, MGP, NOTCH2, NT5E, PHEX, PLOD2, PLOD3, PLS3, PP1B, PYCR1, RECQL4, RUNX2, SAMD9, SEC24D, SERPINF1, SERPINH1, SLC34A1, SLC34A3, SLC39A13, SLC9A3R1, SPARC, TGFBR1, TGFBR2, TMEM38B, TNFRSF11B, VDR, WNT1, WNT16, ZMPSTE24** |
| **ABCC9, ACP5, AMER1, ANKH, ANO5, AXIN1, BMP1, CA2, CLCN7, CTSK, FAM123B, FAM20C, FERMT3, GJA1, HPGD, IKBKG, LEMD3, LRP4, LRP5, OSTM1, PLEKHM1, PTDSS1, SLC2A1, SNX10, SOST, TBXAS1, TCIRG1, TGFBI, TNFRSF11A, TNFRSF11B, TNFSF11** |
### Supplementary Table 2. List of GWAS hits used for calculation of genetic risk score (GRS)

| Chr. | Position | dbSNP ID | beta  | Effect allele | Study ID  | Association | Mapped Genes               |
|------|----------|----------|-------|---------------|-----------|-------------|-----------------------------|
| 1    | 170521376| rs913257*| 0.0118| G             | GCST006979| Heel        | GORAB                      |
| 2    | 54659707 | rs4233949*| 0.0746| C             | GCST006288| Heel        | C2orf73, SPTBN1           |
| 2    | 119613444| rs188303909| 0.16  | T             | GCST005545| Spine       | EN1, MARCO                 |
| 2    | 166618262| rs1968294 | -0.0632| T             | GCST005350| Spine, neck | GALNT3                     |
| 4    | 994414   | rs3755955 | -0.06  | A             | GCST001482| Spine       | IDUA                       |
| 4    | 996165   | rs6831280 | -0.0804| A             | GCST005348| Spine, neck | IDUA                       |
| 7    | 120965465| rs10668066| -0.17  | G             | GCST006288| Heel        | WNT16                      |
| 7    | 120969769| rs2908004 | 0.1039 | A             | GCST002492| Limb, skull | WNT16                      |
| 10   | 54427825 | rs1373004*| -0.06  | T             | GCST001482| Spine       | LNCARD                      |
| 10   | 89468953 | rs17173698| 0.0391 | G             | GCST006979| Heel        | PAPSS2                     |
| 11   | 46880592 | rs149082597| 0.1427| G             | GCST006979| Heel        | LRP4, LRP4-AS1             |
| 11   | 46897253 | rs2306032*| -0.0457| C             | GCST005348| Spine, neck | LRP4                       |
| 11   | 46989771 | rs6485702*| 0.0326 | T             | GCST006979| Heel        | LRP4                       |
| 11   | 58920492 | rs116918730| 0.0353| C             | GCST006979| Heel        | FAM111A                    |
| 11   | 67809268 | rs36027301*| -0.0280| C             | GCST006979| Heel        | TCIRG1                     |
| 11   | 67814943 | rs140191063*| -0.1299| G             | GCST006979| Heel        | TCIRG1                     |
| 11   | 68157342 | rs141889567| 0.2261| T             | GCST006979| Heel        | LRP5                       |
| 11   | 68174189 | rs4988321*| 0.0557 | G             | GCST006979| Heel        | LRP5                       |
| 11   | 68201295 | rs3736228*| -0.08  | T             | GCST001482| Spine       | LRP5                       |
| 12   | 49373410 | rs61758378*| 0.1126| T             | GCST006979| Heel        | WNT1                       |
| 13   | 43148546 | rs138818878*| -0.0439| C             | GCST006979| Heel        | TNFSF11                    |
| 14   | 54417522 | rs17563  | 0.0343 | A             | GCST006979| Heel        | BMP4                       |
| 16   | 347063   | rs117208012| 0.0747| C             | GCST006979| Heel        | AXIN1                      |
| 16   | 354353   | rs142097791| 0.1722| G             | GCST006979| Heel        | AXIN1                      |
| 16   | 359953   | rs2301522 | 0.0297 | A             | GCST006288| Heel        | AXIN1                      |
| 16   | 1532463 | rs13336428*| 0.0134| G             | GCST006979| Heel        | PTHX4, RPS3AP2             |
| 17   | 41773814 | rs1107748*| 0.063  | C             | GCST003389| Hip         | LINC02594                  |
| 17   | 41789965 | rs7220711*| 0.071  | G             | GCST003389| Hip         | LINC02594, WHSC1L2P        |
| 17   | 41798824 | rs4792909*| 0.04   | T             | GCST007691| Neck        | SOST, WHSC1L2P             |
| 18   | 60052034 | rs117028614*| -0.1794| A             | GCST006979| Heel        | TNFRSF11A                  |
| 18   | 60066888 | rs2957137*| -0.0442| A             | GCST005348| Spine, neck | TNFRSF11A, RPL17P44       |
| 18   | 60082093 | rs3018362*| -0.07  | A             | GCST000181| Hip, spine  | TNFRSF11A, RPL17P44       |

