Starting Genetic Imaging Analyses with SOLAR-Eclipse

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To participate in the demo

- Please register and get HCP account
- http://hcpx-demo.humanconnectome.org
- The univariate demo
  - **http://tinyurl.com/mvyxcmq**
  - or
  - https://hcpx-demo.humanconnectome.org/app/template/UnivariatePolygenic.vm
- The bivariate demo
  - **http://tinyurl.com/ltqf8sj**
  - or
  - https://hcpx-demo.humanconnectome.org/app/template/GeneticCorrelation.vm

Introduction

- What is SOLAR-Eclipse
- Downloading and installing SOLAR-Eclipse
- Creating a solar analysis directory
  - Pedigree file
  - Phenotype file
- Common analyses types
  - Heritability
  - Genetic Correlations
    - Get this file: www.mdbrain.org/personalpages/peter/lpa.tar

SOLAR-Eclipse

- Extension of SOLAR for imaging genetics
- Developed for multiplatform (pc/mac/linux)
  - –Genetic analysis of discreet and continuous traits
  - –Supports All Common Genetic Analyses for Continuous and Discrete traits.
    - Heritability
    - Genetic Correlation
    - Quantitative trait Linkage
    - GWAS analyses in related and unrelated samples
  - –Supports uni-and-multivariate analyses
  - –Supports discrete and continuous covariates

Main Strength – Genetic Analysis in Family and Twin Samples

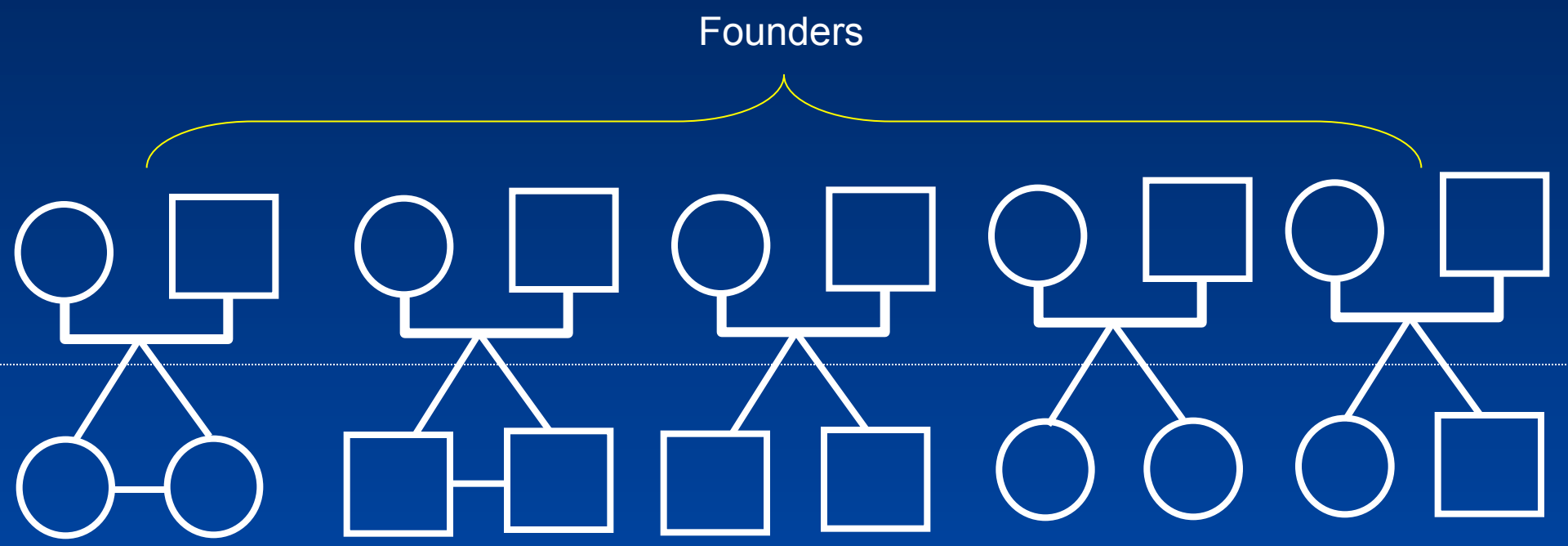
Downloading/Installing SOLAR

- Get it from NITRC website
  - http://www.nitrc.org/projects/se_linux/
  - Use the linux version for most of the features
    - Latest Apple/PC versions are compiled at request
- Email your user name to get the registration code.
  - solar@txbiomedgenetics.org
  - This code will work on any machine with the same user name
  - Registering for a specific domain is also available
- Manual/Instructional videos at http://www.mdbrain.org/solareclipse/

Develop Analysis Plan

- Background
  - Lipo-Protein A (LAP) stimulates lipid metabolism.
  - Specific polymorphisms may influence LPA1/2 levels measured in blood
- Questions
  - Are LPA1/2 concentrations in blood influenced by additive genetic effects?
  - To what extent is white matter integrity measured through DTI-FA determined by additive genetic effects?
  - What extent are the same genetic factors influencing both white matter integrity and LPA levels?
- Approach
  - Measure DTI-FA and [LPA1/2] in blood in N=60/50 MZ/DZ twin pairs.
  - Measure heritability of average FA values
  - Measure heritability of [LPA]
  - Calculate pleiotropy between two traits!

Our Twin Pedigree



Making a pedigree

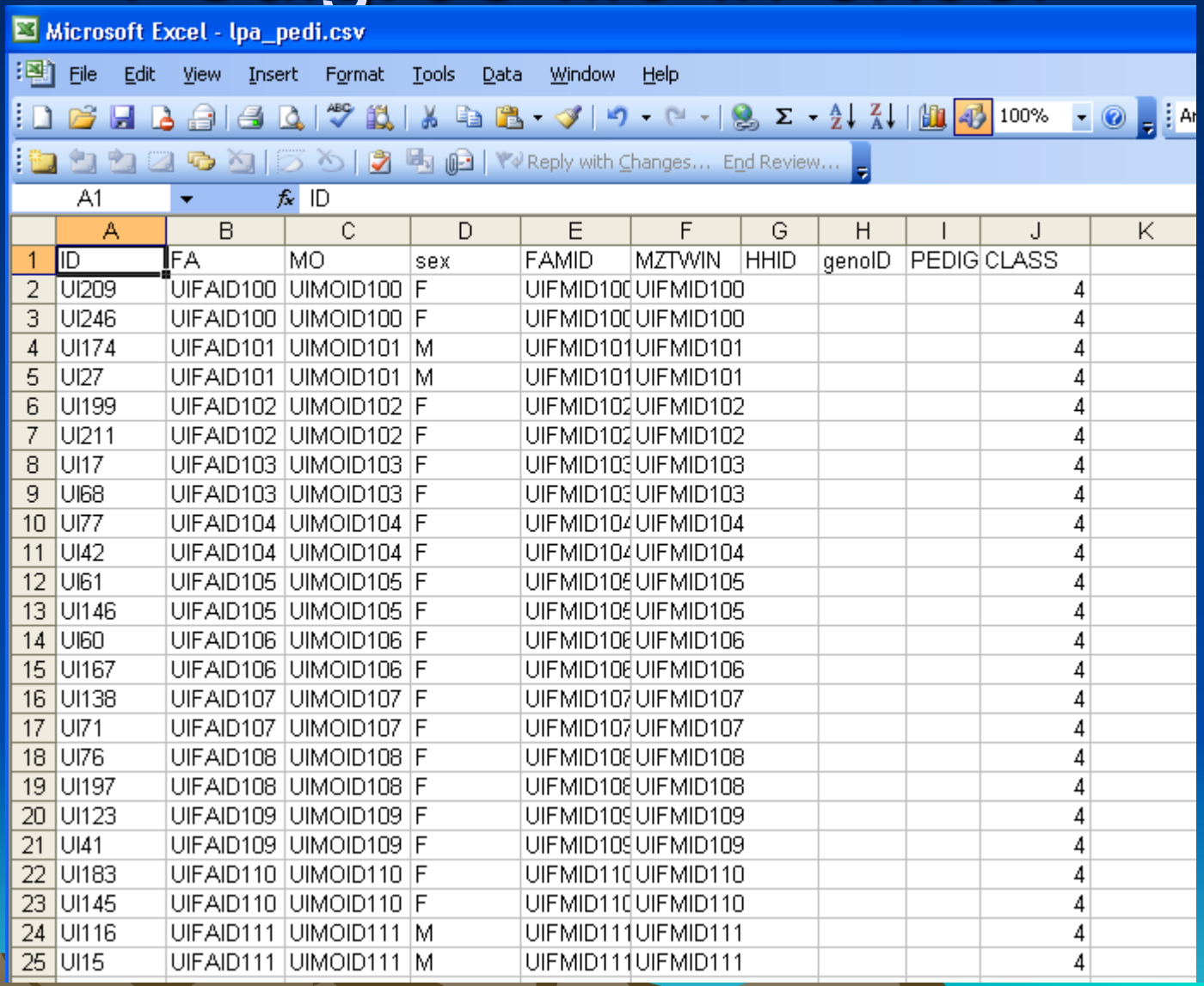
- A very important step.
- Pedigree contains “genetic information” based on relatives.
  - Expressed as kinship matrix
  - MZ twins share 100% genetic variance
  - DZ twins share 50% of genetic variance
- SOLAR pedigree must have “founders”
  - Founders are the basis of the pedigree and are assumed unrelated
  - Founder only pedigrees can be used for GWAS analysis of unrelated individuals.

