Welcome to cardiogenomics lab
University of Antwerp

Program for today

10-12 am: Genomics and sequencing in the year 2023
How did we evolve from chromosomal analysis to whole genome analysis ? Bart Loeys
How does the current genomic sequencing technology work ? Arvid Suls
What is the GEMS project ? Lotte Van Den Heuvel

12-13 pm: lunch

13-14:30 pm: The use of iPSC in vascular research - Joe Davis – Melanie Perik

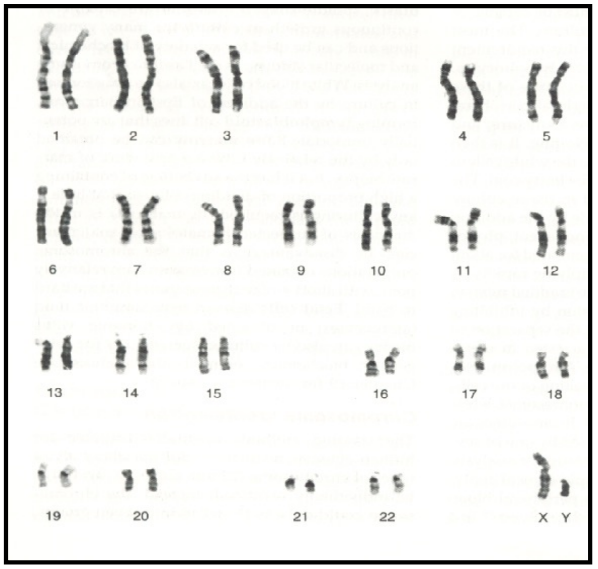
14:30-15:00 pm: coffee and the break

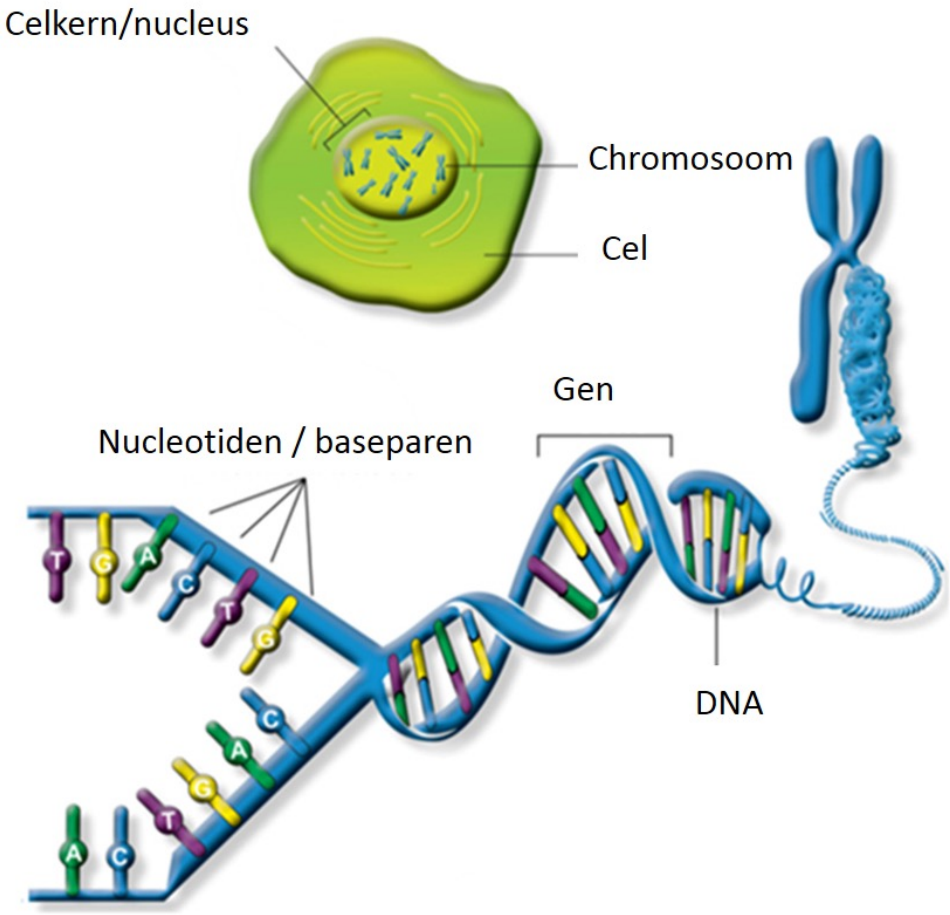
15-16:30 pm: Aorta research in mouse models - Lucia Buccioli - Irene Valdivia Callejon

How did we evolve from chromosomal analysis to whole genome analysis ?

Bart Loeys

What is DNA?

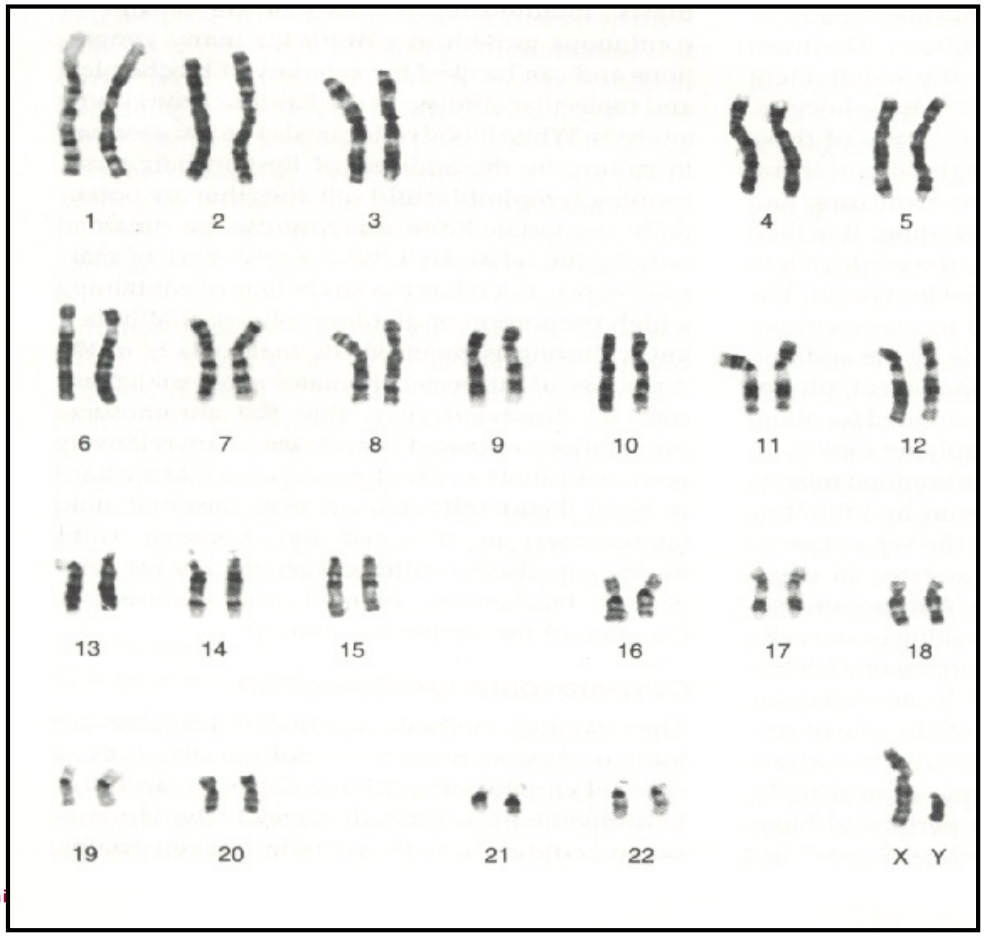

1 2 3 4 5
6 7 8 9 10 11 12
13 14 15 16 17 18
19 20 21 22 X Y


Celkern/nucleus
Chromosoom
Cel
Gen
Nucleotiden / baseparen
DNA

Our genetic material…

DNA	English language
A,G,C,T	a,b,c,d,e,f,…x,y,z
codon	word
gene	sentence, paragraph
chromosome	book
genome	encyclopedia
exome	all important sentences

Our genetic material…



1 genome

46 chromosomes

21.000 genes

3 billion nucleotides/basepairs

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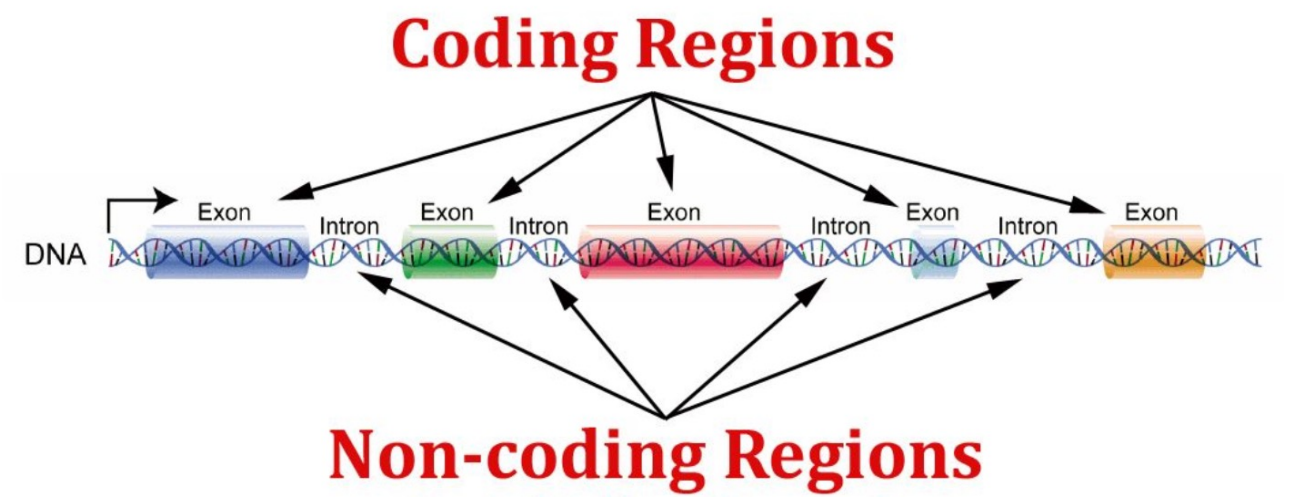
Coding versus non-coding



1-2%

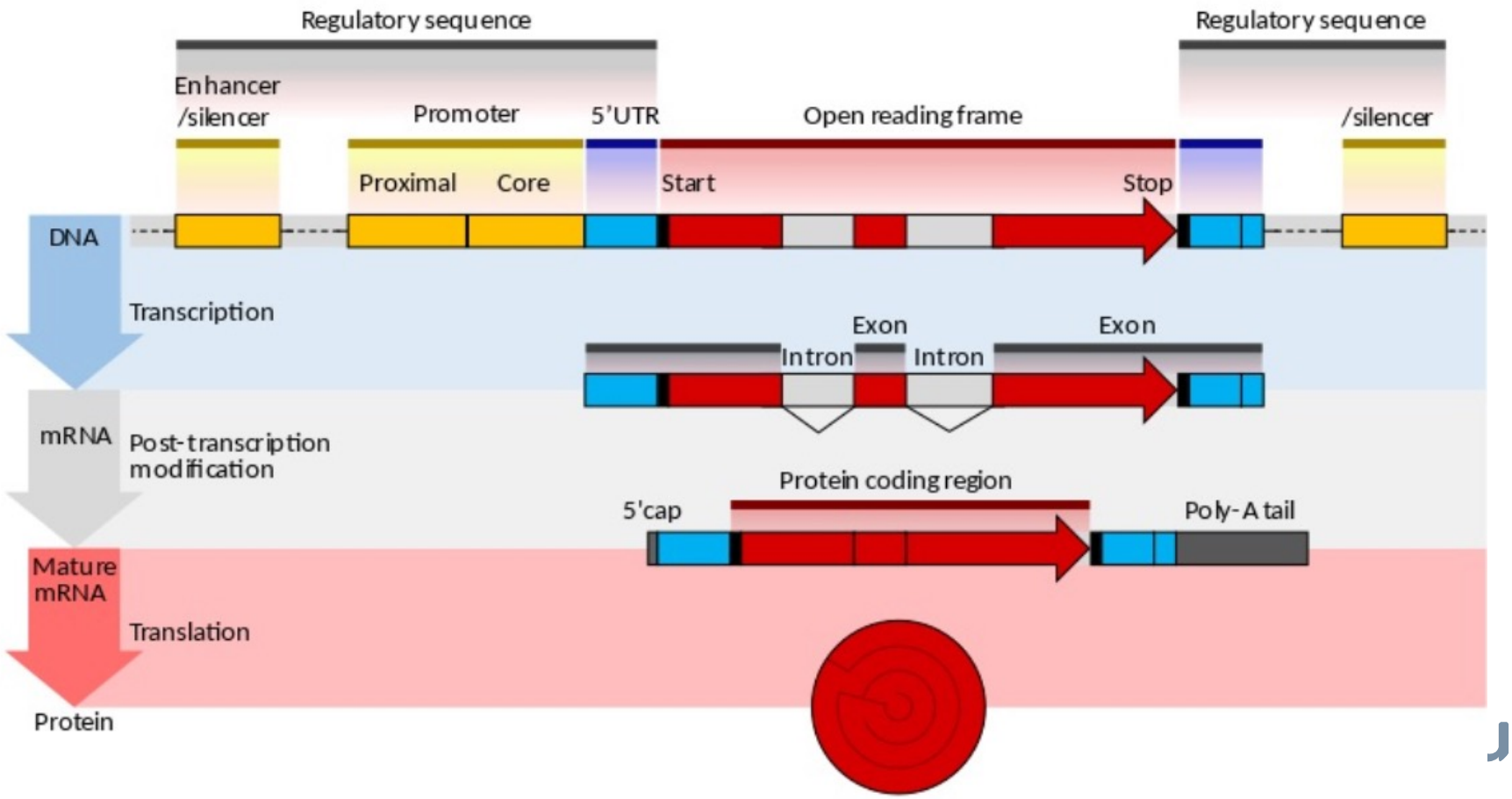
98%

All coding regions together -> EXOOM: 3 million nucleotides

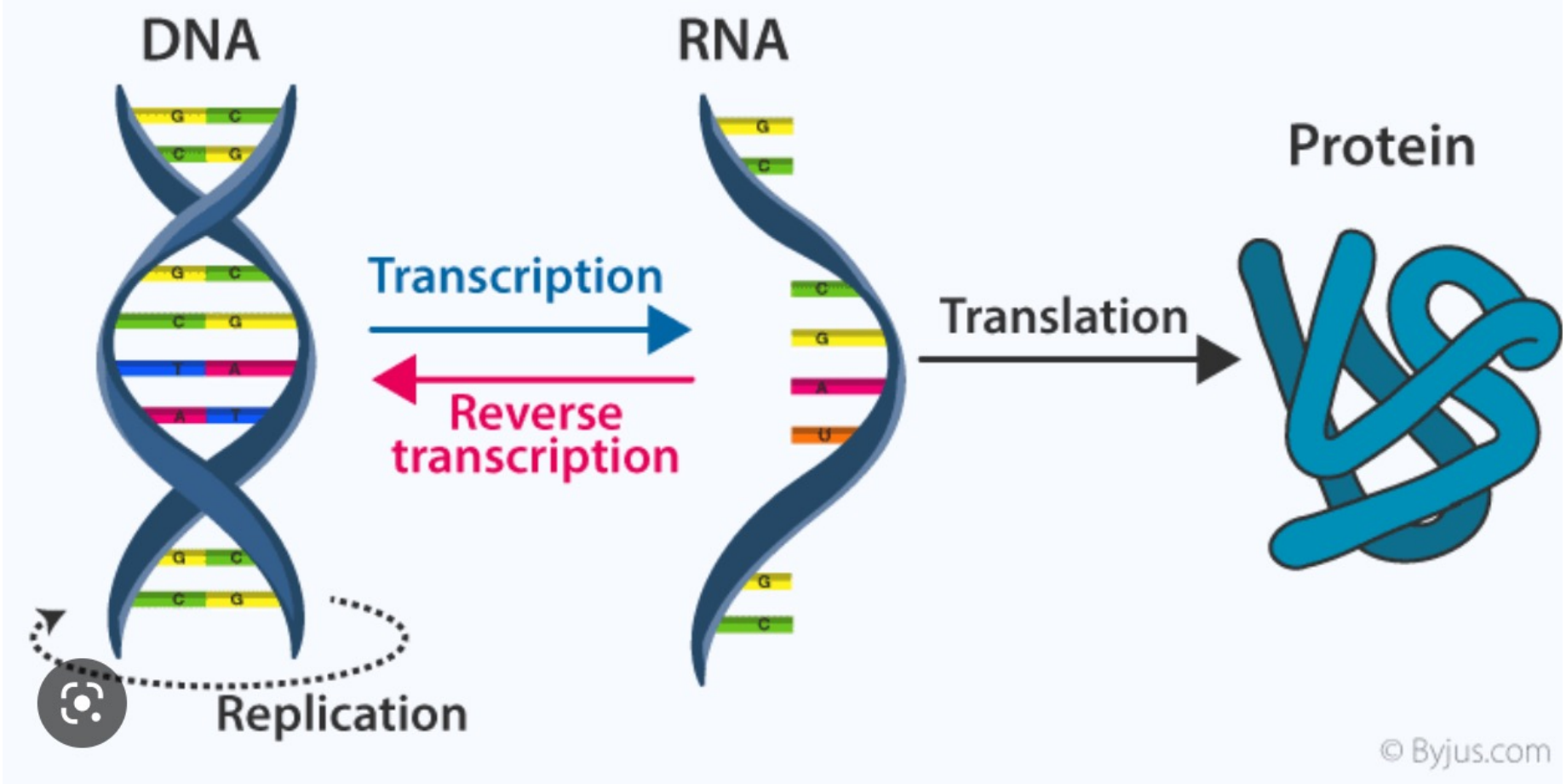
All coding and non-coding regions -> GENOOM: 3 billion

CODING	NON-CODING
DNA in genome contain protein coding genes	Other types of DNA not coding for proteins
1%	99%
exons	Regulatory regions, non-coding RNA, introns, repeating sequences, telomers
Encodes for proteins	Not encoding for proteins
Transcription to mRNA	Transcription to tRNAs, rRNAs, other regulatory RNAs
Important for cell function	Controls gene activity

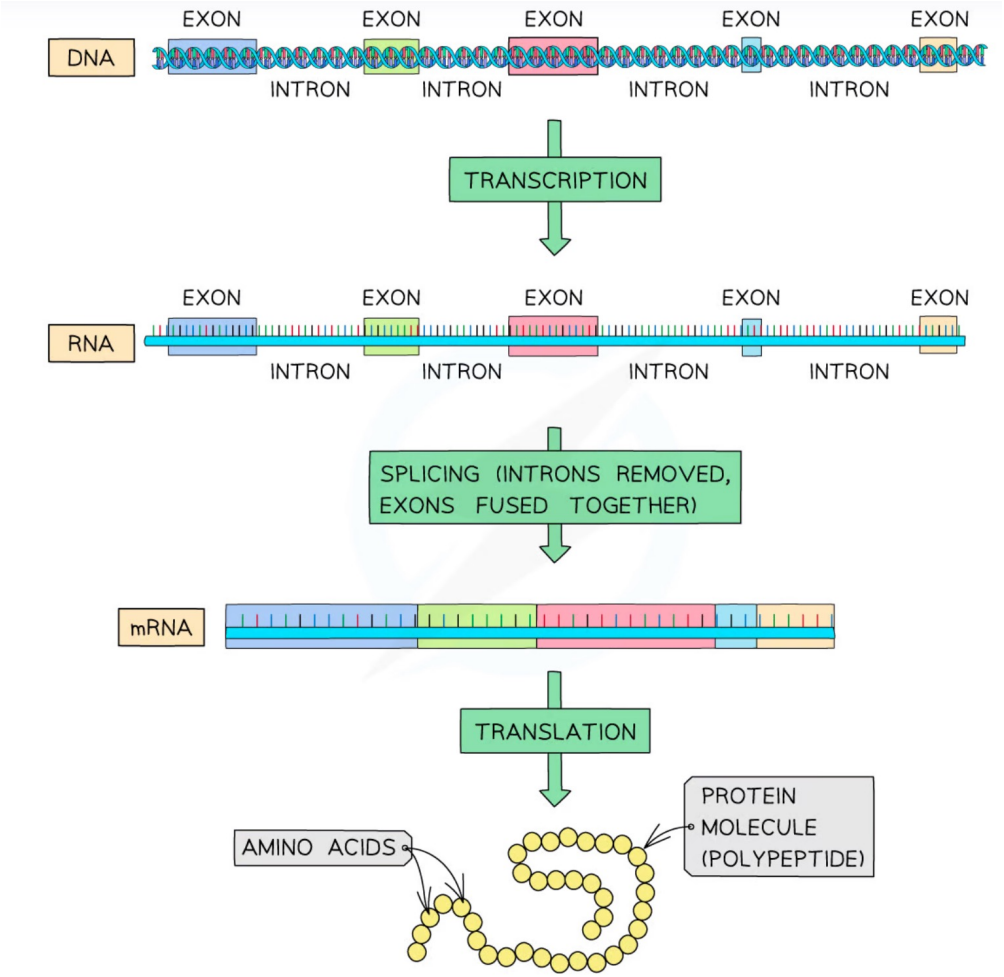
The structure of a gene



The central dogma in genetics

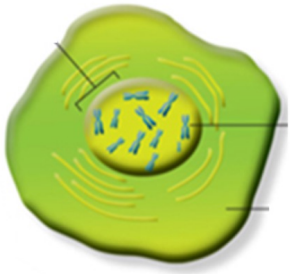


From DNA to mRNA to protein

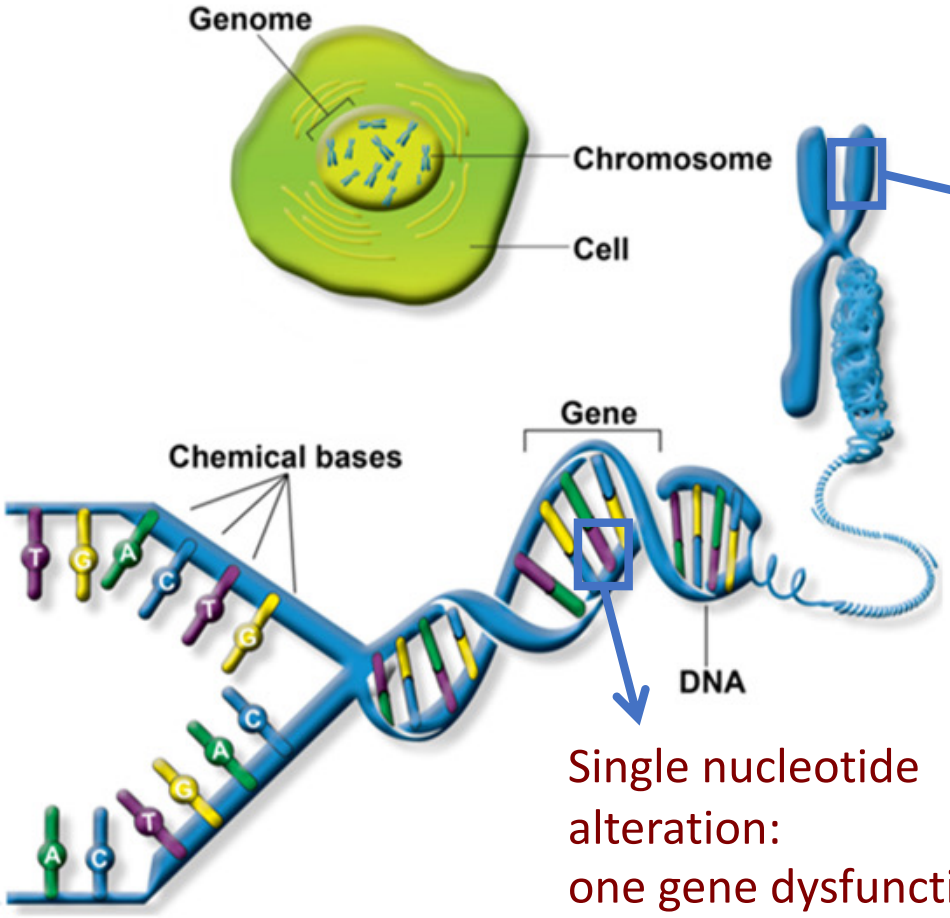


DNA: the recipe for our body

=



Genetic alteration…



Chromosome deletion/duplication: many genes missing or extra

Single nucleotide alteration: one gene dysfunction

Kennis / Erva

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DNA sequenering

DNA Mutations – pathogenic variants

NOW BIG DOG BIT TED

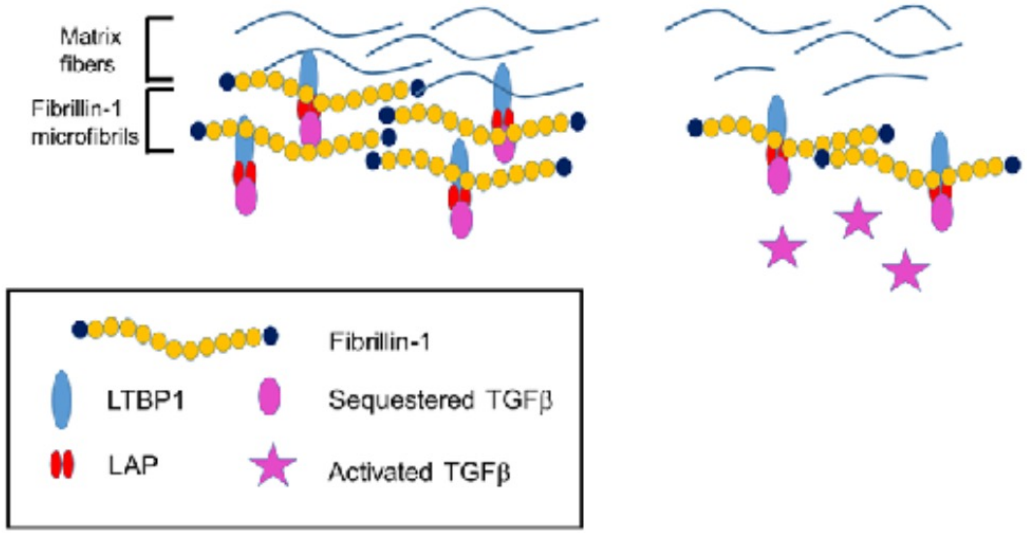
Change one letter (B->J): NOW BIG DOG **J**IT TED

Remove one letter (B): NOW /IGD OGB ITT ED

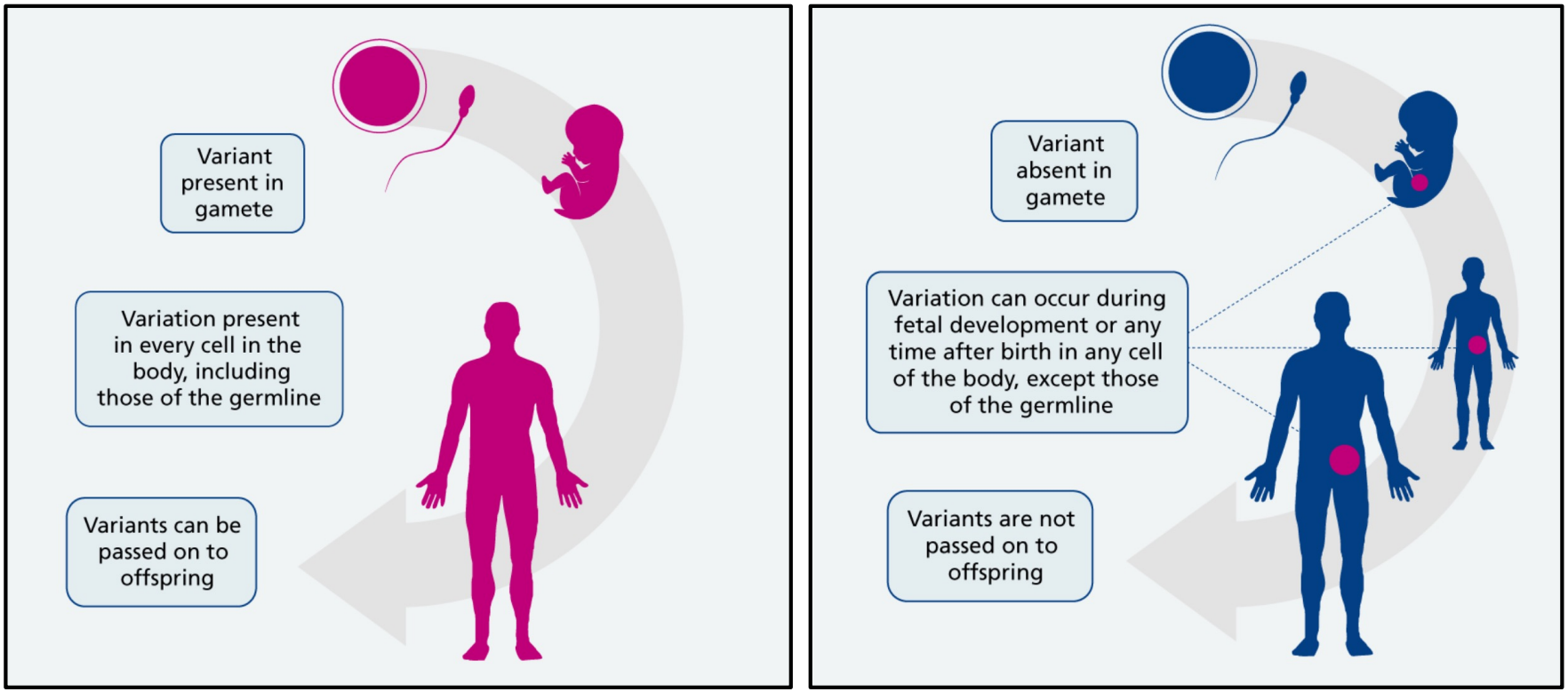
Add one letter (G): NOW BIG GDO GBI TTE D

