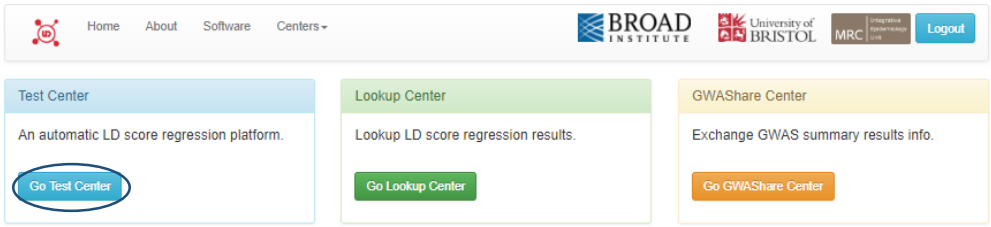
LDSC LD Score Regression

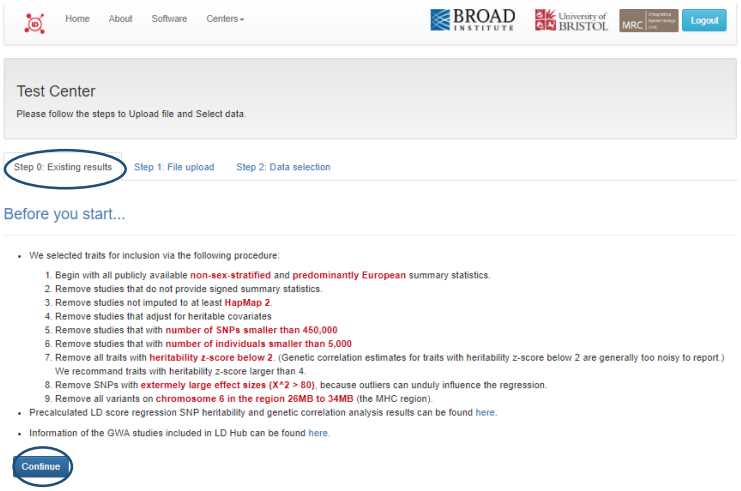
1. Go to the LD Hub website: http://ldsc.broadinstitute.org/ . Click “Get Started with LD Hub”.

2. Click “Sign in with your Google account”. Use the following account and password: **Account** jaxpolygenicity@gmail.com **password** jaxpolygenicity2018, or use your personal gmail account.

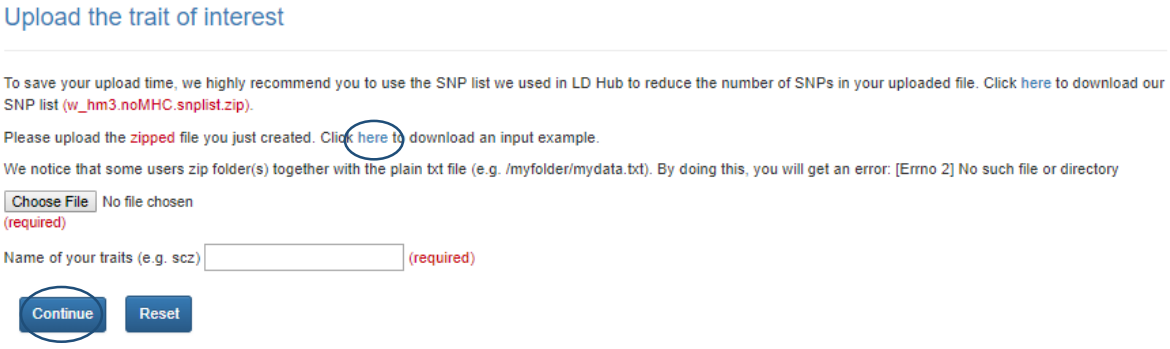
3. Go to the “Test Center” tab:



4. You are now on the “Step 0” tab. Click continue:



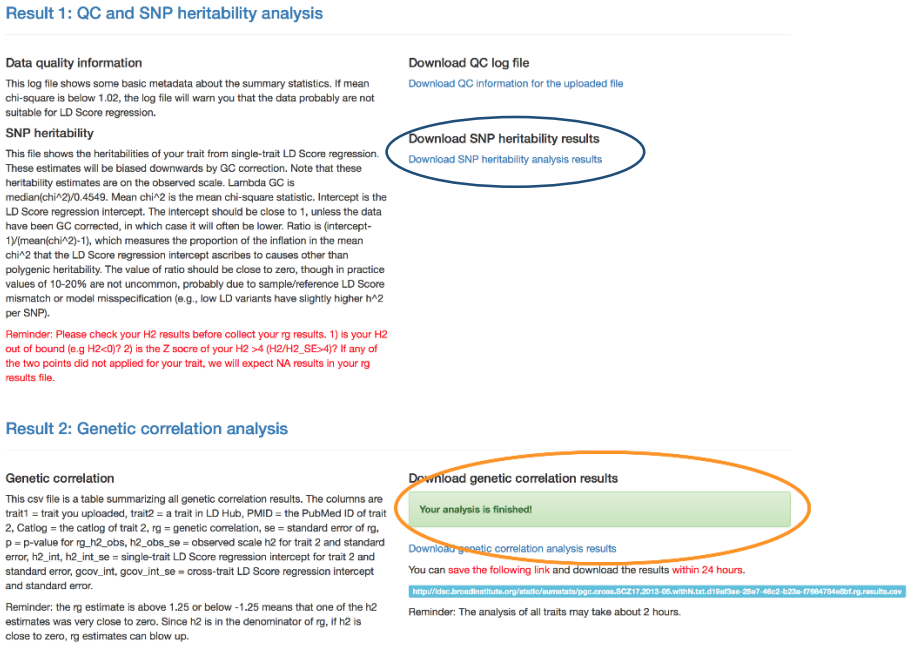
5. You are now on the “Step 1: File upload” tab. **Download the zip file** provided as an example (see picture below, or follow this link http://ldsc.broadinstitute.org/static/example/pgc.cross.scz.withN.zip; zip file name: pgc.cross.scz.withN.zip); this file contains the summary statistics for an early GWAS of schizophrenia. Next, **upload the zip file*** and name the trait (e.g. schizophrenia). Click continue:



*If you are working on a Mac device, your file may get unzipped; make sure you upload a zip file.

6. You are now on the “Step 2: Data selection” tab. Select one or two traits that you may be interested in from the database. For example, you can select “smoking_behavior”, which will calculate genetic correlations between schizophrenia and several smoking-related phenotypes.

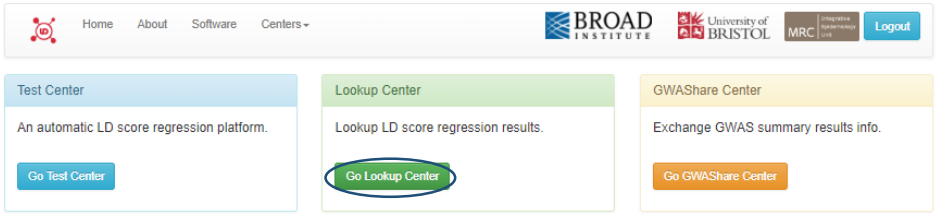
7. You have now been directed to a new tab: “Analysis in progress”. Be Careful! Do not close the window or click on any links before **both** the SNP heritability and genetic correlation analyses are done (see below). *This step should take less than two minutes, but will fluctuate depending on the internet connection and the number of traits selected.*



8. Download or open in new tabs the SNP heritability and genetic correlation results. You can also download the QC (quality control) log file if you want to find out what filters have been applied to the file you uploaded.

9. What is the SNP heritability (“total observed scale h2”) of schizophrenia? Does it show any significant genetic correlations (r_g) with, for example, any smoking related traits?

