Genetics first for primary immunodeficiencies

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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







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10000

4200

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HiSeq 2000

1

Genome sequencing: All variation in one experiment!



Genome with 'all' variation

TATCTTACTTCCACCACATAATCTACGAACTATCAATGTTTATGATGGTCA GTTTGTTAACAAGTGATTTGAATCTGATA<u>ATG</u>CGAAGAGTTGCTAATAATG GCAAAAATACAAAAAATCTTGGATTCTATCGATAACAGCCGAGGTGCCAAT TACAAATAAAAAGCTTACTTTGGATACTTTGACAGGTGGACACTCAAAAGA TGCGAAGTTATATTAATGGCAAACGTATTCCTGAGACTGCCAGAGCTGTAA TCTATGAATAAAACTGGCTTTATTGAAGTACCATCTTACATTTTAAACAAG TGTTGTCTTTTATAAACCACGTTACGAAAGATAACATACTCAAAAGTCTTCA AAGCTTTTCTAACATATATCAAAAGTGATCATAATTCTGAAAATCCTTATA GATTTAGCACAGAAGAATGGATATTTAACCTTGGCTCCTAATTTCGGTGAT CTAATATCCAATCTGGTATAATAAAAAGATCAGAAGGGT ACAATTTGCACATCTTTT ATCACAATA CAAATCCCCATGTGCCAATCTCGAACAAGCTTTGATTATGAACT CACGAAA AAAATTCTATAACAAGCAATCCAATGTTCGGCTTGGTCCAAGATCAAATAC AATAAGTTATATAGACGACAAAATTATACATATAACGATGCGTTGGTGATT



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	
A T O C T T C O C A A G A C T C A A A A A A A A A A A A A A A A	

Variant calling and variant annotation

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447					14 110FF F FM T				-	1011014	14 14 FYON F			7.00							·

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 Mid-face retraction	12/13 13/13	43/43 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	

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 Arts¹, Bart van Lier¹, Marloes Steehouwer¹, Petra de Vries¹, Rick de Reuver¹, Nienke Wieskamp¹, Geert Mortier², Koen Devriendt³, Marta Z Amorim⁴, Nicole Revencu⁵, Alexa Kidd⁶, Mafalda Barbosa⁷, Anne Turner⁸, Janine Smith⁹, Christina Oley¹⁰, Alex Henderson¹¹, Ian M Hayes¹², Elizabeth M Thompson¹³, Han G Brunner¹, Bert B A de Vries¹ & Joris A Veltman¹

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)

Exome-wide analysis

Increased risk for incidental findings (extra counseling)



Exome-wide analysis

Increased risk for incidental findings (extra counseling)



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 \rightarrow 62 of all 254 patients (24%)
- Exome-wide analysis \rightarrow additional 10 patients (4%)



Diagnostic yield per pathogen

% of patients with genetic diagnosis per pathogen or autoimmunity



Diagnostic yield per immune-defect

% of patients with genetic diagnosis per immunophenotypic 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 <u>RNAseq</u> can identify transcriptional profile of almost any cell type AND: <u>epitope</u> recognition can be achieved in <u>same experiment</u>!
- CITE-seq: Stoekius et al. (Nat Meth. 2017)



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

Acknowledgements

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- 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

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



WES PRIMARY IMMUNODEFICIENCIES DG29

Median coverage	% covered >10x	% covered >20x	OMIM disease ID	
204,2	100	100	601495	
159,4	99	99	617241	
128,1	99	95	608233	
160,6	100	99	254600	
139,2	95	93	25	
162,6	100	99	608971	rt
113,1	95	84	26	sis (if the gene panel analysis
132,3	99	96	208900	
110,5	99	95	610799	ate reports
73,6	99	92	615593	sis (if the gene panel analysis
75,4	99	97	312863	
207,5	100	99	602450;606593	
86,2	97	89	12	Radboudumc
	Median coverage 204,2 159,4 128,1 160,6 139,2 162,6 113,1 132,3 110,5 73,6 75,4 207,5 86,2	Median coverage % covered >10x 204,2 100 159,4 99 128,1 99 160,6 100 139,2 95 162,6 100 113,1 95 132,3 99 110,5 99 73,6 99 207,5 100 86,2 97	Median coverage % covered >10x % covered >20x 204,2 100 100 159,4 99 99 128,1 99 95 160,6 100 99 139,2 95 93 162,6 100 99 113,1 95 84 132,3 99 96 110,5 99 95 73,6 99 92 75,4 99 97 207,5 100 99 86,2 97 89	Median coverage % covered >10x % covered >20x % cove

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
- <u>Recessive</u> disorders:
 - Compound heterozygous hits only in small number of candidate genes
 - In consanguineous families, a lot of those are homozygous
- Sporadic cases for <u>dominant</u> 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



Next generation sequencing basics



Exome enrichment prior to sequencing



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! <u>Disadvantage:</u>

-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

<u>Women</u> with recognizable syndrome							
Bacterial long infections	Scoliosis; Marfanoid habitus; pectus exc.						

• Mutations in *MST1R* in 4/11 patients

Research project 1:



Family 1: patients 1 and 2

Individual	Complaints	MST1R mutation
1.1	Unknown	DNA N/A
1.11	Unknown	DNA N/A
	Pectus excavatum,	
	occasional pneumonia,	
11.1	throat clearing with phlegm	V900M/wt
II.II (patient 1)	pNTM, pectus excavatum	V900M/wt
	and scoliosis	
11.111	Unaffected	wt/wt
II.IV	Chronic cough	V900M/wt
II.V (patient 2)	pNTM, pectus excavatum	V900M/wt
	and scoliosis	
II.VI	Unaffected	V900M/wt
111.1	Unaffected	wt/wt
111.11	Unaffected	wt/wt
111.111	Unaffected	V900M/wt
III.IV	Unaffected	V900M/wt
III.V	Unaffected	wt/wt
III.VI	Unaffected	V900M/wt
111.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
11.11	Pneumonia, susceptible to chest infections, throat clearing	p.M1383T/wt
111.1	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
11.1	Unaffected	wt/wt
II.II (patient 4)	pNTM, pectus excavatum and scoliosis	p.D176N/wt
111.1	Unaffected	p.D176N/wt

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

Research project 2:

Familial autoimmune defects (SOCS4)



Arts P. et al. Journal of Internal Medicine 2015

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

Exome sequencing for PIDs

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

Advantage:

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-Requires good counseling, chance of incidental findings;

-Not all genes are 100% completely sequenced

Mutated genes

