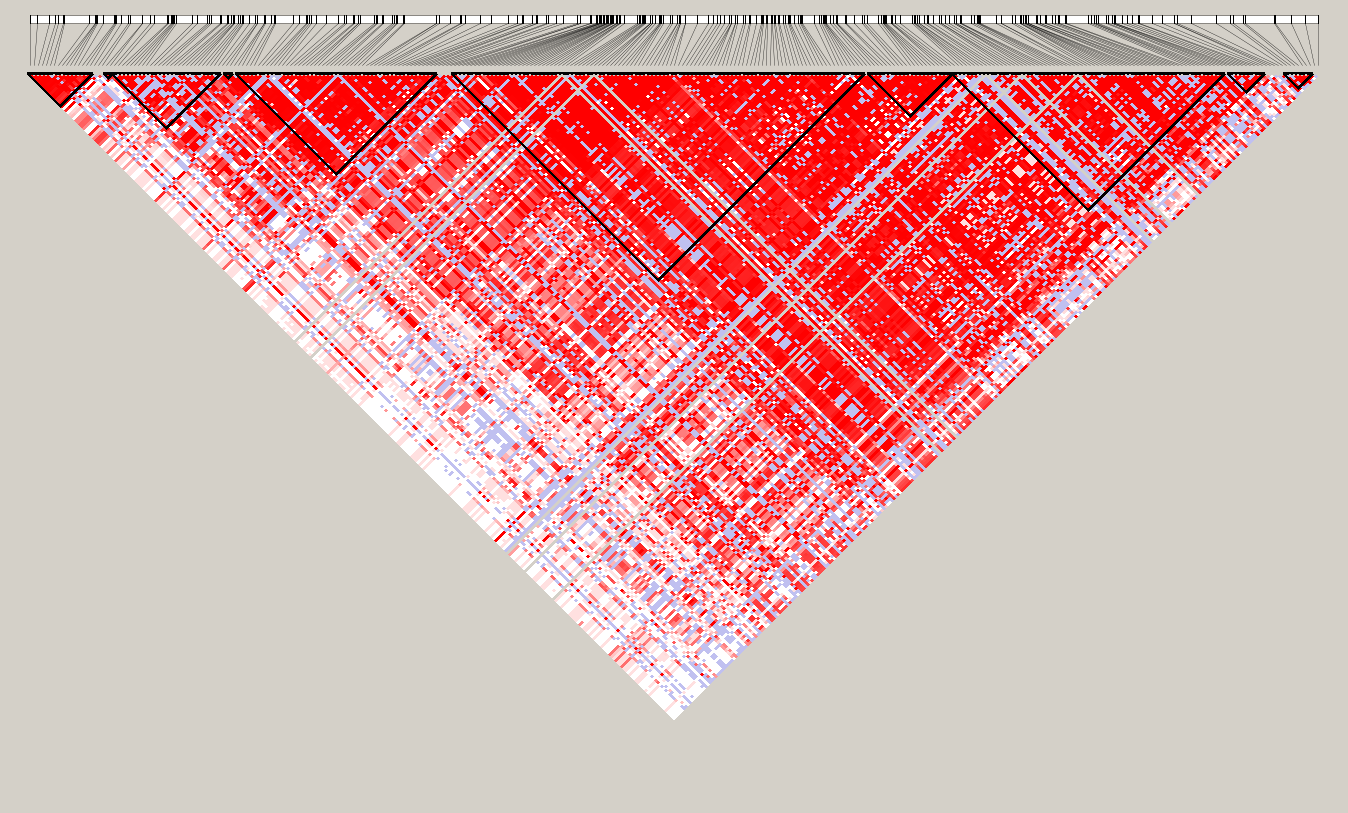
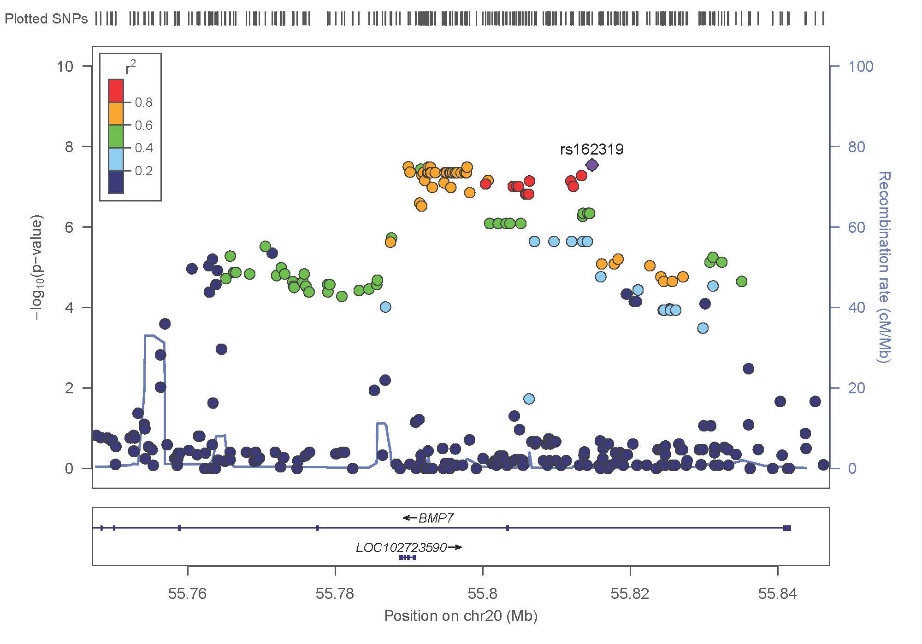
**Supplementary Materials**

