Automated visualization of biomarkers for pyrimidine and urea cycle disorders in pathways

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Supervisor: Denise Slenter & Egon Willighagen
01-07-2019

Maastricht University

Diagnosis of metabolic disorders

1 Lee JJY, et al. (2018) Genetics in medicine
2 Burton BK. (1998) Pediatrics

Diagnosis of metabolic disorders

- IEMs → genetic disorders leading to a defect in metabolic pathways [1]
  - Variable clinical presentation [1]

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Diagnosis of metabolic disorders

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- Challenging diagnosis [1,2]
  - Overlapping symptoms
  - Difficulty interpreting metabolite concentrations

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- IEMs → genetic disorders leading to a defect in metabolic pathways [1]
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- Challenging diagnosis [1,2]
  - Overlapping symptoms
  - Difficulty interpreting metabolite concentrations
- Visualization in pathways
  - Changes at a process level
- For this internship → Pyrimidine and Urea cycle disorders

1 Lee JJY, et al. (2018) Genetics in medicine
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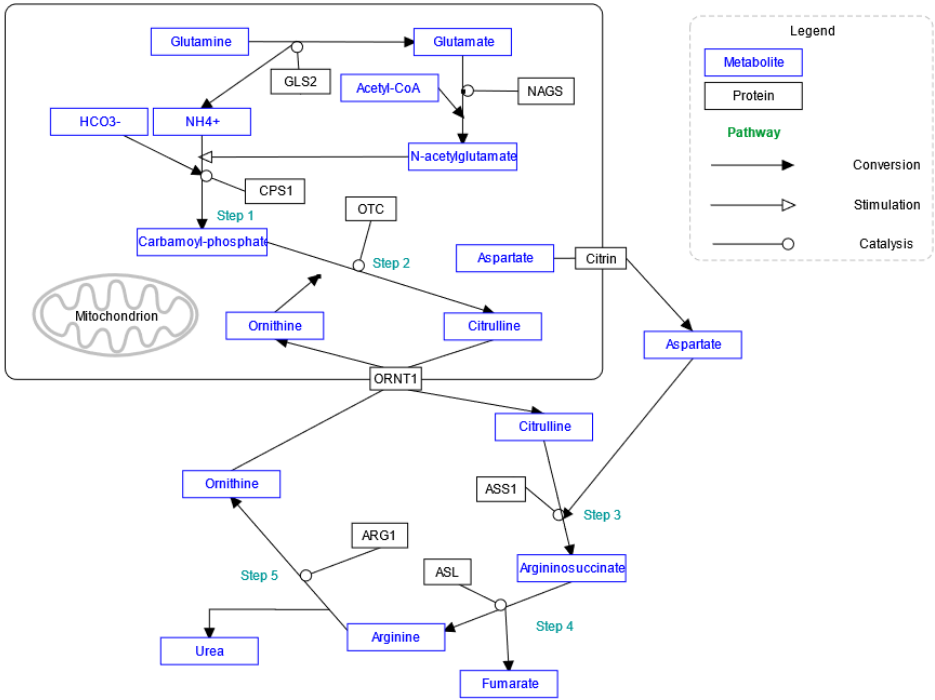
Urea cycle - UCD

Image adapted from WikiPathways:WP4571_104803

1 Barmore W, et al. (2019) https://www.ncbi.nlm.nih.gov/books/NBK513323/
2 Mew NA, et al. (2003) https://www.ncbi.nlm.nih.gov/books/NBK1217/
3 Häberle J, et al. (2012) Orphanet journal of rare diseases

Urea cycle - UCD

- Removal of toxic ammonia [1,2]
  - In the liver
  - 5 step process



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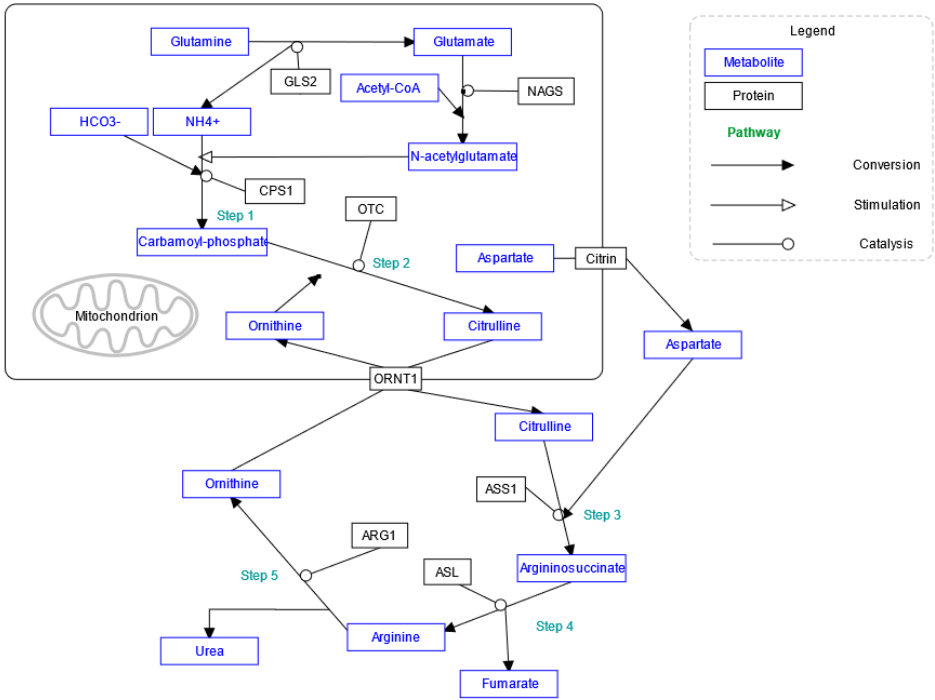

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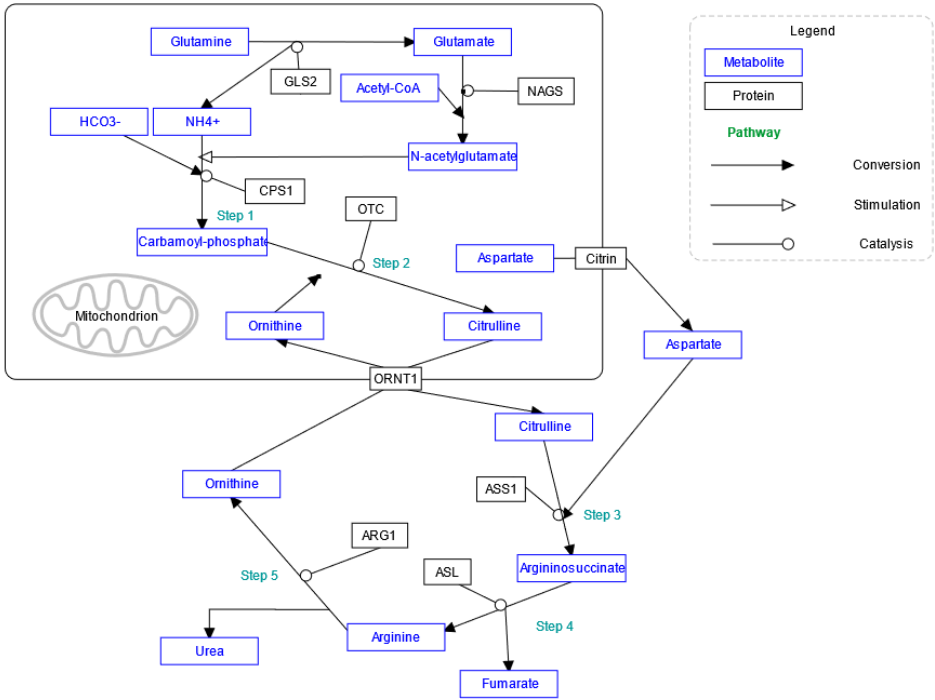
Urea cycle - UCD

- Removal of toxic ammonia [1,2]
  - In the liver
  - 5 step process
- Urea cycle disorders [2]
  - Enzyme or transporter defects
- Diagnosis [3]
  - Hyperammonia
  - Amino acids
  - Orotic acid



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Pyrimidine metabolism - PMD

- Nitrogen containing carbon ring structures [1]
  - Thymine, cytosine and uracil

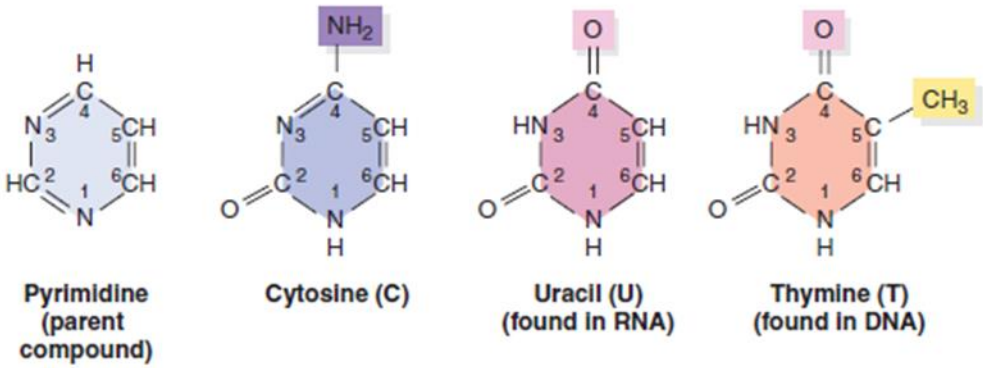

Image: Russell PJ. (2010) iGenetics

1 Löffler M, et al. (2005) Trends in Molecular Medicine

Pyrimidine metabolism - PMD

- Nitrogen containing carbon ring structures [1]
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- Metabolism in 4 parts [1,2]
  - *De novo* synthesis
    - From glutamine to UMP

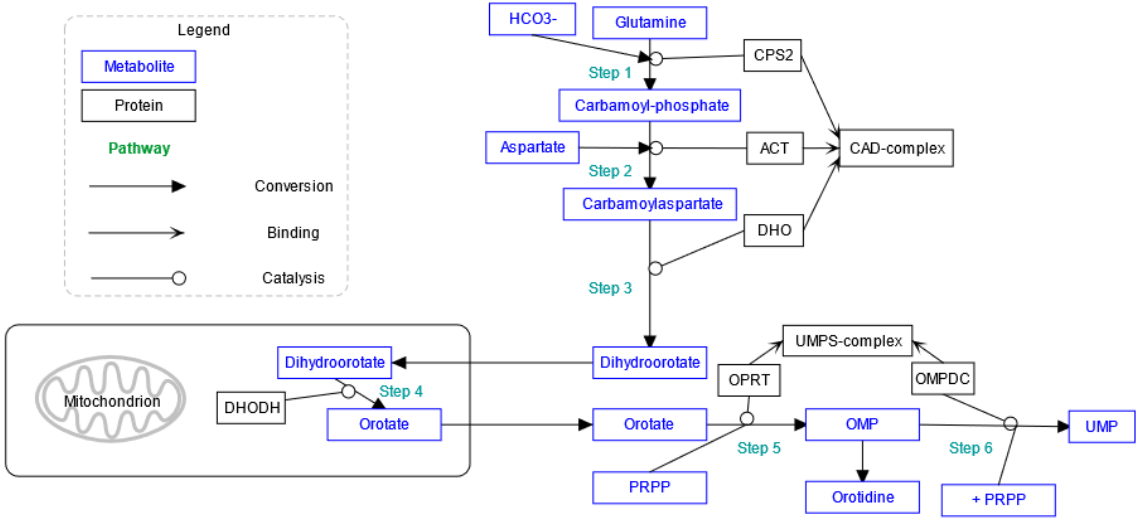

Image adapted from WP4225_104804
1 Löffler M, et al. (2005) Trends in Molecular Medicine
2 Moffat BA, et al. (2002) Arabidopsis Book

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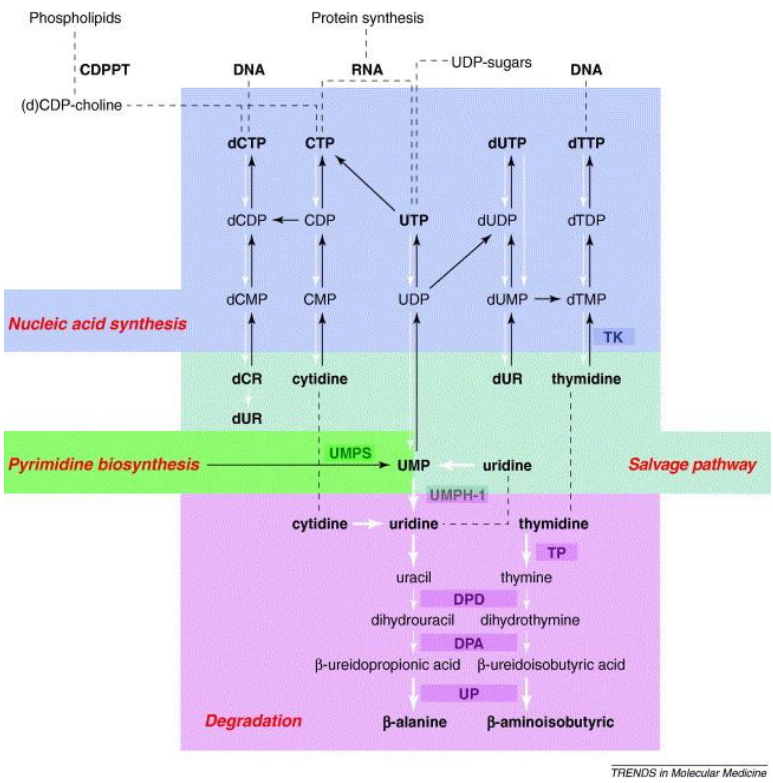

Image: Löffler M, et al. (2005) Trends in Molecular Medicine
Image machine readable version WikiPathways:WP4225

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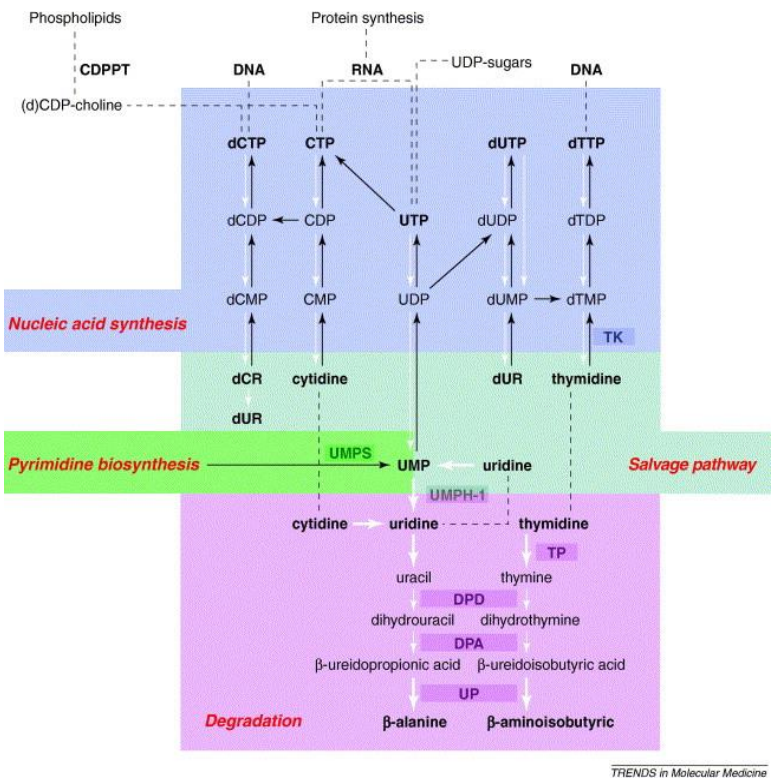
  - Enzyme defects



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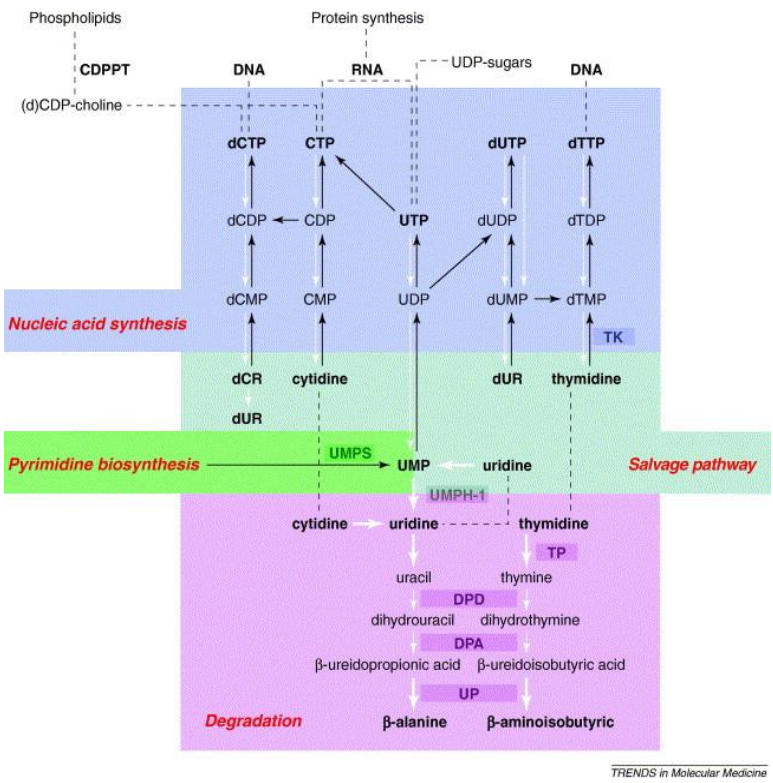
  - Enzyme defects
- Diagnosis [3]
  - Based on metabolite markers



