University Medical Center Groningen

Whole genome sequencing for neonates in trouble

Rapid whole genome sequencing
in severely ill neonates and young children.

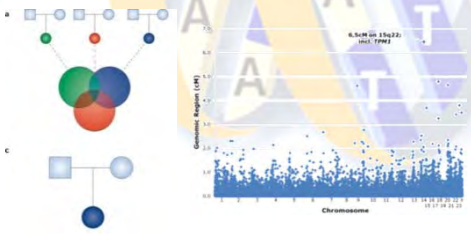
Jan Jongbloed
Genome Diagnostics
Department of Genetics
UMCG Groningen

Introduction

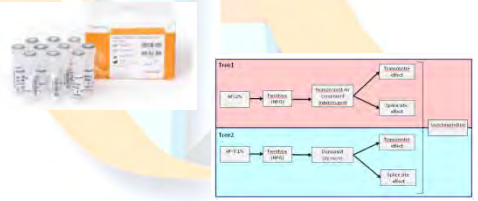
Gene panel based resequencing

ABCC9, ACTC1, ACTN2, ANKRD1, BAG3, CALR3, CRYAB, SRP3/MLP, DES, DMD, DSC2, DSG2, DSP, EMD, GLA, JPH2, JUP, LAMA4, LAMP2, LMNA, MYBPC3, MYH6, MYH7, MYL2, MYL3, MYPN, MYOZ1, MYOZ2, PKP2, PLN, PRKAG2, PSEN1, PSEN2, RBM20, RYR2, SCN5A, SGCD, TAZ, TBX20, TCAP, TMEM43, TNNC1, TNNI3, TNNT2, TPM1, TTN, VCL, ZASP

Exome Sequencing



Whole Genome Sequencing



Introduction; why WGS?

GOAL: Rapid genetic diagnostics in severly ill neonates at the NICU and IC

Why?

- Routine molecular testing time consuming
- Rapid (cyto)genetic testing is limited
- Results (often) too late to aid decision making

Relevance:

- Early diagnosis can prevent/limit unnecessary (invasive) diagnostics
- Facilitates complementary diagnostics/treatment
- Early diagnosis is important for the parents

Introduction; who?

Included: new-borns and severely ill young children in ICU with suspected genetic disease

- Unexplained and severe neurological manifestations; intractable seizures, severe neonatal onset movement disorder
- Suspected metabolic disorder
- Unexplained syndromal manifestations with multiple congenital anomalies
- Acute liver failure
- Other acute disease status

Prerequisites

- No clear acquired cause/explanation (high a-priori risk for monogenetic disorder)
- Blood/DNA is available from both parents

Routine diagnostics

Standard procedures:

- Array-CGH
- Consultants related to clinical genetics, child neurology/cardiology, metabolic diseases, etc.
- MRI, EEG, echocardiogram, etc.
- Biochemical testing
- (when appropriate) targeted gene panel tests

Introduction

Pilot project UMCG Pediatrics and Genetics

RESEARCH ARTICLE

DIAGNOSTICS

Rapid Whole-Genome Sequencing for Genetic Disease Diagnosis in Neonatal Intensive Care Units

Carol Jean Saunders,[1,2,3,4,5]* Neil Andrew Miller,[1,2,4]* Sarah Elizabeth Soden,[1,2,4]* Darrell Lee Dinwiddie,[1,2,3,4,5]* Aaron Noll,[1] Noor Abu Alnadi,[4] Nevene Andraws,[3] Melanie LeAnn Patterson,[1,3] Lisa Ann Krivohlavek,[1,3] Joel Fellis,[6] Sean Humphray,[6] Peter Saffrey,[6] Zoya Kingsbury,[6] Jacqueline Claire Weir,[6] Jason Betley,[6] Russell James Grocock,[6] Elliott Harrison Margulies,[6] Emily Gwendolyn Farrow,[1] Michael Artman,[2,4] Nicole Pauline Safina,[1,4] Joshua Erin Petrikin,[2,3] Kevin Peter Hall,[6] Stephen Francis Kingsmore[1,2,3,4,5]†

Saunders, Sci Transl Med. 2012;4:154ra135

Introduction

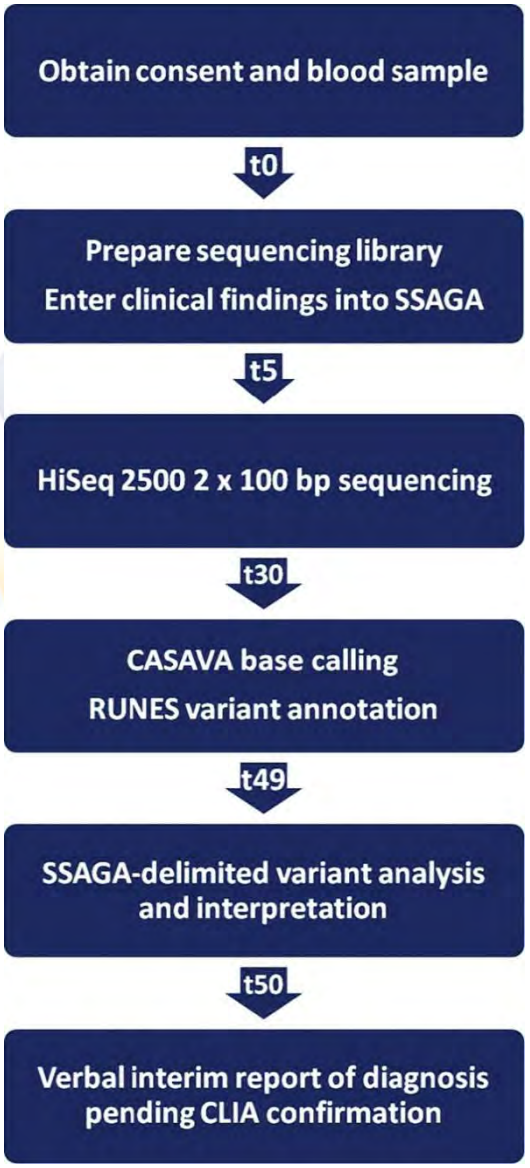
RESEARCH ARTICLE

DIAGNOSTICS

Rapid Whole-Genome Sequencing for Genetic Disease Diagnosis in Neonatal Intensive Care Units

Carol Jean Saunders,[1,2,3,4,5]* Neil Andrew Miller,[1,2,4]* Sarah Elizabeth Soden,[1,2,4]* Darrell Lee Dinwiddie,[1,2,3,4,5]* Aaron Noll,[1] Noor Abu Alnadi,[4] Nevene Andraws,[3] Melanie LeAnn Patterson,[1,3] Lisa Ann Krivohlavek,[1,3] Joel Fellis,[6] Sean Humphray,[6] Peter Saffrey,[6] Zoya Kingsbury,[6] Jacqueline Claire Weir,[6] Jason Betley,[6] Russell James Grocock,[6] Elliott Harrison Margulies,[6] Emily Gwendolyn Farrow,[1] Michael Artman,[2,4] Nicole Pauline Safina,[1,4] Joshua Erin Petrikin,[2,3] Kevin Peter Hall,[6] Stephen Francis Kingsmore[1,2,3,4,5†]

- 50 hour genetic diagnosis applying "Rapid WGS"
- 2 children retrospectively studied and (known) mutation identified
- 5 children (incl. 2 sibs) prospectively studied:
  3/4 (probably) solved
- WGS parents and/or sibs; diagnosis acquired easier and quicker



Introduction

NEWS IN FOCUS

BIOPIRACY Protocol will stop exploitation — and create red tape p.14 | **BOTANY** Forensic chemistry to stop South Africa's plant thieves p.17 | **ASTRONOMY** Telescope data bounty sparks access debate p.18 | **ASTRONOMY** Physicists debate future of Argentina's cosmic-ray observatory p.20

CONRAD BOEHMER/VOA LOVISITY

The genomes of ill newborns can be sequenced in less than 24 hours to give clinicians a rapid diagnosis.

GENOMICS

Fast sequencing saves newborns

Rapid analysis of infant genomes is aiding diagnosis and treatment of inexplicably ill babies.

and healthy. Had physicians sent his DNA off for a conventional genomic test, the diagnosis could have taken more than a month — by which time he would probably have died.

The boy is one of 44 sick infants whose genomes Kingsmore's group has sequenced using a process that can provide a diagnosis in as little as 24 hours. In 28 of these cases, the researchers have been able to diagnose the baby's condition. And in about half of these, they have been able to recommend changes in treatment, Kingsmore reported on 19 September at the Genomics of Common Diseases meeting in Potomac, Maryland. On 6 October, his group will kick off a larger project to sequence hundreds of babies' genomes. It will be the first of four newborn-sequencing studies that each received multimillion-dollar grants from the US National Institutes of Health (NIH) in September 2013. The studies will address both the feasibility and the ethics of a process that could soon become standard for inexplicably ill newborns.

Over the next five years, Kingsmore's group will sequence the genomes of 500 sick babies from the Children's Mercy Hospital NICU and compare the infants' clinical outcomes with those of 500 NICU babies who are diagnosed using conventional genetic and metabolic tests. The researchers will assess whether rapid sequencing allows babies to avoid unnecessary tests and unhelpful treatments, and whether it helps parents to make decisions about care when the child is diagnosed as having a fatal disease. Even when an infant does die, Kingsmore says, a genome sequence and diagnosis can provide closure to parents and give more information about the genetic conditions they carry.

Kingsmore calls the rapid sequencing technique a 'factory' approach, in which four or five specialists each perform one step of the process — from the blood draw to the final

which time he would probably have died.