**Figure S1.** The quantile-quantile (Q-Q) plot showing the observed distribution of –log10 (P values), obtained from the GWAS, in black, against the expected distribution under the null hypothesis of no association (diagonal line, in red).



GWAS, genome-wide association study.

**Figure S2.** Regional association plot and LD plot for imputed region on chromosome 20 (±50 kb from rs6127972). (**a**) *P*-values (-log10) are plotted against the genomic position of each SNP, with the genes in the region depicted below. The LD (*r2*) between rs6127972 and other SNPs are indicated with different colors. Recombination rate in cM per Mb using HapMap controls. The most significant association was for rs162391 (*P* = 2.86 x 10-8). (**b**) LD plot (*r2*) based on genotyped and imputed SNPs in this region for 215 NHW case-parent trios.

b.

a.

cM centimorgan; LD, linkage disequilibrium; Mb, megabase; NHW, non-Hispanic White; SNP, single nucleotide polymorph

**Table S1.** Association results for χ2 test for case/control study for 194 (148 males and 46 females) cases and 333 controls (253 males and 80 females). Each sample represents a different set of female controls in the analysis.

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| CHR | SNP | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | Sample 6 | Sample 7 | Sample 8 | Sample 9 | Sample 10 |
| *P* | *P* | *P* | *P* | *P* | *P* | *P* | *P* | *P* | *P* |
| 2 | rs2860732 | 0.1117 | 0.0919 | 0.1228 | 0.12300 | 0.1117 | 0.13500 | 0.0919 | 0.07498 | 0.13480 | 0.24580 |
| 5 | rs4976789 | 0.1879 | 0.2131 | 0.2131 | 0.31120 | 0.1879 | 0.55030 | 0.2405 | 0.34600 | 0.55030 | 0.74290 |
| 7 | rs1983511 | 0.0606 | 0.0687 | 0.0776 | 0.09819 | 0.0874 | 0.17740 | 0.0982 | 0.09819 | 0.13680 | 0.20610 |
| 7 | rs10254116 | 0.0036 | 0.0043 | 0.0050 | 0.00678 | 0.0050 | 0.01010 | 0.0056 | 0.00648 | 0.00648 | 0.02299 |
| 7 | rs10262453 | 0.00007 | 0.00009 | 0.00011 | 0.00030 | 0.00011 | 0.00042 | 0.00017 | 0.00028 | 0.00025 | 0.00114 |
| 7 | rs4723276 | 0.00008 | 0.00010 | 0.00013 | 0.00023 | 0.00012 | 0.00022 | 0.00019 | 0.00014 | 0.00023 | 0.00098 |
| 7 | rs10251775 | 0.1400 | 0.1557 | 0.1725 | 0.21040 | 0.1907 | 0.26560 | 0.1907 | 0.14760 | 0.27730 | 0.30330 |
| 8 | rs6992848 | 0.4841 | 0.4346 | 0.4841 | 0.55410 | 0.4346 | 0.46610 | 0.4194 | 0.37410 | 0.62920 | 0.53580 |
| 9 | rs55917620 | 0.6892 | 0.6715 | 0.6277 | 0.51860 | 0.6715 | 0.79940 | 0.6715 | 0.79940 | 0.89380 | 0.99130 |
| 9 | rs7853336 | 0.5626 | 0.5794 | 0.5794 | 0.73740 | 0.5432 | 0.74160 | 0.5081 | 0.71530 | 0.57940 | 0.60260 |
| 10 | rs17476559 | 0.8833 | 0.9310 | 0.8833 | 0.71950 | 0.9310 | 0.49980 | 0.8833 | 0.76660 | 0.82740 | 0.76660 |
| 11 | rs936460 | 0.8505 | 0.8097 | 0.8266 | 0.63570 | 0.9086 | 0.44890 | 0.8266 | 0.48190 | 0.47360 | 0.44890 |
| 15 | rs1873150 | 0.5298 | 0.5298 | 0.5839 | 0.47800 | 0.6398 | 0.52980 | 0.5839 | 0.52980 | 0.69750 | 0.58390 |
| 17 | rs1467288 | 0.2331 | 0.2331 | 0.2331 | 0.10950 | 0.2331 | 0.13490 | 0.2331 | 0.27520 | 0.12690 | 0.21830 |
| 17 | rs17688881 | 0.7032 | 0.7395 | 0.7032 | 0.95490 | 0.7120 | 0.97740 | 0.5990 | 0.81380 | 0.92850 | 0.79500 |
| 18 | rs12458289 | 0.7534 | 0.7534 | 0.6961 | 0.49000 | 0.7534 | 0.44500 | 0.7534 | 0.64100 | 0.64100 | 0.53780 |
