Doctoral Dissertation Proposal Defense Data-adaptive SNP-set-based Association Tests of Longitudinal Traits

Yang Yang, M.S

UTSPH

Dec 15, 2014

Table of Contents

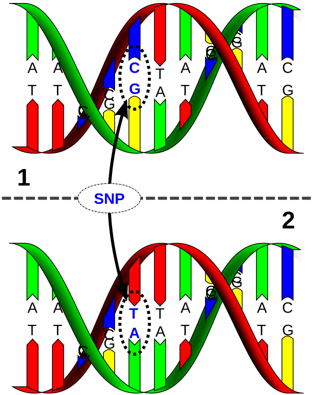
1. **Background**
   - Introduction to GWAS
   - Gene-based association test
   - Test of a single covariate
   - Test of a group of covariates
2. **Test of the contrast**
3. **Regression Diagnostics**
4. **Appendix**
   - Types of sums of squares
   - Eqvivalance of LRT and F-test
5. **References**

Background

- Introduction to GWAS
- Gene-based association test
- Longitudinal data analysis strategy
- Gene-set/Pathway based association test

Introduction to GWAS

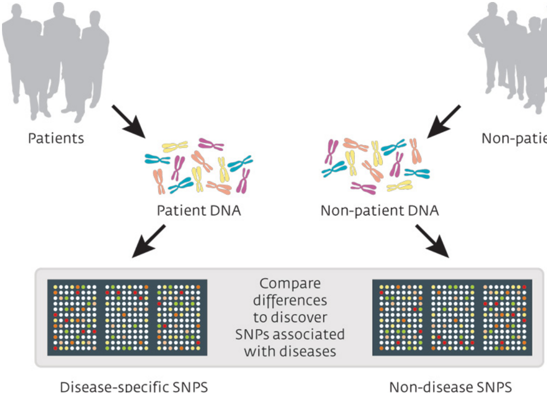
What is SNP?



A Single Nucleotide Polymorphism (SNP) is a DNA sequence variation occurring commonly within a population (e.g. 1%) in which a single nucleotide A, T, C or G in the genome (or other shared sequence) differs between members of a biological species or paired chromosomes.

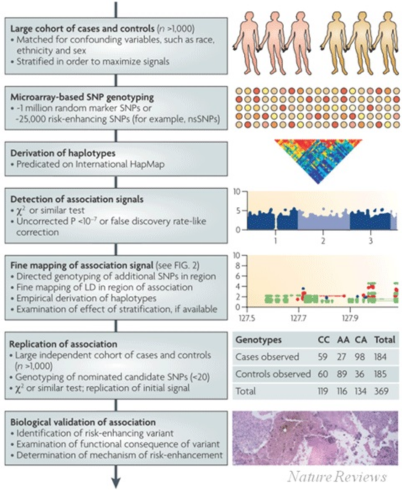
Introduction to GWAS

A simple flowchart



Introduction to GWAS

A more detailed flowchart



Large cohort of cases and controls (*n* >1,000)
- Matched for confounding variables, such as race, ethnicity and sex
- Stratified in order to maximize signals

Microarray-based SNP genotyping
- ~1 million random marker SNPs or ~25,000 risk-enhancing SNPs (for example, nsSNPs)

Derivation of haplotypes
- Predicated on International HapMap

Detection of association signals
- χ^2 or similar test
- Uncorrected P <10^{-7} or false discovery rate-like correction

Fine mapping of association signal (see FIG. 2)
- Directed genotyping of additional SNPs in region
- Fine mapping of LD in region of association
- Empirical derivation of haplotypes
- Examination of effect of stratification, if available

Replication of association
- Large independent cohort of cases and controls (*n* >1,000)
- Genotyping of nominated candidate SNPs (<20)
- χ^2 or similar test; replication of initial signal