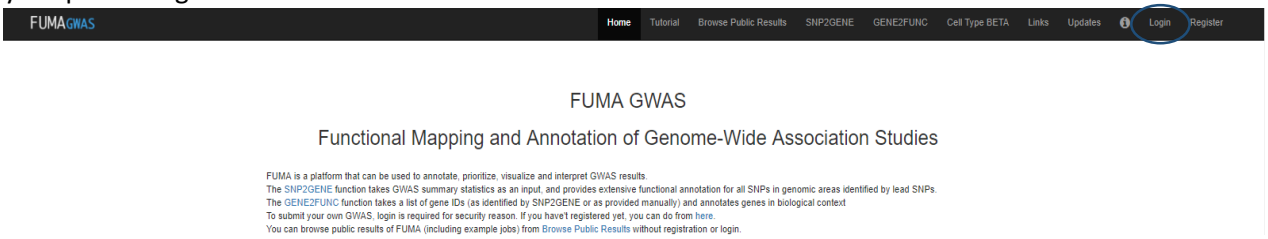
10. Now go to the “Lookup Center” tab. Here you can browse what is the SNP-heritability of your favorite trait from the database, and lookup pairs of genetic correlations.



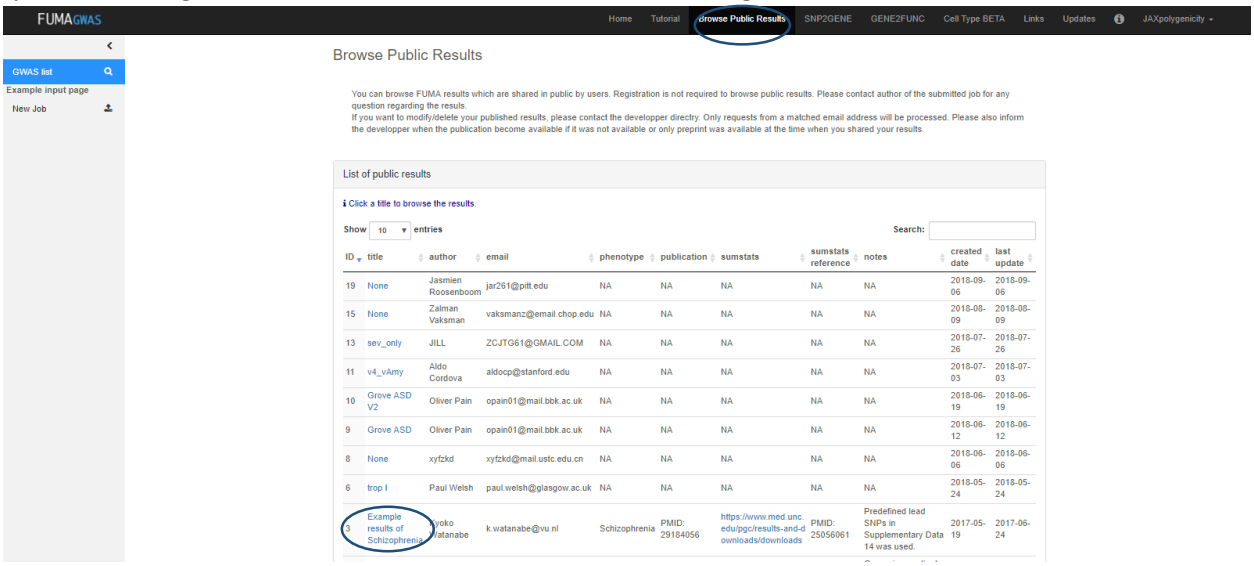
FUMA GWAS Functional Mapping and Annotation of Genome-Wide Association Studies