| 20 | rs1884302 | 0.0278 | 0.0316 | 0.0278 | 0.02236 | 0.0316 | 0.02236 | 0.0214 | 0.02236 | 0.02667 | 0.03179 |
| 20 | rs230191 | 0.0427 | 0.0301 | 0.0478 | 0.04790 | 0.0301 | 0.09093 | 0.0236 | 0.03811 | 0.06664 | 0.05963 |
| 20 | rs230217 | 0.0134 | 0.0076 | 0.0117 | 0.00642 | 0.0076 | 0.00463 | 0.0057 | 0.00408 | 0.00968 | 0.00839 |
| 20 | rs145473835 | 0.1878 | 0.1878 | 0.1878 | 0.44740 | 0.1878 | 0.44740 | 0.1878 | 0.28240 | 0.18780 | 0.18710 |
| 20 | rs6127972 | 0.0088 | 0.0050 | 0.0064 | 0.00416 | 0.0050 | 0.00406 | 0.0037 | 0.00416 | 0.00864 | 0.00561 |
| 20 | rs115085047 | 0.6975 | 0.6975 | 0.6975 | 0.19010 | 0.6975 | 0.19010 | 0.6975 | 0.18950 | 0.69750 | 0.18950 |
| 20 | rs6014954 | 0.0071 | 0.0039 | 0.0061 | 0.00514 | 0.0039 | 0.00501 | 0.0028 | 0.00430 | 0.00801 | 0.00441 |
| 20 | rs230218 | 0.0029 | 0.0015 | 0.0025 | 0.00130 | 0.0015 | 0.00109 | 0.0011 | 0.00109 | 0.00295 | 0.00176 |
| 20 | rs17404303 | 0.0410 | 0.0262 | 0.0410 | 0.06010 | 0.0289 | 0.06852 | 0.0325 | 0.05869 | 0.09861 | 0.03584 |
| 20 | rs162316 | 0.8284 | 0.6625 | 0.8284 | 0.78570 | 0.7857 | 0.82840 | 0.7026 | 0.74370 | 0.66250 | 0.82840 |
| 20 | rs13038779 | 0.1750 | 0.1750 | 0.1750 | 0.19160 | 0.1318 | 0.18150 | 0.1595 | 0.16560 | 0.27920 | 0.18150 |
| 20 | rs12480759 | 0.5754 | 0.5862 | 0.6217 | 0.68380 | 0.5862 | 0.56450 | 0.5517 | 0.49730 | 0.46530 | 0.60200 |
| 21 | rs9977003 | 0.0733 | 0.0733 | 0.0814 | 0.04716 | 0.0752 | 0.02748 | 0.0658 | 0.07524 | 0.02359 | 0.03022 |
| X | rs4831031 | 0.4896 | 0.5369 | 0.4029 | 0.58660 | 0.4449 | 0.88730 | 0.5369 | 0.63870 | 0.58660 | 0.38640 |
| X | rs6629161 | 0.6822 | 0.5864 | 0.5864 | 0.65670 | 0.6822 | 0.73130 | 0.6087 | 0.70650 | 0.51810 | 0.60870 |
| X | rs7061921 | 0.9989 | 0.9178 | 0.8944 | 0.87160 | 0.8944 | 0.95930 | 0.8419 | 0.65830 | 0.65470 | 0.88790 |
| X | rs5926995 | 0.3336 | 0.3336 | 0.3336 | 0.28140 | 0.3336 | 0.35920 | 0.3336 | 0.25210 | 0.20990 | 0.37540 |
| X | rs7888988 | 0.9929 | 0.9929 | 0.9929 | 0.96930 | 0.9236 | 0.83310 | 0.9929 | 0.91940 | 0.96110 | 0.85190 |
| X | rs5908293 | 0.4753 | 0.4326 | 0.3921 | 0.76730 | 0.4753 | 0.82980 | 0.4326 | 0.87370 | 0.84760 | 0.93600 |
| X | rs66603953 | 0.6716 | 0.7281 | 0.7281 | 0.56550 | 0.6174 | 0.97000 | 0.7281 | 0.72810 | 0.96710 | 0.84080 |
| X | rs56248124 | 0.4890 | 0.6663 | 0.4890 | 0.43710 | 0.5446 | 0.28250 | 0.5446 | 0.52960 | 0.25700 | 0.50000 |
| X | rs41313408 | 0.5409 | 0.6010 | 0.5409 | 0.21860 | 0.6010 | 0.28730 | 0.6010 | 0.37100 | 0.54090 | 0.66460 |
| X | rs5984388 | 0.4872 | 0.5247 | 0.4309 | 0.39710 | 0.5635 | 0.53580 | 0.4872 | 0.45110 | 0.51930 | 0.28370 |
| X | rs34360385 | 0.0542 | 0.0366 | 0.0320 | 0.02422 | 0.0418 | 0.04498 | 0.0477 | 0.05818 | 0.04498 | 0.04837 |
| X | rs2573826 | 0.4346 | 0.4663 | 0.5137 | 0.44830 | 0.5480 | 0.38760 | 0.4483 | 0.33200 | 0.65700 | 0.23850 |
| X | rs7472191 | 0.5868 | 0.5472 | 0.5472 | 0.52440 | 0.5089 | 0.95630 | 0.6275 | 0.90810 | 0.84370 | 0.97600 |
| X | rs67804731 | 0.4608 | 0.4608 | 0.3932 | 0.66750 | 0.4608 | 0.60840 | 0.4261 | 0.43290 | 0.49010 | 0.25800 |
| X | rs17149475 | 0.8271 | 0.8351 | 0.8351 | 0.89310 | 0.8351 | 0.92330 | 0.7506 | 0.92330 | 0.83510 | 0.90710 |

