(Supplementary material)

Common variants at the promoter region of the *APOM* confer a risk of rheumatoid arthritis

Hea-Jin Hu[1,2*], Eun-Heui Jin[3*], Seon-Hee Yim[1,4], So-Young Yang[1,2], Seung-Hyun Jung[1,2], Seung-Hun Shin[1,2], Wan-Uk Kim[5], Seung-Cheol Shim[6], Tai-Gyu Kim[2] and Yeun-Jun Chung[1,2]

[1]Integrated Research Center for Genome Polymorphism, [2]Department of Microbiology, The Catholic University of Korea School of Medicine, Seoul, Korea; [3]Genome Research Center for Immune Disorders, School of Medicine, Wonkwang University, Iksan, Korea; [4]Department of Medical Humanities and Social Sciences, [5]Department of Internal Medicine, The Catholic University of Korea School of Medicine, Seoul, Korea; [6]Division of Rheumatology, Department of Internal Medicine, Eulji Medi-Bio Research Institute, Eulji University School of Medicine, Daejeon, Korea.

Supplementary Table 1. The GWAS result around the *APOM* gene (6p21.33)

Probe set_id	SNP	Chr	Position[a]	Allele 1/2	Genotype count						Allele 1 Frequency		OR (95%CI)	*P*-values
					Case			Control						
					11	12	22	11	12	22	Case	Control		
SNP_A-2036273	rs9267247	6	31,563,813	A/C	3	35	62	42	209	346	0.21	0.25	0.81 (0.56-1.16)	0.2489
SNP_A-4261049	rs2516399	6	31,589,278	C/T	6	37	57	13	175	412	0.25	0.17	1.66 (1.15-2.39)	0.007269
SNP_A-2260321	rs3131631	6	31,592,662	C/G	73	24	3	449	138	13	0.85	0.86	1.11 (0.73-1.68)	0.6261
SNP_A-2225620	rs2734573	6	31,602,717	A/C	50	41	9	262	272	64	0.71	0.67	0.84 (0.6-1.17)	0.2993
SNP_A-1899463	rs3115537	6	31,605,814	C/G	86	13	0	518	59	3	0.93	0.94	1.18 (0.64-2.17)	0.5979
SNP_A-4297469	rs3093978	6	31,606,476	G/T	1	13	86	3	77	518	0.08	0.07	1.09 (0.61-1.92)	0.7785
SNP_A-1801089	rs2516478	6	31,606,716	A/G	4	25	71	18	182	400	0.17	0.18	0.92 (0.61-1.38)	0.6894
SNP_A-1861524	rs2071593	6	31,620,778	A/G	0	11	89	0	94	506	0.06	0.08	0.64 (0.33-1.25)	0.1887
SNP_A-4234652	rs3219183	6	31,624,342	C/G	0	10	90	0	86	514	0.05	0.07	0.64 (0.32-1.28)	0.2042
SNP_A-2301199	rs13215091	6	31,636,669	C/T	90	10	0	514	85	1	0.95	0.93	0.64 (0.32-1.27)	0.1976
SNP_A-2313167	rs1799964	6	31,650,287	C/T	6	32	62	38	200	361	0.22	0.23	0.97 (0.68-1.39)	0.8785
SNP_A-2222440	rs1052248	6	31,664,560	A/T	5	27	68	36	217	346	0.19	0.24	0.73 (0.5-1.06)	0.09952
SNP_A-2098264	rs9348876	6	31,683,255	A/G	1	20	79	3	136	461	0.11	0.12	0.89 (0.54-1.47)	0.6529
SNP_A-4225289	rs2857697	6	31,693,198	A/G	46	37	15	188	265	136	0.66	0.54	0.66 (0.48-0.89)	0.006829
SNP_A-1867270	rs2736172	6	31,698,877	C/T	16	42	41	174	274	146	0.37	0.52	1.73 (1.28-2.35)	0.0003701
SNP_A-1912648	rs1046089	6	31,710,946	C/T	53	33	14	213	272	115	0.70	0.58	0.64 (0.47-0.88)	0.005843
SNP_A-2280626	rs2255741	6	31,713,146	C/T	86	13	1	493	104	3	0.93	0.91	0.81 (0.46-1.43)	0.4691
SNP_A-2098024	rs760293	6	31,719,756	C/T	84	14	1	477	117	6	0.92	0.89	0.74 (0.43-1.27)	0.2747
SNP_A-2248558	rs2077102	6	31,719,819	A/C	7	23	70	26	210	364	0.19	0.22	0.82 (0.56-1.2)	0.2999
SNP_A-1879350	rs805301	6	31,726,100	G/T	15	39	46	155	281	163	0.35	0.49	0.57 (0.42-0.78)	0.0004024
SNP_A-1951804	rs805300	6	31,726,546	C/T	1	14	85	3	114	482	0.08	0.10	0.79 (0.45-1.37)	0.4
SNP_A-2274274	**rs805297**	**6**	**31,730,585**	**C/A**	**21**	**51**	**28**	**238**	**272**	**60**	**0.47**	**0.66**	**2.28 (1.65-3.14)**	**5.202E-07**
SNP_A-2268461	rs2242655	6	31,735,428	C/G	5	23	70	20	209	361	0.17	0.21	0.76 (0.5-1.13)	0.1768
SNP_A-2309804	rs707974	6	31,737,478	C/T	0	18	82	3	75	521	0.09	0.07	1.31 (0.76-2.26)	0.3244
SNP_A-4258142	rs805268	6	31,746,157	G/T	1	13	86	3	104	493	0.08	0.09	0.81 (0.46-1.43)	0.4691
SNP_A-2253073	rs2142234	6	31,747,108	A/G	1	6	93	10	98	492	0.04	0.10	0.41 (0.2-0.85)	0.01683

