Supplement for:

Genome-Wide Meta-Analysis Increases to 71 the Number of Confirmed Crohn's Disease Susceptibility Loci

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Supplementary Table 1 - Index GWAS studies used for the meta-analysis, with genotyping platform and numbers of cases and controls from each centre for which (post quality control) genome-wide association study data were available.

Index GWAS	*Crohn's disease cases*	*Healthy controls*	*GWAS platform*
Early Onset	1,689	6,197	Illumina HumanHap550
German	479	1,145	Illumina HumanHap550
USA (Cedars-Sinai)	925	2,882	Illumina HumanHap300
Belgium	537	913	Illumina HumanHap300
USA (NIDDK)	956	982	Illumina HumanHap300
UK (WTCCC)	1,747	2,937	Affymetrix GeneChip 500
TOTAL	6,333	15,056	

Supplementary Table 2 - Country of origin of samples used in the replication experiment, with employed genotyping platform.

REPLICATION - country	Crohn's disease cases	Healthy controls	Genotyping platform
Australia	1,357	1,923	Sequenom iPlex
Belgium	1,282	1,682	SNPlex/Taqman
France	414 trios		SNPlex/Taqman
Germany	3,808	2,747	SNPlex/Taqman
Israel	444	376	SNPlex/Taqman
Italy	921	899	SNPlex/Taqman
Netherlands	1,101	269	SNPlex/Taqman
New Zealand	514	457	Sequenom iPlex
Spain	325	987	SNPlex/Taqman
Sweden	724	992	SNPlex/Taqman
UK	3,243	2,431	Sequenom iPlex
USA (Cedars-Sinai)	1,172	501	SNPlex/Taqman
USA (NIDDK)	803	762	Illumina Goldengate
TOTAL	15,694	14,026	

Supplementary Table 3 – Odds ratios (OR) and risk allele frequencies (RAF) for the 71 SNPs listed in Table 1 and 2.

Column **Repl. Heterogeneity** lists the Breslow-Day heterogeneity chi-square with 11 degrees of freedom. To avoid any bias due to winner's curse we performed this test in replication datasets only (available for 35/71 loci). No significant heterogeneity was observed after correction for multiple tests. For the Belgian and Cedar_2 GWAS sample, which had high genomic inflation factors (see **Supplementary Figure 1**), PCA-corrected *P*-values are shown (**P_corr**) besides the uncorrected *P*-values (**P_uncorr**) from **Table 1 and 2.** These *P*-value columns are also highlighted by light red shading. The comparison demonstrates that the observed associations do not arise due to population stratification. **Chr.:** chromosome

This supplementary table is available for download as an Excel file.

Supplementary Table 4 – Raw allele counts and empirical variance for the 71 SNPs listed in Table 1 and 2.

Chr.: chromosome; **A/B**: counts per allele (aligned to + strand of NCBI's build 36) for healthy controls (**CTRL**) and Crohn's disease patients (**CASE**); **EMPVAR**: empirical variance. The empirical variance is calculated as:

1) Conversion of genotypes to allelic dosages, which are the weighted sum of the genotype class probabilities, e.g. if "A" is the reference allele and genotype probabilities are 0.8 AA 0.1 Aa and 0.1 aa, then the dosage is calculated as

$$2\times0.8 + 1\times0.1 + 0\times0.1 = 1.7$$

2) Calculation of dosages for all individuals.
3) Calculation of the mean dosage for all individuals.
4) Calculation of the square of the deviation from the mean for each individual dosage.
5) Sum the squared deviations and division by twice the number of individuals.

This supplementary table is available for download as an Excel file.

Supplementary Table 5 – Details on 1000G coding SNPs (cSNP) that are in LD with the SNPs listed in Table 1 and 2.

Chr.: chromosome; Position (**pos**) are shown according to NCBI's dbSNP build 130.

		lead SNP				coding SNP				
No.	**Table 1Ʃ н l**	**ŘbSNP ID**	**/HR.**	**Ǉos (dbSNP 130)**	**Risk allele - Allele frequency in control population**	**dbSNP ID**	**pos (dbSNP 130)**	**distance to lead SNP [kb]**	**RefGene**	**amino acid substitution**
1	6	rs780093	2p23	27,742,603	T - 0.418	rs1260326	27,730,940	11.66	*GCKR*	L446P
2	7	rs10495903	2p21	43,660,422	T - 0.129	rs35720761; rs7578597	43,373,481; 43,586,327	286.94; 74.10	*THADA*	C1605Y; T1187A
3	10	rs6738825	2q33	198,605,140	A - 0.473	rs1064213	198,658,485	53.35	*PLCL1*	V667I
4	14	rs2549794	5q15	96,270,305	C - 0.409	rs2549782	96,256,756	13.55	*ERAP2*	K392N
5	21	rs4077515	9q34	138,386,317	T - 0.411	same			*CARD9*	S12N
						rs3812571	138,395,115	8.80	*SNAPC4*	H799Q
6	29	rs8005161	14q35	87,542,348	T - 0.119	rs1805078	87,520,523	21.83	*GALC*	R184C
						rs3742704	87,547,635	5.29	*GPR65*	I231L
7	31	rs151181	16p11	28,398,018	G - 0.386	rs180743	28,415,145	17.13	*APOB48R*	P419A
						rs181206	28,420,904	22.89	*IL27*	L119P
						rs7498665	28,790,742	392.72	*SH2B1*	T484A
8	33	rs12720356	19p13	10,330,975	G - 0.084	same			*TYK2*	I684S
9	35	rs281379	19q13	53,906,086	A - 0.487	rs601338; rs602662	53,898,486; 53,898,797	7.60; 7.29	*FUT2*	**W154X**; G258S
						rs2287922	53,924,038	17.95	*RASIP1*	R601C
10	36	rs4809330	20q13	61,820,030	G - 0.709	rs3208008	61,796,554	23.48	*RTEL1*	Q1042H
11	37	rs181359	22q11	20,258,641	T - 0.203	rs2298428	20,312,892	54.25	*YDJC*	A263T
12	1	rs11209026	1p31	67,705,958	G - 0.932	same			*IL23R*	R381Q
13	2	rs2476601	1p13	114,179,091	G - 0.907	same			*PTPN22*	W602R
14	5	rs7554511	1q32	199,144,185	C - 0.726	rs296520	199,147,601	3.42	*C1orf106*	R453C
15	6	rs3792109	2q37	233,849,156	A - 0.529	rs2241880	233,848,107	1.05	*ATG16L1*	T300A
16	7	rs3197999	3p21	49,696,536	A - 0.297	same			*MST1*	R703C
						rs1050450	49,369,838	326.70	*GPX1*	P200L
						rs34762726	49,664,214	32.32	*BSN*	A741T
17	9	rs12521868	5q31	131,812,292	T - 0.422	rs1050152	131,704,219	108.07	*SLC22A4*	L503F
18	13	rs1799964	6p21	31,650,287	C - 0.209	rs2259435	31,604,894	45.39	*MCCD1*	E42K
						rs2229094	31,648,535	1.75	*LTA*	C13R
19	24	rs11564258	12q12	40,792,300	A - 0.025				*MUC19*	
20	25	rs3764147	13q14	43,355,925	G - 0.245	same			*C13orf3* 1	I254V
21	27	rs2872507	17q21	35,294,289	A - 0.458	rs2345480; rs2305479	35,315,722; 35,315,743	21.43; 21.45	*GSMDL*	P289S; G282R
						rs11557467	35,282,160	12.13	*ZPBP2*	S173I
22	28	rs11871801	17q21	37,824,298	A - 0.756	rs665268	37,975,555	151.26	*MLX*	Q233R

Supplementary Table 6: Positional candidate genes mapping within regions of confirmed association for Crohn's disease. Three *in silico* techniques were used to further highlight genes of interest (see main text for more details):

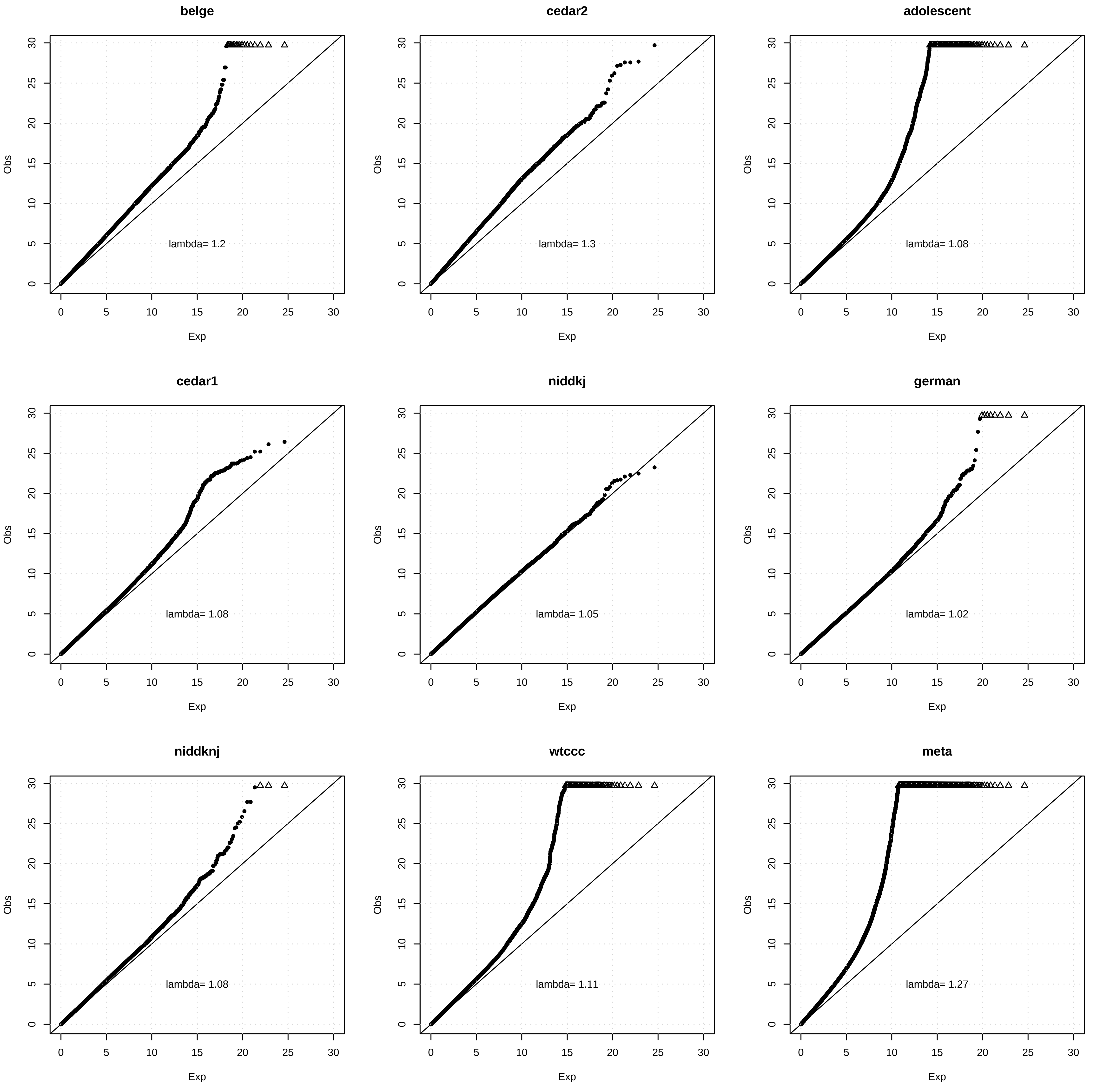
(1) 1000 Genomes Project and HapMap databases were searched for coding SNPs in linkage disequilibrium with our most associated SNP.

(2) GRAIL software was used to identify connectivity between genes mapping to different loci.

(3) An eQTL database was searched to identify loci within which the focal SNP correlated with gene expression with LOD≥5.0 (for details see **Supplementary Note**).

This supplementary table is available for download as an Excel file.