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Differential diagnosis

- Challenging
- Urea cycle disorders → accumulation carbamoyl phosphate → enters *de novo* synthesis of pyrimidines → altered pyrimidine metabolite concentrations [1,2]

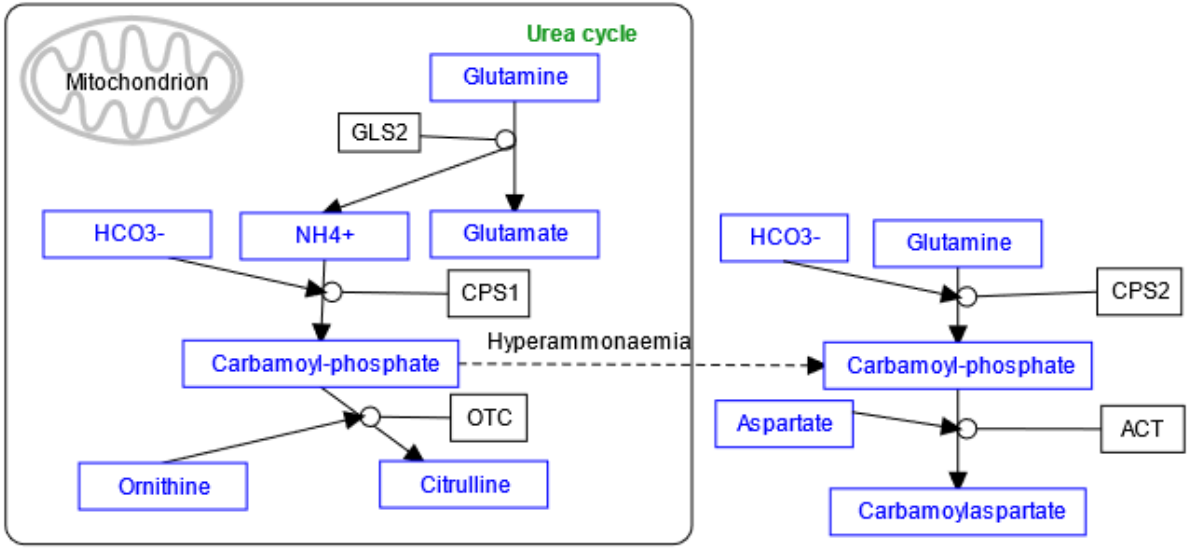


Image adapted from WP4225_104804

1 Leonard JV, Morris AA. (2002) Seminars in neonatology;
2 Burrage LC, et al. (2019) Genetics in medicine

Research question

How can metabolomics data from urine and blood plasma be visualized in an automated manner to enable the interpretation of biomarkers related to pyrimidine and urea cycle defects?

Workflow

Workflow

Two disease types for which differentiation is challenging

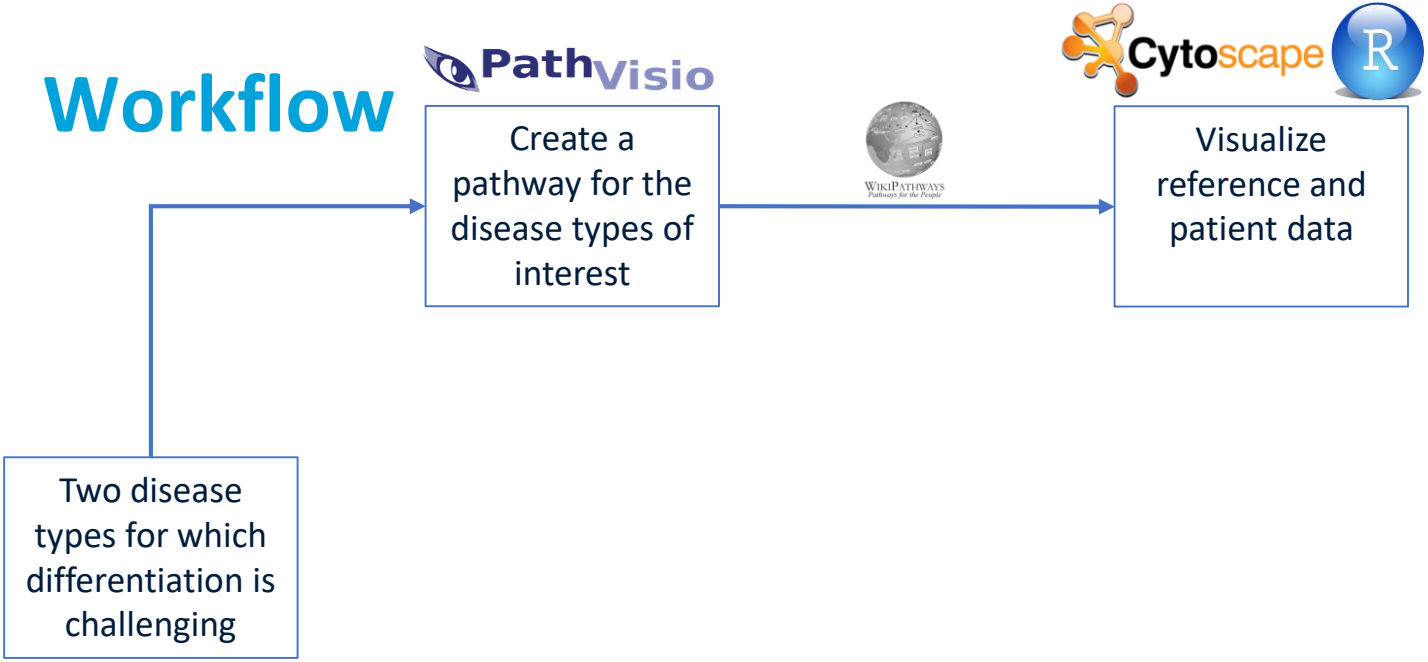
Workflow

PathVisio

Create a pathway for the disease types of interest

Two disease types for which differentiation is challenging

Workflow


PathVisio
Create a pathway for the disease types of interest
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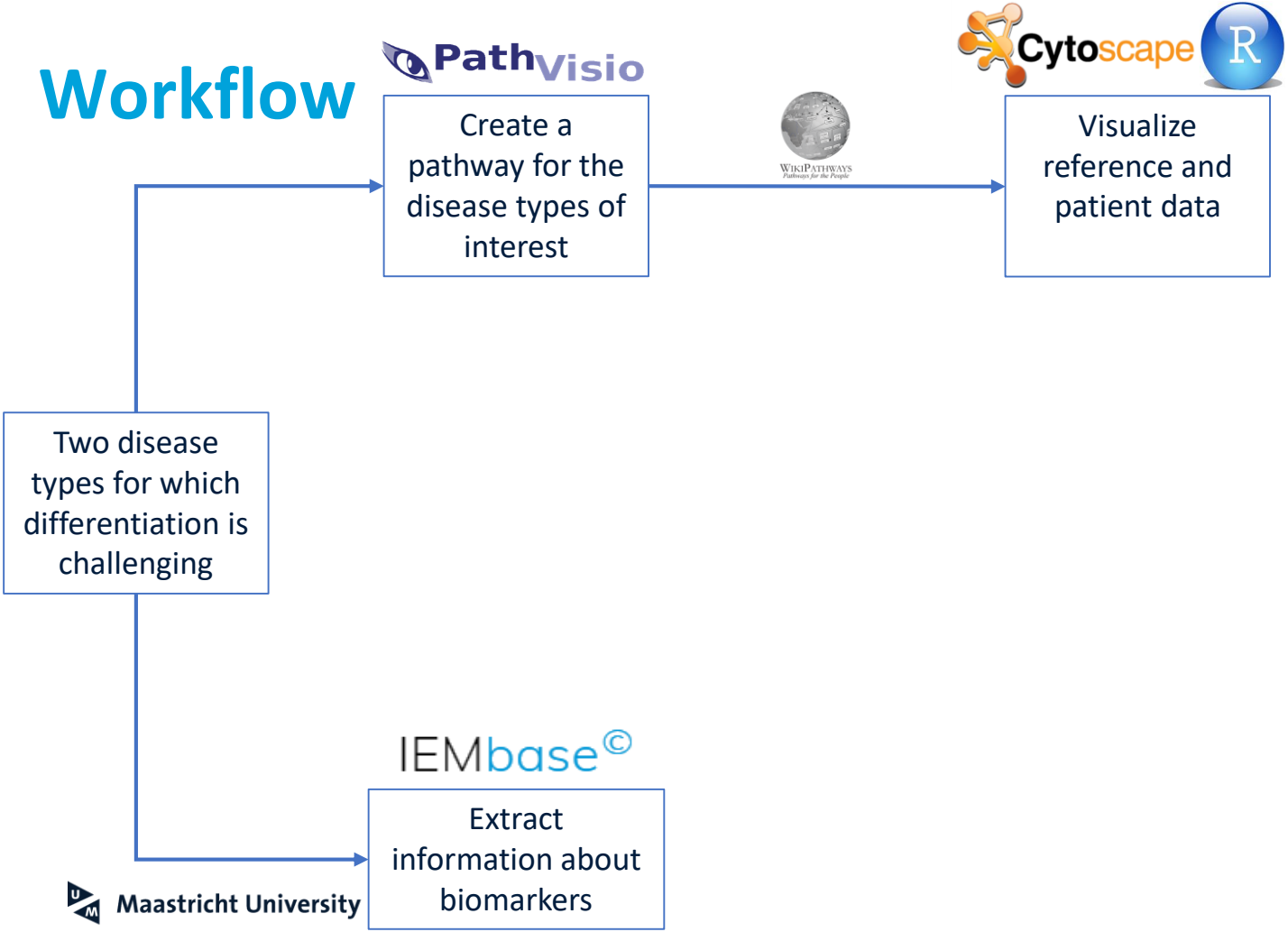
Pathways for the People
Cytoscape
R
Visualize reference and patient data
Two disease types for which differentiation is challenging

Workflow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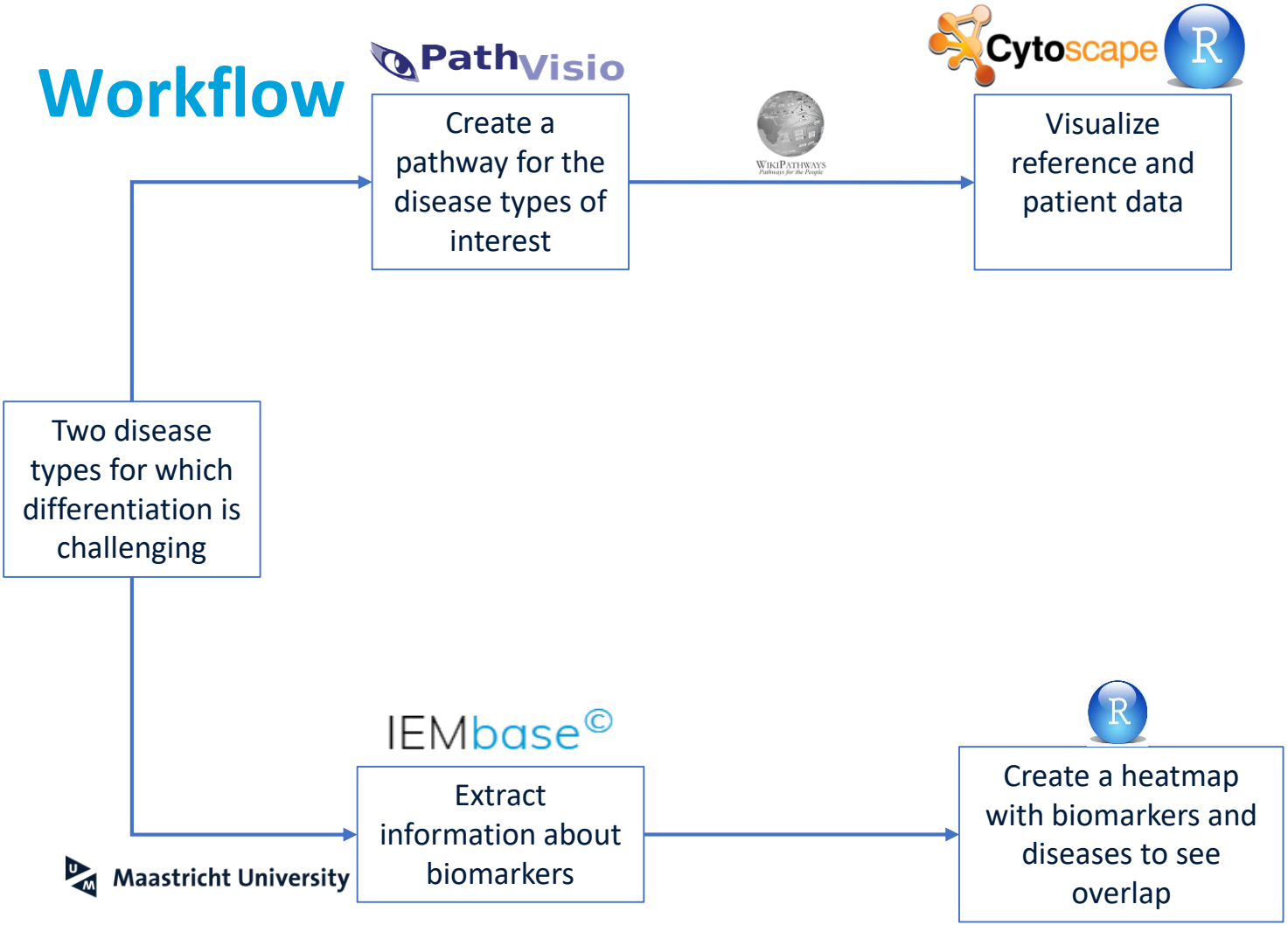
IEMbase©
Extract information about biomarkers
Maastricht University

Workflow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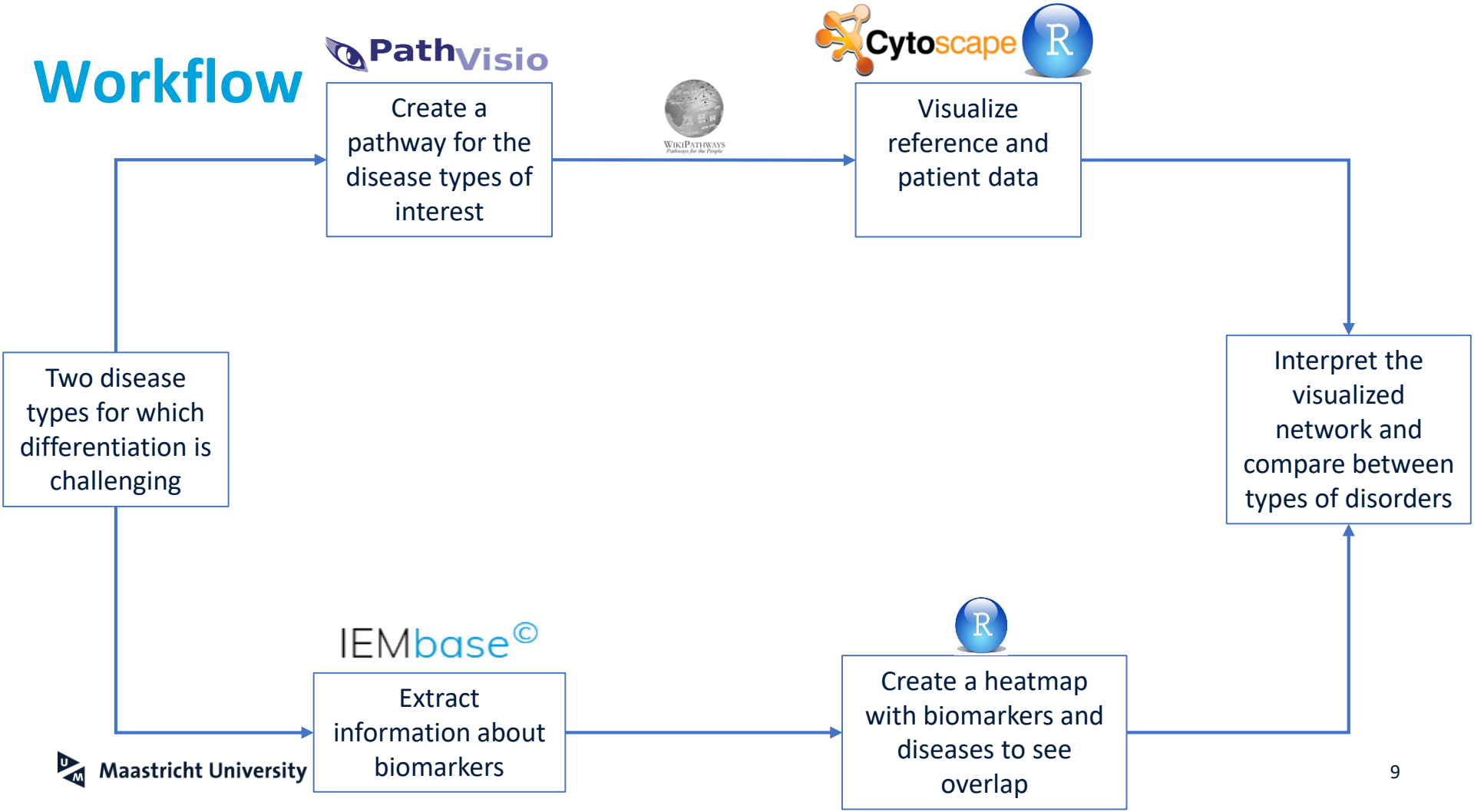
Extract information about biomarkers
Create a heatmap with biomarkers and diseases to see overlap
Maastricht University