FBN1 HI and DN classification

	Normal	Haplo-insufficiency (HI)
DNA	No abnormalities	Most commonly nonsense, frameshift, splice site or deletion
Protein	Normal	Not made or degraded



Germline verus somatic

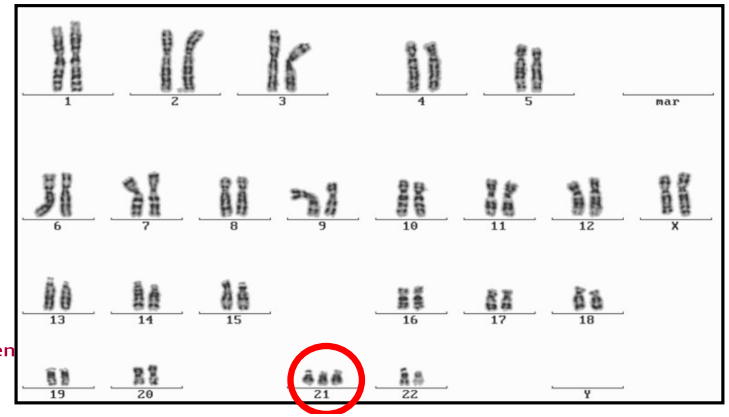


Genetic (R)evolution

1970-

Karyotyping

Down syndrome



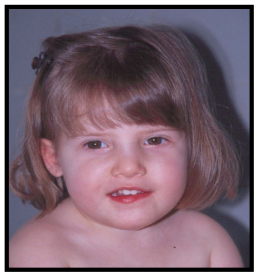
Trisomy 21

Genetic (R)evolution

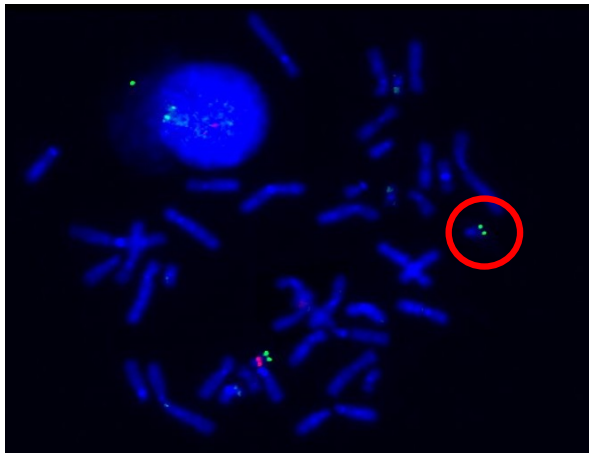
1990-

Karyotyping

FISH



Velocardiofacial syndrome

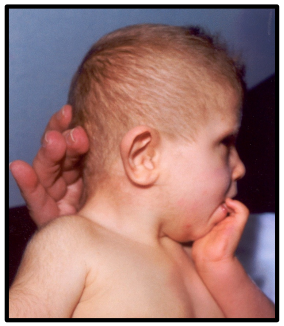


Del 22q11

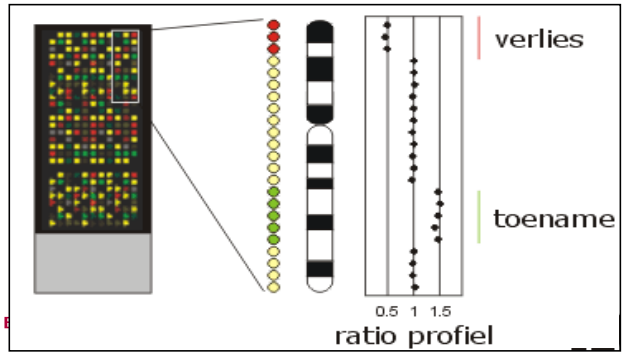
Genetic (R)evolution

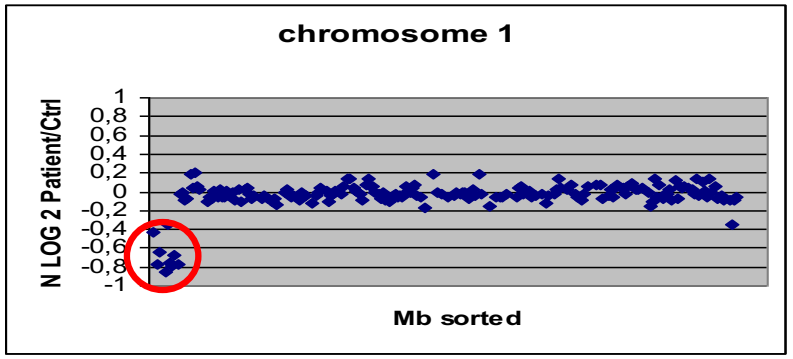
2000-

Karyotyping FISH Array



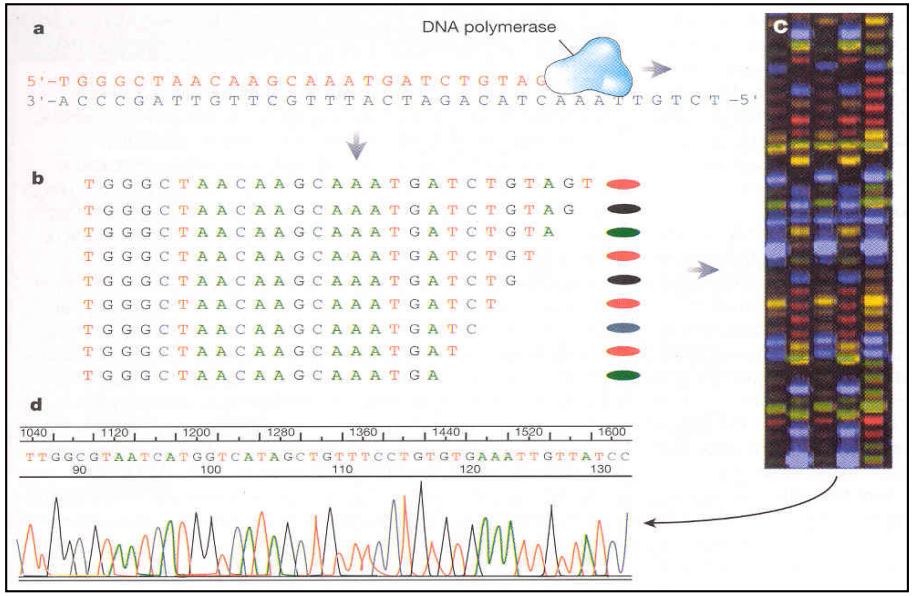
1p36 deletion syndrome





Genetic (R)evolution

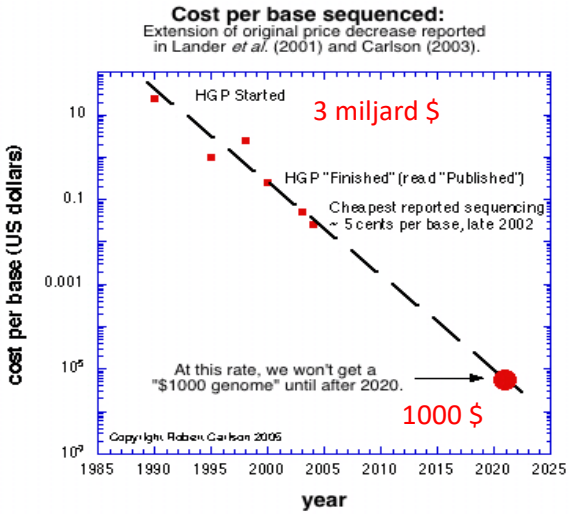
Karyotyping FISH Array Sanger sequencing

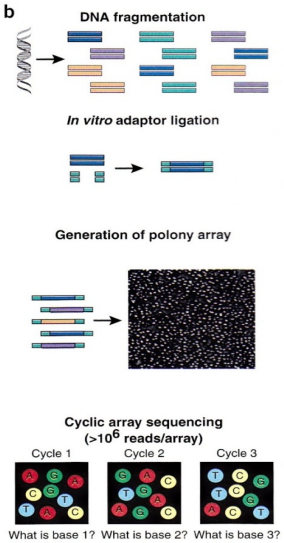


Genetic (R)evolution

2010-

Karyotyping FISH Array Sanger sequencing **Next generation sequencing (NGS)**





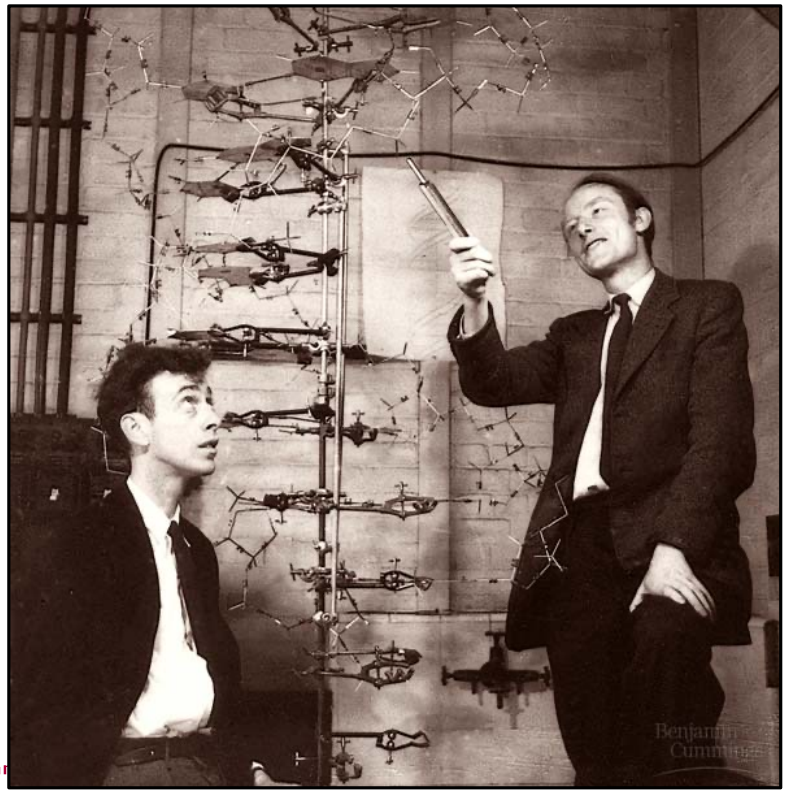
Genetic (R)evolution

- goal international initiative (°1990): determine sequence of total human genome (3 x 10^9 bp) before 2005
- intial 'working draft' sequence: published 2001
- 'final' sequence (95% of total sequence - 99.9% accuracy): april 2003
- precise localisation and structural unraveling of the 21.000 human genes

http://www.ncbi.nlm.nih.gov

Celera genomics

Genetics: “bench” or “bedside” ?



Science or health care?

Genetic technology is advancing

- PAST

Gene A → Gene B → … → Genetic Result

$ $ $

Sanger sequencing

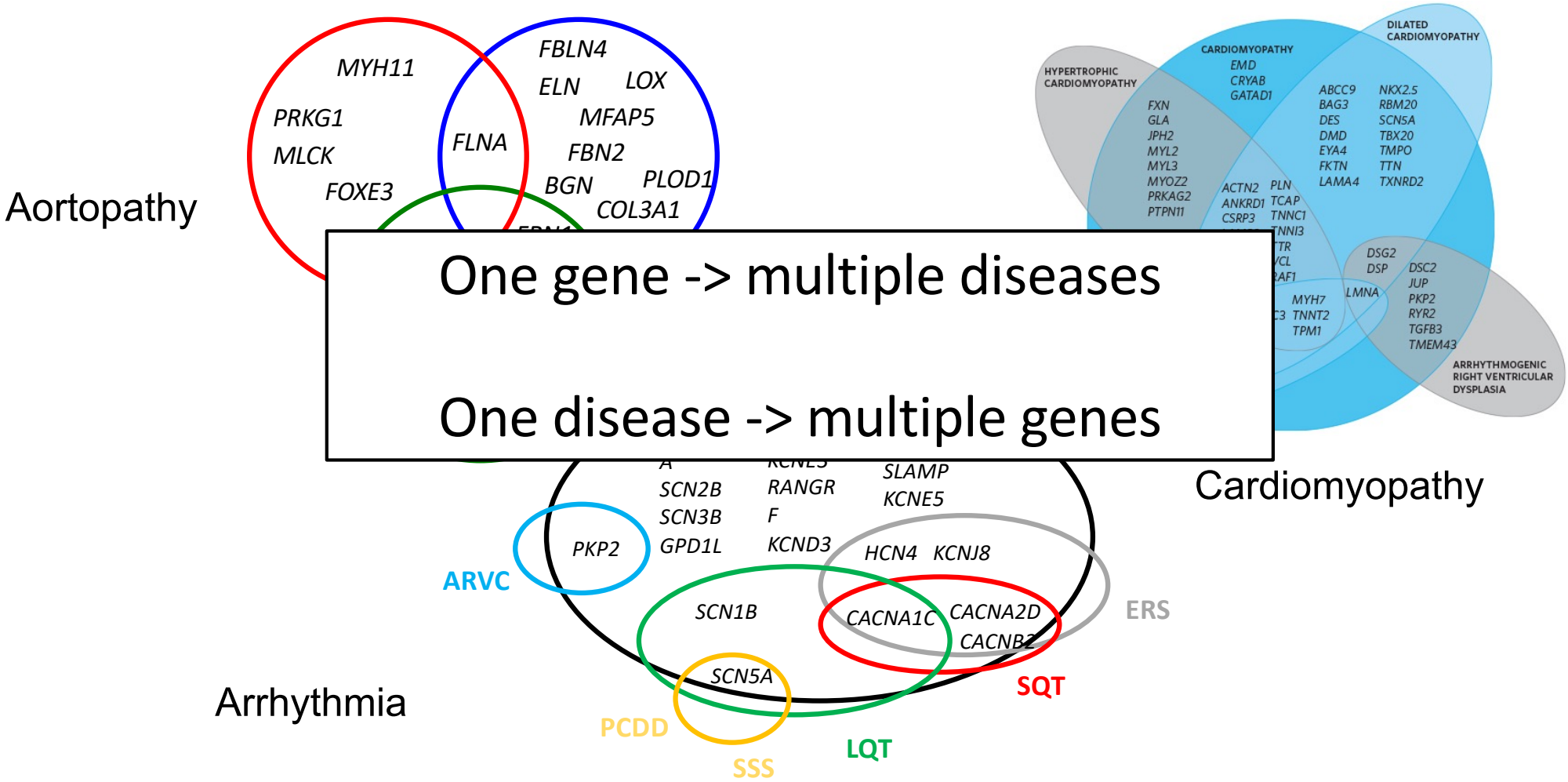
- PRESENT

Gene A
Gene B
Gene C
…

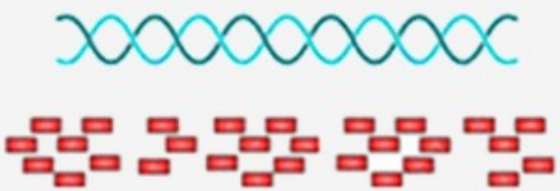
$

Next Generation Sequencing (NGS)

Cardiogenetic testing

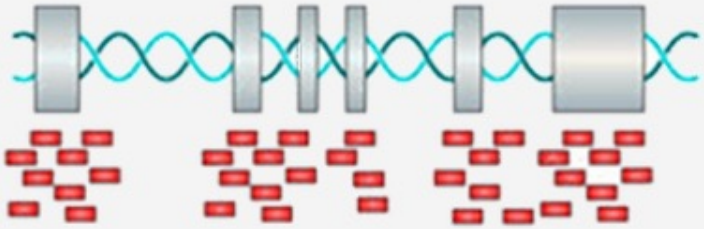


Whole genome sequencing



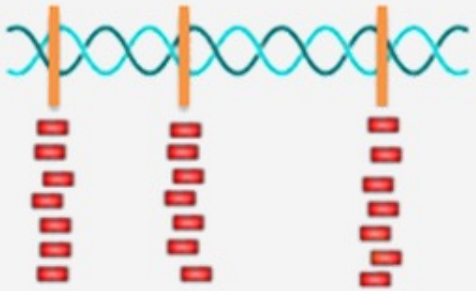
- Sequencing region : whole genome
- Sequencing Depth: >30X
- Covers everything – can identify all kinds of variants including SNPs, INDELs and SV.

Whole exome sequencing



- Sequencing region: whole exome
- Sequencing Depth : >50X ~ 100X
- Identify all kinds of variants including SNPs, INDELs and SV in coding region.
- Cost effective

Targeted sequencing



- Sequencing region: specific regions (could be customized)
- Sequencing Depth : >500X
- Identify all kinds of variants including SNPs, INDELs in specific regions
- Most Cost effective

University of Antwerp
GENOMED | Genomics in Medicine
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Next-generation sequencing Revolutionized Genetic Research

VASCERN

Dr Arvid Suls

Saturday 21st of January

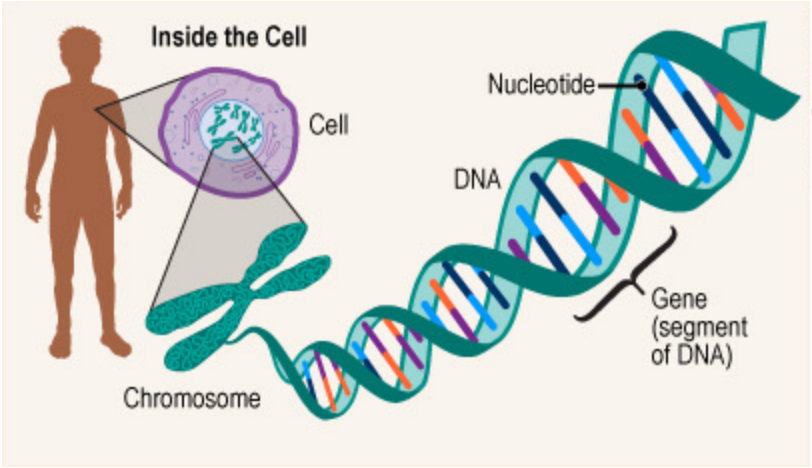
Centrum Medische Genetica Antwerpen

UZA

Human Genome

Sequence of 3.1Gb (billion) bases or letters (A C G T)

(= building instructions)



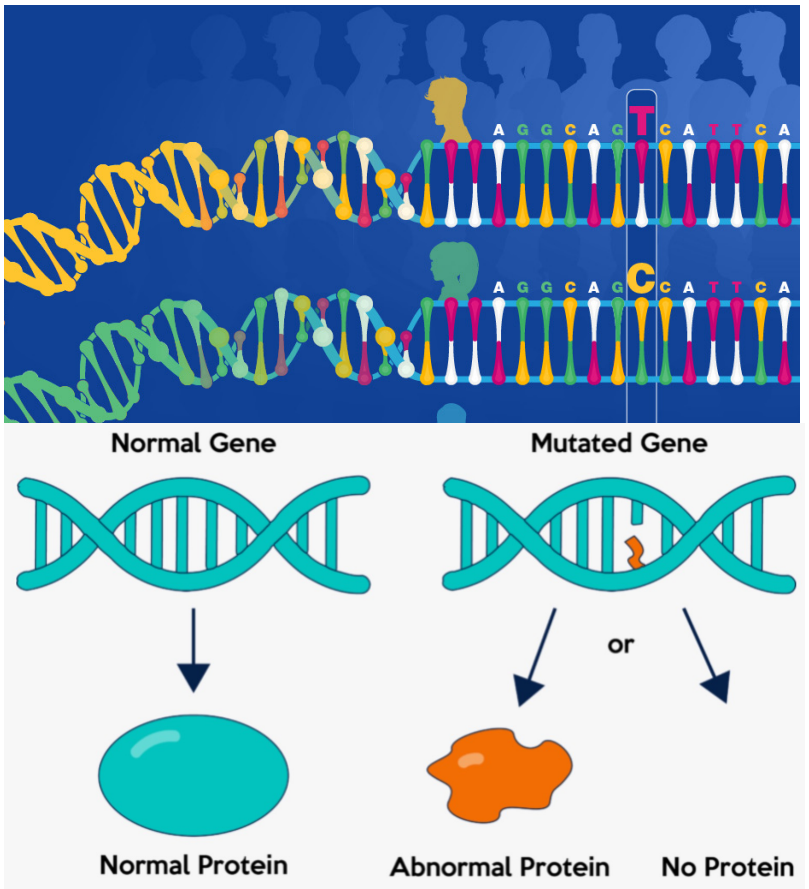
Genetic disorder research

Find causal error(s) in the sequence

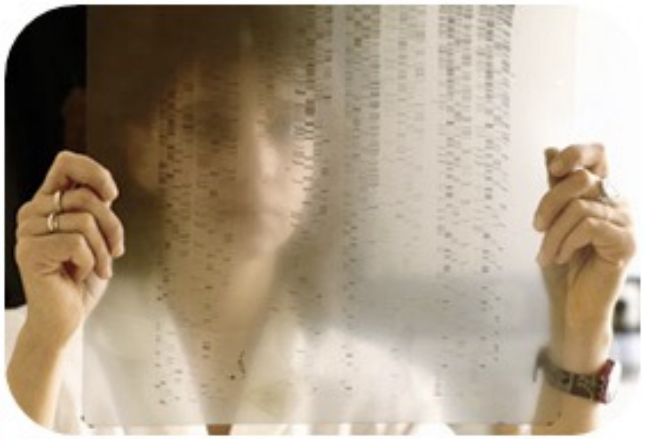
(= find needle in a haystack)

➔Comparison of sequence between healthy and affected persons

➔Powerful technologies needed

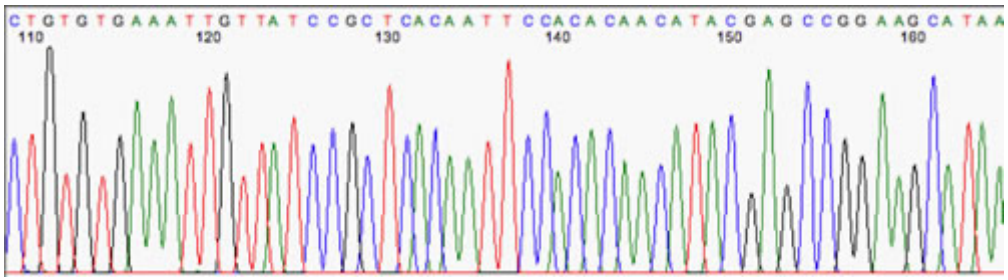


Pre Next-Generation sequencing technologies



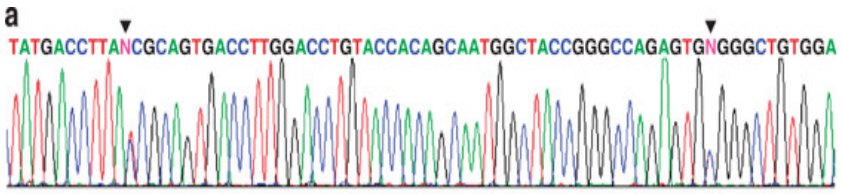
Chain-termination (1980s)
Autoradiography on X-ray film

Dye-termination (1990s)
Automatic capillary sequencer
= Sanger Sequencing



Pre Next-Generation sequencing technologies

- **Sanger sequencing**
  - Check one gene at a time
  - One human genome: >15 years
  - Cost: ~3 billion €



➔ Manual analysis of sequences

➔ Genetic research was limited to study candidate genes/pathways

Next-Generation Sequencing technologies (2005-…)

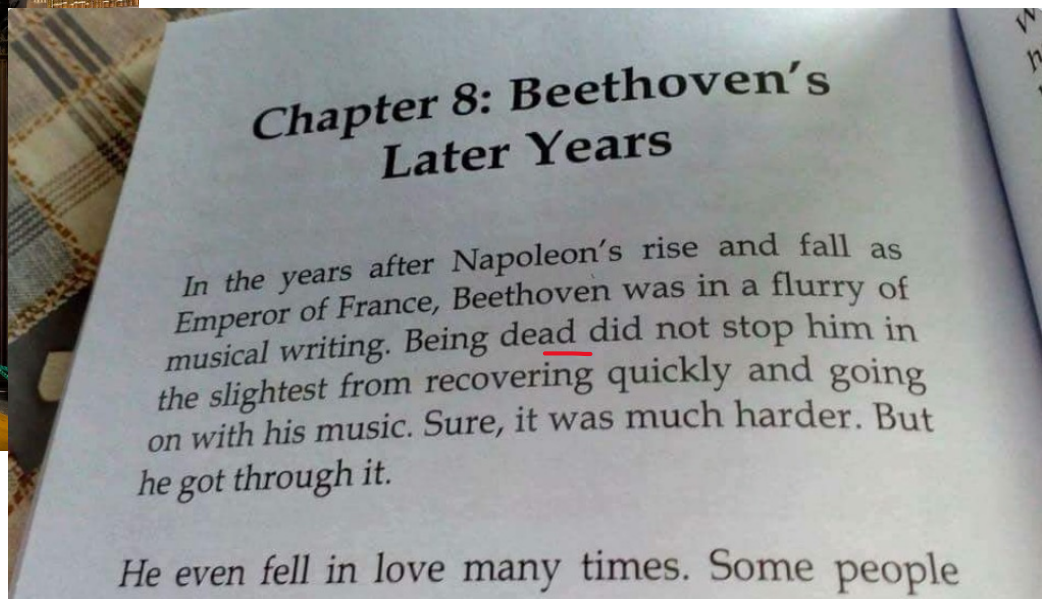
- **Massive parallel sequencing**
  - Sequence all genes or even full genome at a time
  - Multiple individuals at a time

- **Illumina NovaSeq X (released in 2022)**
  - 64 full human genomes in 48h
  - Cost: ~200 €

➔ Hypothesis free genetic research is possible (computational analysis)

Finding a typo in a library

Sanger Sequening = one person reading
NGS = billion persons join forces to read



How does Next-Generation Sequencing work?

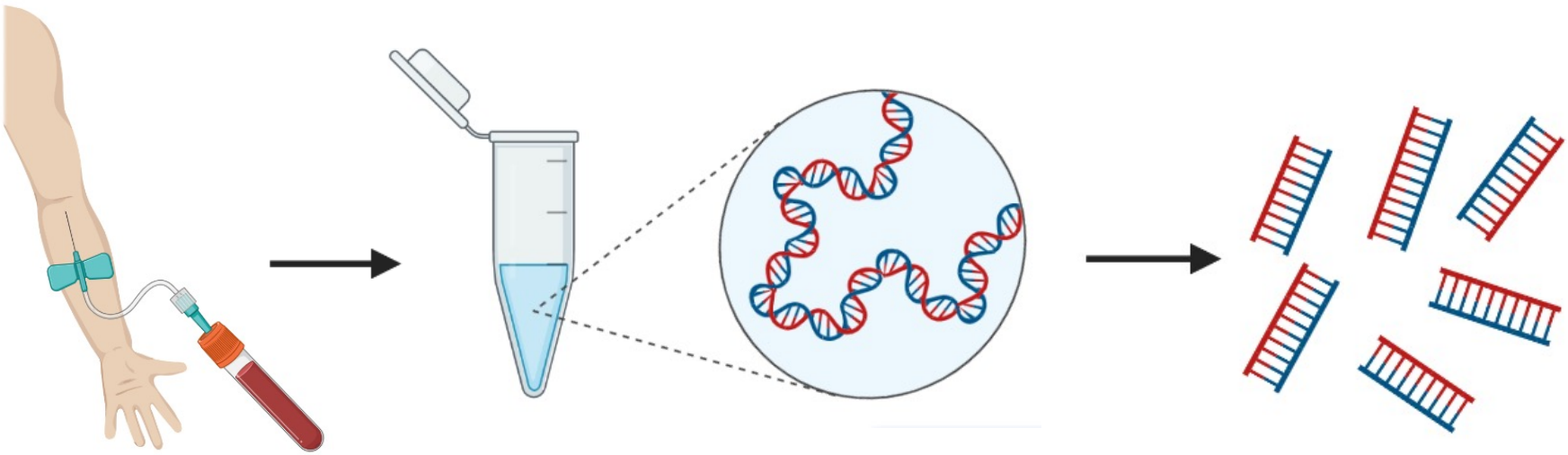
1. **Library preparation**

2. **Library hybridisation and amplification on flow cell**

3. **Library sequencing**

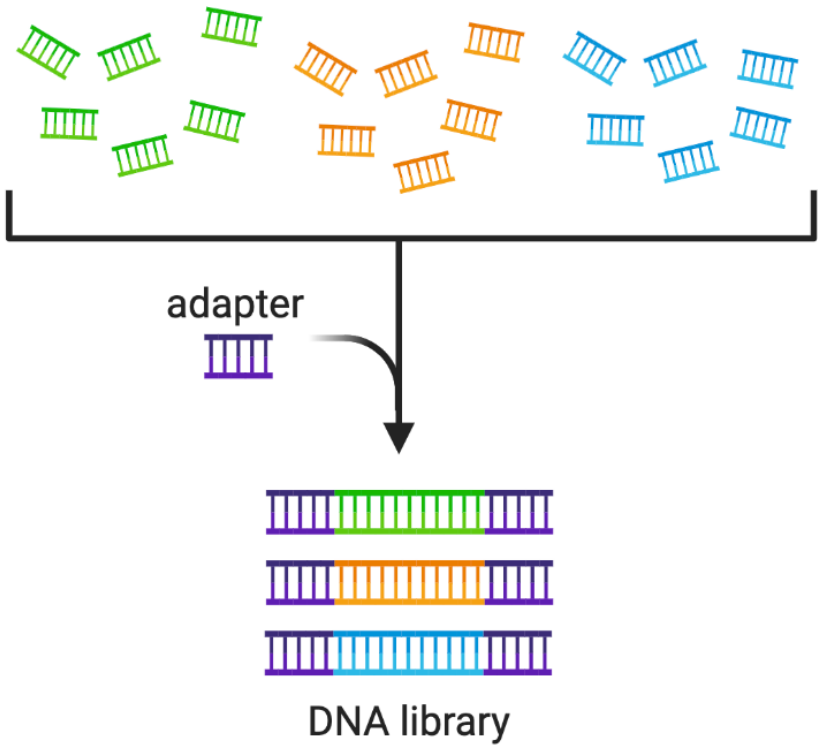
4. **Alignment and data analysis**

Library preparation (1) – extraction/fragmentation



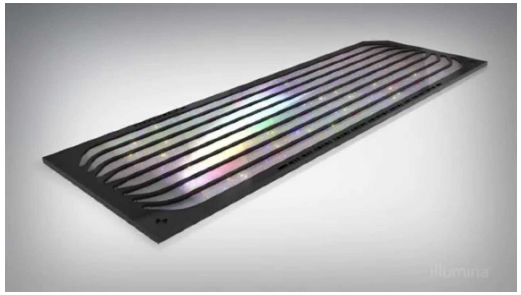
Biorender was used to generate the figures.

