

Supplementary Material

A genome-wide association study of northwestern Europeans involves the CNP signaling pathway in the etiology of human height variation

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De novo genotyping

Genotypes for twelve SNPs were generated using Taqman Allelic discrimination (Applied Biosystems Inc., Foster City, CA, USA). Ten SNPs (rs10472828, rs4362677, rs1552590, rs8009692, rs16970909, rs139909, rs6717918, rs5757949, rs12894275 and rs10898392) were available at www.appliedbiosystems.com as pre-designed assays (C__1237975_10, C_26146185_10, C__1182137_10, C_31535030_10, C_33719154_10, C____13406_10, C__207953_10, C__2487571_20, C_31068339_10 and C_31327595_10). The other two SNPs, rs2804845 and rs9522262, were custom assays ordered at www.appliedbiosystems.com. (Rs2804845 forward primer TCTGGTAAGGGTGTGGAGGG, reverse primer TGCCTTAGGTAGAATTGGAACAAACA, probe 1 TTGTCCCAAGCTTAATAT, probe 2 TTTGTCCCAAGTTTAATAT. Rs9522262 forward primer TCTGAATGACAAGGACTCTGCTAGA, reverse primer GCTCAAATACATCGCTTGAAAGCAA, probe1 TTCTGAAGTTAGAGCTTC, probe2

TTCTGAAGTTACAGCTTC). The PCR reaction mixture included 1-2 ng of genomic DNA in a 2 µl volume and the following reagents: FAM and VIC probes (200 nM), primers (0.9 µM), 2x Taqman PCR master mix (Applied Biosystems Inc). PCR cycling reaction were performed in 384 wells PCR plates in an ABI 9700 PCR system (Applied Biosystems Inc) and consisted of initial denaturation for 15 minutes at 95° C, and 40 cycles with denaturation of 15 seconds at 95° C and annealing and extension for 60 seconds at 60° C. Results were analyzed by the LightCycler® 480II System using the LightCycler® 480 Software release 1.5.0 version 1.5.0.39 (Roche Diagnostics GmbH, Mannheim, Germany).

Table 1. Results in 10,074 Dutch and German samples for SNPs with reported height association in humans from the stage one meta-analysis.

SNP	CHR	POS	Closest Gene	2nd Closest Gene	Effect Allele	Other Allele	Mean Frequency	Effect	SE	P-value
rs11809207	1	26393727	CATSPER4	CNKSR1	A	G	0.1911	0.0346	0.019	0.06839
rs6686842	1	41303458	SCMH1	SLFNL1	T	C	0.432	0.0302	0.0147	0.04044
rs12735613	1	118685496	SPAG17	WDR3	A	G	0.2041	-0.0452	0.0185	0.01478
rs11205277	1	148159496	SF3B4	SV2A	A	G	0.5544	-0.0376	0.0153	0.01381
rs678962	1	170456512	DNM3	C1orf105	T	G	0.7875	-0.0157	0.0185	0.3953
rs2274432	1	182287568	C1orf19	GLT25D2	A	G	0.3656	0.0643	0.0156	3.87E-05
rs1390401	1	225864573	ZNF678	JMJD4	A	G	0.8108	0.0373	0.0189	0.04891
rs3791679	2	55950396	EFEMP1	PNPT1	A	G	0.7623	0.0203	0.0177	0.2517
rs3791675	2	55964813	EFEMP1	PNPT1	T	C	0.239	-0.0223	0.0178	0.2096
rs6724465	2	219652090	NHEJ1	IHH	A	G	0.0971	-0.0557	0.025	0.02577
rs10935120	3	135715782	CEP63	ANAPC13	A	G	0.3112	-0.0053	0.0162	0.742
rs6440003	3	142576899	ZBTB38	ACPL2	A	G	0.4726	0.0634	0.0148	1.81E-05
rs6763931	3	142585523	ZBTB38	ACPL2	A	G	0.4726	0.0627	0.0148	2.21E-05
rs724016	3	142588260	ZBTB38	ACPL2	A	G	0.5275	-0.0627	0.0148	2.22E-05
rs16896068	4	17553938	LCORL	NCAPG	A	G	0.1433	-0.0616	0.0211	0.003484
rs6830062	4	17626828	LCORL	NCAPG	T	C	0.8565	0.0616	0.0211	0.003516
rs1812175	4	145794294	HHIP	ANAPC10	A	G	0.1547	-0.0537	0.0206	0.009014
rs1492820	4	145869471	HHIP	ANAPC10	A	G	0.554	0.0426	0.0153	0.005266
rs10472828	5	32924575	C5orf23	NPR3	T	C	0.4403	-0.073	0.0148	8.10E-07
rs12198986	6	7665058	BMP6	C6orf151	A	G	0.4738	0.018	0.0147	0.2223
rs10946808	6	26341366	HIST1H1D	HIST1H4F	A	G	0.7216	0.0583	0.0167	0.000469
rs2844479	6	31680935	AIF1	NCR3	A	C	0.6691	0.0032	0.0161	0.8438
rs185819	6	32158045	TNXB	CREBL1	T	C	0.5108	-0.0304	0.0148	0.03993
rs1776897	6	34302989	HMGA1	C6orf1	T	G	0.9123	-0.1259	0.0265	2.07E-06
rs2814993	6	34726871	C6orf106	SPDEF	A	G	0.1508	0.0706	0.0208	0.000682
rs4713858	6	35510763	PPARD	FANCE	A	G	0.1461	-0.054	0.0211	0.01038
rs314277	6	105514355	LIN28B	HACE1	A	C	0.1416	0.0352	0.022	0.1103