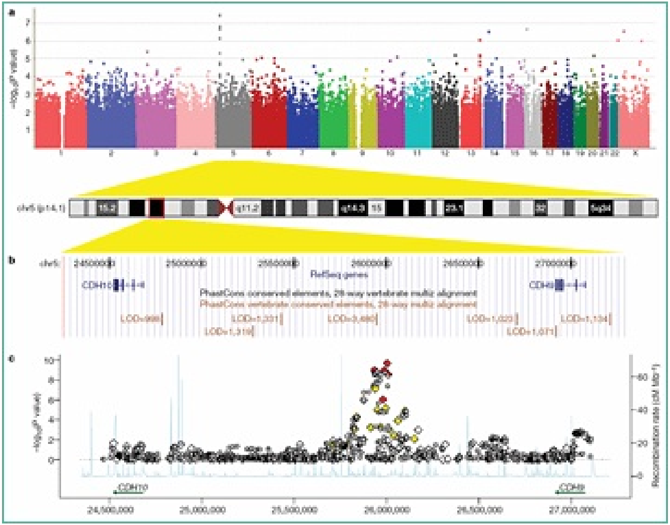
Genotypes	CC	AA	CA	Total
Cases observed	59	27	98	184
Controls observed	60	89	36	185
Total	119	116	134	369

Biological validation of association
- Identification of risk-enhancing variant
- Examination of functional consequence of variant
- Determination of mechanism of risk-enhancement

Nature Reviews

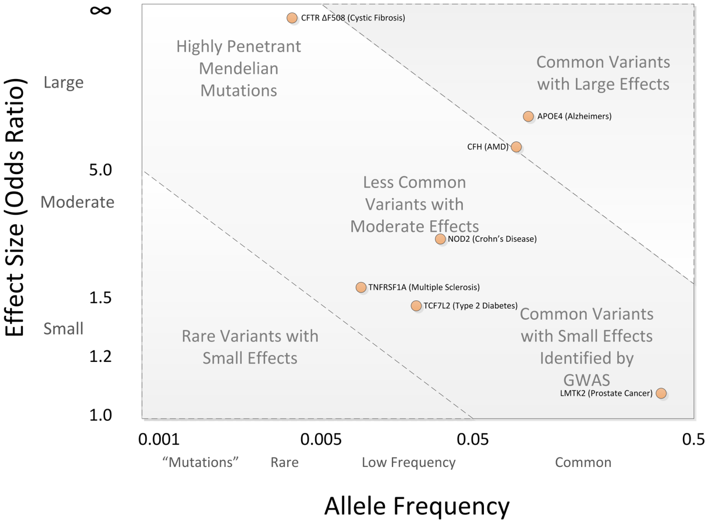
Introduction to GWAS

How does GWAS result look like?



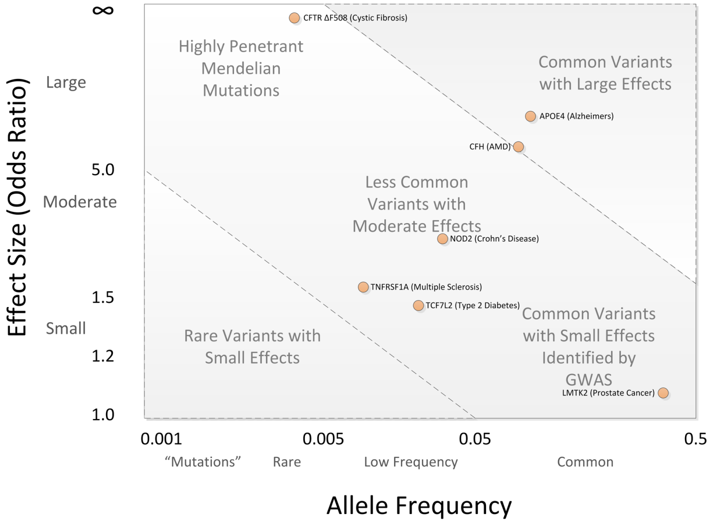
Introduction to GWAS

Common variants and rare variants



Gene-based association test

Common variants and rare variants



Global test

The ANOVA table

Source of variation	Sum of squares	Degrees of freedom
Regression	SSR(x_1)	1
	SSR($x_2 \vert x_1$)	1
	$\vdots$	$\vdots$
	SSR($x_k \vert x_{k-1}, x_{k-2}, \cdots, x_1$)	1
	SSR	k
Error	SSE	n-(k+1)
Total	SST	n-1