SNP_A-4292023	rs805267	6	31,747,736	A/G	1	14	85	4	114	482	0.08	0.10	0.78 (0.45-1.35)	0.3659
SNP_A-4240894	rs9378164	6	31,751,501	A/G	1	15	84	1	112	487	0.09	0.10	0.85 (0.49-1.47)	0.5538
SNP_A-2257984	rs376510	6	31,796,179	C/T	94	5	1	490	102	8	0.97	0.90	0.35 (0.16-0.76)	0.008265
SNP_A-4279910	rs2299851	6	31,826,581	C/T	71	20	7	370	204	19	0.83	0.80	0.81 (0.55-1.21)	0.313
SNP_A-2057497	rs707939	6	31,834,667	G/T	18	48	31	222	280	98	0.43	0.60	1.96 (1.44-2.67)	2.012E-05
SNP_A-1985618	rs707937	6	31,838,993	C/G	50	35	15	227	299	74	0.68	0.63	0.8 (0.58-1.11)	0.1863
SNP_A-4290666	rs480092	6	31,872,878	C/T	4	26	69	41	227	320	0.17	0.26	0.59 (0.4-0.87)	0.008086
SNP_A-1828154	rs2075800	6	31,885,925	A/G	31	48	21	102	282	216	0.55	0.41	1.76 (1.3-2.38)	0.0002455
SNP_A-1867908	rs2763979	6	31,902,571	C/T	55	33	12	210	278	105	0.72	0.59	0.59 (0.43-0.82)	0.00155
SNP_A-2217703	rs11965547	6	31,944,130	C/T	72	22	6	375	202	22	0.83	0.79	0.79 (0.53-1.17)	0.234
SNP_A-4235594	rs486416	6	31,964,049	A/G	85	12	3	497	103	0	0.91	0.91	1.03 (0.6-1.77)	0.9057
SNP_A-2188055	rs9267673	6	31,991,658	A/G	1	5	94	6	101	492	0.04	0.09	0.36 (0.17-0.79)	0.01112
SNP_A-2225939	rs644045	6	31,991,936	C/T	83	14	3	469	130	1	0.90	0.89	0.87 (0.52-1.45)	0.5851
SNP_A-1923640	rs2072633	6	32,027,557	A/G	24	51	25	120	302	178	0.50	0.45	1.19 (0.88-1.61)	0.2713

[a] Based on NCBI human genome build 36.3 (hg18).
Platform: Affymetrix SNP array 5.0

