mGWAS-Explorer Tutorial

-- COVID 19 & T2D Case Studies

Computer and Browser Requirements

- A personal computer with an Internet connection
- An up-to-date web browser that supports HTML5 with JavaScript enabled, such as Google Chrome (v50+), Firefox (3.0+), and Internet Explorer (9.0+)
- We recommend a ≥2 GHz CPU, 4 GB physical RAM with at least 2 GB free and a minimum of a 15-inch screen with a screen resolution of 1,280 × 800 or higher
- A mouse with scrolling support is required for network visualization

Goal for this tutorial

- Demonstrate the practical application of mGWAS-Explorer by using two case studies:
  - COVID-19
  - Type 2 diabetes

COVID-19 Case Study

Motivation

- The host genetic variation is known to influence the severity of SARS-CoV-2 infection [1].
- And the blood metabolome reveals biomarkers for disease prediction and classification [2].
- However, understanding mechanisms that link genetic variation to metabolism and clinical endpoint remains an important challenge.

1. Kousathanas, A.; Pairo-Castineira, E.; Rawlik, K.; Stuckey, A.; Odhams, C.A.; Walker, S.; Russell, C.D.; Malinauskas, T.; Wu, Y.; Millar, J.; et al. Whole genome sequencing reveals host factors underlying critical Covid-19. Nature 2022, doi:10.1038/s41586-022-04576-6.
2. Sindelar, M.; Stancliffe, E.; Schwaiger-Haber, M.; Anbukumar, D.S.; Adkins-Travis, K.; Goss, C.W.; O'Halloran, J.A.; Mudd, P.A.; Liu, W.C.; Albrecht, R.A.; et al. Longitudinal metabolomics of human plasma reveals prognostic markers of COVID-19 disease severity. Cell reports. Medicine 2021, 2, 100369, doi:10.1016/j.xcrm.2021.100369.

Objectives

Therefore, we applied mGWAS-Explorer to a list of SNPs identified from a GWAS of severe COVID-19 [1] to provide insights into shared genetic architecture of diseases and intermediate metabolic phenotypes.

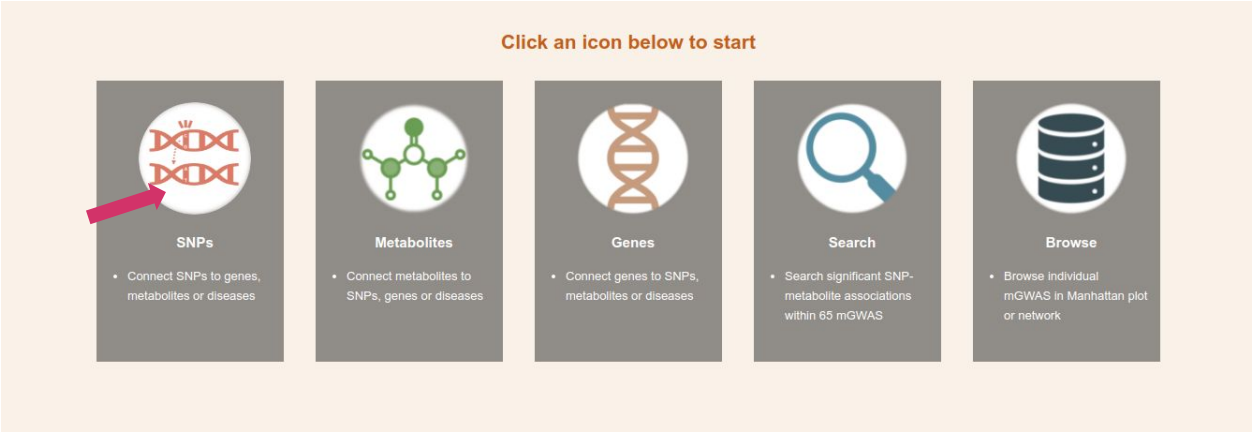
1. Ellinghaus, D.; Degenhardt, F.; Bujanda, L.; Buti, M.; Albillos, A.; Invernizzi, P.; Fernández, J.; Prati, D.; Baselli, G.; Asselta, R.; et al. Genomewide Association Study of Severe Covid-19 with Respiratory Failure. The New England journal of medicine 2020, 383, 1522-1534, doi:10.1056/NEJMoa2020283.

Methods

- Pre-processing:
  - Suggestive significant association p-value threshold (1×10^{-5})
  - LD clumping were performed to identify the independent signals by using the *ieugwasr* package
- Analysis overview:
  - Upload a list of SNPs
  - Network building
  - Network analytics
    - Cross-phenotype association analysis

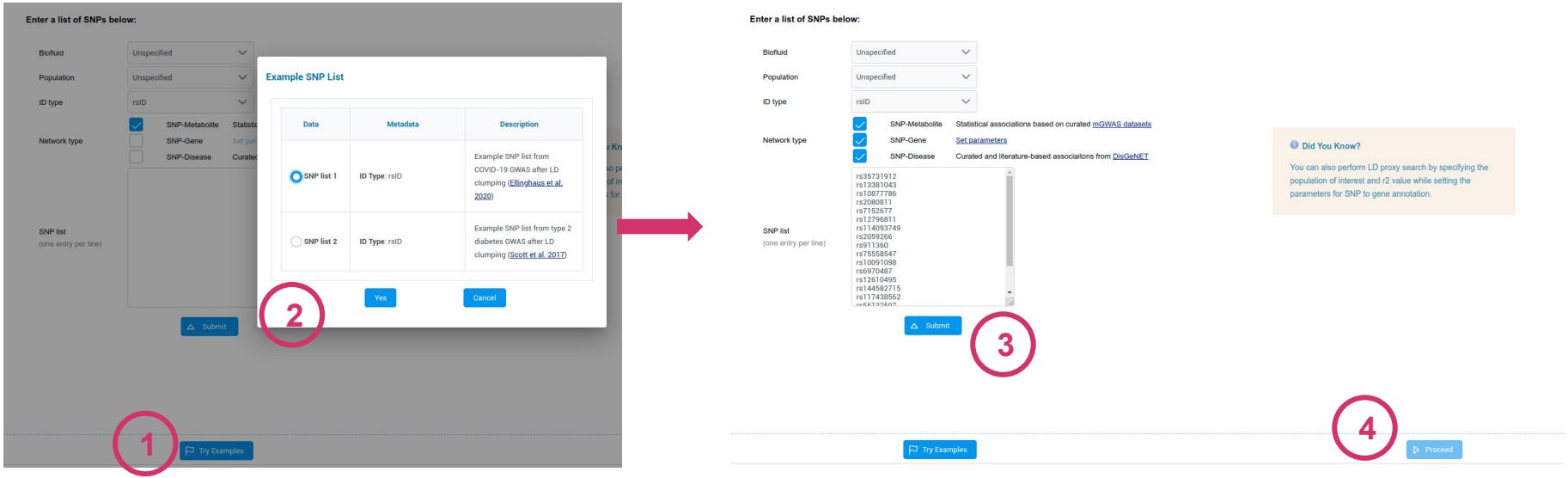
Starting up

- Go to the mGWAS-Explorer homepage (www.mgwas.ca).
- For SNP list input, click the “SNPs” button to enter the data upload page.