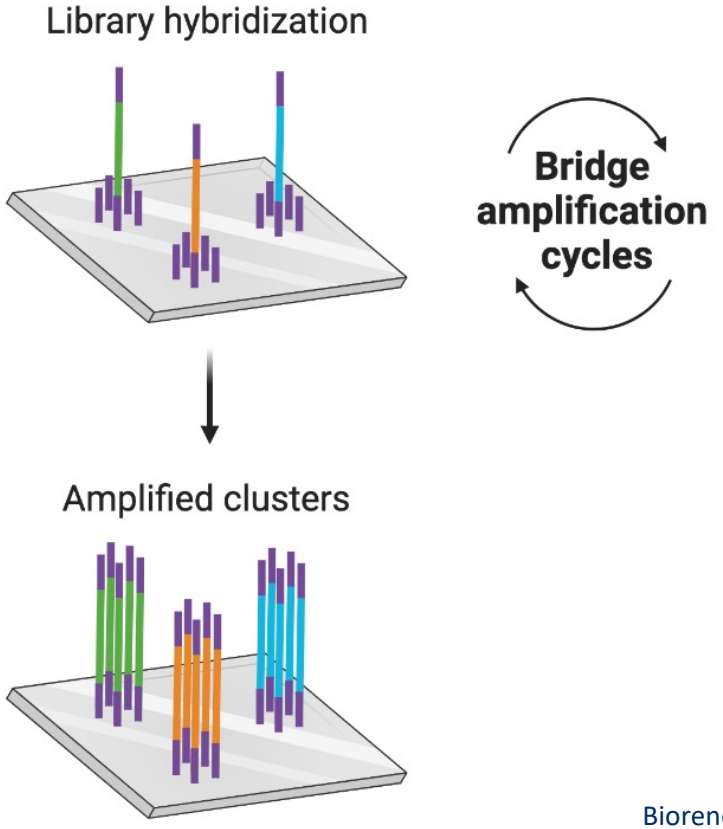
Library preparation (2) – adaptor ligation



Biorender was used to generate the figures.

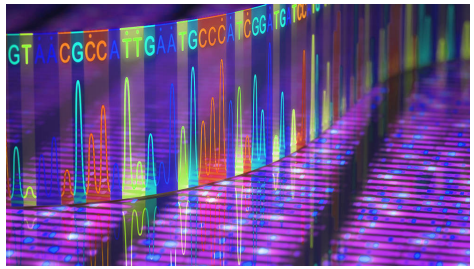
Library hybridisation and amplification on the flow cell

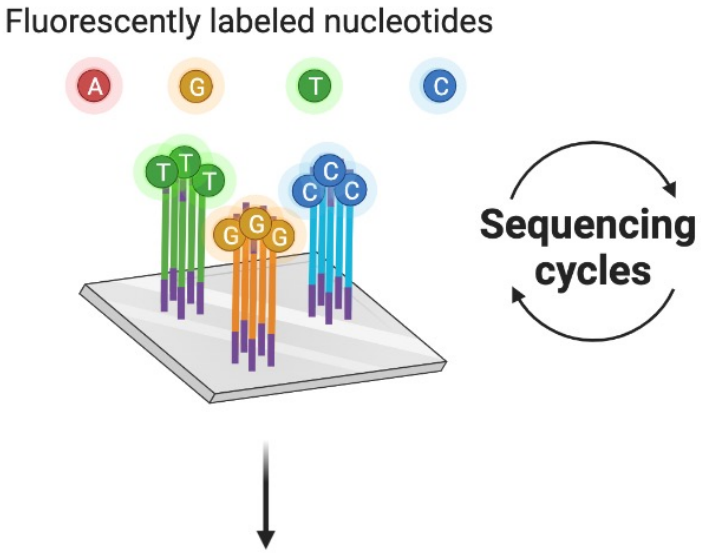




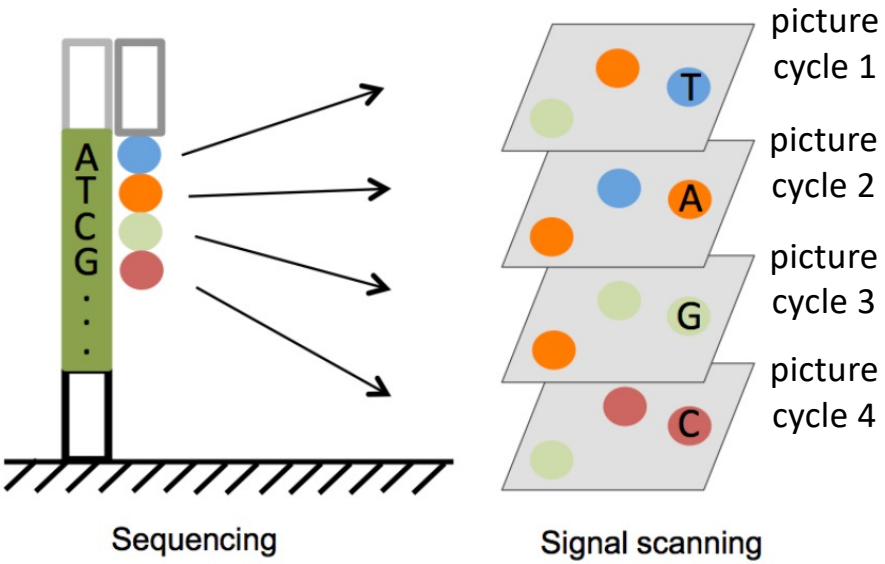
Biorender was used to generate the figures.

DNA library sequencing

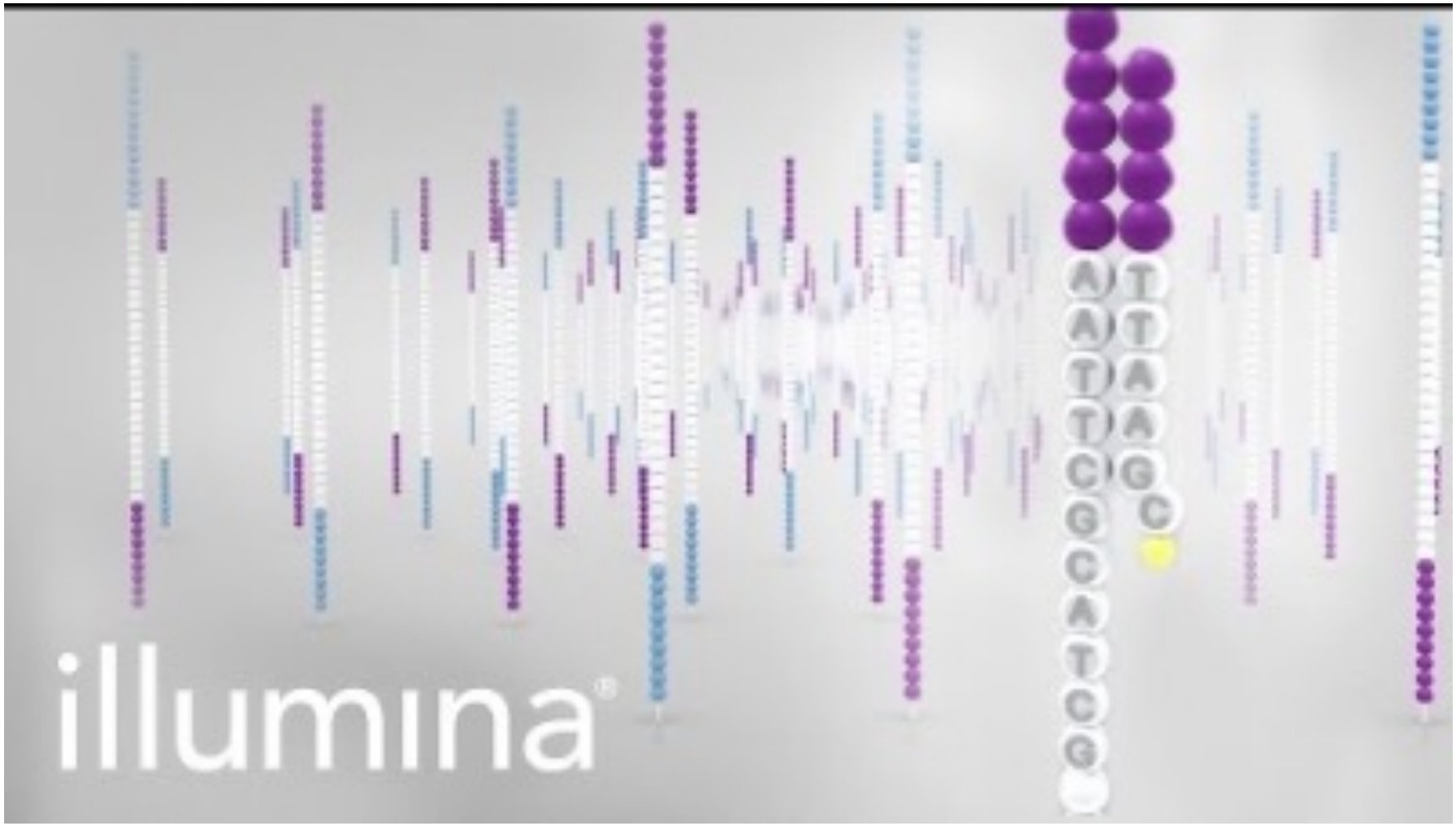




Data collection (= picture of lightspots taken per cycle)

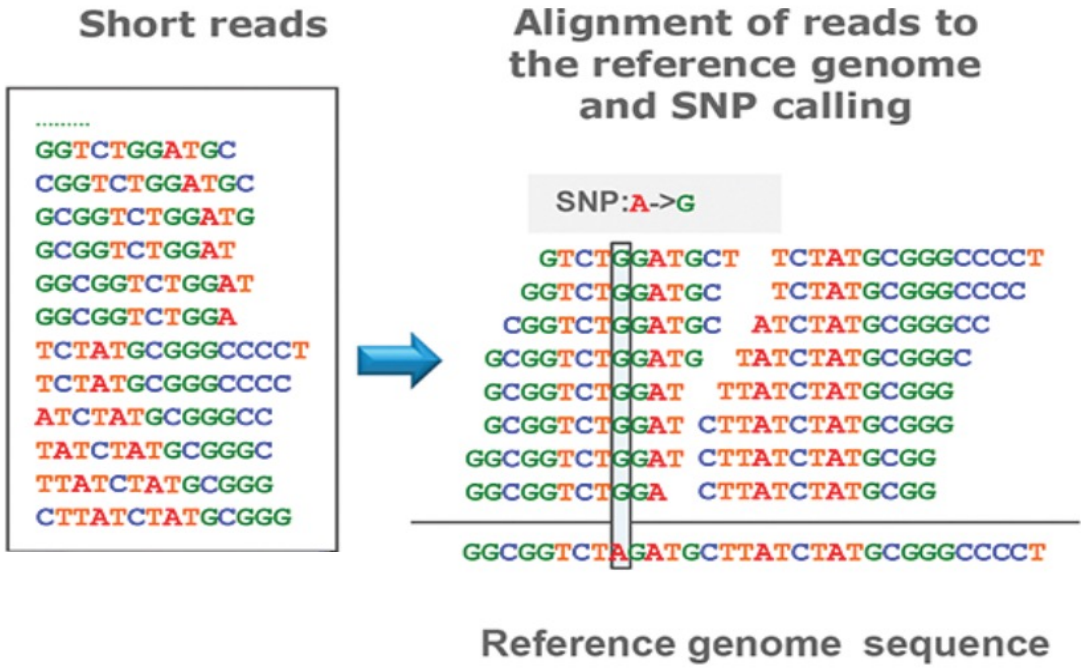


Sequencing read = TAGC…


illumina®

How are variants identified?

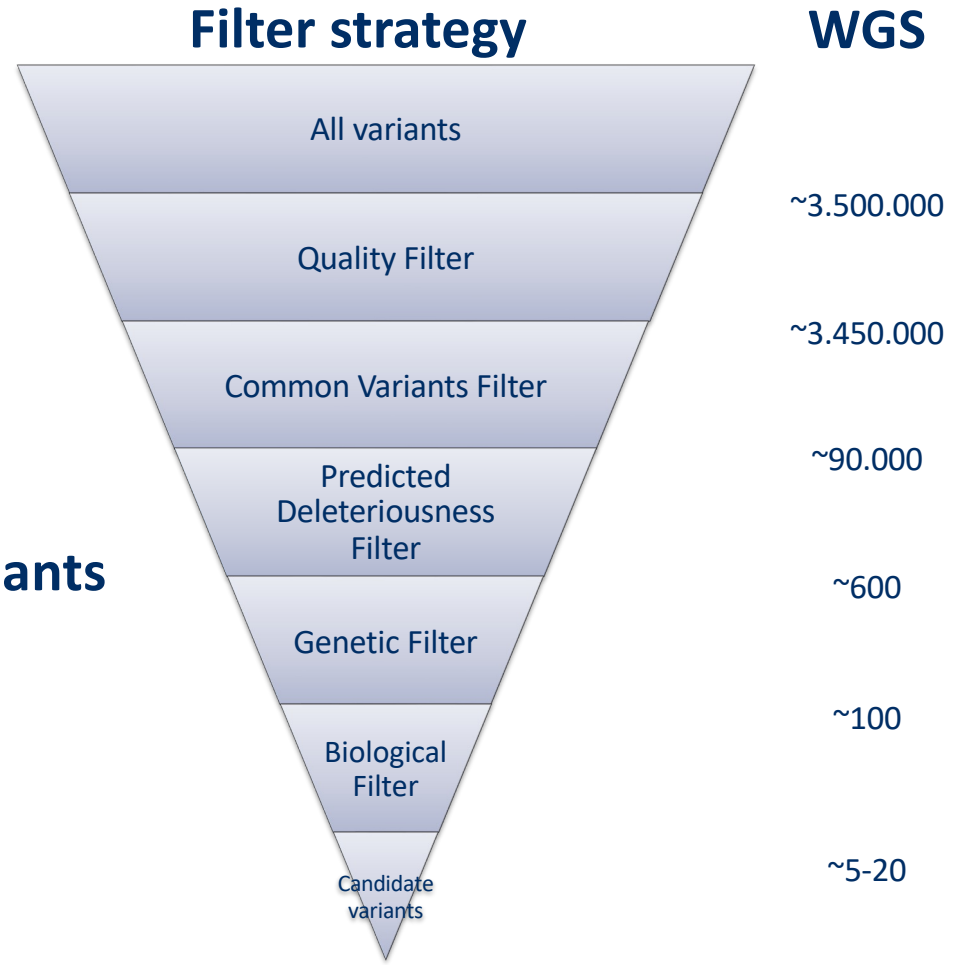
- **Compare with reference genome (= healthy individual)**



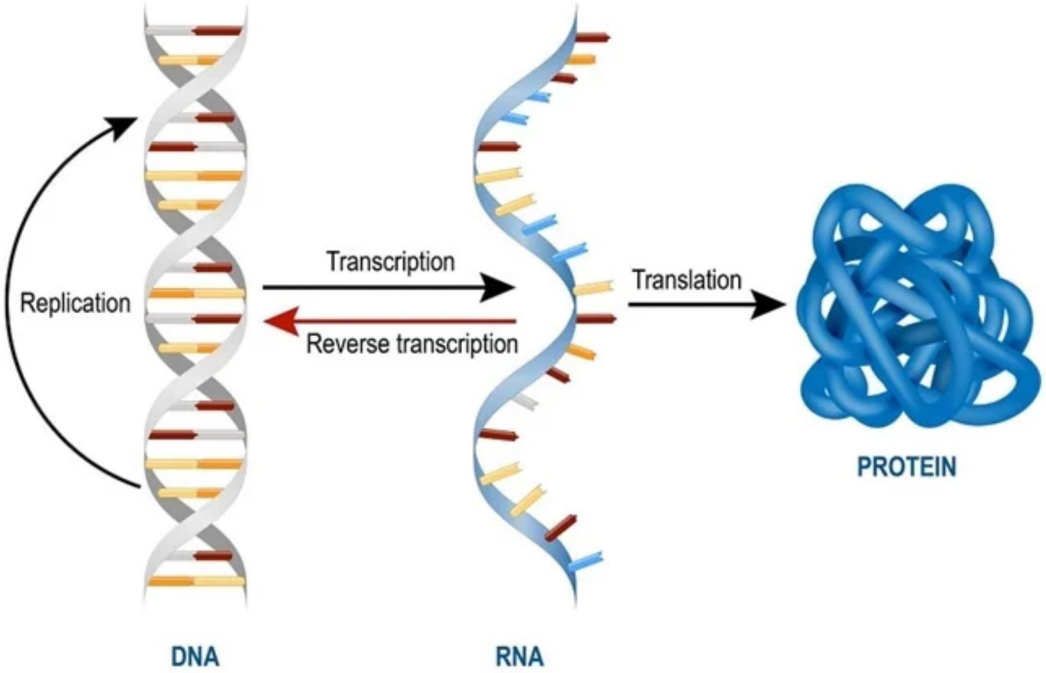
Interpretation of the data

Many variants are harmless

➔ use filter strategies to reduce the number of candidate causal variants



WGS (= DNASeq) versus RNASeq

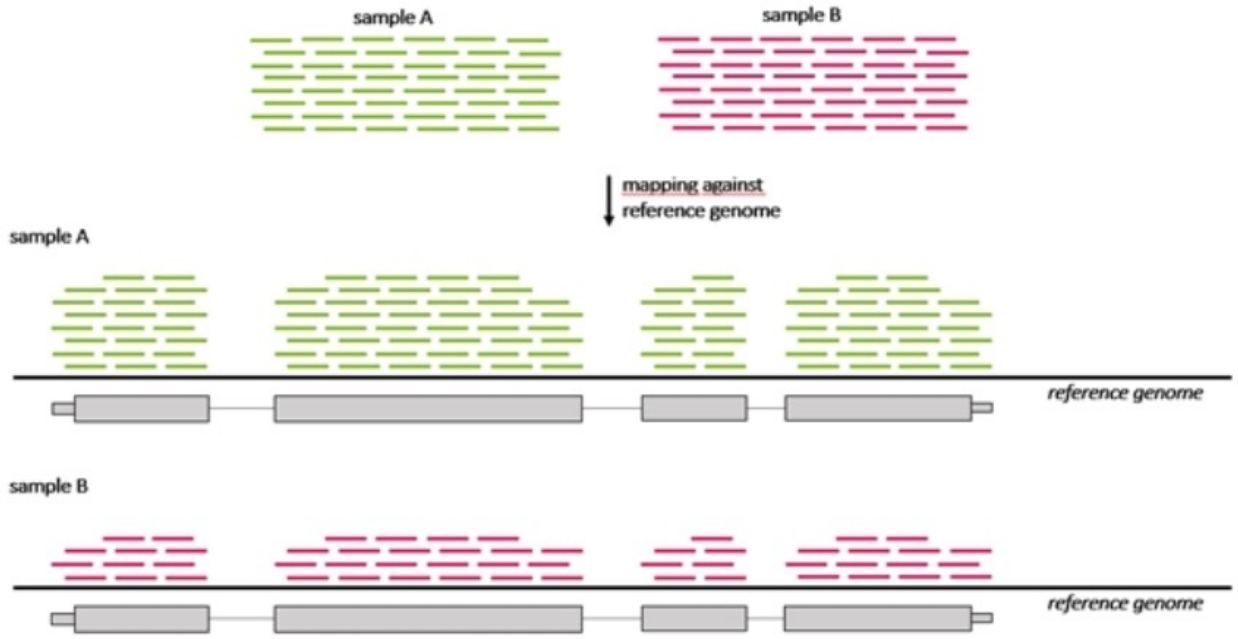


➔ RNA is a measure for which blocks (and how many) are produced by a cell.

➔ WGS= reading vs RNASeq= counting

RNASeq – differential expression analysis

= determine changes in amount of RNA between patient and controls by counting NGS reads



Changes #RNA molecules could pinpoint the cause

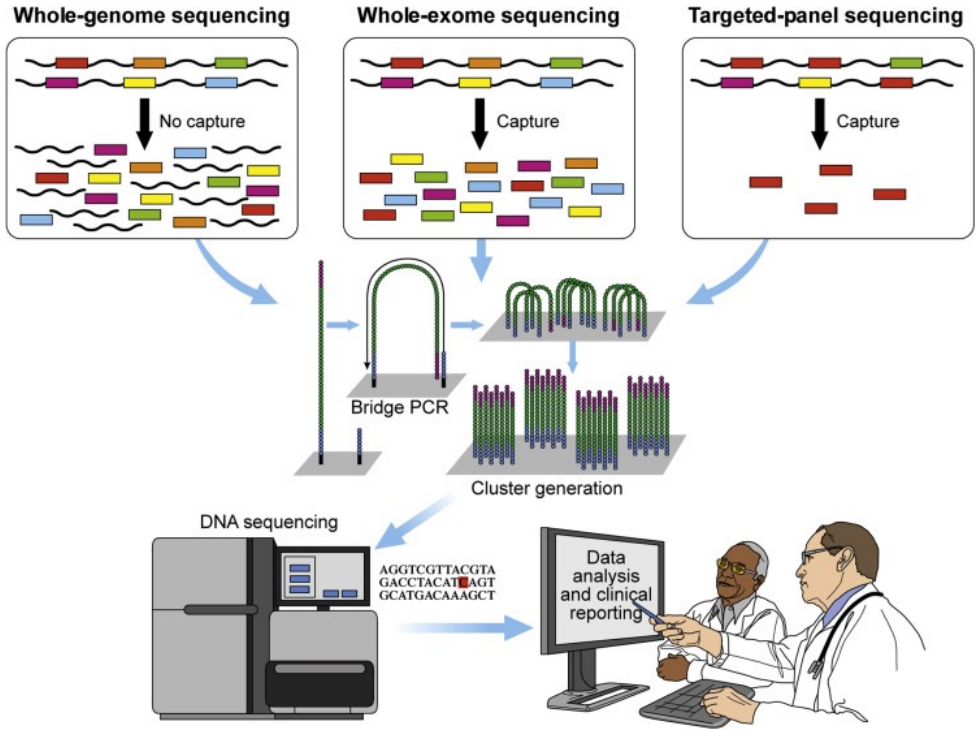
Example WES in research setting: Brain aneurysm

Current situation:

- **No gene panel available**
- **First degree relatives are at risk**
- **Genetic basis mostly unknown**

Research:

- **Cohort of 400 patients**
- **WES to find variants in**
  - Known genes
  - Unknown genes



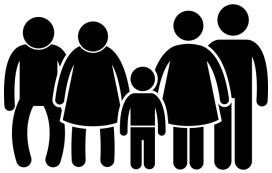
WES in research setting: Brain aneurysms

- **Filter approach depending on family**

De novo or AR variants

- Young individual
- Both unaffected parents available

Shared variants



- Familial cases
- At least 2 DNA samples available

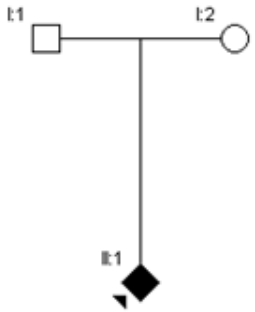
No filtering based on family members possible

- No family members available

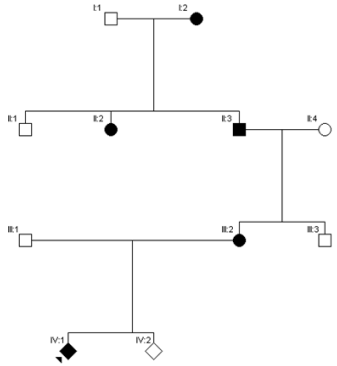
WES in research setting: Brain aneurysms

- **Filter approach depending on familial situation**

De novo or AR variants?



Shared variants?

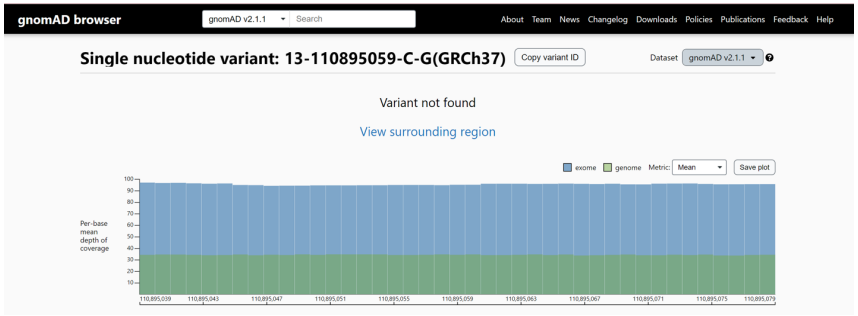


No filtering based on family members possible

WES in research setting: Brain aneurysms

- **Filter rare variants that are predicted to be pathogenic**

Databases of healthy persons



Prediction programs

- Effect on protein function?
- Conservation during evolution?

Protein prediction	0.105 SIFT	0.966 POLYPHEN	0.49 MUT TASTER	0.0 MUT ASSESSOR
	-2.69 FATHMM	-3.19 PROVEAN	0.582 VEST3	23.5 CADD
	0.636 DANN	0.922 FATHMM MKL	-- EIGEN PHRED	5.14 GERP
	1.026 PHYLO P	0.997 PHASTCONS	14.114 SIPHY	0.232 EVE

WES in research setting: Brain aneurysms

- **Investigation of promising variants**

OMIM: data base of disease causing genes

➢ **Is this gene already known to cause disease?**

Alternative titles; symbols

COLLAGEN OF BASEMENT MEMBRANE, ALPHA-2 CHAIN

HGNC Approved Gene Symbol: COL4A2

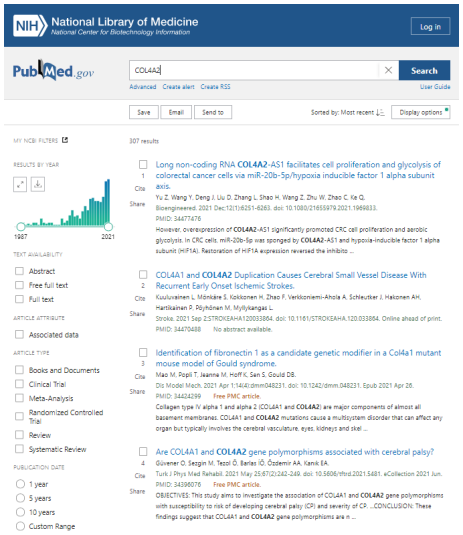
Cytogenetic location: 13q34 Genomic coordinates (GRCh38): 13:110,307,283-110,513,208 (from NCBI)

Gene-Phenotype Relationships

Location	Phenotype	Phenotype MIM number	Inheritance	Phenotype mapping key
13q34	Brain small vessel disease 2	614483	AD	3
	{Hemorrhage, intracerebral, susceptibility to}	614519		3

PubMed: medical and scientific publications

➢ **What research has been done on this gene?**

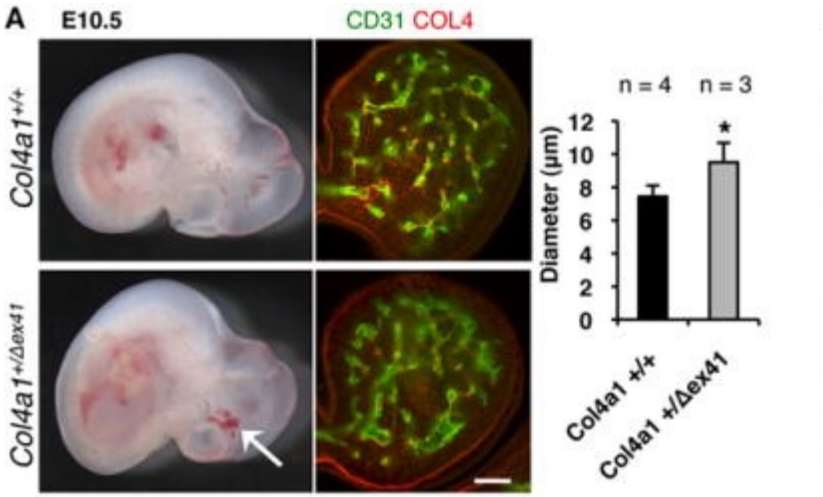


WES in research setting: Brain aneurysms

- **Investigation of promising variants**

Animal models of gene dysfunction

➢ What happens if this gene does not work well?