Supplementary Table 2. Pairwise linkage disequilibrium among four SNPs in *APOM* with 48 Korean subjects

	SNP1 (rs1266078)	SNP2 (rs805297)	SNP3 (rs9404941)	SNP4 (rs805296)
SNP1 (rs1266078)				
SNP2 (rs805297)	0.096	r^2		
SNP3 (rs9404941)	0.010	0.061	r^2	
SNP4 (rs805296)	0.304	0.029	0.021	r^2

Supplementary Table 3. Summary of information for the subjects of rheumatoid arthritis (RA) and healthy control

Study set	Genotyping method		Number	Female (%)	Age (Mean ± SD)	Status *	RF (%)	Anti-CCP (%)	BD (%)	BE (%)	JSN (%)
GWAS discovery set	Affymetrix 5.0	RA	100	81.0	54.1 ± 11.4	+	72 (72.0)	54 (63.5)	N	N	N
						-	28 (28.0)	31 (36.5)			
						N	0	15			
		Control	600	76.0	52.4 ± 9.1						
Replication set	TaqMan assay & RFLP	RA	578	81.1	53.2 ± 12.0	+	389 (70.7)	309 (84.4)	85 (35.6)	177 (75.0)	164 (69.2)
						-	161 (29.3)	57 (15.6)	154 (64.4)	59 (25.0)	73 (30.8)
						N	28	212	339	342	341
		Control	711	63.6	52.3 ± 12.9						

RF: Rheumatoid factor, Anti-CCP: anti-cyclic citrullinated peptide, BD: Bone deformity, BE: Bone erosion, JSN: Joint space narrowing
*+,positive; -, negative; N, no information

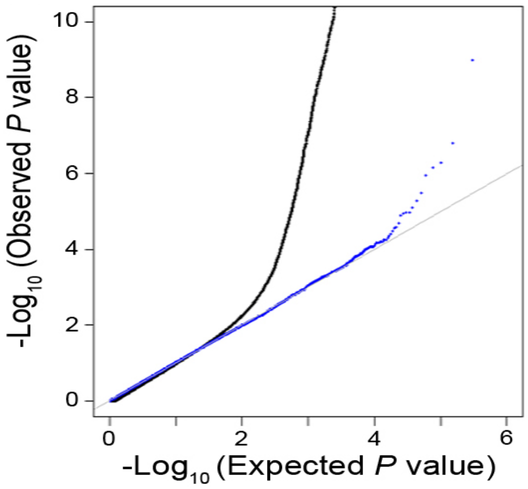
Supplementary Table 4. Primer sequences used for *APOM* gene amplification, sequence analysis and genotyping

Application	Name	Primer/probe sequence (5' → 3')	Region
PCR analysis	PF1	CCTAGTAGGGACCACAGATGCAC	5' flanking region and exon1
	PR1	TCCAGTCATCCTAGGCCATC	
	EF2	GGGGTTTACCCAGGTTGACT	exon2 ~ 6
	ER2	GGAGACAAACCCCAGAATGA	
Sequencing	SR1	GCTTGGGCGACAGAGACT	5' flanking region
	SF1	CCATGTGCAAGTCTTTGCTA	5' flanking region
	SF2	TTGAGTGAATGTGTCAGTGAA	5' flanking region
TaqMan analysis	C___7514748_10	ABI assay-on-demand C___7514748_10 [a]	rs805297
	TF2	AACAAAATTTGGGTGACCAGTATATTAGAGTTTA	rs1266078
	TR2	CAACAGAACGAGACTCCCATCT	
	probe2V	VIC-TGTAATGTTTGAATTTTTT	
	probe2F	FAM-AATGTTTGCATTTTTT	
RFLP analysis	RF1	CAGTTAGGGGTTGGTGGTGT	rs9404941 and rs805296
	RR1	TGAGCCCAGGAGTTCAAAAC	

[a] Predesigned Assay-on-demand from Applied Biosystems.

