

Data supplement for Levey et al., Reproducible Genetic Risk Loci for Anxiety: Results From ~200,000 Million Veteran Program Program Participants. Am J Psychiatry (doi: 10.1176/appi.ajp.2019.19030256)

FIGURE S1. Manhattan Plot for EA Anxiety/Panic Case-Control GWAS

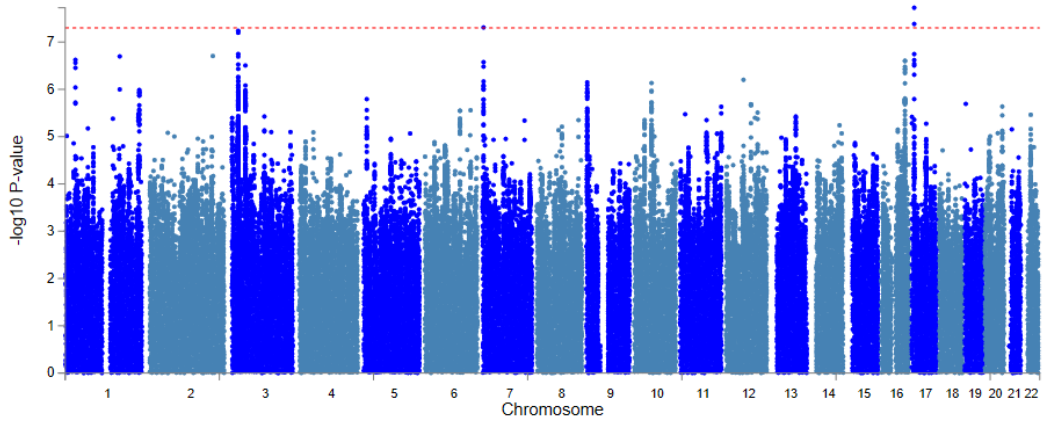


FIGURE S2. Manhattan plot for AA Anxiety/Panic Case-Control GWAS

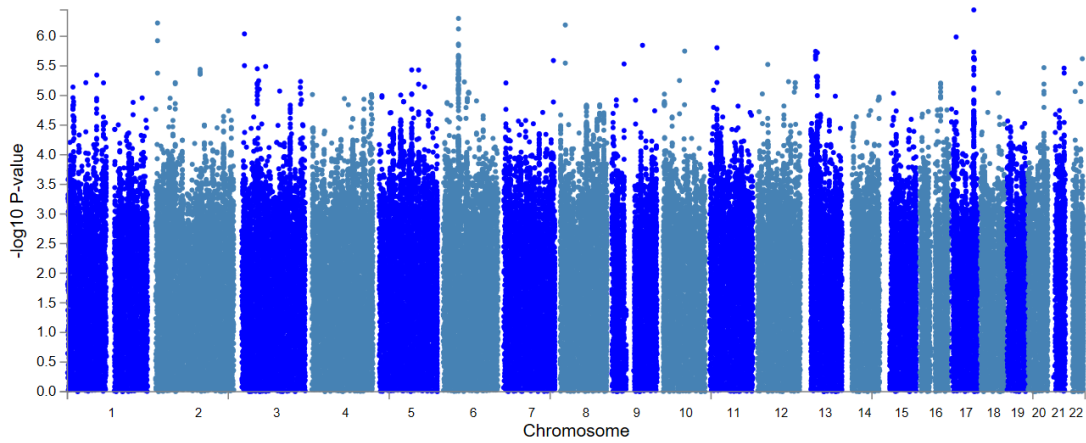


FIGURE S3. Conditional Analysis with mtCOJO using the PGC MDD2 summary statistics (excluding 23andMe). Top is the original GWAS of GAD-2, bottom is the conditional analysis. In most cases signal was reduced by an order of magnitude; peaks on chromosomes 3 and 6 remain GWS.

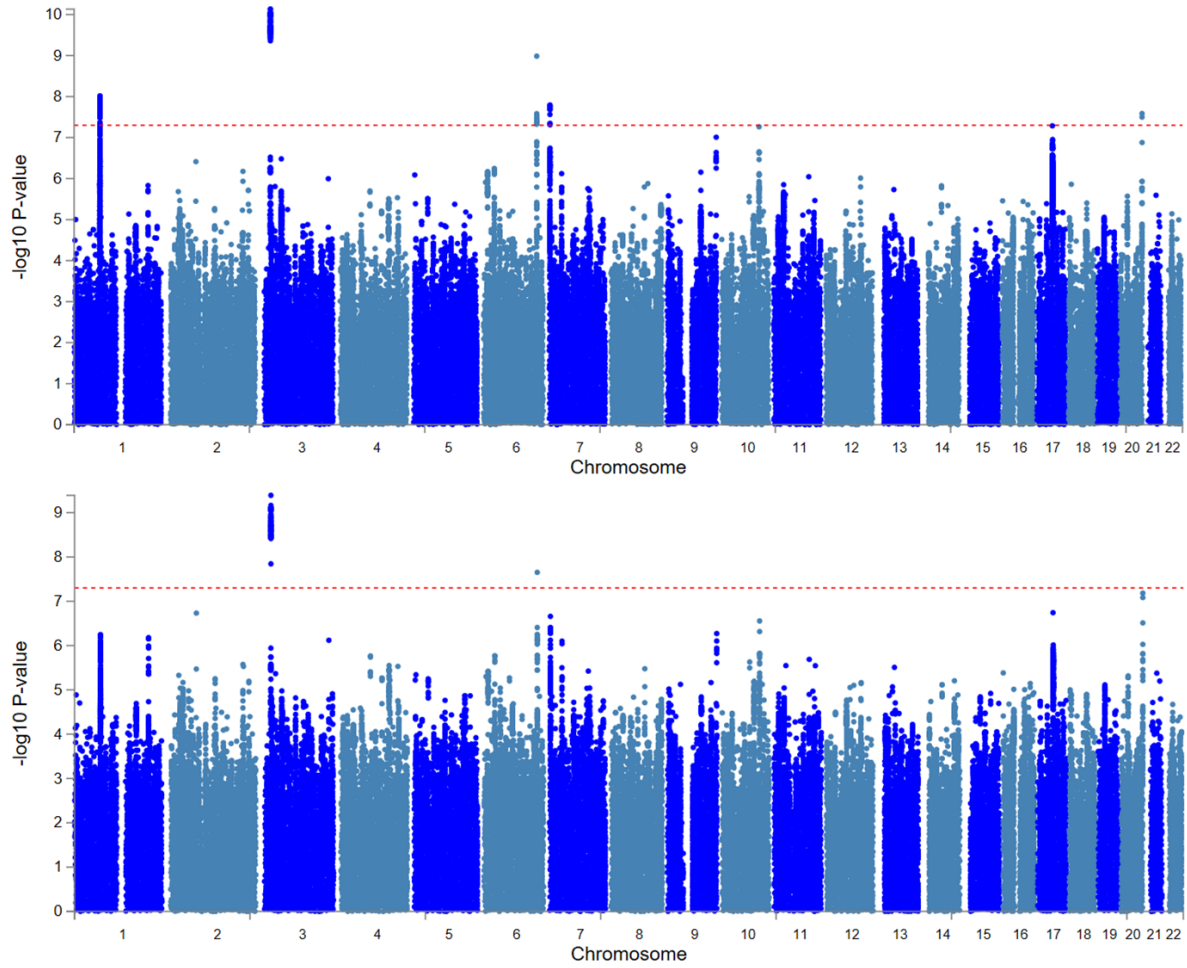
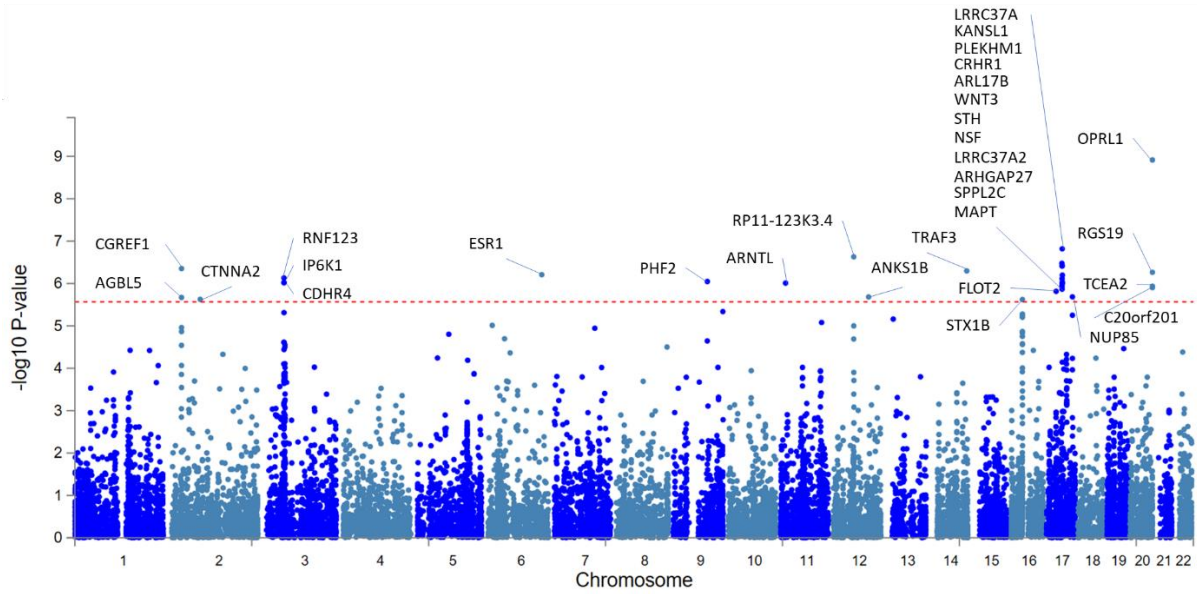