The boy is one of 44 sick infants whose genomes Kingsmore's group has sequenced using a process that can provide a diagnosis in as little as 24 hours. In 28 of these cases, the researchers have been able to diagnose the baby's condition. And in about half of these, they have been able to recommend changes in treatment, Kingsmore reported on 19 September at the Genomics of Common Diseases meeting in Potomac, Maryland. On 6 October, his group will kick off a larger project to sequence hundreds of babies' genomes. It will be the first of four newborn-sequencing

Pilot:

Aim: provisional genetic diagnosis using Whole Genome Sequencing within 72 hours

- Start set-up of project: September 2013
- Start inclusion of patients: May 2014
- Severly ill newborns/young children
- Expected 25-30 patients per year
- Evaluation 1 year after inclusion of first patient

Pilot:

- Whole Genome Sequencing
- Platform: Illimuna HiSeq 2500 (rapid run)
- Analysis of ~2800 genes from the Clinical Genomic Database (CGD) that are “clinically actionable”
- Exclude “late onset” disease genes
- Use HPO terms for filtering

Logistics

Consult clinical genetics

Patient selection ↔ Phenotype

Pretest counseling

Consent and DNA isolation

↓

Genome Sequencing

↓

(Now max 1 month; goal several days)

Data analysis
In house pipeline/Cartagenia
Filtering CGD genes/phenotype

↓

Multidisciplinary consultation

↓

Preliminary results:
Candidate gene/genes

No → *Follow-up*

Posttest counseling

Yes ↓

Initiate complementary tests
(Sanger confirmation; examine parents; other (metabolic) tests)

Logistics lab:

Hour 0-1 → Hour 1-5 → Hour 5-39 → Hour 39 -68 → Hour 68 -72

QIAamp DNA Blood Mini Kit

Nextera DNA library preparation

Sequencing:
Illumina HiSeq 2500
4 lanes 100 bp paired end
Rapid run

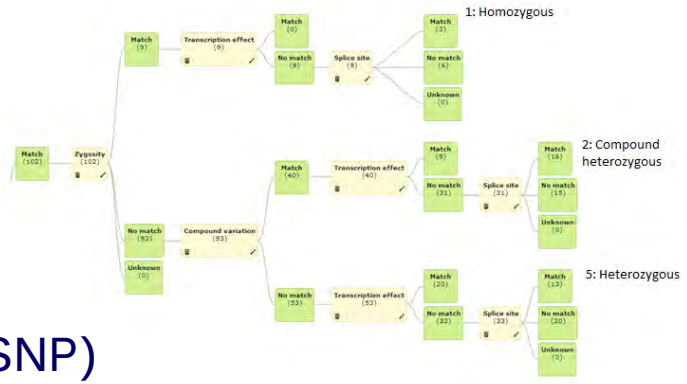
Data Processing:
In house pipeline
GCC

Variant filtering/ interpretation:
Cartagenia

Filtering strategy

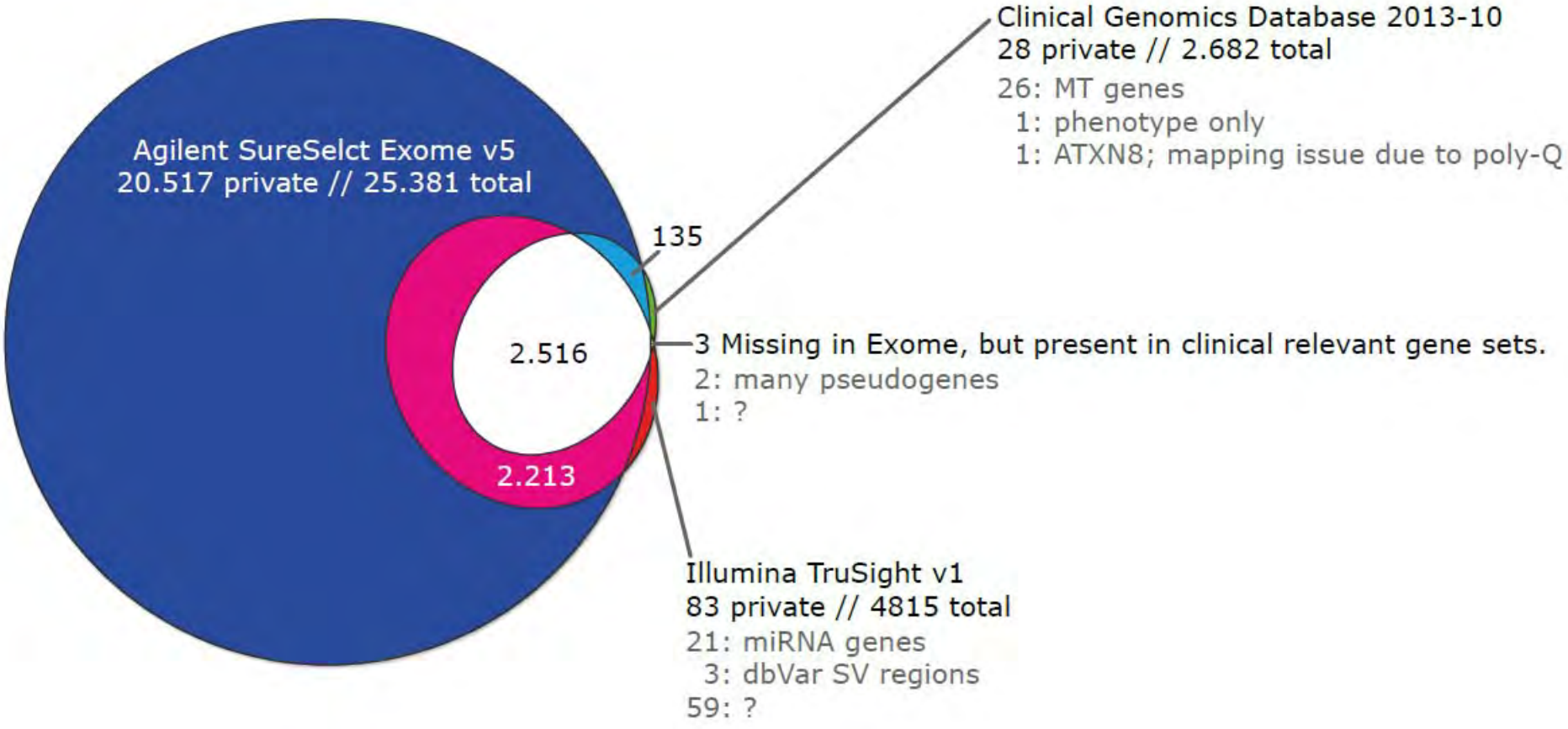
Cartagenia filtering:

- BED-file with 2800 genes from CGD
- Coverage >5
- Filtering trees
  1. <MAF 0.02 (GoNL; ESP6500; 1000G; dbSNP)
  2. HPO phenotype(s)
  3a. homozygosity, compound heterozygosity, transcriptional effect, splice site (-/+ 5 bp)
  3b. Filtering rare (*de novo*) variants <0.1%, transcriptional effect, splice site (-/+ 5 bp)



