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SUPPLEMENTARY INFORMATION

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Meta-analysis of genome-wide association studies for neuroticism in 449,484 individuals identifies novel genetic loci and pathways

Mats Nagel[1,2,11], Philip R. Jansen[1,3,11], Sven Stringer[1], Kyoko Watanabe[1], Christiaan A. de Leeuw[1], Julien Bryois[4], Jeanne E. Savage[1], Anke R. Hammerschlag[1], Nathan G. Skene[5], Ana B. Muñoz-Manchado[5], 23andMe Research Team[6], Tonya White[3], Henning Tiemeier[3,7], Sten Linnarsson[5], Jens Hjerling-Leffler[5,8], Tinca J. C. Polderman[1], Patrick F. Sullivan[4,9,10], Sophie van der Sluis[1,2,12] and Danielle Posthuma[1,2,12]*

[1]Department of Complex Trait Genetics, Center for Neurogenomics and Cognitive Research, Amsterdam Neuroscience, Vrije Universiteit Amsterdam, Amsterdam, the Netherlands. [2]Department of Clinical Genetics, Section of Complex Trait Genetics, Amsterdam Neuroscience, VU University Medical Center, Amsterdam, the Netherlands. [3]Department of Child and Adolescent Psychiatry, Erasmus University Medical Center, Rotterdam, the Netherlands. [4]Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden. [5]Laboratory of Molecular Neurobiology, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden. [6]A list of members and affiliations appears at the end of the paper. [7]Department of Social and Behavioral Science, Harvard T.H. Chan School of Public Health, Boston, MA, USA. [8]UCL Institute of Neurology, Queen Square, London, UK. [9]Department of Genetics, University of North Carolina, Chapel Hill, NC, USA. [10]Department of Psychiatry, University of North Carolina, Chapel Hill, NC, USA. [11]These authors contributed equally: Mats Nagel, Philip R. Jansen. [12]These authors jointly supervised this work: Sophie van der Sluis, Danielle Posthuma. *e-mail: d.posthuma@vu.nl

Supplementary Information for

Meta-Analysis of Genome-wide Association Studies for Neuroticism in 449,484 Individuals Identifies Novel Genetic Loci and Pathways

Mats Nagel[1,2†], Philip R Jansen[1,3†], Sven Stringer[1], Kyoko Watanabe[1], Christiaan A de Leeuw[1], Julien Bryois[4], Jeanne E Savage[1], Anke R Hammerschlag[1], Nathan G. Skene[5], Ana B Muñoz-Manchado[5], the 23andMe Research Team[6], Tonya White[3], Henning Tiemeier[3,8], Sten Linnarsson[5], Jens Hjerling-Leffler[5,6], Tinca JC Polderman[1], Patrick F Sullivan[4,9,10], Sophie van der Sluis[1,2§], Danielle Posthuma[1,2§*]

Affiliations:

1 Department of Complex Trait Genetics, Center for Neurogenomics and Cognitive Research, Amsterdam Neuroscience, Vrije Universiteit Amsterdam, The Netherlands
2 Department of Clinical Genetics, Section Complex Trait Genetics, Amsterdam Neuroscience, VU University Medical Center, Amsterdam, the Netherlands
3 Department of Child and Adolescent Psychiatry, Erasmus University Medical Center, Rotterdam, the Netherlands
4 Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden
5 Laboratory of Molecular Neurobiology, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden
6 UCL Institute of Neurology, Queen Square, London WC1N 3BG, UK
7 23andMe, Inc., Mountain View, CA, USA
8 Department of Social and Behavioral Science, Harvard TH Chan School of Public Health, Boston, MA, USA
9 Department of Genetics, University of North Carolina, Chapel Hill, USA
10 Department of Psychiatry, University of North Carolina, Chapel Hill, USA

† These authors contributed equally to this work
§ These authors jointly supervised this work
*Correspondence should be addressed to: Danielle Posthuma: Department of Complex Trait Genetics, Vrije Universiteit Amsterdam, De Boelelaan 1085, 1081 HV, Amsterdam, The Netherlands. Phone: +31 20 598 2823, Fax: +31 20 5986926, d.posthuma@vu.nl

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1. Phenotype measurement

1.1 UK Biobank – Neuroticism, *depressed affect*, and *worry*

To operationalize neuroticism, we used raw item level data obtained from the UK Biobank study (application number 16406). Neuroticism was measured with 12 dichotomous ("yes"/"no") items of the Eysenck Personality Questionnaire Revised Short Form (EPQ-RS[1]). All 12 items are displayed in the table below. The EPQ-RS neuroticism scale has been shown to have reliability >0.8[1] and correlates strongly (r = 0.85) with the neuroticism score from the NEO Five-Factor Inventory (NEO-FFI)[2], which was predominantly used in the GPC1 sample.

During the first visit of the UK Biobank data collection (2006-2010) the EPQ-RS neuroticism scale was included in the touch-screen assessment held at the UKB assessment centers. Participants with valid responses to <10 items were excluded from our analyses. A weighted neuroticism sum-score was calculated by adding up individual valid item responses, and dividing that sum by the total number of valid responses. Higher sum-scores indicate higher levels of neuroticism.

In total, N=372,903 participants remained after excluding participants with >2 missing responses, who did not have valid genetic data, or were not of European descent. The distribution of item-level missingness is shown in **Supplementary Fig. 2a**. The distribution of the sum-score, after excluding participants, is shown in **Supplementary Fig. 2b**. The mean age in the resulting sample was 56.7 years (SD=8.0), and 54.0% was female. The mean score was 0.35 (SD=0.27), and was higher in females compared to males (Cohen's d=0.30).

Individual items of the EPQ-RS neuroticism questionnaire used to measure neuroticism in the UK Biobank sample (N=372,903), and missingness and endorsement rates				
		Endorsement rate (%)		
Item (UKB field ID)	**Nmissing**	**Total**	**Males**	**Females**
Does your mood often go up and down? (1920)	5,340	44.68	42.27	46.73
Do you ever feel 'just miserable' for no reason? (1930)	3,370	42.57	35.35	48.72
Are you an irritable person? (1940)	11,200	28.25	30.84	26.04
Are your feelings easily hurt? (1950)	6,715	55.20	44.94	63.95
Do you often feel 'fed-up'? (1960)	4,111	40.11	37.49	42.34
Would you call yourself a nervous person? (1970)	5,504	23.47	19.44	26.94
Are you a worrier? (1980)	5,956	56.24	47.45	63.74
Would you call yourself tense or 'highly strung'? (1990)	6,998	17.55	15.21	19.56
Do you worry too long after an embarrassing experience? (2000)	10,452	47.44	39.87	53.95
Do you suffer from 'nerves'? (2010)	8,641	21.18	21.43	20.97
Do you often feel lonely? (2020)	3,111	17.89	14.67	20.63
Are you often troubled by feelings of guilt? (2030)	5,235	28.50	21.77	34.26
		Mean (SD)		
Neuroticism sum-score	-	0.35 (0.27)	0.31 (0.27)	0.39 (0.27)
Depressed affect	14,946	1.45 (1.42)	1.29 (1.38)	1.58 (1.45)
Worry	24,684	1.16 (1.25)	1.01 (1.22)	1.28 (1.27)
Note: In red font: the 4 items belonging to the *depressed affect* cluster. In blue font: the 4 items belonging to the *worry* cluster.				

Scores on 4 EPQ-RS items (red font) were summed to obtain scores for the cluster *depressed affect*. Similarly, scores on 4 other EPQ-RS items (blue font) were summed to obtain scores for the cluster *worry*. For a detailed discussion of the clusters we refer to Nagel et al[2]. Briefly, following item-level GWAS, genetic correlations between the 12 EPQ neuroticism items were computed. Subsequently, hierarchical clustering analysis was performed on the genetic correlations between these 12 items, revealing two clear clusters, that replicated in a second, independent sample. In the item-cluster analyses, only participants with complete scores on all 4 items were included, resulting in N=357,957 and N=348,219 for *depressed affect* and *worry*, respectively. The mean score for the *depressed affect* cluster was 1.45 (SD=1.42), and was higher in females compared to males (Cohen's *d*=0.20). The mean score for the *worry* cluster was 1.16 (SD=1.25), and was higher in females compared to males (Cohen's *d*=0.22). Age, sex, townsend deprivation index, genotyping array and the first 10 European-based genetic principal components were included in the analysis as covariates.

1.2 23andMe - Neuroticism

For the neuroticism meta-analysis, we used neuroticism GWAS summary statistics from a subset of 23andMe participants (N=59,206), described in more detail elsewhere[3].

In the 23andMe sample, neuroticism was measured as a quantitative trait using an adapted version of the Big Five Inventory (BFI[4]). Participants were asked to indicate on a 5-point Likert scale (ranging from 'strongly disagree' to 'strongly agree') whether the 8 statements shown below applied to them. The BFI facet scales (including the neuroticism scale) have been shown to have good reliability and agree well with self-reports on the Revised NEO Personality inventory[5].

1) I am someone who is depressed, often feels sad
2) I am someone who is relaxed, handles stress well (R)
3) I am someone who can be tense
4) I am someone who worries a lot
5) I am someone who is emotionally stable, not easily upset (R)
6) I am someone who can be moody
7) I am someone who remains calm in tense situations (R)
8) I am someone who gets nervous easily

Note: items were evaluated on a 5-point Likert scale ('Disagree strongly' to 'Agree strongly'). R = Reverse-coded item.

The final sample size, consisting of participants with valid BFI scores and valid genetic data, who were genetically unrelated and of >97% European ancestry, consisted of N=59,206 participants, 49% female. For our meta-analysis, we used GWA summary statistics. Item-level information was not available from 23andMe. Age, sex and the first 5 genetic principal components were included as covariates in the GWAS analyses.

1.3 Genetics of Personality Consortium – Neuroticism

We used summary statistics of neuroticism from the first GPC personality meta-analysis (GPC1, http://www.tweelingenregister.org/GPC/)[6], on 10 discovery cohorts (SardiNIA (Italy), NTR/NESDA (the Netherlands), ERF (the Netherlands), SAGE (United States of America), HBCS (Finland), NAG/IRPG (Australia), QIMR(Australia), LBC1936 (United Kingdom), BLSA

(United States of America), and EGPUT (Estonia)), including in total N=17,375 participants of European descent. All samples are described in detail in the original publication[6].

In most GPC1 samples, neuroticism was measured as a quantitative trait using the sums of scores on 12 items (5-point Likert scale; 'Strongly disagree' to 'Strongly agree'; listed below) of the NEO-FFI[7] (otherwise, the related instrument NEO-PI-R was used).

1) I'm not a worrier (R)
2) I often feel less than others
3) When I'm under pressure, sometimes I can't handle it
4) I rarely feel lonely or sad (R)
5) I often feel tense or nervous
6) Sometimes I feel completely worthless
7) I rarely feel anxious (R)
8) I often get mad about the way people treat me
9) When things go wrong, I often get discouraged and feel like giving up
10) I'm rarely sad or depressed (R)
11) I often feel helpless and then want someone to solve my problems
12) Sometimes I'm so embarrassed I don't know where to put it

Note: items were evaluated on a 5-point Likert scale ('Strongly disagree' to 'Strongly agree'). R = Reverse-coded item.

If <4 item scores were missing, data on invalid items were imputed by taking an individual's average score on valid items. Participants were excluded from analyses if they had invalid scores on >3 items[6].

Sample-specific information on the % females, mean age (distinguished by sex), and mean scale scores (distinguished by sex) is given in detail in the original publication[6]. In the original analyses, age and sex were included as covariates in all analyses, and population stratification effects were corrected by applying genomic control in each sample before meta-analysis.

1.4 UK Biobank – Depression

To operationalize depression, we used raw data on two depression items obtained from the UK Biobank study (application number 16406). Depression was operationalized by adding up the scores on two continuous items (both evaluated on a 4-point Likert scale; 'Not at all' to 'Nearly every day'; items are listed below), resulting in a continuous depression score (as used previously[8]). Only participants with scores on both items were included in the analyses, resulting in N=362,696.

The distribution of the depressive symptoms operationalization is shown in **Supplementary Fig. 6**. The mean age in the resulting sample was 56.7 years (SD=8.0), and 54.0% was female. The mean score was 2.55 (SD=1.09), and was higher in females compared to males (Cohen's *d*=0.06). Age, sex, townsend deprivation index, genotyping array and the first 10 European-based genetic principal components were included in the analysis as covariates.

Individual items of the EPQ-RS Neuroticism questionnaire used to measure neuroticism in the UK Biobank sample (N=372,903), and missingness and endorsement rates				
		Mean (SD)		
Item	**$N_{missing}$**	**Total**	**Males**	**Females**
Over the past two weeks, how often have you felt down, depressed or hopeless? (UKB field ID: 2050)	0	1.29 (0.61)	1.25 (0.57)	1.32 (0.63)
Over the past two weeks, how often have you had little interest or pleasure in doing things? (UKB field ID: 2060)	0	1.26 (0.60)	1.27 (0.60)	1.26 (0.60)
Depressive symptoms (SUM)	0	2.56 (1.09)	2.52 (1.05)	2.59 (1.11)

We note that the UKB data base does not contain a true clinical case-control measure of depression. Multiple researchers (e.g., Howard et al.[9], Wray et al.[10]) have created case-control phenotypes by combining answers to multiple questions. We note that this often leads to a (much) lower sample size than our depression operationalization. For instance, Howard et al. studied three different case-control phenotypes for depression: based on ICD-10 (N_{cases}=8,276, $N_{controls}$=217,584, N_{total}=225,860), a "probable depression" phenotype (N_{cases}=30,603, $N_{controls}$=143,916, N_{total}=174,519), and a "broad depression" phenotype (N_{cases}=113,769, $N_{controls}$=208,811, N_{total}=322,580). Using the two items to construct a measure of depressive symptoms, we obtained a depression score for N=362,696 subjects, which exceeds the sample size available for the various case-control operationalizations by a considerable 40,116 to 188,177 subjects. The genetic correlation between our meta-analysis results and results of a

meta-analysis in which the "probable depression" phenotype was used, is very high (r_g=0.930, SE=0.010, see Section 2.7 below).

1.5 23andMe – Depression

For the depression meta-analysis, we used Depression GWAS summary statistics from a subset of 23andMe participants (N=307,354), described in more detail elsewhere[11]. This concerns a case-control sample. Four self-report survey items were used to determine case-control status (see below; response categories: "Yes", "No", "I don't know"). Cases were defined as replying affirmatively to at least one of these questions, and not replying negatively to previous ones. Controls replied negatively to at least one of the questions, and did not report being diagnosed with depression on previous ones.

1) Have you ever been diagnosed by a doctor with any of the following psychiatric conditions? (Depression) – from: Your Medical History survey
2) Have you ever been diagnosed with clinical depression? – from: Research Snippet
3) Have you ever been diagnosed with or treated for any of the following conditions? (Depression) – from: Health Intake
4) In the last 2 years, have you been newly diagnosed with or started treatment for any of the following conditions? (Depression) – from: Health Follow-up survey

The final sample size, consisting of participants with valid case-control status for depression and valid genetic data, who were genetically unrelated and of >97% European ancestry, consisted of N=307,354 participants, 48% female (cases: 62 % female; controls: 44% female). In our meta-

analysis, we used GWA summary statistics. Item-level information was not made available. Age, sex and the first 5 genetic principal components were included as covariates in the GWAS analyses.

1.6 Psychiatric Genetics Consortium – Depression

For the depression meta-analysis, we used summary statistics from the latest published PGC meta-analysis on depression (http://www.med.unc.edu/pgc/results-and-downloads)[12], which included data from 8 cohorts (Bonn/Mannheim, GAIN, GenRED, GSK, MDD2000, MPIP, RADIANT, STAR*D), covering N=18,759 participants of European descent. This concerns a case-control sample. Cases had a DSM-IV lifetime (sometimes (early onset) recurrent) major depression disorder (MDD) diagnosis, either established through structured diagnostic interviews or clinician-administered DSM-IV checklists. Most cases were ascertained from clinical sources, while controls were randomly selected from population resources and screened for lifetime history of MDD[12]. Details on the clinical definition of a case are given in the original publication[12]. Age, sex and five principal components reflecting ancestry were used as covariates in the original meta-analysis.

2. Supplementary Results

2.1 GWAS results per cohort and genetic correlations between cohorts included in the neuroticism meta-analysis

For each of the three cohorts (UKB, 23andMe, and GPC1) an individual GWAS was run and inspected for inflation possibly due to insufficiently corrected population stratification, by inspecting the lambda inflation factor as well as the LD Score regression (LDSC) intercept. A lambda >1 suggest an inflation of the genetic effects which can be due to both spurious and genuine effects, and is likely to increase with sample size[13]. An LDSC intercept >1 suggests that there is spurious association, and an intercept <1.10 is generally considered to suggest that the signal is mostly due to genuine association effects. For the UKB cohort, the genomic inflation factor λ_{GC} computed using LDSC, was 1.60 (**Supplementary Table 1**), suggesting inflation in median test statistic compared to the expected distribution. The LDSC intercept of 1.03 for UKB, however, suggested that the observed inflation in genetic signal is mostly due to polygenicity and unlikely to be driven by population stratification[14]. Specifically, the LDSC ratio of 0.021 indicated that as much as 97.9% of the observed inflation could be ascribed to true polygenicity and large sample size. We also conducted an additional GWAS for neuroticism in the UKB cohort, with 30 instead of 10 genetic principal components as covariates, to control more rigorously for subtle population stratification than in the first analysis. The genomic inflation factor λ_{GC} remained unchanged (1.60), suggesting that the inflation of lambda observed in the original GWAS for neuroticism was not due to insufficiently controlled ancestry.

For the (much smaller) 23andMe and GPC1 samples, λ_{GC} was close to 1 (1.06 and 1.02, respectively) indicating only minor inflation. QQ-plots of the individual cohorts (**Supplementary Fig. 7**) also did not suggest issues concerning population stratification.

Genetic correlations between the GWAS results from the three cohorts included in the meta-analysis (**Supplementary Table 1)** were calculated with LDSC. An estimated r_g that is out of bounds (< -1.25 or > 1.25, LDSC does not limit correlations to -1 and 1) usually means that one of the h^2 estimates was very close to zero. We do also note that to the best of our knowledge the statistical properties of LDSC have not been tested in the context of large discrepancies in sample sizes (one sample > 300,000 and the other < 100,000), and that there is a need for novel tools that can calculate genetic correlations based on summary statistics using current sample sizes and currently available SNP platforms.

Estimates of r_g were high and ranged from 0.829 – 1.074. As expected, the SNP-based h^2 was lowest in the smallest cohort (GPC1: 0.048, SE=0.027), and very similar in the UKB (0.106, SE=0.004) and 23andMe cohorts (0.116, SE=0.016), as well as in the meta-analyses (h^2=0.100, SE=0.003).

The λ_{GC} of the meta-analysis was 1.65. Traditionally, an observed λ_{GC} that deviates from 1 has been interpreted as inflation in test statistics that may be due to systematic bias (such as insufficiently controlled population stratification). However, with the availability of larger sample sizes (e.g. N > 100,000) the value of λ_{GC} is expected to increase in case of high polygenicity. See Yang et al.[14] who through theory, simulation and real data analysis show that in the absence of population structure and other technical artefacts, but in the presence of true polygenic inheritance, substantial genomic inflation is expected, with the size of the inflation

depending on sample size, heritability, linkage disequilibrium structure and the number of causal variants.

Indeed, high values of λ_{GC} are frequently observed in recent large-sample GWAS studies for diverse and polygenic traits, including height[15] (λ_{GC} = 2.7 in European ancestry studies for common markers, N = 381,625), BMI[16] (λ_{GC} = 1.53, N = 234,069), bone mineral density[17] (λ_{GC} = 1.37, N = 142,487), schizophrenia[18] (λ_{GC} = 1.47, N=82,315), and educational attainment[19] (λ_{GC} = 1.28, N = 293,723) and both the UKB λ_{GC} of 1.60 and the λ_{GC} of 1.65 of the full meta-analysis thus seems to fit with what is observed in other traits for which large sample sizes are available.

However, to allow comparisons across studies of different sample sizes and interpretation of the extent of inflation we also calculated λ_{1000}. λ_{1000} can be used for case-control studies (see Freedman et al., 2017[20]) and is the expected λ_{GC} for a sample size of 1000 cases and 1000 controls given the λ_{GC} and N of the original study. It can be calculated as follows:

$$\lambda_{1000} = 1 + (\lambda_{obs} - 1) * \frac{(\frac{1}{N_{cases}} + \frac{1}{N_{controls}})}{\frac{1}{N_{cases,1000}} + \frac{1}{N_{controls,1000}}}$$

We adjusted this formula to calculate the expected λ_{1000} in a non- case-control setting:

$$\lambda_{1000} = 1 + (\lambda_{obs} - 1) * \frac{(\frac{1}{N_{obs}})}{\frac{1}{1000}}$$

where λ_{obs} and N_{obs} are the observed λ_{GC} and the observed sample size in the original analysis.

When we use this formula to calculate the expected λ for N=1000, N=10,000 and N=100,000, given our total observed sample size of N_{obs}=499,484 and λ_{obs}=1.652, we obtained λ_{1000}=1.001, λ_{10000}=1.015, and λ_{100000}=1.145, respectively, showing that 1) the expected λ_{GC} would be much closer to 1 if the sample size was smaller, and 2) the expected λ_{GC} increases considerably with increasing N, as is expected for a highly polygenic trait.

Apart from the above, we also calculated λ_{GC} outside of LDSC, using an R script and basing our calculation on all SNPs (whereas LDSC only uses HapMap SNPs). We implemented the formula: $\lambda_{GC} = median(\chi^2)/qchisq(0.5,1)$. This resulted in a substantially lower value of 1.24. This lower value is likely due to the addition of a relatively large chunk of non-HapMap SNPs and mostly rarer variants that have much smaller effect sizes, and indicates lower inflation when considering all SNPs. However, we believe the LDSC λ_{GC} should be used when comparing our results to other studies that also used the LDSC λ_{GC}.

2.2 Prediction of neuroticism in three independent hold-out samples

To estimate the variation in neuroticism that could be explained by our GWA meta-analysis in independent samples, we re-ran our GWA meta-analysis for neuroticism three times, each time excluding a UKB hold-out sample of N=3,000 randomly drawn individuals. We then calculated polygenic scores (PGS) using two methods: PRSice (*P*-value thresholding and clumping) and LDpred[21]. In the first hold-out sample, the PGS explained at most 3.8% (P=2.12×10^{-28}) of the variance in neuroticism. In the second hold-out sample, the PGS explained at most 3.0% (P=1.88×10^{-22}) of the variance in neuroticism. In the third hold-out sample, the PGS explained at

most 4.2% (P=1.39×10^{-30}) of the variance in neuroticism. In all three samples, the PGS reached a maximum prediction accuracy for an infinitesimal prior, indicating a highly polygenic signal. We refer to **Supplementary Table 14** for the full results for all three hold-out samples and for all thresholds.

2.3 GWA meta-analysis in METAL

The direction of the lead SNPs across the 3 cohorts was largely coherent. Of the 170 lead SNPs, 43 (38) had a positive (negative) direction of effect in all three samples. Another 38 (40) had positive (negative) effects in both the UKB and the 23andMe sample, but were not observed in the smaller GPC1 sample. Furthermore, 10 lead SNPs were observed in all samples, but with varying direction of effects (for 6 out of these 10, the direction in the GPC1 sample differed from the other two samples). One of the 170 lead SNPs was only observed in the UKB sample. Overall, we conclude that most lead SNPs had concordant direction of effects across the three cohorts, strengthening our confidence in the results (see **Supplementary Tables 2 and 3** for all results)

2.4 Genomic risk loci and functional annotations

The meta-analysis resulted in 170 independent lead SNPs (r^2 < 0.1) in 138 independent genomic risk loci (loci < 250 KB apart were merged into 1) (**Supplementary Table 3**). The borders of the genomic risk loci were defined by taking all independent GWS SNPs at r^2 <0.6 and then determining all SNPs that were in LD with one of the independent GWS SNPs, as detailed in **Online Methods** (we provide an overview of lead SNPs reported earlier for neuroticism in **Supplementary Table 4**). Annotated SNPs are all SNPs that are located in a risk locus and that

are in LD with one of the independent GWS SNPs. After inspection of the regional plots (LocusZoom) of all genomic loci (n=138) initially identified in SNP-based analysis, we decided that 2 loci had low confidence, as these appeared to be driven by a single, or only a few SNPs. To further examine whether these loci are plausible, we conducted a number of checks. First, we compared allele frequencies of the lead SNPs in these loci in UKB data to the allele frequency reported by 1000 Genomes and TOPMED, as a large difference could indicate genotyping errors (**Supplementary Table 5**). Secondly, the SNP association *P*-values for the lead SNPs were compared across the three individual cohorts to see whether the signal was exclusively driven by a single cohort. Finally, differential LD across datasets might be another indicator of genotyping errors. We gathered all SNPs in LD (r^2>0.6) with the lead SNPs in the 1000 Genomes data, and compared the LD in the 1000 Genomes data with LD (for the same combinations of SNPs) in the UKB data (**Supplementary Table 6**). For the 2 loci, there was only one lead SNP between 1 to 5 other GWS SNPs. The two lead SNP *P*-values were 8.05×10^{-9} and 2.87×10^{-8} and were mostly driven by the UKB sample. However, this was also the largest sample (N_{UKB}= 372,903; $N_{23andMe}$=59,206; N_{GPC1}=17,375). For both lead SNPs, the *P*-value from the meta-analyses was slightly lower than the *P*-value for that SNP in UKB, indicating that there was also signal in the 23andme and GPC1 samples. MAF of the lead SNPs were comparable to the MAF in 1000G and TOPMED (**Supplementary Table 5**). The lead SNPs did not have many LD proxies in our meta-analyses (where UKB was used as a reference to calculate LD), yet these same SNPs also do not have a lot of LD proxies in the independent reference sample of 1000G. There were thus no clear indications of genotyping errors of these SNPs, yet we felt that the current evidence for these 2 loci is based on very few SNPs and therefore we have less confidence in these loci, and

count the total number of genomic loci for neuroticism as 138-2=136. We do provide information on these loci (risk loci 105 and 114) in **Supplementary Tables 5-6**.

All chromosomes except chromosome 21 (chromosome X was not included in the analysis) included one or more risk loci. The strongest signal was on chromosome 17 (risk locus 126), overlapping an inversion that was previously associated with neuroticism. Despite the presence of this inversion and the resulting long-range LD in this locus, there were 25 independent (r^2<0.6) significant SNPs and 3 lead SNPs (r^2<0.1; rs77804065; rs199447; rs17698176). rs77804065 is in the inversion, but rs199447 and rs17698176 are more distal and are both in intronic regions of *NSF*. The closest exonic SNP rs199533 has an r^2 of 0.991 with lead SNP rs19944 and is also in *NSF*, and has a CADD score of 13.16 suggesting it is likely deleterious. This SNP is a synonymous coding SNP yet acts as an eQTL for multiple genes across several tissues.

The second most associated locus (locus 130, **Supplementary Table 3**) on chromosome 18 includes 2 lead SNPs. The top lead SNP is rs11082011 (2.54×10^{-23}), which is in an intronic region of the *CELF4* gene. The second lead SNP in this locus is rs17570807 and is intergenic, 5458 bp from *MIR4318* (**Supplementary Table 12**). There are no exonic variants in this locus, but there are several intronic SNPs in the *CELF4* gene with high CADD scores. *CELF4* is the only mapped protein-coding gene in this locus. Full details of all neuroticism risk loci and functional implications are listed in **Supplementary Table 12**.

Partitioned heritability showed enrichment of several functional genomic categories (**Supplementary Table 13**), with the strongest enrichment in conserved regions (Enrichment of 13.791, SE= 1.420, h^2=.359, P=5.14×10^{-16}). FUMA[22] annotated all 17,794 SNPs that were in LD (r^2>=0.6) with one of the independent significant SNPs and allowed further inspection of

specific coding variants. The most likely genetic variants to have a substantial outcome on a phenotype are those that have a direct consequence on a protein, specifically variants located in coding exons that have a nonsynonymous change resulting in a change to the protein's amino acid sequence (ExNS SNPs). Of the annotated 17,794 SNPs in GWS loci, we identified 70 ExNS located in 47 unique genes (**Table 1**, **Supplementary Table 12**). Eleven genes included more than 1 ExNS: *SPPL2C* (8 ExNS), *MAPT* (6 ExNS), *FAM120AOS* (3 ExNS), *ANKK1* (3 ExNS), *GNL3*, *ITIH1*, *RPS6KL1, ZNF646, CHR1*, *KANSL1* and *ASXL3* (all 2 ExNS). All ExNS variants were in high LD (r^2>= 0.6) with one of the GWS lead SNPs, and 46 were GWS themselves. 25 ExNS had CADD scores above 12.37[23] (**Table 1**), and 19 ExNS were likely to affect binding sites.

2.5 Mapped genes by FUMA based on GWS SNPs in risk loci and gene based analysis

We used the three gene-mapping strategies implemented in FUMA to select genes of interest based on GWS SNPs in the 136 risk loci (**Supplementary Table 15**). For positional mapping, we mapped SNPs in the risk loci and in LD with the independent GWS SNPs to genes using a window of 10 Kb, resulting in 283 mapped genes. eQTL mapping resulted in 369 genes, of which 159 were outside of risk loci, and chromatin interaction mapping resulted in 119 genes, of which 6 were outside of the risk loci. MAGMA gene-based test resulted in 336 significantly associated genes, and 203 were also mapped by at least one the FUMA gene mapping strategies. Of the resulting set of 599 unique genes, 120 had a pLI >0.90, indicating that these were extremely intolerant to loss of function mutations (**Supplementary Table 15**). Of the 50 genes that were implicated by all four strategies, *DRD2* had the lowest gene-based P-value (3.43×10^{-24})

2.6 Gene-set analyses

We conducted gene-set analysis on a total of 7,323 gene-sets: 7,246 sets derived from the MsigDB, 53 tissue specific gene-sets and 24 cell specific gene-sets. The threshold for significance of gene-sets was thus set at $0.05/7{,}323=6.83\times10^{-6}$ and competitive testing was used throughout (**Online Methods**).

Gene-set analysis using gene-sets from MsigDB version 6.0 in MAGMA resulted in 7 associated gene-sets (**Supplementary Table 17-18**). We then used conditional gene-set analyses to determine which MsigDB gene-sets represent independent associations (**Online Methods**), which reduced this to three gene-sets that showed independent interaction and were driving the association with the other gene-sets (**Supplementary Fig. 16, Supplementary Table 19**). Of the 1,402 genes in the *neurogenesis* gene-set, 51 were implicated by positional, eQTL, or chromatin interaction mapping and 49 genes had a GWS gene-based *P*-value. Of the 12 genes in the *behavioral response to cocaine* set, 3 genes were implicated by positional, eQTL, or chromatin interaction mapping and also had a GWS gene-based *P*-value. Of the 219 genes in the *axon part* set, 11 genes were implicated by positional, eQTL, or chromatin interaction mapping and 13 genes had a GWS gene-based *P*-value (**Supplementary Table 16**). When conditioning these gene-set association of neuroticism on the subclusters *depressed affect* and *worry,* we observed that the association with 'axon-part' only remained after conditioning for *depressed affect* ($P=2.42\times10^{-6}$), but not on *worry* ($P=0.0013$). This suggests that items from the *worry* subcluster largely explain the involvement of this gene-set in neuroticism, whereas this gene-set is not likely to play a role in items related to depressed affect.

We additionally conducted gene-set analysis on 53 sets of genes that were tissue-specific, based on GTEx gene-expression values (**Online Methods**). We found Bonferroni corrected significant

association for 6 tissue specific gene-sets and all of these were brain tissues, with the strongest association in brain frontal cortex (**Supplementary Tables 17-18)**.