rs4549631	6	127008001	C6orf173	RSPO3	T	C	0.5088	-0.049	0.0147	0.000863
rs4896582	6	142745570	GPR126	VTA1	A	G	0.2914	-0.0759	0.0164	3.56E-06
rs3748069	6	142809326	GPR126	VTA1	A	G	0.7192	0.0744	0.0163	5.01E-06
rs798544	7	2729628	GNA12	AMZ1	T	C	0.2814	-0.0554	0.0163	0.000701
rs849141	7	28151616	JAZF1	CREB5	A	G	0.3055	0.0631	0.0161	8.97E-05
rs2040494	7	92094841	CDK6	FAM133B	T	C	0.5314	0.0772	0.0148	1.81E-07
rs2282978	7	92102346	CDK6	FAM133B	T	C	0.6571	-0.0656	0.0156	2.66E-05
rs10958476	8	57258362	PLAG1	CHCHD7	T	C	0.7686	-0.036	0.0181	0.04642
rs9650315	8	57318152	CHCHD7	PLAG1	T	G	0.1205	-0.1006	0.0239	2.64E-05
rs7846385	8	78322734	PXMP3	ZFH4	T	C	0.7056	-0.0397	0.0163	0.015
rs10512248	9	97299524	PTCH1	FANCC	T	G	0.6659	-0.0328	0.0161	0.04165
rs4743034	9	108672174	ZNF462	RAD23B	A	G	0.2342	-0.0024	0.0174	0.8902
rs1042725	12	64644614	HMG2A	C12orf31	T	C	0.4813	-0.0594	0.0148	6.28E-05
rs8756	12	64646019	HMG2A	C12orf31	A	C	0.4997	-0.0595	0.0148	6.00E-05
rs11107116	12	92502635	SOCS2	MRPL42	T	G	0.2334	0.048	0.0173	0.005645
rs3116602	13	50009356	DLEU7	RNASEH2B	T	G	0.8139	0.1152	0.0193	2.32E-09
rs7153027	14	91496975	TRIP11	FBLN5	A	C	0.5705	-0.0179	0.0148	0.2268
rs8007661	14	91529711	TRIP11	FBLN5	T	C	0.3902	0.0327	0.016	0.04124
rs2562784	15	82077496	SH3GL3	ADAMTSL3	A	G	0.785	-0.0301	0.0182	0.09693
rs10906982	15	82359162	ADAMTSL3	SH3GL3	A	T	0.5194	0.0437	0.0146	0.002851
rs8041863	15	87160693	ACAN	HAPLN3	A	T	0.4557	0.0205	0.0155	0.1867
rs4533267	15	98603794	ADAMTSL3	LASS3	A	G	0.2825	0.0291	0.0164	0.07607
rs3760318	17	26271841	CENTA2	C17orf42	A	G	0.355	-0.0641	0.0155	3.49E-05
rs4794665	17	52205328	C17orf67	DGKE	A	G	0.5171	0.0284	0.0148	0.05391
rs757608	17	56852059	C17orf82	TBX2	A	G	0.3424	0.0462	0.0157	0.00317
rs4800148	18	18978326	CABLES1	RBBP8	A	G	0.8083	0.0859	0.019	5.91E-06
rs8099594	18	45245158	DYM	C18orf32	A	G	0.6635	0.0264	0.0161	0.1019
rs12986413	19	2121954	DOT1L	AP3D1	A	T	0.5218	-0.0268	0.0148	0.07117
rs967417	20	6568893	BMP2	C20orf42	A	G	0.4523	-0.0264	0.0148	0.07423
rs6060369	20	33370575	UQCC	FAM83C	T	C	0.6019	-0.0653	0.0154	2.18E-05

