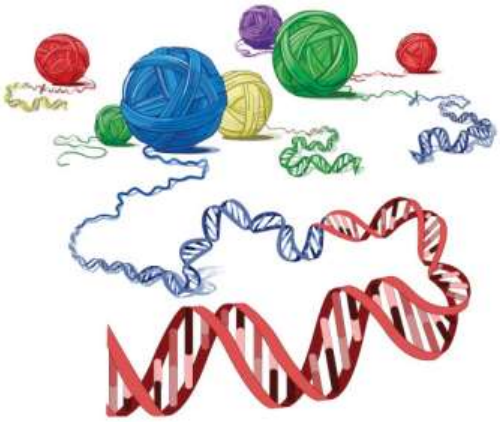


SLIPI, Strömstad
5.Sept 2019

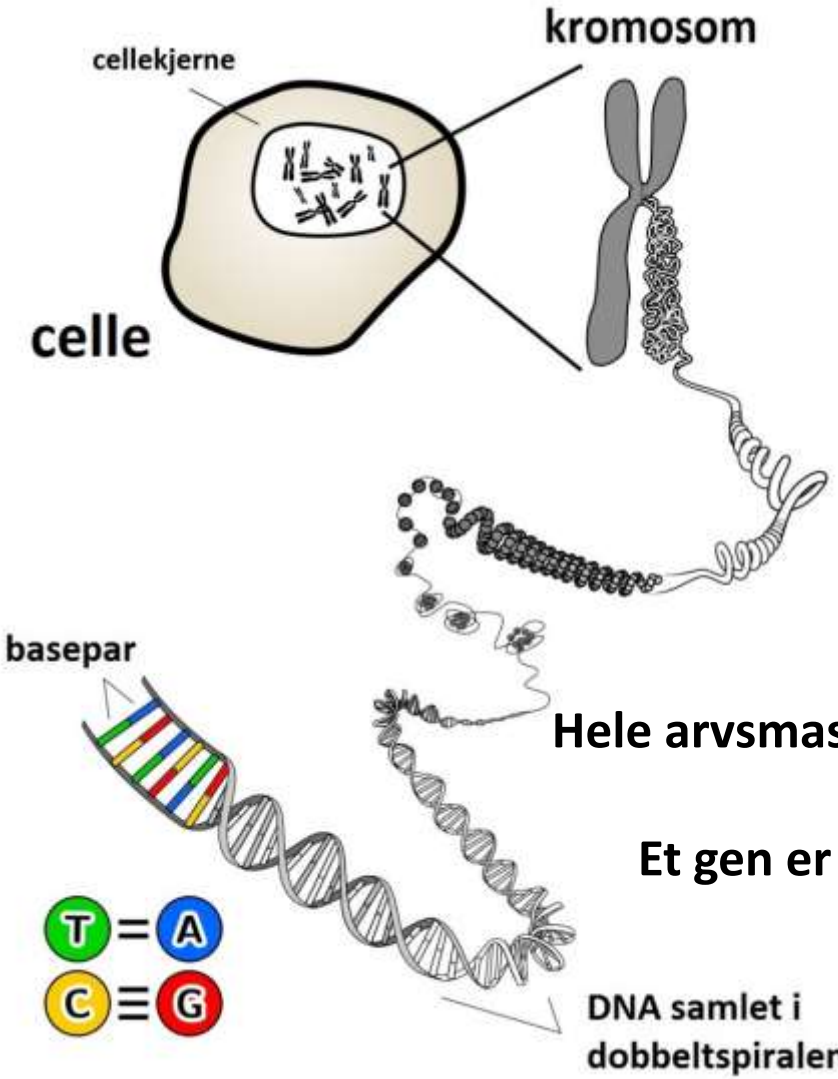
GENetikk & PID

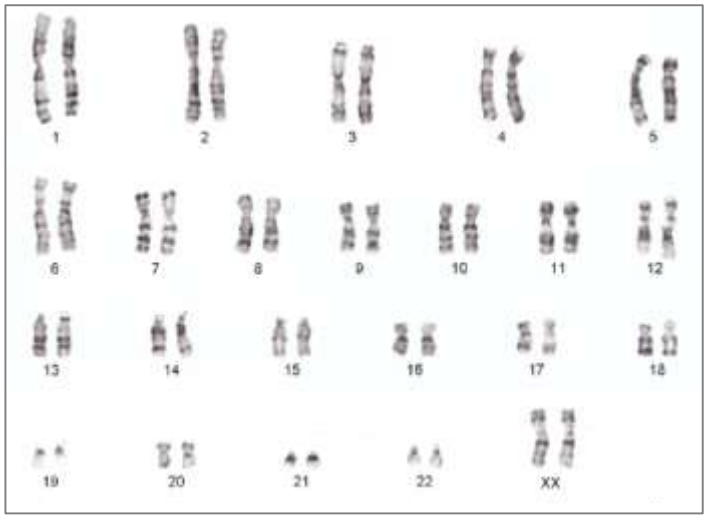
Asbjørg Stray-Pedersen MD, PhD

Norwegian National Unit for Newborn Screening
Division of Pediatric and Adolescent Medicine
Oslo University Hospital, Norway

Norwegian Society of Medical Genetics
THE NORWEGIAN MEDICAL ASSOCIATION

Fra kromosomer til genomer



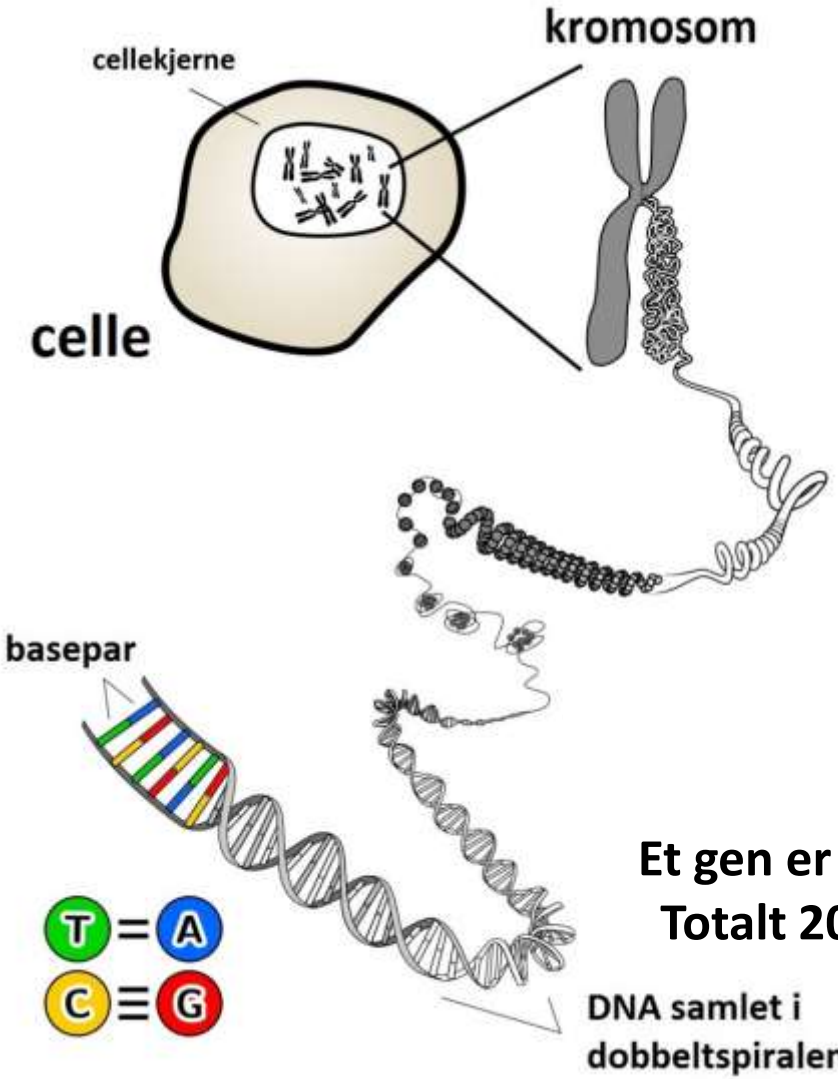


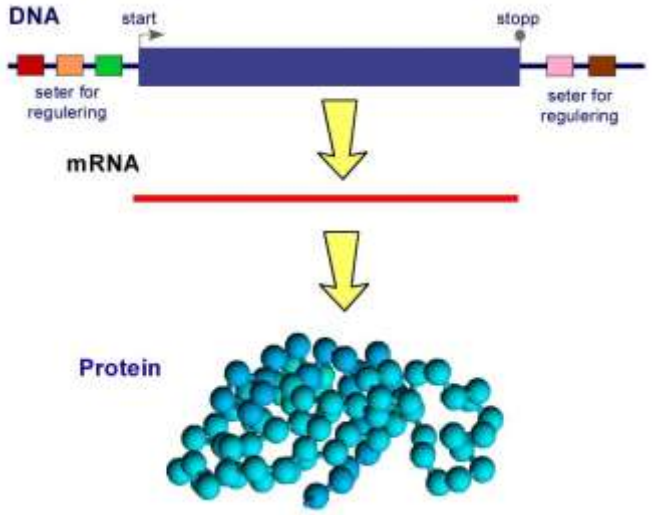
46 kromosomer = 23 par

Hele arvsmassan = genomet = 3 000 000 000 basepar x 2

Et gen er en del av DNA som koder for et protein

Fra kromosomer til genomer





Et gen er en del av DNA som koder for et protein

Totalt 20 000 gener som utgjør 1% av genomet

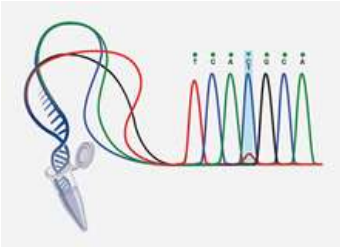
= exomet

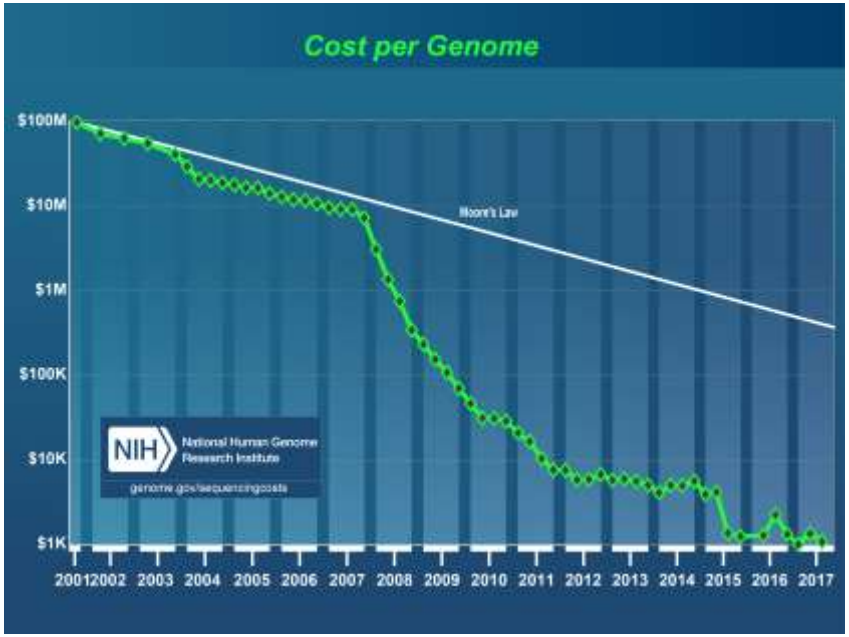
Fra kromosomer til genomer

Fra mikroskopi

& sekvensering ett og ett gen

til test av mange gener i én analyse





"Raskere, bredere, billigere"

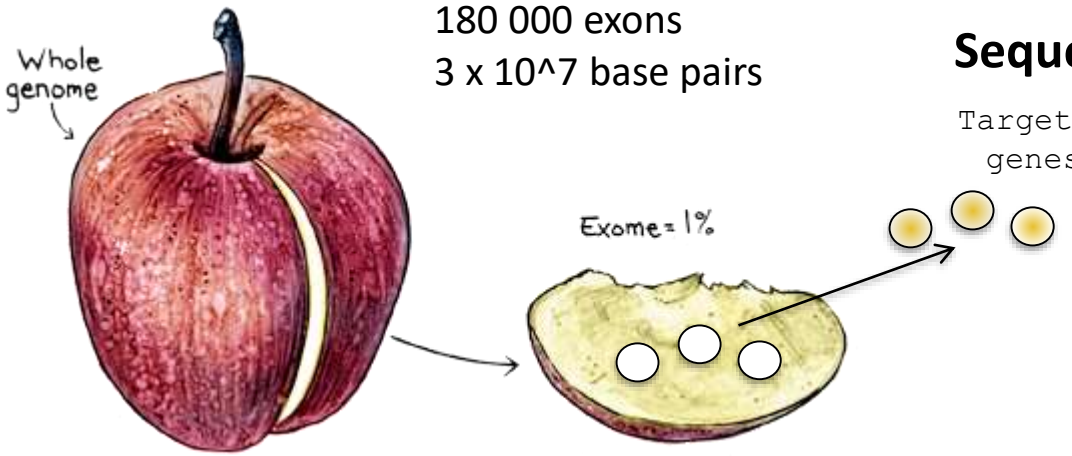
Fra kromosomer til genomer

Genome sequencing, WGS

3 x 10^9 base pairs

Exome sequencing, WES

180 000 exons
3 x 10^7 base pairs



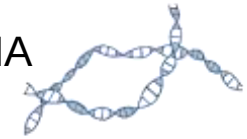
Sequencing of selected genes

~20 000 genes in total
ca. 7000 disease genes

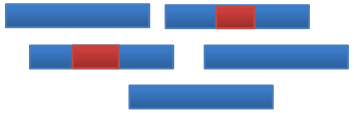
> 4 mill variants differs between 2 persons

1 variant can cause disease ⟶ How to find the needle in the haystack?