CARTAGENIA.com

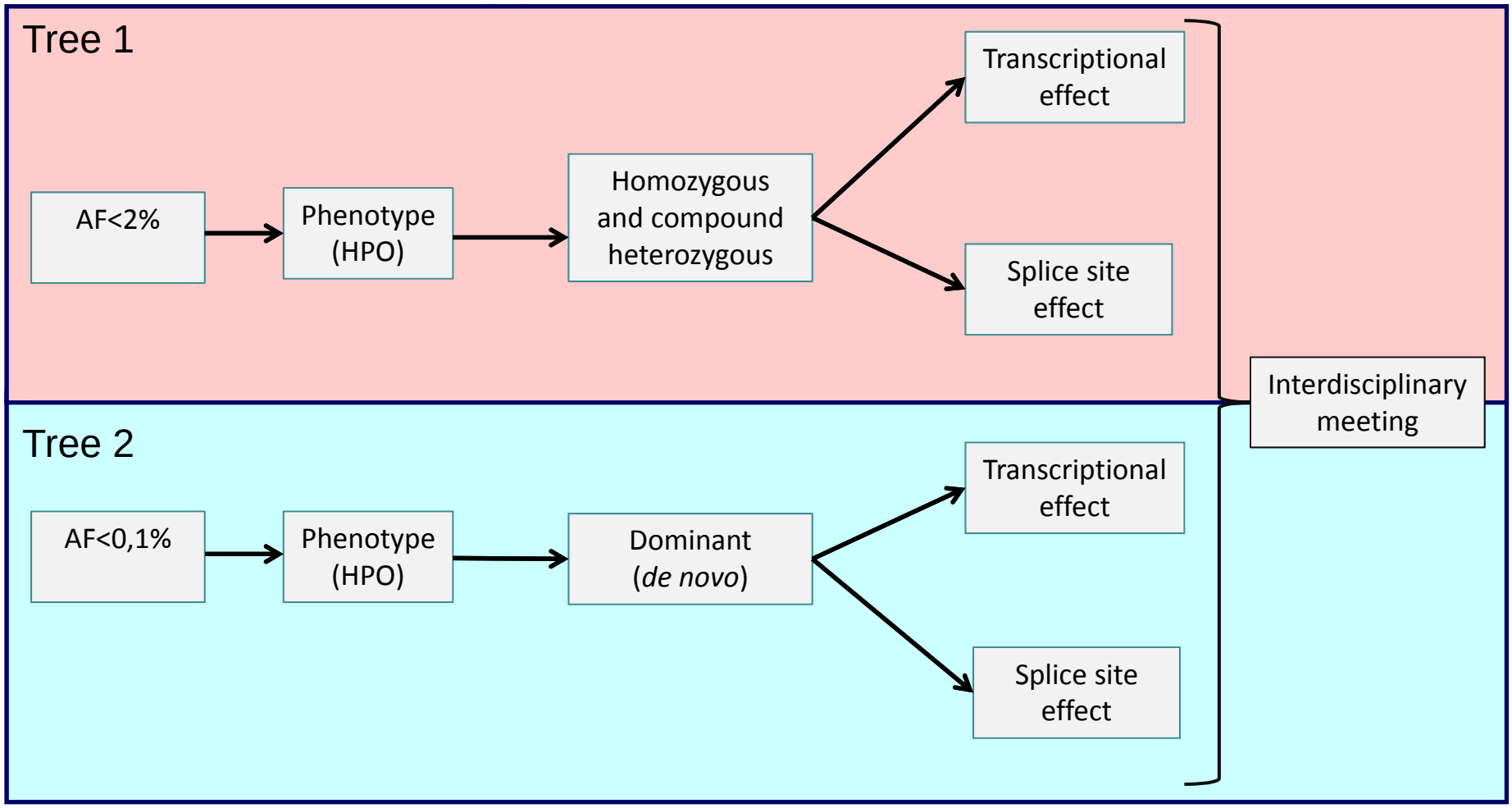
~2800 clinically actionable genes



36 late onset genes excluded

GENE	CONDITION
AIP	Pituitary adenoma, familial isolated
ALK	Neuroblastoma, susceptibility to, 3
APC	Familial adenomatous polyposis; Gardner syndrome; Desmoid disease, hereditary
AXIN2	Oligodontia-colorectal cancer syndrome
BAP1	Tumor predisposition syndrome
BMPR1A	Polyposis syndrome, hereditary mixed, 2; Polyposis, juvenile intestinal
BRCA1	Breast-ovarian cancer, familial, susceptibility to, 1; Pancreatic cancer, susceptibility to
CDH1	CDH1-related cancer
CDK4	Melanoma, cutaneous malignant, susceptibility to, 3
CDKN2A	Melanoma, familial; Melanoma-pancreatic cancer syndrome
CEBPA	Acute myeloid leukemia, familial
CHEK2	Li-Fraumeni syndrome 2
CTHRC1	Barrett esophagus/Esophageal adenocarcinoma
CTNNA1	Hereditary diffuse gastric cancer, familial
DICER1	DICER1 syndrome
EGFR	Acute myeloid leukemia, familial; Lung cancer, familial, susceptibilty to
FH	Hereditary leiomyomatosis and renal cell cancer
FLCN	Birt-Hogg-Dube syndrome; Pneumothorax, primary spontaneous
GATA2	Dendritic cell, monocyte, B and natural killer lymphoid deficiency; Emberger syndrome; Myelodysplastic syndrome; Acute myeloid leukemia, familial; Chronic neutropenia associated with monocytopenia, evolving to myelodysplasia and acute myeloid leukemia
KIT	Gastrointestinal stromal tumor
MAX	Pheochromocytoma
MLH1	Colorectal cancer, hereditary nonpolyposis, type 2; Mismatch repair cancer syndrome; Endometrial cancer; Muir-Torre syndrome
MLH3	Colorectal cancer, hereditary nonpolyposis type 7; Endometrial carcinoma
MSH2	Colorectal cancer, hereditary nonpolyposis, type 1; Endometrial cancer; Mismatch repair cancer syndrome; Muir-Torre syndrome
MSH3	Endometrial carcinoma
MSH6	Colorectal cancer, hereditary nonpolyposis type 5; Mismatch repair cancer syndrome; Endometrial cancer
MUTYH	Familial adenomatous polyposis, 2; Colorectal adenomatous polyposis, autosomal recessive, with pilomatricomas
NF2	Neurofibromatosis type II
PAX5	Pre-B cell acute lymphoblastic leukemia
PDGFRA	Gastrointestinal stromal tumor
PMS2	Colorectal cancer, hereditary nonpolyposis type 4; Mismatch repair cancer syndrome
PRKAR1A	Pigmented nodular adrenocortical disease, primary, 1; Carney complex; Myxoma, intracardiac; Acrodysostosis 1, with/without hormone resistance
RAD51D	Ovarian cancer, familial, susceptibility to
STK11	Peutz-Jeghers syndrome
TMEM127	Pheochromocytoma
TP53	Li-Fraumeni syndrome; Choroid plexus papilloma; Ependymoma, intracranial; Osteogenic sarcoma; Breast cancer, familial; Hepatoblastoma; Non-Hodgkin lymphoma; Adrenocortical carcinoma; Colorectal cancer

Filtering Cartagenia



Validation rapid WGS; criteria

-> 5 DNA samples included; 4 sequenced

- Starting material: Test samples fulfill criteria nextera protocol
  *optimized for 50 ng (2.5 ng/µl) DNA
  *absorbance ratio values of 1.8–2.0 (Qubit and Nanodrop)

- Resulting DNA library quality:
  *size distribution ~250 bp - 450 bp
  *no primer peaks
  *quantity at least 10 µl, 5nM

- Nextera DNA sample preparation finished within 4 hours

- Coverage:
  Mean coverage 30x genome wide
  80% of genes of interest covered at least 20x

- genotypes based on the sequencing results should be 99% concordant with cytoSNP arrays results.

Validation DNA prep:

Hour 0-1 → Hour 1-5 → Hour 5-39 → Hour 39 -68 → Hour 68 -72

Validated and SOP

Sequencing:
Illumina HiSeq 2500
4 lanes 100 bp paired end
Rapid run

Variant filtering/
interpretation:
Cartagenia

Validated and SOP:
Nextera sample prep
within 4 hours

Data Processing:
In house pipeline
GCC

Validation 4 samples WGS

Coverage:

Sample	mean coverage	covered at 20x
B	32x	92,30%
C	33x	89,40%
D	32x	83,00%
F	29x	72,55%

Validation WGS:

Hour 0-1 → Hour 1-5 → Hour 5-39 → Hour 39 -68 → Hour 68 -72

QIAamp DNA Blood Mini Kit

Nextera DNA preparat

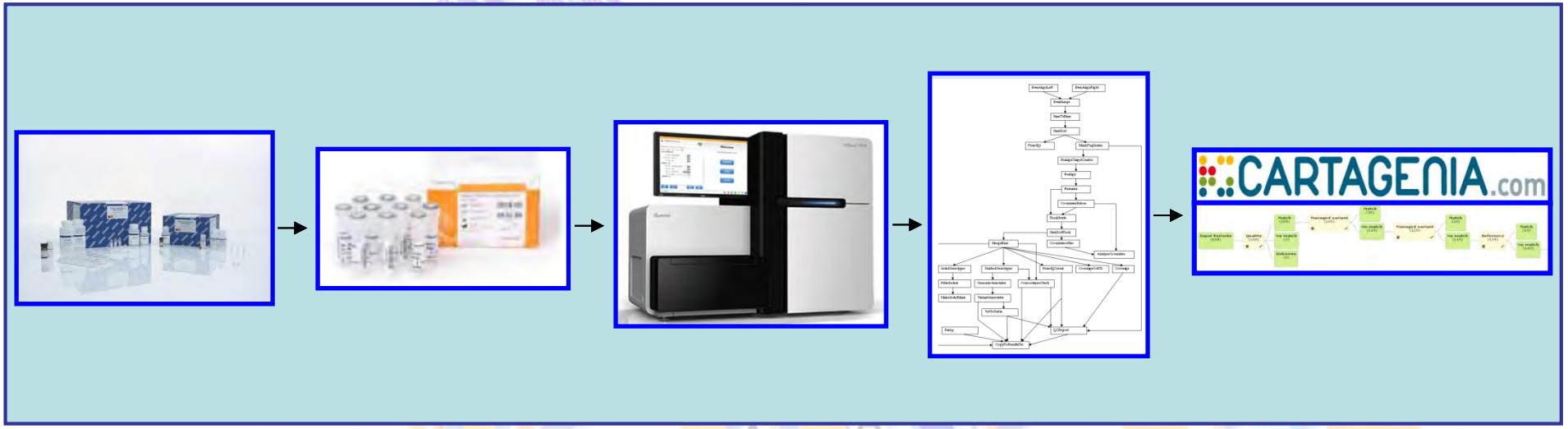
Validated and SOP:
4 samples with known mutation
mean coverage 30x
Genome wide
>80% of CGD genes
Covered >20x

Processing:
use pipeline GCC

Variant filtering/ interpretation:
Cartagenia

Validation data processing:

Hour 0-1 → Hour 1-5 → Hour 5-39 → Hour 39 -68 → Hour 68 -72



QIAamp DNA Blood Mini Kit

Nextera DNA library preparation

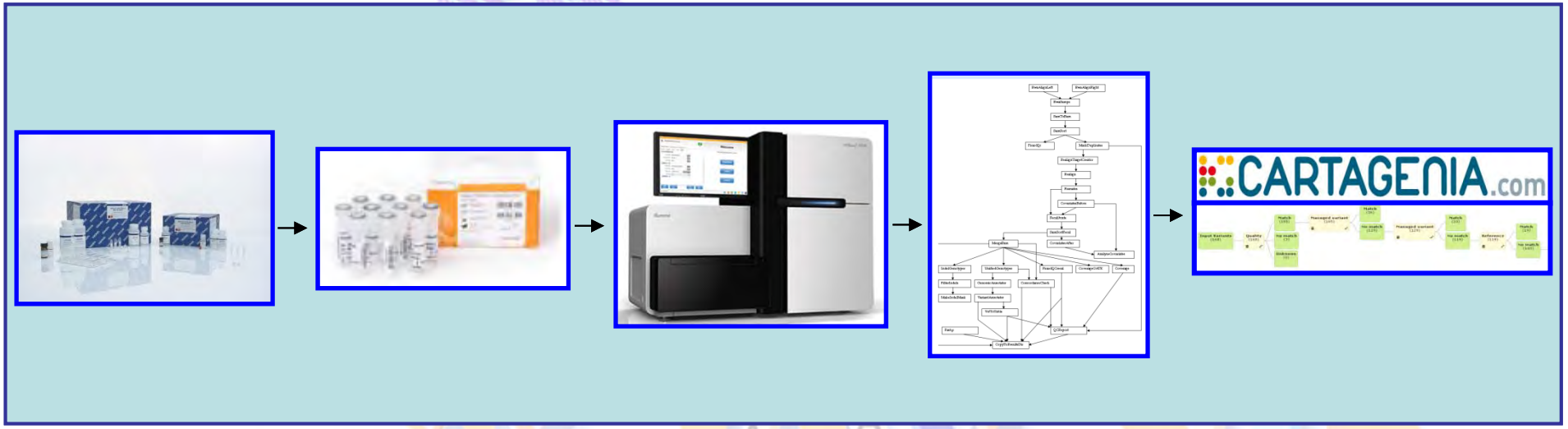
Sequencing:
Illumina HiSeq 2500
4 lanes 100 bp paired end
Rapid ru