Supplementary Figure 1 - Quantile-quantile (Q-Q) plot of the association test statistic for all eight constituent GWAS data sets and the meta analysis (meta). For a detailed description of the constituent GWAS see **Supplementary Table 1**. For the Q-Q plots, the Cedar's sample (USA ("Cedars Sinai") in **Supplementary Table 1**) was divided into the two sub-GWAS ("cedar1" and "cedar2"). For the USA (NIDDK) sample, a separate Q-Q plot is shown for the Jewish ("niddkj") and for the non-Jewish ("niddknj") sub-GWAS. Plots were calculated for all 953,241 SNPs that passed the quality control. For better scaling the y-axis was limited to a maximum chi^2 of 30 and the SNPs with higher chi^2 values are forming a "plateau" along the top. The over-dispersion of the association test statistic was estimated to be λ_{GC}=1.27 for the meta-analysis. The shaded region is the 95% concentration band that is formed by calculating, for each order statistic, the 2.5th and 97.5th centiles of the respective distribution under the null hypothesis.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lambda= 1.2
cedar2
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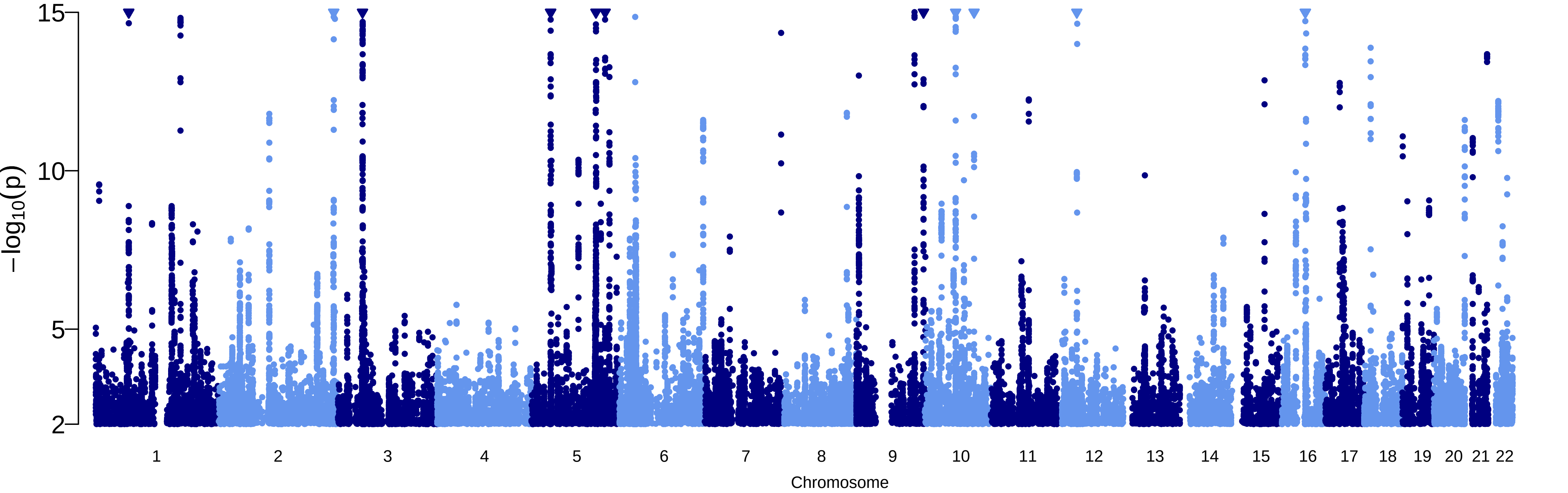
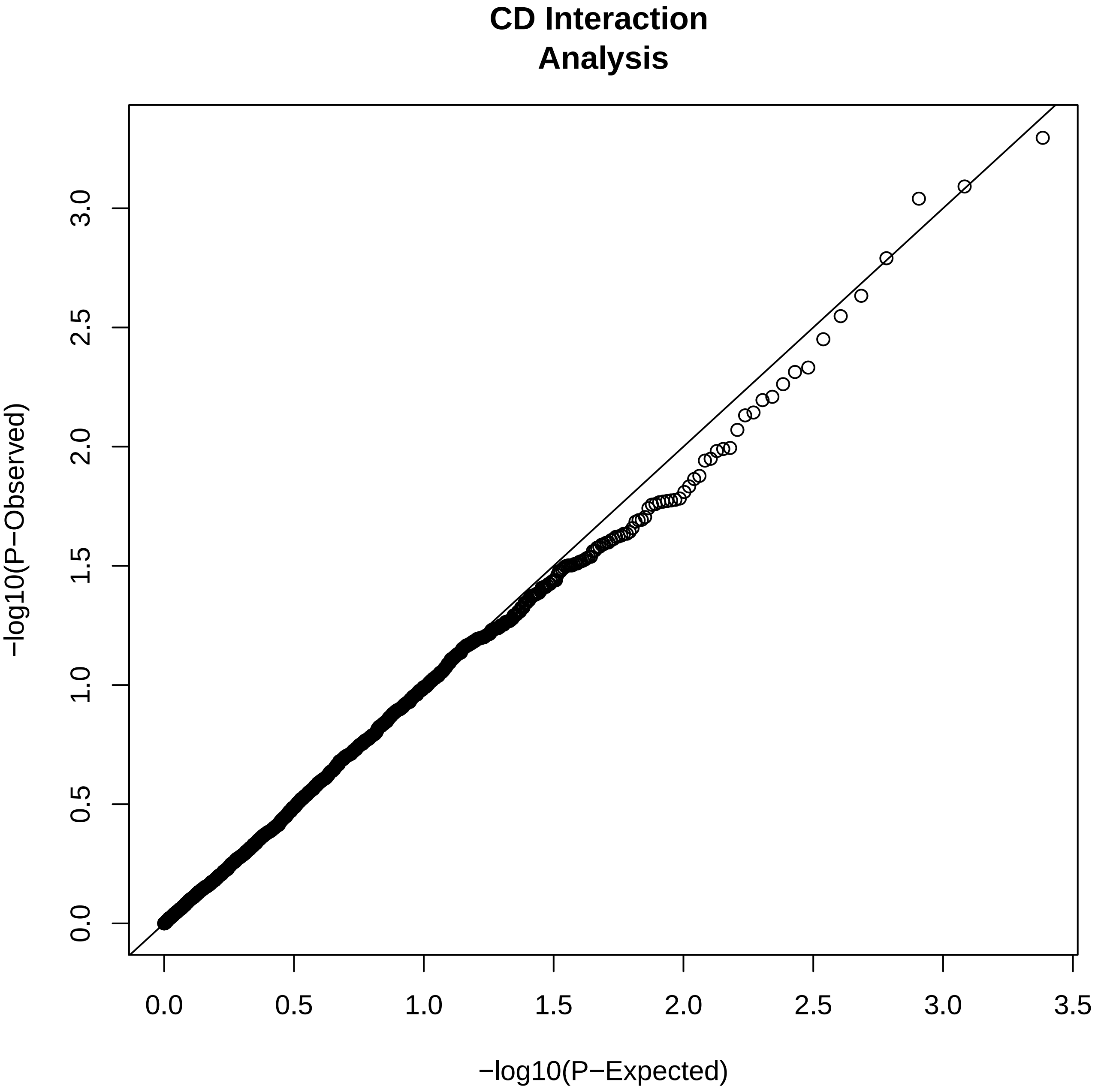
lambda= 1.02
niddknj
lambda= 1.08
wtccc
lambda= 1.11
meta
lambda= 1.27
Obs
Exp
0
5
10
15
20
25
30

Supplementary Figure 2 – Results of association analysis ("Manhattan Plot"). The negative common logarithm of the *P*-values for the test statistic using single-SNP Z scores of the genome-wide association study are shown according to chromosome. Only markers that passed the quality criteria were used for plotting (n=953,241). Marker positions are in NCBI's build 36 (hg18).


15
10
5
2
−log10(p)
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
Chromosome

Supplementary Figure 3 - Quantile-quantile (Q-Q) plot of interaction analysis for the 71 SNPs listed in Table 1 and 2. For a detailed description of the analysis see **Online Methods**. No deviation from the null is observed and no results were significant when considering the number of tests performed.


CD Interaction
Analysis
−log10(P−Observed)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
−log10(P−Expected)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5

Supplementary Figure 4 - Regional plots of Table 1 and 2. Regional Plots of the negative decadic logarithm of the *P*-values obtained in the GWAS in a window of ~250 kb around each of the 71 SNPs displayed in **Table 1 and 2**. The SNP ID of the index SNP is given above each plot and this SNP is marked by a large blue-filled symbol. The magnitude of linkage disequilibrium with the central SNP is reflected by the fill color of the symbols using the measure $\mathbf{r^2}$ (for color coding see legend in the upper right corner of each plot). Recombination activity (**cM/Mb**) is depicted by a blue line. Positions and gene annotations are according to NCBI's build 36 (hg18). Plots are ordered according to their order in **Table 1 and 2**.

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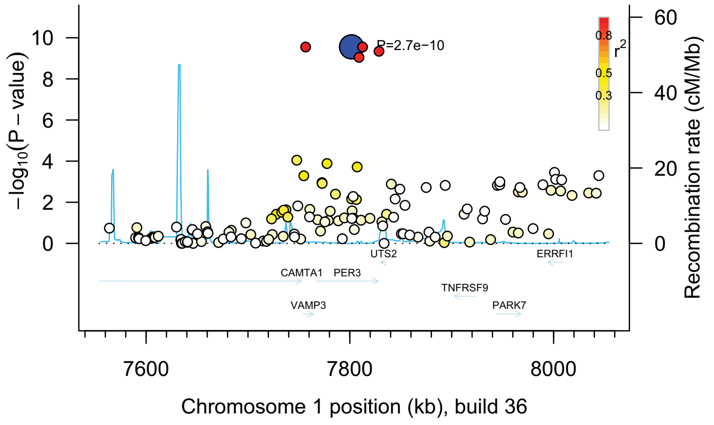

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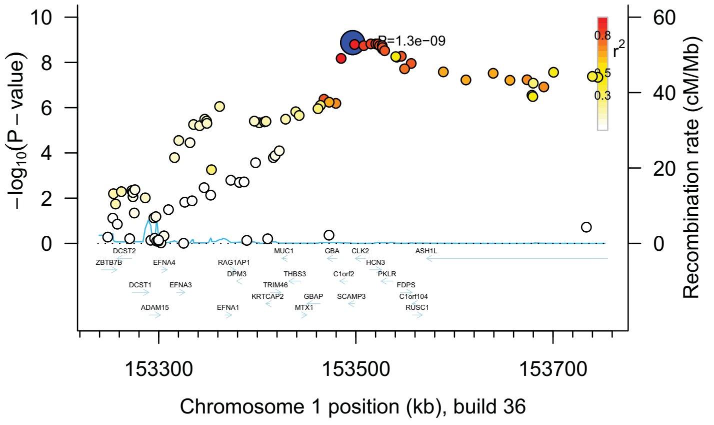

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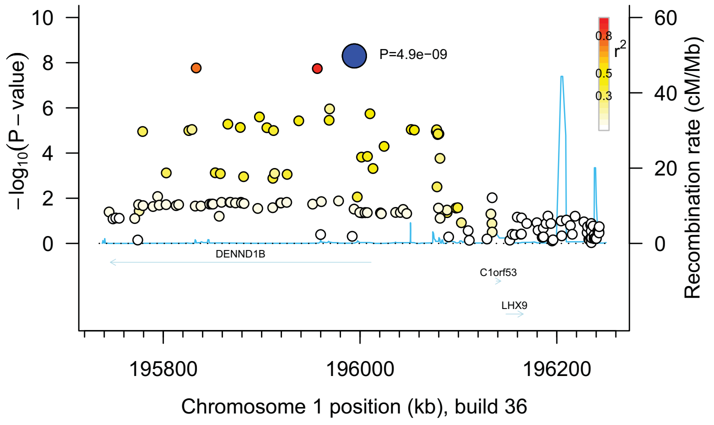

