**Supplementary Table S1.** Descriptive statistics for the three cohorts used in the study.

|  |  |  |  |
| --- | --- | --- | --- |
|  | TwinsUK WGS | TwinsUK GWAS | EPIC GWAS |
| Subjects (N) | 1,268 | 1,610 | 13,749 |
| Age (years), mean (SD) | 47.4 (10.8) | 47.4 (14.4) | 62.3 (9.2) |
| Age range (years) | 18.4-74.6 | 18.3-79 | 42-82 |
| Height (cm), mean (SD) | 162.6 (5.9) | 164.3 (8) | 166.8 (9.1) |
| Weight (kg), mean (SD) | 66.1 (12.5) | 67.7 (12.9) | 74.4 (13.2) |
| Male (%) | 0 | 15.5 | 45.3 |
| VOS (m/s), mean (SD) | 1656.9 (48.9) | 1647.2 (47.3) | 1634 (41.2) |
| BUA (dB/MHz), mean (SD) | 75.9 (17.3) | 76.8 (18.4) | 80.3 (19.1) |
| Fracture rate (%) | 28.1 | 28.6 | 14.2 |
| Variants analysed\* | 9,844,025 | 10,535,635 | 11,393,289 |
| Subjects (N) | 1,268 | 1,610 | 13,749 |

N, number; SD, standard deviation; kg, kilogram; m/s, metres per second; dB/MHz, decibel per megahertz.

\*After genotype imputation and removal of variants with MAF <0.005.

**Supplementary Table S2.** Variants in the *WNT16* gene region associated with BUA at the genome-wide significance level (*P*<*5×10-8*) in the TwinsUK WGS discovery cohort.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  | TwinsUK WGS discovery | | TwinsUK/EPIC GWAS replication | | Meta-analysis | |
| Variant | EA | OA | EAF | Beta | *P* | Beta | *P* | Beta (SE) | *P* |
| rs10276111 | T | C | 0.35 | 0.23 | 4.9E-8 | 0.13 | 1.8E-27 | 0.14 (0.01) | 4.6E-33 |
| rs10233441 | A | T | 0.36 | 0.24 | 4.6E-9 | 0.14 | 7.2E-30 | 0.15 (0.01) | 3.1E-36 |
| rs112901683 | AC | A | 0.36 | 0.24 | 7.2E-9 | 0.14 | 6.5E-30 | 0.15 (0.01) | 3.8E-36 |
| rs2952556 | A | G | 0.37 | 0.25 | 3.0E-9 | 0.14 | 7.9E-30 | 0.15 (0.01) | 2.7E-36 |
| rs7809135 | C | T | 0.36 | 0.24 | 1.2E-8 | 0.14 | 1.7E-29 | 0.15 (0.01) | 1.4E-35 |
| rs114816639 | T | C | 0.35 | 0.23 | 3.4E-8 | 0.13 | 7.0E-28 | 0.14 (0.01) | 1.4E-33 |
| rs6961083 | G | A | 0.36 | 0.24 | 5.0E-9 | 0.14 | 4.6E-30 | 0.15 (0.01) | 2.1E-36 |
| rs7778200 | C | T | 0.36 | 0.24 | 1.1E-8 | 0.14 | 2.2E-29 | 0.15 (0.01) | 1.8E-35 |
| rs10241888 | A | G | 0.35 | 0.23 | 2.6E-8 | 0.13 | 6.9E-28 | 0.14 (0.01) | 1.2E-33 |
| rs2707521 | T | C | 0.37 | 0.24 | 4.7E-9 | 0.14 | 2.5E-29 | 0.14 (0.01) | 1.2E-35 |
| rs1547960 | G | A | 0.36 | 0.24 | 1.2E-8 | 0.14 | 3.8E-30 | 0.15 (0.01) | 2.9E-36 |
| rs1547959 | C | T | 0.36 | 0.24 | 1.3E-8 | 0.14 | 7.4E-30 | 0.15 (0.01) | 6.5E-36 |
| rs6980043 | T | C | 0.36 | 0.24 | 1.6E-8 | 0.14 | 1.8E-29 | 0.15 (0.01) | 1.8E-35 |
| rs6947246 | A | G | 0.36 | 0.24 | 1.1E-8 | 0.14 | 7.1E-30 | 0.15 (0.01) | 5.5E-36 |
| rs6952851 | G | A | 0.36 | 0.24 | 1.4E-8 | 0.14 | 4.3E-30 | 0.15 (0.01) | 3.7E-36 |
| rs10231005 | A | C | 0.36 | 0.24 | 1.2E-8 | 0.14 | 2.3E-30 | 0.15 (0.01) | 1.6E-36 |
| rs76042375 | A | G | 0.05 | 0.55 | 3.5E-8 | 0.11 | 3.0E-5 | 0.14 (0.03) | 4.3E-8 |
| rs2707518 | T | G | 0.39 | 0.25 | 3.9E-9 | 0.17 | 1.8E-42 | 0.17 (0.01) | 1.7E-49 |
| rs2908007 | G | A | 0.40 | 0.23 | 3.5E-8 | 0.17 | 2.9E-45 | 0.17 (0.01) | 1.2E-51 |

EA, effect allele; OA, other allele; EAF, effect allele frequency; SE, standard error.

