

# ENIGMA2 | Protocol For Association Testing Using Unrelated Subjects

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Before we start, you need to download and install some required programs (which you may already have). The required programs are: R, ssh client, mach2qtl. Links to the download sites are available below. Please address any questions to: [enigma2helpdesk@gmail.com](mailto:enigma2helpdesk@gmail.com).

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- R can be downloaded here: <http://cran.stat.ucla.edu/>
- An ssh client can be downloaded here (though there are many to choose from): <http://www.chiark.greenend.org.uk/~sgtatham/putty/download.html>.
- Download mach2qtl here: <http://www.sph.umich.edu/csg/abecasis/MACH/download/> (run `tar -zxvf mach2qtl.tar.gz` to decompress the files and then type “make all” in the same directory to build. You will then have an executable called mach2qtl that you should add to your path.)

The following protocol can be split into three general categories based on cohort type. If you have a sample of unrelated, healthy subjects please follow the directions under Method A. If you have a sample of unrelated subjects with a mix of healthy controls and diagnosed patients please follow Method B. If you have a sample of related individuals, please follow Method C.

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## Method A:

### Protocol for groups with population-based cohorts (healthy subjects only)

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You will need three files to run the association analysis (described below). We recommend you keep these files in your working directory. Please, make sure to have exactly the same header labels and in the same order as shown below so that the commands used in this protocol need not to be changed:

- [LandRvolumes.csv](#) Which contains your imaging phenotypes (after quality control) for the entire sample (healthy cohorts only, without patients). Make sure that the SubjectID’s in this file are in the proper format (i.e. that they match the format of the individual subject ID’s given in the IID column of the [SubCortCovs\\_nopatients.csv](#) file).
  - Make sure that missing values and individual volume measures that were excluded from the analysis during QC in the LandRvolumes.csv are coded as “NA” without the quotes. Note that we originally suggested marking these values with an “x” in the imaging protocol. The following R scripts handle excluded values better if they are marked with NA. Please do a “find and replace” in your favorite text editor for “x” and replace it with “NA” (again all without quotes).
  - **FSL FIRST Users:** The ICV values reported in your LandRvolumes.csv file is actually just a ratio, in order to convert it to a volume measurement (and make it comparable to the ICV measure given in FreeSurfer) you need to multiply each value by the template volume. If you used the default template in FSL FIRST (most likely this is true of everyone) then multiply each

value in the ICV column by 1827243. You can do this easily in a spreadsheet program like Excel or on the Linux command line using awk (remember to save it back as a CSV file).

**NOTE (1):** Missing values in both files: [SubCortCovs\\_nopatients.csv](#) and [LandRVolumes.csv](#) must be coded as “NA” (without the quotation marks -> “”).

SubjID	Lthal	Rthal	Lcaud	Rcaud	Lput	Rput	Lpal	Rpal	Lhippo	Rhippo	Lamyg	Ramyg	Laccumb	Raccumb	ICV
Subj1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Subj2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...

- [SubCortCovs\\_nopatients.csv](#) A spreadsheet generated using Excel or your favourite spreadsheet program, which contains the following columns: Family ID, Individual ID, age, sex and dummy covariates: i.e. a covariate to control for different MR acquisitions, if applicable, remember also that this part of the protocol is for cohorts with healthy subjects only. Save this spreadsheet as a comma delimited (.csv) text file called [SubCortCovs\\_nopatients.csv](#). The spreadsheet should look like this:

FID	IID	Age	Sex	Dummy1	Dummy2..
Fam1	Subj1	...	...	...	...
Fam2	Subj1	...	...	...	...

**NOTE (2):** Sex must be specified as follows: (Males=1, Females=2), and “FID” and “IID” should be named exactly the same in all files.

- The third file is [HM3mds2R.mds.csv](#) (a spreadsheet containing the following columns: individual ID (IID), 4 MDS components (C1, C2, C3 and C4), and PLINK’s assigned solution code (SOL)).

FID	IID	SOL	C1	C2	C3	C4
Fam1	Subj1	...	...	...	...	...
Fam2	Subj2	...	...	...	...	...