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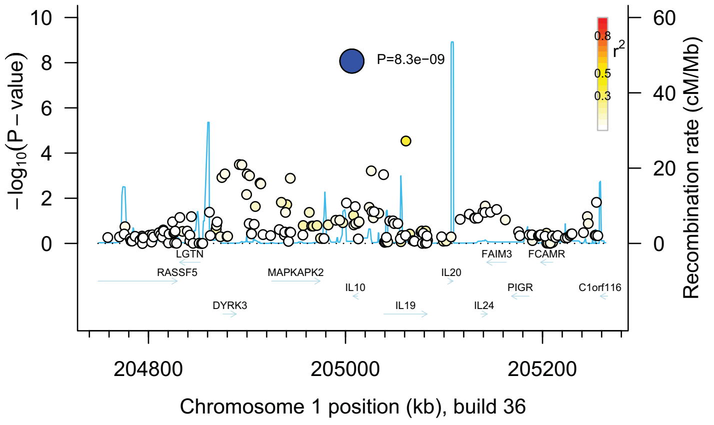

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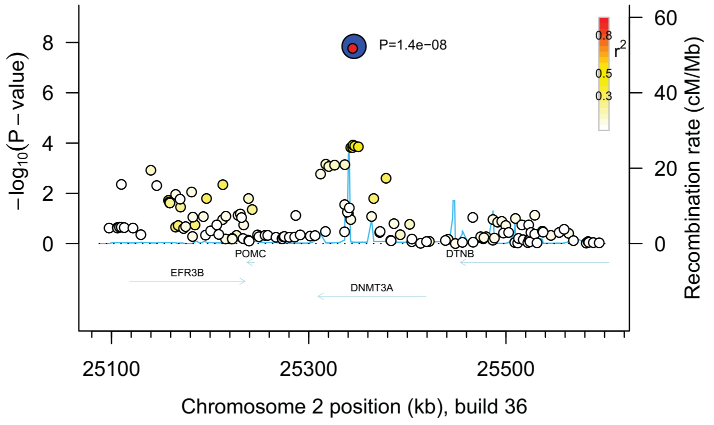

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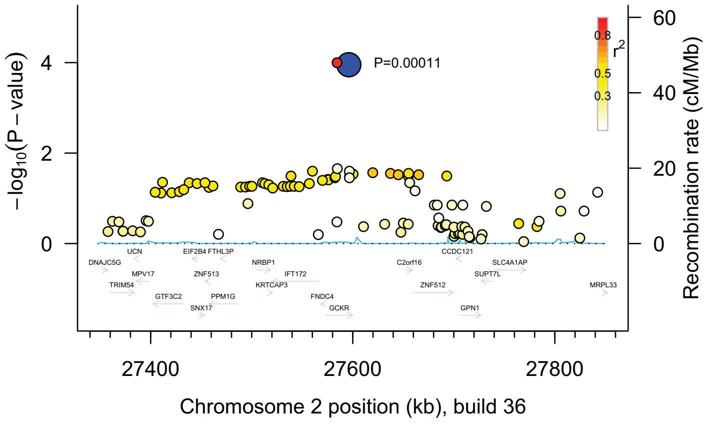

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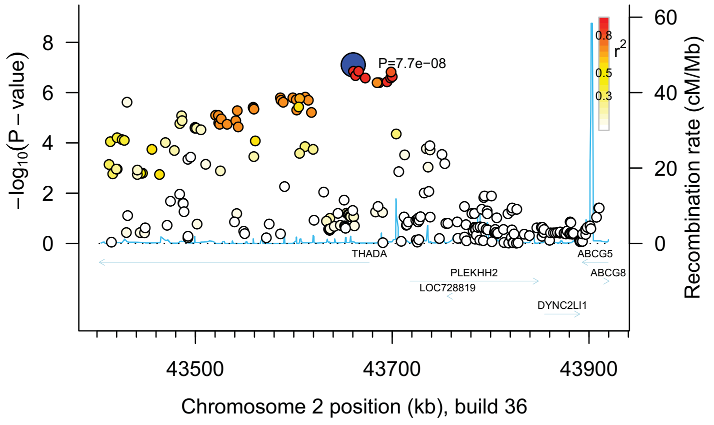

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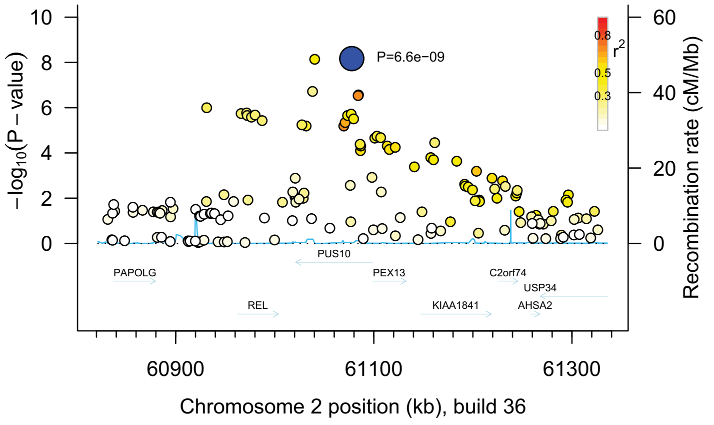

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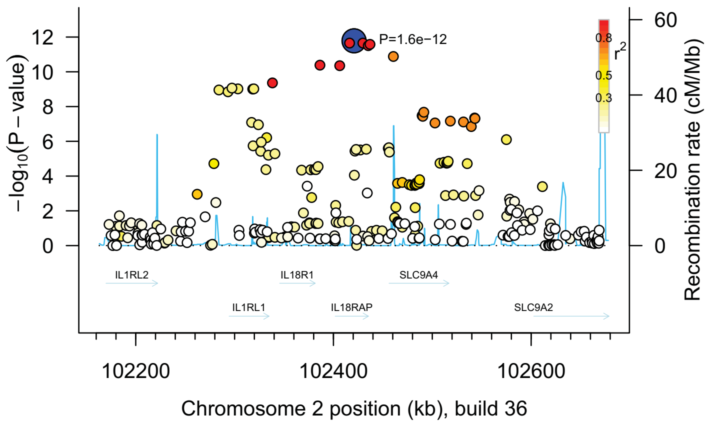

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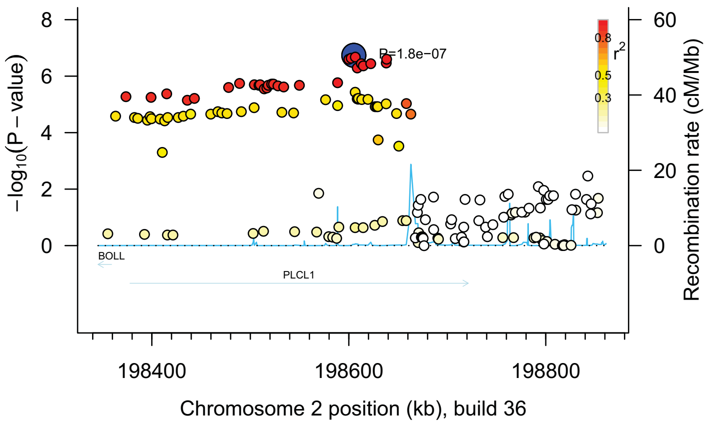

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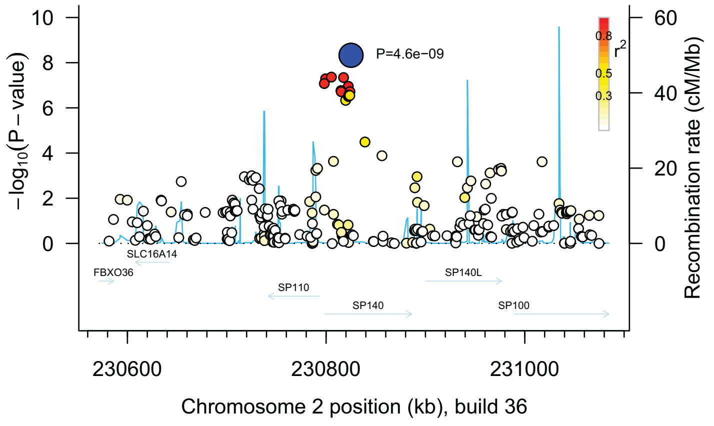

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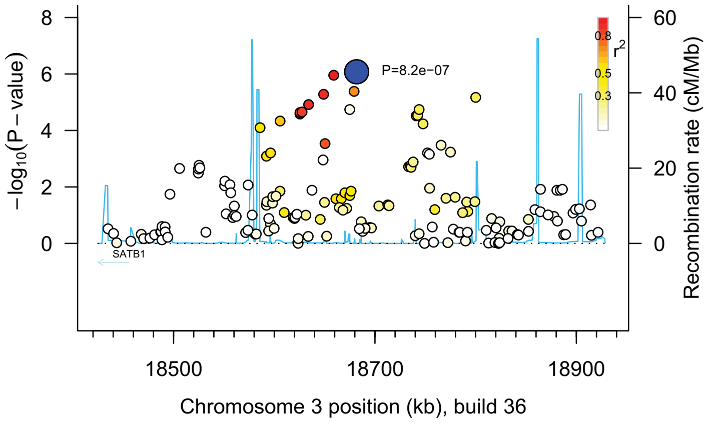

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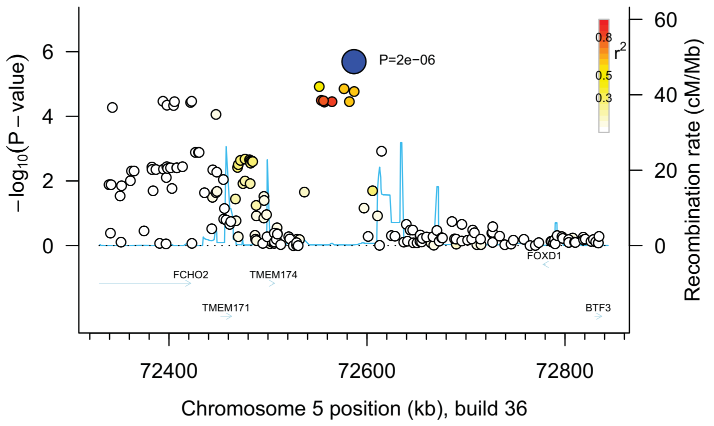

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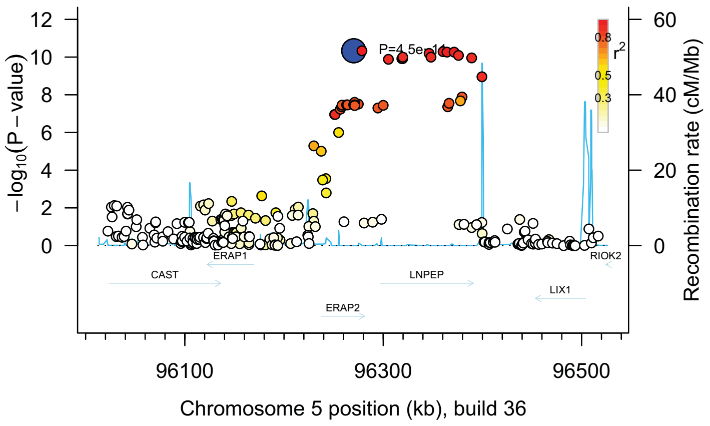

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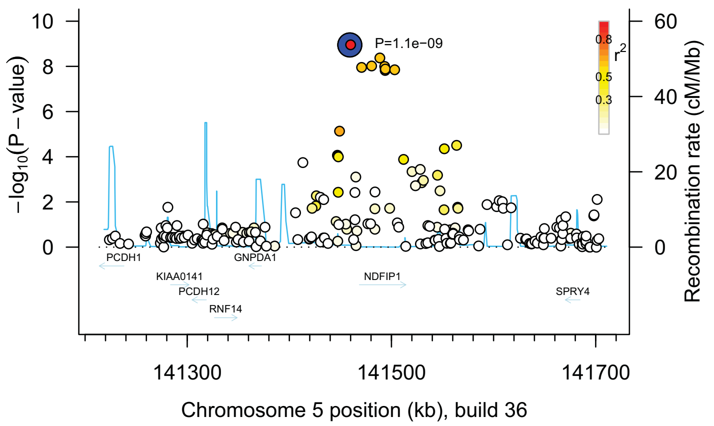