Cell-type specific gene-set analysis, using brain cell type expression data drawn from scRNAseq data from mouse brain[24], showed significant association with 5 cell types: medium spiny neurons, serotonergic neurons, interneurons and dopaminergic neuroblasts, and embryonic midbrain nucleus neurons (**Supplementary Table 21**).

We note that the fact that cell type expression data was derived not from humans but from mice, inevitably raises the question whether the results are applicable to the human species. Further research on the comparability of this method is required, yet studies thus far indicate that the results of this analysis are likely to generalize to human cells. First, only genes with a one-to-one mapping between mice and human were included in the analyses. Secondly, previous studies indicate that gene expression clusters not by species but by tissue or cell type[24]. Finally, for schizophrenia results have been shown to replicated in human single-cell data[24]. Importantly, the use of mouse cell-type expression data rather than human data has specific advantages. For instance, post-mortem gene expression data in human cells is often of lower quality than mouse expression data, e.g., because time between death and measurement is much shorter in mice. In addition, only single nuclei sequencing is possible in humans, whereas isolation of whole cells (excluding distal neurites) is possible in mice. For an extensive discussion, we refer to Skene et al.[24].

While the translation from human personality and mood disorders to animal models is challenging, the relevance of animal, and specifically mouse, models for the genetic and neuroscientific study of human psychiatric traits like neuroticism, and to neuroticism-related traits, such as anxiety and depression, is generally acknowledged[25–29].

2.7 GWAS meta-analysis results of secondary phenotypes, not previously published

2.7.1 Depression

Meta-analysis of the depression GWAS in UKB, 23andMe and PGC was carried out in METAL[30]. As the UKB GWAS concerned a continuous operationalization of the depression phenotype, while 23andMe and PGC used case-control phenotypes, the odds ratio from the 23andMe and PGC summary statistics were converted to log odds, reflecting the direction of the effect. The meta-analysis was then performed on the *P*-value of each SNP using a sample size-weighted fixed-effects analysis. The genetic signal correlated moderately between the three samples (r_g range: 0.61 – .80; **Supplementary Table 22**).

The quantile-quantile (Q-Q) plot of the genome-wide meta-analysis on 688,809 subjects showed high inflation (λ=1.44) and mean χ^2 statistic (1.55) **(Supplementary Fig. 8**; **Supplementary Table 22**, also for the 3 samples separately**)**. The LD Score regression (LDSC)[31] intercept (1.007; SE=0.010) was consistent with inflation due to true polygenicity and large sample size[14]. The LDSC SNP-based heritability (h^2_{SNP}) of depression was 0.040 (SE=0.002).

Our depression phenotype is a continuous one, based on two Likert scale items. A true case-control phenotype is not present in the UKB data base, but other researchers have proposed case-control operationalizations for depression by combining answers to multiple items (e.g., Howard et al.[9], Wray et al.[10]). To test whether our GWAS meta-analysis results are very different from results that we would have obtained with a case-control phenotype, we ran an additional meta-analysis but now with the 'probable depression' case-control phenotype as proposed by Howard et al.[9]. The genetic correlation between our meta-analysis results, and the meta-analysis results

based on that case-control phenotype was very high (r_g=0.930, SE=0.010). This motivated us to continue with our continuous measure of depression, as this score was available in a considerably larger sample (i.e., 362,696 versus 174,519, respectively).

The depression GWAS meta-analysis identified 1,960 genome-wide significant (GWS) SNPs ($P<5\times10^{-8}$) (**Supplementary Table 24**). FUMA extracted 49 independent lead SNPs, which were mapped to 45 independent genomic loci harboring 173 genes (**Supplementary Table 28**). MAGMA[32] identified 119 GWS genes (**Supplementary Table 16**), of which 61 overlapped with genes implicated by SNP-based analyses, resulting in a total of 234 genes associated with depression.

2.7.2 Neuroticism subcluster: Depressed affect

The quantile-quantile (Q-Q) plot of the genome-wide analysis on 357,957 subjects and 10,847,151 SNPs showed high inflation (λ=1.51) and mean χ^2 statistic (1.67) **(Supplementary Table 23)**. The LDSC[31,33] intercept (1.038; SE=0.010) was consistent with inflation due to true polygenicity and large sample size. The LDSC SNP-based heritability (h^2_{SNP}) of *depressed affect* was 0.089 (SE=0.003).

The *depressed affect* GWAS analysis identified 5,240 GWS SNPs ($P<5\times10^{-8}$) (**Supplementary Table 25**). FUMA extracted 75 independent lead SNPs (see **Supplementary information** for definition of lead SNPs), which were mapped to 66 independent genomic loci harboring 246 genes (**Supplementary Table 29**). MAGMA[32] identified an additional 94 unique genes (**Supplementary Tables 16)**, resulting in a total of 340 genes associated to *depressed affect*.

2.7.3 Neuroticism subcluster: Worry

The quantile-quantile (Q-Q) plot of the genome-wide analysis on 348,219 subjects and 10,847,151 SNPs showed high inflation (λ=1.49) and mean χ^2 statistic (1.65) **(Supplementary Table 23)**. The LDSC[31,33] intercept (1.021; SE=0.010) was consistent with inflation due to true polygenicity and large sample size. The LDSC SNP-based heritability (h^2_{SNP}) of *worry* was 0.090 (SE=0.003).

The *worry* GWAS analysis identified 6,382 GWS SNPs ($P<5\times10^{-8}$) (**Supplementary Table 26**). FUMA extracted 73 independent lead SNPs (see **Supplementary information** for definition of lead SNPs), which were mapped to 68 independent genomic loci harboring 332 genes (**Supplementary Table 30**). MAGMA[32] identified an additional 115 unique genes (**Supplementary Table 16)**, resulting in a total of 447 genes associated to *worry*.

2.8 SNP-based heritability of the neuroticism item clusters

The h^2_{SNP} estimates of the subclusters (both .09; see **Supplementary Table 23**) are only slightly lower than the h^2_{SNP} estimate of the total sum-score (.10). Generally, if one sums items that are genetically correlated, then the signal-to-noise ratio (in this case: the ratio of genetic variance to the total variance, i.e., the heritability) will increase with the number of items, i.e., we expect higher h^2_{SNP} if we sum more genetically correlated items. Therefore, the finding that the h^2_{SNP} of the 4 items is only slightly lower as the h^2_{SNP} of the 12 items confirms their genetic homogeneity. It is crucial to note that the higher h^2_{SNP} of the sum of the 12 items does *not* necessarily imply that gene-finding studies using this sum score as dependent variable have enhanced power to identify underlying genetic variants, as higher h^2_{SNP} does not imply larger single SNP effect sizes.

2.9 Functional annotation results for the two neuroticism item clusters

Of the 9,888 (10,161) SNPs in high LD with one of the independent significant SNPs for the *depressed affect* (*worry*) cluster, most were intronic (*depressed affect*: 5,387 = 54.9%; *worry*: 4,707 = 46.3%) or intergenic (*depressed affect*: 2,249 = 22.9%; *worry*: 3,351 = 33.0%; **Supplementary Table 31-33; Supplementary Fig. 15**). For *depressed affect* 1.1% (n = 104) of the SNP was exonic, with 49 SNPs being non-synonymous (ExNS) and 1 splicing variant (**Supplementary Table 34**). For *worry* 1.33% (n = 135) of the SNPs were exonic, of which 66 were non-synonymous, and 1 was a splicing variant (**Supplementary Table 34**). Of all ExNS SNPs, 22 overlapped between the clusters, of which 19 are located in a well-known inversion on chromosome 17[8]. Although some ExNS SNPs were also identified in the meta-analysis of the neuroticism sum-score (27 and 39 for *depressed affect* and *worry*, respectively), we found 12 ExNS SNPs that were specifically associated to the *depressed affect* cluster, and 15 ExNS SNPs that are uniquely associated to the *worry* cluster. This suggests that among those SNPs that are highly likely to have functional consequences, the clusters are 1) distinct and 2) adding information to the results of neuroticism sum-score analysis.

As an example, we highlight here for each cluster one cluster-specific ExNS SNP, not identified for the neuroticism sum-score, that may be a viable candidate for functional follow-up studies. For *depressed affect*, the ExNS SNP with the highest CADD score (35) was rs45510500, located in exon 40 of *KIAA1109*. rs45510500 is a missense mutation that leads to an amino acid change of Arginine to Tryptophan, of which the T allele is associated with a lower score on the *depressed affect* cluster. For the *worry* cluster, one of the SNPs with the highest CADD score (26.9) is rs13072536, in exon 3 of *ITIH1*, and is a missense mutation resulting in a Isoleucine to

Asparagine change. The T allele is associated to a lower score on the *worry* cluster. rs13072536 has a regulome database score of 1f, meaning that it is likely to affect binding and to affect expression of a gene target.

2.10 Genetic correlation analysis

Neuroticism has been phenotypically linked to social, and both mental and physical health-related outcomes[34–37], and genetically to mental and physical health-related outcomes. We used LDSC to calculate genetic correlations between neuroticism and 35 traits from large GWAS studies for which summary statistics were publicly available (an overview of the studies used in this analysis is given in **Supplementary Table 36**). To be able to compare the genetic correlational pattern of neuroticism to that of depression, *depressed affect* and *worry*, we also calculated the genetic correlations of these three traits to the 35 external traits. These are all shown in **Figure 4** of the main text and **Supplementary Table 37**.

The r_g's between our 4 traits are shown in **Supplementary Table 23**. Note that the r_g between depression and *depressed affect* was .895, while the r_g's of depression and *depressed affect* with *worry* were only r_g=.582 and r_g=.629, respectively. The r_g's of depression and *depressed affect* with other traits strongly mirrored each other (correlation between their r_g's is r=.98; r=.70 was between r_g's of depression and *worry*), validating the *depressed affect* cluster.

We observed 11 Bonferroni significant genetic correlations for neuroticism (α=0.05/(4×35); $P<3.6\times10^{-4}$) (**Fig. 4**; **Supplementary Table 37)**. We observed 17 Bonferroni significant genetic correlations for depression, 15 for *depressed affect*, and 13 for *worry*.

The genetic distinctness of the two clusters *depressed affect* and *worry* is apparent from the differences in their genetic relations to other traits. For instance, *worry* is significantly correlated

to anorexia, schizophrenia and bipolar disorder, and to childhood body mass index (BMI) and childhood obesity, while *depressed affect* is not. *Depressed affect*, in turn, is significantly related to ADHD, waist-to-hip ratio, ever smoking, smoking cessation, longevity, age of having first child and number of children, while *worry* is not. For waist circumference, BMI, and hip circumference their genetic correlations are even in opposite directions.

Corroborating previous reports[3,8,36], neuroticism was genetically correlated to various psychiatric traits (r_g range: .20-.82) and to subjective well-being (r_g= -.68). Interestingly, the r_g's of neuroticism with anorexia, schizophrenia, and bipolar disorder seem to originate mainly from the *worry* cluster, suggesting that the genetic link between neuroticism and these disorders may be founded on shared worry/tense/irritable features. In contrast, the r_g of neuroticism with ADHD seems to originate mainly from the *depressed affect* cluster, which is in concordance with a large body of literature on the relation between ADHD and depression. Notably, neuroticism did not significantly correlate to e.g. BMI- and smoking-related phenotypes, while the two clusters did, albeit in opposite directions. Together, these r_g-based findings corroborate the complexity of the neuroticism phenotype, and illustrates how insight can be gained by distinguishing genetically homogeneous sub-dimensions.

2.11 Mendelian randomization

To investigate whether the observed genetic correlations between the four studied traits and the 35 external traits for which large-scale summary statistics were available (**Supplementary Table 39**) could be explained by directional associations, we performed Mendelian randomization (MR) based on GWAS summary statistics, using generalized summary-data-based Mendelian randomization (GSMR[38]). The MR analysis of neuroticism and depression showed strong

bidirectional associations (neuroticism on depression: b_{xy}=0.488, $P<1\times10^{-300}$; depression on neuroticism: b_{xy}=0.934, $P=1.87\times10^{-252}$), implying that neurotic traits increase the liability to depression, while depression in turn results in a more neurotic personality. Neuroticism also showed significant causal relations with three other psychiatric disorders, namely anxiety disorder (strong unidirectional positive effect going from neuroticism to anxiety), ADHD and schizophrenia (both positive and bidirectional). These directional associations between neurotic traits and psychiatric disorders imply that neurotic personality characteristics are both a causal factor and a consequence of psychiatric illnesses. The former suggests that preventive treatment of neurotic tendencies (e.g., reduction of negative beliefs through cognitive behavioral therapy) may decrease the risk of developing psychiatric problems. Indeed, prior studies indeed have shown that individuals scoring high on neuroticism tend to have worse health outcomes compared to individuals low on neuroticism[39].

Analyzing directions of causation for the 21 traits that had significant genetic correlations to at least one of the four phenotypes in LD Score regression (LDSC) analyses (**Supplementary Table 37**), we observed mostly bidirectional negative causal effects for educational attainment (all phenotypes except *worry*, b_{xy} range: -0.326 – -0.165, $P<2.98\times10^{-5}$) and IQ (neuroticism and *depressed affect*, b_{xy} range: -0.324 – -0.086, $P<1.92\times10^{-5}$; **Supplementary Table 39**). Positive bidirectional causal associations were observed between schizophrenia and all four phenotypes (b_{xy} range: 0.608 – 1.654, $P<1.25\times10^{-9}$), indicating that increased liability to neurotic traits and depression causes increased liability to schizophrenia, and vice versa. In addition, significant positive unidirectional associations were observed in the reverse analyses between BMI and both

depression and *depressed affect* (depression, b_{xy}=0.061, P=4.96×10^{-12}, *depressed affect*, b_{xy}=0.049, P=5.35×10^{-6}), suggesting that obesity enhances the probability to develop depression. Interestingly, the *depressed affect* and *worry* clusters showed distinct patterns of causality which partly resembled the observed differences in genetic correlations by LDSC. For instance, bidirectional negative (rather than positive) associations were observed between *worry* and BMI (forward: b_{xy}=-0.236, P=2.10×10^{-10}; reverse: b_{xy}=-0.123, P=4.87×10^{-15}), while BMI only affected *depressed affect* positively (as noted above) and not the other way around (b_{xy}=0.033, P=0.39). These results suggest that a lower liability to *worry* may decrease the probability to become obese, whereas a lower liability to the other sub dimension of neuroticism, *depressed affect*, does not cause decreased liability to obesity. We note that several GSMR analyses showed significant causality between traits that did not show a significant genetic correlation by LDSC, which may be explained by the use of significant variants as instrumental variable in GSMR analysis instead of using all SNPs in LDSC. An overview of the results can be found in **Supplementary Table 39**.

2.12 Drug targets

We explored whether genes implicated by the GWAS and GWGAS results may provide potential targets for pharmacotherapy treatment by searching the drug-gene interaction database (DGIdb[40,41]), a large online collection of drug-interaction databases (**Online Methods**). First, we selected genes associated with any of the four traits, and searched for known interactions with existing FDA approved therapeutic compounds. This resulted in 67 unique genes with reported drug interactions with 491 unique pharmaceuticals (average 7-8 compounds per gene, **Supplementary Table 40**). The *HRH1* gene that was implicated in depression showed the most

reported interactions (130 interactions), of which most were antagonists of the gene-product (n=124, 95.4%). *HRH1* codes for the histamine receptor H1, an important neurotransmitter in the central nervous system[42] . Among all interactions with the *HRH1* gene, the highest DGIdb score (i.e. the number of distinct sources mentioning the interaction) was found for Loratadine (score=16) which is a frequently used antihistamine medication for the treatment of common allergies. In addition, a large number of known interactions (n=104) was observed for the *DRD2* gene, coding the dopamine receptor 2 (implicated in all four traits), of which most interactions were antagonistic (n=65, 62.5%). These results are in line with extensive previous literature on the role of dopamine receptor in psychiatric disorders[43]. When comparing results between the four phenotypes, we observed a number of potentially druggable genes specific for neuroticism and depression/depressed affect, but not for worry, including CACNA1E (5 interactions) and NOS1 (1 interaction). However, we note that this search mainly results in finding interactions that have already been extensively studied in psychiatric disorders, while many more yet untargeted genes may exist. For this reason, in order to find novel drug targets, we performed a search in 10 databases in DGIdb of potentially druggable genes based on the type of target coded by the gene. We found a large number of potential interactions based on the gene-target (**Supplementary Fig. 17, Supplementary Table 41**), covering 168 unique genes, of which the large majority (n=126, 75%) had not previously been reported to interact with approved medication.

These results show that GWAS is able to identify a large set of potentially clinically relevant genes. Indeed, genes that have been identified by GWAS have been shown to have a higher success rate to make it to the approved drug market[44]. Several limitations, however, still exist that need to be overcome to translate these findings into direct clinical use. Given the wealth of

genes that are currently identified, prioritizing genes for therapeutic intervention becomes challenging. Performing trial-and-error clinical investigations of potential gene-targets based on these results is expensive and time-consuming, and thus not a feasible solution. We argue that one possible way to select which gene targeting may be most effective, is by identifying 'core genes' that play a central role in gene networks[45], which requires improved insight into underlying connections between genes (i.e. gene co-expression or protein-interactions). Furthermore, as has been pointed out previously[44], it is unclear which genes mapped by GWAS actually play a causal role, and the inclusive approach of including all mapping methods may lead to an overestimation of the actual number of targetable genes. In addition, given small effect sizes of these genes and the highly polygenic architecture of psychiatric disorders, we argue that targeting single genes may only provide limited results, and we may require multi-targeted therapy in order to yield effective therapies.

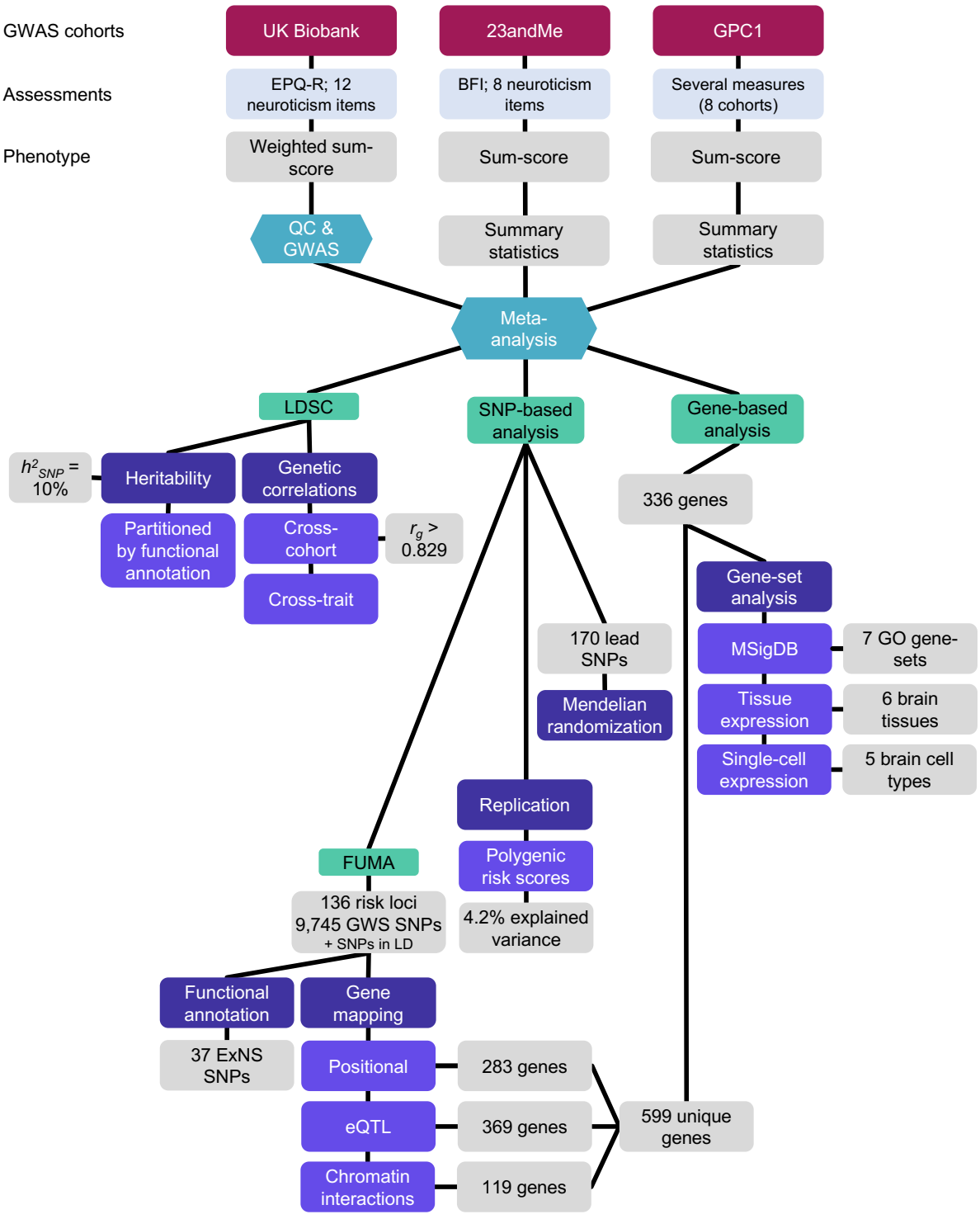
2.13 Comparison with Luciano et al. (2017)

Here we provide a brief discussion of how our results compare to those presented in a study conducted in the same period and which was published while our manuscript was under consideration[46]. We present a brief overview of which loci overlap with those reported in Luciano et al. and which can still be considered novel. To this end, we checked for overlap between the loci identified in the current study, and the 90 non-overlapping loci reported for the 116 independent GWS SNPs reported by Luciano et al. (we refer to their Supplementary Materials for details). Of the 124 loci initially reported in the current study as being novel, 51 overlap with a locus as reported by Luciano et al., while 73 loci identified in our study are not

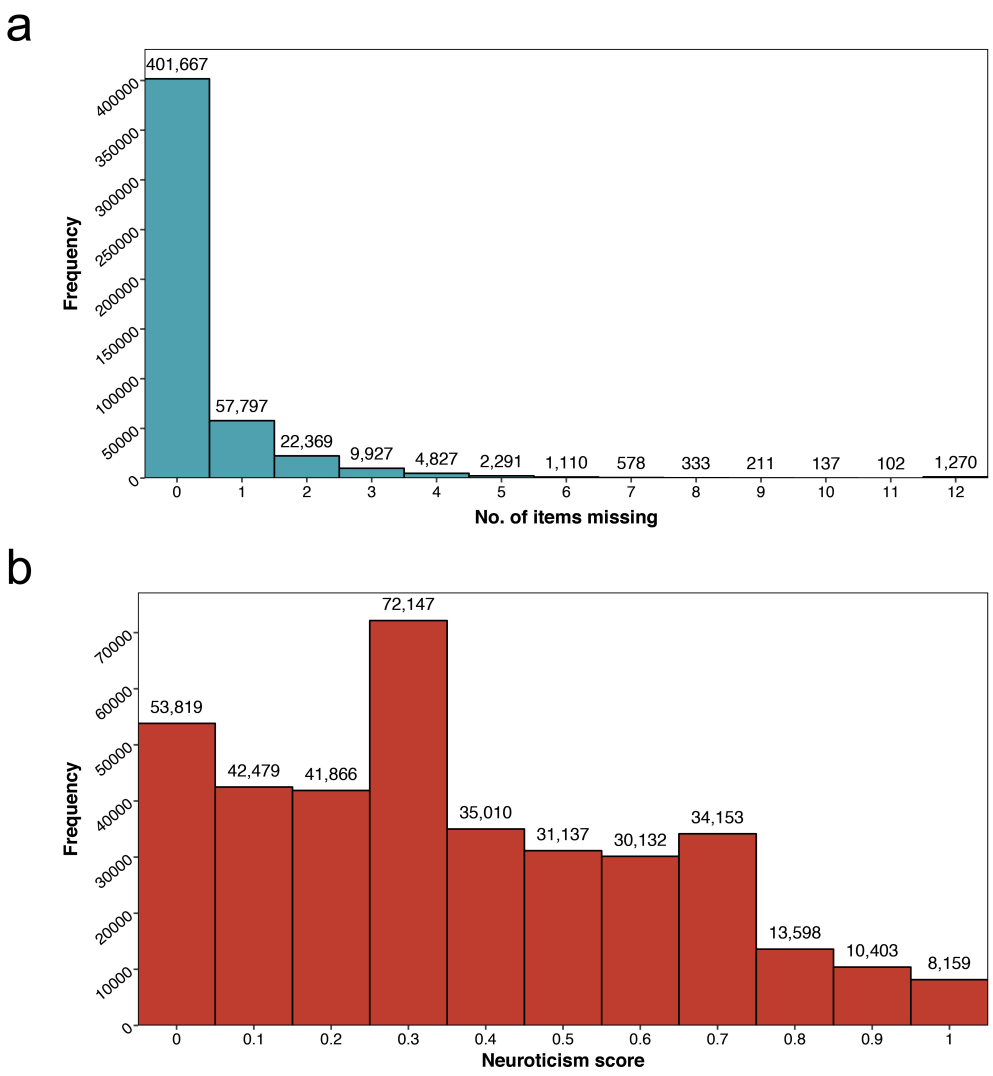
reported in their study, i.e. are novel (**Supplementary Table 42**). Reversely, of the 90 loci reported in Luciano et al., 24 were not GWS in our current, larger sample.

The surplus of novel loci in our study compared to that of Luciano et al., is likely due to a difference in sample size (N=329,821 in the discovery sample of Luciano et al. versus N=449,484 in the current study) and ensuing increased statistical power of the current study. In addition, subtle differences in selection of SNPs, covariates and/or phenotype operationalization may have prevented some SNPs to exceed the genome-wide significance threshold in Luciano et al., whereas they did in our study (obviously, the reverse is likely to be true as well).

Supplementary Figures



Supplementary Fig. 1. Flow chart of analyses conducted in the study of neuroticism
Schematic representation of the analyses conducted for neuroticism. Additional analyses performed on the depression, *depressed affect*, and *worry* phenotypes are not included in this chart.



Supplementary Fig. 2. Distribution of missingness and weighted sum-score for neuroticism in the UK biobank sample

(a) Distribution of the number of items missing. Individuals with invalid responses to more than two items missing were excluded from further analysis. **(b)** For the remaining participants, the neuroticism sum-score was established by summing the individual item responses and dividing by the total number of completed items for that participant. The distributions of these scores is shown in panel b.

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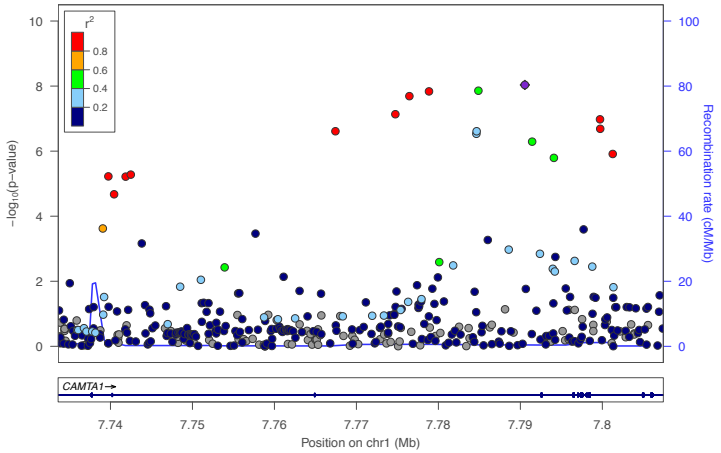

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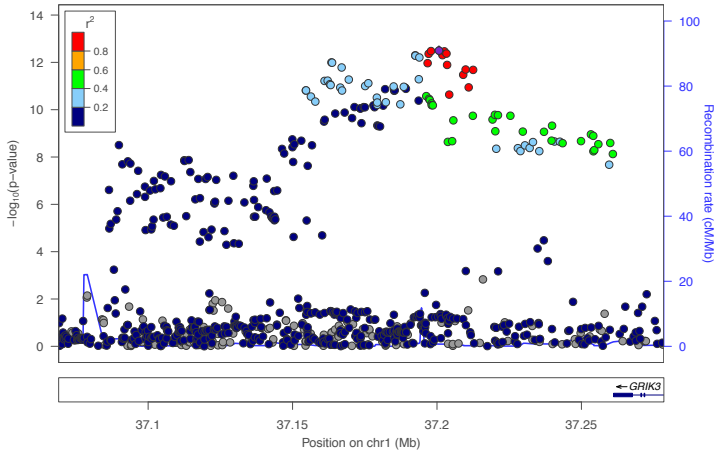

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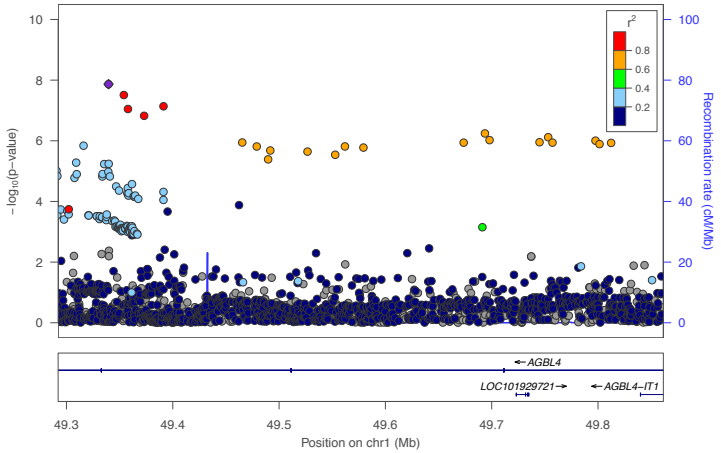

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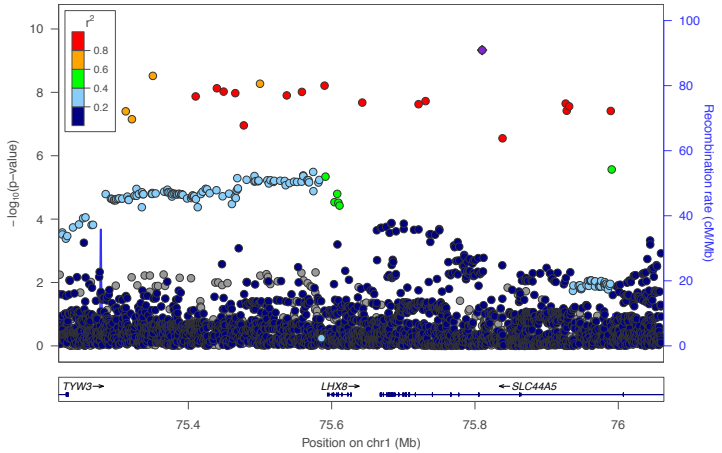

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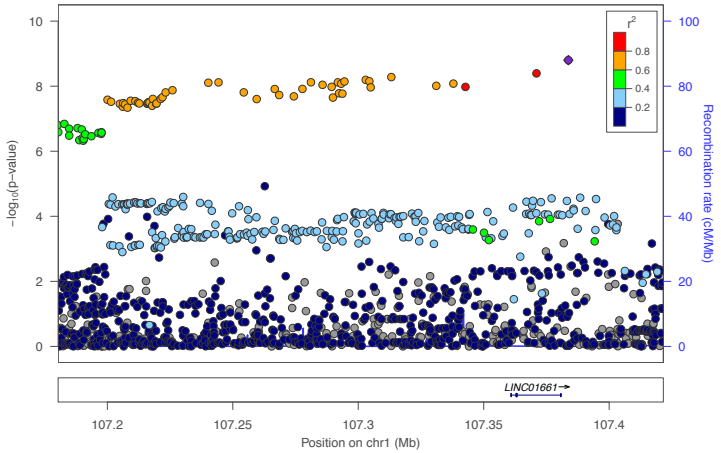

