

PostGWAS Intermediate Bioinformatics Online Course

Fine-mapping and visualisation course

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Course organisation

3 sessions :

- 1 Visualisation
- 2 Fine-Mapping
- 3 Consolidation of 2 previous session

Part. 1 : Visualisation

Visualisation of GWAS: Learning Objectives

- Overview of figures used in GWAS in different level.
- Understand when to use, how to read and analyze figures.
- Detected bias in your GWAS result using figures.

Visualisation of GWAS : Learning Outcomes

- Used various platform or software to plot figures :
 - ▶ Manhattan plot
 - ▶ QQ plot
 - ▶ regional plot
 - ▶ forest plot.
 - ▶ Phenotype distribution by genotype
- understand how to interpret and used figures.

Introduction

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 - ▶ Raw data of sequencing from array.

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 - ▶ example : FUMA, locuszoomV2

Little reminder

Summary statistics

Summary statistics :

- first line :header descriptive of each column.

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chr rs n_miss allele1 af beta se p_wald  
1 1:920733:T:C 0 T 0.098 -1.454952e+00 1.977754e+00 4.622846e-01
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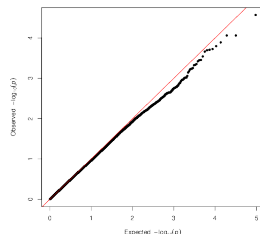
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QQ-Plot

Global visualisation : QQ-plot

Quantile Quantile plot

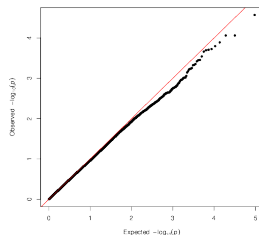
- The QQ plot is a graphical representation of the deviation of the observed P values from the null hypothesis.



Global visualisation : QQ-plot

Quantile Quantile plot

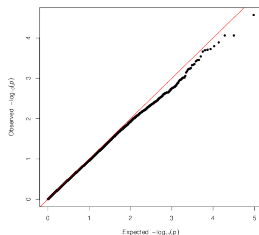
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- Purpose : Determine if there are a likely a large number of false positive



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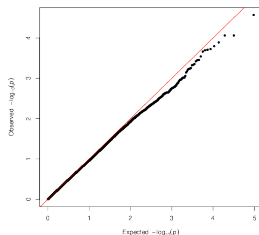
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- Logic : Main of SNPs are not associated with your phenotype only a very, very small number of SNPs should be associated with our trait.



Global visualisation : QQ-plot

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- Purpose : Determine if there are a likely a large number of false positive
- Logic : Main of SNPs are not associated with your phenotype only a very, very small number of SNPs should be associated with our trait.
- Figure : P-values for each SNP are sorted from largest to smallest and plotted against expected values.



Global visualisation : QQ-plot

- P-value distribution should follow a random law, for 100 tests, P-value

source : https://jnmaloof.github.io/BIS180L_web/slides/11_QQPlots.html#1

Global visualisation : QQ-plot

- P-value distribution should follow a random law, for 100 tests, P-value
 - ▶ We expect 1 test (1%) to have a p-value of ≤ 0.01

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Global visualisation : QQ-plot

- P-value distribution should follow a random law, for 100 tests, P-value
 - ▶ We expect 1 test (1%) to have a p-value of ≤ 0.01
 - ▶ We expect 5 test (5%) to have a p-value of ≤ 0.05

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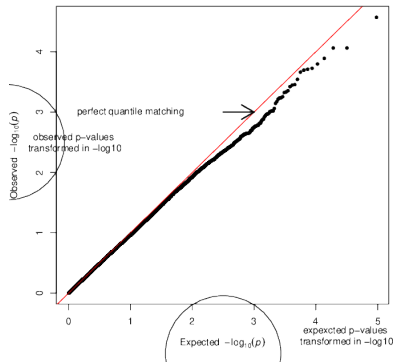
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 - ▶ We expect 50 test (50%) to have a p-value of ≤ 0.50
- We can computed expected p-value using quantile distribution for each SNPs using quantile.

