NGS in daily practice in Autoinflammatory diseases

Center for Autoinflammatory diseases and Immunodeficiencies, Clinics of Pediatrics and Rheumatology

«G. Gaslini» Childrens' Hospital
Genova, Italy

Disclosures

Novartis, Sobi

For consultancies, unrestricted grants and speakers' fee.

Inherited Autoinflammatory diseases

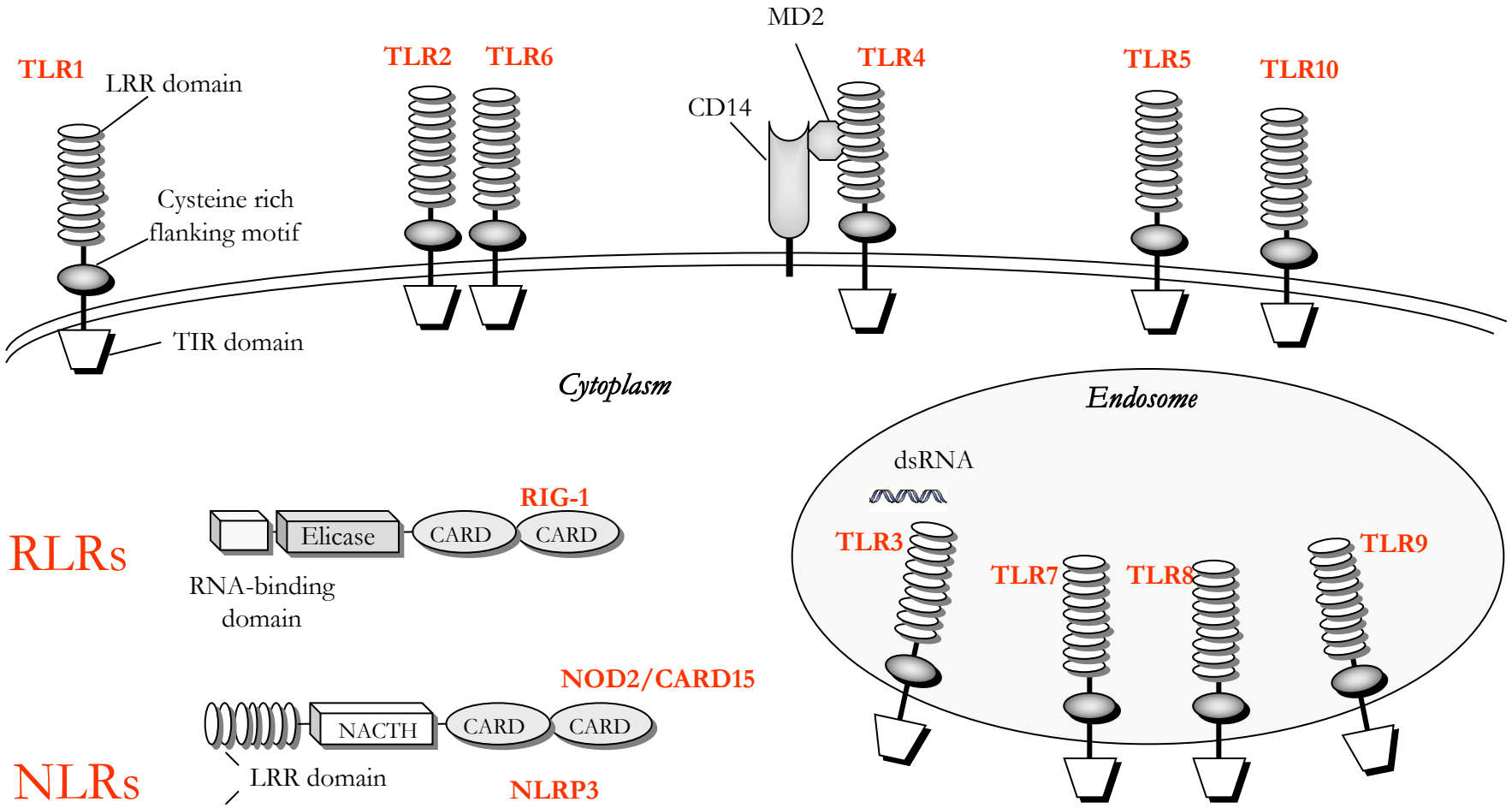
Monogenic diseases caused by mutations of genes involved in the innate immune response *leading to sterile inflammation*

First gene identified in 1997

No class II HLA-association and/or gender disproportion

Absence of (pathogenic) auto-antibodies and/or antigen – specific T cells

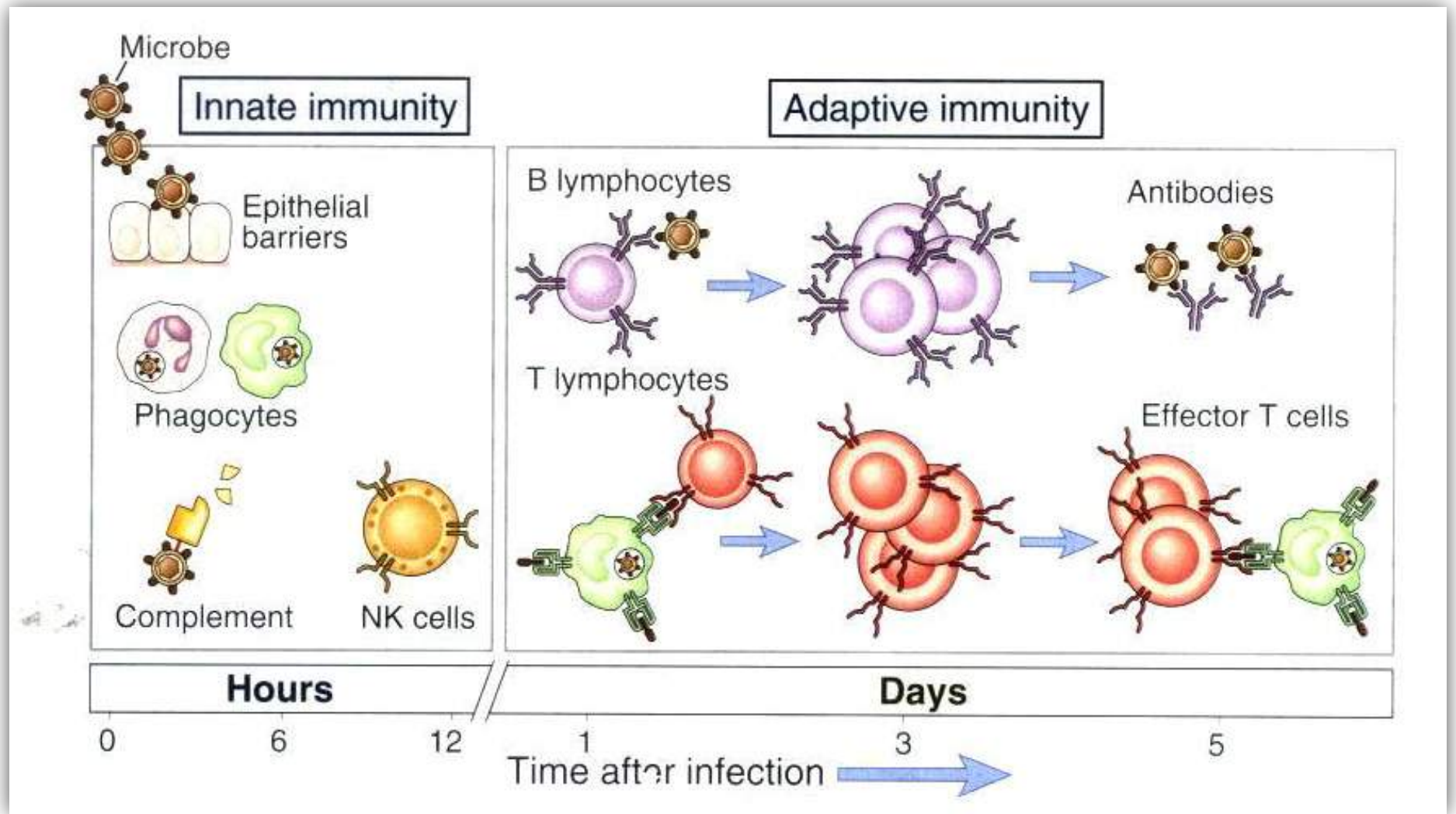
Pattern-recognition receptors



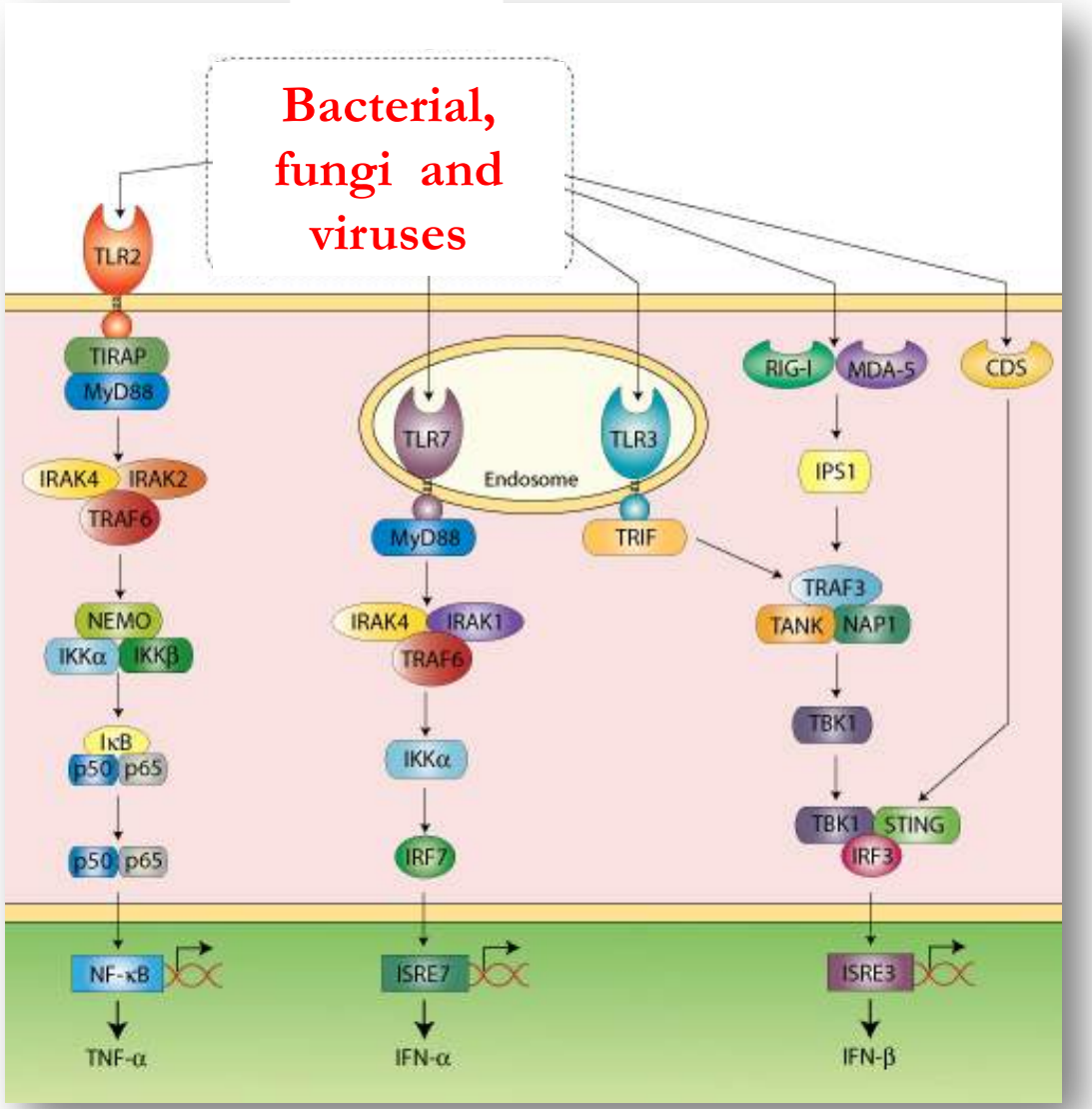
Pathogens associated molecular patterns (PAMPs)

Damage associated molecular patterns (DAMPs)

