**PLINK - Covariate Analysis**

*###plink –bfile plink –maf 0.05 –geno 0.05 –write-snplist*

*###plink --bfile plink --freq 🡪*

*###plink --file plink --r2 --out LD\_same*

Split Master Phenotype file into subsets, preserving the first 6 columns. Each subset is a unique phenotypic group with related phenotypes associated with the group.

I transformed the data from a csv file to a txt file. I edited the txt file for each phenotypic group to make them compatible with the --make-pheno command in plink. (FID, IID, phenotypes for remainder of columns).

*cat bp.txt | cut -f1,2,7- > bp\_trimmed.txt*

Using the trimmed phenotype file, make the binary phenotype files to use for association. For this test case, we run this command 6 times (once for each phenotypic group). Create new file name each time.

*plink --file plink --make-pheno bp\_trimmed.txt '\*' --make-bed --out bp\_pheno*

Run associations on all phenotype groups using the new phenotype file created.

Output format: CHR|SNP|BP|NMISS(Non-missing genotypes)|BETA|Std.Err.|R2|T(wald)|P(wald)

*plink --bfile bp\_pheno --pheno bp\_trimmed.txt --all-pheno --assoc --out bp*

Compute mean phenotype values for each genotype for all associations. Outputs standard deviation stratified by genotype as well.

Output format: CHR|SNP|VALUE|G11(value 1st genotype)|G12(value 2nd genotype)|G22(value 3rd)

*plink --bfile bp\_pheno --pheno bp\_trimmed.txt --all-pheno --assoc qt-means --out bp*

Create covariate file (bp\_cov\_edited.txt) to execute covariate analysis against the binary phenotype file

Edit the covariate file to include FID,IID, and all covariates as columns 🡪 Output: plink.cov specific for each phenotype

*plink --bfile bp\_pheno --covar bp\_cov\_edited.txt --recode*

Association using covariates file against each phenotype groups.

Using linear regression to test both quantitative trait and disease trait SNP association and their interactions with covariates. Must list each covariate called.

Output format-CHR|SNP|BP|A1(tested allele)|Test|NMISS|BETA(regression/logistic)|STAT(tstat coef)|P

*plink --bfile bp\_pheno --linear --pheno bp\_trimmed.txt --all-pheno --covar plink.cov --covar-name Age,Group,BMIbaseline, B\_Beta\_blockers, B\_CVmedsAntihypertensive, B\_BPmed, CV\_medications*