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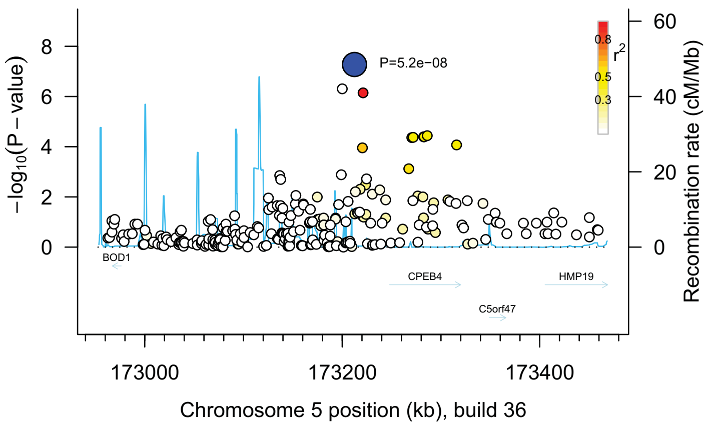

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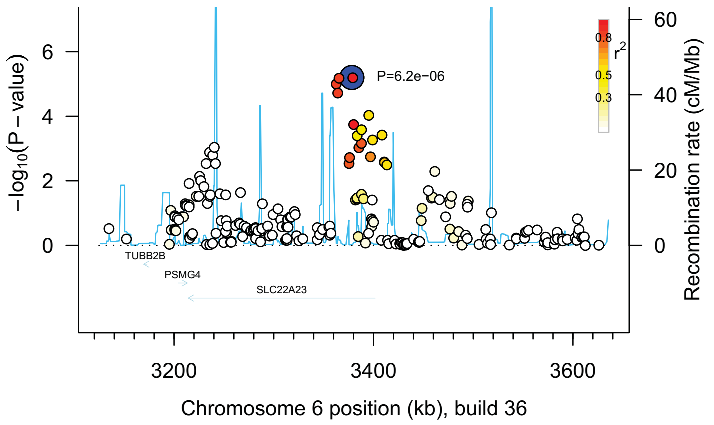

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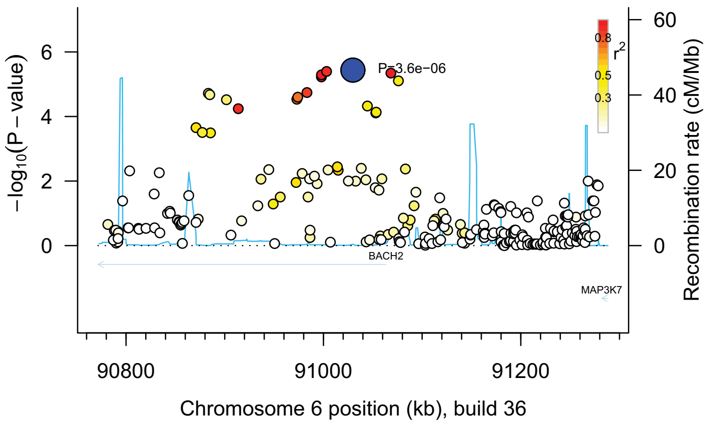

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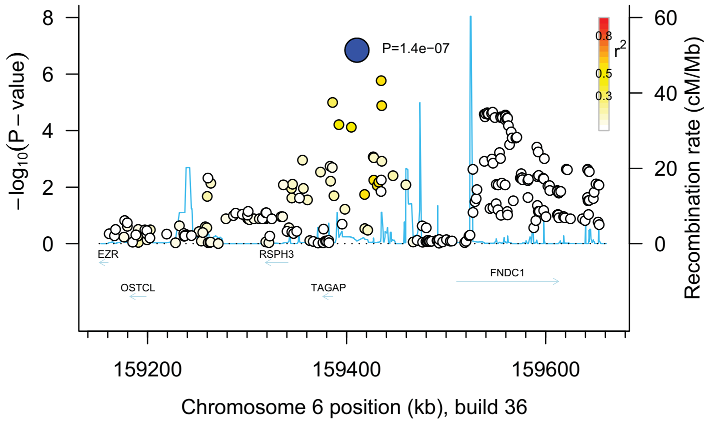

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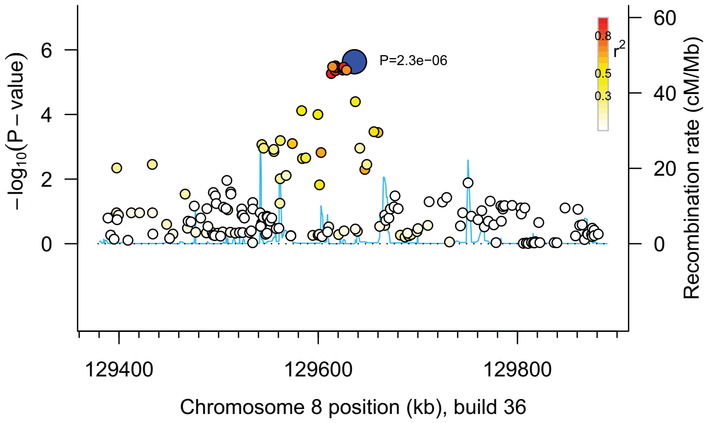

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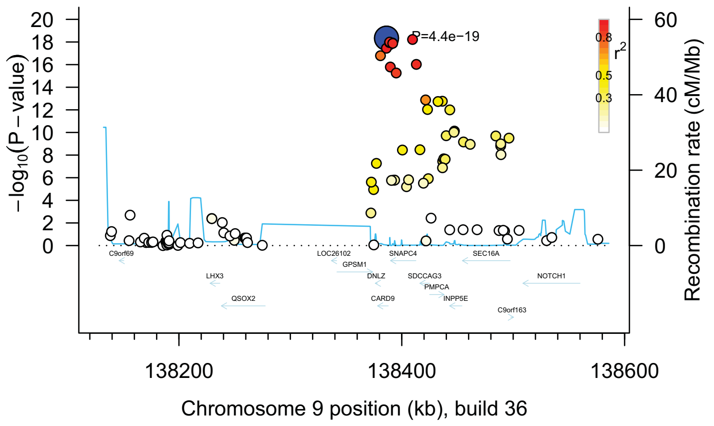

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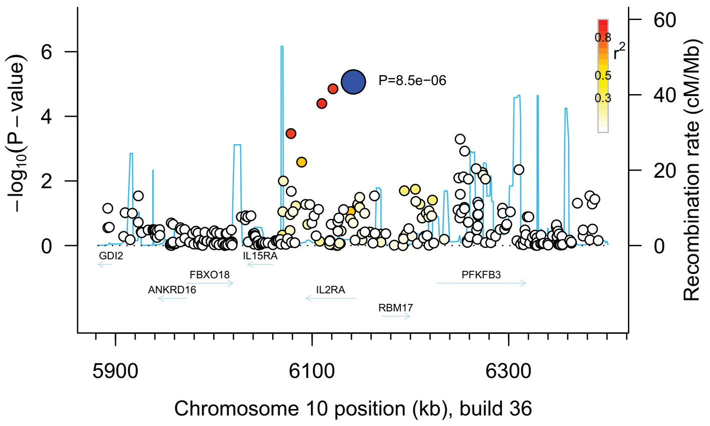

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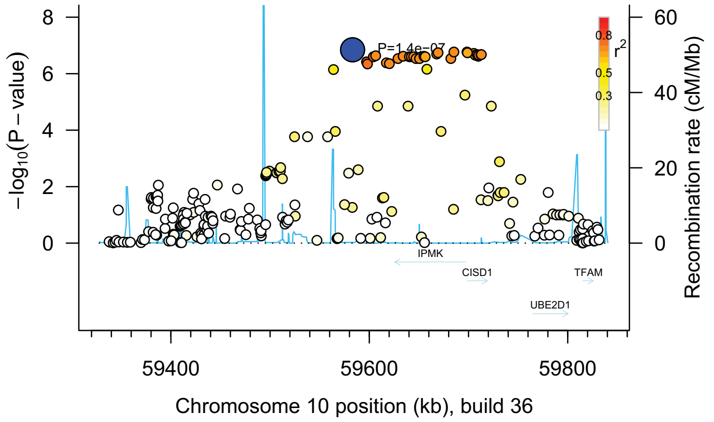

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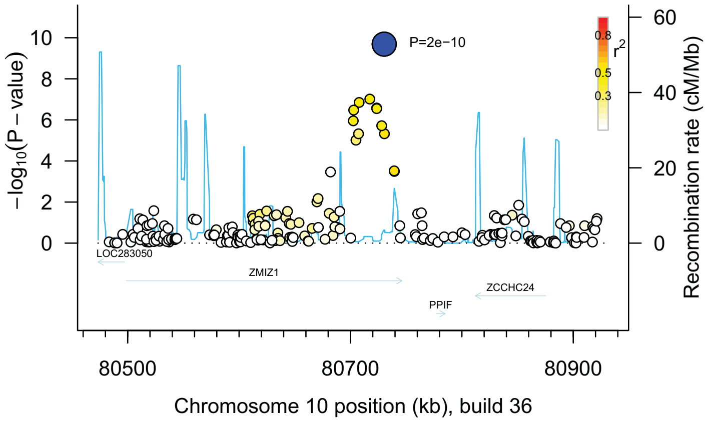