Actually is a very simple process

ID	FA	MO	sex	FAMID	MZTWIN	HHID	genoID	PEDIGREE NUMBER	CLAS
UI209	UIFAID100	UIMOID100	F	UIFMID100	UIFMID100				
UI246	UIFAID100	UIMOID100	F	UIFMID100	UIFMID100				

- Best done in Excel
- Start coding one subject per line.
- ID – subject id (your choice)
- FA/MO are IDs for parents
  - will have to be included as founders even if they don't have phenotypes
- FAMID – optional family id
- MZTWIN – identifier for MZ twins. A twin pair is defined as subjects with same identifier.

Pedigree file in excel



	A	B	C	D	E	F	G	H	I	J	K
1	ID	FA	MO	sex	FAMID	MZTWIN	HHID	genoID	PEDIG	CLASS	
2	UI209	UIFAID100	UIMOID100	F	UIFMID100	UIFMID100				4	
3	UI246	UIFAID100	UIMOID100	F	UIFMID100	UIFMID100				4	
4	UI174	UIFAID101	UIMOID101	M	UIFMID101	UIFMID101				4	
5	UI27	UIFAID101	UIMOID101	M	UIFMID101	UIFMID101				4	
6	UI199	UIFAID102	UIMOID102	F	UIFMID102	UIFMID102				4	
7	UI211	UIFAID102	UIMOID102	F	UIFMID102	UIFMID102				4	
8	UI17	UIFAID103	UIMOID103	F	UIFMID103	UIFMID103				4	
9	UI68	UIFAID103	UIMOID103	F	UIFMID103	UIFMID103				4	
10	UI77	UIFAID104	UIMOID104	F	UIFMID104	UIFMID104				4	
11	UI42	UIFAID104	UIMOID104	F	UIFMID104	UIFMID104				4	
12	UI61	UIFAID105	UIMOID105	F	UIFMID105	UIFMID105				4	
13	UI146	UIFAID105	UIMOID105	F	UIFMID105	UIFMID105				4	
14	UI60	UIFAID106	UIMOID106	F	UIFMID106	UIFMID106				4	
15	UI167	UIFAID106	UIMOID106	F	UIFMID106	UIFMID106				4	
16	UI138	UIFAID107	UIMOID107	F	UIFMID107	UIFMID107				4	
17	UI71	UIFAID107	UIMOID107	F	UIFMID107	UIFMID107				4	
18	UI76	UIFAID108	UIMOID108	F	UIFMID108	UIFMID108				4	
19	UI197	UIFAID108	UIMOID108	F	UIFMID108	UIFMID108				4	
20	UI123	UIFAID109	UIMOID109	F	UIFMID109	UIFMID109				4	
21	UI41	UIFAID109	UIMOID109	F	UIFMID109	UIFMID109				4	
22	UI183	UIFAID110	UIMOID110	F	UIFMID110	UIFMID110				4	
23	UI145	UIFAID110	UIMOID110	F	UIFMID110	UIFMID110				4	
24	UI116	UIFAID111	UIMOID111	M	UIFMID111	UIFMID111				4	
25	UI15	UIFAID111	UIMOID111	M	UIFMID111	UIFMID111				4	

Lpa_pedi.csv. CSV stands for comma separated value file format

Continued

ID	FA	MO	sex	FAMID	MZTWIN	HHID	genoID	PEDIGREE NUMBER	CLASS
UI209	UIFAID100	UIMOID100	F	UIFMID100	UIFMID100				4
UI246	UIFAID100	UIMOID100	F	UIFMID100	UIFMID100				4

- HHID
  - Household id. Use for study of household effects
  - Use it if you have twins living in different households
- genoID
  - Is not commonly used
- Pedigree Number
  - Is not commonly used in humans
- CLASS
  - Important for mega-genetic analysis. Covariates and other normalizations are performed per class
  - If you plan to combine multiple studies assign them non-overlapping class valus

Code founders like this

ID	FA	MO	sex	FAMID	MZTWIN	HHID	genoID	PEDIGREE	CLASS
UIFAID1			M	UIFMID1					4
UIFAID2			M	UIFMID2					4

- Founders are identified as “orphaned” subjects.
  - Make sure you have the right gender
- Save the file in the .csv format.
  - On the mac use “Windows CSV” option
  - Otherwise, “^L” is used to identify end of line

Fire up solar-eclipse

```
[peterk@medusa LPA]$
[peterk@medusa LPA]$
[peterk@medusa LPA]$
[peterk@medusa LPA]$ solar
```

Solar command starts the tcl shell with R-like interface

```
SOLAR Eclipse version 7.5.3 (Experimental), last updated on May 13, 2014
Copyright (c) 1995-2014 Texas Biomedical Research Institute
Enter help for help, exit to exit, doc to browse documentation.

solar> ls
```

Solar inherits all bash commands, so “ls” gives listing of directory

```
lpa_pedi.csv  lpa_pheno.csv
solar>
```

Lpa_pedi is the file we just created. Lpa_pheno doesn’t exist yet!

heron : peterk | 10.0.4.76 : | login1 : petr | test_medx : bash | 10.0.4.76 : petr

Load your pedigree

```
[peterk@medusa LPA]$
[peterk@medusa LPA]$
[peterk@medusa LPA]$
[peterk@medusa LPA]$ solar

SOLAR Eclipse version 7.5.3 (Experimental), last updated on May 13, 2014
Copyright (c) 1995-2014 Texas Biomedical Research Institute
Enter help for help, exit to exit, doc to browse documentation.

solar> ls
lpa_pedi.csv  lpa_pheno.csv  phenotypes.info
solar> load pedi lpa_pedi.csv
Loading pedigree data from the file lpa_pedi.csv ...
solar> ls
house.gz  lpa_pedi.csv  lpa_pheno.csv  pedigree.info  pedindex.cde  pedindex.out  phenotypes.info  phi2.gz
solar>
```

"Load pedi" command with the name of the file

Several new files will be created

heron : peterk | 10.0.4.76 : | login1 : petr | test_medx : bash | 10.0.4.76 : petr

SOLAR creates several internal files that re-arrange pedigree in computation friendly format

Hint – phi2.gz is the kinship matrix in text format that can be used for other packages

Obtaining phenotypes

- DTI-FA values we calculated using ENIGMA-DTI pipeline
  - http://enigma.ini.usc.edu/protocols/dti-protocols/
  - Average whole brain FA values were used
- LPA 1 and 2 levels were obtain from blood
  - 12 hour fasting
  - Measured using Cardio IQ™ Ion Mobility assay
    - Unknown to me and hence presumed very accurate

