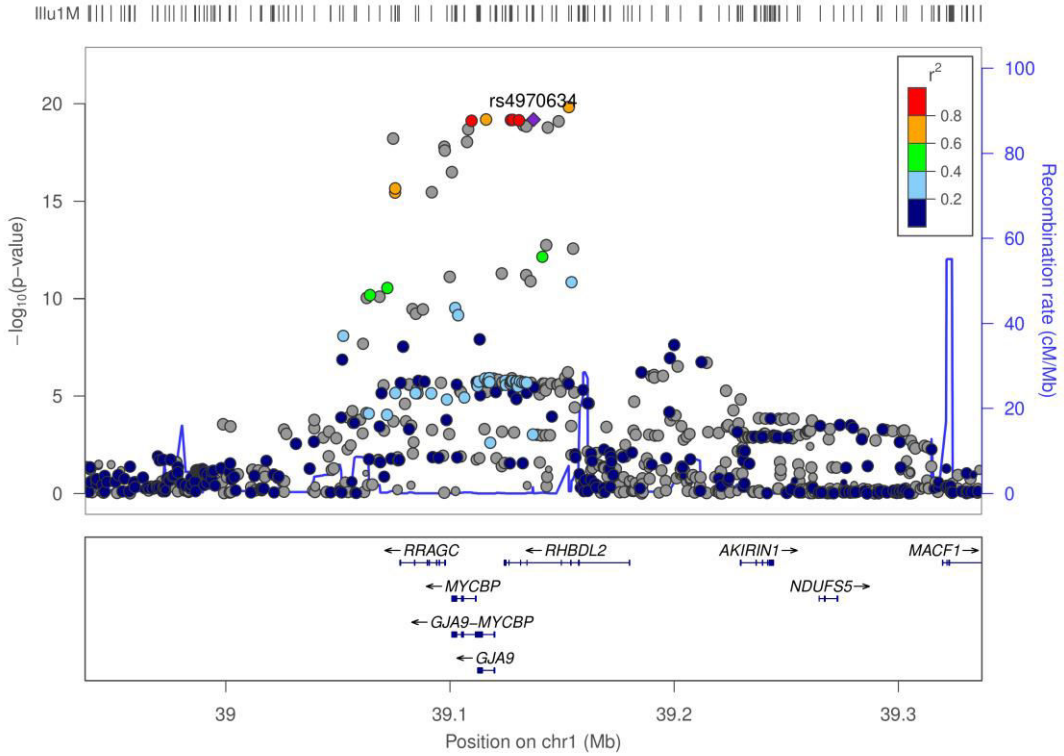
FM-pipeline

This is a pipeline for finemapping using GWAS summary statistics, implemented in Bash as a series of steps to furnish an incremental analysis. As depicted in the diagram below



LocusZoom plot showing Regional association for chr1:39114617-39614617

where our lead SNP rs4970634 is in LD with many others, the procedure attempts to identify causal variants from region(s) showing significant SNP-trait association.

The process involves the following steps, 1. Extraction of effect (beta)/z statistics from GWAS summary statistics (.sumstats), 2. Extraction of correlation from the reference panel among overlapped SNPs from 1 and the reference panel containing individual level data. 3. Information from 1 and 2 above is then used as input for finemapping.

The measure of evidence is typically (log10) Bayes factor (BF) and associate SNP probability in the causal set.

Software included in this pipeline are listed in the table below.

Name	Function	Input	Output	Reference
CAVIAR	finemapping	z, correlation matrix	causal sets and probabilities	Hormozdiari, et al. (2014)
CAVIARBF	finemapping	z,	BF and	Chen, et al.

		correlation matrix	probabilities for all configurations	(2015)
GCTA	joint/conditional analysis	.sumstats, reference data	association results	Yang, et al. (2012)
FM-summary	finemapping	.sumstats association results	updated results	Huang, et al. (2017)
JAM	finemapping	beta, individual reference data	Bayes Factor of being causal	Newcombe, et al. (2016)
LocusZoom	regional plot	partial .sumstats	.pdf/.png plots	Pruim, et al. (2010)
fgwas	functional GWAS			Pickrell (2014)
finemap	finemapping	z, correlation matrix	causal SNPs and configuration	Benner, et al. (2016)

so they range from regional association plots via LocusZoom, joint/conditional analysis via GCTA, functional annotation via fgwas to dedicated finemapping software including CAVIAR, CAVIARBF, an adapted version of FM-summary, R2BGLiMS/JAM and finemap. One can optionally use a subset of these for a particular analysis by specifying relevant flags from the pipeline's settings.

INSTALLATION

On many occasions, the pipeline takes advantage of the GNU parallel.