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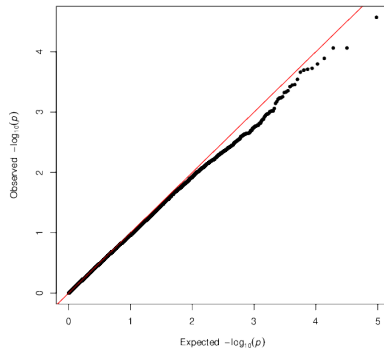
Quantile Quantile plot

- defined in x axis by expected p-value transformed using $-\log_{10}$
- defined in y axis by observed p-value transformed using $-\log_{10}$
- red line represent perfect quantile matching



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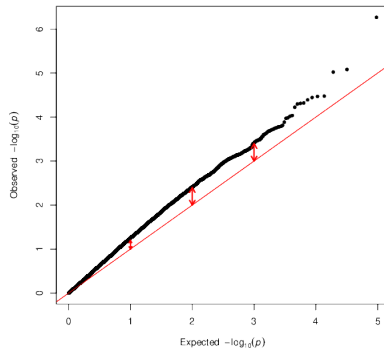
- example QQ-plot where points fit with perfect quantile matching : fit with red line
- no significant positions less than 5×10^{-8} ($-\log_{10} p > 7.3$)



Global visualisation : QQ-plot

QQ plot showed a bias compared to red line :

- inflation of p-value compared to null model
- cause :
 - ▶ genetics structure
 - ▶ allele frequency are not control and lot of low maf < 0.01 %



Global visualisation : QQ-plot and inflation factors

- The genomic inflation factor estimates the amount of inflation by comparing observed test statistics across all genetic variants to those expected under the hypothesis of no effect.

$$\chi^2 = q_{\chi}^2(1 - P, 1)$$

with P p-value and q_{χ}^2 quantile function of χ^2 with 1 degrees of freedom

$$\lambda = \text{median}(\chi^2)/0.456$$

Global visualisation : QQ-plot and inflation factors

- The genomic inflation factor estimates the amount of inflation by comparing observed test statistics across all genetic variants to those expected under the hypothesis of no effect.
- The genomic inflation factor λ is defined as the ratio of the median of the empirically observed distribution of the test statistic to the expected median, thus quantifying the extent of the bulk inflation and the excess false positive rate.

$$\chi^2 = q_{\chi}^2(1 - P, 1)$$

with P p-value and q_{χ}^2 quantile function of χ^2 with 1 degrees of freedom

$$\lambda = \text{median}(\chi^2)/0.456$$

Global visualisation : QQ-plot and inflation factors

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- The genomic inflation factor λ is defined as the ratio of the median of the empirically observed distribution of the test statistic to the expected median, thus quantifying the extent of the bulk inflation and the excess false positive rate.
- commonly accepted that $\lambda < 1.1$ are acceptable

$$\chi^2 = q_{\chi}^2(1 - P, 1)$$

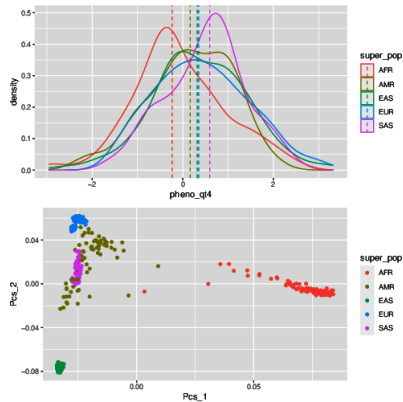
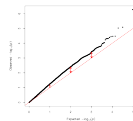
with P p-value and q_{χ}^2 quantile function of χ^2 with 1 degrees of freedom

$$\lambda = \text{median}(\chi^2)/0.456$$

Global visualisation : QQ-plot and inflation factors

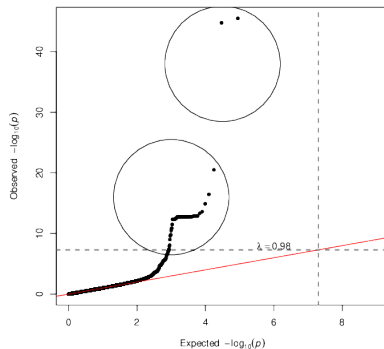
QQ plot showed a bias :

- Inflation factors is 1.4
- Median of phenotype showed a relation between genetics structure and phenotype distribution.
- explanation :
 - ▶ genetics generated from 1000 genome with 5 populations, phenotypes simulated using a bias using population
 - ▶ GWAS done using plink without structure correction
- correction of bias :
 - ▶ running GWAS using a linear mixed models using relatedness or Principal Component
 - ▶ correct P-value using $P_c = P/\lambda$