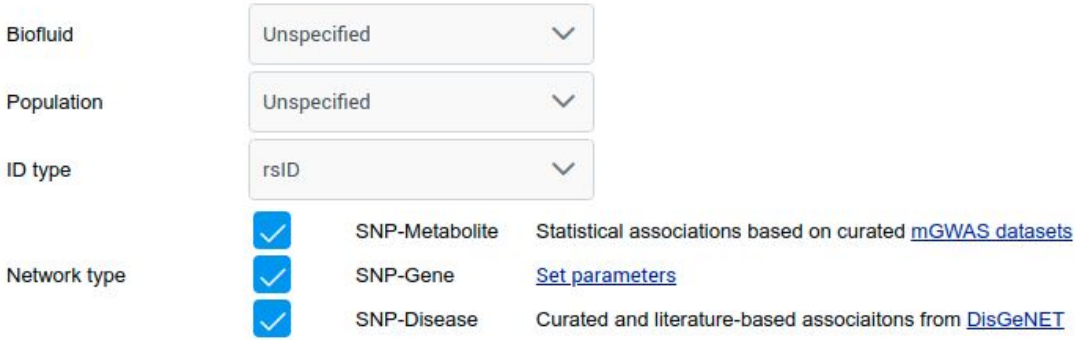


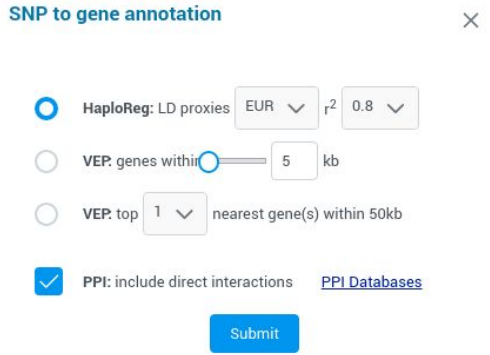
Data upload

1. Click the “**Try Examples**” button
2. Click “**Yes**” button in the pop-up window (default is SNP list 1)
3. Click “**Submit**” button to upload the data
4. Click “**Proceed**” button to the Network Builder page

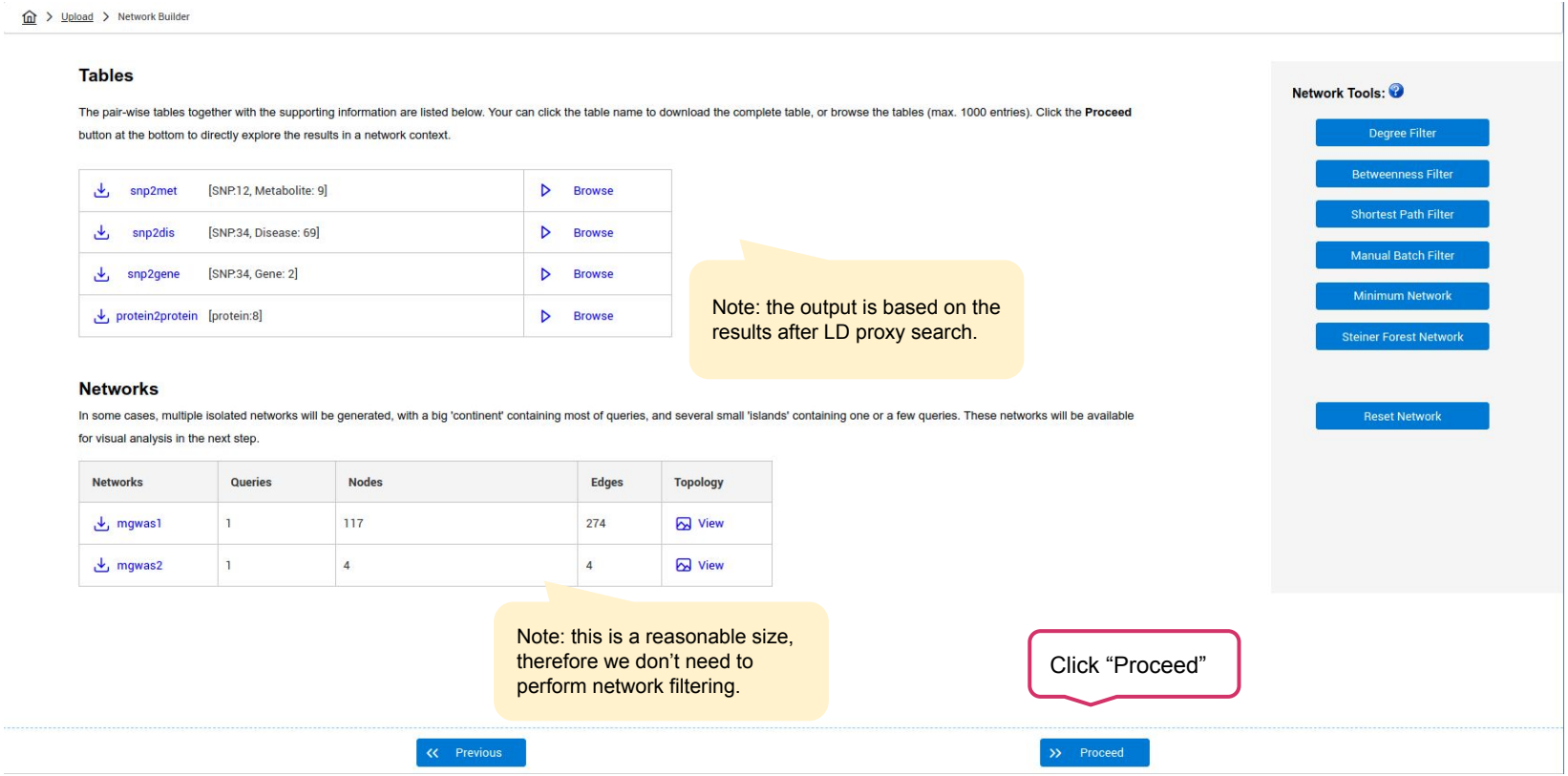


- Note: here are the parameters automatically entered for the COVID-19 case study:
  - Biofluid: “Unspecified”
  - Population: “Unspecified”
  - ID type: “rsID”
  - SNP annotation:
    - “EUR” and “r^2=0.8” are entered for LD proxy search
    - Include PPI
  - Network type:
    - “SNP-Metabolite”
    - “SNP-Gene”
    - “SNP-Disease”