Innate Immunity



The Innate Immunity



Clinical classification of Autoinflammatory diseases

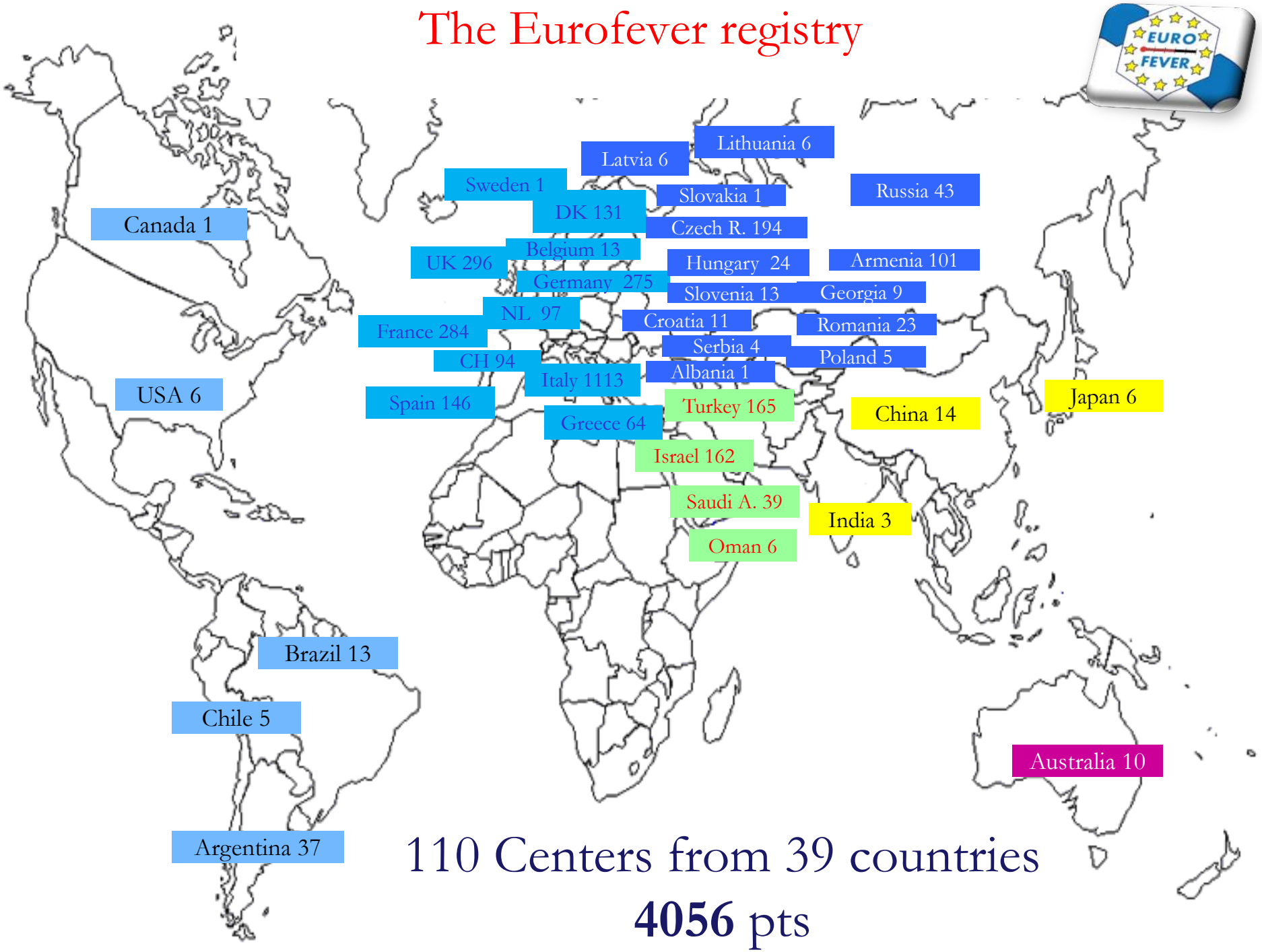
Clinical presentation	Disease (gene)	Inheritance	Year
Periodic fevers	FMF (MEFV)	AR	1997
	MVK (MVK)	AR	1998
	TRAPS (TNFRSF1A)	AD	1999
Systemic inflammation and urticarial rash	FCAS/MWS/CINCA(NLRP3)	AD	2000
	FCAS2 (NLRP12)	AD	2008
	NLRC4-associated disease (NLRC4)	AD	2014
Sterile inflammation of joints/bone/skin	Blau's syndrome (CARD15)	AD	2001
	PAPA (PSTPIP1)	AD	2002
	COPA (COPa)	AR	2015
	Majeed syndrome (LPIN2)	AR	2005
	DIRA (IL1RN)	AR	2009
	DITRA (IL36RN)	AR	2011
	CAMPS (CARD14)	AD	2012
Panniculitis/lypodistropy	CANDLE (PSMB8, PSMA3, PSMB4, PMSB10)	AR	2010
	ORAS (FAM105B)	AD	2016
Inflammatory bowel disease	Early-onset IBD (IL10, IL10RA, IL10RB)	AR	2012
Polyarteritis nodosa and early stroke	ADA2 deficiency (CERC1)	AR	2014
Vasculopathy and ulcers	SAVI (TMEM173)	AD	2014
	A20 halpoinsuficiency (TNFAIP3)	AD	2016
Autoinflammation with immune-deficiency	HOIL-1 deficiency (RBCK1, HOIL-1)	AD	2014
	PLAID/APLAID (PLCγ2)	AD	2015

Clinical classification of inherited and multifactorial autoinflammatory diseases (AID)

	Inherited AID (gene, transmission)	Multifactorial AID
Clinical presentation		
Recurrent episodes of inflammmation	FMF (*MEFV*, AR) TRAPS (*TNFRSF1A*, AD) MVK (*MVK*, AR)	PFAPA Recurrent idiopathic pericarditis Mollaret syndrome (recurrent meningitis)
Systemic inflammation and urticarial rash	CINCA/NOMID (*NLRP3*, AD) Muckle-Wells/FCAS (*NLRP3*, AD) FCAS2 (*NLRP12*, AD)	SoJIA, adult onset Still disease Schnitzler's syndrome Delayed pressure urticaria
Sterile inflammation of skin/bone/joints	PAPA (*CD2BP1,* AD) DIRA (*IL1RN,* AR) DITRA (*IL36RN*, AR) Majeed syndrome (*LPIN2*, AR) Blau's syndrome (*CARD15,* AD) CAMPS (*CARD14, AD*)	CRMO, SAPHO Gout and pseudogout HLA-B27 Spondiloarthopathy Reactive arthritis, Sweet syndrome Generalized pustular psoriasis Hallopeau acrodermatitis
Panniculitis/lypodistropy	Nakajo-Nishimura, JMP, CANDLE (*PSMB8,* AR)	Erythema nodosum and panniculitis
Inflammatory bowel disease	Early-onset IBD (*IL10, IL10RA, IL10RB)*	Crohn's disease
Polyarteritis nodosa	Adenosine deaminase 2 deficiency (*CERC1,*AR)	ANCA-negative PAN
Vasculopathy and ulcers	SAVI (*TMEM173*, AD)	SLE
Hemophagocytic lymphohistiocytosis	FHL1 (Unknown) FHL2 (*PFR1*/perforin 1, AR) FHL3 (*UNC13D*/Munc 13-4, AR) FHL4 (*STX11*/syntaxin 11, AR) FHL5 (*STXB2*/syntaxin binding protein, AR) NLRC4-MAS (NLRC4, AD)	SoJIA-associated MAS Infectious-associated MAS

Adapted from Federici et al, Frontiers in Immunology 2014

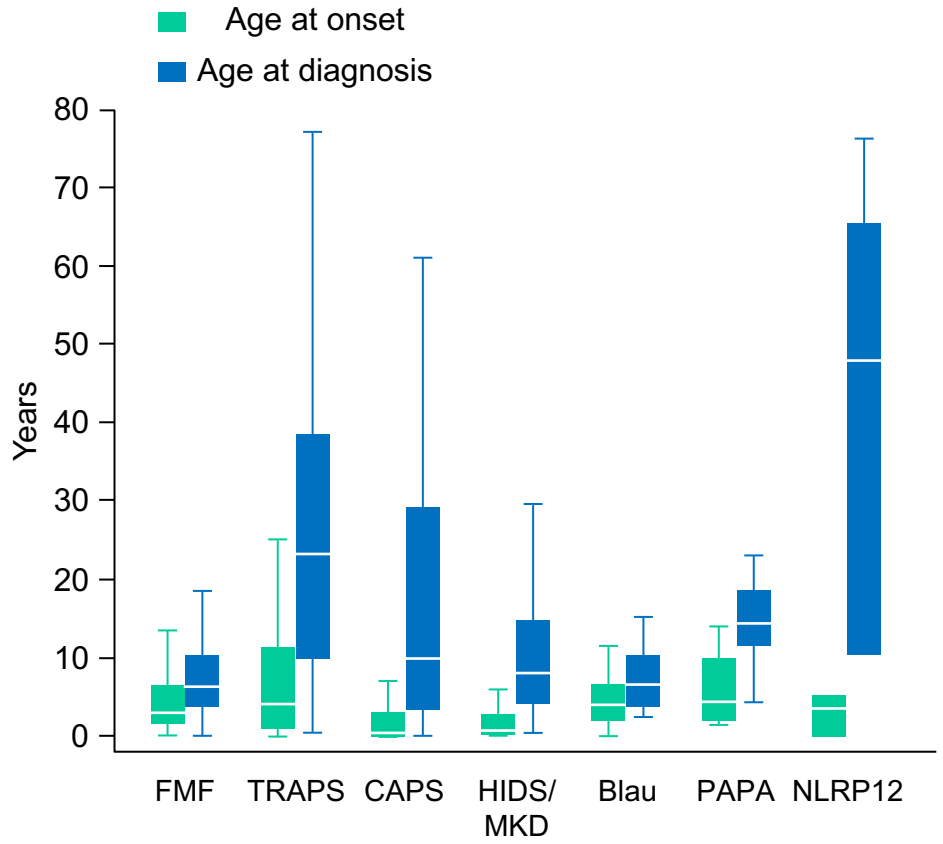
The Eurofever registry



110 Centers from 39 countries

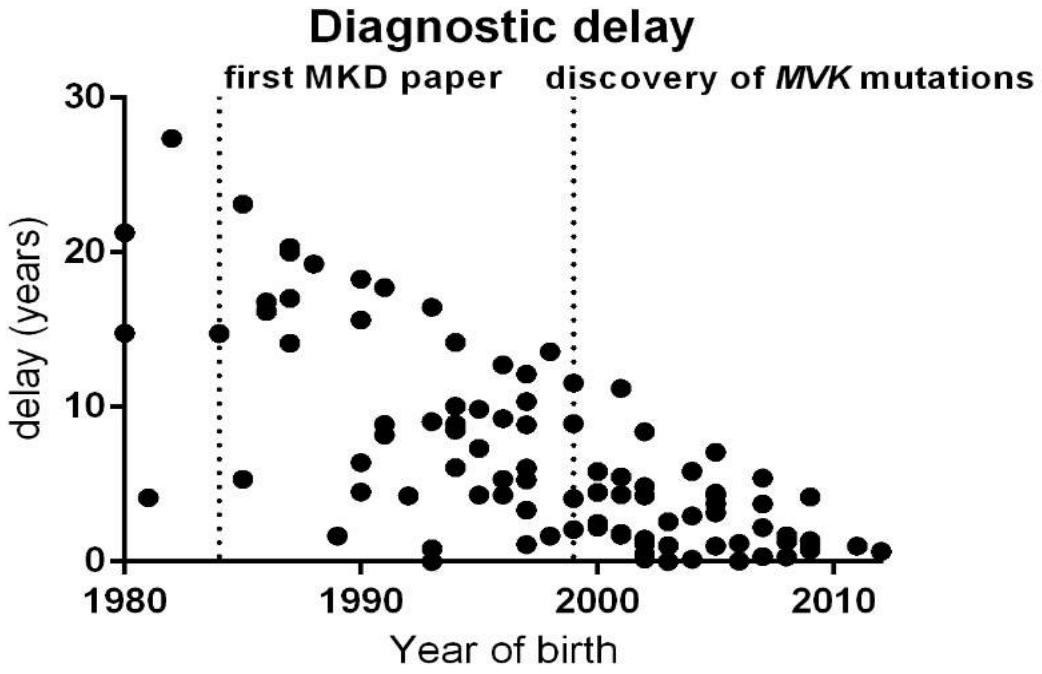
4056 pts

Diagnostic delay (1/2)