Global visualisation : QQ - plot

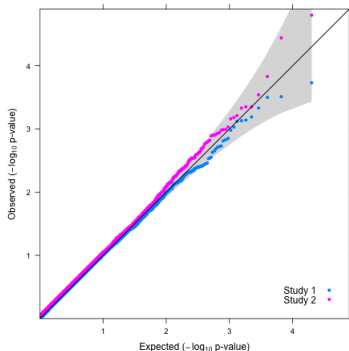
QQ plot indicated also if you have a
some significant SNPs



Global visualisation : QQ - plot

Resources in R : qqman, fastman R-library

- A Fancier QQ Plot by Matthew Flickinger :
https://genome.sph.umich.edu/wiki/Code_Sample:_Generating_QQ_Plots_in_R

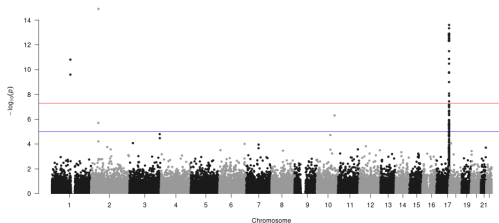


Resource using interface web :
FUMA, LocusZoom V2

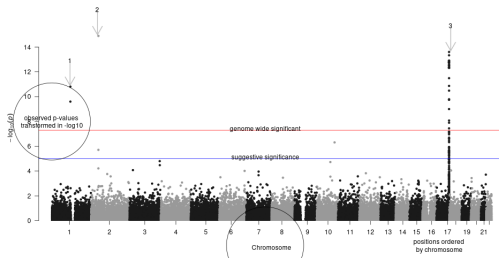
Manhattan plot

Global visualisation : Manhattan plot

Manhattan plots represent the P values of the entire GWAS on a genomic scale. The P values are represented in genomic order by chromosome and position on the chromosome (x axis). The value on the y axis represents the $-\log_{10}$ of the P value.



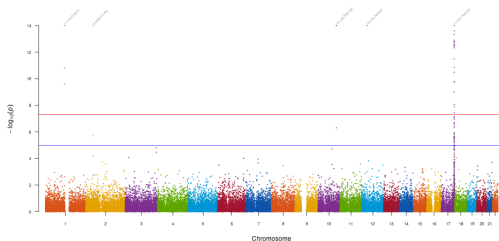
Global visualisation : Manhattan plot



- x axis : result ordered by chromosome and positions
- y axis : $-\log_{10}(P)$
- horizontal red line : significance at 5×10^{-8}
- horizontal blue line : suggestive significant at 10^{-5}
- arrows 1,2 showed low support of result (need to do a zoom of region to confirm)
- arrows 3, "tower" high support of result
- example build using qqman library

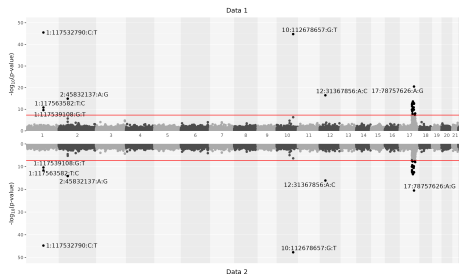
Global visualisation: Manhattan plot - other example

Using fastman library, give more custom option as annotation of lead snps.



Global visualisation: Manhattan plot - other example

Using hudson library, allowed to used mirror Manhattan plot with two data set and annotations of lead snps.



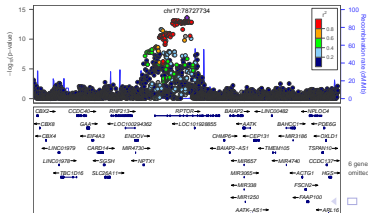
Resource using interface web : fuma, locus zoom V2.

Regional plot

Local visualisation: Regional Association Plot

Pre-defined area of the genome extracted where you plot p-value with annotation, Id...

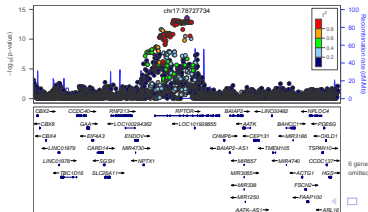
- A regional association plot is essentially a zoomed-in Manhattan plot.
- Allowing the researcher to look at associations in a specific region of the genome
- Analyse how is support lead SNP by other SNPs using LD between Lead SNPs and snps in windows



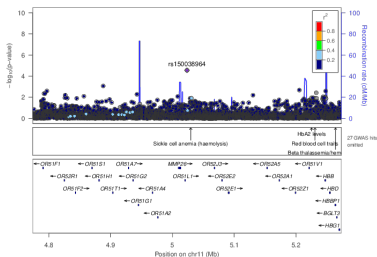
Local visualisation: Regional Association Plot

Pre-defined area of the genome extracted where you plot p-value with annotation, ld...

