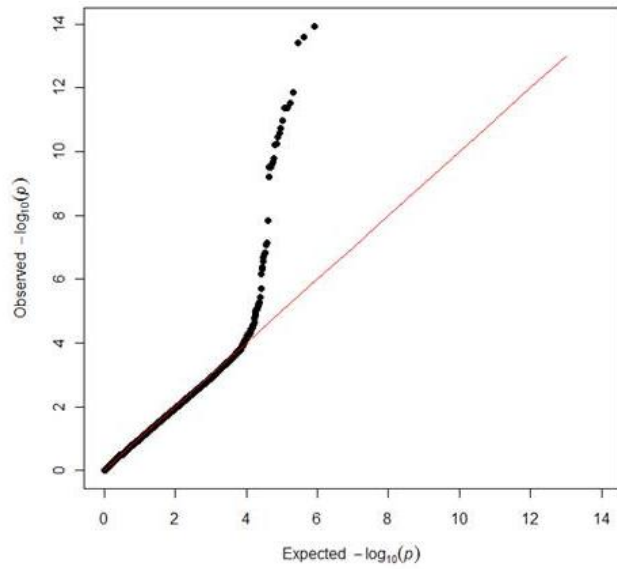


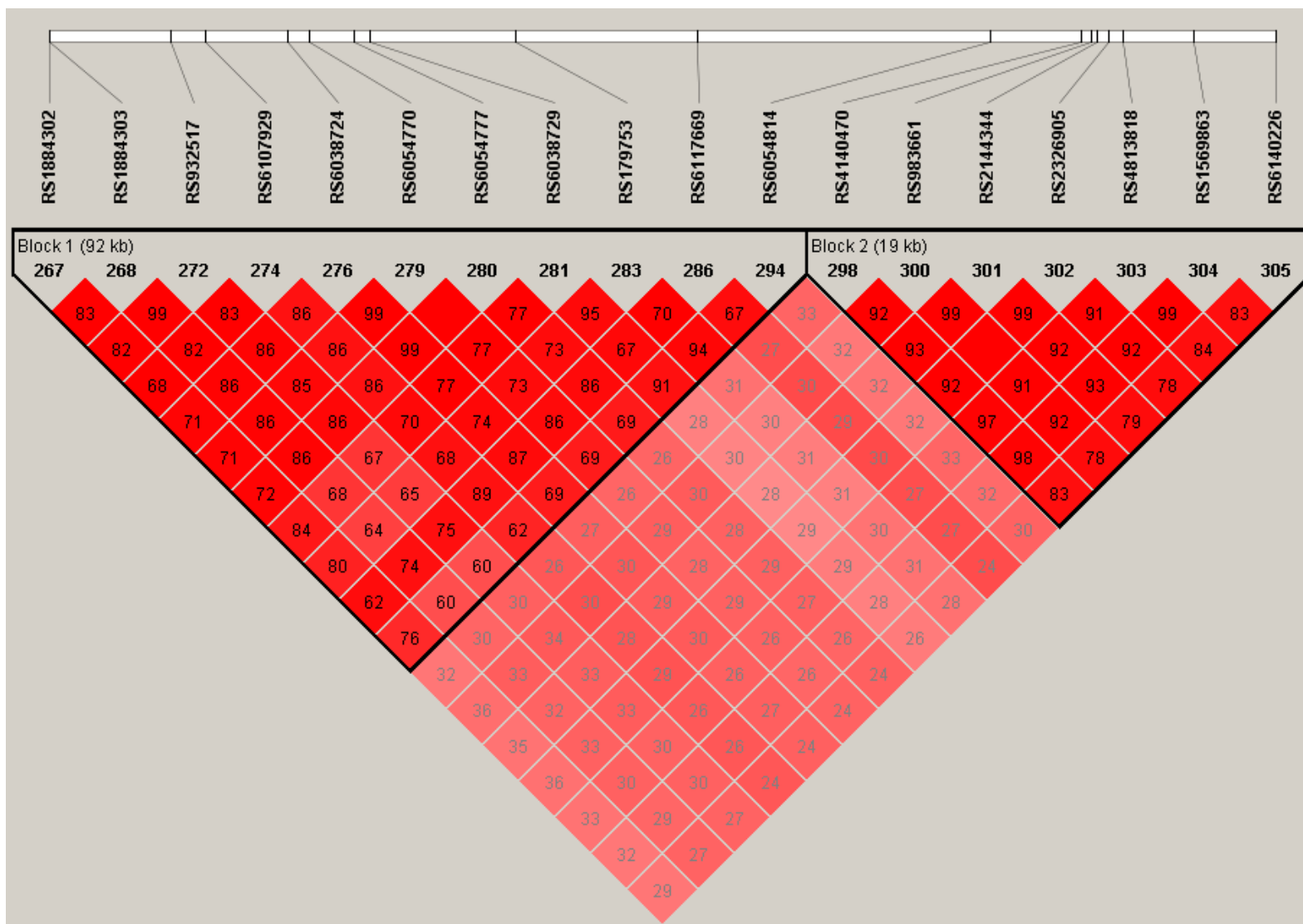
A genome-wide association study identifies susceptibility loci for non-syndromic sagittal craniosynostosis near *BMP2* and within *BBS9*.