Genomic DNA

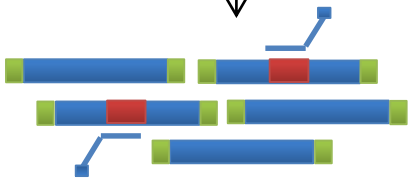


Construct shotgun library



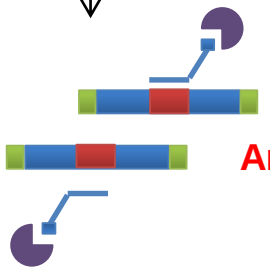
Repair ends; add adaptors

Hybridization



Wash

Pulldown



Amplification based:

DNA fragment Adaptor Probe

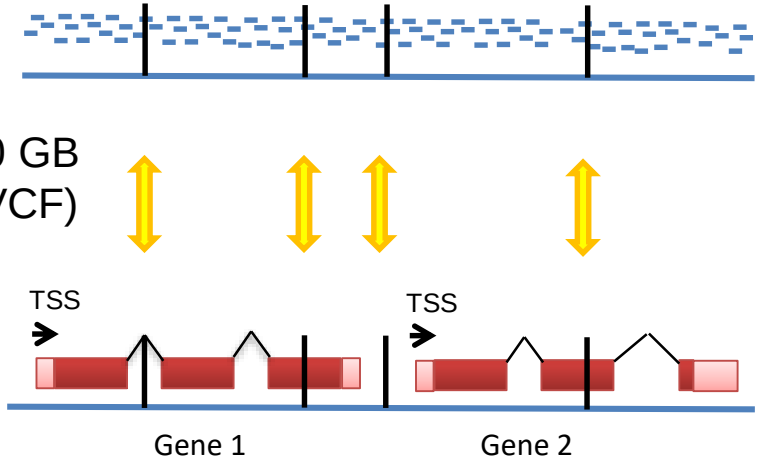
Capture bead TSS: Transcription start site

Coding exons UTR Mutation Short-read alignment

PCR free

WGS

Mapping
Alignment,
Variant calling
Final data +/-150 GB
(FASTQ, BAM, VCF)



WES & targeted panel

Mapping
Alignment,
Variant calling
Final data +/-10 GB

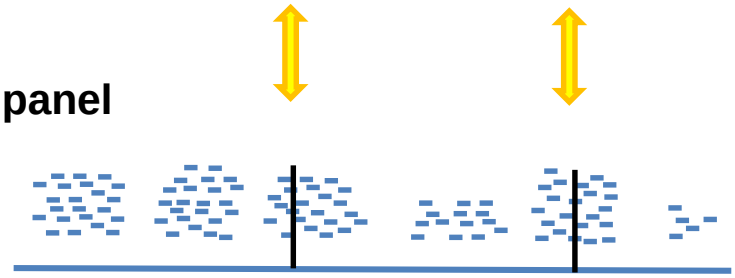
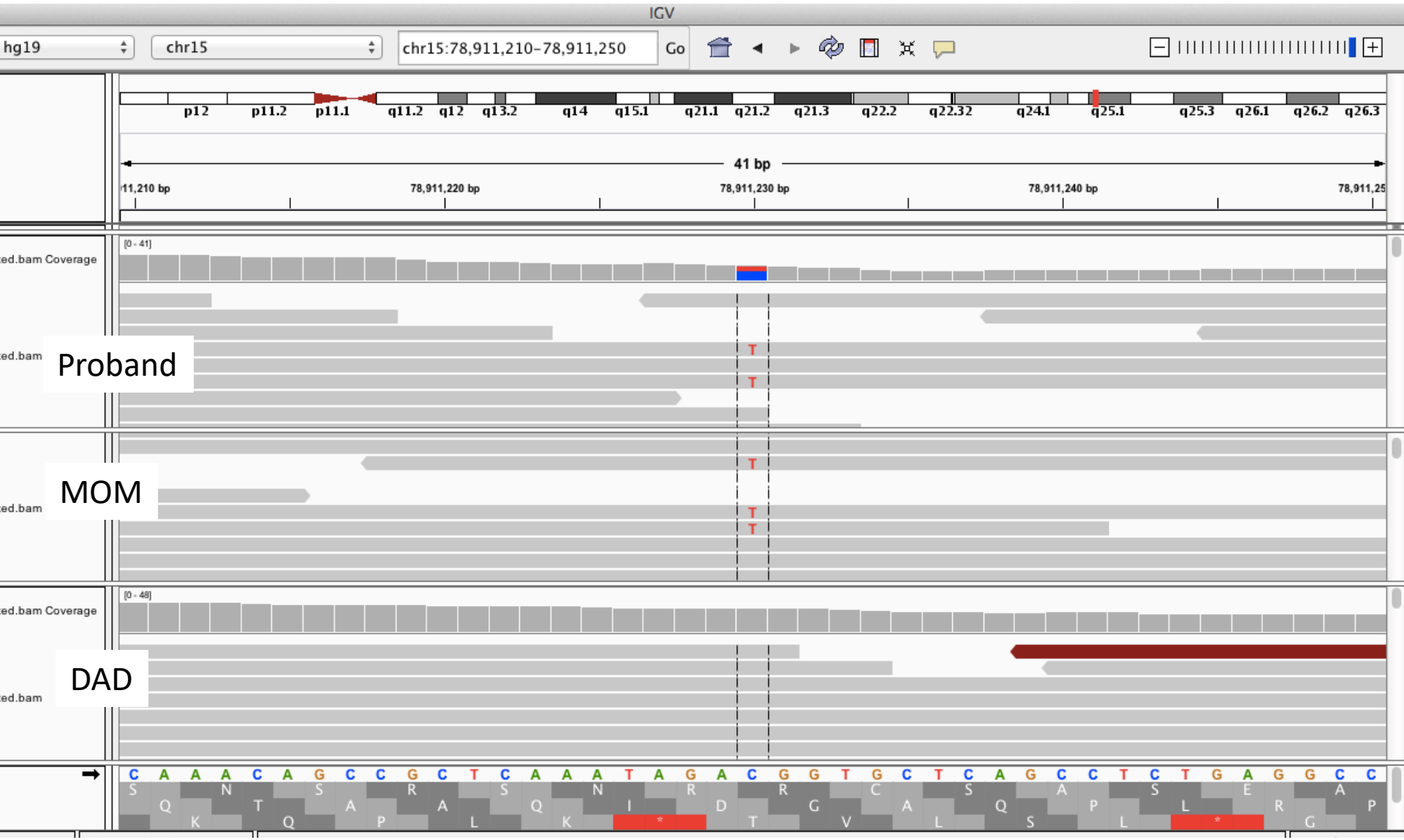
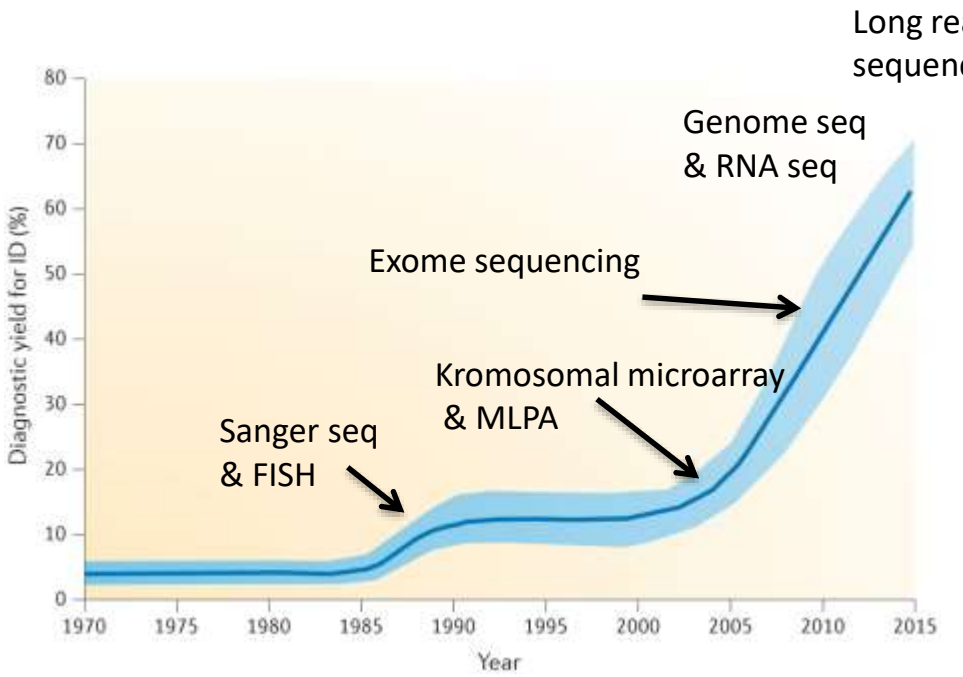


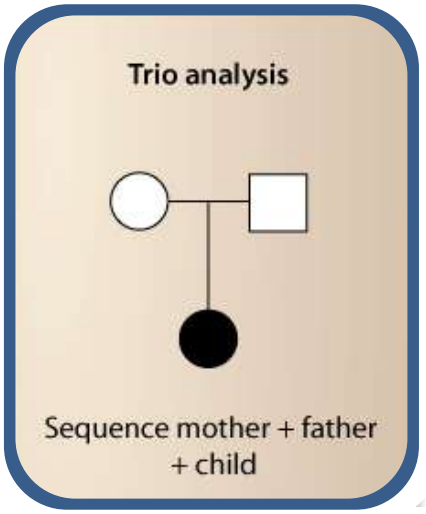
Figure modified from Meyts et al. J Allergy Clin Immunol, 2016;138:957-969

Sequence display

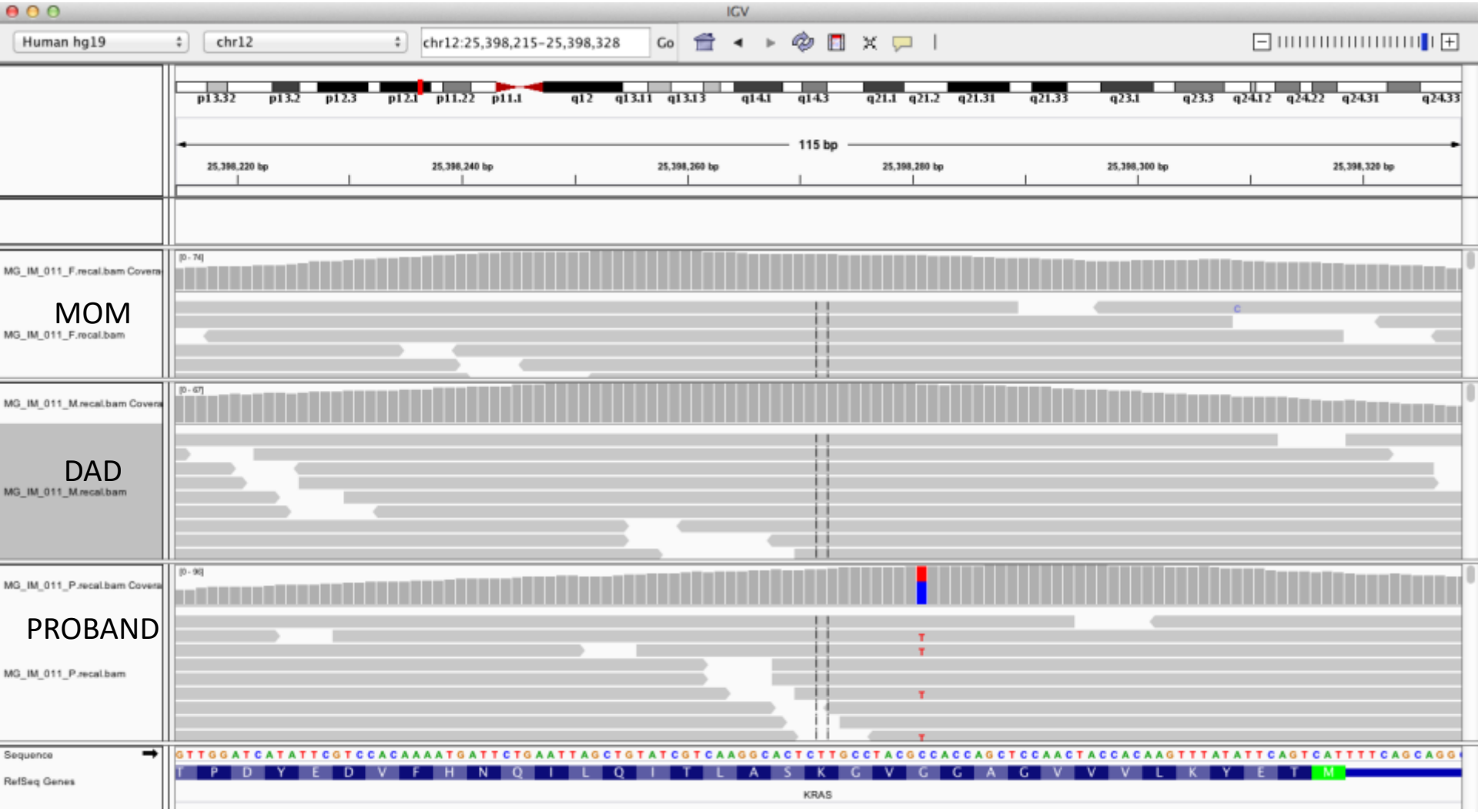

IGV
hg19
chr15
chr15:78,911,210-78,911,250
Go
p12
p11.2
p11.1
q11.2
q12
q13.2
q14
q15.1
q21.1
q21.2
q21.3
q22.2
q22.32
q24.1
q25.1
q25.3
q26.1
q26.2
q26.3
41 bp
78,911,220 bp
78,911,230 bp
78,911,240 bp
[0 - 41]
[0 - 48]
Proband
MOM
DAD

Diagnostic yield has increased with technology





Genetic studies in intellectual disability and related disorders
Lisenka E. L. M. Vissers, Christian Gilissen & Joris A. Veltman
Nature Reviews Genetics **17**, 9–18 (2016)



***KRAS* 47%, c.38G>A, p.Gly13Asp, rs112445441**

RAS associated lymphoproliferative disease *Lanzarotti N et al, Blood 2014*
A continuum from RALD to JMML Niemela JE et al Blood 2011, Calvo K et al, Blood 2015

Pitfalls of phenotype-specific panels

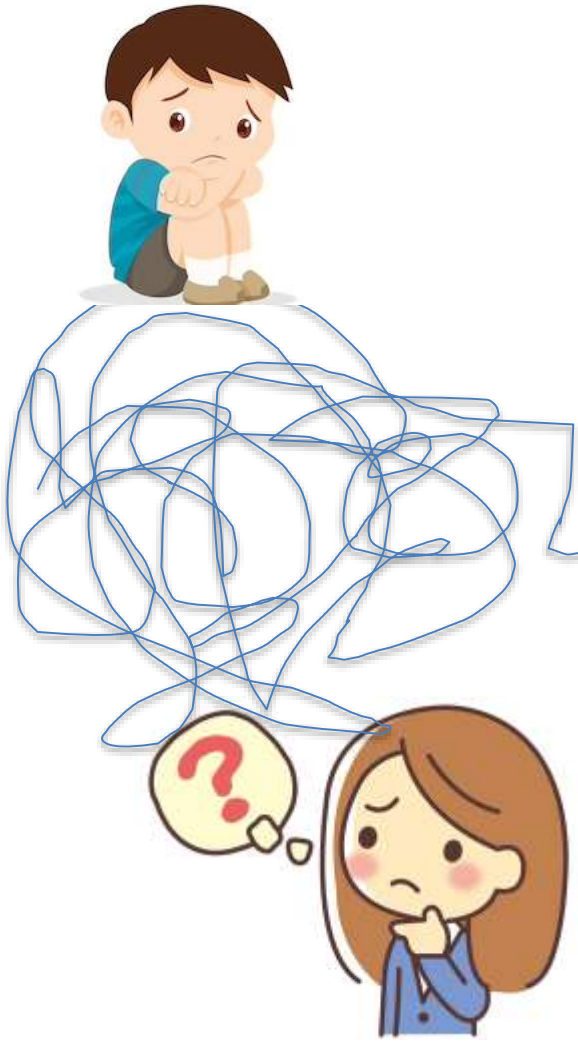
3y old daughter of non-consanguineous parents

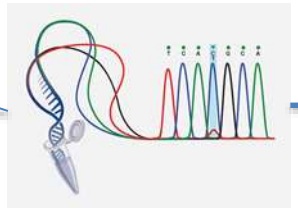
- From birth: recurrent otitis, recurrent pneumonia
- 1y: steroid-dependent ITP
- Recurrent cellulitis
- Persistent hepatosplenomegaly and lymphadenopathy
- Persistent thrombocytopenia (30,000 cells/uL)
- Recurrent pericarditis and pleuritis with pancytopenia

Laboratory evaluation:

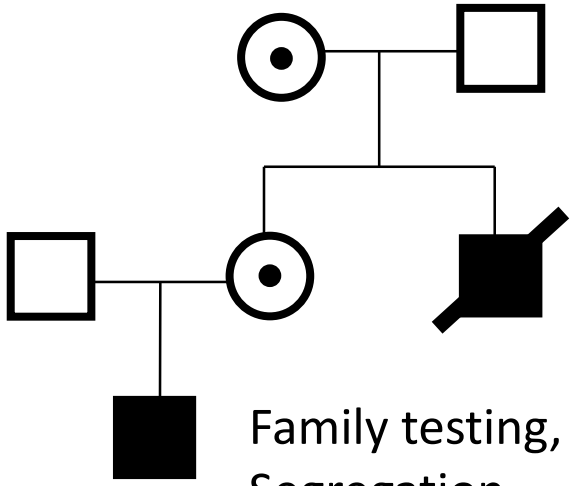
- IgG 17 g/L, IgA 1 g/L, IgM 1,7 g/L
- DNT 4%
- Monocytosis 600 – 2,000/uL
- Vit B12 > 2000 pg/ml
- **ALPS panel: no mutations detected , WES: *KRAS* het c.38G>A, p.Gly13Asp**

Before





1 variant



Family testing,
Segregation

Now

Variant 1
Variant 2
Variant 3
Variant 4
Variant 5
Variant ?

Annotation:
Custom-designed or Various approved software available (Cartagenia)
Conservation scores, prediction (Polyphen, SIFT, LRT, Mutation Taster, CADD)
Frequencies in-house database, external: GnomAD etc.

Variant 1
Variant 2
Variant 3
Variant 4
Variant 5
Variant ?