- A regional association plot is essentially a zoomed-in Manhattan plot.
- Allowing the researcher to look at associations in a specific region of the genome
- Analyse how is support lead SNP by other SNPs using LD between Lead SNPs and snps in windows
- To generated your regional you can use :
 - ▶ locus zoom stand alone : statics, R, python 2.0
 - ▶ locus zoom v2 : java or graphic interface, interactive



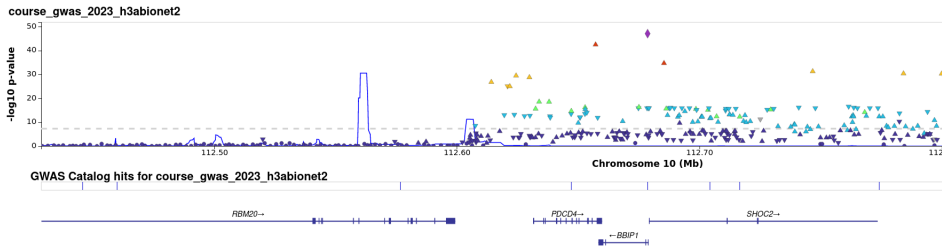
Local visualisation : LocusZoom example



If you lead SNPs has a low support : few SNPs has a low p-value on the region, you need to check for instance, frequency of your positions, plot phenotypes compared to genotype; if positions had been genotyped need to check cluster of luminosity compared to genotyping.

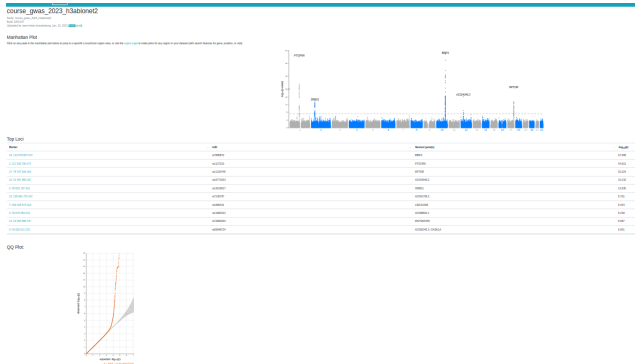
Local visualisation: Locus Zoom V2

Locus zoom v2 : web interface. using local visualisation gave information relative to gene in region, GWAS catalog and Linkage disequilibrium between lead SNPs and SNPs in the region. LD used come from external data set : 1000 Genome project



Local visualisation: Locus Zoom V2

Locus zoom V2 give an easy interactive using way to visualise and explore data, build also QQ plot, best result, Manhattan plot and regional plot, but less option to customise

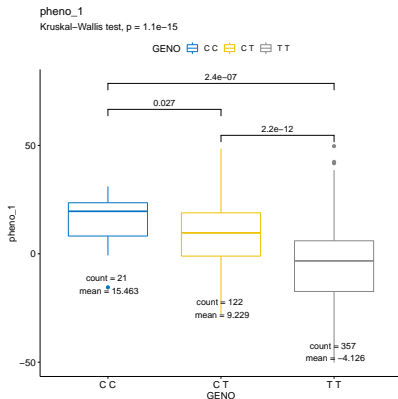


see example : <https://my.locuszoom.org/gwas/91333/>

Positions visualisation

Positions visualisation : distribution phenotypes by genotype

Plot distribution of your phenotype by genotype : give an indication how your variable follows your genotype : follow an additive model? are they properly distributed between genotypes?



Position visualisation : Cluster plot

genotype calling with array:

- steps consist to transform image (raw data) in genotype using intensity, each positions individuals defined by two level of intensity.
- Algorithm to defined genotype used intensity transformed at each locus and each individual.
- error of cluster, or level intensity can have impact of false positive on your GWAS result.
- Positions had been genotyped and are significant can be checked.
- Plot of cluster plot will depend array : illumina, Affymetrix and algorithm /software used (i.e. different output).