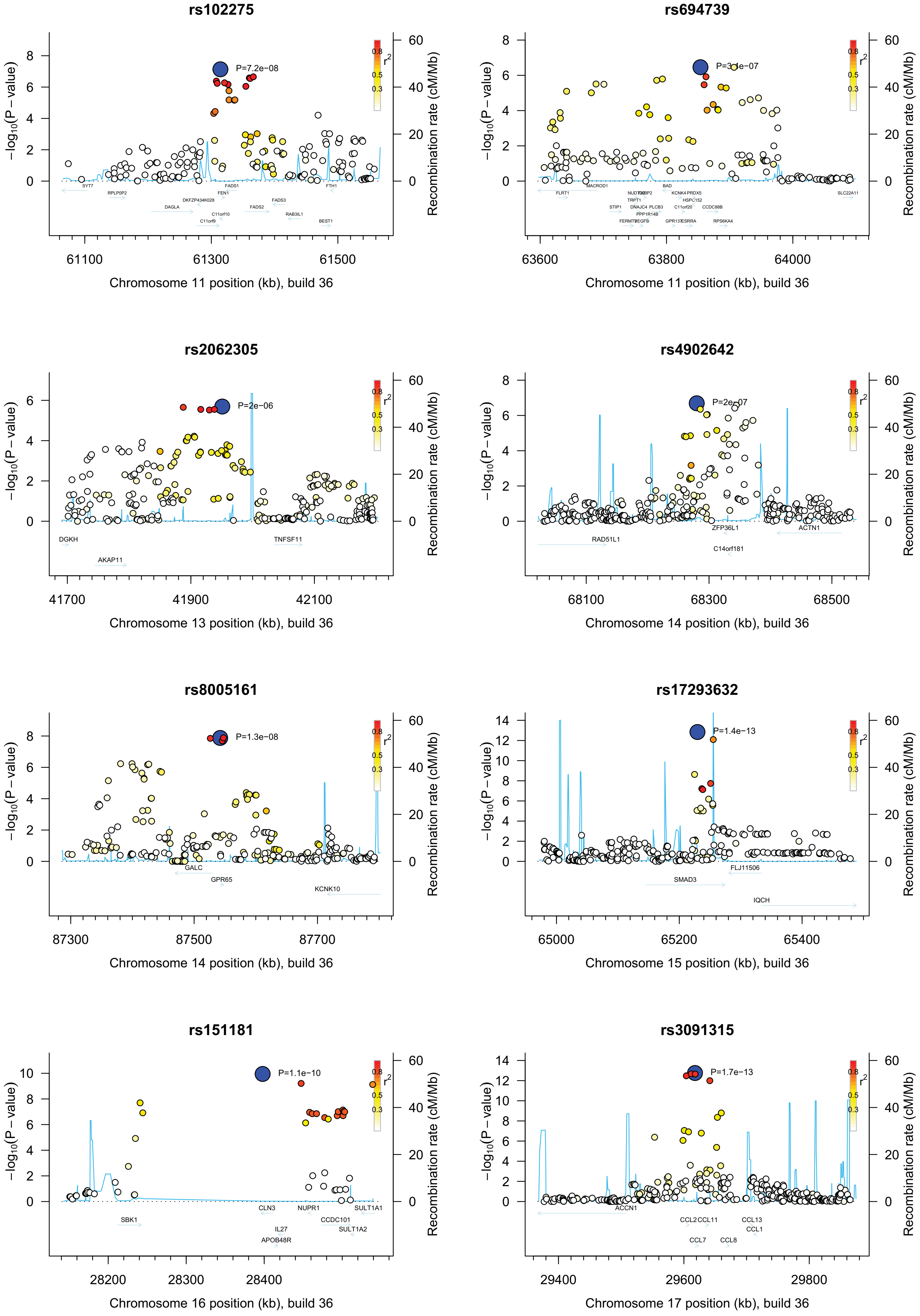
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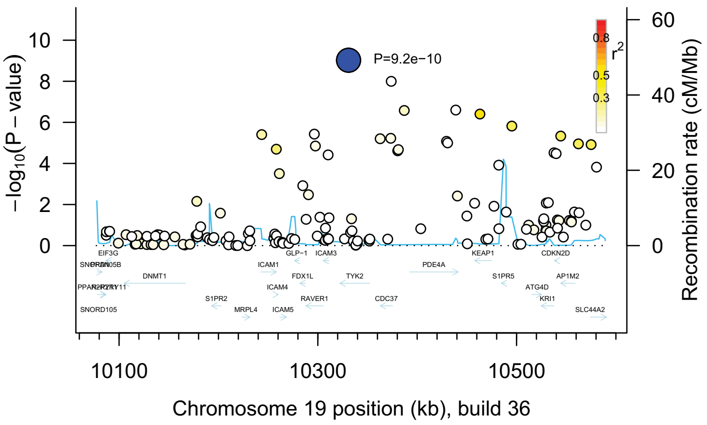
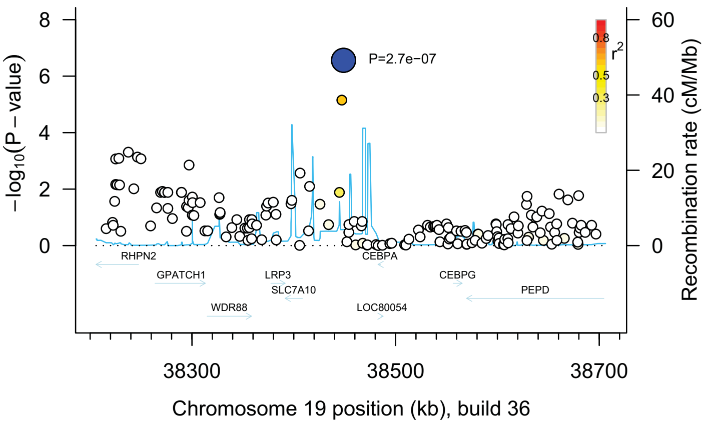
60