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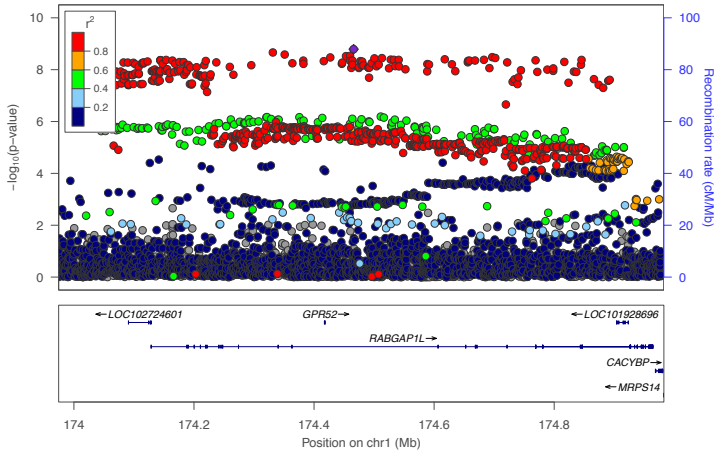

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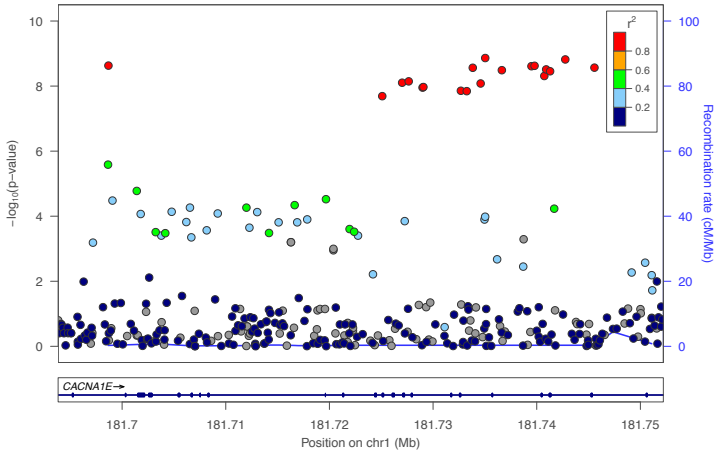

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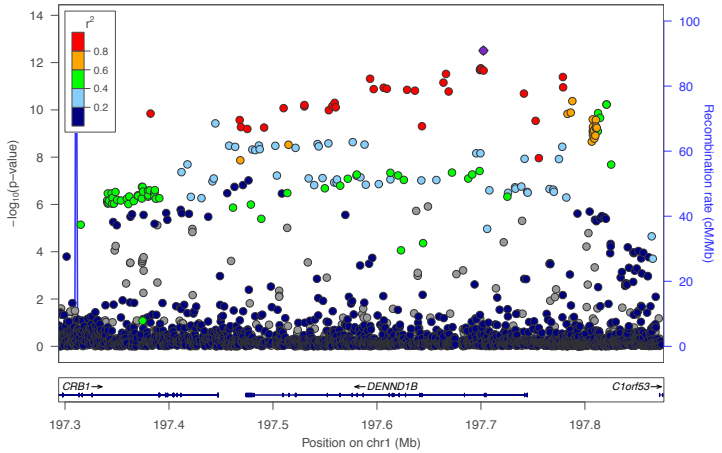

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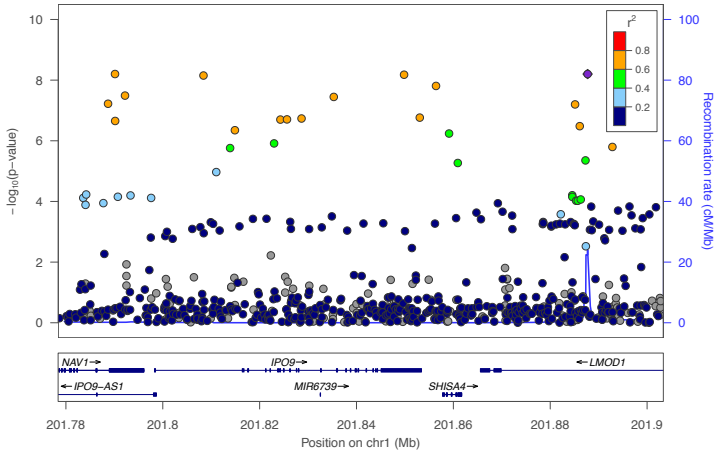

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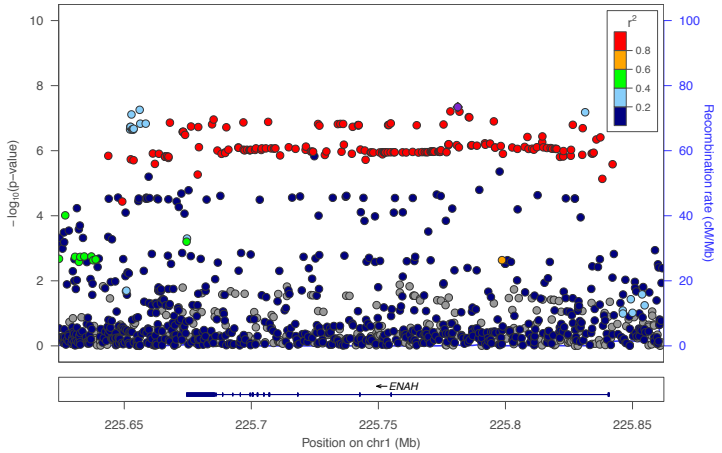

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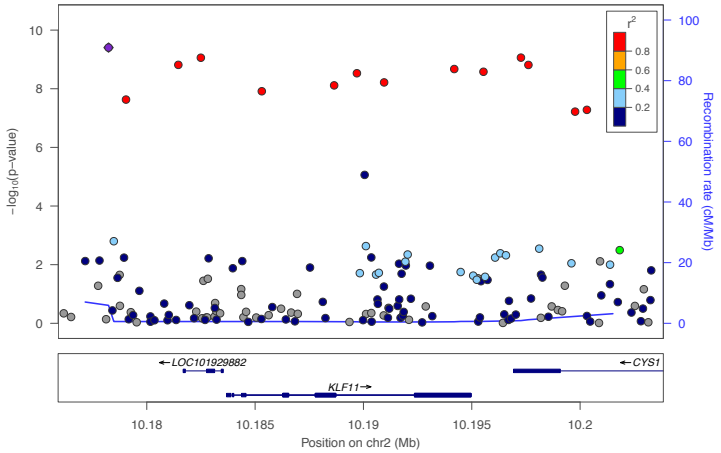

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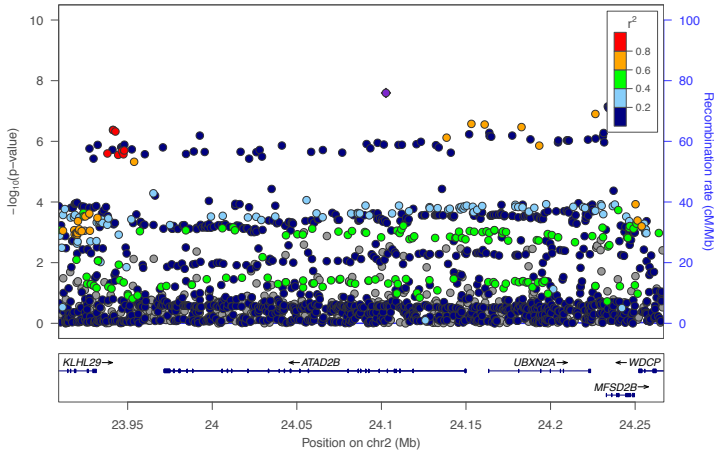

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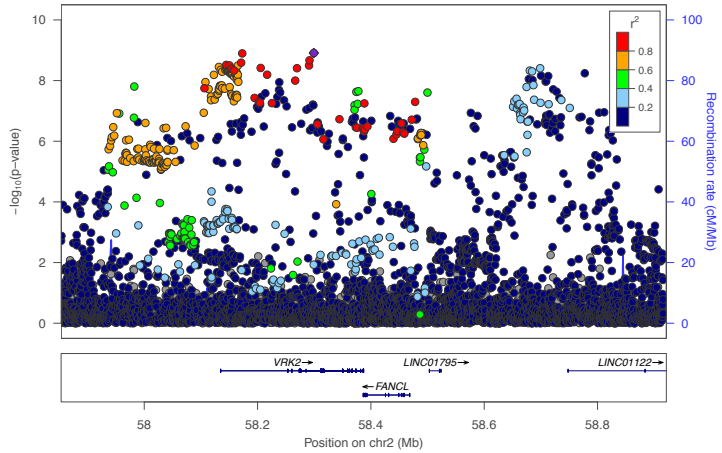

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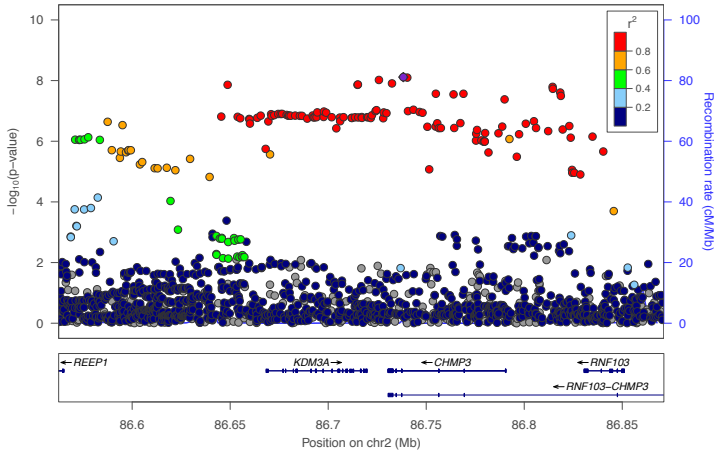

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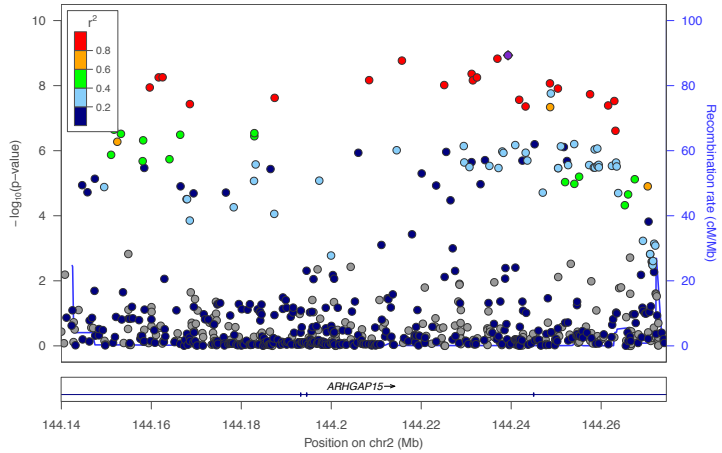

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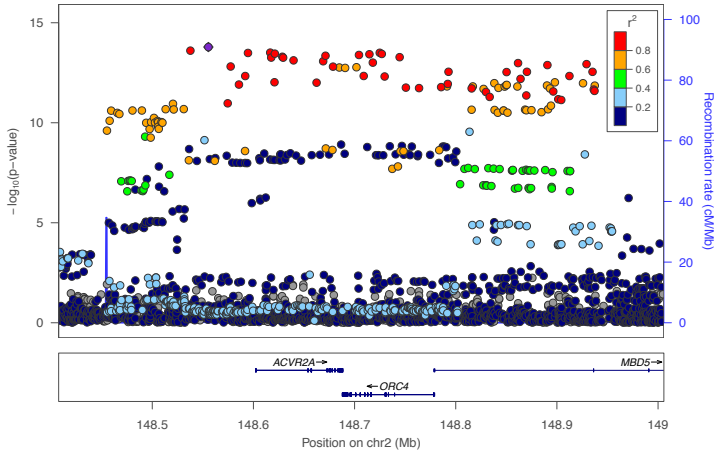

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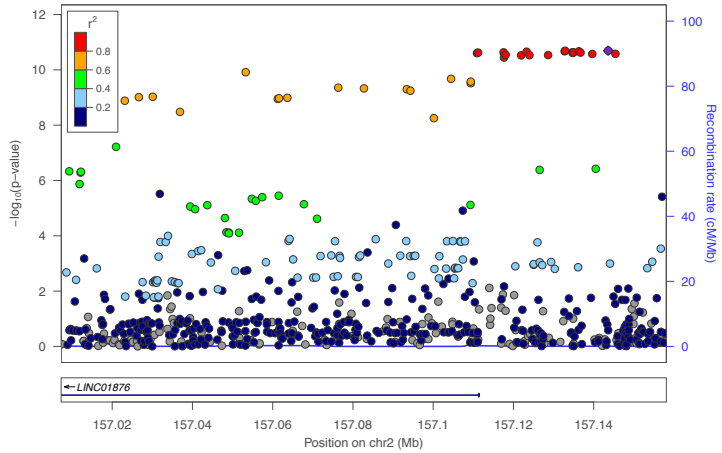

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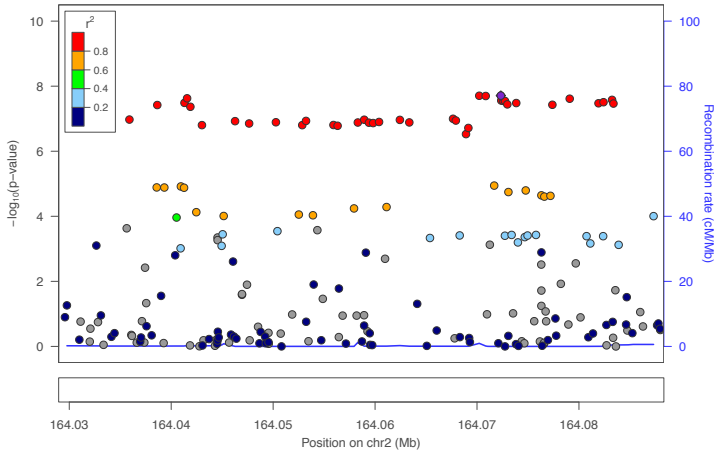

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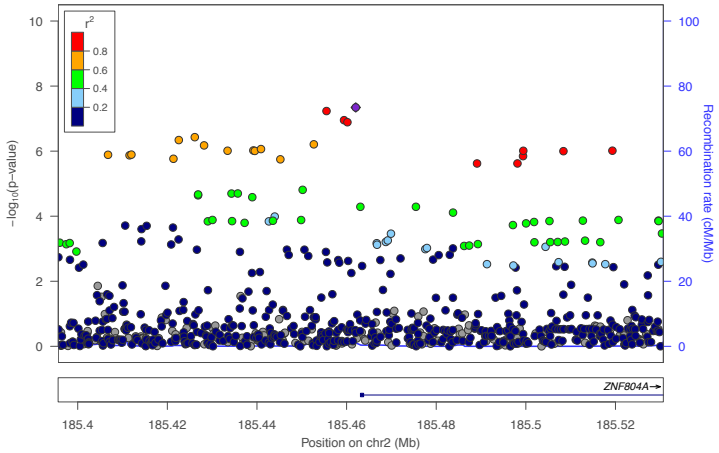

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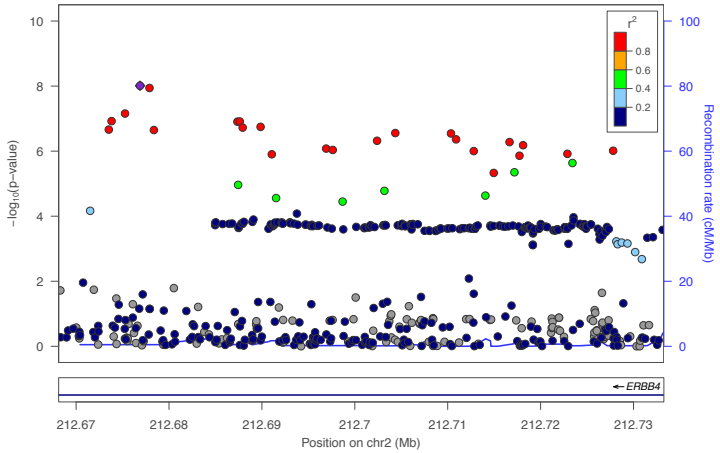

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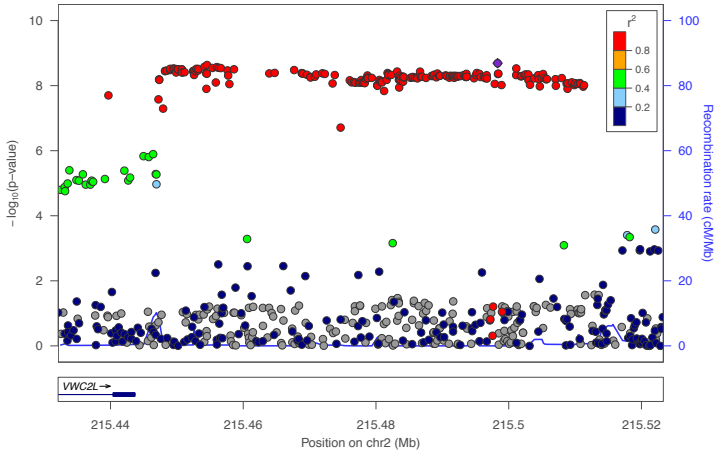

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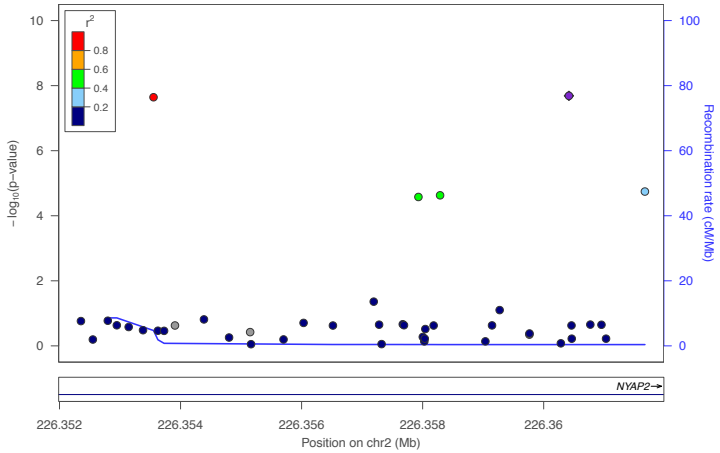

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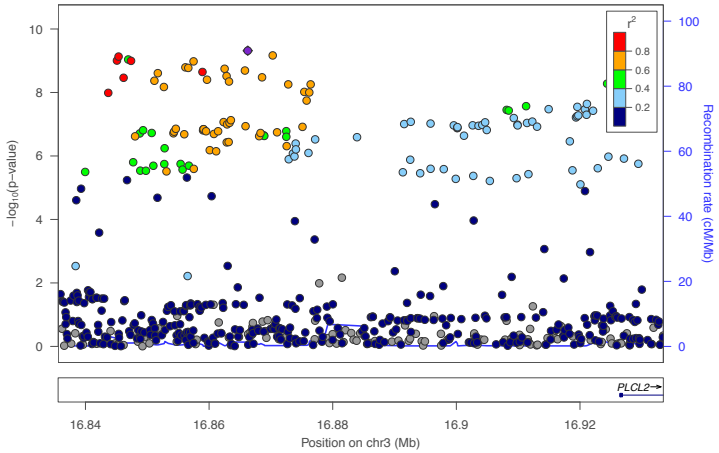

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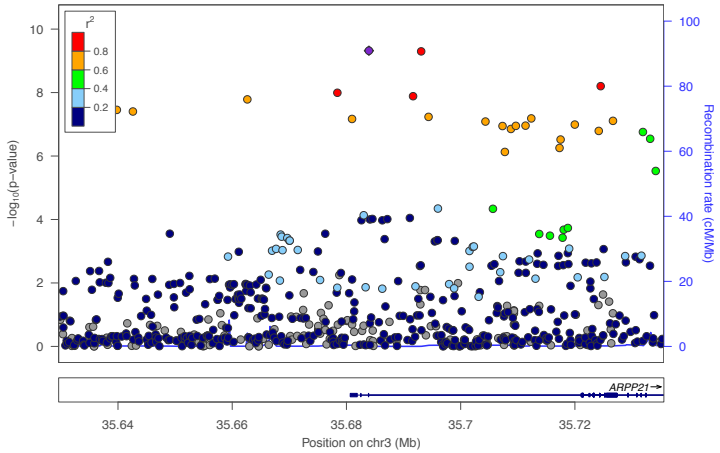

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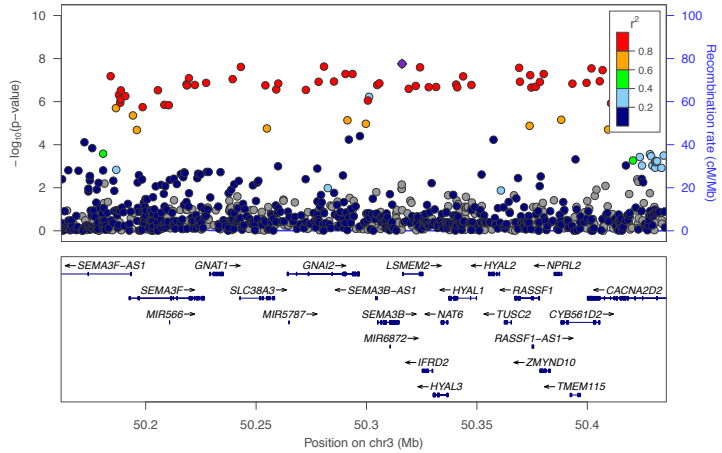

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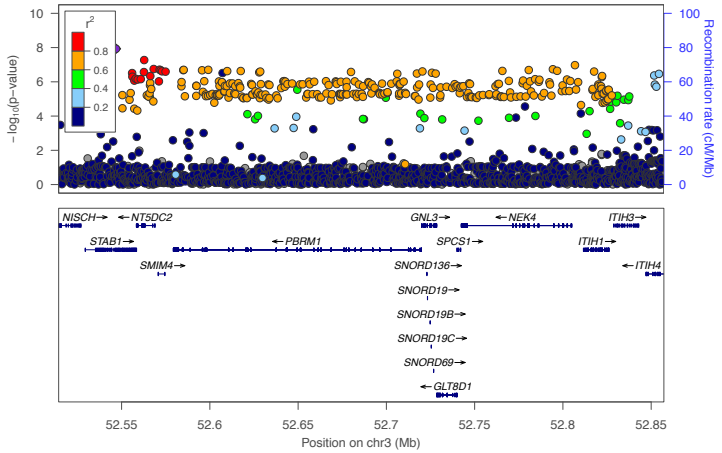

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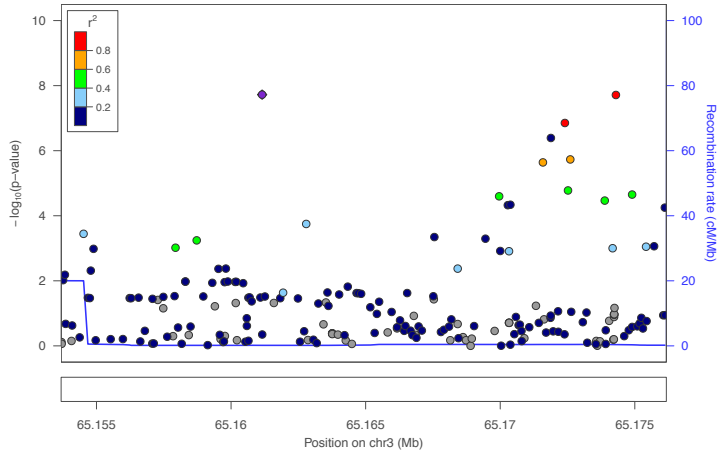

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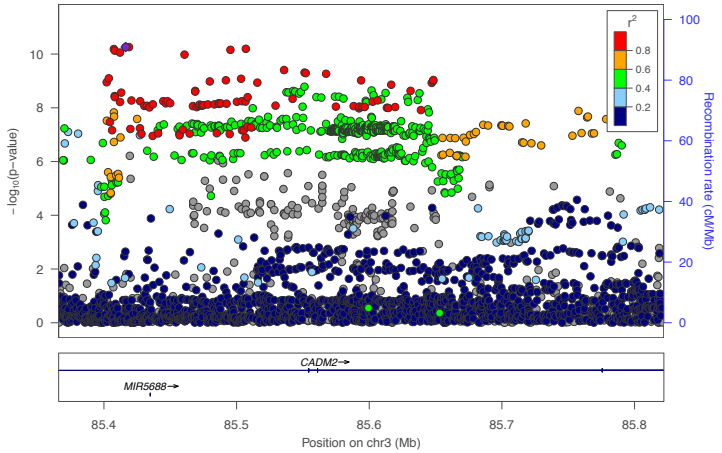

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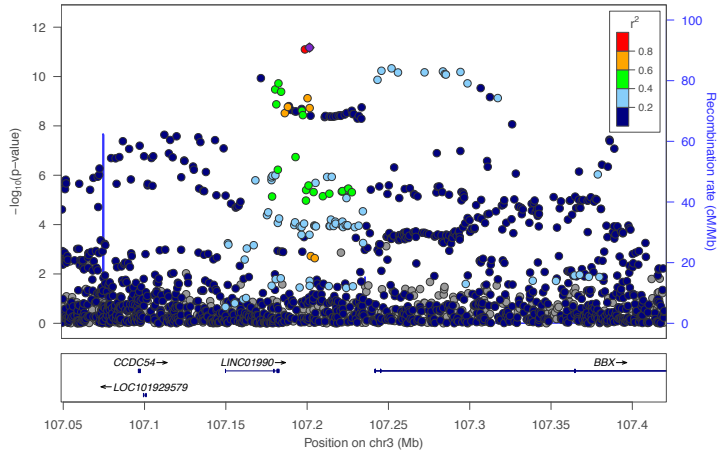

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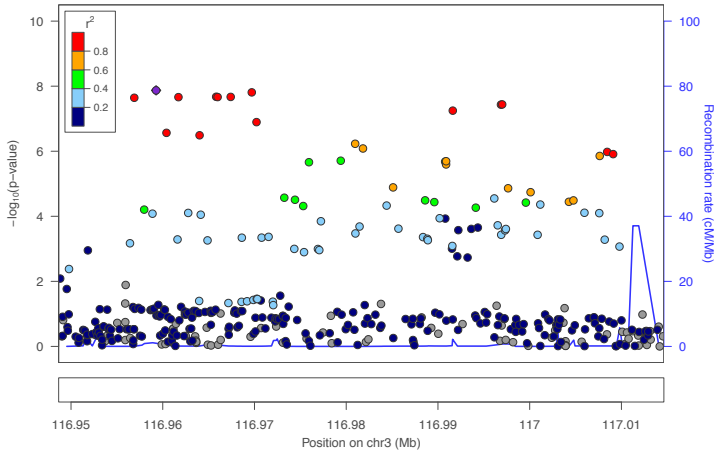

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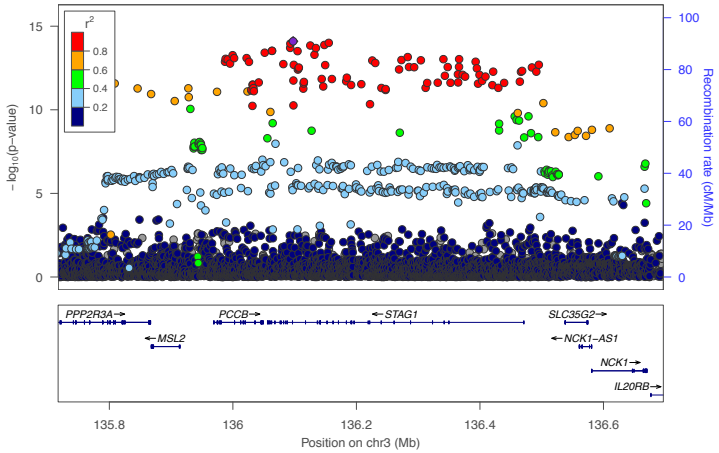

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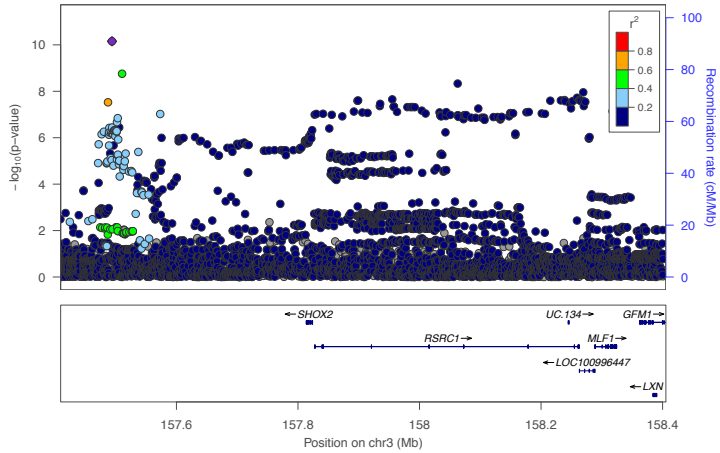

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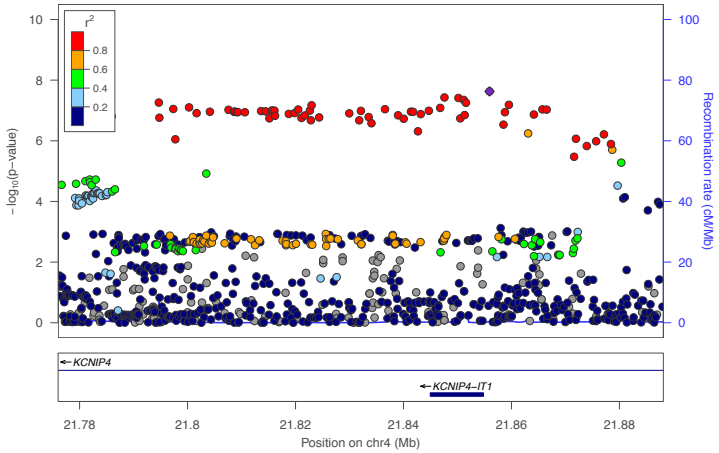

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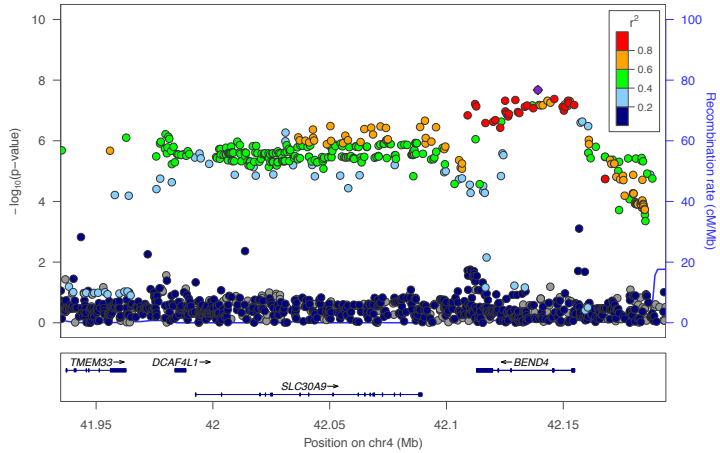

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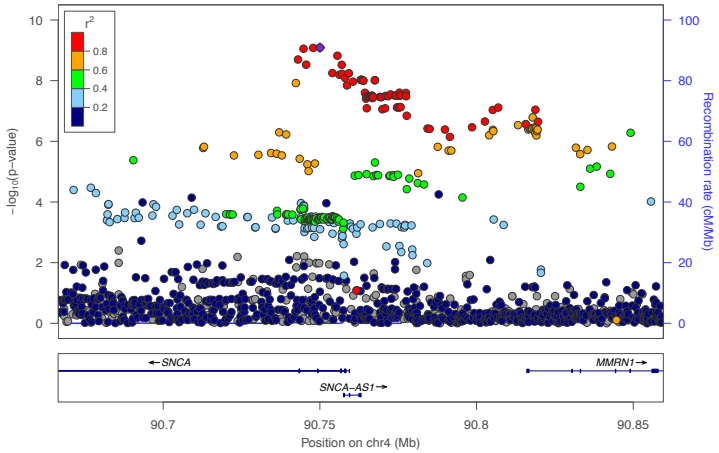

