An atlas connecting shared genetic architecture of human diseases and molecular phenotypes provides insight into COVID-19 susceptibility

iCPAGdb Web App Supplement

Thomas J. Balmat, Research Computing, Duke University

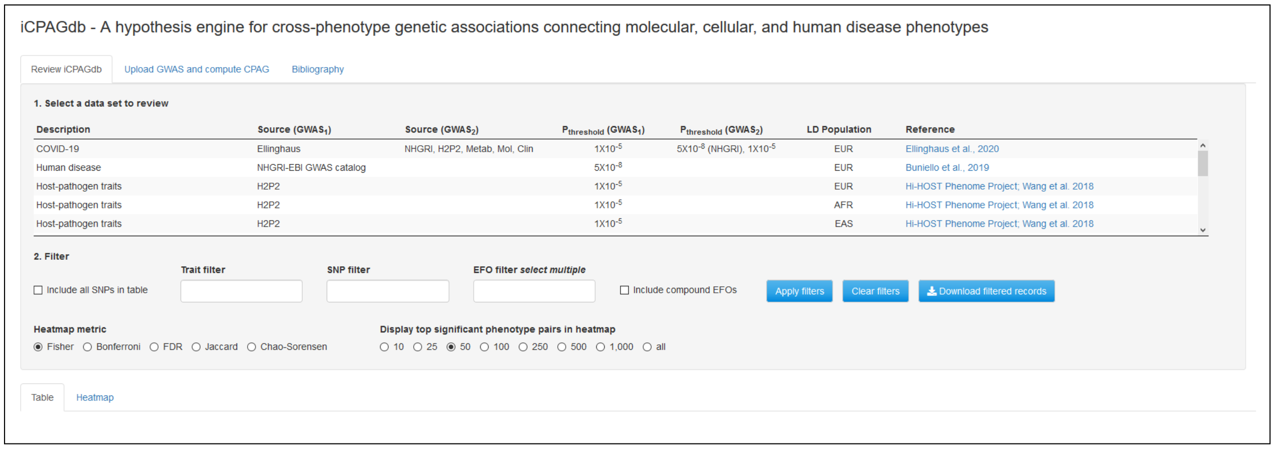
Two primary objectives guided the design of the iCPAGdb web app (the web app): quick accessibility of precomputed cross-phenotype analysis of GWAS (CPAG) results for select GWAS studies and the ability to discover new cross-phenotype relationships between researcher uploaded GWAS data sets and those maintained in the iCPAGdb database (the database). We will refer to the browsing and upload modes simply as "review" and "compute," respectively. Once the data content and public interaction objectives were established, architecture design choices were made, drawing on experience gained while developing a web app for a prior GWAS project (Wang et al., 2018). Primary software components for the web portion of iCPAGdb are the R statistical programming language (R Core Team, 2020), the R package *Shiny* for interaction of web pages with R scripts (Chang et al., 2020), *Shiny Server* as a 24/7 multi-user platform to make Shiny apps publicly accessible (RStudio, 2020), the database environment *SQLite* for efficient querying of GWAS and CPAG results (Hipp, 2020), and the R package *RSQLite* to execute SQL queries from within R scripts (Muller et al., 2020). CPAG computations, as described in the iCPAGdb section of the paper and (Wang et al., 2015), are implemented in the Python programming language, external to the web app, and are executed using parameterized system calls constructed from values supplied by the user through on-screen controls. The results of a CPAG execution are read by the R script, processed, and presented to the viewer in various tables and graphs on a web page. Shiny conducts the interaction between web pages, R scripts, and the CPAG functions. The SQLite database is primarily used by the CPAG functions, but is also a source of identifiers and other values used to populate selection lists within the web app.