Cristina M. Justice, Garima Yagnik, Yoonhee Kim, Inga Peter, Ethylin Wang Jabs, Monica Erazo, Xiaoqian Ye, Edmond Ainehsazan, Lisong Shi, Michael L. Cunningham, Virginia Kimonis, Tony Roscioli, Steven A. Wall, Andrew O.M. Wilkie, Joan Stoler, Joan T. Richtsmeier, Yann Heuzé, Pedro A. Sanchez-Lara, Michael F. Buckley, Charlotte M. Druschel, James L. Mills, Michele Caggana, Paul A. Romitti, Denise M. Kay, Craig Senders, Peter J. Taub, Ophir D. Klein, James Boggan, Marike Zwienenberg-Lee, Cyril Naydenov, Jinoh Kim, Alexander F. Wilson, Simeon A. Boyadjiev

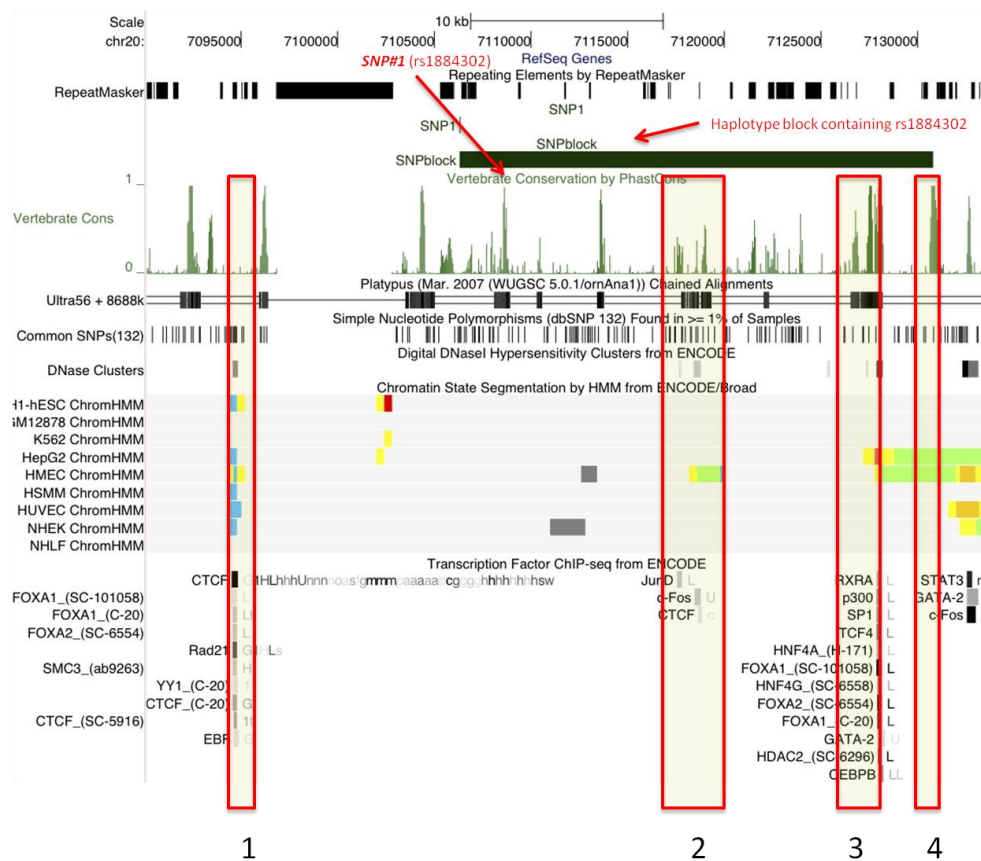
SUPPLEMENTARY INFORMATION



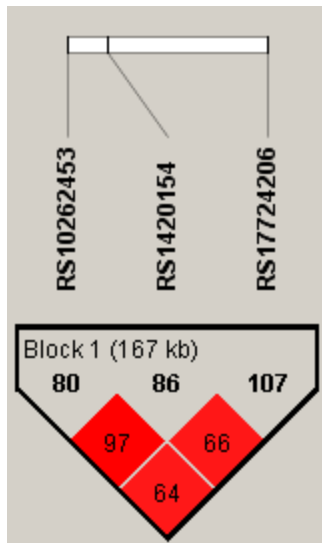
Supplementary Figure 1. The quantile-quantile plot of the observed P-values (obtained from the GWAS, in black) compared to the expected P-values (under the null hypothesis, in red)



Supplementary Figure 2. Linkage disequilibrium pattern (r^2) for genome-wide significant ($P < 5 \times 10^{-8}$) SNPs on 20p12.13



Supplementary Figure 3: Conserved regions on chromosome 20 within or adjacent to the associated haplotype block. All regional boundaries were defined based on human genome version hg19. Region 1 (ch20:7094394-7095215) is a possible insulator with multiple transcription factor (TF) binding sites. Region 2 (ch20:7118172-7119146) is hypersensitive to DNase and contains potential TF binding sites. Region 3 (ch20:7127018-7128451) is highly conserved among vertebrates with weak enhancer marks and several TF site. Region 4 (ch20:7130609-7131082) also demonstrates excellent conservation, but no known TF binding. Regions 2-4 are all within the haplotype block, while region 1 is telomeric of it (Figure courtesy of Douglas Mortlock, Vanderbilt University)



Supplementary Figure 4. Linkage disequilibrium pattern (r^2) for genome-wide significant ($P < 5 \times 10^{-8}$) SNPs on 7p14.3

Supplementary Table 1: Results from two-way gene-gene interaction tests.

SNP1	SNP2	Epistatic <i>P</i> -value	SNP1			SNP2		
			chr.	bp ^a	MAF ^b	chr.	bp ^a	MAF ^b
rs142092	rs761647	0.001495	20	7041432	0.47	2	113989575	0.40
rs2207719	rs761647	0.002801	20	7040386	0.47	2	113989575	0.36
rs8017520	rs7793862	0.003839	14	77440635	0.30	7	33270743	0.11
rs9933242	rs1160006	0.004746	16	51053221	0.34	1	231799950	0.43
rs6054814	rs7753	0.009449	20	7146501	0.36	3	198254194	0.47

^amap positions from dbSNP build 130; ^bMAF, minor allele frequencies in the discovery cohort

Supplementary Table 2: All discovery SNPs $P < 1.0 \times 10^{-5}$ and replication SNPs.