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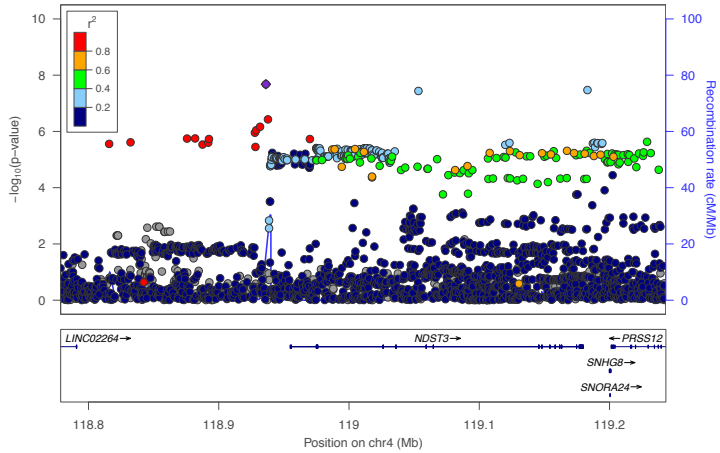

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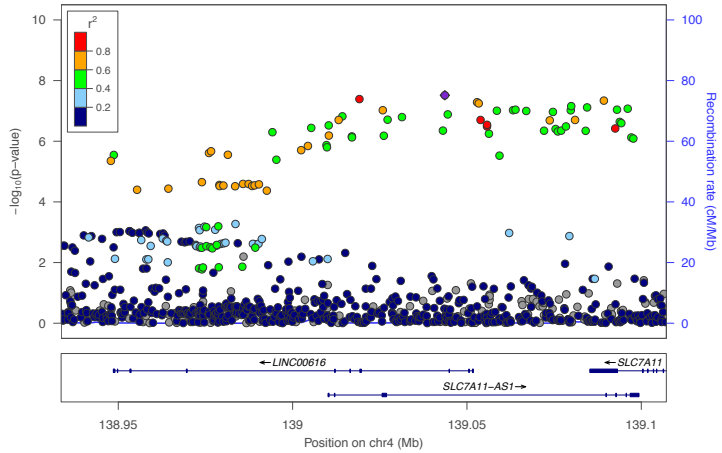

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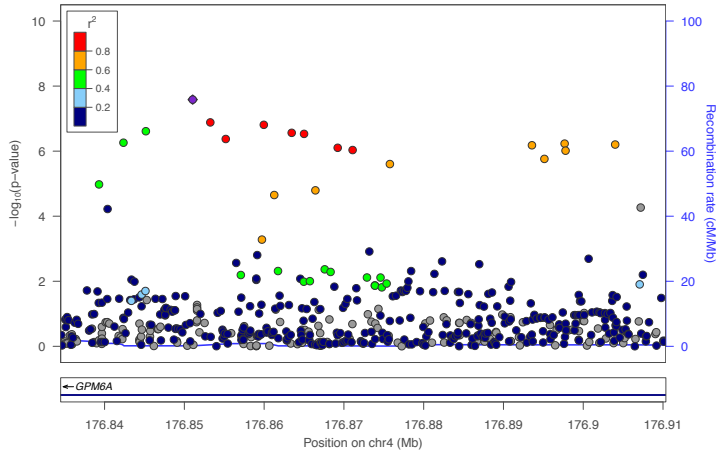

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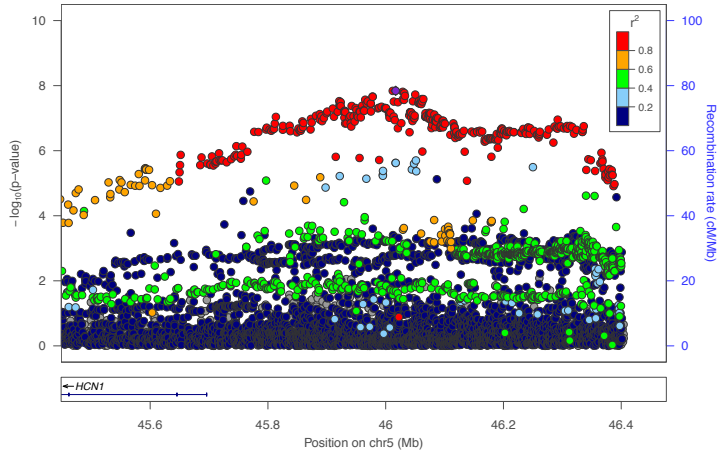

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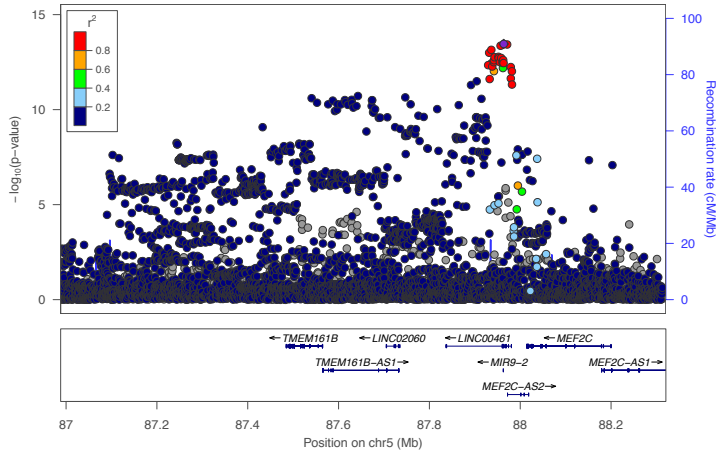

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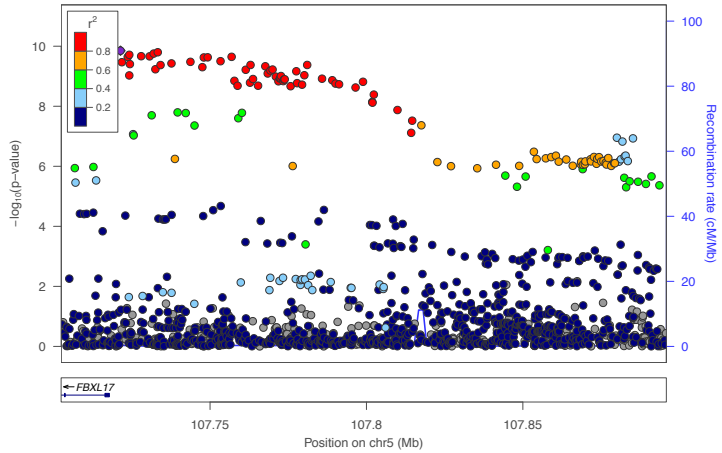

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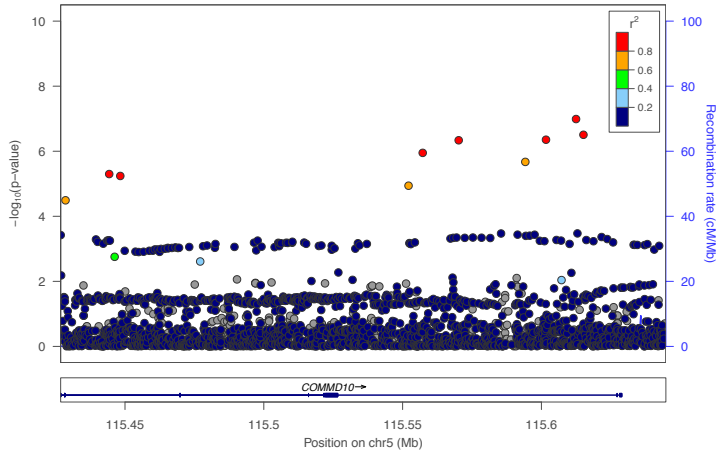

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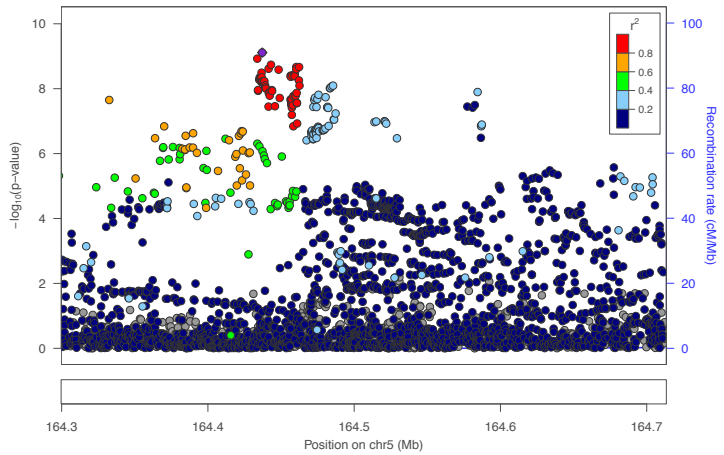

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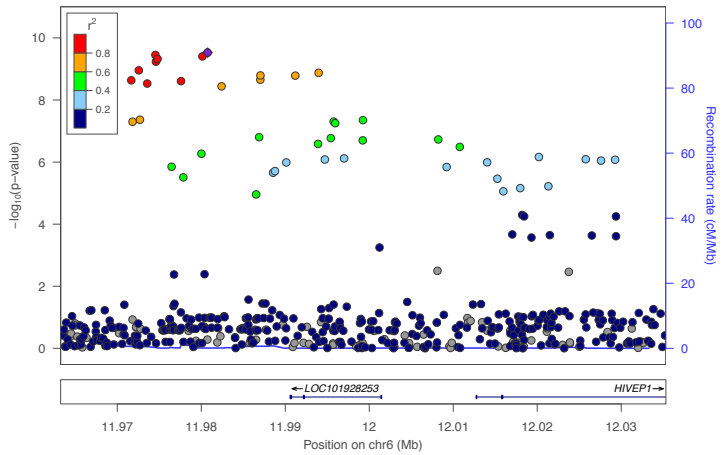

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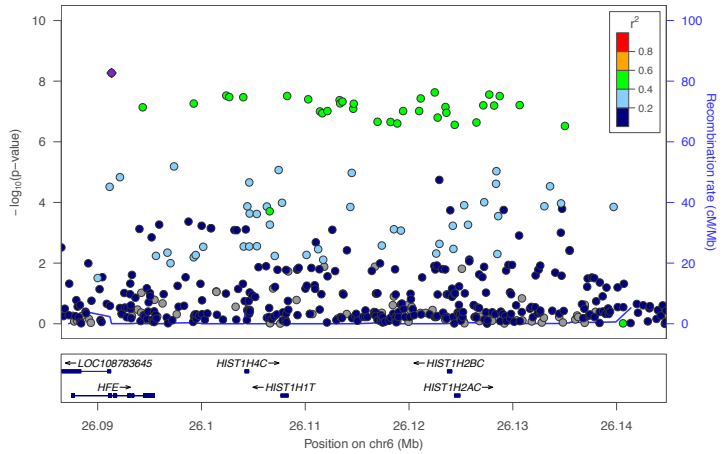

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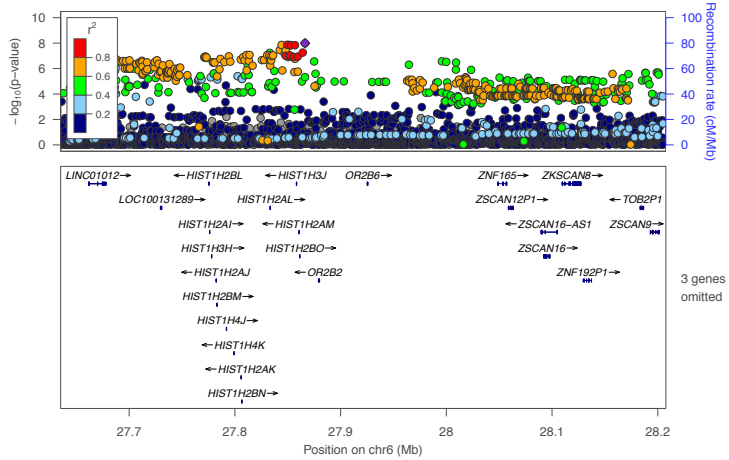

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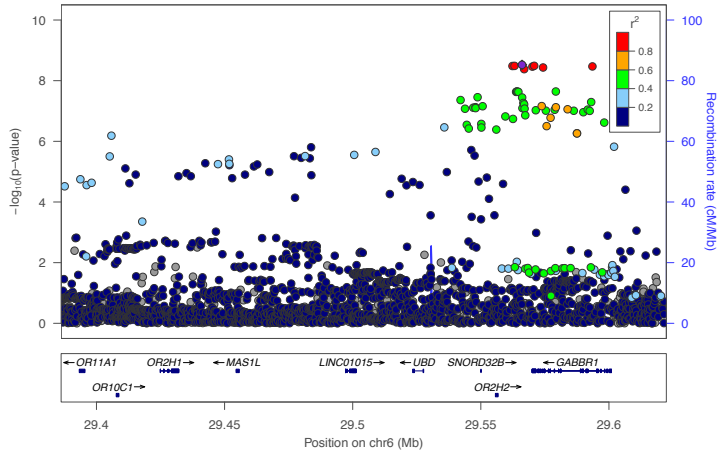

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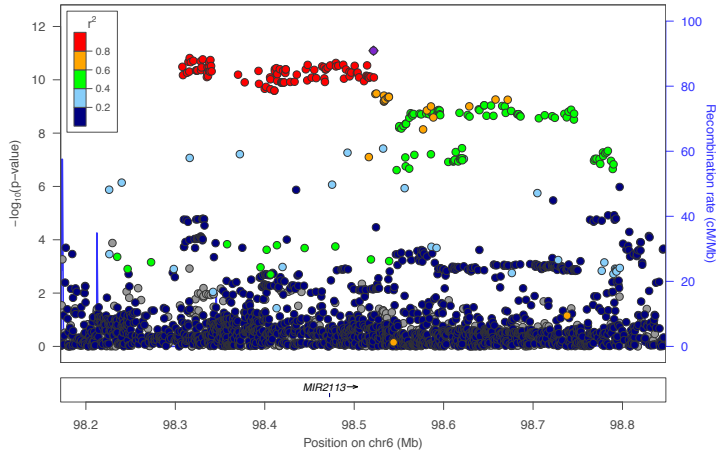

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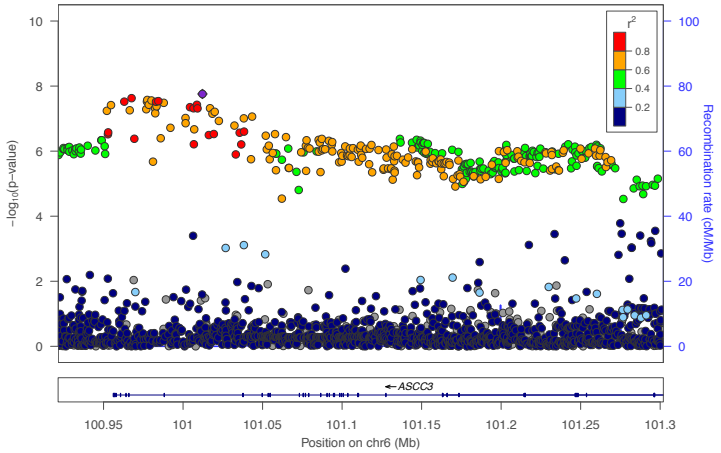

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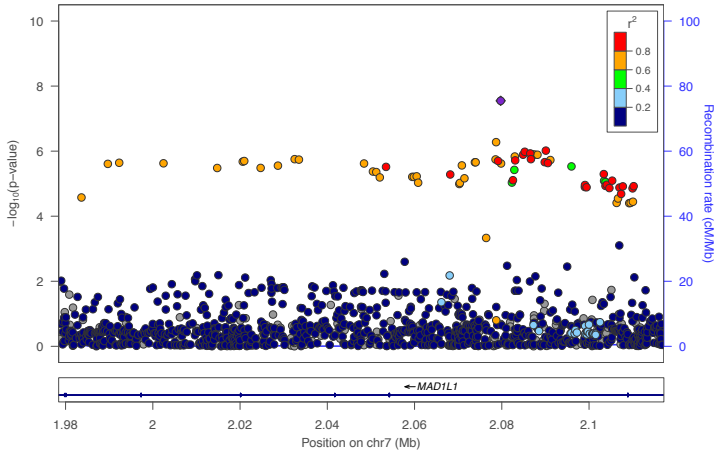

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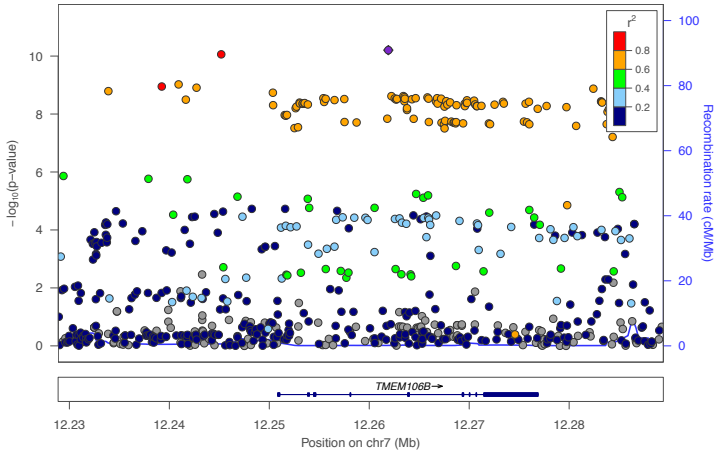

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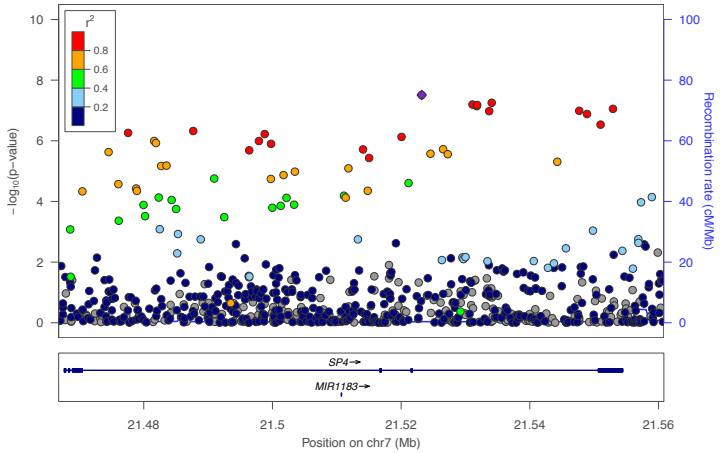

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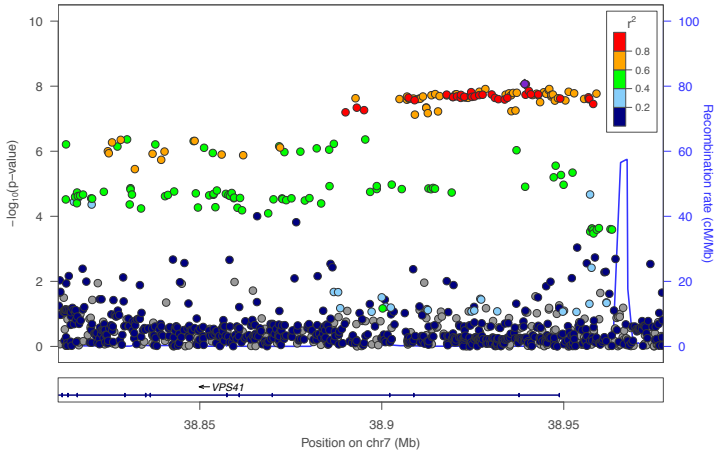

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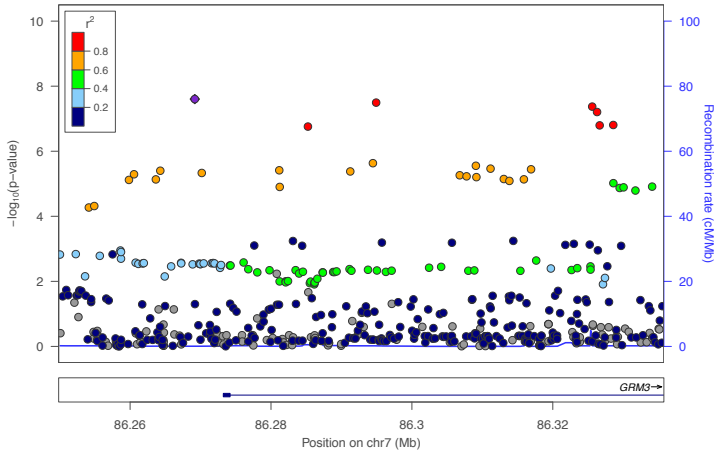

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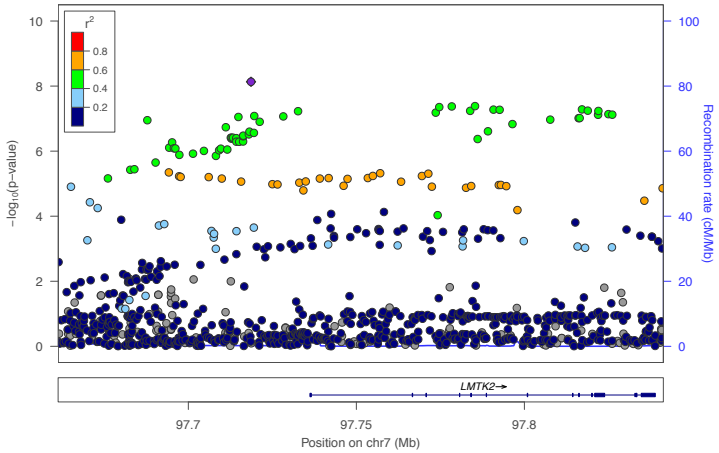

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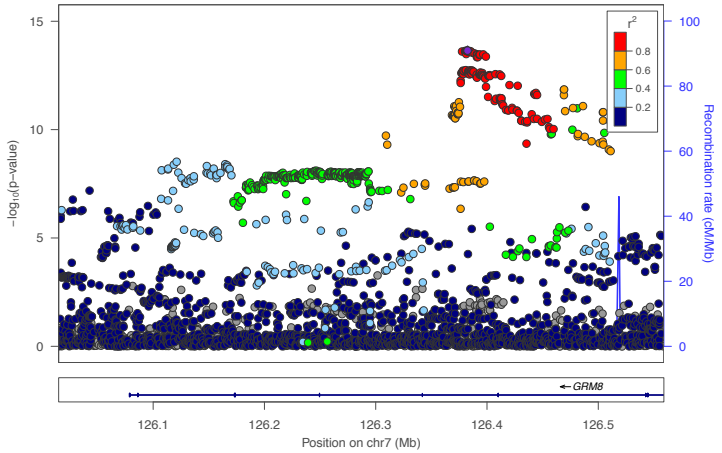

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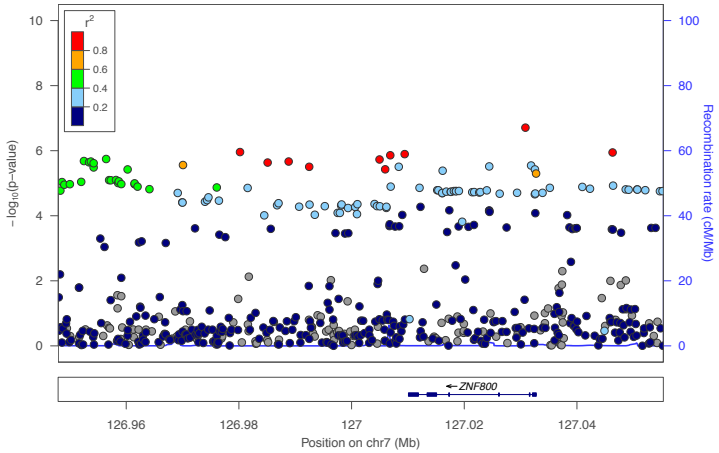

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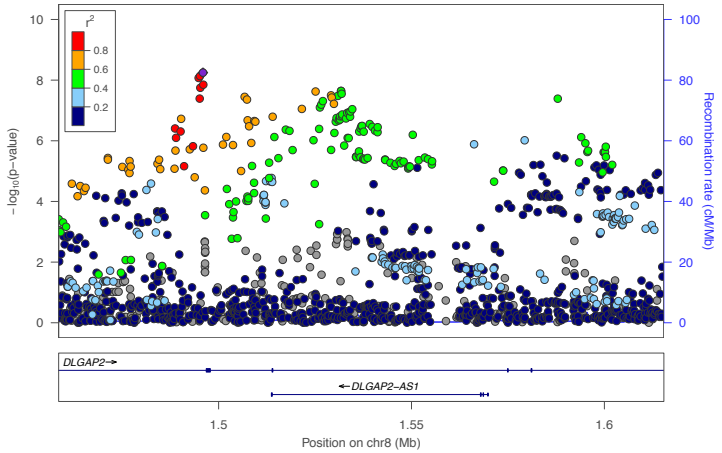

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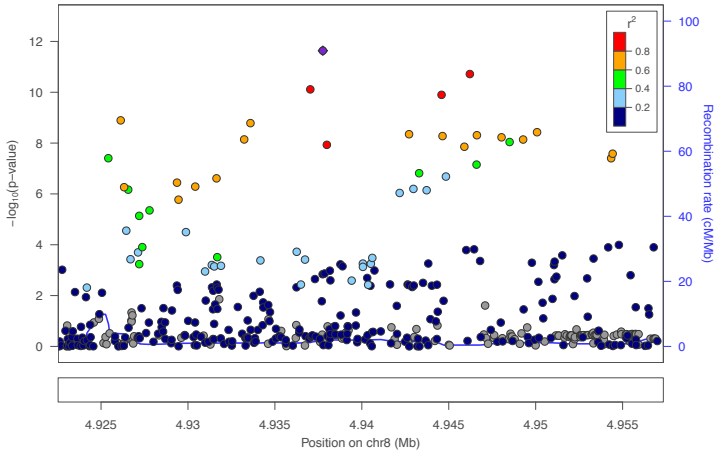

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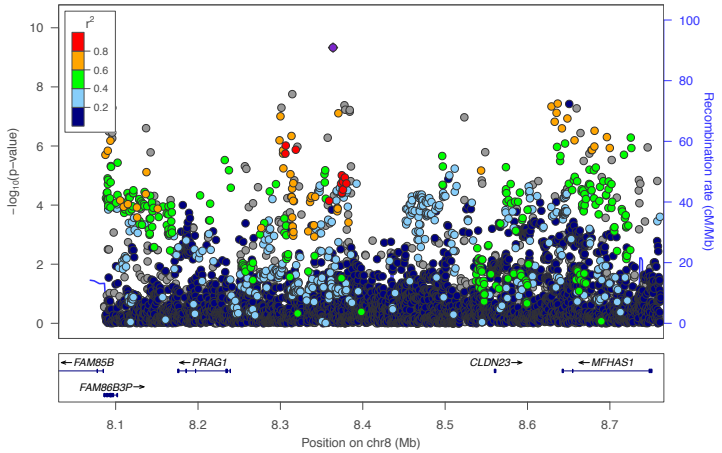

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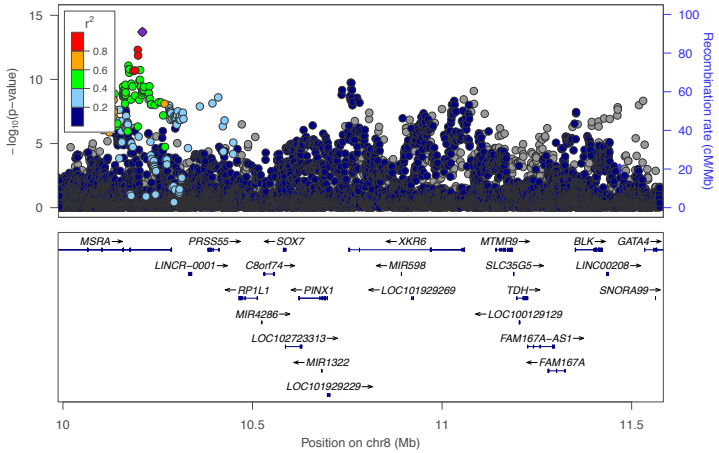

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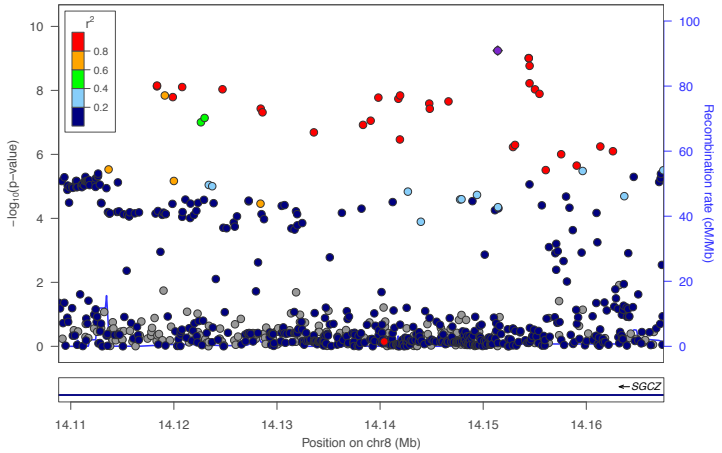

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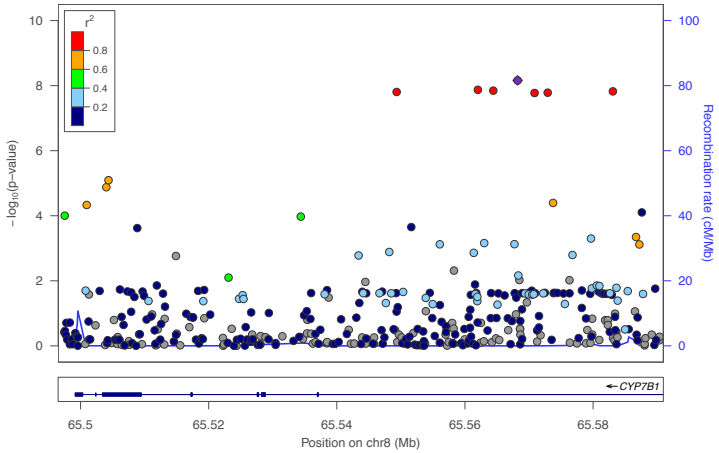

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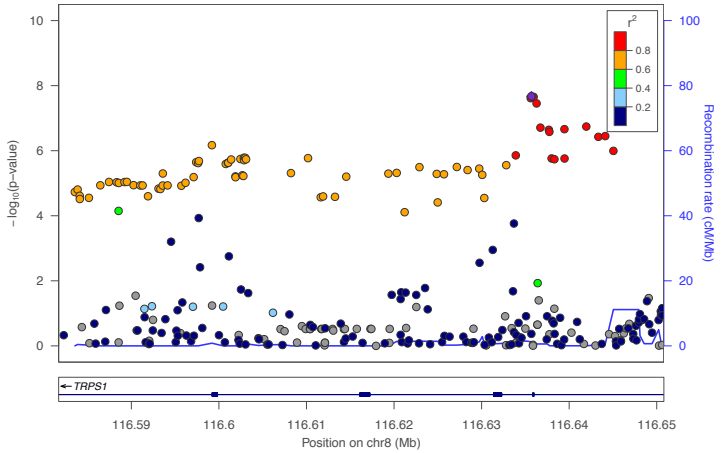