**NOTE (3):** If you have no dummy covariates (or more than 1 dummy covariate) the commands below should still work (just add the extra dummy covariates to the end where indicated below).

These three files: [LandRVolumes.csv](#), [SubCortCovs\\_nopatients.csv](#) and [HM3mds2R.mds.csv](#) will be read into R to generate PED and DAT files that will be used for association with mach2qtl.

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The following R script assumes your files are all kept in the same folder, which is also the working directory of R.

R

```

getwd() #Check that you are in the correct directory
SubCort <- read.table("LandRvolumes.csv", colClasses=c("character", rep("numeric",15)),
sep=",", header=T); #Read in the phenotypes file
Covs <- read.table("SubCortCovs_nopatients.csv", colClasses=c(rep("character",2),
rep("numeric",2)), sep=",", header=T); #Read in the covariates file

SubCort$IID = SubCort$SubjID #This just renames a column for easier merging
SubCort$SubjID = NULL
SubCortCovs <- merge(SubCort, Covs, by="IID"); #Merge into a single dataframe
SubCortCovs$AgeSq <- SubCortCovs$Age*SubCortCovs$Age; #add an age^2 term
SubCortCovs$Mthal <- rowMeans(SubCortCovs[,c("Lthal","Rthal")]); #calculate mean Thalamus
SubCortCovs$Mcaud <- rowMeans(SubCortCovs[,c("Lcaud","Rcaud")]); #calculate mean Caudate
SubCortCovs$Mput <- rowMeans(SubCortCovs[,c("Lput","Rput")]); #calculate mean Putamen
SubCortCovs$Mpal <- rowMeans(SubCortCovs[,c("Lpal","Rpal")]); #calculate mean Pallidum
SubCortCovs$Mhippo <- rowMeans(SubCortCovs[,c("Lhippo","Rhippo")]); #calculate mean
Hippocampus
SubCortCovs$Mamyg <- rowMeans(SubCortCovs[,c("Lamyg","Ramyg")]); #calculate mean Amygdala
SubCortCovs$Maccumb <- rowMeans(SubCortCovs[,c("Laccumb","Raccumb")]); #calculate mean
Accumbens

mds.cluster <- read.table("HM3mds2R.mds.csv", colClasses=c(rep("character",2),
rep("numeric",5)), sep=",", header=T); #Read in the MDS components
mds.cluster$SOL <- NULL; #Remove the "SOL" column in the MDS components since this is not
a covariate to be included
merged_temp <- merge(SubCortCovs, mds.cluster, by=c("FID","IID")); #Merge the MDS and
other covariates

merged_ordered <- merged_temp[,c("FID", "IID", "Sex", "Lthal", "Lcaud", "Lput", "Lpal",
"Lhippo", "Lamyg", "Laccumb", "Rthal", "Rcaud", "Rput", "Rpal", "Rhippo", "Ramyg",
"Raccumb", "Mthal", "Mcaud", "Mput", "Mpal", "Mhippo", "Mamyg", "Maccumb", "ICV", "Age",
"AgeSq", "C1", "C2", "C3", "C4")] #Create data frame with left, and right and average
volumes, and all relevant covariates. Please ADD the names of dummy covariates for
different scanners/acquisitions, if you have any. For instance (see below):
#merged_ordered <- merged_temp[,c("FID", "IID", "Sex", "Lthal","Lcaud", "Lput", "Lpal",
"Lhippo", "Lamyg", "Laccumb", "Rthal","Rcaud", "Rput", "Rpal", "Rhippo", "Ramyg",
"Raccumb", "Mthal","Mcaud", "Mput", "Mpal", "Mhippo", "Mamyg", "Maccumb", "ICV", "Age",
"AgeSq", "C1", "C2", "C3", "C4", "Dummy1", "Dummy2"...)]

numcovs <- length(colnames(merged_ordered))-24; #Calculate the number of Covariates(ICV,
age, age2, population stratification (4 MDS components), dummy covariate for different
scanners/acquisitions).

merged_ordered[,1:(24+numcovs)][is.na(merged_ordered[,1:(24+numcovs)])] <- "x" #recode
"NAs" into "x", to comply with required association format

## * * * * * ##
##Create two PED files containing 21 traits (7 x Left, 7 x Right and 7 x Mean Hemispheric
Volumes): One PED file for Males-Only and another for Females-Only.
## * * * * * ##
merged_MF_ordered <- merged_ordered; #Create a Males+Females variable

merged_MF_ordered$Sex -> merged_MF_ordered$SexPED; #Rename Sex column as SexPED Variable

merged_MF_ordered_combined$SexPED -> merged_MF_ordered_combined$Sex; #Create a SexCOV
Variable