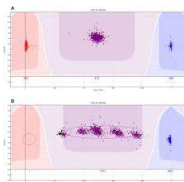


Figure: source : Bianco, 2020, plos one

Meta analysis

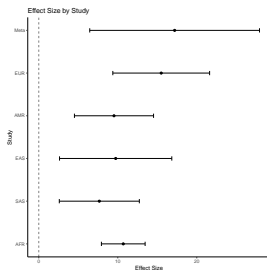
- Meta-analysis is a set of methods that allows the quantitative combination of data from multiple studies.
- Meta-analysis gave you a beta, se and p-value.
- QQ-plot, Manhattan plot and Regional Association plot can be done on summary statistics
- for regional plot or Fine mapping: you need to use adapted data to compute Linkage disequilibrium.
- forest plot is a representation at one position gave you distribution of your various beta and se

Meta Analysis in GWAS

Meta analysis : forest plot

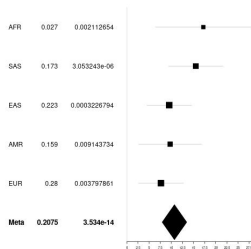
A forest plot, is a graphical display of estimated results from a number of scientific association at one positions running same phenotype from different population

- give your indication how different summary follow same trend.
 - ▶ Effect size.
 - ▶ standard error.
 - ▶ highlight meta result.



Meta analysis : forest plot, other example

Used forestplot library for R - 3.6, we add information as Allele frequency and P-value



Conclusion

Conclusion

Visualisation of result :

- Visualisation in GWAS is a step essential and complementary of statistics method to :
 - ▶ understand your data, extract data of interest
 - ▶ research bias
 - ▶ publications
- Softwares and approaches are more or less easy and more or less configurable.

Conclusion : resources

Softwares / library	Scale	Data	Platforms
qqman / hudson / fastman	QQ - plot or/and Manhattan plot	Summary statistics / annotation	R - library
CGT-VL / Z-browse/ Fuma / locus zoom V2	QQ - plot or/and Manhattan plot / regional plot	Summary statistics	web interface
Locus Zoom Stand alone	Regional plot	Summary statistics / ld	R/python script
R / forestplot	Positions / meta analyse	Summary statistics	R
boxplot / ggplot2	Positions :pheno geno	phenotype / genotype	R
	QQ - plot or/and		
gwaslab	Manhattan plot / regional plot	Summary statistics	python python, Interactive,
Assocplots	Manhattan / QQ plot	Summary statistics	statics
Genesis tool	POPULATION structure	genotype	java / interactive

Thank you for your attention

contact : jean-tristan.brandenburg@wits.ac.za

Global visualisation : QQ - plot in practice

```
1 # import library
2 library(data.table) ## allow to read big file using fread function
3 data_sumstat<-fread('assoc/result.plink') # reading summary statistics
4 # identify colums contains p-value
5 head(data_sumstat, 2)
6 # dimension of data
7 dim(data_sumstat)
8 # extraction of column contains p_value (P for plink or p_wald for gemma)
9 pvector<-data_sumstat$P
10 # clean na value, null value and allow finite vale
11 pvector <- pvector[!is.na(pvector) & !is.nan(pvector) & !is.null(pvector) & is.finite(
    pvector) & pvector < 1 & pvector > 0]
```

Global visualisation : QQ - plot in practice

```
1 # sort and transform p -value
2 o = -log10(sort(pvector, decreasing = FALSE))
3 # computed value expected, pppoints probability points for the continuous sample quantile
4 e = -log10(ppoints(length(pvector)))
5 # plot of expected points and observed in y-axis
6 plot(e,o, xlab='Expected', ylab='Observed')
7 # add red line
8 abline(0, 1, col = "red")
```

Global visualisation : QQ - plot in pratice

using qqman library (qq2.r)

```
1 # import library
2 library(data.table) ## allow to read big file using fread function
3 library(qqman)
4 data_sumstat<-fread('assoc/result.plink') # reading summary statistics
5 ## save your plot
6 png("qq.png") ## function to save can be png, tiff, pdf, avoid used pdf all points will
   be plot and file can reach a big size.
7 qq(data_sumstat$P) ## implemented same line than before.
8 dev.off() # save plot end
```

Global visualisation : QQ - plot in practice

computed genomic inflation factor (λ)

```
1 # import library
2 library(data.table) ## allow to read big file using fread function
3 data_sumstat<-fread('assoc/result.plink') # reading summary statistics
4
5 chisq <- qchisq(1-data_sumstat$P[!is.na(data_sumstat$P)],1) # transform p in chisq using
  density
6 lambda<-median(chisq)/qchisq(0.5,1) # computed lambda
```