SNP ^a	Gene	Allele	Chr.	Position	Discovery p-value	Case ^b	Control ^b	Passed replication assay?	Replication p-value	Replicated p<0.05?
rs1884302	345 kb 3' of BMP2	C/T	20	7,054,289	1.1×10^{-14}	172	547	passed	4.4×10^{-31}	Yes
rs1884303	345 kb 3' of BMP2	G/A	20	7,054,353	2.6×10^{-14}			not selected		
rs932517	357 kb 3' of BMP2	C/T	20	7,066,235	4.0×10^{-14}			not selected		
rs6038729	376 kb 3' of BMP2	C/A	20	7,085,757	1.4×10^{-12}			not selected		
rs6054770	370 kb 3' of BMP2	A/G	20	7,079,752	3.0×10^{-12}			not selected		
rs6038724	368 kb 3' of BMP2	C/T	20	7,077,738	4.2×10^{-12}			not selected		
rs6054777	375 kb 3' of BMP2	A/G	20	7,084,244	4.2×10^{-12}			not selected		
rs6107929	360 kb 3' of BMP2	G/A	20	7,069,672	1.1×10^{-11}	172	547	passed	9.0×10^{-25}	Yes
rs179753	391 kb 3' of BMP2	C/T	20	7,099,968	1.9×10^{-11}	167	542	passed	5.0×10^{-24}	Yes
rs2326905	449 kb 3' of BMP2	C/T	20	7,158,137	2.6×10^{-11}			not selected		
rs6140226	465 kb 3' of BMP2	T/C	20	7,174,483	3.4×10^{-11}	172	548	passed	1.4×10^{-11}	Yes
rs6117669	408 kb 3' of BMP2	A/G	20	7,117,791	5.7×10^{-11}			not selected		
rs6054814	437 kb 3' of BMP2	G/T	20	7,146,501	6.1×10^{-11}	171	545	passed	4.1×10^{-20}	Yes
rs10262453	BBS9	C/A	7	33,222,564	1.6×10^{-10}	172	548	passed	3.5×10^{-14}	Yes
rs4140470	446 kb 3' of BMP2	G/T	20	7,155,479	2.2×10^{-10}	171	543	passed	1.3×10^{-12}	Yes
rs1420154	BBS9	T/C	7	33,257,456	2.5×10^{-10}	171	546	passed	2.7×10^{-13}	Yes
rs983661	447 kb 3' of BMP2	G/A	20	7,156,508	3.0×10^{-10}			not selected		
rs2144344	448 kb 3' of BMP2	G/A	20	7,156,971	3.0×10^{-10}			not selected		
rs1569863	457 kb 3' of BMP2	A/G	20	7,166,508	3.0×10^{-10}			not selected		
rs4813818	450 kb 3' of BMP2	A/G	20	7,159,515	6.1×10^{-10}			not selected		
rs17724206	BBS9	A/G	7	33,390,286	1.5×10^{-9}			failed		
rs7649443	DLG1	C/T	3	198,416,218	7.5×10^{-9}	171	545	passed	0.2	No
rs12152266	DLG1	T/C	3	198,395,758	8.3×10^{-9}			not selected		
rs2207584	447 kb 3' of BMP2	G/A	20	7,156,149	8.6×10^{-9}			not selected		
rs9826379	DLG1	C/T	3	198,311,645	1.6×10^{-7}	172	547	passed	0.22	No
rs2341184	DLG1	G/A	3	198,415,178	1.7×10^{-7}			not selected		
rs7793096	BBS9	A/G	7	33,249,102	1.7×10^{-7}			not selected		
rs10254116	BBS9	C/T	7	33,204,014	2.0×10^{-7}	161	529	passed	4.5×10^{-11}	Yes
rs12536844	BBS9	A/G	7	33,510,003	2.8×10^{-7}			not selected		
rs1009355	BBS9	A/G	7	33,185,288	4.2×10^{-7}	170	539	passed	1.7×10^{-10}	Yes
rs1894872	BBS9	C/T	7	33,226,684	4.9×10^{-7}			not selected		
rs4634034	18 kb 5' of ARFGAP3	G/A	22	41,504,357	7.1×10^{-7}			failed		
rs17597256	1.6 kb 5' of DLG1	T/C	3	198,252,251	2.0×10^{-6}			not selected		
rs4716412	52 kb 3' of LINC00574	T/C	6	169,996,971	3.6×10^{-6}	172	547	passed	0.0478	No ^c
rs2904698	CNOT7	G/A	8	17,134,884	3.8×10^{-6}	167	530	passed	0.298	No
rs11854994	SHC4	A/G	15	46,986,684	5.1×10^{-6}	170	545	passed	0.3	No
rs1029335	VPS37A	C/T	8	17,151,419	5.5×10^{-6}			not selected		
rs142092	332 kb 3' of BMP2	G/A	20	7,041,432	6.0×10^{-6}	171	545	passed	7.9×10^{-12}	Yes
rs7641	DLG1	C/G	3	198,254,379	7.1×10^{-6}	170	544	passed	0.113	No
rs7638423	DLG1	A/G	3	198,258,753	7.1×10^{-6}			not selected		
rs7818793	VPS37A	T/C	8	17,155,159	8.6×10^{-6}	162	531	passed	0.174	No
rs1108723	ADCK1	G/A	14	77,400,981	8.6×10^{-6}	171	545	passed	0.787	No
rs7457112	CNTNAP2	A/C	7	146,507,435	8.8×10^{-6}	163	540	passed	3.2×10^{-4}	No ^c
rs6805920	DLG1	C/A	3	198,446,726	9.3×10^{-6}	169	541	passed	0.394	No
rs1941500	LOC100130480	A/G	18	6,537,131	9.5×10^{-6}	169	541	passed	0.336	No
rs4522429	PDILT	A/G	16	20,293,616	9.8×10^{-6}	171	535	passed	0.0234	No ^c