Program flow 1 outlines the major processing steps for review mode. Basically, the user requests an existing CPAG result set from which a corresponding table and heatmap are generated and displayed. Various filtering and graph construction controls are available for iterative sub-setting of data and selection of significance measure and number of top significant phenotype pairs to plot. The "Download" button enables the researcher to make a local copy of records appearing in the currently displayed results table. Important packages used in this mode are DT (Xie et al., 2020) for construction of and interaction with tables and ggplot2 (Wickham, 2016), plotly (Sievert, 2020), and heatmaply (Galili et al., 2017) for basic plotting, interactive plotting (hover labels), and heatmap generation, respectively.

Program flow 1, review mode

1. Query available precomputed CPAG results and construct table with selectable rows, one per study
2. Present table in web app
3. When table row is selected, read corresponding study results, prepare table of results and heatmap for review
4. Interact with user, iterating through on-screen control settings to sort, filter, and modify results table while synchronizing heatmap with tabular data

Although the packages employed are relatively full-featured and robust, several custom algorithms had to be developed to overcome specific limitations of various functions of these packages when used with our data. For example, the clustering algorithm used by the heatmaply() function to add dendrogram lines to a graph generated errors when used with the distance matrix computed by the base R distance() function. A substitute vector distance algorithm was developed to overcome domain errors reported by heatmaply(). Figure 1 shows an example review session.[1] The table in the upper section of the form lists precomputed CPAG results available for review. Below that are the filter and graph controls. When a row in the CPAG table is selected, a table of corresponding results appears in the "Table" tab (bottom of the image) and a corresponding heatmap that relates pairs of inter-GWAS phenotypes by the selected significance measure (Fisher, Bonferroni, etc.) appears in the "Heatmap" tab. Figures 2 and 3 show the table and heatmap that appear after clicking the "Molecular traits vs. Human disease" row of the selection table, which is accessed by scrolling down.



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1. Select a data set to review

Description	Source (GWAS$_1$)	Source (GWAS$_2$)	$P_{threshold}$ (GWAS$_1$)	$P_{threshold}$ (GWAS$_2$)	LD Population	Reference
COVID-19	Ellinghaus	NHGRI, H2P2, Metab, Mol, Clin	$1X10^{-5}$	$5X10^{-8}$ (NHGRI), $1X10^{-5}$	EUR	Ellinghaus et al., 2020
Human disease	NHGRI-EBI GWAS catalog		$5X10^{-8}$		EUR	Buniello et al., 2019
Host-pathogen traits	H2P2		$1X10^{-5}$		EUR	Hi-HOST Phenome Project; Wang et al. 2018
Host-pathogen traits	H2P2		$1X10^{-5}$		AFR	Hi-HOST Phenome Project; Wang et al. 2018
Host-pathogen traits	H2P2		$1X10^{-5}$		EAS	Hi-HOST Phenome Project; Wang et al. 2018