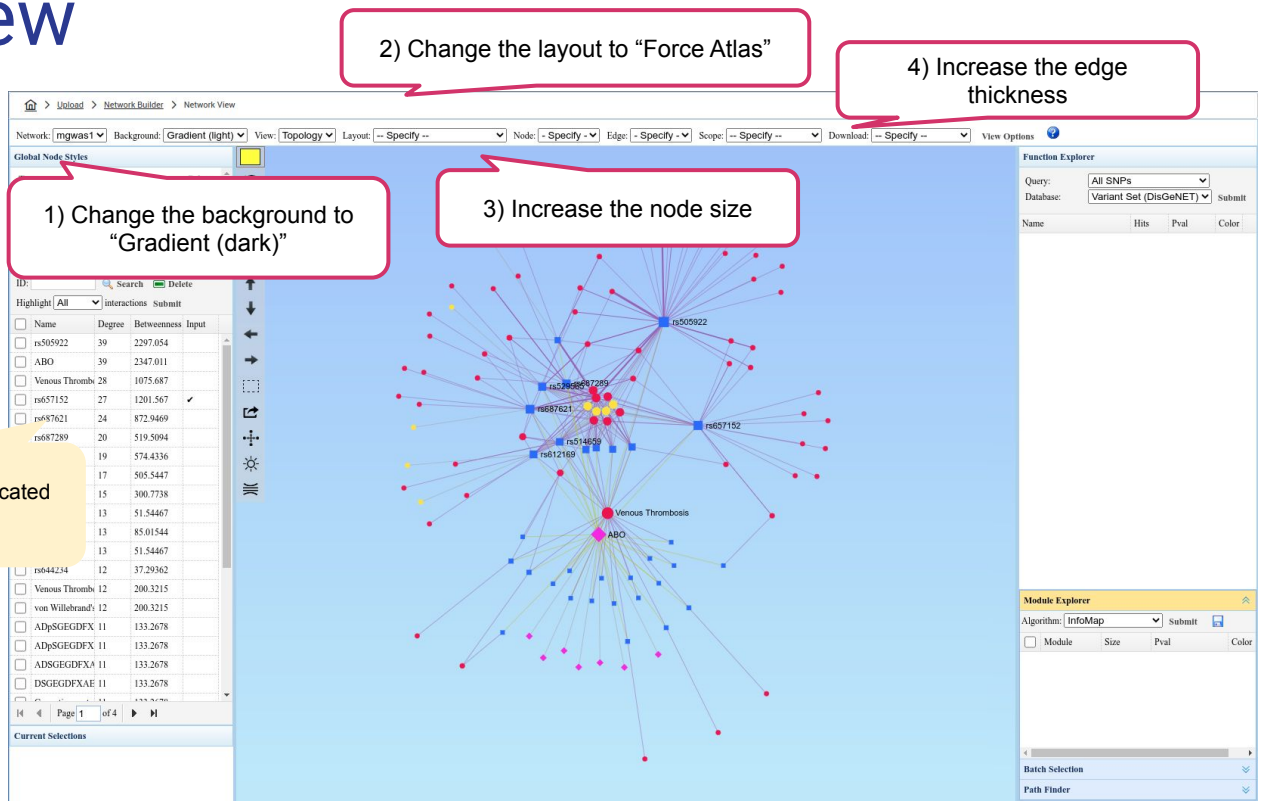


Network building



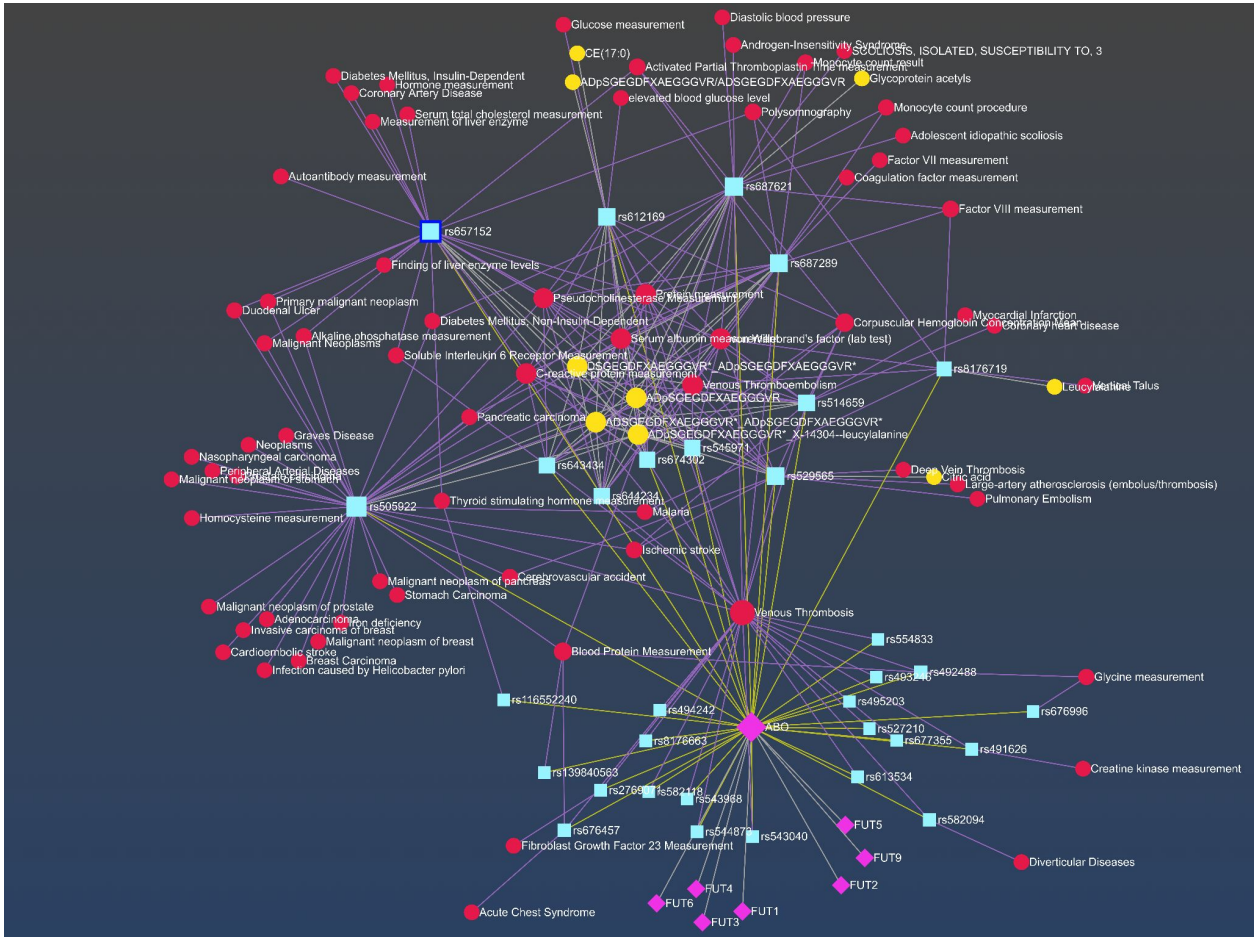
Network overview

Here, we perform some basic customization to better visualize the network.



