Modeling the genetics and metabolism of heart failure

A multi-omics story in two parts

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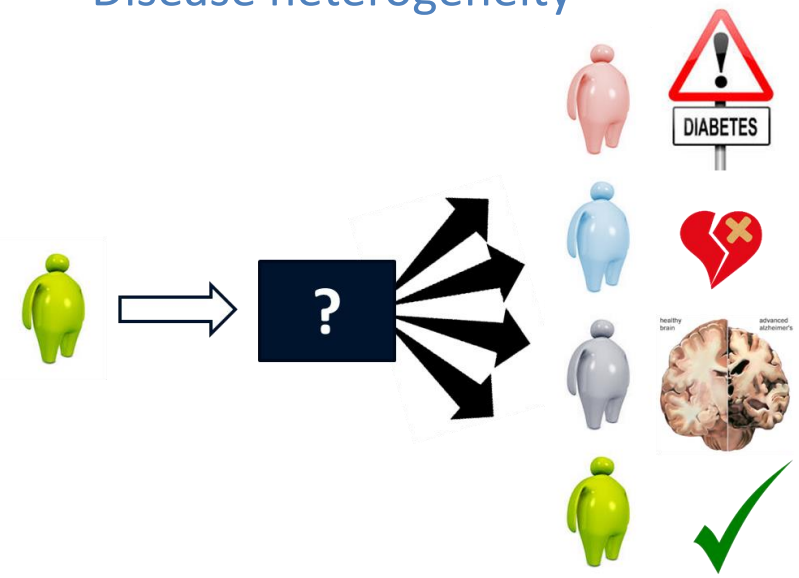
1

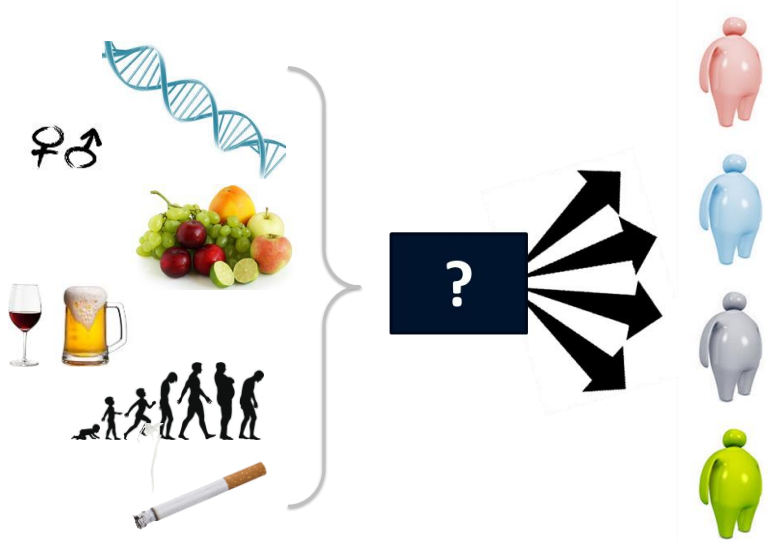
2

Disease heterogeneity



3

Disease heterogeneity: multi-factorial



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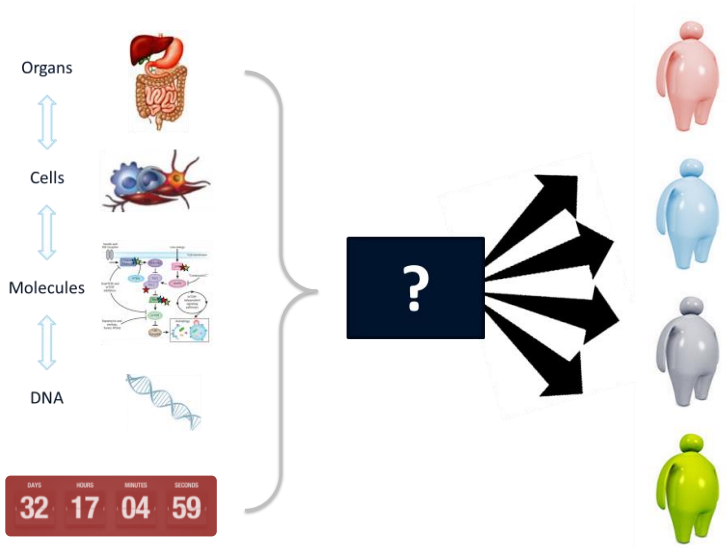
Disease heterogeneity: multi-scale



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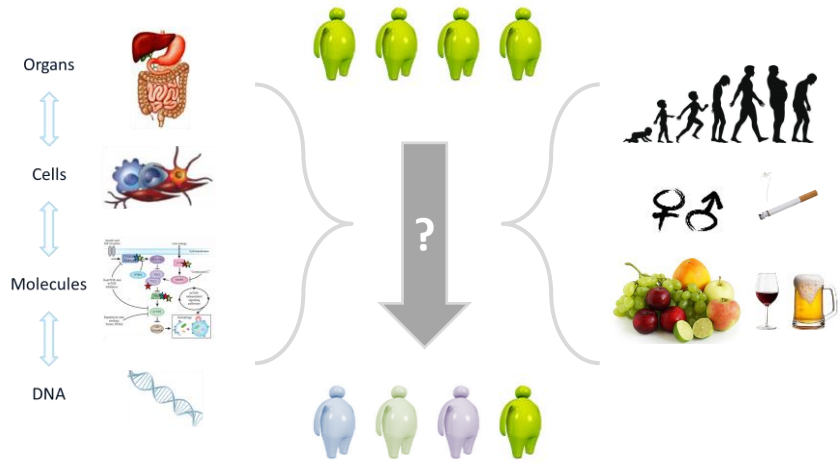
Systems Biology to understand **and** predict disease development



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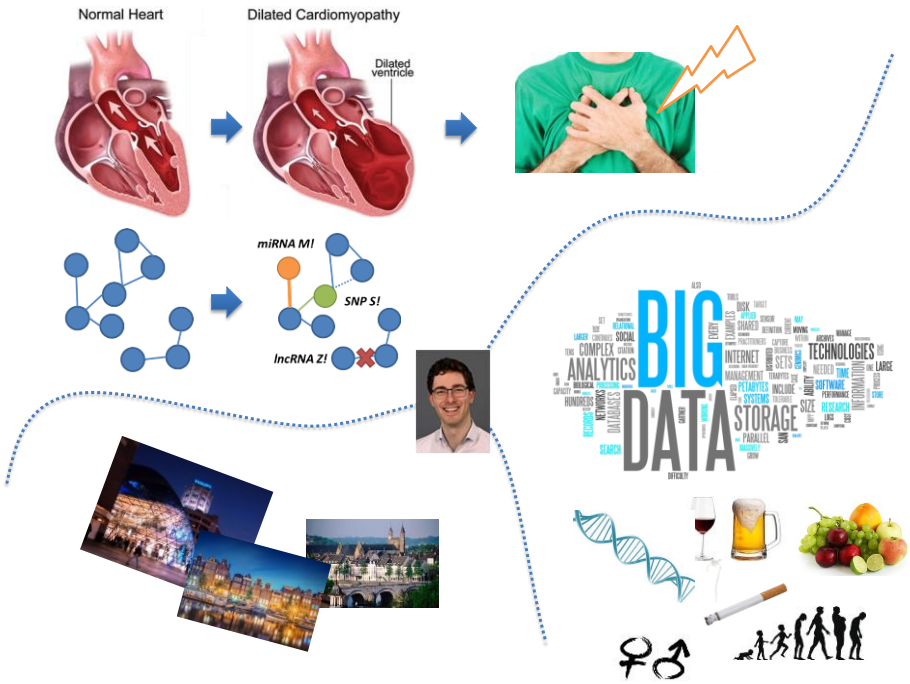
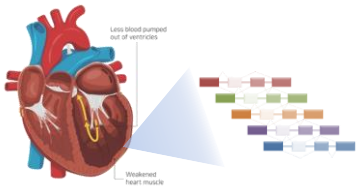


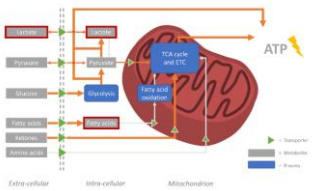
7

Outline

1. Genetic control of gene transcription in heart failure



2. Modeling the metabolism of the failing heart



8

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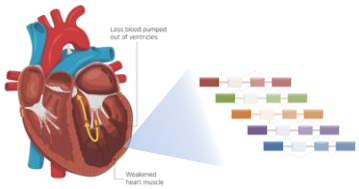

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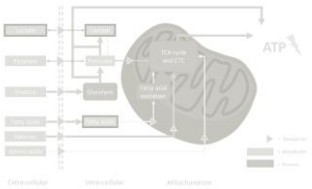
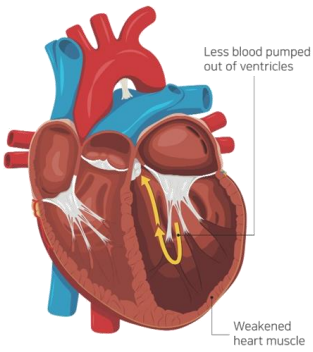
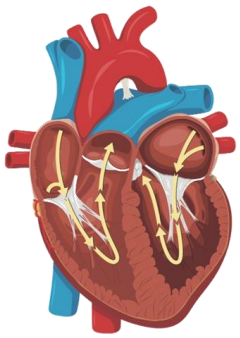


9

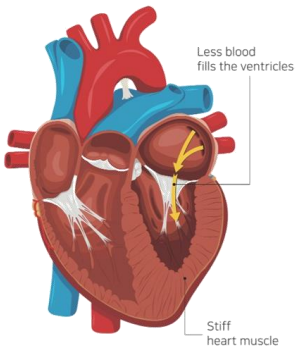
What is heart failure?