Variant filtering/ interpretation:
Cartagenia

Validated and SOP:
Sequencing genotypes 99% concordant with cytoSNP arrays

Validation data analysis:

Hour 0-1 → Hour 1-5 → Hour 5-39 → Hour 39 -68 → Hour 68 -72



QIAamp DNA Blood Mini Kit

Nextera DNA library preparation

Sequencing:
Illumina HiSeq 2500
4 lanes 100 bp paired end
Rapid run

Data Processing:
In house pipeline
GCC

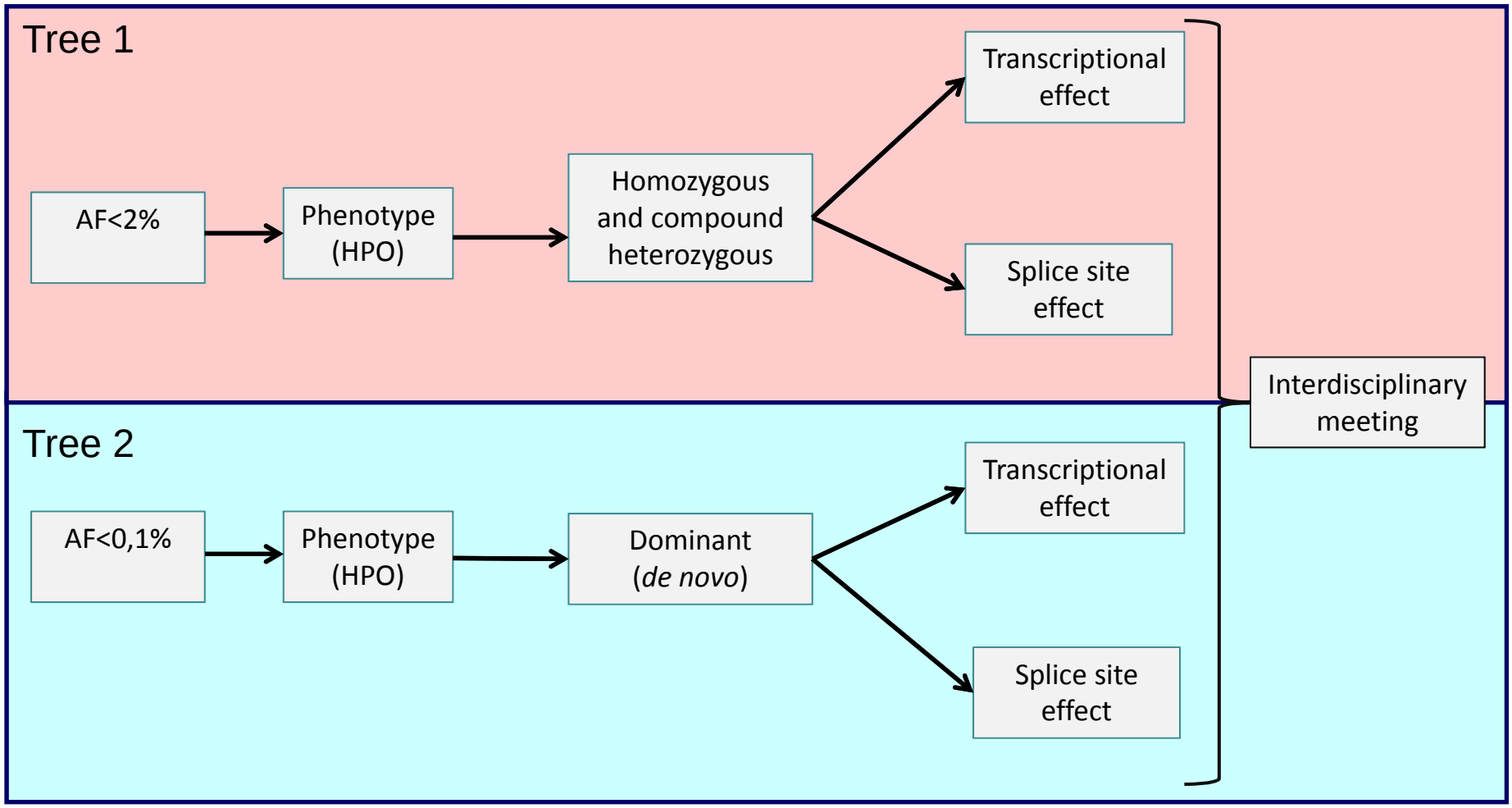
Variant filtering/ interpretation:
Cartagenia

Validation filtering strategy

CARTAGENIA.com

- The pathogenic mutation in 30 patient previously detected with exome sequencing should be correctly detected in Cartagenia
- The amount of genes/variants left over after running a classification trees in Cartagenia should not be >20
- The tree should be applicable to different types of diseases and inheritance modes
- Comparing the genotype calls based on NGS with Sanger sequencing results. All pathogenic mutations confirmed by sanger sequencing, need to be called for each patient.

Filtering Cartagenia



Validation filtering strategy

CARTAGENIA.com

- The pathogenic mutation(s) in 20 patient previously detected with exome sequencing should be correctly detected in Cartagenia

  *20/20 patients pathogenic mutations identified
  *average coverage of respective nucleotide(s): 35x (min. 8x; max. 90x)

- The amount of genes/variants left over after running a classification trees in Cartagenia should not be more than 20

- The tree should be applicable to different types of diseases and inheritance modes

  *Every kind of disease and the recessive, compound heterozygote and dominant enheritance mode could be correctly analyzed with this tree.

- Comparing the genotype calls based on NGS with Sanger sequencing results. All pathogenic mutations confirmed by Sanger sequencing, need to be called from each patient.

  * The SNV and indel calling pipeline called every variant correctly

Validation filterering strategy:

Hour 0-1 → Hour 1-5 → Hour 5-39 → Hour 39 -68 → Hour 68 -72

QIAamp DNA
Blood Mini Kit

Nextera DNA library
preparation

Sequencing:
Illumina HiSeq 2500
4 lanes 100 bp paired enc
Rapid run

Data
In house pipeline
GCC

Validated and SOP:
Mutation detected in
4 WGS and 30 exome
samples
using filtering pipelne

Logistics

Consult clinical genetics

Patient selection ↔ Phenotype

Pretest counseling

Consent en DNA isolation

↓

Genome Sequencing

↓

(Now max 1 month; goal several days)

Data analysis
In house pipeline/Cartagenia
Filtering CGD genes/phenotype

↓

Multidisciplinary consultation

↓

Preliminary results:
Candidate gene/genes

No → *Follow-up*

Posttest counseling

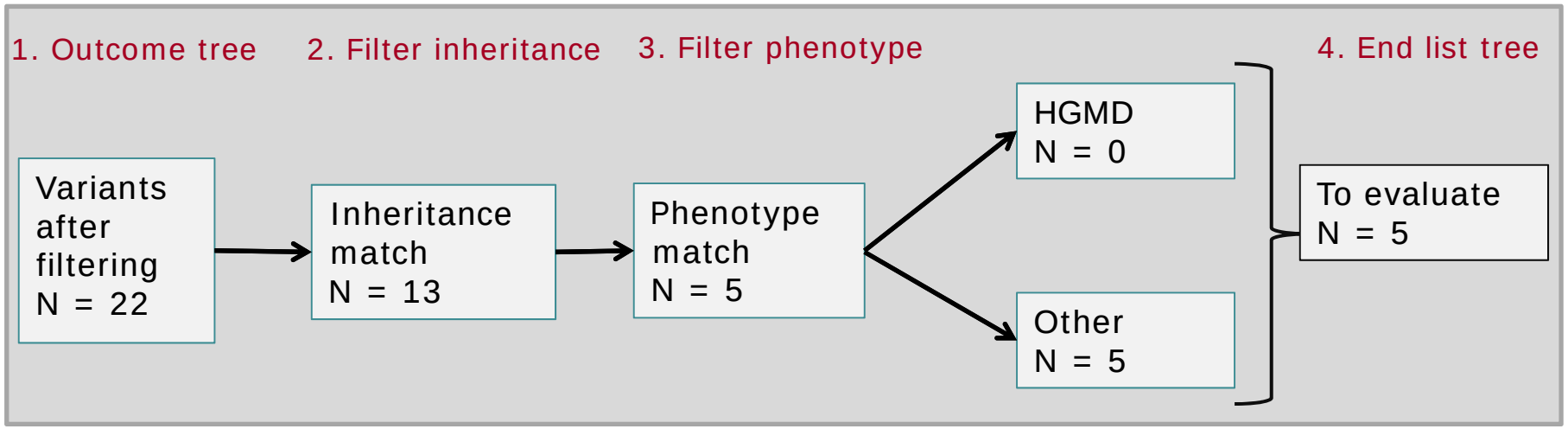
Yes ↓

Initiate complementary tests
(Sanger confirmation; examine parents; other (metabolic) tests)

Interdisciplinary meeting

- technician, lab specialist, clinical geneticist, bioinformatician and if possible paediatrician
- Evaluate each variant based on OMIM annotation/disease, check inheritance match and phenotypes

- Evaluate incidental findings: if necessary consult review board installed for this purpose