**Table S2.** Genotypic transmission disequilibrium test analysis results under an additive, dominant, and recessive models for SNPs with a GWAS allelic transmission disequilibrium test *P*-value < 5 x 10-8.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| CHR | SNP | BP (hg19) | Allelic | |  | Additive | |  | Dominant | |  | Recessive | |
| OR | *P* |  | OR | *P* |  | OR | *P* |  | OR | *P* |
| 20 | rs6127972 | 55797057 | 2.17 | 4.41 x 10-8 |  | 2.17 | 9.38 x 10-8 |  | 2.25 | 0.0003 |  | 2.57 | 7.10 x 10-6 |
| 25 | rs6259097 | 89022047 | 0.34 | 6.22 x 10-9 |  | 0.34 | 2.98 x 10-8 |  | 0.33 | 1.8 x 10-7 |  | 0.18 | 0.0182 |
| 25 | rs2522623 | 91626534 | 0.45 | 1.76 x 10-8 |  | 0.45 | 4.14 x 10-8 |  | 0.55 | 0.0008 |  | 0.13 | 2.91 x 10-6 |
| 25 | rs2573826 | 91628577 | 0.45 | 3.31 x 10-8 |  | 0.45 | 7.36 x 10-8 |  | 0.56 | 0.0012 |  | 0.14 | 3.22 x 10-6 |
| 25 | rs2754857 | 91632746 | 0.44 | 1.09 x 10-8 |  | 0.44 | 2.73 x 10-8 |  | 0.53 | 0.0005 |  | 0.14 | 3.22 x 10-6 |
| 25 | rs781716 | 154994995 | 2.44 | 4.71 x 10-8 |  | 2.44 | 1.45 x 10-8 |  | 2.67 | 2.19 x 10-6 |  | 2.73 | 0.0002 |

BP, base pair; CHR, chromosome; GWAS, genome-wide association study; OR, odds ratio; SNP, single nucleotide polymorphism.