Systolic Dysfunction



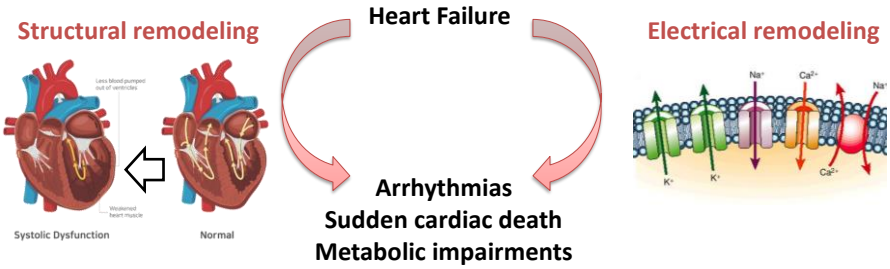
Normal



Diastolic Dysfunction

10

Heart failure ↔ complications



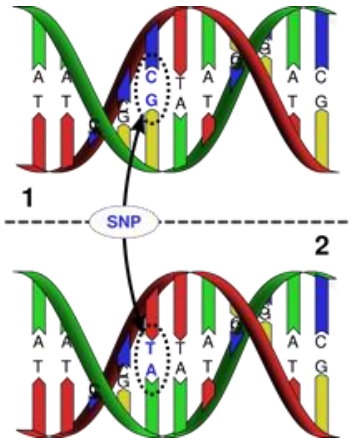
- *Complications are patient specific*
- *What explains this difference?*

Genetics?

11

Genetic association studies

- SNP (single nucleotide polymorphism):
  - A variation in a single nucleotide that occurs at a specific position in the genome
- Example SNP:
  - Base C may appear in most individuals
  - Base T occurs in some individuals
  - C an T are called the "alleles" of the SNP
- We all have two copies of every chromosome (and every gene!)



Allele C

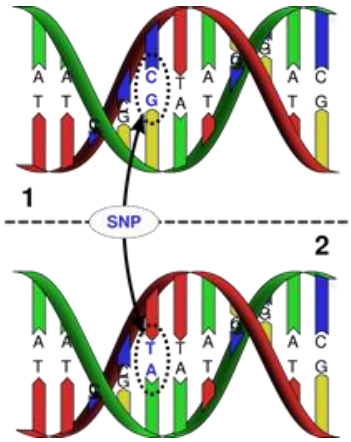
Allele T

Genotype = CT

12

Genetic association studies

- Variations in the DNA affect
  - Disease development
  - Response to pathogens, chemicals, drugs
- How to find these variations?
  - Genotyping of individuals
  - Comparing e.g. cases versus controls



Genotype = CT

13

Genetic association studies

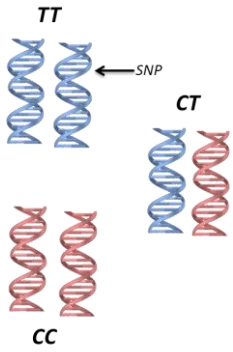
Example: *E-cadherin* gene SNP and prostate cancer

	Cases	*Controls*
TT or CT	61	84
CC	21	104
Total	82	188

$OR_{TT/CT\ vs.\ CC} = 3.6$

Conclusion: the 'T' allele is associated with prostate cancer (3.6-fold increased risk)

Source: Verhage *et al.* Int J Cancer 2002;100:683-5 (adapted)

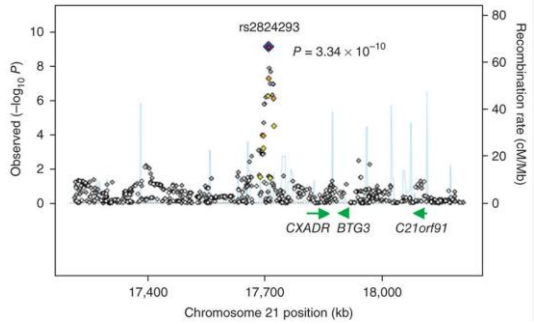


Source: Verhage et al. (2002)

14

Genome-wide association studies

- **GWAS =**
  - Genotype thousands of variants in a population of cases and controls
  - Genetic association for each variant
- **GWAS** have identified many genetic variants associated with complex traits and diseases
  - *Example below: susceptibility to arrhythmias after MI*

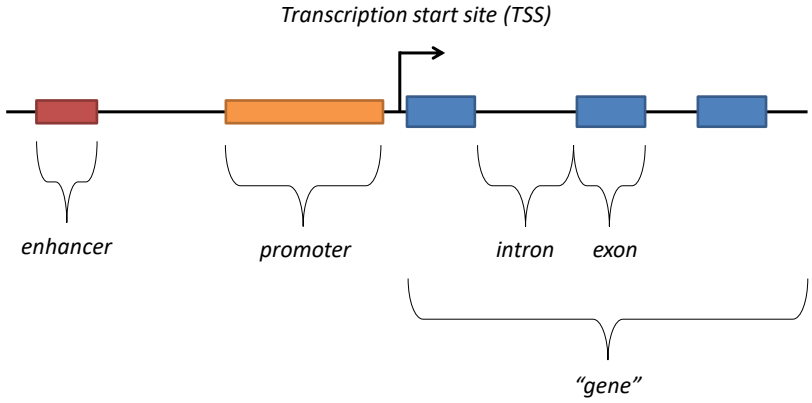


Genes?
Mechanism?

Bezzina, Pazoki, et al., Nat Gen (2010)

15

Genetic control of gene transcription



16

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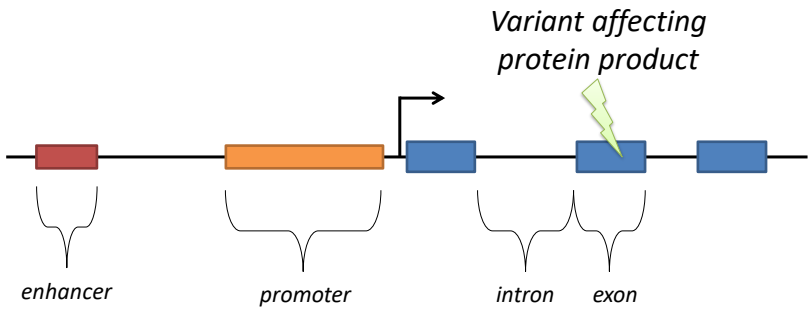
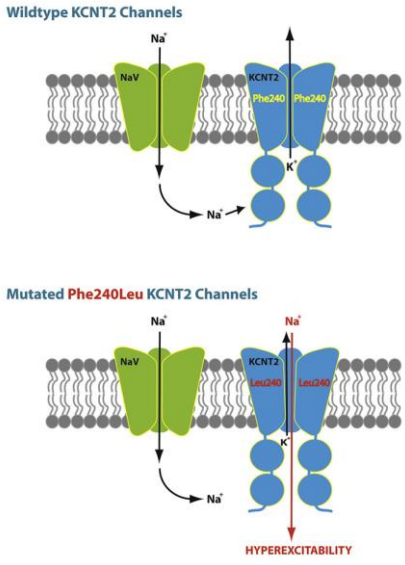


17

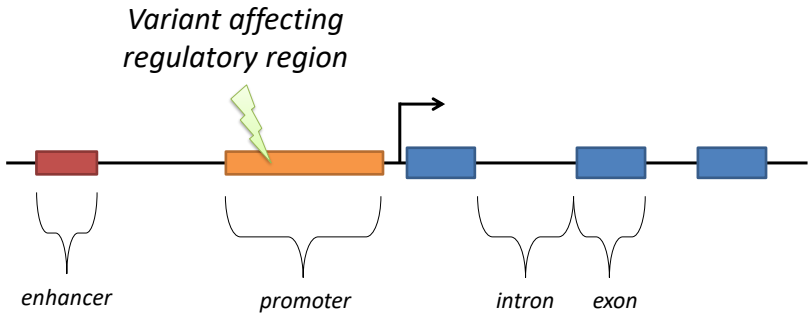
Genetic variants in exons can influence protein structure



Gururaj et al. (2017)

18

Genetic control of gene transcription



19

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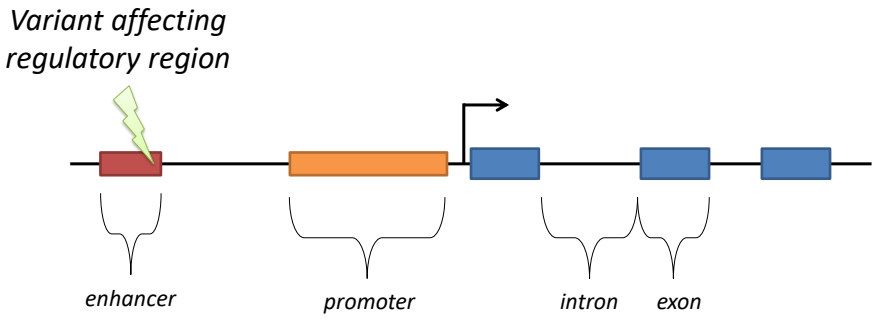
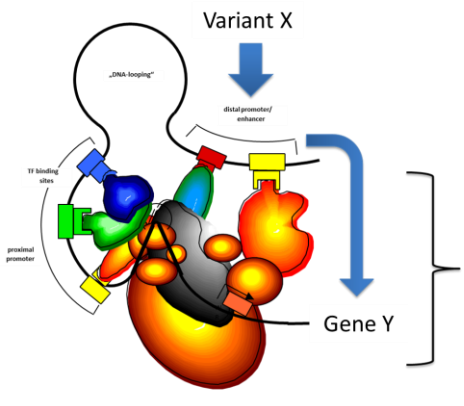
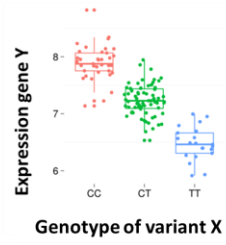