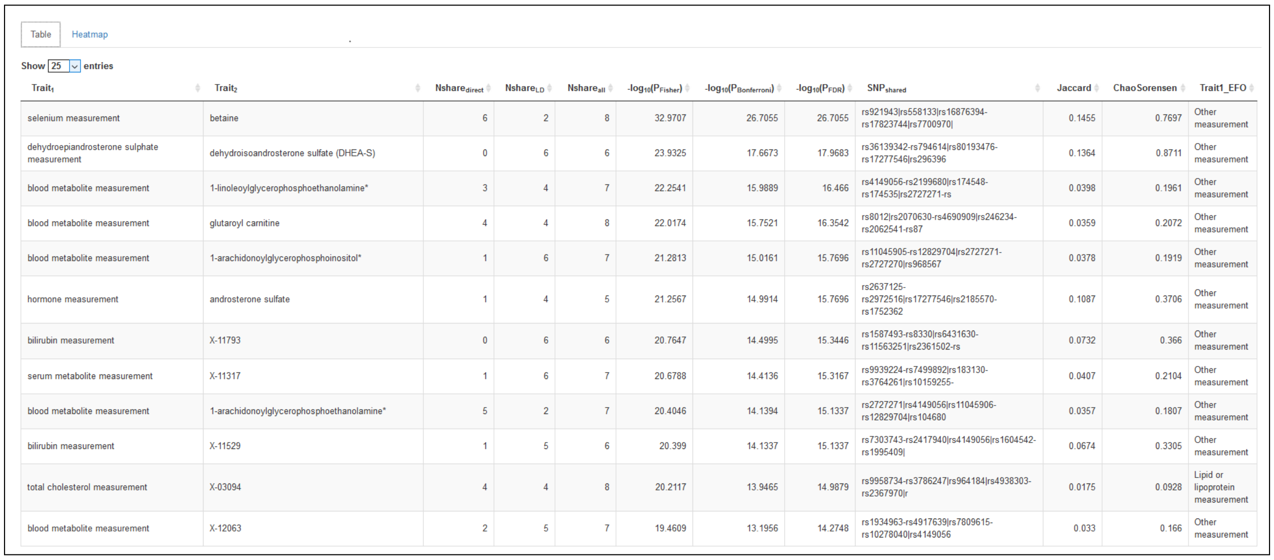
2. Filter

☐ Include all SNPs in table
Trait filter
SNP filter
EFO filter *select multiple*
☐ Include compound EFOs
Apply filters | Clear filters | Download filtered records

Heatmap metric
[✓ Fisher] ○ Bonferroni ○ FDR ○ Jaccard ○ Chao-Sorensen

Display top significant phenotype pairs in heatmap
○ 10 ○ 25 [✓ 50] ○ 100 ○ 250 ○ 500 ○ 1,000 ○ all

Figure 1: iCPAGdb web app Review tab



Show 25 entries

Trait$_1$	Trait$_2$	Nshare$_{direct}$	Nshare$_{LD}$	Nshare$_{all}$	-log$_{10}$(P$_{Fisher}$)	-log$_{10}$(P$_{Bonferroni}$)	-log$_{10}$(P$_{FDR}$)	SNP$_{shared}$	Jaccard	ChaoSorensen	Trait1_EFO
selenium measurement	betaine	6	2	8	32.9707	26.7055	26.7055	rs921943|rs558133|rs16876394-rs17823744|rs7700970|	0.1455	0.7697	Other measurement
dehydroepiandrosterone sulphate measurement	dehydroisoandrosterone sulfate (DHEA-S)	0	6	6	23.9325	17.6673	17.9683	rs36139342-rs794614|rs80193476-rs17277546|rs296396	0.1364	0.8711	Other measurement
blood metabolite measurement	1-linoleoylglycerophosphoethanolamine*	3	4	7	22.2541	15.9889	16.466	rs4149056-rs2199680|rs174548-rs174535|rs2727271-rs	0.0398	0.1961	Other measurement
blood metabolite measurement	glutaroyl carnitine	4	4	8	22.0174	15.7521	16.3542	rs8012|rs2070630-rs4690909|rs246234-rs2062541-rs87	0.0359	0.2072	Other measurement
blood metabolite measurement	1-arachidonoylglycerophosphoinositol*	1	6	7	21.2813	15.0161	15.7696	rs11045905-rs12829704|rs2727271-rs2727270|rs968567	0.0378	0.1919	Other measurement
hormone measurement	androsterone sulfate	1	4	5	21.2567	14.9914	15.7696	rs2637125-rs2972516|rs17277546|rs2185570-rs1752362	0.1087	0.3706	Other measurement
bilirubin measurement	X-11793	0	6	6	20.7647	14.4995	15.3446	rs1587493-rs8330|rs6431630-rs11563251|rs2361502-rs	0.0732	0.366	Other measurement
serum metabolite measurement	X-11317	1	6	7	20.6788	14.4136	15.3167	rs9939224-rs7499892|rs183130-rs3764261|rs10159255-	0.0407	0.2104	Other measurement
blood metabolite measurement	1-arachidonoylglycerophosphoethanolamine*	5	2	7	20.4046	14.1394	15.1337	rs2727271|rs4149056|rs11045906-rs12829704|rs104680	0.0357	0.1807	Other measurement
bilirubin measurement	X-11529	1	5	6	20.399	14.1337	15.1337	rs7303743-rs2417940|rs4149056|rs1604542-rs1995409|	0.0674	0.3305	Other measurement
total cholesterol measurement	X-03094	4	4	8	20.2117	13.9465	14.9879	rs9958734-rs3786247|rs964184|rs4938303-rs2367970|r	0.0175	0.0928	Lipid or lipoprotein measurement
blood metabolite measurement	X-12063	2	5	7	19.4609	13.1956	14.2748	rs1934963-rs4917639|rs7809615-rs10278040|rs4149056	0.033	0.166	Other measurement