Table 2. Results of 12 SNPs representing new putative height loci from the stage one meta-analysis in 6912 Dutch and Swedish individuals of the stage two meta-analysis and in the combined stage one and stage two meta-analysis (n=16,986)

SNP	CHR	POS	Closest Gene	2nd Closest Gene	Effect Allele	Other Allele	Stage one data P-value	Stage two data P-value	Combined data P-value
rs6717918	2	232863354	DIS3L2	ALPP	T	C	4.46E-06	0.0001469	3.40E-09
rs139909	22	39027527	TNRC6B	ADSL	T	C	4.53E-07	0.02242	1.78E-07
rs10472828	5	32924575	C5orf23	NPR3	T	C	8.13E-07	0.02055	3.40E-07
rs10898392	11	84842399	TMEM126B	TMEM126A	T	G	4.16E-06	0.04063	2.78E-06
rs5757949	22	39150097	SGSM3	MKL1	T	C	2.02E-06	0.07042	3.94E-06
rs12894275	14	102971177	MARK3	CKB	A	G	3.21E-06	0.2071	3.74E-05
rs16970909	17	73702089	AFMID	TK1	T	G	1.07E-07	0.66	7.36E-05
rs4362677	3	159150051	SHOX2	RSRC1	A	G	2.06E-06	0.4977	0.0001763
rs2804845	10	36182751	FZD8	CX40.1	T	C	4.92E-06	0.9404	0.001608
rs1552590	2	18451268	RDH14	NT5C1B	T	C	6.73E-07	0.3352	0.003004
rs9522262	13	110984284	C13orf16	ARHGEF7	C	G	3.52E-06	0.3186	0.01459
rs8009692	14	72364752	DPF3	WDR21A	T	C	3.30E-06	0.1132	0.03429

Table 3. Sex and age adjusted effect sizes (with standard errors) in cohort specific height standard deviations for two SNPs near *NPPC* associated with height

SNP (Effective allele)	Stage One Meta-analysis				Stage Two Meta-analysis				Gudbjartsson et al. ¹ (Suggestive signals)			Overall combined Meta-analysis	
	RS-I	RS-II	ERF	PopGen-KIEL	EPOS	LASA	NTR/NESDA	GOOD	Iceland	Netherlands	European Americans	Beta (SE)	P-value
rs6717918 (T)	0.076 (0.024)	0.116 (0.039)	0.088 (0.067)	0.067 (0.057)	0.031 (0.040)	0.050 (0.061)	0.068 (0.023)	0.14 (0.05)	NA	NA	NA	0.076 (0.012)	3.4×10 ⁻⁹
rs6718438 (T)	0.077 (0.024)	0.117 (0.040)	0.098 (0.066)	0.032 (0.058)	NA	NA	0.071 (0.023)	0.13 (0.05)	0.041 (0.012)	0.073 (0.035)	0.068 (0.052)	0.058 (0.009)	8.4×10 ⁻¹²

¹ Gudbjartsson *et al.* (2008) *Nat. Genet.*, **40**, 609-615.

