Genome-Wide Association Study of Treatment Response to Venlafaxine XR in Generalized Anxiety Disorder

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Supplementary Materials

Methods

Subjects

Participants enrolled in an 18-month relapse prevention outpatient study which included three treatment phases (Rickels et al., 2010): a 6-month open-label venlafaxine XR flexible-dose treatment phase (75–225 mg day^{-1}; Phase I), a 6-month, randomized, double-blind, placebo-controlled relapse phase (Phase II), and a final 6-month, randomized, double-blind, placebo-controlled relapse phase (Phase III). The first phase (Phase I) was used to conduct primary pharmacogenetic analyses. The data for this study were collected between 2005 and 2009.

The present study required subjects to be 18 years or older and meet the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria for GAD. Diagnoses of GAD were determined by the Structured-Clinical Interview (SCID) (First et al., 1995) and psychiatric evaluation. Subjects also needed a score of ≥ 20 on the Hamilton Anxiety Scale (HAM-A) (Gjerris et al., 1983) at screen and at baseline, and a score of ≥ 4 on the Clinical Global Impression (CGI)-Severity scale(Guy, 1976). Exclusion criteria included any current

DSM-IV anxiety spectrum diagnosis at the threshold but not sub-threshold level, current or past history of bipolar disorder, schizophrenia, other psychotic disorders, and dementia. Furthermore, severely depressed subjects with a HAM-D score of > 18, as well as suicidal patients with a score of ≥ 2 on the suicide item of the HAM-D were excluded. Finally, subjects with a major depressive episode and substance abuse or dependence in the past 6 months were excluded. Patients were not excluded for occasional recreational drug or alcohol use in order for our sample to reflect the general GAD population. Patients on a low daily dose of benzodiazepine anxiolytics or hypnotics were allowed to enter the trial and were tapered off their medication over the first 12 weeks of treatment with venlafaxine XR.

Patients were recruited at the University of Pennsylvania Medical Center with approval and oversight by the Institutional Review Board of the University of Pennsylvania. All participants gave written informed consent before performing any study procedures. Eligible patients were screened over a 4- to 28-day period and then started on venlafaxine XR 37.5 mg for 1 week, followed by 75 mg day^{-1} for the second week. After the second week, flexible dosing within the range of 75–225 mg day^{-1} was applied. The study aimed to increase the patient's daily dose to 225 mg by week 8, unless this outcome was precluded by adverse events or the patient was in remission. Number of remaining pills was counted for each individual to measure adherence to medication.

Overall, 156 patients (European-Americans [EA] $n = 112$; African-Americans $n = 41$; others $n = 3$) were evaluated for treatment response to venlafaxine XR. However, due to ethnic differences in allele frequencies and consequent population stratification, only the EA population ($n = 112$) was used in the pharmacogenetic analysis. The HAM-A score was used as a primary outcome measure, with treatment response defined as HAM-A reduction of ≥ 50%. Remission

was defined as a HAM-A score of ≤ 7. The CGI of Improvement (CGI-I) score at 6 months was used as a secondary outcome measure. Improvement was defined as a CGI-I score of ≤ 2, and remission was defined as a CGI-I score of 1. This study used the last observation carried forward imputation method to account for missing data. Unresponsive patients, with a CGI-I score of ≥ 4, were discontinued.

Genotype and Quality Controls

Table 1A shows the 98 European samples with common SNPs of 266,820 which remained for further statistical analysis.

Results

Table 2A shows a list of the most significant 9 SNPs with p-values less than 1.0E-5, minor/major alleles, minor allele frequency (MAF), and odds ratio (OR) for each phenotype at week 24. None of SNPs were identified with genome-wide significance ($1.9x10^{-7}$ by Bonferroni correction) at week 24. We found that 9 SNPs were significantly associated with categorical outcomes (HAM-A response/remission, CGI-I response/remission) at week 24. Samples with major allele of exm1187524, rs6712232, rs75965524, and rs6918679 responded to the treatment significantly better than those with minor alleles (ORs >10). Likewise, samples with major allele of rs3013580, rs2454517, rs3020264, rs10914746, rs7984972 remitted significantly better than those with minor alleles (ORs ≈ 4~6). Table 3A shows a list of the most significant 8 SNPs with p-values less than $1.0x10^{-5}$ at week 12. None of SNPs were identified with genome-wide significance ($1.9x10^{-7}$ by Bonferroni correction) at week 12. Patients with major allele of 5 SNPs (rs3819595, rs539853, rs76778048, rs2070419, and exm827696) had significantly better responses to treatment than those with minor allele, and the minor allele of 3 SNPs (rs7152947, rs8020289, rs13129231) involved better remission than those with major allele at week 12.

Table 4A shows a list of significant SNPs associated with at least one of the 5 outcomes including a HAM-A score at week 24 (p-value < $1.0x10^{-5}$). The identified four genes were previously linked to psychiatric disorders: *CSMD2* (Rs10914746) was associated with alcohol dependence (Edwards et al., 2012); *NEGR1* (rs977145) was related to depression and bipolar disorder(Maccarrone et al., 2013), as well as cognitive and behavioral traits of bulimia nervosa (BN)(Gamero-Villarroel et al., 2015); *MCPH1* (rs2454517 and rs3020264) was linked to anxiety and major depressive disorder(Ishitobi et al., 2014); and *POLG* (exm1187524) was associated with bipolar disorder(Chen et al., 2010). Table 5A shows a list of 8 significant SNPs associated with at least one of the 5 outcomes at week 12. Among them, expression of *PCP4* (rs3819595) was related to depression/alcohol dependence(Iwamoto et al., 2004; Teyssier et al., 2011). Figures 1-10 show Manhattan plots of genome-wide association analyses of each outcome at week 24 and 12.

Tables

Table 1A: Demographic characterization of clinical sample (*n*=98) by age, sex, and race.

Age		
	Mean	**SD**
	50.8	15.56
Sex		
	Count	**Percent**
Female	59	60.20%
Male	39	39.80%
Race		
	Count	**Percent**
European Americans	98	100%

Table 1B: Clinical Data for responders and non-responders at 24 weeks.

	N	Age (mean)	Baseline HAM-A	Week 24 HAM-A
Week 24 Responders	78	50.2	24.19	4.48
Week 24 Non-Responders	20	53.4	23.35	17.60

Table 2A: Results of each GWAS p-value < 0.00001 at week 24[a].