Chr. – chromosome, Position – chromosomal position GRCh37 genome build, dbSNP ID – SNP identifier an asterisk (*) mark SNPs used for GRS because of high coverage in all samples, Beta – effect strength and BMD direction denoted by sign, Study ID – identifier of study as mentioned in GWAS catalogue, Association – BMD used in the denoted study,Mapped genes – Genes overlapping the variant. If a variant is intergenic, the closest 5' and 3' genes are listed.
Supplementary Table 3. List of class IV and V disease causing variants (DCVs) in EOOP patients.

| Index | Gene | DNA level | Protein level | Status | Allele Frequency | Pathogenicity Evaluation | Segregation | ACMG class | Reference |
|-------|------|-----------|---------------|--------|-----------------|--------------------------|-------------|------------|-----------|
| 1     | ALPL | NM_000478.5:c.407G>A | p.Arg136His | Het    | 0.000128        | 13/0/5 (likely pathogenic) | N           | IV         | 10094560  |
| 2     | ALPL | NM_000478.5:c.436G>A | p.Glu146Lys  | Het    | 0.000114        | 15/0/3 (uncertain significance) | N           | IV         | 26432670  |
| 3     | ALPL | NM_000478.5:c.436G>A | p.Glu146Lys  | Het    | 0.000114        | 15/0/3 (uncertain significance) | N           | IV         | 26432670  |
| 4†    | ALPL | NM_000478.5:c.212G>A | p.Arg71His   | Het    | 0.000004        | 18/0/0 (likely pathogenic) | N           | IV         | 11438998  |
|       | ALPL | NM_000478.5:c.571G>A | p.Glu191Lys  | Het    | 0.002474        | 13/0/5 (uncertain significance) | N           | IV         | 1409720   |
| 5     | ALPL | NM_000478.5:c.1283G>C | p.Arg428Pro  | Het    | 0               | 18/0/0 (likely pathogenic) | N           | V          | 17253930  |
| 6     | ALPL | NM_000478.5:c.1309+198_1548del | p.? | Het    | 0               | 1/0/0 (pathogenic) | N           | V          |           |
| 7     | ALPL | NM_000478.5:c.872A>T   | p.Lys346Met  | Het    | 0.021970        | 18/0/0 (likely pathogenic) | N           | IV         | 31267001  |
| 8     | ALPL | NM_000478.5:c.558G>A   | p.Trp186*    | Het    | 0.00009         | 6/0/2 (pathogenic) | N           | V          | 10094560  |
| 9     | CASR | NM_000388.3:c.577C>T   | p.Gln193*    | Het    | 0               | 6/1/2 (pathogenic) | N           | V          |           |
| 10    | COL1A1| NM_000088.3:c.148C>T   | p.Arg50*     | Het    | 0               | 3/0/1 (Pathogenic) | N           | V          | 28725987  |
| 11    | COL1A1| NM_000088.3:c.581del   | p.Gly194Valfs*71 | Het    | 0               | 1/0/0 (pathogenic) | N           | V          |           |
| 12    | COL1A1| NM_000088.3:c.595del   | p.Gln199fs   | Het    | 0               | 1/0/0 (pathogenic) | N           | V          | 29946973  |
| 13    | COL1A1| NM_000088.3:c.696+1G>T | p.? (splicing) | Het    | 0               | 5/0/0 (pathogenic) | N           | V          | 30715774  |
| 14    | COL1A1| NM_000088.3:c.697G>C   | p.Gly233Arg  | Het    | 0               | 16/0/0 (uncertain significance) | N           | V          | 29946973  |
| 15    | COL1A1| NM_000088.3:c.838G>A   | p.Gly280Ser  | Het    | 0               | 1/0/14 (uncertain significance) | N           | V          |           |