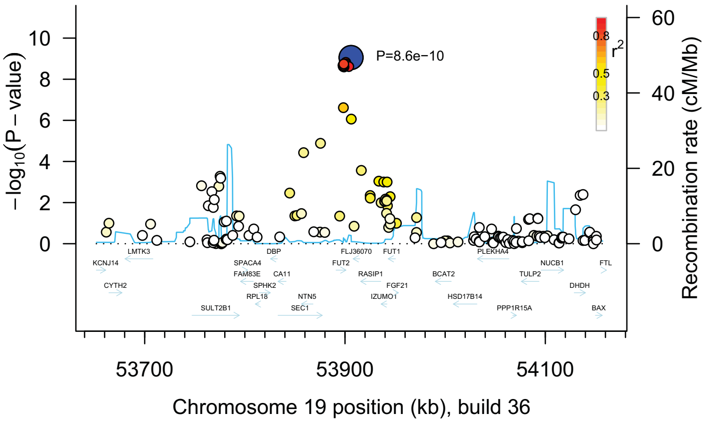
rs12720356



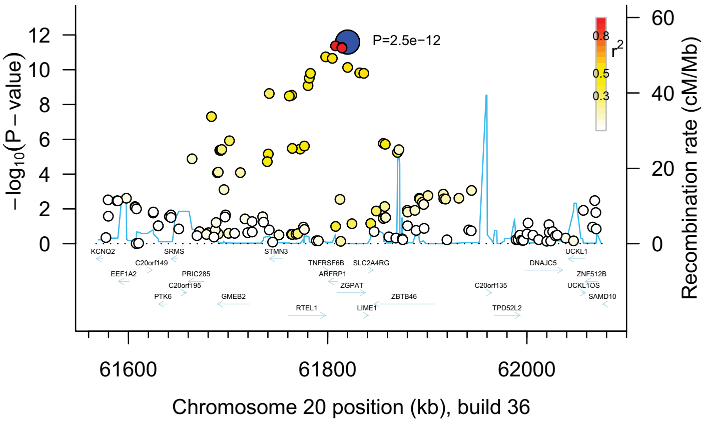
rs736289



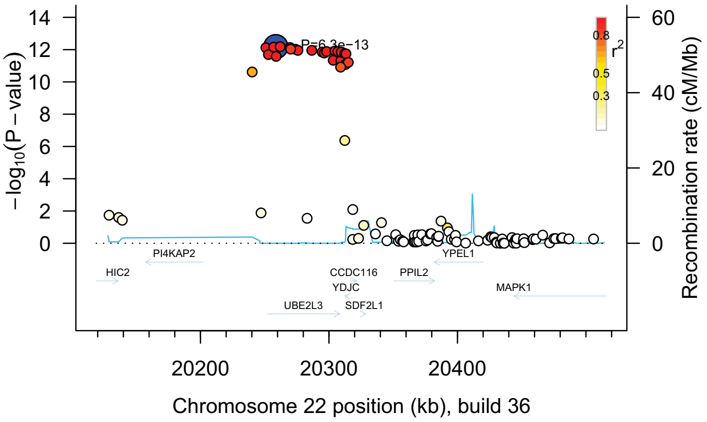
rs281379



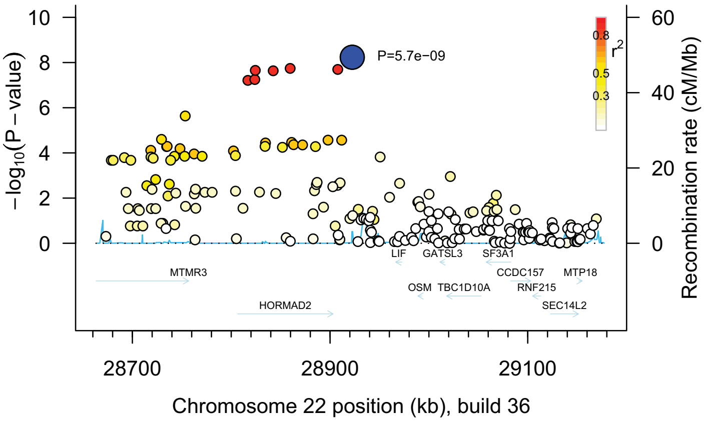
rs4809330



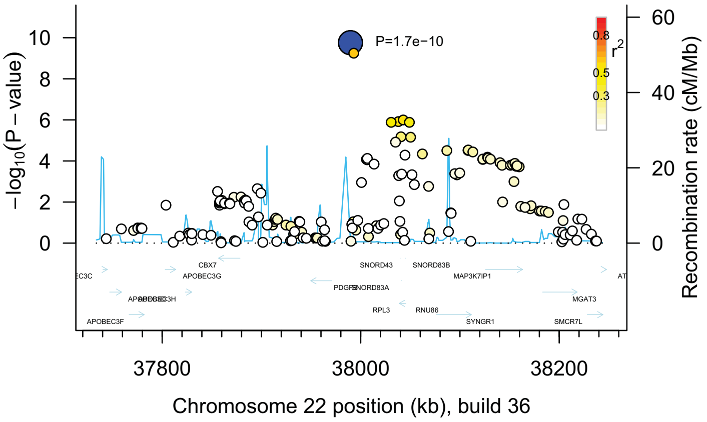
rs181359

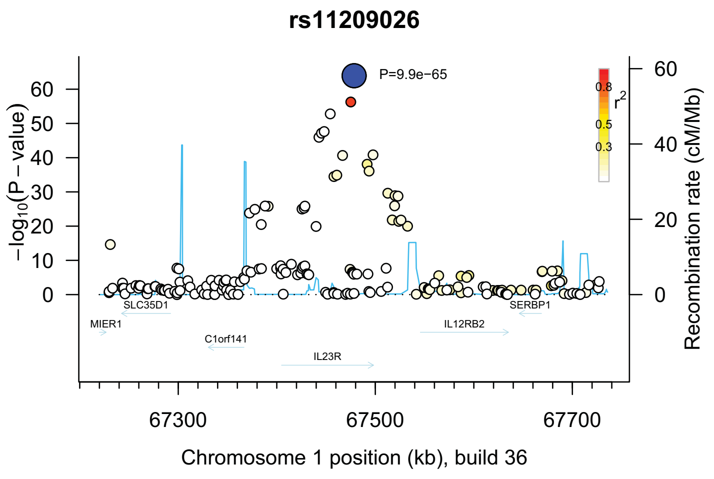


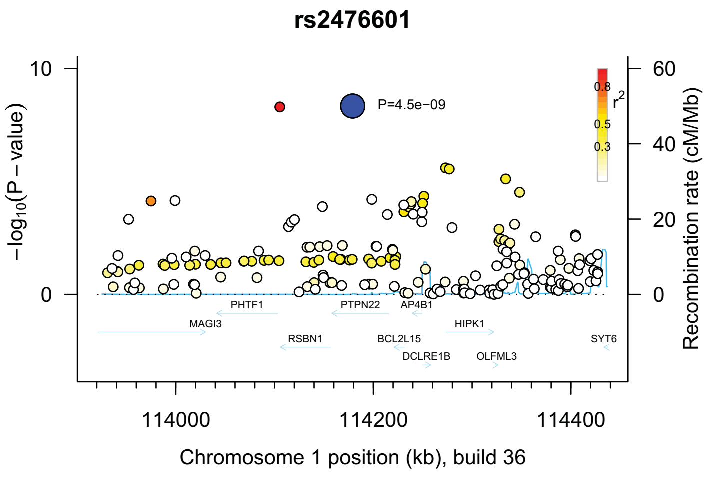
rs713875

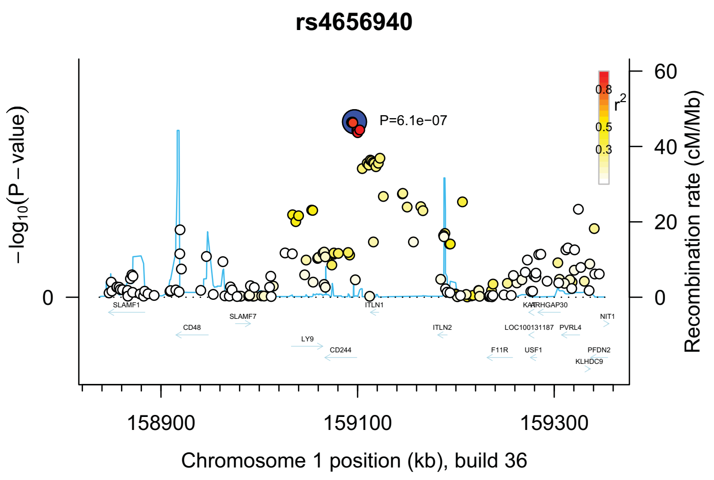


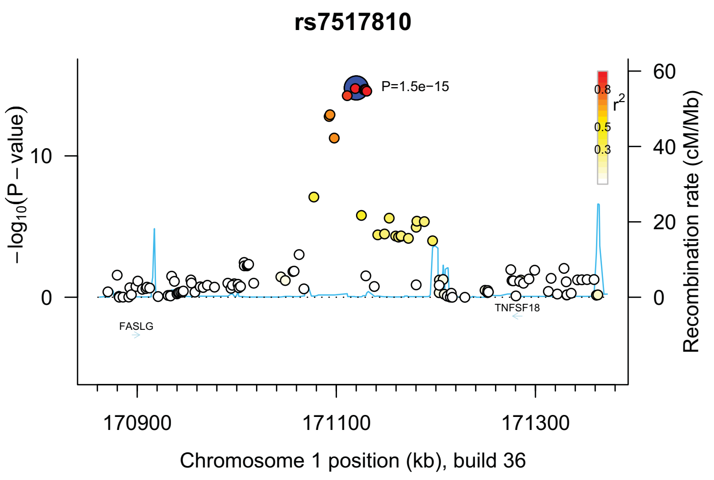
rs2413583

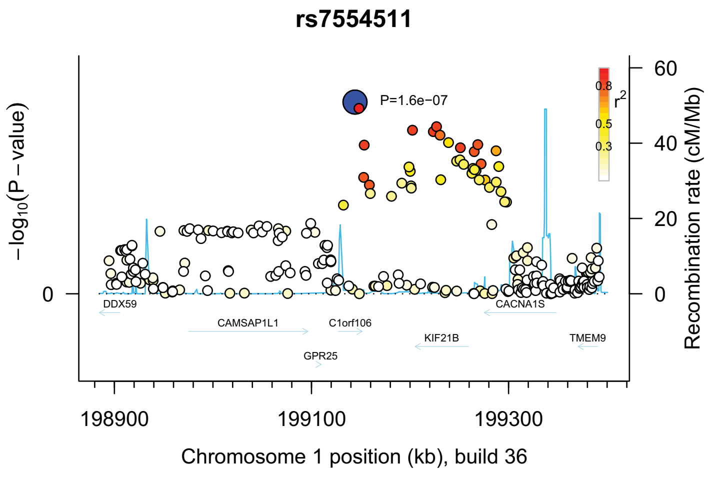


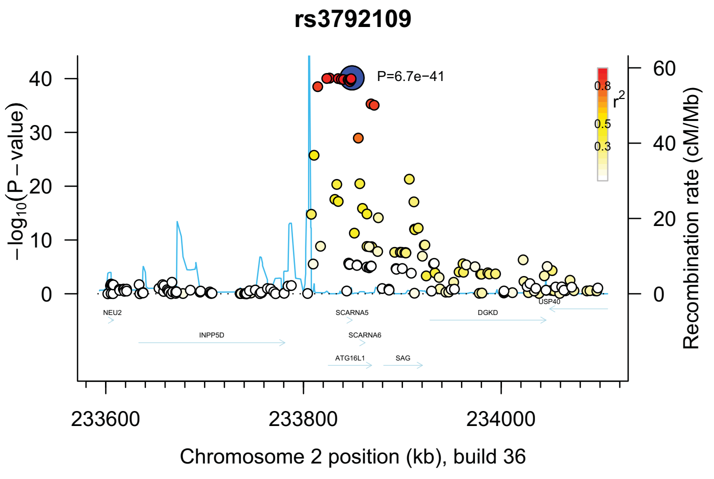

rs11209026
P=9.9e−65
−log10(P − value)
60
50
40
30
20
10
0
Recombination rate (cM/Mb)
60
40
20
0
0.8
0.5
0.3
r2
SLC35D1
MIER1
C1orf141
IL23R
IL12RB2
SERBP1
67300
67500
67700
Chromosome 1 position (kb), build 36

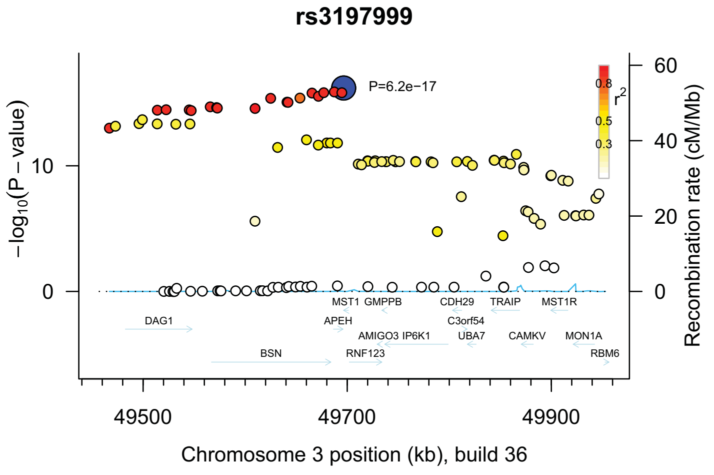

rs2476601
P=4.5e−09
−log10(P − value)
10
0
Recombination rate (cM/Mb)
60
40
20
0
0.8
0.5
0.3
r2
PHTF1
PTPN22
AP4B1
MAGI3
HIPK1
RSBN1
BCL2L15
SYT6
DCLRE1B
OLFML3
114000
114200
114400
Chromosome 1 position (kb), build 36

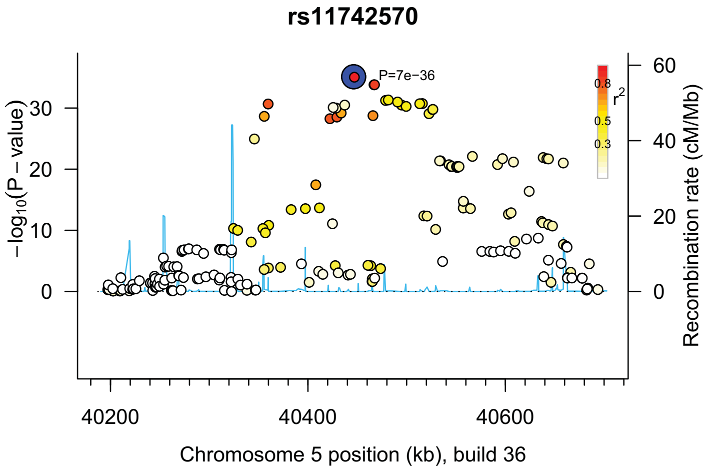

rs4656940
P=6.1e−07
−log10(P − value)
0
Recombination rate (cM/Mb)
60
40
20
0
0.8
0.5
0.3
r2
SLAMF1
ITLN1
KAT ARHGAP30
SLAMF7
NIT1
CD48
ITLN2
LOC100131187
PVRL4
LY9
CD244
F11R
USF1
PFDN2
KLHDC9
158900
159100
159300
Chromosome 1 position (kb), build 36


rs7517810
P=1.5e−15
−log10(P − value)
10
0
Recombination rate (cM/Mb)
60
40
20
0
0.8
0.5
0.3
r2
TNFSF18
FASLG
170900
171100
171300
Chromosome 1 position (kb), build 36

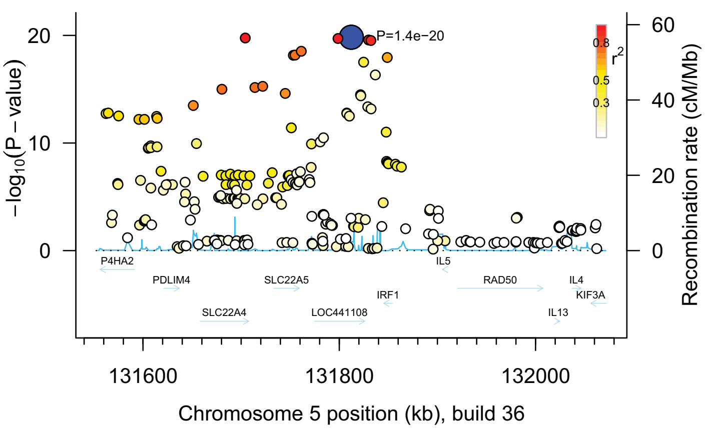

rs7554511
P=1.6e−07
−log10(P − value)
0
Recombination rate (cM/Mb)
60
40
20
0
0.8
0.5
0.3
r2
DDX59
CACNA1S
CAMSAP1L1
C1orf106
KIF21B
TMEM9
GPR25
198900
199100
199300
Chromosome 1 position (kb), build 36


rs3792109
P=6.7e−41
−log10(P − value)
40
30
20
10
0
Recombination rate (cM/Mb)
60
40
20
0
0.8
0.5
0.3
r2
USP40
NEU2
SCARNA5
DGKD
INPP5D
SCARNA6
ATG16L1
SAG
233600
233800
234000
Chromosome 2 position (kb), build 36

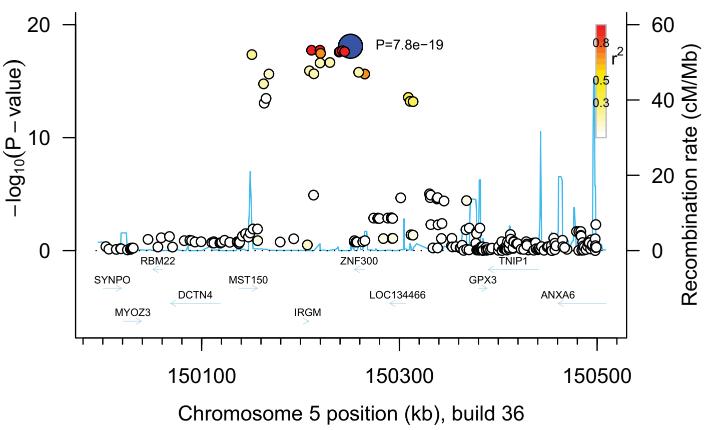

rs3197999
P=6.2e−17
−log10(P − value)
10
0
Recombination rate (cM/Mb)
60
40
20
0
0.8
0.5
0.3
r2
MST1
GMPPB
CDH29
TRAIP
MST1R
DAG1
APEH
C3orf54
AMIGO3
IP6K1
UBA7
CAMKV
MON1A
BSN
RNF123
RBM6
49500
49700
49900
Chromosome 3 position (kb), build 36


rs11742570
P=7e−36
−log10(P − value)
30
20
10
0
Recombination rate (cM/Mb)
60
40
20
0
0.8
0.5
0.3
r2
40200
40400
40600
Chromosome 5 position (kb), build 36