	SNP	CHR	BP	Minor/Major allele	MAF in cases	MAF in controls	CHISQ[b]	P-value	OR
RESPONSE									
	rs2307441	15	89861826	G/A	0.02	0.22	21.99	2.74E-06	14.38
CGI RESPONSE									
	rs6712232	2	72131032	G/A	0.02	0.22	20.99	4.62E-06	14.28
	rs75965524	2	236830138	G/A	0.02	0.22	21.3	3.93E-06	14.47
	rs6918679	6	75532577	A/G	0.03	0.28	24.29	8.29E-07	11.97
	rs3013580	13	67903616	G/A	0.19	0.59	22.68	1.91E-06	6.23
REMISSION									
	rs2454517	8	6473758	G/A	0.21	0.53	20.74	5.27E-06	4.36
	rs3020264	8	6488577	A/G	0.17	0.48	21.55	3.44E-06	4.70
CGI REMISSION									
	rs10914746	1	34039844	G/A	0.14	0.43	19.77	8.72E-06	4.63
	rs7984972	13	32008008	A/G	0.24	0.57	19.85	8.39E-06	4.17

a: None of SNPs were identified with genome-wide significance (1.9x10E-7 by Bonferroni correction)

b : statistics of Chi-square test

Table 3A: Results of each GWAS p-value < 0.00001 at week 12[a].

	SNP	CHR	BP	Minor/Major allele	MAF in cases	MAF in controls	CHISQ[b]	P-value	OR
RESPONSE									
	rs3819595	21	41282698	A/G	0.08	0.35	21.07	4.43E-06	6.45
CGI RESPONSE									
	rs539853	1	40194903	A/G	0.07	0.34	19.53	9.89E-06	7.00
	rs76778048	6	68929932	A/C	0.03	0.25	19.91	8.14E-06	10.20
	rs2070419	21	32933024	A/C	0.13	0.47	20.73	5.28E-06	6.09
REMISSION									
	rs7152947	14	77292093	G/A	0.42	0.11	21.58	3.39E-06	0.17
	rs8020289	14	77293520	A/G	0.42	0.12	19.68	9.14E-06	0.19
CGI REMISSION									
	rs13129231	4	114352881	G/A	0.39	0.10	20.57	5.76E-06	0.17
	rs2236295	10	64564892	A/C	0.21	0.52	20.71	5.34E-06	4.14

a: None of SNPs were identified with genome-wide significance (1.9x10E-7 by Bonferroni correction)

b : statistics of Chi-square test

Table 4A: Results of p-values of the significant SNPs across results of GWAS for five outcomes at week 24 (at least one p-value <1.0E-5).

SNP	CHR	BP	A1	RESPONSE	CGI RESPONSE	REMISSION	CGI REMISSION	HAM-A	GENE
rs10914746	1	34039844	G	0.0024	0.0275	2.40E-05	8.72E-06	0.00049	CSMD2
rs977145	1	71929289	A	0.0033	0.0020	0.00013	0.00333	6.40E-06	NEGR1
rs6712232	2	72131032	G	2.67E-05	4.62E-06	0.01366	0.00662	4.27E-05	
rs75965524	2	236830138	G	2.31E-05	3.93E-06	0.00145	0.00605	0.001	AGAP1
rs6918679	6	75532577	A	0.0002	8.29E-07	0.01183	0.00506	0.0120	
rs2454517	8	6473758	G	0.0024	0.0040	5.27E-06	2.89E-05	0.0005	MCPH1
rs3020264	8	6488577	A	0.0325	0.0651	3.44E-06	0.00013	0.0020	MCPH1
rs7853333	9	34065659	A	0.0021	0.0069	0.00191	0.00506	3.72E-06	
rs7984972	13	32008008	A	0.0171	0.0707	0.00044	8.39E-06	0.0047	
rs3013580	13	67903616	G	3.93E-05	1.91E-06	0.00023	0.00018	0.0002	
Rs2307441	15	89861826	G	2.74E-06	0.00058	0.00050	0.00017	0.0006	POLG

Table 5A: Results of p-values of the significant SNPs across results of GWAS for five outcomes at week 12 (at least one p-value <1.0E-5).

SNP	CHR	BP	A1	RESPONSE	CGI RESPONSE	REMISSION	CGI REMISSION	HAM-A	GENE
Rs2236295	10	64564892	A	0.0152	0.0265	0.0008	5.34E-06	0.0204	ADO
rs76778048	6	68929932	A	0.0011	8.14E-06	0.0493	0.0338	0.0004	
rs13129231	4	114352881	G	0.0337	0.0123	0.0006	5.76E-06	0.0163	
rs2070419	21	32933024	A	3.16E-05	5.28E-06	0.1411	0.03473	0.0002	
rs3819595	21	41282698	A	4.43E-06	0.0003	0.0078	0.0074	0.0048	PCP4
rs539853	1	40194903	A	4.87E-05	9.89E-06	0.0006	0.0014	0.0092	
rs7152947	14	77292093	G	0.0007	0.0088	3.39E-06	0.0008	0.0083	
rs8020289	14	77293520	A	0.0019	0.0245	9.14E-06	0.0016	0.0272	C14orf166B

Figure 1. Manhattan plot of GWAS for HAM-A response at week 24.