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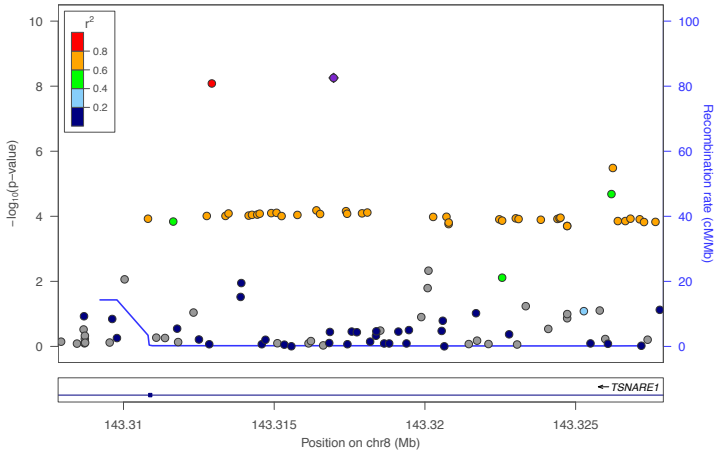

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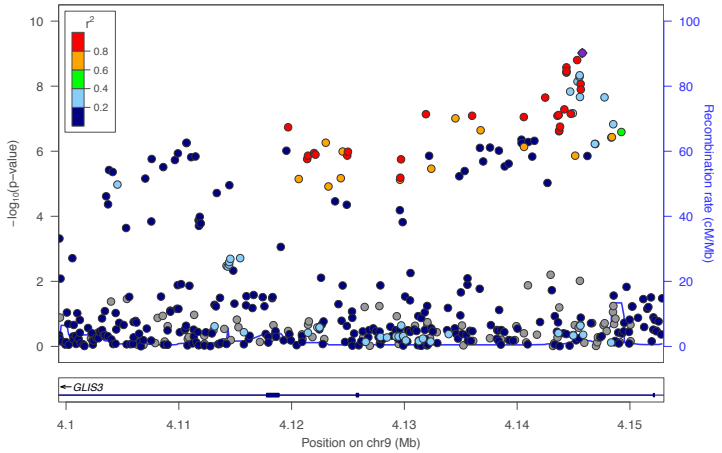

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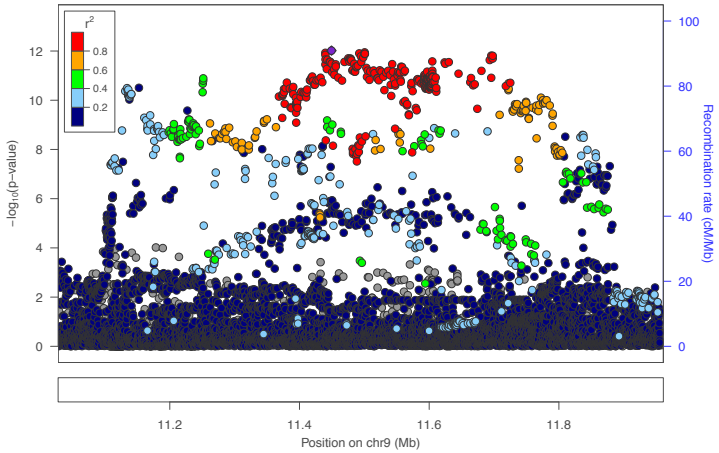

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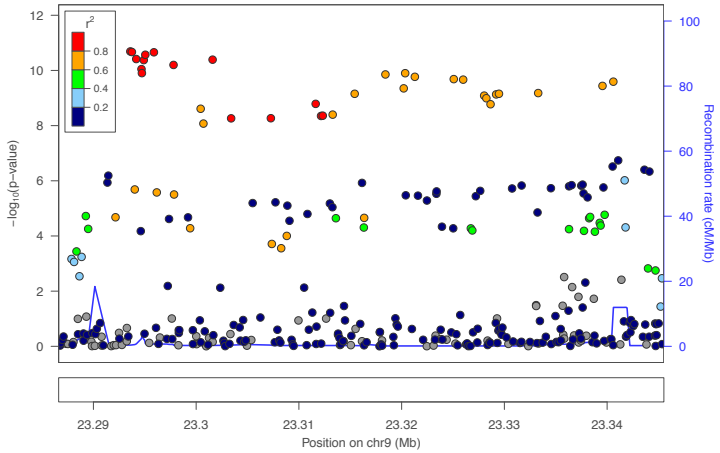

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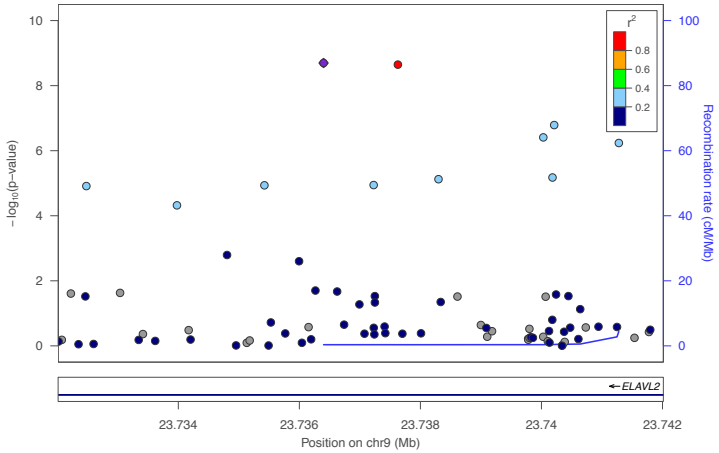

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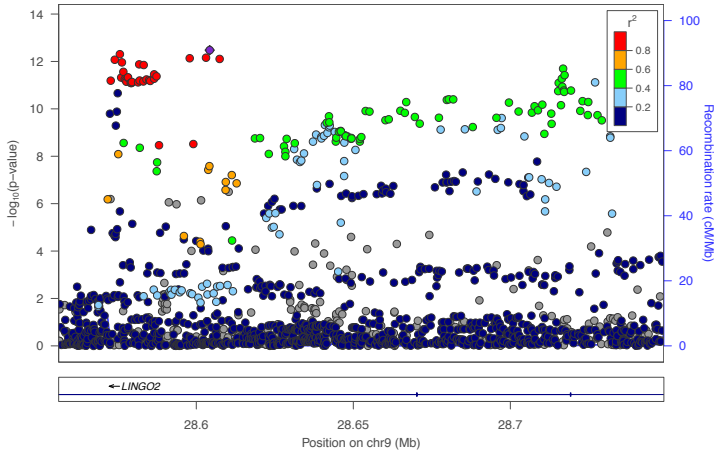

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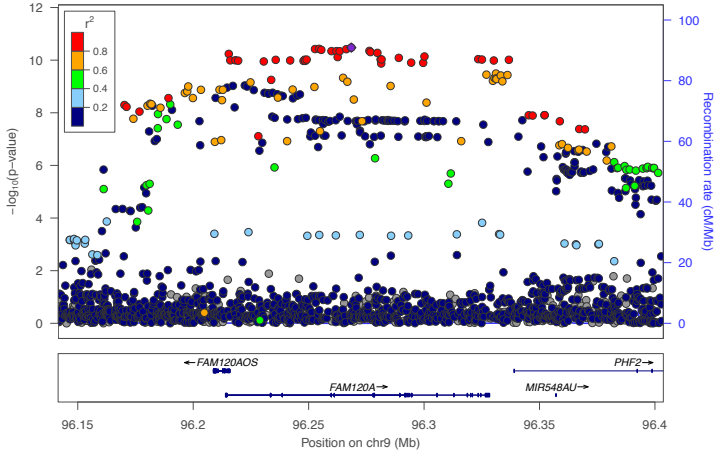

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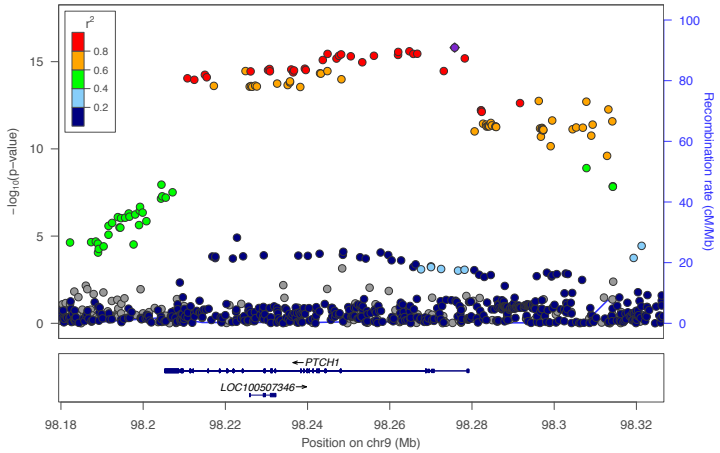

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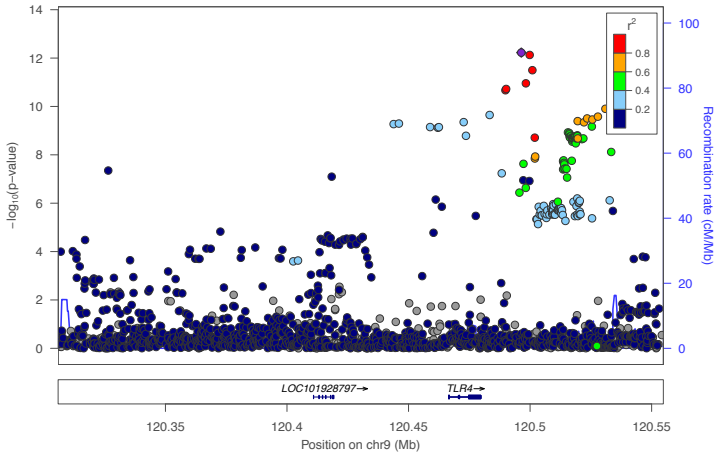

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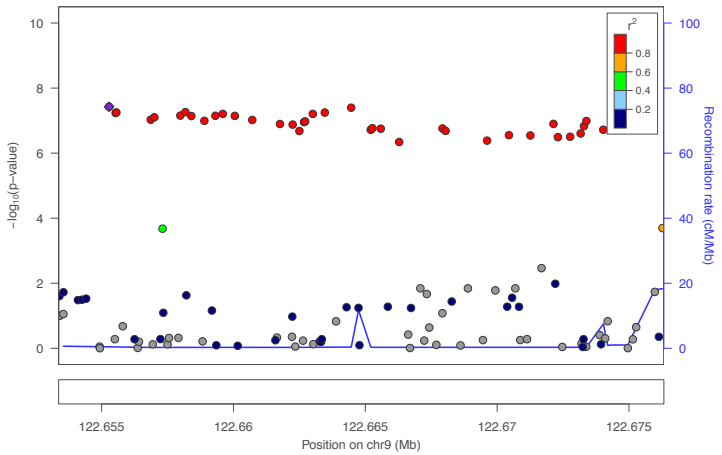

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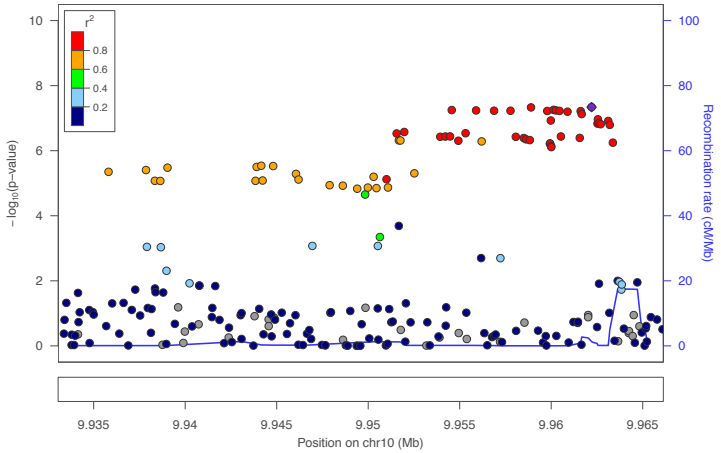

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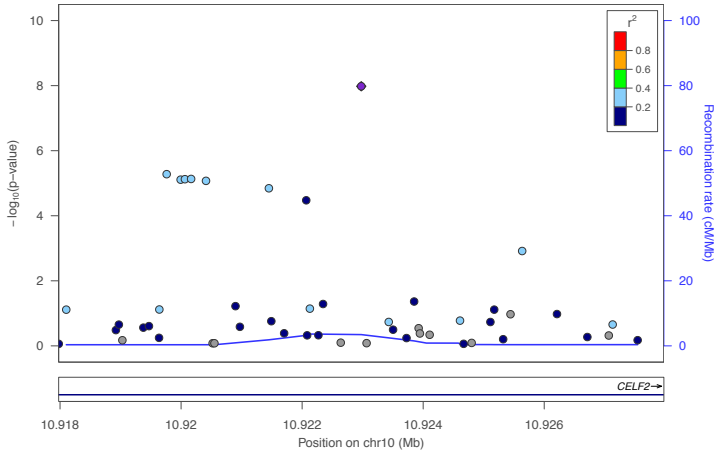

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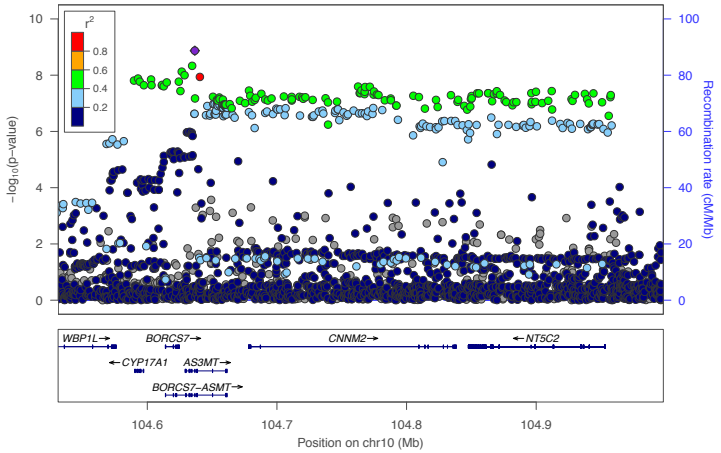

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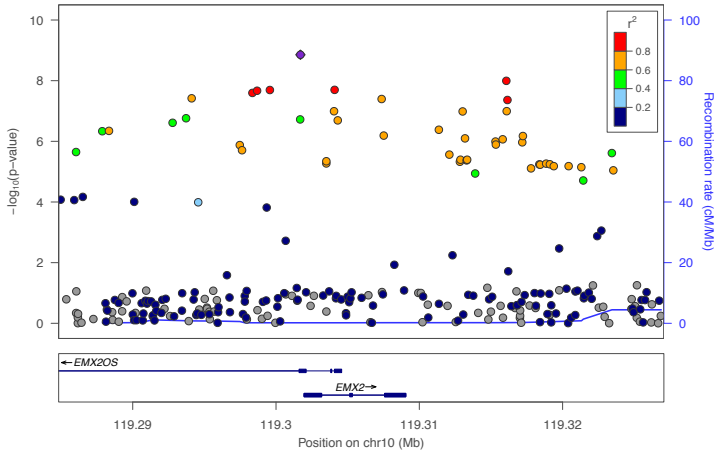

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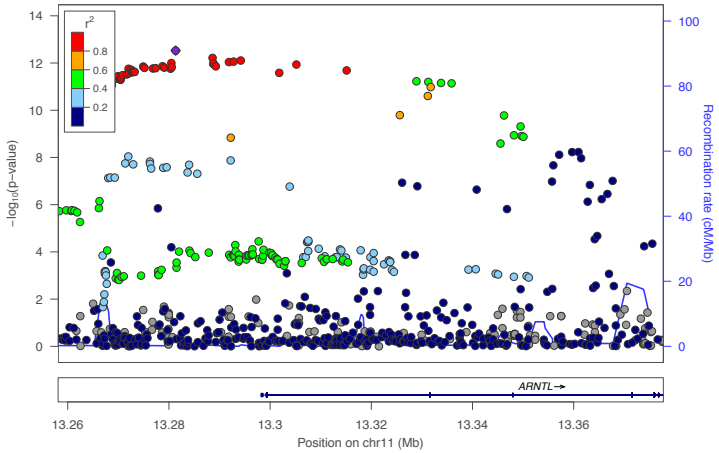

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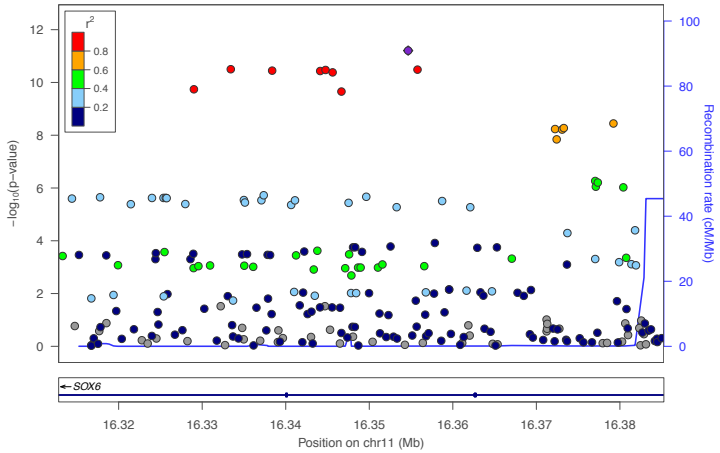

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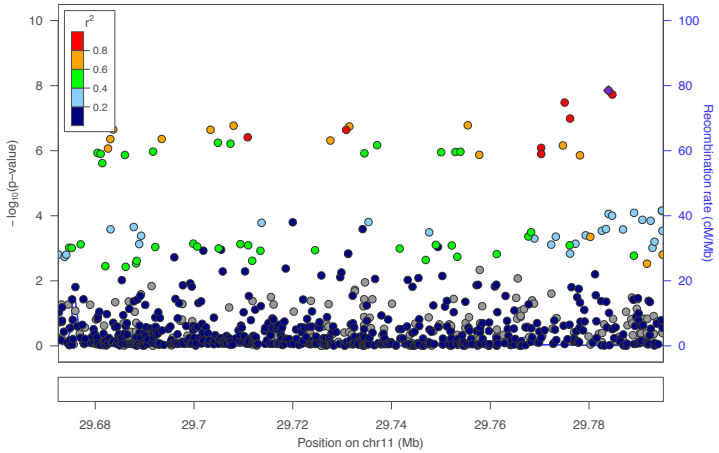

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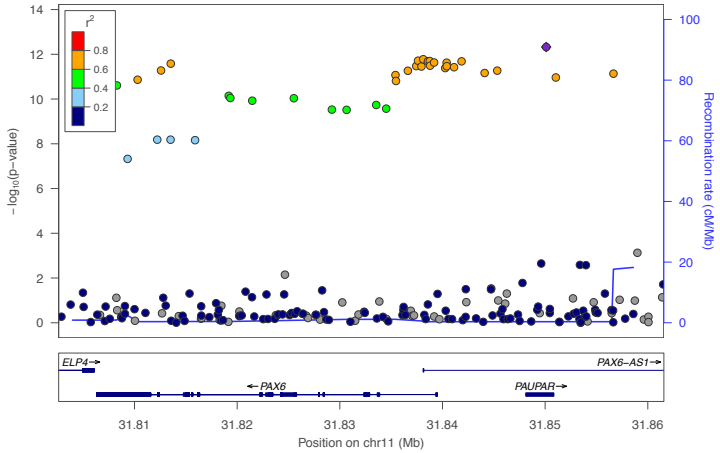

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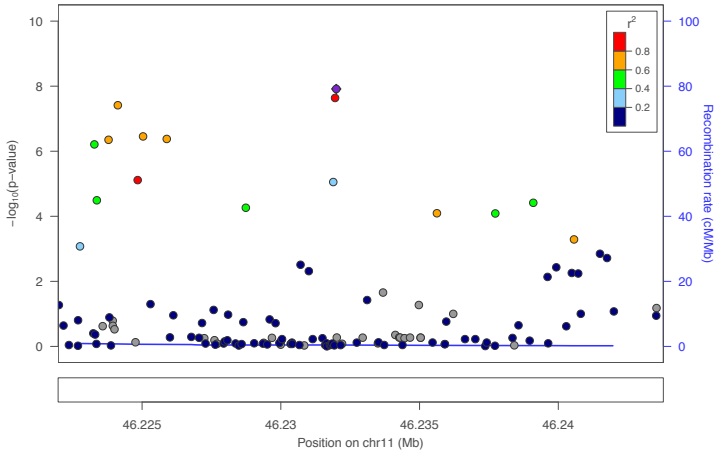

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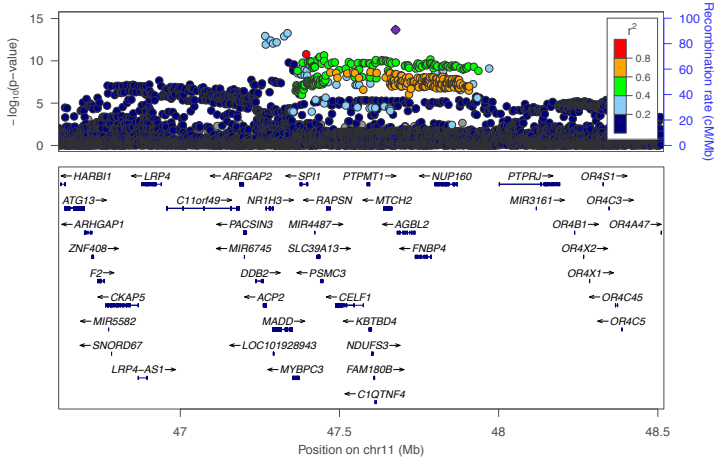

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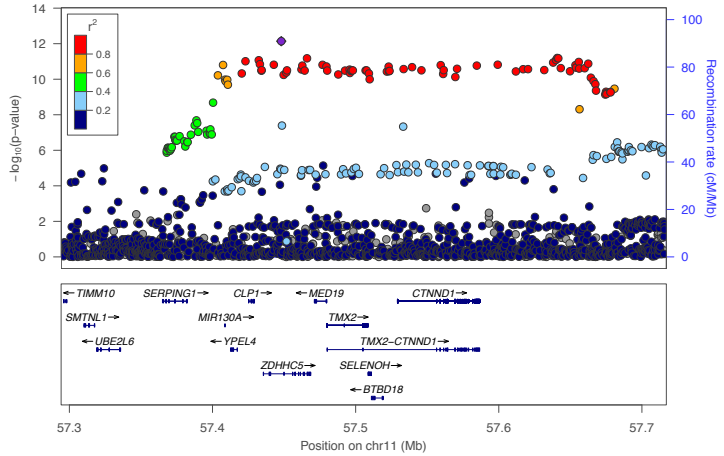

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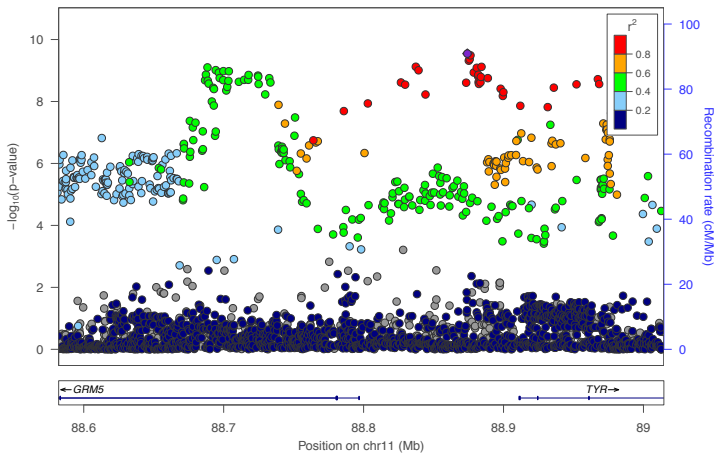

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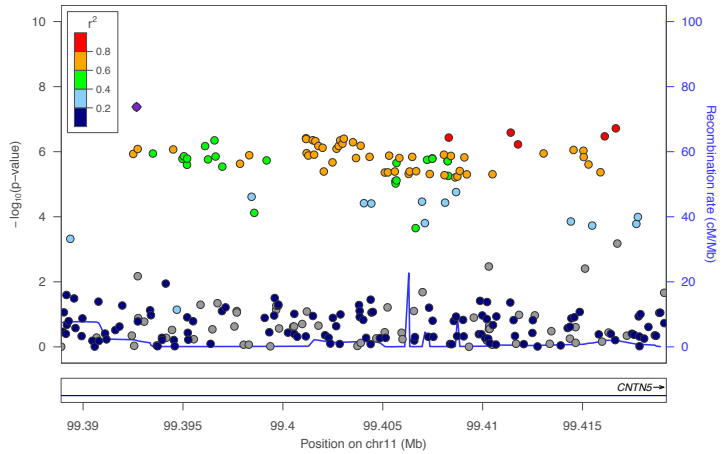

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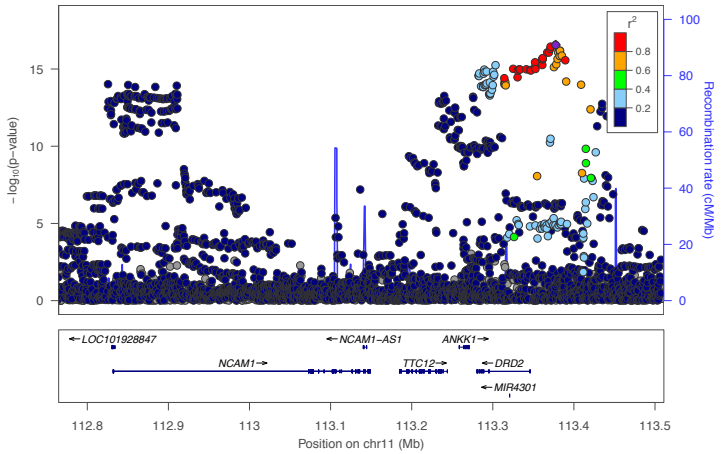

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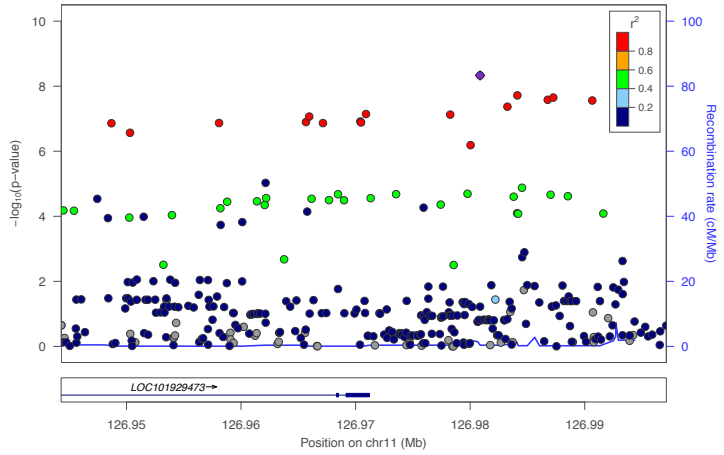

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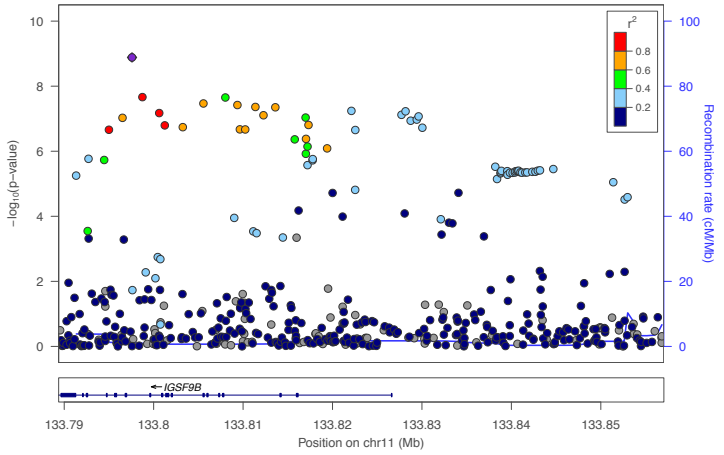

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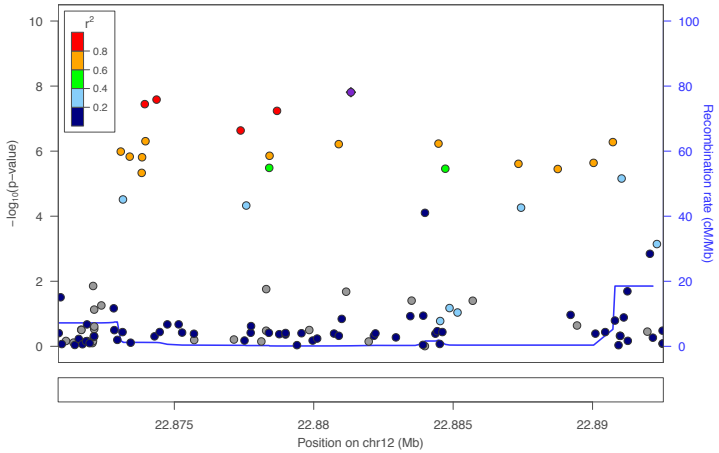

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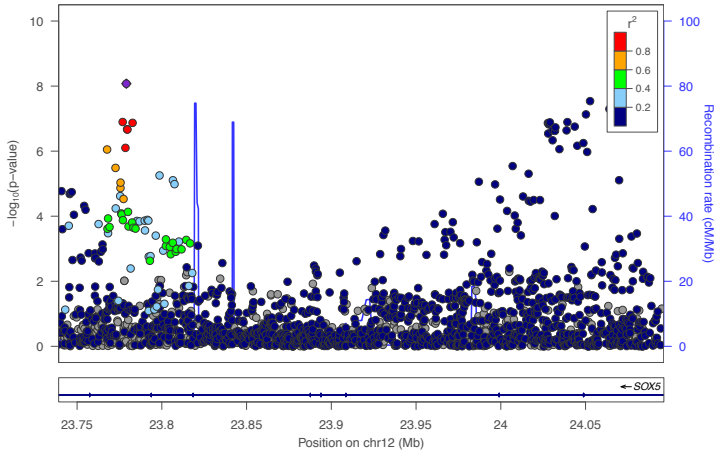

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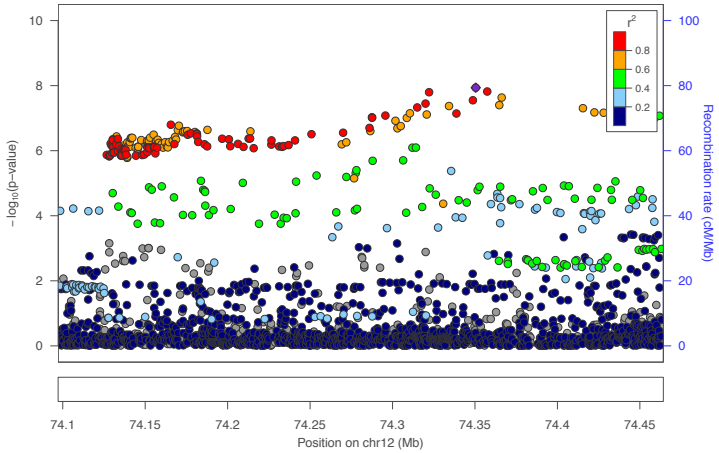

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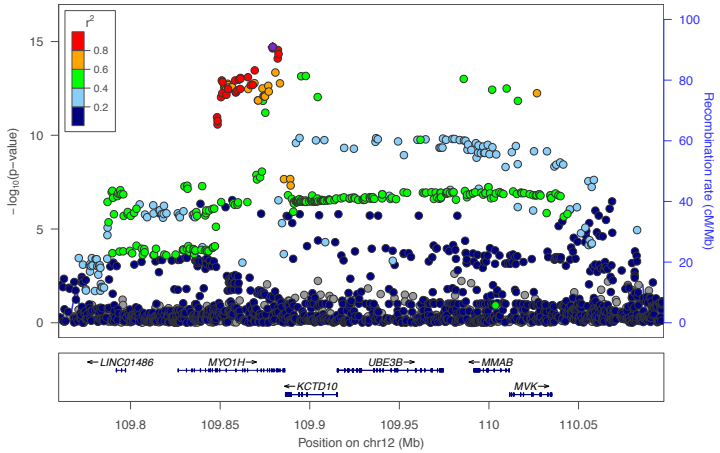