where

$SSR = SSR(x_1) + SSR(x_2|x_1) + \cdots + SSR(x_k|x_{k-1}, x_{k-2}, \cdots, x_1) = \hat{\beta}'\mathbf{X}'\mathbf{y} - n\bar{y}^2$

$SSE = \mathbf{y}'\mathbf{y} - \hat{\beta}'\mathbf{X}'\mathbf{y}$ (for the full model)

$SST = \mathbf{y}'\mathbf{y} - n\bar{y}^2$ (stays the same for all models)

Global test

Under the null hypothesis, $SSR/\sigma^2 \sim \chi^2_k$ and $SSE/\sigma^2 \sim \chi^2_{n-k-1}$ are independent. Therefore we have

$$TS = \frac{SSR/k}{SSE/(n-k-1)} \sim F_{k,n-k-1}$$

$p - value = Pr(F_{k,n-k-1} > TS)$.

Global test

Example:

$$mgp_i = \beta_0 + hp_i\beta_1 + wt_i\beta_2 + \varepsilon_i$$

$H_0 : \beta_1 = \beta_2 = 0$, H_1:at least one $\beta \neq 0$

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
hp	1	678.37	678.37	100.86	0.0000
wt	1	252.63	252.63	37.56	0.0000
Residuals	29	195.05	6.73		

$$TS = \frac{(678.37 + 252.63)/2}{195.05/29} = 69.21 > F_{2,29,0.95} = 3.33$$

Thus, we reject the null at 0.05 significance level and conclude that at least one β_1 and β_2 is not equal to 0.

Global test

Example cont.

The overall F statistic is also available from the output of `summary()`

```
> summary(fit.all)

Call:
lm(formula = mpg ˜ hp + wt, data = mtcars)
Residuals:
   Min     1Q Median     3Q    Max
-3.941 -1.600 -0.182  1.050  5.854

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept) 37.22727    1.59879  23.285  < 2e-16 ***
hp          -0.03177    0.00903  -3.519  0.00145 **
wt          -3.87783    0.63273  -6.129 1.12e-06 ***
---

Signif. codes:  0 *** 0.001 ** 0.01 * 0.05 . 0.1   1

Residual standard error: 2.593 on 29 degrees of freedom
Multiple R-squared:  0.8268, Adjusted R-squared:  0.8148
F-statistic: 69.21 on 2 and 29 DF,  p-value: 9.109e-12
```

Testing of single β_j

Once we have determined that at least one of the regressors is important, a natural next question might be which one(s)?

Important considerations:

- Is the increase in the regression sums of squares sufficient to warrant an additional predictor in the model?
- Additional predictors will increase the variance of $\hat{y}$ - include only predictors that explain the response (note: we may not know this through hypothesis testing as confounders may not test significant but would still be necessary in the regression model).
- Adding an unimportant predictor may increase the residual mean square thereby reducing the usefulness of the model.

Testing of single β_j

$$y_i = \beta_0 + x_{i1}\beta_1 + \cdots + x_{ij}\beta_j + \cdots + x_{ik}\beta_k + \varepsilon_i$$

- Question to answer: does one particular variable of interest significantly affect the prediction of **y** when the other independent variables presented in the model?
- $H_0 : \beta_j = 0$, $H_1 : \beta_k \neq 0$
- $TS = \frac{\hat{\beta}_j}{\hat{se}(\hat{\beta}_j)} \sim t_{n-k-1}$, reject H_0 if $|TS| > t_{n-k-1,1-\alpha/2}$
- This is a **partial test** because $\hat{\beta}_j$ depends on all of the other predictors x_i, for $i \neq j$, that are in the model. Thus, this is a test of the contribution of x_j given other predictors in the model.