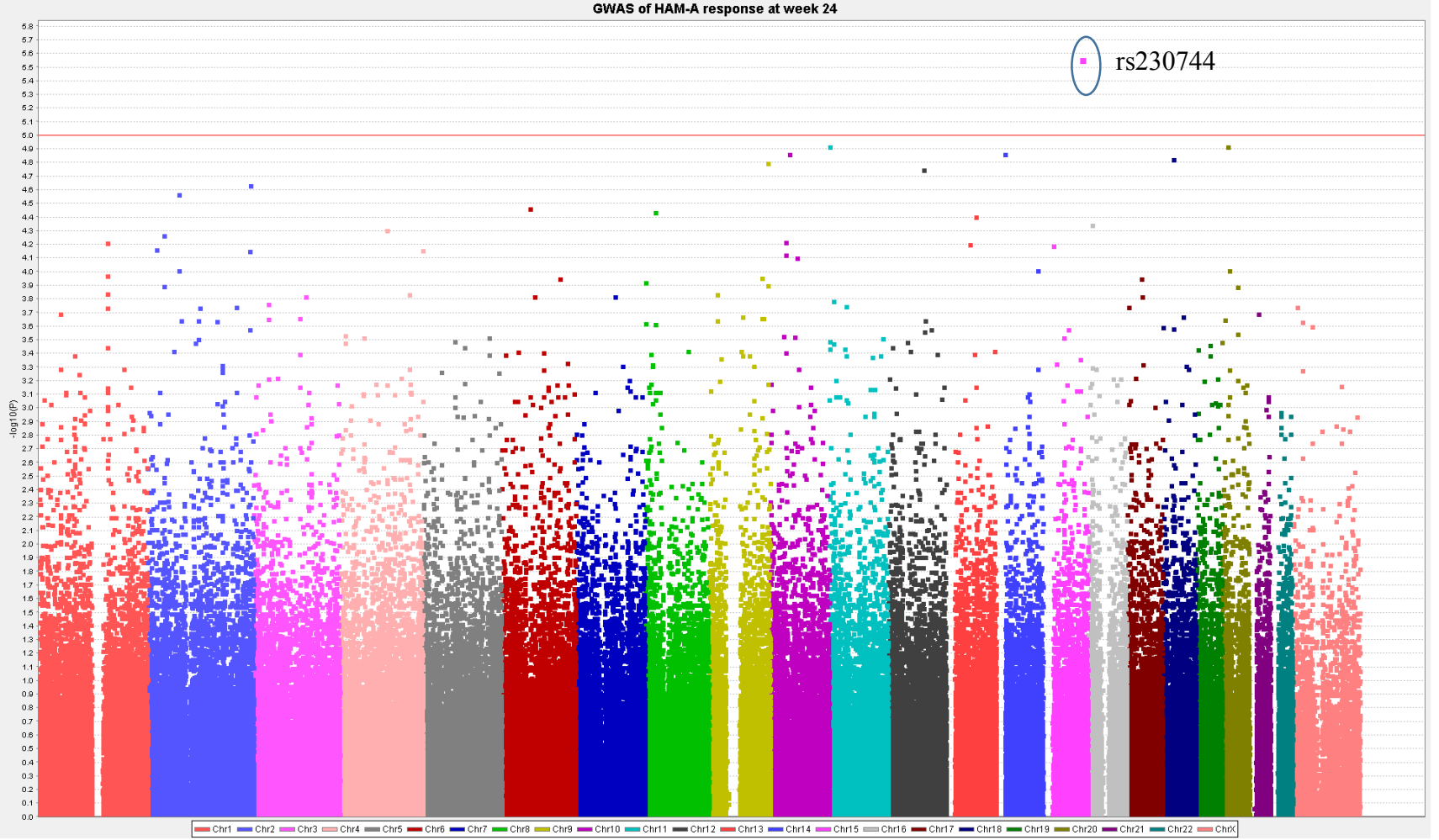


Figure 2. Manhattan plot of GWAS for HAM-A remission at week 24.

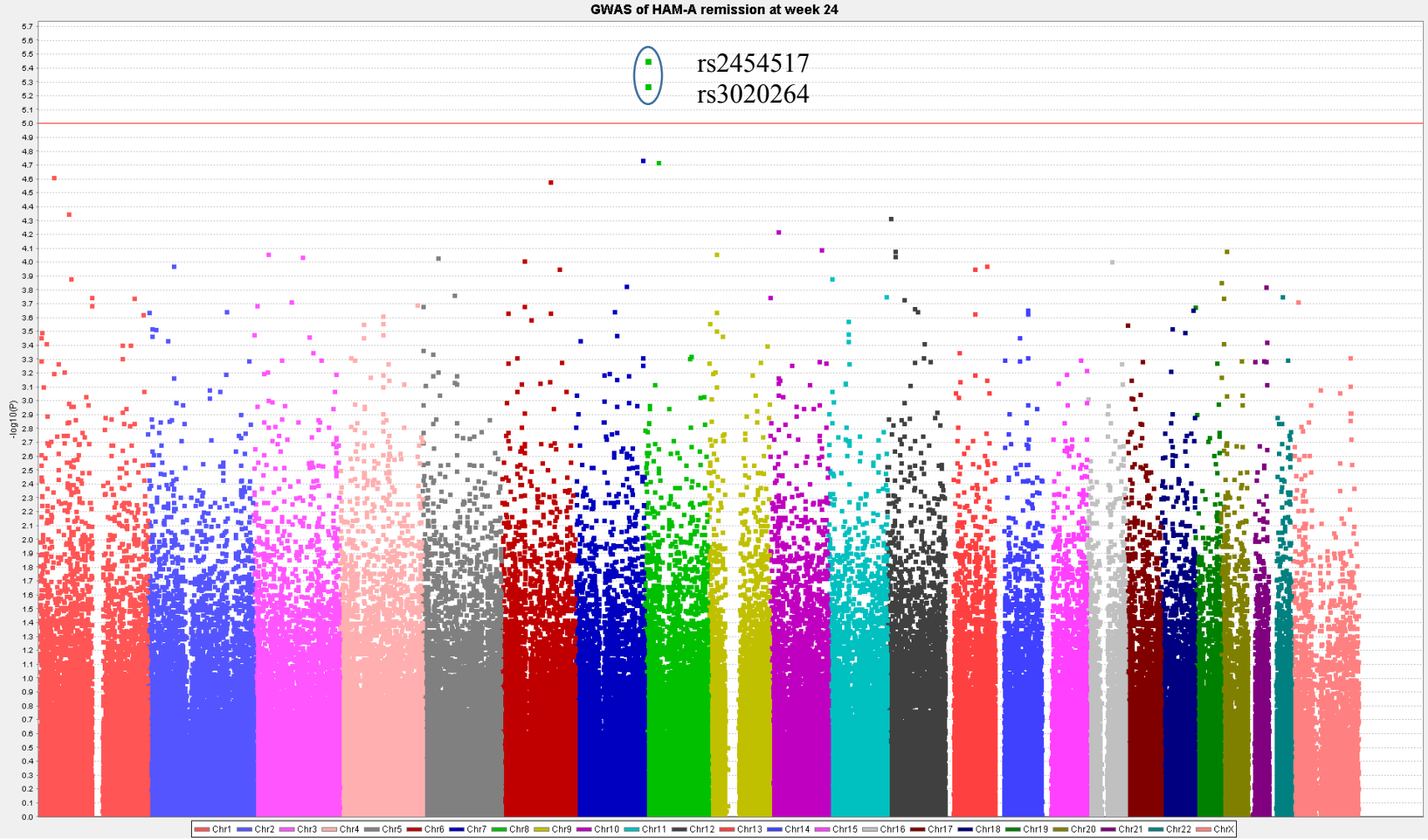


Figure 3. Manhattan plot of GWAS for CGI response at week 24.

