Genetics first for primary immunodeficiencies

Alexander Hoischen, PhD
Department of Human Genetics, & Department of Internal Medicine
Radboud University Medical Center, Nijmegen, The Netherlands

Contact:
alexander.hoischen@radboudumc.nl
www.radboudumc.nl/en/immunogenomics

@ahoischen

Human Genetics Nijmegen

Radboudumc

Full disclosure

I am a geneticist NOT an immunologist
…and know almost nothing about cytometry

…and still I was asked to provoke some thoughts with ‘bold genetics statements’ at the NVC

…and I cannot write Dutch – sorry!

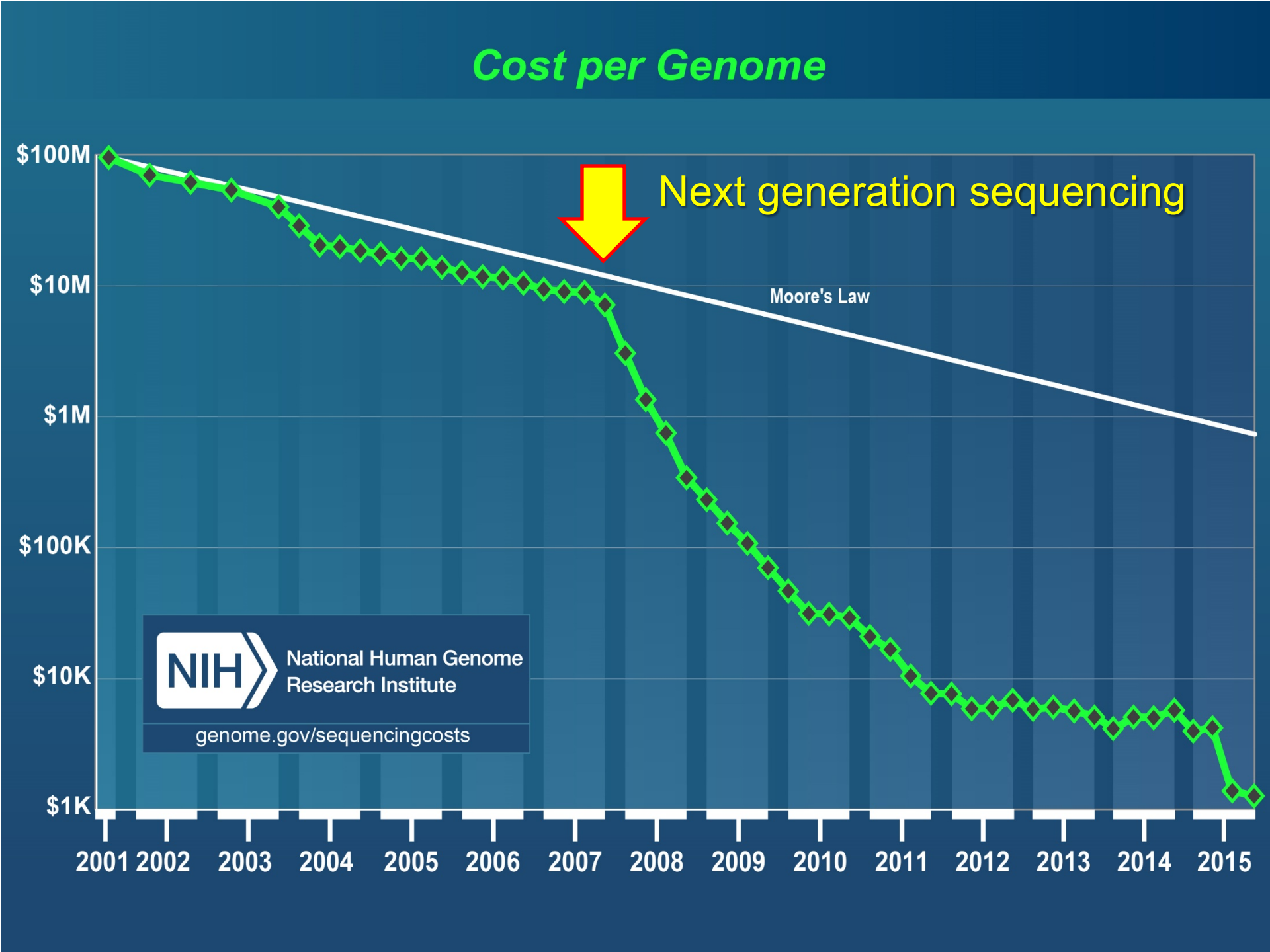
Finding the answer in the genome

6 billion nucleotides
46 chromosomes

2 people differ
at **XXX** positions

1 variation (mutation)
can result in disease

Cost per Genome



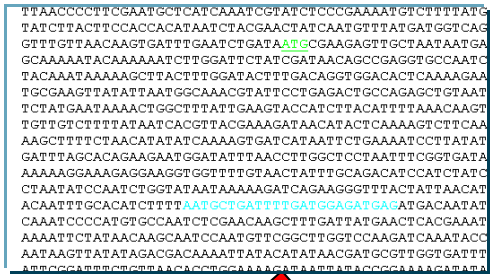

From art….

To industry….

Genome sequencing: All variation in one experiment!

DNA from
blood/saliva

Genome
with ‘all’ variation

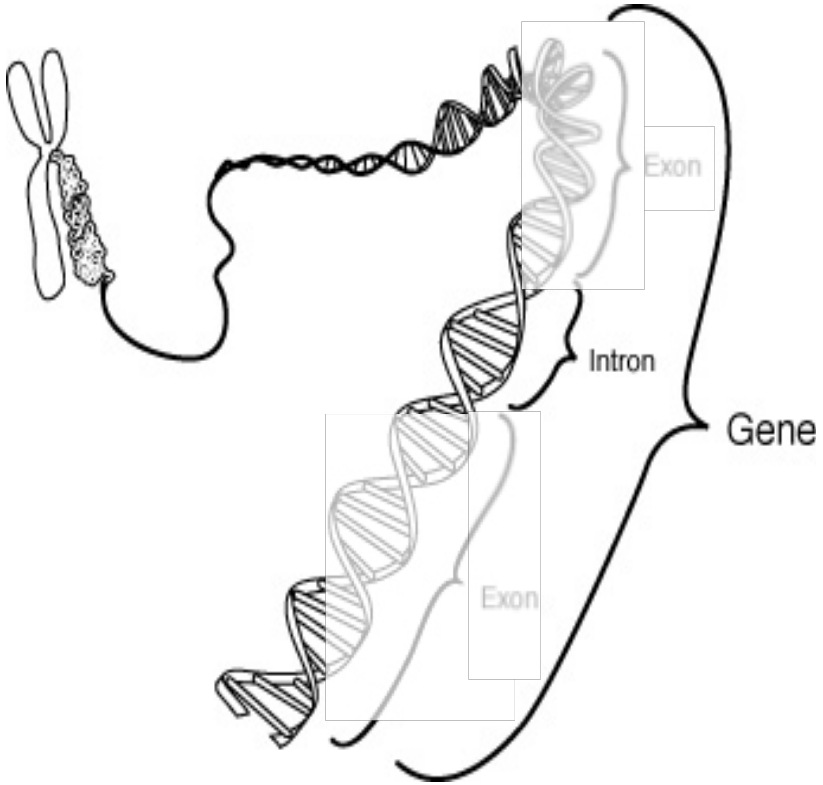


Important:
- Accuracy
- Speed
- Price

Exome sequencing; Practicing for genomes

‘Exome’ (all exons of a genome)