20

Genetic variant modulating expression levels





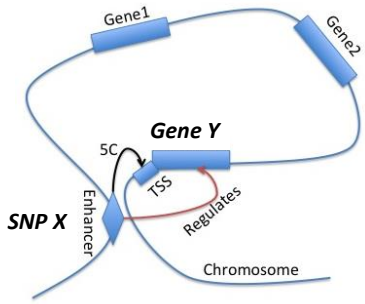
Expression quantitative trait locus
(eQTL)
=
in silico association between genotype and gene expression level within a specific population

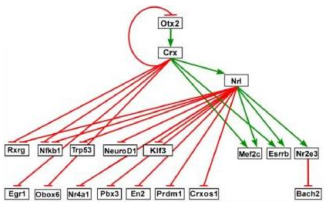
- Method: Linear regression (*GenABEL, MATRIXEQTL* R)
- *cis* (= local) effects focused (sample size)

21

What are *cis* & *trans* eQTLs

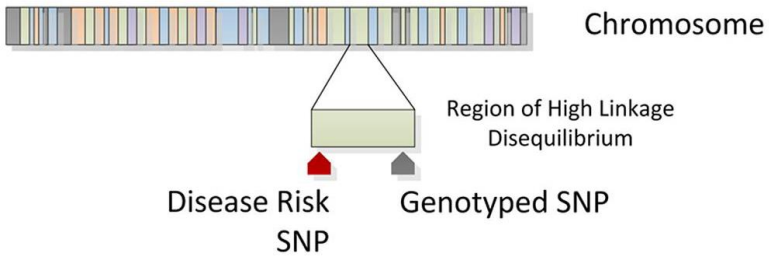
- *trans* eQTL: **SNP X** with **Gene Y**
  - **SNP X** not within 1 megabase of **Gene Y**
  - **SNP X** and **Gene Y** on different chromosomes
- **Distant interactions**
  - **SNP X** could be in a distant regulatory element (interactions between chromosomes)
  - **SNP X** linked to a transcription factor
- Expect small effect sizes → power issues in all but the largest studies





22

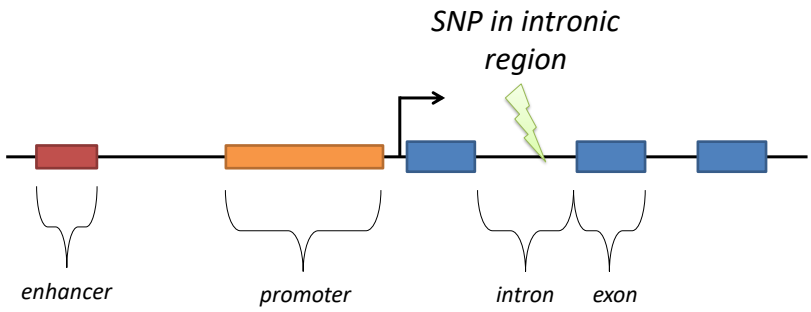
Linkage disequilibrium and eQTLs



- LD = the non-random association of alleles at different loci (i.e. $\rho_{AB} \neq \rho_A \rho_B$)
  - *Often calculated as the square of correlation coefficient: r^2*
  - *Often visualized in GWAS Manhattan plots*
- Indirect association due to LD structure: an eQTL SNP may or may not be the causal SNP

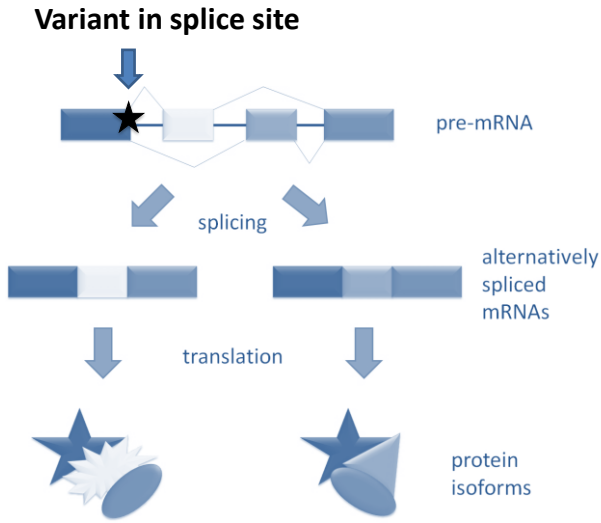
23

Genetic control of gene transcription



24

Genetic variants regulate exon usage



25

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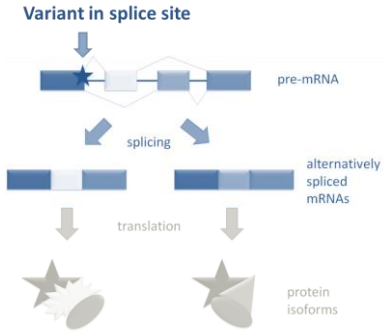
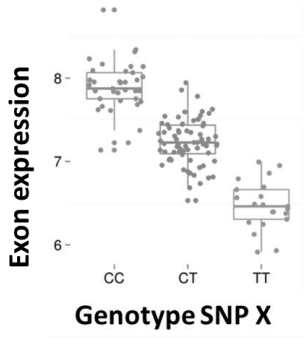




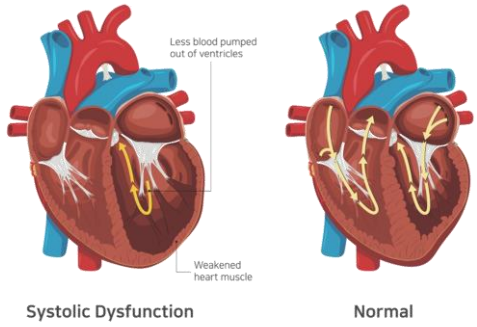
Splicing quantitative trait locus
(sQTL)
=
in silico association between genotype and alternative splicing within a specific population

26

Research: genetics of transcription and splicing in DCM

Samples: Left ventricle
- **108** non-diseased donor hearts
- **97** dilated cardiomyopathy (DCM) hearts

Data:
- RNA-seq: 16,219 unique mRNA levels
- Genotyping: 2 million common variants (SNPs)



Adriaens, Koopmann et al. (2014)

Heinig, Adriaens, Schaefer et al. (2017)

27

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Data:
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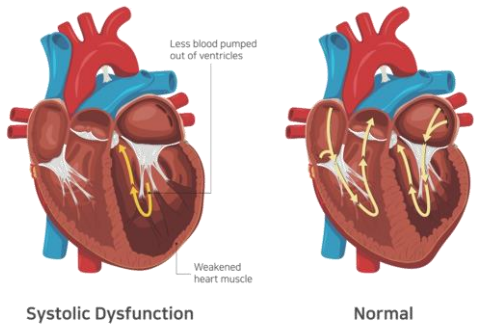
Research questions:
- **Which variants modulate gene expression? (eQTL)**
- **Which variants modulate splicing? (sQTL)**
- **Do these differ between DCM and controls?**



Adriaens, Koopmann et al. (2014)

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28

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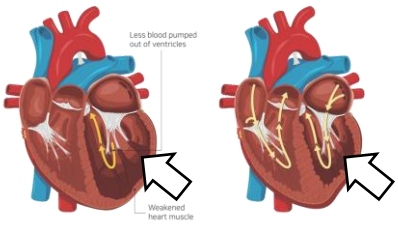
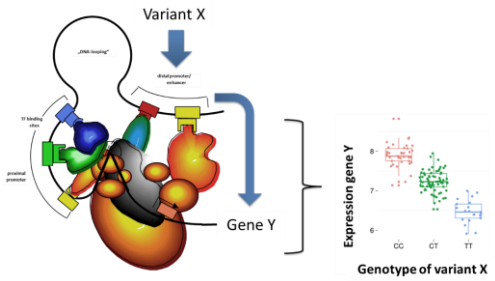


Transcriptomics
Genomics



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29

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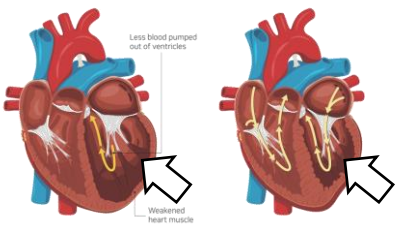

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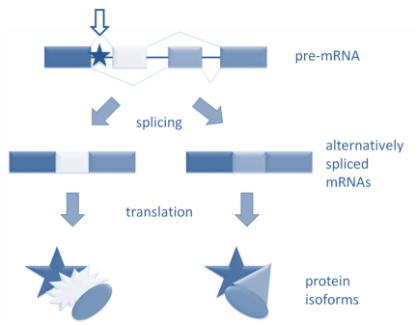
Genomics