Supplementary Figure 1

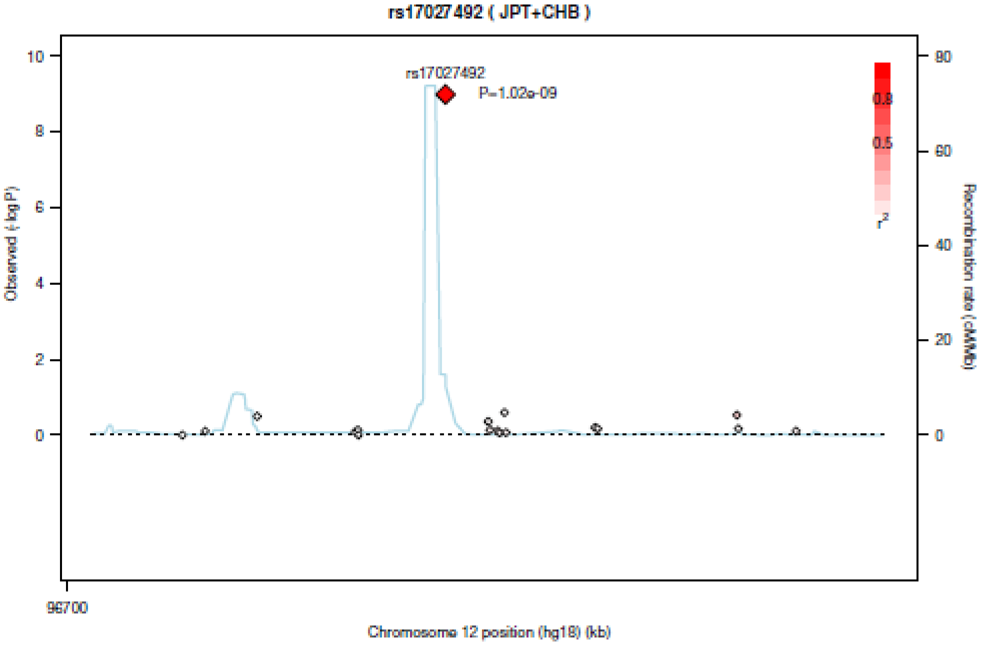
Quantile-Quantile plot (QQ-plot) of *P*-values obtained from the genome-wide association study (GWAS). The QQ-plot is drawn with a logarithmic scale. The expected *P*-values are presented on the x-axis and the observed *P*-values are on the y-axis. The black curve is for the *P*-values obtained from the logistic regression test using the whole SNPs before SNP quality control and the blue curve is for the *P*-values after quality control.



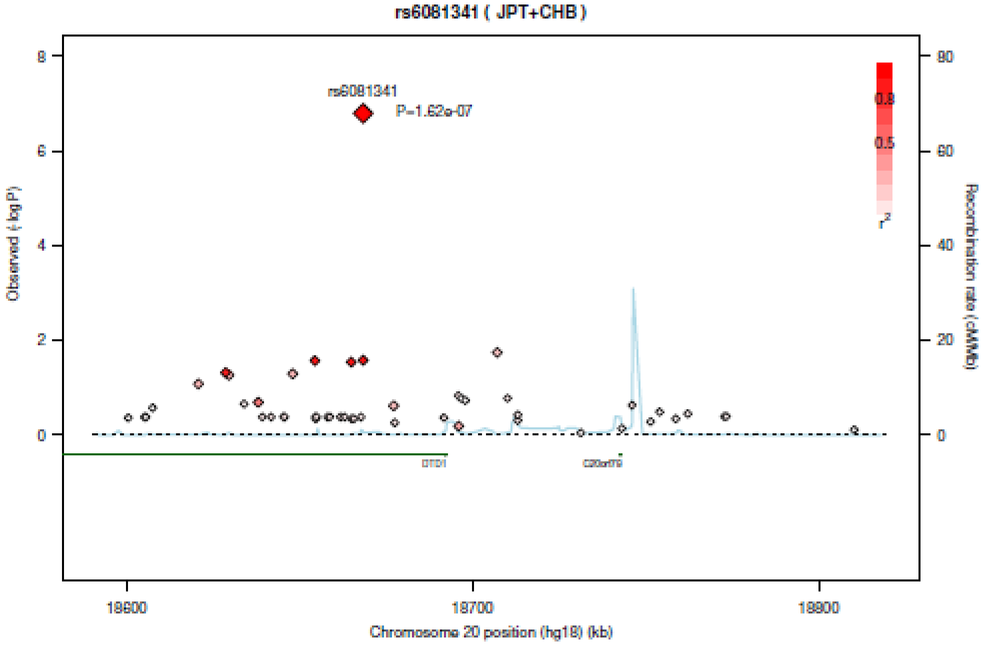
Supplementary Figure 2.