Figure 2: iCPAGdb web app. Table generated by selecting a precomputed CPAG result.

[1]Screen-shots were taken from the iCPAGdb website, http://cpag.oit.duke.edu

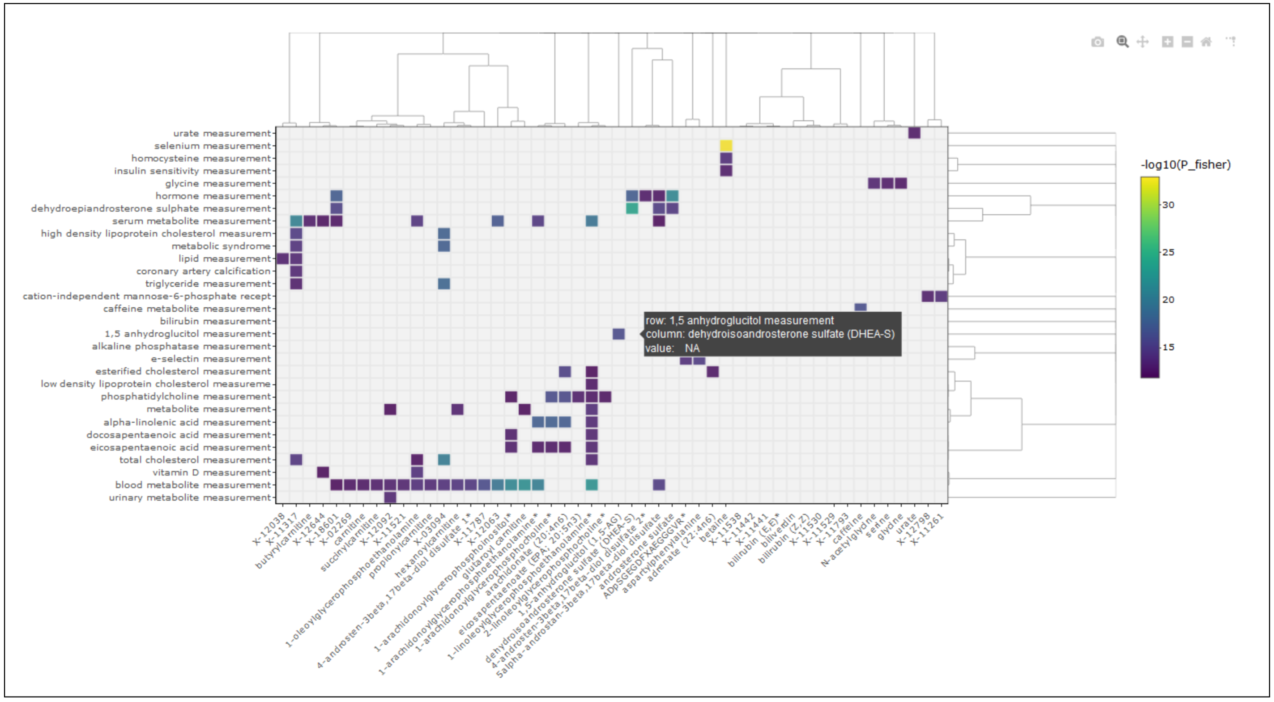


Figure 3: iCPAGdb web app. Heatmap generated by selecting a precomputed CPAG result.