Global visualisation : QQ - plot

Other resources

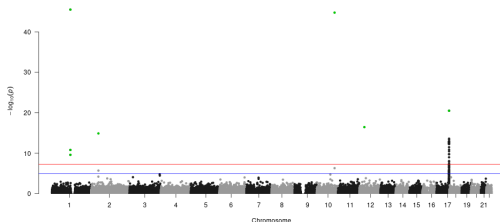
- fastman (R-library) :
<https://github.com/kaustubhad/fastman>

```
1 # import library
2 library(data.table) ## allow to read big
  file using fread function
3 #devtools::install_github('kaustubhad/
  fastman')
4 library(fastman)
5 data_sumstat<-fread('assoc/result.plink') #
  reading summary statistics
6 # identify colums contains p-value
7 head(data_sumstat, 2)
8 # dimension of data
9 fastqq (data_sumstat$P)
```

Global visualisation : Manhattan plot - exercise

using qqman library. qqman offer few custom option

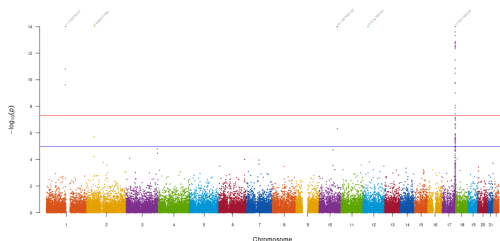
```
1 # import library
2 library(qqman)
3 library(data.table)
4 library(data.table) ## allow to read big file using fread function
5 data_sumstat<-fread('assoc/asso_phenoqt2.gemma') # reading summary statistics
6 #identify columns contains p-value
7 head(data_sumstat, 2)
8 # dimension of data
9 dim(data_sumstat)
10 png('figure/man_qqman.png', width = 480*2, height = 480)
11 ## used mamhattan function of qq with argument chro header, bp header, p header and snp
    header
12 manhattan(datagemma,chr = "chr",bp = "ps",p = "p_wald", snp='rs')
13 dev.off()
```



Global visualisation : Manhattan plot - other example

using fastman library. give more custom option as annotation of lead SNPs.

```
1 # import library
2 library(data.table) ## allow to read big file using fread function
3 #devtools::install_github('kaustubhad/fastman')
4 library(fastman)
5 data_sumstat<-fread('assoc/asso_phenoqt2.gemma') # reading summary statistics
6 # identify columns contains p-value
7 head(data_sumstat, 2)
8 # dimension of data
9 png('qq_fastman.png')
10 fastman(data_sumstat, chr = "chr", bp = "ps", p = "p_wald", snp="rs",annotatePval=5E-8)
11 dev.off()
```



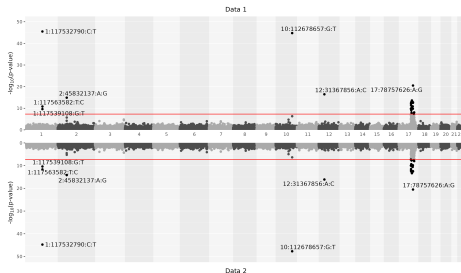
Position visualisation : Cluster plot

```
Index,Name,Chr,Position,AA Freq,AB Freq,BB Freq,Call Freq,Minor Freq,10% GC,50% GC
1,200610-1,MT,2757,0.9935668,0.00,0.006433167,0.997149,0.006433167,0.2633592,0.2637034
2,200610-10,MT,6753,0.9957234,0.00,0.00427655,1,0.00427655,0.3727026,0.3727026
3,200610-100,MT,15173,0.02068474,0.00,0.9793153,0.9992872,0.02068474,0.2195367,0.2195367
```

Global visualisation : Manhattan plot - exercises

using hudson library library. give mirror Manhattan plot, annotation of snps

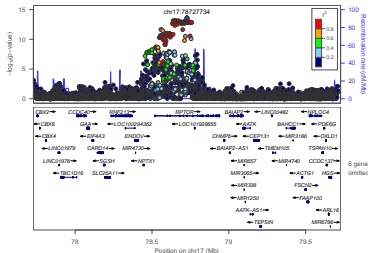
```
1 # import library
2 library(data.table) ## allow to read big file using fread function
3 library(hudson)
4 data_sumstat_1<-fread('assoc/asso_phenoqt1.gemma') # reading summary statistics
5 data_sumstat_2<-fread('assoc/asso_phenoqt2.gemma') # reading summary statistics
6 # data frame, must contain SNP, CHR, POS, pvalue, optional Shape
7 # select of column of interest and change name, order is important.
8 data_sumstat_1<-data_sumstat_1[,c('rs', 'chr','ps', 'p_wald')];names(data_sumstat_1)<-c("
  SNP","CHR","POS", "pvalue")
9 data_sumstat_2<-data_sumstat_2[,c('rs', "chr",'ps', 'p_wald')];names(data_sumstat_2)<-c("
  SNP","CHR","POS", "pvalue")
10 # Create a basic plot with Bonferroni lines and highlighting using the toy gwas datasets
11 gmmirror(top=data_sumstat_2, bottom=data_sumstat_1, tline=5*10**-8, bline=5*10**-8,
  toptitle="Data 1", bottomtitle="Data 2", highlight_p = c(5*10**-8, 5*10**-8),
  annotate_p = c(5*10**-8, 5*10**-8), highlighter="black", file="man_hudson")
```