Besides (sub)set of software listed in the table above, the pipeline requires qctool 2.0, PLINK 1.9, and the companion program LDstore from finemap's website need to be installed.

The pipeline itself can be installed in the usual way,

```
git clone https://github.com/jinghuazhao/FM-pipeline
```

The setup is in line with summary statistics from consortia where only RSid are given for the fact that their chromosomal position may be changed over different builds. To remedy this, we use information from UCSC, e.g.,

```
wget http://hgdownload.soe.ucsc.edu/goldenPath/hg19/database/snp150.txt.gz
gunzip -c snp150.txt.gz | \
awk '{split($2,a,"_");sub(/chr/,"",a[1]);print a[1],$4,$5}' | \
sort -k3,3 > snp150.txt
```

Note that JAM requires Java 1.8 so call to Java -jar inside the function needs to reflect this, not straightforward with `install_github()` from `devtools` but one needs to clone the package, modify the R source code and then use

```
git clone https://github.com/pjnewcombe/R2BGLiMS
### change java to java-1.8 in R2BGLiMS/R/R2BGLiMS.R
R CMD INSTALL R2BGLiMS
```

Implementations have been done for the finemapping software along with LocusZoom and GCTA; support for fgwas is still alpha tested. To facilitate handling of grapahics, e.g., importing them into Excel, pdftopng from xpdf is used.

We use Stata and Sun grid engine (sge) for some of the data preparation, which would become handy when available.

USAGE

Before start, settings at the beginning of the script need to be changed and only minor change is expected after this. The syntax of pipeline is then simply

```
bash fmp.sh <input>
```

Inputs

--- GWAS summary statistics ---

These include the following columns,

Column	Name	Description
1	SNP	RSid
2	A1	Effect allele
3	A2	Other allele
4	freqA1	A1 frequency
5	beta	effect estimate
6	se	standard error of effect
7	P	P-value
8	N	sample size
9*	chr	chromosome
10*	pos	position

This format is in line with joint/conditional analysis by GCTA. Note the last two columns are not always available but can be obtained from UCSC as above; see below for example use.

--- Reference panel ---

The pipeline uses a reference panel in a .GEN format, taking into account directions of effect in both the GWAS summary statistics and the reference panel. Its development will facilitate

summary statistics from a variety of consortiua as with reference panels such as the HRC and 1000Genomes.

A .GEN file is required for each region, named such that chr{chr}_{start}_{end}.gen, together with a sample file. For our own data, a utility program in Stata is written to generate such files from their whole chromosome counterpart using SNPinfo.dta.gz which has the following information,

chr	snpid	rsid	pos	FreqA2	info	type	A1	A2
1	1:54591_A_G	rs561234294	54591	.0000783	.33544	0	A	G
1	1:55351_T_A	rs531766459	55351	.0003424	.5033	0	T	A
...	...	...	...	...	...	...	...	...

Optionally, a file is specified which contains samples to be excluded from the reference panel; one leaves it unspecified when not needed. In line with qctool -excl-samples option, it contains a list of individuals corresponding to ID_2 of the sample file rather than ID_1 and ID_2.

--- The lead SNPs ---

Given these, one can do away with Stata and work on a text version for instance SNPinfo.txt. An auxiliary file called `st.bed` contains chr, start, end, rsid, pos, r corresponding to the lead SNPs specified and r is a sequence number of region. As GCTA conditional/joint analysis requires whole chromosome reference the counterpart is HRC.do. Note in this case the snpid and rsid variables are called rsid and RSnum instead; both porgrams filter SNPs on minor allele count and measure of imputation quality. As it is very slow, we use .bgen instead see the section on WHOLE-GENOME CONDITIONAL/JOINT ANALYSIS below.

Outputs

The output will involve counterpart(s) from individual software, i.e., .set/post, caviarbf, .snp/.config, .jam/.top

Software	Output type	Description
CAVIAR	.set/.post	causal set and probabilities in the causal set/posterior probabilities
CAVIARBF	.caviarbf	causal configurations and their BFs
FM-summary	.txt	additional information to the GWAS summary statistics
JAM	.jam/.top	the posterior summary table and top models containing selected SNPs
finemap	.snp/.config	top SNPs with largest

log10(BF) and top
configurations as with their
log10(BF)

It is helpful to examine directions of effects together with their correlation which is now embedded as with finemap.

WHOLE-GENOME CONDITIONAL/JOINT ANALYSIS

As the pipeline works on regions defined by lead SNPs, it is desirable to have a genomewide counterpart and currently this is possible with GCTA and we have a script called gcta-slct.sh which accepts a single sumstats file, and only a minor change is required, namely,

```
gcta-slct.sh <input>
```

The file `id3.txt.gz` contains SNP IDs, snpid, and rsid which correspond to variant IDs in the reference, (ordered) snpid and reference sequence IDs. By doing so, we can deal with duplicate chromosomal positions commonly seen in reference data. Optionally, exclusion lists for SNPs and samples can be incorporated. At the end of the script, it also shows how the relevant information was generated in our analysis.