Workflow


PathVisio
Create a pathway for the disease types of interest
WikiPathways
Cytoscape
R
Visualize reference and patient data
Interpret the visualized network and compare between types of disorders
Two disease types for which differentiation is challenging
IEMbase©
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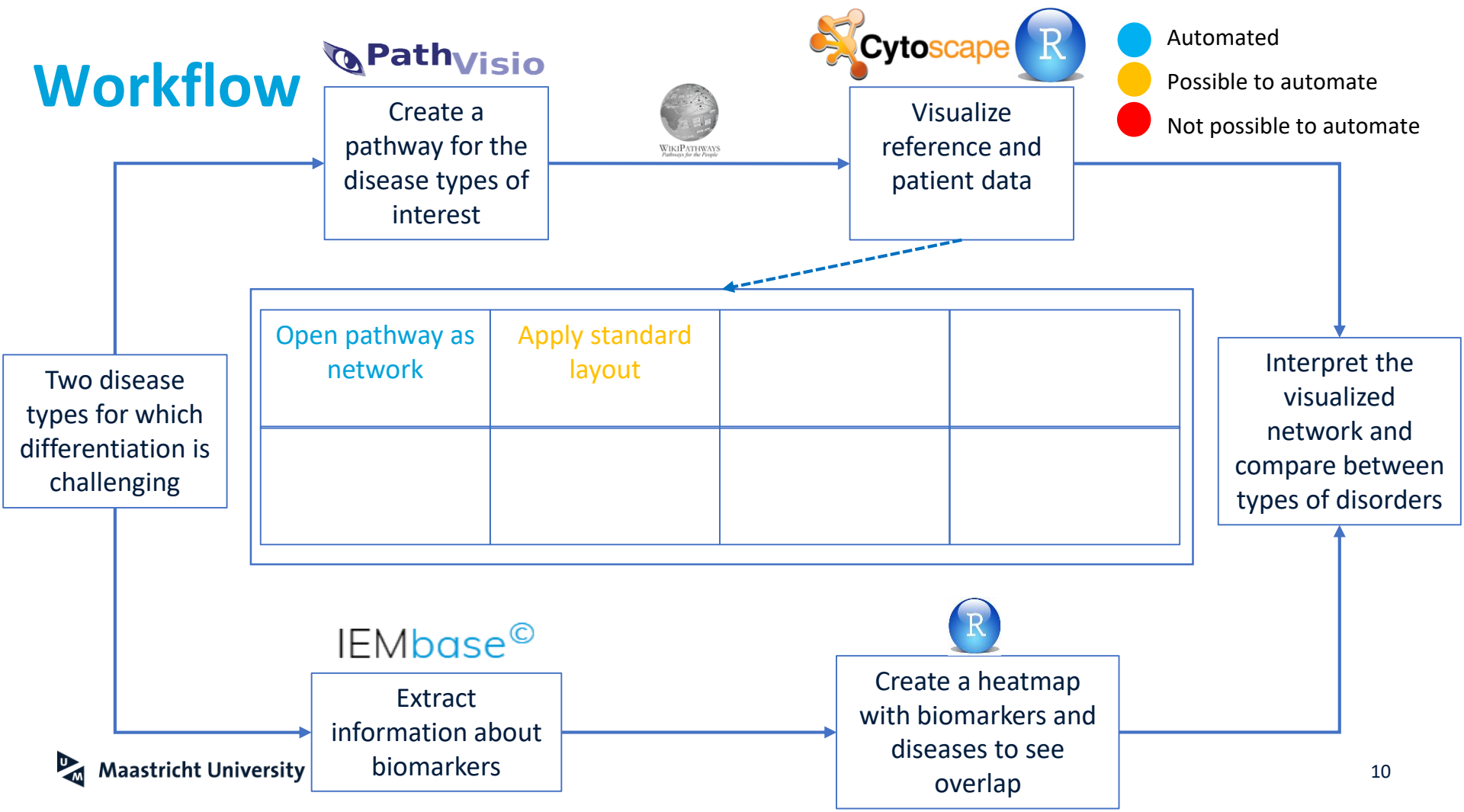
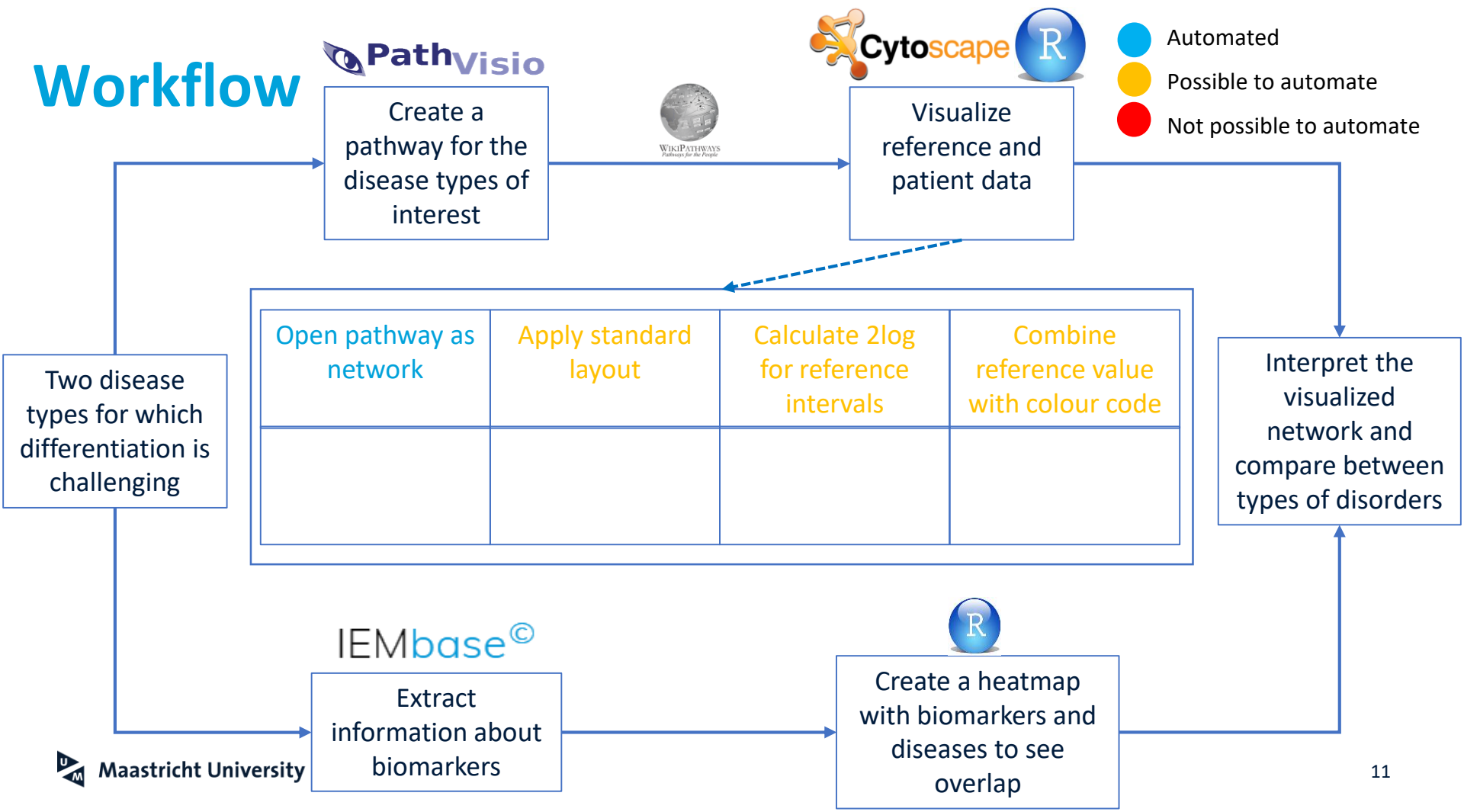

PathVisio
Cytoscape
R
Automated
Possible to automate
Not possible to automate
Create a pathway for the disease types of interest
WikiPathways
Pathways for the People
Visualize reference and patient data
Open pathway as network
Apply standard layout
Two disease types for which differentiation is challenging
Interpret the visualized network and compare between types of disorders
IEMbase©
R
Extract information about biomarkers
Create a heatmap with biomarkers and diseases to see overlap
Maastricht University

Workflow



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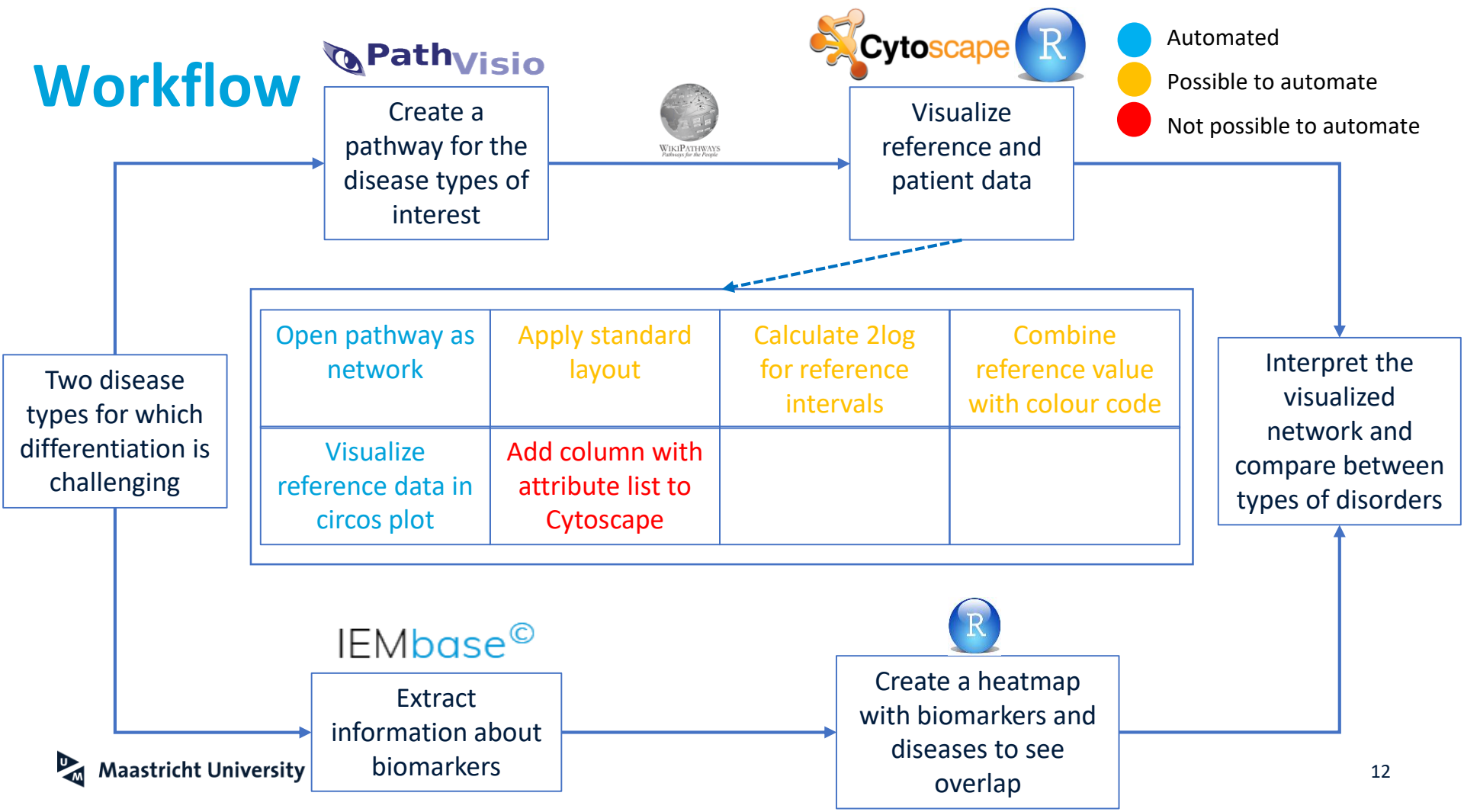
Workflow


PathVisio
Cytoscape
R
Automated
Possible to automate
Not possible to automate
Create a pathway for the disease types of interest
WikiPathways
Visualize reference and patient data
Open pathway as network
Apply standard layout
Calculate 2log for reference intervals
Combine reference value with colour code
Visualize reference data in circos plot
Add column with attribute list to Cytoscape
Two disease types for which differentiation is challenging
Interpret the visualized network and compare between types of disorders
IEMbase©
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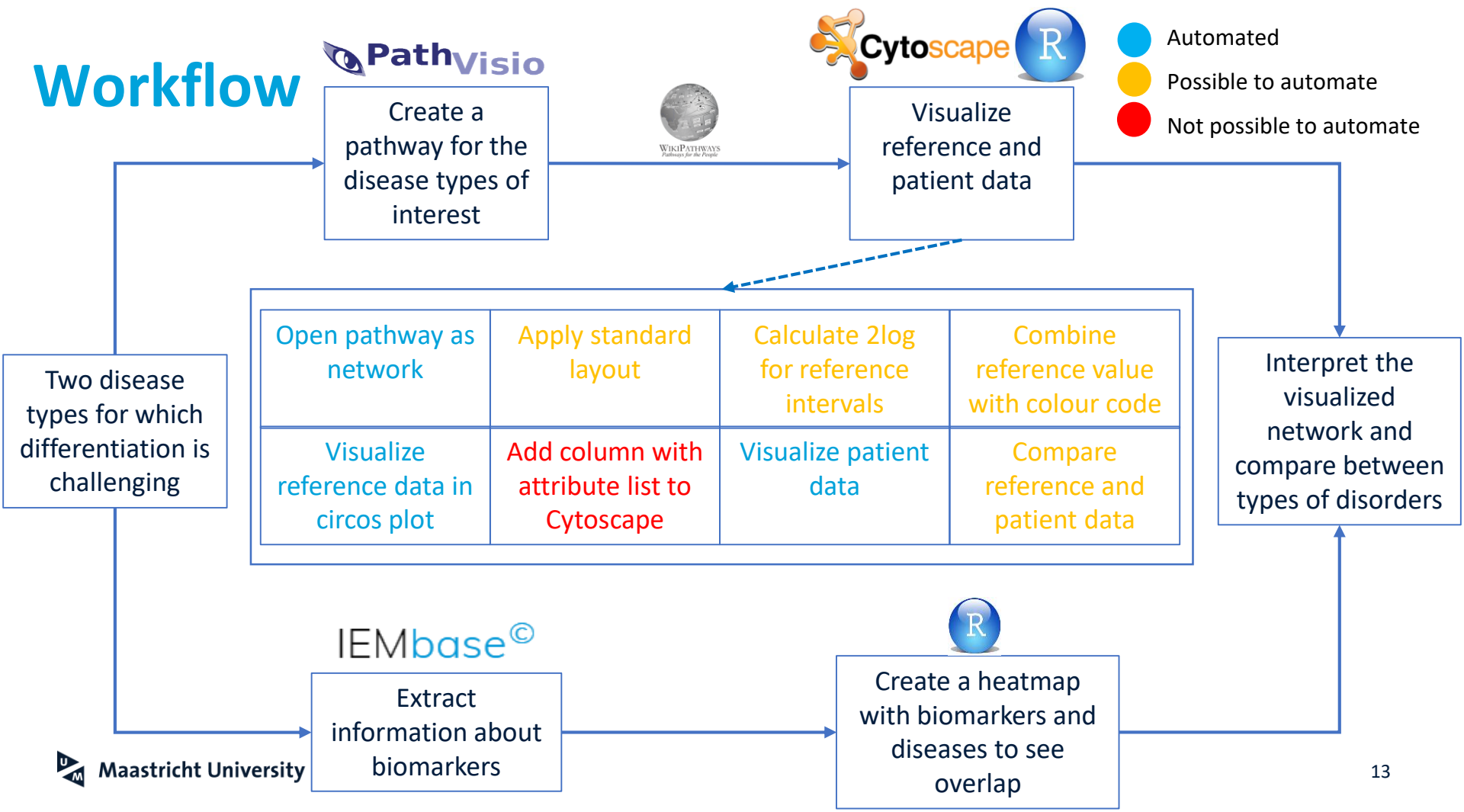