Van: Jeanne et al. Circulation. 2015

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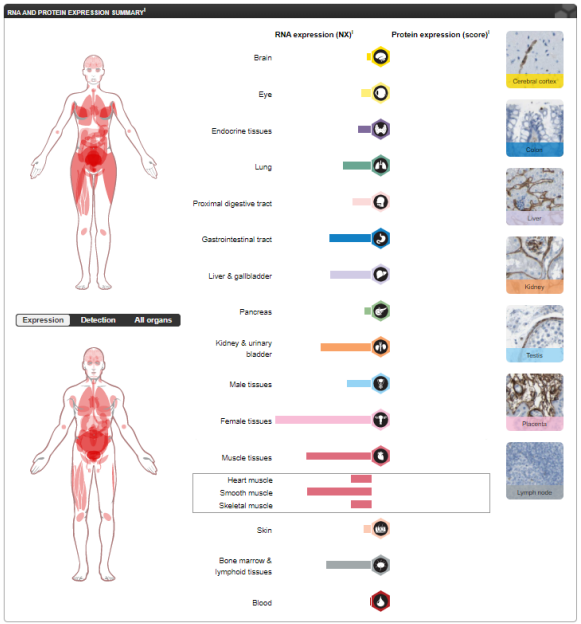
Gene expression map

➢ Where does this gene has a function?



WES in research setting: Brain aneurysms

- **Further research**

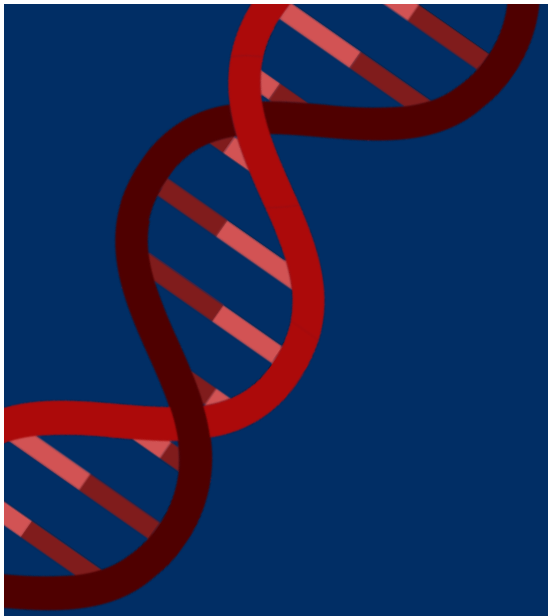
Functional testing

➢ **Can we prove that this variant causes this disease?**

- Animal models
- Cell models
- …

Publication

➢ **Do other researchers find the same or similar variants in this gene?**



GEMS

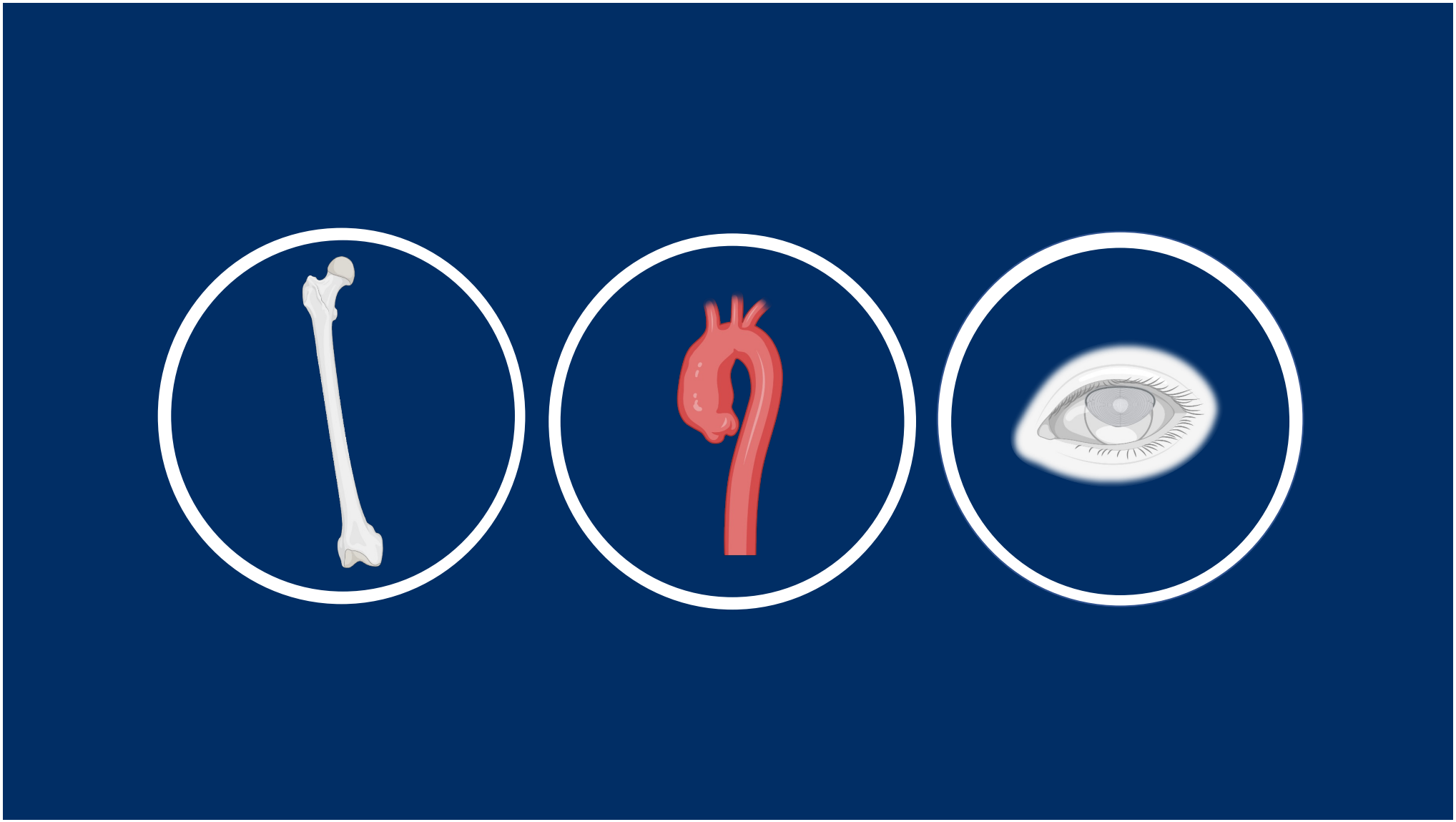
Genome-wide epistasis for cardiovascular severity in Marfan study design:
patient organization driven research

Lotte Van Den Heuvel

VASCERN exchange visit 21/01/2023

Marfan Syndrome

1/3000 - 5000

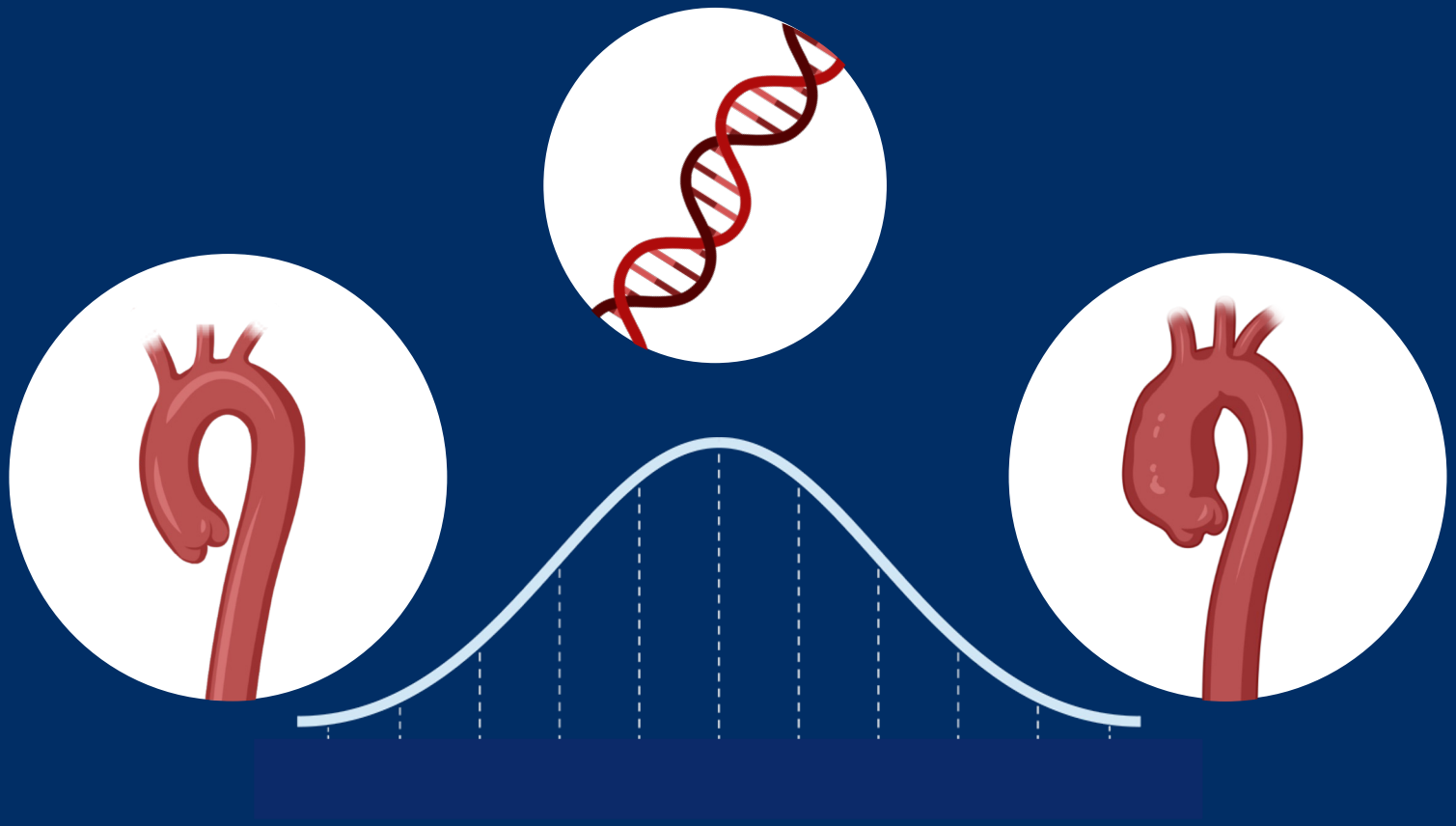


Thoracic aortic aneurysm dissection

80%
mortality

Need for new therapies

FBN1 c.7754T>C p.Ile2585Thr



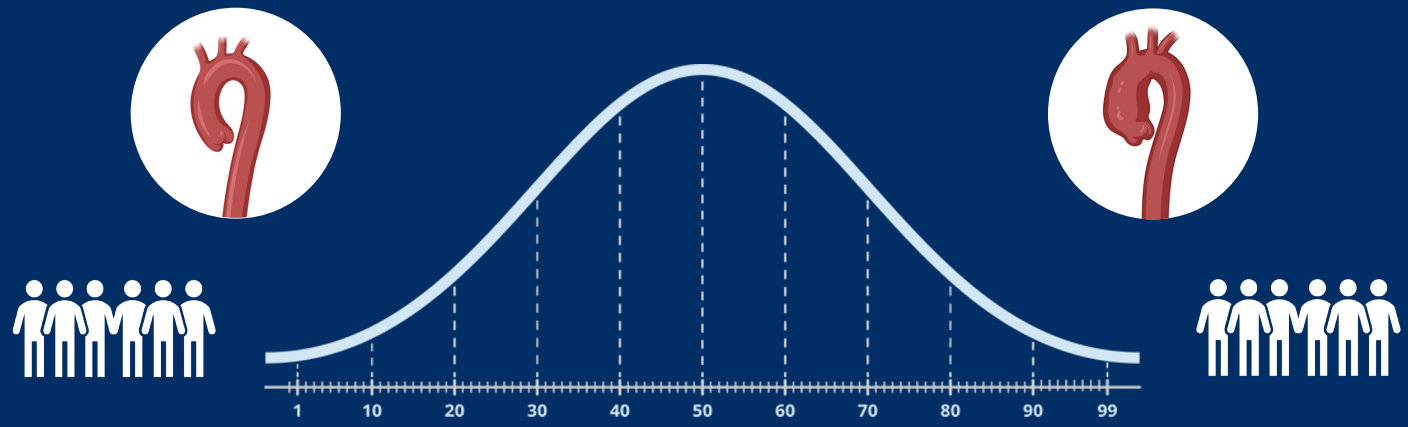
GEMS study

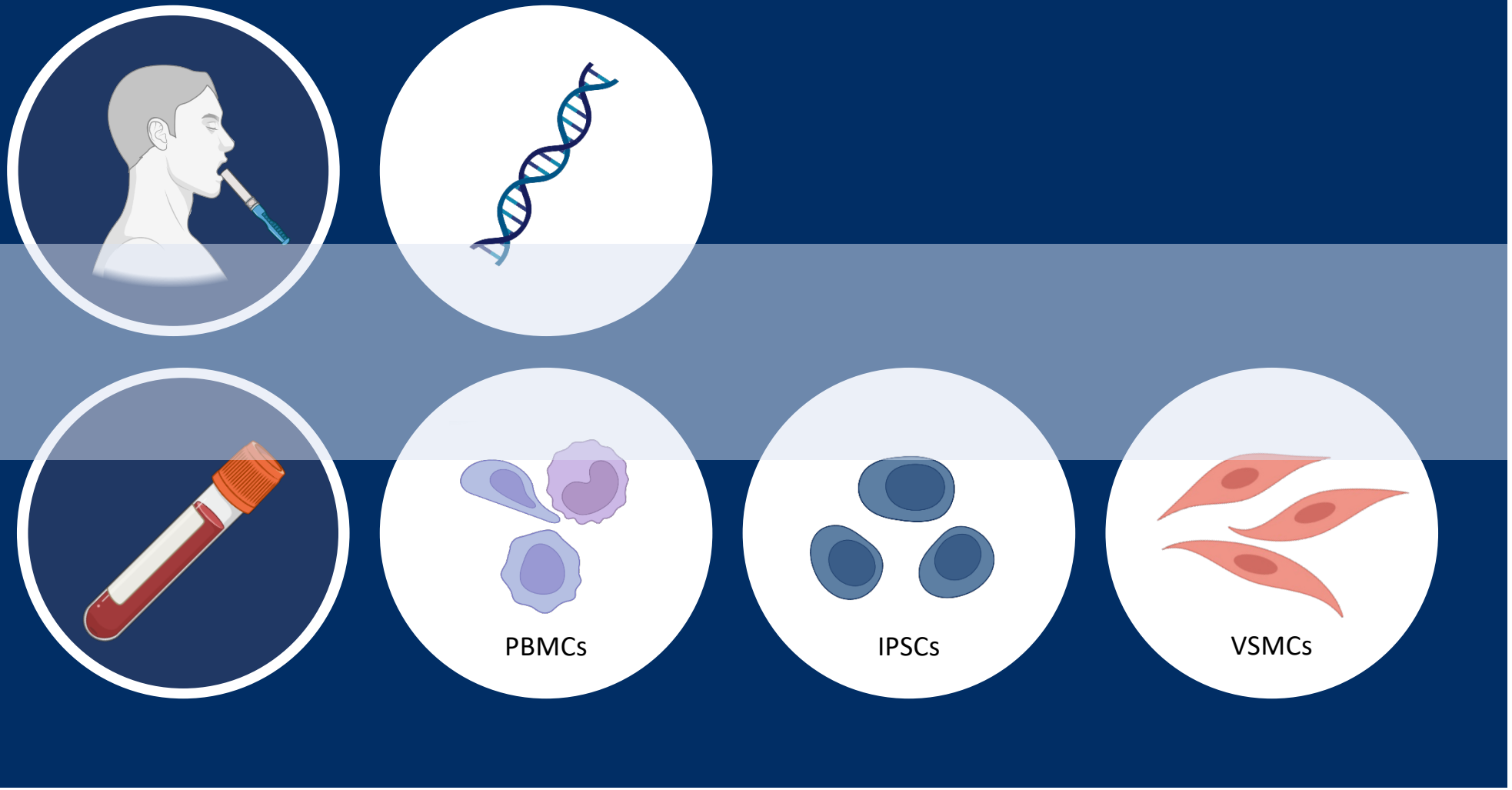
Most common missense variant: *FBN1* p.Ile2585Thr

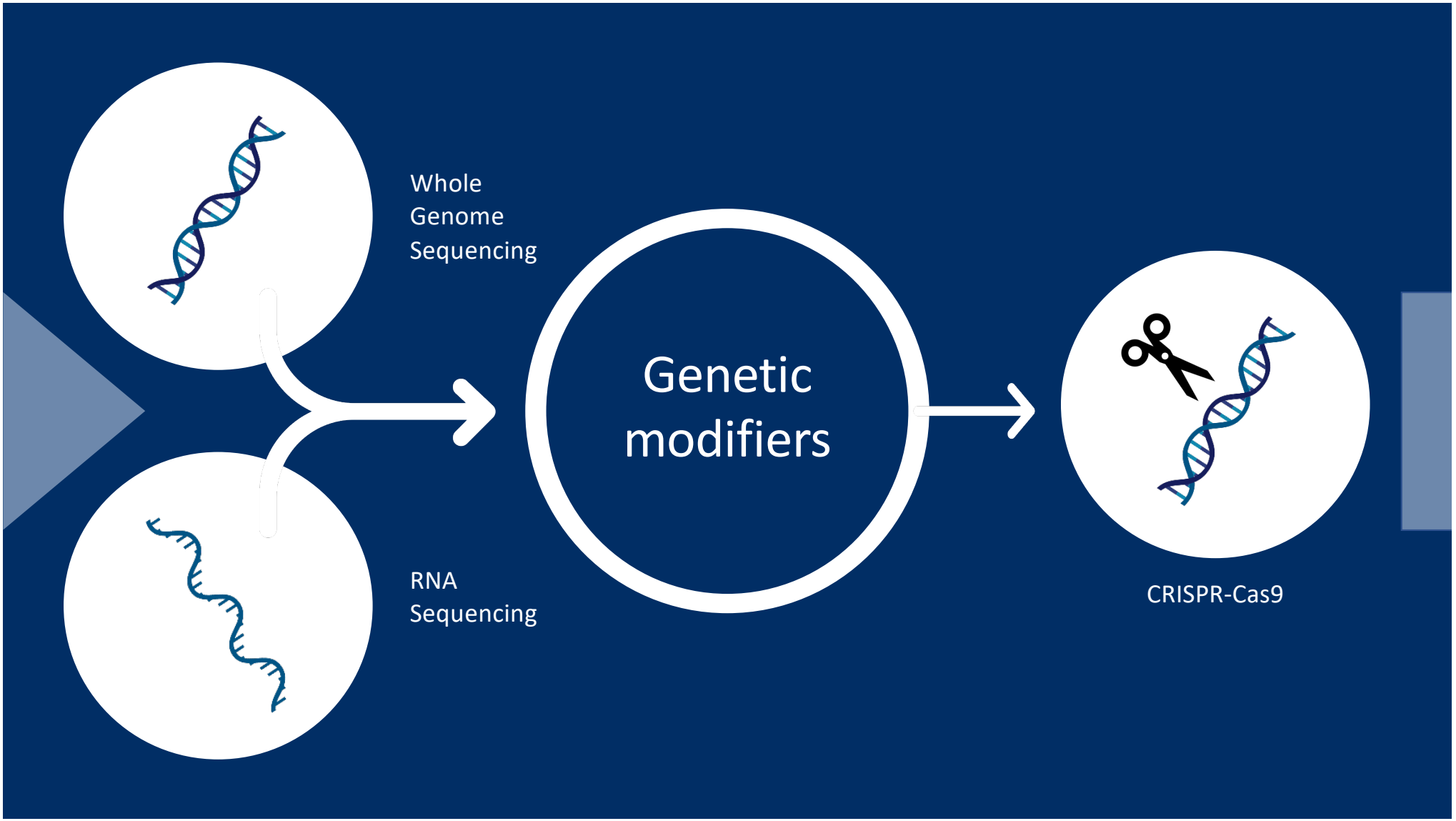
Cohort: > 200 mutation carriers

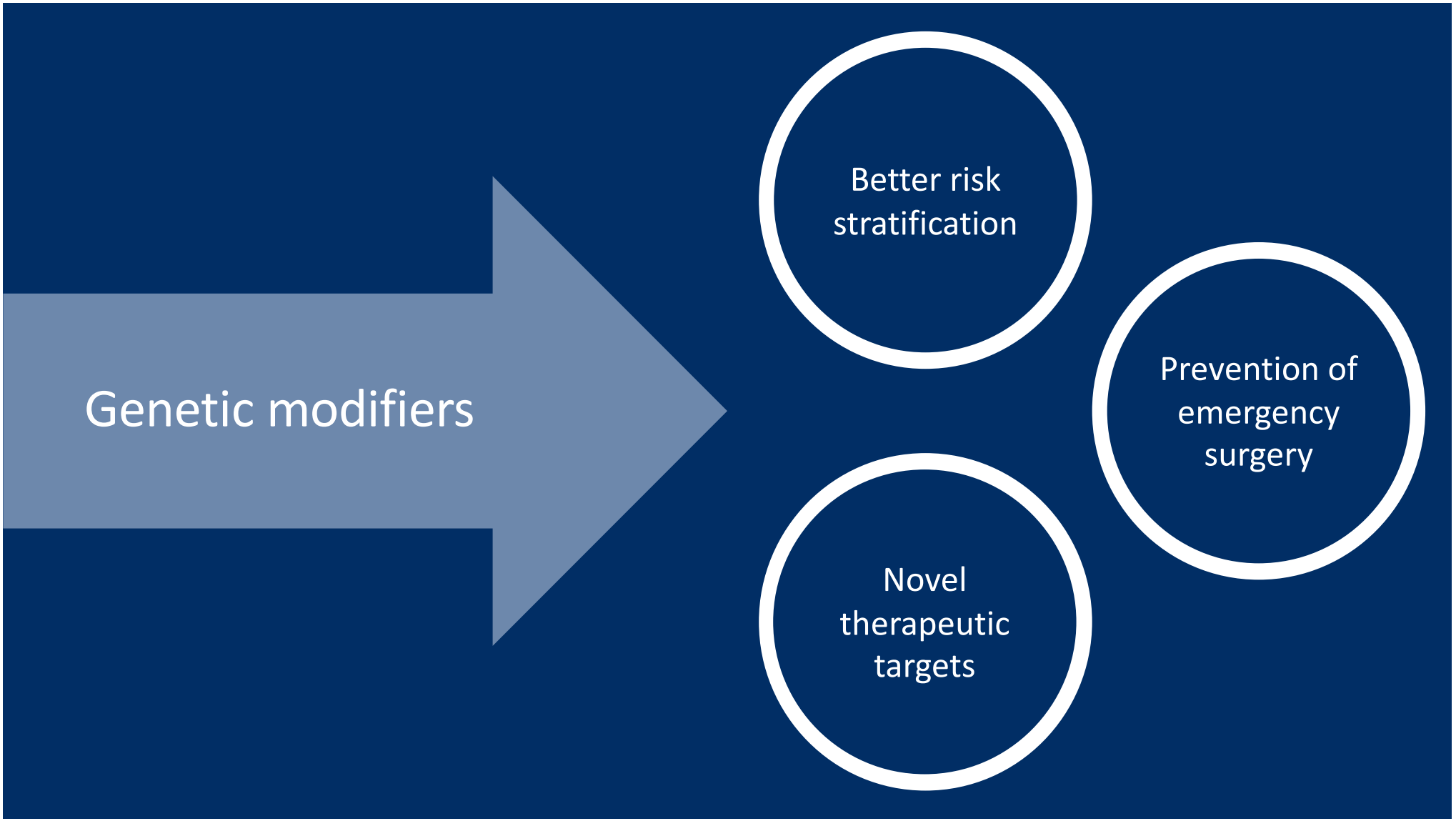
GnomAD: asymptomatic cases

Clinical phenotyping: extreme variability demonstrated




PBMCs
IPSCs
VSMCs


Whole
Genome
Sequencing
RNA
Sequencing
Genetic
modifiers
CRISPR-Cas9


Genetic modifiers
Better risk stratification
Prevention of emergency surgery
Novel therapeutic targets

Better risk stratification

Prevention of emergency surgery

Novel therapeutic targets

Mimic a protective modifier

Try to minimize the effect of an aggrevating modifier

Need for many patients

Patient organization driven

GEMS WebApp for enrollment

Discovery of **genetic modifiers** of the phenotypical cardiovascular variability in **Marfan syndrome**

Lotte Van Den Heuvel

University of Antwerp

GHENT UNIVERSITY

fwo

UZA

UZ GENT

innoviris .brussels
we fund your future

Fondation 101 Génomes
Genomics for Rare Diseases

European Reference Networks | Vascular Diseases (VASCERN)

Marfan Europe Network

CONTACTGROEP MARFAN NEDERLAND

ABSM

my innovation center. brussels
supported by Microsoft

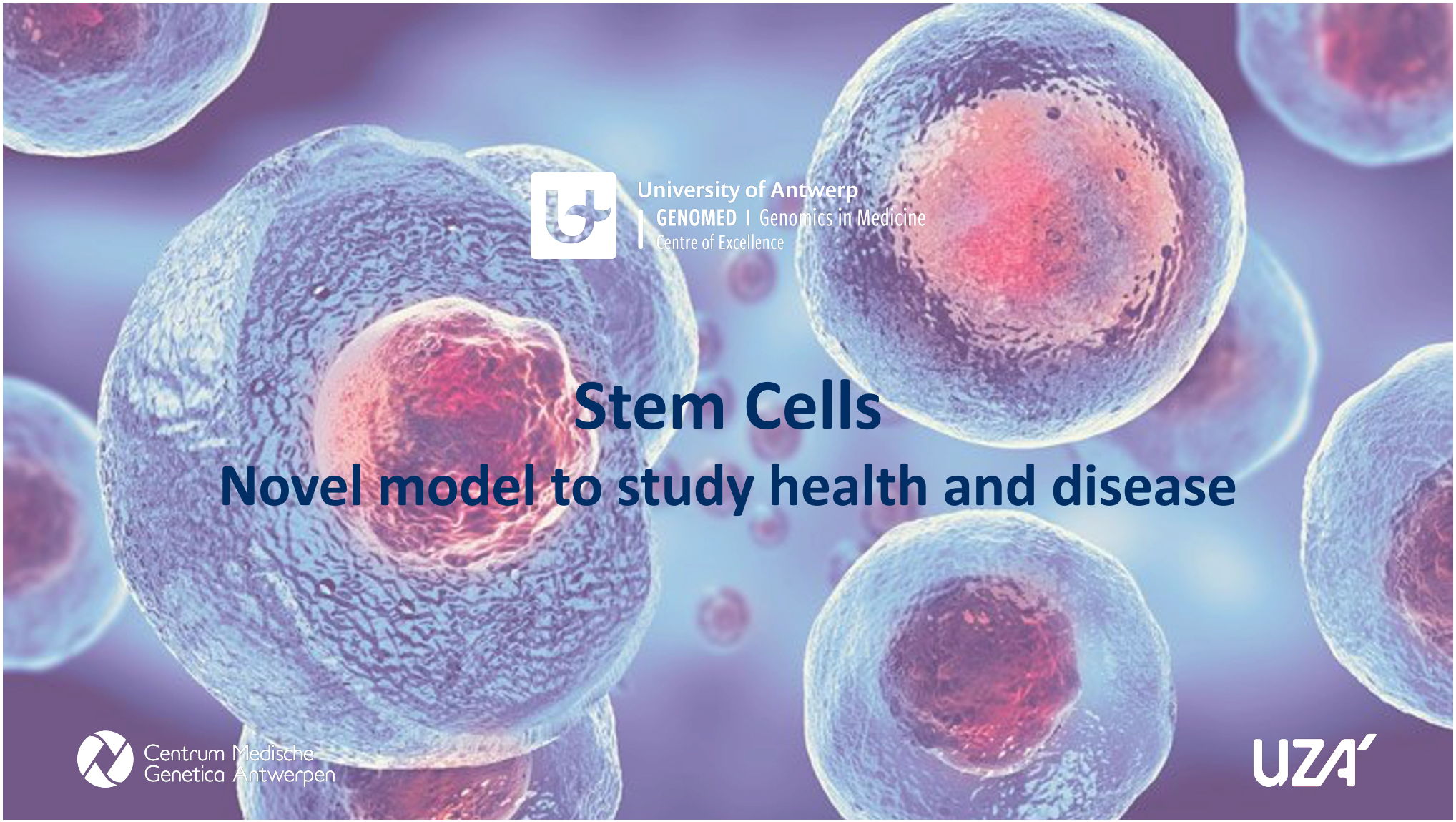
den_i.lu
syndrome de Marfan

marfans

Fonds Moulaert-Laloux

Thank you!