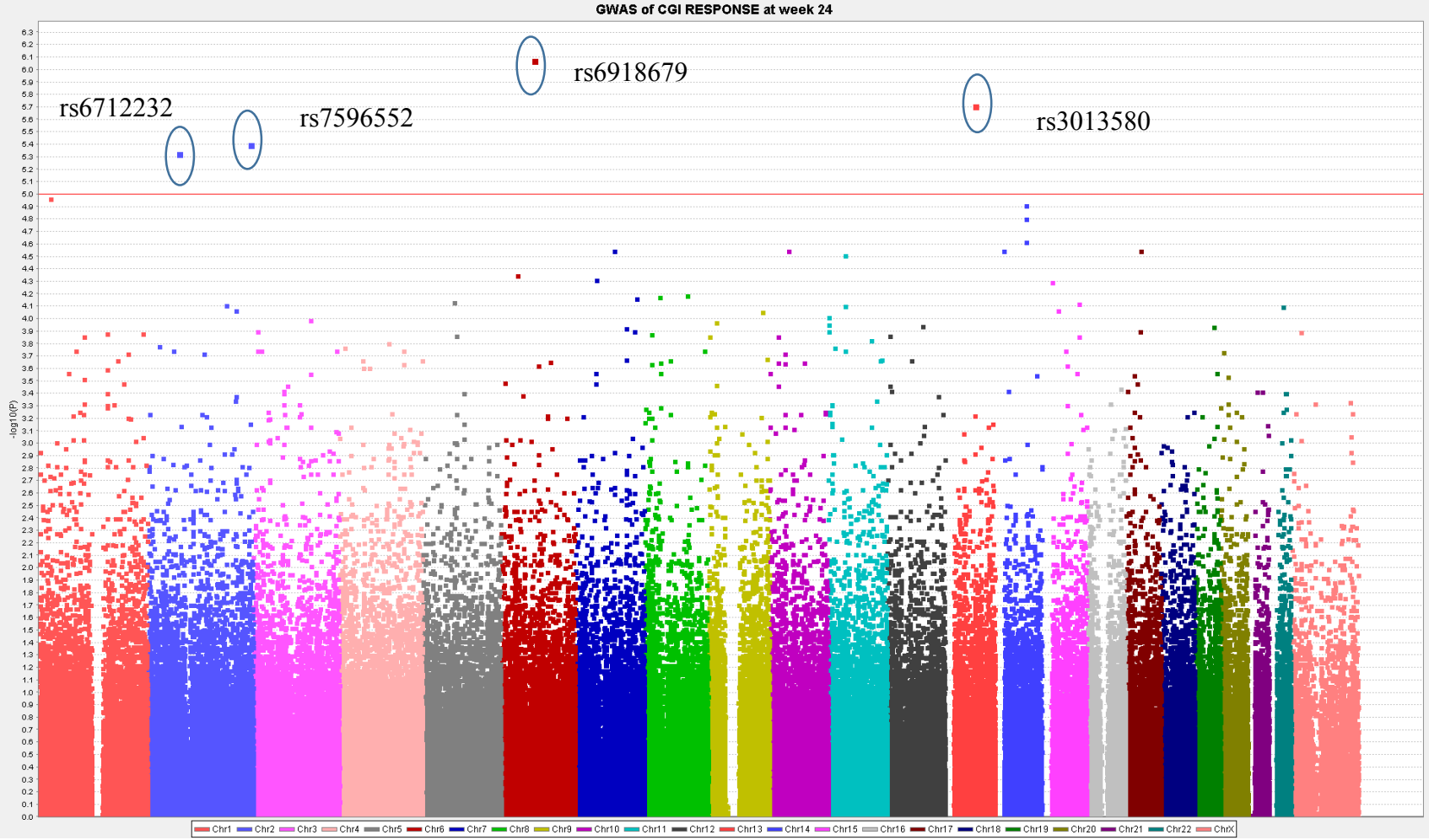


Figure 4. Manhattan plot of GWAS for CGI remission at week 24.