Program flow 2 outlines the major processing steps for compute mode. Figure 4 shows the corresponding "Upload and Compute" tab of the app. In this mode, the user browses files on a local computer, selects a properly formatted GWAS result file of interest (containing, for a single phenotype, SNPs and GWAS significance, p, values), specifies format and column configuration, then uploads the file. Next, CPAG computation parameter values, including iCPAGdb GWAS set to be crossed with, significance thresholds for filtering, and linkage disequilibrium (LD) population are specified. When "Compute CPAG" is pressed, the R script composes a system level command to execute the CPAG (Python) function. The future() function of the R future package (Bengtsson, 2020) combined with a delaying pipe from the promises package (Cheng, 2020) execute CPAG operations asynchronously, waiting on completion before resuming R script execution. An important consideration is that the default behavior of R is to execute instructions synchronously, so that one instruction is completed in entirety prior to another beginning. This is problematic in a multi-user setting when long-running computations are executed. Typical CPAG execution time ranges from thirty seconds to ten minutes, making synchronous execution problematic. Although (the open source version of) Shiny Server accommodates multiple simultaneous connections, it allocates a single R process to each application. iCPAGdb is an application. Therefore, within the group of simultaneous iCPAGdb users, at most one is being serviced at any given time. Others must wait until that user's CPAG analysis completes. But, by executing the CPAG function from within a future() call and promise pipe sequence, R will spawn an individual, asynchronous process for executing the CPAG function, enabling multiple, simultaneous users. Of course, this has to be programmed and scripts must be adjusted to account for the delayed, and impromptu nature of results being returned. In addition to one CPU being allocated for each asynchronous CPAG call, the CPAG function itself executes as a multi-threaded process on multiple CPUs. It is important to consider expected load (number of simultaneous users and number of CPUs used in parallel) when configuring a server to be used for public compute services.

Program flow 2, compute mode

Upload a GWAS section

Researcher:

1. Browses local computer for a GWAS file and uploads it
2. Specifies file structure (delimiter type, GWAS SNP and significance columns)

Compute section

Researcher:

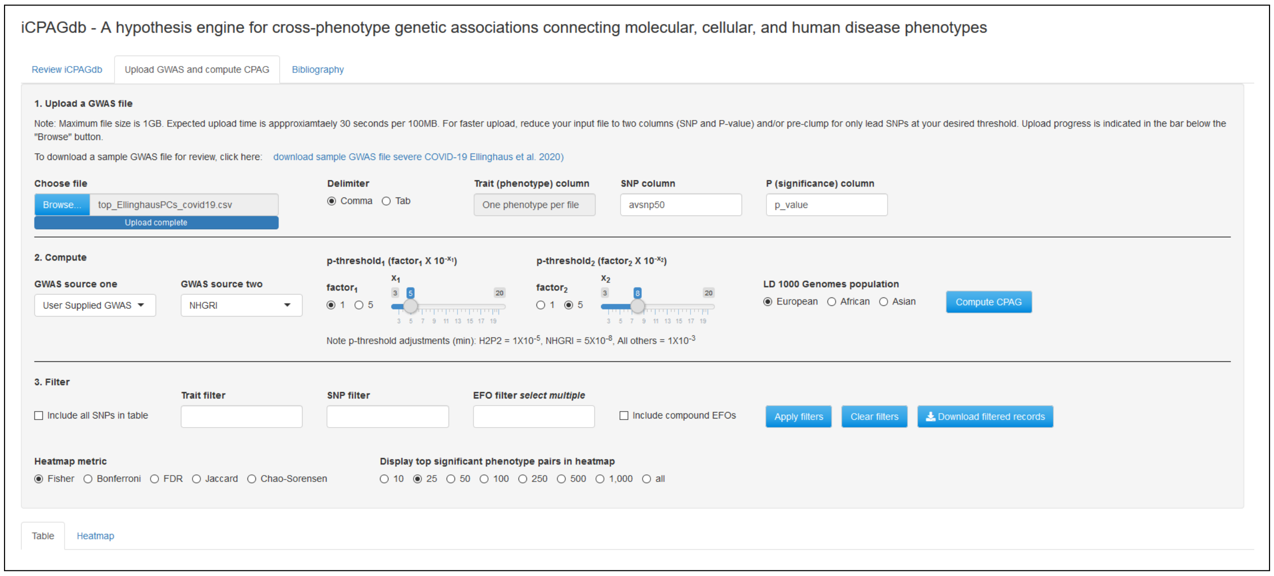
3. Selects iCPAGdb GWAS study (GWAS source two) to be used for investigation of cross-GWAS associations
4. Selects p-thresholds (to filter phenotypes and SNPs) for each GWAS and specifies a linkage disequilibrium (LD) population
5. Clicks “Compute CPAG”