Scan and share me with the Marfan community!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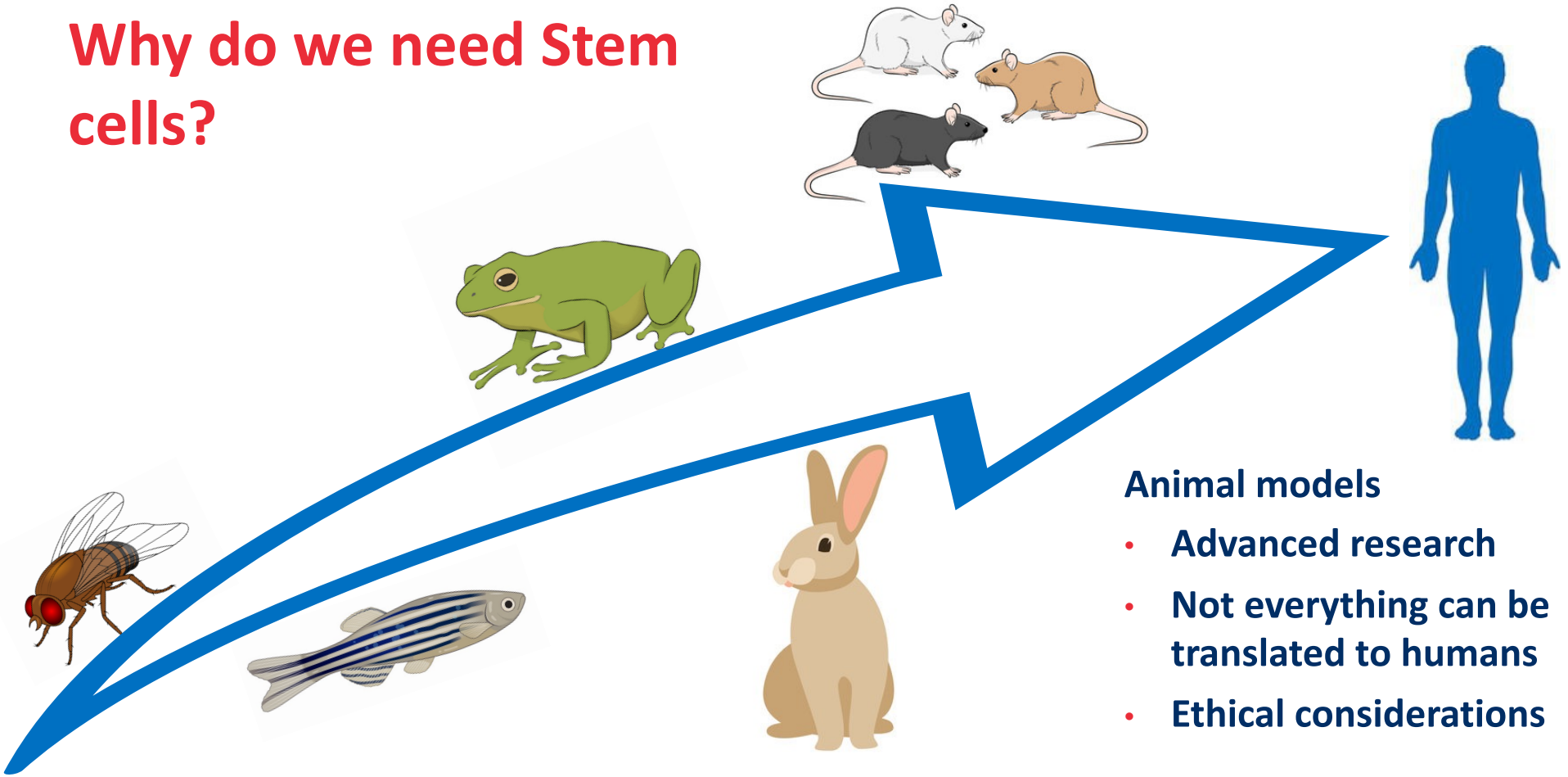
Stem Cells
Novel model to study health and disease
Centrum Medische
Genetica Antwerpen
UZA

Why do we need Stem cells?



Animal models

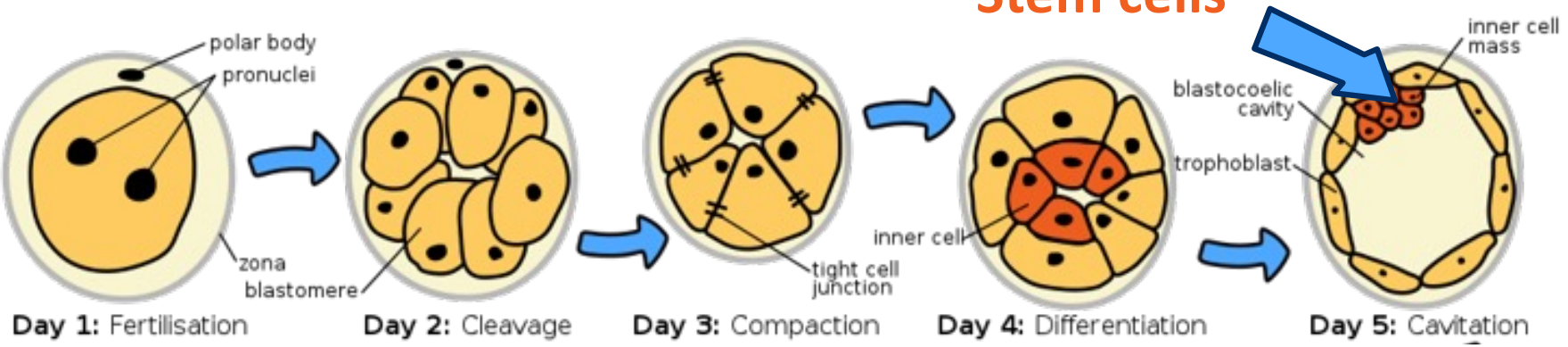
- Advanced research
- Not everything can be translated to humans
- Ethical considerations

Why do we need Stem cells?

- Study human disease in humans?
- Ethically Correct!
- Difficult for brain or aorta?

Stem Cells!

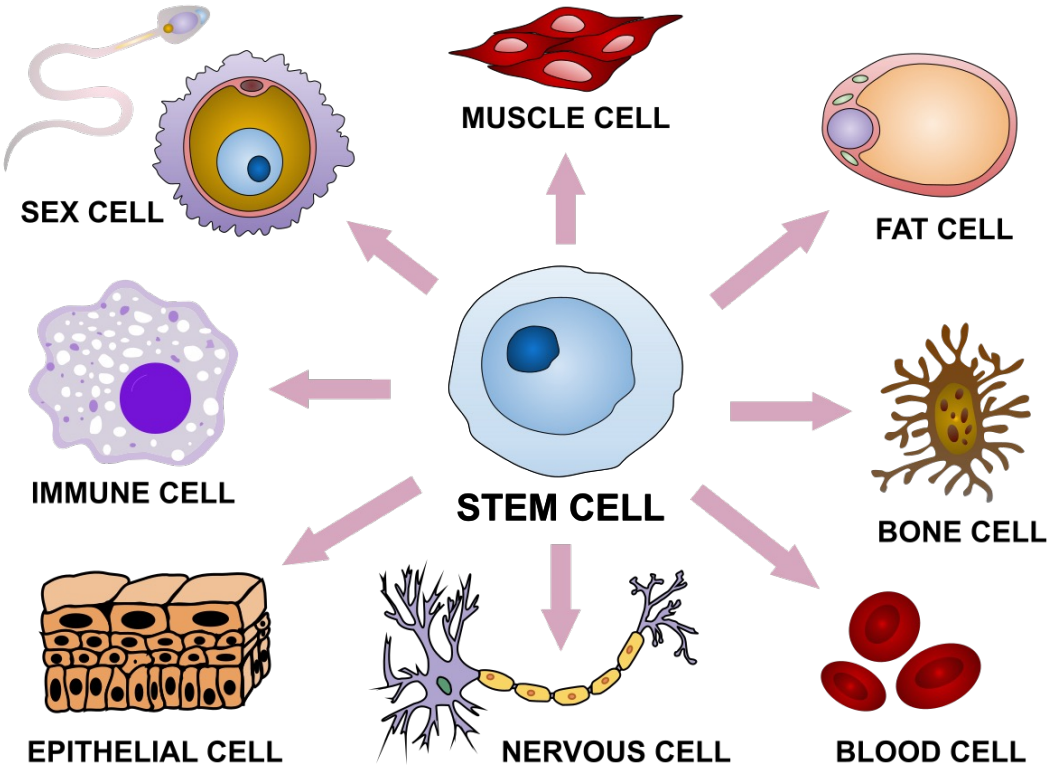
Stem cells



The Beginning of Stem cell research

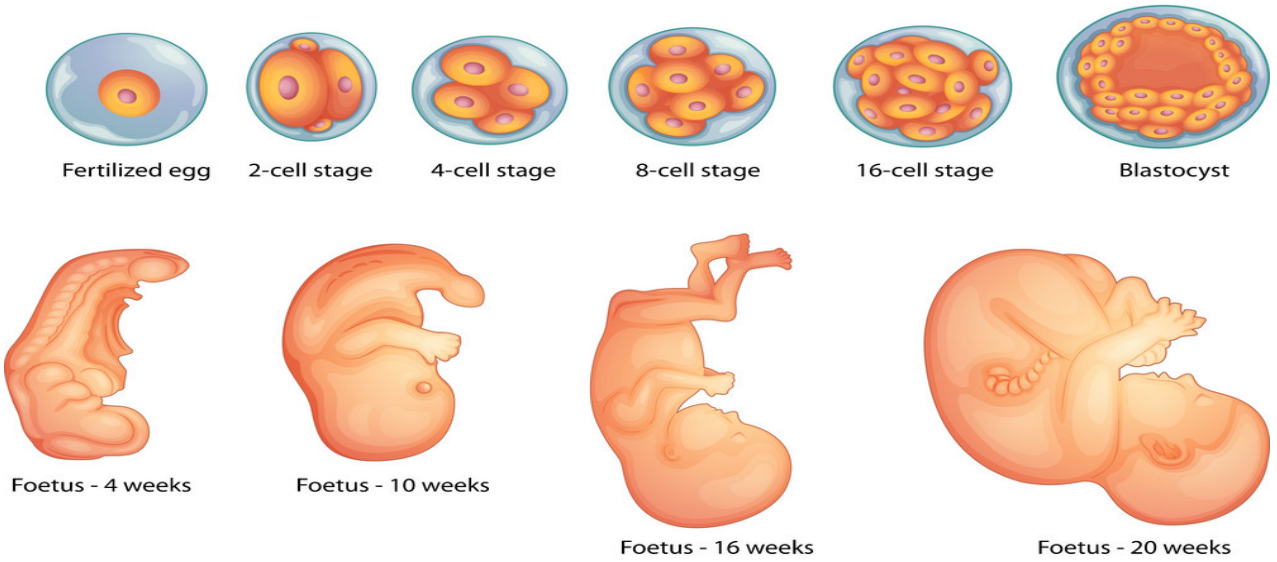
Stem cells arise during the embryonic development

What is a Stem Cell?



- **Embryonic Stem Cells**
- **The body's raw material – all cells with specialized functions are generated**

Embryonic Stem Cells



- **Limited number of cells**
- **Extracted from donated IVF eggs**
- **Ethically challenging!**

The Breakthrough!

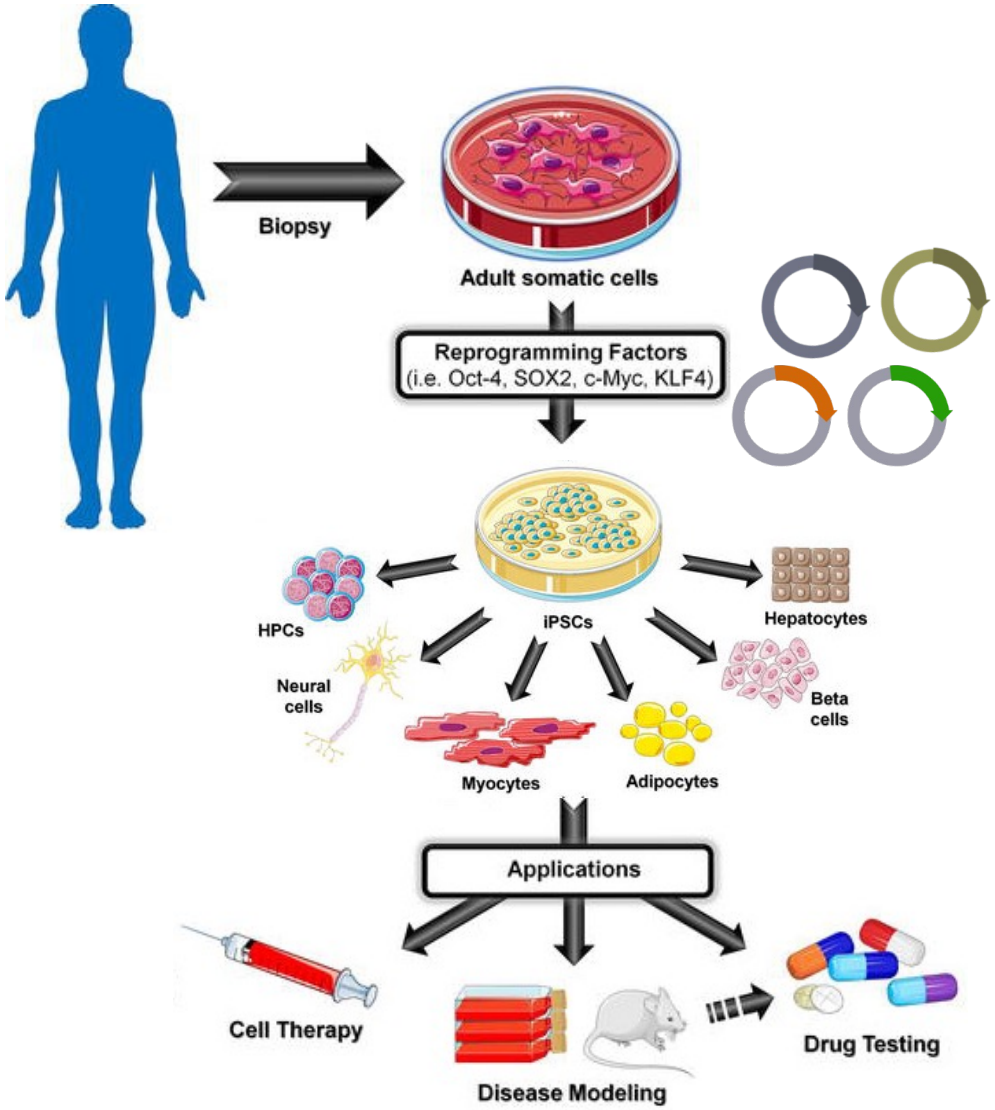
The Nobel Prize in Physiology or Medicine 2012

Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors

Kazutoshi Takahashi [1], Shinya Yamanaka

Induction of pluripotent stem cells from adult human fibroblasts by defined factors

Kazutoshi Takahashi [1], Koji Tanabe, Mari Ohnuki, Megumi Narita, Tomoko Ichisaka, Kiichiro Tomoda, Shinya Yamanaka

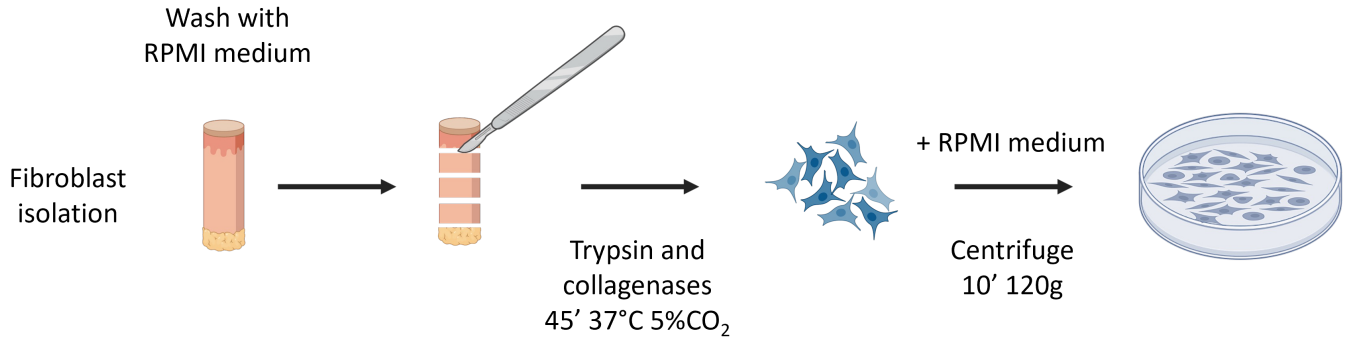


Induced Pluripotent Stem Cells (iPSCs)

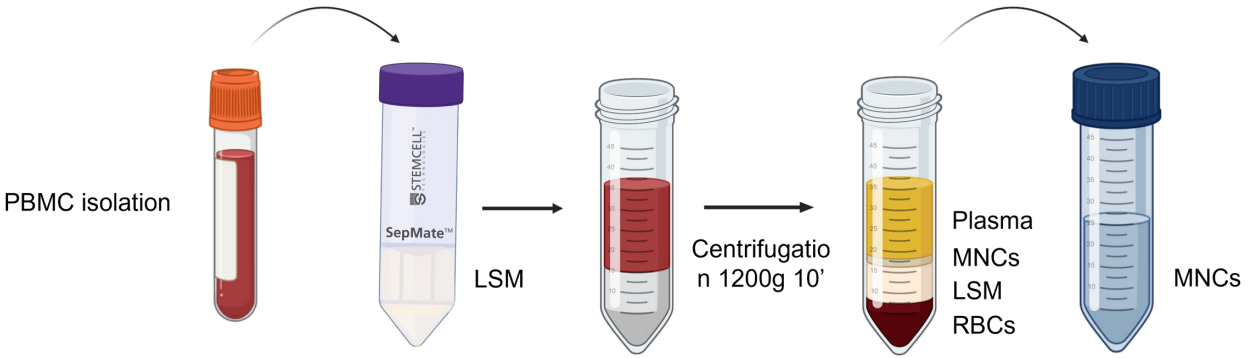
- No need for invasive collection of cells
- More ethical model
- Limitless supply
- Patient genetic background
- Multitude of applications

How do we do it?

- **Fibroblasts**

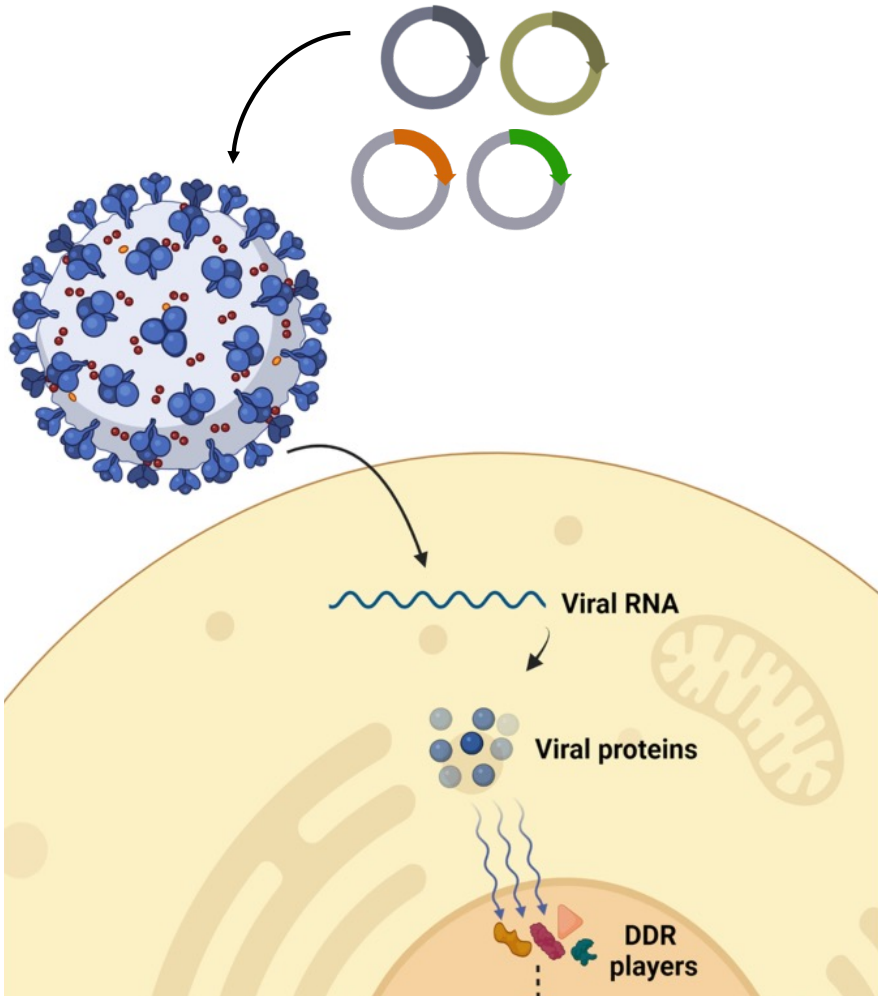


- **PBMC isolation**



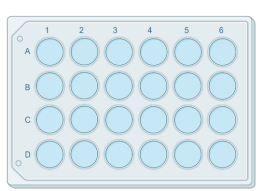
Transduction of Somatic cells

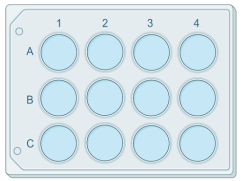
- **Introducing the Yamanaka factors**
  - CytoTuneTM iPS 2.0 Sendai reprogramming kit

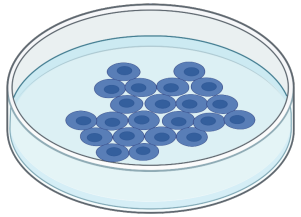


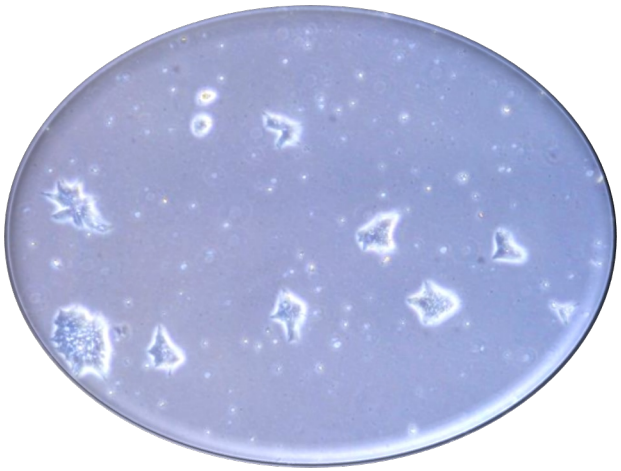
Selection and culturing of iPSCs

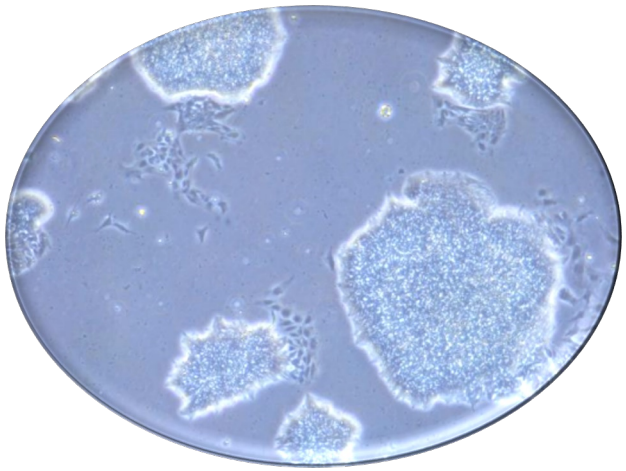
Three months, Seven selection rounds

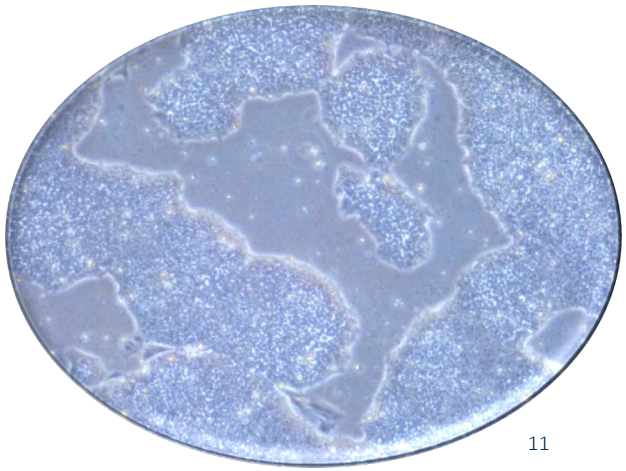






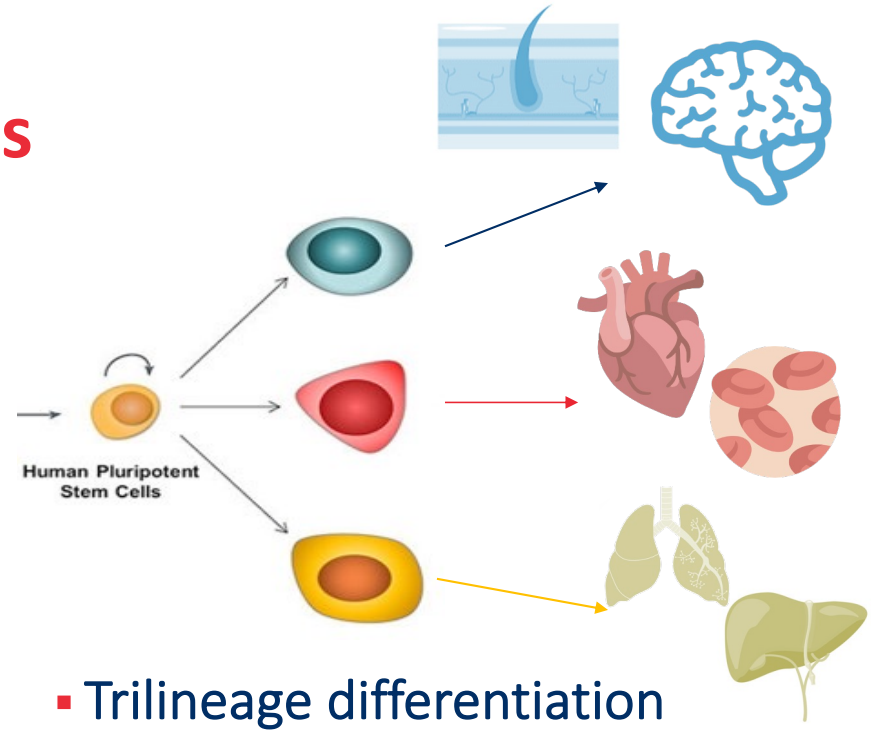






Validation of the resulting iPSCs

- Morphology – rounded, uniform colonies

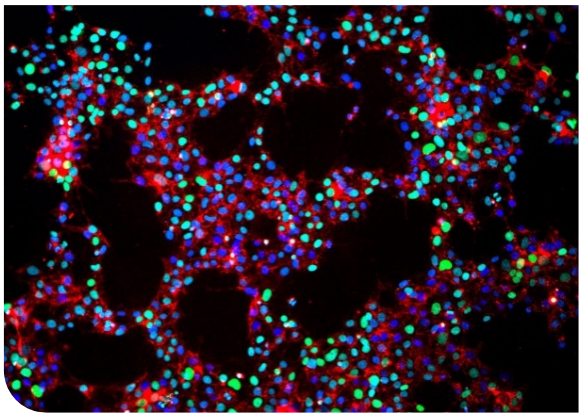


- Trilineage differentiation – Stem cell characteristics

Validation of the resulting iPSCs

- DNA – genomic stability
- No additional mutations

- RNA – absence of Sendai virus
- Independently pluripotent



- Fluorescent staining – presence of iPSC proteins

Congratulations!

- We have generated and validated an iPSC cell line!

Generation of two induced pluripotent stem cell (iPSC) lines (BBANTWi006-A, BBANTWi007-A) from Brugada syndrome patients carrying an SCN5A mutation

Eline Simons [1], Aleksandra Nijak [1], Bart Loeys [1], Maaike Alaerts [1]

Generation and validation of an iPSC line (BBANTWi008-A) from a Loeys-Dietz Syndrome type 3 patient

Joe Davis Velchev [1], Aline Verstraeten [1], Josephina Meester [1], Peter Ponsaerts [2], Julie Richer [3], Maaike Alaerts [1], Bart Loeys [4]

Generation of a human TGFB3-hIPSC line, BBANTWi010-A, from a Loeys-Dietz syndrome type V patient

Melanie Perik [1], Aline Verstraeten [1], Aleksandra Nijak-Paeske [1], Laura Rabaut [1], Lut Van Laer [1], Bart Loeys [2]

Generation of an induced pluripotent stem cell (iPSC) line (BBANTWi009-A) from a Meester-Loeys syndrome patient carrying a BGN mutation

Pauline De Kinderen [1], Laura Rabaut [1], Anne Hebert [1], Peter Ponsaerts [2], Melanie Perik [1], Josephina A N Meester [3]

Thank you!