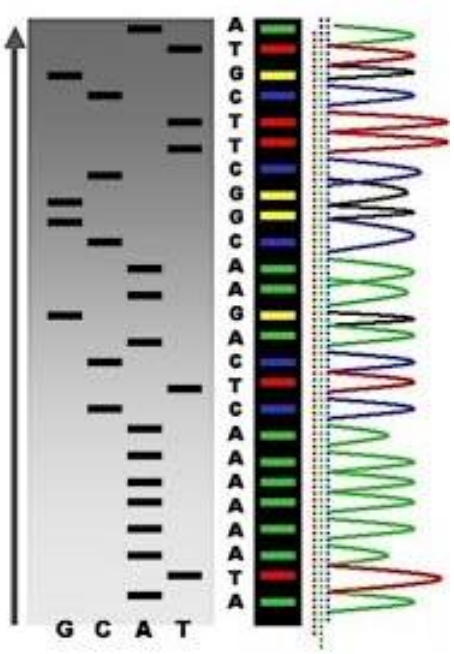
~1% of the human genome



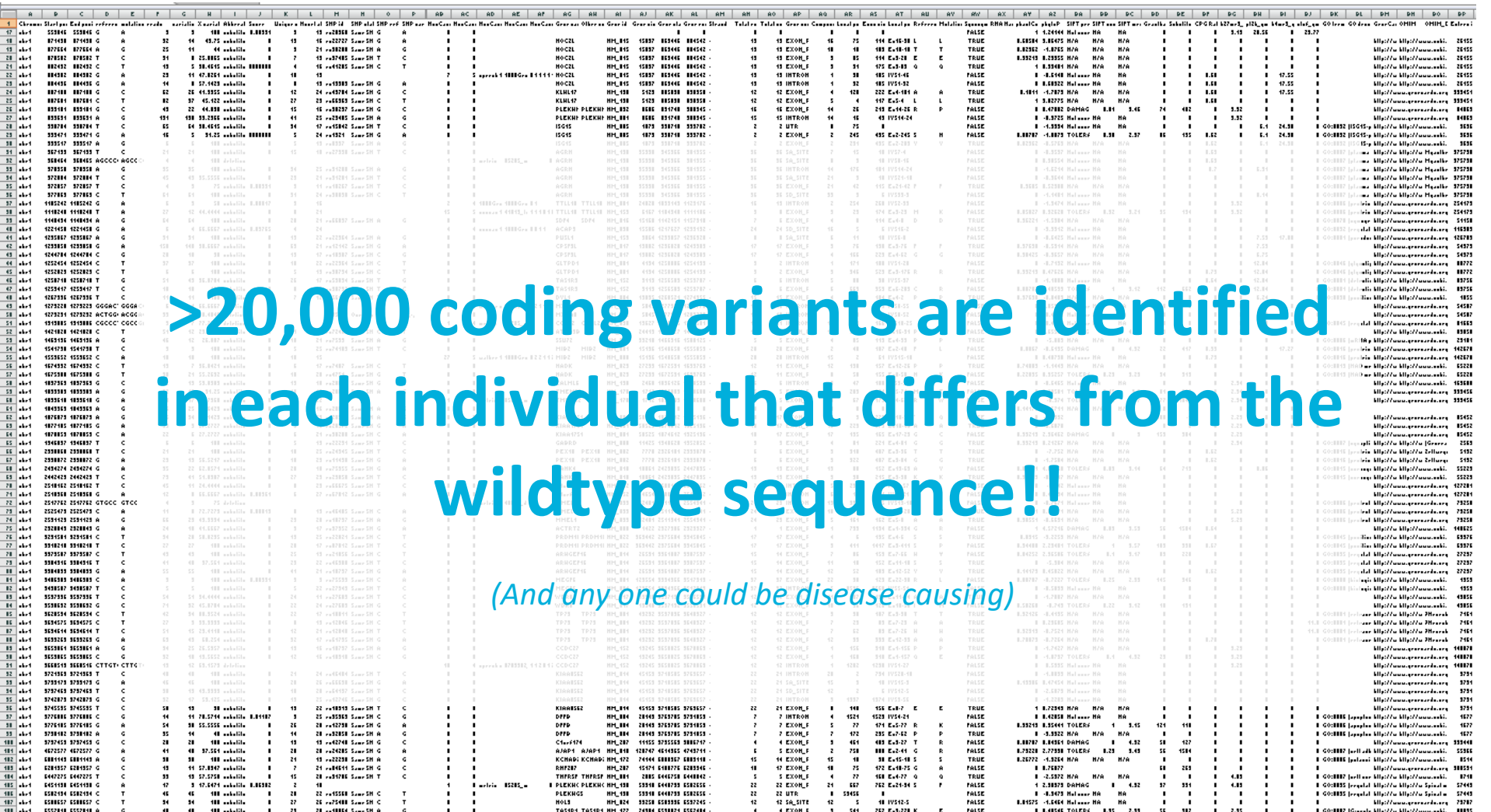
‘All’ coding sequences of a human genome (>200,000 exons), sequenced and analyzed in **one** experiment

Revolution in reading DNA

Sanger sequencing	Exome sequencing
1 gene per test	



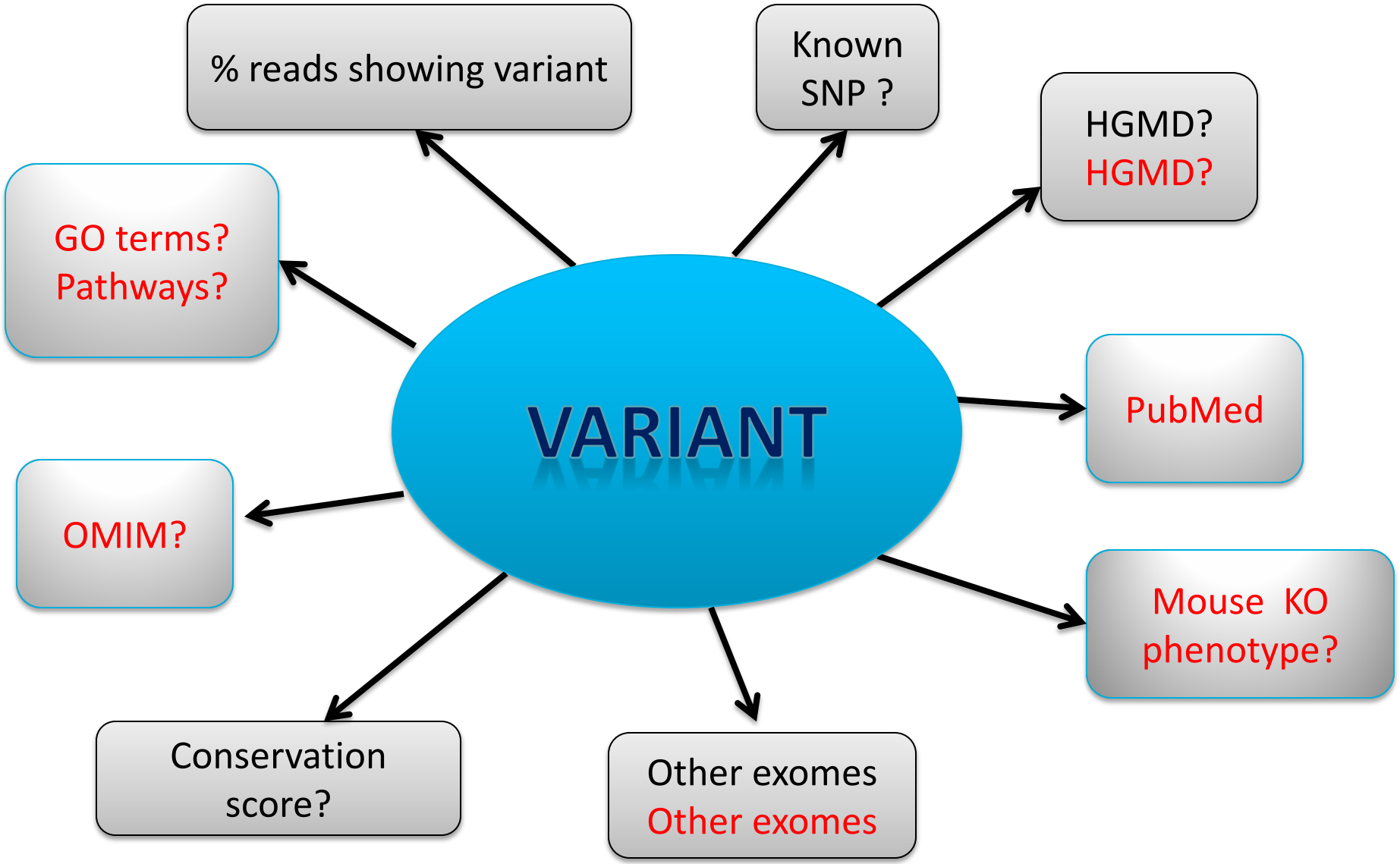
Variant calling and variant annotation



>20,000 coding variants are identified in each individual that differs from the wildtype sequence!!

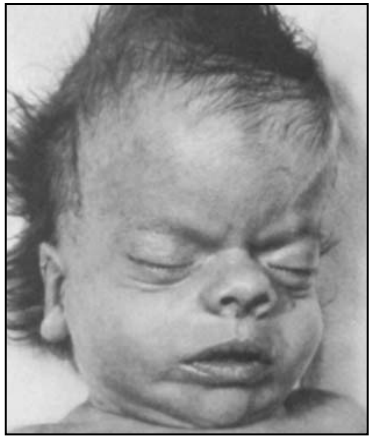
(And any one could be disease causing)

What do we know about this position in the genome?



Key to success: Well-defined clinical collection

Schinzel-Giedion Syndrome
Dominant sporadic disease



Am J Med Genet. 1978;1(4):361-75.

	Our study	Literature: 46 cases
Neurodevelopmental anomalies		
Developmental delay	11/11	39/39
Seizures	12/13	32/35
➡ Vision impairment	8/9	11/12
➡ Hearing impairment	8/9	8/11
➡ **Craniofacial features**		
Prominent forehead	12/13	43/43
Mid-face retraction	13/13	46/46
➡ Short, upturned nose	13/13	40/42
Low-set ears	12/13	37/39
Structural anomalies		
Genital	13/13	35/38
➡ Hydronephrosis or vesicoureteral reflux	13/13	42/45
Cardiac defect	7/13	20/35
➡ Characteristic skeletal malformations	11/11	

Based on: Lehman, A.M. *et al. AJMG A. (2008)*

NGS-based disease gene identification: Filter & combine

Variants	Patient 1	Patient 2	Patient 3	Patient 4	Mean	Genes with variants in all samples
Total called	22,916	22,602	22,152	19,528	21,800	
Exonic + SpliceSites(SS)	12,196	12,255	11,796	10,498	11,686	
Non-synonymous (NS) + SS	5,556	5,618	5,427	4,802	5,351	
Novel, private variants	**180**	**186**	**154**	**172**	**173**	