Lets make a phenotype file

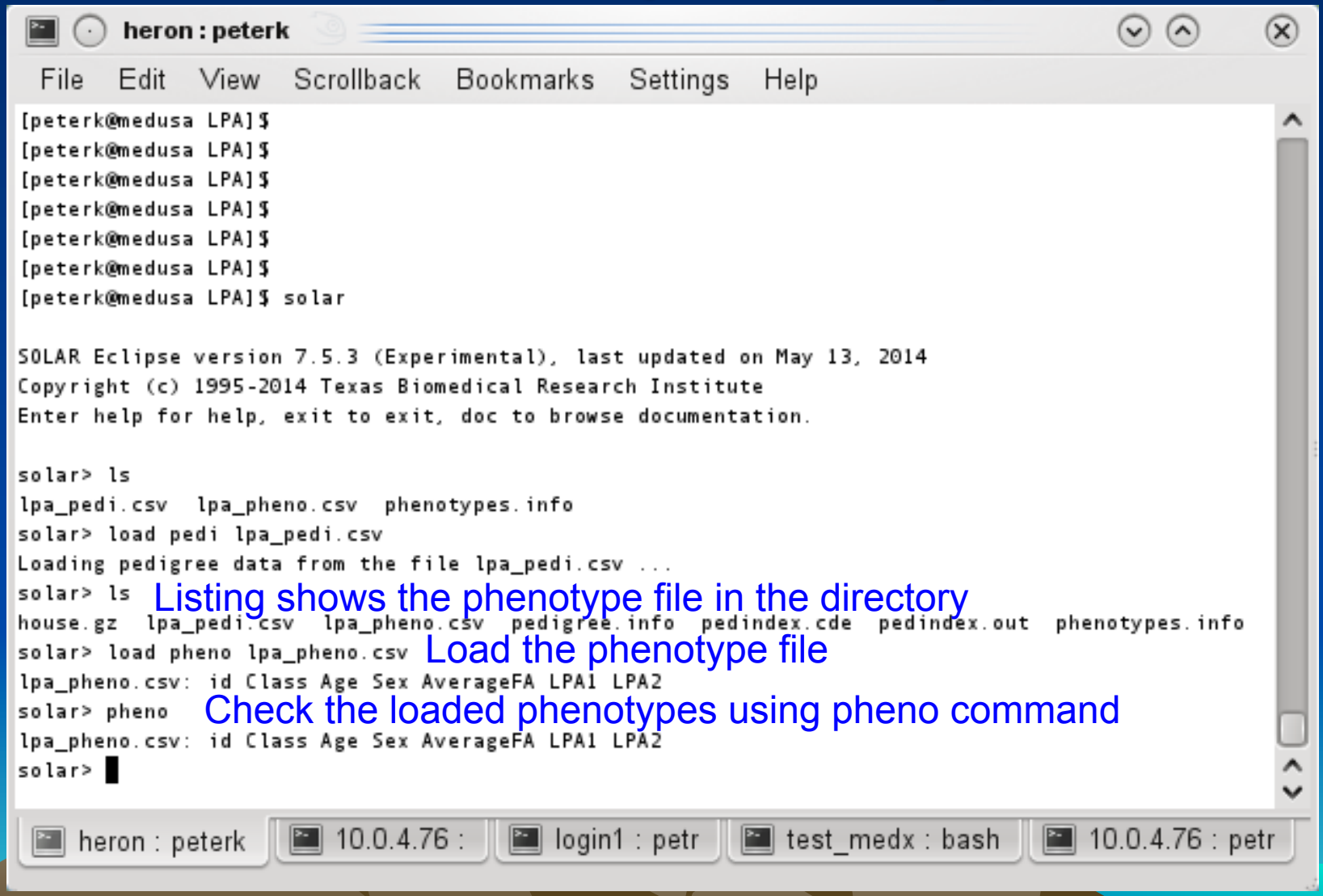
- Phenotypes are stored in a simple csv format
- Subject ID should match IDs in pedigree file
- Multiple phenotypes files can be loaded at once and merged in memory
  - Convenient for storing fixed factors such as sex and age for the entire pedigree
- Load phenotype command is
  - “load pheno file_name.csv”
  - http://www.mdbrain.org//solareclipse/solar_commd.html
  - Type “pheno” to check that phenotypes were loaded

Phenotype file

- Let put average FA values measured from DTI
- Blood-levels of two LPA proteins LPA1 and LPA2
- Add sex and gender
- Simple CSV format.

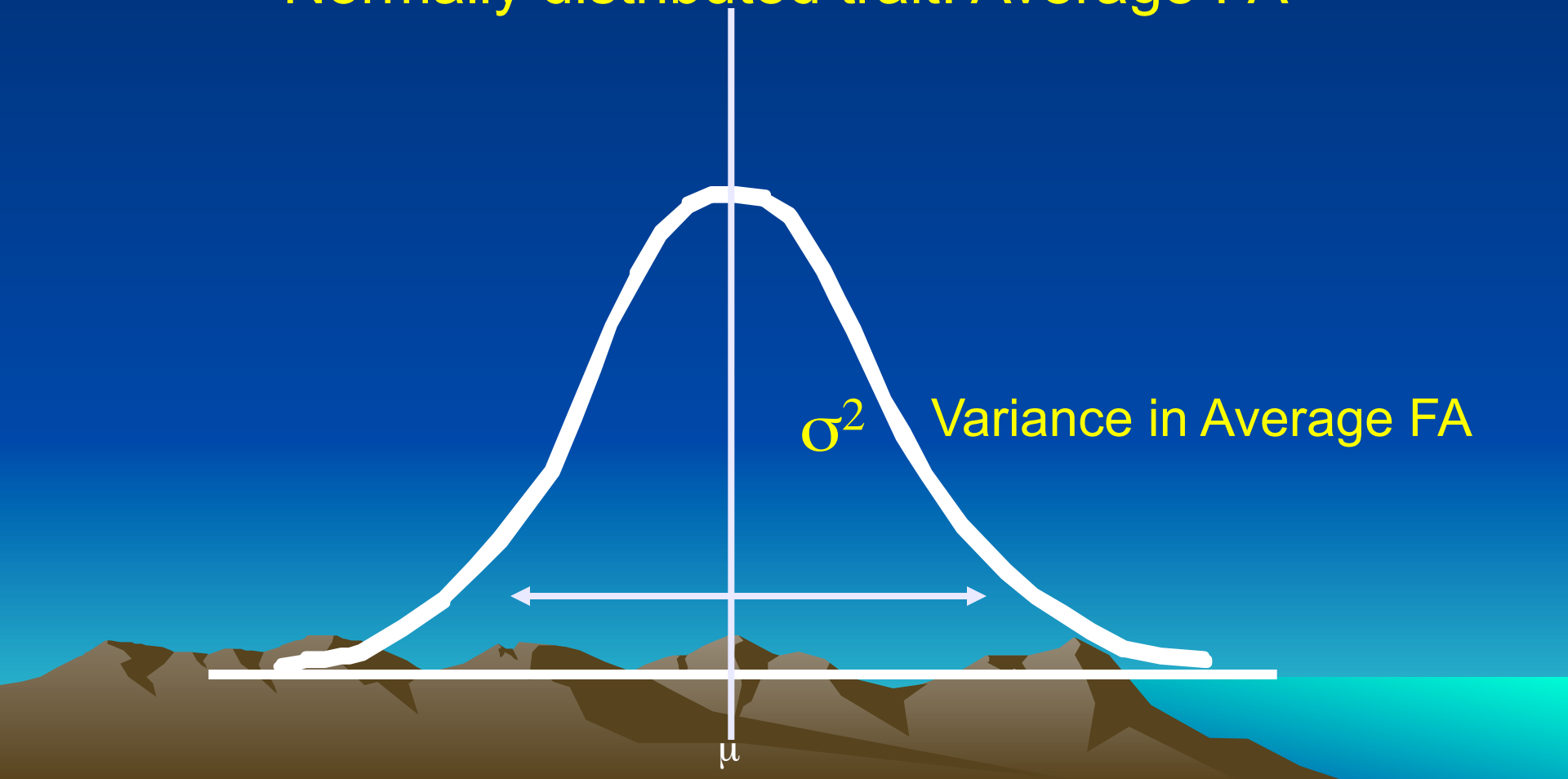
id	Age	Sex	AverageFA	LPA1	LPA2
UI101	38	F	0.489995	6.27615	1.191184
UI102	38	F	0.486044	5.73763	1.05002
UI103	45	F	0.475386	6.42397	1.13037
UI10	35	F	0.455976	5.40412	1.11781
UI104	19	F	0.493612	6.20166	1.01562
UI105	56	F	0.457284	6.03059	1.14993
UI106	35	F	0.471036	1.96892	1.05707
UI107	36	F	0.498711	6.31608	1.17105
UI108	32	F	0.485737	6.12398	1.07816

Load the phenotype file



Calculating heritability

- What is heritability?