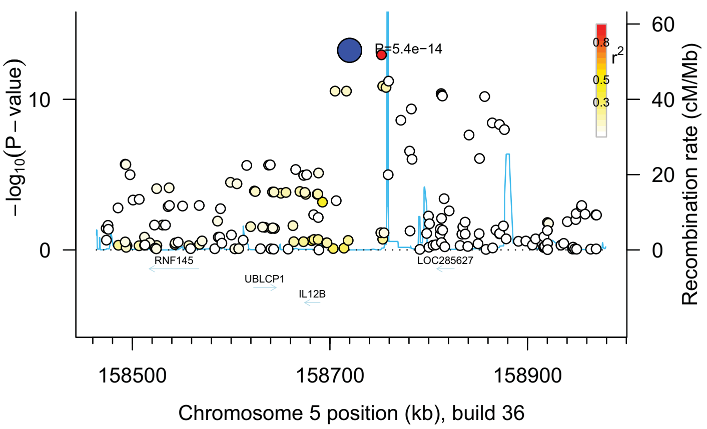
rs12521868



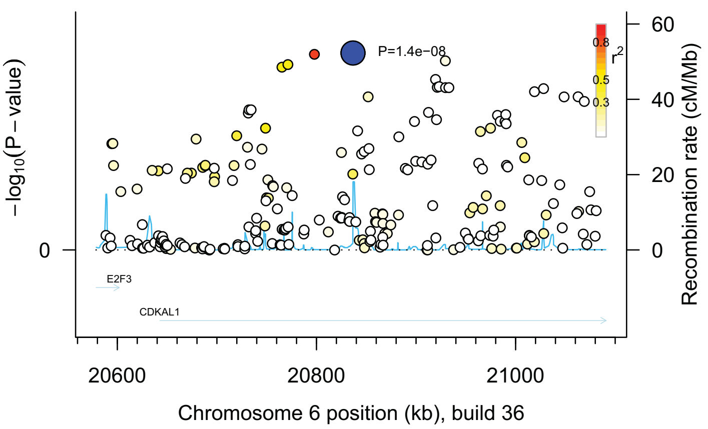
rs7714584



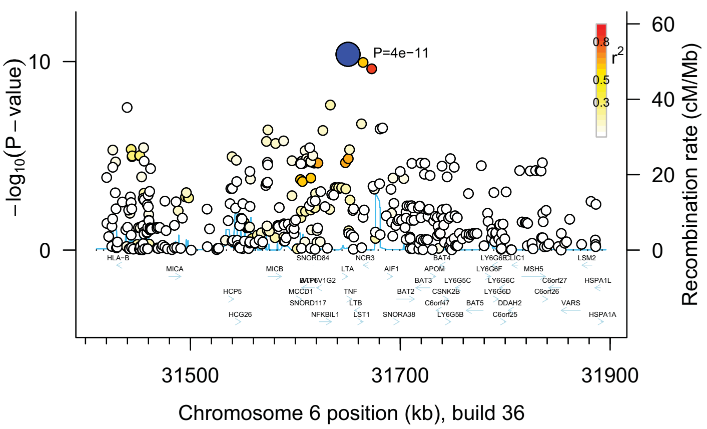
rs6556412



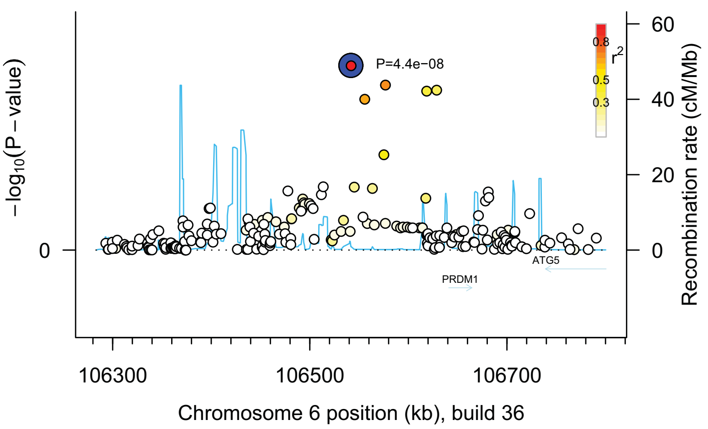
rs6908425



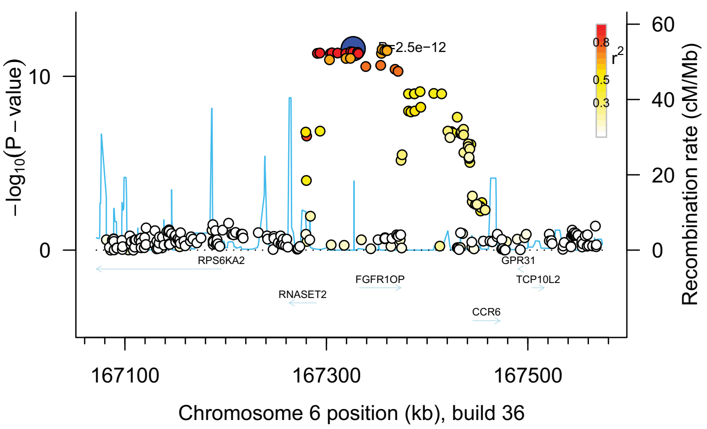
rs1799964



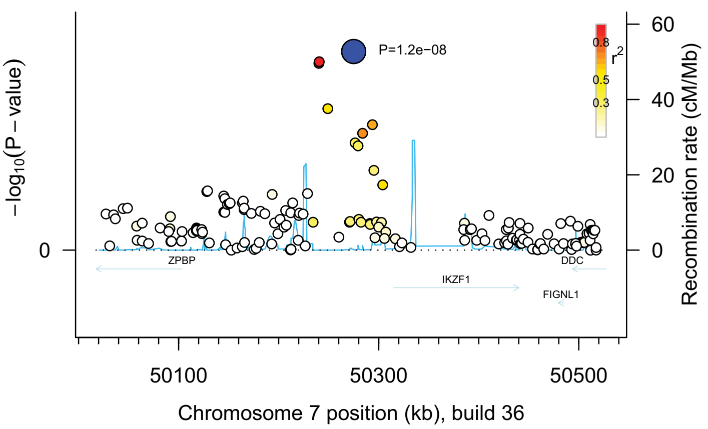
rs6568421



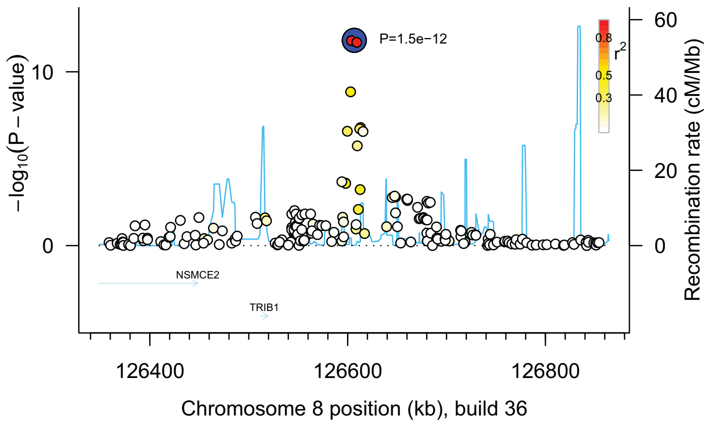
rs415890



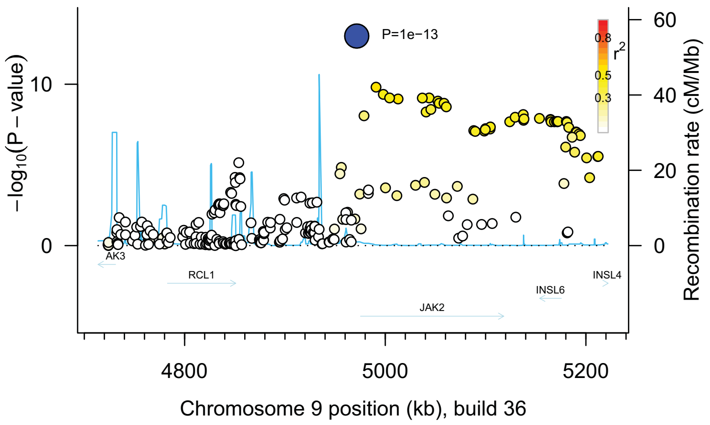
rs1456896



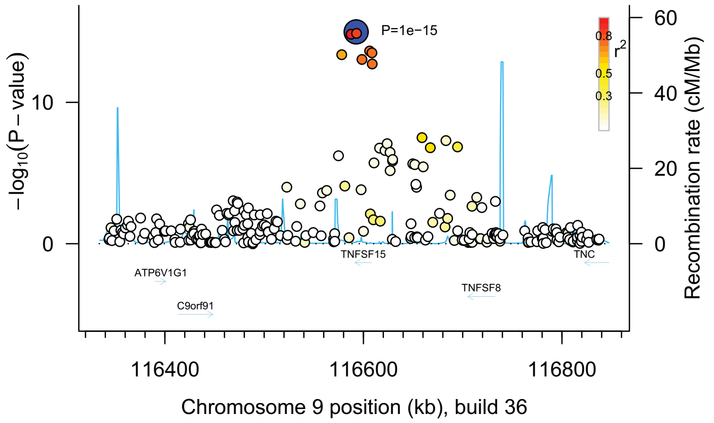
rs4871611



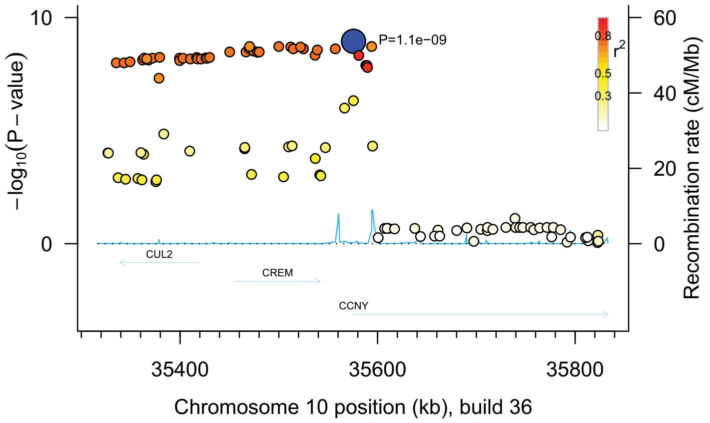
rs10758669



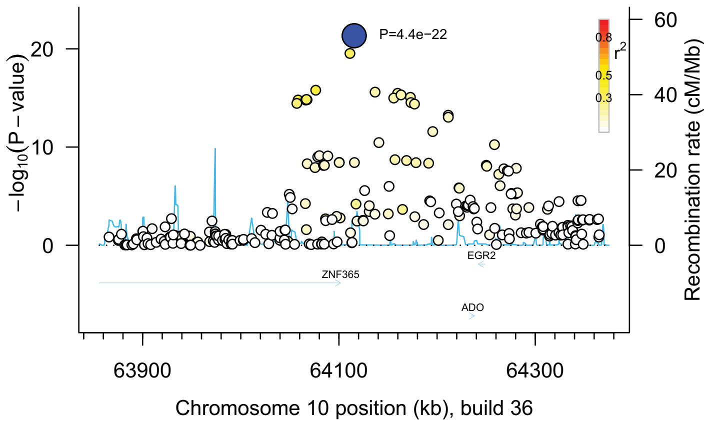
rs3810936



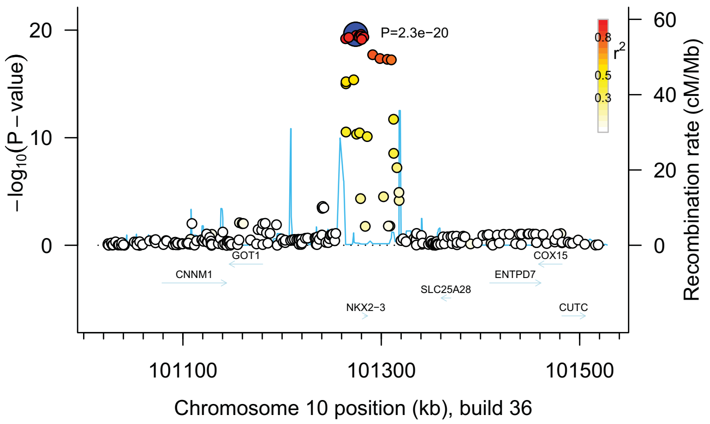
rs12242110



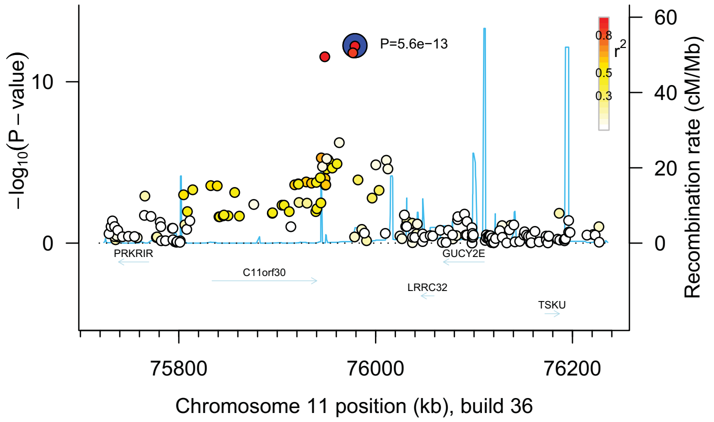
rs10761659



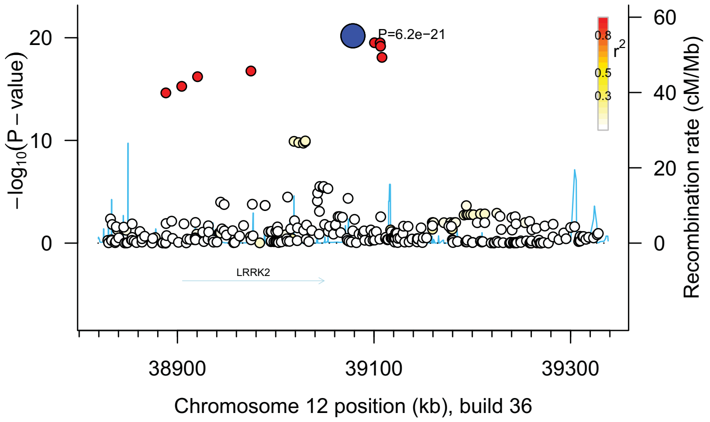
rs4409764

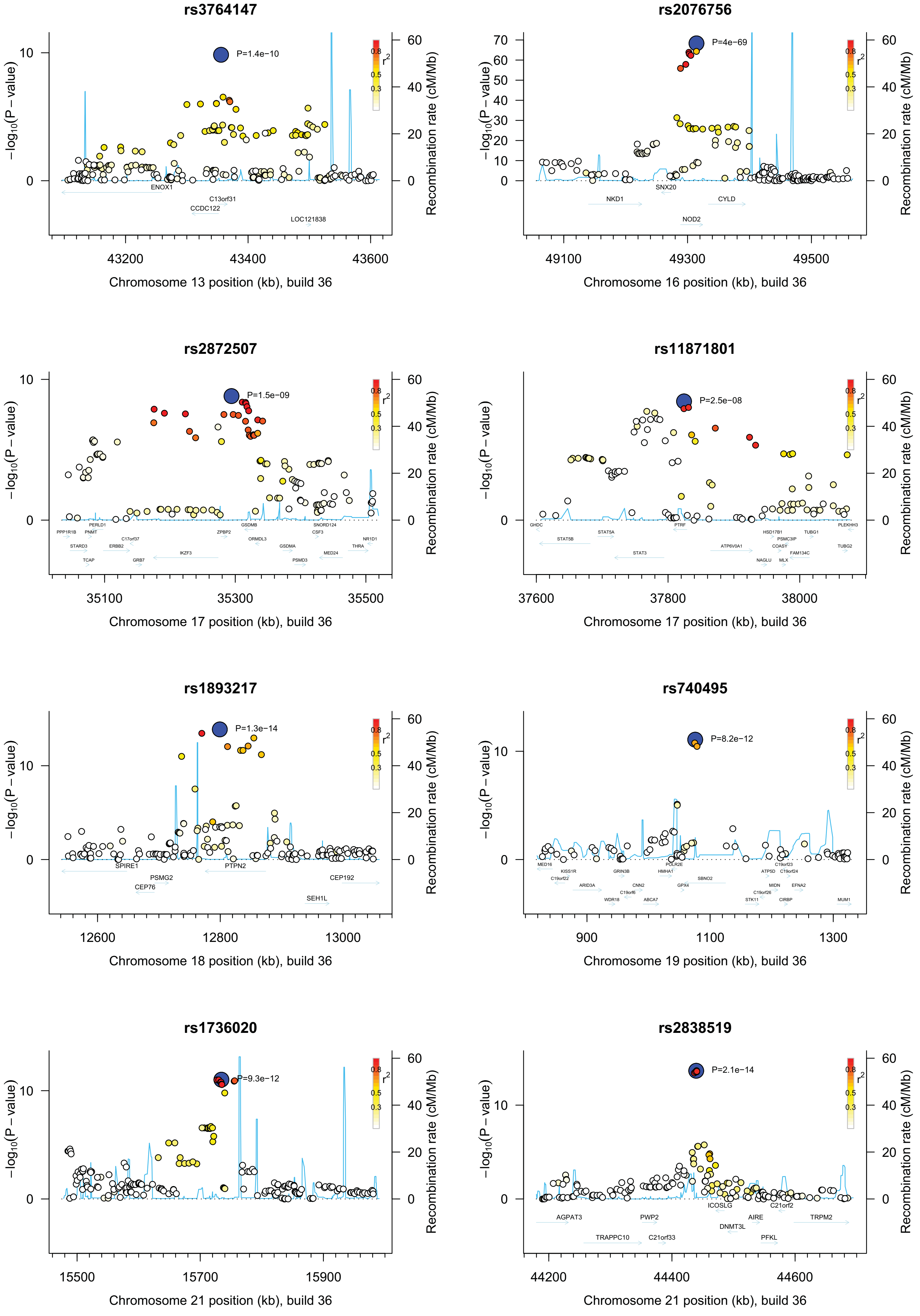


rs7927997



rs11564258





rs3764147

rs2076756

rs2872507

rs11871801

rs1893217

rs740495

rs1736020

rs2838519

Supplementary Note – eQTL Analysis

Regional annotation via eQTL analysis

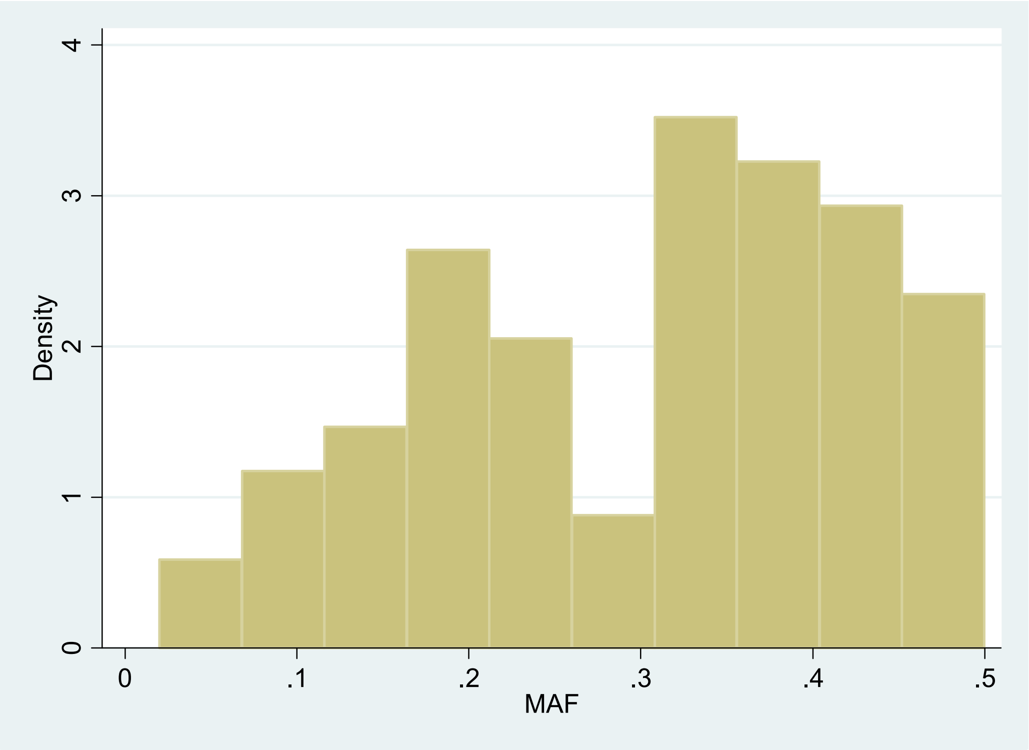
The effects on expression of neighboring genes of the 71 SNPs listed in Table 1 and 2 (of the main manuscript) were studied using the Dixon *et al.* transcriptome data, based on Epstein-Barr virus–transformed lymphoblastoid cell lines from 400 children, as described previously [**1**]. The Dixon *et al.* study genotyped subjects using the Illumina HumanHap300 platform and measured gene expression levels on 54,675 transcripts representing 20,599 genes using the Affymetrix U133 Plus 2.0 GeneChip. For each SNPs listed in Table 1 and 2 of the main manuscript, if it was not genotyped in the Dixon *et al.* study, the best proxy based on CEU r^2 was examined instead (see **Table I**). A LOD score threshold of 5 was used to declare a significant association between SNP genotypes and gene expression levels.

To evaluate the significance of the eQTL findings with the CD-associated SNPs, we compared the observed number of *cis* eQTLs yielding LOD scores >5, and the corresponding number among a randomly selected sets of 71 frequency-matched SNPs. The coordinates of the transcripts were provided by Dixon *et al.* (NCBI's 35 assembly), so the coordinates of the SNPs that we used were also based on NCBI 35 assembly. The allele frequency distribution of the 71 SNPs are shown in **Figure I**. The 71 CD SNPs produced seven eQTLs with LOD >5. In 1000 simulated experiments, only 1/1000 produced more than seven eQTLs with LOD >5 (see **Figure II**). Therefore, there is a significant enrichment of cis-operating eQTLs among the CD-associated loci.