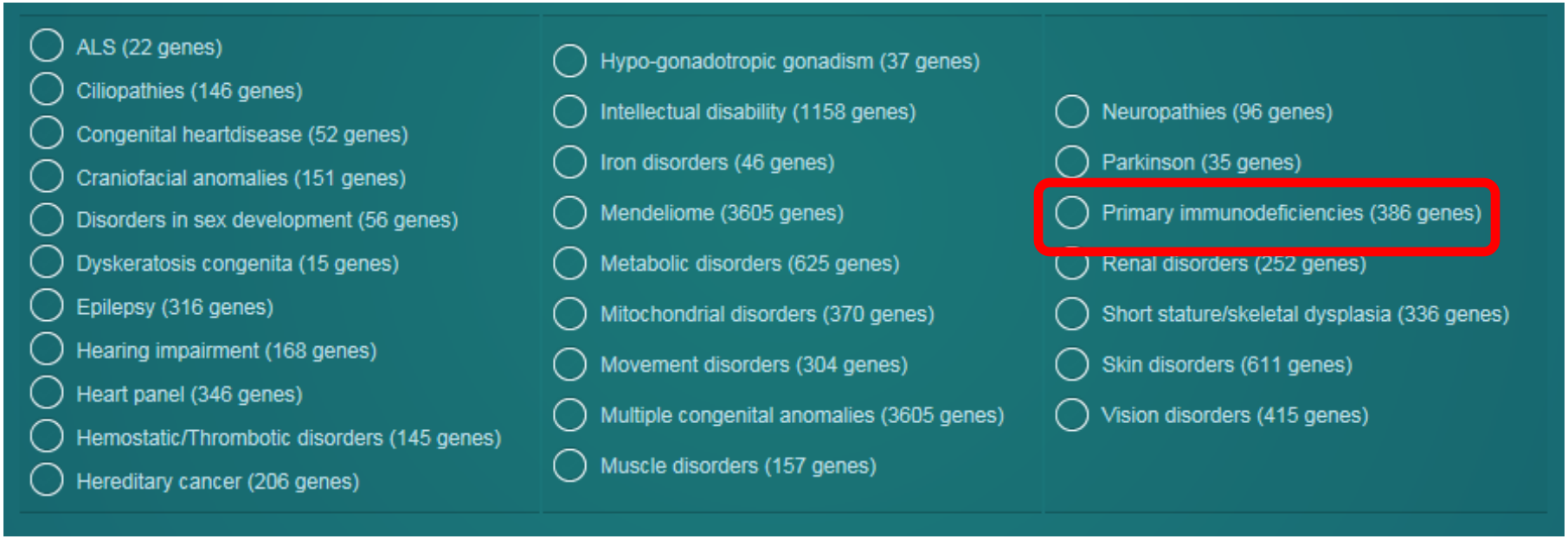
nature genetics

De novo mutations of *SETBP1* cause Schinzel-Giedion syndrome

Alexander Hoischen[1,14], Bregje W M van Bon[1,14], Christian Gilissen[1,14], Peer Arts[1], Bart van Lier[1], Marloes Steehouwer[1], Petra de Vries[1], Rick de Reuver[1], Nienke Wieskamp[1], Geert Mortier[2], Koen Devriendt[3], Marta Z Amorim[4], Nicole Revencu[5], Alexa Kidd[6], Mafalda Barbosa[7], Anne Turner[8], Janine Smith[9], Christina Oley[10], Alex Henderson[11], Ian M Hayes[12], Elizabeth M Thompson[13], Han G Brunner[1], Bert B A de Vries[1] & Joris A Veltman[1]

Exome sequencing is used in diagnostics

- 30,000 exomes analyzed for 27 heterogeneous diseases



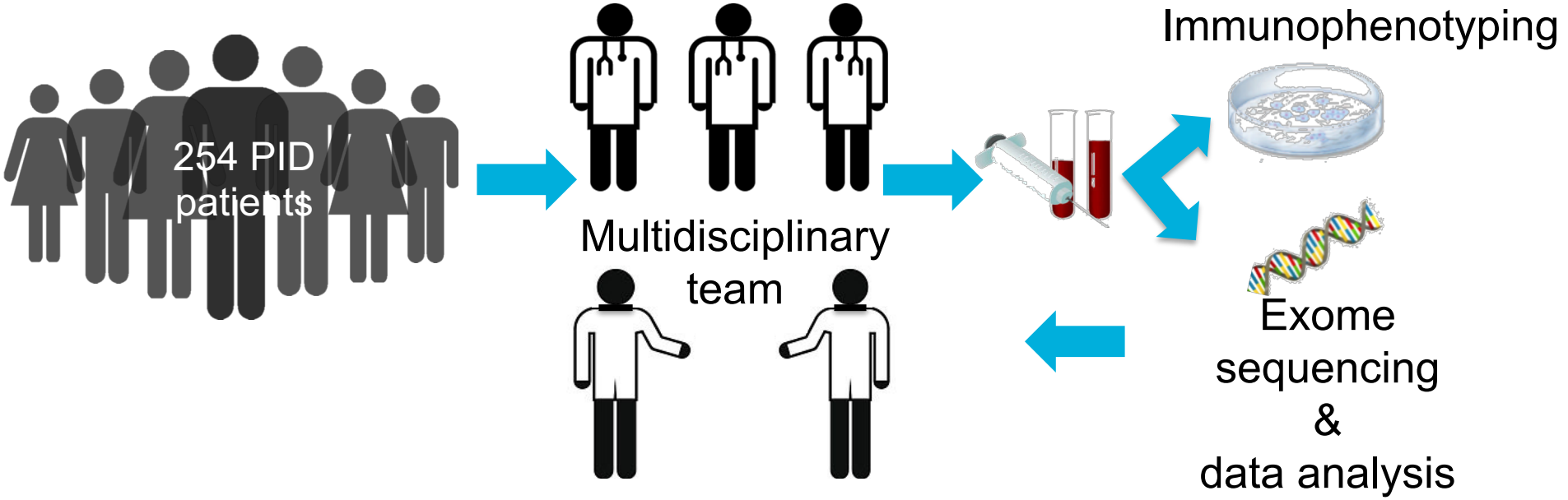
- 2 step analysis (opt-in/opt-out options):
  - *In silico* gene panel analysis (i.e. only known genes)
  - Genome-wide analysis (via clinical geneticist)

Primary immunodeficiency (PID)

- **Inborn defect;** the immune system is unable to effectively defend the host against:
  - Infectious agents (possibly part of human microbiota)
    - Bacteria
    - Viruses
    - Fungi
  - Auto-immune
    - Self-antigens
  - Auto-inflammatory

- Prevalence 1:500; **genetic and phenotypic very heterogeneous**
- **Hypothesis: a significant # of patients have monogenic disease**
- Understanding the disease mechanism directly influences treatment

Exome sequencing in routine diagnostics



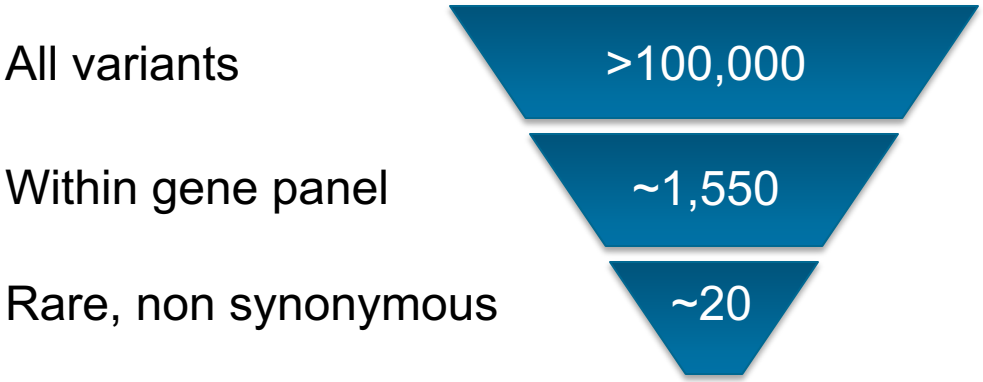
- **Two step approach in exome sequencing**

1. **Gene panel analysis**
   Find disease causing mutations in PID known genes

2. **Exome-wide analysis**
   Find (potential) novel causes of PIDs

Gene panel analysis

- *In silico* filter applied to only disclose variants in known genes
  - Gene list quarterly updated (currently 386 genes)



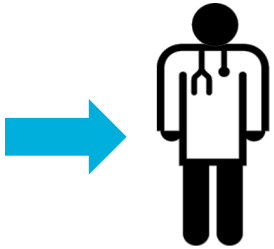
- Per patient ~20 variants evaluated for pathogenicity (class 1-5*)
- Diagnostic yield of 24% (62/254 patients)

*Richards S et al. Genet. Med. **2015**; 17:405–424.

Exome-wide analysis

- Increased risk for incidental findings (extra counseling)

192 diagnosis negative patients



Clinical geneticist

Exome-wide analysis

- Increased risk for incidental findings (extra counseling)

152 consent for exome-wide

192 diagnostic negative patients

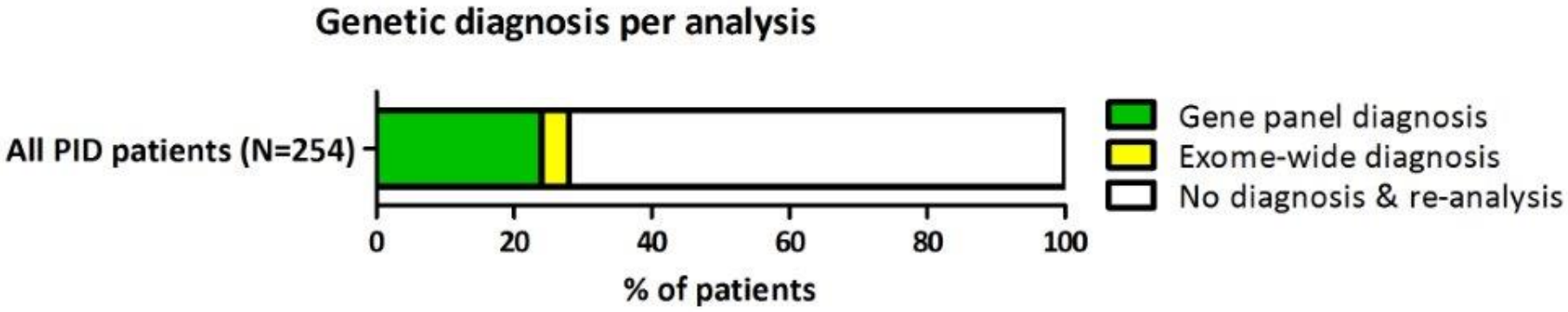
36 no consent for exome-wide (yet)

- Filter for rare, non-synonymous variants.