^aSNPs in red were initially selected for replication

^bnumber of cases and controls with genotype called

^cSNPs with opposite direction of effect between discovery and replication analysis

Supplementary Table 3. Results of association analysis of sNSC

chr.	SNP	Alleles ^a	RAF ^c	GWAS		Replication		Meta-analysis		
				OR (95% CI)	<i>P</i> -value	OR (95% CI)	<i>P</i> -value	OR (95% CI)	<i>P</i> -value	H <i>P</i> ^d
20	rs1884302	<u>C</u> /T	0.30	4.58 (2.99-6.97)	1.1 X 10 ⁻¹⁴	4.30 (3.33-5.56)	4.4 X 10 ⁻³¹	4.38 (3.51-5.45)	1.1 X 10 ⁻³⁹	0.81
20	rs6107929	<u>G</u> /A	0.20	3.82 (2.52-5.79)	1.1 X 10 ⁻¹¹	3.72 (2.87-4.83)	9.0 X 10 ⁻²⁵	3.75 (3.01-4.67)	5.0 X 10 ⁻³²	0.92
20	rs179753	<u>C</u> /T	0.30	3.92 (2.55-6.03)	1.9 X 10 ⁻¹¹	3.59 (2.78-4.63)	5.0 X 10 ⁻²⁴	3.67 (2.95-4.57)	3.4 X 10 ⁻³¹	0.73
20	rs6140226	T/ <u>C</u>	0.49	0.24 (0.15-0.38)	3.4 X 10 ⁻¹¹	0.41 (0.31-0.53)	1.4 X 10 ⁻¹¹	0.36 (0.29-0.45)	1.2 X 10 ⁻¹⁸	0.05
20	rs6054814	<u>G</u> /T	0.30	3.74 (2.45-5.72)	6.1 X 10 ⁻¹¹	3.15 (2.45-4.04)	4.1 X 10 ⁻²⁰	3.29 (2.65-4.08)	2.8 X 10 ⁻²⁷	0.49
20	rs4140470	G/ <u>T</u>	0.51	0.28 (0.18-0.42)	2.2 X 10 ⁻¹⁰	0.40 (0.30-0.51)	1.3 X 10 ⁻¹²	0.36 (0.29-0.45)	1.5 X 10 ⁻¹⁹	0.16
20	rs142092	<u>G</u> /A	0.31	2.39 (1.62-3.53)	6.9 X 10 ⁻⁶	2.35 (1.84-3.02)	7.0 X 10 ⁻¹²	2.36 (1.92-2.91)	7.5 X 10 ⁻¹⁶	0.95
7	rs10262453	C/ <u>A</u>	0.31	0.20 (0.11-0.34)	1.6 X 10 ⁻¹⁰	0.26 (0.18-0.37)	3.5 X 10 ⁻¹⁴	0.24 (0.17-0.32)	5.6 X 10 ⁻²⁰	0.44
7	rs1420154	T/ <u>C</u>	0.31	0.21 (0.12-0.36)	2.5 X 10 ⁻¹⁰	0.28 (0.19-0.40)	2.7 X 10 ⁻¹³	0.25 (0.19-0.34)	3.1 X 10 ⁻¹⁹	0.38
7	rs10254116	C/ <u>T</u>	0.33	0.25 (0.14-0.44)	2.0 X 10 ⁻⁷	0.33 (0.24-0.47)	4.5 X 10 ⁻¹¹	0.31 (0.23-0.41)	2.2 X 10 ⁻¹⁵	0.44
7	rs1009355	A/ <u>G</u>	0.46	0.38 (0.26-0.56)	4.2 X 10 ⁻⁷	0.44 (0.34-0.57)	1.7 X 10 ⁻¹⁰	0.42 (0.34-0.52)	2.2 X 10 ⁻¹⁵	0.56