- mGWAS-Explorer discovered that the input SNP **rs657152** at the ***ABO*** locus is in high LD (r^2>0.8) with multiple other SNPs in this region.

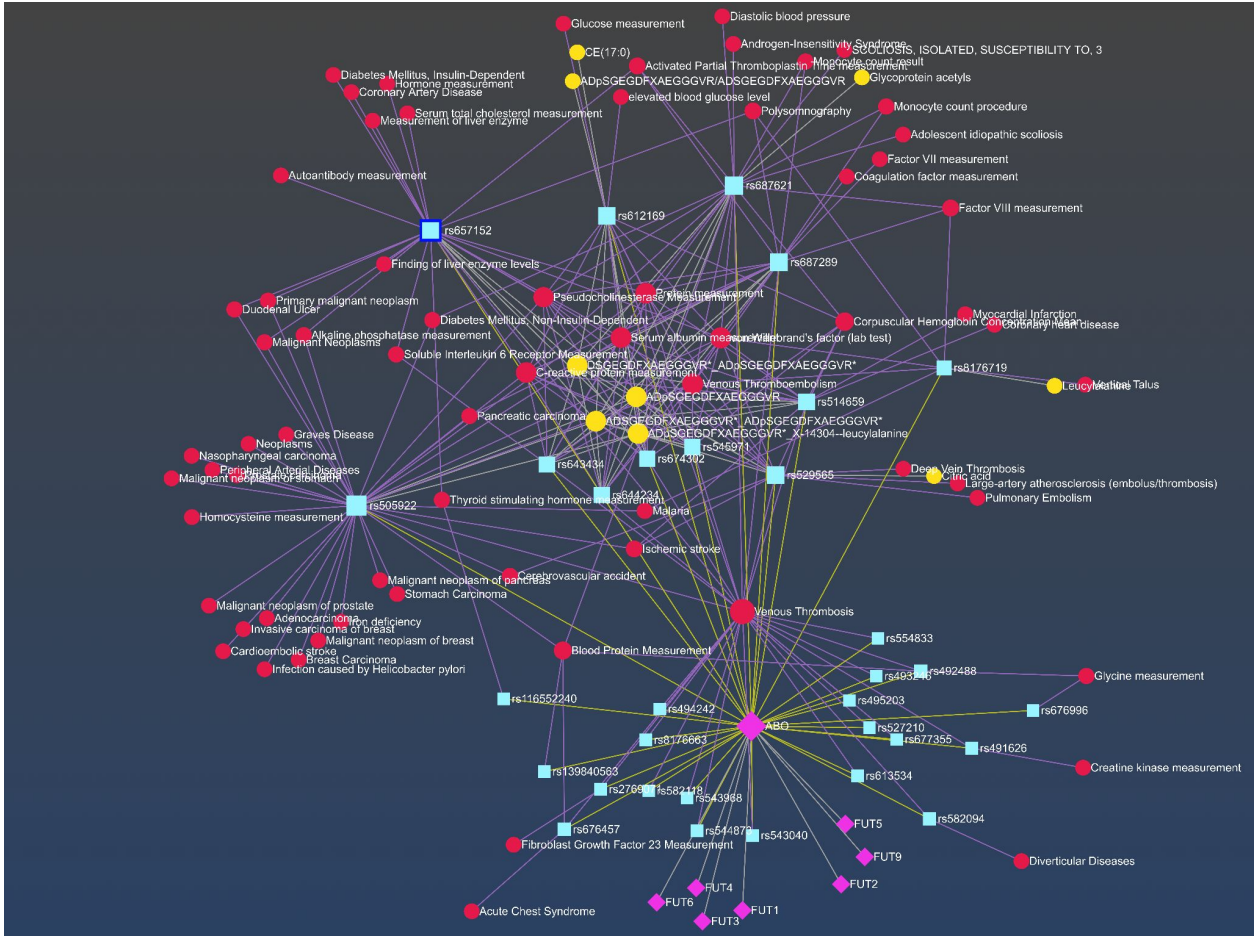
- The input SNP and SNPs in high LDs are associated with multiple metabolites and other human diseases, such as citric acid, malaria, ischemic stroke, and venous thrombosis.



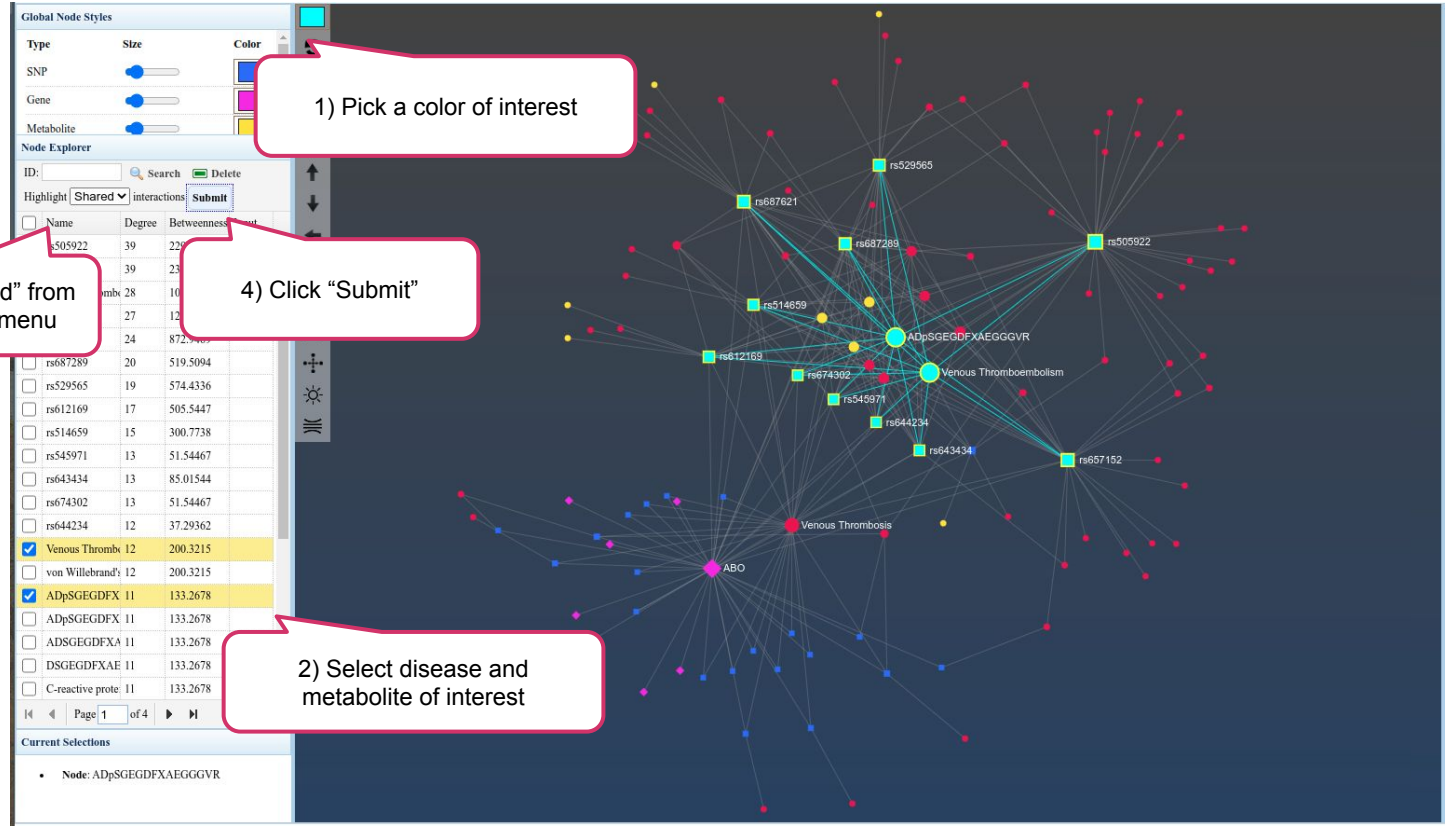
- Interestingly, mGWAS-Explorer identified shared ***ABO*** variants between **COVID-19**, **fibrinogen A-α peptides** (e.g., ADpSGEGDFXAEGGGVR) and **venous thromboembolism**.