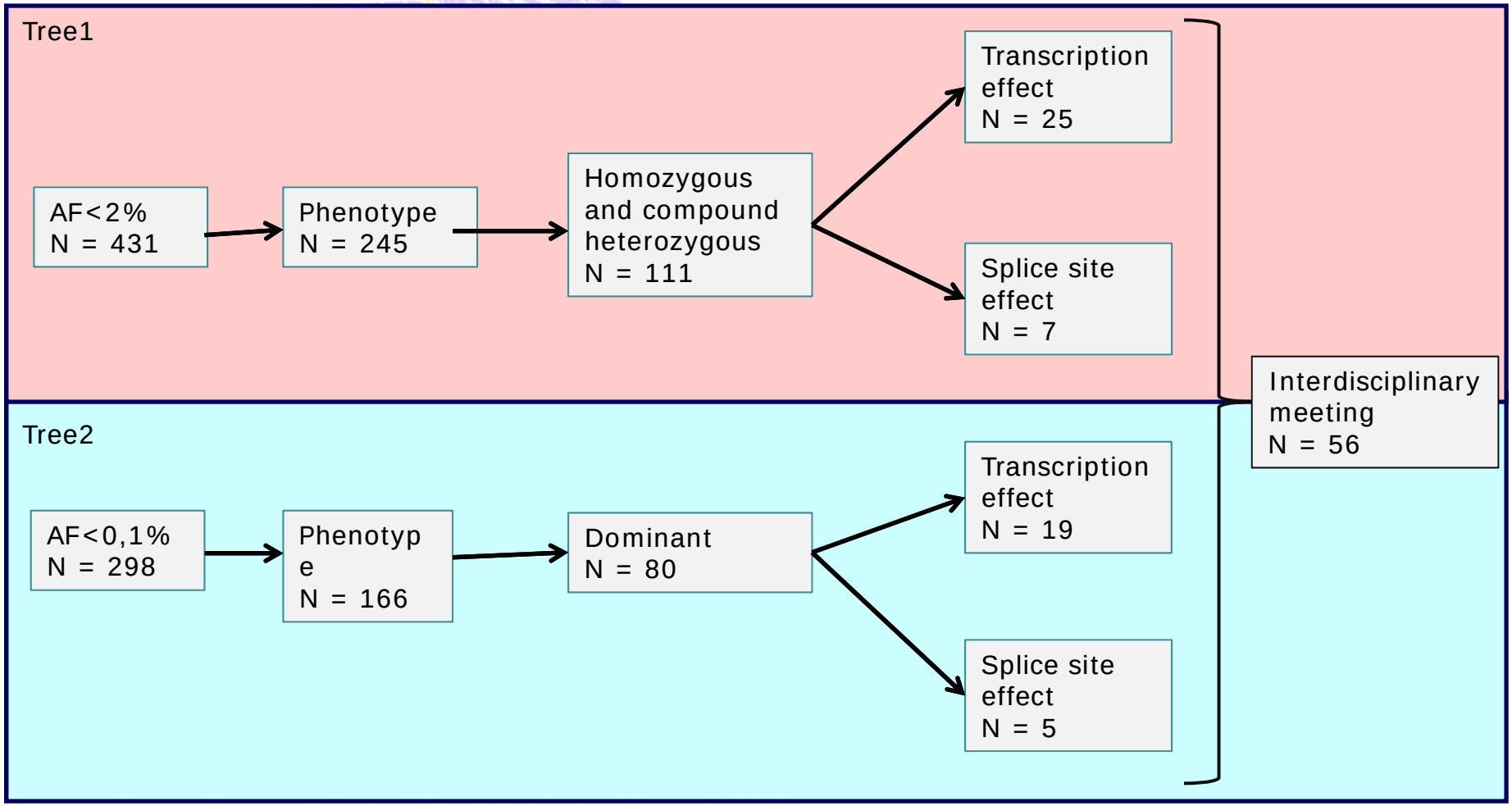
Validation patient (WES en WGS)!

- Girl, 2 years old, psychomotoric retardation, developmantal delay, epilepsy

Filtering trees

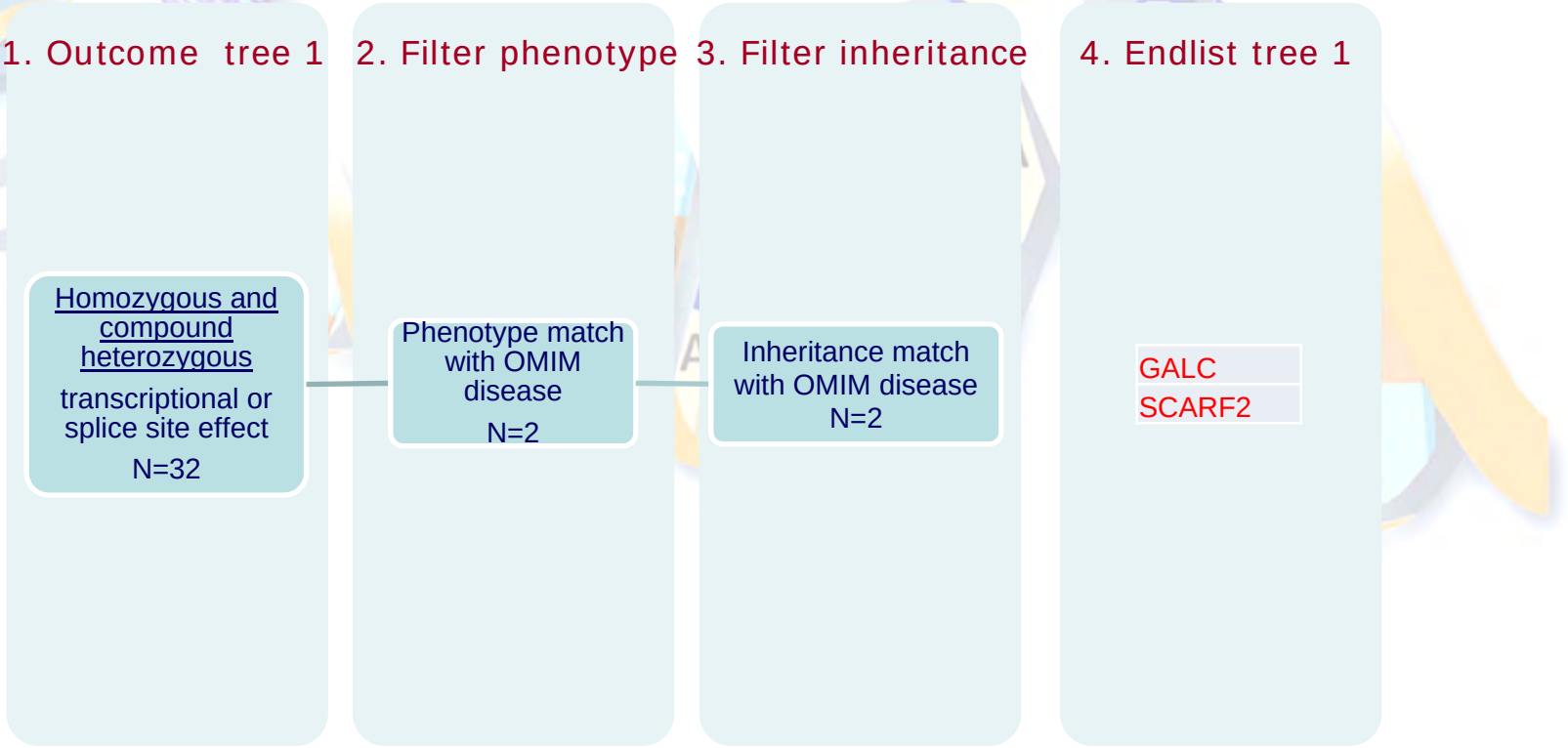
start with **5200** variants after filtering for CGD genes

Phenotype: HP:0002011 Abnormality of the central nervous system



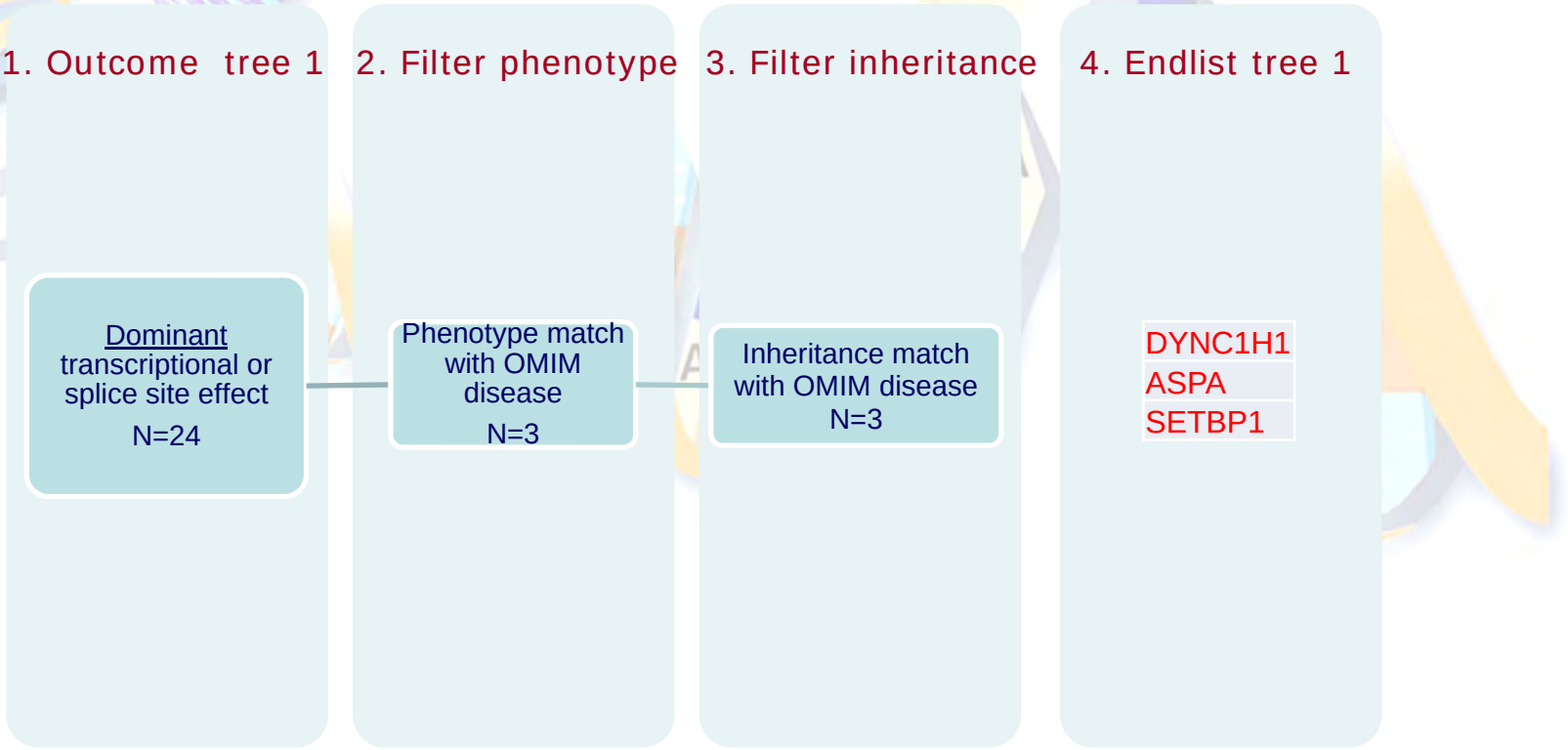
Interdisciplinary meeting

- technician, lab specialist, clinical geneticist, bioinformatician and if possible paediatrician
- Evaluate each variant based on OMIM annotation/disease, check inheritance match and phenotypes



Interdisciplinary meeting

- technician, lab specialist, clinical geneticist, bioinformatician and if possible paediatrician
- Evaluate each variant based on OMIM annotation/disease, check inheritance match and phenotypes



Validation patient!