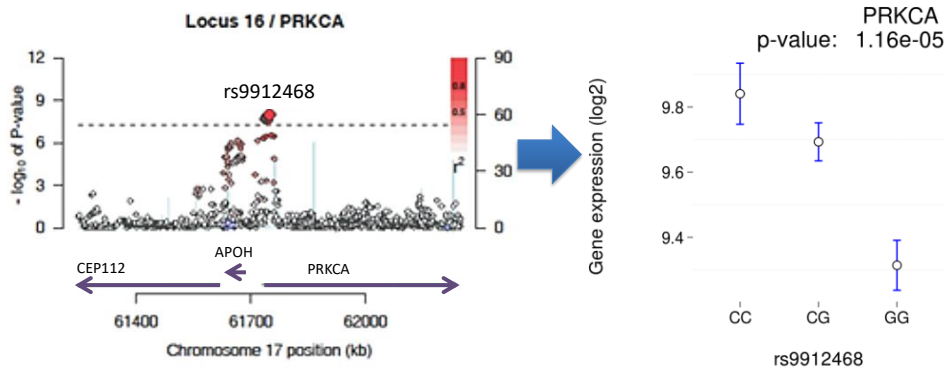


Differential splicing and expression contributes to the DCM phenotype and is genetically controlled for known and novel genes

Heinig, Adriaens, Schaefer et al. (2017)

30

Usage example: eQTLs for known GWAS loci



GWAS result from Sotoodehnia et al. (2010)

- ***rs9912468***: associated with QRS prolongation (effect allele = G)
- **Protein kinase C alpha**: regulator of cardiac contractility and Ca^{2+} handling in myocytes

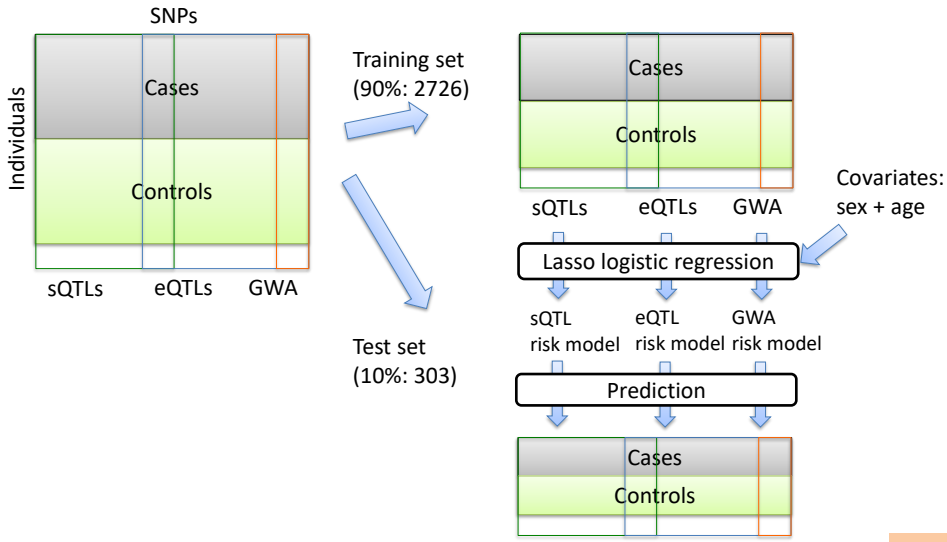
Adriaens, Koopmann et al. (2014)

31

Question:
what would you do next?

32

DCM genetic risk prediction



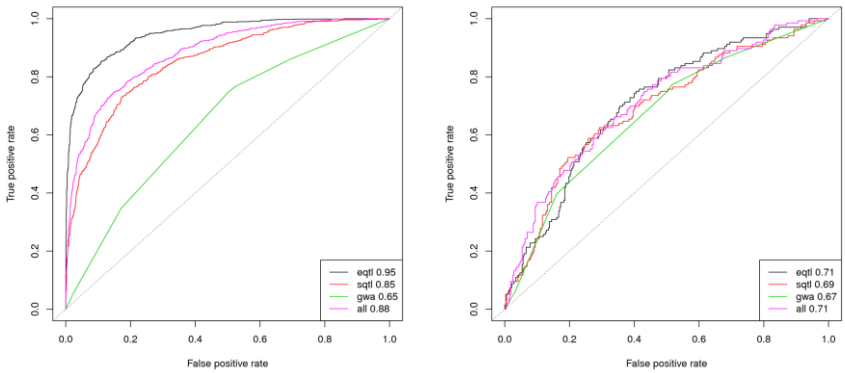
GWAS data from: Meder et al. Eur Heart J 2014

33

DCM genetic risk prediction

Training set (90%)

Test set (10%)



LASSO (glmnet  *)*

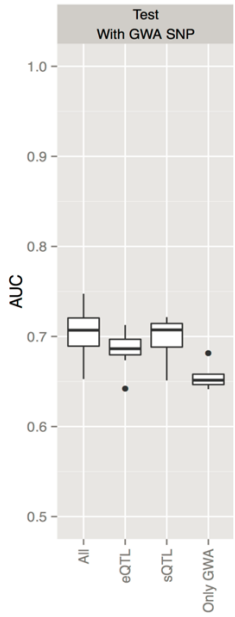
Heinig, Adriaens, Schaefer et al. (2017)

34

DCM genetic risk prediction

Better prediction

Random prediction



Combining
1. Co-variates (age, sex)
2. Genotype of DCM GWA SNP (rs9262636)
3. Genotypes of SNPs modulating expression (eQTLs)
4. Genotypes of SNPs modulating splicing (sQTLs)

In single predictive model leads to better prediction

Heinig, Adriaens, Schaefer et al. (2017)

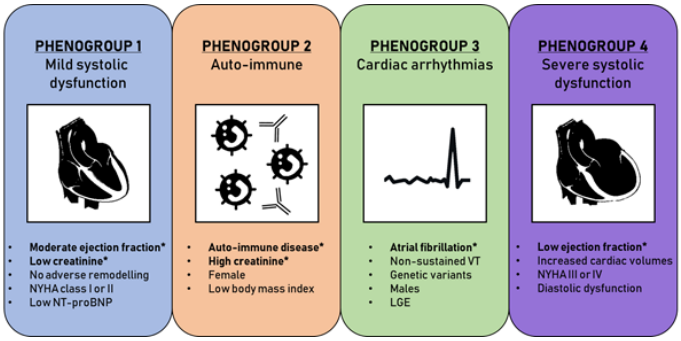
35

Question:
what would you do next?

36

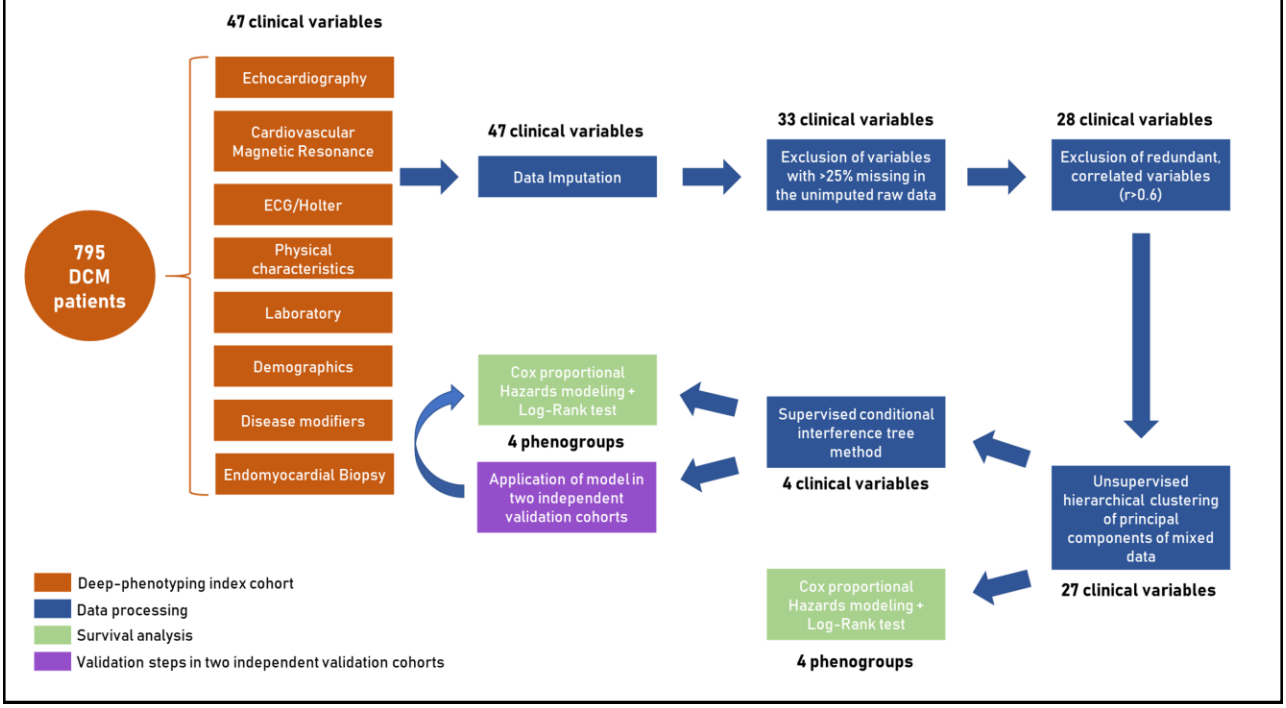
Research: DCM cohort in Maastricht

- Many clinical parameters available for more extensive subtyping:
  - Machine learning resulted in 4 distinct phenotypic clusters ("phenogroups")
- **Questions:**
  - **Which genes show differences in eQTLs and sQTLs between phenogroups?**
  - **In which processes and pathways are the corresponding genes involved?**
- Using RNA-seq of EMBs (n = 76)