1. Go to the FUMA website: http://fuma.ctglab.nl

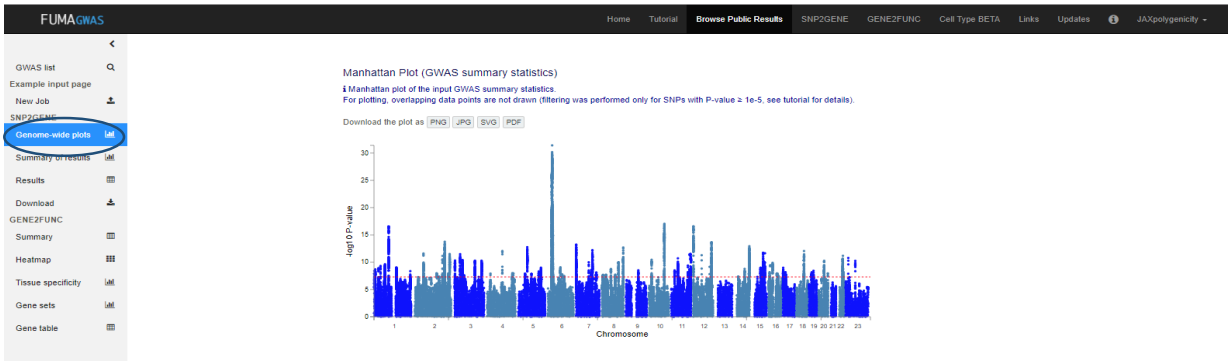
2. Log in: **Account** jaxpolygenicity@gmail.com **password** jaxpolygenicity2018. You can also register with your personal gmail account.



3. Go to the "Browse Public Results" tab and select "Example results of Schizophrenia". Use the following questions as a guidance to learn more about the variants and genes associated with this trait.



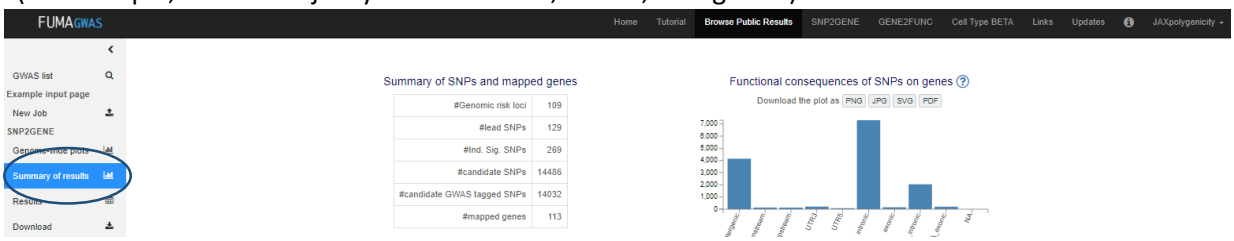
4. Go to the "Genome-wide plots" tab; you can find the Manhattan plot of the GWAS results. Are there any variants surpassing the GWAS significant threshold (P-value = 5 x 10^{-8}, or –log10 P-value = 7.3)?



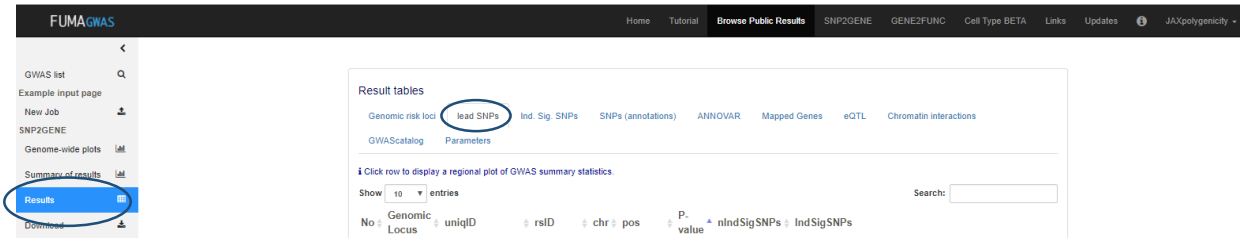
5. In the "Genome-wide plots" tab, scroll down to "MAGMA Gene-Set Analysis". Can you identify some of the biological functions (pathways) associated with schizophrenia?