Toplak et al, *ARD* 2012

Diagnostic delay (2/2)

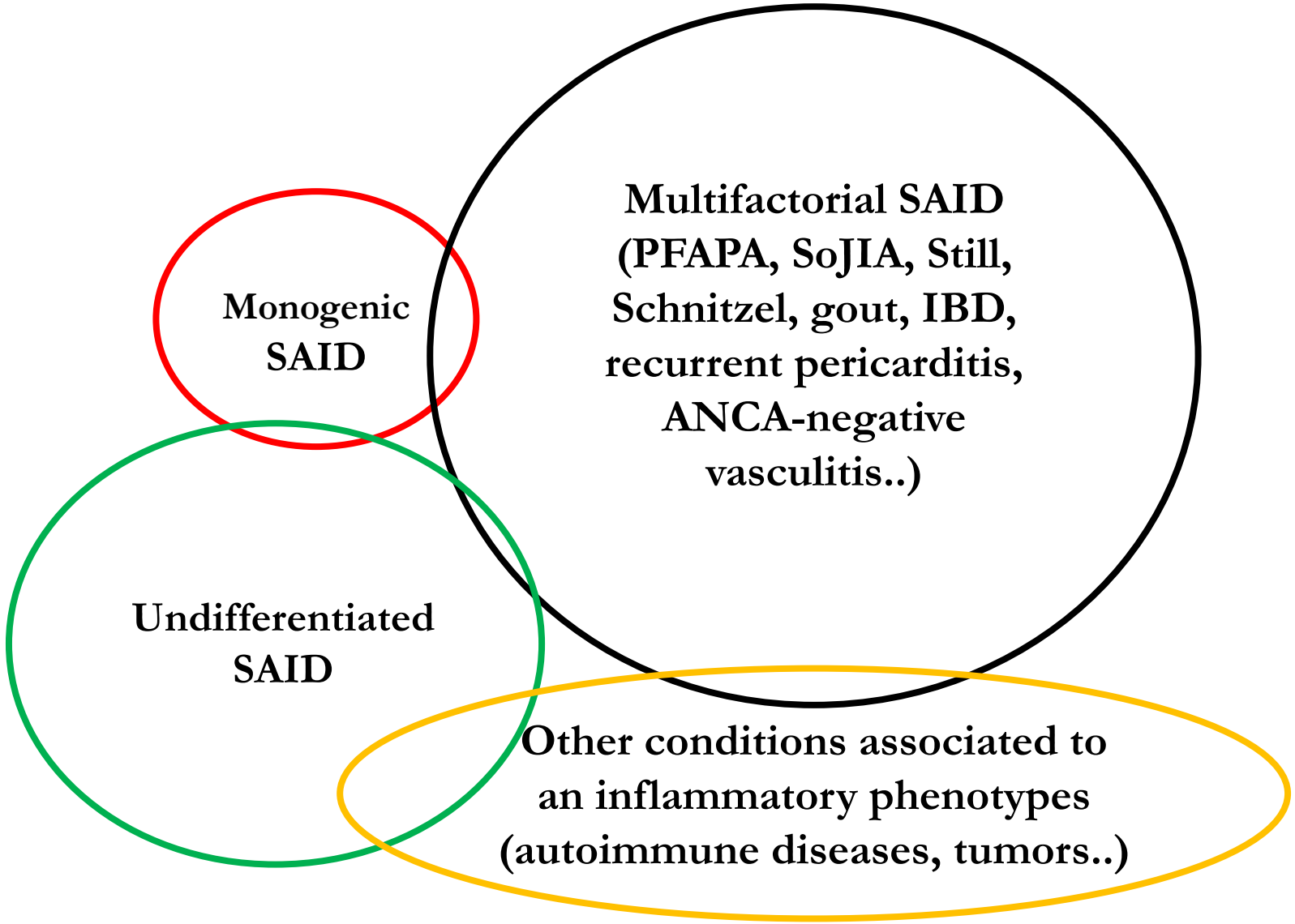


Ter Haar et al, A&R 2016

Agenda

- How do we define a monogenig SAID?
- New genetic tools and possible pittfals
- Do we still need clinical classification criteria in the NGS era?
- Possible role of NGS in the identification of undefined, orphan patients

The expanding spectrum of Autoinflammatory diseases



How we define a monogenic AID?

Defining monogenic AID

Clinical picture + Genetic analysis

Defining monogenic AID

Clinical picture

- *Typical* (classical phenotype)
- *Atypical* (unexpected phenotypes

+

Genetic analysis

- *Confirmatory*
- *Not confirmatory* -
  incomplete (heterozygous for AR disease, e.g. FMF),
  - polymorphisms, low penetrance variants
  - negative
  - "false negative" (somatic mosaicisms, deletions, duplications)
  - redundant (more than 1 gene)

International Society for Systemic AutoInflammatory Diseases

Infevers

This web site was supported by the EU 5th framework

A HGVS affiliated database

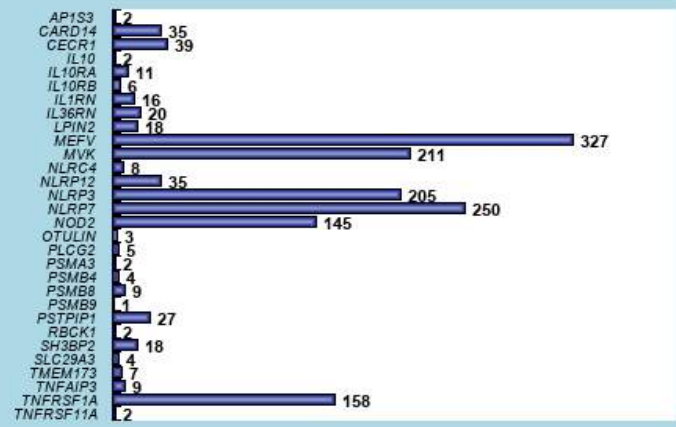
References : 2003 2004 2008 2014

Editor in chief: Isabelle Touitou

The registry of Hereditary Auto-inflammatory Disorders Mutations

AP1S3	*CARD14*	*CECR1*	*IL10*	*IL10RA*
_	PRP/PSORS2	DADA2	IL10 deficiency	IL10R1 deficiency
IL10RB	*IL1RN*	*IL36RN*	*LPIN2*	*MEFV*
IL10R2 deficiency	DIRA	DITRA	Majeed Syndrome	FMF
MVK	*NLRC4*	*NLRP12*	*NLRP3*	*NLRP7*
MKD/DSAP/RP	AIFEC	NAPS12	CAPS	HYDM1
NOD2	*OTULIN*	*PLCG2*	*PSMA3*	*PSMB4*
CD/BS/EOS	AIPDS	APLAID/PLAID	CANDLE/PRAAS	CANDLE/PRAAS
PSMB8	*PSMB9*	*PSTPIP1*	*RBCK1*	*SH3BP2*
JMP/NNS/CANDLE	CANDLE/PRAAS	PAPA	PBMEI	Cherubism
SLC29A3	*TMEM173*	*TNFAIP3*	*TNFRSF11A*	*TNFRSF1A*
H syndrome	SAVI	AISBL	TRAPS11	TRAPS

Current number (1581) of sequence variants in the database



Director of the publication Isabelle Touitou
Project Manager Florian Milhavet
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[IntraNet]

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Last update: 2014-10-29 3:41 PM

Guidelines for the genetic diagnosis of hereditary recurrent fevers

Y Shinar,[1] L Obici,[2] I Aksentijevich,[3] B Bennetts,[4] F Austrup,[5] I Ceccherini,[6] J M Costa,[7] A De Leener,[8] M Gattorno,[9] U Kania,[10] I Kone-Paut,[11] S Lezer,[12] A Livneh,[13] I Moix,[14] R Nishikomori,[15] S Ozen,[16] L Phylactou,[17] L Risom,[18] D Rowczenio,[19] T Sarkisian,[20] M E van Gijn,[21] M Witsch-Baumgartner,[22] M Morris,[23] H M Hoffman,[24] I Touitou[25]

Ann Rheum Dis 2012;**71**:1599–1605.

3 types of mutations

1. Clearly pathogenic variants
2. Variants of uncertain significance
3. Polymorphisms

Table 1 Recommendations for the screening and interpretation of sequence variants for the genetic diagnosis of HRFs

Disease	Gene	Reference sequence/LRG	Sequence variants	Exons 1	2	3	4	5	6	7	8	9	10	11
FMF	*MEFV*	NM_000243.2/ LRG_190	Screening*		X	X		X					X	
			Category†		p.E148Q, ***p.E167D***, ***p.T267I***, *p.R202Q*	p.P369S, *p.R408Q*		p.F479L				p.I591T	**p.M680I**, **p.M694V**, **p.M694I**, **p.V726A**, ***p.A744S***, ***p.R761H***, ***p.I692del***, p.K695R	
MKD	*MVK*	NM_000431.2/LRG_156	Screening*		X	X	X	X	X	X	X	X	X	X
			Category†		***p.H20P***	*p.S52N*						**p.I268T**, **p.S272F**		**p.V377I**
TRAPS	*TNFRSF1A*	NM_001065.3/LRG_193	Screening*		X	X	X							
			Category†		**p.C59R (C30R)**, **p.C62Y (C33Y)**	**p.D71del (D42del)**, **p.T79M (T50M)**, **p.C81Y (C52Y)**, **p.C84Y (C55Y)**, **p.C102W (C73W)**, p.P75L (P46L)	p.R121Q (R92Q)							
CAPS	*NLRP3*	NM_001243133.1 or NM_004895.4 /LRG_197	Screening*			X								
			Category†			**p.R260W**, **p.D303N**, **p.L305P**, **p.E311K**, **p.T348M**, **p.L353P**, **p.A439V**, p.V198M, *pQ703K*								

A complete list of HRF gene variants is available in Infevers, the registry of autoinflammatory mutations: http://fmf.igh.cnrs.fr/ISSAID/infevers/.
The latest reference sequence should be used. LRG, locus reference genomic sequences.
*In bold: minimum set of exons recommended to screen; in grey, other exons most commonly screened for routine diagnosis of HRFs.
†In bold and normal letters: minimum set of clearly pathogenic sequence variants recommended to screen; in bold and italics: example of rare clearly pathogenic sequence variants suggested to be screened; in normal letters: sequence variants of uncertain significance; in italics and greyed examples of common single-nucleotide polymorphisms not to be reported; in parentheses: common names of TNFRSF1A mutations.
CAPS, cryopyrin-associated periodic syndrome; FMF, familial Mediterranean fever; HRFs, hereditary recurrent fevers; MKD, mevalonate kinase deficiency; TRAPS, tumour necrosis factor receptor-associated periodic syndrome.