The $-log_{10}$ (P-value) of SNPs identified from GWAS as strongly associated with RA. SNPs are represented with diamond dots and the most associated SNP was marked with the largest diamond. The strength of LD relationship (r^2) between the most associated SNP and the other SNPs is presented with red color intensities based on the JPT + CHB Hapmap data. The background recombination rate curve is drawn with the JPT + CHB Hapmap data (light blue curve).

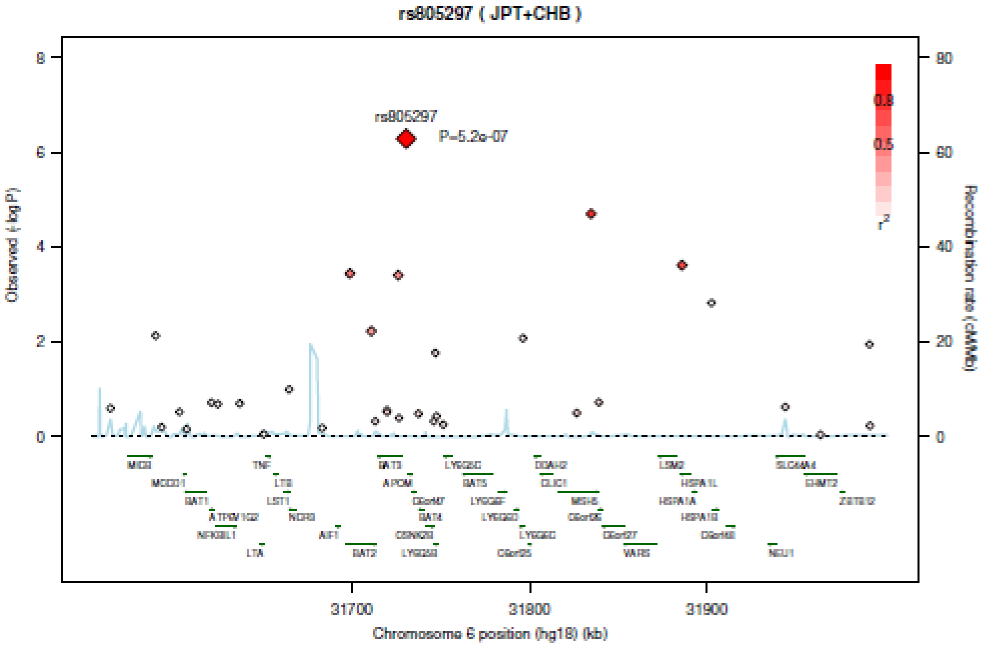
a