- Fibrinogen play a role in forming the blood clots.

- Therefore, the associations between ***ABO*** variants with **fibrinogen** may suggest that ***ABO*** influences COVID-19 via regulating **thrombosis**, which provided a functional explanation for the observed association of ***ABO*** with COVID-19 risk.



How to identify the shared SNPs between a disease and a metabolite?

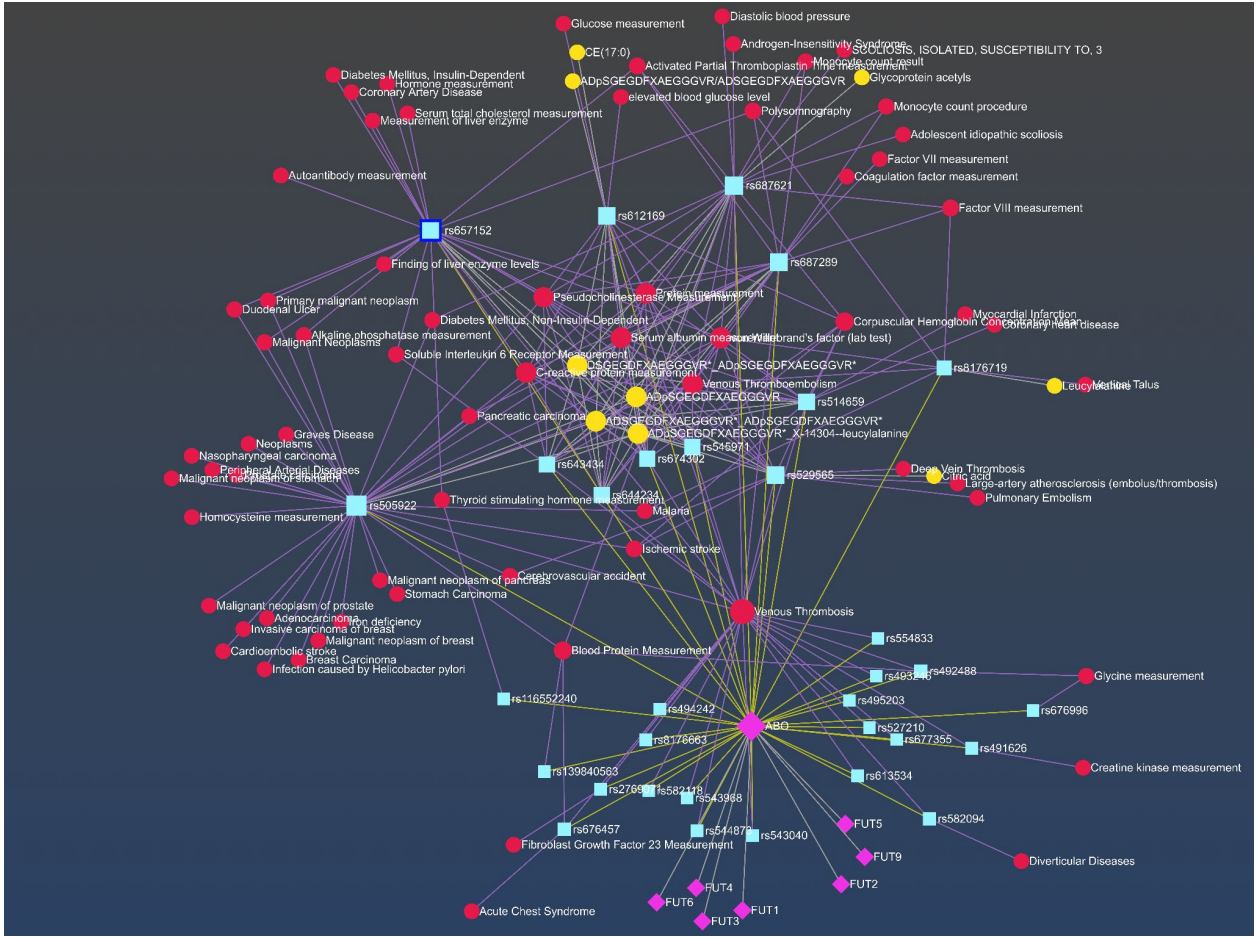


Cross-phenotype association analysis

- Indeed, studies have reported that COVID-19 is associated with an increased risk of thromboembolism.

- Therefore, we sought to investigate whether the association between **fibrinogen A-α peptides** associated loci could provide additional insights into underlying pathophysiologic mechanisms of COVID-19.

Let's take the list of the **fibrinogen A-α peptides** and start from the **Metabolite** module.