^aminor allele/major allele; ^bfrom HapMap CEU, minor allele frequency/major allele frequency; ^cRAF (risk allele frequency) calculated from controls; ^d*P*-value for heterogeneity between the discovery and replication cohorts. Underlined alleles are overtransmitted in the sNSC cohort.

Supplementary Table 4. gTDT analysis results based on additive, dominant and recessive models for GWAS SNPs in Supp. Table 3

SNPs	Genetic model	Coefficient gTDT	OR	Lower OR (95%CI)	Upper OR (95%CI)	P-value
rs1884302 (Chr20)	additive	1.521	4.577	2.994	6.996	2.12E-12
	dominant	1.814	6.135	2.630	14.310	2.69E-05
	recessive	1.712	5.539	3.277	9.363	1.63E-10
rs6107929 (Chr20)	additive	1.341	3.821	2.521	5.793	2.69E-10
	dominant	1.434	4.195	2.199	8.004	1.36E-05
	recessive	1.598	4.944	2.754	8.878	8.68E-08
rs179753 (Chr20)	additive	1.367	3.923	2.550	6.034	4.92E-10
	dominant	1.734	5.661	2.413	13.280	6.76E-05
	recessive	1.476	4.377	2.549	7.516	8.74E-08
rs6140226 (Chr20)	additive	-1.418	0.242	0.154	0.382	1.04E-09
	dominant	-1.517	0.219	0.131	0.369	1.03E-08
	recessive	-1.803	0.165	0.050	0.544	3.10E-03
rs6054814 (Chr20)	additive	1.319	3.741	2.446	5.720	1.13E-09
	dominant	1.557	4.743	2.135	10.540	1.32E-04
	recessive	1.476	4.377	2.549	7.516	8.74E-08
rs4140470 (Chr20)	additive	-1.289	0.276	0.180	0.422	3.01E-09
	dominant	-1.424	0.241	0.146	0.398	2.92E-08
	recessive	-1.450	0.235	0.091	0.605	2.71E-03
rs142092 (Chr20)	additive	0.871	2.389	1.619	3.525	1.15E-05
	dominant	0.553	1.739	1.023	2.956	0.04096
	recessive	1.465	4.329	2.355	7.957	2.39E-06
rs10262453 (Chr7)	additive	-1.623	0.197	0.113	0.343	9.28E-09
	dominant	-1.645	0.193	0.105	0.354	1.04E-07
	recessive	-2.634	0.072	0.010	0.536	0.01023
rs1420154 (Chr7)	additive	-1.571	0.208	0.121	0.356	1.07E-08
	dominant	-1.602	0.202	0.112	0.363	9.88E-08
	recessive	-2.587	0.075	0.010	0.562	0.01167
rs10254116 (Chr7)	additive	-1.386	0.250	0.142	0.440	1.57E-06
	dominant	-1.433	0.239	0.125	0.457	1.56E-05
	recessive	-1.907	0.149	0.034	0.640	0.0105
rs1009355 (Chr7)	additive	-0.966	0.380	0.258	0.562	1.14E-06
	dominant	-1.082	0.339	0.204	0.562	2.79E-05
	recessive	-1.086	0.338	0.168	0.677	2.24E-03

Supplementary Table 5. Imputed SNPs on 3q29 reaching genome-wide significance ($P < 5 \times 10^{-8}$)