Local visualisation : LocusZoom stand alone

Locus zoom give gene info, Id with lead snps. Option for region, can be rsid or region of interest. we selected chr start and end.

```
1 #we format file in epact
2 echo -e "#CHROM\tBEGIN\tEND\tMARKER_ID\tPVALUE" > data/data_regplot.epact
3 sed 'id' data/data_regplot.gemma | awk '{print $1"\t"$3"\t"$3"\t"$2"\t"$12}' >> data/
  data_regplot.epact
4 # we run locuszoom using database from locus zoom
5 locuszoom/bin/locuszoom --build hg19 --epacts data/data_regplot.epact --source 1000
  G_Nov2014 --pop EUR --chr 17 --start 77727734 --end 79727734
6 # we run locuszoom using database from locus zoom add locus zoom
```



Position visualisation : Cluster plot

MT 2755 200610-1 A G 0/0:1:0.263703:-0.000443399:0.0505827:2.85271:0.268731:3.12144:0.0597945:24607:115

Meta analysis : forest plot

format and build data set using different summary statistic for a specific position

```
1 library(data.table)
2 DataAFR<-fread('data/AFR_pheno.gemma');
3 DataEUR<-fread('data/EUR_pheno.gemma')
4 DataAMR<-fread('data/AMR_pheno.gemma')
5 DataEAS<-fread('data/EAS_pheno.gemma')
6 DataSAS<-fread('data/SAS_pheno.gemma')
7 DataMeta<-fread('data/metal_res_metal.format')
8 chr=17; bp<-7872773
9 DataAFR_chrbp<-DataAFR[DataAFR$chr==chr & DataAFR$ps==bp,c('allele1', 'allele0', 'af', '
  beta', 'se', 'p_wald')];names(DataAFR_chrbp)<-c('A1', 'A2', 'AF', 'mean', 'SE', 'P')
10 DataSAS_chrbp<-DataSAS[DataSAS$chr==chr & DataSAS$ps==bp,c('allele1', 'allele0', 'af', '
  beta', 'se', 'p_wald')];names(DataSAS_chrbp)<-c('A1', 'A2', 'AF', 'mean', 'SE', 'P')
11 DataEAS_chrbp<-DataEAS[DataEAS$chr==chr & DataEAS$ps==bp,c('allele1', 'allele0', 'af', '
  beta', 'se', 'p_wald')];names(DataEAS_chrbp)<-c('A1', 'A2', 'AF', 'mean', 'SE', 'P')
12 DataAMR_chrbp<-DataAMR[DataAMR$chr==chr & DataAMR$ps==bp,c('allele1', 'allele0', 'af', '
  beta', 'se', 'p_wald')];names(DataAMR_chrbp)<-c('A1', 'A2', 'AF', 'mean', 'SE', 'P')
13 DataEUR_chrbp<-DataEUR[DataEUR$chr==chr & DataEUR$ps==bp,c('allele1', 'allele0', 'af', '
  beta', 'se', 'p_wald')];names(DataEUR_chrbp)<-c('A1', 'A2', 'AF', 'mean', 'SE', 'P')
14 DataMeta_chrbp<-DataMeta[DataMeta$CHRO==chr & DataMeta$BP==bp,c('Allele1', 'Allele2', '
  Freq1', 'Effect', 'StdErr', 'P-value')];names(DataMeta_chrbp)<-c('A1', 'A2', 'AF', 'mean', 'SE', 'P')
15 ## DataMeta doesn't have same ref / alt we switch beta / af
16 DataMeta_chrbp$mean<- DataMeta_chrbp$mean;DataMeta_chrbp$AF <- 1 - DataMeta_chrbp$AF
17 Data_reschrbp<-rbind(DataAFR_chrbp,DataSAS_chrbp,DataEAS_chrbp, DataAMR_chrbp,DataEUR_
  chrbp,DataMeta_chrbp)
18 Data_reschrbp<-cbind(Pop=c('AFR', 'SAS', 'EAS', 'AMR', 'EUR', 'Meta'), Data_reschrbp, is.
  summary=c(F,F,F,F,F,T))
19 Data_reschrbp$index<-nrow(Data_reschrbp):1
20 # we computed lower and upper using 95%
21 Data_reschrbp$lower<- Data_reschrbp$mean-1.96 * Data_reschrbp$SE
22 Data_reschrbp$upper<- Data_reschrbp$mean+1.96 * Data_reschrbp$SE
```