Table 4. SNP selection criteria and genotype imputation parameters used

Genotyping			SNP Inclusion criteria					Association				
Study	Platform Chip(s)	Calling Algorithm	MAF	Call Rate	P-test HWE	Included SNPs	Imputation Method	Total # imputed SNPs	Software	Imputation Quality metric	MAF	Included SNPs
RS-I	Illumina HumanHap 550	Beadstudio Genecall	≥ 1%	≥ 98%	≥ 10 ⁻⁶	512349	MACH	2,543,887	MACH2QTL	(O/E)σ ² ≥ 0.05	≥ 5%	2,170,858
RS-II	Illumina HumanHap 550 Duo	Beadstudio Genecall	≥ 1%	≥ 98%	≥ 10 ⁻⁶	495,478	MACH	2,543,835	MACH2QTL	(O/E)σ ² ≥ 0.05	≥ 5%	2,169,897
ERF	Illumina HumanHap 300	Beadstudio Genecall	≥ 1%	≥ 98%	≥ 10 ⁻⁶	305,093	MACH	2,543,887	ProABEL	(O/E)σ ² ≥ 0.05	≥ 5%	2,145,565
KIEL	Affymetrix 500K GeneChip	BRLMM	≥ 1%	≥ 97%	≥ 10 ⁻⁶	378,163	MACH	2,543,835	MACH2QTL	(O/E)σ ² ≥ 0.05	≥ 5%	2,168,132
NTR-NESDA	Perlegen 600K	Perlegen Propriety	≥ 1%	≥ 95%	≥ 10 ⁻⁵	495,478	IMPUTE	2,543,887	SNPTEST	Proper_info ≥ 0.4	≥ 5%	12
GOOD	Illumina HumanHap 610	Beadstudio Genecall	≥ 1%	≥ 98%	≥ 10 ⁻⁶	554,085	MACH	2,543,887	MACH2QTL	(O/E)σ ² ≥ 0.05	≥ 5%	12
EPOS	TaqMan Assays	ABI Taqman 7900HT	≥ 1%	≥ 90%	≥ 10 ⁻⁴	12	NA	NA	PLINK	NA	≥ 5%	12
LASA	TaqMan Assays	ABI Taqman 7900HT	≥ 1%	≥ 90%	≥ 10 ⁻⁴	12	NA	NA	PLINK	NA	≥ 5%	12

Legends to Supplementary Figures

Figure 1. Regional association plot for the loci near the *TNRC6B* gene. P-values are results from the stage one meta-analysis. The color scheme of SNPs in are red for strong LD ($r^2 \geq 0.8$), orange for moderate LD ($r^2 \geq 0.5$ and < 0.8), yellow for weak LD ($r^2 \geq 0.2$ and < 0.5) and white for limited or no LD ($r^2 < 0.2$). Blue peaks indicate recombination rates.

Figure 2. Regional association plot for the loci near the *NPR3* gene. P-values are results from the stage one meta-analysis. The color scheme of SNPs in are red for strong LD ($r^2 \geq 0.8$), orange for moderate LD ($r^2 \geq 0.5$ and < 0.8), yellow for weak LD ($r^2 \geq 0.2$ and < 0.5) and white for limited or no LD ($r^2 < 0.2$). Blue peaks indicate recombination rates.