Candidate genes

tree	Over-erving	dbSNP	omimlink	Gene	cDNA	Protein	HGMD/OMIM Disease	Alamut	CADD
2	dom (het)	rs150428209	http://omim.org/entry/609806	**HMBS**	c.962G>A	p.R321H	Porphyria (HGMD)	Likely benign	9.6
2	dom (het)	rs142041344	http://omim.org/entry/608034	**ASPA**	c.10T>C	p.C4R	Canavan disease (HGMD)	Likely benign	5.8
1	rec	rs11300320	http://omim.org/entry/606890	**GALC**	c.1162-4delT		Krabbe disease	Likely benign	3?
1	rec	rs5844419	http://omim.org/entry/613619	**SCARF2**	c.2304dupC	p.A768fs	Van den Ende-Gupta syndrome	VOUS*	5?
2	dom (het)		http://omim.org/entry/600112	**DYNC1H1**	c.5140G>A	p.A1714T	Mental retardation, autosomal dominant 13	Likely pathogenic	35
2	dom (het)	rs200957852	http://omim.org/entry/611060	**SETBP1**	c.682_683insTCTT	p.T228fs	Schinzel-Giedion midface retraction syndrome	Likely pathogenic**	10?

*ESP: MAF EA: 0.014
MAF AA: 0.027

**ESP:
- comparable insertion known
- in 1/2 isoforms
- does not fit with phenotype

Validation patient

Candidate genes

tree	Over-erving	dbSNP	omimlink	Gene	cDNA	Protein	HGMD/OMIM Disease	Alamut	CADD
2	dom (het)	rs150428209	http://omim.org/entry/609806	**HMBS**	c.962G>A	p.R321H	Porphyria (HGMD)	Likely benign	9.6
2	dom (het)	rs142041344	http://omim.org/entry/608034	**ASPA**	c.10T>C	p.C4R	Canavan disease (HGMD)	Likely benign	5.8
1	rec	rs11300320	http://omim.org/entry/606890	**GALC**	c.1162-4delT		Krabbe disease	Likely benign	3?
1	rec	rs5844419	http://omim.org/entry/613619	**SCARF2**	c.2304dupC	p.A768fs	Van den Ende-Gupta syndrome	VOUS*	5?
2	dom (het)		http://omim.org/entry/600112	**DYNC1H1**	c.5140G>A	p.A1714T	Mental retardation, autosomal dominant 13	Likely pathogenic	35
2	dom (het)	rs200957852	http://omim.org/entry/611060	**SETBP1**	c.682_683insTCTT	p.T228fs	Schinzel-Giedion midface retraction syndrome	Likely pathogenic**	10?

*ESP: MAF EA: 0.014
MAF AA: 0.027

**ESP:
- comparable insertion known
- in 1/2 isoforms
- does not fit with phenotype

Real patients

Validation interpretation procedure 6 patients:

- 5/6 patients pathogenic mutation identified
- 1/6 patiënts not.
  Reason: gene not yet known disease related in CGD database.
- in half of patients incidental findings.

Incidental findings:

Different situations:

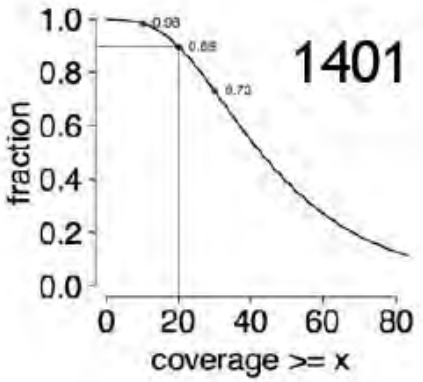
1. Finding (P/LP) explains part of phenotype; reported and confirmed

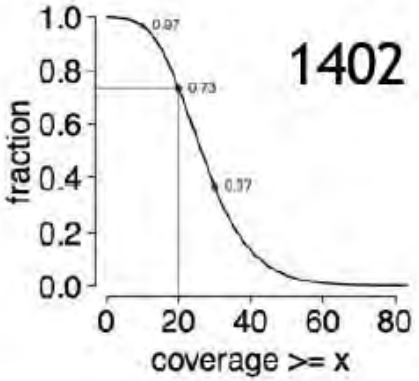
2. Clealrt actionable finding (P/LP) for patient or family; reported and confirmed

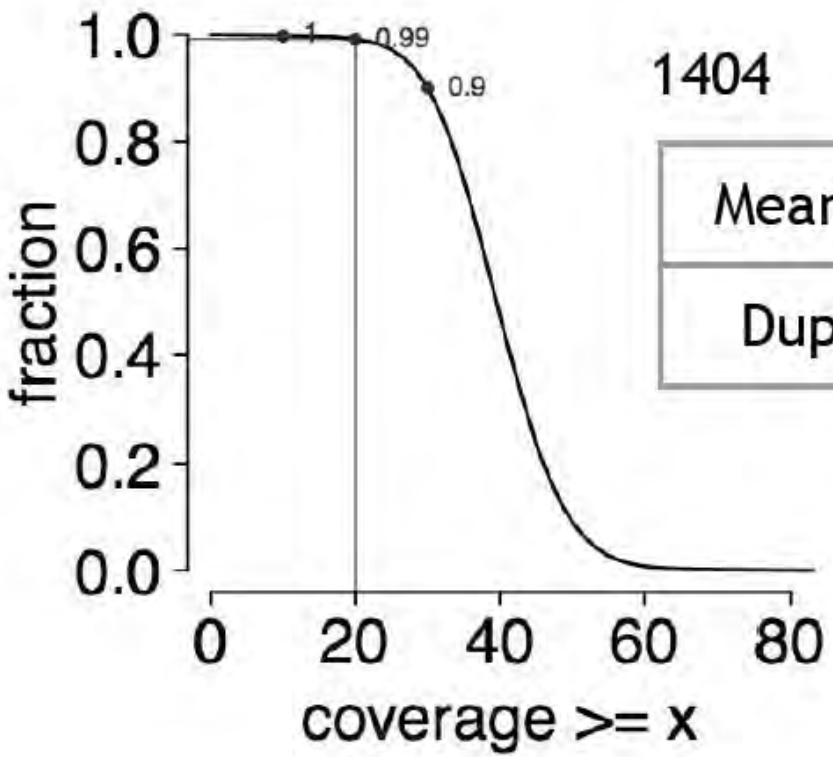
3. With respect to VUS (class 3); not reported unless convincingly related to phenotype.

4. remainder: discussed within multidisciplinary meeting, also when this concerns VUS (class 3), but association with phenotype convincing.