FIGURE S4. GWAS for European Americans (list of significant genes in Table S3)

GAD-2



Case-Control

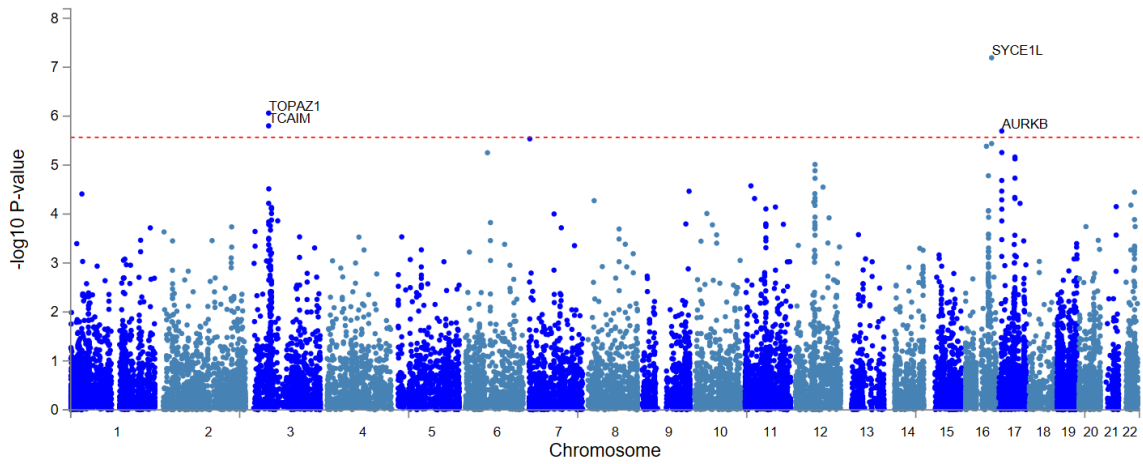
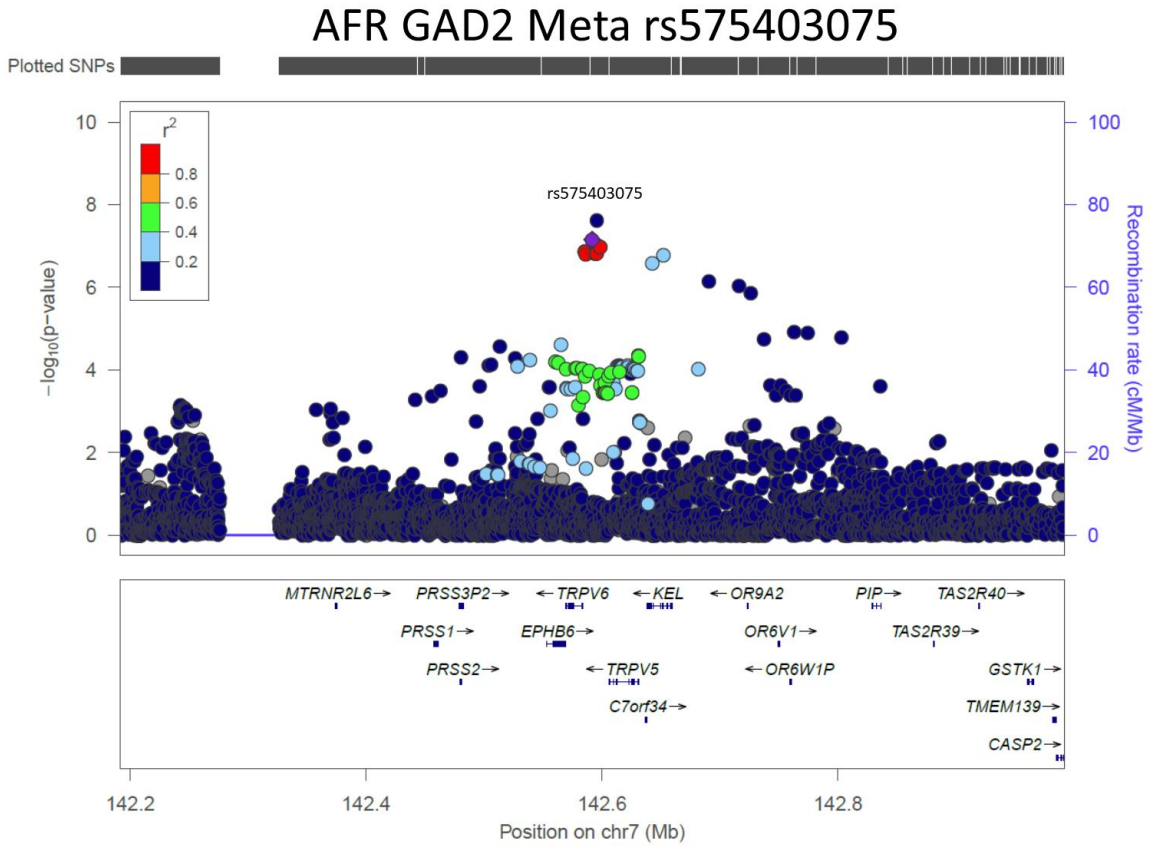
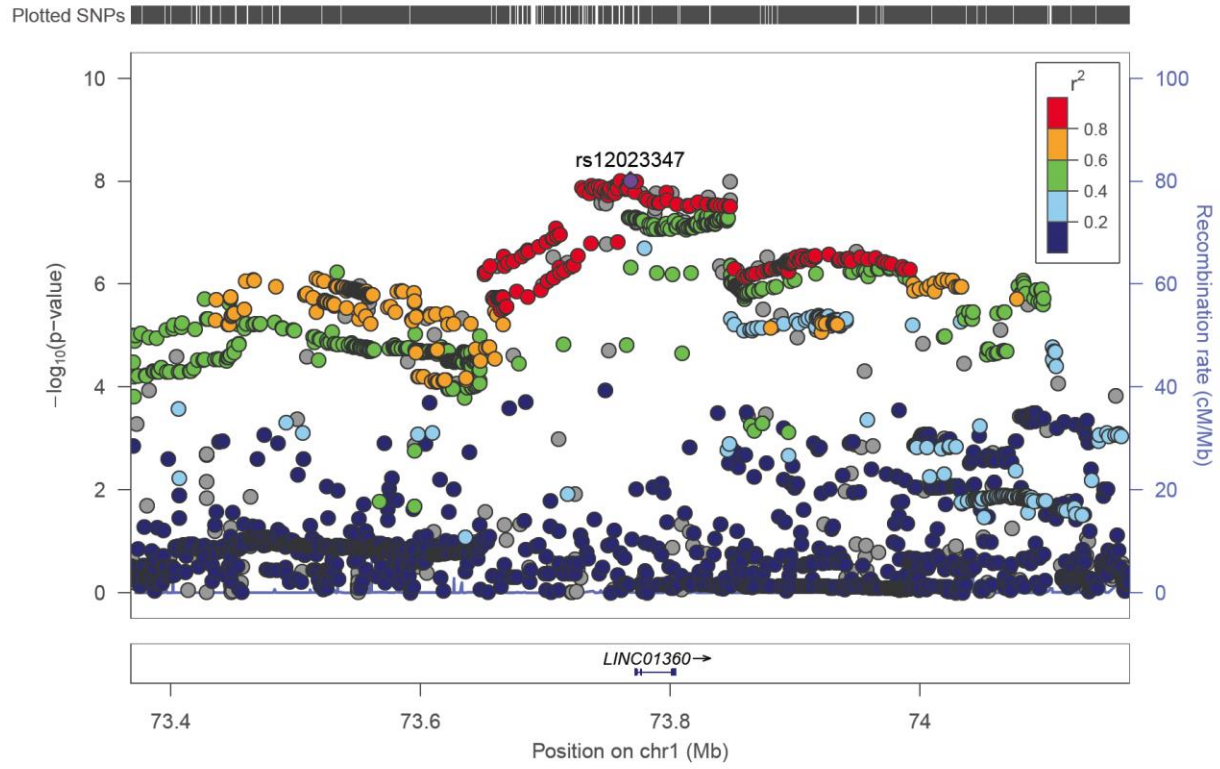