Start from the Metabolite Module

Follow similar workflow as the SNP module:

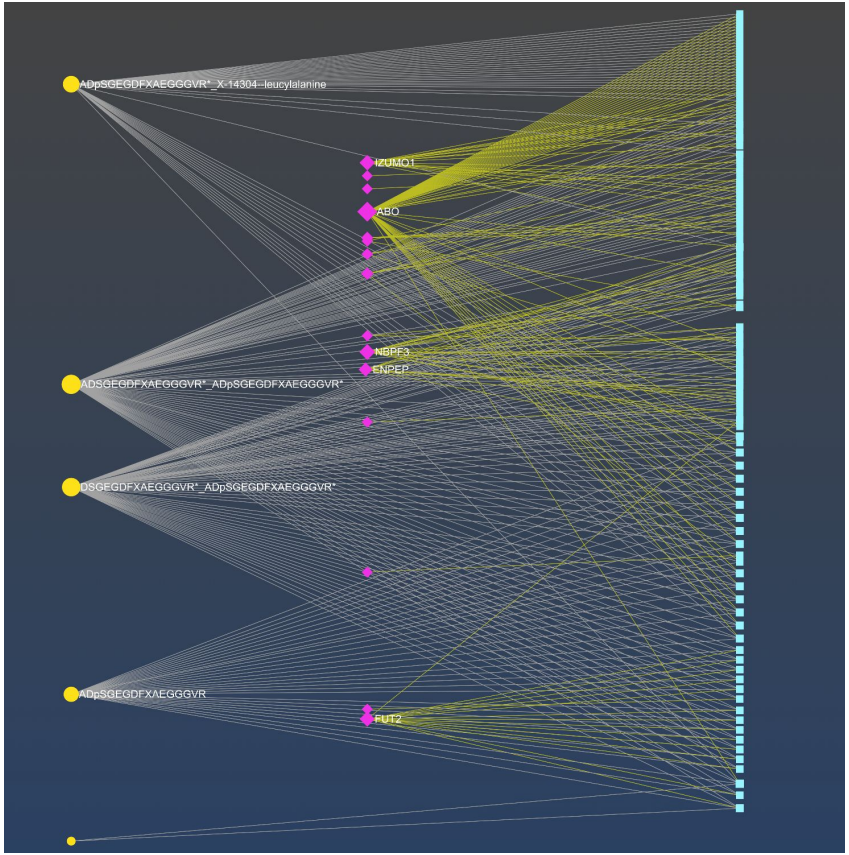
- Data Upload page: Click “Try Example” → “Yes” → “Submit” → “Proceed”
- Network Builder page: Click “Proceed”

Network result

- mGWAS-Explorer revealed variants at *ENPEP* and *FUT2* genes are associated with levels and/or ratios of fibrinogen A-α peptides.

- In fact, *ENPEP* was discovered to be a candidate co-receptor for the coronavirus SARS-CoV-2 [1].

- And, individuals with an inactivating *FUT2* mutations were more likely to develop a less severe form of the COVID-19 disease [2].



1) Lange, C.; Wolf, J.; Auw-Haedrich, C.; Schlecht, A.; Boneva, S.; Lapp, T.; Horres, R.; Agostini, H.; Martin, G.; Reinhard, T.; et al. Expression of the COVID-19 receptor ACE2 in the human conjunctiva. Journal of medical virology 2020
2) Mankelow, T.J.; Singleton, B.K.; Moura, P.L.; Stevens-Hernandez, C.J.; Cogan, N.M.; Gyorffy, G.; Kupzig, S.; Nichols, L.; Asby, C.; Pooley, J.; et al. Blood group type A secretors are associated with a higher risk of COVID-19 cardiovascular disease complications. EJHaem 2021

In summary

mGWAS-Explorer supports the evidence that *ABO*, *ENPEP* and *FUT2* may be candidate genes and discovered fibrinogen A-α peptides as potential biomarkers for COVID-19 disease.

Type 2 Diabetes Case Study

Motivation

- Around 250 genomic regions have been associated to type 2 diabetes (T2D) susceptibility in genome-wide association studies [1].
- Some studies have highlighted the link to metabolomic profiles [2].

1. Langenberg, C.; Lotta, L.A. Genomic insights into the causes of type 2 diabetes. Lancet (London, England) 2018, 391, 2463-2474, doi:10.1016/s0140-6736(18)31132-2.
2. Lotta, L.A.; Pietzner, M.; Stewart, I.D.; Wittemans, L.B.L.; Li, C.; Bonelli, R.; Raffler, J.; Biggs, E.K.; Oliver-Williams, C.; Auyeung, V.P.W.; et al. A cross-platform approach identifies genetic regulators of human metabolism and health. Nature Genetics 2021, 53, 54-64, doi:10.1038/s41588-020-00751-5.

Objectives

Therefore, we applied mGWAS-Explorer to a list of SNPs from a published GWAS of T2D [1] with an attempt to examine shared genetic signals with circulating metabolites.

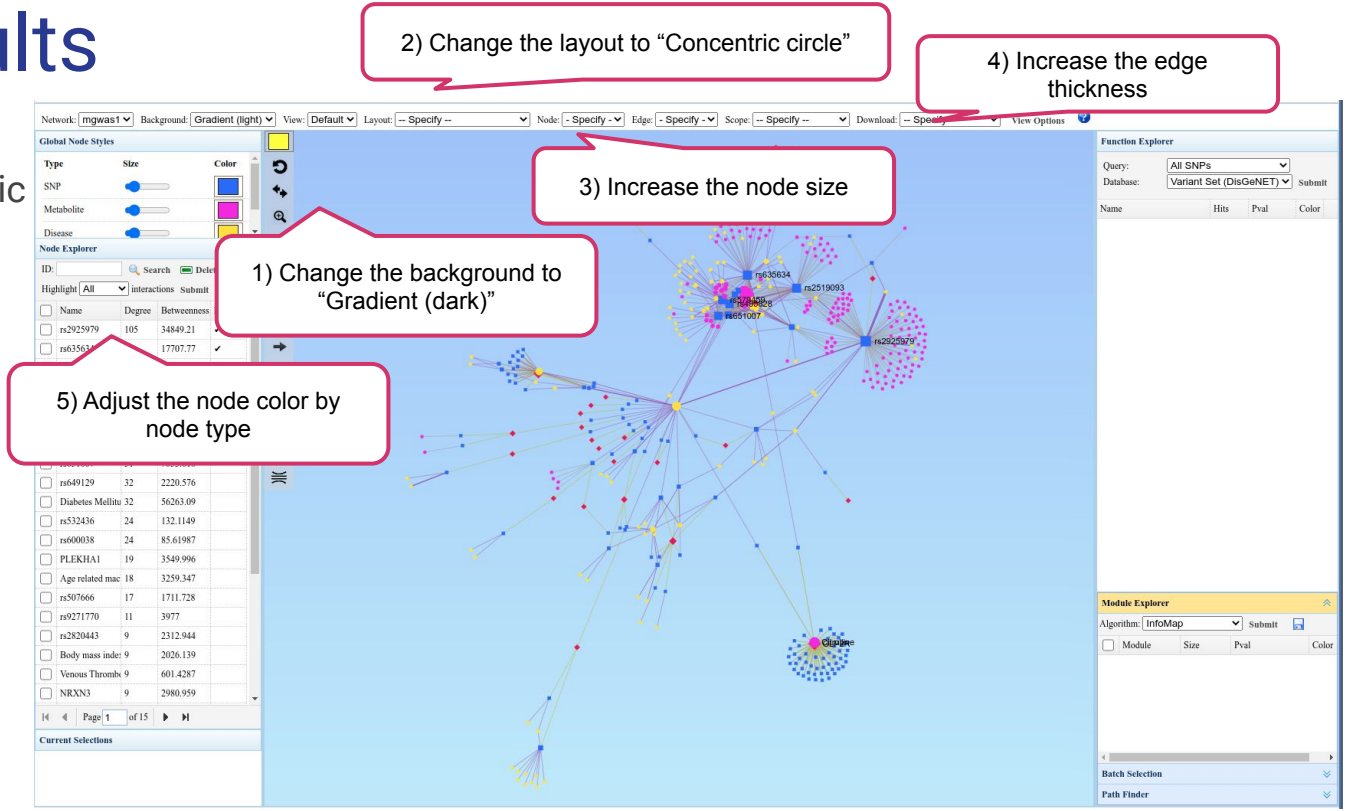
1. Scott, R.A.; Scott, L.J.; Mägi, R.; Marullo, L.; Gaulton, K.J.; Kaakinen, M.; Pervjakova, N.; Pers, T.H.; Johnson, A.D.; Eicher, J.D.; et al. An Expanded Genome-Wide Association Study of Type 2 Diabetes in Europeans. Diabetes 2017, 66, 2888-2902, doi:10.2337/db16-1253.