Guidelines for the evaluation of pathogeneticy and reporting of sequence variants

Class Description

1. Clearly not pathogenic
2. Unlikely to be pathogenic
3. Unknown significance (VUS)
4. Likely to be pathogenic
5. Clearly pathogenic

Class **Wording** to include within reports

1. **Not** pathogenic → "Common" polymorphism, therefore not reported
2. **Unlikely** to be pathogenic → Diagnosis not confirmed molecularly
3. **Uncertain** pathogenicity → **Does not** confirm or exclude diagnosis
4. **Likely** to be pathogenic → **Consistent** with the diagnosis
5. Predicted to be **pathogenic** → This result **confirms** the diagnosis

British society of Medical Genetics

Agenda

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- New genetic tools and possible pittfals
- Do we still need clinical classification criteria in the NGS era?
- Possible role of NGS in the identification of undefined, orphan patients

Sanger → → Next Generation Sequencing

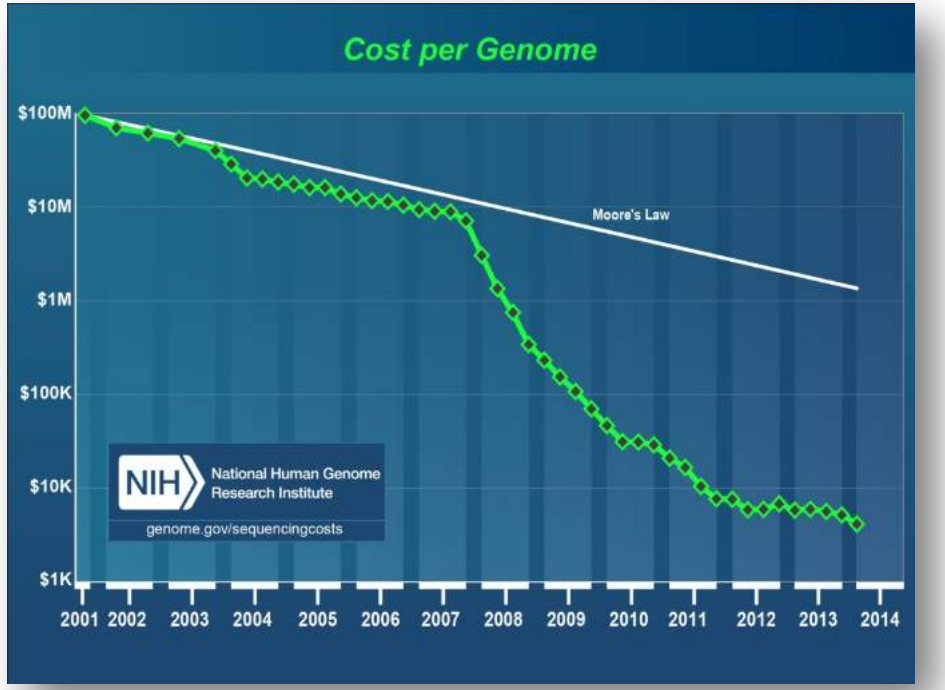
1977	•Sanger: Dideoxy Chain Termination
1986	•Hood et al., Fluorescently labeled ddNTPs, Partial Automation
1990	•NIH begins Human Genome Project,
2001	•HGP/Celera draft assembly published Nature / Science
2004	•Next-Gen Sequencing (454 Roche)
2006	•First Solexa Sequencer, Genome Analyzer 1G/Run

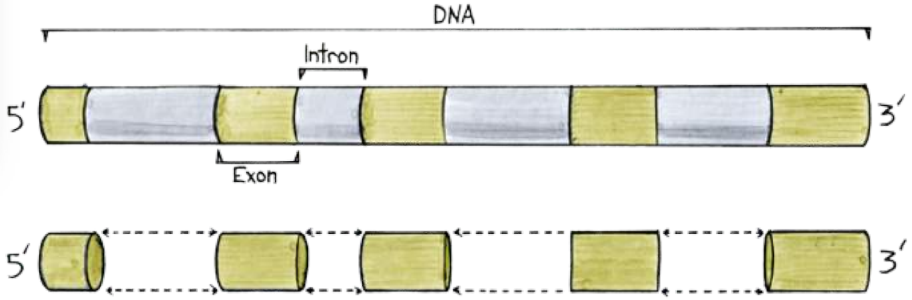
- 1990 – 2003
- "shotgun"

J.Craig Venter
2007

James Watson

NIAID



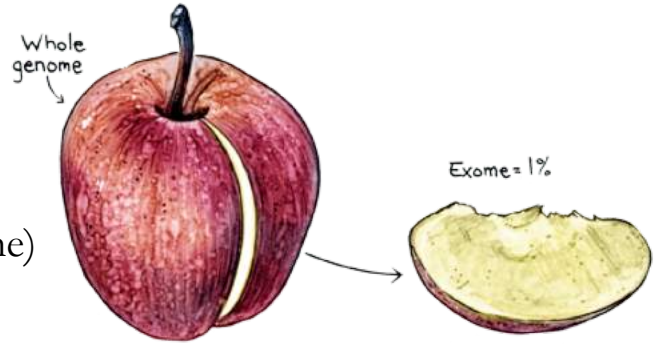


Genome sequencing

vs

Exome sequencing

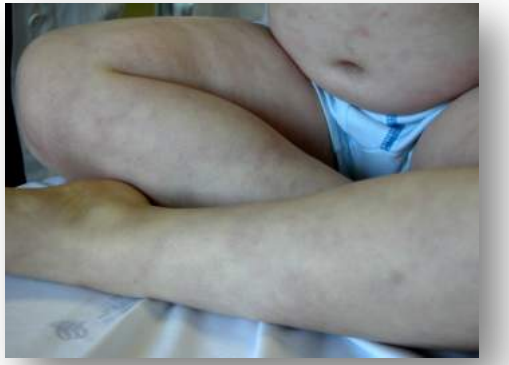
(85-90 % of human genetic diseases involve the exome)

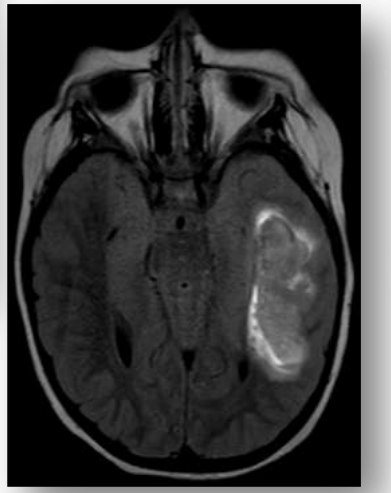


Possible applications

- Identification of new diseases

ADA-2 deficieny





The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Early-Onset Stroke and Vasculopathy Associated with Mutations in ADA2

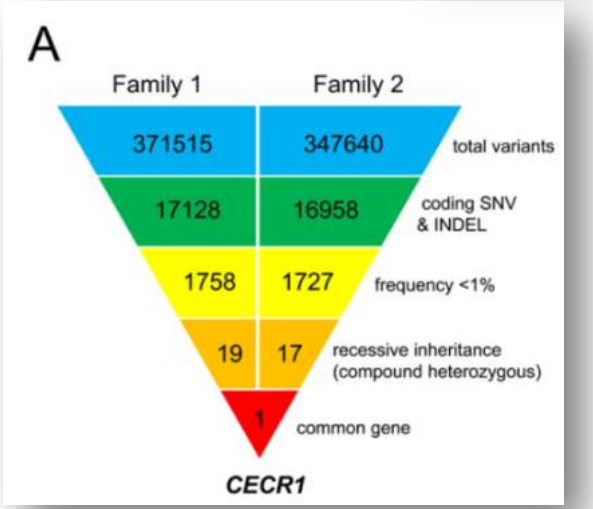
Q. Zhou, D. Yang, A.K. Ombrello, Andrey V. Zavialov, C. Toro, Anton V. Zavialov, D.L. Stone, J.J. Chae, S.D. Rosenzweig, K. Bishop, K.S. Barron, H.S. Kuehn, P. Hoffmann, A. Negro, W.L. Tsai, E.W. Cowen, W. Pei, J.D. Milner, C. Silvin, T. Heller, D.T. Chin, N.J. Patronas, J.S. Barber, C.-C.R. Lee, G.M. Wood, A. Ling, S.J. Kelly, D.E. Kleiner, J.C. Mullikin, N.J. Ganson, H.H. Kong, S. Hambleton, F. Candotti, M.M. Quezado, K.R. Calvo, H. Alao, B.K. Barham, A. Jones, J.F. Meschia, B.B. Worrall, S.E. Kasner, S.S. Rich, R. Goldbach-Mansky, M. Abinun, E. Chalom, A.C. Gotte, M. Punaro, V. Pascual, J.W. Verbsky, T.R. Torgerson, N.G. Singer, T.R. Gershon, S. Ozen, O. Karadag, T.A. Fleisher, E.F. Remmers, S.M. Burgess, S.L. Moir, M. Gadina, R. Sood, M.S. Hershfield, M. Boehm, D.L. Kastner, and I. Aksentijevich

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Mutant Adenosine Deaminase 2 in a Polyarteritis Nodosa Vasculopathy

Paulina Navon Elkan, M.D., Sarah B. Pierce, Ph.D., Reeval Segel, M.D., Tom Walsh, Ph.D., Judith Barash, M.D., Shai Padeh, M.D., Abraham Zlotogorski, M.D., Yackov Berkun, M.D., Joseph J. Press, M.D., Masha Mukamel, M.D., Isabel Voth, M.D., Philip Hashkes, M.D., Liora Harel, M.D., Vered Hoffer, M.D., Eduard Ling, M.D., Ph.D., Fatos Yalcinkaya, M.D., Ozgur Kasapcopur, M.D., Ming K. Lee, Ph.D., Rachel E. Klevit, D.Phil., Paul Renbaum, Ph.D., Ariella Weinberg-Shukron, B.Sc.Med., Elif F. Sener, Ph.D., Barbara Schormair, Ph.D., Sharon Zeligson, M.Sc., Dina Marek-Yagel, Ph.D., Tim M. Strom, M.D., Mordechai Shohat, M.D., Amihood Singer, M.D., Alan Rubinow, M.D., Elon Pras, M.D., Juliane Winkelmann, M.D., Mustafa Tekin, M.D., Yair Anikster, M.D., Ph.D., Mary-Claire King, Ph.D., and Ephrat Levy-Lahad, M.D.



NEJM, February 19th , 2014

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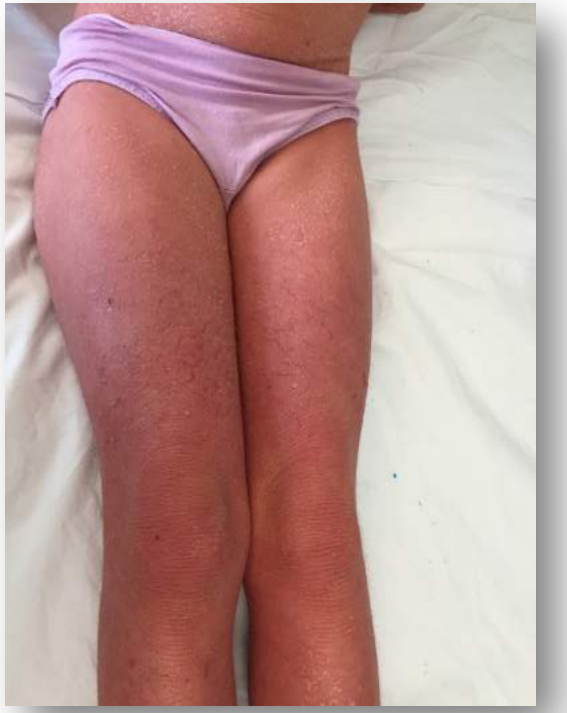
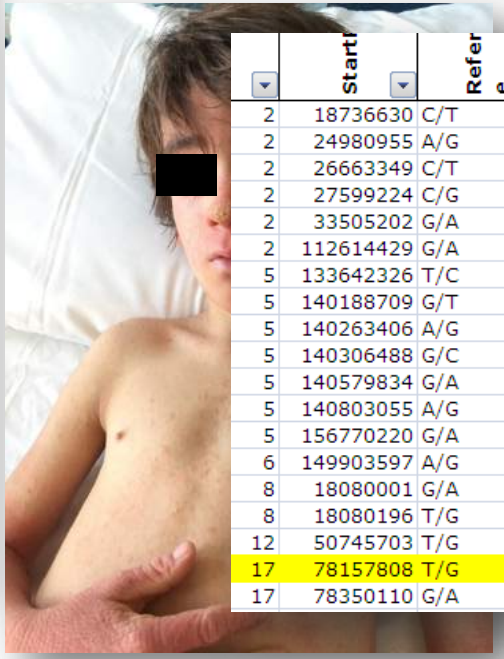
Possible applications

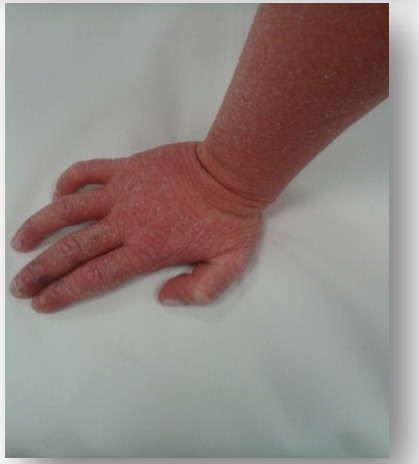
- Identification of new diseases
- Identification of unexpected or novel phenotypes for genes already associated to diseases