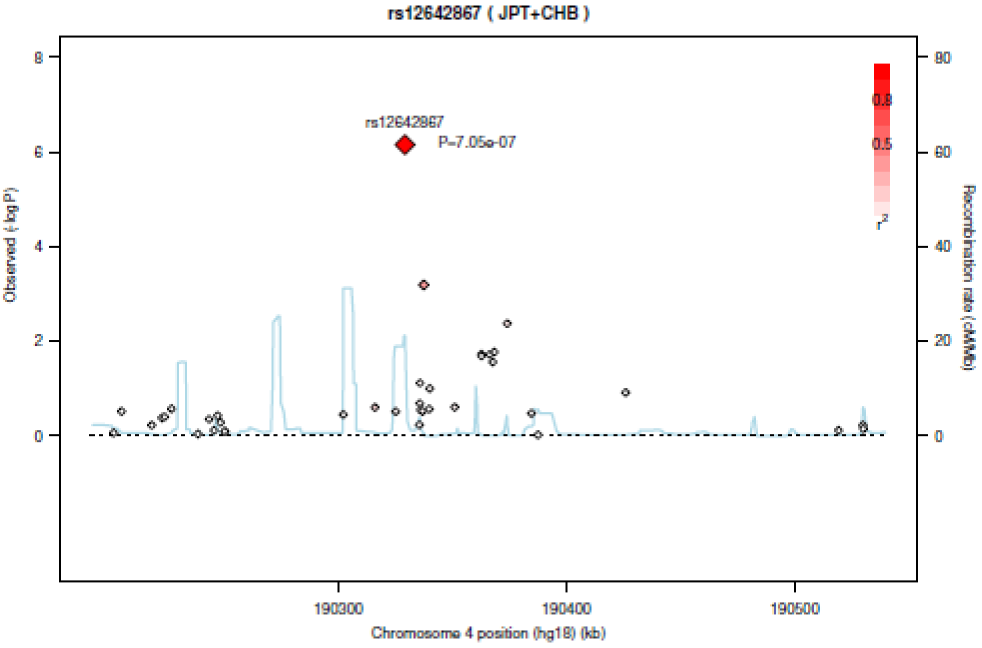
b



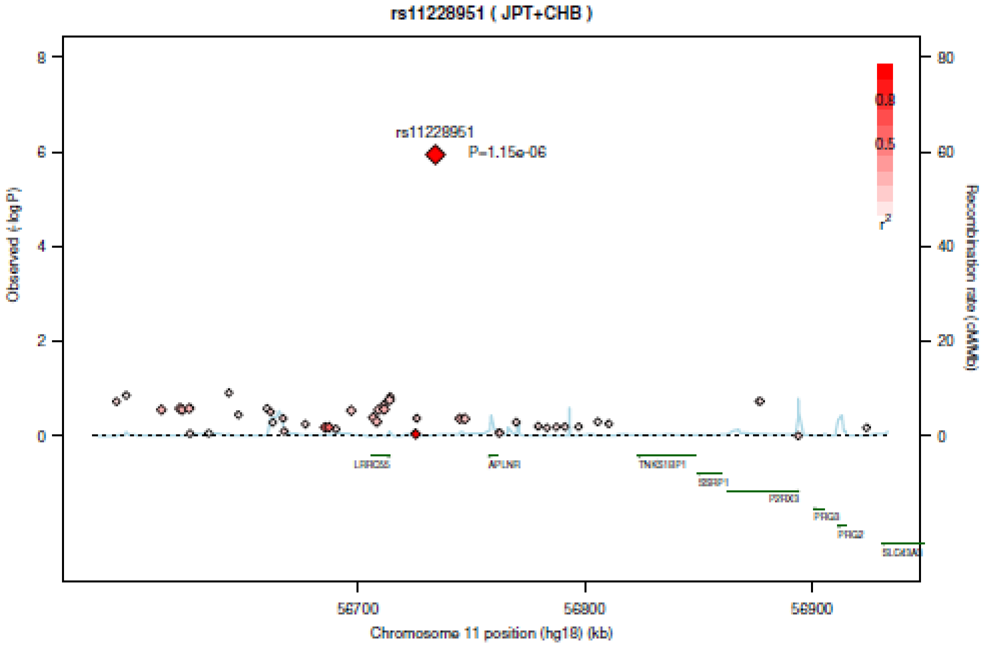
c


rs805297 (JPT+CHB)
rs805297
P=5.2e-07
Observed (-logP)
Recombination rate (cM/Mb)
0.8
0.5
r²
8
6
4
2
0
80
60
40
20
0
MICB
MCCD1
BAT1
ATP6V1G2
NFKBIL1
LTA
TNF
LTB
LST1
NCR3
AIF1
BAT2
BAT3
APOM
C6orf47
BAT4
CSNK2B
LY6G5B
LY6G5C
BAT5
LY6G6F
LY6G6D
LY6G6C
C6orf25
DDAH2
CLIC1
MSH5
C6orf26
C6orf27
VARS
LSM2
HSPA1L
HSPA1A
HSPA1B
C6orf48
NEU1
SLC44A4
EHMT2
ZBTB12
31700
31800
31900
Chromosome 6 position (hg18) (kb)