As it is very time-consuming for interactive use, on our system we resort to sge, e.g.,

```
qsub -S /bin/bash -V -N HRC -cwd -e HRC.err -o HRC.out -pe make 10 -q all.q
gcta-slct.sh HRC
```

so the job is sent to the clusters instead. In this case, we specifies the shell (-S) with environment variables (-V), error message file (-e), log file (-o), threads (-pe) and queue (-q) whereas the last item is argument to `gcta-slct.sh` itself. If you system supports for GNU parallel, the syntax is similar.

The use of gene list from the analysis can compare to feeding SNPs and their p values from a GWAS into VEGAS2v2 as illustrated with vegas2v2.sh where `interceptBed` utility from the bedtools package is used. Note that instead of the 1000Genomes reference provided, we use our own.

Some changes are required for the command-line version of VEGAS2v2 and noted at the of the script. We don't have experiences with the pathway analysis option from command-line or https://vegas2.qimrberghofer.edu.au/. Nevertheless, as indicated in the original VEGAS paper, Liu et al. (2010), > If a gene contains only one causal variant, then the inclusion of a large number of nonsignificant markers into the gene-based > test will dilute this gene's significance.``

and we perhaps would see an analogy here. However, more broadly software in PW-pipeline can be used and in terms of LD information PASCAL will be useful.

EXAMPLE

We show how to set up for BMI GWAS summary data as reported by the GIANT consortium, Locke, et al. (2015),

```
# GWAS summary statistics
wget
http://portals.broadinstitute.org/collaboration/giant/images/1/15/SNP_gwas_mc
_merge_nogc.tbl.uniq.gz
gunzip -c SNP_gwas_mc_merge_nogc.tbl.uniq.gz |
awk 'NR>1' | \
join -11 -23 - snp150.txt | \
awk '($9!="X" && $9!="Un")' > bmi.txt

# A list of 97 SNPs
R --no-save <<END
library(openxlsx)
xlsx <- "https://www.nature.com/nature/journal/v518/n7538/extref/nature14177-
s2.xlsx"
snps <- read.xlsx(xlsx, sheet = 4, colNames=FALSE, skipEmptyRows = FALSE,
cols = 1, rows = 5:101)
snplist <- sort(as.vector(snps[,1]))
write.table(snplist, file="97.snps", row.names=FALSE, col.names=FALSE,
quote=FALSE)
END

# st.bed
grep -w -f 97.snps snp150.txt | \
sort -k1,1n -k2,2n | \
awk -vflanking=250000 '{print $1,$2-flanking,$2+flanking,$3,$2,NR}' > st.bed
```

where we download the GWAS summary statistics adding SNP positions in build 37 rather than 36. The list of SNPs can also be used to generate st.bed as above.

We illustrate use of 1000Genomes data, available as FUSION LD reference panel, with 1KG.sh to generate `SNPinfo.dta.gz` and st.do to generate the required data.

ACKNOWLEDGEMENTS

The work was motivated by finemapping analysis at the MRC Epidemiology Unit and inputs from authors of GCTA, finemap, JAM, FM-summary as with participants in the Physalia course `Practical GWAS Using Linux and R` are greatly appreciated. In particular, the utility program in Stata was adapted from p0.do (which is still used when LD_MAGIC is enabled) originally written by Dr Jian'an Luan and computeCorrelationsImpute2forFINEMAP.r by Ji Chen from the MAGIC consortium who also provides code calculating the credible set based on finemap configurations. Earlier version of the pipeline also used GTOOL.

SOFTWARE AND REFERENCES

CAVIAR

Hormozdiari F, et al. (2014) Identifying Causal Variants at Loci with Multiple Signals of Association. Genetics, 44, 725–731

CAVIARBF

Chen W, et al. (2015) Fine Mapping Causal Variants with an Approximate Bayesian Method Using Marginal Test Statistics. Genetics 200:719-736.

FM-summary

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GCTA

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JAM

Newcombe PJ, et al. (2016) JAM: A Scalable Bayesian Framework for Joint Analysis of Marginal SNP Effects. Genet Epidemiol 40:188–201

LocusZoom

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fgwas

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Benner C, et al. (2016) FINEMAP: Efficient variable selection using summary data from genome-wide association studies. Bioinformatics 32, 1493-1501

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GIANT paper

Locke AE, et al. (2015) Genetic studies of body mass index yield new insights for obesity biology. Nature 518(7538):197-206. doi: 10.1038/nature14177