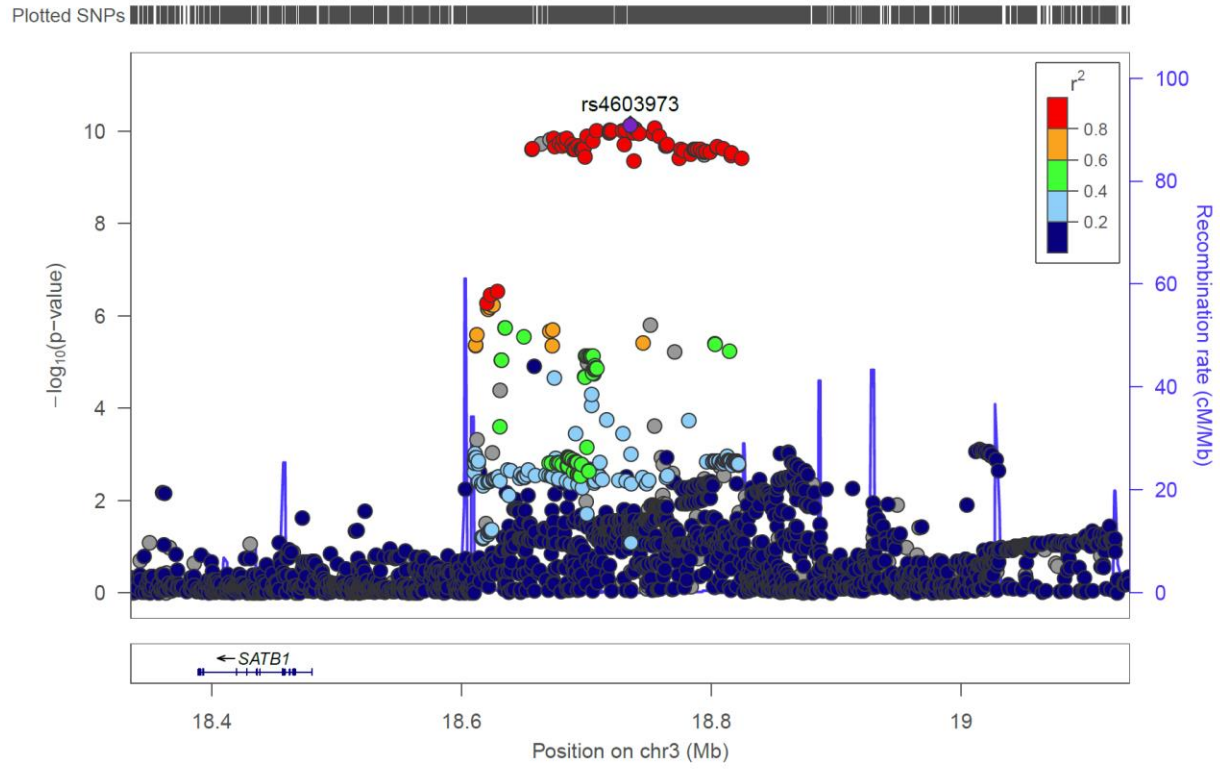
FIGURE S5. Regional Manhattan Plots for GAD-2 GWAS