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Pathways

1 Kutmon M, et al. (2015) Plos computational biology
2 Blau N, et al. (2014) Physician's Guide to the Diagnosis, Treatment, and Follow-Up of Inherited Metabolic Diseases, Ch 4
3 UniProt Consortium (2019) Nucleic Acids Research
4 Morgat A, et al. (2017) Nucleic Acids Research
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6 OMIM (2019-04-12) https://omim.org/

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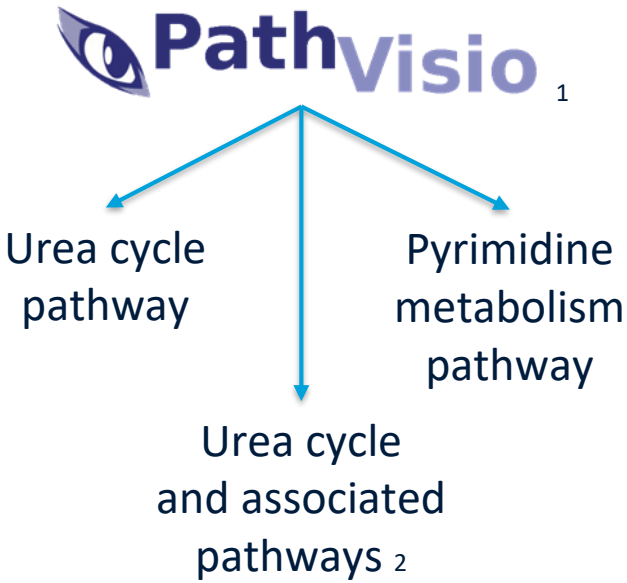

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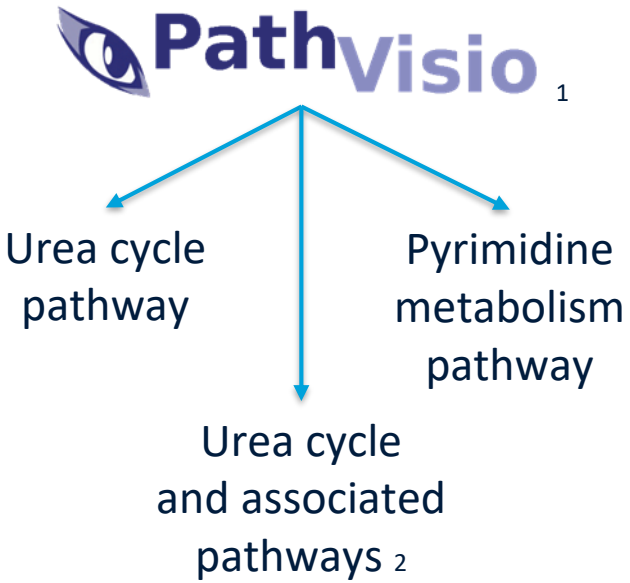

- Proteins annotated with Uniprot IDs 3

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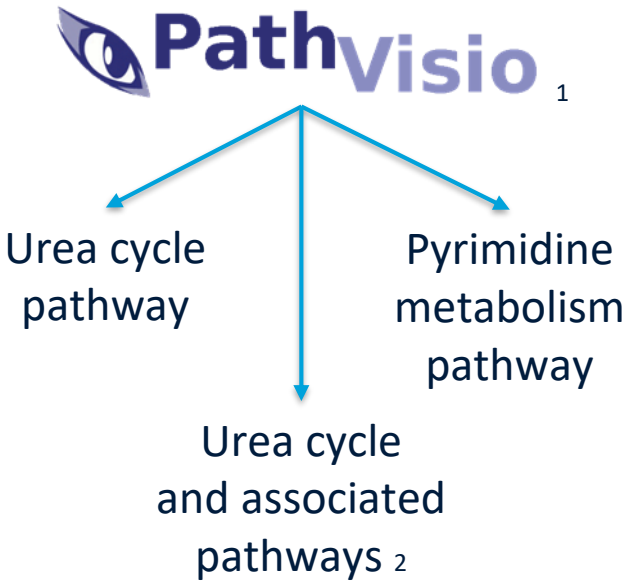

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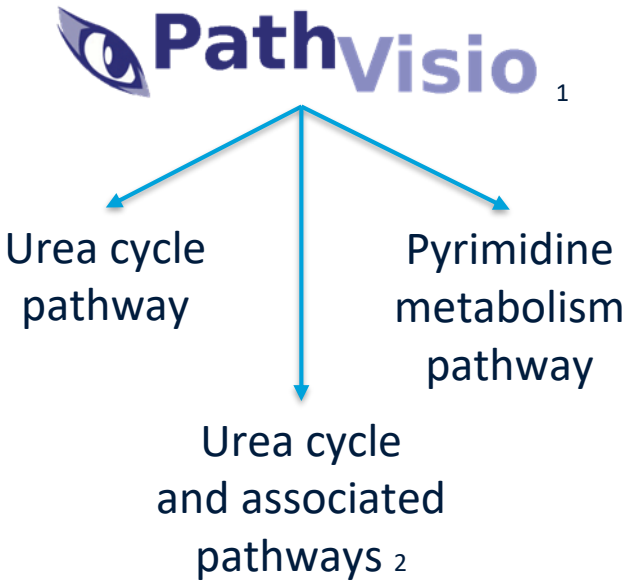

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  - With charge needed for reaction

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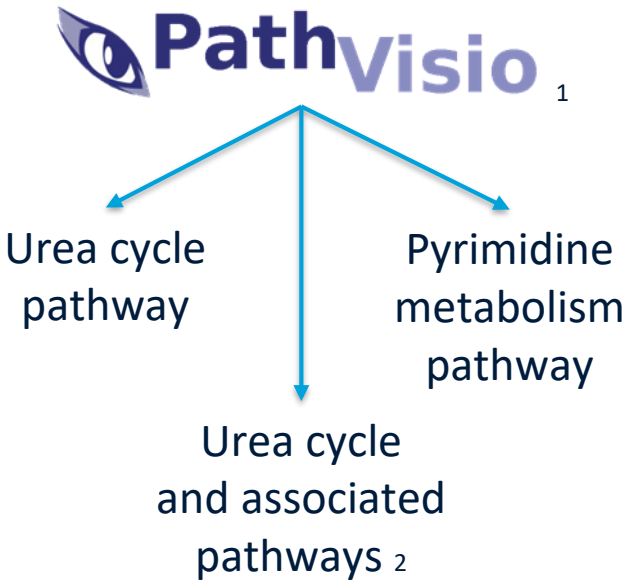

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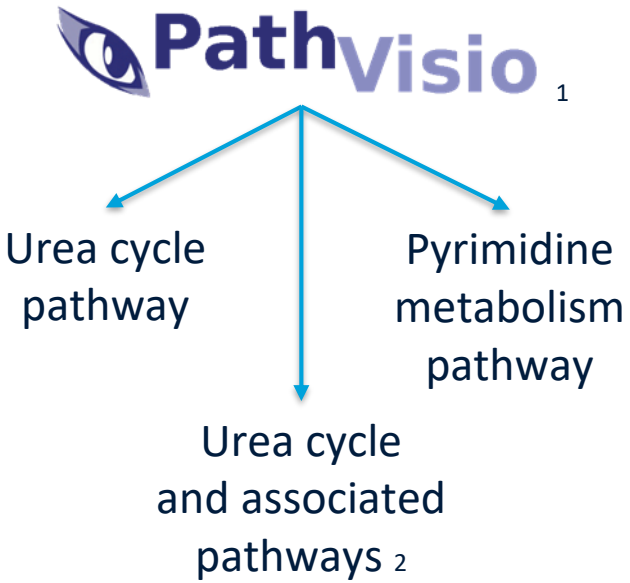

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- Pathway nodes for links between pathways

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Pathways

PathVisio [1]

Urea cycle pathway

Pyrimidine metabolism pathway

Urea cycle and associated pathways

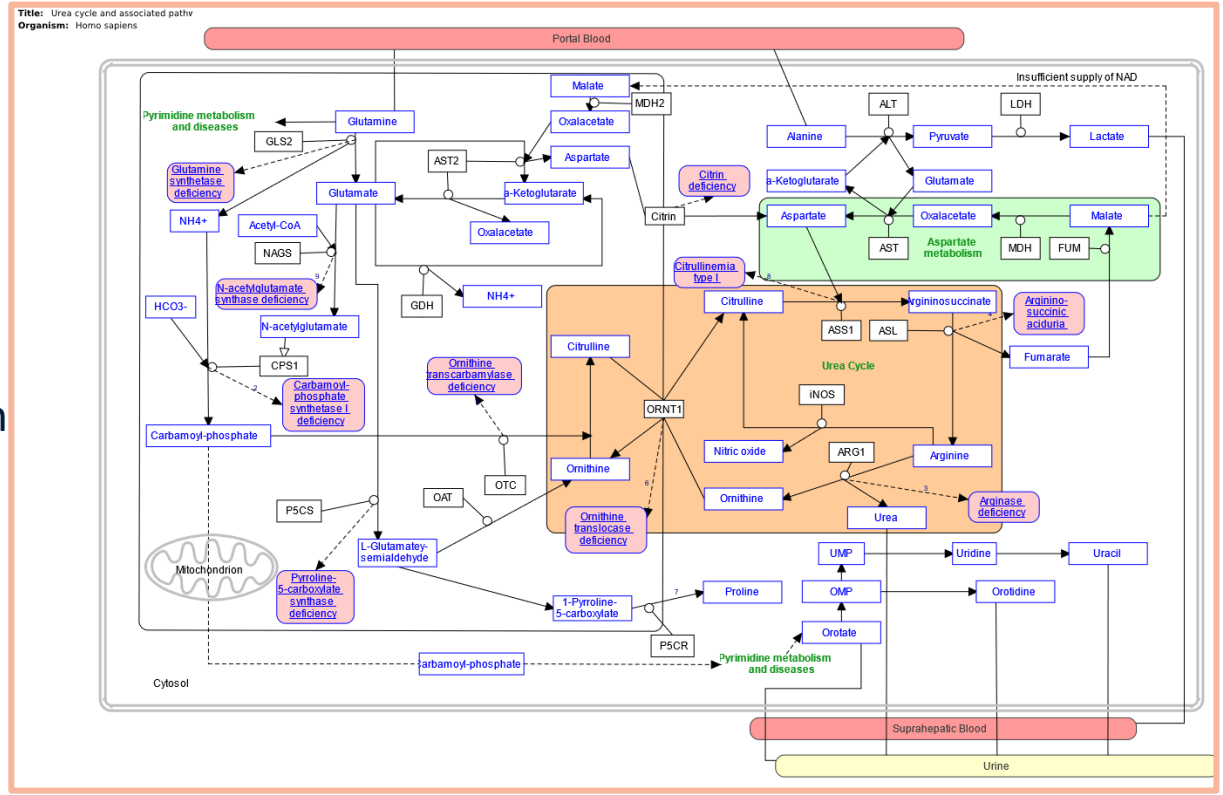

Image: WikiPathways:WP4595_104830
Pathway based on: Blau N, et al. (2014) Physician's Guide to the Diagnosis, Treatment, and Follow-Up of Inherited Metabolic Diseases, Ch 4
1 Kutmon M, et al. (2015) Plos computational biology

Biomarkers

Image: WikiPathways:WP4583_104797

1 Lee JJY, et al. (2018) Genetics in medicine; last accessed: 2019-04-16
2 Kutmon M, et al. (2015) Plos computational biology

Biomarkers

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Biomarker information [1]

Image: WikiPathways:WP4583_104797

1 Lee JJY, et al. (2018) Genetics in medicine; last accessed: 2019-04-16

2 Kutmon M, et al. (2015) Plos computational biology

Biomarkers

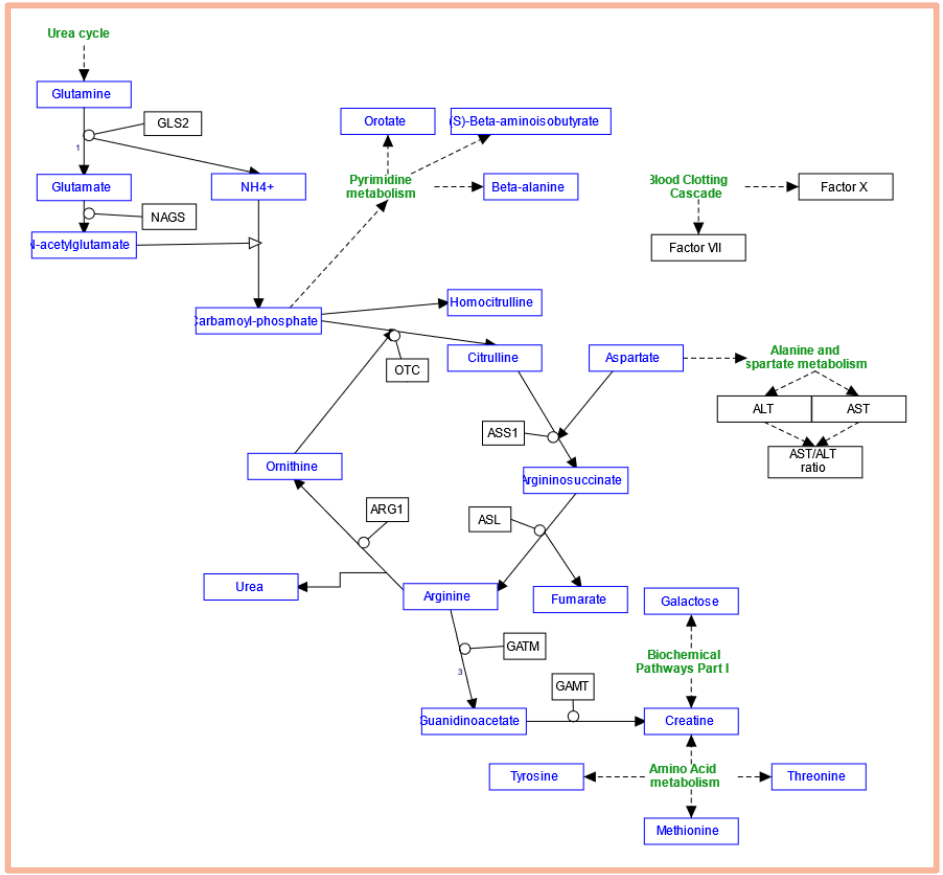
IEMbase©

Biomarker information [1]

PathVisio [2]

Urea cycle disorders biomarker pathway

Pyrimidine disorders biomarker pathway



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Biomarkers

IEMbase©

Biomarker information [1]

PathVisio [2]

Urea cycle disorders biomarker pathway

Pyrimidine disorders biomarker pathway

Challenges:
Mapping between HMDB and ChEBI ID

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Biomarkers

IEMbase©

Biomarker information [1]

[2]

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Challenges:
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Problems with charge and zwitterions

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Challenges:
Mapping between HMDB and ChEBI ID

↓

Problems with charge and zwitterions

↓

File with IDs in pathways and IDs uncharged biomarkers

1 Lee JJY, et al. (2018) Genetics in medicine; last accessed: 2019-04-16
2 Kutmon M, et al. (2015) Plos computational biology

Heatmap

1 RStudio Team (2015) http://www.rstudio.com/

Heatmap

[1]

Create heatmap with RStudio 1.1.442 (R-version 3.6.0)

1 RStudio Team (2015) http://www.rstudio.com/

Heatmap

[1]

Create heatmap with RStudio 1.1.442 (R-version 3.6.0)