**Table S3.** Candidate gene list and number of SNPs

from array found in each gene region.

|  |  |
| --- | --- |
| Genea | Number of SNPsb |
| *ALPL* | 37 |
| *ALX3* | 2 |
| *ALX4* | 16 |
| *BBS9* | 52 |
| *BMP2* | 3 |
| *BMP4* | 7 |
| *BMP7* | 12 |
| *BMPER* | 42 |
| *CDC45* | 5 |
| *ESCO2* | 4 |
| *FAM20C* | 13 |
| *FBN1* | 10 |
| *FGF9* | 6 |
| *FGFR1* | 13 |
| *FGFR2* | 39 |
| *FREM1* | 79 |
| *GLI3* | 48 |
| *IFT122* | 1 |
| *IFT140* | 7 |
| *IFT43* | 9 |
| *IGF1R* | 104 |
| *IL11RA* | 4 |
| *IMPAD1* | 2 |
| *IRX5* | 1 |
| *KRAS* | 2 |
| *MASP1* | 20 |
| *MEGF8* | 1 |
| *MSX2* | 1 |
| *NTRK2* | 79 |
| *POR* | 16 |
| *POSTN* | 4 |
| *RAB23* | 1 |
| *RECQL4* | 1 |
| *RUNX2* | 20 |
| *SCARF2* | 3 |
| *SFRP2* | 3 |
| *SKI* | 15 |
| *SMAD6* | 15 |
| *SMO* | 4 |
| *SMURF1* | 6 |
| *SPRY1* | 1 |
| *SPRY4* | 3 |
| *STAT3* | 12 |
| *TCF12* | 16 |
| *TGFBR1* | 7 |
| *TGFBR2* | 52 |
| *WDR19* | 6 |
| *WDR35* | 4 |

SNP, single nucleotide polymorphism

atranscript including untranslated regions obtained from

[www.genome.ucsc.edu](http://www.genome.ucsc.edu), GRCh37/hg19

bonly SNPs with a minor allele frequency>15% and missing in <5 individuals were considered.