Cloud basert software & storage

Variant evaluation

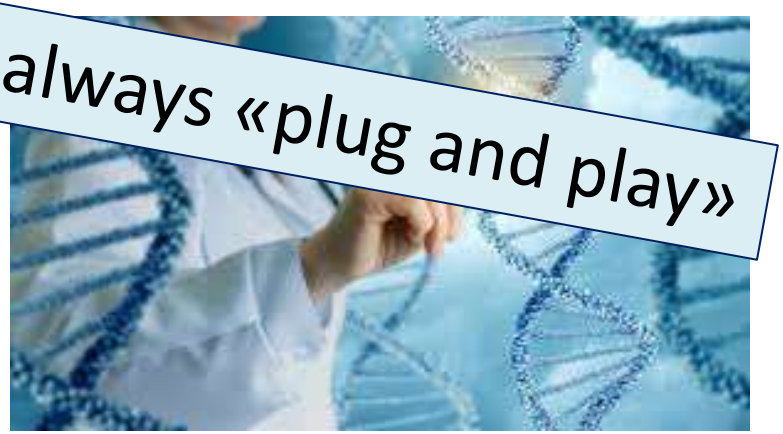
Gene test Results

Annotation:
Custom-designed or Various approved software available (Cartagenia)
Conservation scores, prediction (Polyphen, SIFT, LRT, Mutation Taster, CADD)
Frequencies in-house database, external: GnomAD etc.

Evaluation of gene variants

- Raw data (Fastq) **need safe storage**
- Aligned files (BAM) visualisation in IGV and Alamut **need flexible storage**
- Annotated files (csv) **need software (cloud based vs. local server)**

- Confirmation of the variant with Sanger seq or other method
- Familial segregation testing
- **Variant interpretation:**
  - **Curated databases: HGMD, ClinVar**
  - **Frequency databases: GnomAD**
  - **Publications: pubmed, google**
- Variant classification using the ACMG criteria
- Functional testing



gnomAD

genome aggregation database

http://gnomad.broadinstitute.org

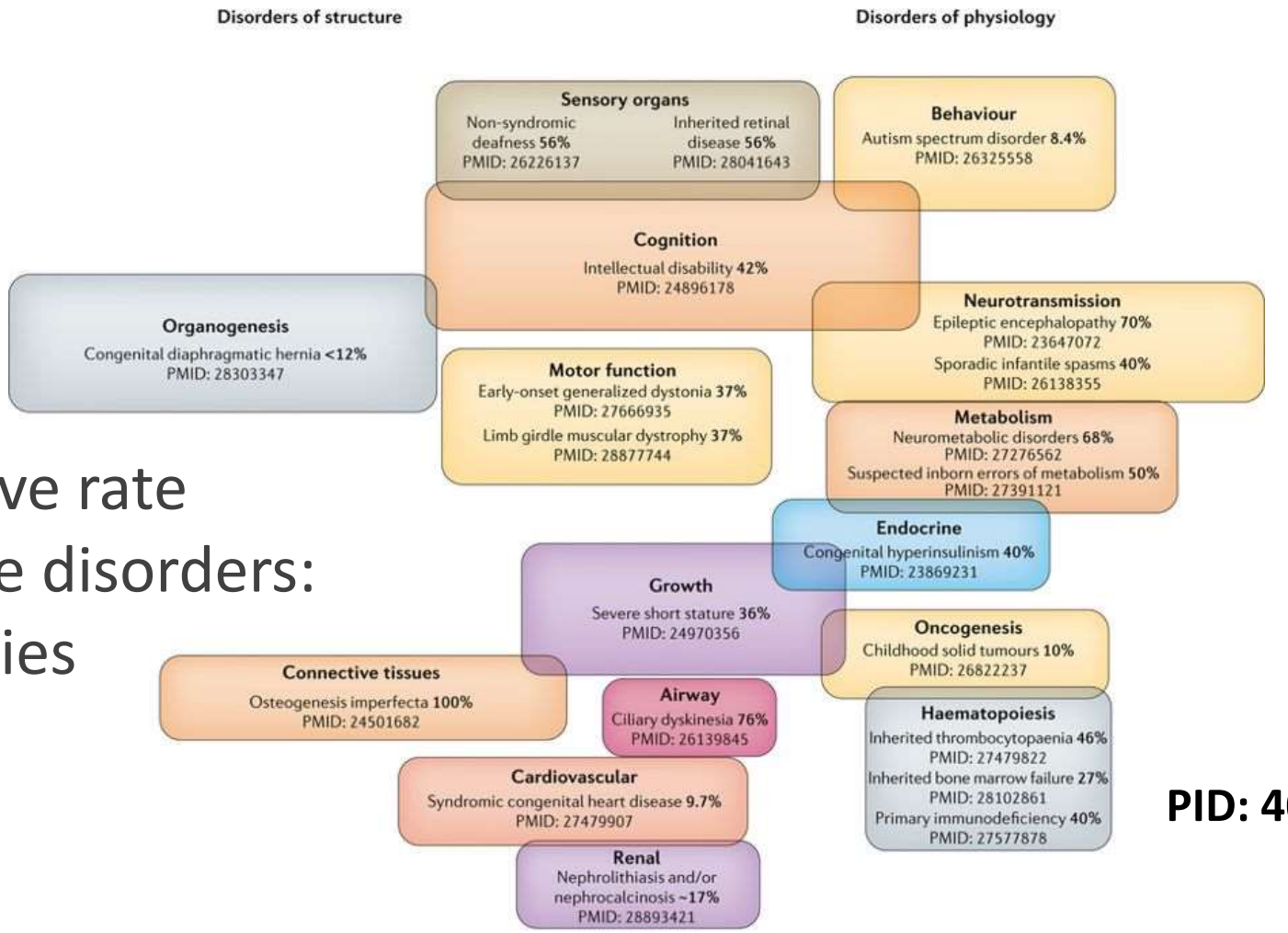
KRAS c.38G>A, p.Gly13Asp

gnomAD
genome aggregation database

Population Frequencies

Population	Allele Count	Allele Number	Number of Homozygotes	Allele Frequency
African	0	16070	0	0.000
Latino	0	34438	0	0.000
Ashkenazi Jewish	0	9966	0	0.000
East Asian	0	18348	0	0.000
European (Finnish)	0	21602	0	0.000
European (non-Finnish)	0	112628	0	0.000
Other	0	6106	0	0.000
South Asian	0	30248	0	0.000
Female	0	114648	0	0.000
Male	0	134758	0	0.000
Total	**0**	**249406**	**0**	**0.000**

Diagnostic yield has increased with technology



Solve rate rare disorders: varies

PID: 40%

Primary Immunodeficiency Disorders (PIDs)

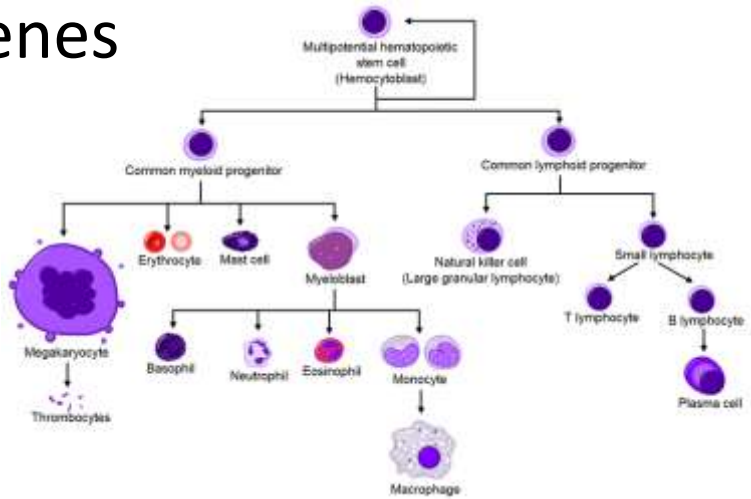
= Heterogeneous group:

- 300 different disease genes known
- Variable severity, different inheritance pattern
- Specific diagnosis may direct treatment
- ~50% patients without genetic diagnosis
- Many undiscovered disease genes

Primary
Immune
Deficiency
Disorders

Congenital defects
in the immune system
and other bone marrow defects

"Experiments of nature"



J Allergy Clin Immunol. 2016 Jul 16.

Strategy for our genetic analysis

- approaching **all** PID phenotypes,

  immunodysregulation, autoinflammatory diseases

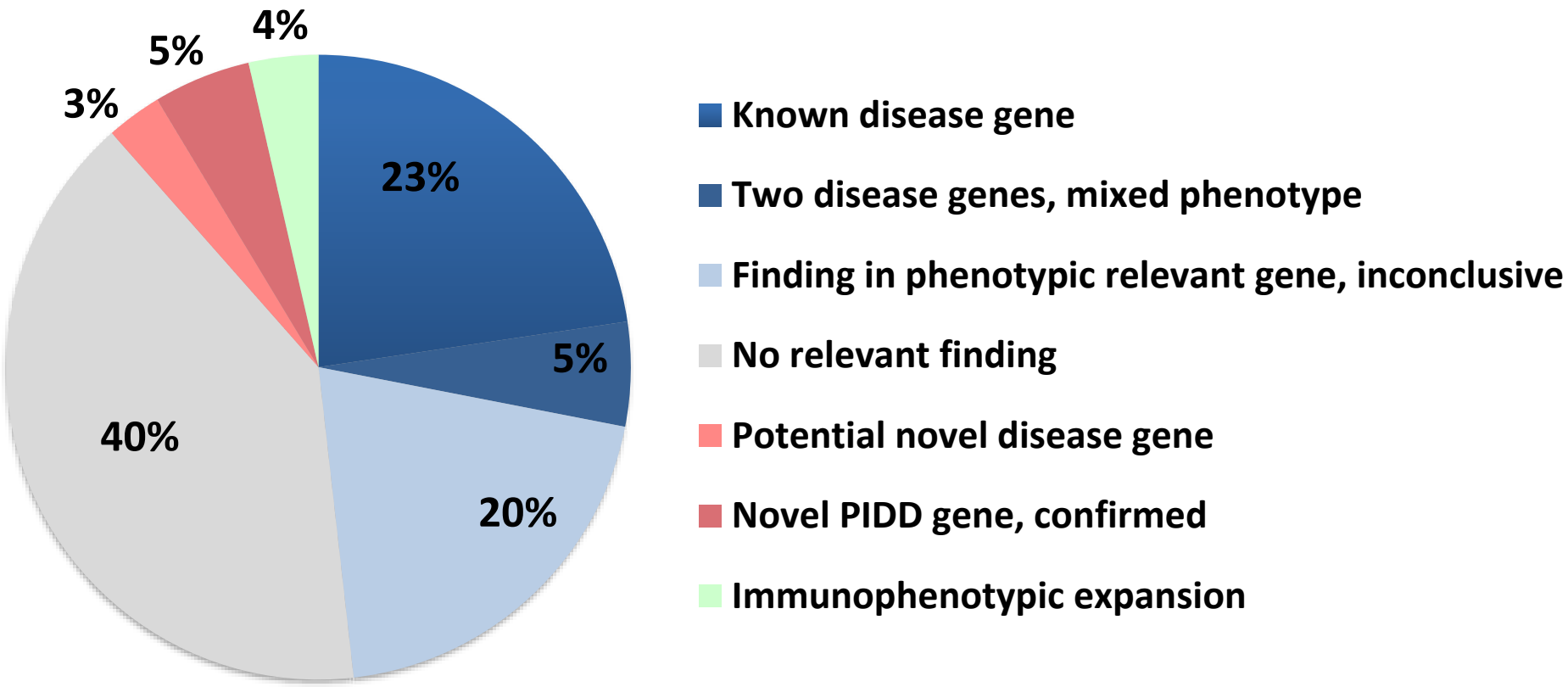
  as well as **inherited bone marrow failure syndromes**
- WES will yield unbiased and novel insights
- Proband sample
- +/- affected & unaffected family members
- PID gene list (330 genes, expanded to >440)
- All known disease causing genes
- **All** genes
- Copy number analyses in tandem with WES
  - Traditional diagnostic arrays
  - Customized exon tiling array
  - Bioinformatic prediction of CNV from WES data

Included PID families WES tested Oslo and Houston:

Clinical phenotypes[a] per family	CMG-BCM[b] Houston, USA	Oslo, Norway	Total
Antibody deficiency	14	2	16
Autoimmune disease	27	5	33
Autoinflammatory disorders	7	8	15
SCID	6	4	10
Combined of selective T cell defects	40	16	56
Common variable immunodeficiency (CVID)	13	7	20
Defects in Innate immunity	14	7	21
Lymphoproliferative or NK cell defects	53	9	62
Bone marrow failure or neutrophil defects	12	17	29
Syndromal PIDDs	14	3	17
Sum ready WES analyzed families	200	78	278
Total number of individuals WES analyzed	400	83	483

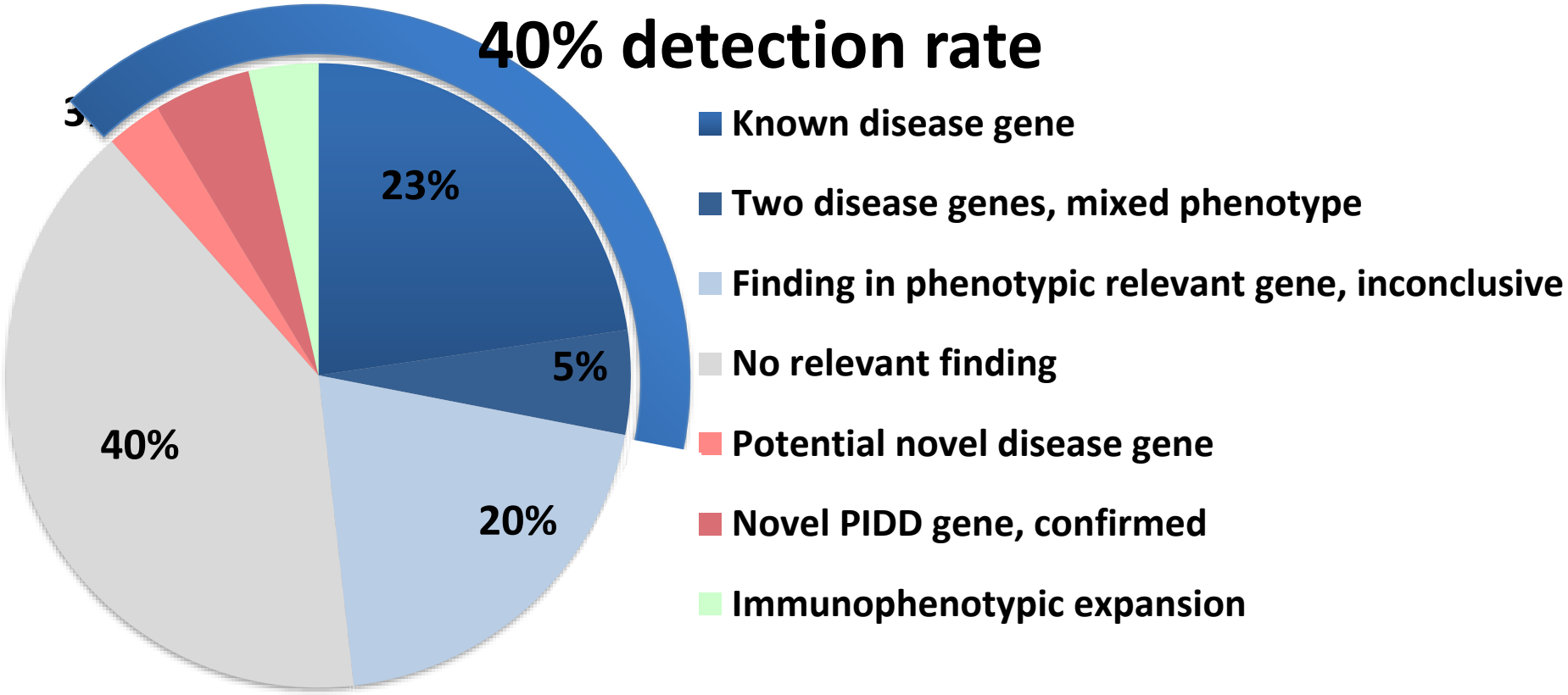
J Allergy Clin Immunol. 2016 Jul 16.