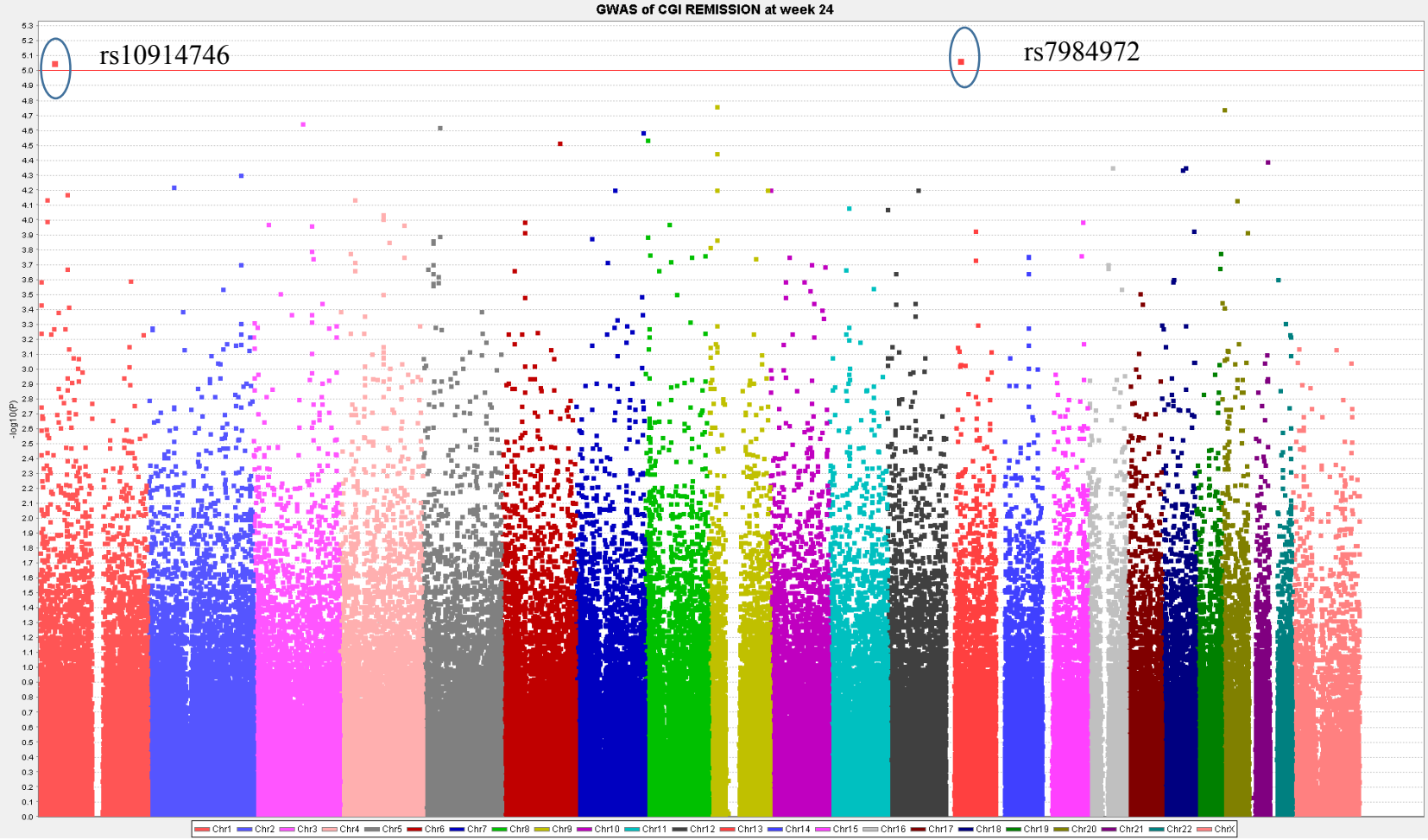


Figure 5. Manhattan plot of GWAS for HAM-A response at week 12.

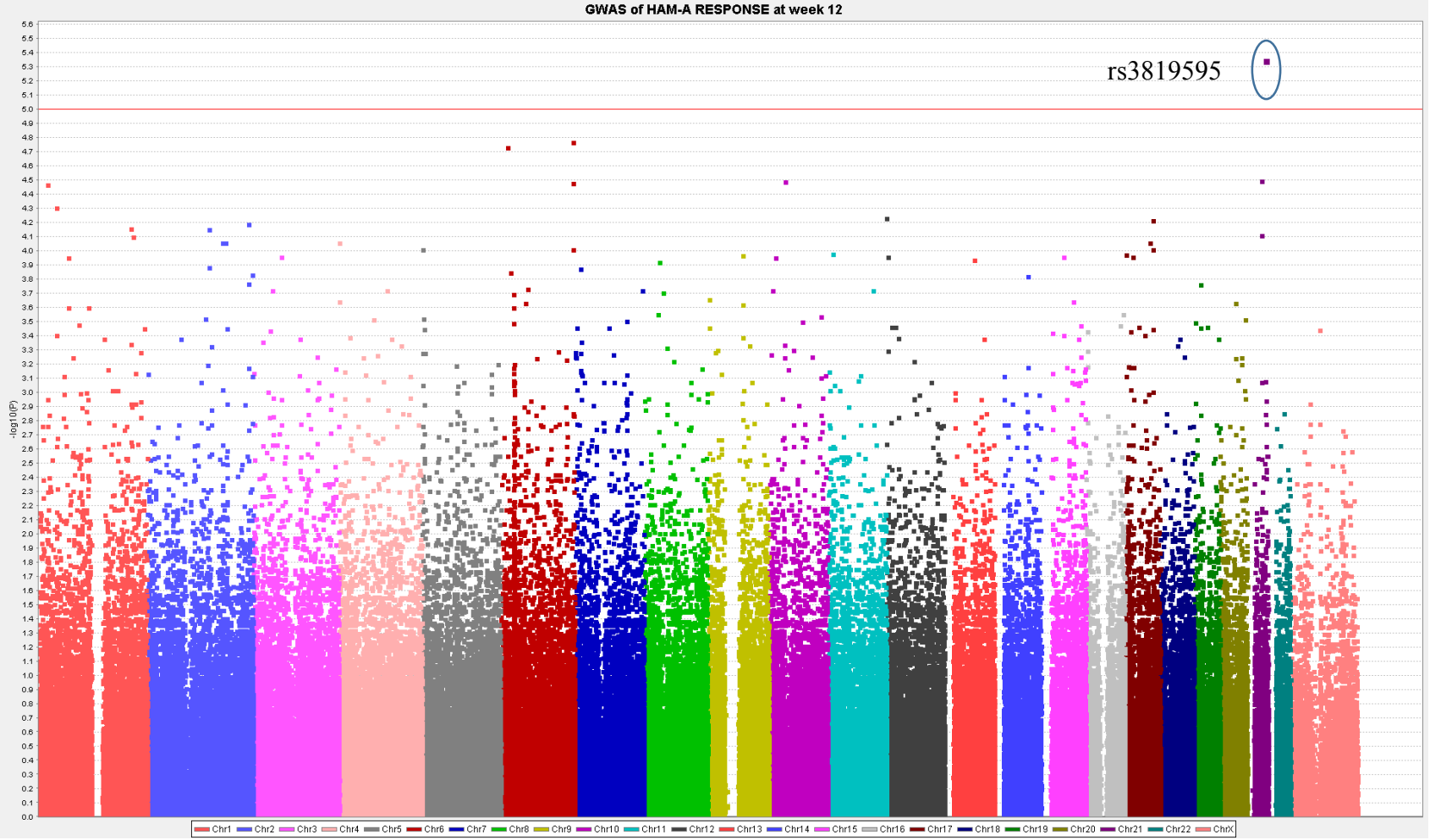


Figure 6. Manhattan plot of GWAS for HAM-A remission at week 12.