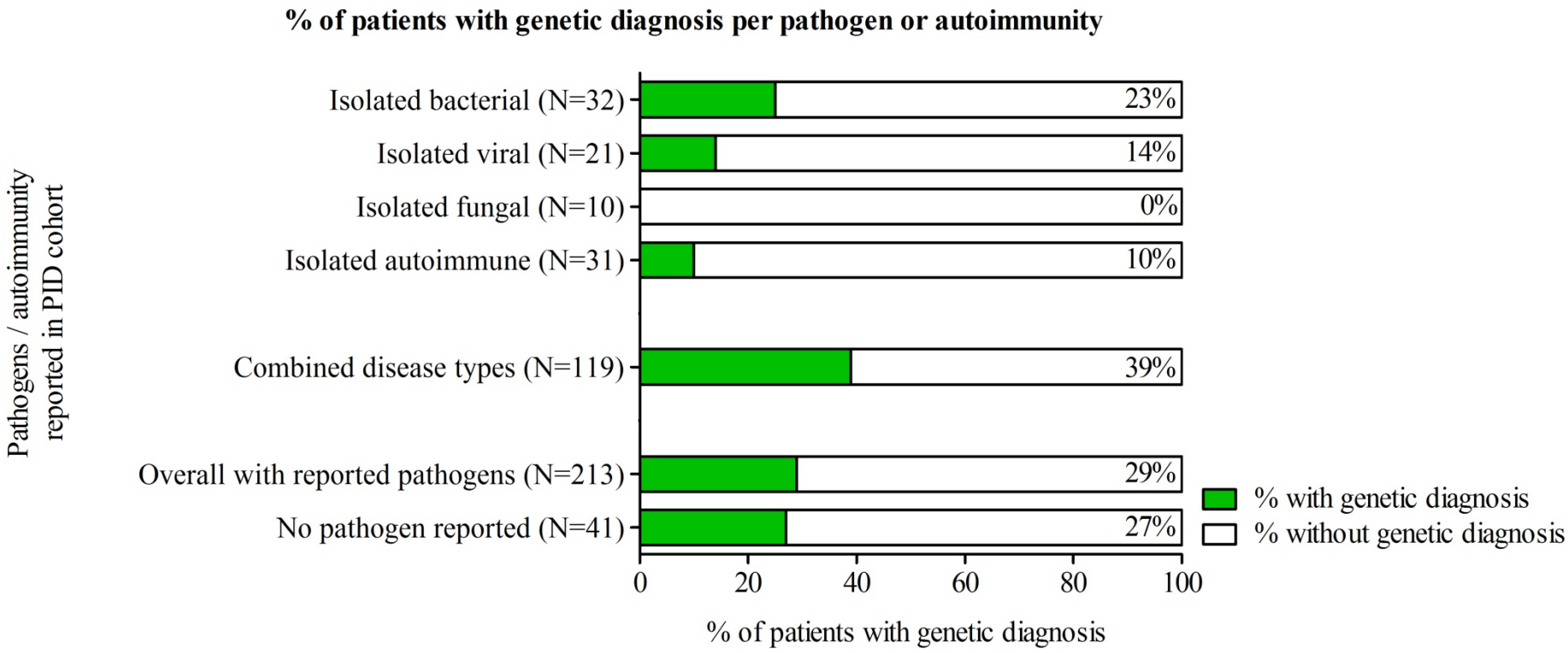
- Special focus on:
  - Recently published genes
  - Genes involved in immunological pathways
  - Mouse KO phenotypes
  - Gene function in specific tissues (e.g. lung, skin, blood)
  - Copy number variants (CNVs)

Diagnostic yield of exome sequencing

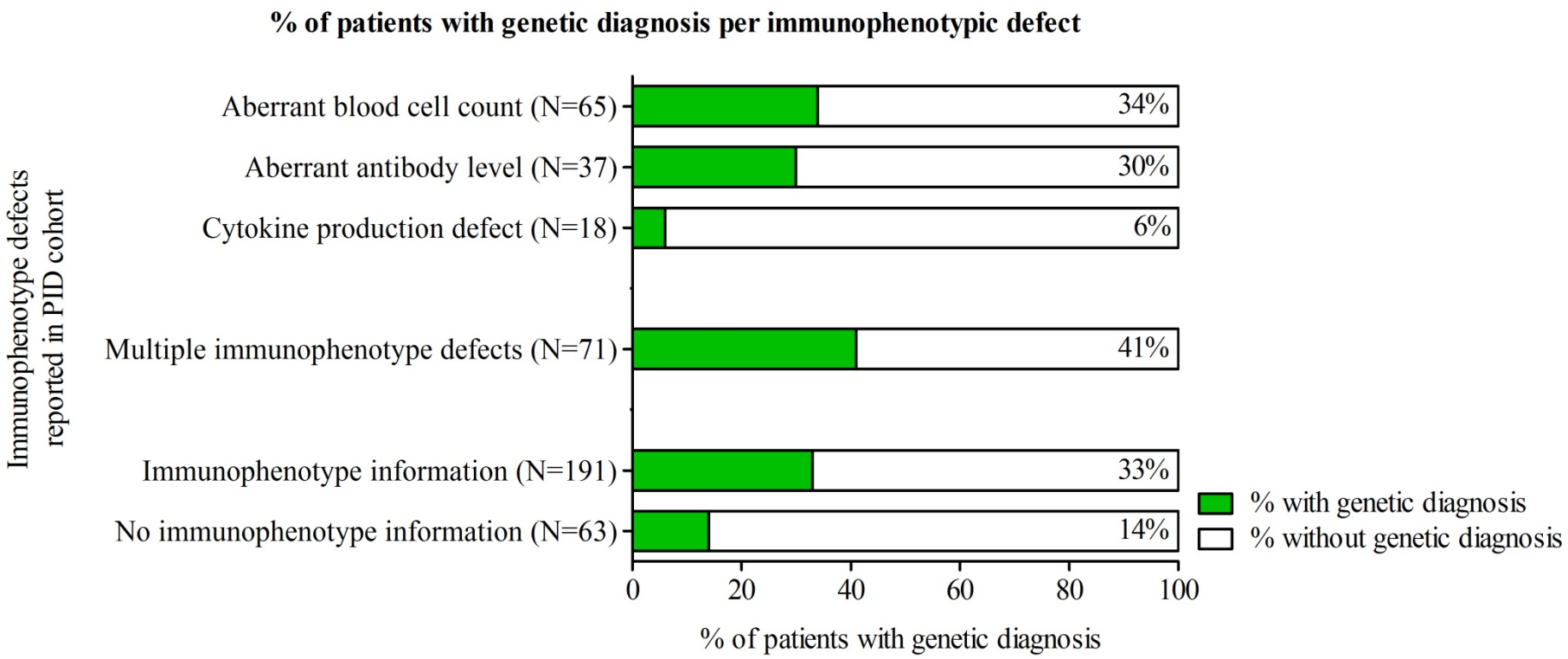
- Gene panel analysis→ 62 of all 254 patients (24%)
- Exome-wide analysis→ additional 10 patients (4%)



Diagnostic yield per pathogen



Diagnostic yield per immune-defect



A molecular diagnosis can change treatment

- **72/254 (28%)** patients a molecular diagnosis was achieved
  - For 19 patients (26%) no change of treatment possible at the moment
  - For **27 patients (38%) with SCID or ICF → bone marrow transplantation** best treatment option.
  - For **26 patients (36%)** the genetic diagnosis defined **targeted therapeutic options (or better prophylaxis)**

Examples:

- Abatacept for patients with *CTLA4* mutations
- Anti-TNF treatment for patients with *CECR1* mutations
- Glutamine supplementation & IFN-γ treatment for patients with *CARD11* mutations
- Avoidance of fava beans & specific drugs for patient with *G6PD* mutation

Why do we only solve 28% of PID cases?

- Very very heterogeneous patient population
- Diagnostic interpretation very conservative
- For a new gene we need >1 patients with mutations
- Mutation present but not interpreted as pathogenic
  - RNAsequencing soon added to assist interpretation
- Di-genic; multi-factorial inheritance?
- Non-coding mutations (missed by exome sequencing)?

Summary exome sequencing

- Exome sequencing →diagnostic yield in 28% of PID patients

- Higher yield in patients with:
  - Consanguineous parents
  - Early onset disease
  - More severe disease (infections & blood cell defects)
  - Severe sporadic disease, analyze: patient-parent trio
  - Extensive (molecular) phenotyping available

- Genetic diagnosis led to altered treatment for several patients e.g. *CTLA4 & LRBA (abatacept), IL1RA (anakinra*)

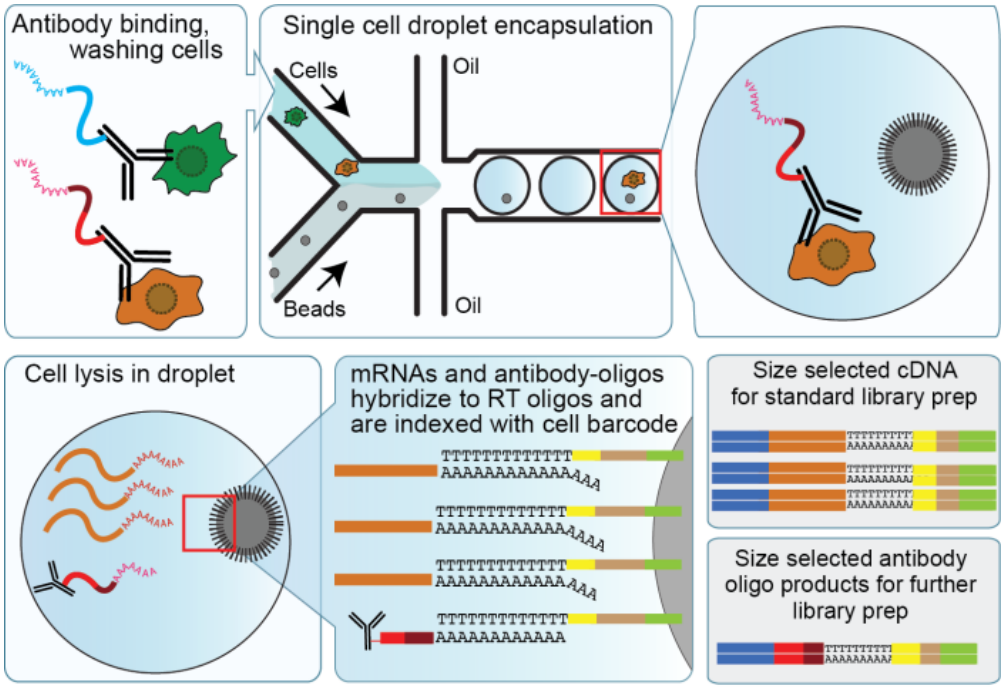
Why genetics first?