```

```

merged_MF_ordered$Sex[merged_MF_ordered$Sex==1] <- 0; #recode males from "1" into "0", in
the sex covariate.
merged_MF_ordered$Sex[merged_MF_ordered$Sex==2] <- 1; #recode females from "2" into "1",
in the sex covariate.

merged_MF_ordered <- merged_MF_ordered[,c("FID", "IID", "SexPED", "Lthal", "Lcaud",
"Lput", "Lpal", "Lhippo", "Lamyg", "Laccumb", "Rthal", "Rcaud", "Rput", "Rpal", "Rhippo",
"Ramyg", "Raccumb", "Mthal", "Mcaud", "Mput", "Mpal", "Mhippo", "Mamyg", "Maccumb",
"ICV", "Age", "Sex", "AgeSq", "C1", "C2", "C3", "C4")] #Create an ordered data frame
with left and hemisphere volumes, as well as mean volumes and covariates. If you have
additional dummy covariates to accommodate different scanners you will need to modify
this command in order to work properly. For an example, see below:
#merged_ordered <- merged_temp[,c("FID", "IID", "SexPED", "Lthal", "Lcaud", "Lput",
"Lpal", "Lhippo", "Lamyg", "Laccumb", "Rthal", "Rcaud", "Rput", "Rpal", "Rhippo", "Ramyg",
"Raccumb", "Mthal", "Mcaud", "Mput", "Mpal", "Mhippo", "Mamyg", "Maccumb", "ICV", "Age",
"Sex", "AgeSq", "C1", "C2", "C3", "C4", "Dummy1", "Dummy2"...)]

pedfile=as.data.frame(c(merged_MF_ordered[1:2], rep(0, length(merged_MF_ordered[1])), rep(0,
length(merged_MF_ordered[1])), merged_MF_ordered[3:24], merged_MF_ordered[25:(numcovs+25)]
)); #Create a pedfile variable containing all individuals in the sample.
write.table(pedfile, "MalesFemales_subcortCov_NP.ped", quote=F, col.names=F, row.names=F);
#Write out MalesFemales_subcortCov_patientonly.ped file

##Males+Females combined DAT file - Without ICV
write.table(cbind(c(rep("T", 21), "S", rep("C", (numcovs))), c("Lthal", "Lcaud", "Lput", "Lpal", "
Lhippo", "Lamyg", "Laccumb", "Rthal", "Rcaud", "Rput", "Rpal", "Rhippo", "Ramyg", "Raccumb", "Mthal
", "Mcaud", "Mput", "Mpal", "Mhippo", "Mamyg", "Maccumb", colnames(merged_MF_ordered) [25:(numcov
s+25)])), "subcort_SexCov_NP_nICV.dat", col.names=F, row.names=F, quote=F); # Generate a DAT
file that skips ICV

##Males+Females combined DAT file - With ICV
write.table(cbind(c(rep("T", 21), rep("C", numcovs+1)), c("Lthal", "Lcaud", "Lput", "Lpal", "Lhip
po", "Lamyg", "Laccumb", "Rthal", "Rcaud", "Rput", "Rpal", "Rhippo", "Ramyg", "Raccumb", "Mthal", "M
caud", "Mput", "Mpal", "Mhippo", "Mamyg", "Maccumb", colnames(merged_MF_ordered) [25:(numcovs+25
)])), "subcort_SexCov_NP_wICV.dat", col.names=F, row.names=F, quote=F); # Generate a DAT file
that includes ICV as a covariate

merged_M_Ordered <- subset(merged_ordered, Sex==1); #Create a MALES ONLY subset
pedfile=as.data.frame(c(merged_M_Ordered[1:2], rep(0, length(merged_M_Ordered[1])), rep(0, le
ngth(merged_M_Ordered[1])), merged_M_Ordered[3:24], merged_M_Ordered[25:(numcovs+24)]));
#Create a pedfile variable containing Males-only.

write.table(pedfile, "Males_subcortCov_NP.ped", quote=F, col.names=F, row.names=F); #Write
out Males_subcortCov_NP.ped file

merged_F_Ordered <- subset(merged_ordered, Sex==2); #Create a FEMALES ONLY subset
pedfile=as.data.frame(c(merged_F_Ordered[1:2], rep(0, length(merged_F_Ordered[1])), rep(0, le
ngth(merged_F_Ordered[1])), merged_F_Ordered[3:24], merged_F_Ordered[25:(numcovs+24)]));
#Create a pedfile variable containing Females-only.

write.table(pedfile, "Females_subcortCov_NP.ped", quote=F, col.names=F, row.names=F); #Write
out Females_subcortCov_NP.ped file