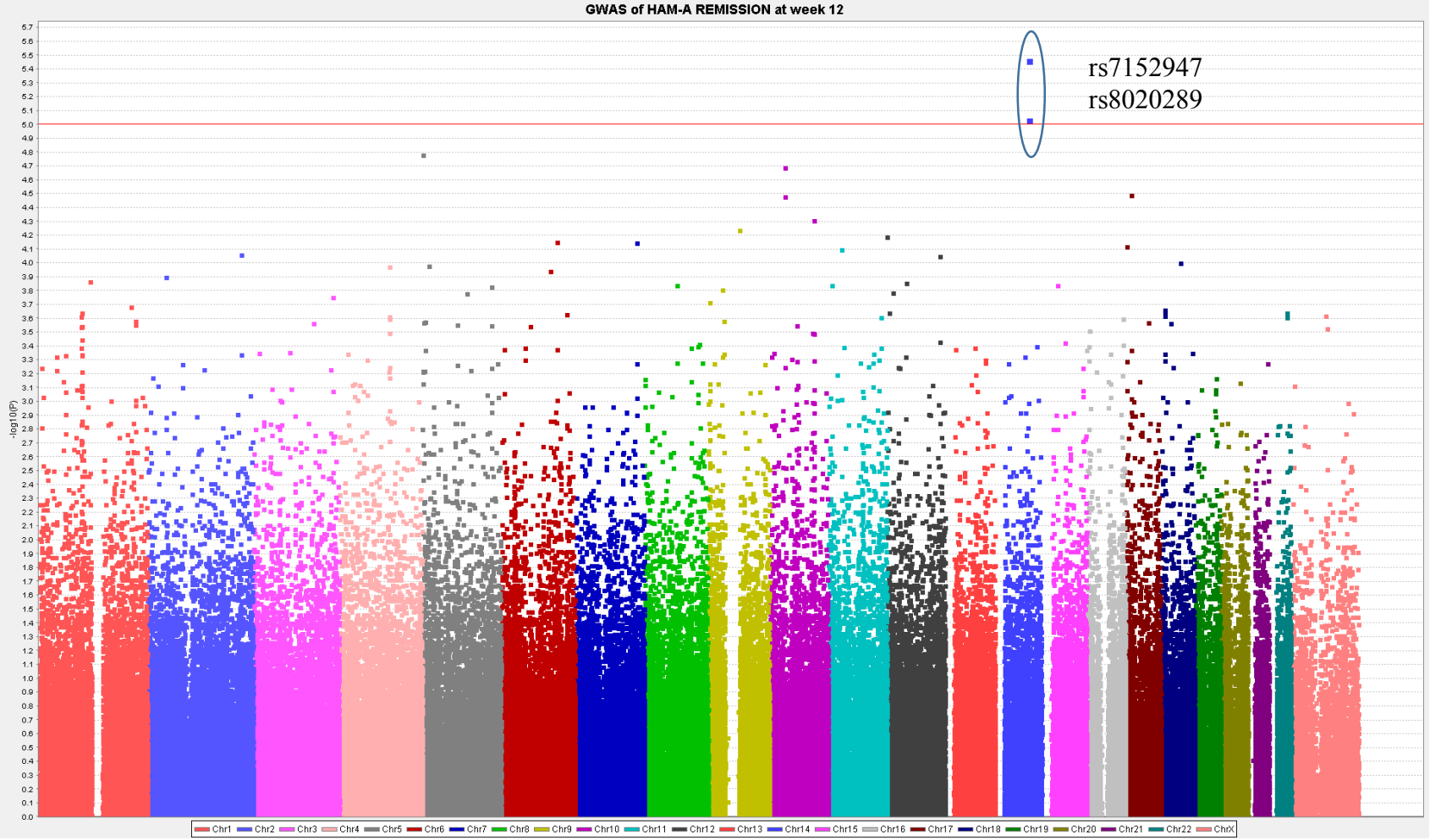


Figure 7. Manhattan plot of GWAS for CGI response at week 12.