WES results for 278 families with PID Analyzed at CMG BCM Houston & OUS Oslo:



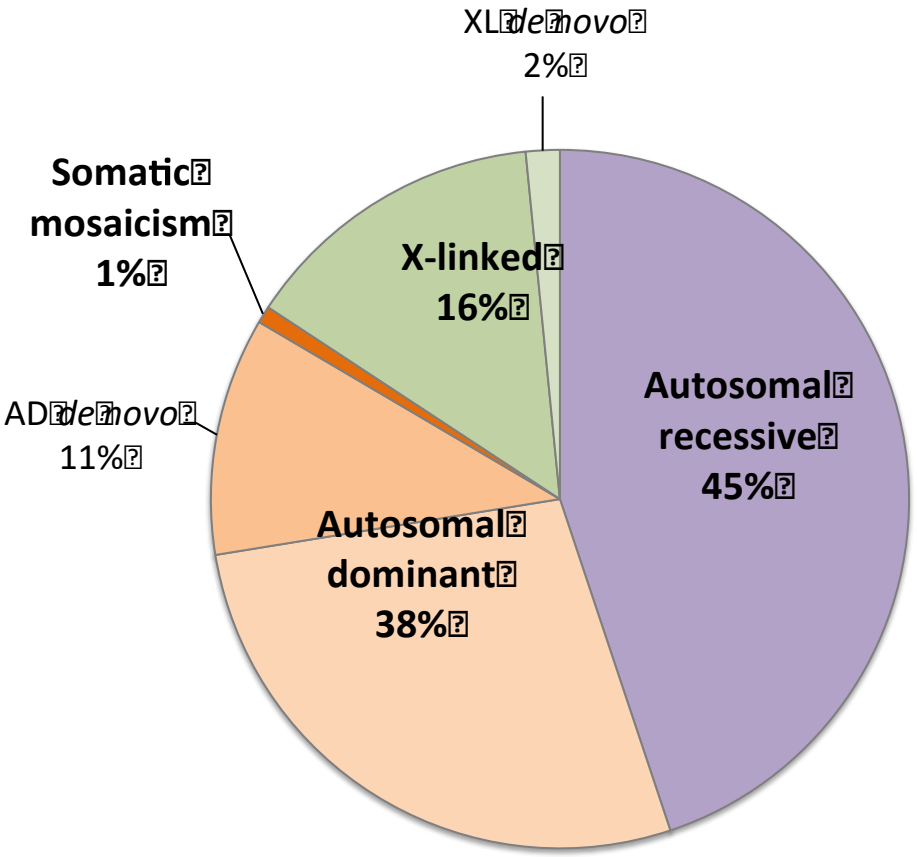
J Allergy Clin Immunol. 2016 Jul 16.

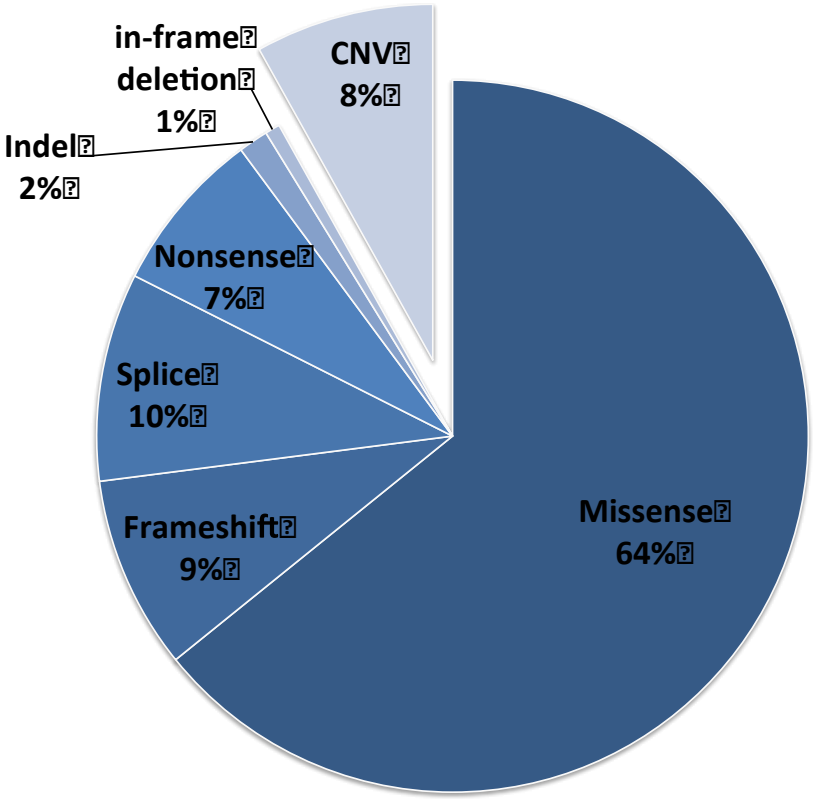
WES results for 278 families with PID Analyzed at CMG BCM Houston & OUS Oslo:



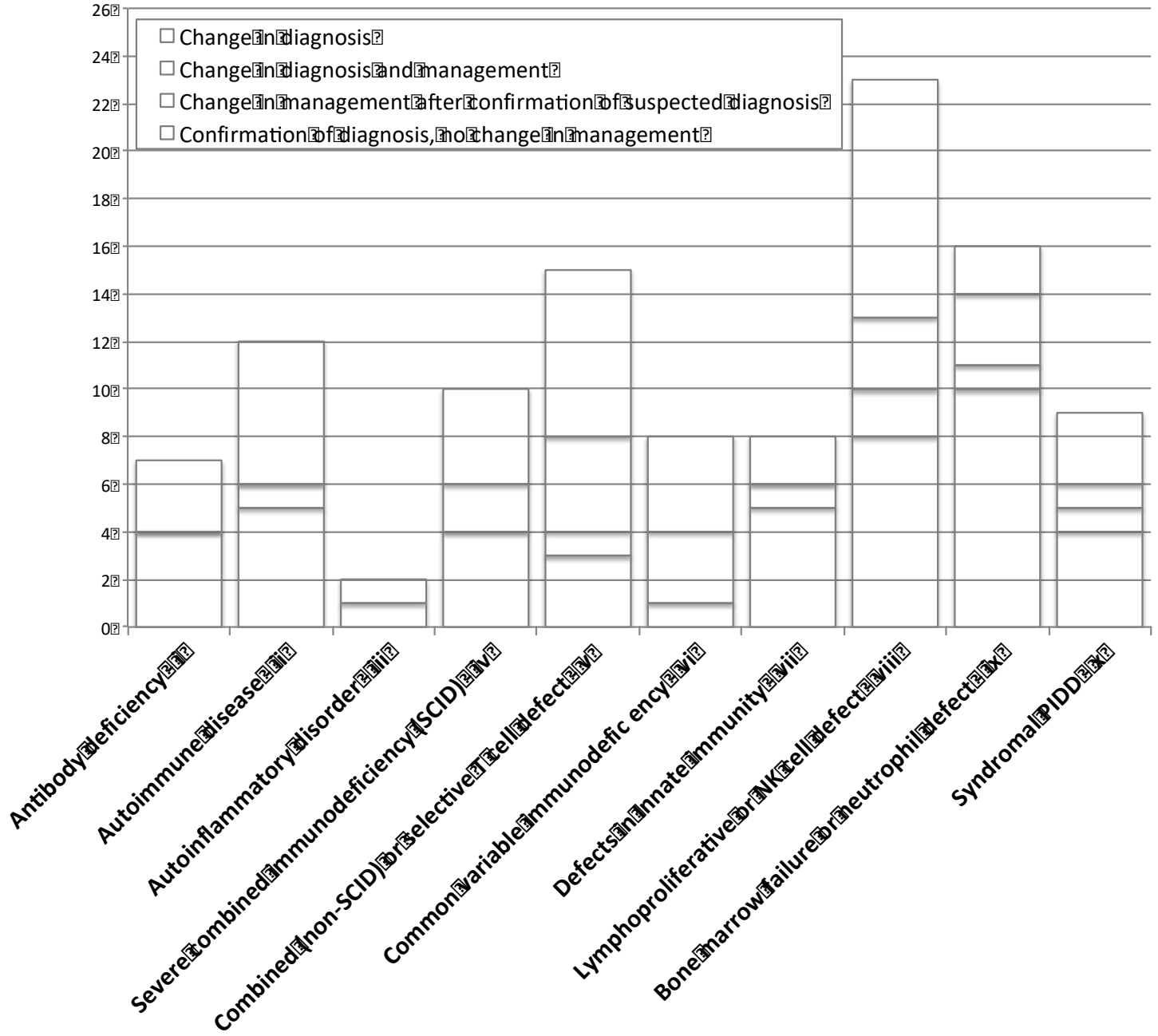
J Allergy Clin Immunol. 2016 Jul 16.

Inheritance patterns and variants detected





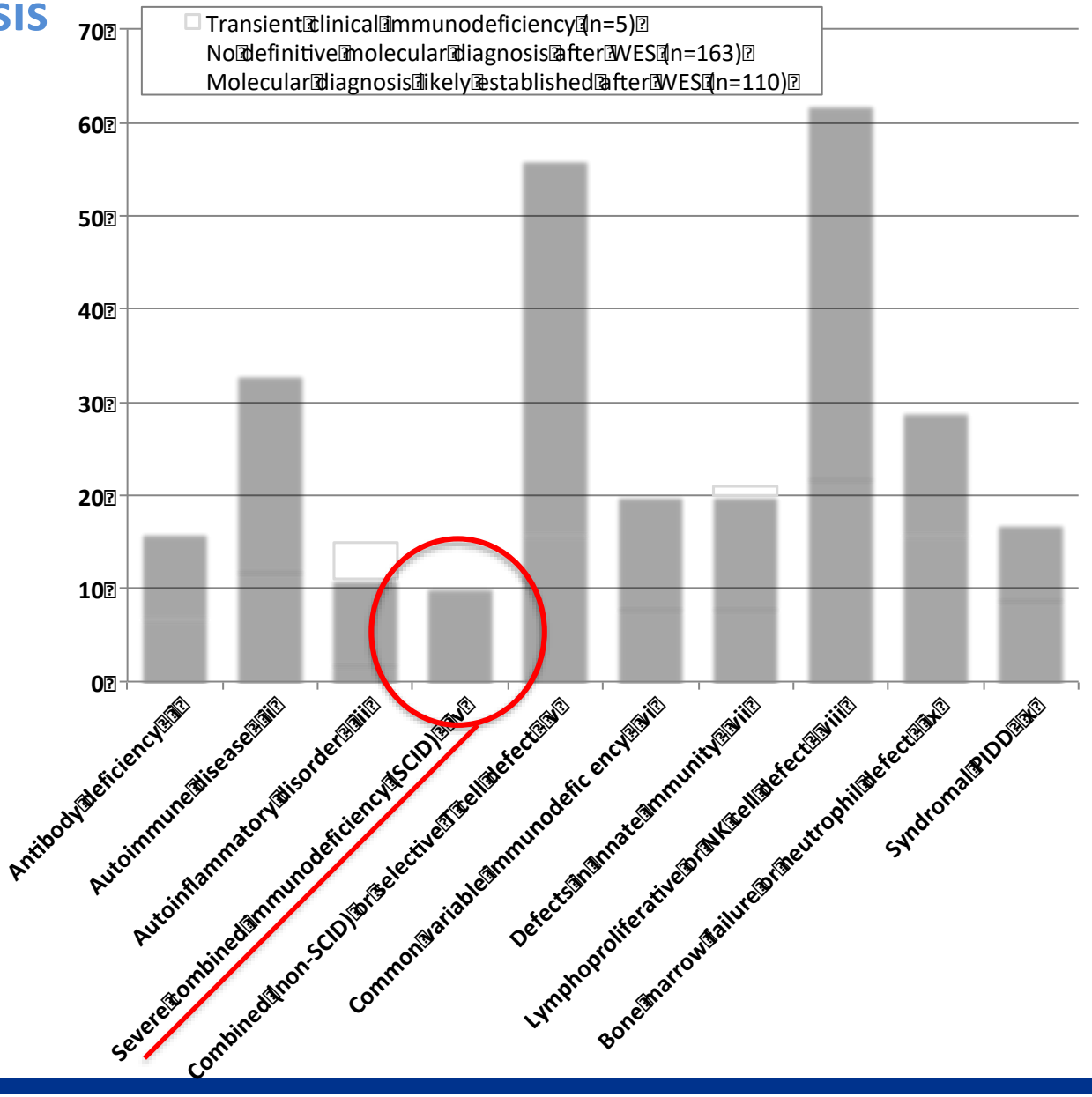
J Allergy Clin Immunol. 2016 Jul 16.


Change in diagnosis
Change in diagnosis and management
Change in management after confirmation of suspected diagnosis
Confirmation of diagnosis, no change in management
26
24
22
20
18
16
14
12
10
8
6
4
2
0
Antibody deficiency [i]
Autoimmune disease [ii]
Autoinflammatory disorder [iii]
Severe Combined Immunodeficiency (SCID) [iv]
Combined (non-SCID) or Selective T cell defect [v]
Common Variable Immunodefic ency [vi]
Defects in Innate Immunity [vii]
Lymphoproliferative or NK cell defect [viii]
Bone marrow failure or neutrophil defect [ix]
Syndromal PIDD [x]

Molecular diagnosis established:

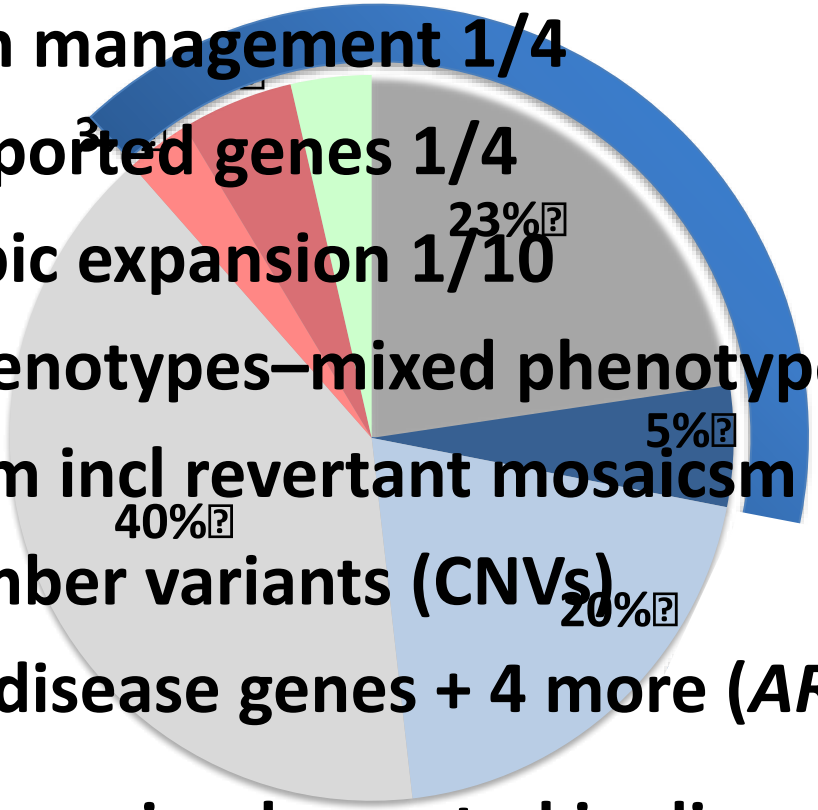
Overall: 40%

SCID: 100%



Out of the 110 'solved' by WES:

- Change in diagnosis: 1/2
- Change in management 1/4
- Newly reported genes 1/4
- Phenotypic expansion 1/10
- Double genotypes–mixed phenotypes
- Mosaicism incl revertant mosaicsm
- Copy number variants (CNVs)
- 16 novel disease genes + 4 more (*ARPC1B, CDC42*)