```

```
## * * * * * ##
##Create two DAT files: With and without ICV as a Covariate including ALL Volumes, Left,
Right and Mean##
## * * * * * ##
```

```
##Without ICV
write.table(cbind(c(rep("T",21),"S",rep("C", (numcovs-
1))),c("Lthal","Lcaud","Lput","Lpal","Lhippo","Lamyg","Laccumb","Rthal","Rcaud","Rput","R
pal","Rhippo","Ramyg","Raccumb","Mthal","Mcaud","Mput","Mpal","Mhippo","Mamyg","Maccumb",
colnames(merged_ordered)[25:(numcovs+24)]),"subcort_NoSexCov_NP_nICV.dat",col.names=F,ro
w.names=F,quote=F); # Generate a DAT file that skips ICV
```

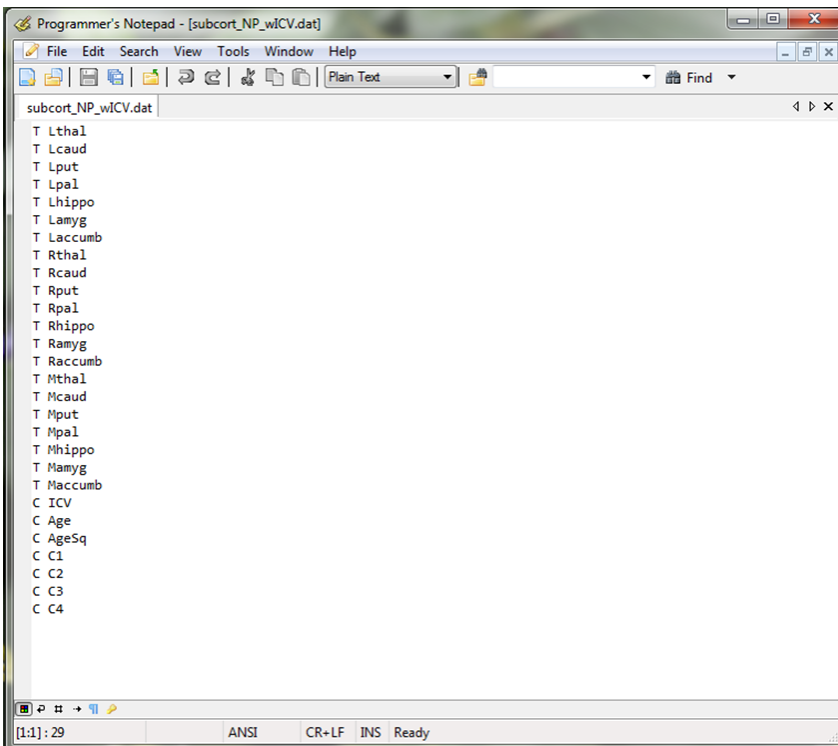
```
##With ICV
write.table(cbind(c(rep("T",21),rep("C",numcovs)),c("Lthal","Lcaud","Lput","Lpal","Lhippo
","Lamyg","Laccumb","Rthal","Rcaud","Rput","Rpal","Rhippo","Ramyg","Raccumb","Mthal","Mca
ud","Mput","Mpal","Mhippo","Mamyg","Maccumb",colnames(merged_ordered)[25:(numcovs+24)])),
"subcort_NoSexCov_NP_wICV.dat",col.names=F,row.names=F,quote=F); # Generate a DAT file
that includes ICV as a covariate
```