| 16    | COL1A1| NM_000088.3:c.910dup   | p.Arg304Profs*6 | Het    | 0               | 1/0/0 (pathogenic) | N           | V          | 22987783  |
| 17    | COL1A1| NM_000088.3:c.1054_1056+2del | p.? (splicing) | Het    | 0               | 1/0/0 (pathogenic) | N           | V          | 33716164  |
| 18    | COL1A1| NM_000088.3:c.1613del  | p.Lys538Argfs*3 | Het    | 0               | 1/0/0 (pathogenic) | Y           | V          | 29946973  |
| 19    | COL1A1| NM_000088.3:c.1614+1G>A | p.? (splicing) | Het    | 0               | 5/0/0 (pathogenic) | N           | V          | 17078022  |
| 20    | COL1A1| NM_000088.3:c.1884del  | p.Gly629Alafs*137 | Het    | 0               | 1/0/0 (pathogenic) | N           | V          |           |
| 21    | COL1A1| NM_000088.3:c.2073del  | p.Gly692Valfs*74 | Het    | 0.000004        | 1/0/0 (pathogenic) | N           | V          | 9443882   |
| 22    | COL1A1| NM_000088.3:c.2101G>A  | p.Gly701Ser  | Het    | 0               | 16/0/0 (likely pathogenic) | de novo     | V          | 17078022  |
|   | Gene  | Chromosome | Mutation | Protein Effect | Heterogeneity | Pathogenicity |   |   |
|---|-------|------------|----------|----------------|---------------|---------------|---|---|
| 23 | COL1A1 | NM_000088.3:c.2311C>T | p.Pro771Ser | Het | 0.00004 | 11/0/5 (likely pathogenic) | N | V |
| 24 | COL1A1 | NM_000088.3:c.2612del | p.Pro781fs | Het | 0 | 1/0/0 (pathogenic) | N | V |
| 25 | COL1A1 | NM_000088.3:c.2850dup | p.Pro951fs | Het | 0 | 1/0/0 (pathogenic) | N | V |
| 26 | COL1A1 | NM_000088.3:c.2612del | p.Pro781fs | Het | 0 | 1/0/0 (pathogenic) | N | V |
| 27 | COL1A1 | NM_000088.3:c.2850dup | p.Pro951fs | Het | 0 | 1/0/0 (pathogenic) | N | V |
| 28 | COL1A1 | NM_000088.3:c.2990_2997del | p.Pro977Hisfs*12 | Het | 0 | 1/0/0 (pathogenic) | N | V |
| 29 | COL1A1 | NM_000088.3:c.3649_3651del | p.Arg1220del | Het | 0 | 1/0/0 (pathogenic) | N | IV |
| 30 | COL1A1 | NM_000089.3:c.577G>A | p.Gly193Ser | Het | 0 | 11/0/1 (likely pathogenic) | N | V |
| 31 | COL1A2 | NM_000089.3:c.874G>A | p.Gly292Ser | Het | 0 | 18/0/0 (uncertain significance) | N | V |
| 32 | COL1A2 | NM_000089.3:c.1009G>A | p.Gly337Ser | Het | 0 | 18/0/0 (pathogenic) | Y | V |
| 33 | COL1A2 | NM_000089.3:c.1522G>A | p.Gly508Ser | Het | 0.000008 | 18/0/0 (likely pathogenic) | N | V |
| 34 | COL1A2 | NM_000089.3:c.2123G>A | p.Arg724Glu | Het | 0.000662 | 15/0/3 (uncertain significance) | N | IV |
| 35 | COL1A2 | NM_000089.3:c.2171G>A | p.Gly724Glu | Het | 0 | 18/0/0 (uncertain significance) | Y | V |
| 36 | COL1A2 | NM_000089.3:c.2314G>A | p.Gly772Ser | Het | 0 | 18/0/0 (likely pathogenic) | Y | V |
| 37 | COL1A2 | NM_000089.3:c.3313G>A | p.Gly1105Ser | Het | 0.000938 | 15/0/3 (uncertain significance) | N | V |
| 38 | ENPP1 | NM_006208.2:c.2344C>T | p.Arg782* | Het | 0 | 5/1/3 (pathogenic) | N | IV |
| 39 | ENPP1 | NM_006208.2:c.2330A>G | p.His777Arg | Het | 0.000141 | 11/0/7 (uncertain significance) | Y | IV |
| 40 | EXT2 | NM_207122.1:c.536+1G>A | p.? (splicing) | Het | 0 | 5/0/0 (pathogenic) | N | V |
| 41 | LMNA | NM_170707.2:c.544C>T | p.Glu182* | Het | 0 | 7/0/2 (pathogenic) | N | V |
| 42 | LRP5 | NM_002335.2:c.523C>T | p.Arg175Trp | Het | 0.000010 | 16/1/1 (likely pathogenic) | N | IV |
| 43 | LRP5 | NM_002335.2:c.1007G>T | p.Cys336Phe | Het | 0 | 17/1/0 (uncertain significance) | Y | IV |