NGS now implemented in diagnostics for PID

Copy number variants in WES tested PID patients

Gender	Age	Diagnosis pre-WES (PIDD subgroup)	PIDD gene involved	Inheritance pattern for disease	Mutation type	Size of CNV	State	Affected family members
Male	13y	Hoyeraal Hreidarssons syndrome, X linked (ix)	***DCK1***	XL	Duplication	14 kb	Hemi	3
Male	NA	Combined immunodeficiency (v)	***DOCK8***	AR	Deletion	355 kb	Hom	2
Female	5y	HLH and NK cell defect (viii)	***DOCK8***	AR	Deletion	84 kb	Hom	1
Female	34y	Fanconi anemia, mild (ix)	***FANCA***	AR	Deletion	22-24.6 kb	Het	2
Male	30y	Agammaglobulinemia (i)	***IKZF1***	AD	Deletion	16.8 kb (exons 4-5)	Het	1
Female	2y	SCID, later debut (iv)	***IL7R***	AR	Deletion	224 kb (exon 3)	Het	1
Male	16y	Immunodeficiency, X linked, extensive warts (v)	***MAGT1***	XL	Deletion	16 kb	Het	2
Female	8y	Immunodeficiency, progressive bone marrow failure. Short stature, dysmorphic facial features. (x)	***MYB***	AD	Deletion	3.4 Mb	Het	1
Female	71y	Chronic Granulomatous Disease (ix)	***NCF1***	AR	Deletion	15 kb	Hom	1
Male	6y	T and B cell deficiency and neutropenia (iv)	***PGM3***	AR	Deletion	1.24 Mb	Het	3
Female	4y	Immunosseous dysplasia (x)	***SMARCAL1***	AR	Deletion	1.6 kb	Hom	1
Male	49y	Dyskeratosis Congenita, progressive bone marrow failure, Short telomere lengths (ix)	***TERC***	AD	Deletion	3 kb	Het	1

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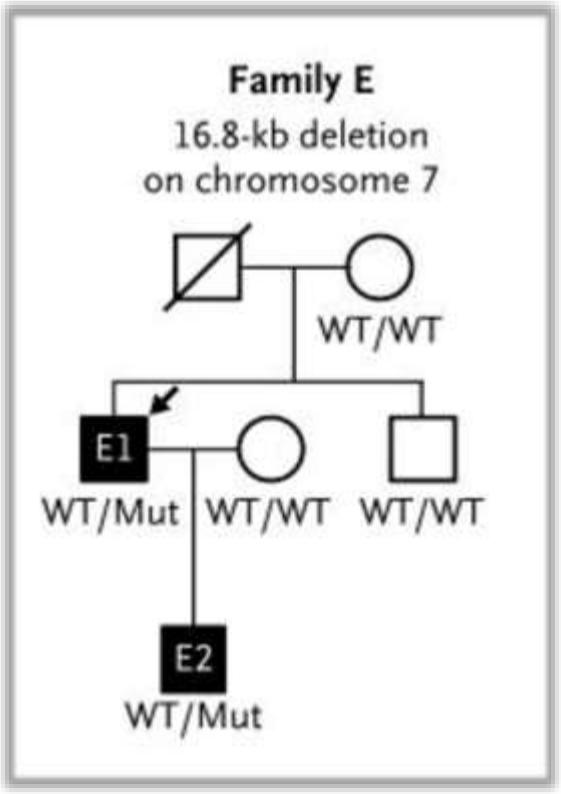
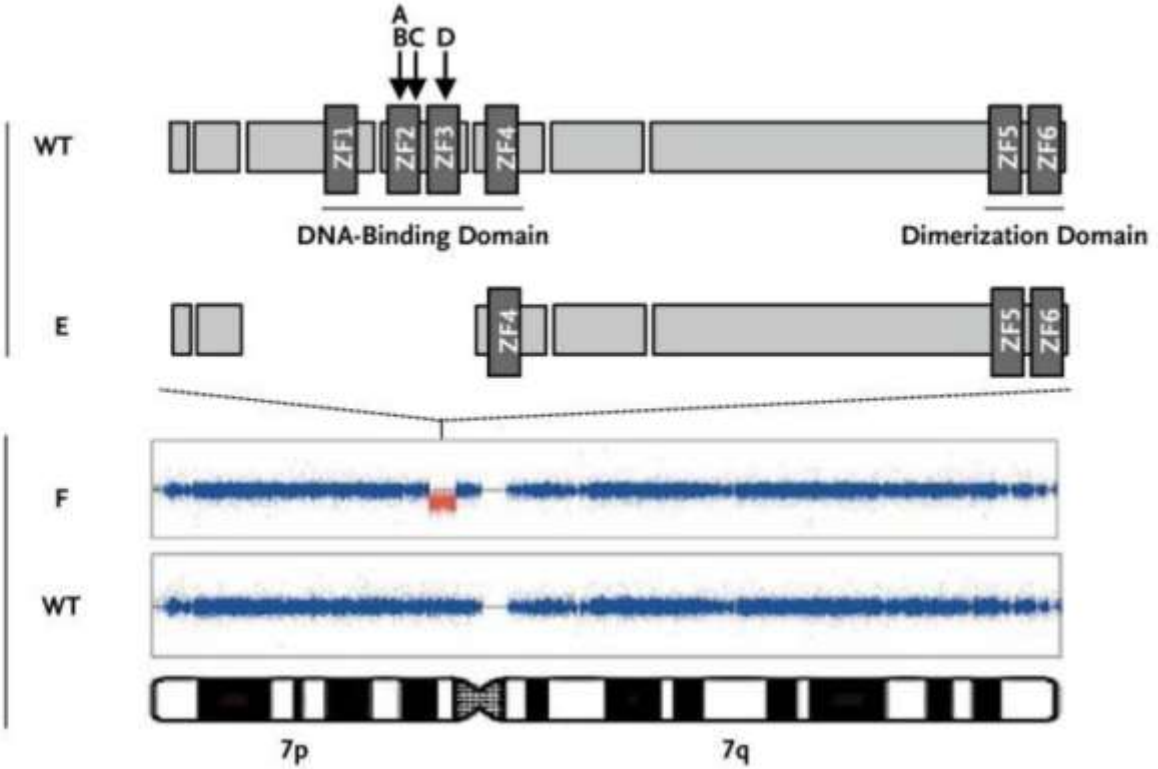


IKZF1 deletion

16.8 kb in-frame deletion exons 4 and 5,
loss of zinc fingers 1, 2, and 3
in the DNA binding domain

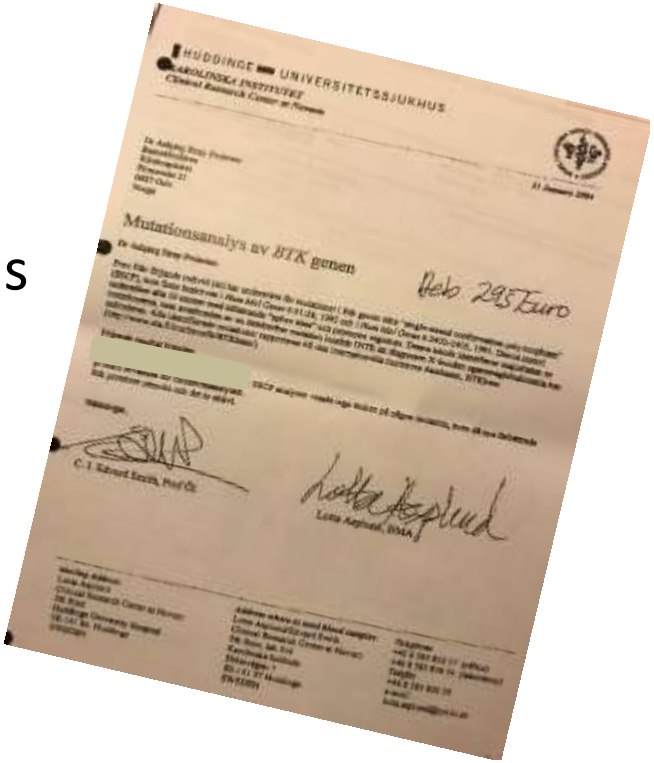


Loss of B Cells in Patients with Heterozygous Mutations in IKAROS
Keuhn et al N Engl J Med. 2016 Mar 17; 374(11): 1032–1043

IKZF1 deletion

Father

- **Recurrent infections from 1st month of life**
- Pneumococcal infections
- Low IgG at 4 years old
- SclgG since 5 y of age
- Few B cells (CD19+)
- ***BTK* tested 4 times!**
- "Agammaglobulinemia"


HUDDINGE UNIVERSITETSSJUKHUS
KAROLINSKA INSTITUTET

Mutationsanalys av *BTK* genen

Beb 295 Euro

IKZF1 deletion

Father

- **Recurrent infections from 1st month of life**
- Pneumococcal infections
- Low IgG at 4 years old
- ScIg since 5 y of age
- Few B cells (CD19+)
- ***BTK* tested 4 times!**
- "Agammaglobulinemia"

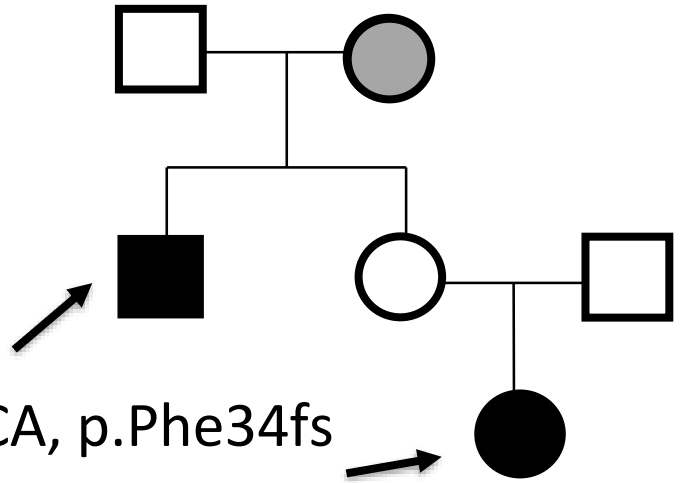
Son

- **No symptoms at 7 years old** when he received the molecular diagnosis
- IgG 4.2 g/L
- B cells low
- No vaccination response
- Started ScIg Hizentra
- **Presymptomatic testing in the child was allowed since it had consequences for treatment**

Molecular finding directing treatment

Two affected, uncle and niece with

- pure cell anemia, thrombocytopenia,
- atypical infections
- and enteritis/colitis in the family
- Whole exome sequencing:
- *CTLA4* (NM_005214) c.101_102insCATCA, p.Phe34fs
- = CTLA4 deficiency
- autoimmune cytopenia and immunodeficiency fits

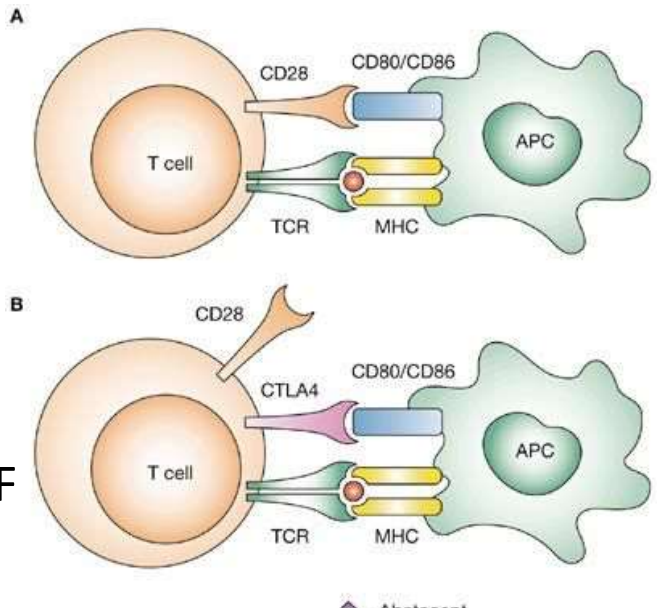


- Directive for treatment Abatacept : CTLA4-Ig
- Hb 5 -> Hb14, and improved health condition

Molecular finding directing treatment

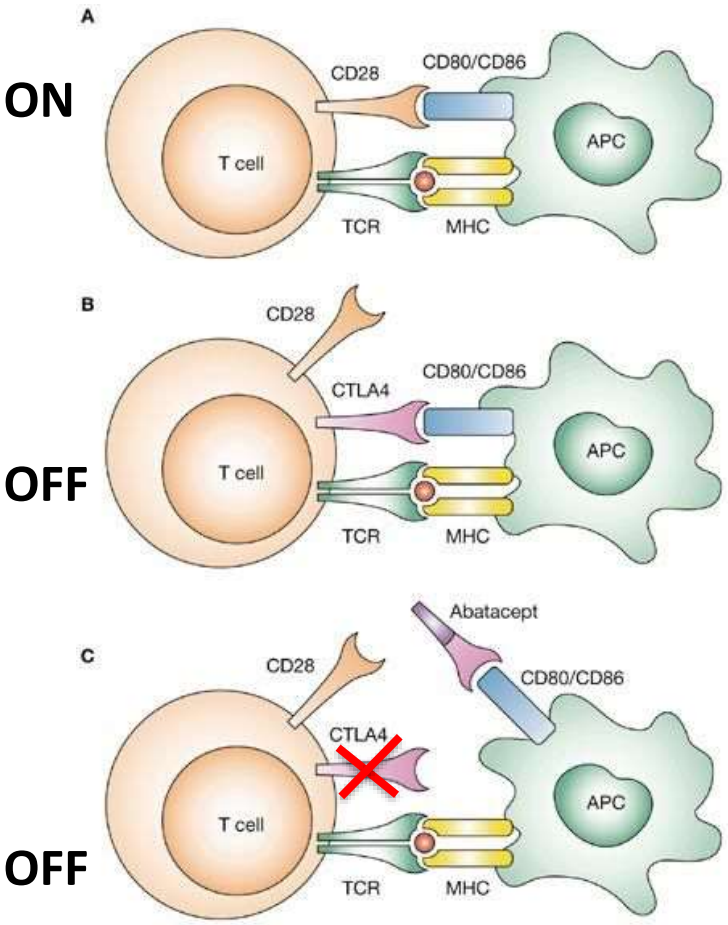
ON

OFF



- **CTLA4** switches autoimmunity off, CD28 turns it on.
- Reduced CTLA4 -> increased autoimmunity

Molecular finding directing treatment



- **CTLA4** switches autoimmunity off, CD28 turns it on.
- Reduced CTLA4 -> increased autoimmunity

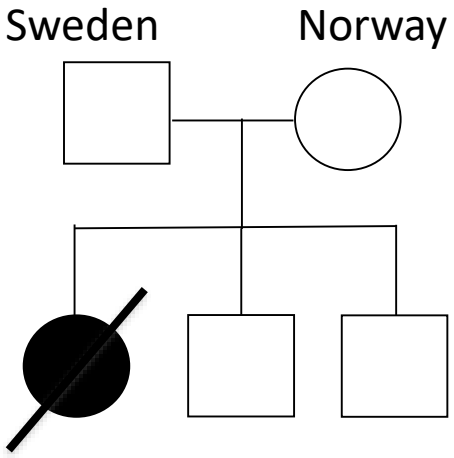
- **Abatacept** CTLA4-Ig is a fusion protein composed of the Fc region of the immunoglobulin IgG1 fused to the extracellular domain of CTLA4
- Turns the autoimmunity off

CTLA4 deficiency with Abatacept treatment

Severe autoinflammatory disorder?

- Extramedullary hematopoiesis from birth with
- ‘blueberry muffin’ lesions
- Rash changed to maculo/papolous exanthema, migrating
- Anemia, low platelets – transfusion dependent
- Developed hepatosplenomegaly during first weeks of life
- Fever and infections
- NOMID suspected, received Anakinra, non-responsive
- CRP, SR and Ferritin elevated
- Developed HLH. Treated with HLH-2004 protocol: steroids, etopocid, sandimmune/cyclosporine
- Yenan&Henter: no HLH gene mutations
- Newcastle – no causative molecular diagnosis, likely sec HLH.
- Maternal Haplo graft, died 1 week after HSCT

Severe autoinflammatory disorder?