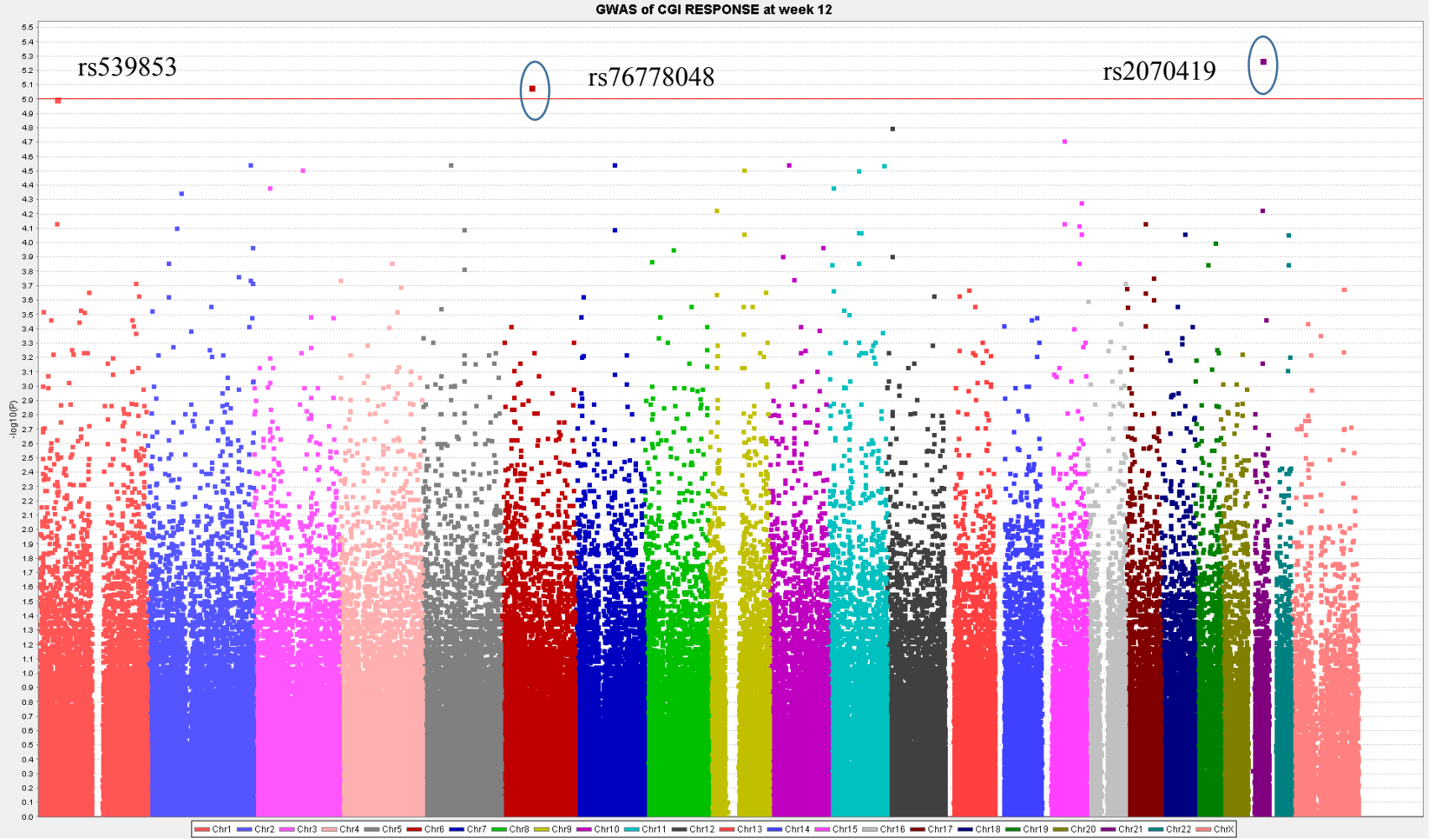


Figure 8. Manhattan plot of GWAS for CGI remission at week 12.

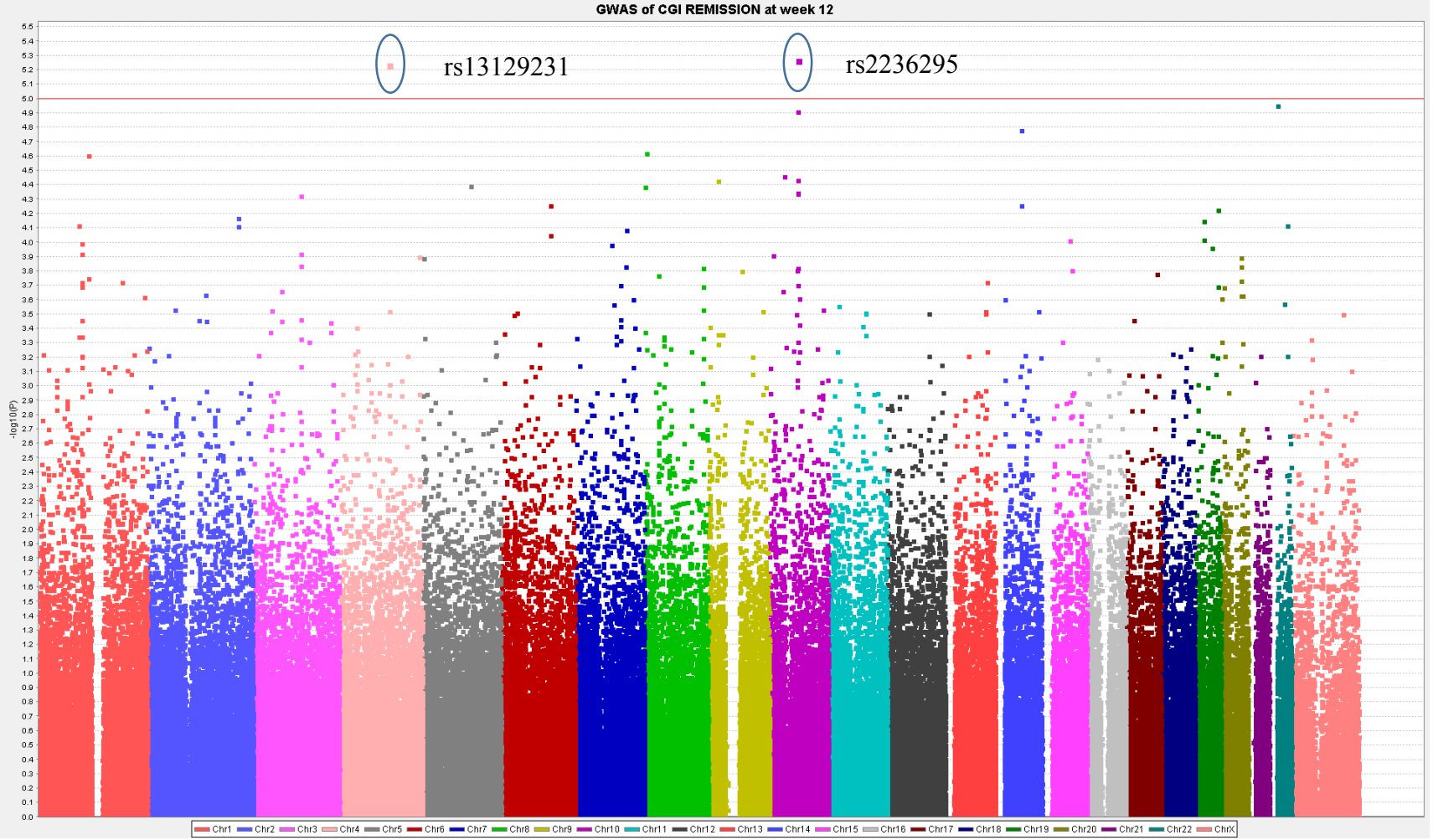


Figure 9. Manhattan plot of GWAS for HAM-A reduction at week 24.