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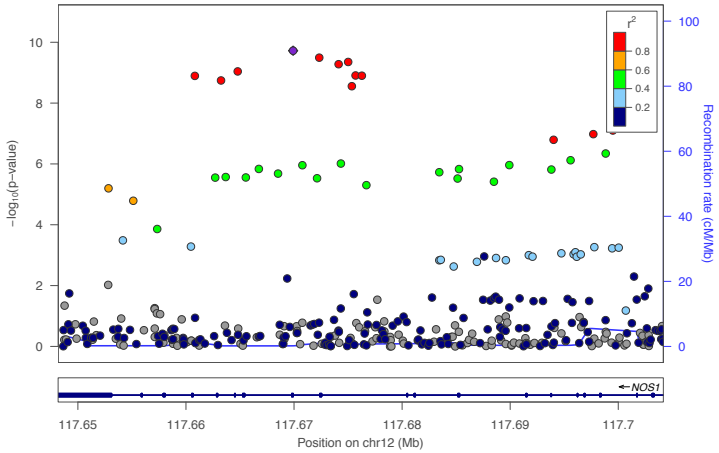

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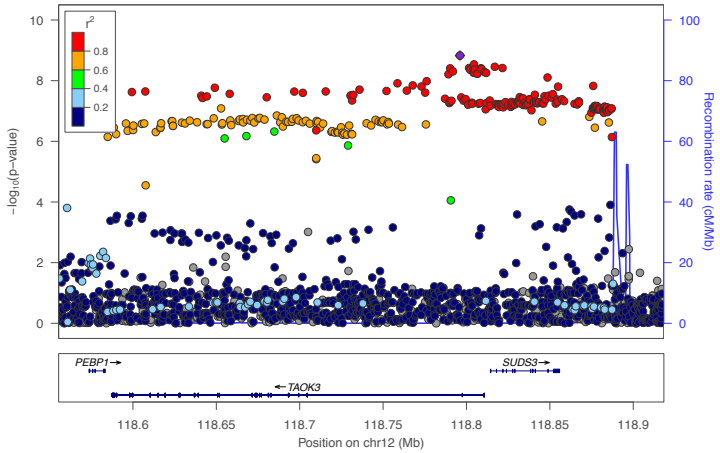

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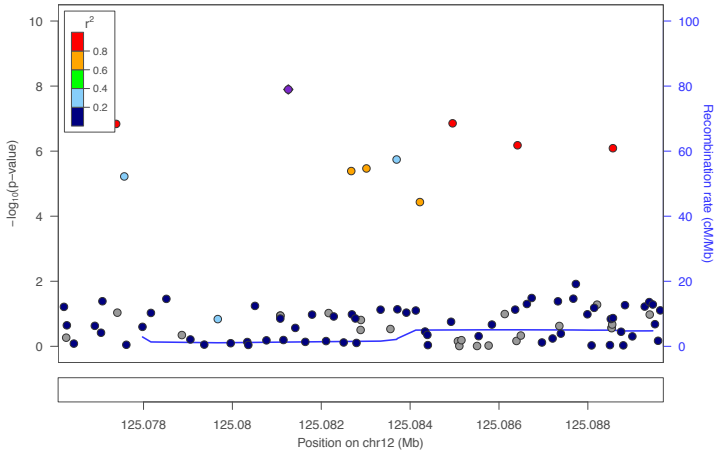

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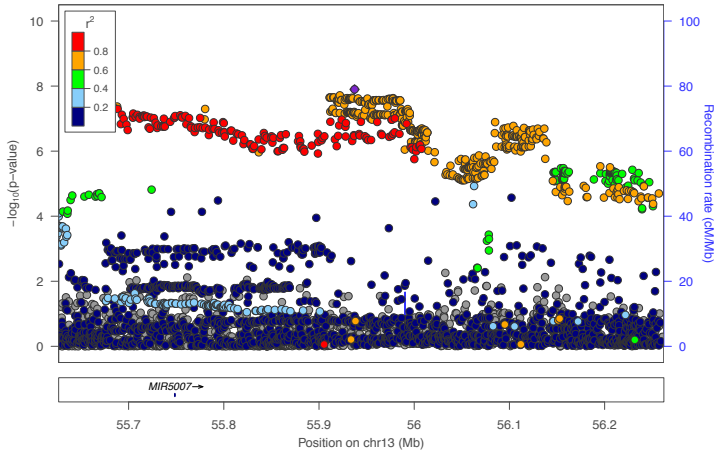

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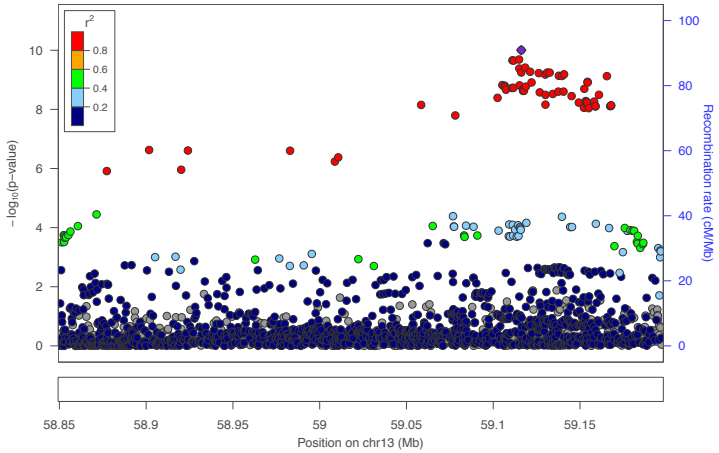

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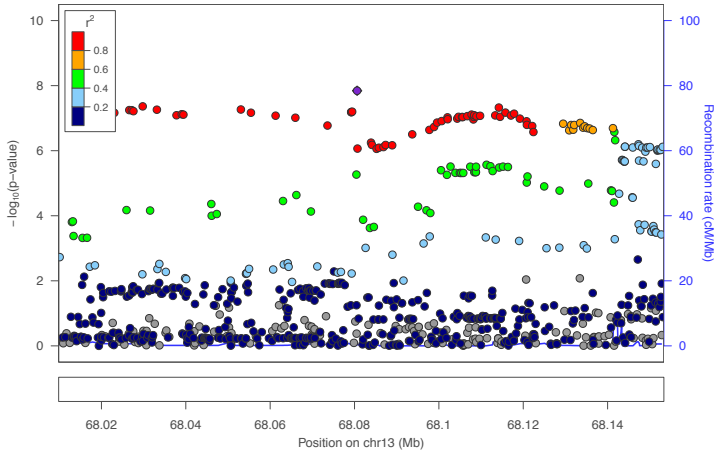

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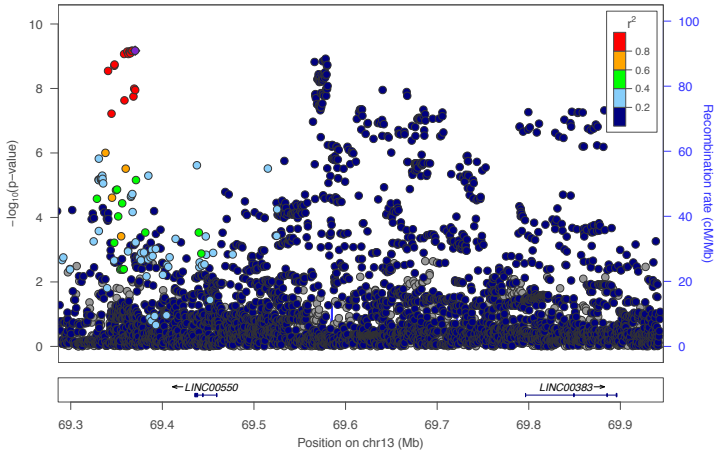

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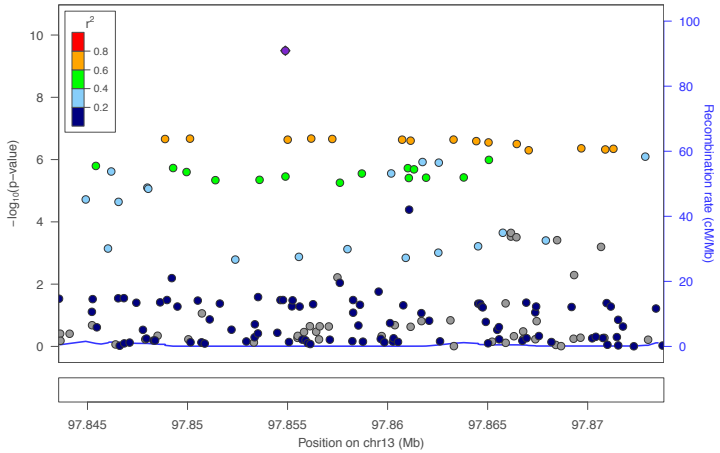

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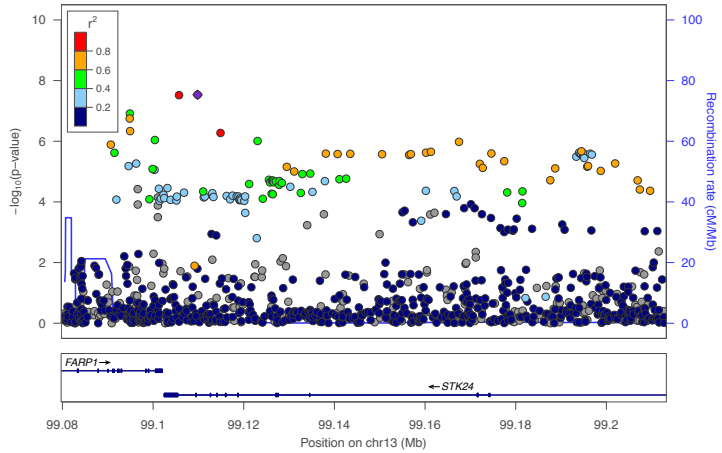

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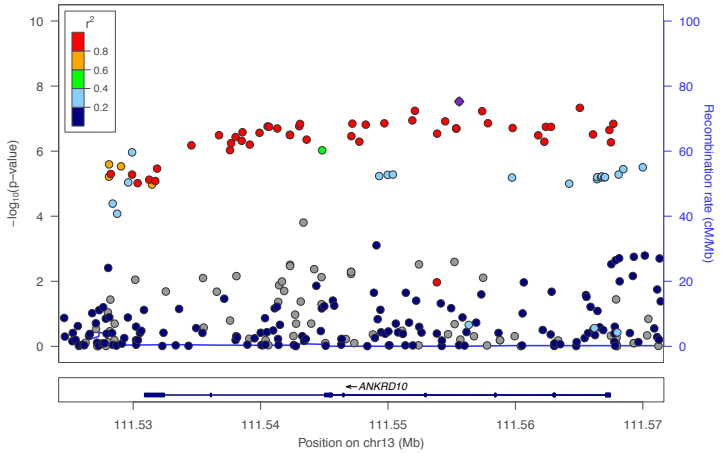

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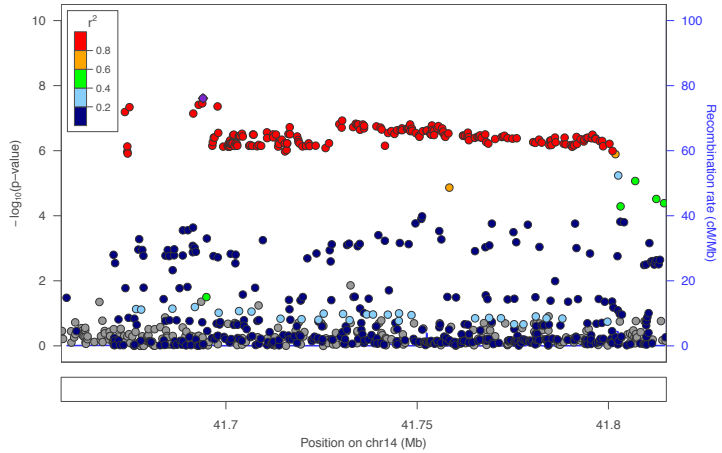

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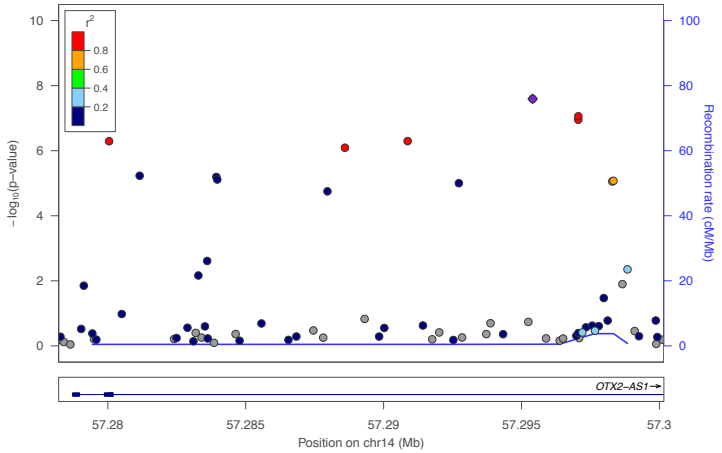

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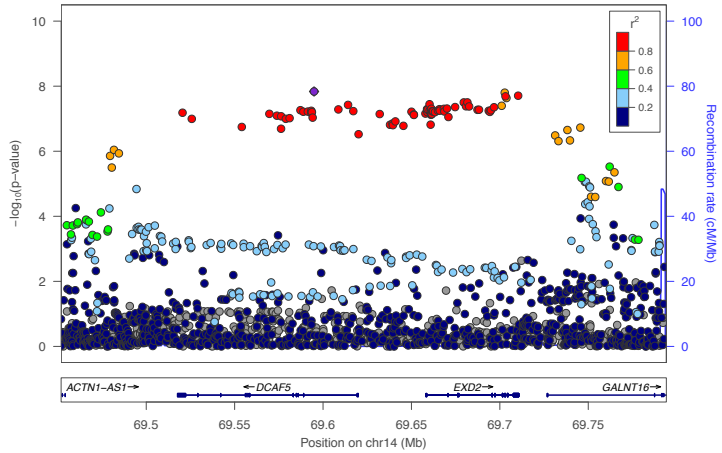

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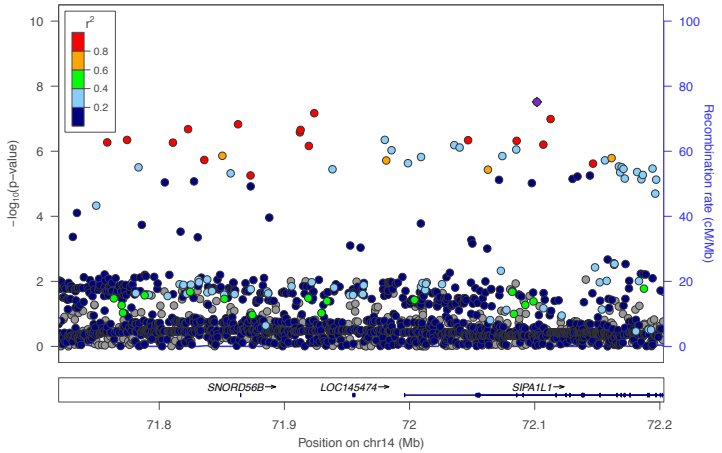

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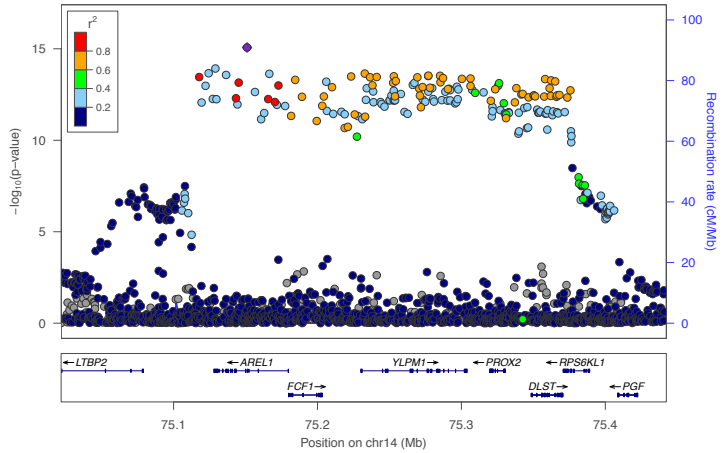

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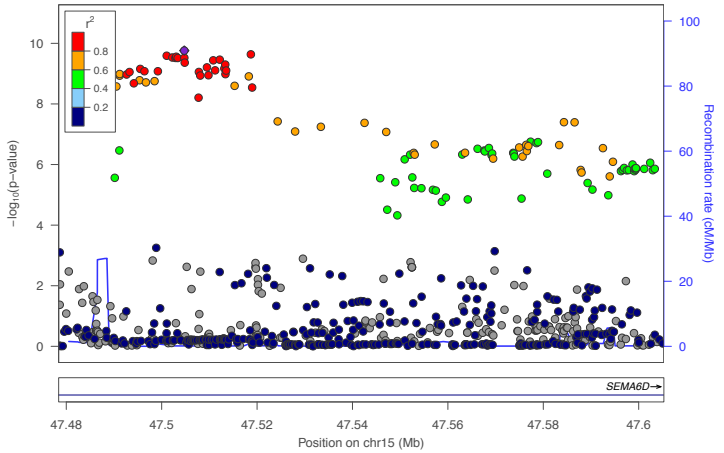

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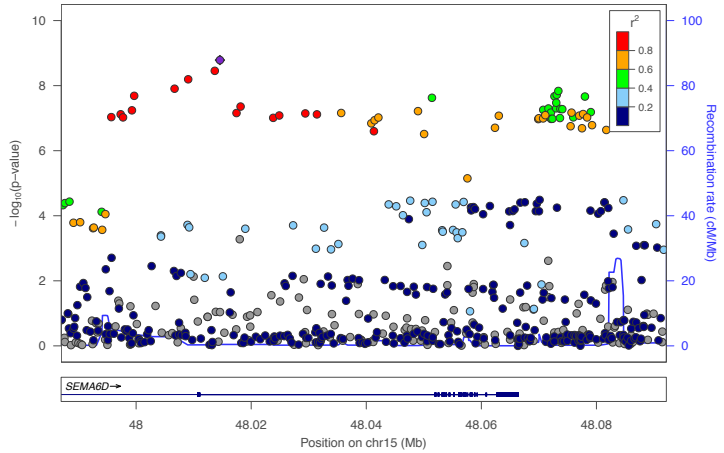

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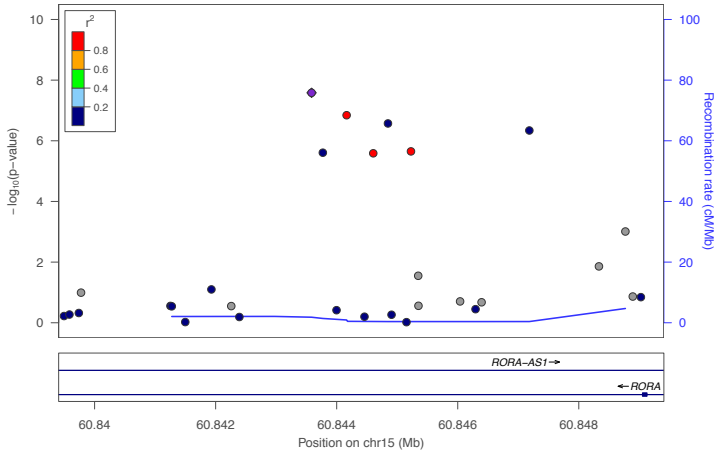

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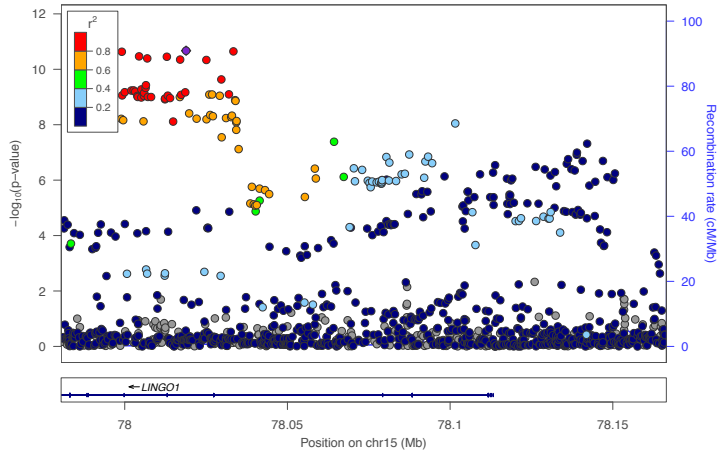

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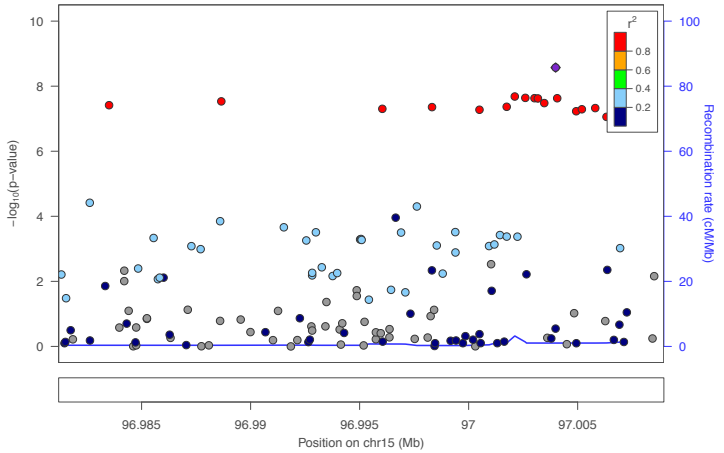

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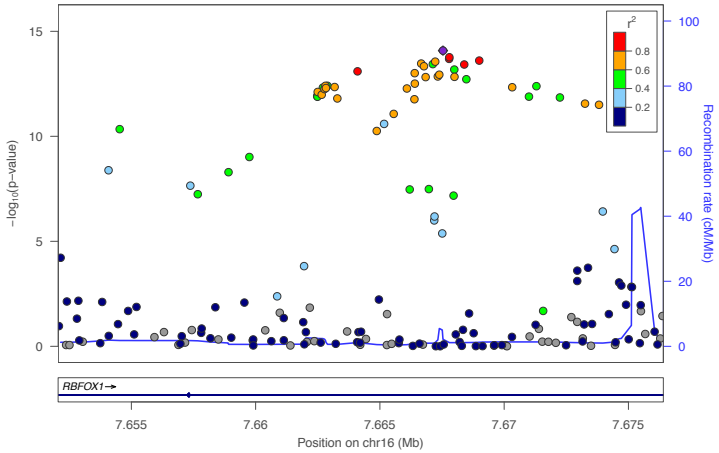

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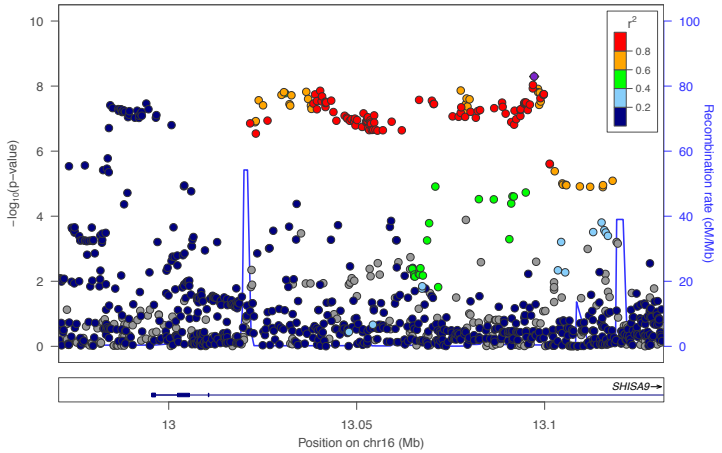

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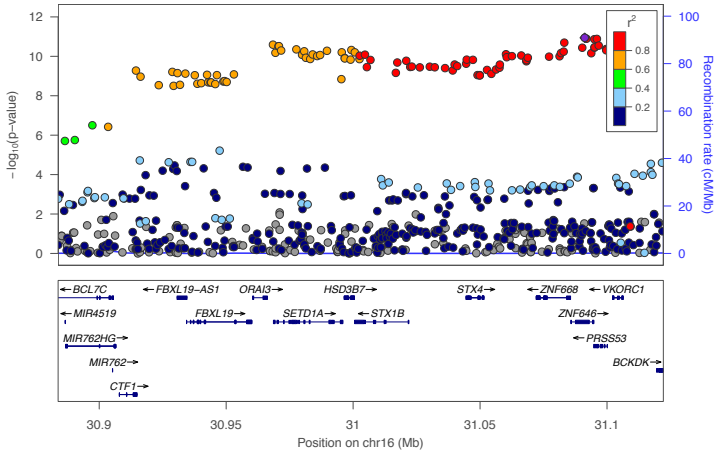

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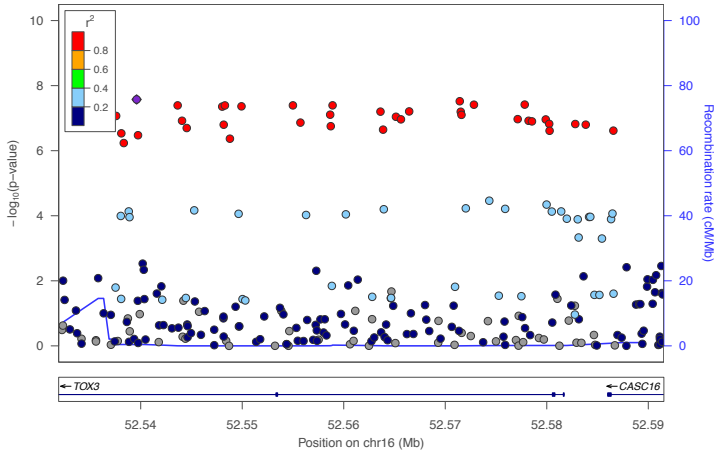

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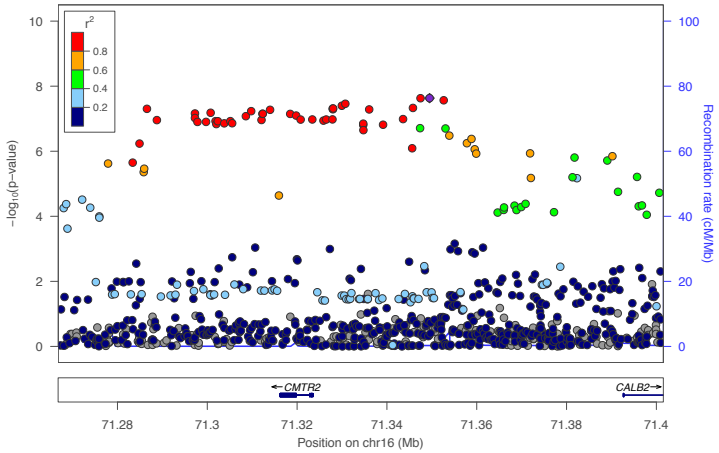

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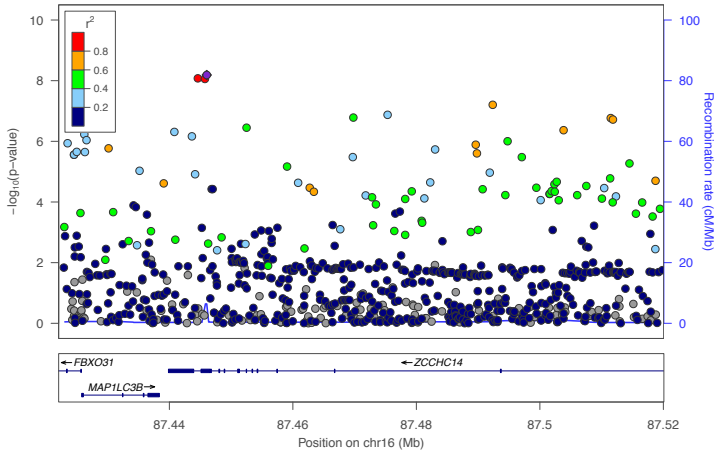

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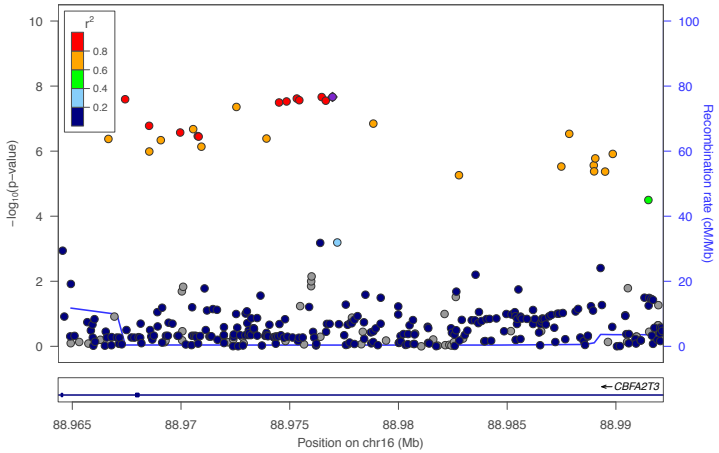

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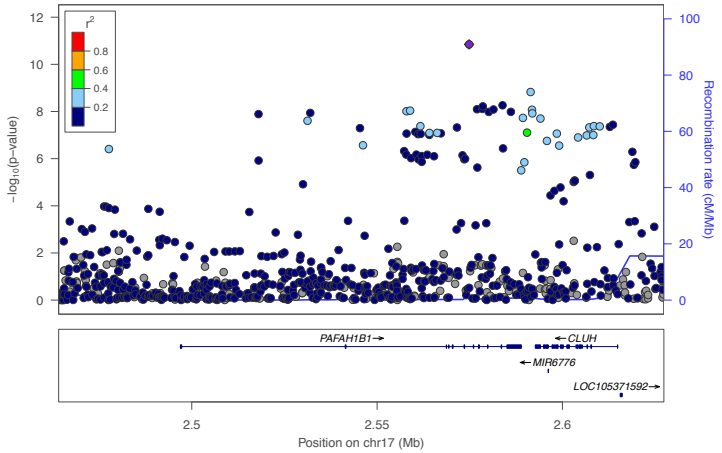

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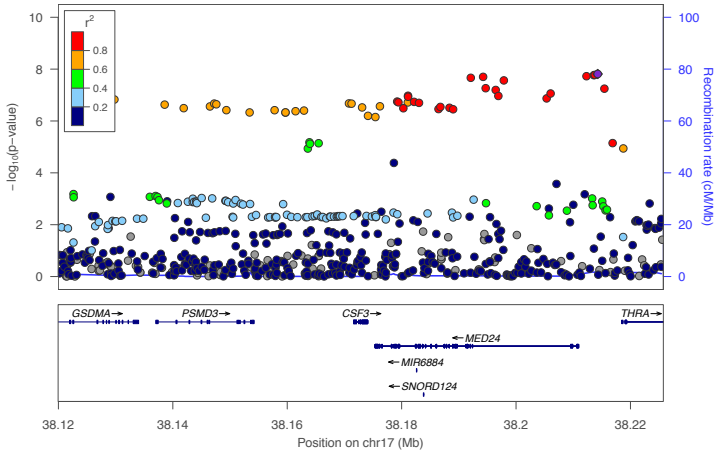