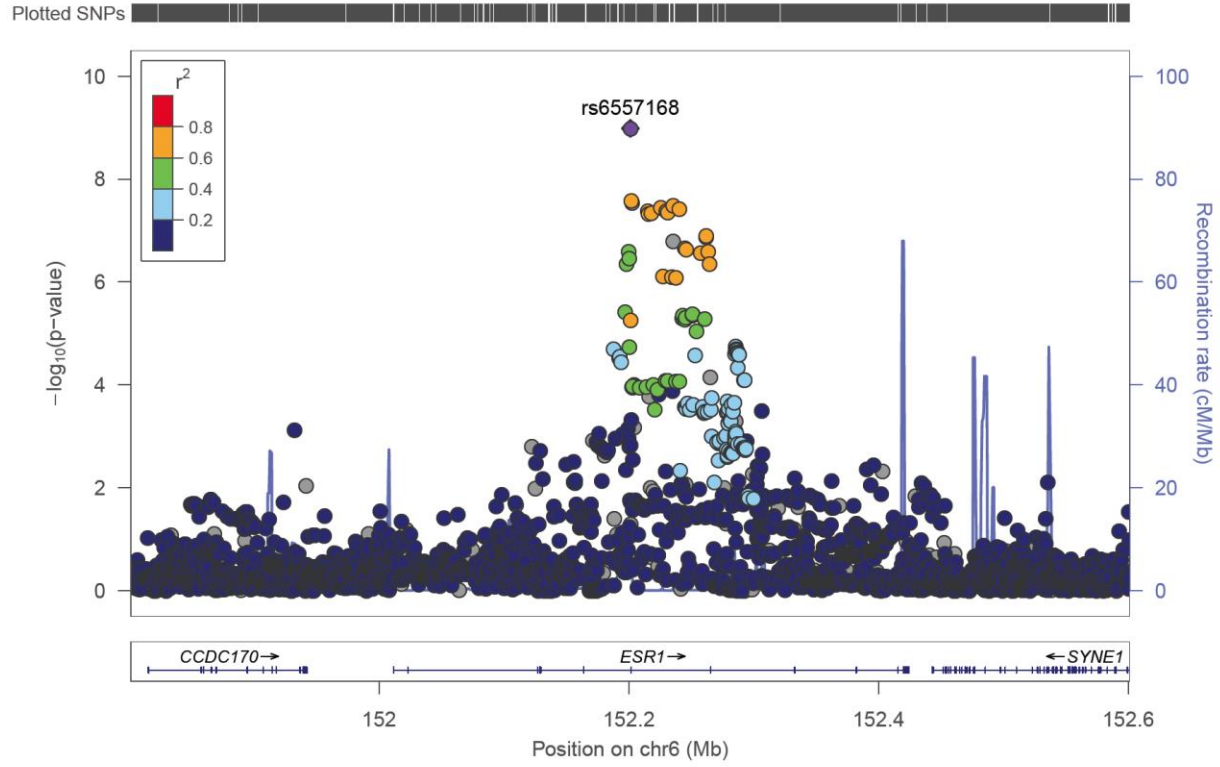
EA GAD2 Meta rs12023347



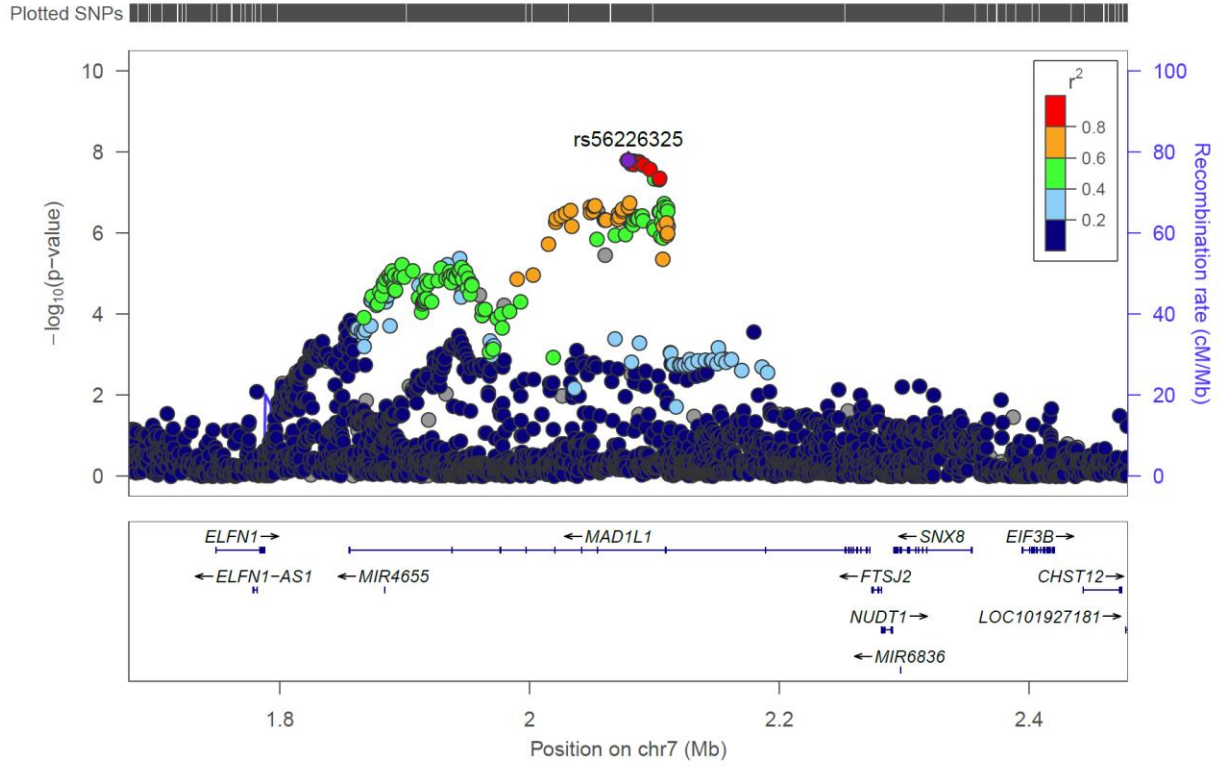
EA GAD2 Meta rs4603973



EA GAD2 Meta rs6557168



EA GAD2 Meta rs56226325



EA GAD2 Meta rs6090040

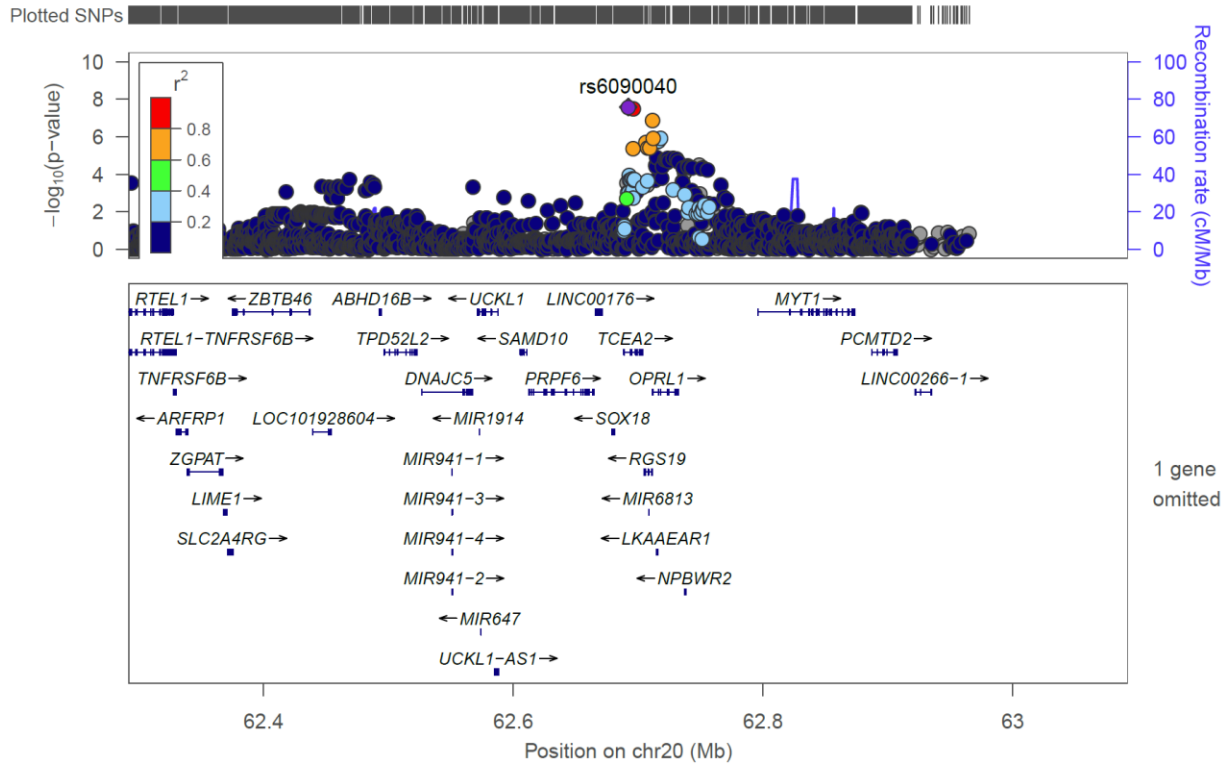


FIGURE S6. Fine mapping analysis conducted in PAINTOR v3. Upper box plots the posterior probabilities on the y axis and the SNP position on the x axis. The second box represents the annotation used, which comes from fetal brain tissue.