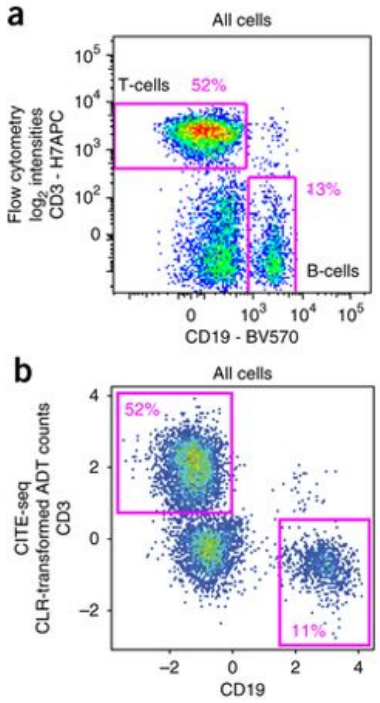
- Classical approach to rare (heterogenous) diseases:
  - Establish a clinical diagnosis on phenotyping, symptoms, molecular phenotypes
  - Genetics used to confirm diagnosis or as 'last escape'
  - This is changing in many fields to **'genetics first'**
    - e.g. neurology, oncology, …)

- Genome sequencing will cost only 100$/sample within 3-5years
- Sequencing is trivial – interpretation is key (multidisciplinary expert teams needed)
- Genetics allows a more educated clinical care & follow-up
- Genetics first works best with good (molecular) phenotyping

Other ways in which genomics may change medicine?

- Single-cell sequencing is booming!
- **Single-cell RNAseq can identify transcriptional profile of almost any cell type AND: epitope recognition can be achieved in same experiment!**
- CITE-seq: Stoekius et al. (Nat Meth. 2017)





scRNAseq data of each cell!

- Could flow cytometry be complemented (or replaced) by sequencing?

Acknowledgements

- Peer Arts
- Elanur Yilmaz
- Tuomo Mantere
- Simone Kersten
- Simon van Reijmersdaal
- Marloes Steehouwer
- Maartje van der Vorst
- Lisenka Vissers
- Christian Gilissen
- Han Brunner

- Janneke Schuurs-Hoejmakers
- Annet Simons
- Wendy van Zelst-Stams

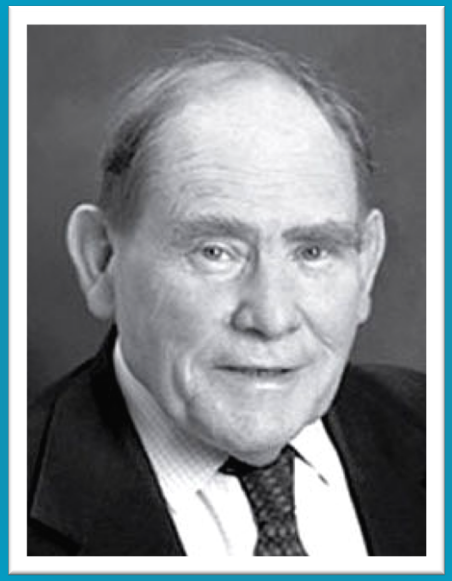
- Mihai Netea
- Frank van de Veerdonk
- Martin Jaeger
- Theo Plantinga
- Jaap ten Oever

- Esther Hoppenreijs

- Michiel van de Flier
- Stefanie Henriet
- Koen van Aerde

- Hans Koenen

And many others!



Sydney Brenner, 2002 Nobel Prize Winner

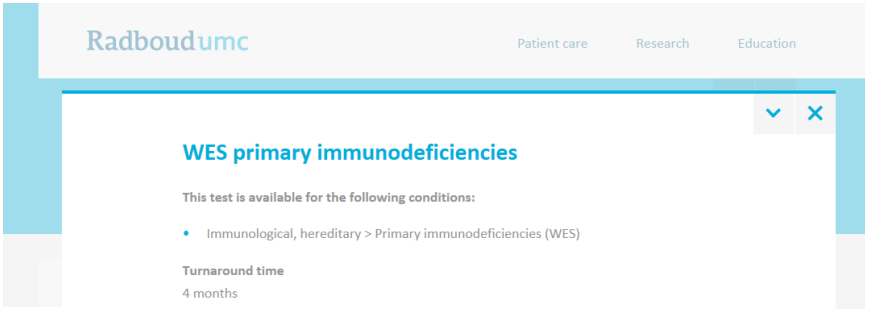
"Progress in science depends on new techniques, new discoveries, and new ideas, probably in that order."

Thesis online:
http://books.ipskampprinting.nl/thesis/peer-arts/

Primary immunodeficiencies –
from genetic basis to therapeutic targets

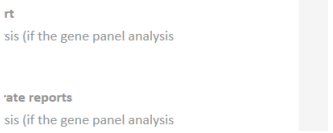
Request diagnostic exome sequencing

- https://order.radboudumc.nl/en/products/wes-primary-immunodeficiencies?c=4063



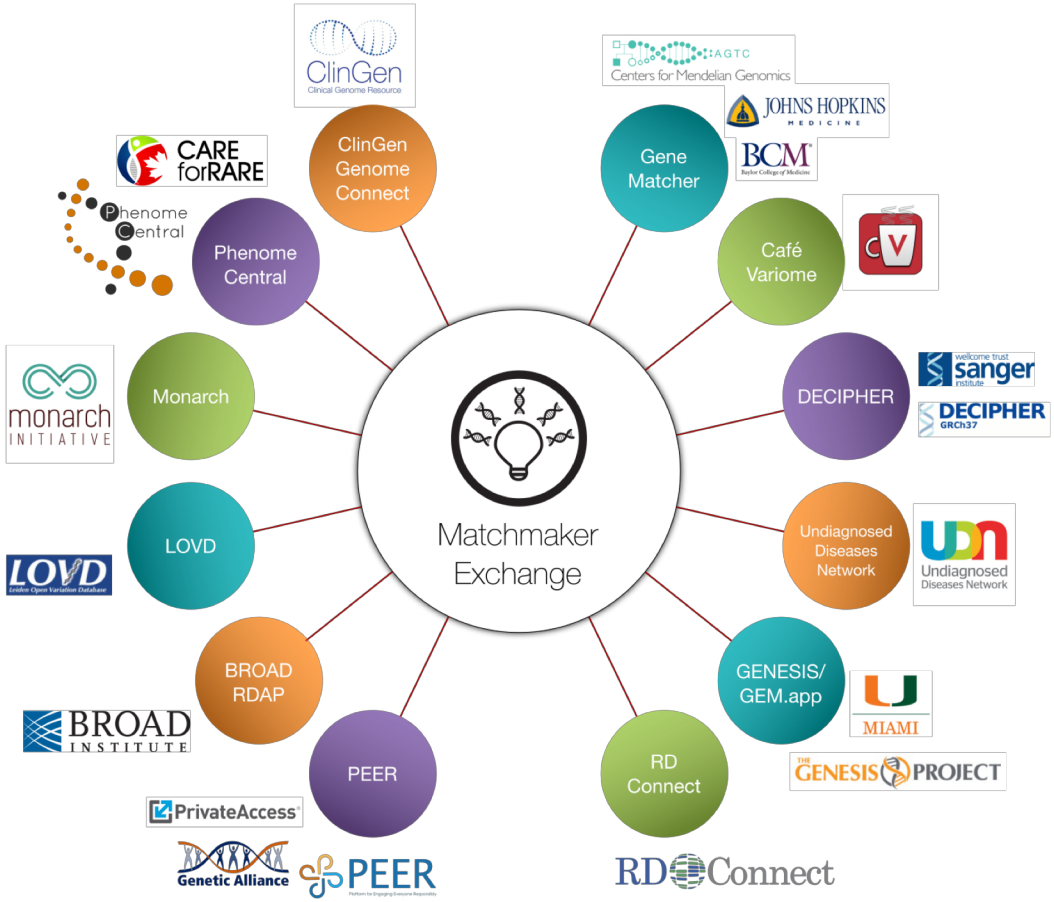
WES PRIMARY IMMUNODEFICIENCIES DG29

Gene	Median coverage	% covered >10x	% covered >20x	OMIM disease ID
IGHM	204,2	100	100	601495
NDNL2	159,4	99	99	617241
AP3B1	128,1	99	95	608233
MPO	160,6	100	99	254600
RLTPR	139,2	95	93	25
IL7R	162,6	100	99	608971
SH2B3	113,1	95	84	26
ATM	132,3	99	96	208900
IRAK4	110,5	99	95	610799
TNFRSF4	73,6	99	92	615593
IL2RG	75,4	99	97	312863
LIG4	207,5	100	99	602450;606593
IL2	86,2	97	89	12



Genomic matchmaking

- You may not have the 2nd patient with mutations in gene X, but somebody else may have!