↓

Determination if biomarker was present for each disease

1 RStudio Team (2015) http://www.rstudio.com/

Heatmap

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Create heatmap with RStudio 1.1.442 (R-version 3.6.0)

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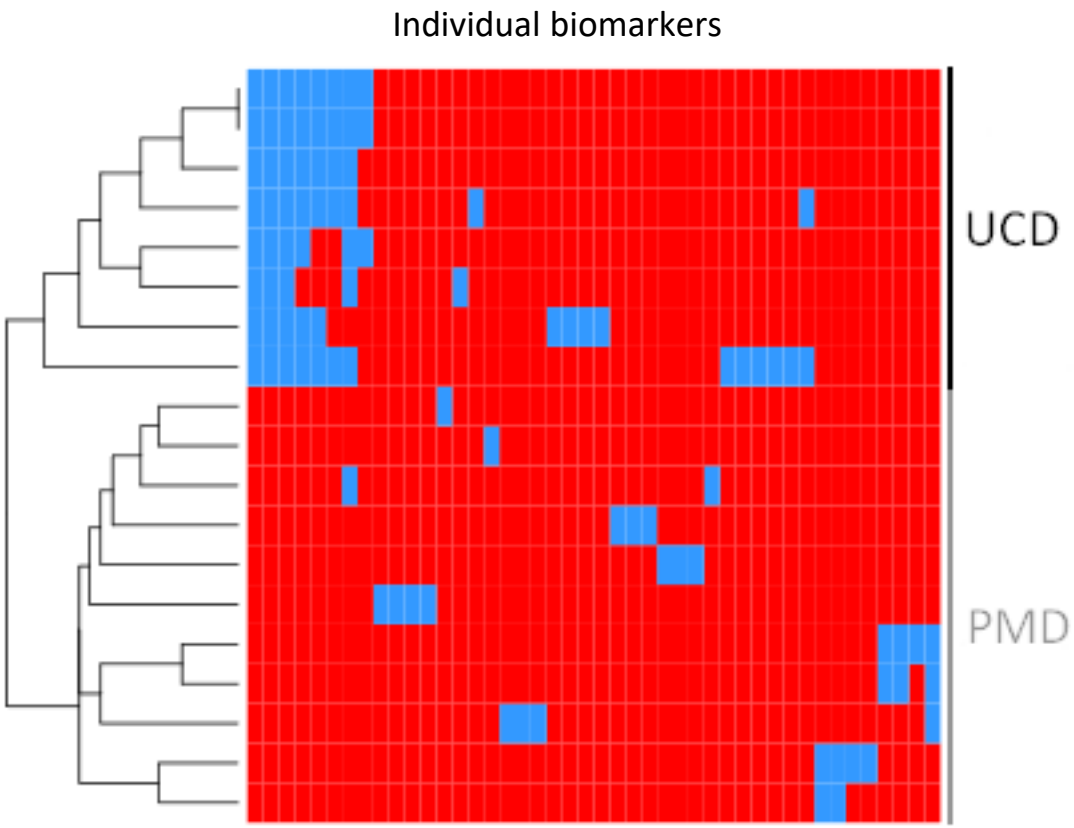
Determination if biomarker was present for each disease



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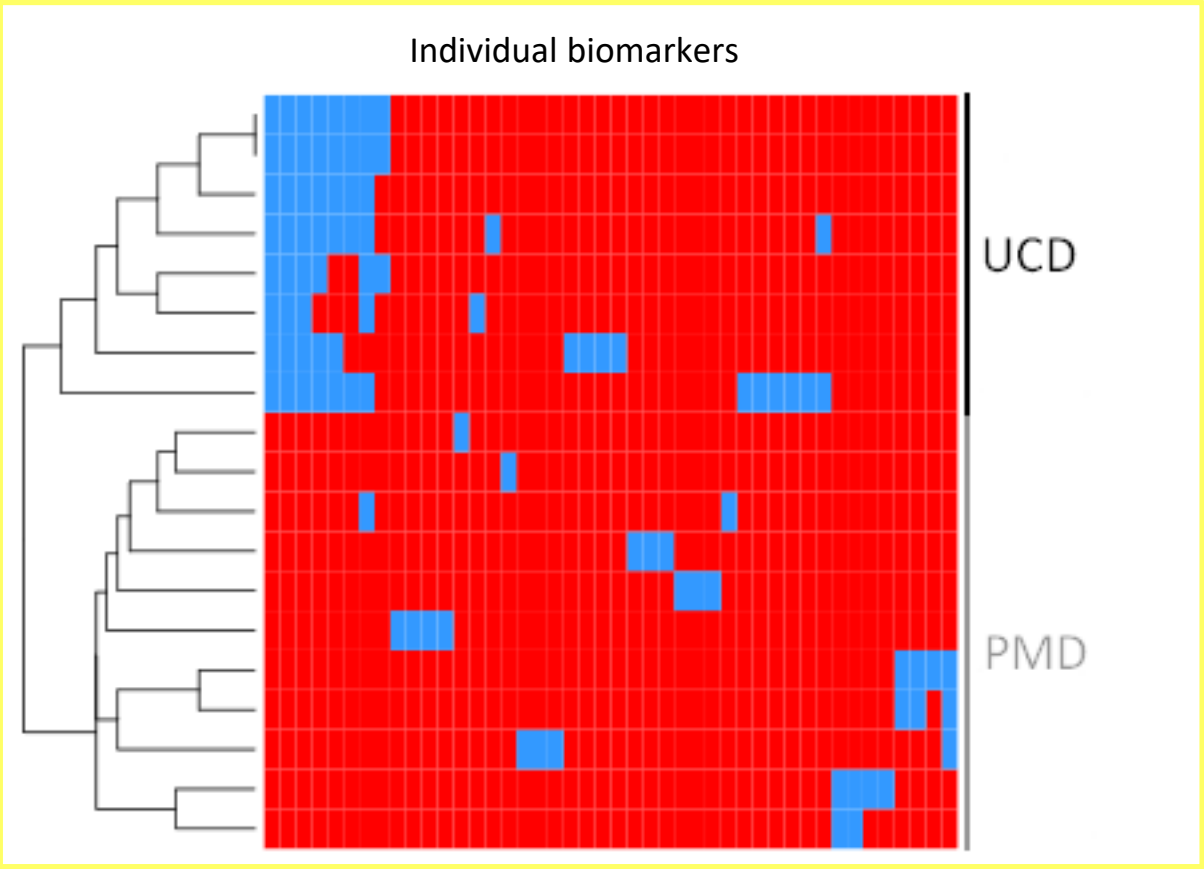
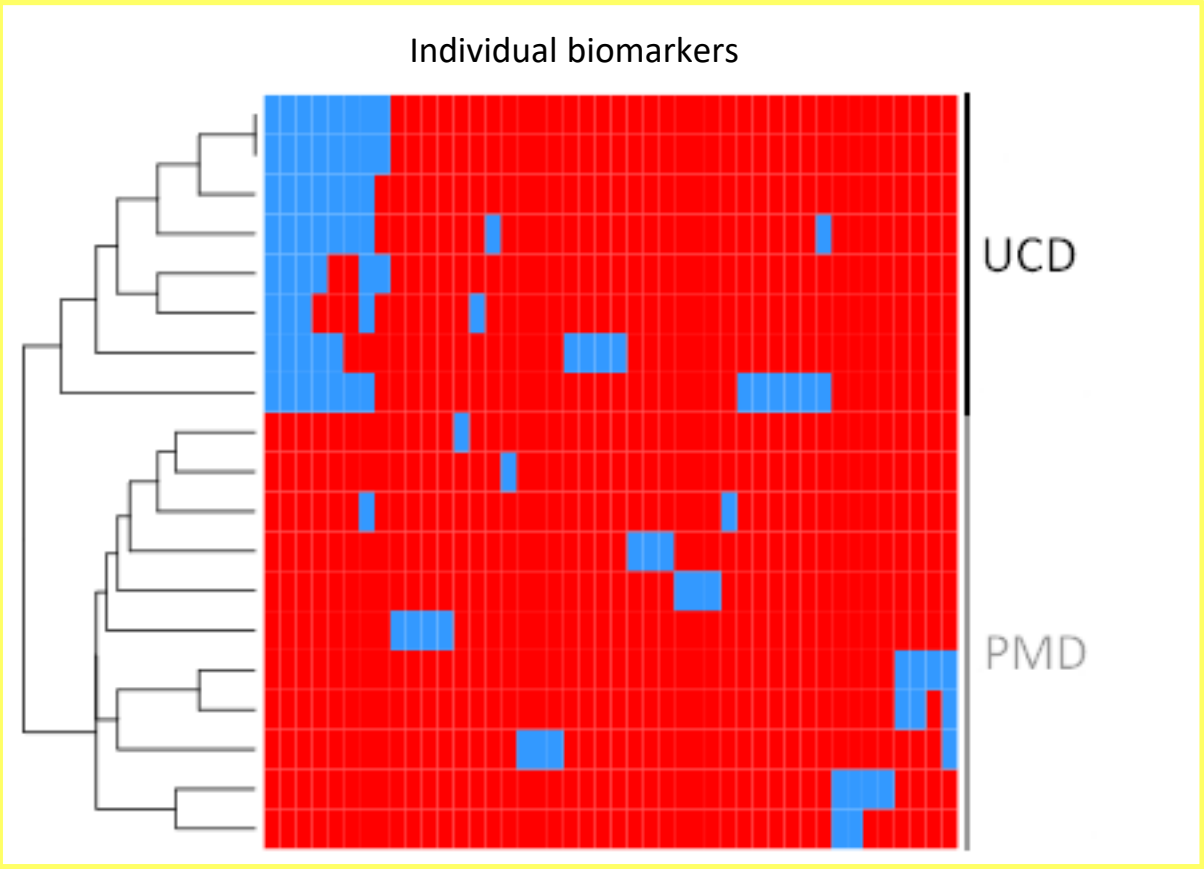
Heatmap



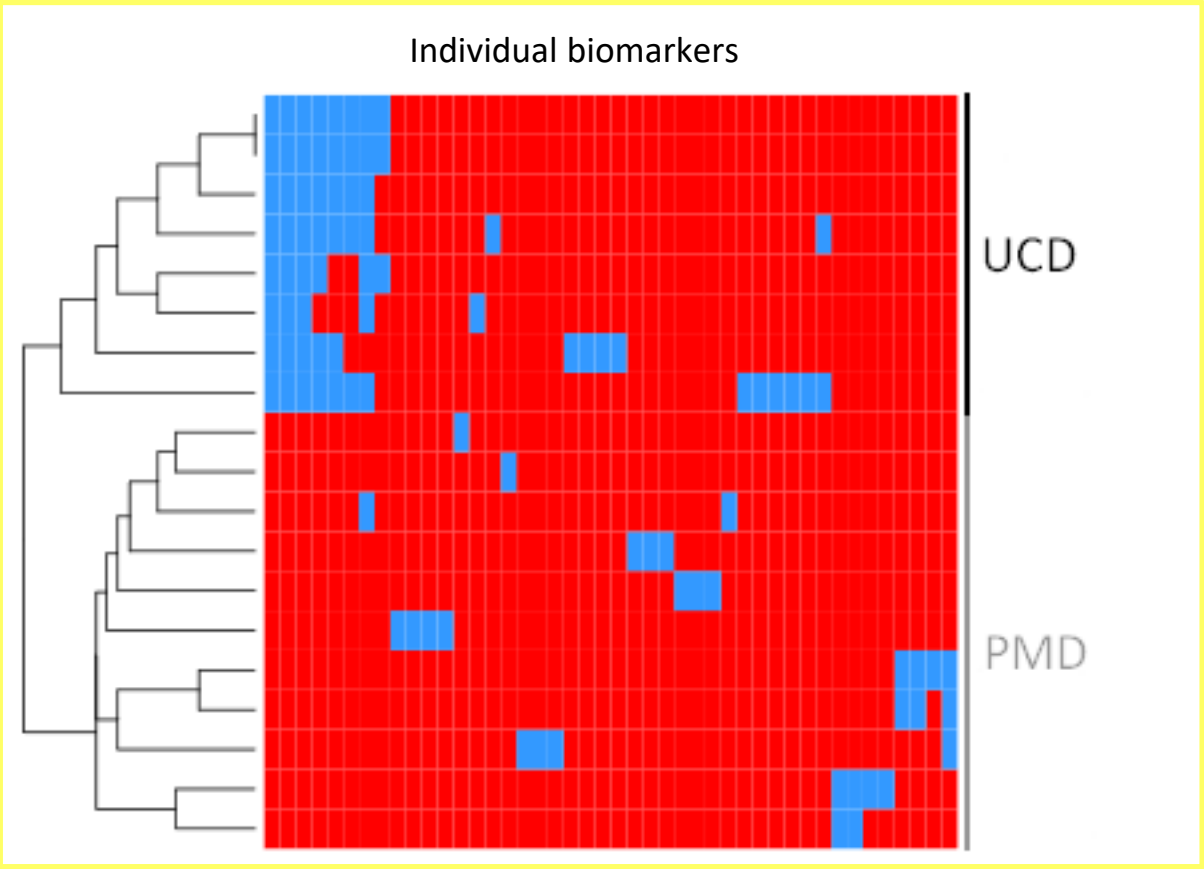
Heatmap

- Disease types form clusters



Heatmap

- Disease types form clusters
- UCD
  - 3 amino acids for all disorders
  - Several others overlap



Heatmap

- Disease types form clusters
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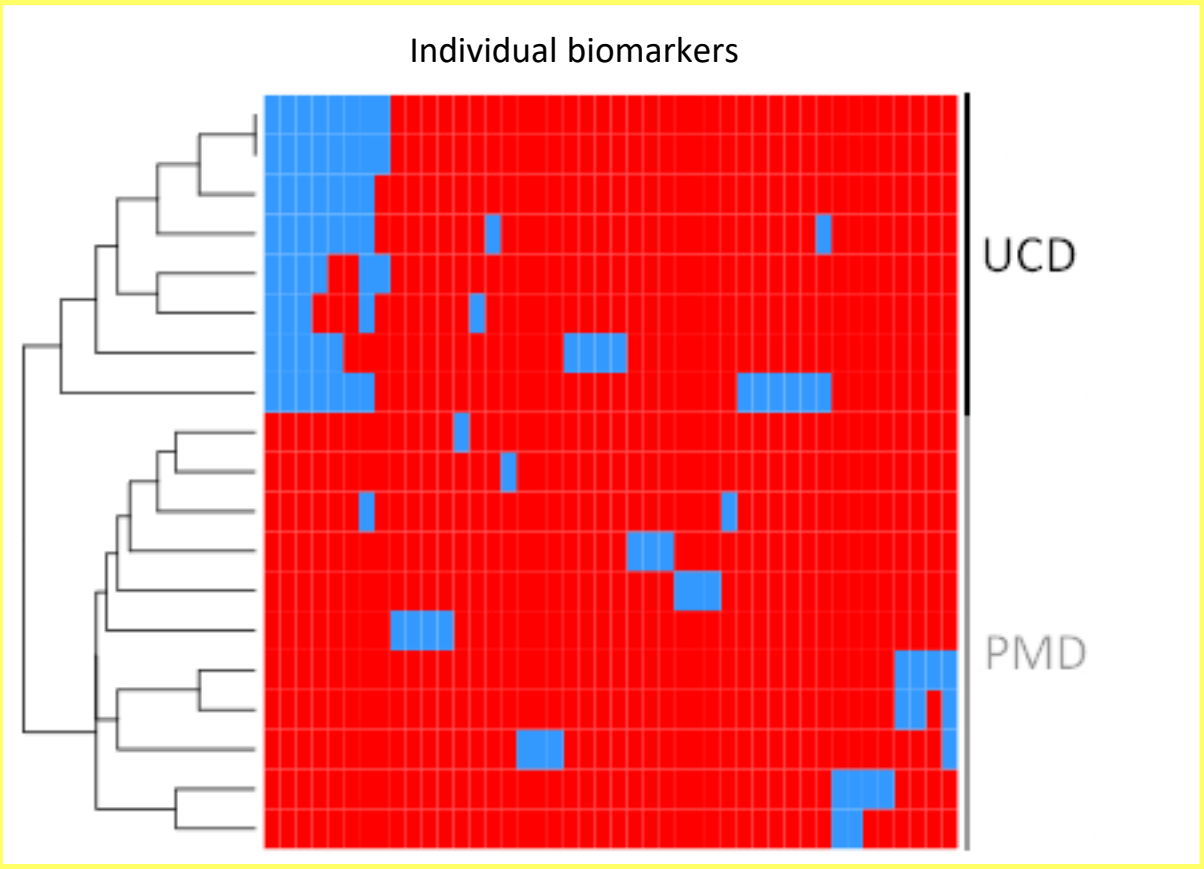
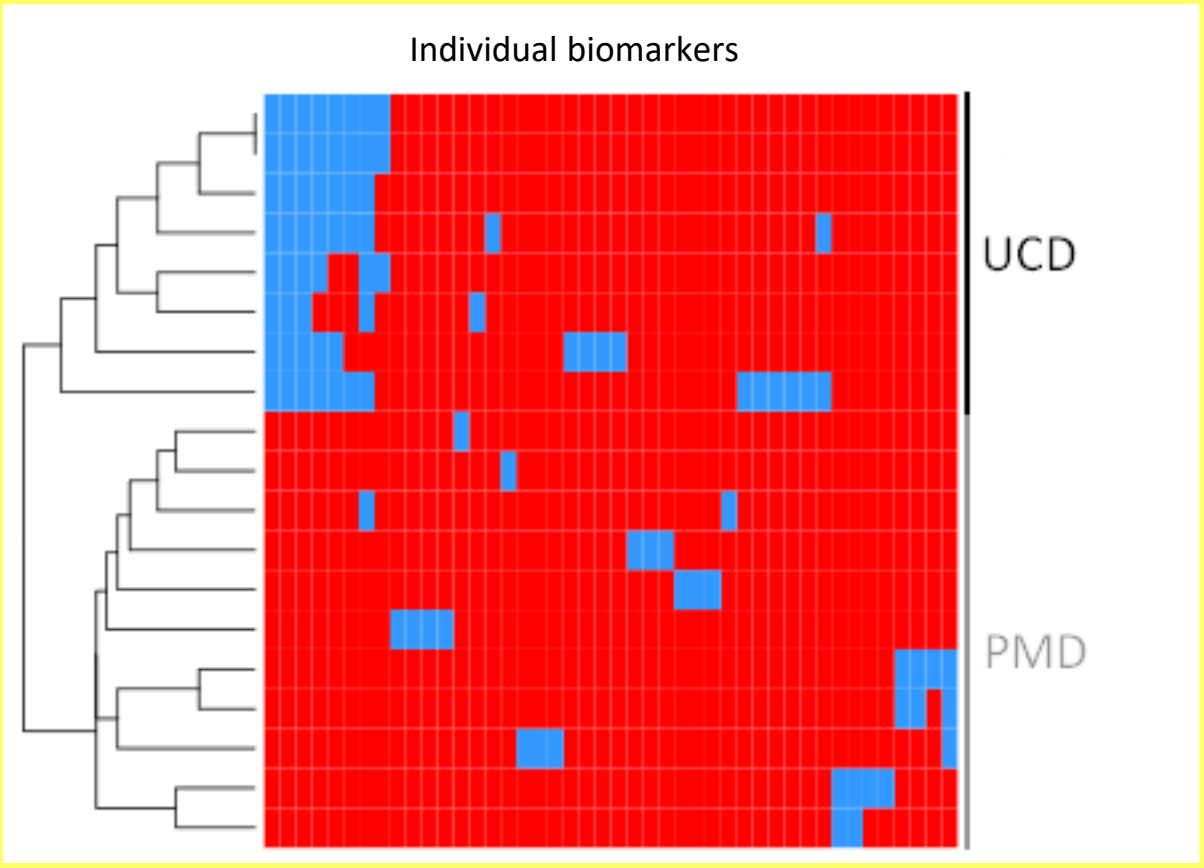
- PMD
  - Hardly any overlap