6. Next, scroll down to "MAGMA Tissue Expression Analysis". Where are the majority of the genes expressed?

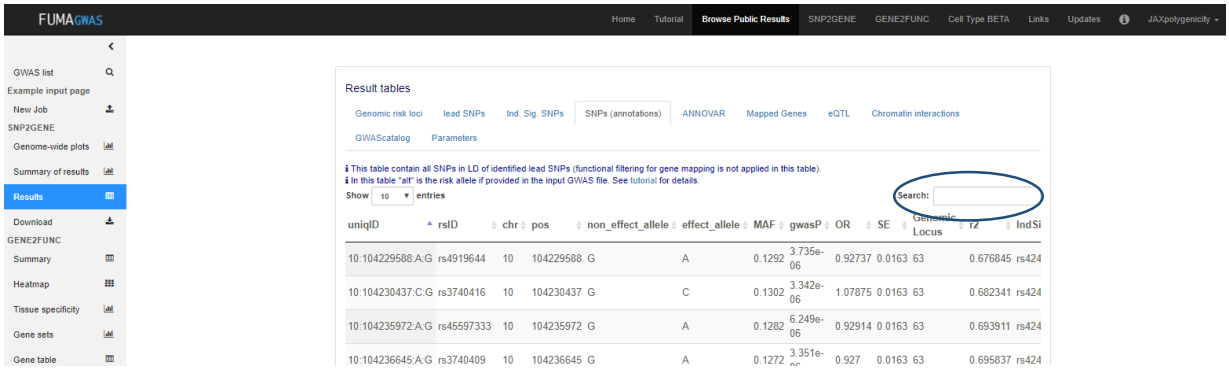
7. Go to the “Summary of results” tab. How many genomic risk loci have been associated with schizophrenia? What are the most common functional consequences of the annotated SNPs on genes (for example, are the majority of the intronic, exonic, intergenic?)



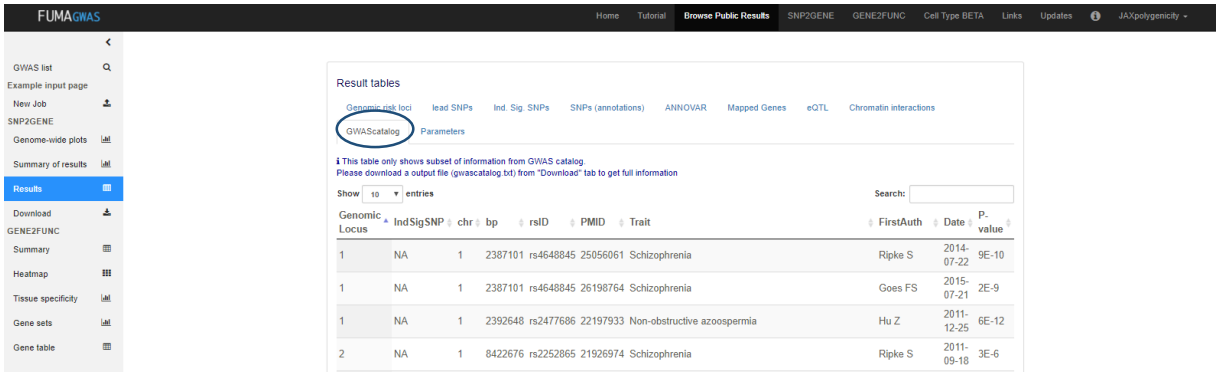
8. Go to the “Results” section, and select the “lead SNPs” tab. How many lead SNPs have been associated with schizophrenia?



9. Go to the “SNPs (annotations)” tab. You can search for your favorite gene (for example, *SLC39A8*) and check if this gene is associated with schizophrenia.



10. Lastly, go to the “GWAScatalog” tab. Are the SNPs associated with schizophrenia candidate SNPs for other traits from independent GWAS studies?