Human genetic paradigms are changing

Sporadic cases can become easier then big families

- Assuming 200-500 rare non-synonymous variants/exome

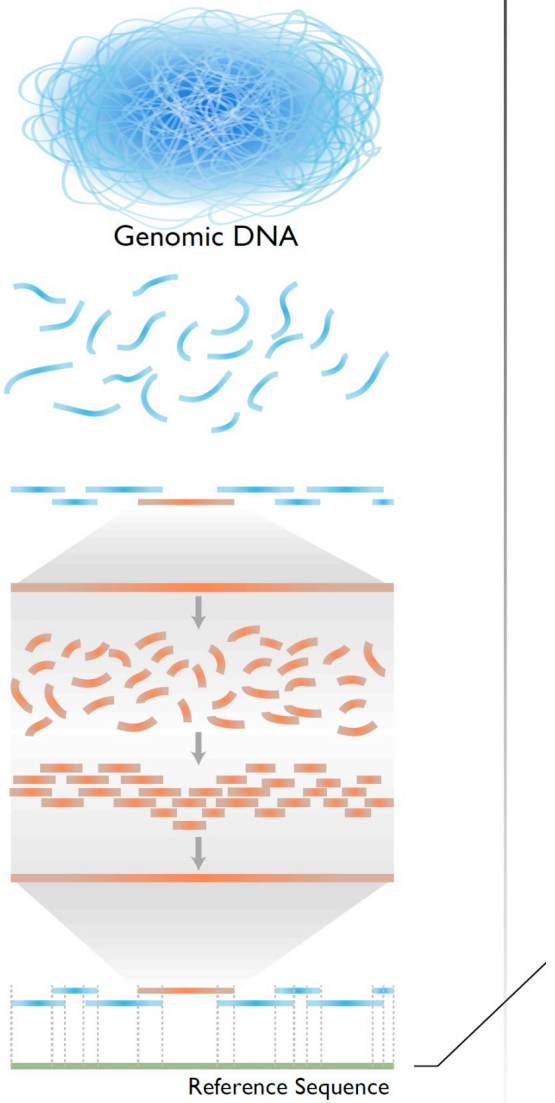
- Recessive disorders:
  - Compound heterozygous hits only in small number of candidate genes
  - In consanguineous families, a lot of those are homozygous

- Sporadic cases for dominant disease: *de novo* mutations 'easy to identify'

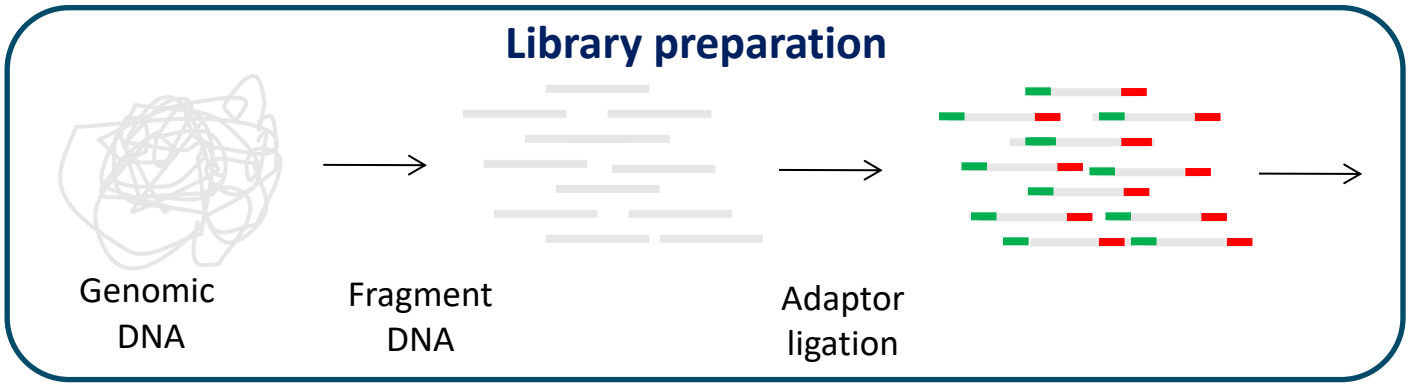
- Segregation in larger families happens in larger blocks – i.e. difficult to pinpoint the causative variant

Human Genome Sequencing

Generating a Reference Genome Sequence (e.g., Human Genome Project)

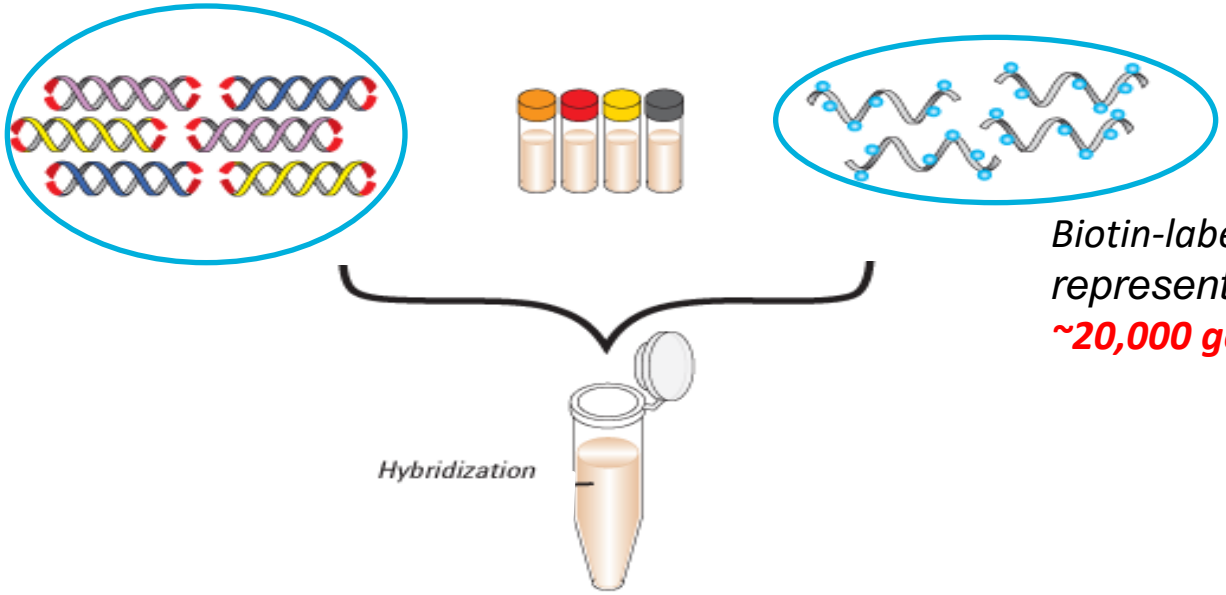


Next generation sequencing basics



Exome enrichment prior to sequencing

Fragmented DNA



Biotin-labelled "Baits" representing all exons of ***~20,000 genes***

Exome sequencing for PIDs

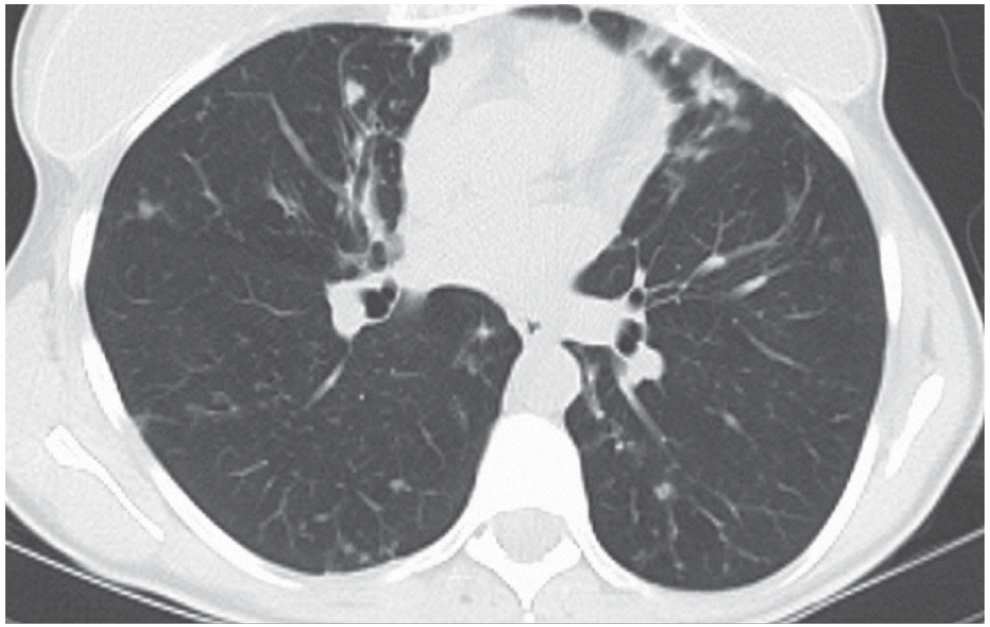
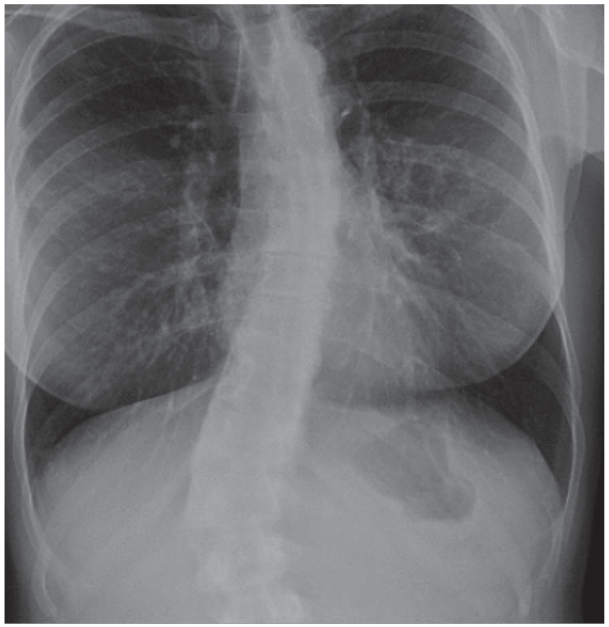
To understand rare immune disorders, one test to find the molecular defect