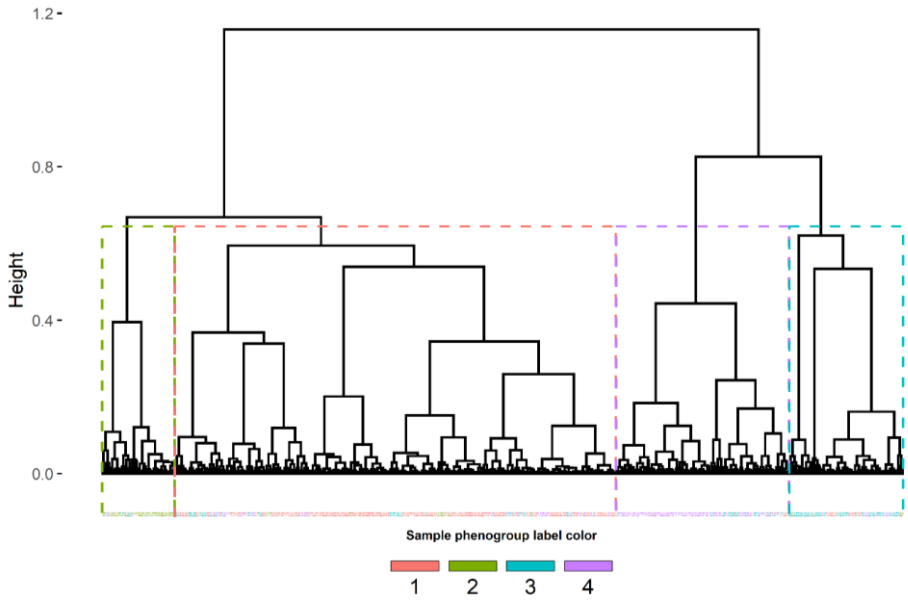


Verdonschot et al. (2020)

37



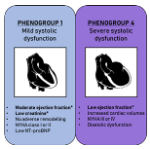
38



39

Severe versus mild systolic dysfunction

- 96 unique genes that are significantly differentially imbalanced between phenogroup 4 and 1
- Gene Ontology enrichment analysis:

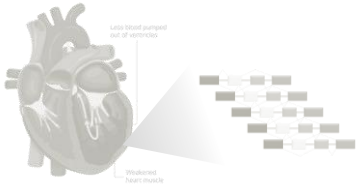


Term	***P-value***
cyclosporin A binding	6.00E-04
muscle structure development	9.60E-04
establishment of protein localization to membrane	1.15E-03
negative regulation of oxidative phosphorylation	1.44E-03
electron transport chain	9.35E-03
fat cell differentiation	1.05E-02
regulation of actin filament-based movement	1.50E-02
cellular response to stress	1.68E-02
response to calcium ion	1.70E-02
mitochondrial respiratory chain complex assembly	1.76E-02

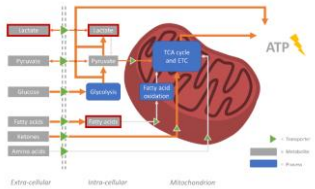
40

Outline

1. Genetic control of gene transcription in heart failure

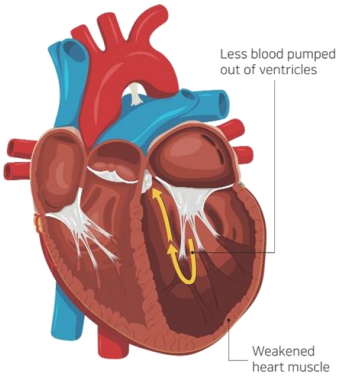


2. Modeling the metabolism of the failing heart

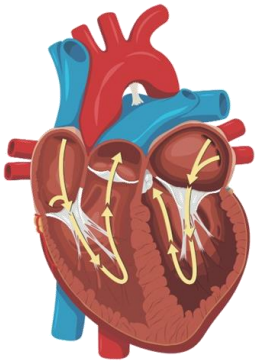


41

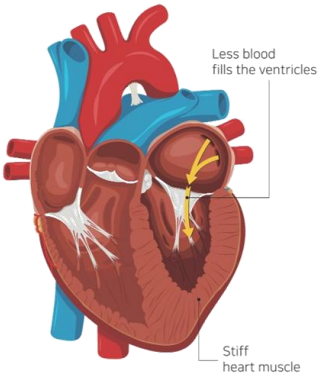
Recap: what is heart failure?



Systolic Dysfunction



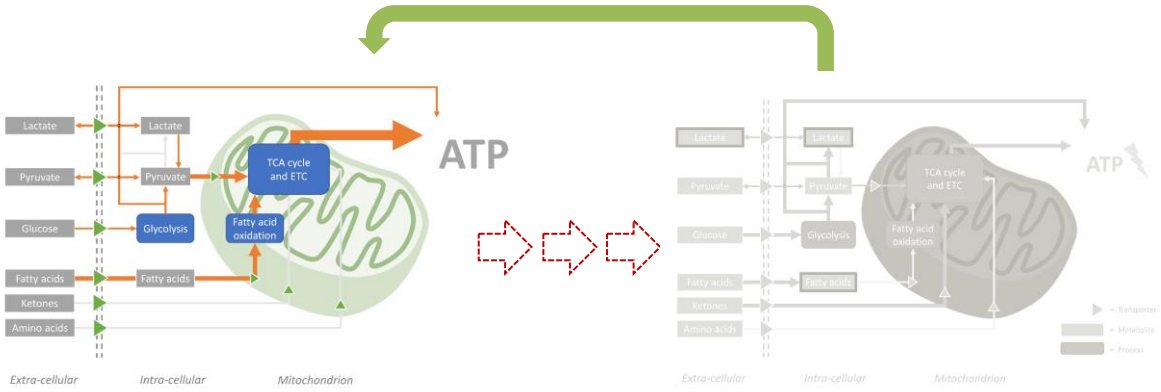
Normal



Diastolic Dysfunction

42

Loss of metabolic flexibility



Healthy heart

Failing heart

43

Restoring metabolic flexibility?

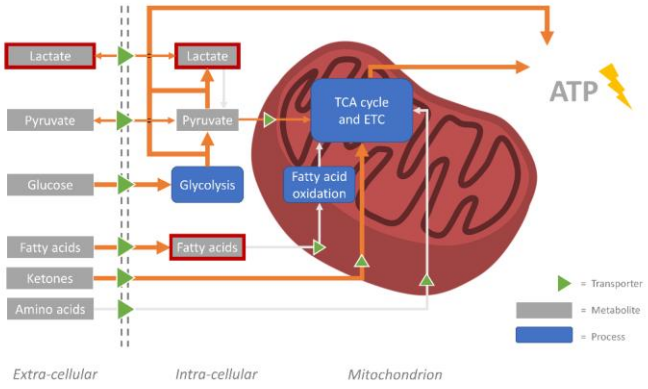
- Clinical trials aimed at restoring metabolic flexibility have so far led to mixed results
- Patient-to-patient differences are currently poorly understood

→ Targeted metabolic therapies have therefore not seen clinical implementation yet

44

Diagnosing loss of metabolic flexibility

- To diagnose, we need to determine **metabolic fluxes**
  - **Fluxomics: reaction fluxes of all known metabolic reactions**
  - Identify which pathways differ between patients
- Ideally: *in vivo* tracer studies to measure metabolic fluxes:
  - Problem 1: expensive and low sensitivity
  - Problem 2: some impairments only appear under stress



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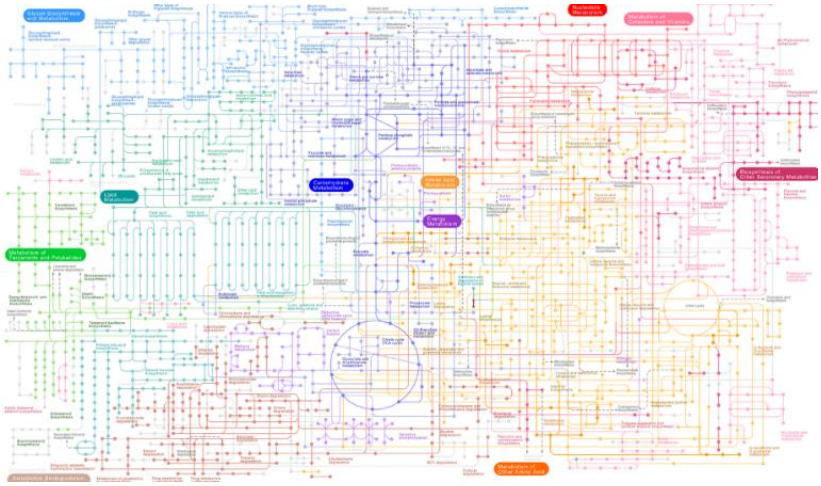
Question: what would you do next?