**Supplementary Table S3.** Genetic loci associated with QUS parameters at the suggestive level (*5×10-8* < *P* < *5×10-7*) in the gender-combined meta-analysis.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  |  |  |  | TwinsUK WGS | | TwinsUK GWAS | | EPIC GWAS | | Meta-analysis | |
| Trait | Locus | Variant | Closest gene | EA | OA | EAF | Beta | *P* | Beta | *P* | Beta | *P* | Beta (SE) | *P* |
| BUA | 2q14.2 | rs76588671 | *LOC101927709* | C | T | 0.16 | 0.11 | 0.03 | 0.02 | 0.76 | 0.08 | 3.8E-7 | 0.08 (0.02) | 1.8E-7 |
|  | 8p23.1 | rs4512344 | *XKR6/*  *MTMR9* | A | C | 0.49 | 0.11 | 0.005 | -0.04 | 0.54 | 0.05 | 5.5E-6 | 0.06 (0.01) | 3.9E-7 |
|  | 10q21.1 | rs6480949 | *MBL2* | T | C | 0.11 | 0.02 | 0.77 | -0.10 | 0.06 | -0.10 | 1.9E-7 | -0.09 (0.02) | 3.6E-7 |
|  | 15q26.2 | rs34408206 | *MCTP2* | G | A | 0.31 | 0.09 | 0.03 | 0.07 | 0.08 | 0.06 | 3.3E-6 | 0.06 (0.01) | 1.6E-7 |
|  | 19q13.11 | rs1559088 | *RHPN2/*  *GPATCH1* | T | C | 0.25 | 0.09 | 0.04 | 0.09 | 0.07 | 0.07 | 1.6E-6 | 0.07 (0.01) | 8.2E-8 |
| VOS | 2q14.2 | rs537474121 | *EN1* | - | GTCGT | 0.02 | 0.29 | 0.05 | 0.28 | 0.05 | 0.25 | 5.0E-6 | 0.25 (0.05) | 1.9E-7 |
|  | 7q21.3 | rs4448201 | *C7orf76* | G | C | 0.33 | 0.05 | 0.21 | -0.07 | 0.12 | -0.07 | 1.1E-8 | -0.06 (0.01) | 2.3E-7 |
|  | 10q21.1 | rs11003050 | *MBL2* | A | G | 0.11 | -0.02 | 0.71 | -0.10 | 0.08 | -0.10 | 5.0E-7 | -0.09 (0.02) | 3.8E-7 |
|  | 19q13.11 | rs58636263 | *GPATCH1* | - | T | 0.34 | 0.21 | 2.4E-5 | 0.06 | 0.27 | 0.06 | 5.1E-5 | 0.07 (0.01) | 3.8E-7 |

EA, effect allele; OA, other allele; EAF, effect allele frequency; SE, standard error.