Oheim et al. Genetic Diagnostics in Routine Osteological Assessment of Adult Low Bone Mass Disorders
| Case | Gene | Mutation | p.Amino Acid Change | Allele | Value | Significance | Decision |
|------|------|----------|---------------------|--------|-------|--------------|----------|
| 49   | LRP5 | NM_002335.2:c.1067C>T | p.Ser356Leu | Het | 0.000007 | 17/1/0 (likely pathogenic) | Y V 16252235 |
| 50   | LRP5 | NM_002335.2:c.1265C>T | p.Ala422Val | Het | 0.00002390 | 17/1/0 (likely pathogenic) | N IV 25711638 |
| 51   | LRP5 | NM_002335.2:c.1709G>A | p.Arg570Gln | Het | 0 | 17/1/0 (likely pathogenic) | Y V 15346351 |
| 52   | LRP5 | NM_002335.2:c.2009C>T | p.Pro670Leu | Het | 0.00002 | 16/1/1 (likely pathogenic) | N IV |
| 53   | LRP5 | NM_002335.2:c.2134G>A | p.Val712Met | Het | 0.000024 | 15/1/2 (likely pathogenic) | Y IV 33118644 |
| 54   | LRP5 | NM_002335.2:c.2234C>A | p.Ala745Glu | Het | 0.0009 | 13/1/4 (uncertain significance) | Y IV 27486893 |
| 55   | LRP5 | NM_002335.2:c.2254C>T | p.Ala745Glu | Het | 0 | 16/1/1 (likely pathogenic) | N V 25384351 |
| 56   | LRP5 | NM_002335.2:c.2377G>A | p.Gly793Arg | Het | 0 | 17/1/0 (likely pathogenic) | Y IV 29594386 33716164 |
| 57   | LRP5 | NM_002335.2:c.2413C>T | p.Arg805Trp | Het | 0.000016 | 14/1/3 (likely pathogenic) | Y IV 19324841 |
| 58   | LRP5 | NM_002335.2:c.3245A>G | p.Tyr1082Cys | Het | 0.000075 | 17/1/0 (likely pathogenic) | Y IV 33118644 |
| 59   | LRP5 | NM_002335.2:c.3336_3337del | p.Ile1112Metfs*25 | Het | 0 | 1/0/0 (pathogenic) | N V |
| 60   | LRP5 | NM_002335.2:c.3403C>T | p.Arg1135Cys | Het | 0 | 18/0/0 (likely pathogenic) | N IV 25711638 |
| 61   | LRP5 | NM_002335.2:c.3835C>T | p.Arg1279Cys | Het | 0 | 16/1/1 (uncertain significance) | Y IV 33118644 |
| 62   | LRP5 | NM_002335.2:c.3985G>A | p.Glu1329Lys | Hom | 0.000004 | 17/1/0 (uncertain significance) | N IV 25711638 |
| 63   | LRP5 | NM_002335.2:c.4475C>T | p.Thr1492Met | Het | 0.000036 | 12/2/4 (uncertain significance) | Y IV 30452590 |
| 64   | LRP5 | NM_002335.2:c.4565C>T | p.Pro1522Leu | Het | 0.000003 | 16/1/1 (likely pathogenic) | N IV |
| 65   | NOTCH2 | NM_024408.3:c.6657del | p.Pro2219fs | Het | 0 | 12/2/4 (uncertain significance) | N V |
| 66   | PLS3 | NM_005032.6:c.1512-1G>T | p.Ala507Thr | Het | 0 | 3/1/0 (pathogenic) | N V 31678489 |
| 67   | PLS3 | NM_005032.6:c.1760+2T>A | p.? (splicing) | Het | 0 | 3/1/0 (pathogenic) | N V |
| 68   | PLS3 | NM_005032.6:c.766C>T | p.Arg256* | Het | 0 | 5/0/0 (pathogenic) | N V 28748388 |
| 69   | PLS3 | NM_005032.6:c.1658T>A | p.Leu553* | Het | 0 | 5/0/0 (pathogenic) | Y V |
| 70   | SLC34A3 | NM_080877.2:c.575C>T | p.Ser192Leu | Het | 0.00046 | 9/0/9 (uncertain significance) | N IV 16358215 |
| 71   | SLC34A3 | NM_080877.2:c.995T>C | p.Leu332Pro | Het | 0.000052 | 9/1/8 (uncertain significance) | Y IV |
| Allele Frequency | Description | Frequency | Pathogenicity Evaluation | Final ACMG Classification | Reference IDs |
|------------------|-------------|-----------|--------------------------|---------------------------|---------------|
| 0.002271         | NM_080877.2.c.756G>A | Heterozygous | 0/0/2 (likely benign) | Y | V | 16849419 |
| 0.000008         | NM_005430.3.c.350T>G | Heterozygous | 13/0/5 (uncertain significance) | N | IV | 30404864 |
| 0.000008         | NM_005430.3.c.730G>T | Heterozygous | 7/0/4 (Uncertain Significance) | N | IV | 23499309 33716164 |
| 0.000502         | NM_005430.3.c.754G>C | Heterozygous | 11/0/7 (uncertain significance) | N | V | 30404864 |
| 0.000004         | NM_005430.3.c.929C>T | Heterozygous | 14/0/5 (uncertain significance) | N | V | 30404864 |
| 0.000004         | NM_005430.3.c.1093_1102delinsC | Heterozygous | 18/0/0 (uncertain significance) | N | V | 30404864 |
| 0.000004         | NM_001127464.2.c.10240del | Heterozygous | 1/0/0 (pathogenic) | N | V | 32671420 |
| 0.000004         | NM_001127464.2.c.10664del | Heterozygous | 1/0/0 (pathogenic) | Y | V | 32671420 |