Sanger seq with normal results prior to WES:

NLRP3, IL1RN, LPIN2, NOD2, MEFV, TNFRSF1A, PSTPIP1, MVK, PSMB8

aCGH normal

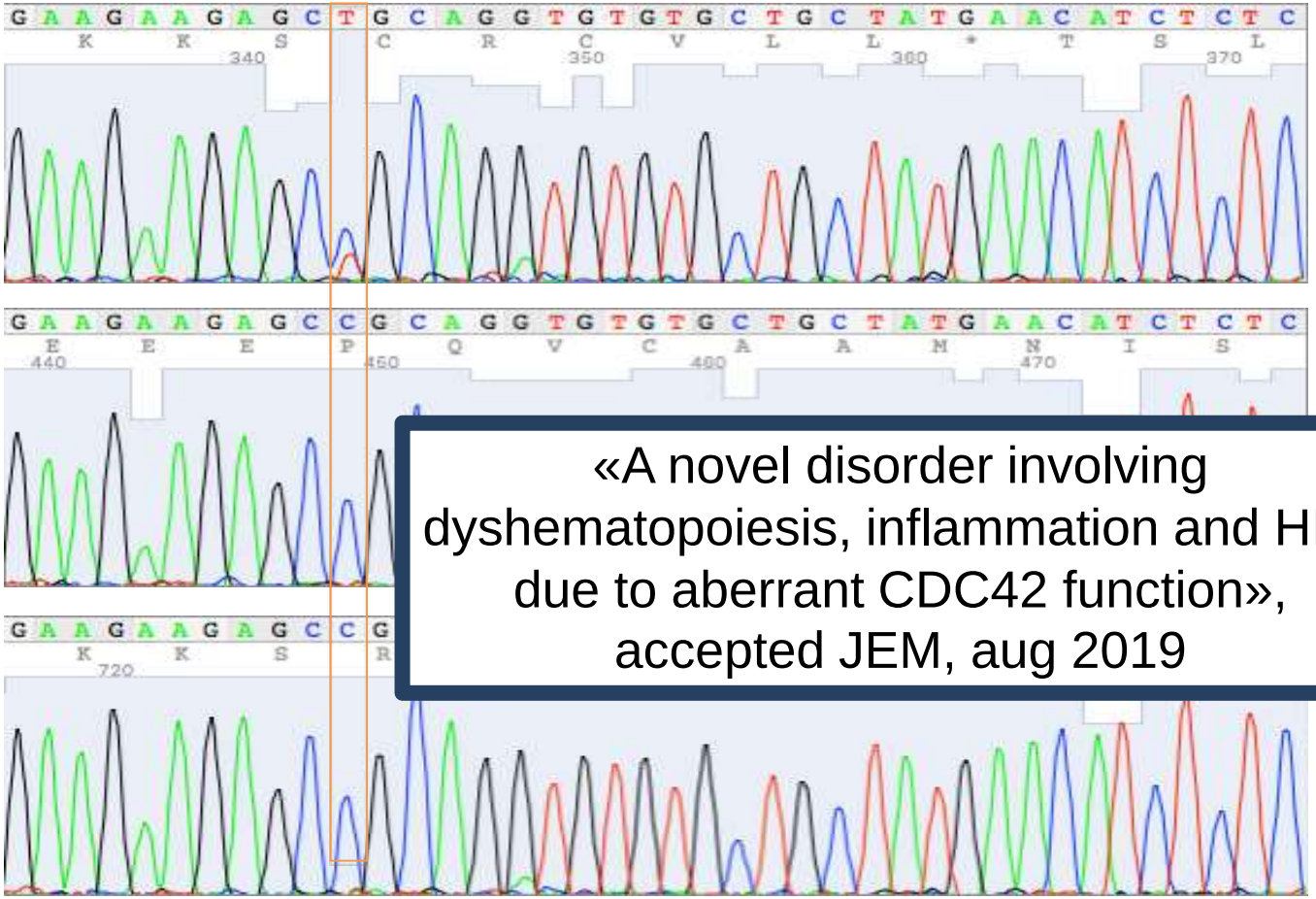
Heterozygous *de novo* (not inherited) variant
CDC42 (NM_001791) c.556C>T, p.Arg186Cys

CDC42

(Proband): C>T
[C/T]

(Mother): C
[C/C]

(Father): C
[C/C]



«A novel disorder involving dyshematopoiesis, inflammation and HLH due to aberrant CDC42 function», accepted JEM, aug 2019

Heterozygous *de novo* (not inherited) variant
CDC42 (NM_001791) c.556C>T, p.Arg186Cys

«Tinder» for geneticists

- Decipher DECIPHER GRCh37
- Gene Matcher GENE MATCHER
- **Matchmaker Exchange**

Global Alliance for Genomics & Health

Collaborate. Innovate. Accelerate.

What have we learned

- WES and WGS are efficient methods to establish a genetic diagnosis.
- Genomic approach -> Unexpected findings
- **Discover novel genes**, novel disease mechanisms
- One obstacle: **Turn-around-time**
- **Success factor #1: Dynamic team lab/clinician.**
- **Structural abnormalities** may be detected on WGS,
- **Copy number variants** may be detected on WES, also smaller CNVs, given a bioinformatic pipeline
- **New recommendations for molecular diagnostics…..**

J Allergy Clin Immunol. 2016 Jul 16.

Diagnostics PIDD – Recommendations:

1

<u>Rapid</u> molecular testing w/limited capture kit

targeting for

a. **Common** disease causing genes
b. **Actionable** gene variants
c. genes causing **severe phenotypes** requiring a rapid molecular diagnosis
   - Low TRECs on Newborn Screening for SCID
   - Lymphoproliferative disorders. Bone marrow failure syndromes

lighter, quicker, cheaper

2

WES or WGS diagnostics

a. **Gene candidate list**, large & frequently updated list
b. **Diagnostics: Variants in other disease causing OMIM genes**
c. **Research:** Variants in other genes

Novel genes, unexpected findings

Rask identifisering
av alvorlig medfødte
behandlbare tilstander hos barn
ved bruk av ny teknologi
i nyfødtscreening

3 mm
3 ul fullblod

~ 30 ng DNA

Variant of uncertain significance (VUS)

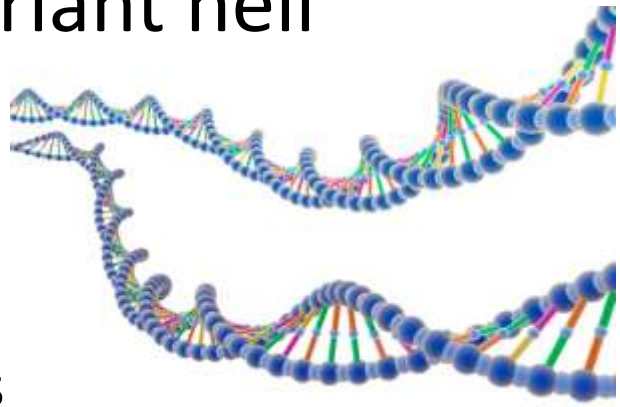
Pathogenic	**5**
Likely pathogenic	**4**
Variant of uncertain significance	**3**
Likely benign	**2**
Benign	**1**

VUS is NOT a yellow light!
Most likely a benign, rare variant
Functional validation is essential

ACMG criteria: Genet Med. 2015 May; 17(5): 405–424.

The American College of Medical Genetics and Genomics (ACMG)

How to avoid variant hell



- In the lab,
- Clinical genetics,
- Genetic counselor,
- Other physicians/pediatricians,
- For patient/families

Sharing of data incl Scandinavian variants, Uniform/harmonized format/software for sharing of variant data with classification

.

As of 2019 < 50% with PIDD receives a molecular diagnosis using next generation sequencing techniques

MIND THE GAP

Be aware of:

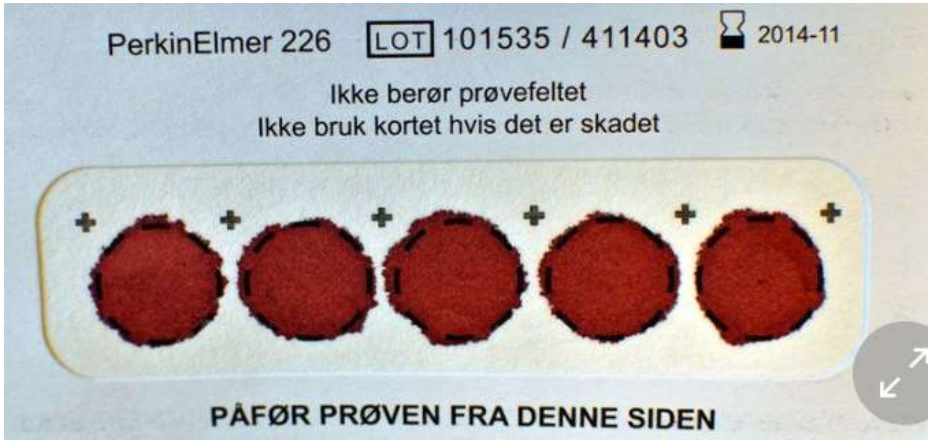
- **Copy number variants (CNVs)**
- **Other structural variants**
- **Mosaicism**
- **‘Silent’ mutations**

Why extended genetic testing?

Answers and actions:

- Specific molecular diagnosis
- Improved follow-up
- Targeted treatment
- Personalized medicine
- Prenatal diagnostics

GENetikk & SCID



Norwegian National Unit for Newborn Screening

- Nationwide service
- 60.000 newborns/year

Located at Oslo University Hospital

- Mandate to screen for severe and treatable congenital disorders

Norwegian National Unit for Newborn Screening testing 25 diseases as of 2019:

- Aminoacidopathies
  - PKU, MSUD, TYR I, HCY
- Organic acidurias
  - PROP, MMA, IVA, HCS/MCD,
  - βKT, GA1, BIOT, HMG
- Beta-oxidation & carnitine cycle defects
  - MCAD, LCHAD, VLCAD, TFP,
  - CUD, CPTI, CPTII, CACT, GA2
- Endocrinopathies
  - CH, CAH
- Cystic fibrosis
- SCID pilot sept 2015, nationwide jan 2018

Newborn SCID screening algorithm

DNA from filtercard punch

1.Tier test

RT-PCR*

TRECs

Re-run if **TRECs/µl <25**

TRECs + Betaactin

After re-run, if

TRECs/µl <20

and Betaactin normal:

2.Tier

RAPID

NGS results ready
within 2-3 working days
USING **SAME** FILTERCARD

Next generation sequencing (NGS)
Targeted panel
SCID and T cell deficiency genes

**Douek et al Lancet. 2000;355:1875–1881*

We were lucky

”Tobias is now healthy thanks to a heel stick”

Tobias was saved from Severe Combined Immunodeficiency since he participated in a Newborn Screening pilot project.

Norwegian Newspaper A-magasinet 1.june 2017

Newborn SCID screening Norway

Pilot project - prospective :

- *Written* consent
- Sept 2015 – Dec 2017
- 21 000 TREC tested
- 10 cards gene panel tested
- 3 newborns with SCID
  - ***IL2RG* defect** -> transplanted
  - ***RAG2 leaky*** -> transplanted
  - ***RMRP***-cartilage hair hypoplasia and total Hirschprung

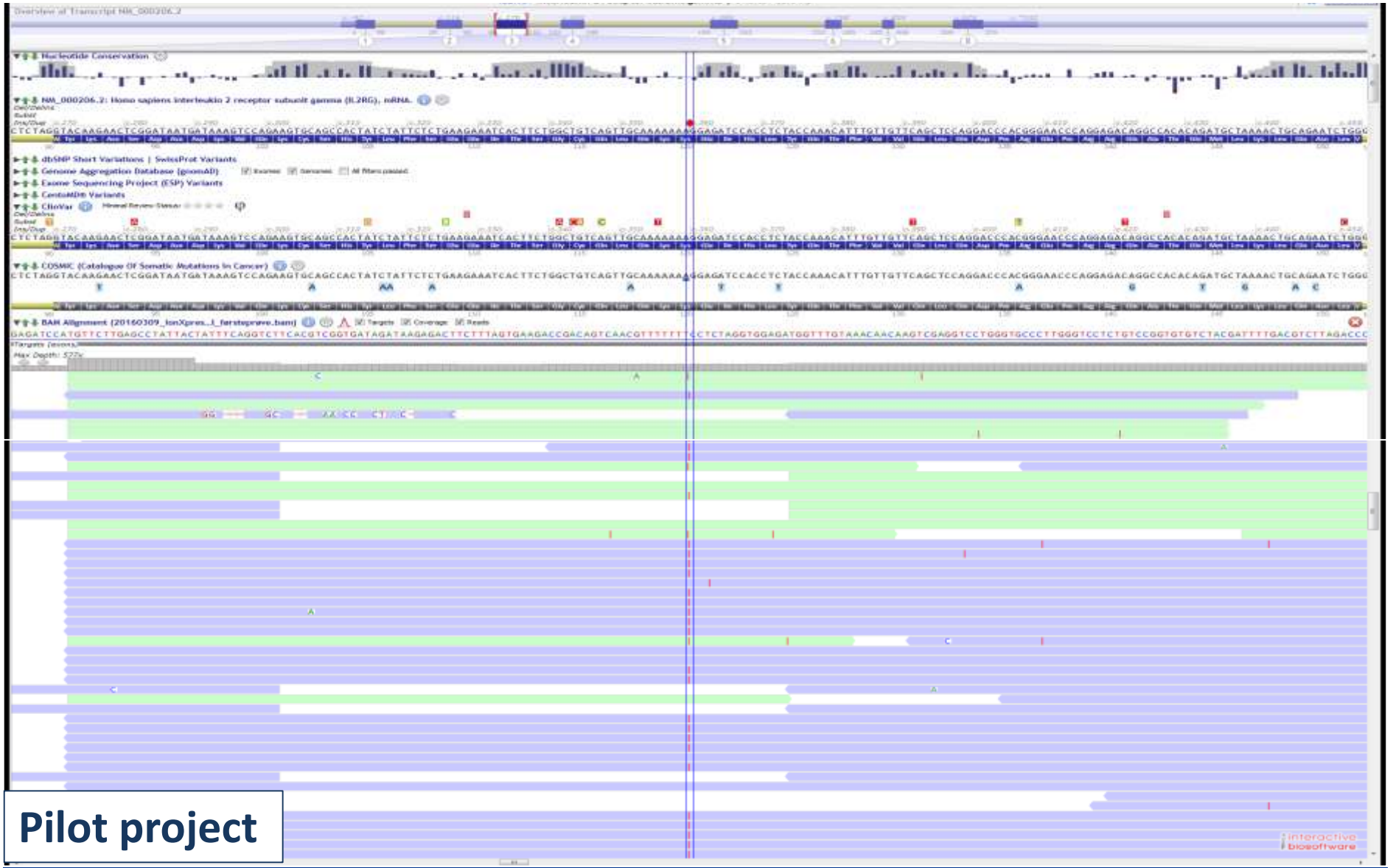
Nationwide screening:

- Informed consent
- Started 1.Jan 2018
- >88 000 as of 5.Sept 2019
- 29 cards gene panel tested
- 4 with SCID:
- **Artemis, radiosensitive type T-B-NK+ SCID** -> transplanted
- **JAK3 defect** -> transplanted
- **NBN T-B-NK+ SCID**
- **IL2RG defect**

New patients referred from hospitals outside pilot region
Retrospective study of patients with known immunodeficiency

TRECs/µl: 0,5 - 0 - 0

NM_000206.2(IL2RG):