**Supplementary Table S4.** Genetic loci associated with QUS parameters at the suggestive level (*5×10-8* < *P* < *5×10-7*) in the gender-stratified meta-analysis.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  |  |  |  | TwinsUK WGS | | TwinsUK GWAS | | EPIC GWAS | | Meta-analysis | |
| Trait | Locus | Variant | Closest gene | EA | OA | EAF | Beta | *P* | Beta | *P* | Beta | *P* | Beta (SE) | *P* |
| *Female-specific* | | | | | | | | | | | | | | |
| BUA | 6q25.1 | rs1038304 | *CCDC170* | A | G | 0.49 | 0.13 | 6.8E-4 | 0.03 | 0.44 | 0.07 | 2.1E-5 | 0.07 (0.01) | 3.4E-7 |
|  | 11q14.2 | rs533931 | *TMEM135* | A | G | 0.11 | -0.11 | 0.07 | -0.10 | 0.15 | -0.12 | 2.7E-6 | -0.12 (0.02) | 2.8E-7 |
|  | 17q23.2 | rs4968539 | *BCAS3* | C | T | 0.27 | 0.05 | 0.24 | 0.04 | 0.45 | 0.11 | 6.1E-8 | 0.10 (0.02) | 1.6E-7 |
|  | 22q11.21 | rs9606132 | *SEPT5* | C | G | 0.17 | -0.12 | 0.02 | -0.11 | 0.03 | -0.09 | 2.3E-5 | -0.10 (0.02) | 2.9E-7 |
| VOS | 6q22.33 | rs7741021 | *RSPO3* | C | A | 0.48 | 0.09 | 0.02 | 0.03 | 0.43 | 0.08 | 1.8E-6 | 0.08 (0.01) | 2.4E-7 |
|  | 8p22-p21.3 | rs2659678 | *PSD3/*  *LOC100128993* | T | C | 0.47 | 0.06 | 0.15 | 0.08 | 0.06 | 0.07 | 4.2E-6 | 0.07 (0.01) | 4.3E-7 |
|  | 19q13.11 | rs58636263 | *GPATCH1* | - | T | 0.33 | 0.21 | 2.4E-5 | 0.07 | 0.28 | 0.08 | 1.3E-4 | 0.09 (0.02) | 3.1E-7 |
| *Male-specific* | | | | | | | | | | | | | | |
| BUA | 1q31.3 | rs10921528 | Intergenic | G | C | 0.34 | ND | ND | -0.23 | 0.03 | -0.10 | 4.1E-7 | -0.10 (0.02) | 1.2E-7 |
|  | 7q36.1 | rs12216545 | *GIMAP7* | A | G | 0.38 | ND | ND | -0.11 | 0.28 | -0.10 | 1.5E-7 | -0.10 (0.02) | 1.4E-7 |
|  | 8p23.1 | rs73198271 | *CLDN23/*  *MFHAS1* | T | A | 0.26 | ND | ND | -0.11 | 0.33 | -0.10 | 3.9E-7 | -0.10 (0.02) | 3.8E-7 |
|  | 8p23.1 | rs62494425 | *PPP1R3B/*  *LOC101929128* | G | A | 0.47 | ND | ND | 0.09 | 0.42 | 0.09 | 2.3E-7 | 0.09 (0.02) | 2.5E-7 |
|  | 10q22.3 | rs2104157 | *ZCCHC24* | A | G | 0.28 | ND | ND | 0.18 | 0.10 | 0.10 | 7.2E-7 | 0.10 (0.02) | 3.5E-7 |
|  | 22q13.31 | rs62225284 | *TBC1D22A* | C | T | 0.23 | ND | ND | -0.12 | 0.29 | 0.23 | 2.2E-7 | -0.11 (0.02) | 2.0E-7 |
| VOS | 6q22.33 | rs7741021 | *RSPO3* | C | A | 0.48 | ND | ND | 0.12 | 0.25 | 0.09 | 4.1E-7 | 0.09 (0.02) | 3.1E-7 |
|  | 11q14.2 | rs636159 | *TMEM135* | T | G | 0.32 | ND | ND | ND | ND | -0.10 | 2.8E-7 | -0.10 (0.02) | 4.2E-7 |
|  | 12p11.22 | rs117611356 | *CCDC91* | A | C | 0.02 | ND | ND | 0.21 | 0.56 | 0.34 | 3.5E-7 | 0.07 (0.07) | 4.8E-7 |
|  | 14q32.2 | rs2629514 | *VRK1* | G | A | 0.05 | ND | ND | 0.03 | 0.90 | 0.21 | 1.6E-7 | 0.20 (0.04) | 3.2E-7 |
|  | 18q12.3 | rs4084232 | *SETBP1* | A | T | 0.47 | ND | ND | ND | ND | 0.09 | 2.6E-7 | 0.09 (0.02) | 3.9E-7 |

EA, effect allele; OA, other allele; EAF, effect allele frequency; SE, standard error.

**Supplementary Table S5.** Bioinformatics analysis of variants in strong LD (r2>0.8) with top variant from each novel BUA locus.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Locus | Variant | Relative  position\* | LD with lead variant (r2) | GERP score | Promoter histone marks | Enhancer histone marks | DNAse | eQTL results |
| 8p23.1 | rs439989 | -404 | 0.81 | 2.41 | - | - | - | 25 hits |
|  | rs367543 | 0 | 1 | 2.13 | - | 1 tissue | - | 14 hits |
|  | rs106380 | +930 | 0.97 | 1.94 | - | - | - | 17 hits |
|  | rs12547955 | +1,681 | 0.86 | -2.35 | - | 2 tissues | 1 tissue | 21 hits |
| 11q23.1 | rs1945120 | -16,118 | 0.8 | 2.19 | - | - | 2 tissues | 1 hit |
|  | rs7111561 | -9,310 | 0.8 | 3 | - | - | - | 1 hit |
|  | rs6589301 | -8,873 | 0.8 | 1.5 | - | - | - | 1 hit |
|  | rs6589302 | -8,791 | 0.8 | -3.25 | - | - | 2 tissues | 1 hit |
|  | rs7105119 | -6,071 | 0.8 | 2.74 | - | 1 tissue | - | 1 hit |
|  | rs7952251 | 0 | 1 | 0.24 | - | - | - | 2 hits |
|  | rs202046393 | +42 | 0.96 | ND | - | - | - | 2 hits |
|  | rs7109000 | +1,895 | 0.91 | -2.36 | 2 tissues | 13 tissues | 19 tissues | 1 hit |
|  | rs7121746 | +3,569 | 0.81 | 2.06 | 1 tissue | 3 tissues | 2 tissues | 1 hit |
| 22q11.21 | rs9606138 | -1,555 | 0.98 | -0.69 | 2 tissues | 7 tissues | - | 3 hits |
|  | rs202240051 | 0 | 1 | ND | 2 tissues | 10 tissues | 3 tissues | - |
|  | rs9606139 | +1,355 | 1 | 1.63 | 14 tissues | 9 tissues | 28 tissues | 3 hits |

GERP, genomic evolutionary rate profiling; eQTL, expression quantitative trait locus; ND, no data.

\*Relative to lead variant in locus (hg19).