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**Now, check the files you just produced to make sure they have the correct information. There was a lot of text manipulation we just did, so please make sure to look at the files you created to see if they have the correct number of subjects, correct columns, and correct .dat files.**

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Below is an example of the contents of subcort\_wICV\_NP.dat file:  
less subcort\_wICV\_NP.dat



Here is an example of the Males\_subcortCov\_NP.ped file (all the data is fake):  
less Males\_subcortCov\_NP.ped



```

1forrest24.qimr.edu.au - forrest24 - SSH Secure Shell
File Edit View Window Help
Quick Connect Profiles
80030 8003001 0 0 8207.58 3874.39 4872.66 1712.55 3415.61 821.865 122.607 8206 3791.34 5190.64 1768.71 3437.75 715.078 460.371 7324.16660
80033 8003301 0 0 8440.14 4506.42 5420.83 1922.96 4028.64 854.297 632.812 8206 4163.12 5504.68 1990.2 4025.48 825.029 466.699 7324.166607
80033 8003302 0 0 6686.45 3614.94 NA 1384.28 3026.43 412.119 526.025 5912.84 3908.41 4183.68 1515.59 2044.78 548.965 306.123 7324.1666075
80041 8004101 0 0 7180.05 3313.56 4844.18 1707.01 3766.03 NA 628.857 7555.78 3369.73 4962.04 1503.72 3484.42 844.805 503.086 7324.1666075
80050 8005001 0 0 7184 3925.81 3800.83 NA 3490.75 388.389 337.764 7064.56 4087.18 3986.72 1516.38 3839.59 669.99 145.547 7324.16660759494
80050 8005001 0 0 7184 3925.81 3800.83 NA 3490.75 388.389 337.764 7064.56 4087.18 3986.72 1516.38 3839.59 669.99 145.547 7324.16660759494
80050 8005001 0 0 7184 3925.81 3800.83 NA 3490.75 388.389 337.764 7064.56 4087.18 3986.72 1516.38 3839.59 669.99 145.547 7324.16660759494
80050 8005001 0 0 7184 3925.81 3800.83 NA 3490.75 388.389 337.764 7064.56 4087.18 3986.72 1516.38 3839.59 669.99 145.547 7324.16660759494
80050 8005001 0 0 7176.09 4198.71 4174.98 1391.4 3627.6 655.752 261.035 6986.25 NA 4194.76 1461.01 3710.65 512.578 164.531 7324.166607594
80050 8005001 0 0 7176.09 4198.71 4174.98 1391.4 3627.6 655.752 261.035 6986.25 NA 4194.76 1461.01 3710.65 512.578 164.531 7324.166607594
80050 8005001 0 0 7176.09 4198.71 4174.98 1391.4 3627.6 655.752 261.035 6986.25 NA 4194.76 1461.01 3710.65 512.578 164.531 7324.166607594
80050 8005001 0 0 7176.09 4198.71 4174.98 1391.4 3627.6 655.752 261.035 6986.25 NA 4194.76 1461.01 3710.65 512.578 164.531 7324.166607594
80090 8009001 0 0 7967.9 3643.42 5661.3 1593.11 3778.68 515.742 619.365 7831.05 3479.68 5741.98 1597.85 4144.13 586.934 577.441 7324.1666
80090 8009002 0 0 NA 3240.79 4449.46 1616.04 3270.85 473.027 367.822 6647.7 3198.87 4648.8 1655.6 3272.43 649.424 309.287 7324.1666075949
80092 8009201 0 0 10637.6 5421.62 6485.54 1854.93 4708.12 692.93 459.58 10182.7 5807.64 6271.17 1839.9 5023.74 389.971 283.975 7324.16660
80180 8018002 0 0 6595.49 3957.45 4655.92 1694.36 2900.65 853.506 416.865 6964.1 4186.85 4458.16 1701.47 2778.84 428.73 211.992 7324.1666
80182 8018201 0 0 7720.31 3688.51 5613.84 1863.63 3889.42 711.123 685.02 7503.57 4058.7 5611.46 1900.81 3896.54 712.705 238.887 7324.1666
80332 8033202 0 0 6422.26 3249.49 4842.6 1225.28 3454.37 747.51 520.488 5685.82 3437.75 5117.08 1508.47 3616.52 468.281 321.943 7324.1666
80333 8033302 0 0 7161.86 3820.61 3539 1322.58 3513.69 280.811 212.783 6807.48 3976.44 3739.13 1336.03 3052.53 629.648 542.637 7324.16660