¹ The third row shows a regional plot of the original GWAS. Lead SNPs from the GWAS analysis do not necessarily line up with the highest Bayesian posterior probability. The fourth row contains LD structure of the loci analyzed.

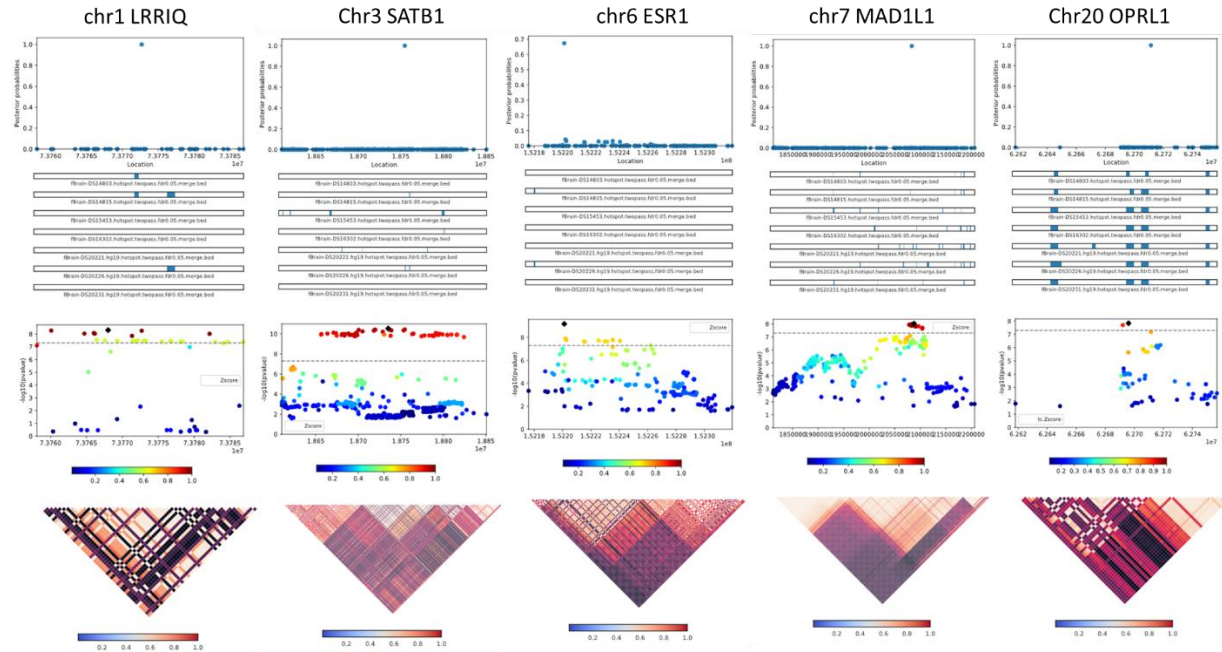


FIGURE S7. Polygenic Risk Scores

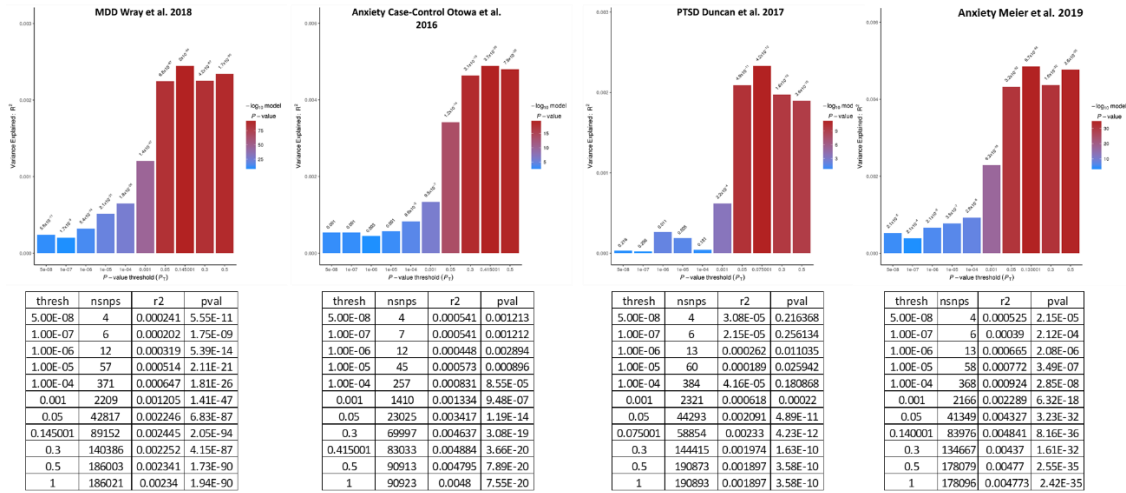


FIGURE S8. Trans-populational meta-analysis of European and American subjects for the GAD-2 trait. Lead SNPs were largely the same except that the signal on chromosome 20 is no longer genome-wide significant and a new signal near CRHR1 on chromosome 17 is now genome-wide significant.