First 5 patients



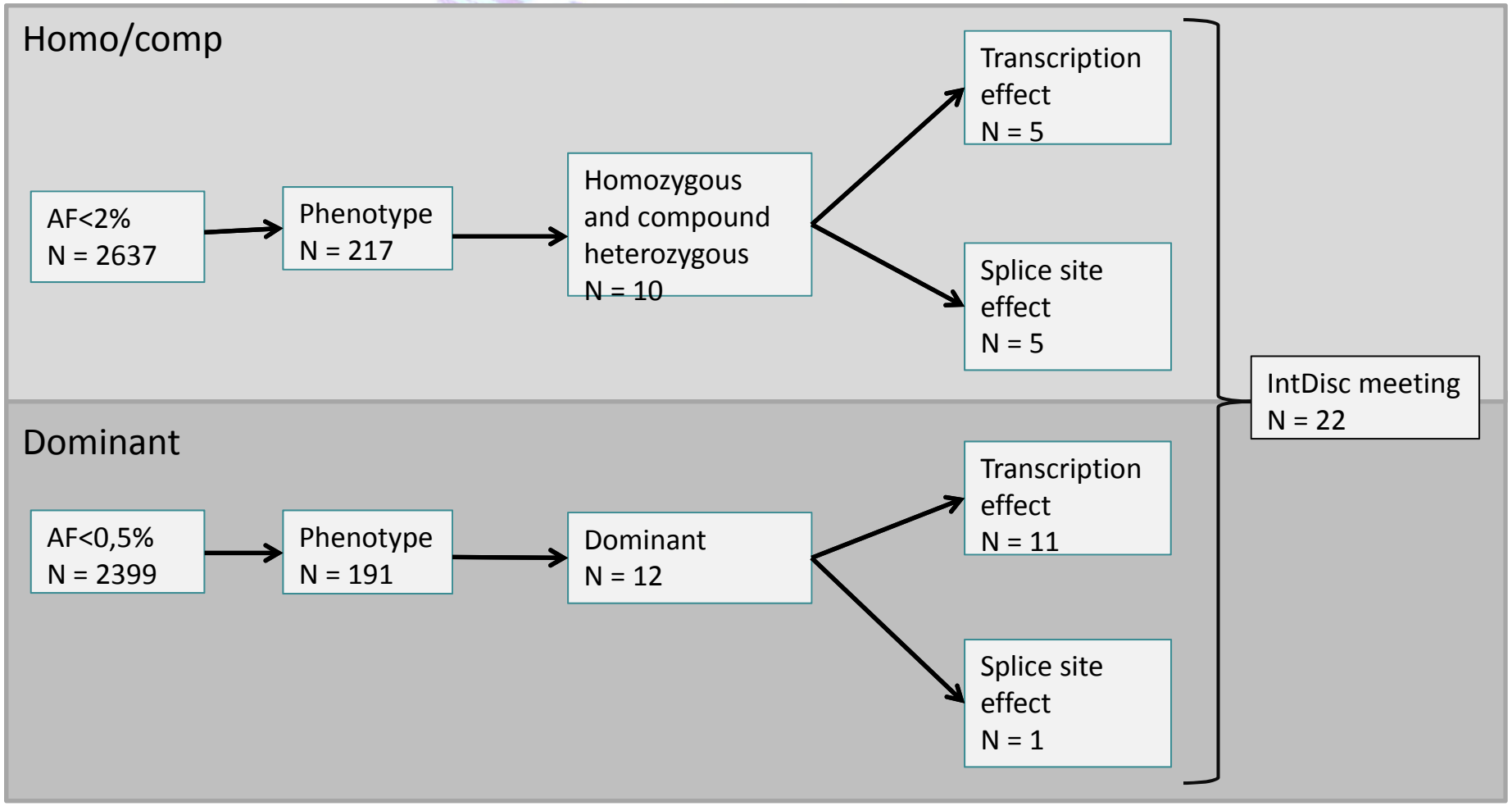




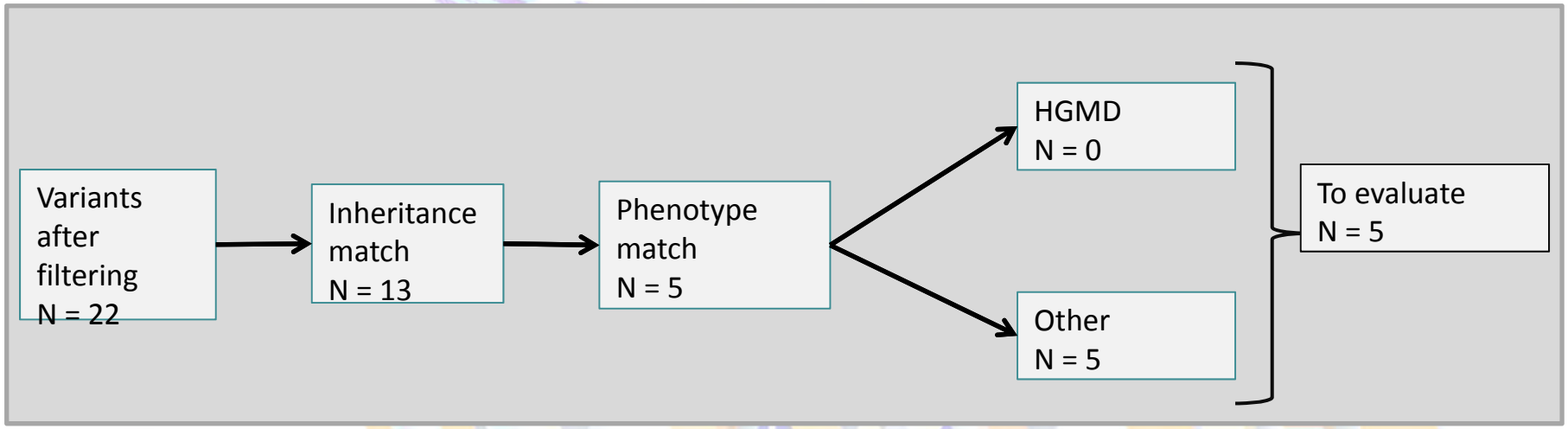
Mean Coverage	39.8
Duplicates (%)	0.9

Example patient

HPO terms HP:0001638 Cardiomyopathy



example patient



5 variants for Sanger sequencing

3 heterozygous variants also present in one of the parents

1 variant “low qual” not confirmed

1 variant not confirmed

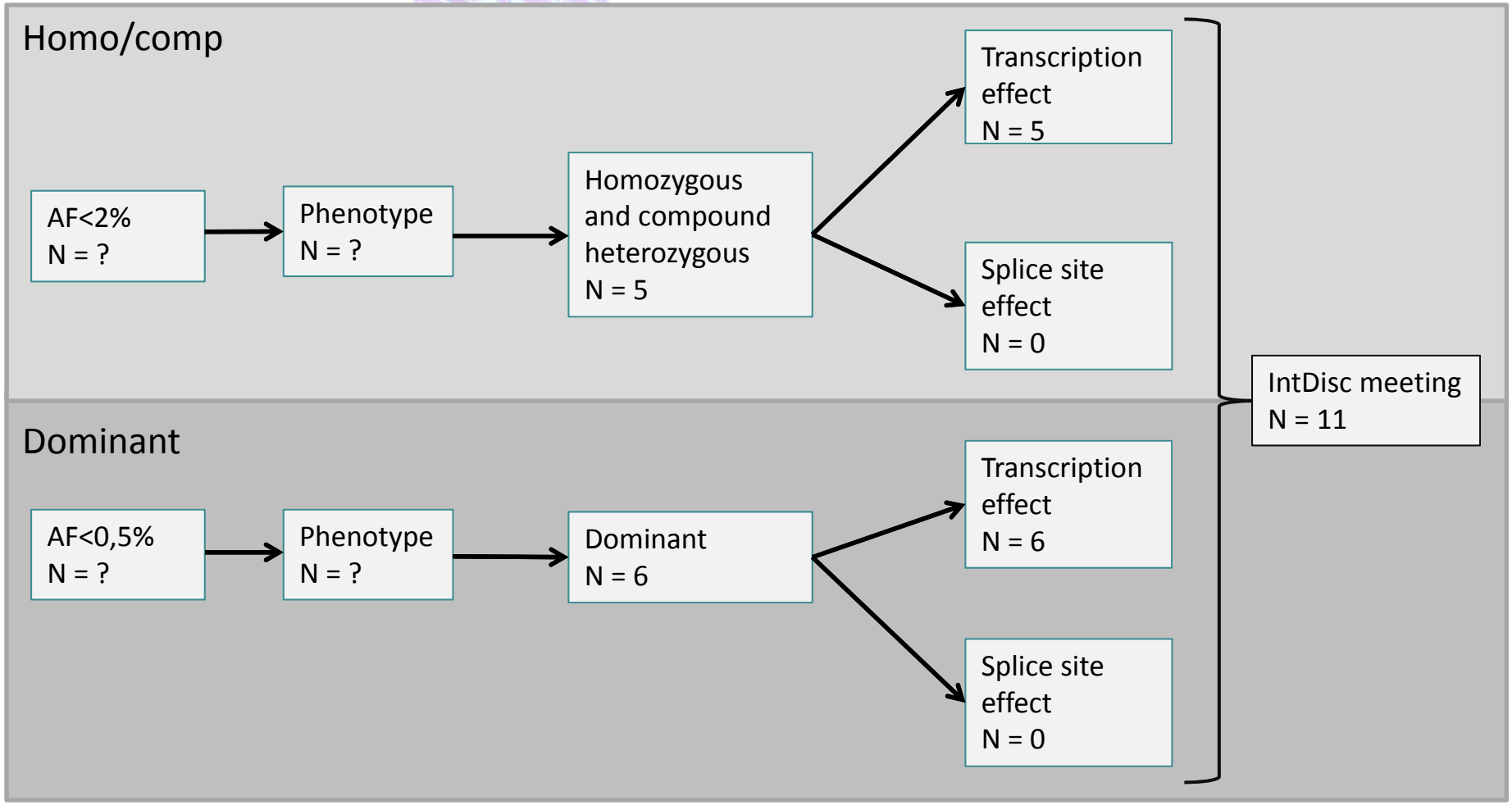
First patients

Patient	Phenotype	Mean coverage	Nr of candidates after filtering	Provisional diagnosis
1	Abnormality of the nervous system Abnormality of movement	51x	5	no
2	Dilated cardiomyopathy (Myopathy)	29x	5	no
3	Cleft palate Abnormality of finger Abnormality of the cerebral ventricles	40x *	0	no

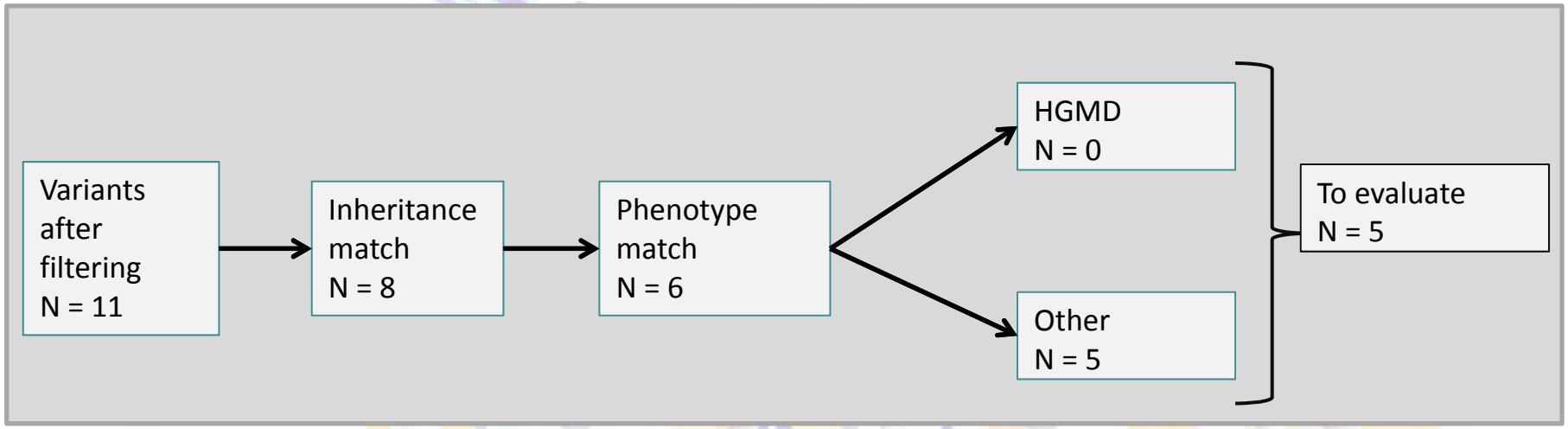
** Switch to different library prep chemistry*