Methods

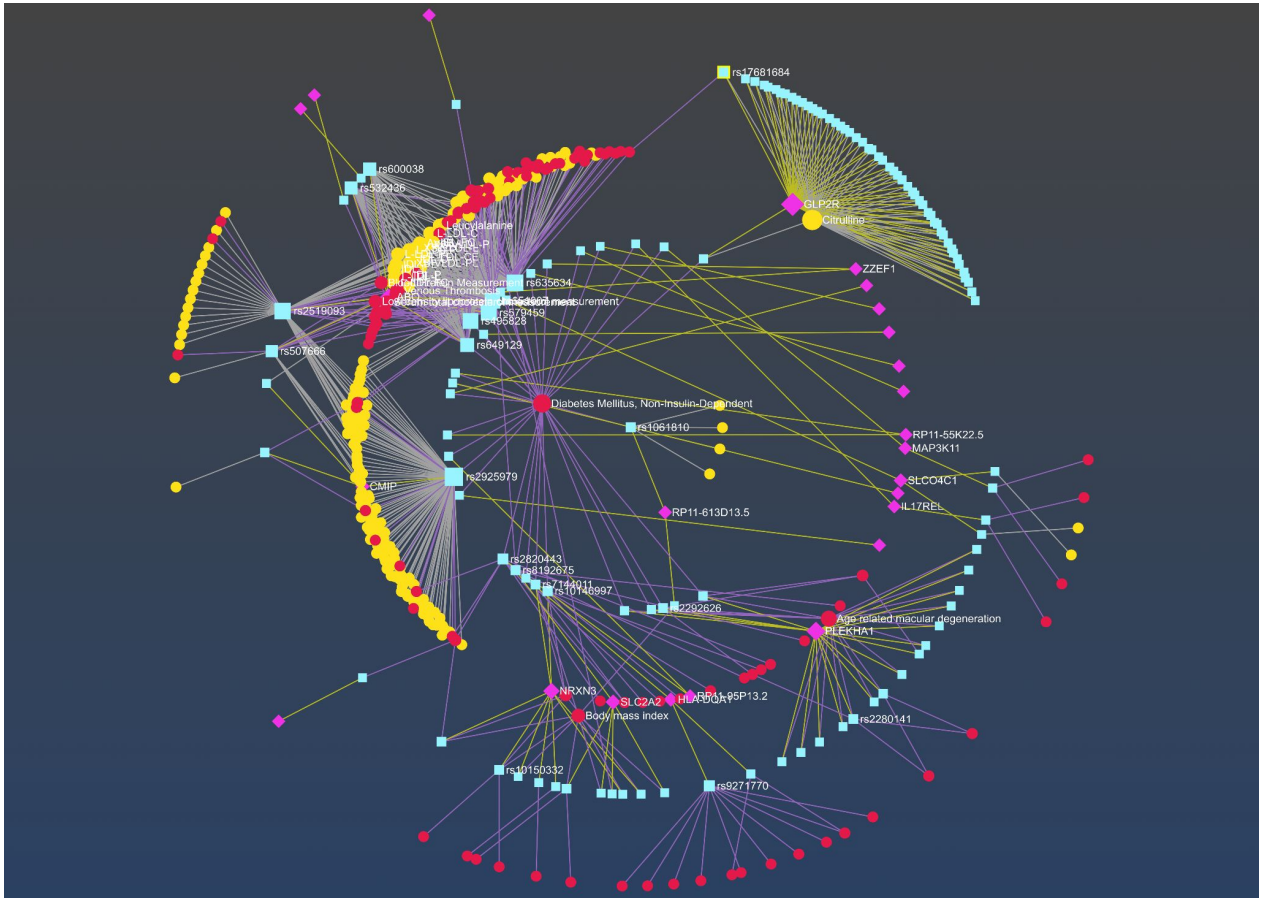
- Pre-processing:
  - Significant association p-value threshold (5×10^{-8})
  - LD clumping were performed to identify the independent signals by using the *ieugwasr* package
- Analysis overview: similar workflow as the COVID-19 case study
  - Upload a list of SNPs → select SNP list 2
  - Network building
  - Network analytics
    - Cross-phenotype association analysis

Network results

Here, we perform some basic customization to better visualize the network.