Heatmap

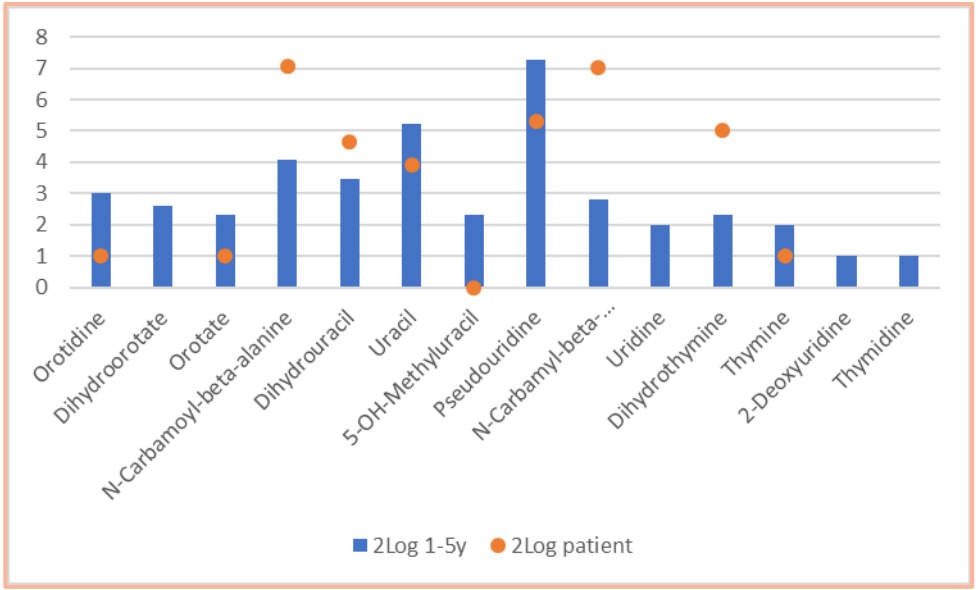
- Disease types form clusters
- UCD
  - 3 amino acids for all disorders
  - Several others overlap
- PMD
  - Hardly any overlap
- Based on these biomarkers differentiation seems possible
  - Not in practice



Possible visualization

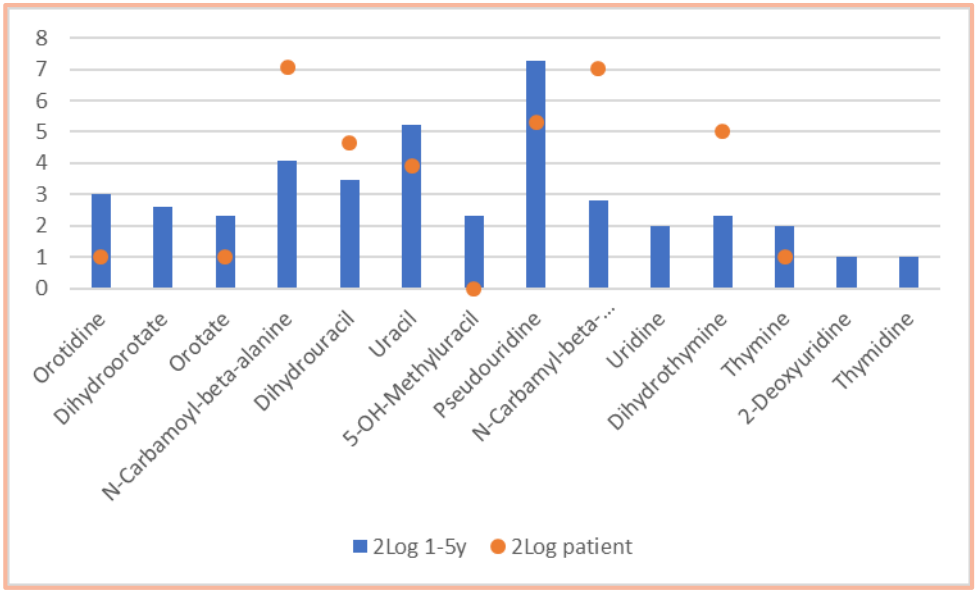
Possible visualization

- Log transformed reference and patient data



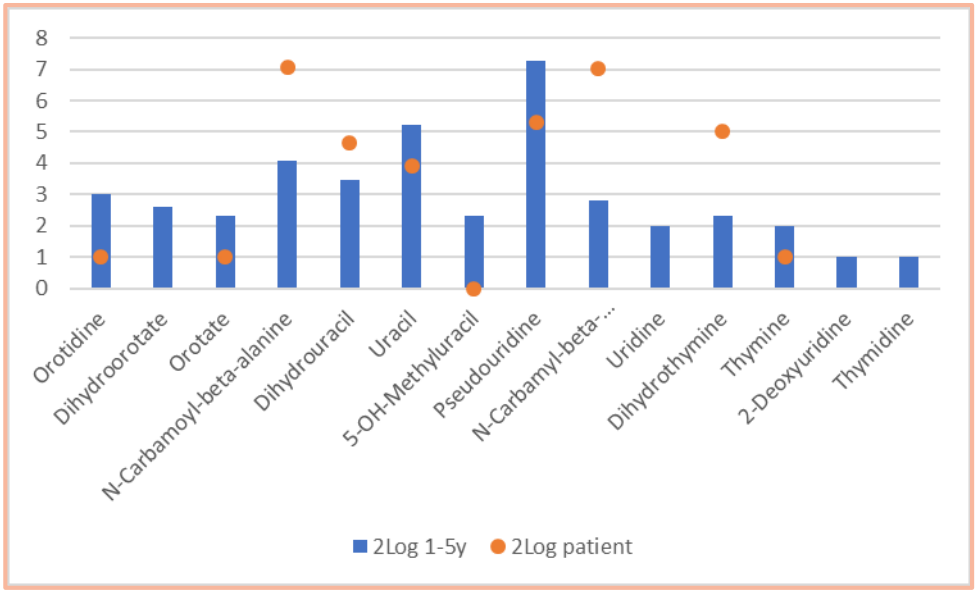
Possible visualization

- Log transformed reference and patient data
- 4 metabolites with increased concentration



Possible visualization

- Log transformed reference and patient data
- 4 metabolites with increased concentration
  - How are they connected?
  - And which protein is involved?



Open network

- Automated
- Possible to automate
- Not possible to automate

1 RStudio Team (2015) http://www.rstudio.com/
2 Shannon P, et al. (2003) Genome research

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R markdown script made with Rstudio [1]

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R markdown script made with Rstudio [1]

→

Open pathway as network in Cytoscape 3.7.1 WP4583/WP4584

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- Not possible to automate

R markdown script made with Rstudio [1]

→

Cytoscape

Open pathway as network in Cytoscape 3.7.1 WP4583/WP4584

→

Apply default style

→

- Grey node colour
- Small anchors
- Hexagons for non-metabolites
- Directionality for edges

1 RStudio Team (2015) http://www.rstudio.com/
2 Shannon P, et al. (2003) Genome research

Reference data

- Automated
- Possible to automate
- Not possible to automate

Reference data

- Automated
- Possible to automate
- Not possible to automate

File with reference data lower and upper limit

File with names in pathway and IDs

Reference data

- Automated
- Possible to automate
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File with reference data lower and upper limit

File with names in pathway and IDs

One file with reference data

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One file with reference data

Manual 2log transformation

Reference data

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- Not possible to automate

File with reference data lower and upper limit

File with names in pathway and IDs

One file with reference data

Manual 2log transformation

Determine range of log values

Reference data

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File with reference data lower and upper limit

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Manual 2log transformation

Determine range of log values

Colour gradient from white to red in 100 steps with hex colour codes

Reference data

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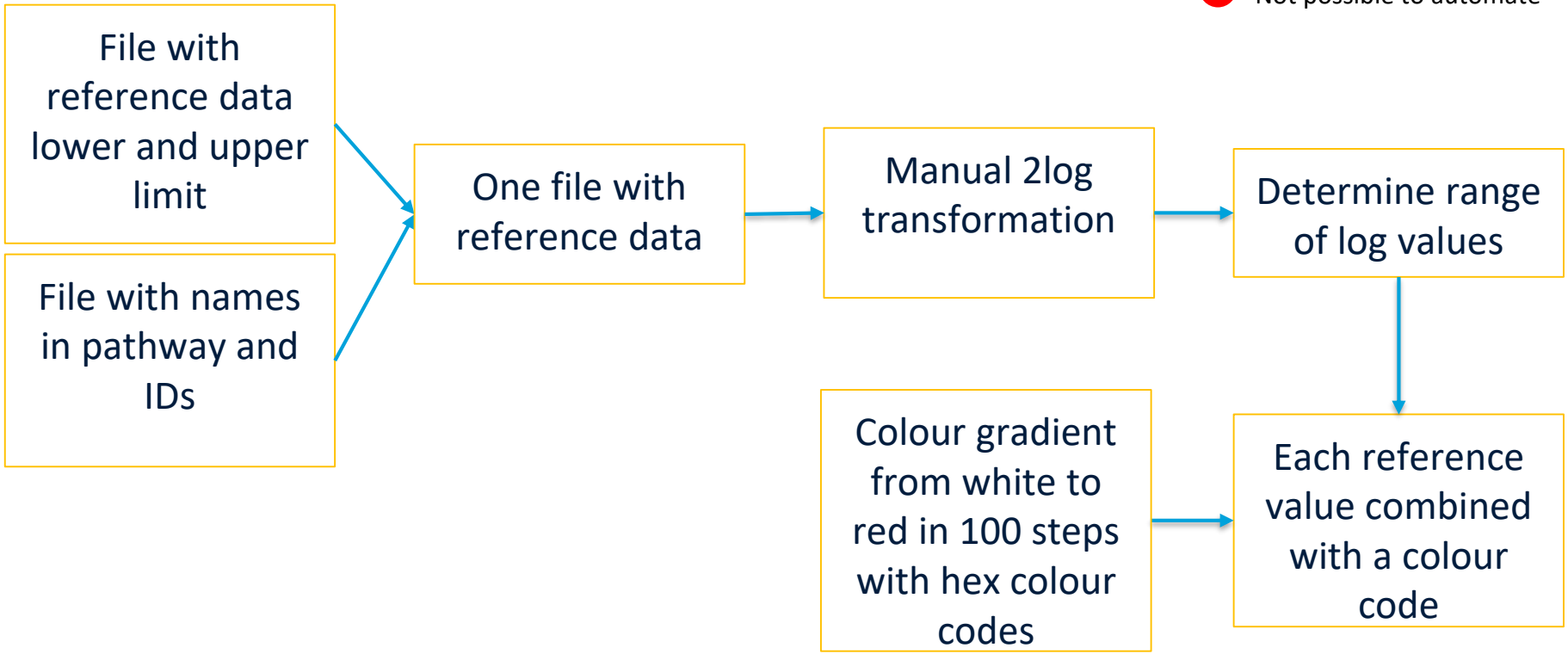

Reference data

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File with reference data lower and upper limit

File with names in pathway and IDs

One file with reference data

Manual 2log transformation

Determine range of log values

Colour gradient from white to red in 100 steps with hex colour codes

Each reference value combined with a colour code

Name	CHEBI	0m-1m L	Log 0m-1m L	Colour code
Threonine	CHEBI:16857	13.1	3.817623	#FFA7A7

Visualize reference data

- Automated
- Possible to automate
- Not possible to automate

Visualize reference data

- Automated
- Possible to automate
- Not possible to automate

Loading circo string into Cytoscape table column

Visualize reference data

- Automated
- Possible to automate
- Not possible to automate

Loading circo string into Cytoscape table column

Start angle 90 degrees

Colour codes are added for the reference values

Value list for distribution in the circo

Attribute list
-
Dependent on manually loaded list column

Visualize reference data

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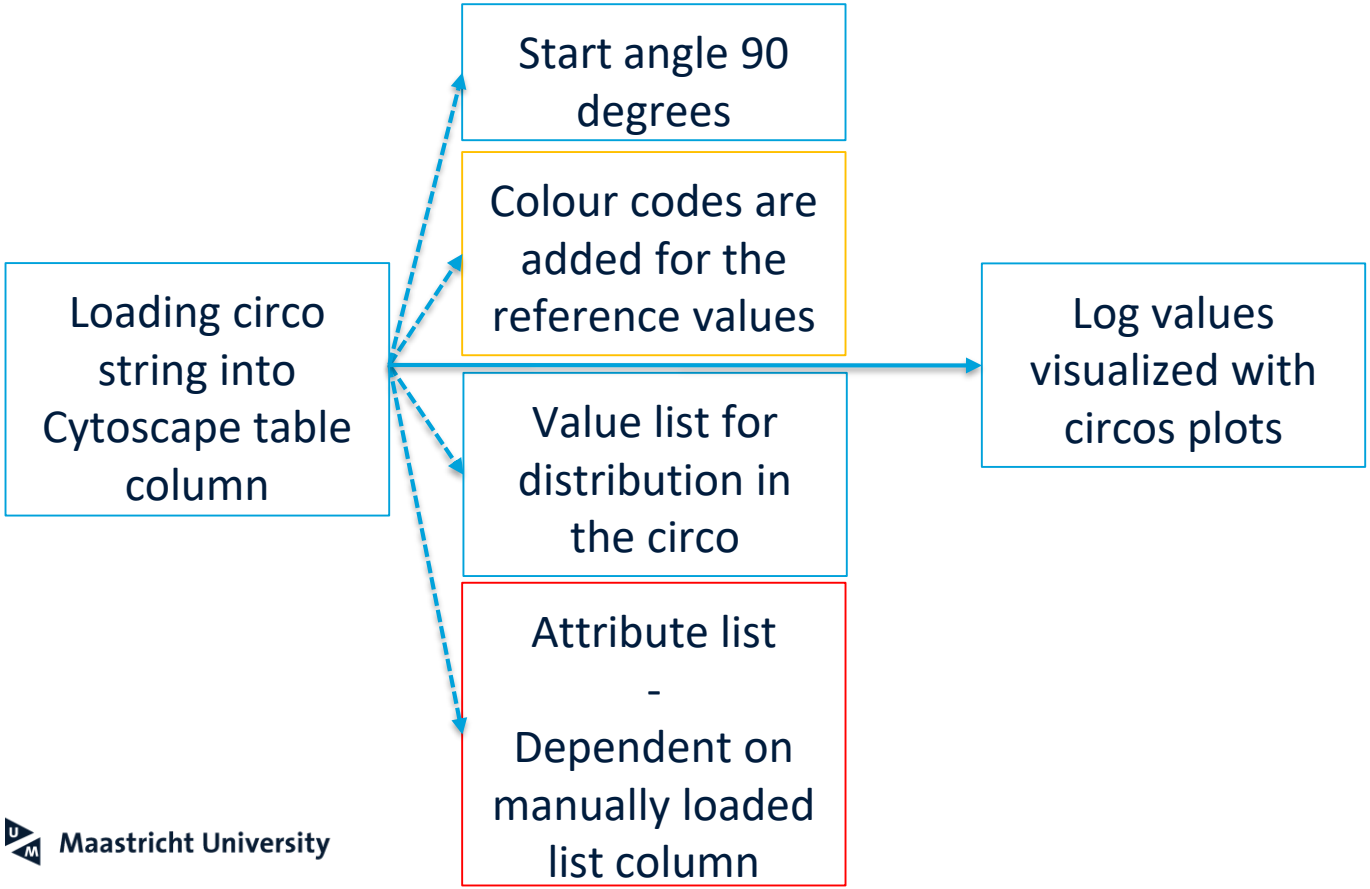
- Possible to automate
- Not possible to automate