80402 8040201 0 0 7230.67 4081.64 5438.32 1814.59 3433.01 1000.63 669.199 7727.43 4323.69 6066.3 1842.28 3962.2 1207.09 305.332 7324.1666
80402 8040202 0 0 9155.21 4366.41 6399.32 2133.37 3604.66 764.912 855.088 9402.8 5099.68 6669.05 2017.88 4405.17 407.373 516.533 7324.1666
80410 8041001 0 0 7739.3 3490.75 4525.4 1694.36 3099.99 688.975 649.424 7758.28 3754.95 5005.55 1740.23 4077.69 329.062 478.564 7324.1666
80414 8041401 0 0 6106.64 3163.27 3648.16 1294.1 2449.78 574.277 232.559 5889.9 3261.36 3766.03 1381.9 2869.8 579.814 276.064 7324.166607
80414 8041402 0 0 8710.98 3196.49 4045.25 1260.09 2854.78 759.375 326.689 6086.87 3410.86 3322.27 1317.83 3118.18 649.424 158.203 7324.16
80414 8041405 0 0 7336.67 3777.89 4339.51 1642.94 3924.23 1619.21 186.68 7255.99 3690.88 4631.4 1669.83 3716.98 734.853 300.586 7324.1666
80491 8049102 0 0 7263.64 4001.75 4879.65 1762.38 2855.44 962.666 408.164 6995.74 3967.73 4147.29 1566.21 2766.97 593.262 280.02 7324.166
80492 8049201 0 0 6861.27 3253.45 4499.3 1473.66 3104.74 687.393 626.484 6461.81 3440.92 4559.41 1415.92 2789.12 662.871 286.348 7324.166
80495 8049501 0 0 7942.59 3158.53 NA 1573.33 3327.01 903.34 484.102 7542.33 3146.66 4835.48 1569.37 2724.26 836.103 495.967 7324.16660759
80495 8049501 0 0 7942.59 3158.53 NA 1573.33 3327.01 903.34 484.102 7542.33 3146.66 4835.48 1569.37 2724.26 836.103 495.967 7324.16660759
80495 8049501 0 0 7491.71 3064.39 NA 1726.79 3359.44 696.094 322.734 7891.96 3062.81 5501.51 1527.45 2922.8 419.238 522.861 7324.16660759
80495 8049501 0 0 7491.71 3064.39 NA 1726.79 3359.44 696.094 322.734 7891.96 3062.81 5501.51 1527.45 2922.8 419.238 522.861 7324.16660759
80495 8049502 0 0 7634.88 3695.62 5107.59 1553.55 3135.59 473.027 235.723 8024.06 3253.45 3478.1 1578.08 2792.29 56.9531 246.797 7324.166
80495 8049502 0 0 7634.88 3695.62 5107.59 1553.55 3135.59 473.027 235.723 8024.06 3253.45 3478.1 1578.08 2792.29 56.9531 246.797 7324.166
80495 8049502 0 0 7634.88 3695.62 5107.59 1553.55 3135.59 473.027 235.723 8024.06 3253.45 3478.1 1578.08 2792.29 56.9531 246.797 7324.166
80495 8049502 0 0 7634.88 3695.62 5107.59 1553.55 3135.59 473.027 235.723 8024.06 3253.45 3478.1 1578.08 2792.29 56.9531 246.797 7324.166
80495 8049502 0 0 7641.21 NA 5138.44 1518.75 3538.21 517.324 313.242 7896.71 3045.41 5113.92 1605.76 3062.02 757.793 306.914 7324.1666075
80495 8049502 0 0 7641.21 NA 5138.44 1518.75 3538.21 517.324 313.242 7896.71 3045.41 5113.92 1605.76 3062.02 757.793 306.914 7324.1666075
80495 8049502 0 0 7641.21 NA 5138.44 1518.75 3538.21 517.324 313.242 7896.71 3045.41 5113.92 1605.76 3062.02 757.793 306.914 7324.1666075
80520 8052001 0 0 6898.45 3154.57 3982.76 1453.89 3098.41 757.793 532.354 6986.25 3264.52 4371.15 1563.84 2849.24 816.328 383.643 7324.166
80520 8052002 0 0 7134.17 3272.43 4029.43 1734.7 3245.54 802.09 525.234 6817.76 3420.35 3980.39 1718.09 3117.39 803.672 399.463 7324.1666
80621 8062101 0 0 7634.09 4167.07 5927.87 1865.21 4025.48 734.853 486.475 7985.3 4525.4 6432.54 1925.33 4302.33 824.238 528.398 7324.1666
80640 8064001 0 0 7348.53 3746.25 5040.35 1515.59 3573.81 685.02 691.348 7438.71 3786.59 5537.9 1413.54 3368.94 499.922 267.363 7324.1666
Males_subcortCov_NP.ped
Connected to forrest24.qimr.edu.au