46

Modeling loss of metabolic flexibility

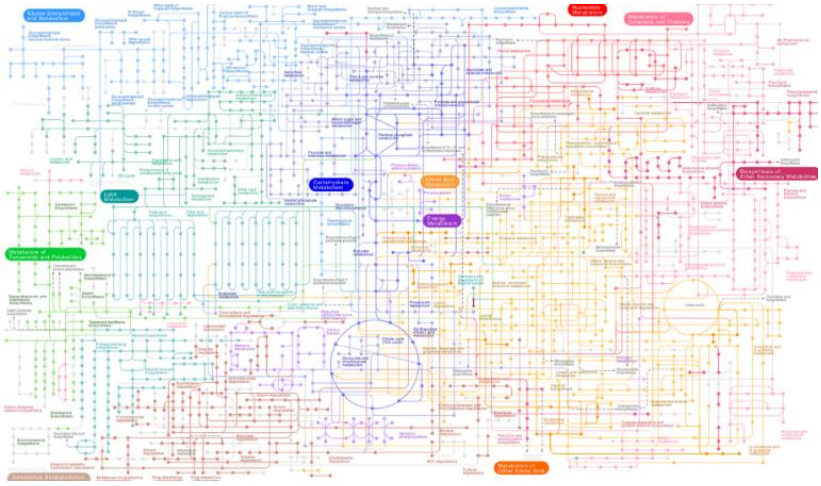
- Genome-scale metabolic models



47

Genome-scale metabolic model (GEM)

- Metabolites
- Reactions
- Stoichiometry
- Directionality of reactions (thermodynamic considerations)
- Enzymes (catalysing a reaction)
- Gene-Protein-Reaction rules
- Mass- and charge-balance of reactions
- Compartmentalisation



48

Genome-scale metabolic model (GEM)

Metabolites

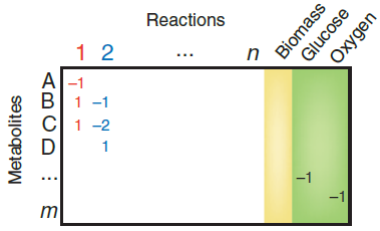
Reactions

Stoichiometry

Reaction: HEX1: hexokinase

ATP	D-glucose	proton	ADP	D-glucose 6-phosphate
atp[c]	+ glc_D[c]	→ h[c]	+ adp[c]	+ g6p[c]
-1	-1	1	1	1

Stoichiometric matrix S:



49

Genome-scale metabolic model (GEM)

Metabolites

Reactions

Stoichiometry

Reaction: HEX1: hexokinase

ATP	D-glucose	proton	ADP	D-glucose 6-phosphate
atp[c]	+ glc_D[c]	→ h[c]	+ adp[c]	+ g6p[c]
-1	-1	1	1	1

c = cytoplasm	*e = extracellular space*
g = Golgi apparatus	*l = lysosome*
m = mitochondrion	*n = nucleus*
r = endoplasmic reticulum	*x = peroxisome*

50

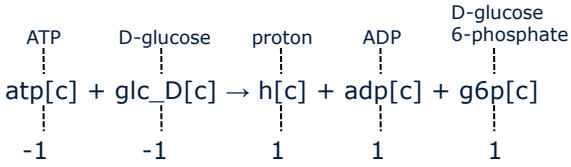
Genome-scale metabolic model (GEM)

Metabolites

Reactions

Stoichiometry

Reaction: HEX1: hexokinase



Flux of reaction

- *has upper and lower bound*
- *often expressed in mmol/gDW/s*
- *gDW = gram dry weight*

51

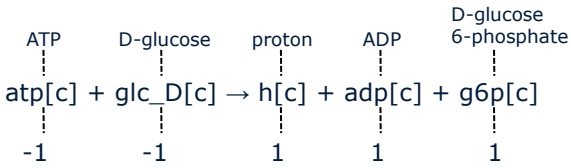
Genome-scale metabolic model (GEM)

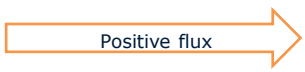
Metabolites

Reactions

Stoichiometry

Reaction: HEX1: hexokinase





Flux of reaction

- *has upper and lower bound*
- *often expressed in mmol/gDW/s*
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52

Genome-scale metabolic model (GEM)

- Metabolites
- Reactions
- Stoichiometry

Reaction: HEX1: hexokinase

ATP	D-glucose	proton	ADP	D-glucose 6-phosphate
atp[c] +	glc_D[c] →	h[c] +	adp[c] +	g6p[c]
-1	-1	1	1	1

Negative flux

Flux of reaction
- *has upper and lower bound*
- *often expressed in mmol/gDW/s*
- *gDW = gram dry weight*

53

Genome-scale metabolic model (GEM)

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atp[c] +	glc_D[c] →	h[c] +	adp[c] +	g6p[c]
-1	-1	1	1	1

→ vs. ↔, irreversible vs. reversible

Hexokinase 1, 2, 3, or 4 (glucokinase) catalyze the reaction

(3098) or (3099) or (3101) or (2645)…

Gene number for hexokinase 1

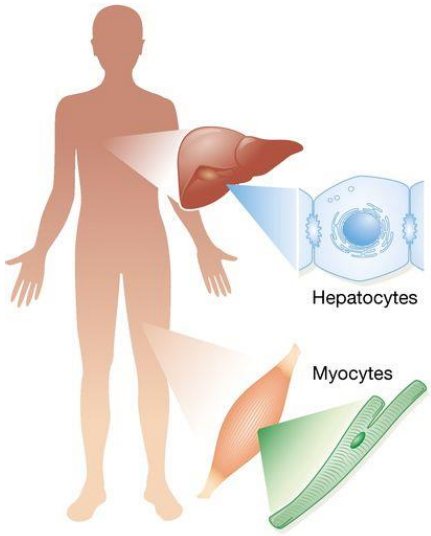
glc_D[e] <=> glc_D[c]

Glucose transport from extracellular space to cytosol

54

The aim of a model is context specific

- GEMs are often organism-specific, but not tissue/cell type specific
- Tissue-specific models include only reactions that are active in the respective tissue
- *Rationale: Reaction is inactive if catalyzing enzyme is not expressed*

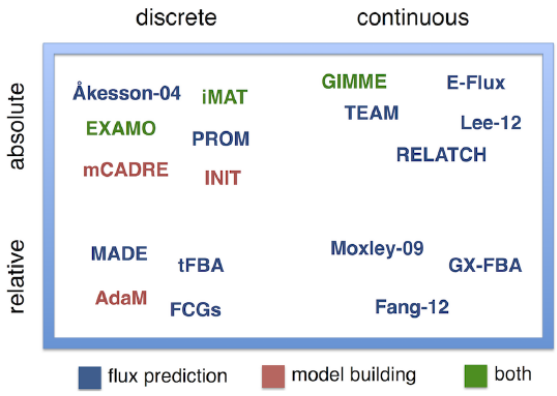


Uhlen, M et al. Mol Sys Bio, 12:862 (2016)

55

Model extraction methods (MEMs)

- Many algorithms have been proposed for building tissue-specific models based on generic models
- Simplest approach: delete genes that are not expressed
  - Typically based on tissue-specific transcriptomics data
- Problems:
  - Cutoff for being not expressed
  - Orphan reactions & dead-ends
  - Need to check metabolic functions

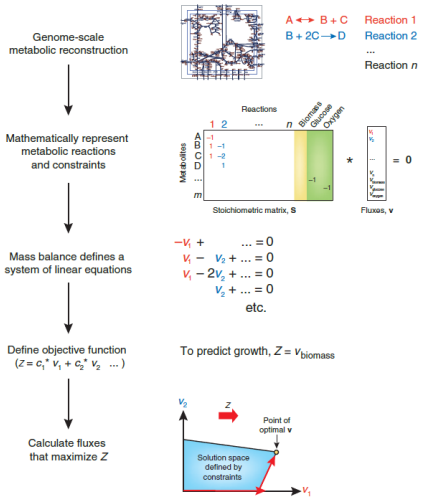


Machado, D et al. PLoS Comp Bio, 10(4):e1003580 (2014)

56

Flux balance analysis

- Used to calculate flow of metabolites through metabolic network
- Predict growth rate of organism or rate of production of given metabolite
- Assumes steady state
- Optimizes a given objective function



Orth JD, et al. Nat Biotech, 28(3):245-8 (2010)

57

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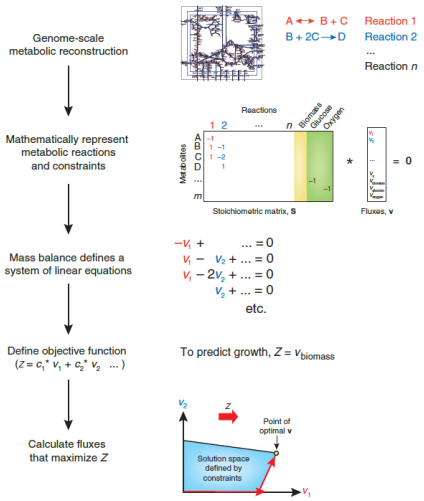

Orth JD, et al. Nat Biotech, 28(3):245-8 (2010)

58

Objective function - Examples

The objective function ≈ the aim of the model

- Biomass reaction (e.g. plants for consumption)
- ATP production (ATP demand reaction)
- Maximize a product of interest (e.g. lysine production)
- …

59

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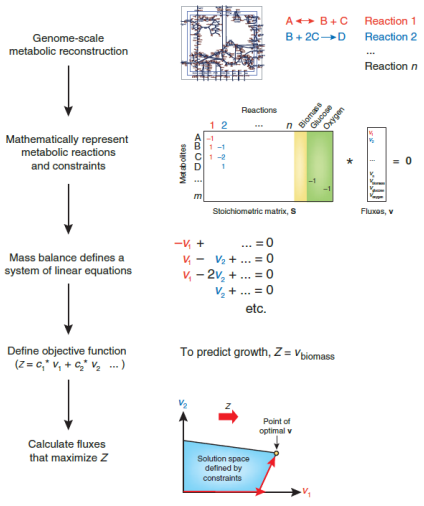

Orth JD, et al. Nat Biotech, 28(3):245-8 (2010)

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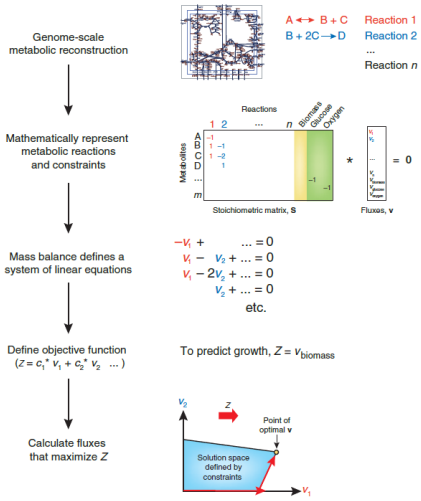