ANSWERS

LDSC

#9. Schizophrenia showed a SNP heritability estimate of 0.57 (SE=0.039). We can identify a positive genetic correlation between schizophrenia and age of smoking onset (r_g=0.24, SE=0.12, P=0.038), and a nominal positive genetic correlation between schizophrenia and smoking initiation (r_g=0.11, SE=0.06, P=0.053).

Log file showing the SNP-heritability results:

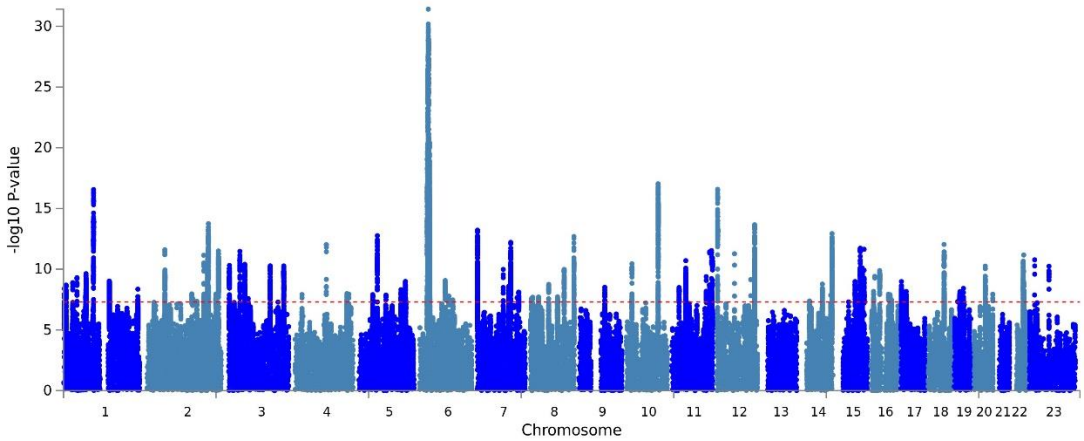
```
*********************************************************************
* LD Score Regression (LDSC)
* Version 1.0.0
* (C) 2014-2015 Brendan Bulik-Sullivan and Hilary Finucane
* Broad Institute of MIT and Harvard / MIT Department of Mathematics
* GNU General Public License v3
*********************************************************************
Call:
./ldsc.py \
--h2 /var/www/ldhub/ldhub_web/static/sumstats/pgc.cross.SCZ17.2013-05.withN.txt.394ab93c-
1a72-4d17-9588-e755ca41d6a3.sumstats.gz \
--ref-ld-chr /root/ldsc/eur_w_ld_chr/ \
--out /var/www/ldhub/ldhub_web/static/sumstats/pgc.cross.SCZ17.2013-
05.withN.txt.394ab93c-1a72-4d17-9588-e755ca41d6a3-h2 \
--w-ld-chr /root/ldsc/eur_w_ld_chr/

Beginning analysis at Sun Sep  9 20:09:30 2018
Reading summary statistics from
/var/www/ldhub/ldhub_web/static/sumstats/pgc.cross.SCZ17.2013-05.withN.txt.394ab93c-1a72-
4d17-9588-e755ca41d6a3.sumstats.gz ...
Read summary statistics for 842651 SNPs.
Reading reference panel LD Score from /root/ldsc/eur_w_ld_chr/[1-22] ...
Read reference panel LD Scores for 1293150 SNPs.
Removing partitioned LD Scores with zero variance.
Reading regression weight LD Score from /root/ldsc/eur_w_ld_chr/[1-22] ...
Read regression weight LD Scores for 1293150 SNPs.
After merging with reference panel LD, 838647 SNPs remain.
After merging with regression SNP LD, 838647 SNPs remain.
Using two-step estimator with cutoff at 30.
Total Observed scale h2: 0.5665 (0.0387)
Lambda GC: 1.2005
Mean Chi^2: 1.2221
Intercept: 1.0074 (0.01)
Ratio: 0.0334 (0.0448)
Analysis finished at Sun Sep 9 20:09:43 2018
Total time elapsed: 12.42s
```