Table I. Genotype-expression correlation analysis on CD-associated SNPs to identify cis-eQTLs. For primary SNPs that were not genotyped in the Dixon *et al.* data set, a proxy SNP was determined for the analysis, and the pairwise linkage disequilibrium (LD) between the primary and proxy SNPs are shown using the measure r^2 (R2). The column "eSNP nearby for same transcript?" shows whether nearby SNPs with stronger eQTL effects are present for the same transcript in the "eQTL?" column.

Chr.	Left	Right	No. $P<10^{-5}$	eQTL?	eSNP nearby for same transcript?	eSNP tested	Same SNP or Proxy
1	204,869,063	205,099,374	1	None		rs3024505	same SNP
1	153,244,553	154,389,854	83	None		rs3180018	same SNP
1	7,660,386	7,891,753	5	None		rs2797685	same snp
1	195,577,400	196,205,063	23	None		rs1998598	same SNP
2	43,301,270	43,795,977	38	None		rs10495903	same SNP
2	230,761,562	230,944,130	13	*SP140* (LOD=8.8)		rs13397985	rs13397985 - R2 = 0.89
2	25,301,554	25,461,262	2	None		rs7583409	rs7583409 - R2 = 1.0
2	197,850,455	198,667,617	47	None		rs1541953	rs1541953 - R2 = 1.0
3	18,582,796	18,857,492	5	None		rs6792314	rs6792314 - R2 = 0.93
5	173,150,790	173,472,805	3	*CPEB4* (LOD=6.1)	rs747472 (LOD=12.0)	rs359457	same SNP
5	72,492,752	72,616,167	1	None		rs7702331	same SNP
5	96,105,158	96,450,821	36	*LRAP* (LOD=47.2)	rs39602 (LOD=50.3)	rs27306	rs27306 - R2 = 1.0
5	141,394,644	141,622,106	10	None		rs11167764	same SNP
6	159,260,927	159,464,567	2	None		rs212388	same SNP
6	90,863,556	91,139,647	6	None		rs1010474	rs1010474 - R2 = 0.92
7	152,992,808	153,135,560	4	None		rs2098112	same SNP
8	129,560,876	129,668,460	11	None		rs6651253	rs6651253 - R2 = 1.0
9	138,274,802	138,544,419	41	*CARD9* (LOD=12.4)	rs11794847 (LOD=13.1)	rs4077515	same SNP
10	80,671,808	80,775,690	11	None		rs1250550	same SNP
10	59,493,900	59,815,801	34	None		rs1698408	rs1698408 - R2 = 0.80
10	6,070,249	6,205,531	1	None		rs122722561	rs122722561 - R2 = 0.93
11	61,283,132	61,441,126	13	*FADS1* (LOD=5.0)	rs174578 (LOD=7.2)	rs102275	same SNP
13	41,724,842	41,999,763	5	*TNFSF11* (LOD=5.9)		rs9594759	rs9594759 - R2 = 0.97
14	87,280,056	87,712,056	17	None		rs3742704	rs3742704 - R2 = 1.0
14	68,226,845	68,387,815	12	None		rs194772	rs194772 - R2 = 0.67
15	65,195,295	65,269,057	13	None		rs16950687	rs16950687 - R2 = 0.88
16	28,202,322	28,935,308	33	*EIF3S8* (LOD=11.3)	rs7189927 (LOD=11.9)	rs4788084	rs4788084 - R2 = 0.89
19	10,261,304	10,495,264	9	None		rs12720356	same SNP
19	53,784,242	53,969,894	13	None		rs676388	rs676388 - R2 = 1.0
22	37,984,993	38,135,698	8	None		rs2413583	same SNP
22	20,141,991	20,393,337	26	None		rs5754217	rs5754217 - R2 = 1.0
22	28,232,382	28,998,308	8	None		rs757024	rs757024 - R2 = 0.90

Figure I. The minor allele frequency (MAF) distribution of the 71 SNPs associated with CD in Table 1 and 2.


Density
MAF