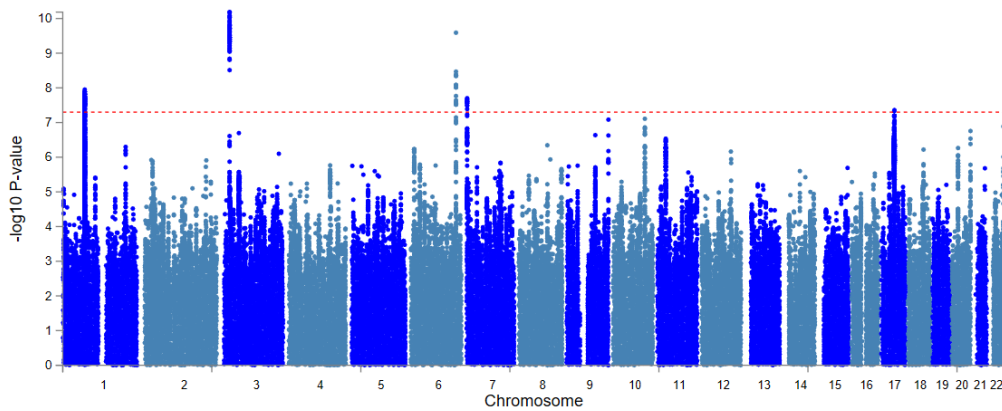


FIGURE S9. QQplot for GAD2 GWAS in European Americans

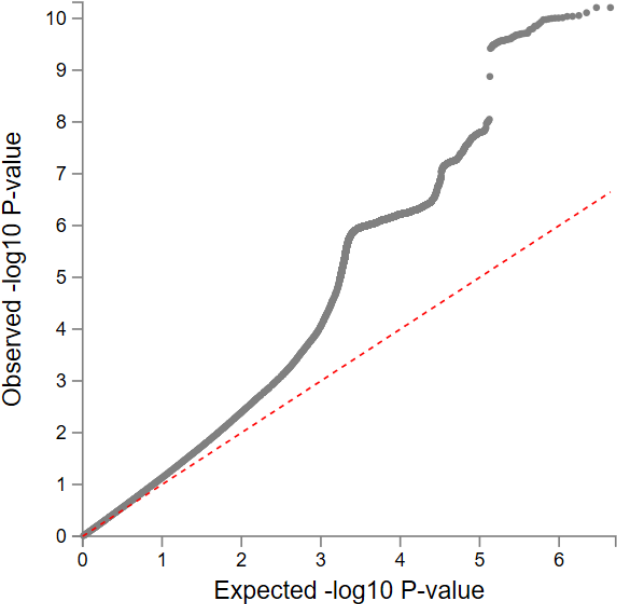


TABLE S1. GAD-2 Phenotype

GAD-2				
Over the last 2 weeks, how often have you been bothered by the following problems? <i>(Use "✓" to indicate your answer)</i>	Not at all	Several days	More than half the days	Nearly every day
1. Feeling nervous, anxious, or on edge	0	1	2	3
2. Not being able to stop or control worrying	0	1	2	3

TABLE S2. Case control sample sizes

	Case	Control	Total
SR-Anx European Americans	28525	163731	192256
SR-Anx African Americans	5664	26410	32074

TABLE S3. Gene-Based Genome-Wide Association (GWGAS) in EAs

EA Meta GWGAS

GENE	CHR	START	STOP	NSNPS	NPARAM	N	ZSTAT	P	SYMBOL
ENSG00000125510	20	62711526	62731996	50	13	175163	5.9748	1.15E-09	OPRL1
ENSG00000176681	17	44370099	44415160	12	3	175163	5.1386	1.38E-07	LRR37A
ENSG00000120071	17	44107282	44302733	1014	6	175163	5.0888	1.80E-07	KANSL1
ENSG00000258830	12	57643392	57690267	72	14	175163	5.0489	2.22E-07	RP11-123K3.4
ENSG00000225190	17	43513266	43568115	157	8	175163	4.9858	3.09E-07	PLEKHM1
ENSG00000120088	17	43699267	43913194	1155	10	175163	4.9557	3.60E-07	CRHR1
ENSG00000228696	17	44352150	44439130	192	6	175163	4.9528	3.66E-07	ARL17B
ENSG00000138028	2	27321757	27341995	47	9	175163	4.9128	4.49E-07	CGREF1
ENSG00000171700	20	62704534	62711323	12	5	175163	4.8871	5.12E-07	RGS19
ENSG00000131323	14	1.03E+08	1.03E+08	316	20	175163	4.8801	5.30E-07	TRAF3
ENSG00000091831	6	1.52E+08	1.52E+08	1673	89	175163	4.8585	5.91E-07	ESR1
ENSG00000108379	17	44839872	44910520	144	26	175163	4.8513	6.13E-07	WNT3
ENSG00000164068	3	49726932	49758962	52	9	175163	4.8205	7.16E-07	RNF123
ENSG00000197724	9	96338689	96441869	519	27	175163	4.79	8.34E-07	PHF2
ENSG00000256762	17	44076616	44077060	1	1	175163	4.7896	8.36E-07	STH
ENSG00000073969	17	44668035	44834830	69	6	175163	4.7797	8.78E-07	NSF
ENSG00000176095	3	49761727	49823975	131	11	175163	4.7768	8.91E-07	IP6K1
ENSG00000187492	3	49828165	49837268	18	7	175163	4.7686	9.28E-07	CDHR4
ENSG00000238083	17	44588877	44633016	14	4	175163	4.7666	9.37E-07	LRR37A2
ENSG00000133794	11	13298199	13408813	307	37	175163	4.757	9.82E-07	ARNTL
ENSG00000171695	20	62714733	62715712	3	1	175163	4.7232	1.16E-06	C20orf201
ENSG00000159314	17	43471275	43511787	115	8	175163	4.7217	1.17E-06	ARHGAP27
ENSG00000185294	17	43922256	43924438	17	2	175163	4.7139	1.22E-06	SPPL2C
ENSG00000171703	20	62681189	62703700	72	17	175163	4.7125	1.22E-06	TCEA2
ENSG00000186868	17	43971748	44105700	822	5	175163	4.6821	1.42E-06	MAPT
ENSG00000132589	17	27206353	27224697	30	6	175163	4.653	1.64E-06	FLOT2
ENSG00000125450	17	73201754	73231853	68	9	175163	4.6278	1.85E-06	NUP85
ENSG00000084693	2	27265232	27293490	46	9	175163	4.6021	2.09E-06	AGBL5
ENSG00000066032	2	79412357	80875905	4914	287	175163	4.5966	2.15E-06	CTNNA2
ENSG00000185046	12	99120235	1E+08	3499	186	175163	4.5933	2.18E-06	ANKS1B
ENSG00000099365	16	31000577	31021949	47	10	175163	4.5538	2.63E-06	STX1B

TABLE S4. Manually curated selection of top pathways from significant functional annotations (N=189 genes) using Ingenuity Pathway Analysis.