Mendelian distribution of severe erytrodermic psoriasis





Start	Refer e	rsID	dbsnp	False	MostI ne	MostI ure
2 18736630	C/T	rs74378191	131		RDH14	missense
2 24980955	A/G	rs150066931	134		NCOA1	missense
2 26663349	C/T	rs143181834	134		DRC1	missense
2 27599224	C/G		NA		SNX17	missense
2 33505202	G/A	rs61754247	129		LTBP1	missense
2 112614429	G/A	rs72936240	131		ANAPC1	stopgain
5 133642326	T/C	rs142248452	134		CDKL3	missense
5 140188709	G/T	rs147674000	134		PCDHA4	missense
5 140263406	A/G	rs376667172	138		PCDHA13	missense
5 140306488	G/C	rs369906778	138		PCDHAC1	missense
5 140579834	G/A	rs61743184	129		PCDHB11	missense
5 140803055	A/G	rs141810253	134		PCDHGA11	missense
5 156770220	G/A	rs140164881	134		FNDC9	missense
6 149903597	A/G	rs1137086	86		GINM1	missense
8 18080001	G/A	rs4987076	113		NAT1	missense
8 18080196	T/G	rs4986783	113		NAT1	missense
12 50745703	T/G	rs201307392	137		FAM186A	missense
17 78157808	T/G		NA		CARD14	missense
17 78350110	G/A	rs148731719	134		RNF213	missense



CARD14: p.L149R
(pustular psoriasis or pityriasis rubra pilaris)

in collaboration with E. Campione (Tor Vergata, Rome)

Possible applications

- Identification of new diseases
- Identification of unexpected or novel phenotypes for genes already associated to diseases
- Identification of somatic mosaicisms in patients with a clinical picture consisting with a given monogenic SAID

Genetically-«negative» patients

- Somatic NLRP3 mosaicism is identified in 70% of "genetically-negative" **CINCA** patients

  N. Tanaka et al A&R 2011

- 12.5% of **MWS** patients

  Nakagawa et al. ARD, 2013

- Myeloid lineage–restricted somatic mosaicism of NLRP3 in **Schnitzler syndrome**

  H. De Koning et al. JACI, 2015

- Somatic NOD2 mosaicism in **Blau syndrome**

  J. De Inocencio et al JACI, 2015

- Gonosomal mosaicism in **TRAPS**

  DM Rowczenio et al Arth Rheumatol, 2016

Possible applications

- Identification of new diseases
- Identification of unexpected or novel phenotypes for genes already associated to diseases
- Identification of somatic mosaicisms in patients with a clinical picture consisting with a given monogenic SAID
- Elaboration of “diagnostic panels” for a complete and fast molecular characterization of patients with suspected SAID

DEVELOPMENT AND DAILY USE OF A DIAGNOSTIC PANELS FOR SAID

Our preliminary experience at
Gaslini Institute

2 questions:

1) Can we use the NGS panel as a reliable diagnostic tool in daily clinical practice?

2) How can we interpret (and report?) the different variants found?

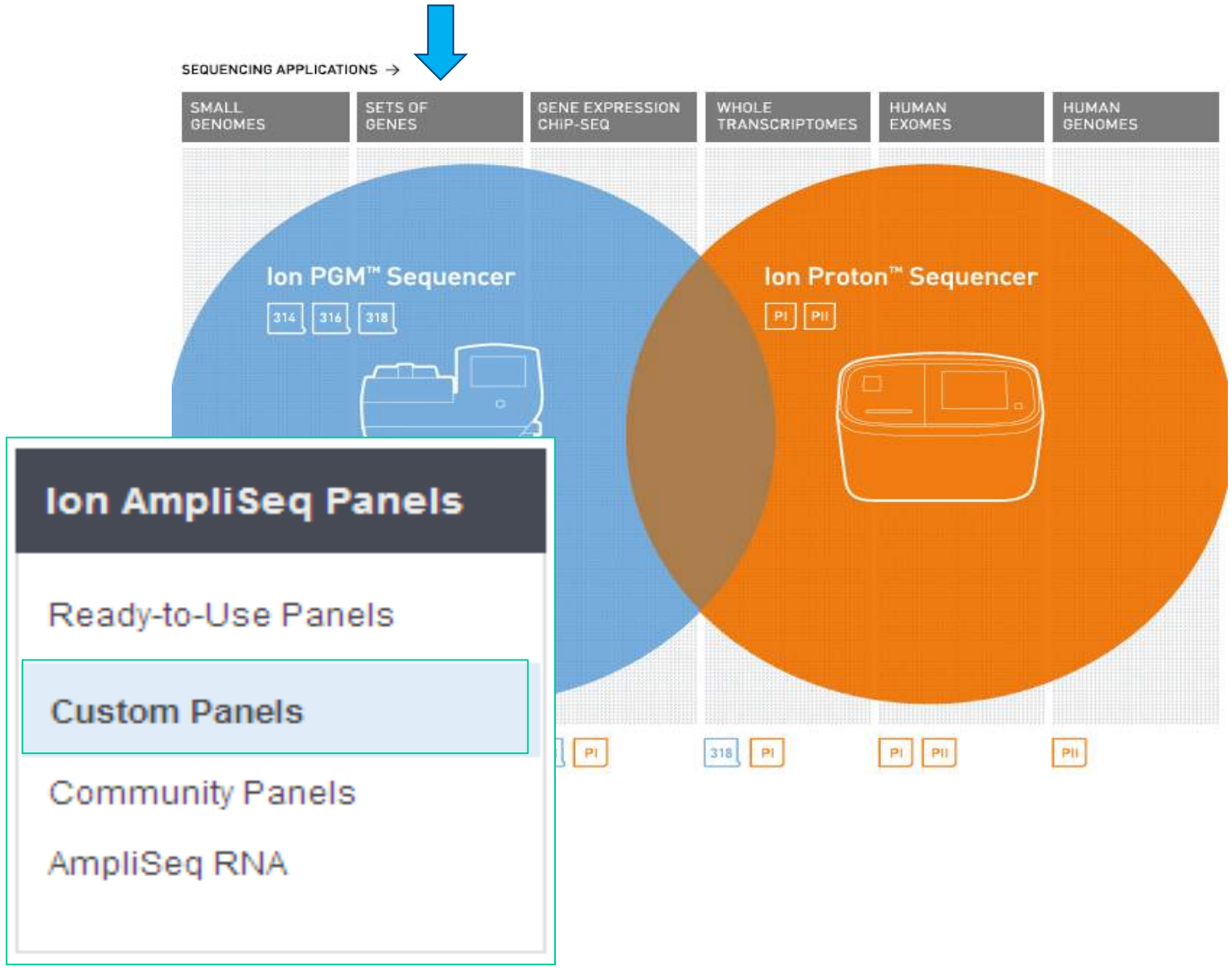
Aims of the study:

1) Validation of a NGS based protocol
2) Evaluation of the impact of the different variants identified on the clinical phenotype

Patients and Methods

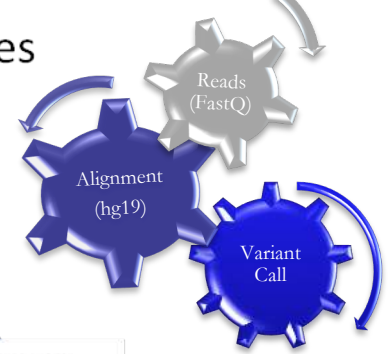
- 2205 patients screened at Gaslini Institute since 2002
- 50 patients with already confirmed monogenic AIDs (15 CAPS, 10 TRAPS, 15 MKD, 12 FMF, 2 NLRP12, 1 PAPA)
- 22 with undifferentiated SAID followed by Gaslini Institute
- Secreened for 10 genes (all exons): MEFV, MVK, TNFRSF1A, NLRP3, NLRP12, NOD2, PSMB8, PSTPIP1, LPNI2, IL1RN

M. Rusmini et al, ARD 2015


SEQUENCING APPLICATIONS →
SMALL GENOMES
SETS OF GENES
GENE EXPRESSION ChIP-SEQ
WHOLE TRANSCRIPTOMES
HUMAN EXOMES
HUMAN GENOMES
Ion PGM™ Sequencer
314
316
318
Ion Proton™ Sequencer
PI
PII
PI
318
PI
PI
PII
PII
Ion AmpliSeq Panels
Ready-to-Use Panels
Custom Panels
Community Panels
AmpliSeq RNA

DNA sequencing by Sanger and variant detection in one of the AIDs genes

Next Generation Sequencing



Raw data (fastQ)

Ion Reporter

Home-made Workflow:
Alignment to Hg19
SNVs and Indels detection
Annotation

CLC-bio Workbench

Alignment to Hg19

Probabilistic Variant Detection

Frameshift and missense variants

Annotation

BWA

Alignment to Hg19

Samtool

.BAM sorting

GATK

Realignment around known indels;
Frameshift and missense variants

SnpEff

Annotation

Comparison of results

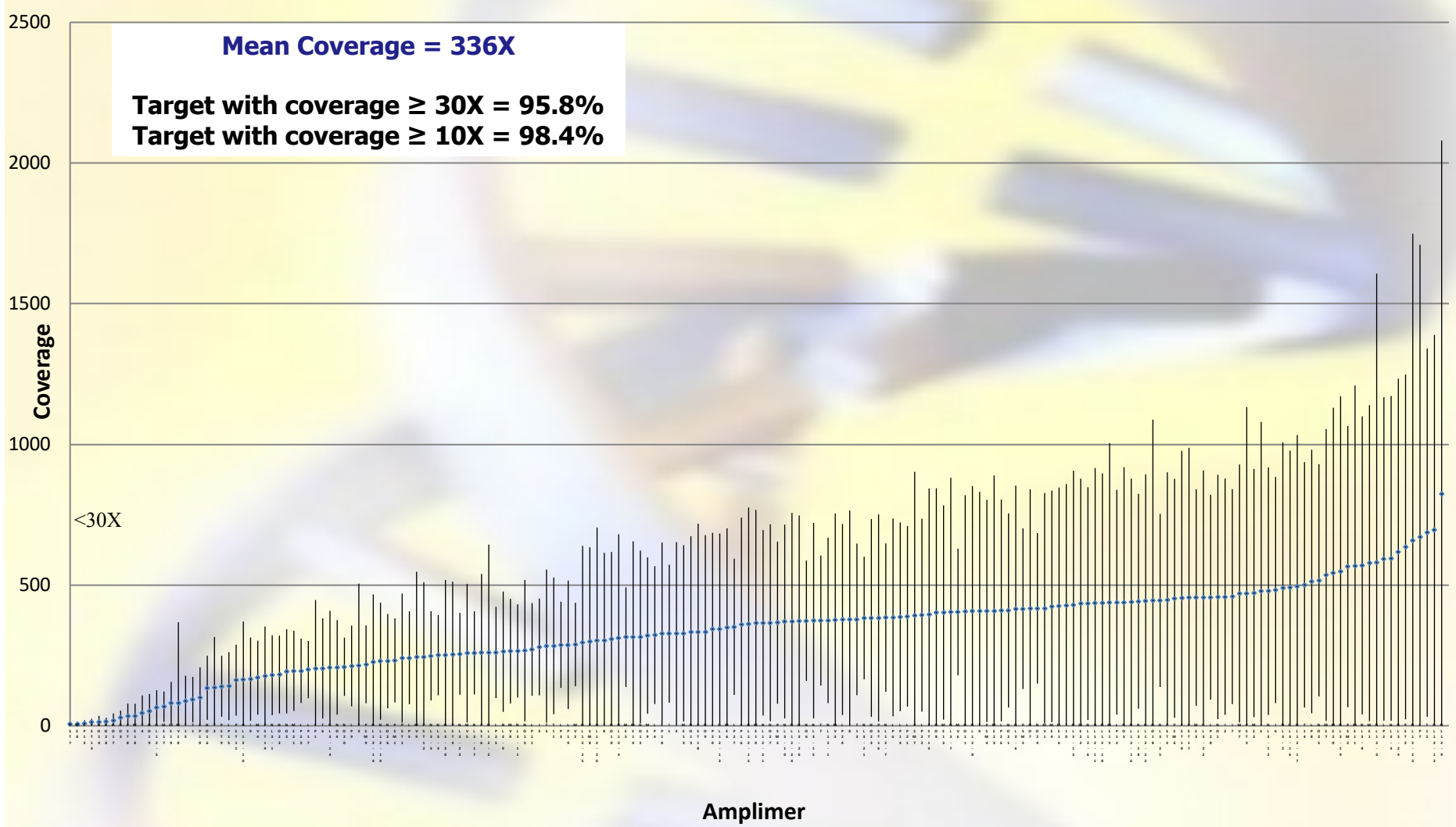
Sanger sequencing validation of unexpected variants