Positions visualisation : distribution phenotypes by genotype

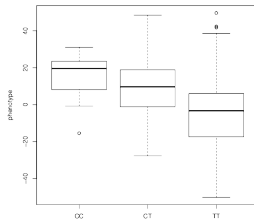
Extract genotype from your plink file in ped file using plink

```
1 plinkbase=data/chr7_77727734_79727734
2 filers=data/17_78727734.bed
3 echo "17          78727734          78727734          17:78727734" > $filers
4 # format in ped file
5 out=data/17_78727734bp
6 plink -bfile $plinkbase --extract range $filers --recode tab --out $out
```

Positions visualisation : distribution phenotypes by genotype

using R to plot distribution phenotypes by genotype

```
1 ## reading pheno
2 pheno<-read.table('data/KGPH3abionetsub_pheno_qt.pheno', header=T)
3 ## reading geno from plink output
4 geno<-read.table('data/17_78727734bp.ped')
5 geno<-geno[,c(1,2,7,8)]
6 names(geno)<-c('FID','IID','G1','G2')
7 geno$genot<-paste(geno$G1,geno$G2,sep='')
8 # name geno header=
9 genopheno<-merge(pheno , geno , by=c(1,2),all=F)
10 # boxplot by genotype
11 boxplot(pheno_1~genot,data=genopheno , xlab='', ylab='phenotype')
```



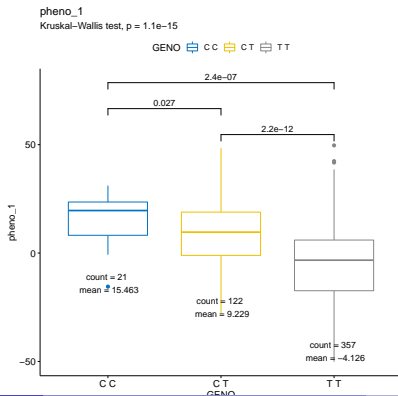
Position visualisation : distribution phenotypes by genotype

Improved figure using "ggpubr" and "gridExtra", script from

h3agwas/annotation/ workflow

possibility to add co-variable with plot of residuals. possibility to do a GxE plot.

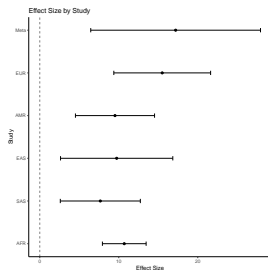
```
1 data=data/KGPH3abionetsub_pheno_qt.pheno
2 outpdf=h3agwas_genos.pdf
3 pheno=pheno_1
4 ./an_plotboxplot.r --ped $out".ped" --data $data --out $outpdf --pheno $pheno
```



Meta analysis : forest plot

Used ggplot2 to build your own forestplot

```
1 library(ggplot2)
2 ggplot(data=Data_reschrbp, aes(y=index, x=mean, xmin=lower, xmax=upper)) +
3   geom_point() +
4   geom_errorbarh(height=.1) +
5   scale_y_continuous(breaks=1:nrow(Data_reschrbp), labels=Data_reschrbp$Pop) +
6   labs(title='Effect Size by Study', x='Effect Size', y = 'Study') +
7   geom_vline(xintercept=0, color='black', linetype='dashed', alpha=.5) +
8   theme_classic()
9   ## save using ggsave
10 ggsave("meta_ggplot.pdf")
```



source : <https://www.r-bloggers.com/2022/09/forest-plot-in-r-quick-guide/>

Meta analysis : forest plot, other example

Used forestplot library for R - 3.6, we add information as Allele frequency and P-value

```
1 library(forestplot)
2 jpeg('forestplot_lib_meta.jpeg')
3 forestplot(Data_reschrpb_2[,c('Pop','AF','P')], mean=Data_reschrpb_2$mean, lower=Data_
  reschrpb_2$lower, upper=Data_reschrpb_2$upper, is.summary=Data_reschrpb$is.summary)
4 dev.off()
```