Categories	Diseases or Functions Annotation	P-value	# Molecules
Cancer, Organismal Injury and Abnormalities	Carcinoma	1.76E-07	156
Cellular Assembly and Organization	Binding of membrane rafts	6.20E-05	2
Carbohydrate Metabolism, Small Molecule Biochemistry	Metabolism of glucose-1-phosphate	6.20E-05	2
Dermatological Diseases and Conditions, Organismal Injury and Abnormalities	Skin lesion	1.21E-04	100
Cancer, Dermatological Diseases and Conditions, Organismal Injury and Abnormalities	Skin tumor	1.69E-04	99
Inflammatory Response	Frequency of plasmacytoid dendritic cells	1.85E-04	2
Organismal Survival	Organismal death	2.26E-04	48
Behavior	Fear conditioning	3.62E-04	3
Gastrointestinal Disease, Hepatic System Disease, Organismal Injury and Abnormalities	Liver lesion	8.84E-04	112
Behavior, Reproductive System Development and Function	Mating	7.80E-03	2

Table S5.

Trait	rg	se	z	p	h2_obs	h2_obs_se
Major depressive disorder	0.7092	0.0395	17.9701	3.34E-72	0.082	0.0047
Depressive symptoms	0.805	0.0524	15.3731	2.48E-53	0.0482	0.0038
Neuroticism	0.7171	0.0469	15.3049	7.09E-53	0.0904	0.0076
Years of schooling 2016	-0.3863	0.0297	-13.0201	9.40E-39	0.1283	0.0047
Intelligence	-0.4535	0.0415	-10.9386	7.53E-28	0.1946	0.0104
Age of first birth	-0.4528	0.0444	-10.2021	1.94E-24	0.0641	0.0036
Subjective well being	-0.4504	0.0478	-9.4221	4.42E-21	0.0251	0.0021
Schizophrenia	0.2684	0.0327	8.2097	2.22E-16	0.4601	0.019
Insomnia	0.4397	0.0545	8.0727	6.88E-16	0.1323	0.0118
College completion	-0.4043	0.0536	-7.5376	4.79E-14	0.08	0.0065
Educational Attainment	-0.3959	0.0527	-7.5153	5.68E-14	0.1071	0.008
Insomnia	0.4727	0.0646	7.3173	2.53E-13	0.0472	0.0048
Neuroticism	0.7451	0.1085	6.8677	6.52E-12	0.0144	0.0034
Years of schooling 2013	-0.3684	0.0539	-6.8291	8.55E-12	0.0841	0.0066
Major depressive disorder	0.4763	0.0727	6.5517	5.69E-11	0.165	0.0259
Number of children ever born	0.269	0.0477	5.6428	1.67E-08	0.0256	0.0018
Smoking Initiation	0.3462	0.0625	5.5433	2.97E-08	0.0738	0.0068
Childhood IQ	-0.4298	0.0815	-5.2714	1.35E-07	0.2806	0.0476
Waist-to-hip ratio	0.1834	0.0374	4.8992	9.62E-07	0.1133	0.0067
Smoking Cessation	-0.4148	0.0871	-4.7624	1.91E-06	0.0602	0.0108
PGC cross-disorder analysis	0.2544	0.0539	4.7219	2.34E-06	0.1711	0.0132
Lung cancer	0.3563	0.0764	4.6642	3.10E-06	0.3122	0.0656
Waist circumference	0.1565	0.0339	4.6192	3.85E-06	0.1215	0.0053
Anxiety	0.7449	0.1643	4.5351	5.76E-06	0.0746	0.0295
Lung cancer (all)	0.3637	0.0805	4.519	6.21E-06	0.1322	0.0295
Body mass index	0.1476	0.0333	4.4281	9.50E-06	0.1898	0.0097
Fathers age at death	-0.3036	0.0731	-4.1521	3.29E-05	0.0389	0.0066
Peak expiratory flow	-0.1105	0.0268	-4.1225	3.75E-05	0.1418	0.0087
Peak expiratory flow	-0.1077	0.0273	-3.9384	8.20E-05	0.1477	0.0093
Obesity class 1	0.1496	0.0395	3.7843	0.0002	0.2174	0.0119
Obesity	0.2302	0.0611	3.7668	0.0002	0.1244	0.0134

Mothers age at death	-0.2959	0.0787	-3.7611	0.0002	0.0351	0.0074
Forced expiratory volume in 1 second	-0.1063	0.0282	-3.77	0.0002	0.178	0.0069
Amyotrophic lateral sclerosis	0.3853	0.1067	3.6102	0.0003	0.0452	0.0123
Forced expiratory volume in 1 second	-0.0978	0.0275	-3.5583	0.0004	0.1669	0.0063
Body fat	0.18	0.0519	3.4667	0.0005	0.1081	0.0087
Coronary artery disease	0.1297	0.0374	3.4711	0.0005	0.0799	0.006
Squamous cell lung cancer	0.3578	0.1062	3.3684	0.0008	0.0398	0.0117
Parents age at death	-0.3047	0.0906	-3.3641	0.0008	0.0292	0.007
Triglycerides	0.1262	0.0381	3.3128	0.0009	0.168	0.0299
Lung cancer (squamous cell)	0.4367	0.1331	3.2808	0.001	0.0487	0.0204
Urate	0.1084	0.0333	3.257	0.0011	0.1772	0.0607
Attention deficit hyperactivity disorder	0.3826	0.126	3.0375	0.0024	0.0698	0.0309
Rheumatoid Arthritis	0.1493	0.0494	3.0244	0.0025	0.1593	0.0273
Lung adenocarcinoma	0.4236	0.1402	3.022	0.0025	0.031	0.0117
Overweight	0.121	0.0406	2.983	0.0029	0.1114	0.0069
Fasting insulin main effect	0.1986	0.0669	2.9683	0.003	0.0713	0.0102
Forced vital capacity	-0.0805	0.0273	-2.9478	0.0032	0.174	0.0062
HDL cholesterol	-0.1359	0.0463	-2.9361	0.0033	0.122	0.0251
Obesity class 2	0.1352	0.0461	2.9294	0.0034	0.1871	0.0127
Forced vital capacity	-0.0768	0.0263	-2.922	0.0035	0.1612	0.0056
Peak expiratory flow	-0.2211	0.0781	-2.8322	0.0046	0.1011	0.0201
Parkinsons disease	-0.1912	0.0677	-2.8263	0.0047	0.3725	0.1155
Age at Menopause	-0.1338	0.0501	-2.6692	0.0076	0.1373	0.0169

TABLE S6. Genetic overlap between GAD-2 trait anxiety and Anxiety/Panic disorder

trait	rg	se	z	p	h2_obs	h2_obs_se
Anxiety/Panic Disorder	0.8557	0.0368	23.2277	2.39E-119	0.0374	0.0037