Figure 3. Regional association plot for the loci with GWS near the *NPPC* gene P-values are results from the combined stage one and stage two meta-analysis together with data from Gudbjartsson et al. (2008) (only 9 SNPs available). The color scheme of SNPs in are red for strong LD ($r^2 \geq 0.8$), orange for moderate LD ($r^2 \geq 0.5$ and < 0.8), yellow for weak LD ($r^2 \geq 0.2$ and < 0.5) and white for limited or no LD ($r^2 < 0.2$). Blue peaks indicate recombination rates.

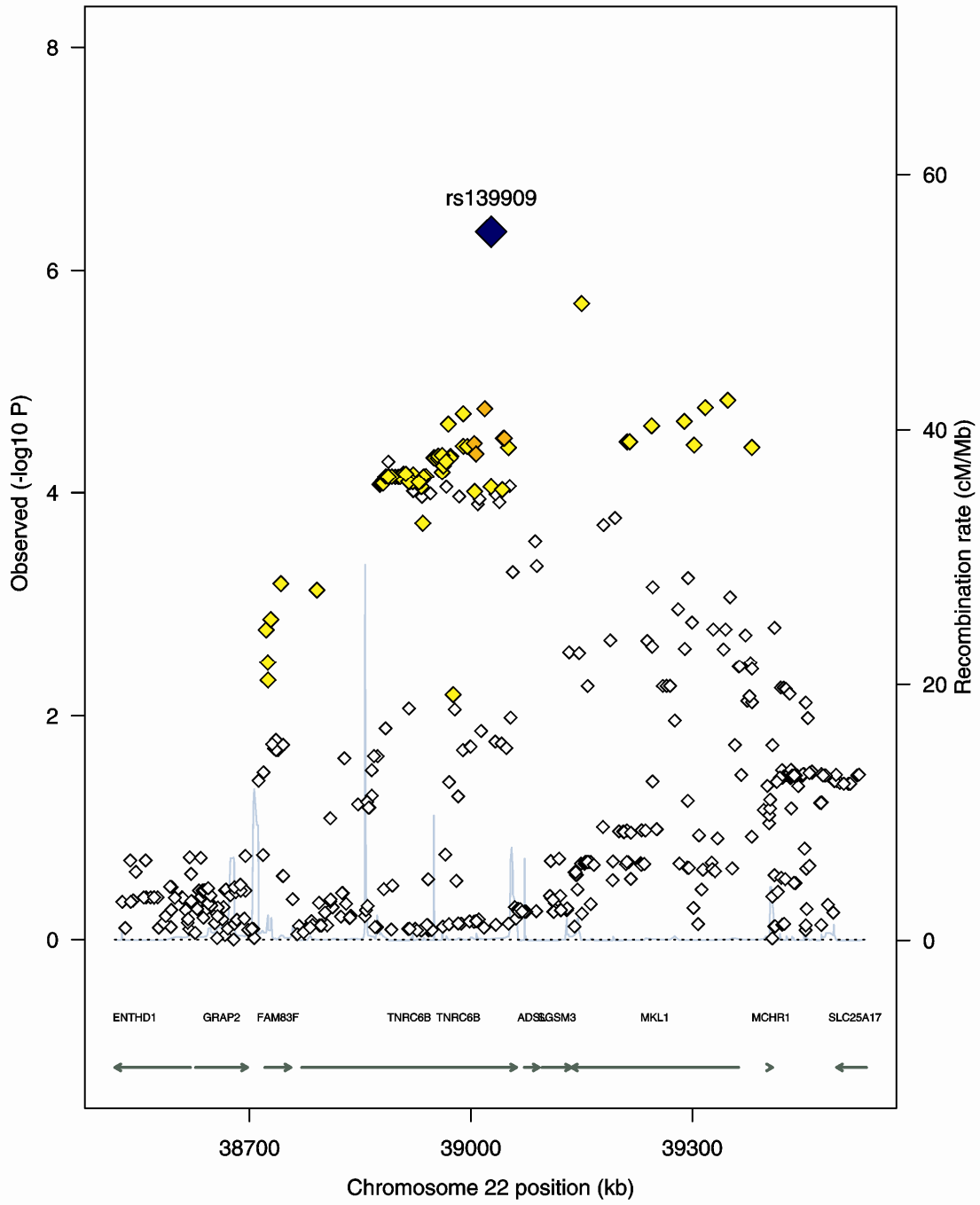


Figure 1

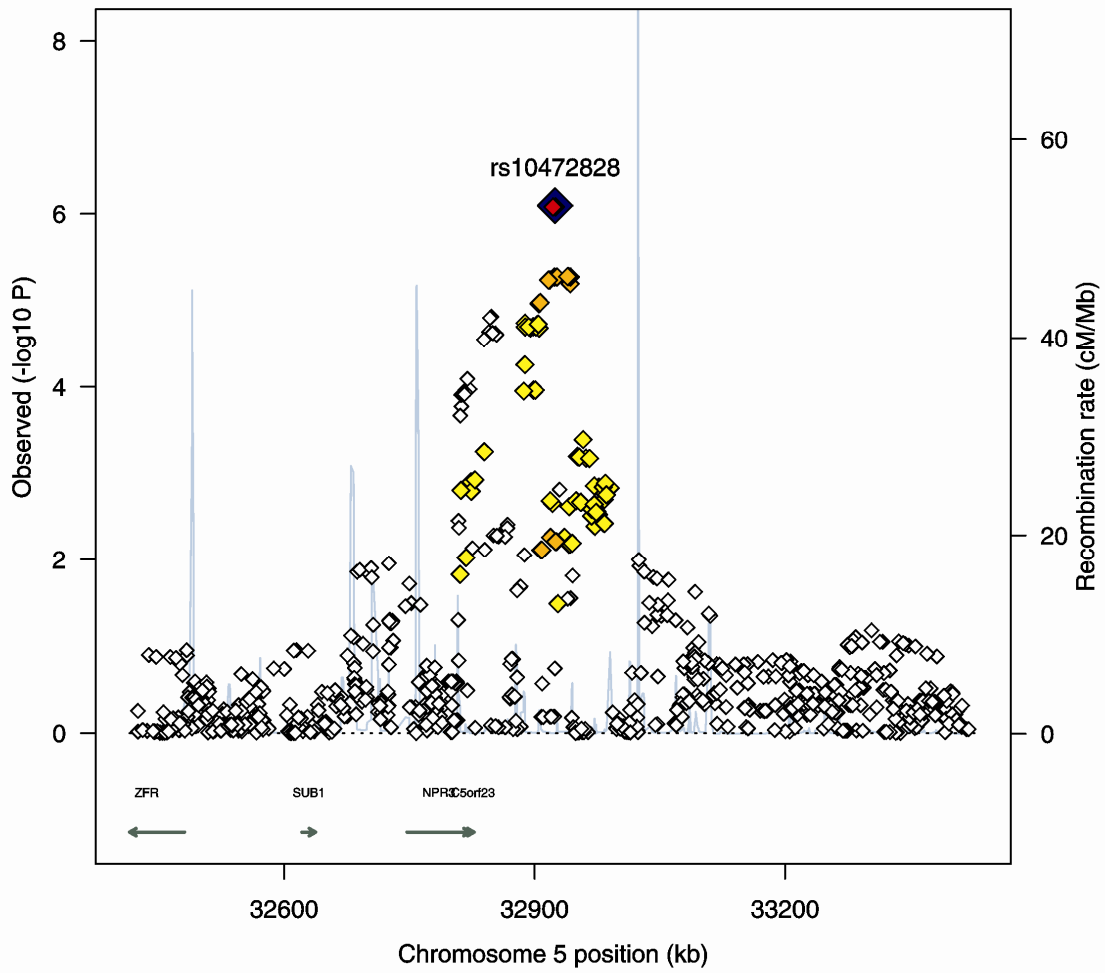


Figure 2

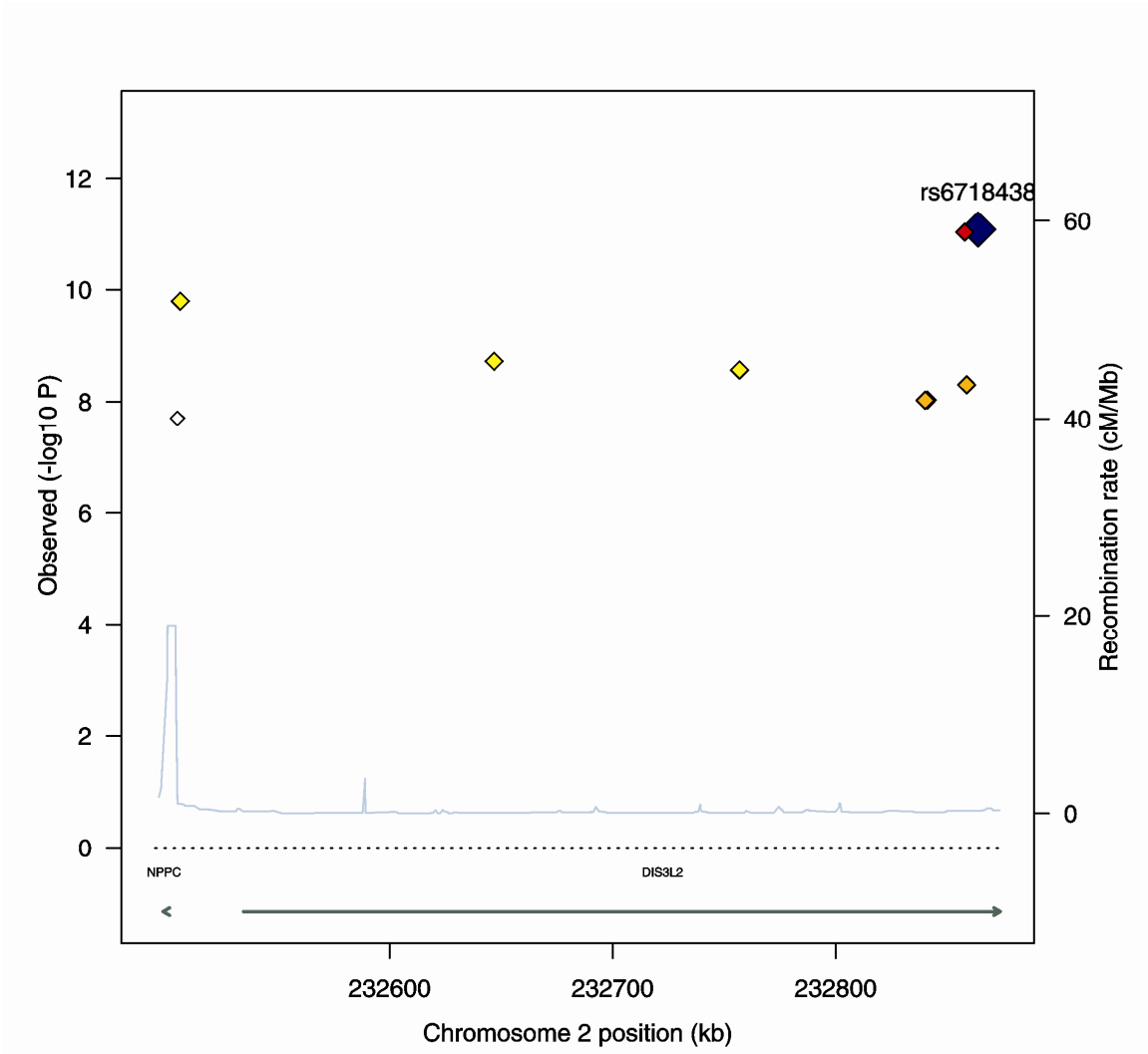


Figure 3