Variance Decomposition

$$\sigma_p^2 = \sigma_g^2 + \sigma_e^2$$

σ_p^2 = Total phenotypic variance

σ_g^2 = Variance due to genetic sources

σ_e^2 = Variance due to environmental source

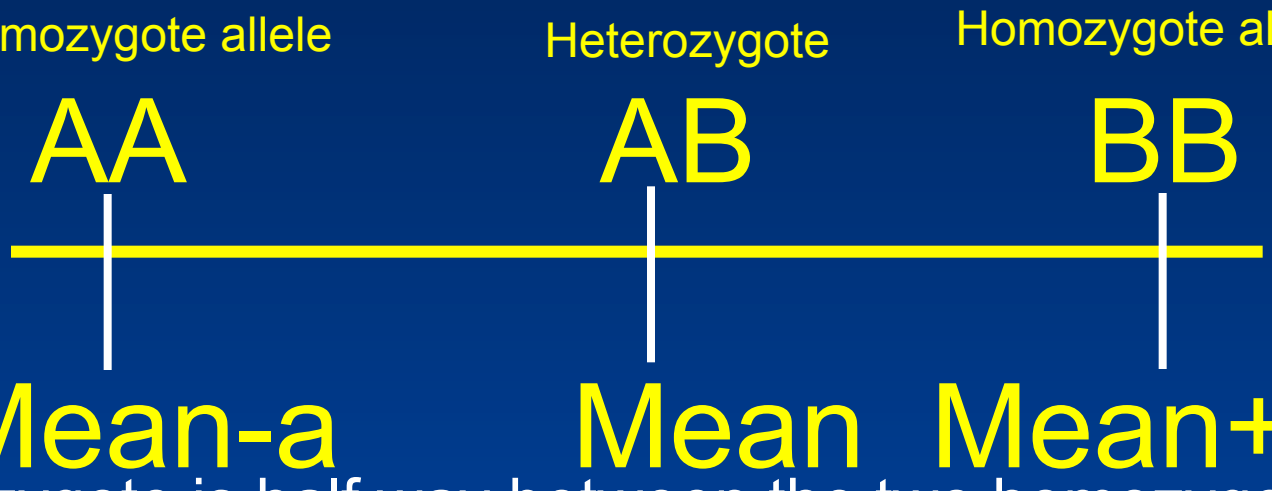
Genetic variance is due to

$$\sigma_g^2 = \sigma_a^2 + \sigma_d^2$$

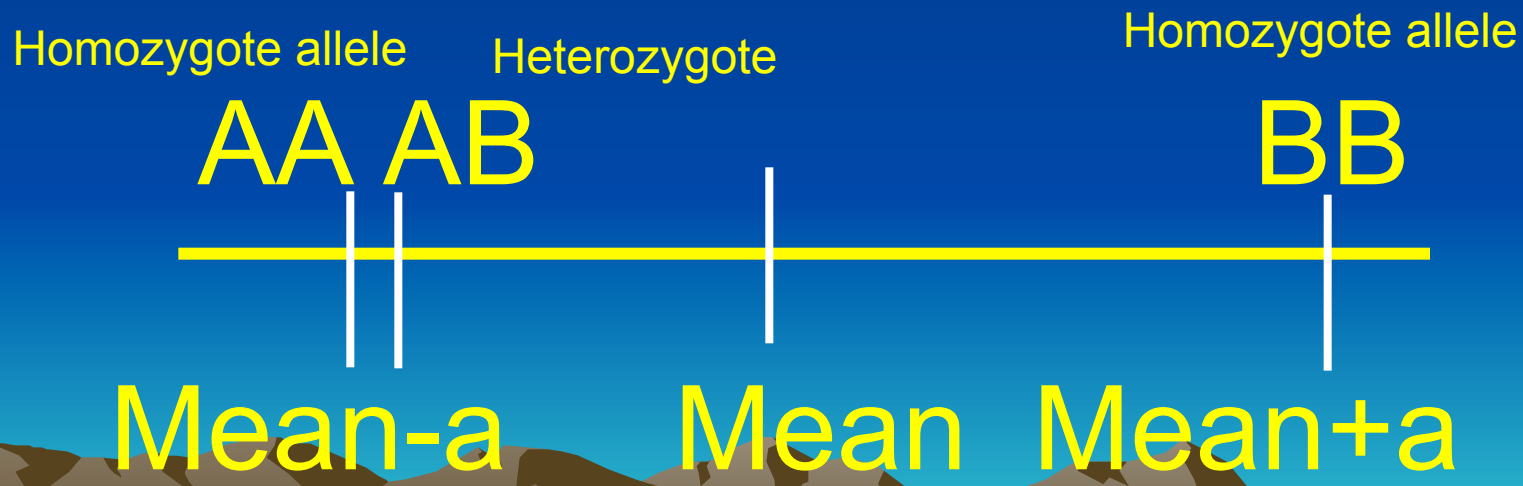
σ_a^2 = Additive genetic variance

σ_d^2 = Dominance variance

Additive genetic effect



heterozygote is half way between the two homozygotes,



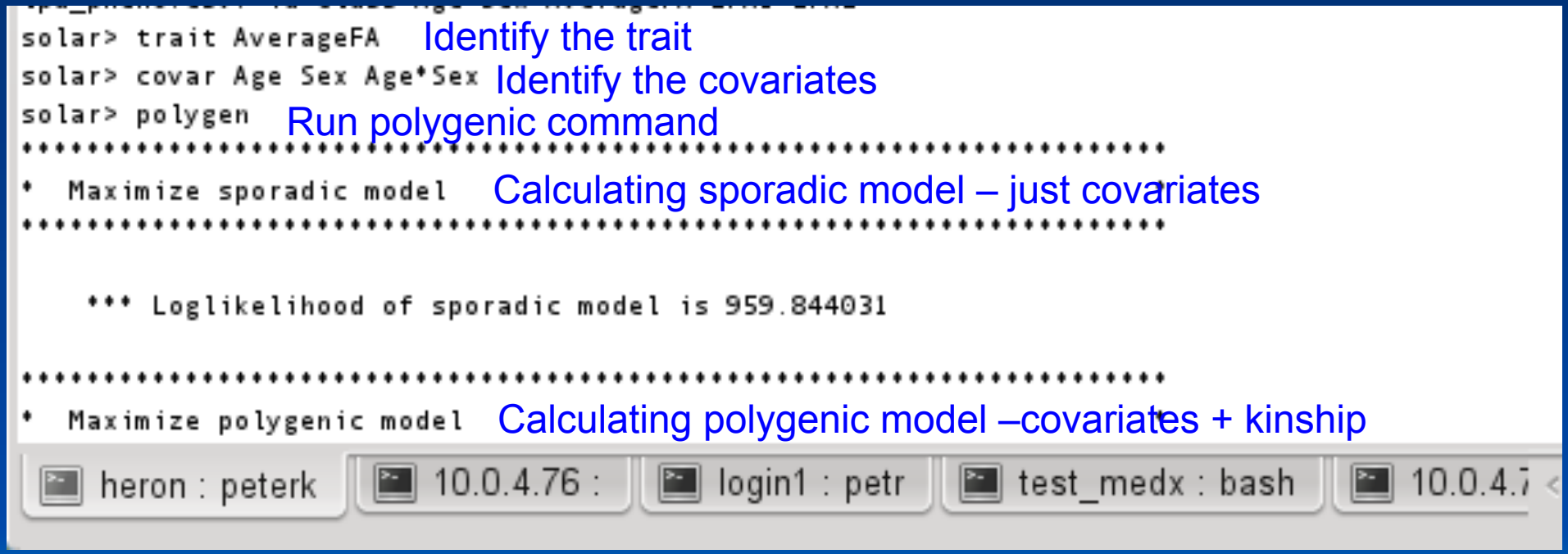
Dominant genetic effect: A dominates B

Additive genetic (narrow sense) heritability

$$h^2 = \frac{\sigma_a^2}{\sigma_p^2}$$

Fraction of the total variance in a trait explained by additive genetic variance

How to calculate it?