Allele frequency is the frequency in non-Finnish Europeans according to GnomAD. Pathogenicity evaluation shows the number of bioinformatic tools predicting pathogenic/unknown/benign effects for the respective variant. The automated verdict from the Varsome software is given in parentheses. The final ACMG classification takes additional aspects into account and is not necessarily identical with the Varsome verdict: III = unknown significance, IV= likely pathogenic, V= pathogenic. References are given as PubMed IDs, EOOP - early-onset osteoporosis, Het - heterozygous, Hom - homozygous, N - no, Y – yes, ų= biallelic variant. Gray rows – variant found in participants with > 10 fractures classified as osteogenesis imperfecta.
### Supplementary Table 4. List of class III variants of unknown significance (VUS) in EOOP patients.

| Index | Gene | DNA level | Protein level | Status | Allele Frequency | Pathogenicity Evaluation | Segregation | ACMG class | Reference |
|-------|------|-----------|---------------|--------|-----------------|--------------------------|-------------|-------------|-----------|
| 83    | BMP1 | NM_006129.4:c.1756C>T | p.Arg586Cys | Het | 0.000029 | 10/1/7 (uncertain significance) | Y           | III         |           |
| 84    | CASR | NM_000388.3:c.3221A>G | p.His715Pro | Het | 0 | 10/0/8 (uncertain significance) | Y           | III | 33716164 |
| 85[^1] | COL1A1 | NM_000088.3:c.437C>T | p.Pro146Leu | Het | 0 | 8/0/8 (uncertain significance) | N           | III | 18996919 |
|       |       | NM_000088.3:c.3556C>G | p.Pro1186Ala | Het | 0.0000354 | 8/0/8 (uncertain significance) | N           | III | 27132807 |
| 86    | COL1A1 | NM_000088.3:c.1200+3A>G | p.?(splicing) | Het | 0.000007 | 0/0/2 (uncertain significance) | N           | III         |           |
| 87    | COL1A1 | NM_000088.3:c.3195T>G | p.Asp1065Glu | Het | 0 | 6/0/10 (uncertain significance) | Y           | III | 29946973 |
| 88    | COL1A1 | NM_000088.3:c.992T>G | p.Asp331Val | Het | 0.000014 | 11/0/5 (likely pathogenic) | N           | III         |           |
| 89    | COL1A2 | NM_000089.3:c.2309C>T | p.Pro770Leu | Het | 0.0001495 | 5/0/13 (uncertain significance) | N           | III | 26264438 |
| 90    | COL1A2 | NM_000089.3:c.3485C>T | p.Thr1162Ile | Het | 0 | 17/0/1 (likely pathogenic) | N           | III         |           |
| 91    | COL1A2 | NM_000089.3:c.4091G>A | p.Cys1364Tyr | Het | 0 | 17/0/1 (likely pathogenic) | N           | III         |           |
| 92    | COL1A2 | NM_000089.3:c.3211C>A | p.Pro1071Thr | Het | 0 | 8/0/1 (uncertain significance) | N           | III         |           |