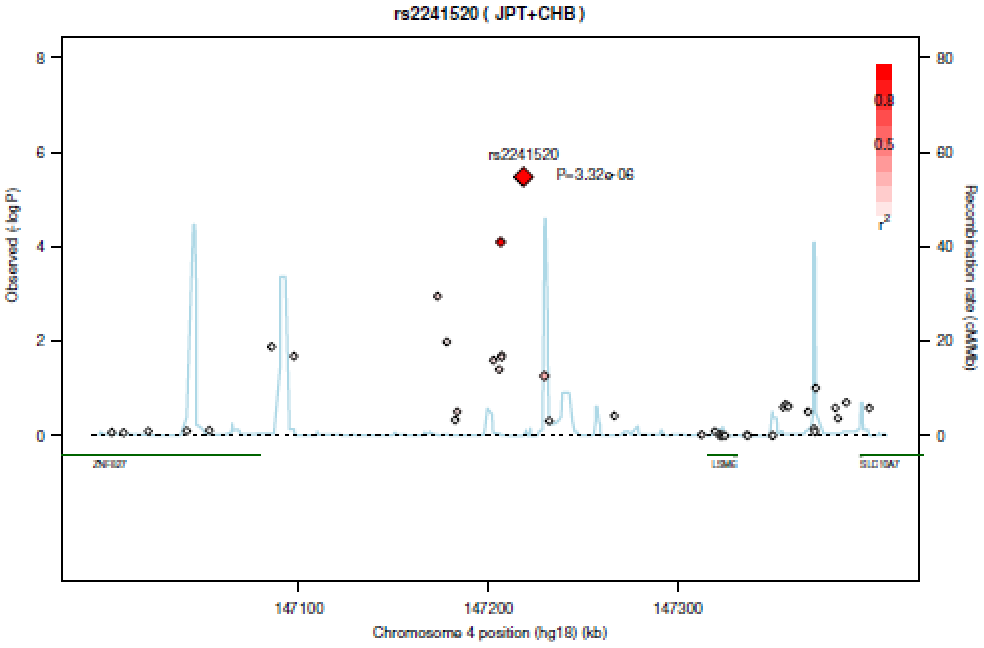
d


rs12642867 (JPT+CHB)
rs12642867
P=7.05e-07
Observed (-logP)
Recombination rate (cM/Mb)
0.8
0.5
r²
8
6
4
2
0
80
60
40
20
0
190300
190400
190500
Chromosome 4 position (hg18) (kb)