Polygenic function tests two GLM models based on their log-likelihood values

Sporadic model assumes no genetic effects (unitary kinship matrix)

Polygenic model assumes genetic effects (non-unitary kinship matrix)

Output

heron : peterk

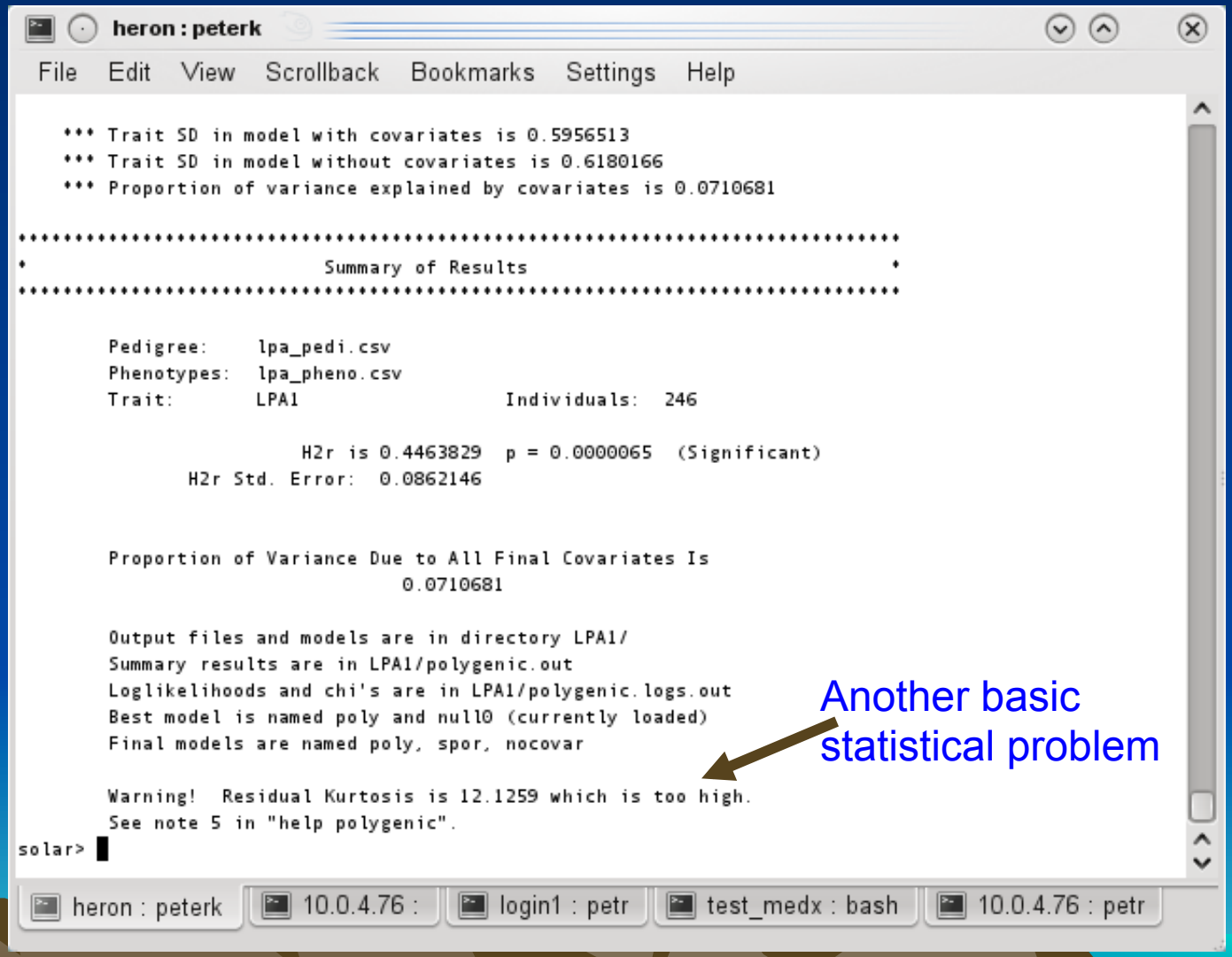
File Edit View Scrollback Bookmarks Settings Help

```
*                          Summary of Results                                  *
**************************************************************************************

        Pedigree:     lpa_pedi.csv
        Phenotypes:   lpa_pheno.csv
        Trait:        AverageFA              Individuals:   246

        WARNING!  Estimated Trait Standard Deviation is 0.01248
        When Trait SD is below 0.5, results are sometimes incorrect
        Multiplying trait by a factor such as 80.2 is recommended

                        H2r is 0.8318344   p = 6.066895e-20   (Significant)
                H2r Std. Error:   0.0346385


        Proportion of Variance Due to All Final Covariates Is
                                0.1987657

        Output files and models are in directory AverageFA/
        Summary results are in AverageFA/polygenic.out
        Loglikelihoods and chi's are in AverageFA/polygenic.logs.out
        Best model is named poly and null0 (currently loaded)
        Final models are named poly, spor, nocovar

        Residual Kurtosis is -0.0206, within normal range
solar>
```