Testing of single β_j

Example cont.:

$$mgp_i = \beta_0 + hp_i\beta_1 + wt_i\beta_2 + \varepsilon_i$$

$H_0 : \beta_2 = 0,\ H_1 : \beta_2 \neq 0$

From the summary of lm $\hat{\beta}_2 = -3.88$, the variance and covariance matrix of the parameter estimates is

```
> vcov(fit.all)
              (Intercept)            hp          wt
(Intercept)  2.5561215917  1.484701e-04 -0.73594515
hp           0.0001484701  8.153566e-05 -0.00376369
wt          -0.7359451464 -3.763690e-03  0.40035167
```

$$TS = \frac{-3.88}{\sqrt{0.40}} = -6.13 < t_{29,0.025} = -2.05$$

Thus, we reject the null and conclude that $\beta_2 \neq 0$.

Testing of a subset of β

$$y_i = \beta_0 + x_{i1}\beta_1 + \cdots + x_{ij}\beta_j + \cdots + x_{ip}\beta_p + \cdots + x_{ik}\beta_k + \varepsilon_i$$

- Often it is of interest to determine whether a group of predictors contribute to predicting y given another predictor or group of predictors that are in the model.
- $H_0 : \beta_j = \cdots = \beta_p = 0$, $H_1 : \beta_l \neq 0$ for at least one $l, l = j, \cdots, p$

Testing of a subset of β

Partition the vector of regression coefficient and **X** matrix as

$$\boldsymbol{\beta} = \begin{bmatrix} \boldsymbol{\beta}_1 \\ \boldsymbol{\beta}_2 \end{bmatrix}, \mathbf{X} = [\mathbf{X_1} | \mathbf{X_2}]$$

Hypotheses of interest: $H_0 : \boldsymbol{\beta}_2 = 0$ v.s. $H_1 : \boldsymbol{\beta}_2 \neq 0$

The model can be written as $\mathbf{y} = \mathbf{X_1}\boldsymbol{\beta}_1 + \mathbf{X_2}\boldsymbol{\beta}_2 + \varepsilon$

$$SSR(\mathbf{X}) = \hat{\beta}'\mathbf{X}'\mathbf{y} \text{ } (k + 1 \text{ degrees of freedom})$$

$$MSE = \frac{\mathbf{y}'\mathbf{y} - \hat{\beta}'\mathbf{X}'\mathbf{y}}{n - k - 1}$$

$$\begin{aligned} SSR(\mathbf{X_2}|\mathbf{X_1}) &= SSR(\mathbf{X}) - SSR(\mathbf{X_1}) \\ &= SSE(reduced) - SSE(full) \text{ } (r \text{ degrees of freedom}) \end{aligned}$$

Under H_0

$$TS = \frac{SSR(\mathbf{X_2}|\mathbf{X_1})/r}{MSE} \sim F_{r,n-k-1}$$

Testing of a subset of β

Example cont.

$$mgp_i = \beta_0 + disp_i\beta_1 + hp_i\beta_2 + qsec_i\beta_3 + wt_i\beta_4 + \varepsilon_i$$

```
fit.sub<-lm(mpg~disp+hp+qsec+wt,data=mtcars)
> summary(fit.sub)

Call:
lm(formula = mpg ~ disp + hp + qsec + wt, data = mtcars)

Residuals:
    Min      1Q  Median      3Q     Max
-3.8664 -1.5819 -0.3788  1.1712  5.6468

Coefficients:
             Estimate Std. Error t value Pr(>|t|)
(Intercept) 27.329638   8.639032   3.164  0.00383 **
disp         0.002666   0.010738   0.248  0.80576
hp          -0.018666   0.015613  -1.196  0.24227
qsec         0.544160   0.466493   1.166  0.25362
wt          -4.609123   1.265851  -3.641  0.00113 **
---
Signif. codes:  0 *** 0.001 ** 0.01 * 0.05 . 0.1   1

Residual standard error: 2.622 on 27 degrees of freedom
Multiple R-squared:  0.8351, Adjusted R-squared:  0.8107
F-statistic: 34.19 on 4 and 27 DF,  p-value: 3.311e-10
```