Example recent patient

Myoclonieen: Myoclonus (HP:0001336); Generalized hypotonia (HP:0001290); Status epilepticus (HP:0002133); Multifocal epileptiform discharges (HP:0010841); Epileptic encephalopathy (HP:0200134)



example patient



5 variants for Sanger sequencing

3 heterozygous variants also present in one of the parents

1 variant “low qual” not confirmed

1 variant not confirmed

example patient

Overerving	Gene (gene)	cDNA (cNome	Protein	HGMD Disease	Inheritanc	Overerving	Fenotype	Inclusie	Classifica	vervolgactie	CADD
Comp	ATN1	c.1500G>T	p.Q500H		AD	Ja	JA	JA	VOUS	bevestigen Sanger	0.019
Comp	ATN1	c.2260G>A	p.V754I		AD	Ja	JA	JA	VOUS	bevestigen Sanger	17.26
Comp/Dom	BRAT1	c.1636G>T	p.V546L		AR	Nee	JA	JA, array	VOUS	bevestigen Sanger	14.31
Comp/Dom	CHMP2B	c.560G>A	p.S187N		AD	Ja	NEE		n.v.t.		10.87
Homo	GRIA3	c.380dupG			X-linked	Ja	NEE, o.b.v. frequentie		n.v.t.		
Comp	RANBP2	c.2173A>G	p.S725G		AD	ja	JA	JA	VOUS		12.79
Comp	RANBP2	c.2351G>A	p.R784K		AD	Ja	JA	JA	VOUS		0.440
Comp/Dom	SERPINI1	c.40A>G	p.S14G		AD	Ja	JA	JA	VOUS		8.923
Comp/Dom	SHANK3	c.829G>A	p.G277R		AD	Ja	JA	JA	VOUS		13.01
Comp/Dom	TSFM	c.817G>A	p.V273I		AR	Nee	JA	JA, array	VOUS		5.311
Comp/Dom	UPB1	c.254C>A	p.A85E	Ureidopropionase	AR	Nee	JA	Biochemisch	VOUS	bevestigen Sanger	14.74

4 variants: confirm with Sanger sequencing

Biochemical test: purines/pyrimidines in urine

3 heterozygous variants with gene known as recessively inherited: check SNP array result

Conclusions

- Technically feasible within one week
- Nextera sample prep not reliable enough; switch to NEB
- Very good coverage
- Interdisciplinary approach has taught us a lot:
  highlighted good and weaker aspects of whole procedure
- Begin to speak and understand each other's language
- Also better procedures/faster for routine diagnostics

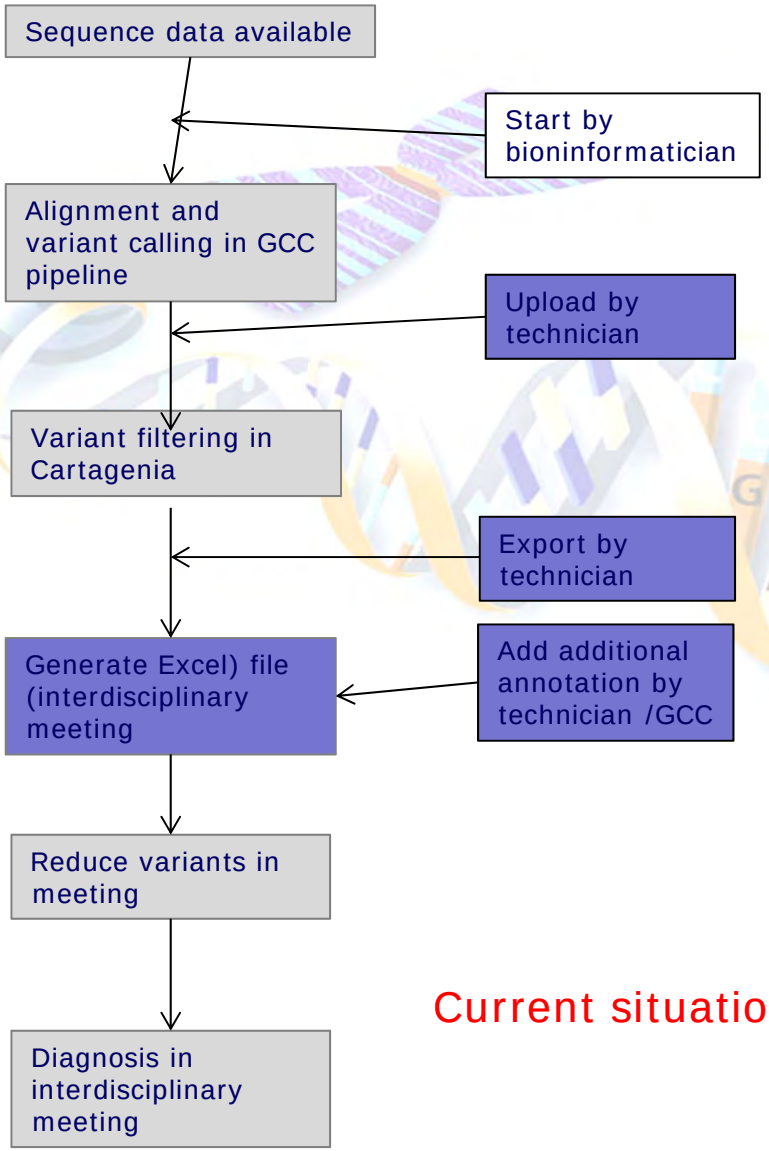
- Just started the full procedure, still work in progress…

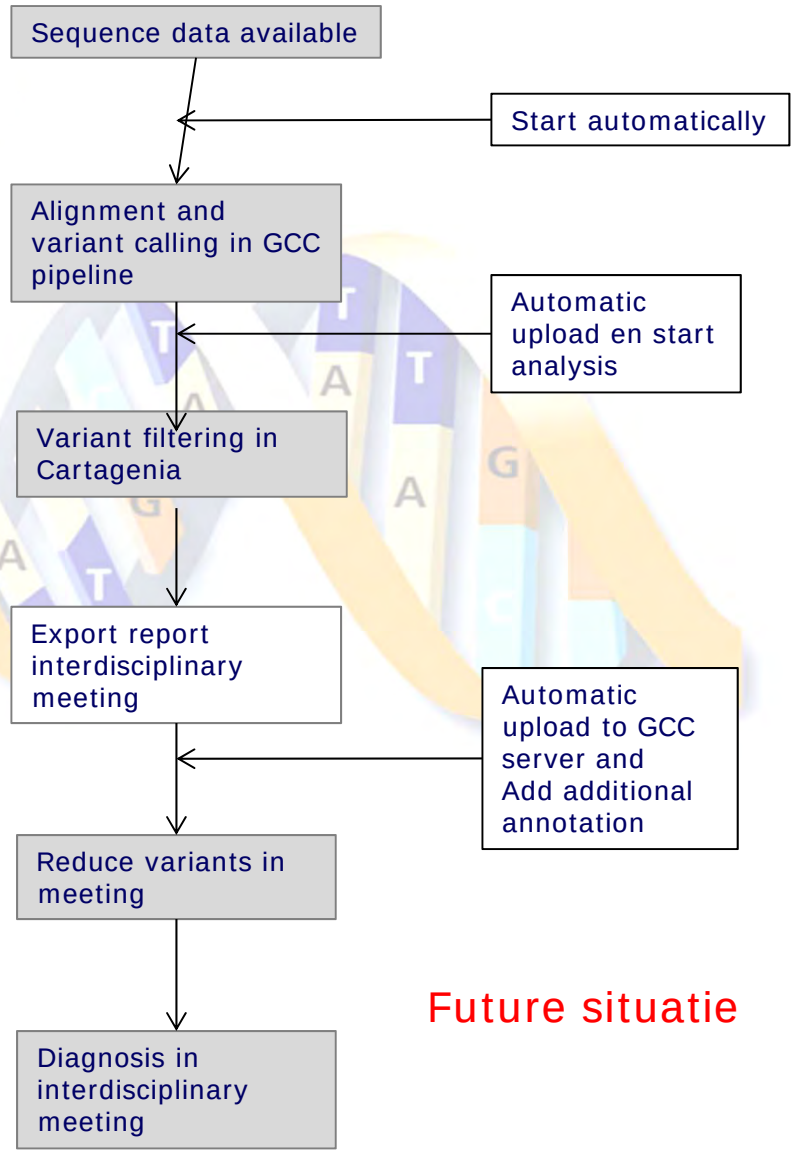
- Aim still: genetic diagnosis within 72 hours

Future Perspectives

- Additional CNV detection in the pipeline
- Additional annotation tools; more automation
- Fast Exome sequencing in trio design or WGS?
- Inclusion (20) additional patients (not rapid screening): patients that died without cause known
  - -Trio's Agilent ID kit
  - -CNV tools integrated
  - -(fibro's available) RNA seq
  - - neg? -> WGS

Data analysis process





Future Perspectives

- Additional CNV detection in the pipeline
- Additional annotation tools; more automation
- Fast Exome sequencing in trio design or WGS?
- Inclusion (20) additional patients (not rapid screening): patients that died without cause known
  - -Trio's Agilent ID kit
  - -CNV tools integrated
  - -(fibro's available) RNA seq
  - - neg? -> WGS

Acknowledgments

Clinical Genetics:
Anne Herkert
Erica Gerkes
Katharina Löhner
Conny van Ravenswaaij-Arts
Patrick Rump
Mieke Kerstjens
Irene van Langen

GCC (Bioinformatics):
Mark de Haan
Gerben van der Vries
Joeri van der Velde
Freerk van Dijk
Pieter Nierincx
Morris Swertz

Genome diagnostics:
Kristin Abbott
Jos Dijkhuisen
Martine Meems-Veldhuis
Renée Niessen
Birgit Sikkema-Raddatz
Martijn Viel

Research:
Cleo van Diemen
Kim de Lange
Lennart Johansson
Pieter van der Vlies
Desirée Weening
Tom de Koning
Rolf Sijmons
Cisca Wijmenga

Pediatrics:
Tom de Koning
Klasien Bergman