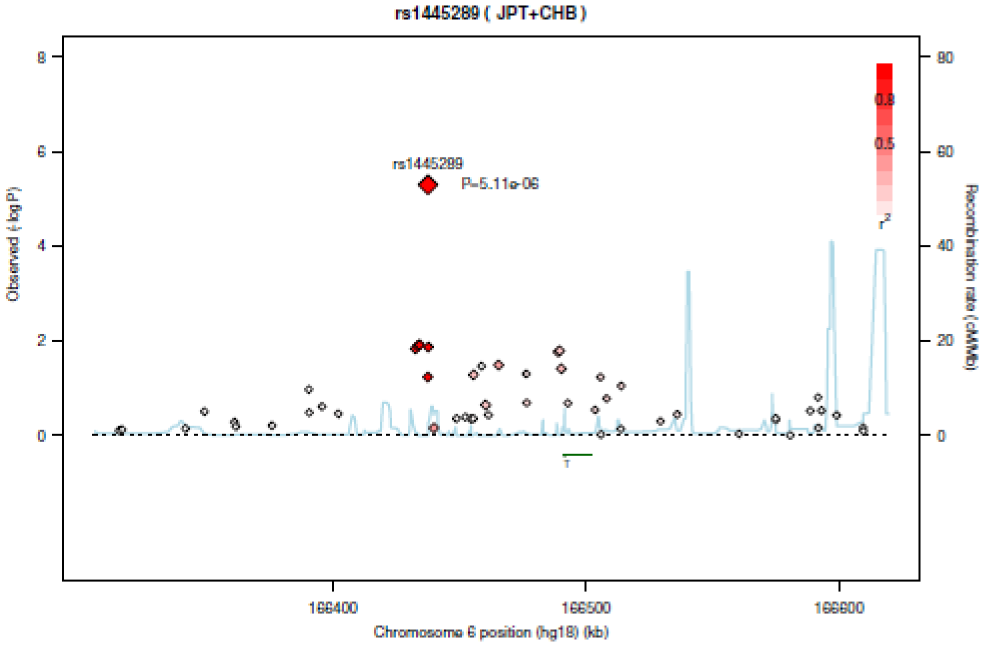
e


rs11228951 (JPT+CHB)
rs11228951
P=1.15e-06
Observed (-logP)
8
6
4
2
0
Recombination rate (cM/Mb)
80
60
40
20
0
0.8
0.5
r^2
LRRC55
APLNR
TNKS1BP1
SSRP1
P2RX3
PRG3
PRG2
SLC43A3
56700
56800
56900
Chromosome 11 position (hg18) (kb)

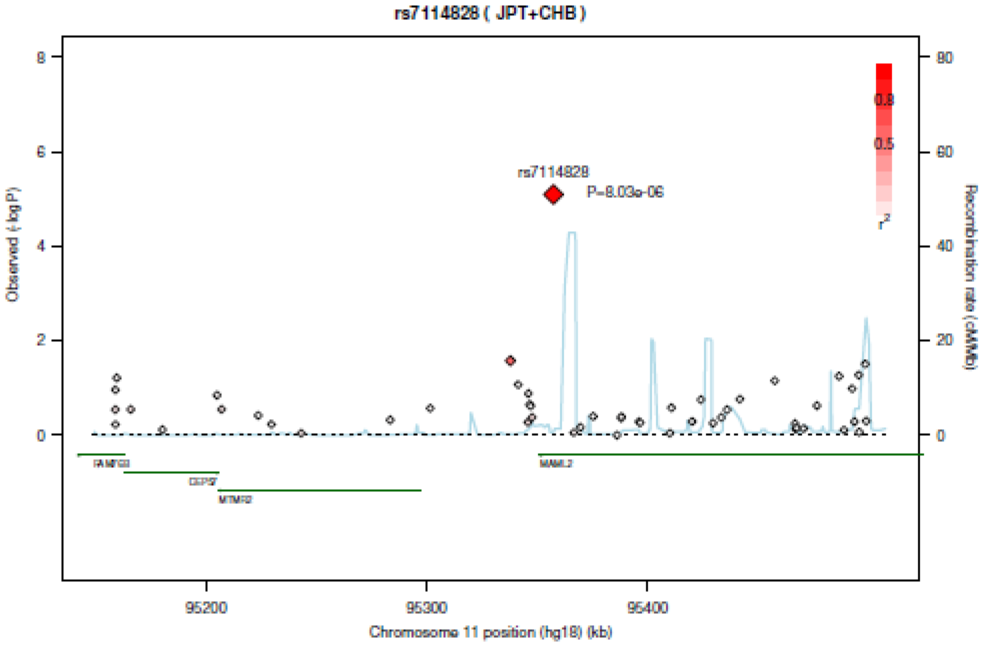
f


rs2241520 (JPT+CHB)
rs2241520
P=3.32e-06
Observed (-logP)
8
6
4
2
0
Recombination rate (cM/Mb)
80
60
40
20
0
0.8
0.5
r^2
ZNF827
LSM6
SLC10A7
147100
147200
147300
Chromosome 4 position (hg18) (kb)

g


rs1445289 (JPT+CHB)
rs1445289
P=5.11e-06
Observed (-log P)
Recombination rate (cM/Mb)
0.8
0.5
r²
0
2
4
6
8
0
20
40
60
80
T
166400
166500
166600
Chromosome 6 position (hg18) (kb)

h


rs7114828 (JPT+CHB)
rs7114828
P=8.03e-06
Observed (-log P)
Recombination rate (cM/Mb)
0.8
0.5
r²
0
2
4
6
8
0
20
40
60
80
FAM76B
CEP57
MTMR2
MAML2
95200
95300
95400
Chromosome 11 position (hg18) (kb)