M. Rusmini ARD 2015

Fig.3

Coverage / amplimer

~1% target could not be included in the design



M. Rusmini et al, ARD 2015

A NGS diagnostic panel for SAID (Rusmini et al, ARD Sept 2015)

	sample1	sample2	sample3	sample4	sample5	sample6	sample7	sample8	sample9	sample10	sample11	sample12	sample13	sample14	sample15	sample16	sample17	sample18
NLRP3	V198M*	I313V*		E567K*	G569A*	R260W*	I572F*	A712S*	G454E	D303N*	I313V*	F443L* ¥	R260W*	I480F*	Q703K*		I313V*	
					I274V													
IL1RN			A106T															
PSMB8		T5N																
TNFRSF1A	R92Q*_h	L447F				L447F	R92Q*		L447F					L421R				
MVK		S52N*			S52N*	S52N*_h									I268T*	c.850delG*	I268T*	D170D*
															V377I*	V377I*	V377I*	I56V*
PSTPIP1			E277D* ¥															
MEFV	R202Q*		R202Q*	R202Q*	E112G			E112G	E112G		R202Q*_h	R202Q*	R202Q*		R202Q*		R202Q*	R202Q*
	E112G		M694V*	E112G	A744S*													
			V726A*															
			E112G															
NOD2	P268S*_h	P268S*	Q945K ¥		P268S*	P268S*	P268S*	P268S*	P268S*_h	P268S*			P268S*				P268S*_h	P268S*
		A946T	A946T ¥			G908R*	V955I*					V955I*		P397L				
		R791Q*	V955I*															
LPIN2	R760G		R760G ¥			P348L*												
	C874F										K387E*				P599L	M358V		
NLRP12	G39V*	G39V*			G39V*_h		G39V*					G39V*					G39V*_h	G39V*
								F402L*	F402L*			F402L*			F402L*			

	sample19	sample20	sample21	sample22	sample23	sample24	sample25	sample26	sample27	sample28	sample29	sample30	sample31	sample32	sample33	sample34	sample35	sample36
NLRP3					V198M*	L369M*					I274V	I274V				I274V	A871T	A871T
						Q703K*											I274V	I274V
IL1RN																		
PSMB8																		
TNFRSF1A										T50M*	C33G*	R92Q*	R92Q*	P46L*				
												C29G*						
MVK	V377I*_h		G142D*	c.22_23ins4nt		S52N*	R124W*	I268T*	V22M*		S52N*		S52N*		S52N*		S52N*	S52N*
	V132I*	V377I*_h		V377I*	V377I*_h		V377I*	V310M*	I268T*									
PSTPIP1				G258A*														
MEFV		R202Q*	R202Q*		E148Q*		R202Q*		G660E	E148Q*	R202Q*	R202Q*	R202Q*	P162Q	M680IGA*_h	M694I*	R761H*	R761H*
			M694V*_h													M680IGA*	E148Q*	R202Q*
																		E148Q*
NOD2	P268S*			P268S*_h					P268S*_h	P268S*	V955I*	P268S*		P268S*	P268S*	P268S*	V955I*	
							N289S*		R702W*		K731R			K731R		K731R		
	E729G					T294S*			c.3016_3017insC		A946T							
			V955I*			V955I*	V955I*		Q945K									
LPIN2					S768R	S768R		S768R			P348L*	E601K*	P626S*					
			I505L		R760G	G428S	R760G											
NLRP12		G39V*	G39V*_h		H304Y*				G39V*_h	F402L*		G39V*_h	G39V*	G39V*			G39V*	G39V*

expected | Expected but detected by not all the tools | unexpected | Unexpected but detected by not all the tools

2 questions:

1) Can we use the NGS panel as a reliable tool for daily clinical practice?

2) How can we interpret (and report?) the different variants found?

Aims of the study

1) Validation of a NGS based protocol
2) **Evaluation of the impact of the different variants identified on the clinical phenotype**

3 groups of patients

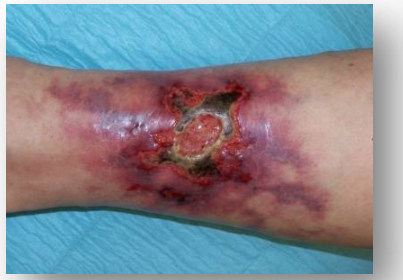
- **Group 1**: patients with a confirmed SAID (typical clinical presentation associated with a confirmatory genetic test) *not presenting other variants/mutations* in the other genes examined;
- **Group 2**: patients with a confirmed SAID (as above) *presenting one or more variants/mutations* in the other genes of the panel;
- **Group 3**: patients with a clinical picture consistent with a given SAID, but with not-confirmatory genetic test (i.e. patients with a clinical picture consistent for FMF-like, but heterozygous for MEFV mutations);

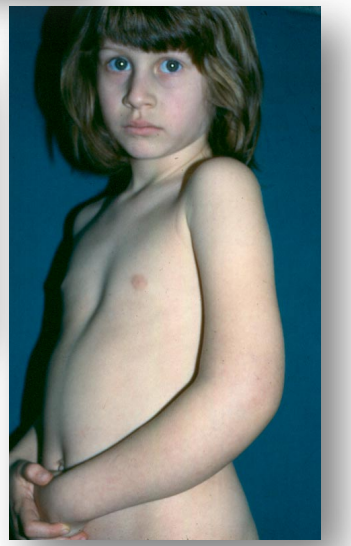
NB: complete clinical data were available for 30 out of 50 patients

A NGS diagnostic panel for SAID (Rusmini et al, ARD Sept 2015)

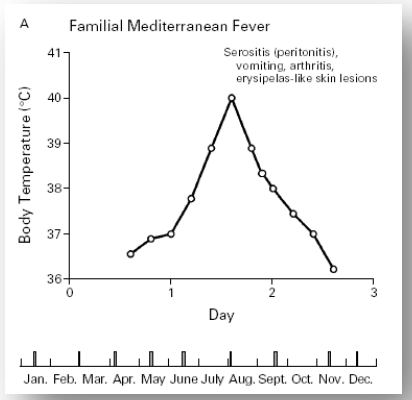
	sample1	sample2	sample3	sample4	sample5	sample6	sample7	sample8	sample
NLRP3	V198M*	I313V*		E567K*	G569A*	R260W*	I572F*	A712S*	G454E
					I274V				
IL1RN			A106T						
PSMB8		T5N							
TNFRSF1A	R92Q*_h	L447F				L447F	R92Q*		L447F
MVK		S52N*			S52N*	S52N*_h			
PSTPIP1			E277D* ¥						
MEFV	R202Q*		R202Q*	R202Q*	E112G			E112G	E112G
	E112G		M694V*	E112G	A744S*				
			V726A*						
			E112G						
NOD2	P268S*_h	P268S*	Q945K ¥		P268S*	P268S*	P268S*	P268S*	P268S*_
		A946T	A946T ¥			G908R*	V955I*		
		R791Q*	V955I*						
LPIN2	R760G		R760G ¥			P348L*			
	C874F								
NLRP12	G39V*	G39V*			G39V*_h		G39V*		
								F402L*	F402L*

	sample19	sample20	sample21	sample22	sample23	sample24	sample25	sample26	sample2
NLRP3					V198M*	L369M*			
						Q703K*			

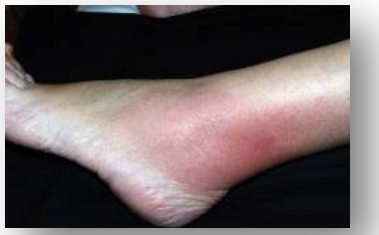




PAPA syndrome



Familial Mediterranean Fever (FMF)



V22M*									
I268T*									
G660E	E148Q*	R202Q*	R202Q*	R202Q*	P162Q	M680IGA*_h	M694I*	R761H*	R761H*
							M680IGA*	E148Q*	R202Q*
									E148Q*
P268S*_h	P268S*	V955I*	P268S*		P268S*	P268S*	P268S*	V955I*	
R702W*		K731R			K731R		K731R		
c.3016_3017insC		A946T							
Q945K									
		P348L*	E601K*	P626S*					
G39V*_h	F402L*		G39V*_h	G39V*	G39V*			G39V*	G39V*

expected | Expected but detected by not all the tools | unexpected | Unexpected but detected by not all the tools

Pts with a confirmatory genetic test + additional variants

	ID	Diagnosis	Genotype	Additional variants/mutations	Atypical clinical features
GROUP 2	FP631	CAPS	NLRP3: **R260W**	NOD2: G908R	
	FP836	CAPS	NLRP3: **I572F**	TNFRSF1A: **R92Q**	
	FP1236	CAPS	NLRP3: **L369M /*Q703K***	NOD2: T294S; LPIN2: G428S	Recurrent pericarditis, chest pain, abdominal pain, non pruriginous skin rash and pomfoid-like swelling of the subcutaneous tissue
	FP1719	CAPS	NLRP3: **F523Y**	NLRP12: **P210L**	
	FP1073	MKD	MVK: **V377I_h**	NLRP3: **V198M** MEFV: ***E148Q*** NLRP12: **H304Y**	
	FP1263	MKD	MVK: **R124W/V377I**	NOD2: N289S	Coxalgia either during fever episodes and intercritical periods
	FP1852	MKD	MVK: **V377I_h**	NOD2: N289S	
	FP451	MKD	MVK: **A147T/ V377I**	MEFV: ***E148Q***	
	FP1281	MKD	MVK: **I268T/ V377I**	NLRP3**: I313V**	
	FP1084	TRAPS	TNFRSF1A**: C33G**	MEFV: ***E148Q*** LPIN2: P348L	
	FP1036	TRAPS	TNFRSF1A: **T50M**	MEFV: ***E148Q*** NLRP12: F402L	
	FP1882	FMF	MEFV: **M694V/M 680I**	NLRP12: F402L	
	FP1883	FMF	MEFV: **R761H/ M680I**	NOD2: G908R	Partial response to colchicine with persistent abdominal pain
	FP1390	PAPA	PSTPIP1: **E277D**	MEFV: **M694V/ V726A**	
	FP953	FCAS2	NLRP12: **H304Y**	NOD2: 1007FS/SNP13	

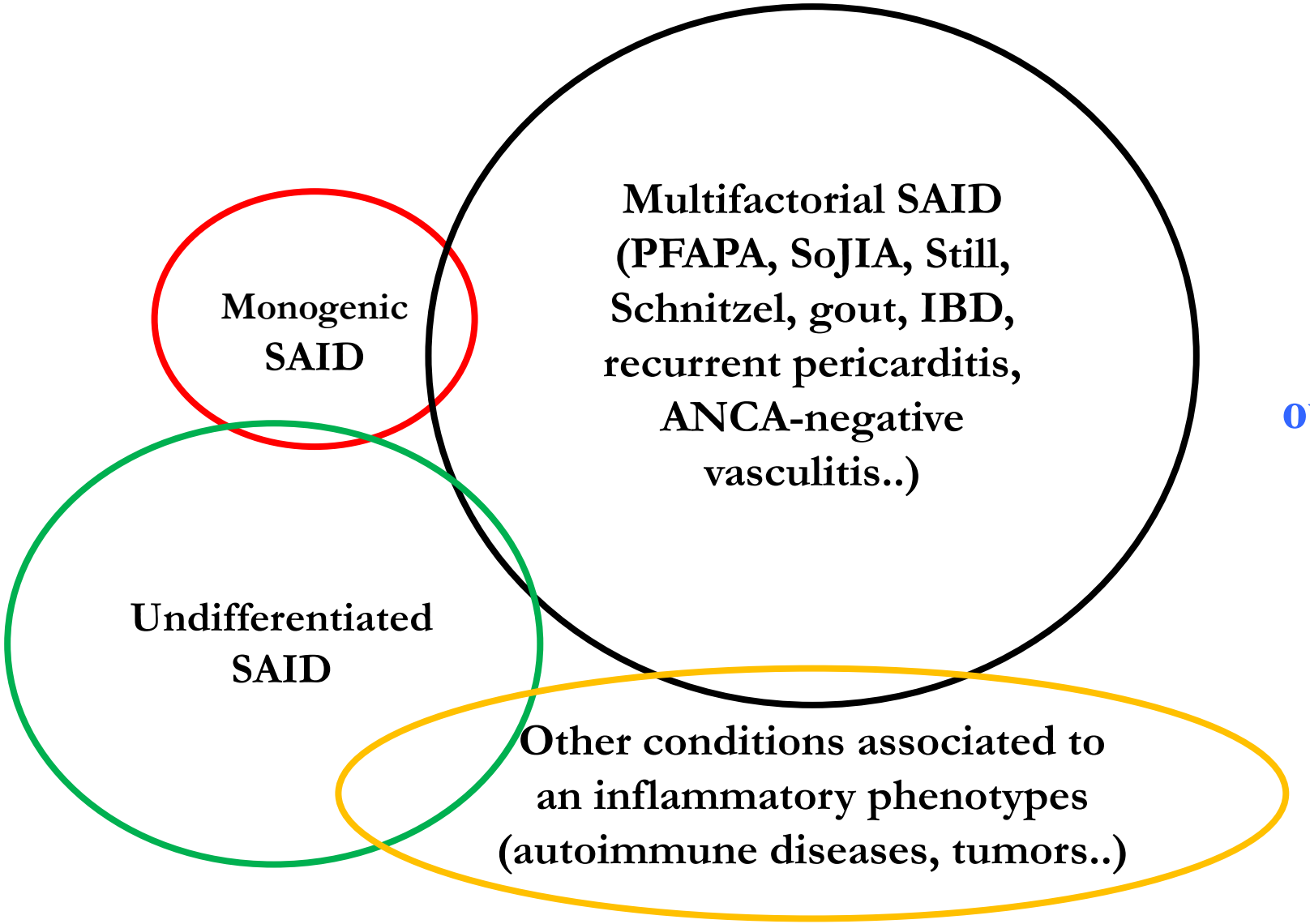
M. Rusmini ARD 2015

Conclusions (i)