TABLE S7. eQTL evidence for top GWS SNPs

uniqID	db	tissue	gene	testedAlle	p	signed_stats	FDR	RiskIncAlle	chr	pos	symbol	eqlMapFilt
20:62706105	GTEx_v7	Brain_Caudate_basal_ganglia	ENSG00000171695	G	9.40E-06	-0.357702	0.00916956	G	20	62706105	C20orf201	1
20:62707527	GTEx_v7	Brain_Caudate_basal_ganglia	ENSG00000171695	T	1.07E-05	0.363151	0.00916956	T	20	62707527	C20orf201	1
20:62709274	GTEx_v7	Brain_Caudate_basal_ganglia	ENSG00000171695	A	2.43E-06	0.380321	0.00916956	A	20	62709274	C20orf201	1
20:62712053	GTEx_v7	Brain_Caudate_basal_ganglia	ENSG00000171695	C	3.95E-06	0.368909	0.00916956	C	20	62712053	C20orf201	1
7:2085165	GTEx_v7	Brain_Cerebellar_Hemispheres	ENSG00000122687	C	6.28E-06	0.297022	8.17E-08	T	7	2085165	FTSJ2	1
7:2085553	GTEx_v7	Brain_Cerebellar_Hemispheres	ENSG00000122687	C	6.28E-06	0.297022	8.17E-08	T	7	2085553	FTSJ2	1
20:62692060	GTEx_v7	Brain_Cerebellar_Hemispheres	ENSG00000171700	C	9.75E-07	0.452831	0.00257718	C	20	62692060	RGS19	1
20:62695931	GTEx_v7	Brain_Cerebellar_Hemispheres	ENSG00000125510	A	2.53E-07	0.444876	0.000539622	A	20	62695931	OPRL1	1
20:62695931	GTEx_v7	Brain_Cerebellar_Hemispheres	ENSG00000171700	A	2.19E-06	0.429128	0.00257718	A	20	62695931	RGS19	1
20:62696024	GTEx_v7	Brain_Cerebellar_Hemispheres	ENSG00000171700	T	6.24E-06	0.424968	0.00257718	T	20	62696024	RGS19	1
20:62706105	GTEx_v7	Brain_Cerebellar_Hemispheres	ENSG00000125510	G	7.56E-08	0.457153	0.000539622	G	20	62706105	OPRL1	1
20:62706105	GTEx_v7	Brain_Cerebellar_Hemispheres	ENSG00000171700	G	5.36E-06	0.410154	0.00257718	G	20	62706105	RGS19	1
20:62707527	GTEx_v7	Brain_Cerebellar_Hemispheres	ENSG00000125510	T	3.04E-07	0.439947	0.000539622	T	20	62707527	OPRL1	1
20:62707527	GTEx_v7	Brain_Cerebellar_Hemispheres	ENSG00000171700	T	8.12E-06	0.404679	0.00257718	T	20	62707527	RGS19	1
20:62709274	GTEx_v7	Brain_Cerebellar_Hemispheres	ENSG00000125510	A	8.76E-08	0.449324	0.000539622	A	20	62709274	OPRL1	1
20:62709274	GTEx_v7	Brain_Cerebellar_Hemispheres	ENSG00000171700	A	2.37E-06	0.418317	0.00257718	A	20	62709274	RGS19	1
20:62712053	GTEx_v7	Brain_Cerebellar_Hemispheres	ENSG00000125510	C	6.82E-08	0.446404	0.000539622	C	20	62712053	OPRL1	1
20:62712053	GTEx_v7	Brain_Cerebellar_Hemispheres	ENSG00000171700	C	1.46E-06	0.420188	0.00257718	C	20	62712053	RGS19	1
20:62692060	GTEx_v7	Brain_Cerebellum	ENSG00000171700	C	9.42E-08	0.435066	0.000132103	C	20	62692060	RGS19	1
20:62695931	GTEx_v7	Brain_Cerebellum	ENSG00000125510	A	5.26E-07	0.388893	0.000621703	A	20	62695931	OPRL1	1
20:62695931	GTEx_v7	Brain_Cerebellum	ENSG00000171700	A	1.88E-08	0.446254	0.000132103	A	20	62695931	RGS19	1
20:62696024	GTEx_v7	Brain_Cerebellum	ENSG00000125510	T	3.42E-05	0.333873	0.000621703	T	20	62696024	OPRL1	1
20:62696024	GTEx_v7	Brain_Cerebellum	ENSG00000171700	T	6.54E-08	0.440536	0.000132103	T	20	62696024	RGS19	1
20:62706105	GTEx_v7	Brain_Cerebellum	ENSG00000125510	G	1.10E-07	0.411089	0.000621703	G	20	62706105	OPRL1	1
20:62706105	GTEx_v7	Brain_Cerebellum	ENSG00000171700	G	5.23E-08	0.435991	0.000132103	G	20	62706105	RGS19	1
20:62707527	GTEx_v7	Brain_Cerebellum	ENSG00000125510	T	1.55E-07	0.413277	0.000621703	T	20	62707527	OPRL1	1
20:62707527	GTEx_v7	Brain_Cerebellum	ENSG00000171700	T	1.08E-07	0.433475	0.000132103	T	20	62707527	RGS19	1
20:62709274	GTEx_v7	Brain_Cerebellum	ENSG00000125510	A	1.04E-07	0.410003	0.000621703	A	20	62709274	OPRL1	1
20:62709274	GTEx_v7	Brain_Cerebellum	ENSG00000171700	A	6.10E-08	0.432145	0.000132103	A	20	62709274	RGS19	1
20:62712053	GTEx_v7	Brain_Cerebellum	ENSG00000125510	C	9.95E-08	0.408897	0.000621703	C	20	62712053	OPRL1	1
20:62712053	GTEx_v7	Brain_Cerebellum	ENSG00000171700	C	1.28E-07	0.420938	0.000132103	C	20	62712053	RGS19	1

TABLE S8. Comorbidities in the SR-ANX cohorts using self-reports of other psychiatric traits. Top value is the number of effected individuals, number below is the proportion of individuals within each group.

	163731	28525
	SR-Anx Control	SR-Anx Case
SR-Depression	23208 14%	20831 73%
SR-ADHD	2208 1%	2343 8%
SR-Bipolar	0 0%	0 0%
SR-PTSD	14023 9%	14305 50%
SR-Eating Disorder	2225 1%	1518 5%
SR-Personality Disorder	914 1%	2159 8%
SR-Schizophrenia	0 0%	0 0%
SR-Social Phobia	0 0%	2888 10%

TABLE S9. Replication of UKB Anxiety lead SNPs

GWA	rsid	CHR	A1	A2	Purves et al. UKBio ²			MVP GAD2 Result*		
					AF	Beta	P	AF	Beta	P
Any Anxiety Disorder Case/Control	rs10809485	9	G	A	0.24	-0.04	3.3 x 10 ⁻¹²	0.2462	-0.012	0.0612
	rs2996471	9	G	T	0.79	0.04	7.8 x 10 ⁻⁹	0.8098	-0.004	0.5604
	rs3807866	7	A	G	0.42	0.03	1.1 x 10 ⁻⁸	0.3981	0.016	0.006008
GAD-7 Severity Case/Control	rs17189482	9	G	T	0.21	-0.04	4.2 x 10 ⁻⁹	0.2028	-0.021	0.002853

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