Visualize reference data

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Loading circo string into Cytoscape table column

Start angle 90 degrees

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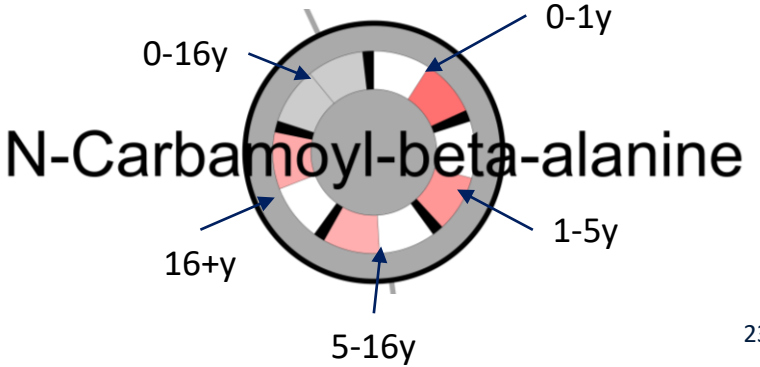
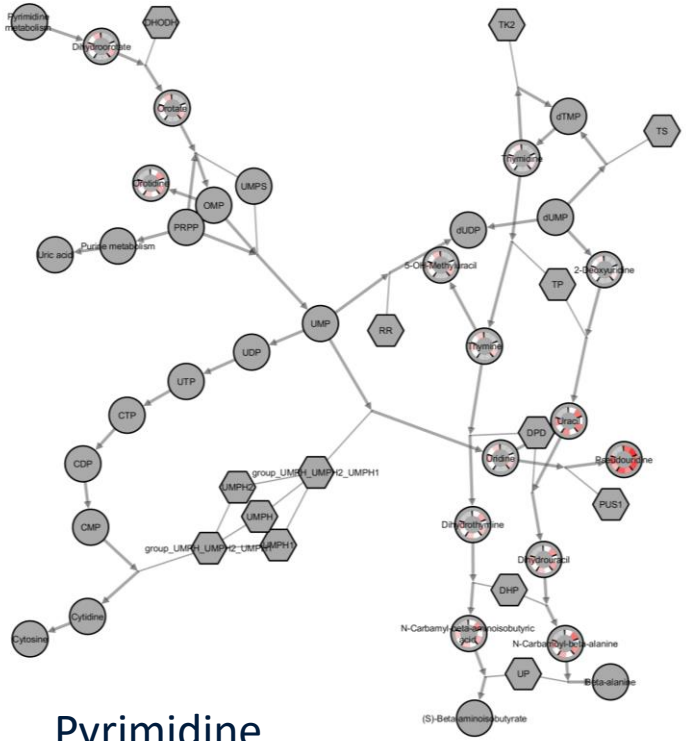
Attribute list
-
Dependent on manually loaded list column

Log values visualized with circos plots



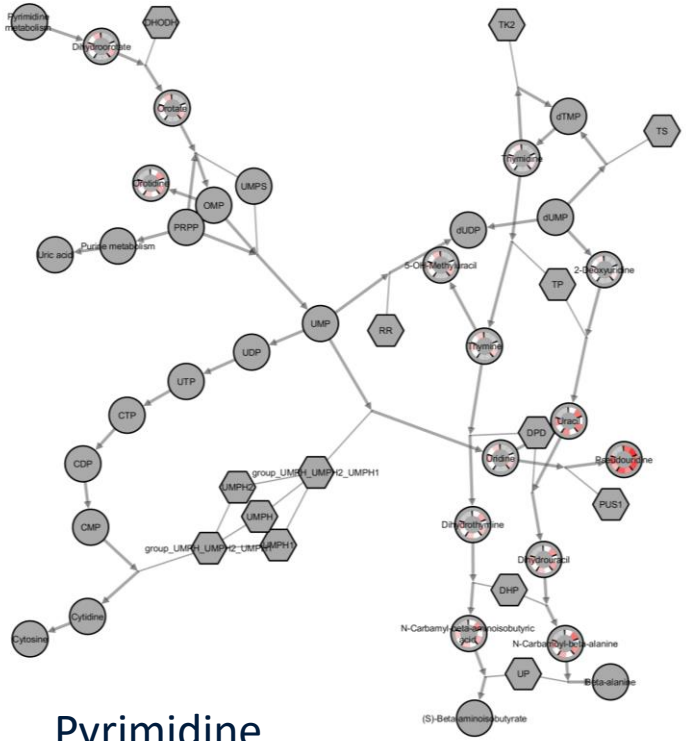
Visualize reference data

Visualize reference data

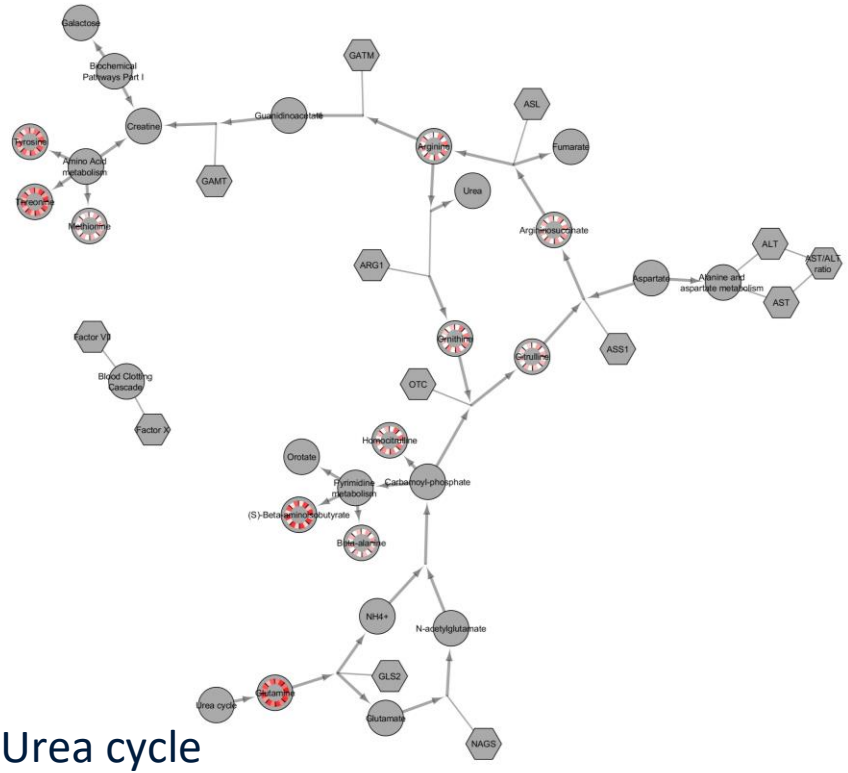


Pyrimidine

Visualize reference data

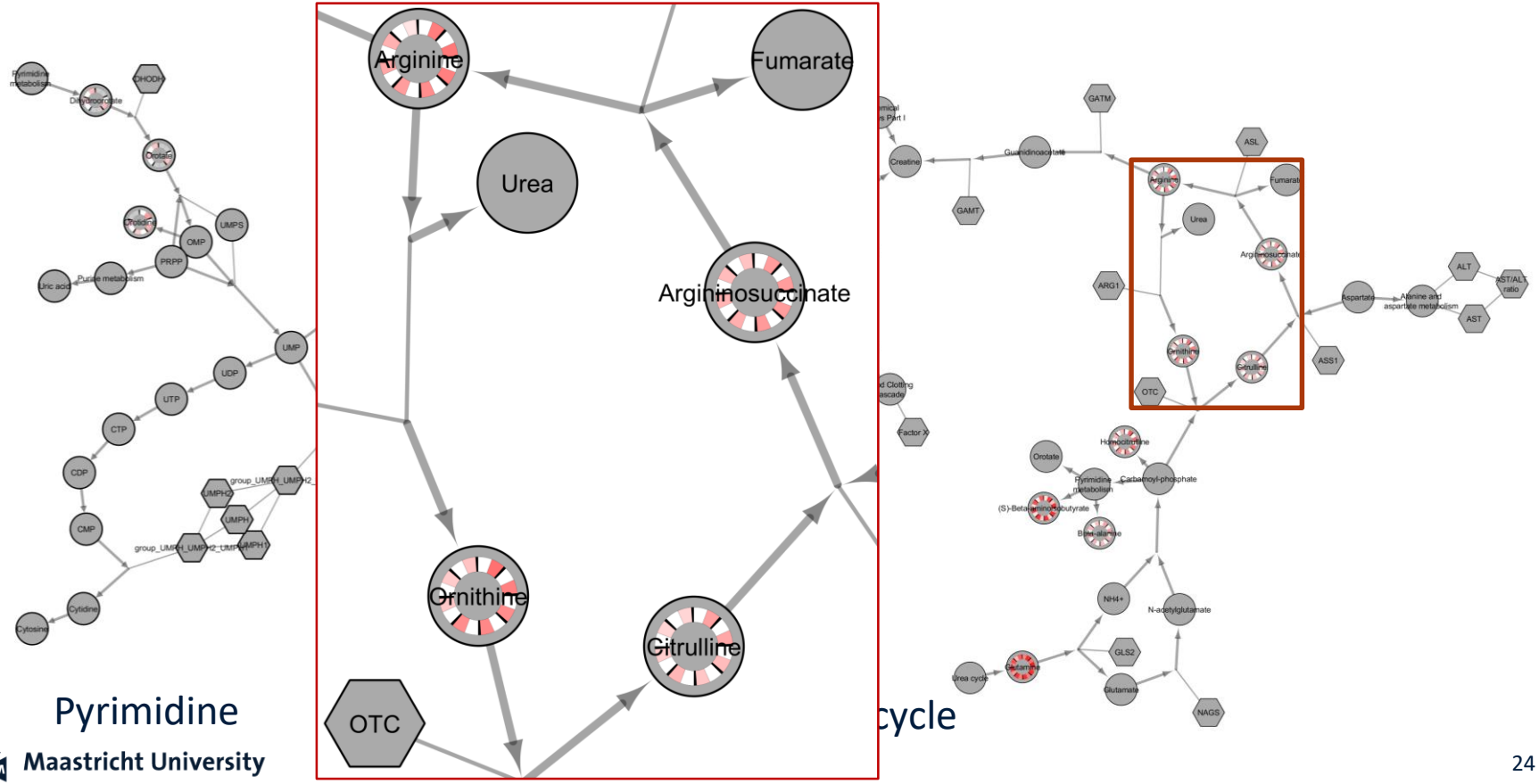


Pyrimidine

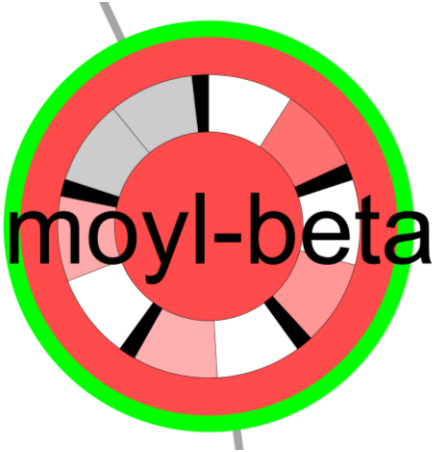


Urea cycle

Visualize reference data



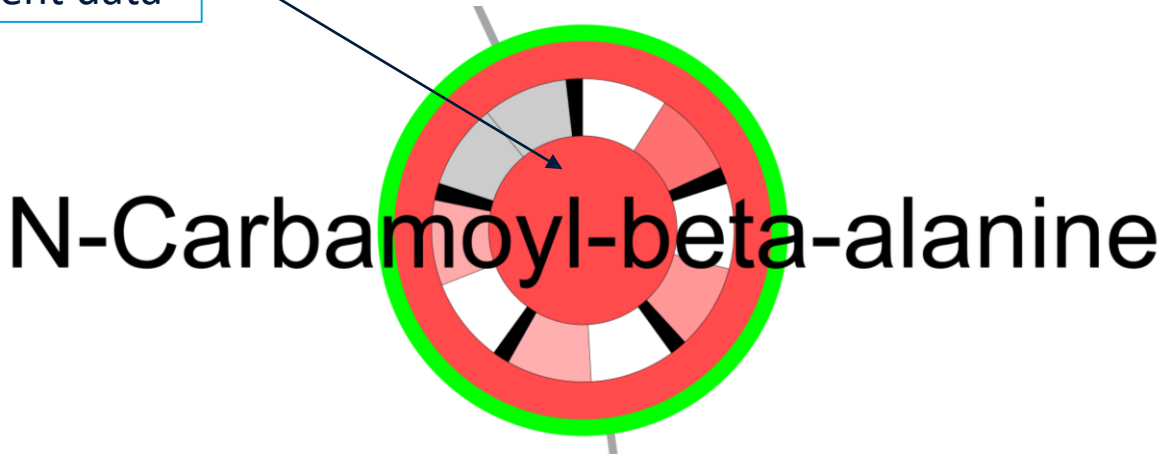
Visualize patient data



N-Carbamoyl-beta-alanine

Visualize patient data

Node fill colour for patient data



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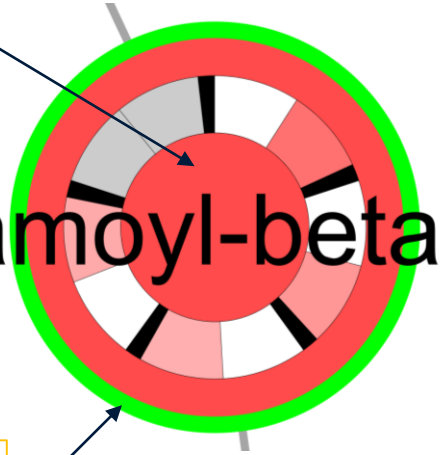

N-Carbamoyl-beta-alanine

Compare to reference data

Challenges automatization

Challenges automatization

- Parts of the workflow not automated

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  - Time restrictions

Challenges automatization

- Parts of the workflow not automated
  - Time restrictions
  - Other issues
    - Cannot add lists to Cytoscape table with Cy3
    - Age of patients not represented in same categories as reference data
    - Challenges with mapping of charged metabolites in pathways

Results

Results

- Data from 12 patients analysed
  - 5 Urea cycle disorder
  - 7 Pyrimidine disorder

Results

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  - 11 Pyrimidine metabolites data available

Results

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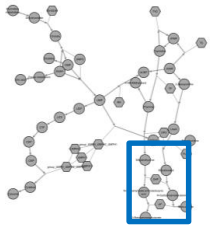
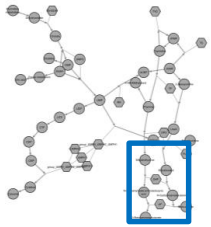
  - 7 Pyrimidine disorder
  - 6 patients Urea cycle data available
  - 11 Pyrimidine metabolites data available
- 3 groups of patients identified
  - Similar parts of network
  - Differences within one group