(from the preliminary Gaslini's experience)

- NGS is a reliable diagnostic tool for SAID
- Careful validation of the method (coverage, best work-flow for variant call) and careful interpretation of the mutations identified
- In monogenic SAID *with confirmatory genetic test* additional variants in other geners does not seems to substantially modified by
- **How is the actual impact of the high number of variants identified by NGS in the daily practice?**

The expanding spectrum of Autoinflammatory diseases



Possible risk of over-interpretation of the variants identified !

A comprehensive clinical and experimental approach to personalized molecular medicine in patients with defined and undefined autoinflammatory disorders (INSAID E-rare Project 2016-2019)

Main goals:

1) to establish consensus among experts on the correct classification of patients with SAID based on the combination of **genetic tests and clinical variables**;
2) to **improve the performance** of genetic diagnosis (new diagnostic panels);
3) to evaluate the **impact of epigenetic factors** in the phenotype of defined SAID, using Familial Mediterranean Fever (FMF) as a prototype;
4) to establish a **multidimensional platform for the identification of new genes and conditions for undifferentiated-orphan patients**. Data and information (clinical, immunomics, proteomics, genetics and epigenetics) sharing among the participating groups will be facilitated by establishing a proper common database and by implementing a pilot bioinformatics platform

Consensus among experts on the correct classification of patients with SAID based on the combination of **genetic tests and clinical variables (WP1)**

To identify **evidence based classification criteria for INHERITED PERIODIC FEVERS and PFAPA** syndrome (80% of children presenting for consultation)

Involvement of BOTH clinicians and geneticists

Development of new classification criteria for inherited periodic fever and PFAPA

EUROFEVER PROJECT

OVERVIEW OF THE PROJECT

First Delphi open survey

↓

Second Delphi survey

↓

Selection of patients from Eurofever Registry

↓

Selection of a panel of Experts for patients evaluation

↓

Patients evaluation by experts

↓

Statistical analysis

Selection of candidate criteria

↓

Consensus Conference

Parallel project runned by geneticists

Classification of pathogeneticity of all variants described for hereditary periodic fevers

ACMG* classification

5. Clearly pathogenic
4. Likely pathogenic
3. Unknown (VUS)
2. Likely benign
1. Benign

*American college of medical genetics and genomics

(Leaders: I. Touitou, I. Ceccherini, M Van Gejn)

E-rare Project: INSAID

The new classification criteria for periodic fevers

Genetic and clinical criteria

CAPS	FMF	TRAPS	MKD	PFAPA
Presence of a *pathogenic NLRP3 variant* and **at least 1** among: • Urticaria like rash • Red eye (conjunctivitis, episcleritis, uveitis) • Neurosensorial hearing loss **OR** Presence of a *genetic variant of NLRP3 of unknown significance* and **at least 2** among: • Urticarial rash • Red eye (conjunctivitis, episcleritis, uveitis) • Neurosensorial hearing loss	Presence of *confirmatory MEFV genotype* and at **least 1** among: • Duration of episodes 1-3 days • Arthritis • Chest pain • Abdominal pain **OR** Presence of *not confirmatory MEFV genotype* and **at least 2** among: • Duration of episodes 1-3 days • Arthritis • Chest pain • Abdominal pain	Presence of a *confirmatory TNFRSF1A genotype* and **at least 1** among: • Duration of episodes ≥ 7 days • Myalgia • Migratory rash • Periorbital oedema • Relatives affected **OR** Presence of *variants of TNFRSF1A of unknown significance* and **at least 2 among**: • Duration of episodes ≥ 7 days • Myalgia • Migratory rash • Periorbital oedema • Relatives affected	Presence of a *confirmatory MVK genotype* and **at least 1** among: • Gastrointestinal symptoms • Cervical lymphadenitis	/

in preparation

Parallel classification of the pathogenicity of all variants associated to periodic fevers by geneticists (paper in publication)

The INSAID project

Improve the diagnostic rate with the NGS technique

The challenging case of undifferentiated SAIDs

Undifferentiated autoinflammatory diseases in the pediatric age

- Patients resembling an autoinflammatory disease, with a negative or non-univocal clinical and genetic characterization (70-80% of patients screened in tertiary centers).
- Most have a clear phenotype of a multifactorial condition (PFAPA → careful selection of the cases!)
- Others recall monogenic SAID (CAPS-like? DADA2-like? etc) but are "negative" at standard genetic analysis → ask more to your geneticists!
- Other display a peculiar and persistent inflammatory phenotype not consistent with any specific disease (new diseases? unusual phenotypes of already identified conditions?)

Undifferentiated AID

- Monogenic AID with an atypical phenotype
- "Double-positive" patients
- Combination of different known and unknown variants ("Harlequin" patients)
- New monogenic diseases
- Different diseases (multifactorial AID or other conditions associated with systemic inflammation, autoimmune diseases, tumors..)

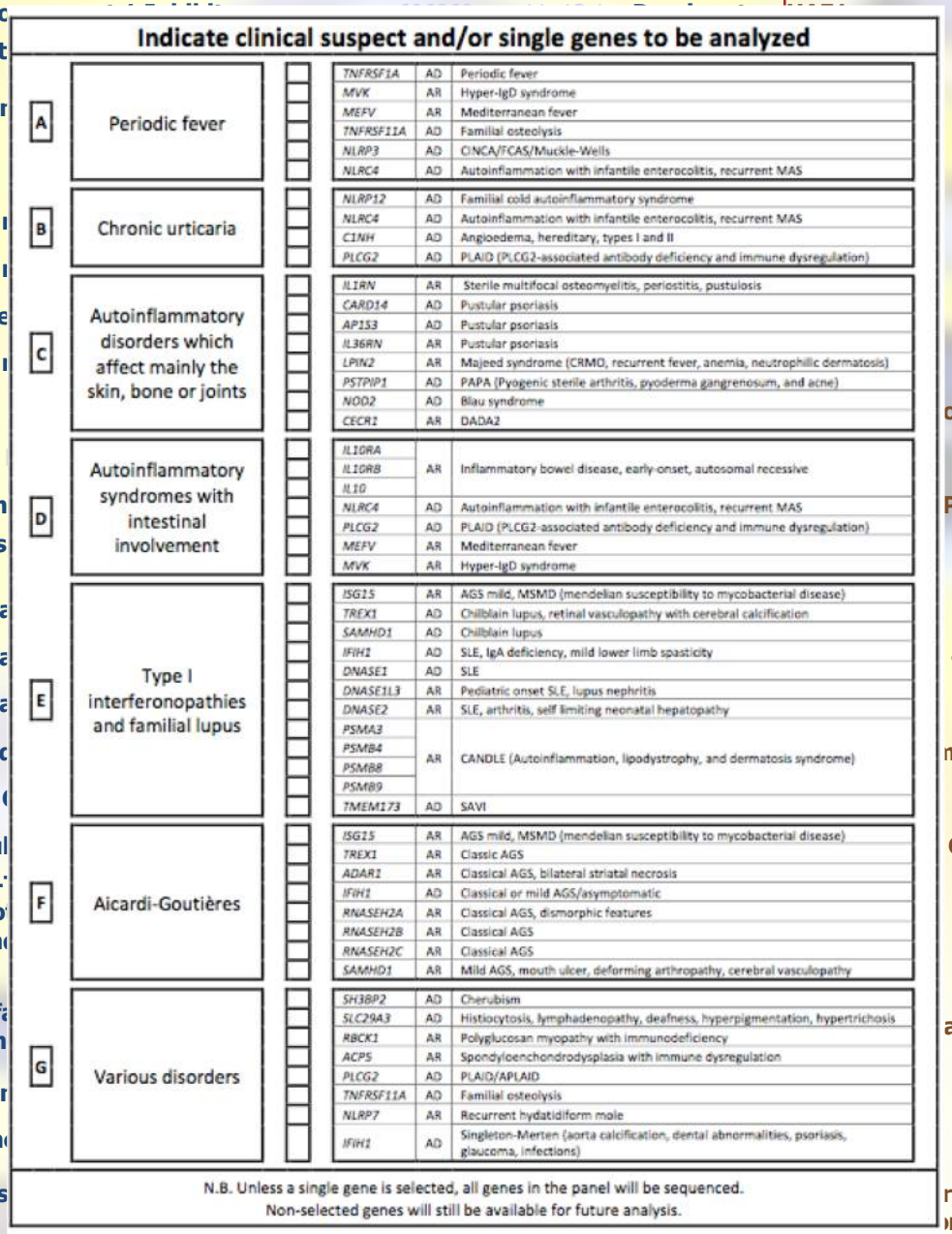
Warning: possible over-interpretation of the results

SAID/infevers genes

AP1S3	Adaptor-related protein complex 1, sigma-3 subunit	615781	2q36.1		
C1NH	Complement Component 1 Inhibitor	606860	11q12.1	Dominant	HAE1
CARD14	Caspase recruitment domain-containing protein 14	607211	17q25.3	Dominant	PSORS2, PRP
CECR1	Cat eye syndrome chromosome region, candidate 1	607575	22q11.2	Recessive	PAN
IL10	Interleukin 10	124092	1q32.1	Recessive	
IL10RA	Interleukin 10 receptor, alpha	146933	11q23.3	Recessive	IBD28
IL10RB	Interleukin 10 receptor, beta	123889	21q22.11	Recessive	IBD25
IL1RN	Interleukin 1 receptor antagonist	147679	2q13	Recessive	OMPP
IL36RN	Interleukin 36 receptor antagonist	605507	2q13	Recessive	PSORP
LPIN2	LIPIN 2	605519	18p11.31	Recessive	Majeed syndrome
MEFV	MEditerranean FeVer	608107	16p13	Recessive	FMF
MVK	Mevalonate Kinase	251170	12q24	Recessive	HIDS, MEVA, POROK3
NLRC4	NLR family, caspase recruitment domain-containing 4	606831	2p22.3	Dominant	AIFEC
NLRP12	NLR pyrin domain containing protein 12	609648	19q13.42	Dominant	FCAS2
NLRP3	NLR pyrin domain containing protein 3	606416	1q44	Dominant	FCAS1 - MWS - CINCA
NLRP7	NLR pyrin domain containing protein 7	609661	19q13.42	Recessive	HYDM1
NOD2	Nucleotide-binding oligomerization domain 2	605956	16q12	Dominant	BLAU syndrome, EOS, IBD1
PLCG2	Phospholipase C, Gamma-2	600220	16q23.3	Dominant	APLAID
PSMB8	Proteasome Subunit, Beta-Type, 8	177046	6p21.32	Recessive	ALDD - NNS - CANDLE - JMP
PSTPIP1	Proline-Serine-Threonine Phosphatase Interacting Protein 1	606347	15q24.3	Dominant	PAPA
RBCK1	Ranbp-Type and C3HC4-Type Zinc Finger-Containing 1	610924	20p13	Recessive	PBMEI
SLC29A3	Solute carrier family 29 (nucleoside transporter), member 3	612373	10q22.1	Recessive	Histiocytosis-lymphadenopathy plus syndrome
SH3BP2	SH3-domain binding protein 2	602104	4p16.3	Dominant	Cherubism
TMEM173	Transmembrane protein 173	612374	5q31.2	Dominant	SAVI
TNFRSF1A	Tumor Necrosis Factor Receptor Super Family 1A	191190	12p13.31	Dominant	Periodic Fever, Familial, Autosomal Dominant
TNFRSF11A	Tumor necrosis factor receptor superfamily, member 11A	603499	18q21.33	Dominant	