| 93    | DVL1  | NM_004421.2:c.1264+1G>A | p.?(splicing) | Het | 0.000004 | 5/0/0 (pathogenic) | N           | III         |           |
| 94    | ENPP1 | NM_006208.2:c.797G>T | p.Gly266Val | Het | 0 | 18/0/0 (likely pathogenic) | N           | III         |           |
| 95    | ENPP1 | NM_006208.2:c.1405T>C | p.Phe469Leu (splicing) | Het | 0.0002642 | 8/0/10 (uncertain significance) | N           | III         |           |
| 96    | FBN1  | NM_000138.4:c.1027G>A | p.Gly343Arg | Het | 0.000184 | 16/0/0 (benign) | N           | III | 17253931 |
| 97    | FBN1  | NM_000138.4:c.3503A>G | p.Asn1168Ser | Het | 0.000072 | 10/0/6 (uncertain significance) | Y           | III | 21907952 |
| 98    | FBN1  | NM_000138.4:c.7661G>A | p.Arg2554Gln | Het | 0.000153 | 10/0/6 (benign) | Y           | III | 21883168 |
| 99    | FBN2  | NM_001999.3:c.7808T>C | p.Phe2603Ser | Het | 0.000089 | 17/0/0 (benign) | N           | III         |           |
| 100   | FBN2  | NM_001999.3:c.7868A>G | p.Gln2629Arg | Het | 0.000028 | 14/0/3 (uncertain significance) | N           | III         |           |
| 101   | FBN2  | NM_001999.3:c.1009A>G | p.Glu337Lys | Het | 0 | 15/0/3 (likely pathogenic) | N           | III         |           |

[^1]: vUS variant
| #  | Gene     | Variant ID   | Allele | Chromosome | Effect        |Allele frequency | Pathogenicity Evaluation | ACMG Classification | Reference (PubMed ID) |
|----|----------|--------------|--------|------------|---------------|------------------|------------------------|----------------------|----------------------|
| 102| LMNA     | NM_170707.2:c.1978A>G | p.Asn660Asp | Het | 0.000124 | 8/0/12 (uncertain significance) | N | III | 30564623 |
| 103| LRP5     | NM_002335.2:c.229G>A | p.Val77Met | Het | 0 | 9/1/8 (uncertain significance) | Y | III |
| 104| LRP6     | NM_002336.2:c.775C>T | p.Arg259Cys | Het | 0.000024 | 15/0/3 (likely benign) | N | III | 33118644 |
| 105| LRP6     | NM_002336.2:c.775C>T | p.Arg259Cys | Het | 0.000024 | 15/0/3 (likely benign) | N | III | 33118644 |
| 106| LRP6     | NM_002336.2:c.3370C>T | p.Arg1124Trp | Het | 0.0000968 | (13/1/4) (likely benign) | N | III |
| 107| PLOD1    | NM_000302.3:c.1015C>T | p.Gln139* | Het | 0 | 5/0/3 (pathogenic) | N | III |
| 108| SLC34A1  | NM_003052.4:c.1708C>T | p.Pro570Ser | Het | 0 | 14/0/4 (uncertain significance) | N | III |
| 109| SLC34A1  | NM_003052.4:c.625G>A | p.Asp209Asn (splicing) | Het | 0 | 9/0/9 (uncertain significance) | N | III |
| 110| TGFBR1   | NM_004612.3:c.1433A>G | p.Asn478Ser | Het | 0 | 17/0/1 (likely pathogenic) | N | III |