Hmm, we have a basic statistical problem

heron : peterk | 10.0.4.76 : | login1 : petr | test_medx : bash | 10.0.4.7

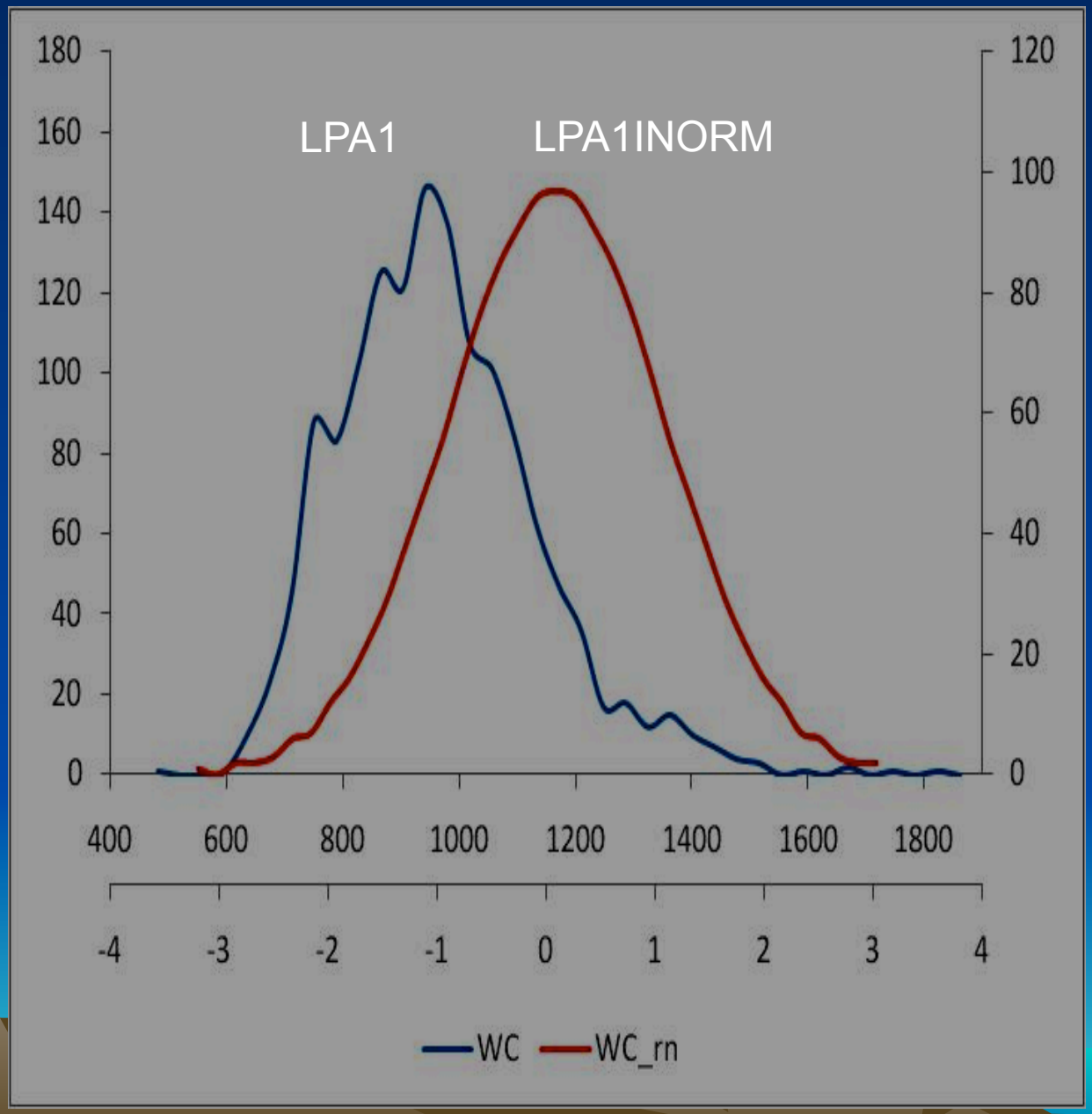
Do the same to LPA1



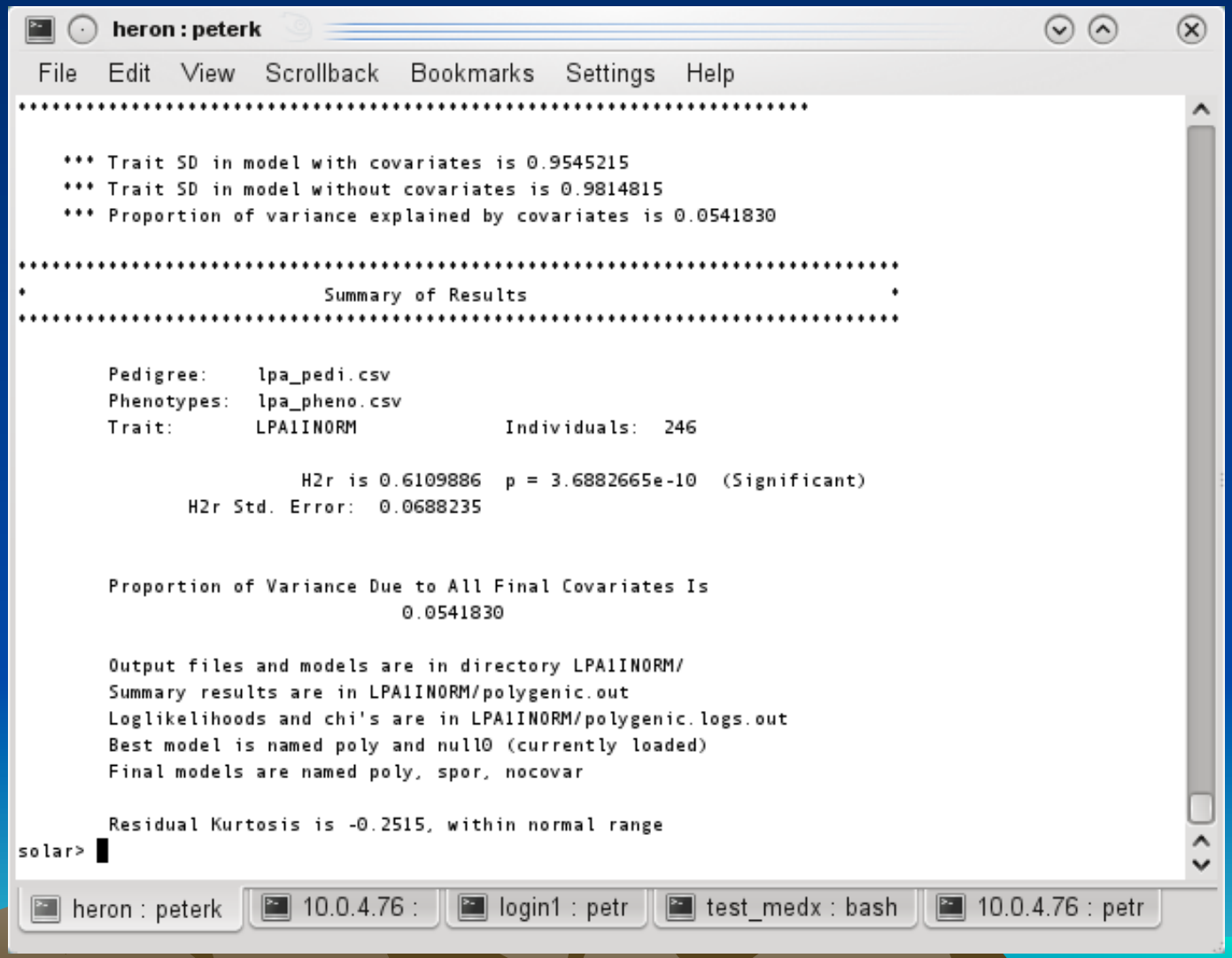
What to do about it?

- Both problems infer lack of normal distribution
- Normality of the distribution has to be enforced for validity of measurements
- Inorm function in solar does it
  - AverageFAINOR = inorm_AverageFA
  - LPA1INORM = inorm_LPA1
- Lets repeat!

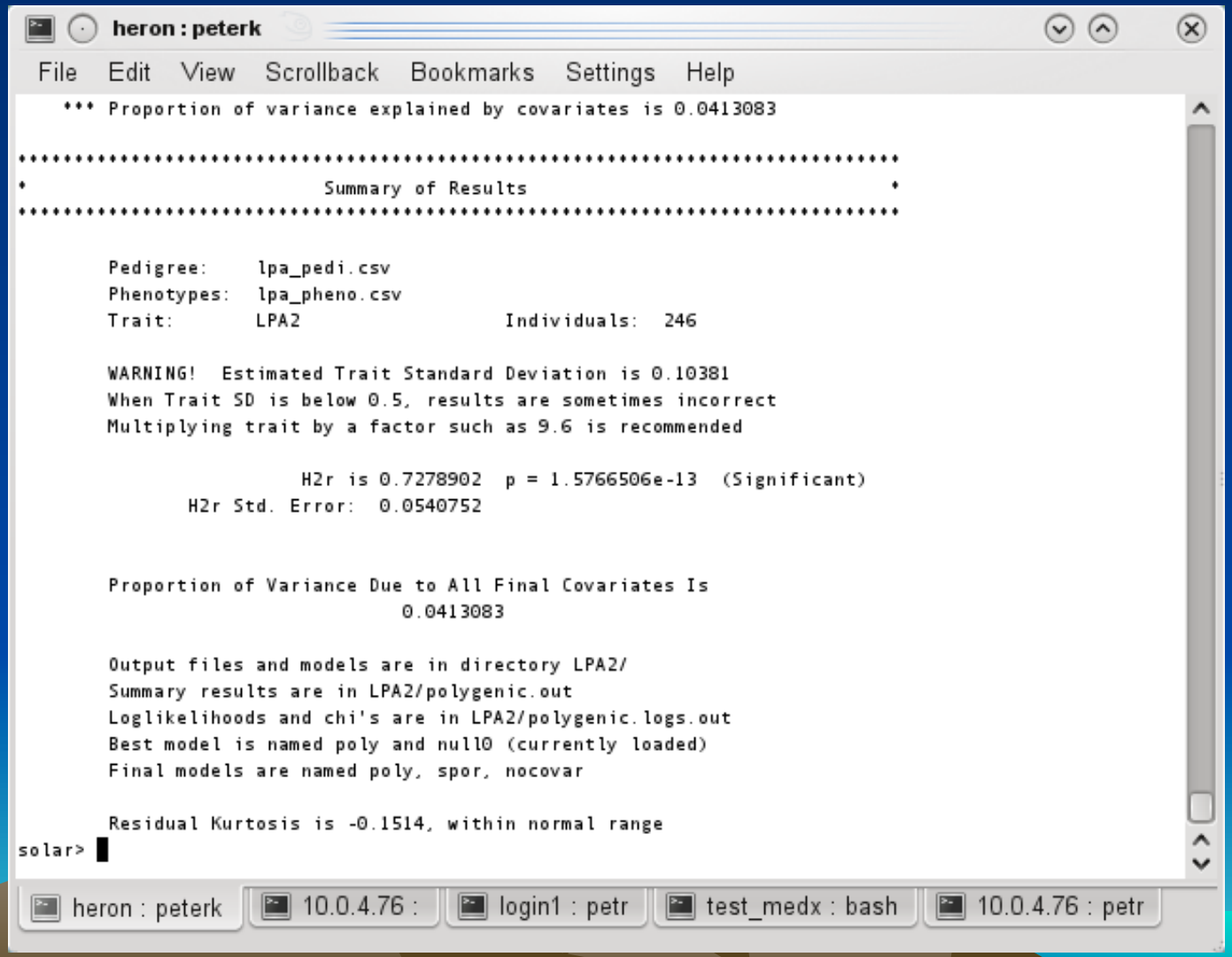
Forcing normal distribution via inverse Gaussian transform.



A-ha, inorm did the trick!



Do the same for LPA2



What do we have

Additive genetic variance explains

- 82% of FA variance
- 61% of the variance in LPA1 protein
- 72% of the variance in LPA2 protein

Now the important part.