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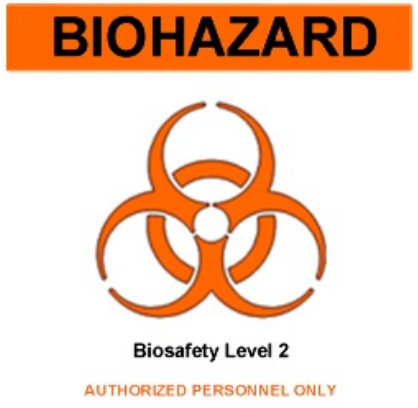
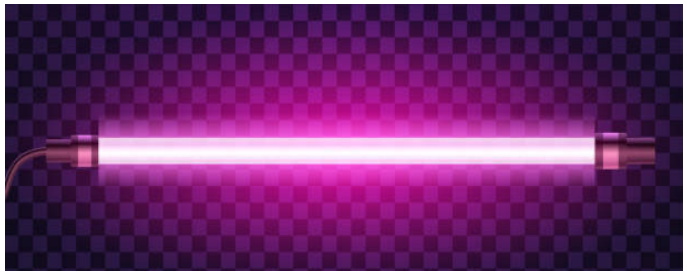
Good laboratory practices

- **Practices to work sterile and safe in the lab**
- **Types of laboratories - L2 lab**
- **Safety**
  - Wear PPE; gloves, lab coat, closed shoes, tied hair



Good laboratory practices

- **Sterile work**
  - No skin exposed
  - Spray and clean all materials
  - Proper waste collection
- **Sensitive cells → Additional care necessary**
- **Aseptic techniques → Prevent Contamination!!**
  - Umonium38
  - EtOH 70%
  - UV light

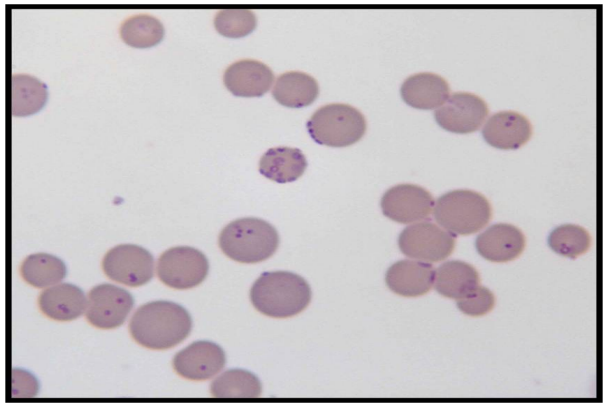


Laboratory contamination

- **Bacteria**

Fungi

Mycoplasma



- **Monitoring your cultures**
- **Monthly Mycoplasma test**


Let's get in the lab!

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Applications of iPSCs

Projects within the Loeys lab

Centrum Medische Genetica Antwerpen

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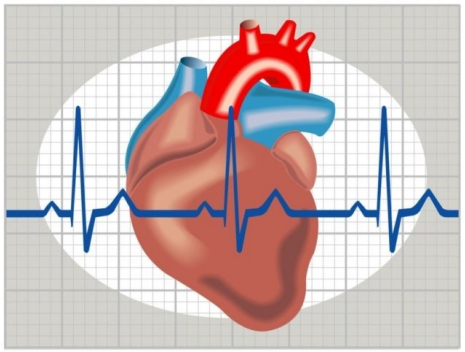
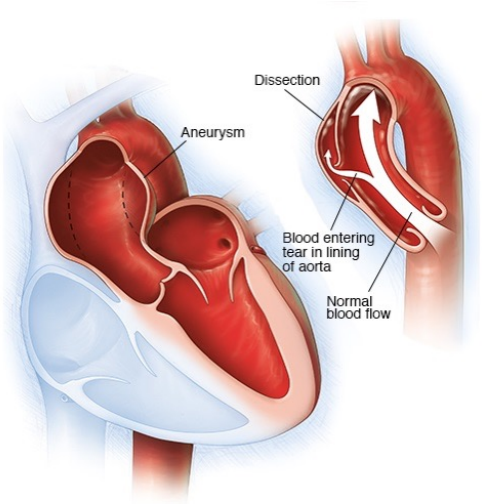
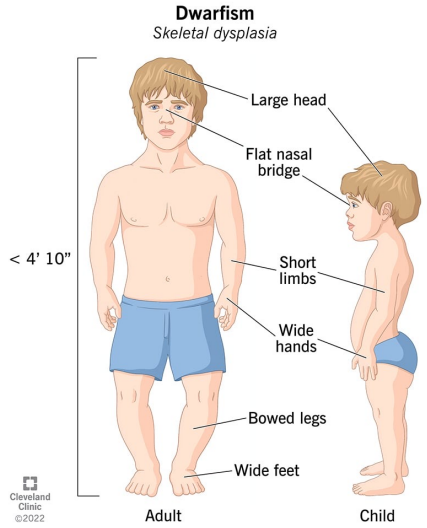
Focus of our group



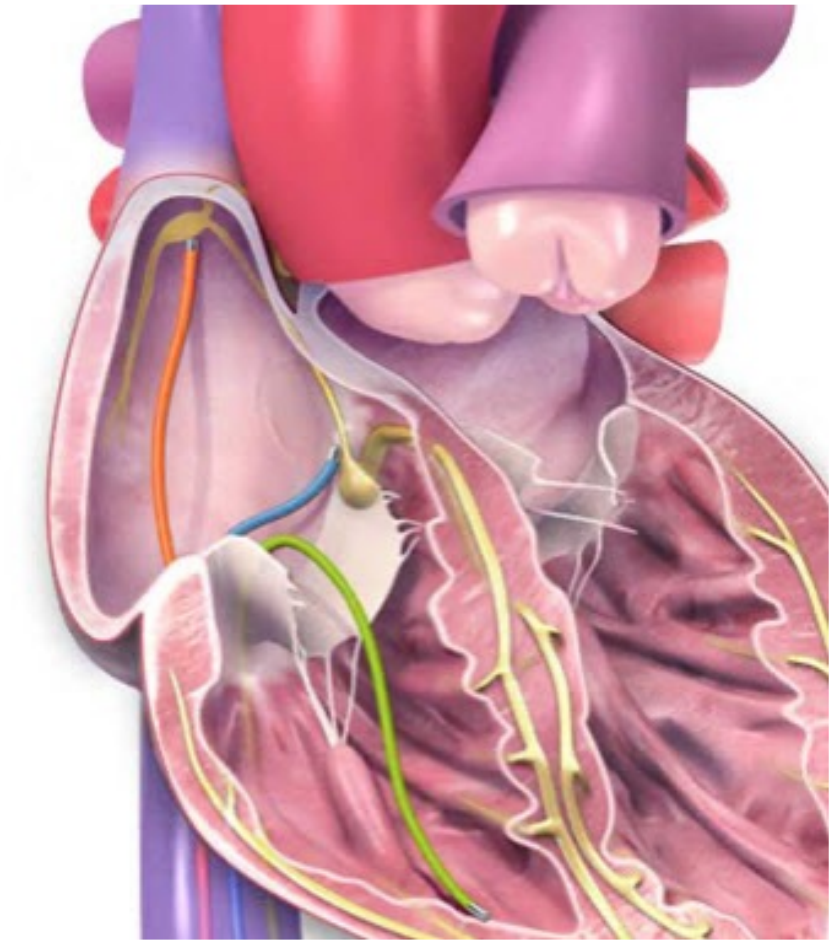
- **Primary Electrical Disorders**
  - Problems with the electrical guidance in the heart
  - Need cardiomyocytes (CMs)



- **Skeletal disorders**
  - E.g. chondrodysplasia
  - Need chondrocytes

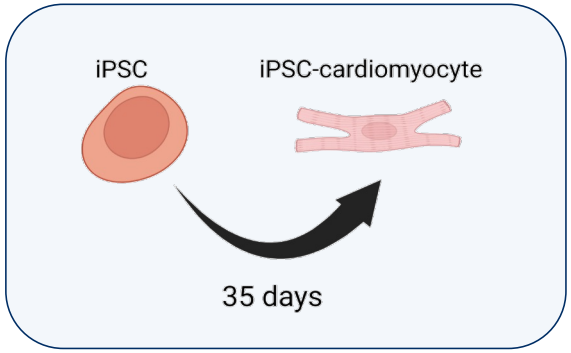


- **Hereditary aortopathies**
  - E.g. MFS, LDS
  - Need Vascular Smooth Muscle Cells (VSMCs)

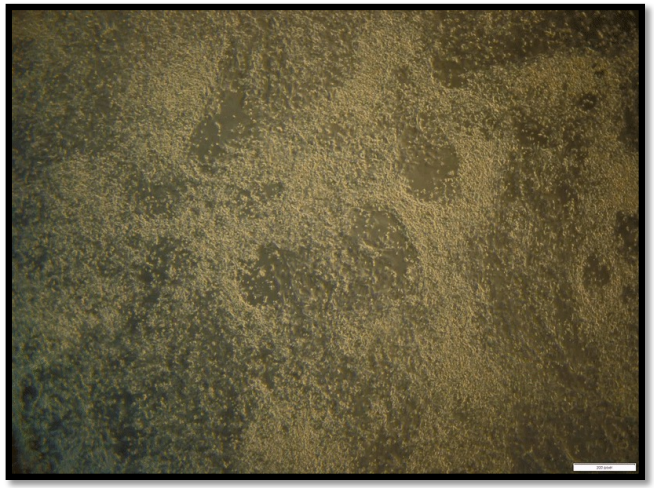


Primary Electrical Disorders

iPSC derived cardiomyocytes (CMs)

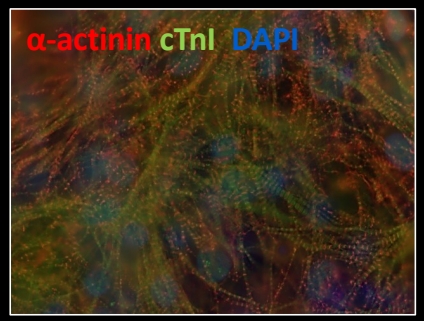


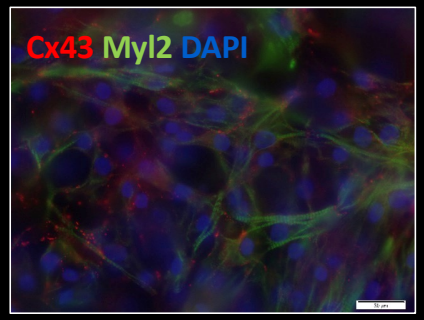
Beating monolayer

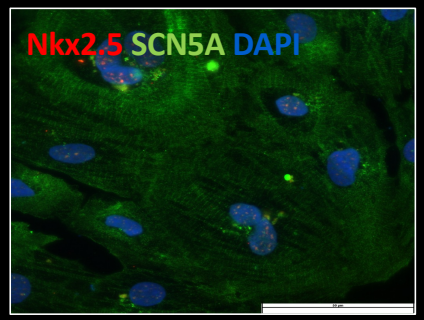


Beating clusters

iPSC-CM - validation

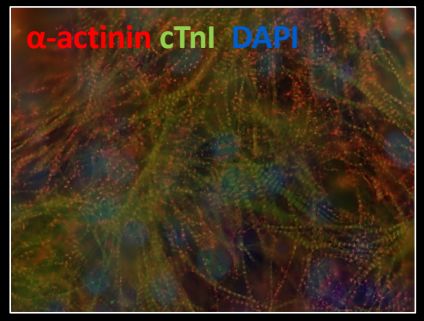


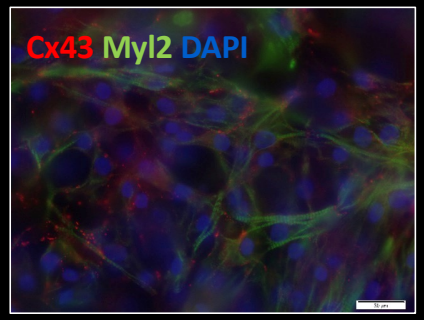


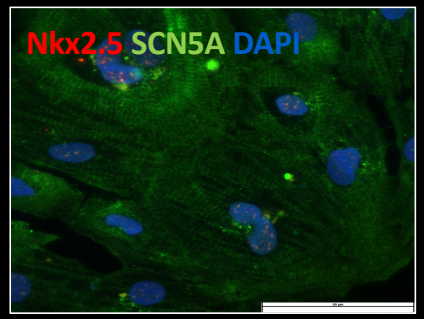


Protein level

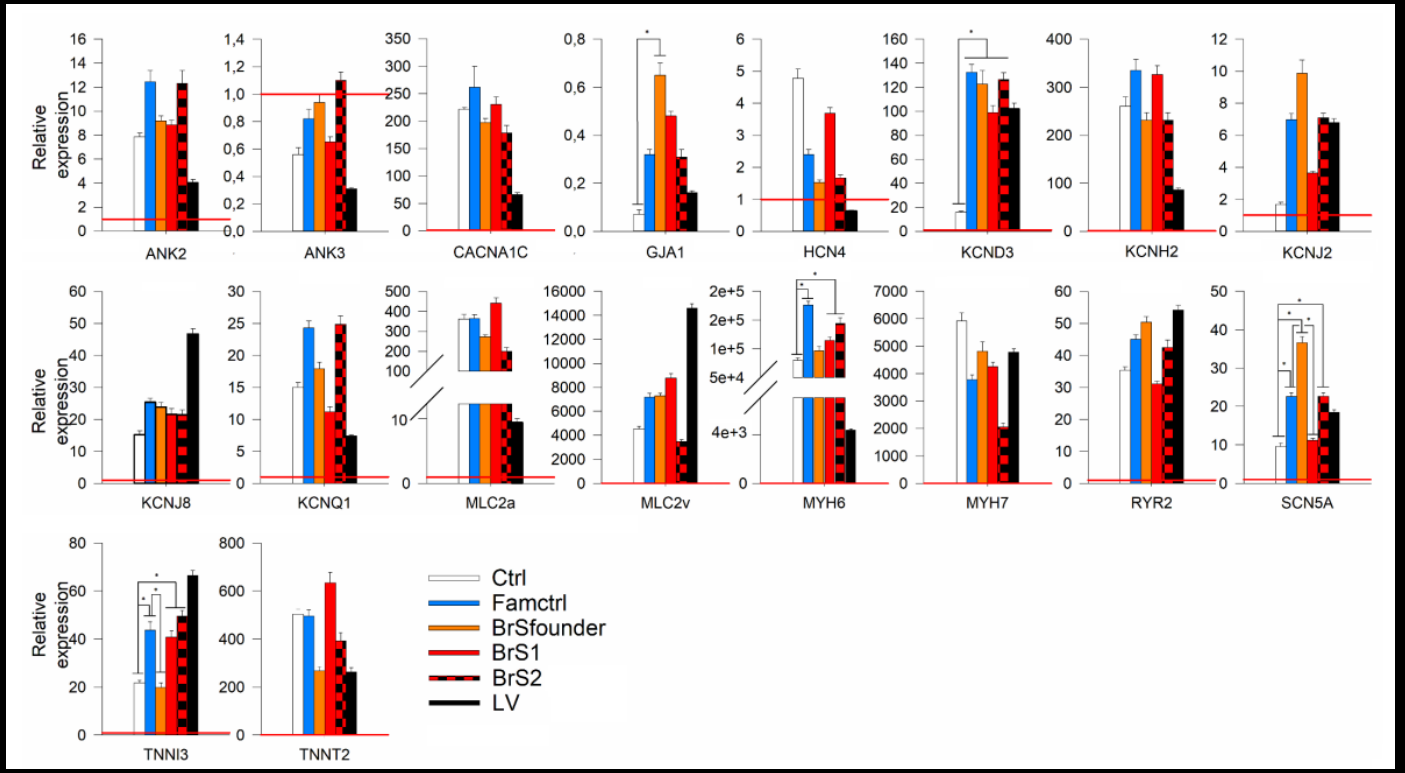
iPSC-CM - validation



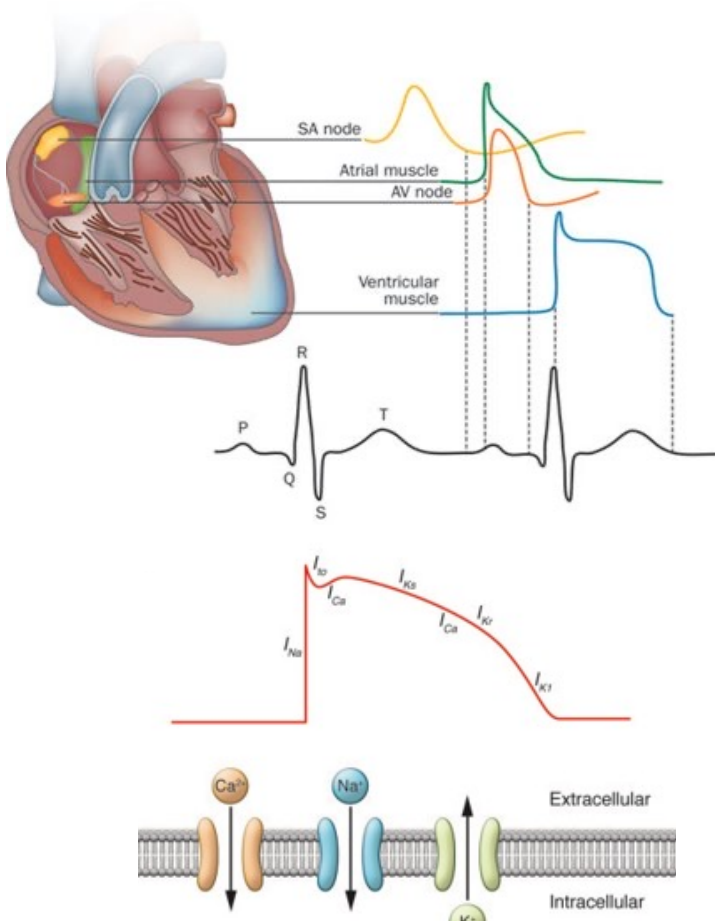




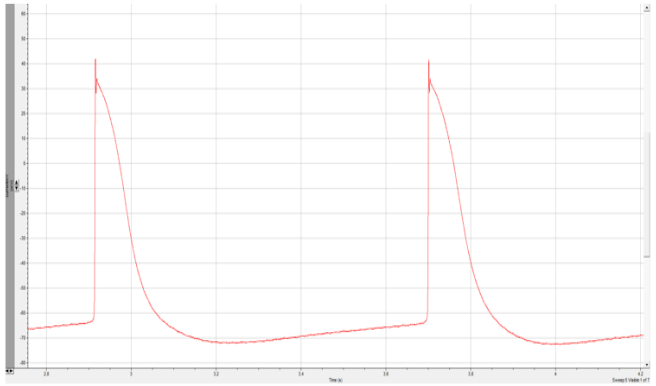
RNA level



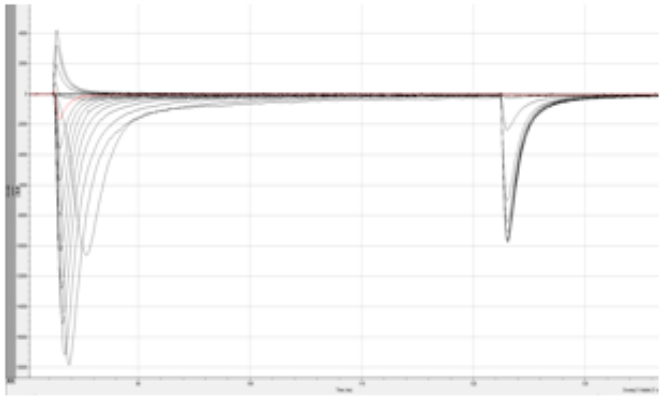
iPSC derived cardiomyocytes (CMs) – functional studies