Advantage:
-All genes in 1 test; generic testing possible for the first time!
Disadvantage:
-Requires good counseling, chance of incidental findings;
-Not all genes are 100% completely sequenced

- Routine diagnostics

- **Research projects to identify novel PID genes**
  1. **Pulmonary mycobacterial infections (MST1R)**
  2. **Familial autoimmune defects (SOCS4)**

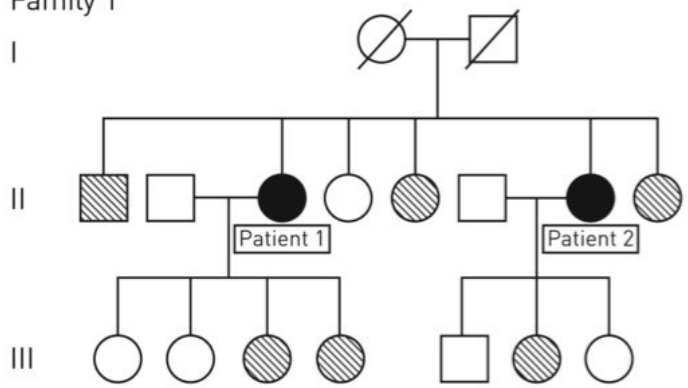
Lady Windermere syndroom

Women with recognizable syndrome	
Bacterial long infections	Scoliosis; Marfanoid habitus; pectus exc.
	

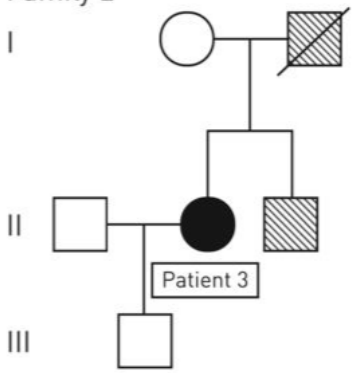
- Mutations in *MST1R* in 4/11 patients

Research project 1:

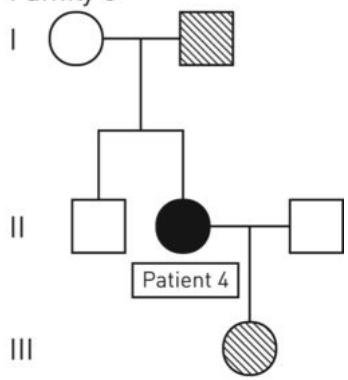
a) Family 1



Family 2



Family 3



Family 1: patients 1 and 2

Individual	Complaints	*MST1R* mutation
I.I	Unknown	DNA N/A
I.II	Unknown	DNA N/A
II.I	Pectus excavatum, occasional pneumonia, throat clearing with phlegm	V900M/wt
II.II (patient 1)	**pNTM, pectus excavatum and scoliosis**	**V900M/wt**
II.III	Unaffected	wt/wt
II.IV	Chronic cough	V900M/wt
II.V (patient 2)	**pNTM, pectus excavatum and scoliosis**	**V900M/wt**
II.VI	Unaffected	V900M/wt
III.I	Unaffected	wt/wt
III.II	Unaffected	wt/wt
III.III	Unaffected	V900M/wt
III.IV	Unaffected	V900M/wt
III.V	Unaffected	wt/wt
III.VI	Unaffected	V900M/wt
III.VII	Unaffected	wt/wt

Family 2: patient 3

Individual	Complaints	*MST1R* mutation
I.I (mother)	Unaffected	wt/wt
I.II (father)	Frequent severe bronchitis, throat clearing with phlegm	DNA N/A (p.M1383T/wt)#
II.I (patient 3)	**pNTM, pectus excavatum and scoliosis**	**p.M1383T/wt**
II.II	Pneumonia, susceptible to chest infections, throat clearing	p.M1383T/wt
III.I	Unaffected	DNA N/A

Family 3: patient 4

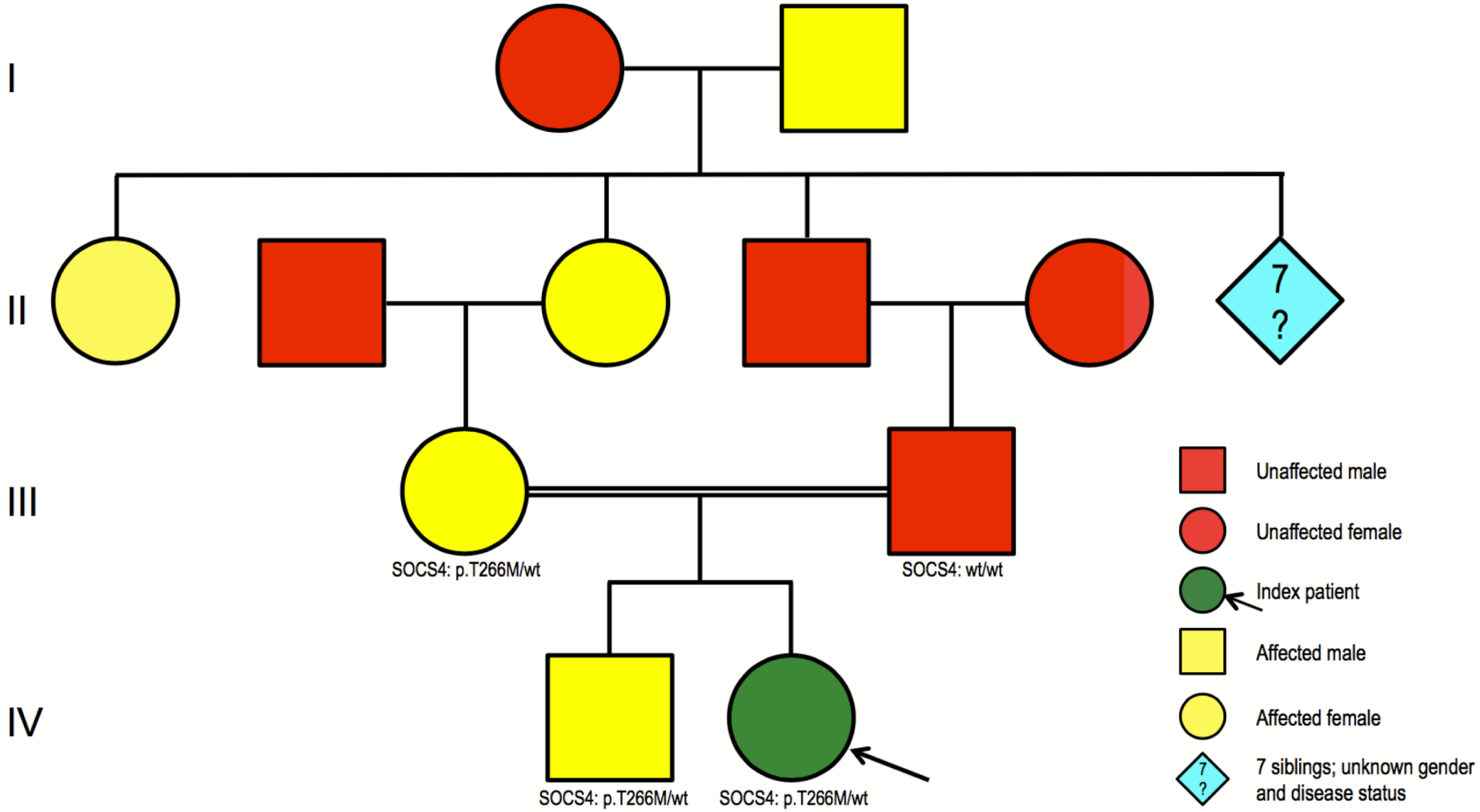
Individual	Complaints	*MST1R* mutation
I.I (mother)	Unaffected	wt/wt
I.II (father)	Pronounced cough >20 years, raspy hoarse voice	p.D176N/wt
II.I	Unaffected	wt/wt
II.II (patient 4)	**pNTM, pectus excavatum and scoliosis**	**p.D176N/wt**
III.I	Unaffected	p.D176N/wt