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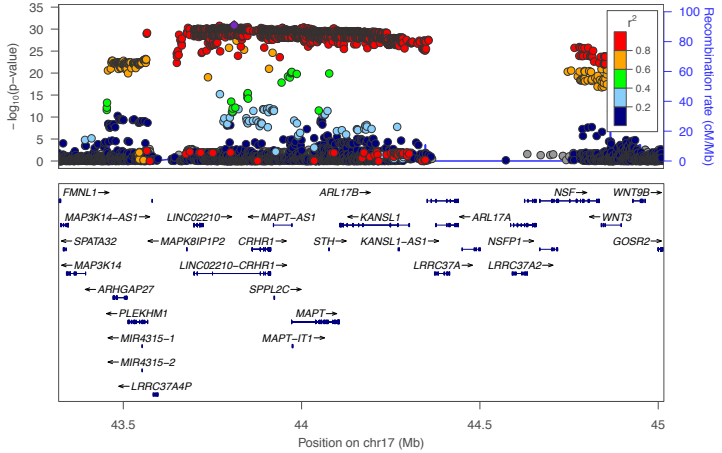

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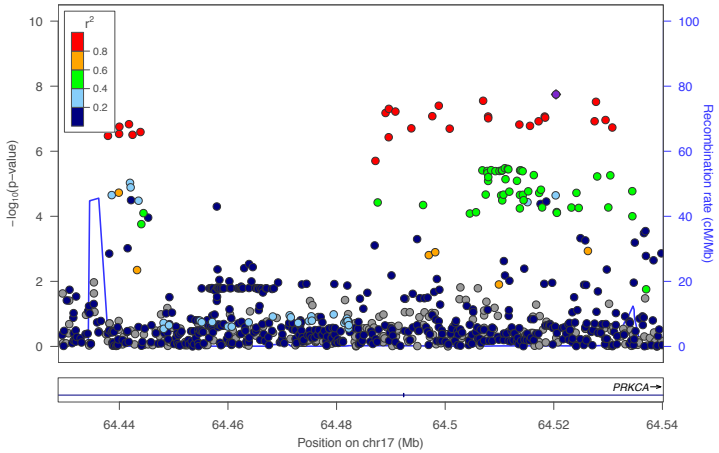

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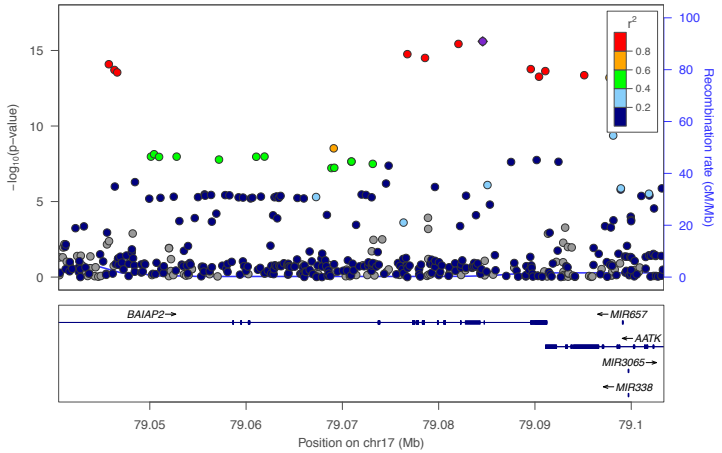

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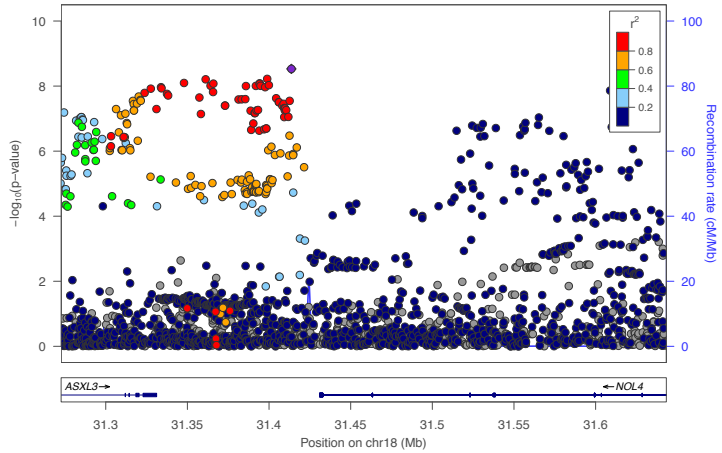

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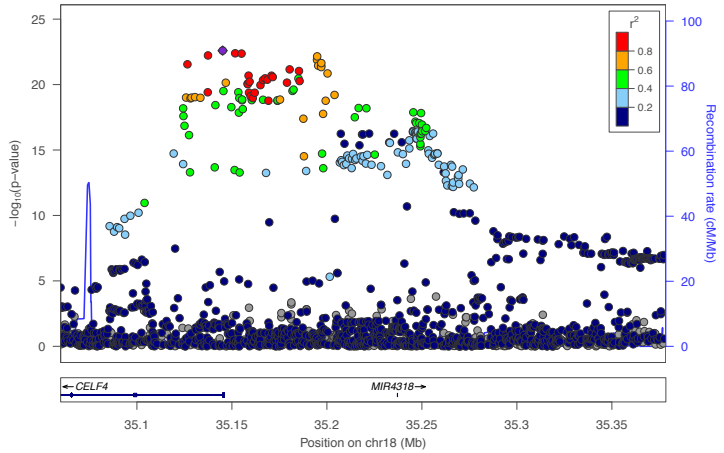

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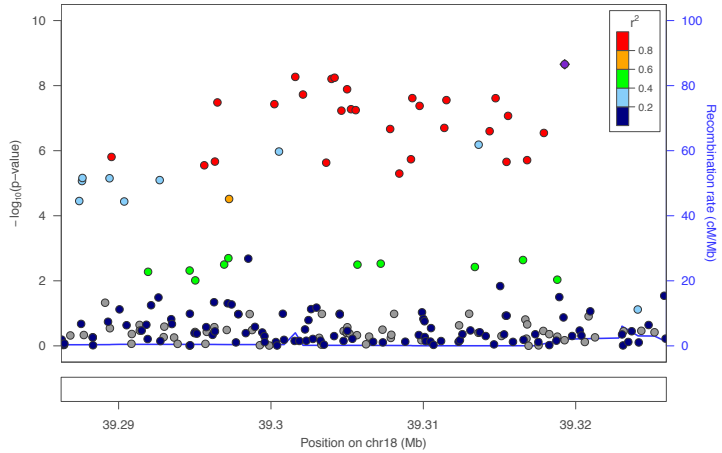

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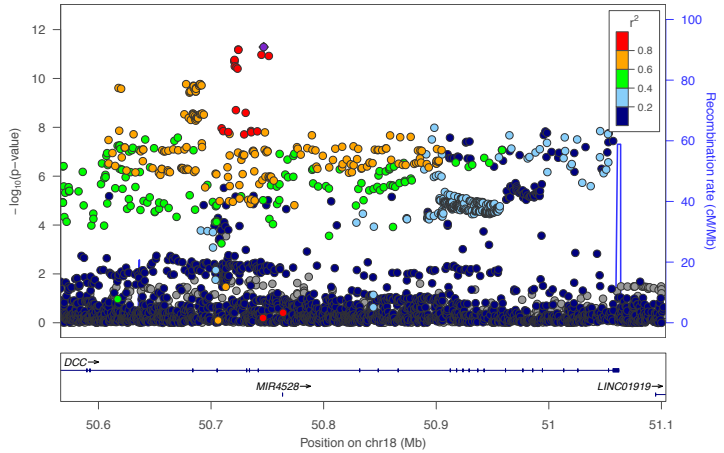

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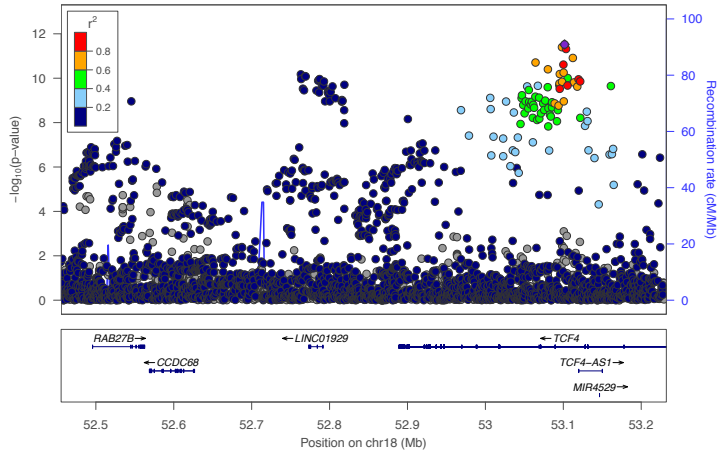

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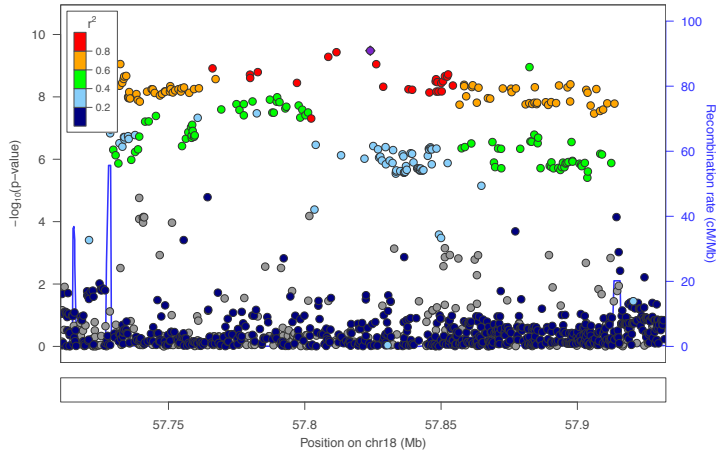

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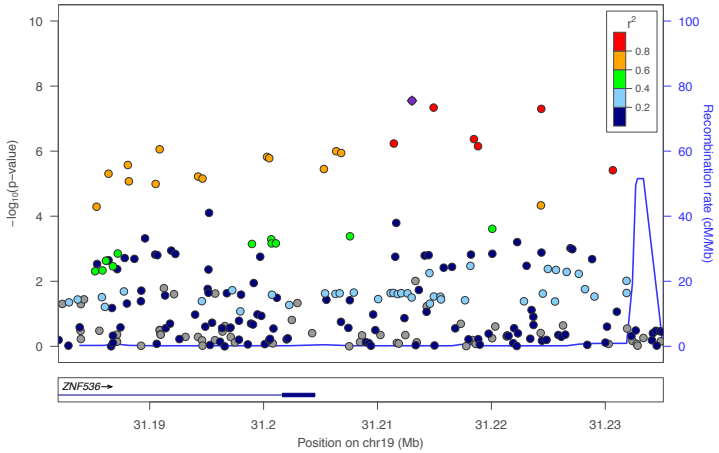

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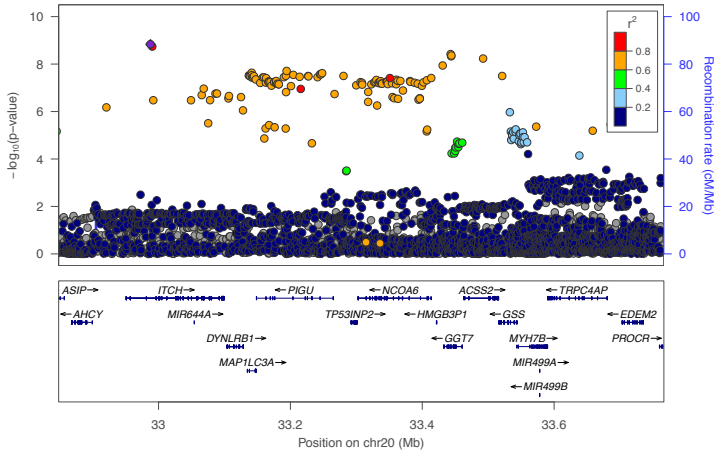

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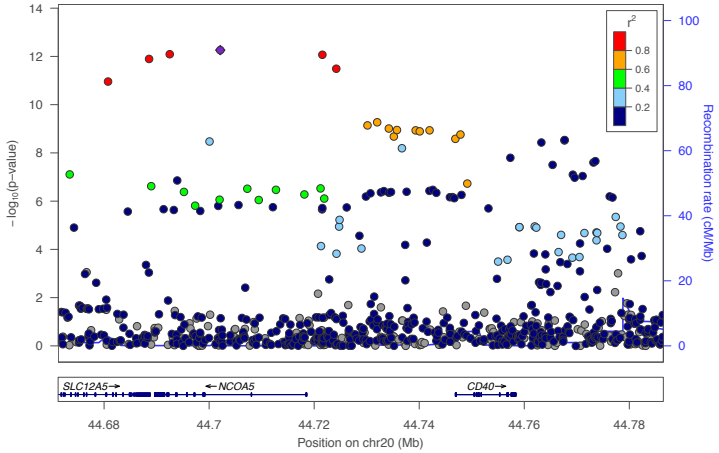

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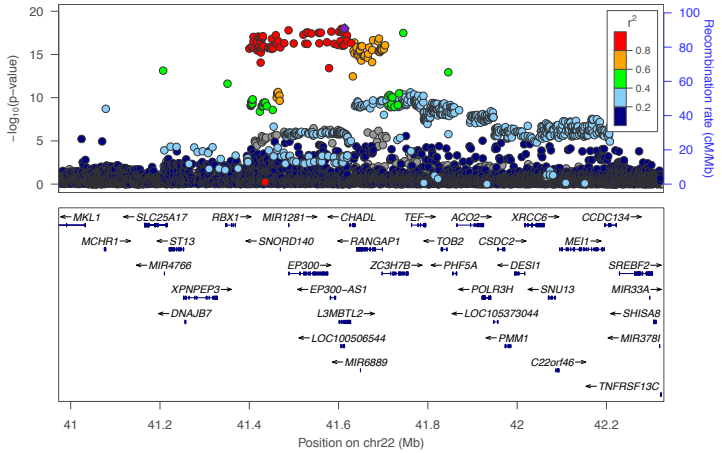
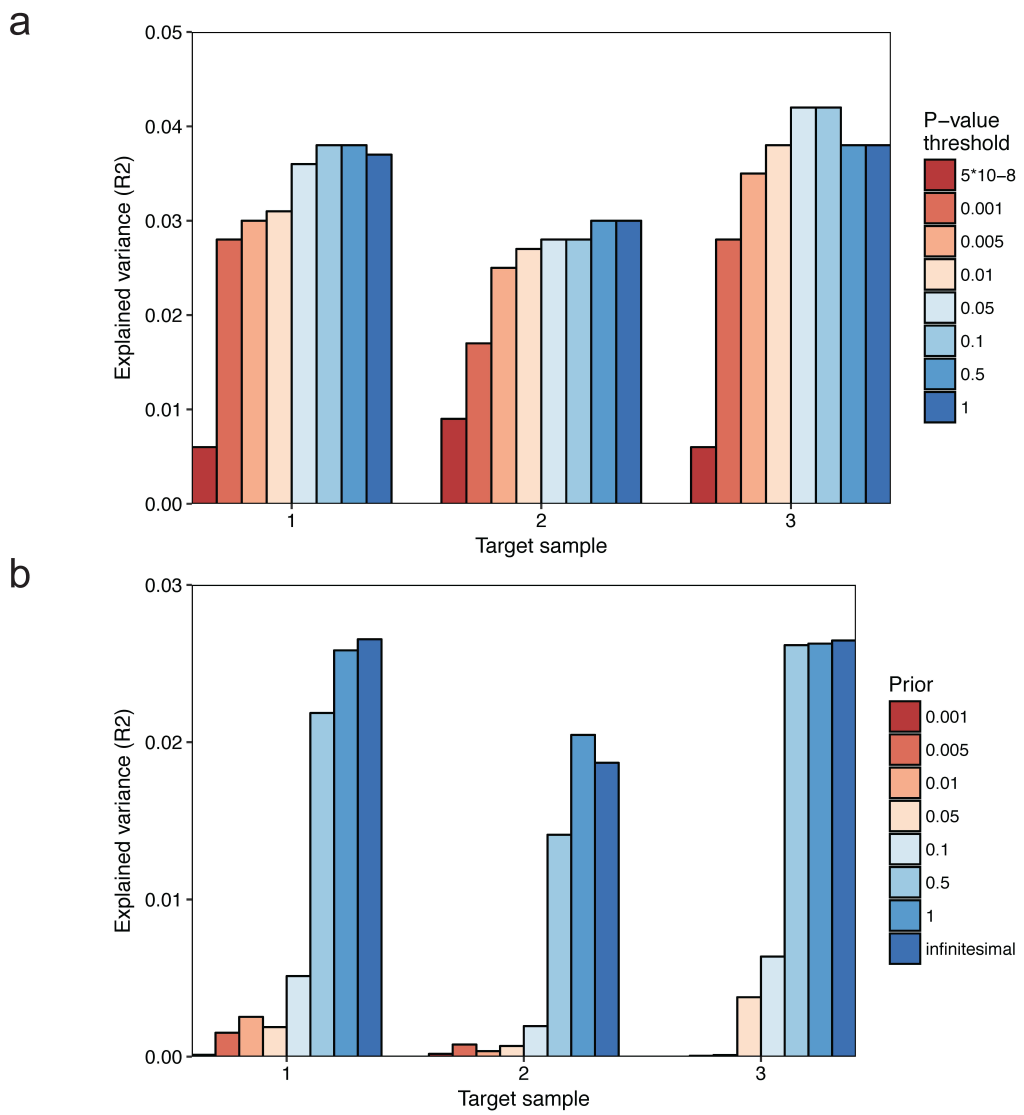


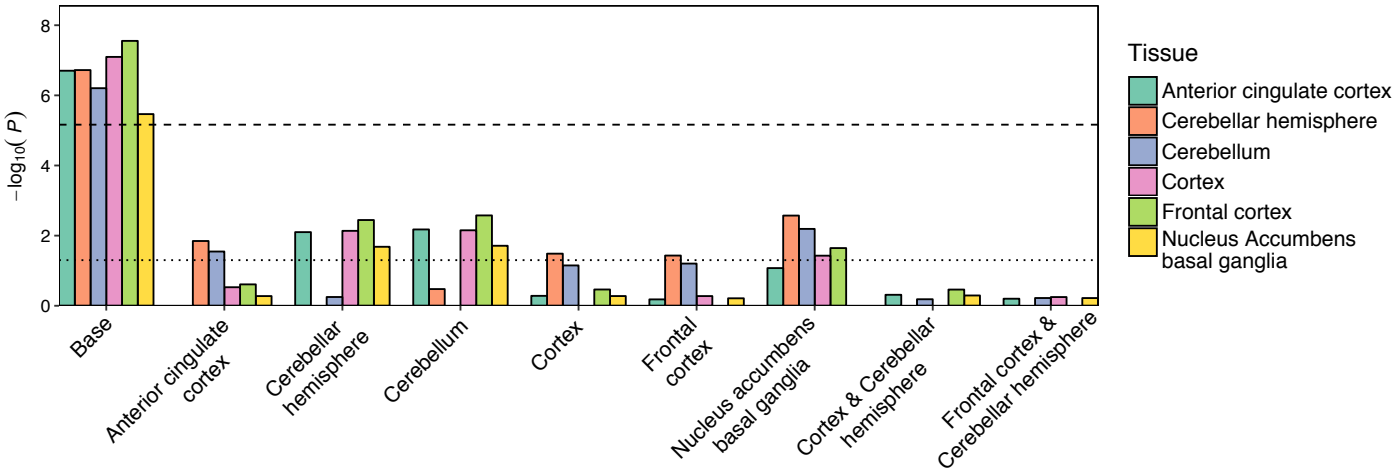
Supplementary Fig. 3. Regional association plots of the meta-analyzed SNP results for neuroticism

Regional plots were created using LocusZoom for all genomic loci identified in the GWAS meta-analysis of neuroticism (N=449,484 individuals). The *y*-axis represents the –log10 transformed SNP *P*-values and the *x*-axis indicates the genomic position. Level of LD between a SNP and the lead SNP (purple) is indicated by the color.

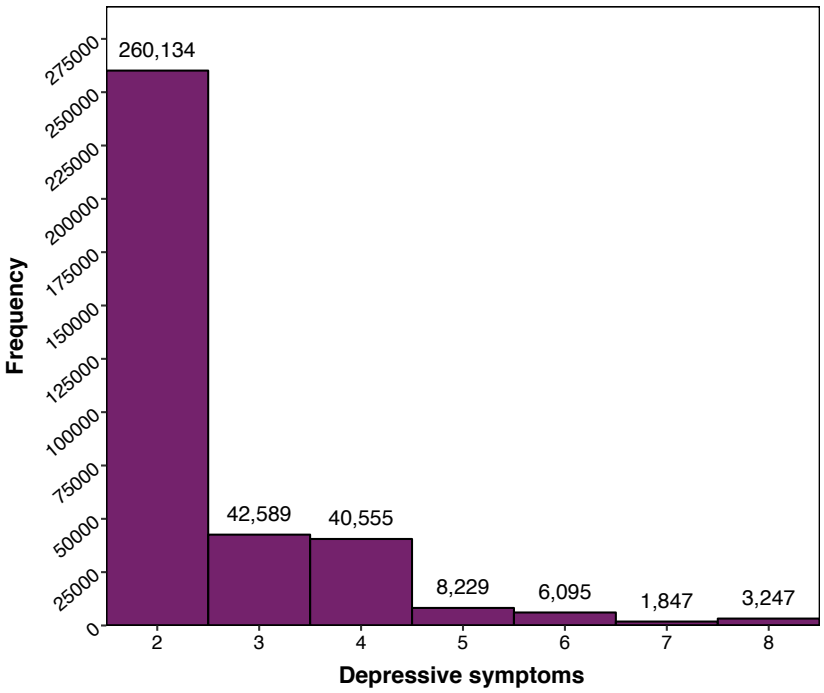


Supplementary Fig. 4. Explained variance in neuroticism by polygenic risk scores (PGS) in 3 holdout samples (N=3,000)

To estimate the variation in neuroticism that could be explained by our GWA meta-analysis in independent samples, we re-ran out GWA meta-analysis for neuroticism three times, each time excluding a UKB hold-out sample of N=3,000 randomly drawn individuals (N = 449,484-3,000 = 446,484). We then calculated polygenic scores (PGS) using two methods: **(a)** PRSice[47] (*P*-value thresholding and clumping) and **(b)** LDpred[21]. See **Supplementary Table 14**.

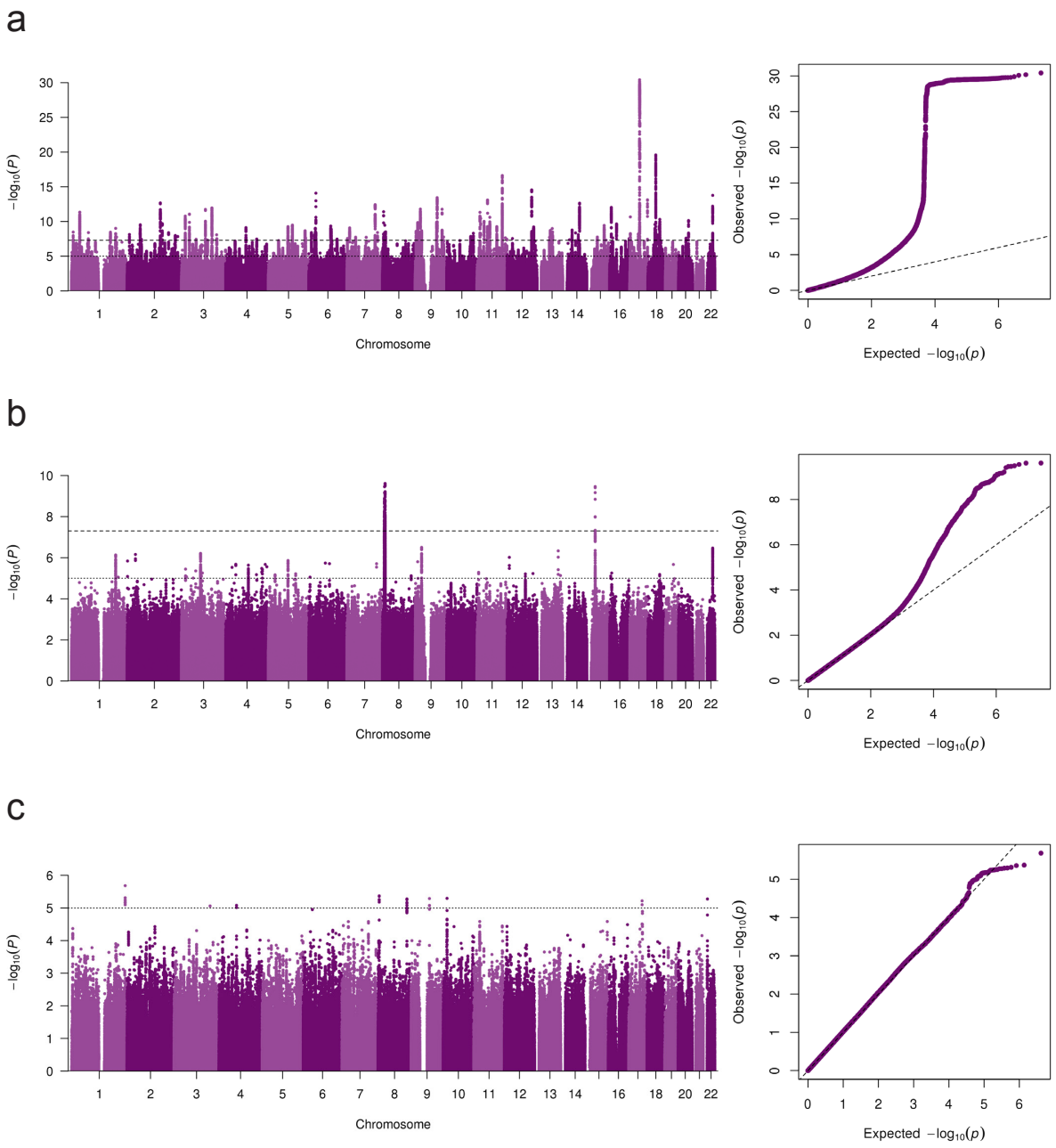


Supplementary Fig. 5. Conditional gene-set analyses on 6 significant brain tissues
Conditional gene-set analysis was conducted in MAGMA, using the gene-based analysis results as input. The dotted line indicates nominal significance at α=0.05; the dashed line indicates the Bonferroni corrected significance threshold (α=0.05/7,299=6.85×10^{-6}) used in the initial gene expression analysis (base).



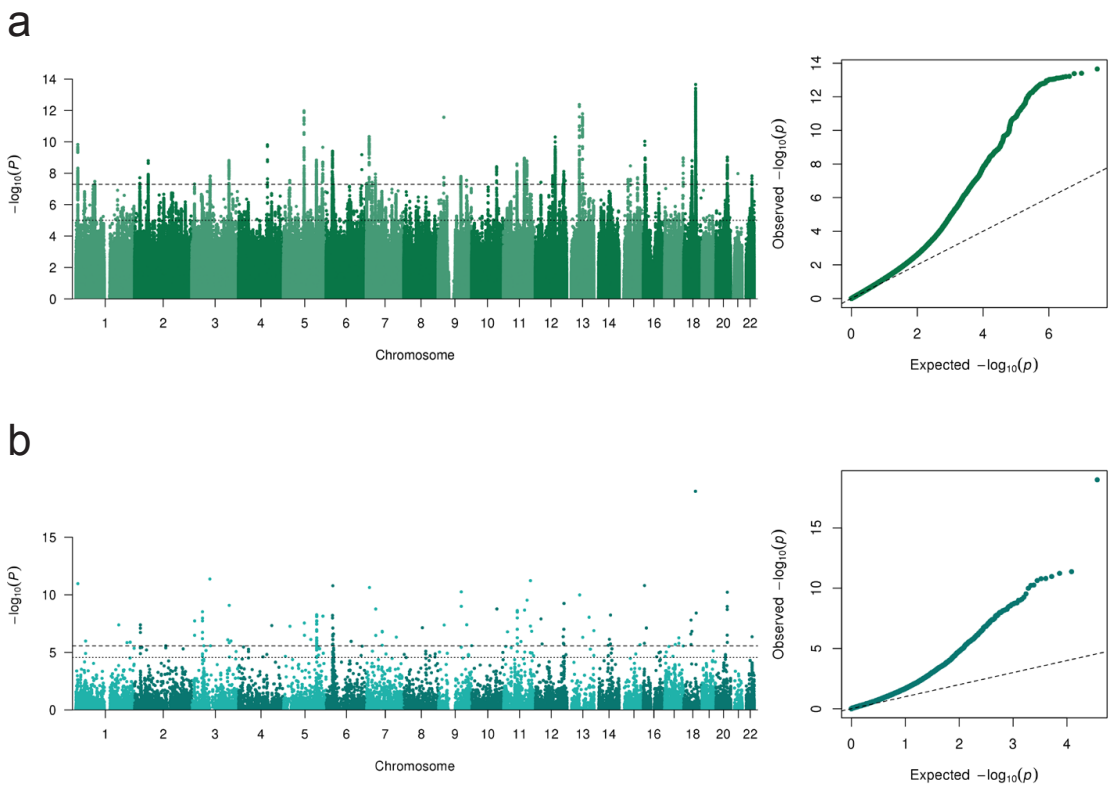
Supplementary Fig. 6. Distribution of the depressive symptoms score in the UK biobank sample

The distribution of the continuous depressive symptoms score that we calculated in the UKB sample. Depression was operationalized by adding up the scores on two continuous items (both evaluated on a 4-point Likert scale), resulting in a continuous depression score. Individuals that did not complete both items were excluded from further analysis. For the remaining participants, the depressive symptoms score was established by summing the individual item responses.