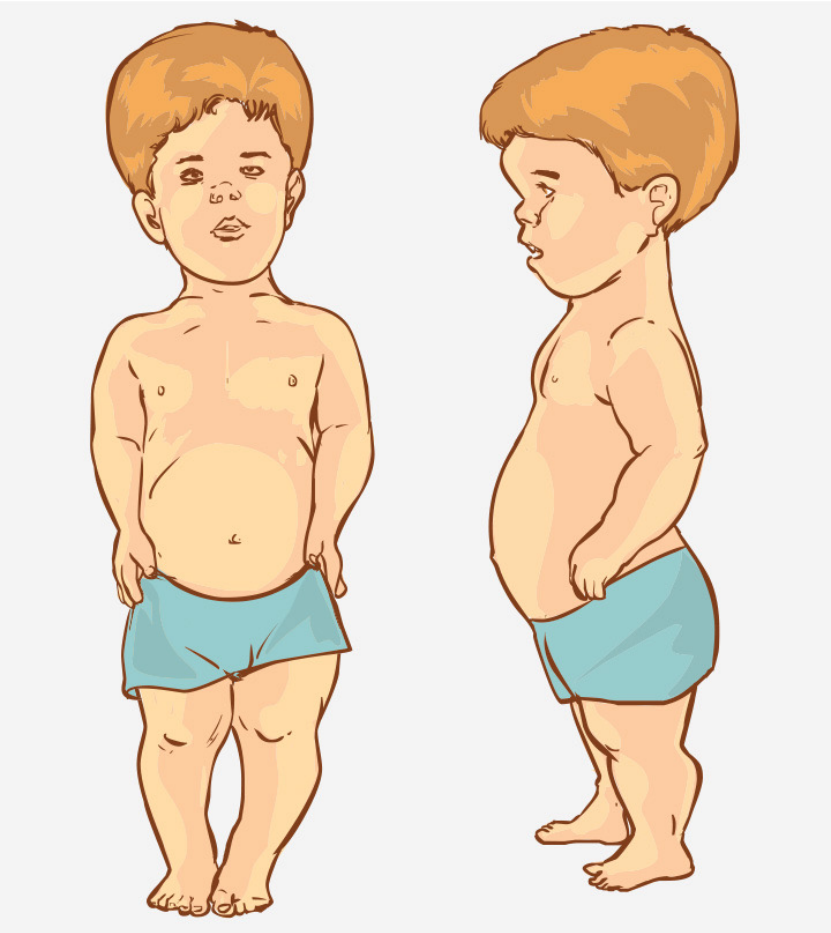


Action potential waveforms



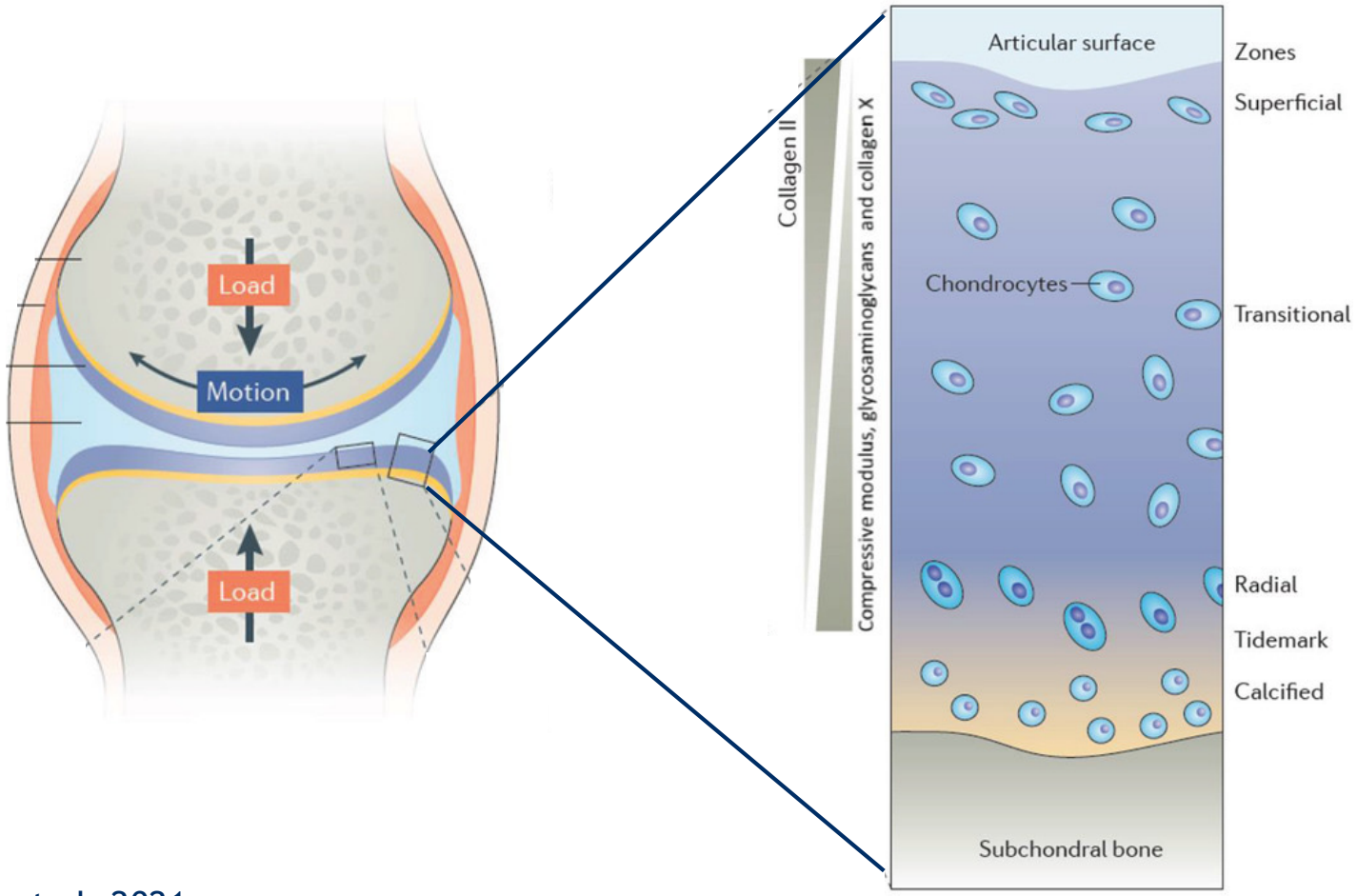
Sodium current waveforms





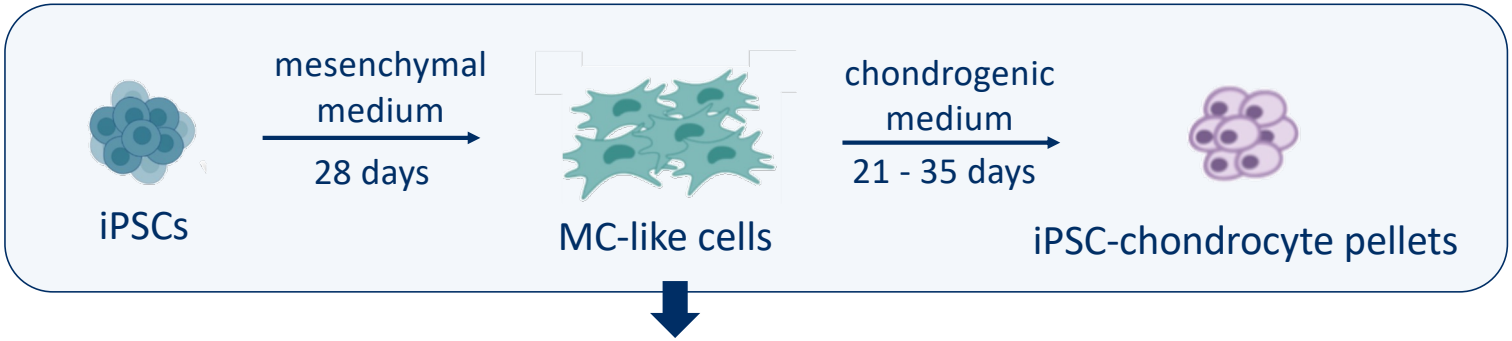
Skeletal disorders

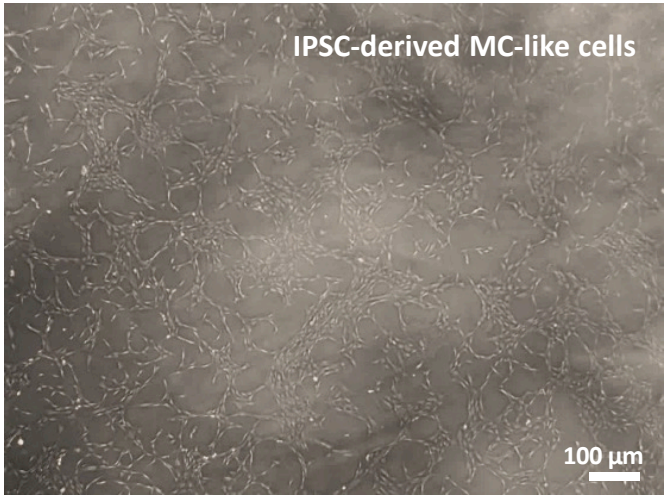
iPSC derived chondrocytes



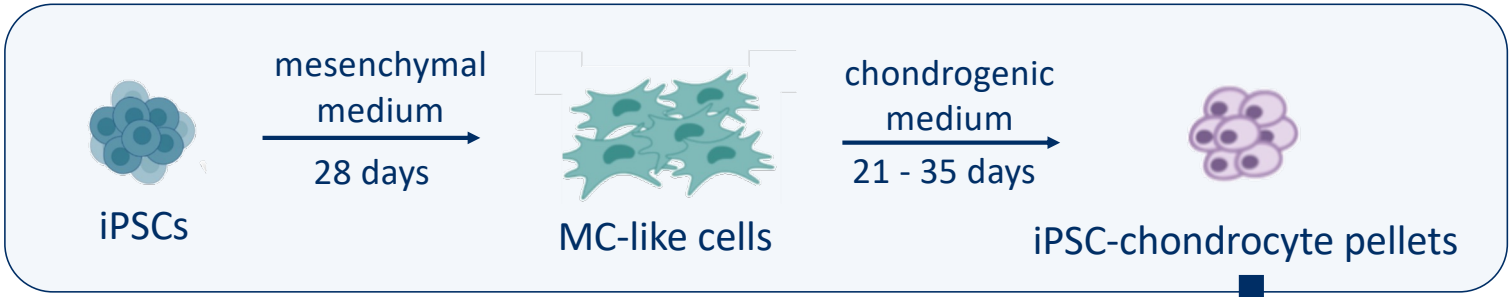
Adapted from Lin et al. 2021

iPSC derived chondrocytes

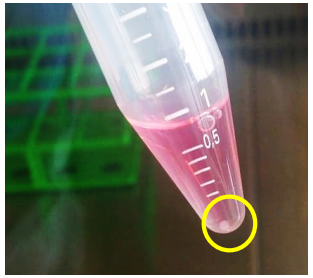




iPSC derived chondrocytes



Macro-pellet

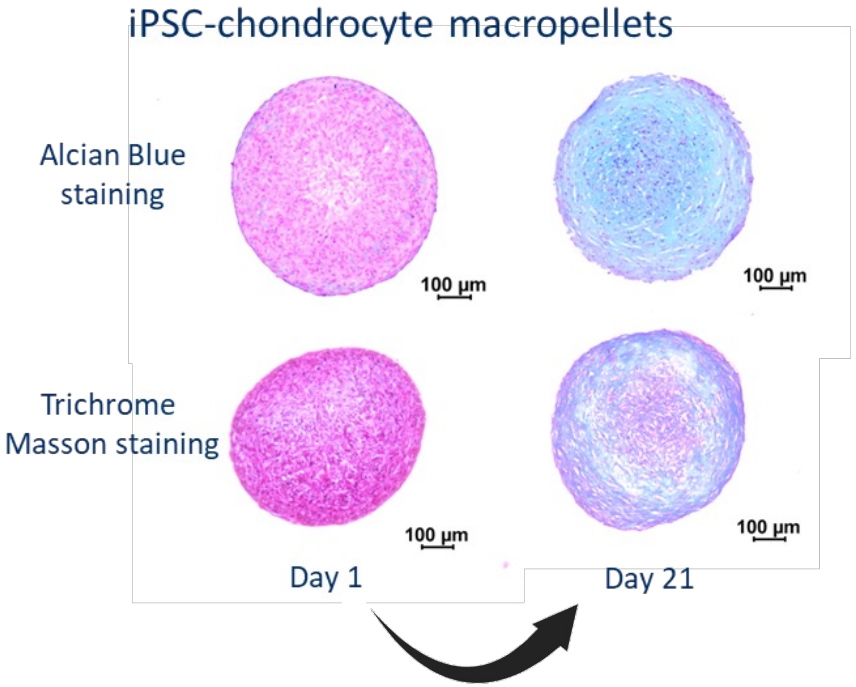


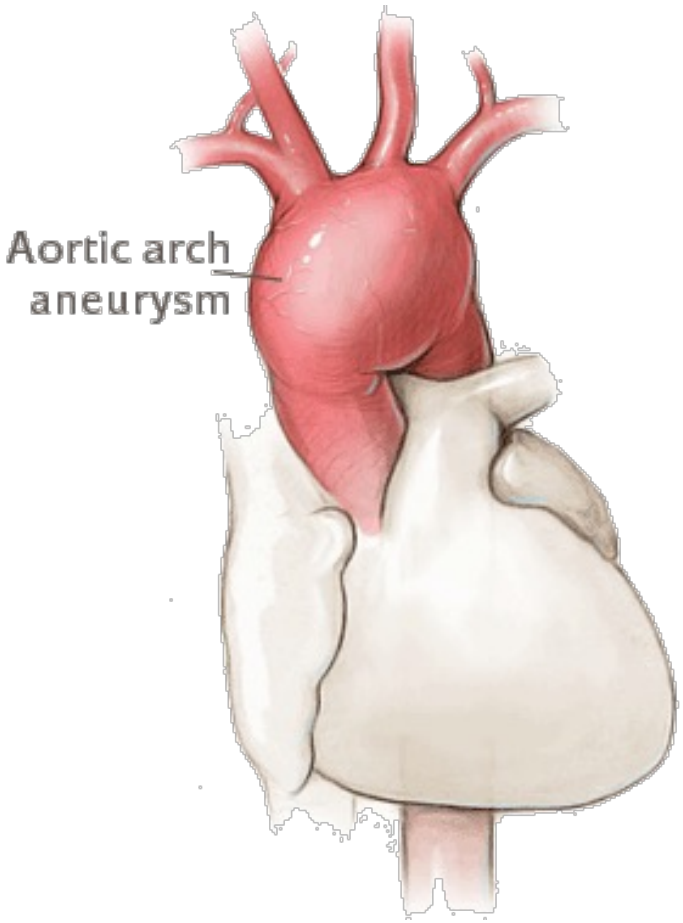
- 15 ml tube
- 200K MC-like cells

Micro-pellet

- 96-well plate
- 50K MC-like cells
- High-throughput purposes

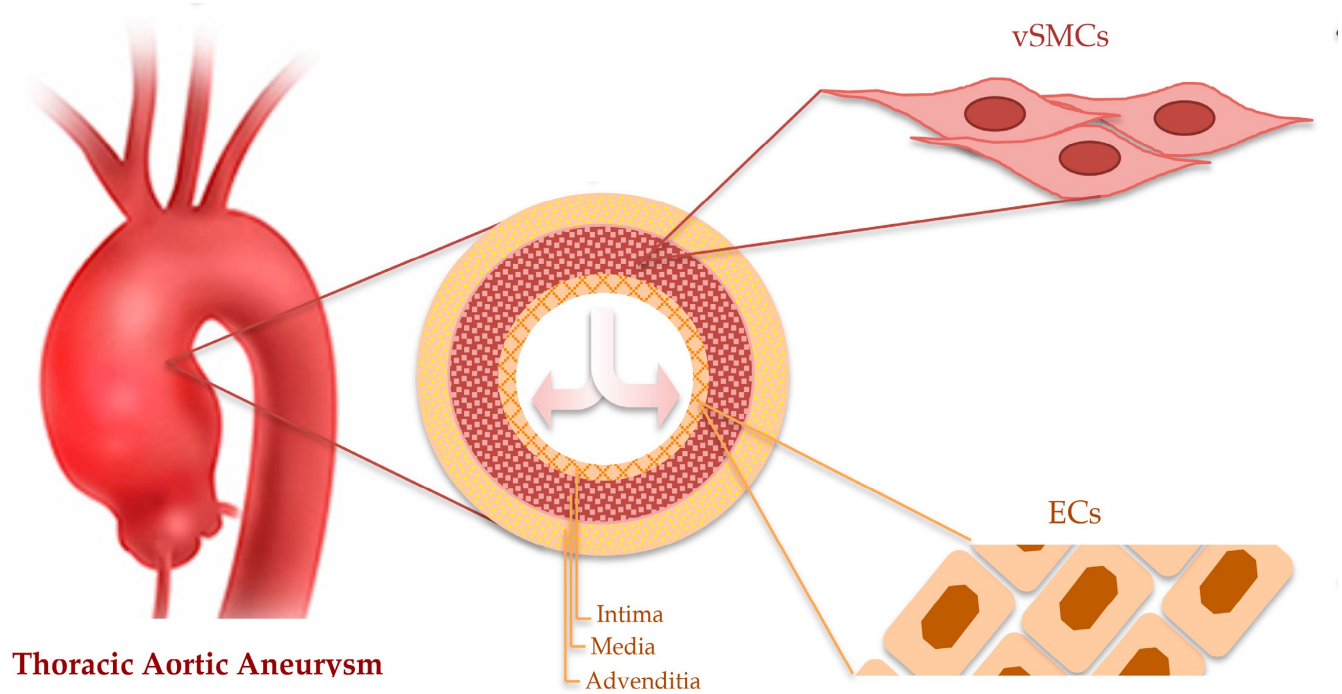
iPSC derived chondrocytes - validation





Hereditary Aortic Diseases

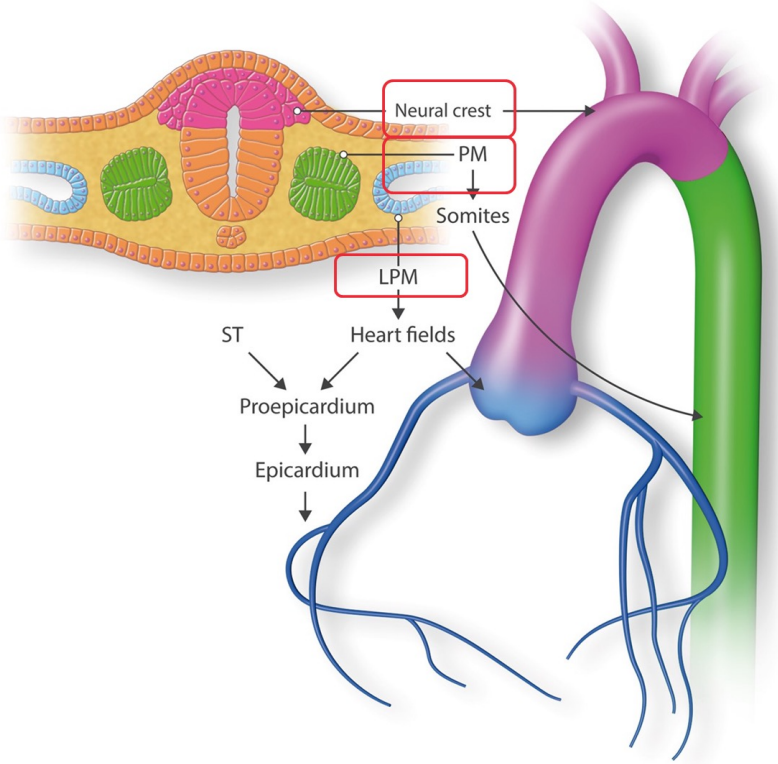
Composition aorta



Adapted from Aschacher et al. 2018.

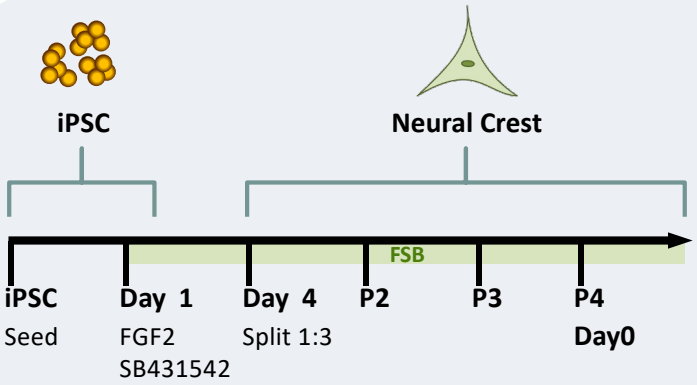
Aortic Vascular Smooth Muscle Cells (VSMCs)

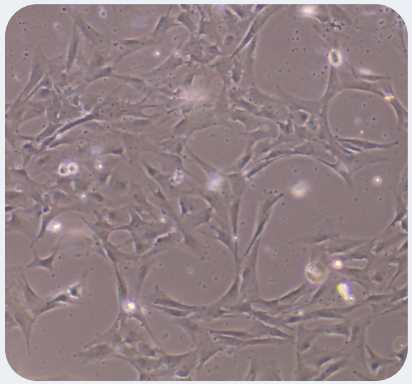
- **Embryonal lineages**
  - Lateral mesoderm (LM)
  - Neural crest (NC)

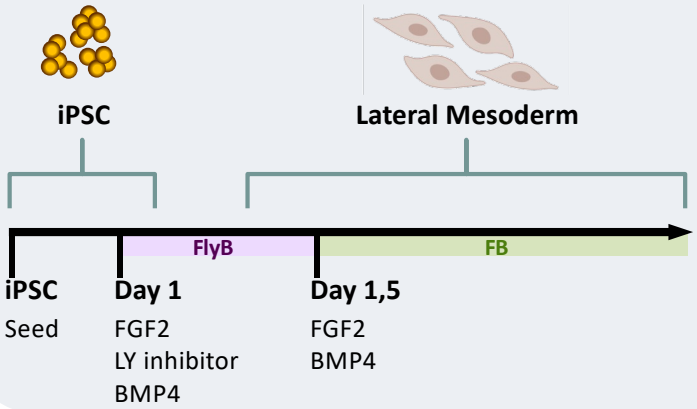


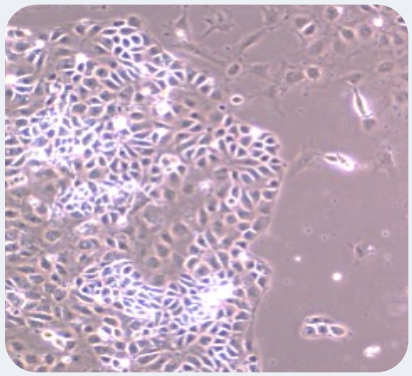
Adapted from Granata et al. 2018.

iPSC derived Vascular Smooth Muscle Cells (VSMCs)

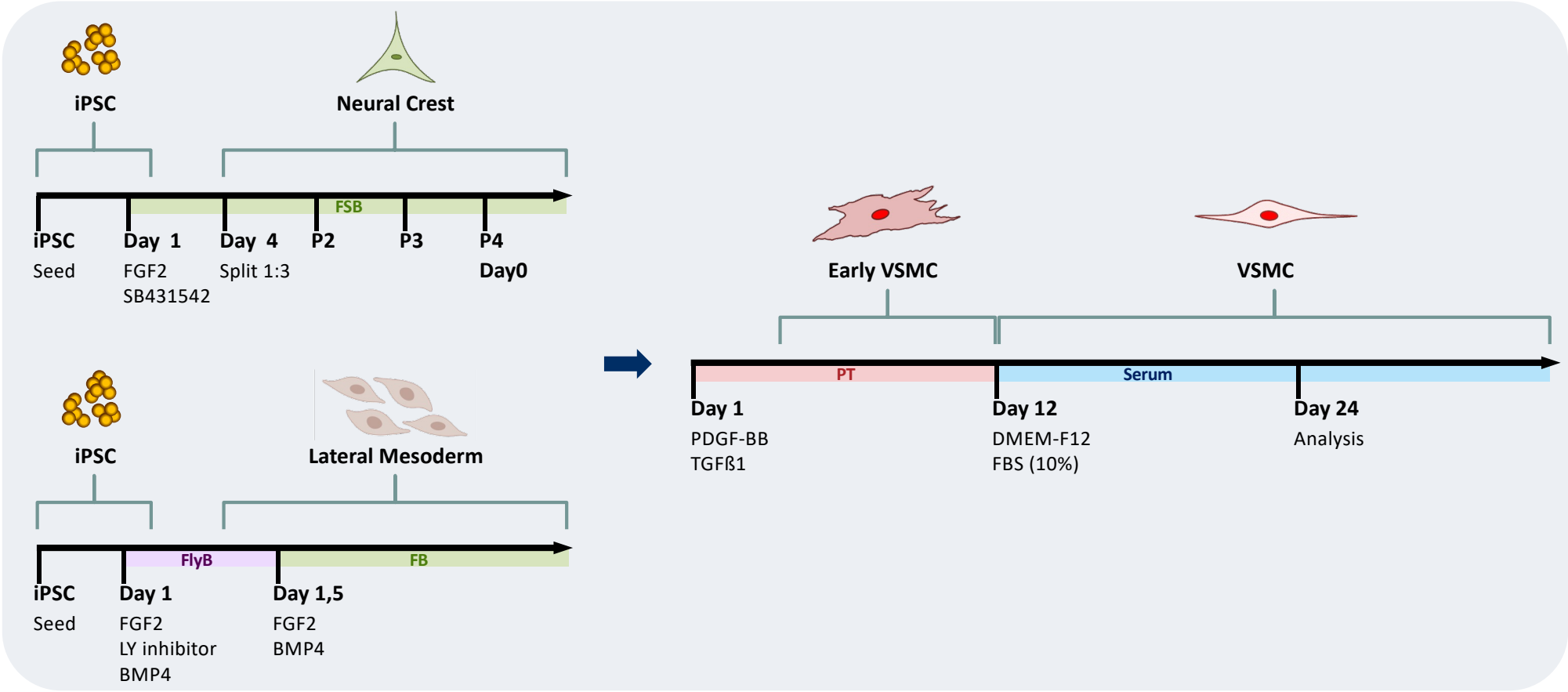






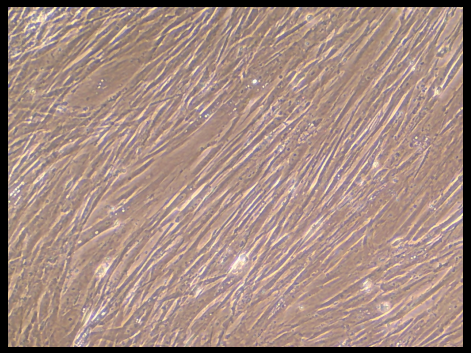


iPSC derived Vascular Smooth Muscle Cells (VSMCs)