Becker K*., Arts P*. et al. Eur Respir J 2017

Radboudumc

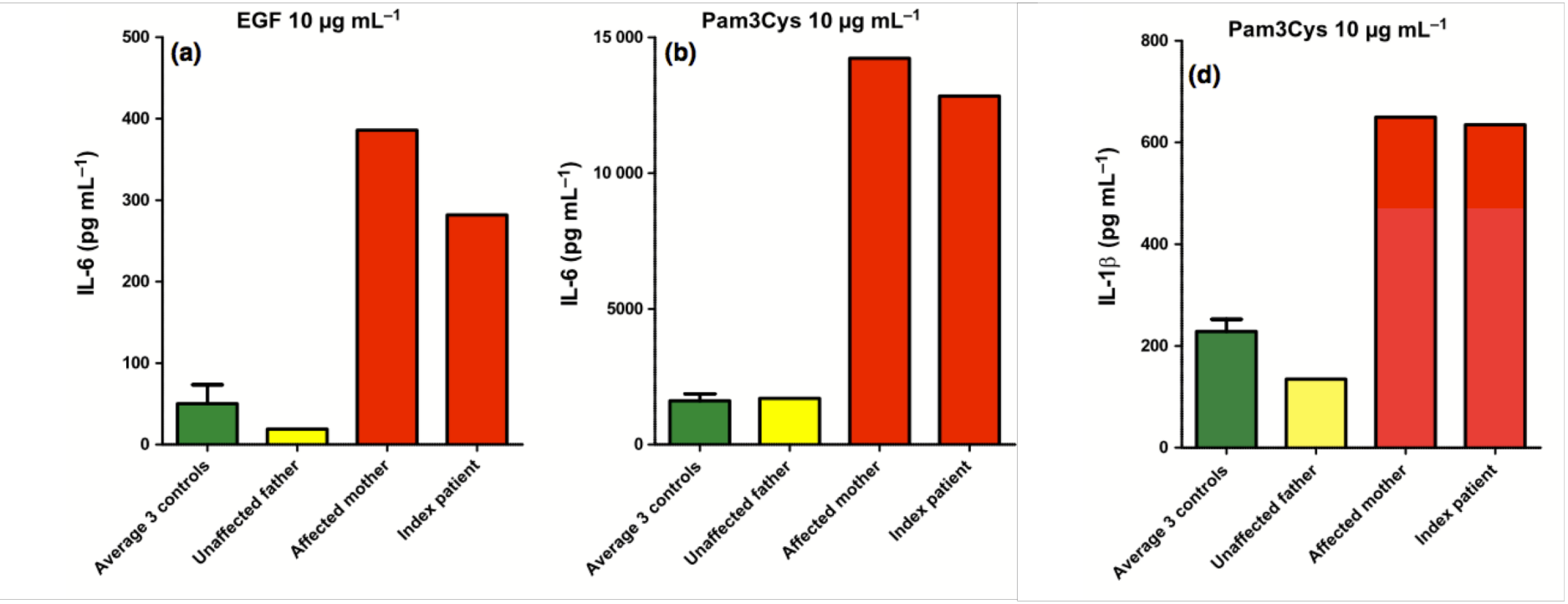
Research project 2:

Familial autoimmune defects (SOCS4)



Research project 2:

Familial autoimmune defects (SOCS4)



In vitro stimulations show that individuals with *SOCS4* variant have higher cytokine response (IL1b and IL6)

*SOCS4: suppressor of cytokine signaling

Arts P. et al. Journal of Internal Medicine 2015

Radboudumc

Exome sequencing for PIDs

To understand rare immune disorders, one test to find the molecular defect

Advantage:

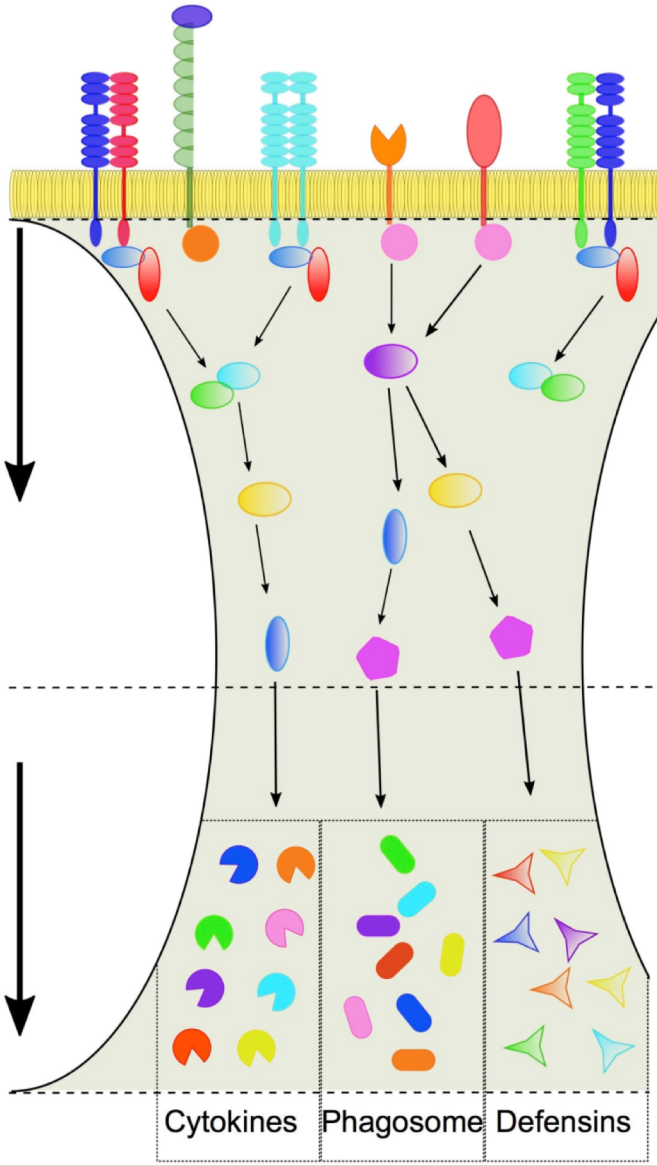
-All genes in 1 test; generic testing possible for the first time!

Disadvantage:

-Requires good counseling, chance of incidental findings;

-Not all genes are 100% completely sequenced

Mutated genes



Pattern Recognition Receptors:
No mutations in 23 genes identified

BLM, CD19, CD247, CD3D, CD3E, CD3G, CD81, CD8A, CLEC4D, CLEC7A, COLEC11, CR2, DDX58, FCN3, IFIH1, MASP2, MBL2, MC2R, NLRP3, NOD2, RPSA, TLR3, TLR4

Adaptors: mutations in 36/115 genes identified

AIRE, AK2, ATM, BLNK, BTK, CARD11, CARD14, CARD9, CASP10, CASP8, CBL, CD79A, CD79B, CDCA7, CDKN2B, CEBPE, CHD7, COPA, CREBBP, DCLRE1C, DHFR, DNMT3B, DOCK8, FADD, FOXN1, FOXP3, GATA2, GFI1, GTF2H5, IKBKB, IKBKG, INSR, IRAK4, IRF7, ISG15, ITCH, ITK, JAK2, JAK3, KMT2D, KRAS, LAMTOR2, LCK, MAL, MEFV, MKL1, MS4A1, MTHFD1, MVK, MYD88, NBN, NFKB1, NFKB2, NFKBIA, NKX2-5, NLRC4, NLRP1, NLRP12, NRAS, PBX1, PIK3CD, PIK3R1, PLCG2, PMM2, PSTPIP1, PTPN11, PTPRC, PTRF, RAC2, RAG1, RAG2, RASGRP2, RBCK1, RECQL4, RFX5, RFXANK, RHOH, RNASEH2B, RNF168, RNF31, RPL5, SH2D1A, SKIV2L, SMARCAL1, SOCS4, SP110, STAT1, STAT2, STAT3, STAT4, STAT5B, STAT6, STIM1, STK4, TALDO1, TBX1, TCIRG1, TERC, TERT, TICAM1, TMEM173, TNFRSF11A, TPP2, TRAF3, TRAF3IP2, TTC37, TTC7A, TYK2, UNC119, UNC93B1, WAS, WRAP53, XIAP, ZAP70, ZBTB24

Effectors: mutations in 23/174 genes identified

ACP5, ACTB, ADA, ADAM17, ADAR, AGA, AICDA, ALG13, ANK1, AP3B1, APOL1, BLOC1S6, C1QA, C1QB, C1QC, C1R, C1S, C2, C3, C4A, C4B, C5, C6, C7, C8A, C8B, C9, CD27, CD40, CD40LG, CD46, CD55, CD59, CECR1, CFB, CFD, CFH, CFHR1, CFHR3, CFHR5, CFI, CFP, CFTR, CIITA, CORO1A, CSF2RA, CSF3R, CTC1, CTLA4, CTSC, CXCR4, CYBA, CYBB, DKC1, DOCK2, ELANE, ELF4, EPG5, ERCC2, ERCC3, F12, FANCA, FAS, FASLG, FCGR1A, FCGR3A, FERMT3, FPR1, G6PC, G6PC3, G6PD, GJC2, HAX1, HBB, HELLS, ICOS, IFNGR1, IFNGR2, IGLL1, IKZF1, IL10RA, IL10RB, IL12B, IL12RB1, IL17F, IL17RA, IL1RN, IL2, IL21R, IL2RA, IL2RG, IL36RN, IL7R, IRF8, ITGA2B, LIG1, LIG4, LPIN2, LRBA, LRRC8A, LTBP3, LYST, MAGT1, MAN2B1, MANBA, MCM4, MPO, MRE11A, NCF1, NCF2, NCF4, NCSTN, NDNL2, NHEJ1, NHP2, NOP10, ORAI1, PARN, PCCA, PCCB, PEPD, PGM3, PIGA, PLG, PNP, PRF1, PRKDC, PRPS1, PSENEN, PSMB8, RAB27A, RFXAP, RMRP, RNASEH2A, RNASEH2C, RSPH9, RTEL1, SAMHD1, SBDS, SERAC1, SERPING1, SLC29A3, SLC35A1, SLC35C1, SLC37A4, SLC39A4, SLC46A1, SPINK5, STX11, STXBP2, TAP1, TAP2, TAPBP, TAZ, TCN2, TFRC, THBD, TINF2, TMC6, TMC8, TNFRSF13B, TNFRSF13C, TNFRSF1A, TNFRSF4, TREX1, TRNT1, UNC13D, UNG, USB1, VPS13B, VPS45, WIPF1