Pilot project

TRECs/µl: 0,5 - 0 - 0

NM_000206.2(IL2RG):

c.[359dupA];[0],p.(Glu121Glyfs*47) hemizygous

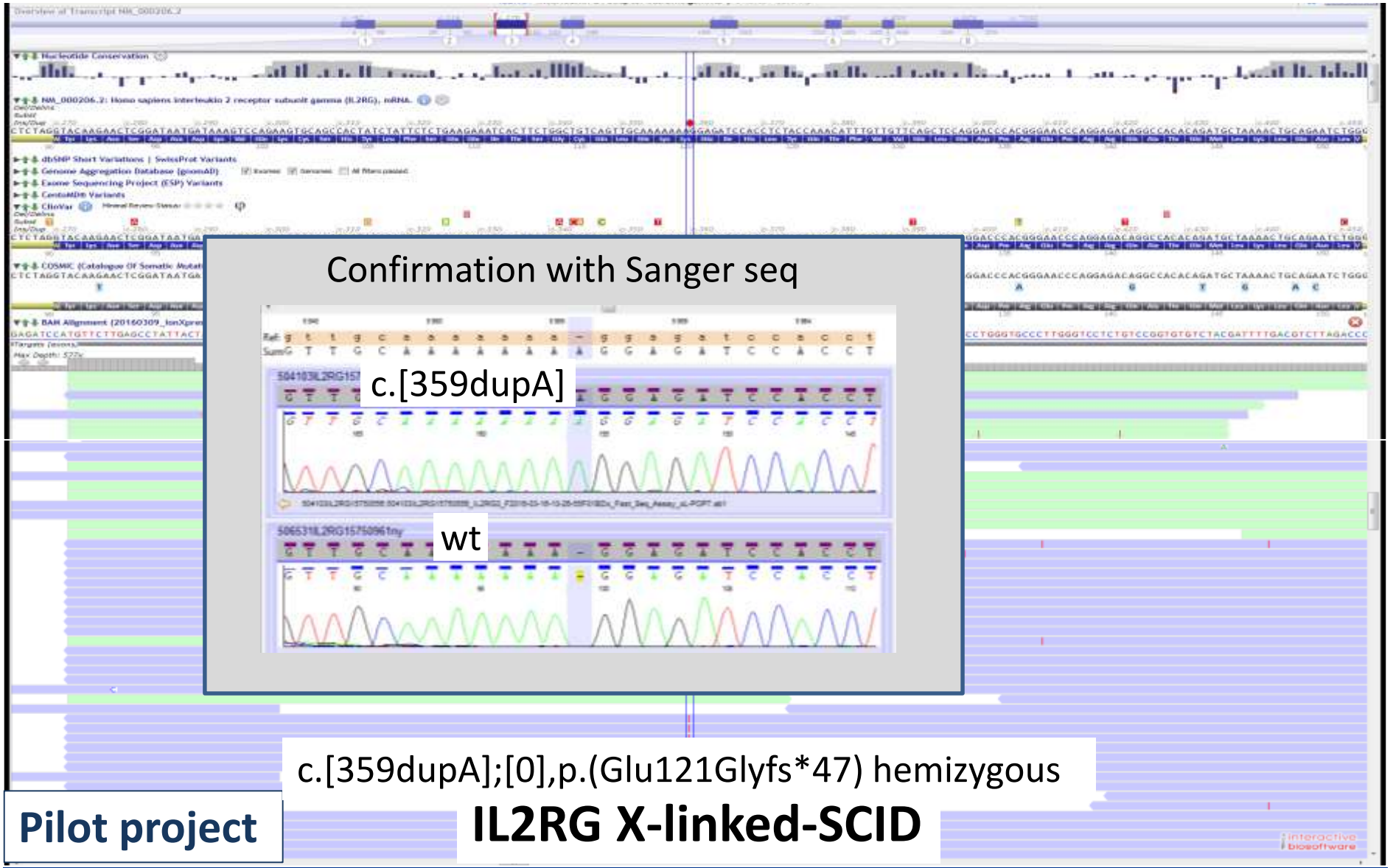
Pilot project

IL2RG X-linked-SCID

Oslo University Hospital

TRECs/µl: 0,5 - 0 - 0

NM_000206.2(IL2RG):

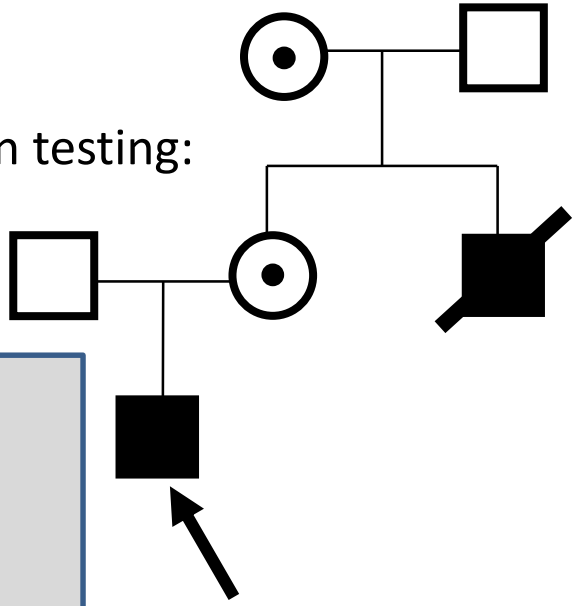


c.[359dupA];[0],p.(Glu121Glyfs*47) hemizygous

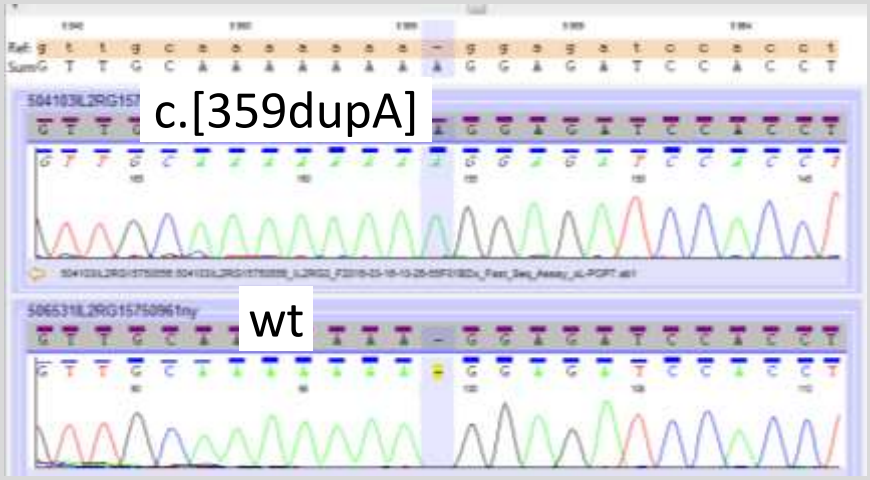
IL2RG X-linked-SCID

Pilot project

And segregation testing:



Confirmation with Sanger seq



c.[359dupA];[0],p.(Glu121Glyfs*47) hemizygous

Pilot project

IL2RG X-linked-SCID

TREC/µl		NICU	Known condition at birth	Gene panel	Findings and history
initial	mean				
0	0	Yes	Intestinal malformation and skeletal dysplasia	Yes	*RMRP*-SCID. Deceased
0	0	No	None	Yes	*IL2RG*-SCID, Transplanted
2	10	Yes	Intestinal malformation and pulmonal hypertension	Yes	No mutation, TRECs normalizing
4	10	No	None	Yes	*RAG2*-SCID, Transplanted
7	9	Yes	Prematurity <1000g	Yes	No mutation
8	24	Yes	Intestinal malformation	No	Reported
12	15	Yes	Congenital heart disease	Yes	No mutation
13	11	Yes	Prematurity <500g	No	Not reported
13	7	Yes	Prematurity <1000g	Yes	No mutation
14	18	Yes	Prematurity 1000g	No	TRECs normalizing
15	19	Yes	Prematurity 1000g, cystic hygroma, polyhydramnios and congenital heart disease	WES	Deceased 2 weeks old, *RIT1*-cardiomyopathy
16	22	Yes	Congenital heart disease	No	TRECs normalizing
16	9	Yes			normalizing
18	16	Yes			nutation
19	15	Yes	Congenital heart disease	No	TRECs normalizing
19	11	Yes	Prematurity <500g	No	Redraw not received
19	30	Yes	Intestinal malformation	No	Not reported
20	19	Yes	Birth asphyxia and meconium aspiration	No	TRECs normalizing
20	24	No	Neonatal reduced health condition	Yes	No mutation, TRECs normalizing
20	24	Yes	Prematurity <1000g and lung disease	No	Monozygous twin with normal TRECs

Low TRECs: 17/20 in NICU

TRECs/µl: 4 - 18 - 7

NM_000536.3(RAG2):

Pilot project

TRECs/µl: 4 - 18 - 7

NM_000536.3(RAG2):

Pilot project

TRECs/µl: 4 - 18 - 7

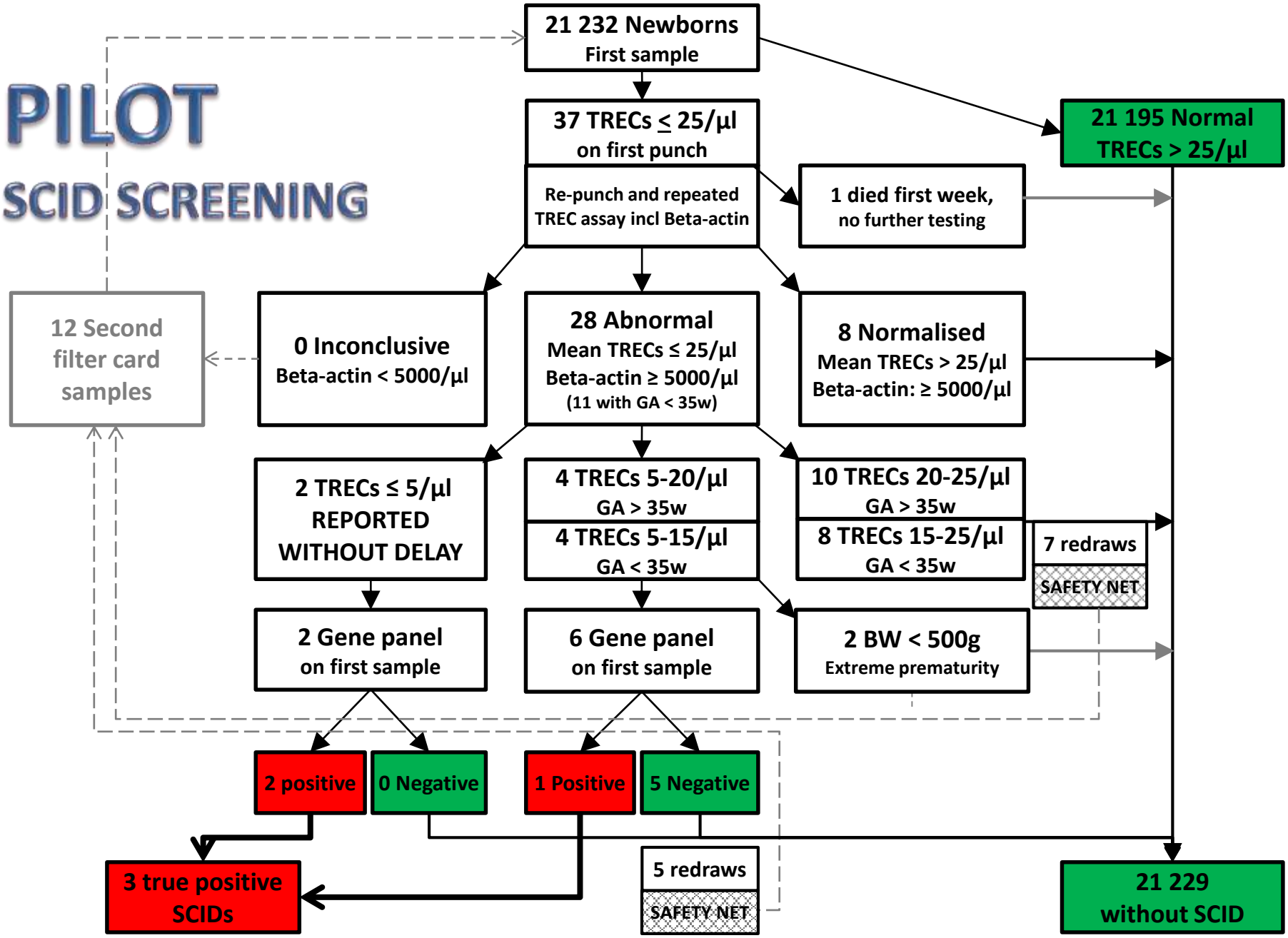
NM_000536.3(RAG2):