Orth JD, et al. Nat Biotech, 28(3):245-8 (2010)

61

Steady-state assumption

Assumption to reduce model complexity:

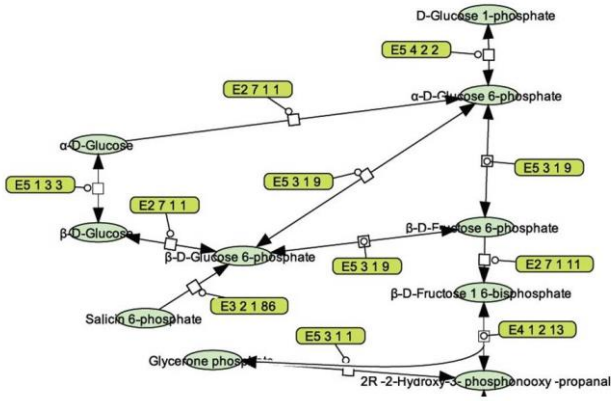
Metabolite concentrations and reaction rates stay constant over time (steady-state)

Benefit:

1. We have to estimate only one value (reaction rate/flux) per reaction instead of a function over time
2. We do not have to care about different metabolite concentrations
3. Introduces a direct dependence between reactions: Production and consumption of each metabolite cancel out

62

Steady-state assumption visualized



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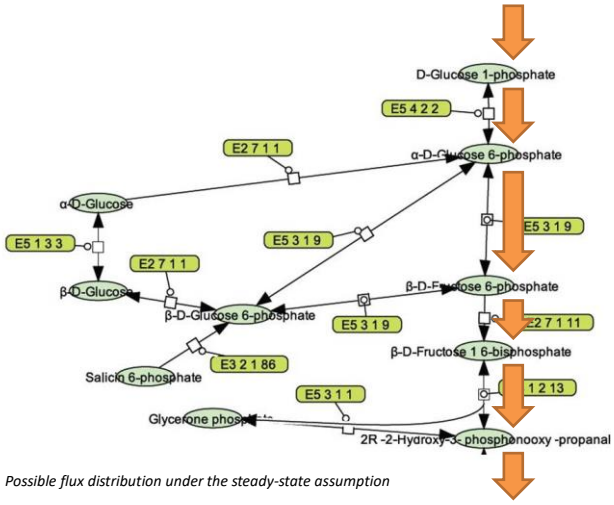
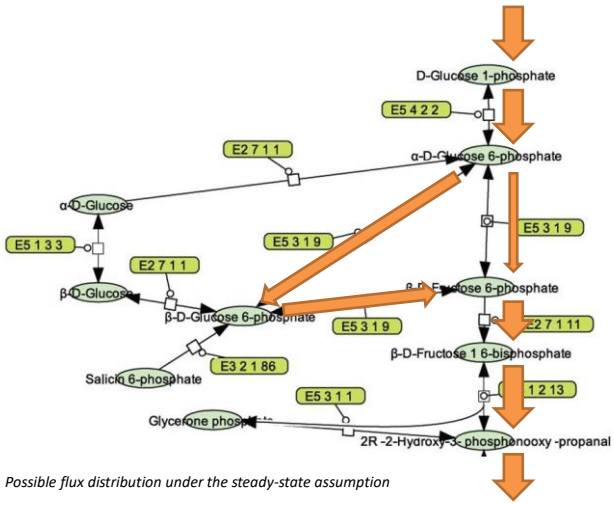
Steady-state assumption visualized



Possible flux distribution under the steady-state assumption

64

Steady-state assumption visualized



Possible flux distribution under the steady-state assumption

65

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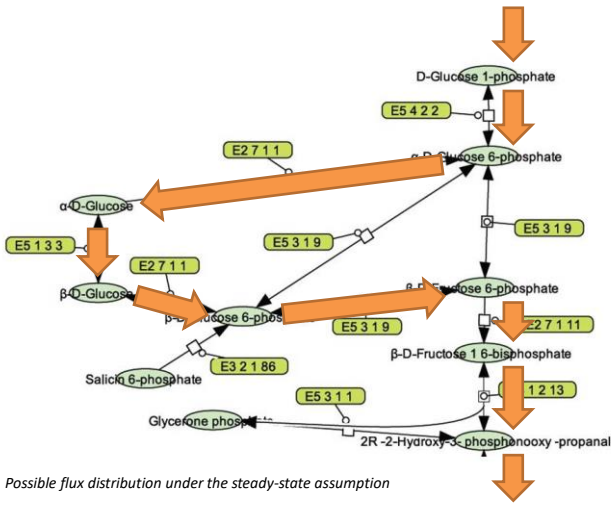

Possible flux distribution under the steady-state assumption

66

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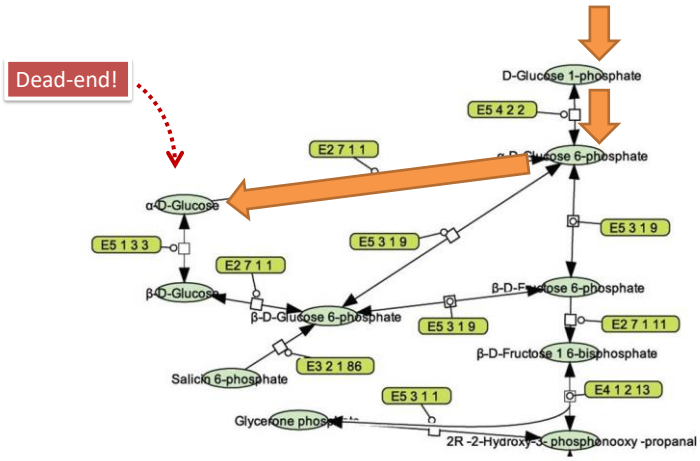
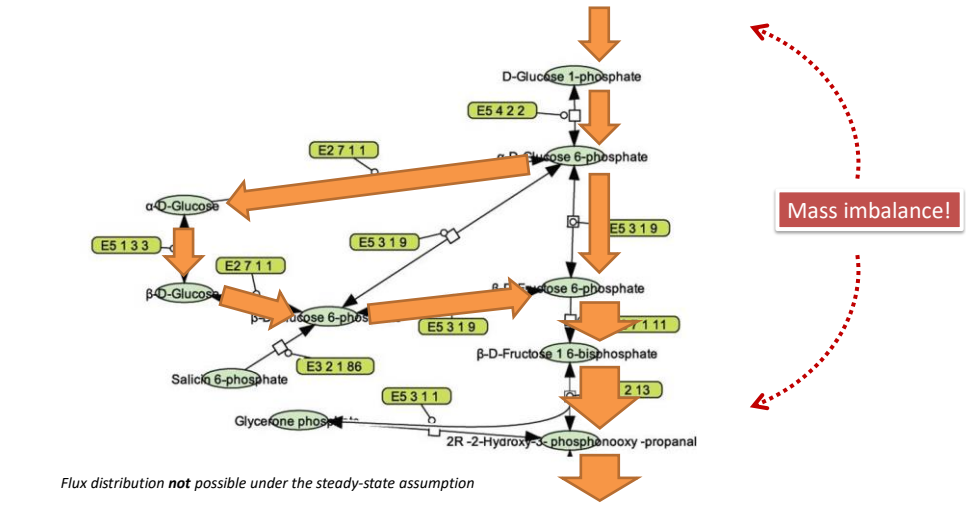


*Flux distribution **not** possible under the steady-state assumption*

67

Steady-state assumption visualized

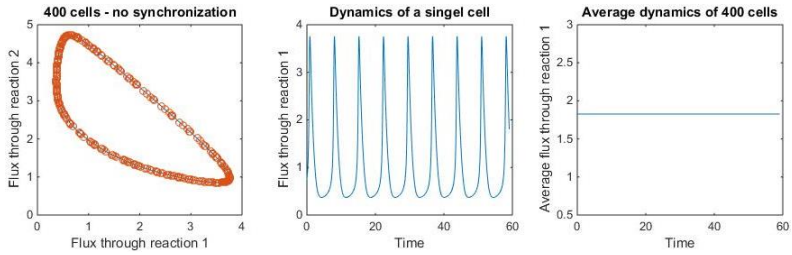


*Flux distribution **not** possible under the steady-state assumption*

68

Possible problem of steady state assump.

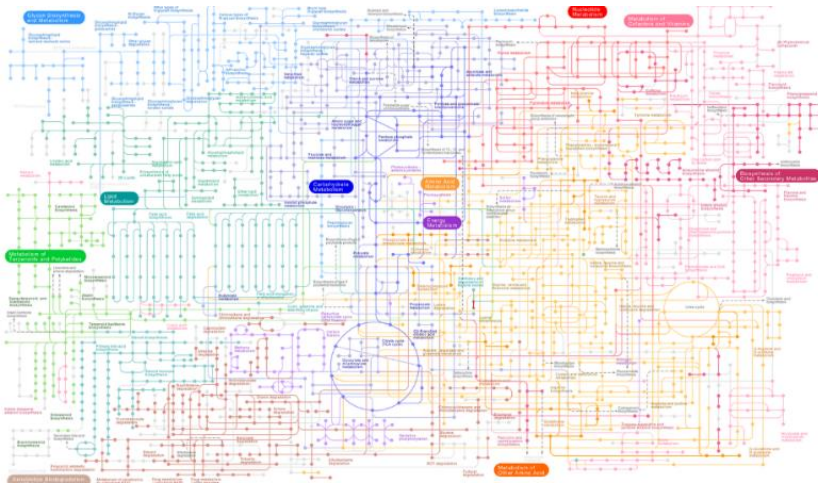
- Cyclic behavior (e.g. limit cycles/periodic fixed points)
- No steady state for single cells
- Consider average of many cells (no synchronization) → steady state reasonable