- Is that variance shared between traits?
  - This would suggest common genetic or environmental cause

Perform genetic correlation

- Use genetic correlation (ρ_G)

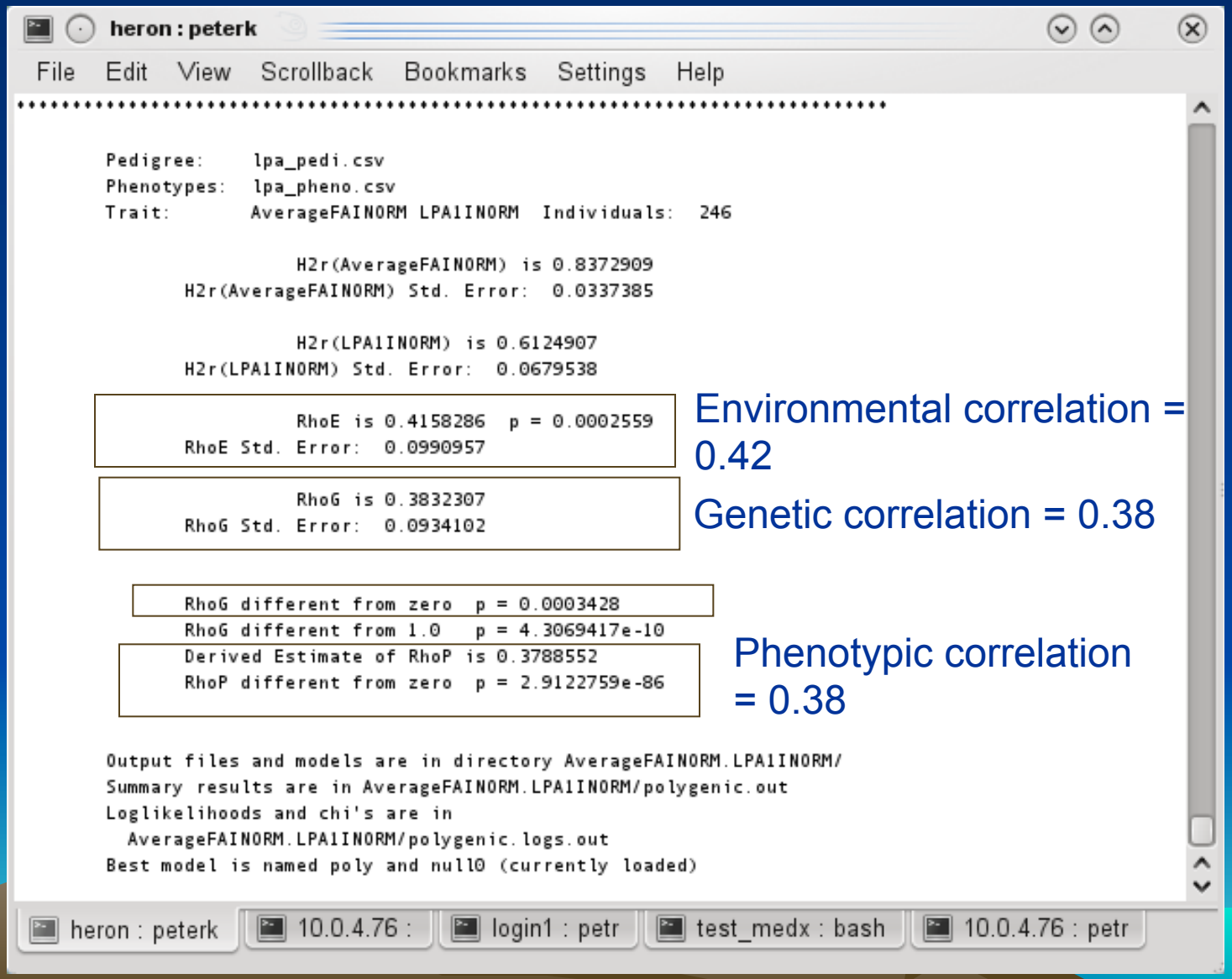
$$r = \sqrt{h_A^2}\sqrt{h_B^2} \cdot \rho_G + \sqrt{1 - h_A^2}\sqrt{1 - h_B^2} \cdot \rho_E$$

  - Pearson's r decomposed into ρ_G and ρ_E
  - ρ_G is the proportion of variability due to shared genetic effects
- To calculate degree of shared genetic variance

Multivariate Analysis in Solar

- Simply use more then one trait
  - trait AverageFAINORM LPA1INORM
  - covar age^1,2#sex
    - Covaries effects of age, age^2, sex and age by sex
  - Polygen –testrhoe –testrhog –testrhop
    - –testrhoe calculates signficance of genetic correlation
    - –testrhog calculates signficance of enviormental correlation
    - –testrhop calculate signficance of the phenotypic (combined) correlation
      - Useful for calculating correlations in family samples