c.[1367C>T](;)[1367C>T], p.(Ala456Val) homozygous

Pilot project

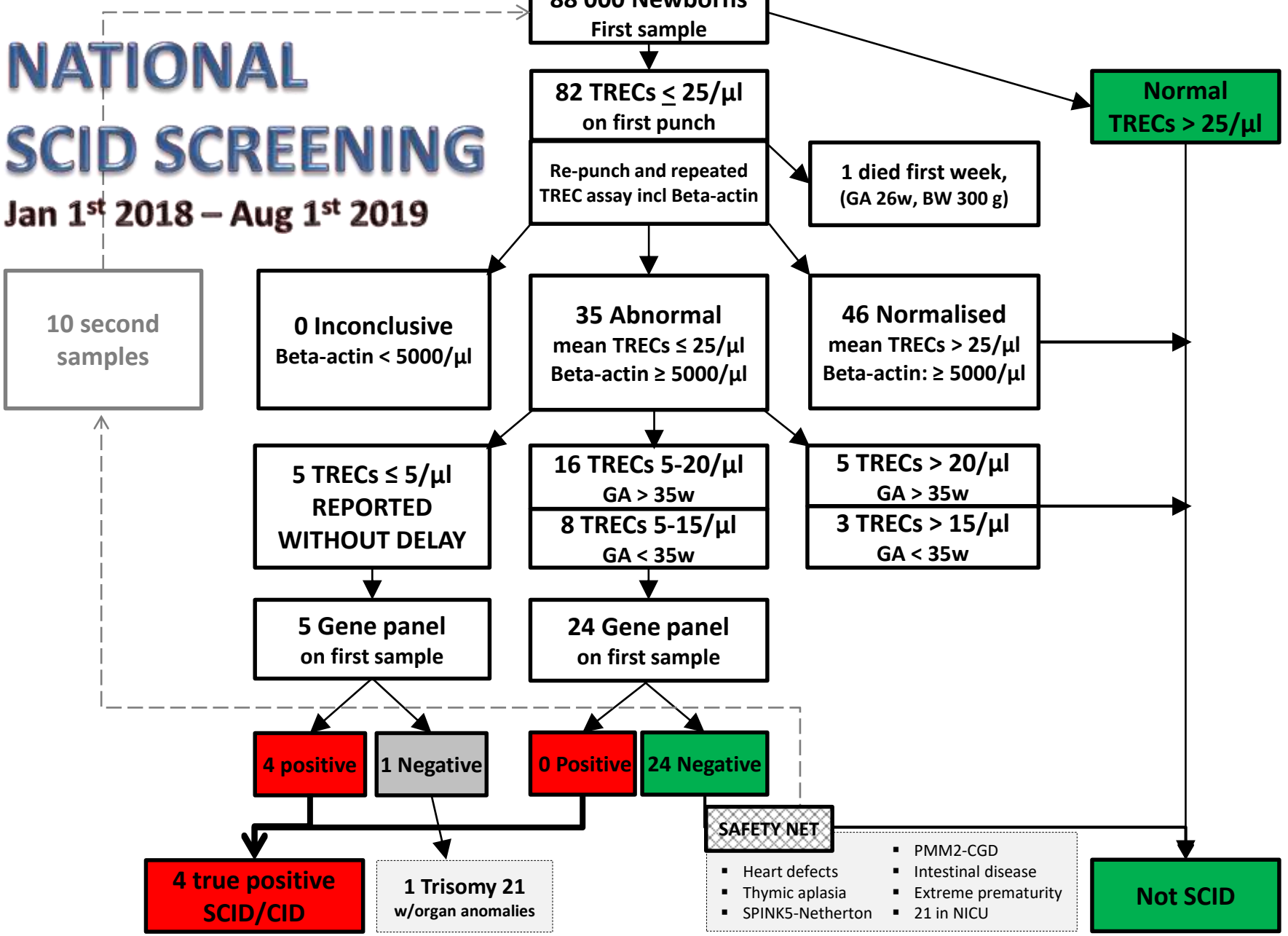
leaky RAG2-SCID

PILOT
SCID SCREENING

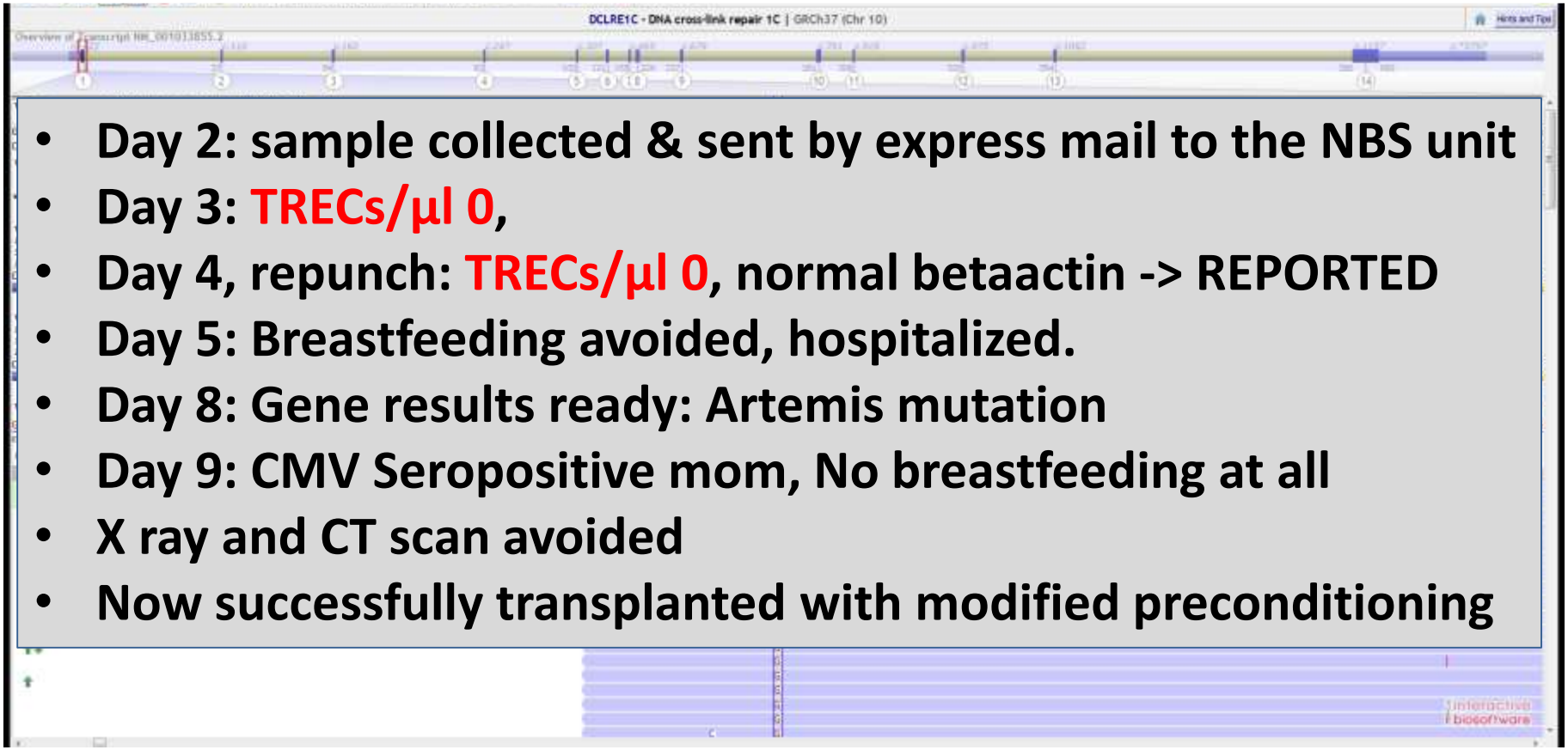


NATIONAL SCID SCREENING

Jan 1st 2018 – Aug 1st 2019



NM_001033855.2(DCLRE1C):



- **Day 2: sample collected & sent by express mail to the NBS unit**
- **Day 3: TRECs/µl 0,**
- **Day 4, repunch: TRECs/µl 0, normal betaactin -> REPORTED**
- **Day 5: Breastfeeding avoided, hospitalized.**
- **Day 8: Gene results ready: Artemis mutation**
- **Day 9: CMV Seropositive mom, No breastfeeding at all**
- **X ray and CT scan avoided**
- **Now successfully transplanted with modified preconditioning**

c.[82C>G](;)[82C>G], p.Ala28Pro homozygous

Artemis, radiosensitive type SCID

Nationwide screening

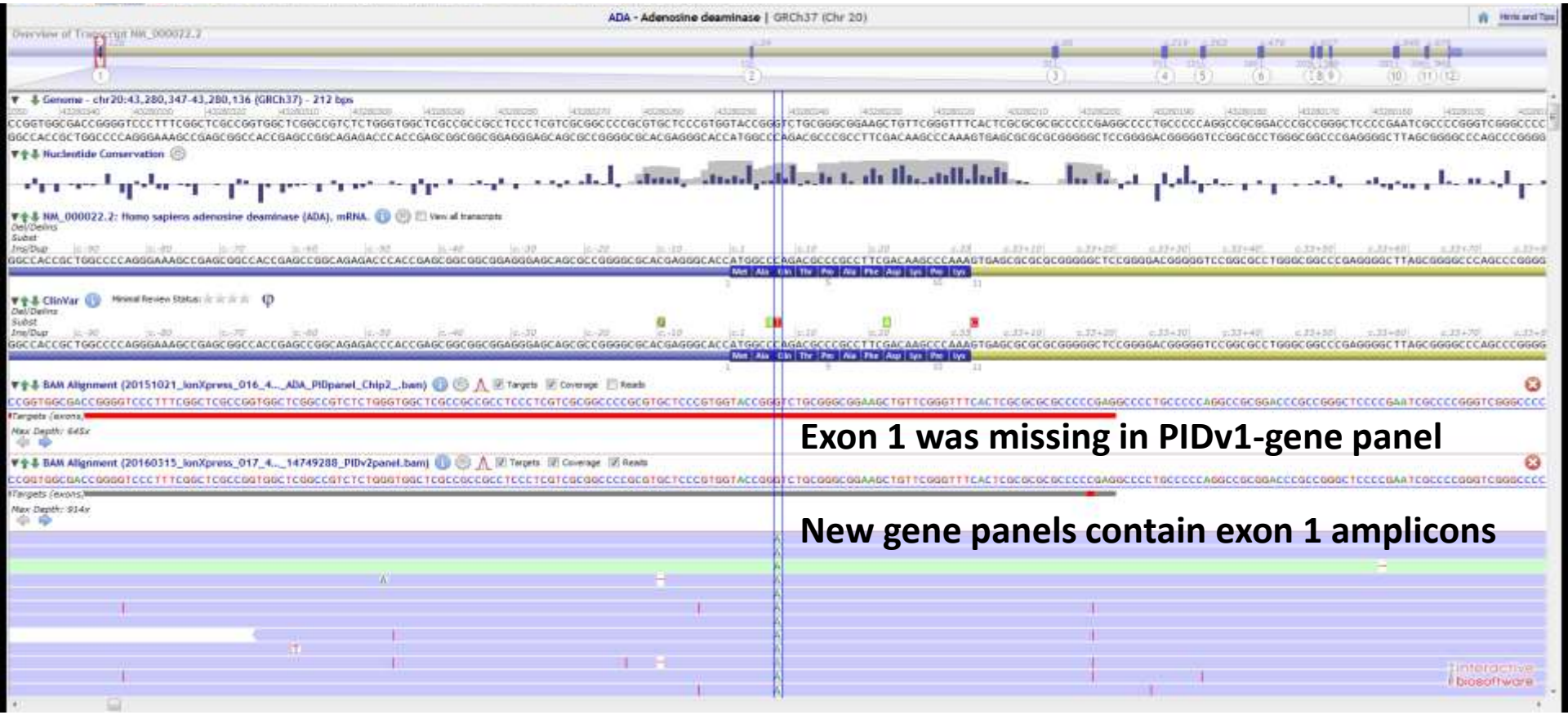
Retrospective TRECs and NGS in patients with known immunodeficiency

	RETROSPECTIVE TRECs and NGS TESTING										
	INDIVIDUALS WITH KNOWN SCID or severe T-cell deficiency									OTHER PIDDs	
Sample ID	KID_1	KID_2	KID_3	KID_4	KID_5	KID_6	KID_7	KID_8	KID_9	KID_10	KID_11
Year	2012	2010	2010	2015	2012	2014	2015	2006	2009	2009	2012
Gender	Male	Female	Female	Female	Female	Male	Male	Female	Male	Male	Female
GA	37	34	38,5	42	40	42	39	38	39	40	38
BW	2954	1999	2618	3360	4135	4420	2484	NA	3445	3775	2950
TRECs/µl	**0**	**0**	**0**	**0**	**11**	**0**	**0**	**0**	**0**	**25.5**	**60**
Gene	***IL2RG***	***LIG4***	***IL7R***	***ADA***	***PGM3***	***JAK3***	***TBX1***	***TBX1***	***TBX1***	***IKZF1***	***RECQL4***
SNV/CNV	c.[924+5G>A];[0]	c.[1341G>T];[482delC]	c.[707-2A>G] HOM	c.[7C>T] HOM	c.[737A>G];[737A>G]	c.1837C>T; c.1695C>A	22q11.21 del	22q11.21 del	22q11.21 del	c.1618388_589+2308del (16.8kb del exons 4-5)	c.2269C>T; ND
Protein	Splice defect	p.Trp447Cys; p.Ala161Valfs*6	Splice defect	p.Gln3*	p.Ala246Gly	p.Arg613*; p.Cys565*	loss	loss	loss	Inframe deletion	p.Gln757*
RefSeq	NM_000206.2	NM_002312.3	NM_002185.3	NM_000022.2	NM_015599.2	NM_000215.3	NM_080647.1	NM_080647.1	NM_080647.1	NM_006060.5	NM_004260.3

Next generation sequencing (NGS) may identify copy number variants (CNVs)

Retrospective

NM_000022.2(ADA):



c.[7C>T];[7C>T], p.Gln3Ter homozygous

ADA-SCID

Retrospective

Retrospective TRECs and NGS in patients with Ataxia Telangiectasia

	INDIVIDUALS WITH KNOWN ATAXIA TELANGIECTASIA						
Sample ID	**AT_1.1**	**AT_1.2**	**AT_2**	**AT_3**	**AT_4**	**AT_5**	**AT_6**
Year	2008	2011	2010	2010	2011	2011	2016
Gender	Male	Male	Female	Female	Male	Male	Female
GA w	36	39	40	40	41	41	40
BW g	3015	3650	3196	3570	2815	4180	2959
TRECs/µl	**4**	**3**	**16**	**7**	**22**	**93**	**27**
gene	**ATM NM_000051.3**						
SNV/CNV	c.[6047A>G] HOM	c.[6047A>G] HOM	c.3245_3247delATC insTGAT] HOM	c.[3245_3247delATC insTGAT] ;[6679C>T]	c.[3245_3247delATC insTGAT] HOM	c.[1564_1565delGA] ;[9023G>A]	c.[5932G>T] ;[9123delC]
protein	p.Asp2016Gly	p.Asp2016Gly	p.His1082fs	p.His1082fs; p.Arg2227Cys	p.His1082fs	p.Glu522Ilefs*43; p.Arg3008His	p.Glu1978Ter; Asn3044fs
Panel	PIDv2	PIDv2	PIDv2	PIDv2	PIDv2	PIDv2	PIDv2

Ataxia Telangiectasia is a progressive neurological disorder without treatment

Retrospective

«Newborn screening is not a test, it's a plan»

2.tier DNA analyses in Newborn Screening (NBS)
=> moving to next generation sequencing (NGS)

CF Cystic Fibrosis: First NBS-NGS disorder

2.tier sequencing of all coding regions of *CFTR*

CFTR carriers are NOT reported

IEM Inborn errors of metabolism

- IEMv1 panel 570 genes
- IEMv2 panel 594 genes
  Incl 33 NBS-IEM genes
- IEMv3 panel 800 genes

PID Primary Immunodeficiency

- PIDv1 panel 266 genes
- PIDv2 panel 264 genes
  Incl ~45 SCID genes
- PIDv3 panel ~700 genes

NBS-NGS core panel 170 genes, incl 50 SCID genes

1.5 – 1.8 ng DNA total

Read depths > 200

Low input - high output!

NBS-NGS core panel 170 genes

“Uniform International Newborn Screening panel”

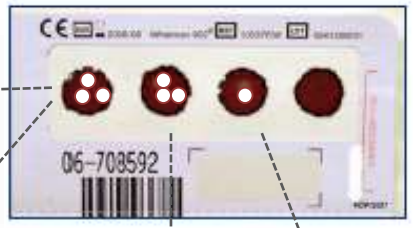
ADA	*AK2*	*ARPC1B*	*ATM*	*BCL11B*	*BLNK*	*BTK*	*CARMIL2*	*CD247*	*CD3D*
CD3E	*CD3G*	*CD40LG*	*CD79A*	*CD79B*	*CD8A*	*CHD7*	*CORO1A*	*DCLRE1C*	*DKC1*
DNMT3B	*DOCK2*	*DOCK8*	*EFL1*	*EPG5*	*EXTL3*	*FOXN1*	*GATA2*	*IGHM*	*IGLL1*
IKBKB	*IKZF1*	*IL2RG*	*IL7R*	*JAK3*	*LAT*	*LCK*	*LIG4*	*LRRC8A*	*LYST*
NBN	*NHEJ1*	*NUDCD3*	*PAX1*	*PGM3*	*PIK3CD*	*PIK3R1*	*PNP*	*PRKDC*	*PTPRC*
RAC2	*RAG1*	*RAG2*	*RAI1*	*RECQL4*	*RMRP*	*SBDS*	*SLC46A1*	*SMARCAL1*	*STAT5B*
STIM1	*TBX1*	*TCF3*	*TP63*	*TTC7A*	*UNC119*	*WAS*	*ZAP70*	*ZBTB24*	
ABCD1	*ACAD8*	*ACADM*	*ACADS*	*ACADSB*	*ACADVL*	*ACAT1*	*ADK*	*AHCY*	*ALDH7A1*
ALDOB	*ARG1*	*ARSA*	*ASL*	*ASS1*	*BCKDHA*	*BCKDHB*	*BCKDK*	*BTD*	*CBS*
CFTR	*CPS1*	*CPT1A*	*CPT2*	*CYP11A1*	*CYP17A1*	*CYP21A1P*	*CYP21A2*	*DBT*	*DLD*
DMD	*ETFA*	*ETFB*	*ETFDH*	*FAH*	*GAA*	*GALE*	*GALK1*	*GALT*	*GAMT*
GATM	*GCDH*	*HADHA*	*HADHB*	*HBB*	*HLCS*	*HMGCL*	*HMGCS2*	*HPD*	*IDUA*
IVD	*LCRB*	*LMBRD1*	*MAT1A*	*MC2R*	*MCCC1*	*MCCC2*	*MMAA*	*MMAB*	*MMACHC*
MMADHC	*MTHFR*	*MTR*	*MTRR*	*MUT*	*NADK2*	*NAGS*	*NPC1*	*NPC2*	*OTC*
PAH	*PCBD1*	*PCCA*	*PCCB*	*PHGDH*	*POR*	*PSAP*	*PSAT1*	*PSPH*	*PTS*
QDPR	*SLC16A1*	*SLC22A5*	*SLC25A13*	*SLC25A15*	*SLC25A20*	*SLC52A2*	*SLC52A3*	*SLC6A8*	*SMN1*
SMN2	*TAT*								

CNVs	Del22q11, DiGeorge syndrome	Del8q12 CHARGE syndrome	Del17p11.2 SmithMagenis syndrome	Del11q, Jacobsen syndrome	Trisomy18 Edwards syndrome	Trisomy21 Downs syndrome
Dosage	*TBX1*	*CHD7*	*RAI1*	*ACAD8*	*EPG5, NPC1*	*JAK3*

AmpliSeq On-Demand

2.Tier genetic testing confirms disease

GSP ®



1.Tier

Mass spectrometry
LC-MS/MS

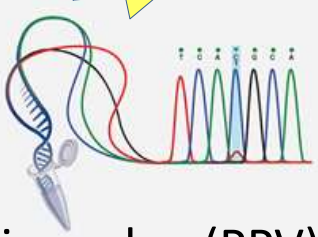
TREC

QuantStudio
RT-PCR

DNA extracted
from filtercard punch

2./3.Tier

RAPID



DNA testing as an integrated part of NBS

In-house implementation of sequencing and increased positive predictive value (PPV)

“Only a blood spot on a paper”..

Minister of Health and Care Services, Bent Høie
in Norwegian Newspaper Dagens Medisin, 2017-12-01

What have we learned from our SCID screening?

2nd tier next generation sequencing in newborn SCID screening

- **reduce number of blood redraws and recalls,**
- **provides rapid molecular confirmation of disease with**
- **implications for follow-up and treatment**
- **May identify leaky SCIDs**
- **Prior to CMV infection**