69

Modeling loss of metabolic flexibility

- Genome-scale metabolic models



70

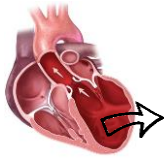
Modeling loss of metabolic flexibility

Like a water supply network!
- *Some places might use more than others*
- *But what goes in, must come out*

71

Modeling loss of metabolic flexibility

- Genome-scale metabolic models
- Activate and deactivate reactions based on **<u>gene activity</u>**

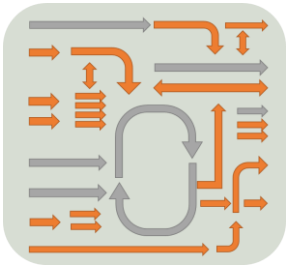


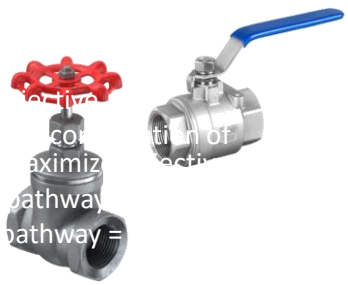
Measure gene activity as proxy for metabolic enzyme activity

Active gene =

Inactive gene =

Personalized model





72

Modeling loss of metabolic flexibility

- Genome-scale metabolic models
- Activate and deactivate reactions based on **gene activity**
- Simulate metabolism for individual
  - Choose objective
  - Find optimal combination of fluxes to maximize objective
  - High flux pathway = active
  - Low flux pathway = less active

73

Modeling loss of metabolic flexibility

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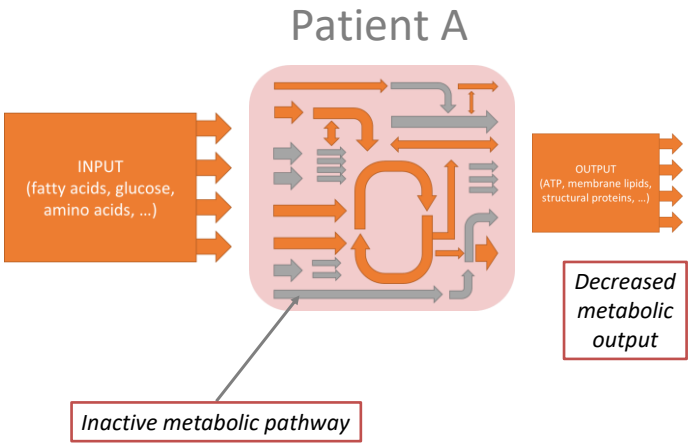

74

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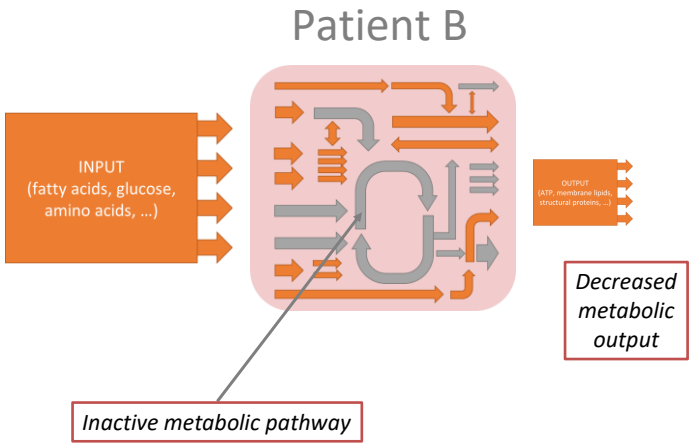
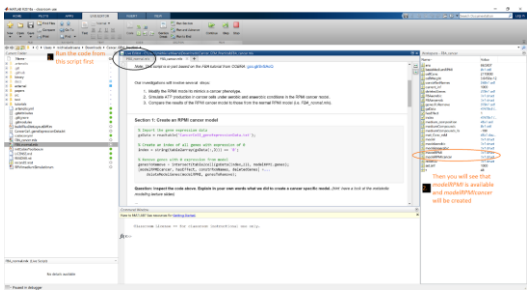
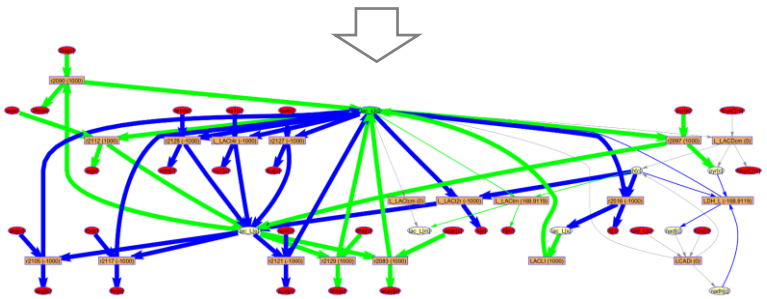


75

Software & tools

- Matlab
  - Python can be an alternative open-source solution for GEM analysis
- CobraToolbox
  - https://opencobra.github.io/cobratoolbox/stable/
  - Model extraction methods
    - Transcriptomics data integration
  - Flux balance analysis



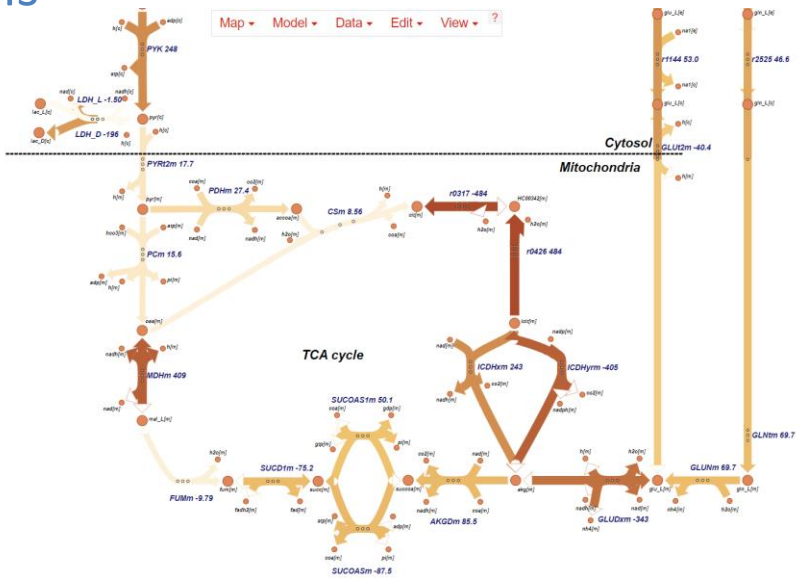


76

Software & tools

- Escher maps:
  - Demo:

https://sbrg.github.io/escher-fba



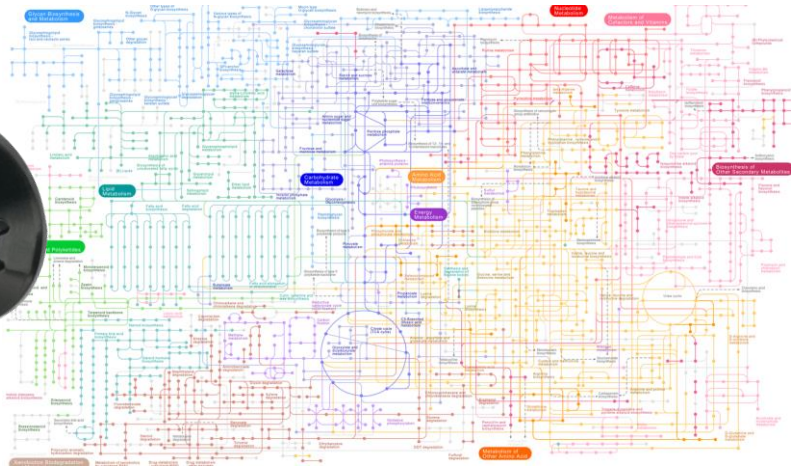
Male DCM cardiomyocyte metabolism simulation

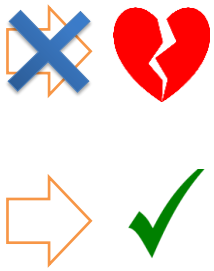
77

Question:
what would you do next?

78

Example: test the effect of different diets





79

Advantages & limitations of GEMs

+ Relatively little information needed
+ Applicable to large networks
+ Quantitative flux estimations

- Only steady state estimation
- Often no unique solutions (large solution space)
- Optimization assumptions (FBA) critical

80

Model databases

BioModels Database

https://www.ebi.ac.uk/biomodels-main/

VIRTUAL METABOLIC HUMAN

https://vmh.uni.lu/

Human Metabolic Atlas

http://www.metabolicatlas.org/

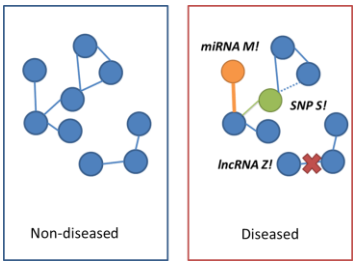
BiGG Models

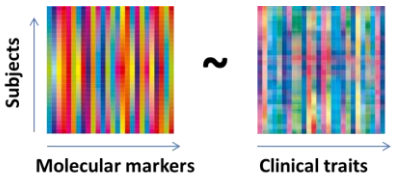
http://bigg.ucsd.edu/

81

Take-home messages

- Systems Medicine involves:
  - Large datasets
  - Multivariate modeling
  - Data-driven aspects complemented by prior knowledge
- To understand and predict disease progression, to support clinical decision making





82

michiel.adriaens@maastrichtuniversity.nl

83