```

Check that the file has the same number of rows as subjects:

```
wc Males_subcortCov_NP.ped
```

Please check all of the files to make sure they have the correct information.

```
#####
```

**Association with Mach2QTL**

You should now have 2 PED files ((Males-only, Females-only) and 2 DAT files (L, R and M volumes, with and without ICV in as a covariate). This is all you will need to run the association on each chunk of chromosome you produced in the imputation section of these protocols. Use the shell script below to that end.

```
#####
```

Replace **highlighted** portions below to customise for your data. This code will generate a script called mach2qtl\_association.sh that you need to tailor to your server/queuing system. The aim is to run association commands in as many chromosome chunks in parallel as possible. The files being generated will be zipped as they are produced to help preserve space.

```
#!/bin/bash

machdir=/home/1KGPref/Mach #give the directory to the imputed output from Mach/minimac
peddatdir=/home/1KGPref #give the dir to the ped and dat files just created
samplename=ADNI #give abbreviated name of your sample, no spaces in the name (i.e. ADNI)
mach2qtlout=/home/1KGPref/mach2qtl_out #make a folder for the output from mach2qtl

#Males-only, Females-only
for group in Males Females; do
#with and without ICV as covariate
for cov in w n; do
```

```

#loop over chromosomes
for ((i=1; i<=23; i++)); do
# loop over 'chunks'
for ((j=1; j<=15; j++)); do
if test -f ${machdir}/chunk"$j"-ready4mach."$i".imputed.dose.gz
then
#Specify the commands, parameters and data files required for association
echo "mach2qtl --datfile ${peddatdir}/subcort_NoSexCov_NP_"$cov"ICV.dat \
--pedfile ${peddatdir}/"$group"_subcortCov_NP.ped \
--infofile ${machdir}/chunk"$j"-ready4mach."$i".imputed.info.gz \
--dosefile ${machdir}/chunk"$j"-ready4mach."$i".imputed.dose.gz \
--samplesize >
${mach2qtlout}/${samplename}_"$group"_"$cov"_ICV_NP_subcort_chr"$i"_"$j".out" >>
mach2qtl_association.sh
#Generate a shell script to zip association results files to be uploaded to the ENIGMA
server
echo "gzip ${mach2qtlout}/${samplename}_"$group"_"$cov"_ICV_NP_subcort_chr"$i"_"$j".out"
>> gzip_results.sh
fi
if [ -f ${machdir}/chunk"$j"-ready4mach."$i".female.imputed.dose.gz ] && [ ${group} ==
"Females" ]
then
#Specify the commands, parameters and data files required for association
echo "mach2qtl --datfile ${peddatdir}/subcort_NP_"$cov"ICV.dat \
--pedfile ${peddatdir}/"$group"_subcortCov_NP.ped \
--infofile ${machdir}/chunk"$j"-ready4mach."$i".female.imputed.info.gz \
--dosefile ${machdir}/chunk"$j"-ready4mach."$i".female.imputed.dose.gz \
--samplesize >
${mach2qtlout}/${samplename}_"$group"_"$cov"_ICV_NP_subcort_chr"$i"_"$j".female.out" >>
mach2qtl_association.sh
#Generate a shell script to zip association results files to be uploaded to the ENIGMA
server
echo "gzip
${mach2qtlout}/${samplename}_"$group"_"$cov"_ICV_NP_subcort_chr"$i"_"$j".female.out" >>
gzip_results.sh
fi
done
if [ -f ${machdir}/chunk"$j"-ready4mach."$i".male.imputed.dose.gz ] && [ ${group} ==
"Males" ]
then
#Specify the commands, parameters and data files required for association
echo "mach2qtl --datfile ${peddatdir}/subcort_NP_"$cov"ICV.dat \
--pedfile ${peddatdir}/"$group"_subcortCov_NP.ped \
--infofile ${machdir}/chunk"$j"-ready4mach."$i".male.imputed.info.gz \
--dosefile ${machdir}/chunk"$j"-ready4mach."$i".male.imputed.dose.gz \
--samplesize >
${mach2qtlout}/${samplename}_"$group"_"$cov"_ICV_NP_subcort_chr"$i"_"$j".male.out" >>
mach2qtl_association.sh
#Generate a shell script to zip association results files to be uploaded to the ENIGMA
server
echo "gzip
${mach2qtlout}/${samplename}_"$group"_"$cov"_ICV_NP_subcort_chr"$i"_"$j".male.out" >>
gzip_results.sh
fi
done