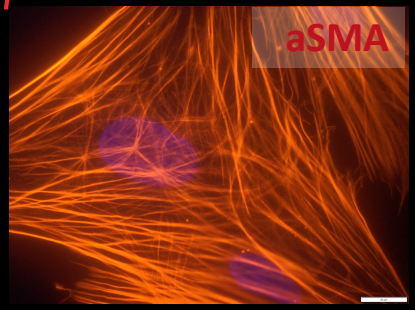


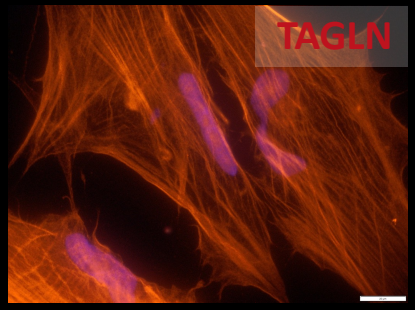
iPSC-VSMC - validation

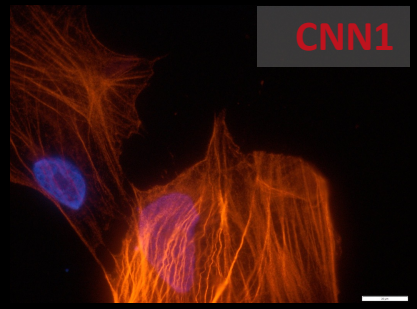
Morphology



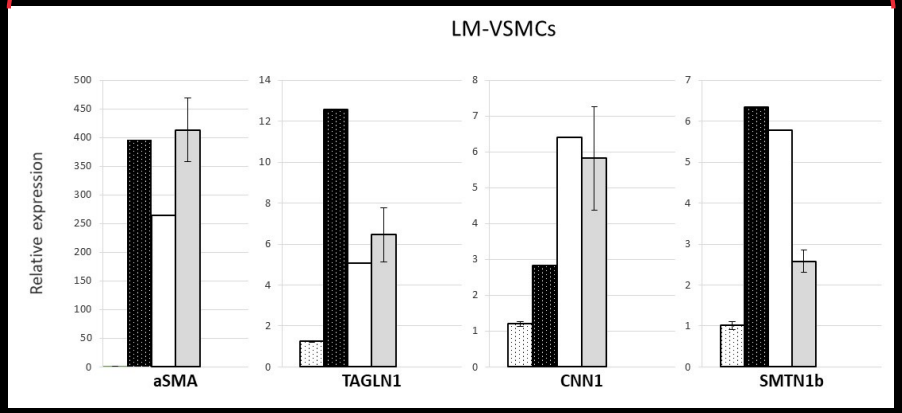
Protein level





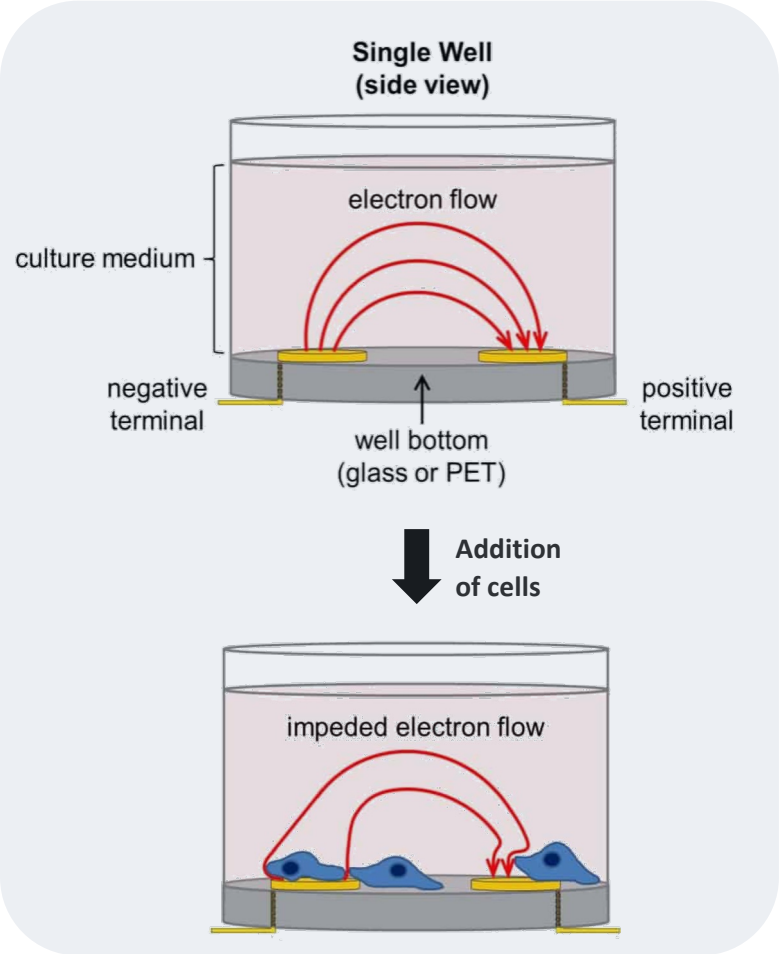


RNA level

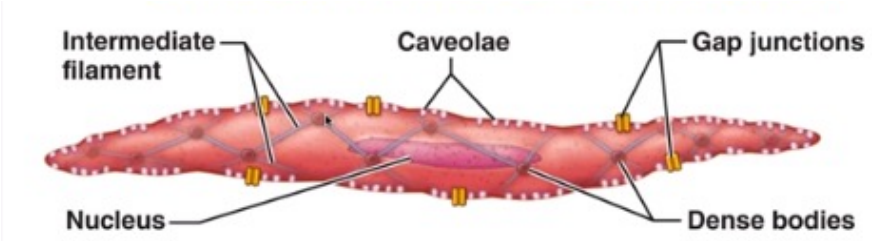


iPSC-VSMC - Functional studies

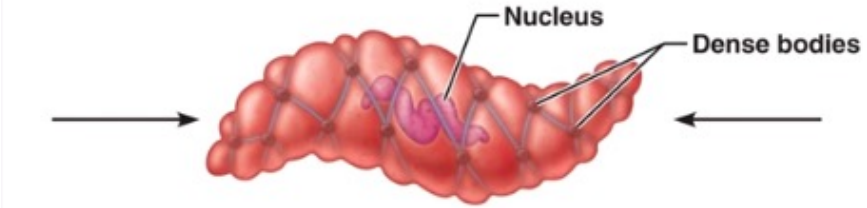
Xcelligence



Relaxed

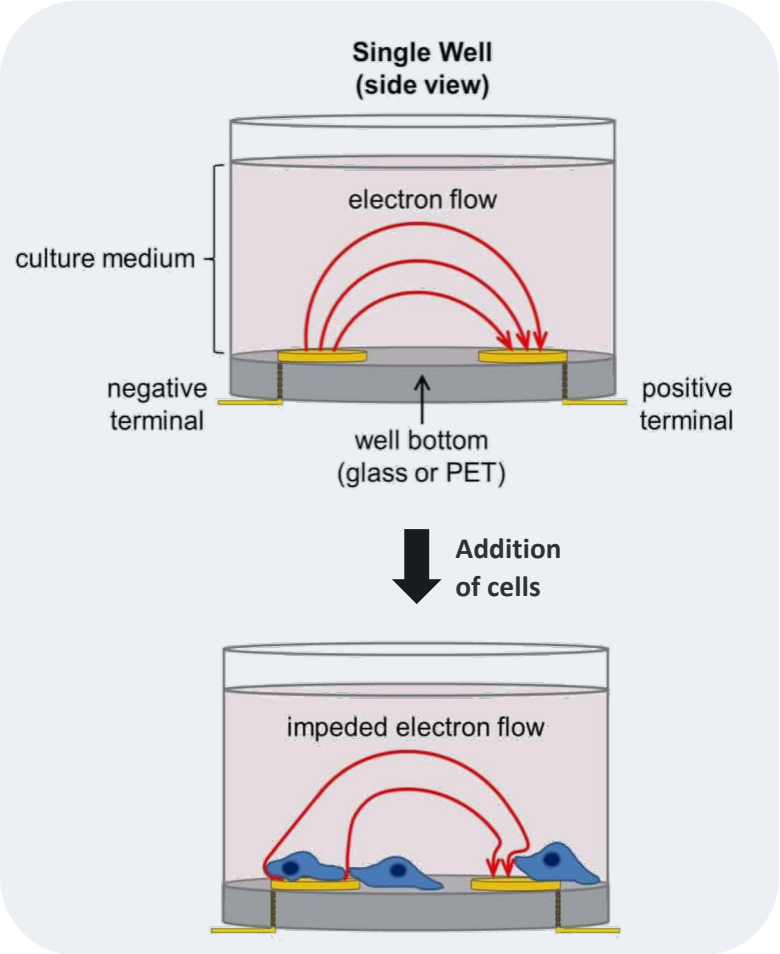


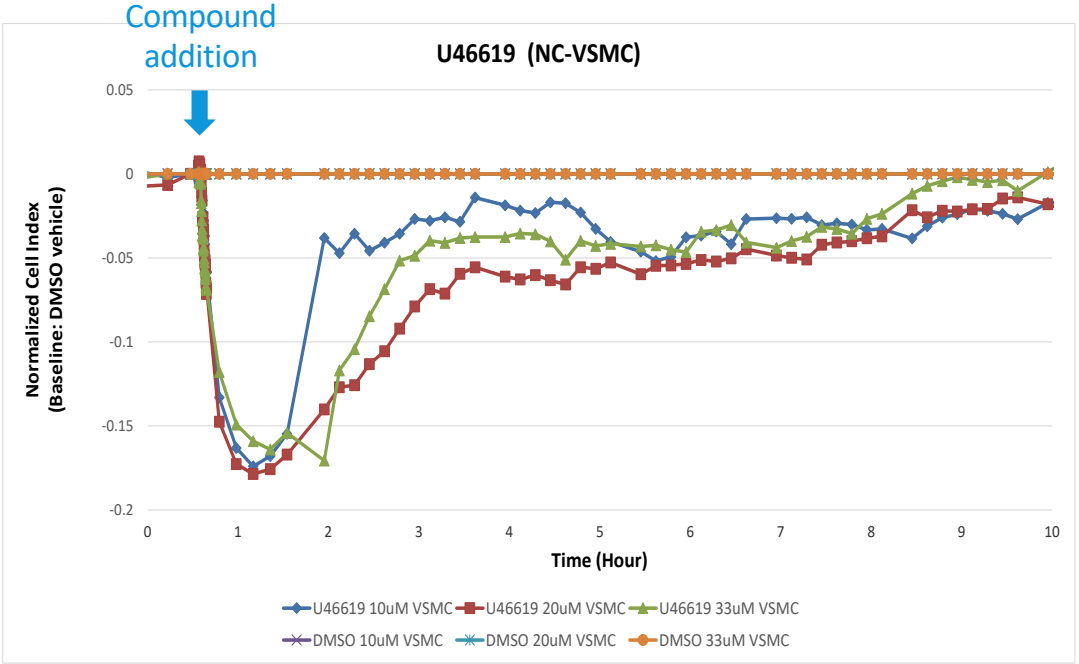
Contracted



iPSC-VSMC - Functional studies

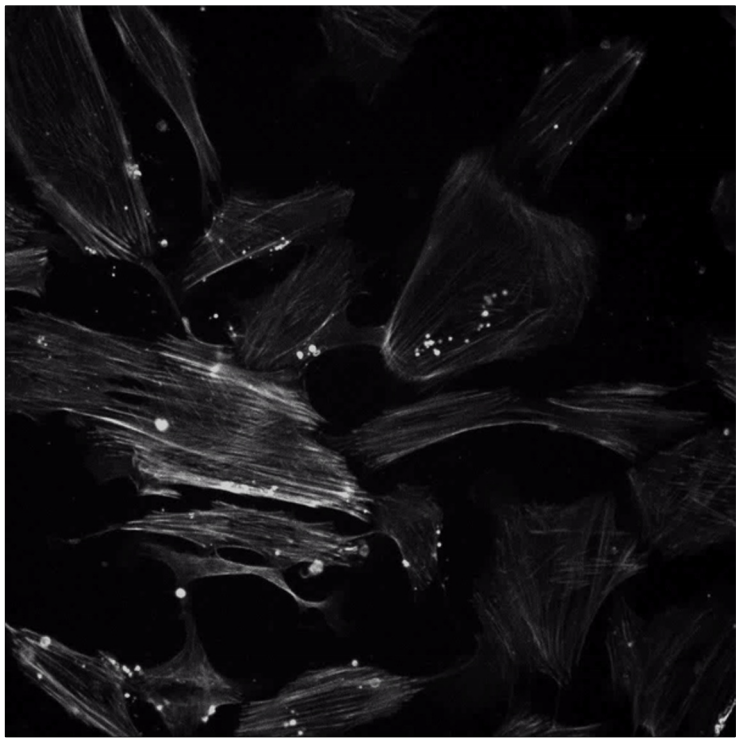
Xcelligence

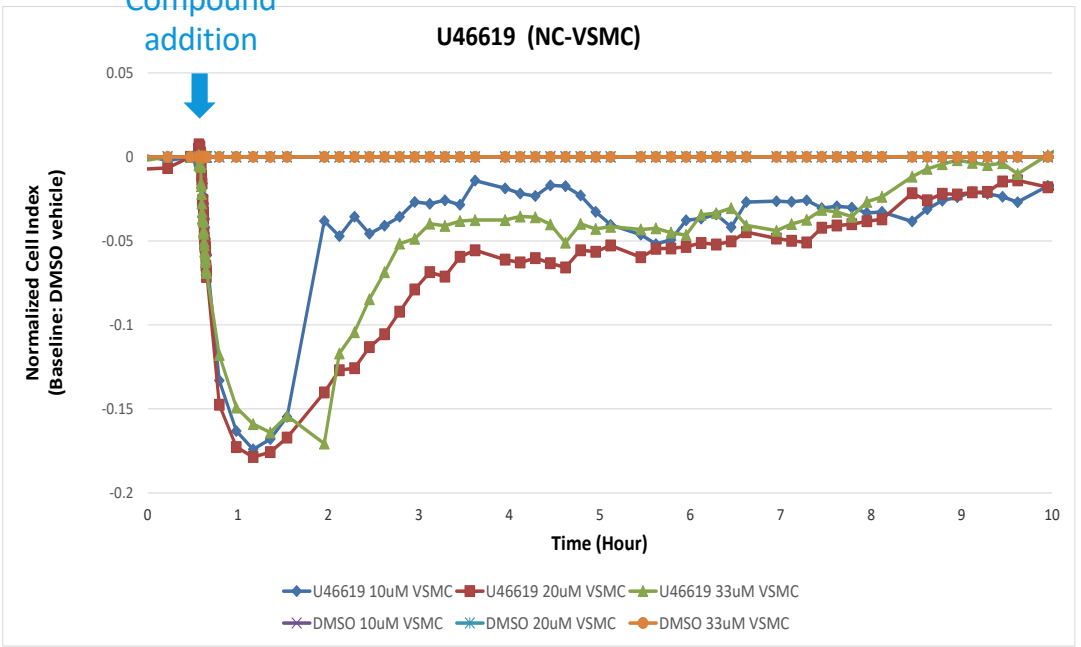




iPSC-VSMC - Functional studies

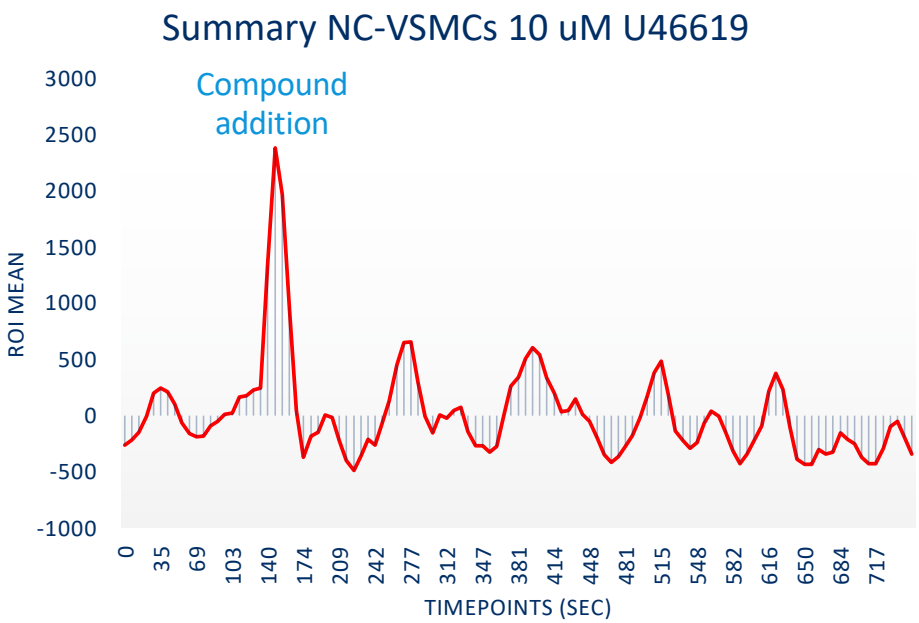
Sir-Actin staining



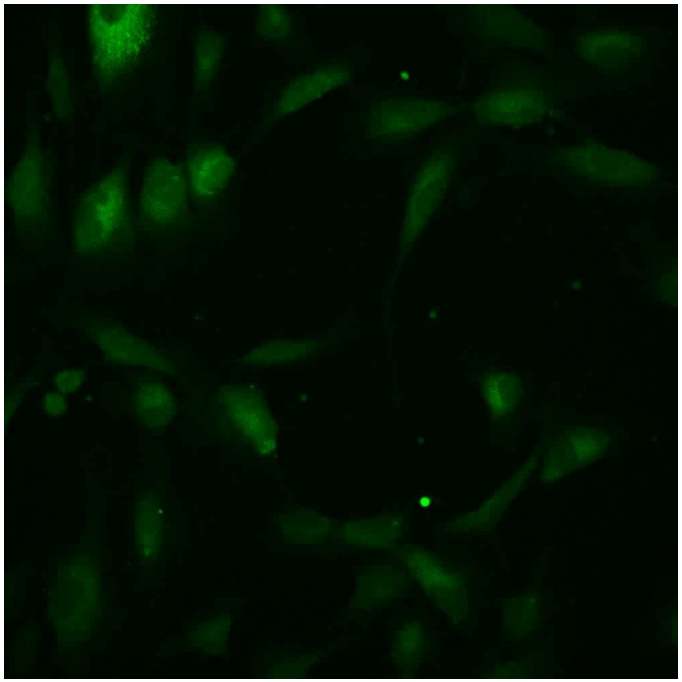


iPSC-VSMC - Functional studies

Calcium Flux: Cal-520 AM dye



Calcium Flux



Current projects with VSMCs

- **Modifier projects**
  - LDS families with *TGFB3* or *SMAD3* mutation
  - Marfan patient cohort
- **Investigation effect of *IPO8* involved in TAAD**
  - Phenotype similar to LDS patients
- **Unravel desease mechanism of *BGN* mutations in TAAD**
  - BGN mutations: Skeletal V.S. TAAD

Thank you!

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Aorta research in mouse models

Irene Valdivia Callejon & Lucia Buccioli

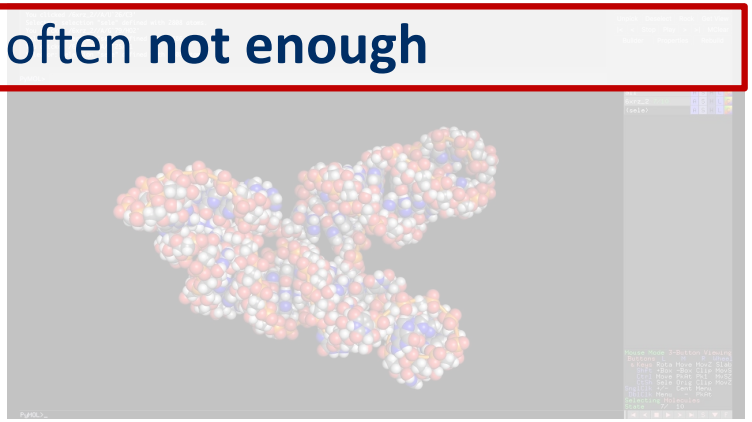
Centrum Medische Genetica Antwerpen

UZA

Why are animal models still necessary in research?

Non-animal alternative methods exist and are widely used: 2D or 3D cell cultures or computer simulations.

However, these options are often **not enough**



Why are animal models still necessary in research?

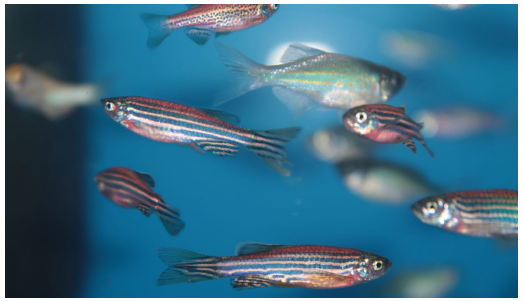
- Complex biological interactions
- Disease progression
- Drug testing
- …

Mechanisms of a **whole living system** that would be unethical, morally unacceptable or technically unfeasible or too difficult to perform in human subjects

The use of animals in research

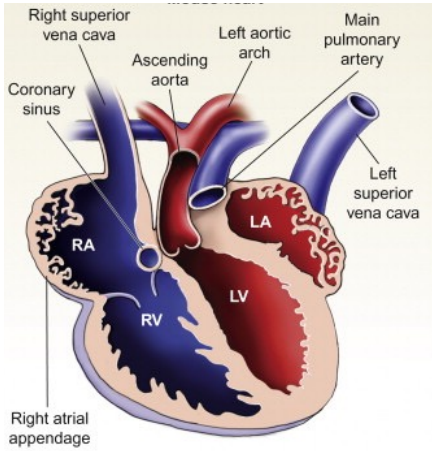
- Over the years, many **animal models** have been developed to **represent human diseases**

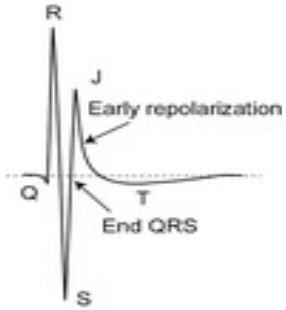
- **Genetically-modified organisms**



The use of animals in research

Mouse





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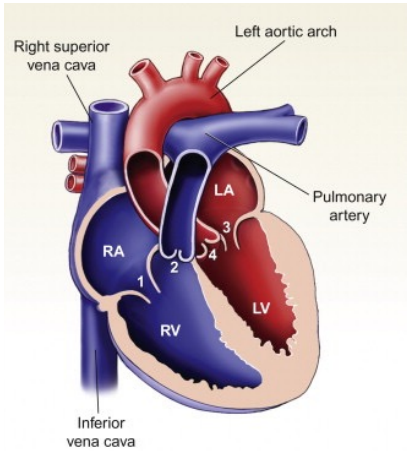
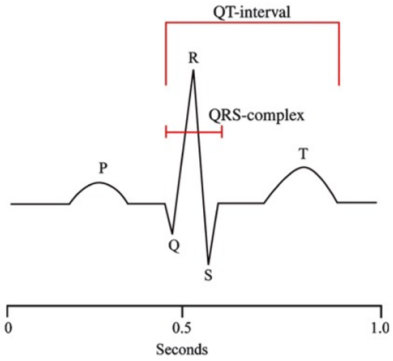
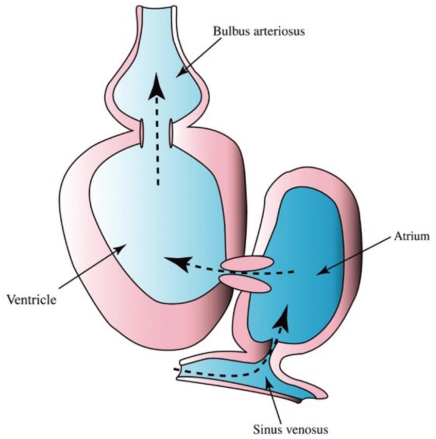
Human

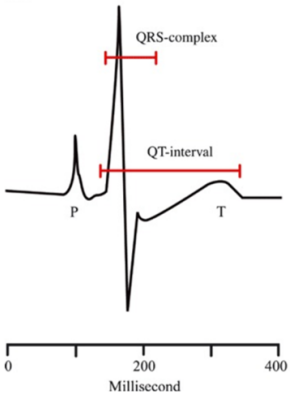




Centrum Medische Genetica Antwerpen

Zebrafish





UZA

The use of animals in research

It triggers important **ethical questions:**

- **Ethical committee approval;**

The 3Rs Principle

- **Strict requirements for the housing, care and veterinary oversight;**

- **People working with animals must have successfully completed accredited trainings;**

The 3Rs Principle

Animal experiments are forbidden if an alternative is reasonably and practically available

Number of animals limited to the lowest amount possible

Perform procedures that result in less pain or suffering

Mice and Rats

- Represent **95% of all laboratory animals** → their physiology and genetic background closely resembles that of people;
- Short life cycle and numerous off-spring;
- The similarities are strong enough to provide a **mammalian system** in which to investigate human diseases.

The study of the aorta in mice

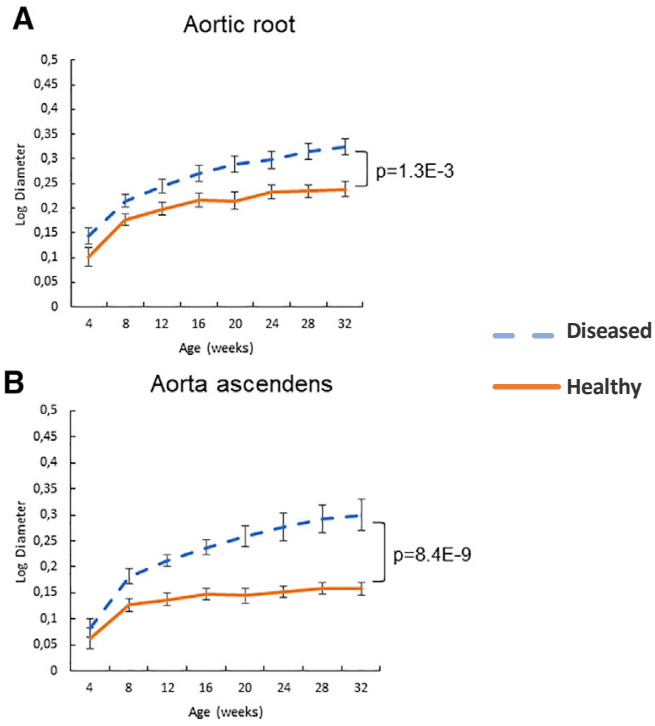
- *in vivo*: by **echocardiography**

- *ex vivo*: aorta **isolation**

Echocardiography in mice

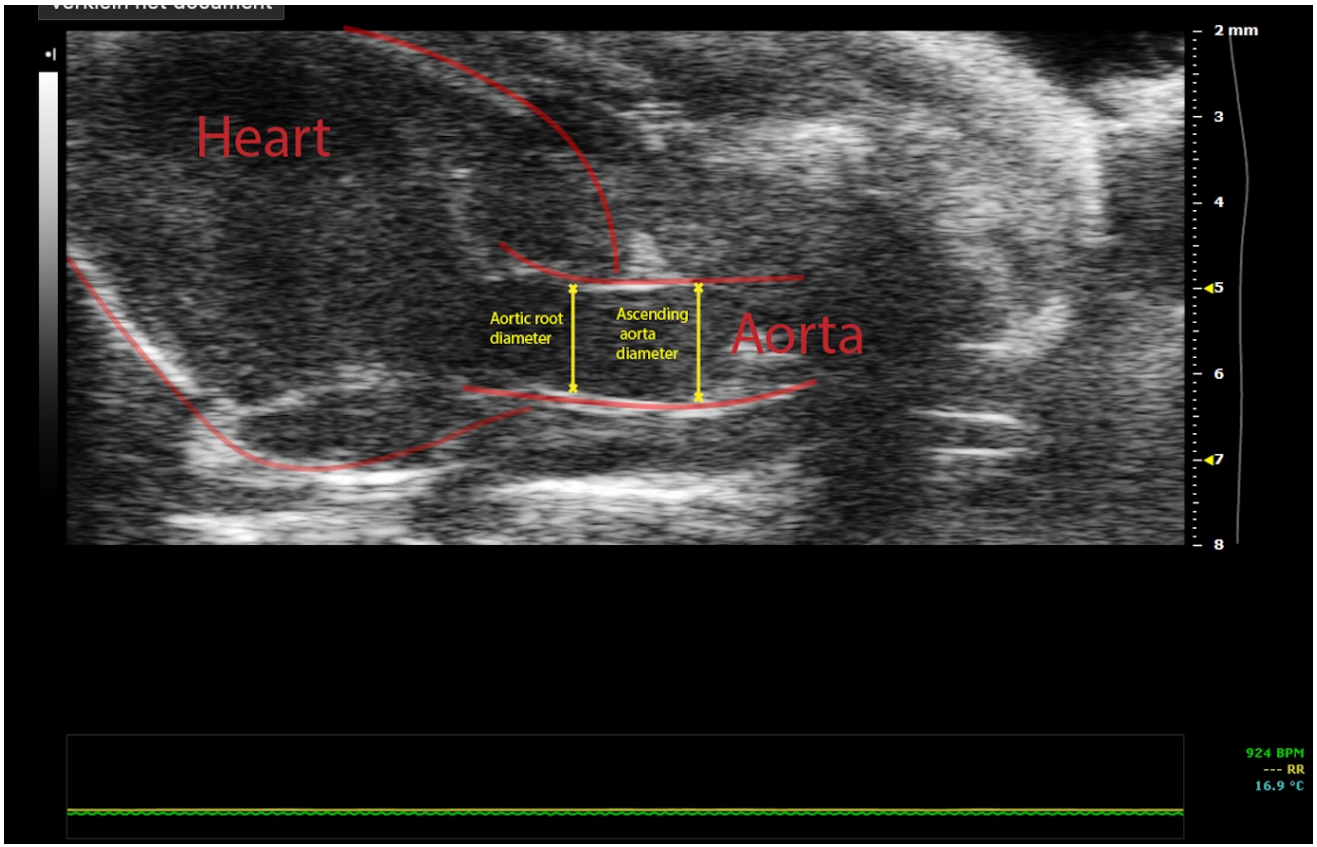
It allows to record videos of internal organs at **different time points**

Pictures are used to measure aortic root and ascending aorta **diameters**



Echocardiography in mice

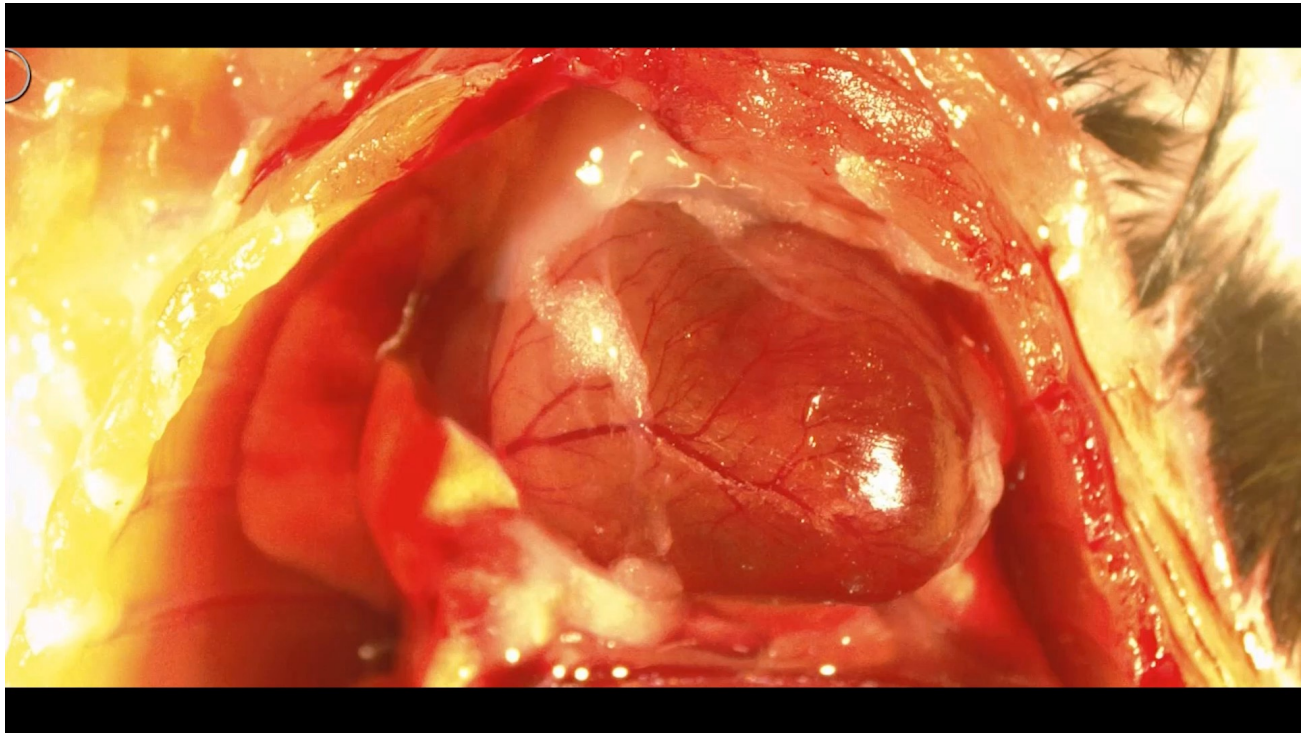
Echocardiography picture analysis



Aorta isolation

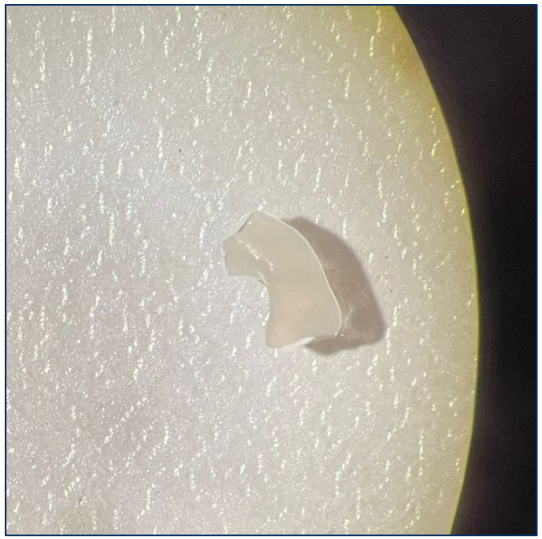
- The mouse is sacrificed by CO_2 inhalation;
- It is dissected by performing a cut from the abdomen to the thorax;
- Blood can be extracted from the heart;
- Aorta is cleaned from fat tissue and isolated:
  - RNA extraction
  - Protein extraction
  - Histological analysis

Aorta isolation

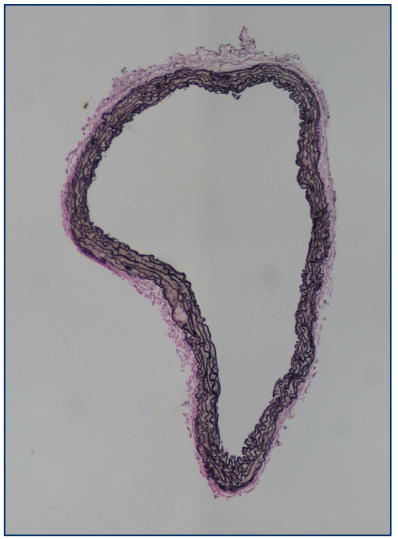


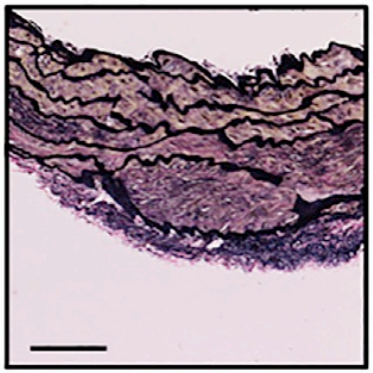
Aorta isolation

RNA and protein extraction

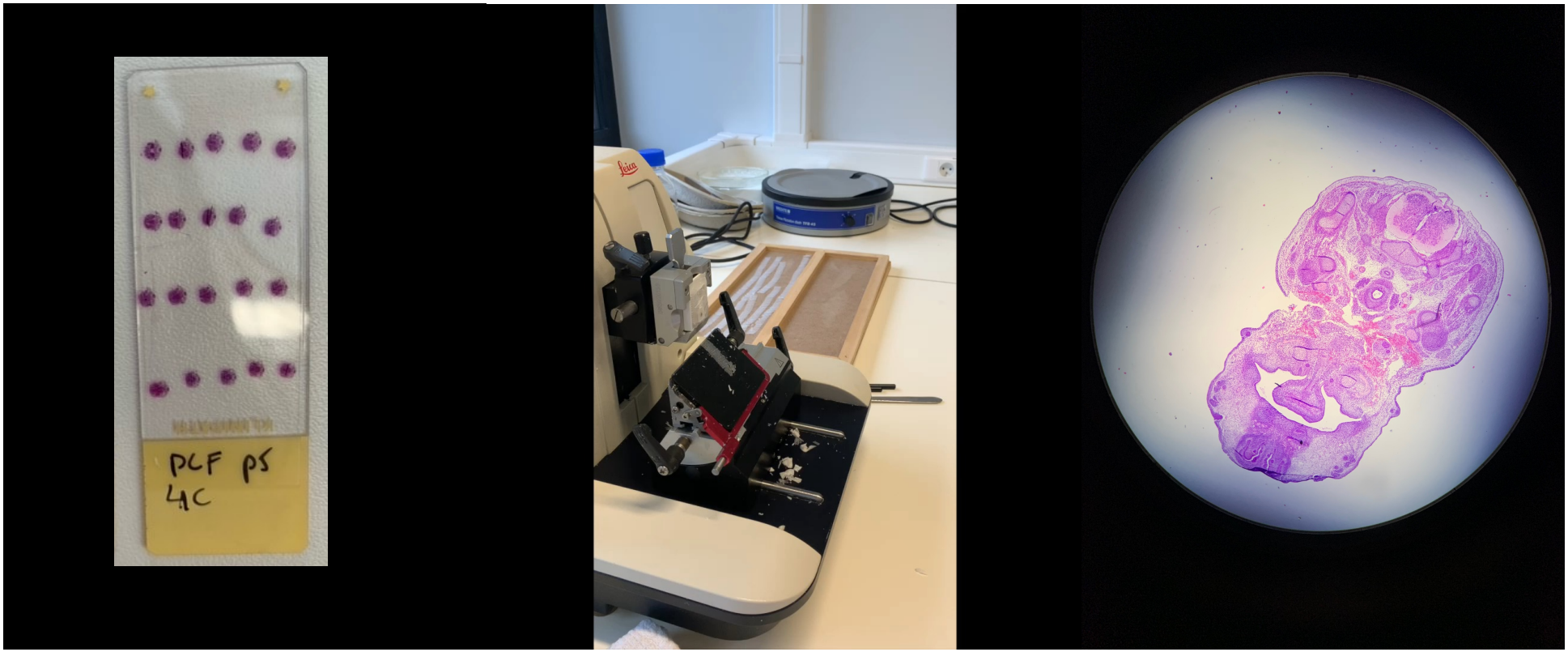


Histology - Aortic wall





Microtome and tissue staining



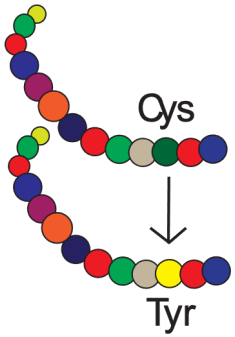
Our mouse models

- **Genetically modified mice**

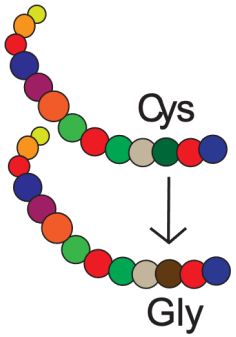
Fbn1 gene

Marfan syndrome

Cys1039Tyr



Cys1041Gly



Smad3 gene

Loeys-Dietz syndrome

Defects on one copy

Deletion of part of the gene in both copies

Our mouse models

Fbn1 $^{C1041G/+}$

(Marfan syndrome)

- Aortic root dilation from 8 weeks of age
- Mild ascending aortic dilation
- Dissections are very rare
- More pronounced in males than in females

Smad3$^{-/-}$

(Loeys-Dietz syndrome type III)

- Ascending aorta and root dilation from 6 weeks
- Rapid aneurysm growth and early death
- More pronounced in males than in females

Our mouse models

Bgn $^{0/-}$

(Meester-Loeys syndrome)

- Thoracic and abdominal aneurysm and dissection
- 50% males die from dissection before 2-3 months
- Males

Ipo8 $^{-/-}$

(Importin-β disorder)

- Ascending aortic dilation
- Mild aortic root dilation
- Dissections can happen from 30 weeks onwards
- More pronounced in males than in females

Importance of the genetic background

C57BL/6N background

Ipo8 $^{-/-}$

Sv129 background

Ascending aortic dilation and mild aortic root dilation

No cardiovascular phenotype!

Study protective mechanisms

Mouse pre-clinical trials

ECD application

- **Relevance**
- **How many animals needed?**
- **Housing**
- **Humane endpoints**

Thank you for your attention!