SNP	chr.	Position	Nearby Gene	Distance (bp) ^a	Allele ^b	OR (95% CI)	<i>P</i> value
rs12374173	3	198300686	DLG1	46858 209158	C/T	0.29 (0.18-0.46)	2.94 X 10 ⁻⁸
rs13099700	3	198303250	DLG1	49422 206594	G/A	0.29 (0.18-0.46)	2.94 X 10 ⁻⁸
rs13317398	3	198308918	DLG1	55090 200926	A/G	0.29 (0.18-0.46)	2.94 X 10 ⁻⁸
rs9826379	3	198311645	DLG1	57817 198199	C/T	0.29 (0.18-0.46)	1.95 X 10 ⁻⁸
rs56309312	3	198314904	DLG1	61076 194940	C/T	0.29 (0.18-0.46)	1.95 X 10 ⁻⁸
rs3906480	3	198317692	DLG1	63864 192152	G/C	0.29 (0.18-0.46)	1.95 X 10 ⁻⁸
rs6782621	3	198323425	DLG1	69597 186419	C/T	0.29 (0.18-0.46)	1.95 X 10 ⁻⁸
rs12631361	3	198325971	DLG1	72143 183873	A/C	0.29 (0.18-0.46)	1.95 X 10 ⁻⁸
rs3773855	3	198352268	DLG1	98440 157576	A/G	0.29 (0.18-0.46)	3.24 X 10 ⁻⁸
rs11185466	3	198353324	DLG1	99496 156520	A/G	0.29 (0.18-0.46)	2.94 X 10 ⁻⁸
rs6804327	3	198355134	DLG1	101306 154710	G/C	0.29 (0.18-0.46)	3.24 X 10 ⁻⁸
rs6771091	3	198355392	DLG1	101564 154452	C/T	0.29 (0.18-0.46)	3.24 X 10 ⁻⁸
rs35624335	3	198367478	DLG1	113650 142366	C/T	0.29 (0.18-0.46)	1.95 X 10 ⁻⁸
rs35429814	3	198376161	DLG1	122333 133683	A/C	0.29 (0.18-0.46)	1.95 X 10 ⁻⁸
rs7642604	3	198376383	DLG1	122555 133461	A/G	0.29 (0.18-0.46)	1.95 X 10 ⁻⁸
rs7634994	3	198376410	DLG1	122582 133434	C/T	0.29 (0.18-0.46)	1.95 X 10 ⁻⁸
rs1868891	3	198386089	DLG1	132261 123755	A/G	0.29 (0.18-0.46)	1.95 X 10 ⁻⁸
rs13070996	3	198390471	DLG1	136643 119373	T/G	0.28 (0.18-0.45)	8.52 X 10 ⁻⁹
rs13081715	3	198391687	DLG1	137859 118157	T/G	0.29 (0.18-0.46)	1.95 X 10 ⁻⁸
rs12152266	3	198395758	DLG1	141930 114086	T/C	0.27 (0.17-0.44)	1.01 X 10 ⁻⁸
rs4491876	3	198401969	DLG1	148141 107875	T/C	0.28 (0.17-0.44)	9.31 X 10 ⁻⁹
rs3821714	3	198402467	DLG1	148639 107377	C/T	0.28 (0.17-0.44)	9.31 X 10 ⁻⁹
rs34207201	3	198406488	DLG1	152660 103356	G/C	0.28 (0.17-0.44)	9.31 X 10 ⁻⁹
rs13080468	3	198409497	DLG1	155669 100347	T/C	0.28 (0.17-0.44)	9.31 X 10 ⁻⁹
rs12630261	3	198409673	DLG1	155845 100171	A/T	0.28 (0.17-0.44)	9.31 X 10 ⁻⁹