Allele frequency is the frequency in Non-Finnish Europeans according to GnomAD. Pathogenicity evaluation shows the number of bioinformatic tools predicting pathogenic/unknown/benign effects for the respective variant. In parenthesis the automated verdict from the Varsome software is given. The final ACMG classification takes additional aspects into account and is not necessarily identical with the Varsome verdict: III = unknown significance, IV= likely pathogenic, V= pathogenic. References are given as Pubmed IDs. *= biallelic variant. Gray rows – variant found in participants with > 10 fractures classified as osteogenesis imperfecta.
### Supplementary Table 5: Biochemical assessment of EOOP study population at baseline.

|                     | Reference values | No DCV (n=312) | DCV (n=82) | Total (n=394) | p value |
|---------------------|------------------|----------------|------------|---------------|---------|
| **Calcium [mmol/L]** | 2.10 – 2.74      | 225 (2.28 (0.15)) | 66 (2.33 (0.17)) | 291 (2.29 (0.16)) | 0.025 |
| **Phosphate [mmol/L]** | 0.77 – 1.50      | 223 (0.95 (0.20)) | 66 (0.94 (0.21)) | 289 (0.95 (0.20)) | 0.654 |
| **Creatinine [mg/dL]** | 0.55 – 1.02      | 182 (0.85 (0.70)) | 58 (0.83 (0.29)) | 240 (0.84 (0.63)) | 0.814 |
| **ALP [U/L]**       | 35.0 – 104.0     | 215 (82.55 (47.64)) | 64 (92.17 (65.91)) | 279 (84.76 (52.42)) | 0.198 |
| **BAP [µg/L]**      | 5.5 – 22.9       | 221 (15.09 (16.03)) | 65 (17.88 (20.00)) | 286 (15.73 (17.01)) | 0.245 |
| **Vitamin D [µg/L]** | 30.0 – 70.0      | 223 (33.8 (11.7)) | 66 (35.5 (12.3)) | 289 (34.2 (11.8)) | 0.287 |
| **PTH [ng/L]**      | 17.0 – 84.0      | 222 (53.5 (27.9)) | 66 (48.4 (26.8)) | 288 (52.3 (27.7)) | 0.191 |
| **Osteocalcin [µg/L]** | 12.0 – 52.1    | 221 (19.8 (10.1)) | 65 (20.7 (10.2)) | 286 (20.0 (10.1)) | 0.568 |
| **DPD [nmol/mmol]** | 2.0 – 7.0        | 161 (6.31 (4.30)) | 57 (6.26 (2.59)) | 218 (6.30 (3.92)) | 0.938 |
| **DPD female [nmol/mmol]** | 3.0 – 7.0    | 64 (7.56 (4.29)) | 25 (6.56 (2.81)) | 89 (7.28 (3.95)) | 0.284 |
| **DPD male [nmol/mmol]** | 2.0 – 5.0    | 97 (5.48 (4.12)) | 32 (6.03 (2.43)) | 129 (5.62 (3.77)) | 0.479 |

EOOP - early-onset osteoporosis, DCV - disease causing variant (ACMG class IV and V), n - number, ALP - alkaline phosphatase, BAP - bone-specific alkaline phosphatase, PTH - parathyroid hormone, DPD - deoxypyridinoline/creatinine
Supplementary Table 6. CADD-score enrichment analysis for variants in high BMD genes

| cohort          | frequency  | impact | EOOP     | EOOP noDCV |
|-----------------|------------|--------|----------|------------|
|                 | all        | <1%    | ultrarare| all        | <1%        | ultrarare  |
| high            | 0.881088   | 0.656651 | 1        | 0.814032   | 0.814032   | 1          |
| moderate        | 0.975689   | 0.975689 | 1        | 0.975689   | 0.975689   | n.d.       |
| moderate + high | 0.814032   | 0.315597 | 0.981577 | 0.794304   | 0.656651   | 1          |
| any             | 0.656651   | 0.697268 | 1        | 0.656651   | 0.814032   | 1          |

| p-value         | n.s.       | p<0.05 | p<0.01   | p<0.001    |

EOOP all = entire EOOP cohort, EOOP noDCV = EOOP without all individuals with a disease-causing variant. Impact as annotated by Annovar. Variant frequency according to gnomAD database. All = all variants, <1% frequency below 1%, ultrarare = variants not found in gnomAD database. P-values were corrected using the Benjamini-Hochberg method.