Log file showing the rg estimates along with the standard error and p-values (highlighted in grey):

trait1	trait2	PMID	Category	ethnicity	note	rg	se	z	p	h2_obs	h2_obs_se	h2_int	h2_int_se	gcov_int	gcov_int_se
scz	Age of smoking initiation	20418890	smoking_I	European	SNPs fr	0.2428	0.1171	2.0728	0.0382	0.0596	0.0185	1.0004	0.0076	-0.0093	0.0063
scz	Cigarettes smoked per day	20418890	smoking_I	European	SNPs fr	0.0171	0.0992	0.1729	0.8628	0.0549	0.0169	1.0101	0.0084	-0.0006	0.0064
scz	Former vs Current smoker	20418890	smoking_I	European	SNPs fr	-0.0009	0.0976	-0.0094	0.9925	0.0531	0.0122	1.0117	0.0087	-0.0008	0.0065
scz	Ever vs never smoked	20418890	smoking_I	European	SNPs fr	0.1129	0.0585	1.9303	0.0536	0.0714	0.0078	1.0038	0.0083	0.0199	0.0059

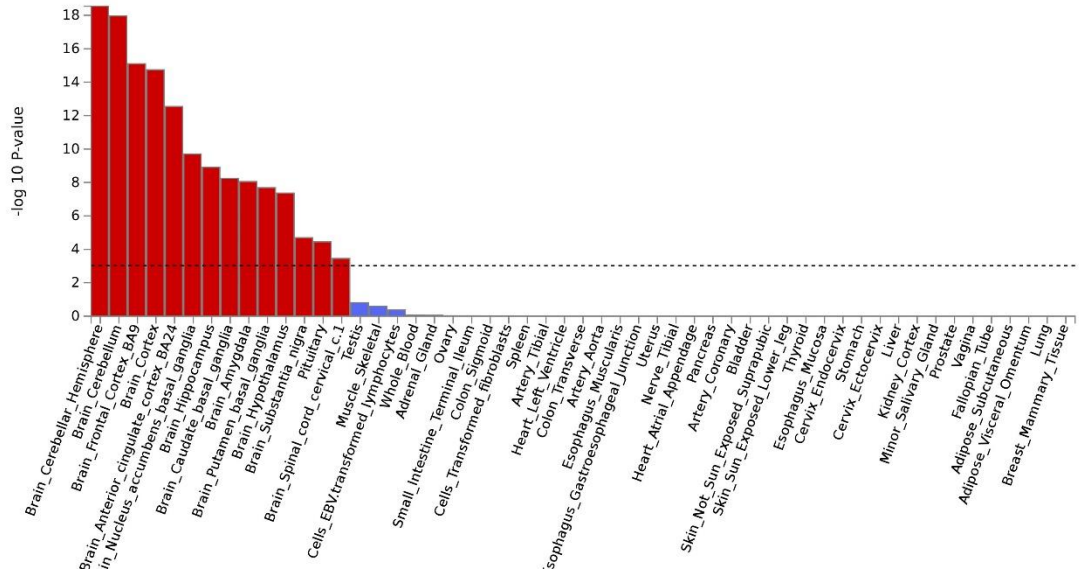
FUMA

#4. Find below the Manhattan plot showing the GWAS results of schizophrenia. There are many genetic variants surpassing the GWAS significant threshold.



#5. We can identify several pathways associated with schizophrenia; these belong to synapse organization and plasticity, and voltage gated calcium channel activity. All of these pathways survive bonferroni correction for multiple testing.

#6. The majority of the genes associated with schizophrenia are expressed in the brain.



#7 and #8. As we can see in the summary of results, there are 129 lead SNPs located in 109 loci. The majority of the annotated candidate SNPs (N=7,304) are intronic.

#10. SNPs associated with schizophrenia have also been associated with multiple other phenotypes, including bipolar disorder, educational attainment, nicotine dependence, among others.