Testing of a subset of β

$H_0 : \beta_1 = \beta_2 = \beta_3 = 0,\ H_1 : \beta_j \neq 0, j = 1, 2, 3$

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
disp	1	808.89	808.89	117.65	0.0000
hp	1	33.67	33.67	4.90	0.0356
qsec	1	6.71	6.71	0.98	0.3321
wt	1	91.15	91.15	13.26	0.0011
Residuals	27	185.64	6.88		

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
wt	1	847.73	847.73	91.38	0.0000
Residuals	30	278.32	9.28		

$$SSR(disp, hp, qsec|wt) = 278.32 - 185.64 = 92.68$$

$$TS = \frac{92.68/3}{6.88} = 4.49 > F_{3,27,0.95} = 2.96$$

Thus we reject the null and conclude that *disp*, *hp* and *qsec* are jointly significant.

Test of the contrast

Many functions in R can be used to test the contrasts.

function	package	description
fit.contrast	{gmodels}	Compute and test arbitrary contrasts for regression objects
contrast.lm	{contrast}	computes one or more contrasts of the estimated regression coefficients
glht	{multcomp}	generalized linear hypothesis test
linear.hypothesis	{car}	Generic function for testing a linear hypothesis

A simple example.

Regression Diagnostics

Frequently used functions provide information used with model diagnostics

fitted.values() Returns fitted values

residuals() Returns residuals

rstandard() Standardized residuals, variance one; residual standardized using overall error variance (9.25)

rstudent() Studentized residuals, variance one; residual standardized using leave-one-out measure of the error variance (9.26)

qqnorm() Normal quantile plot

qqline() Add a line to the normal quantile plot

plot.lm() Given a lm object it produces six diagnostic plots, selected using the ‘which’ argument; default is plots 1-3 and 5

1.Residual versus fitted values

2. Normal quantile-quantile plot

3. $\sqrt{|\text{Standardized residuals}|}$ versus fitted values

4. Cook’s distance versus row labels

5.Standardized residuals versus leverage along with contours of Cook’s distance

plot.lm()	6. Cook's distance versus leverage/(1-leverage) with $\sqrt{|\text{Standardized residuals}|}$ contours
dffits()	Return DFFITS
dfbeta()	Return DFBETAS
covratio()	Return covariance ratio; vector whose ith element is the ratio of the determinants of the estimated covariance matrix with and without data point i
cooks.distance()	Returns Cook's distance
hatvalues()	Diagonal of the hat matrix
influence.measures()	Returns the previous five measure of influence and flags influential points
lm.influence()	Returns four measures of influence:
hat	Diagonal of the hat matrix, measure of leverage
coefficients	Matrix, whose ith row contains the change in the estimated coefficients when the ith case is removed
sigma	Vector, whose ith element contains the estimated of the residual standard error when the ith case is removed
wt.res	Vector of weighted residuals or raw residuals if weights are not set.