```

```

done
done

#Males+Females combined group
for group in MalesFemales; do
#with and without ICV as covariate
for cov in w n; do
#loop over chromosomes
for ((i=1; i<=23; i++)); do
# loop over 'chunks'
for ((j=1; j<=15; j++)); do
if test -f ${machdir}/chunk"$j"-ready4mach."$i".imputed.dose.gz
then
#Specify the commands, parameters and data files required for association
echo "mach2qtl --datfile ${peddatdir}/subcort_SexCov_NP_"$cov"ICV.dat \
--pedfile ${peddatdir}/"$group"_subcortCov_NP.ped \
--infofile ${machdir}/chunk"$j"-ready4mach."$i".imputed.info.gz \
--dosefile ${machdir}/chunk"$j"-ready4mach."$i".imputed.dose.gz \
--samplesize >
${mach2qtlout}/${samplename}_"$group"_"$cov"_ICV_NP_subcort_chr"$i"_"$j".out" >>
mach2qtl_association.sh
#Generate a shell script to zip association results files to be uploaded to the
ENIGMA server
echo "gzip
${mach2qtlout}/${samplename}_"$group"_"$cov"_ICV_NP_subcort_chr"$i"_"$j".out" >>
gzip_results.sh
fi
done
done
done
done

```

The code above will generate two shell script files: “mach2qtl\_association.sh” and “gzip\_results.sh”. Change the permission to make them executable and run “mach2qtl\_association.sh”:

```

chmod +x mach2qtl_association.sh
chmod +x gzip_results.sh

```

**You can run the association script directly (./mach2qtl\_association.sh), but in the interest of time try to split the commands up to run in parallel in the format appropriate for your computing cluster.**

When association has finished running for all chunks, run the [gzip\\_results.sh](#) script to compress the results files and save space (this will make it a lot easier and faster to upload them to the ENIGMA server):

```

./gzip_results.sh

```

Each group has a secure space on the ENIGMA upload server to upload the .info.gz and gzipped association result files. Please contact [enigma2helpdesk@gmail.com](mailto:enigma2helpdesk@gmail.com) to obtain upload information for your group’s data.