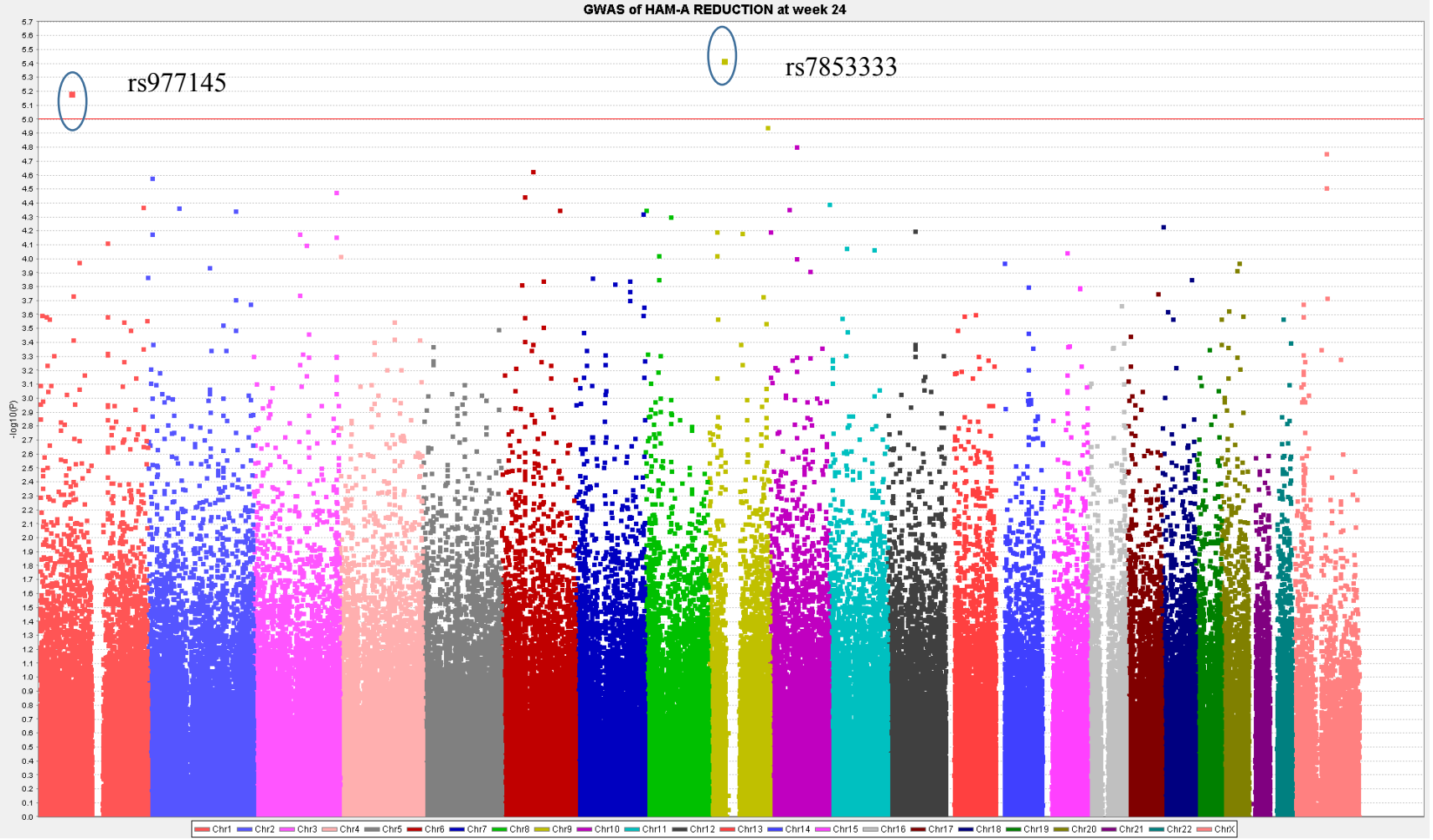
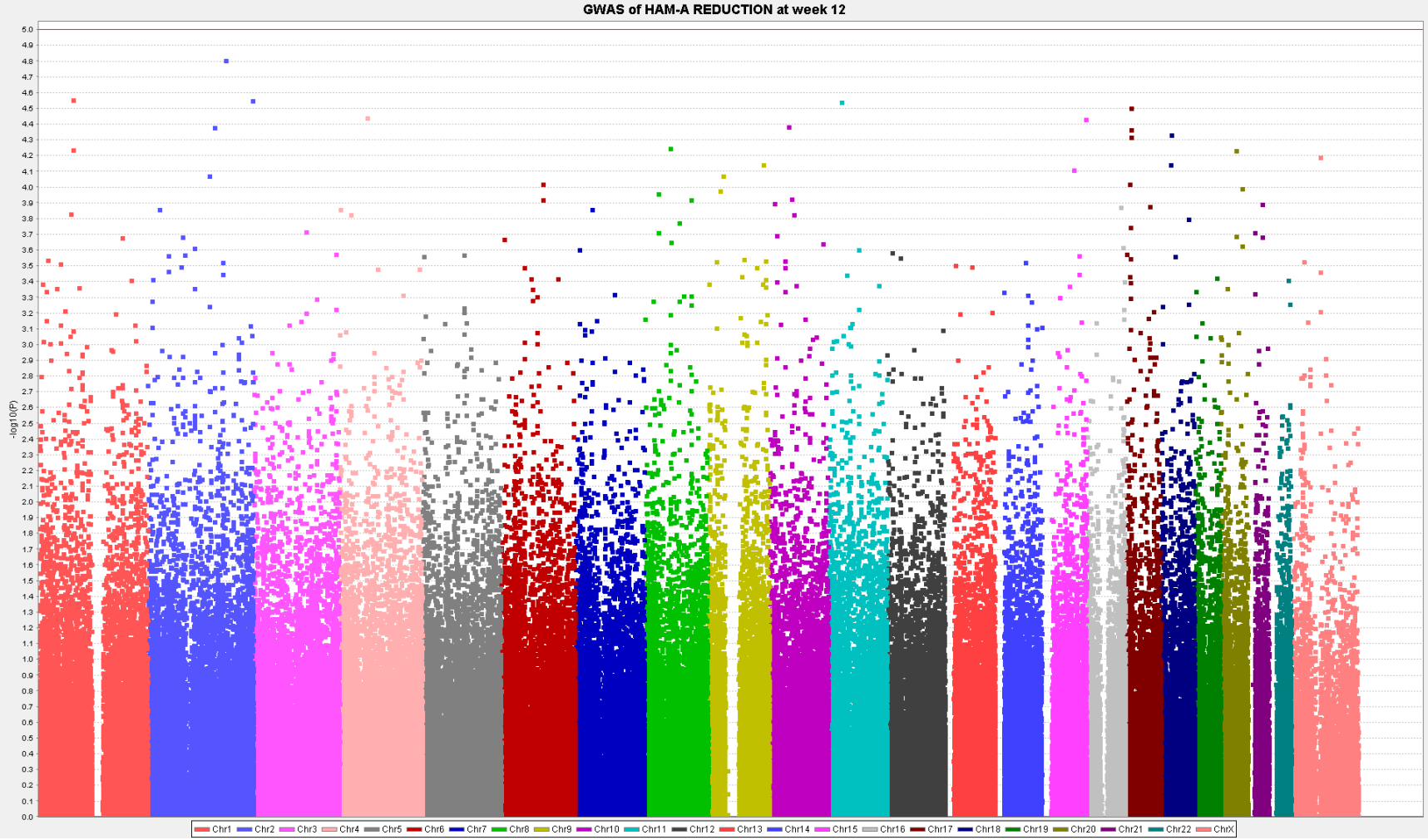


Figure 10. Manhattan plot of GWAS for HAM-A reduction at week 12.



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