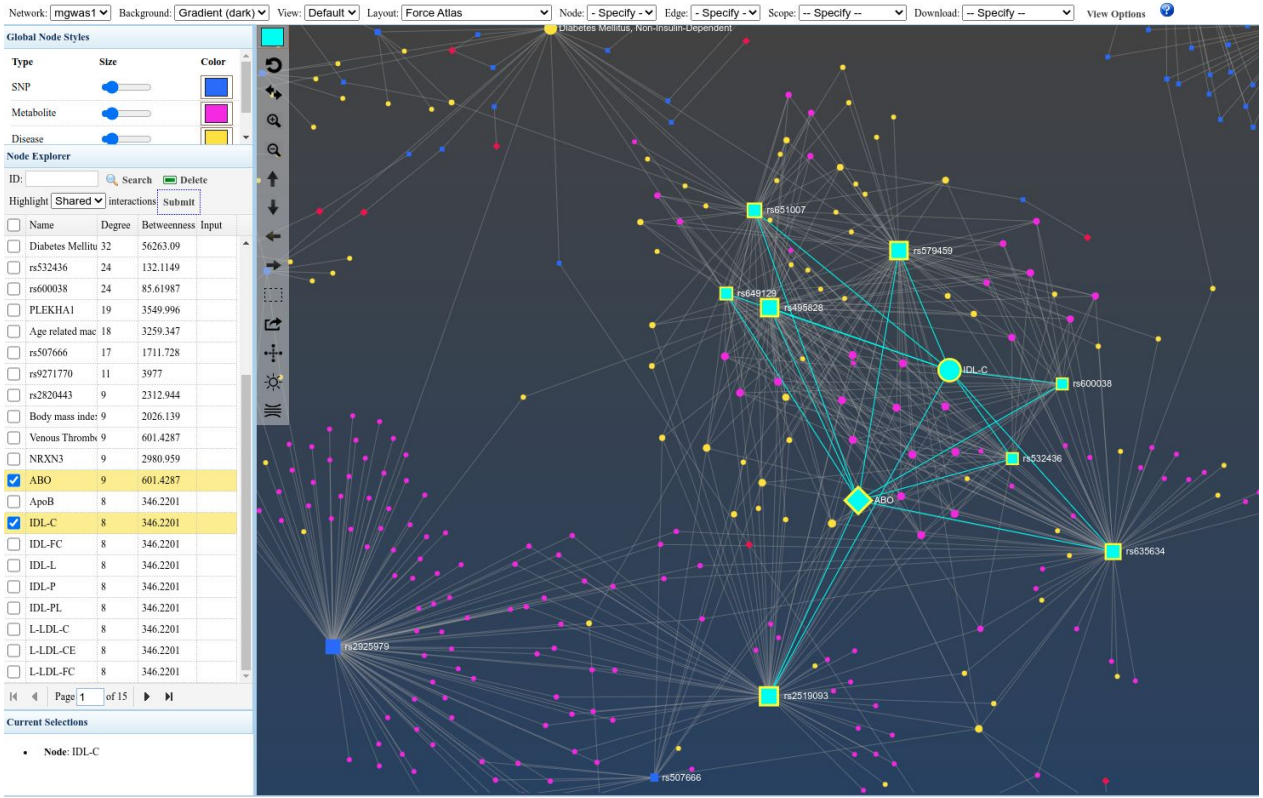


- mGWAS-Explorer confirmed the cross-phenotype associations between **citrulline** metabolite, **T2D**, and **body mass index** and identified the missense **rs17681684** variant for **citrulline** in the ***GLP2R*** gene as reported by Lotta et al. [1].



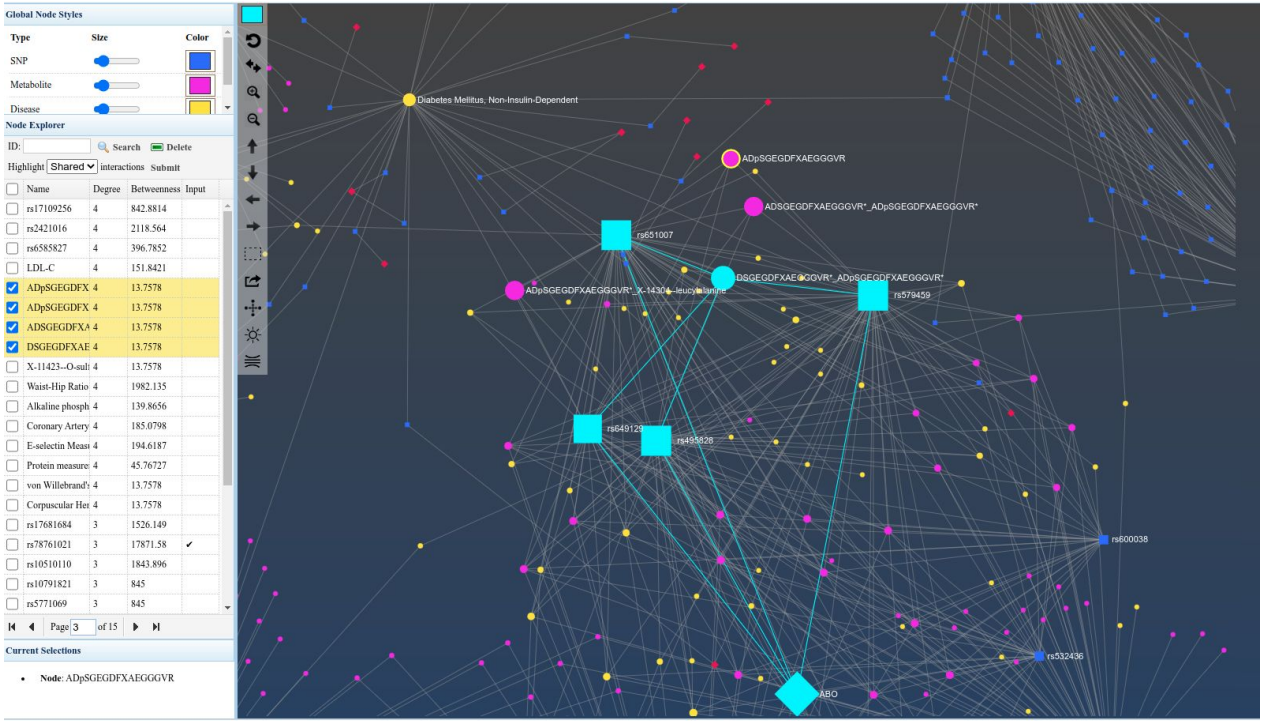
1) Lotta, L.A.; Pietzner, M.; Stewart, I.D.; Wittemans, L.B.L.; Li, C.; Bonelli, R.; Raffler, J.; Biggs, E.K.; Oliver-Williams, C.; Auyeung, V.P.W.; et al. A cross-platform approach identifies genetic regulators of human metabolism and health. Nature Genetics 2021, 53, 54-64, doi:10.1038/s41588-020-00751-5.

- Additionally, we identified shared genetic signals between **T2D**, **coronary artery disease** and **cholesterol** levels at ***ABO*** locus.



- Furthermore, mGWAS-Explorer also revealed **metabolites** levels and their ratios identified in the previous **COVID-19** case study shared associations with **T2D** associated SNPs at ***ABO*** locus.

- In fact, multiple studies have reported comorbidity of T2D and COVID-19 [1].



1) Rajpal, A.; Rahimi, L.; Ismail-Beigi, F. Factors leading to high morbidity and mortality of COVID-19 in patients with type 2 diabetes. Journal of diabetes 2020, 12, 895-908, doi:10.1111/1753-0407.13085.

In summary

In brief, analyzing the T2D cross-phenotype associations with metabolites and other diseases highlighted comorbid conditions with shared genetic signals, illustrating the usefulness of mGWAS-Explorer.

==The End==