Regression Diagnostics

Example:

```
fit<-lm(mpg˜wt,data=mtcars)
#influential points are labeled
par(mfrow=c(2,2))
plot(fit) #returns four diagnostics plot (1-3 and 5)
par(mfrow=c(2,3))
plot(fit,which=1:6) #returns all six diagnostic plots

par(ask=T)
plot(residuals(fit),fitted.values(fit))
qqnorm(residuals(fit));qqline(residuals(fit))
plot(cooks.distance(fit),rownames(fit),type="h")

#influence measures
influence.measures(fit)

#extract influential points, uses $is.inf
inf.temp<-influence.measures(fit)
inf.pts<-which(apply(inf.temp$is.inf,1,any))
mtcars[inf.pts,]

#Influence measures
lm.influence(fit)
```

Regression Diagnostics

```
#Extract points that cause the greatest change in the estimates
lm.inf.coef<-lm.influence(fit)$coefficients
lm.inf.pts<-apply(lm.inf.coef[,2,drop=F],2,
+ FUN=function(x)which.max(abs(x)))

lm.inf.coef[lm.inf.pts,]
#this gives the same results with the diagnostic plots

#Get the five points that cause the greatest
#change in the estimates
lm.inf.pts.top5<-apply(lm.inf.coef,2,
+ FUN=function(x)names(rev(sort(abs(x)))[1:5]))
lm.inf.pts.top5
```

Sums of Squares

- Type I
  - Also called "sequential" sum of squares
  - Can be viewed as the reduction in SSE obtained by adding additional term to a fit that already includes the terms listed before it.
  - Pros: a complete decomposition of the predicted SS for the whole model; Preferable when some factors should be taken out before other factors.
  - Cons:Lack of invariance to order of entry into the model; not appropriate for factorial designs.
- Type II
  - The reduction in SSE due to adding the term to the model after all other terms except those that contain it (interaction terms).
  - Pros: Appropriate for model building and natural choice for regression; Most powerful when no interaction; Invariant to the order when the factors are entered to the model.
  - Cons:Not appropriate for factorial designs
- Type III
  - Effect of each variable is evaluated after all other factors have been accounted for.
  - Pros: Appropriate for unbalanced data;
  - Cons: Testing main effects when interactions presence; not appropriate with missing cells.

LRT and F test

The F-test of the null hypothesis $H_0 : \mathbf{C}\beta = \mathbf{t}$ is a likelihood ratio test (LRT) because the F-ratio is a monotone transformation of the likelihood ratio λ.

Proof:

The log-likelihood is given by

$$\begin{aligned}
\log L(\boldsymbol{\beta}, \sigma^2) &= -\frac{n}{2}\log(2\pi) - \frac{n}{2}\log(\sigma^2) - \frac{1}{2\sigma^2}(\mathbf{y} - \mathbf{X}\beta)'(\mathbf{y} - \mathbf{X}\beta) \\
&= -\frac{n}{2}\log(2\pi) - \frac{n}{2}\log(\sigma^2) - \frac{1}{2\sigma^2} SSE(\mathbf{X}) \\
\lambda = -2\log\frac{\max_{H_0} L(\boldsymbol{\beta})}{\max_{H_1 UH_0} L(\boldsymbol{\beta})} &= -2\log\frac{L(\hat{\boldsymbol{\beta}}_1)}{L(\hat{\boldsymbol{\beta}}_1, \hat{\boldsymbol{\beta}}_2)} \\
&= \frac{SSE(\mathbf{X_1}) - SSE(\mathbf{X_1} + \mathbf{X_2})}{\sigma^2},
\end{aligned}$$

for a fixed value of σ^2.

Since σ^2 is unknown, we can use $\hat{\sigma}^2_{MLE} = SSE(X_1 + X_2)/n$, then

$$F = C * \lambda = \frac{[SSE(\mathbf{X_1}) - SSE(\mathbf{X_1} + \mathbf{X_2})]/r}{SSE(\mathbf{X_1} + \mathbf{X_2})/(n-k-1)} \sim F_{r,n-k-1},$$

where $C = \frac{n-k-1}{nr}$.

References

1. Elizabeth R. Brown, *Introduction to Regression Models*
2. Nicholas Christian, *Statistical Computing in R*
3. Langsrud, ϕ. (2003), ANOVA for Unbalanced Data: Use Type II Instead of Type III Sums of Squares, *Statistics and Computing*, 13, 163-167.