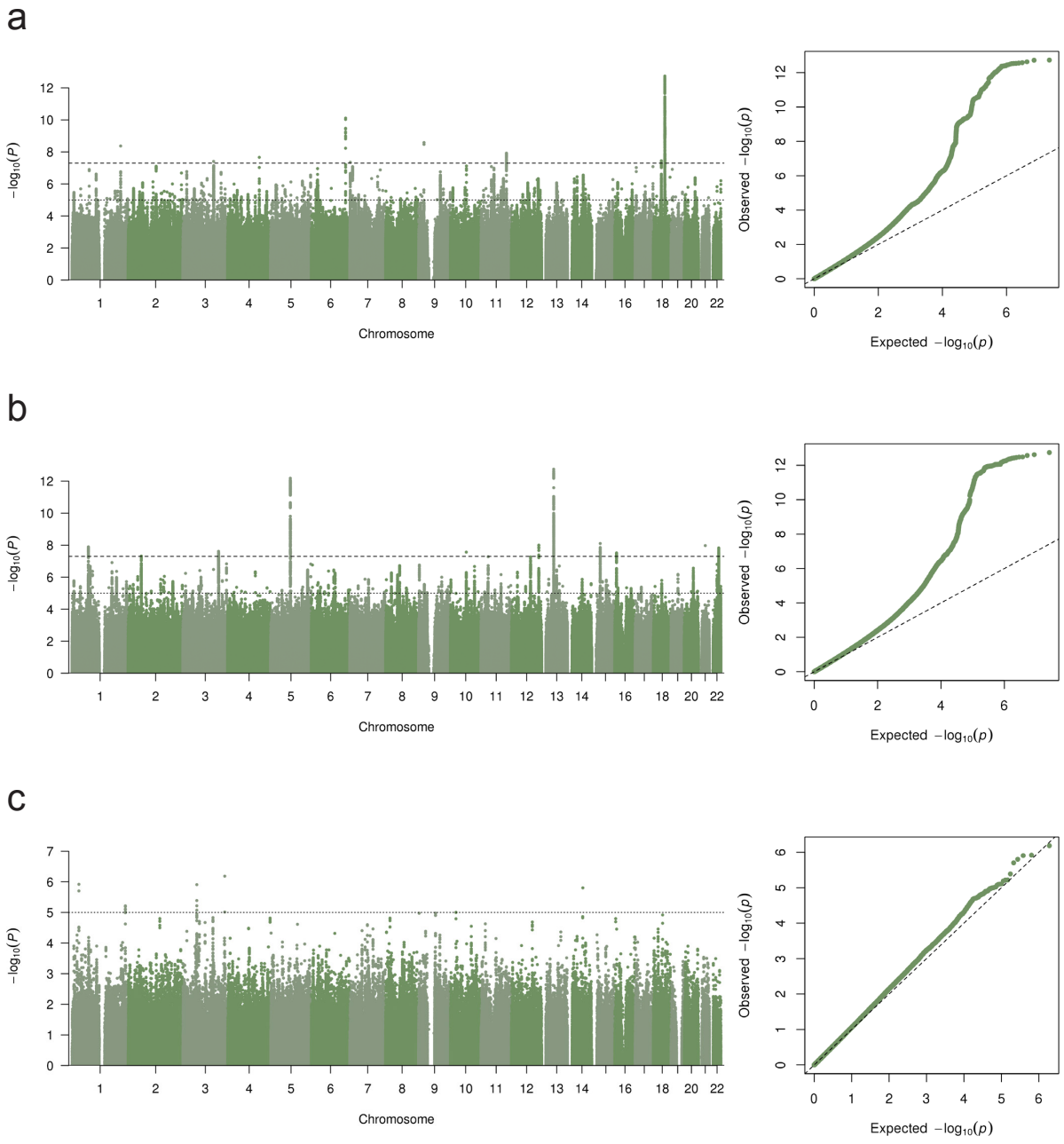
Supplementary Fig. 7. Manhattan and Q-Q plots of SNP-based association with neuroticism in the individual cohorts

SNP Association results from the GWAS on neuroticism in **(a)** UK Biobank (N=372,903 individuals) **(b)** 23andMe (N=59,206 individuals) and **(c)** GPC1 (N=17,375 individuals) cohorts. *P*-values for the UKB cohort were computed using a linear regression model of additive allelic effects and covariates in PLINK (see **Online Methods** for covariates and more information on the 23andMe and GPC1 cohorts). Dashed lines indicate genome-wide significance ($P<5\times10^{-8}$) and dotted lines indicate the 'suggestive' significance threshold ($P<1\times10^{-5}$).



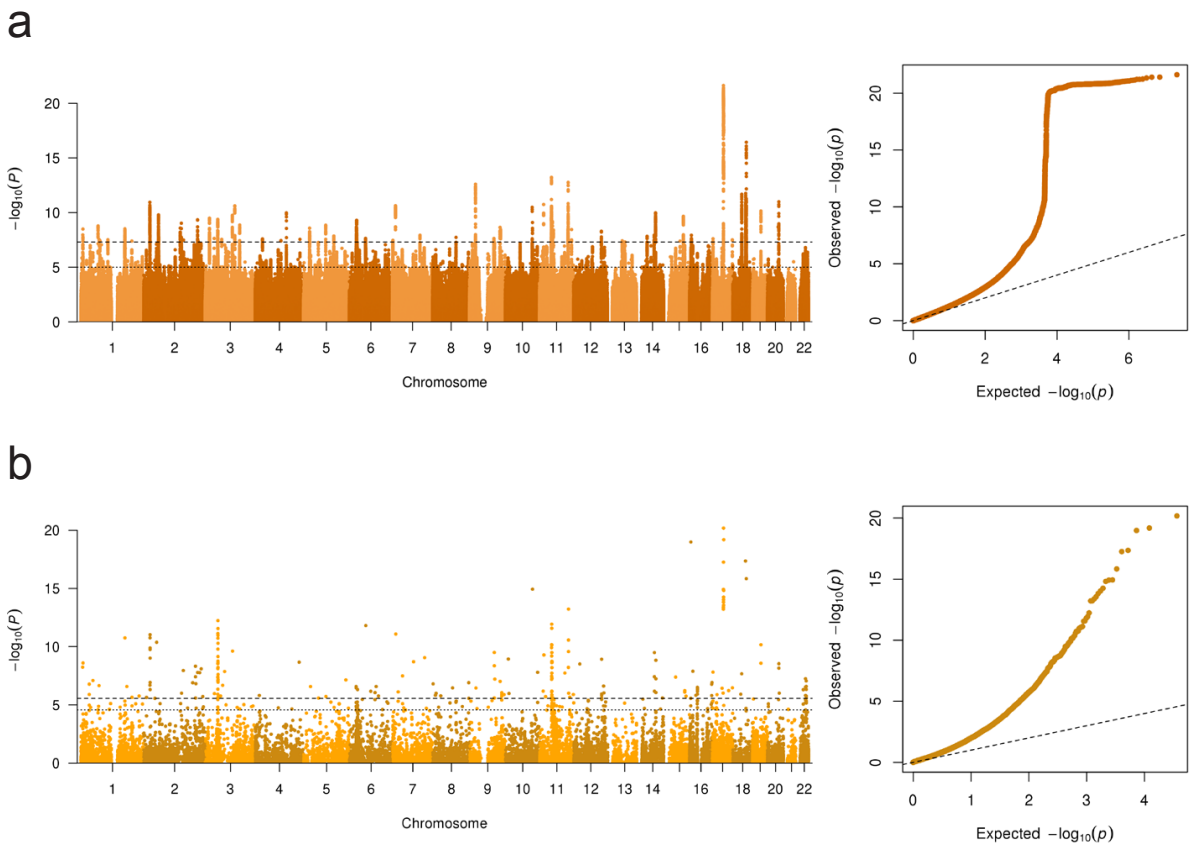
Supplementary Fig. 8. Manhattan and Q-Q plots of SNP- and gene-based association with depression (N=688,809 individuals)

(a) SNP-based association results from the GWA meta-analysis (UK biobank, 23andMe and PGC) on depression. SNP *P*-values were computed in METAL using a two-sided, weighted z-score method. Dashed lines indicate genome-wide significance ($P<5\times10^{-8}$) and dotted lines indicate the 'suggestive' significance threshold ($P<1\times10^{-5}$). An overview of these genome-wide significant (GWS) SNPs and their *P*-values can be found in **Supplementary Table 24**. **(b)** Gene-based association results from the meta-analysis (UK biobank, 23andMe and PGC) on depression. Gene-based *P*-values were computed using MAGMA's gene-based test (where the summary statistics from GWAS were used as input). Dashed lines indicate genome-wide significance ($P<2.75\times10^{-6}$) and dotted lines indicate the 'suggestive' significance threshold ($P<2.75\times10^{-5}$). An overview of these GWS genes and their *P*-values can be found in **Supplementary Tables 16** and **28**.



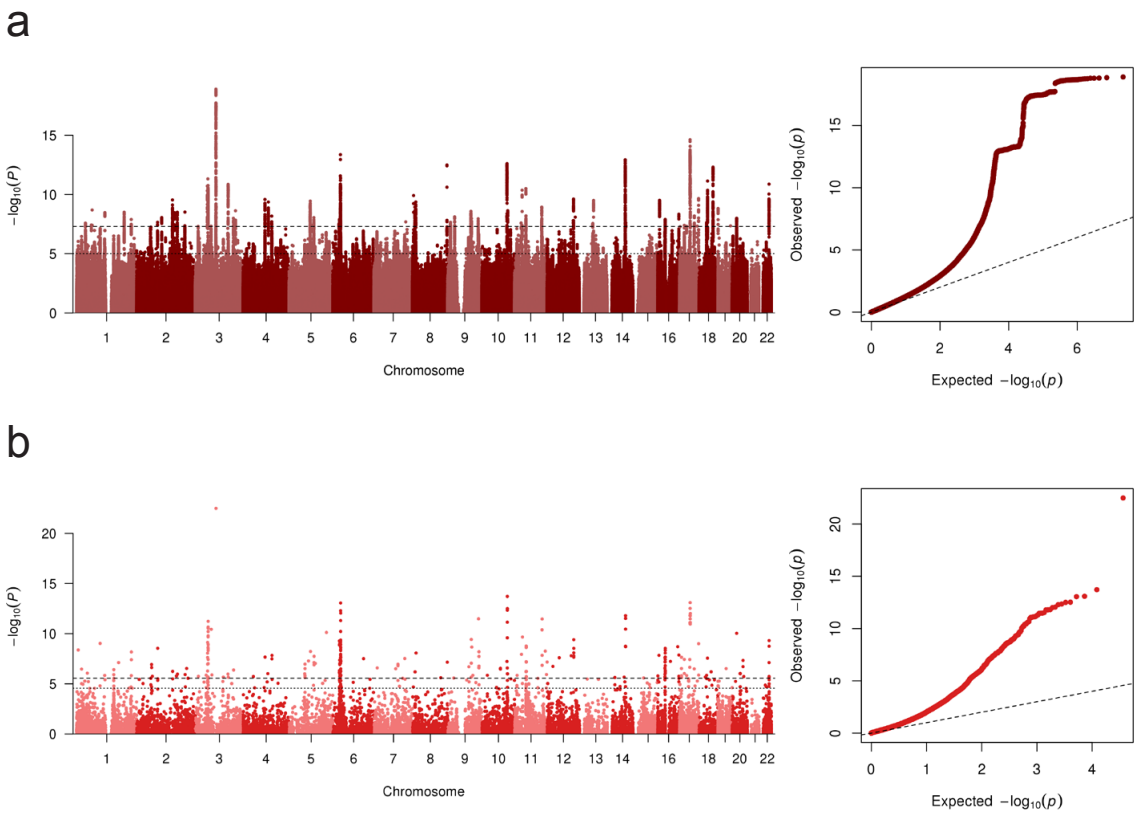
Supplementary Fig. 9. Manhattan and Q-Q plots of SNP-based association with depression in the individual cohorts

SNP Association results from the GWAS on depression in **(a)** UK Biobank (N=362,696 individuals) **(b)** 23andMe (N=307,354 individuals) and **(c)** PGC (N=18,759 individuals) cohorts. *P*-values for the UKB cohort were computed using a linear regression model of additive allelic effects and covariates in PLINK (see **Online Methods** for covariates and more information on the 23andMe and PGC cohorts). Dashed lines indicate genome-wide significance ($P<5\times10^{-8}$) and dotted lines indicate the 'suggestive' significance threshold ($P<1\times10^{-5}$).



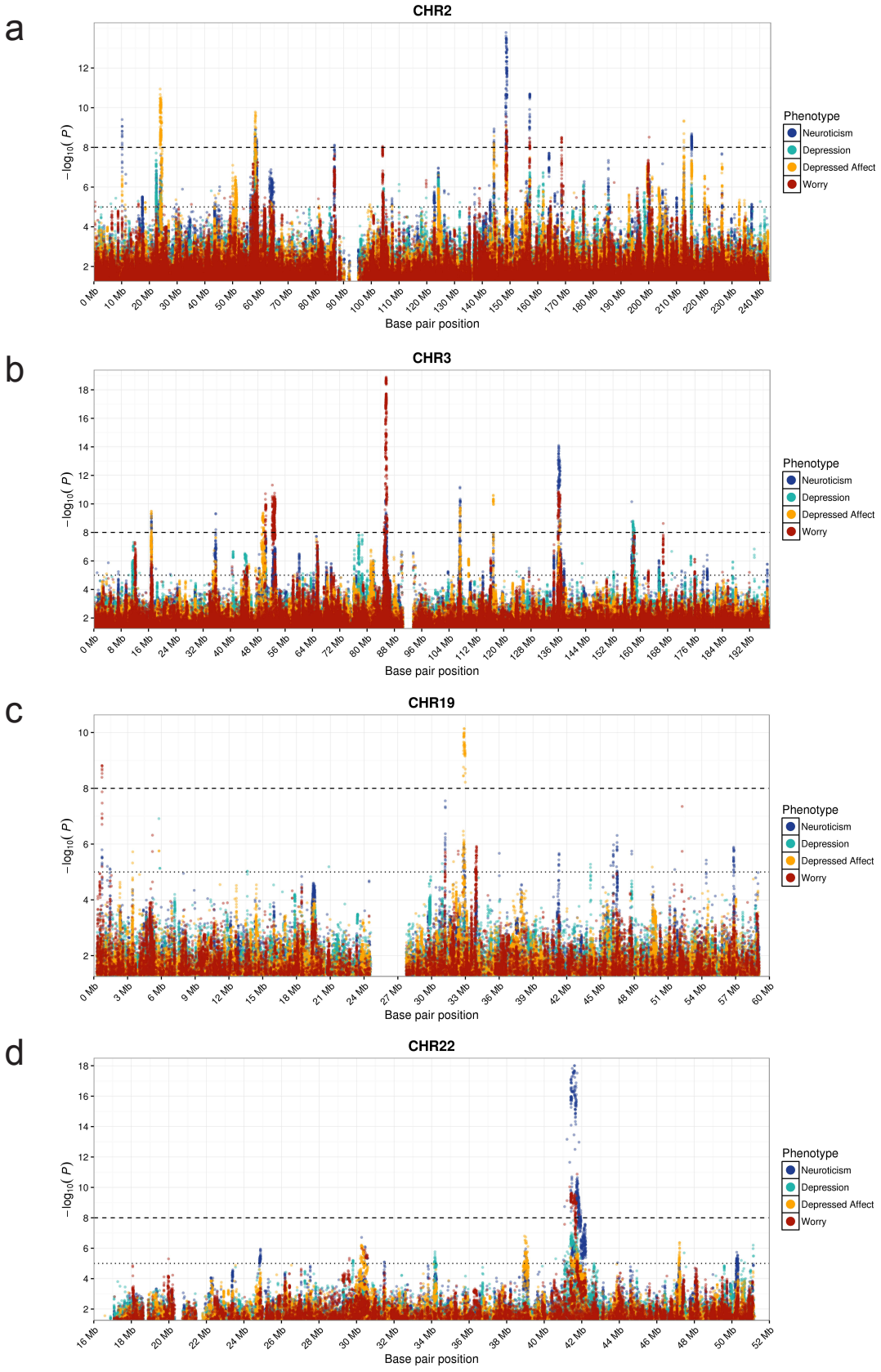
Supplementary Fig. 10. Manhattan and Q-Q plots of SNP- and gene-based association with *depressed affect* (N=357,957 individuals)

(a) SNP-based association results from the GWAS on *depressed affect* in the UK biobank sample SNP *P*-values were computed using a linear regression model of additive allelic effects and covariates in PLINK. Dashed lines indicate genome-wide significance ($P<5\times10^{-8}$) and dotted lines indicate the 'suggestive' significance threshold ($P<1\times10^{-5}$). **(b)** Gene-based association results from the GWAS on *depressed affect* in the UK biobank sample. Gene-based *P*-values were computed in MAGMA using a two-sided, weighted z-score method. Dashed lines indicate genome-wide significance ($P<2.75\times10^{-6}$) and dotted lines indicate the 'suggestive' significance threshold ($P<2.75\times10^{-5}$).



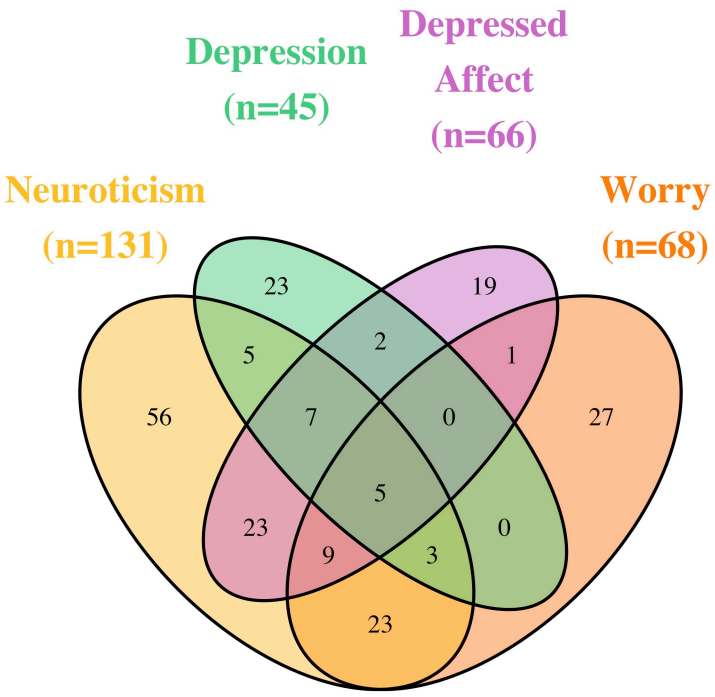
Supplementary Fig. 11. Manhattan and Q-Q plots of SNP- and gene-based association with *worry* (N=348,219 individuals)

(a) SNP-based association results from the GWAS on *worry* in the UK biobank sample. SNP *P*-values were computed using a linear regression model of additive allelic effects and covariates in PLINK. Dashed lines indicate genome-wide significance ($P<5\times10^{-8}$) and dotted lines indicate the 'suggestive' significance threshold ($P<1\times10^{-5}$). **(b)** Gene-based association results from the GWAS on *worry* in the UK biobank sample. Gene-based *P*-values were computed in MAGMA using a two-sided, weighted z-score method. Dashed lines indicate genome-wide significance ($P<2.75\times10^{-6}$) and dotted lines indicate the 'suggestive' significance threshold ($P<2.75\times10^{-5}$).

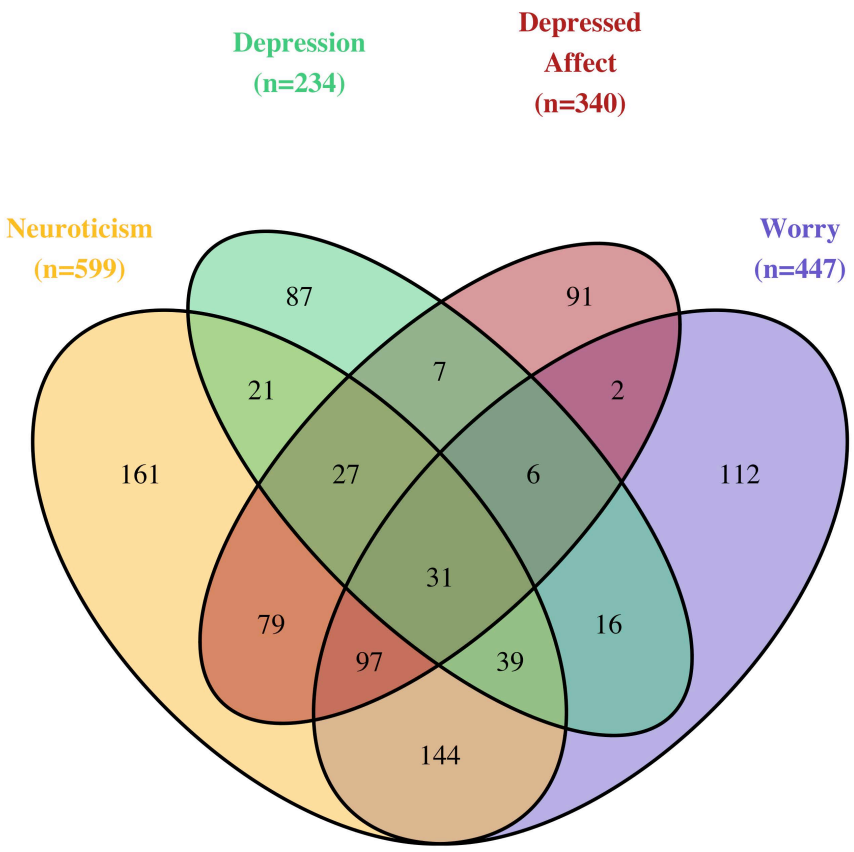

a
CHR2
b
CHR3
c
CHR19
d
CHR22
−log10(P)
Base pair position
Phenotype
Neuroticism
Depression
Depressed Affect
Worry

Supplementary Fig. 12. Manhattan plots showing SNP associations for neuroticism, depression, *depressed affect* and *worry* on 4 chromosomes

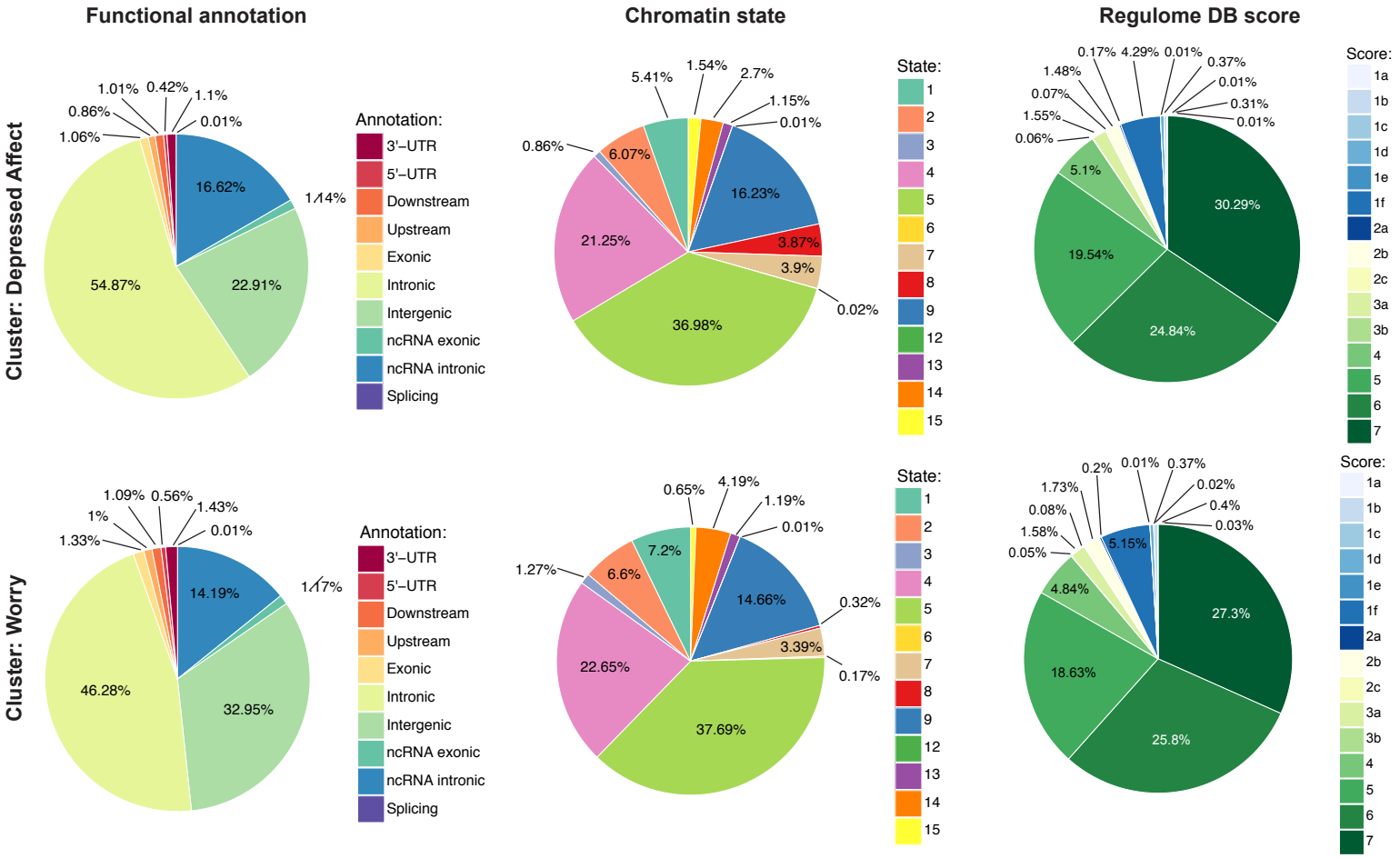
Color-coded SNP-based association results of all 4 phenotypes plotted in the same plot. SNP *P*-values were computed in METAL using a two-sided, weighted z-score method (neuroticism and depression meta-analyses) or linear regression in PLINK (clusters). Dashed lines indicate genome-wide significance ($P<5\times10^{-8}$) and dotted lines indicate the 'suggestive' significance threshold ($P<1\times10^{-5}$). significance threshold ($P<2.75\times10^{-5}$). SNP-based association results on **(a)** chromosome 2, **(b)** chromosome 3, **(c)** chromosome 19 and **(d)** chromosome 22.



Supplementary Fig. 13. Venn diagram showing overlap in associated genomic loci for neuroticism, depression, *depressed affect* and *worry*
Loci for neuroticism include the two low confidence loci, discussed in the **Supplementary Note**.

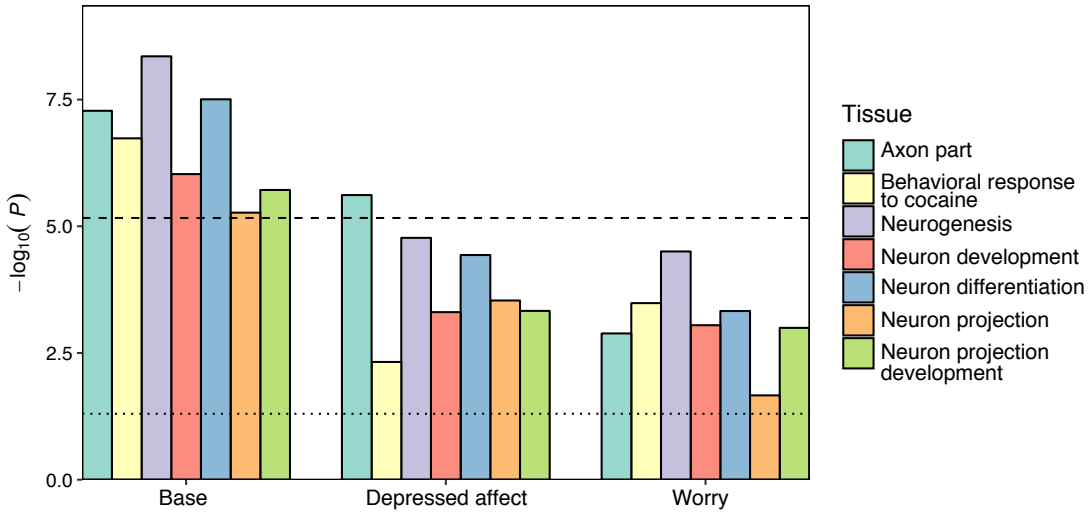


Supplementary Fig. 14. Venn diagram showing overlap in associated genes for neuroticism, depression, *depressed affect* and *worry*

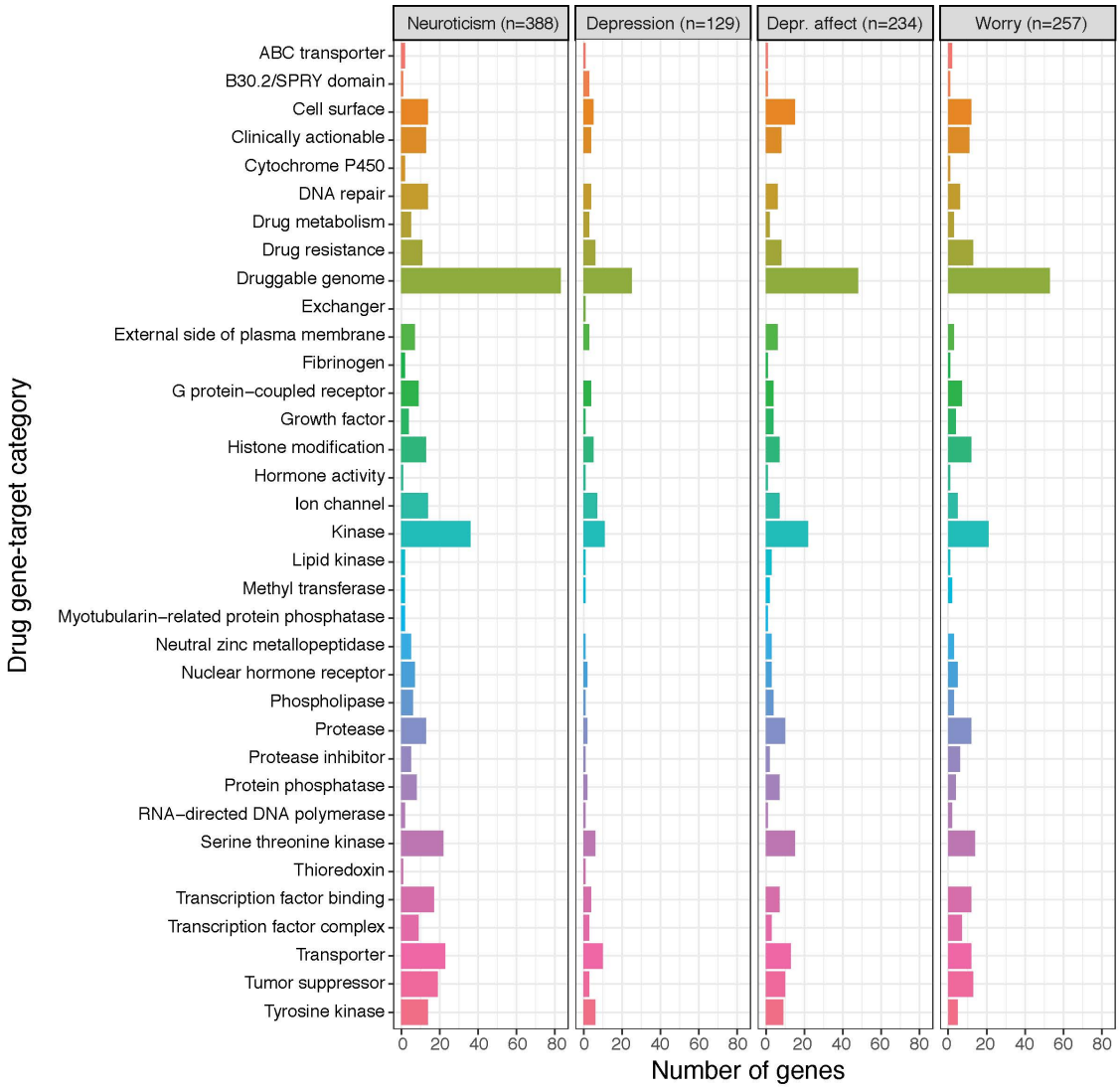


Supplementary Fig 15. Functional categories, chromatin state and regulome DB score for SNPs that were GWS for the *depressed affect* and *worry* clusters.

The functional annotation indicates the functional consequences on the gene to which a given SNP was annotated (**Supplementary Table 31**). The chromatin state refers to the minimum (i.e., most active) chromatin state across 127 tissues for all genome-wide significant SNPs. The lower the regulome DB (database) score, the more likely it is that the SNP has a regulatory function. Coding of the chromatin states and regulome DB scores is presented in **Supplementary Tables 32-33**. Annotation was performed in FUMA[22].



Supplementary Fig. 16. Gene-set analysis conditional on *depressed* affect and *worry*
Association *P*-values of gene-sets for neuroticism, conditional on the scores on the neuroticism item clusters *depressed affect* and *worry*. The initial conditional gene-set analysis (**Supplementary Table 35**) showed that 3 of the 6 gene-sets (axon part, behavioral response to cocaine and neurogenesis) had largely independent associations with neuroticism. Repeating the conditional analyses, now conditioning on *depressed affect* and *worry* scores, respectively, shows that the involvement of 'axon part' in neuroticism may largely originate in the *worry* component of neuroticism (compared to conditioning on *depressed affect*, the *P*-value drops more substantially). Conditional gene-set analyses were conducted in MAGMA using the results of the gene-based analyses as input.



Supplementary Fig. 17. Potentially druggable gene categories for all four traits.
Bar plot showing the number of potentially druggable gene-targets based on data from the drug-gene interaction database[48] (DGIdb). The search in the DGIdb was performed on all genes that were implicated by one of the gene-mapping strategies for each of the four traits. These categories highlight gene-targets that may form a potential drug-target, but which is not necessarily already targeted by currently approved drugs. A full overview of the results is provided in **Supplementary Table 41**.

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