Results for FA and LPA1

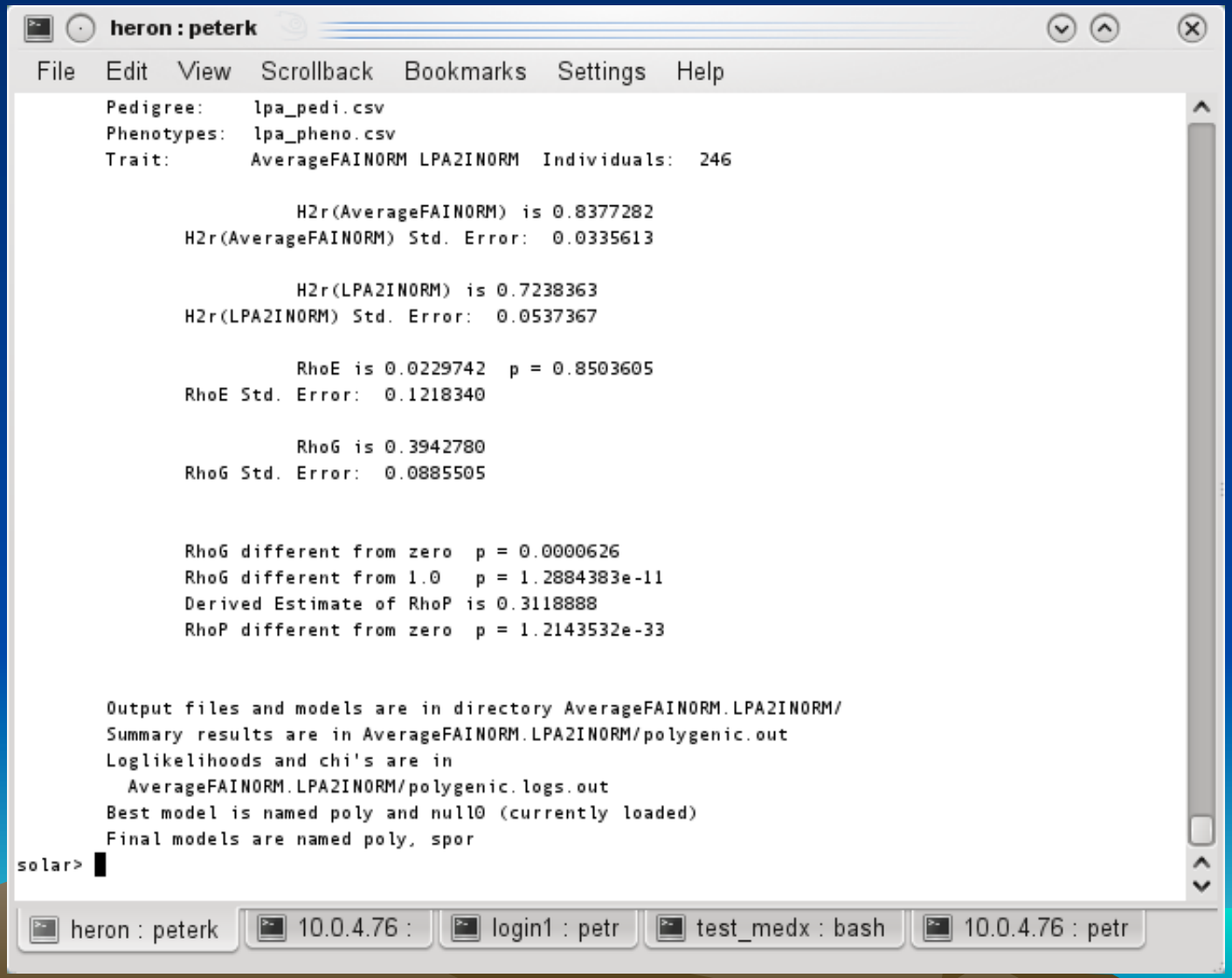


Environmental correlation = 0.42

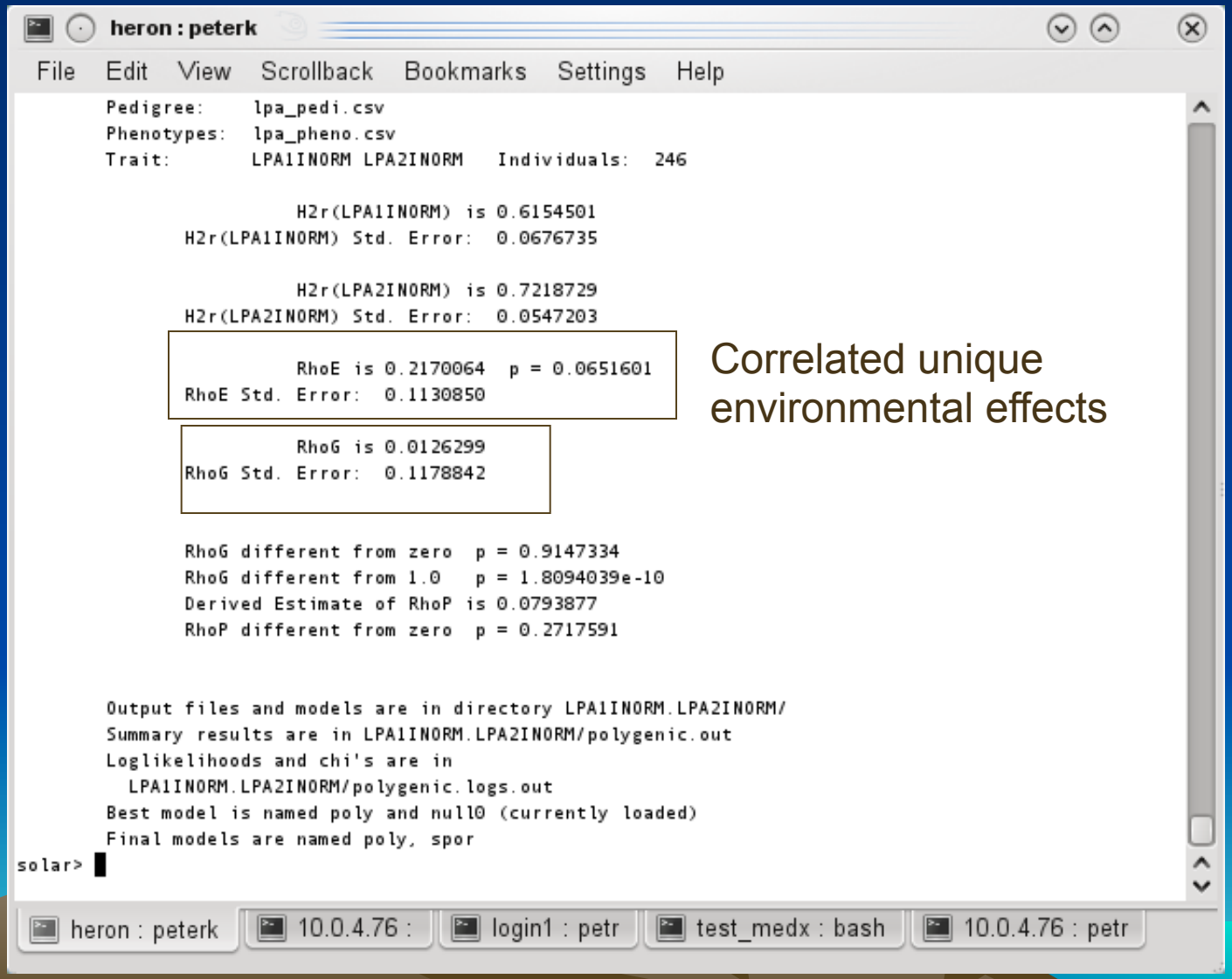
Genetic correlation = 0.38

Phenotypic correlation = 0.38

Results for FA and LPA2



What about LPA1 and LPA2



Correlated unique environmental effects

What have we learned?

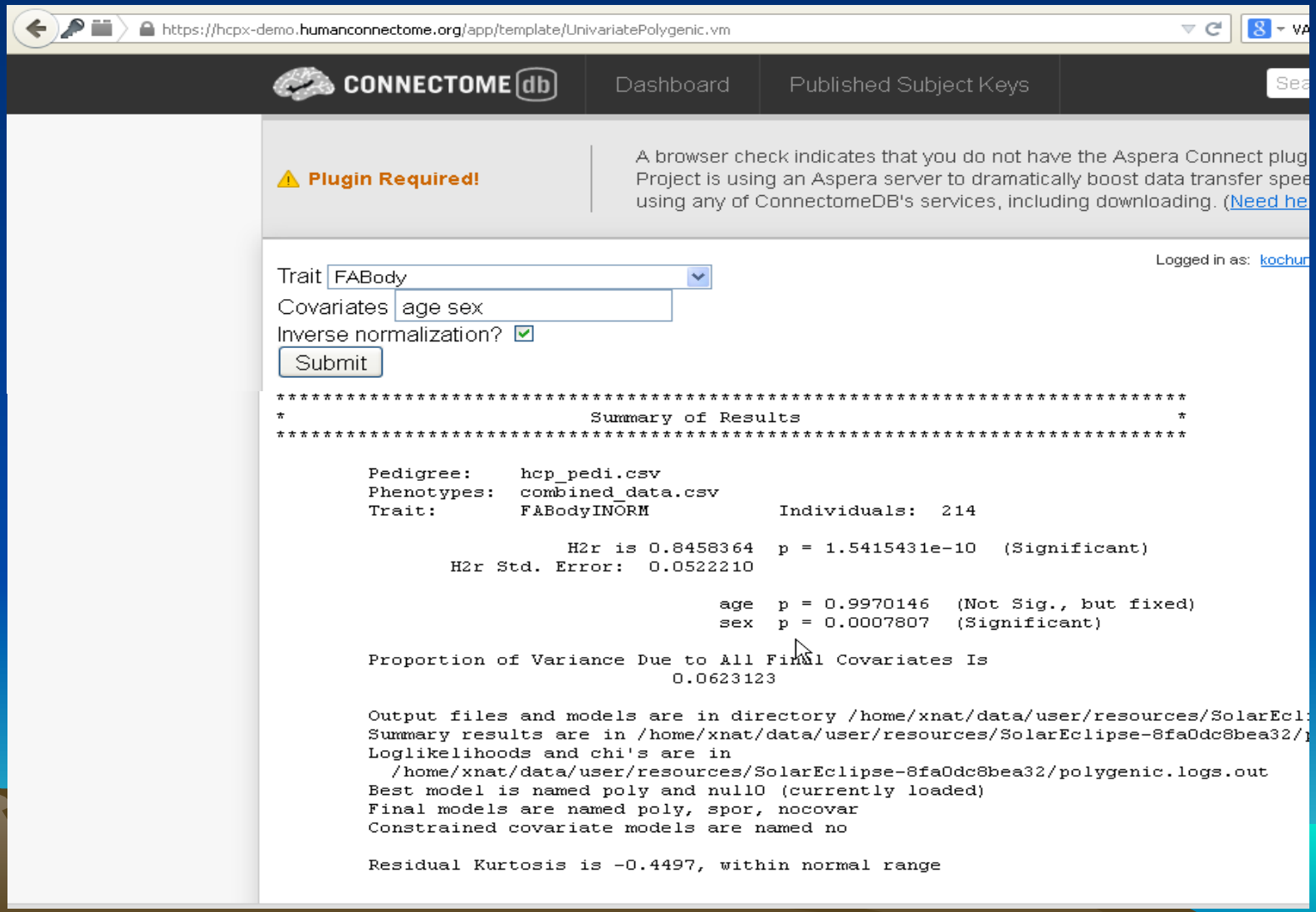
- FA has shared genetic variance with blood volumes of two lipid messenger/receptors
- FA shows shared environmental variance
  - Caused by factors like stress or diet
- The two LPA molecules are genetically independent
  - Encoded by different genes
  - But show some common environmental variance

Contact (pkochunov@gmail.com)

- HCP researchers are running SOLAR-Eclipse as a part of their analysis service
- https://hcpx-demo.humanconnectome.org/app/template/UnivariatePolygenic.vm
  https://hcpx-demo.humanconnectome.org/app/template/GeneticCorrelation.vm
- Visit SOLAR-Eclipse workshop at Imaging Genetic Conference
  - http://www.imaginggenetics.uci.edu/
  - 1/19-20, 2015/ Irvine California
  - To learn how to use SE
    - Voxel-wise genetic analysis
    - Mega and Meta genetic analysis

Human Connectome Web SE

https://hcpx-demo.humanconnectome.org/app/template/UnivariatePolygenic.vm



Human Connectome Web SE

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