rs2341184	3	198415178	DLG1	161350 94666	C/T	0.28 (0.17-0.44)	9.31 X 10 ⁻⁹
rs7649443	3	198416218	DLG1	162390 93626	C/T	0.28 (0.17-0.44)	9.31 X 10 ⁻⁹
rs12632727	3	198416459	DLG1	162631 93385	T/G	0.28 (0.17-0.44)	9.31 X 10 ⁻⁹
rs7616531	3	198424280	DLG1	170452 85564	T/C	0.28 (0.17-0.44)	9.31 X 10 ⁻⁹
rs35136822	3	198425670	DLG1	171842 84174	A/G	0.28 (0.17-0.44)	9.31 X 10 ⁻⁹
rs6782349	3	198433204	DLG1	179376 76640	T/C	0.28 (0.17-0.44)	9.31 X 10 ⁻⁹
rs2044861	3	198433961	DLG1	180133 75883	T/C	0.28 (0.17-0.44)	9.31 X 10 ⁻⁹
rs2044860	3	198434267	DLG1	180439 75577	T/C	0.28 (0.17-0.44)	9.31 X 10 ⁻⁹
rs10461050	3	198436568	DLG1	182740 73276	C/A	0.28 (0.17-0.44)	9.31 X 10 ⁻⁹
rs10461048	3	198437688	DLG1	183860 72156	A/T	0.28 (0.17-0.44)	9.31 X 10 ⁻⁹
rs10489880	3	198445583	DLG1	191755 64261	G/A	0.28 (0.17-0.44)	9.31 X 10 ⁻⁹
rs13077565	3	198451217	DLG1	197389 58627	T/C	0.28 (0.18-0.45)	2.14 X 10 ⁻⁸
rs7624764	3	198455594	DLG1	201766 54250	C/G	0.28 (0.18-0.45)	2.14 X 10 ⁻⁸
rs61394945	3	198458999	DLG1	205171 50845	C/A	0.29 (0.19-0.47)	4.43 X 10 ⁻⁸
rs35112822	3	198460866	DLG1	207038 48978	T/C	0.29 (0.19-0.47)	4.43 X 10 ⁻⁸
rs2340433	3	198461276	DLG1	207448 48568	C/A	0.29 (0.19-0.47)	4.43 X 10 ⁻⁸
rs62282105	3	198463116	DLG1	209288 46728	C/T	0.29 (0.19-0.47)	4.43 X 10 ⁻⁸
rs35665404	3	198466168	DLG1	212340 43676	G/A	0.29 (0.19-0.47)	4.43 X 10 ⁻⁸
rs7619429	3	198483487	DLG1	229659 26357	T/G	0.29 (0.19-0.47)	4.43 X 10 ⁻⁸
rs1073726	3	198485405	DLG1	231577 24439	A/G	0.29 (0.19-0.47)	4.43 X 10 ⁻⁸
rs34121928	3	198487266	DLG1	233438 22578	C/T	0.29 (0.19-0.47)	4.43 X 10 ⁻⁸
rs6800383	3	198489443	DLG1	235615 20401	T/C	0.29 (0.19-0.47)	4.43 X 10 ⁻⁸
rs13072590	3	198496077	DLG1	242249 13767	A/G	0.29 (0.19-0.47)	4.43 X 10 ⁻⁸
rs6779689	3	198498570	DLG1	244742 11274	C/T	0.29 (0.19-0.47)	4.43 X 10 ⁻⁸
rs34305944	3	198499815	DLG1	245987 10029	A/C	0.29 (0.19-0.47)	4.43 X 10 ⁻⁸
rs6782876	3	198505543	DLG1	251715 4301	G/A	0.29 (0.19-0.47)	4.43 X 10 ⁻⁸
rs3915512	3	198506637	DLG1	252809 3207	T/A	0.29 (0.19-0.47)	4.43 X 10 ⁻⁸