The R script:

6. Validates the uploaded file (verifies that specified columns and delimiters are present)
7. Composes a CPAG (Python) function call, using researcher supplied values
8. Creates an asynchronous future() environment for CPAG execution
9. Passes control to the CPAG function and waits on returned (promise piped) results
10. Generates a table of results and heatmap using current on-screen configuration values

Researcher:

11. Reviews and interacts with results as on the Review tab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1. Upload a GWAS file

Note: Maximum file size is 1GB. Expected upload time is appproxiamtaely 30 seconds per 100MB. For faster upload, reduce your input file to two columns (SNP and P-value) and/or pre-clump for only lead SNPs at your desired threshold. Upload progress is indicated in the bar below the "Browse" button.

To download a sample GWAS file for review, click here: download sample GWAS file severe COVID-19 Ellinghaus et al. 2020)

Choose file: top_EllinghausPCs_covid19.csv — Upload complete

Delimiter: [✓ Comma] Tab

Trait (phenotype) column: One phenotype per file

SNP column: avsnp50

P (significance) column: p_value

2. Compute

GWAS source one: User Supplied GWAS

GWAS source two: NHGRI

p-threshold$_1$ (factor$_1$ X 10^{-x_1}): factor$_1$ [✓ 1] 5; x_1 = 5

p-threshold$_2$ (factor$_2$ X 10^{-x_2}): factor$_2$ 1 [✓ 5]; x_2 = 8

Note p-threshold adjustments (min): H2P2 = $1X10^{-5}$, NHGRI = $5X10^{-8}$, All others = $1X10^{-3}$

LD 1000 Genomes population: [✓ European] African Asian

Compute CPAG

3. Filter

Include all SNPs in table

Trait filter

SNP filter

EFO filter *select multiple*

Include compound EFOs

Apply filters | Clear filters | Download filtered records

Heatmap metric: [✓ Fisher] Bonferroni FDR Jaccard Chao-Sorensen

Display top significant phenotype pairs in heatmap: 10 [✓ 25] 50 100 250 500 1,000 all

Table | Heatmap

Figure 4: iCPAGdb web app. Upload and Compute tab..

In initial testing, the app was found to perform efficiently, giving researchers convenient access to both precomputed CPAG results and newly computed values for uploaded GWAS data. Cross-phenotype relationships presented by the app agree with those known to researchers with expert knowledge of data sets employed during verification. It is hoped that the app will become a recognized and useful tool for researchers conducting exploratory cross-phenotype analysis of GWAS.

Complete R and Shiny scripts for the iCPAGdb web app, along with additional design information, are available at https://github.com/tbalmat/iCPAGdb.

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