Results group 1



- Four metabolites connected in the Pyrimidine pathways altered for four patients

Results group 1



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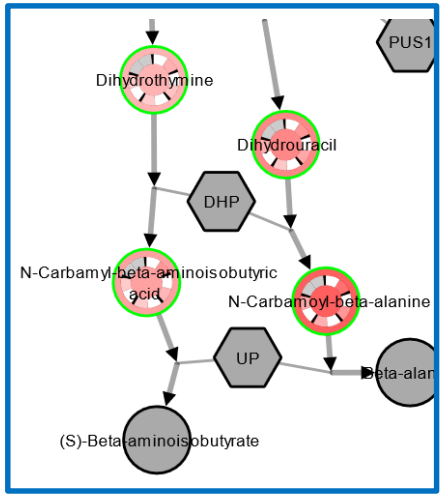
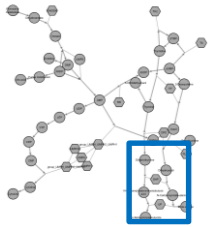


Citrullinemia type I

Results group 1



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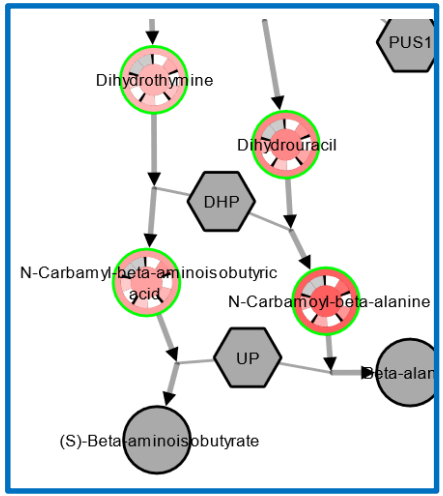

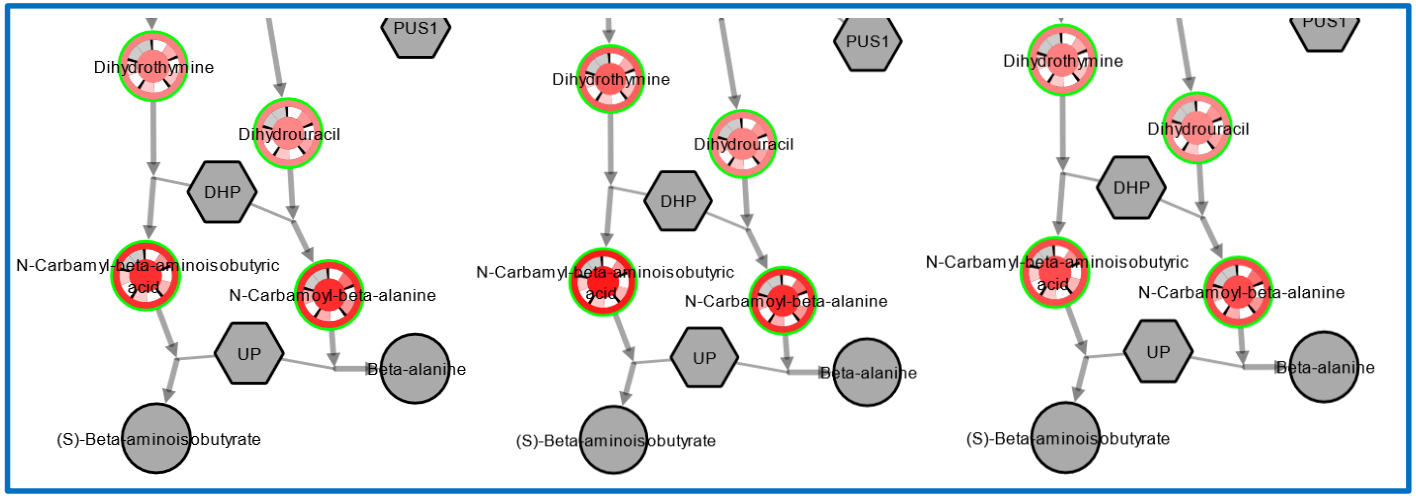

Citrullinemia type I

Beta-ureidopropionase deficiency

Discussion group 1

1 Kuilenburg AB van, et al. (2004) Hum Mol Genet
2 Kuilenburg AB van, et al (2012) BBA- MolecularBasis of Disease

Discussion group 1

- Beta-ureidopropionase deficiency only 4 metabolites upstream from defect altered
  - No other altered metabolites
  - In correspondence with literature [1]

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  - No other altered metabolites
  - In correspondence with literature [1]
- Two metabolites downstream from defect expected to be decreased [2]
  - Part of Urea cycle metabolites → not available
- These metabolites only altered for these and 1 other patient
  - Several other altered metabolites

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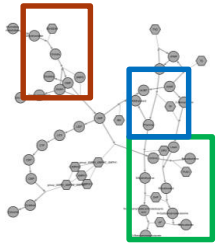
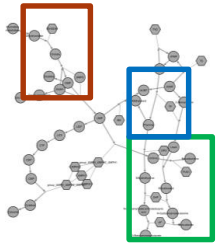
Results group 1



- Patient with Citrullinemia type I
  - 7 altered Pyrimidine metabolites
    - 6 in breakdown section of Pyrimidine metabolism

Results group 1



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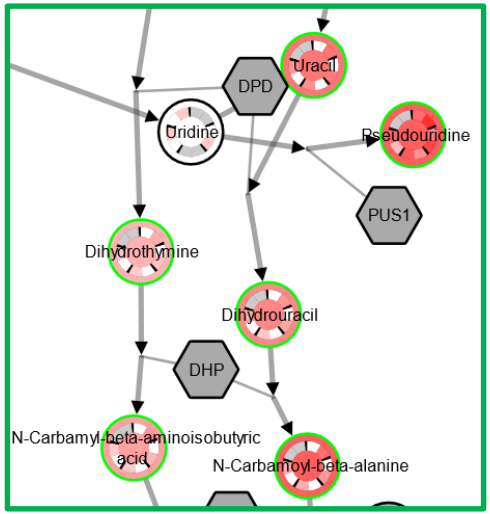
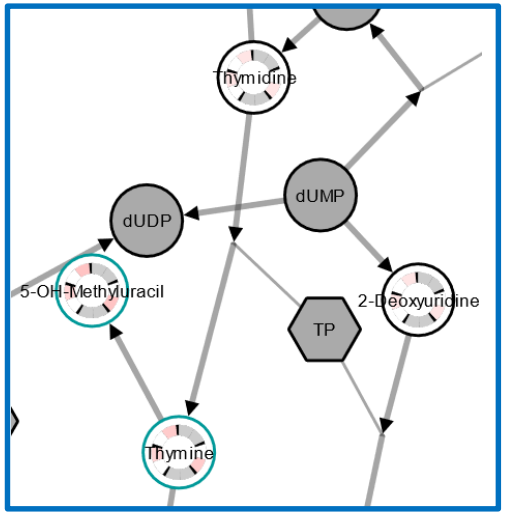
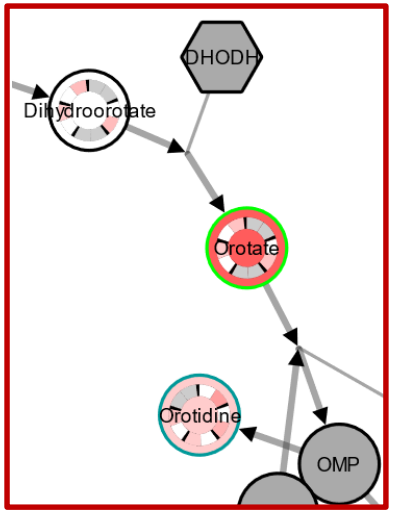
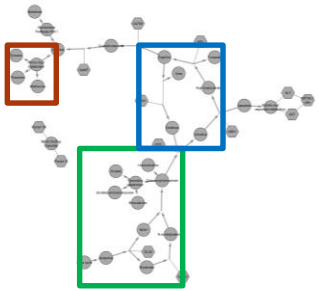




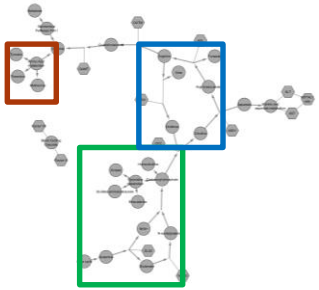


Results group 1



- Patient with Citrullinemia type I
  - 9 altered Urea cycle metabolites
    - 2 metabolites part of Pyrimidine breakdown metabolism
    - Citrulline more than 4 times the reference value

Results group 1



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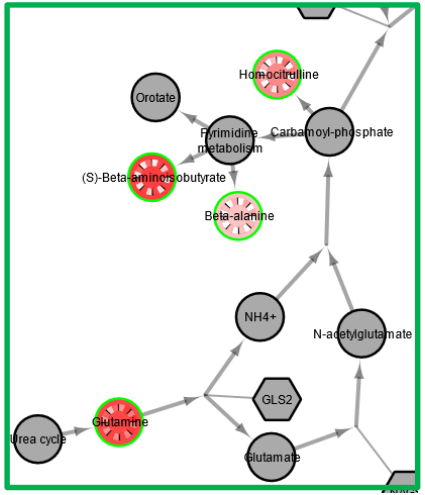

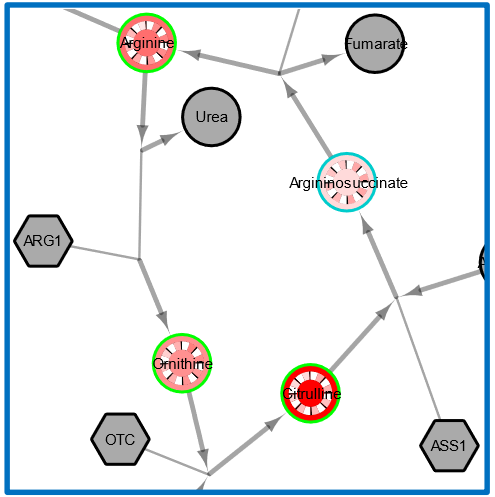

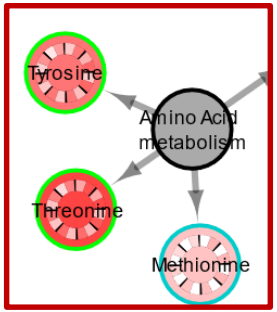

Discussion group 1

1 Burrage LC, et al. (2019) Genet Med
2 Quinonez SC, et al. (1993) GeneReviews

Discussion group 1

- Citrullinemia patient
  - 7 altered Pyrimidine metabolites
  - 9 altered Urea cycle metabolites

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Discussion group 1

- Citrullinemia patient
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  - 9 altered Urea cycle metabolites
  - Of these 8 are part of Pyrimidine degradation
    - 4 more than for beta-ureidopropionase deficiency patients
    - Only 1 found in literature [1]

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  - 7 altered Pyrimidine metabolites
  - 9 altered Urea cycle metabolites
  - Of these 8 are part of Pyrimidine degradation
    - 4 more than for beta-ureidopropionase deficiency patients
    - Only 1 found in literature [1]
  - Citrulline over 4 times the maximum reference value
    - Defect in enzyme that converts citrulline to argininosuccinate
    - Main metabolic alteration for patients [2]

1 Burrage LC, et al. (2019) Genet Med
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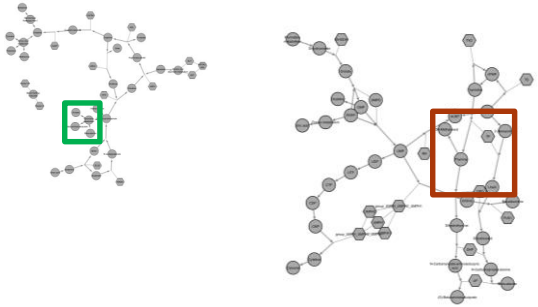
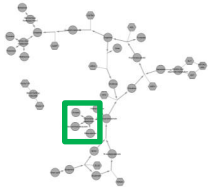
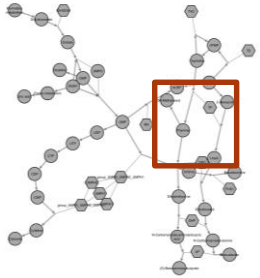
Results group 2



Results group 2





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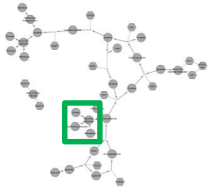

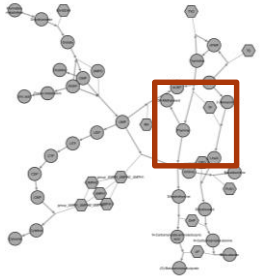

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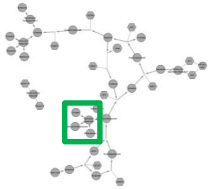

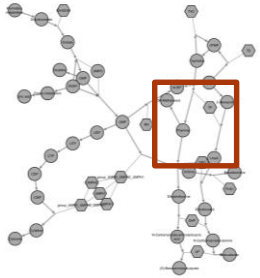

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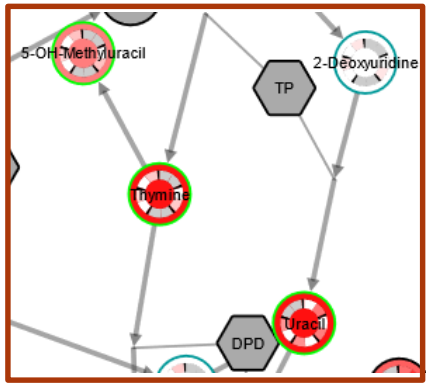

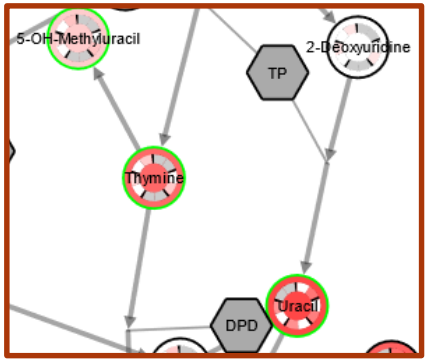

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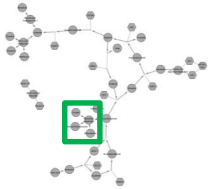

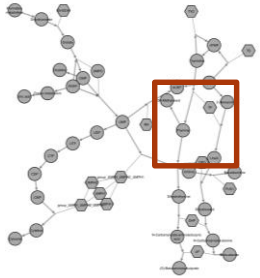

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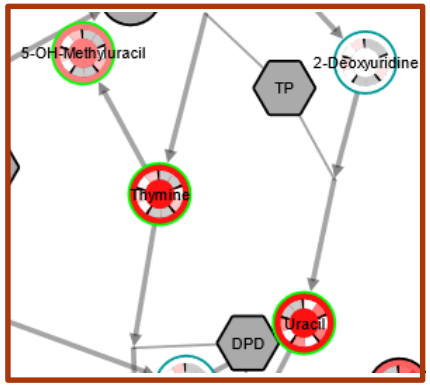

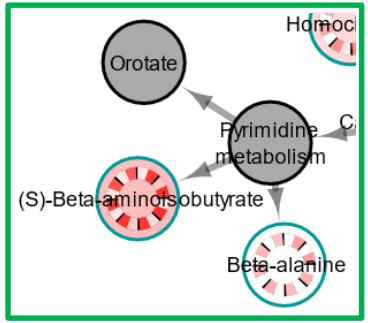

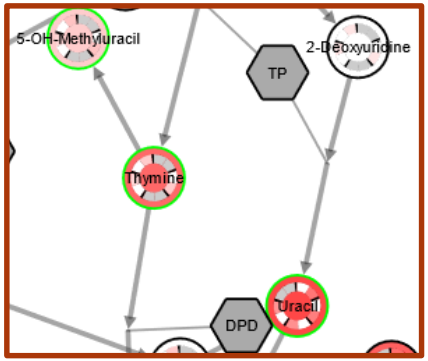

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  - No altered metabolites downstream from the defect
    - Including beta-alanine and (S)-beta-aminoisobutyrate

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- N-acetylglutamate synthetase deficiency
  - No altered Urea cycle metabolites
  - 1 altered Pyrimidine metabolite in *de novo* synthesis

Discussion group 3

1 Fjellstedt E, et al. (2003) Urol Res
2 Burrage LC, et al. (2019) Genet Med

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- After further automation
  - Workflow can help with the diagnosis of different IEMs types

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1 Kutmon M, et al. (2013) PLoS One

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- Addition of transcriptomics data or data on the genetic defect
  - Predict the expected effect on the metabolites

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Questions?