^afrom start of gene||from end of gene; ^bminor allele/major allele

Supplementary Table 6. Sequencing Primers

A. *BMP2* primers

BMP2PCR1FW	5' - AGACTCGTGAGTGTGCCAAG - 3'	BMP2PCR1RV	5' - GGCCAAGCTGCCTCTACA - 3'
BMP2PCR2FW	5' - GCCACCTGGAAGCAGTATTC - 3'	BMP2PCR2RV	5' - TGTTTTCCCAACTTCTTTTCG - 3'

B. *BBS9* cDNA primers

BBS9PCR1FW	5' - ATCACTGGGACCTTGTGAGGCG - 3'	BBS9PCR1RV	5' - CCTGCCTTTTATCGCATCTGTTGC - 3'
BBS9PCR2FW	5' - GATGGTATTTGAGCAGGAGAG - 3'	BBS9PCR2RV	5' - TCTTTGATGATTTTCTGAAGTTC - 3'
BBS9PCR3FW	5' - GTAGCAGTAAGAGTGGGCTGTTTG - 3'	BBS9PCR3RV	5' - CTAATTTTTCACCATTATCCGTAG - 3'
BBS9PCR4FW	5' - CAGGGAGTCAAAGATTTTGCATG - 3'	BBS9PCR4RV	5' - AGCAGATGGATGACCAAAGGCAAC - 3'