**Table S4.** Discovery study, replication study and meta-analysis results.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| CHR | SNPa | BP (hg19) | Location | Allelesb | Discovery | |  | Replication | | | |  | Meta-analysis | | |
| OR | *P* | RAF | Cases | Controls | OR | *P* |  | OR | *P* | Phet |
| 5 | rs4976789 | 177,968,840 | *COL23A1* | G/A | 2.83 | 7.56x10-6 | 0.14 | 193 | 329 | 1.20 | 0.3112 |  | 1.63 | 0.0008 | 0.0046 |
| 7 | rs10254116c | 33,237,489 | *BBS9* | C/T | 1.71 | 0.0002 | 0.35 | 186 | 331 | 1.44 | 0.0068 |  | 1.56 | 6.53x10-6 | 0.3722 |
| 7 | rs10262453c | 33,256,039 | *BBS9* | C/A | 1.79 | 4.48x10-5 | 0.34 | 184 | 318 | 1.62 | 0.0003 |  | 1.70 | 7.46x10-8 | 0.6296 |
| 7 | rs4723276c | 33,259,793 | *BBS9* | C/T | 1.80 | 1.88x10-5 | 0.34 | 188 | 332 | 1.63 | 0.0002 |  | 1.72 | 2.79x10-8 | 0.5373 |
| 18 | rs12458289 | 60,978,586 | *BCL2* | T/G | 3.38 | 5.58x10-6 | 0.11 | 193 | 330 | 0.86 | 0.4900 |  | 1.40 | 0.0468 | 0.0001 |
| 20 | rs230191 | 55,772,683 | *BMP7* | C/T\* | 1.87 | 9.30x10-6 | 0.49 | 191 | 324 | 0.77 | 0.0479 |  | 1.15 | 0.1437 | 5.11x10-6 |
| 20 | rs230217 | 55,796,517 | *BMP7* | G/C | 2.20 | 4.41x10-8 | 0.40 | 188 | 324 | 1.43 | 0.0064 |  | 1.72 | 2.24x10-8 | 0.0315 |
| 20 | rs6127972 | 55,797,057 | *BMP7* | T/G | 2.17 | 4.41x10-8 | 0.40 | 189 | 330 | 1.45 | 0.0042 |  | 1.74 | 1.27x10-8 | 0.0379 |
| 20 | rs6014954 | 55,797,585 | *BMP7* | T/G | 2.17 | 4.41x10-8 | 0.39 | 188 | 324 | 1.44 | 0.0051 |  | 1.72 | 2.40x10-8 | 0.0305 |
| 20 | rs230218 | 55,797,797 | *BMP7* | A/G | 2.17 | 4.41x10-8 | 0.40 | 188 | 326 | 1.52 | 0.0013 |  | 1.78 | 2.61x10-9 | 0.0676 |
| 20 | rs17404303 | 55,803,687 | *BMP7* | T/C | 0.52 | 2.37x10-6 | 0.40 | 190 | 328 | 0.78 | 0.0601 |  | 0.64 | 5.42x10-6 | 0.0375 |
| 20 | rs13038779 | 55,809,657 | *BMP7* | A/G | 0.49 | 3.05x10-6 | 0.30 | 191 | 331 | 0.83 | 0.1916 |  | 0.65 | 4.92x10-5 | 0.0134 |
| X | rs5984388 | 89,000,953 | *CPXCR1,TGIF2LX* | T/C | 0.46 | 12.71x10-7 | 0.25 | 186 | 323 | 0.40 | 0.7172 |  | 0.72 | 0.0027 | 3.92x10-5 |
| X | rs34360385 | 90,407,072 | *TGIF2LX,PABPC5* | T/C | 0.44 | 5.81x10-7 | 0.26 | 191 | 326 | 0.69 | 0.0242 |  | 0.56 | 6.27x10-7 | 0.0504 |
| X | rs2573826 | 91,628,577 | *PCDH11X* | G/A | 0.45 | 3.31x10-8 | 0.32 | 190 | 331 | 1.11 | 0.4483 |  | 0.72 | 0.0014 | 1.06x10-5 |
| X | rs7472191 | 92,245,578 | *PCDH11X,NAP1L3* | A/G | 0.47 | 2.70x10-6 | 0.26 | 190 | 332 | 1.11 | 0.5244 |  | 0.74 | 0.0069 | 0.0002 |
| X | rs67804731 | 154,995,079 | *TMLHE,SPRY3* | G/A | 0.93 | 0.7449 | 0.19 | 188 | 324 | 0.93 | 0.6675 |  | 0.93 | 0.5900 | 0.9978 |
| X | rs17149475 | 154,995,314 | *TMLHE,SPRY3* | G/A | 4.26 | 0.0390 | 0.04 | 193 | 331 | 1.04 | 0.8931 |  | 1.36 | 0.2559 | 0.1047 |

CHR, chromosome; SNP, single nucleotide polymorphism; BP, base pairs; RAF, risk allele frequency calculated from replication study controls; OR, odds ratio; Phet, test of homogeneity of the odds ratios

aSNPs in red font are those that were imputed in the 215 NHW trios discovery sample, and are in linkage disequilibrium to associated SNP

bMinor allele/major allele, \* indicates where minor allele differed between discovery and replication samples.

cSNP associated with sNCS

**Table S5.** GWASTDT analysis results for the top associations identified in the mNCS and sNCS GWAS, and meta-analysis results.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | GWAS mNCS | | | | | | | GWAS sNCS | | | | | | | Meta-analysis | |
| SNP | A1 | A2 | T | U | MAF | OR | *P* | A1 | A2 | T | U | MAF | OR | *P* | Meta-P | Phet |
| rs6127972 | T | G | 150 | 69 | 0.45 | 2.17 | 4.41x10-8 | T | G | 62 | 54 | 0.41 | 1.15 | 0.4576 | 1.16 x 10-6 | 0.0088 |
| rs1884302 | C | T | 86 | 94 | 0.30 | 0.91 | 0.5510 | C | T | 119 | 26 | 0.5 | 4.58 | 1.13x10-14 | 2.42 x 10-6 | 7.95x10-10 |
| rs10262453 | C | A | 134 | 75 | 0.38 | 1.79 | 4.48x10-5 | C | A | 15 | 76 | 0.26 | 0.20 | 1.61x10-10 | 0.9081 | 3.32x10-14 |

GWAS, genome-wide association study; TDT, transmission disequilibrium test; mNCS, metopic nonsyndromic craniosynostosis; sNCS, sagittal nonsyndromic craniosynostosis; SNP, single nucleotide polymorphism; A1, minor allele; A2, major allele; T, transmitted; U, untransmitted; MAF, minor allele frequency; OR, odds ratio; Phet, Cochran’s Q test of heterogeneity.