http://fmf.igh.cnrs.fr/ISSAID/infevers/

SAID/infevers genes



Indicate clinical suspect and/or single genes to be analyzed

A	Periodic fever	*TNFRSF1A*	AD	Periodic fever
		MVK	AR	Hyper-IgD syndrome
		MEFV	AR	Mediterranean fever
		TNFRSF11A	AD	Familial osteolysis
		NLRP3	AD	CINCA/FCAS/Muckle-Wells
		NLRC4	AD	Autoinflammation with infantile enterocolitis, recurrent MAS
B	Chronic urticaria	*NLRP12*	AD	Familial cold autoinflammatory syndrome
		NLRC4	AD	Autoinflammation with infantile enterocolitis, recurrent MAS
		C1NH	AD	Angioedema, hereditary, types I and II
		PLCG2	AD	PLAID (PLCG2-associated antibody deficiency and immune dysregulation)
C	Autoinflammatory disorders which affect mainly the skin, bone or joints	*IL1RN*	AR	Sterile multifocal osteomyelitis, periostitis, pustulosis
		CARD14	AD	Pustular psoriasis
		AP1S3	AD	Pustular psoriasis
		IL36RN	AR	Pustular psoriasis
		LPIN2	AR	Majeed syndrome (CRMO, recurrent fever, anemia, neutrophilic dermatosis)
		PSTPIP1	AD	PAPA (Pyogenic sterile arthritis, pyoderma gangrenosum, and acne)
		NOO2	AD	Blau syndrome
		CECR1	AR	DADA2
D	Autoinflammatory syndromes with intestinal involvement	*IL10RA* *IL10RB* *IL10*	AR	Inflammatory bowel disease, early-onset, autosomal recessive
		NLRC4	AD	Autoinflammation with infantile enterocolitis, recurrent MAS
		PLCG2	AD	PLAID (PLCG2-associated antibody deficiency and immune dysregulation)
		MEFV	AR	Mediterranean fever
		MVK	AR	Hyper-IgD syndrome
E	Type I interferonopathies and familial lupus	*ISG15*	AR	AGS mild, MSMD (mendelian susceptibility to mycobacterial disease)
		TREX1	AD	Chilblain lupus, retinal vasculopathy with cerebral calcification
		SAMHD1	AD	Chilblain lupus
		IFIH1	AD	SLE, IgA deficiency, mild lower limb spasticity
		DNASE1	AD	SLE
		DNASE1L3	AR	Pediatric onset SLE, lupus nephritis
		DNASE2	AR	SLE, arthritis, self limiting neonatal hepatopathy
		PSMA3 *PSMB4* *PSMB8* *PSMB9*	AR	CANDLE (Autoinflammation, lipodystrophy, and dermatosis syndrome)
		TMEM173	AD	SAVI
F	Aicardi-Goutières	*ISG15*	AR	AGS mild, MSMD (mendelian susceptibility to mycobacterial disease)
		TREX1	AR	Classic AGS
		ADAR1	AR	Classical AGS, bilateral striatal necrosis
		IFIH1	AD	Classical or mild AGS/asymptomatic
		RNASEH2A	AR	Classical AGS, dismorphic features
		RNASEH2B	AR	Classical AGS
		RNASEH2C	AR	Classical AGS
		SAMHD1	AR	Mild AGS, mouth ulcer, deforming arthropathy, cerebral vasculopathy
G	Various disorders	*SH3BP2*	AD	Cherubism
		SLC29A3	AD	Histiocytosis, lymphadenopathy, deafness, hyperpigmentation, hypertrichosis
		RBCK1	AR	Polyglucosan myopathy with immunodeficiency
		ACP5	AR	Spondyloenchondrodysplasia with immune dysregulation
		PLCG2	AD	PLAID/APLAID
		TNFRSF11A	AD	Familial osteolysis
		NLRP7	AR	Recurrent hydatidiform mole
		IFIH1	AD	Singleton-Merten (aorta calcification, dental abnormalities, psoriasis, glaucoma, infections)

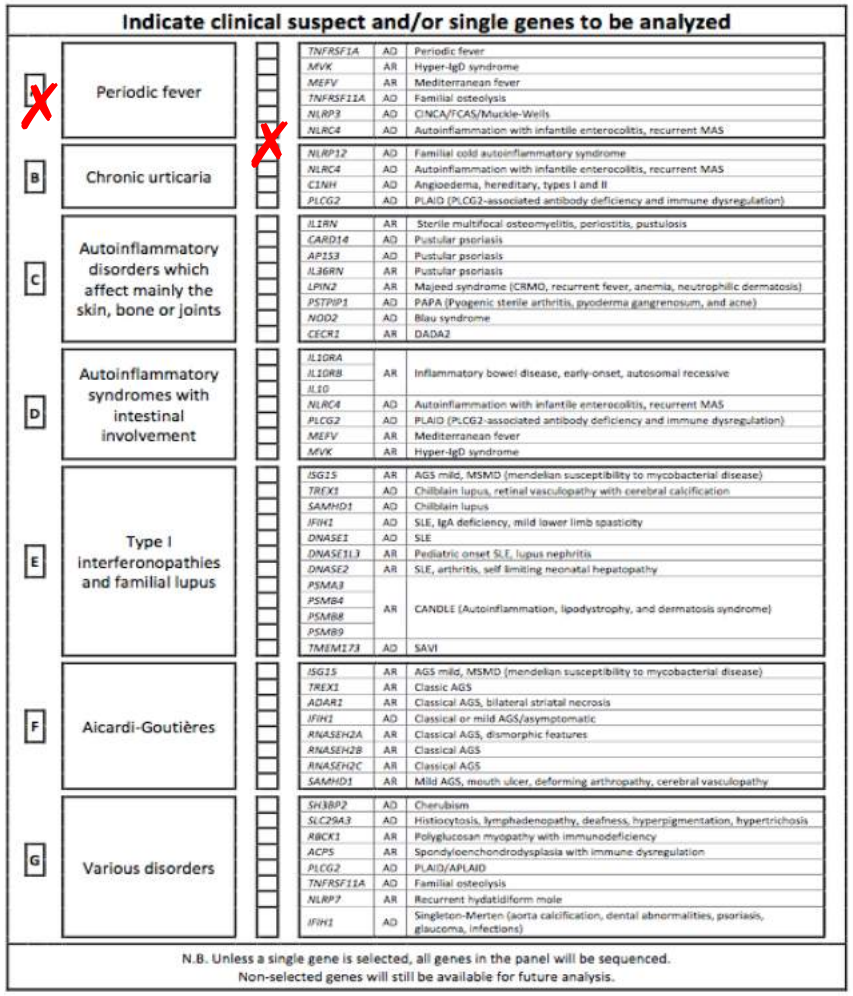
N.B. Unless a single gene is selected, all genes in the panel will be sequenced.
Non-selected genes will still be available for future analysis.

AP1S3	Adaptor-related protein complex 1, sigma-3 subunit	615781	2q36.1	
C1NH	Complement Co			
CARD14	Caspase recruit protein 14			
CECR1	Cat eye syndror candidate 1			
IL10	Interleukin 10			
IL10RA	Interleukin 10 r			
IL10RB	Interleukin 10 r			
IL1RN	Interleukin 1 re			
IL36RN	Interleukin 36 r			
LPIN2	LIPIN 2			ome
MEFV	MEditerranean			
MVK	Mevalonate Kin			POROK3
NLRC4	NLR family, cas containing 4			
NLRP12	NLR pyrin doma			
NLRP3	NLR pyrin doma			- CINCA
NLRP7	NLR pyrin doma			
NOD2	Nucleotide-binc			ne, EOS, IBD1
PLCG2	Phospholipase (			
PSMB8	Proteasome Sul			CANDLE - JMP
PSTPIP1	Proline-Serine-T Interacting Pro			
RBCK1	Ranbp-Type and Containing 1			
SLC29A3	Solute carrier fa transporter), m			athy plus
SH3BP2	SH3-domain bir			
TMEM173	Transmembran			
TNFRSF1A	Tumor Necrosis 1A			r, Familial, minant
TNFRSF11A	Tumor necrosis factor receptor superfamily, member 11A	603499	18q21.33	Dominant

http://fmf.igh.cnrs.fr/ISSAID/infevers/

Patients and methods (I)

Panel design



Indicate clinical suspect and/or single genes to be analyzed

A ✗	Periodic fever	*TNFRSF1A*	AD	Periodic fever
		MVK	AR	Hyper-IgD syndrome
		MEFV	AR	Mediterranean fever
		TNFRSF11A	AD	Familial osteolysis
		NLRP3	AD	CINCA/FCAS/Muckle-Wells
		NLRC4	AD	Autoinflammation with infantile enterocolitis, recurrent MAS
B ✗	Chronic urticaria	*NLRP12*	AD	Familial cold autoinflammatory syndrome
		NLRC4	AD	Autoinflammation with infantile enterocolitis, recurrent MAS
		C1NH	AD	Angioedema, hereditary, types I and II
		PLCG2	AD	PLAID (PLCG2-associated antibody deficiency and immune dysregulation)
C	Autoinflammatory disorders which affect mainly the skin, bone or joints	*IL1RN*	AR	Sterile multifocal osteomyelitis, periostitis, pustulosis
		CARD14	AD	Pustular psoriasis
		AP1S3	AD	Pustular psoriasis
		IL36RN	AR	Pustular psoriasis
		LPIN2	AR	Majeed syndrome (CRMO, recurrent fever, anemia, neutrophilic dermatosis)
		PSTPIP1	AD	PAPA (Pyogenic sterile arthritis, pyoderma gangrenosum, and acne)
		NOD2	AD	Blau syndrome
		CECR1	AR	DADA2
D	Autoinflammatory syndromes with intestinal involvement	*IL10RA*	AR	Inflammatory bowel disease, early-onset, autosomal recessive
		IL10RB		
		IL10		
		NLRC4	AD	Autoinflammation with infantile enterocolitis, recurrent MAS
		PLCG2	AD	PLAID (PLCG2-associated antibody deficiency and immune dysregulation)
		MEFV	AR	Mediterranean fever
		MVK	AR	Hyper-IgD syndrome
E	Type I interferonopathies and familial lupus	*ISG15*	AR	AGS mild, MSMD (mendelian susceptibility to mycobacterial disease)
		TREX1	AD	Chilblain lupus, retinal vasculopathy with cerebral calcification
		SAMHD1	AD	Chilblain lupus
		IFIH1	AD	SLE, IgA deficiency, mild lower limb spasticity
		DNASE1	AD	SLE
		DNASE1L3	AR	Pediatric onset SLE, lupus nephritis
		DNASE2	AR	SLE, arthritis, self limiting neonatal hepatopathy
		PSMA3	AR	CANDLE (Autoinflammation, lipodystrophy, and dermatosis syndrome)
		PSMB4		
		PSMB8		
		PSMB9		
		TMEM173	AD	SAVI
F	Aicardi-Goutières	*ISG15*	AR	AGS mild, MSMD (mendelian susceptibility to mycobacterial disease)
		TREX1	AR	Classic AGS
		ADAR1	AR	Classical AGS, bilateral striatal necrosis
		IFIH1	AD	Classical or mild AGS/asymptomatic
		RNASEH2A	AR	Classical AGS, dismorphic features
		RNASEH2B	AR	Classical AGS
		RNASEH2C	AR	Classical AGS
		SAMHD1	AR	Mild AGS, mouth ulcer, deforming arthropathy, cerebral vasculopathy
G	Various disorders	*SH3BP2*	AD	Cherubism
		SLC29A3	AD	Histiocytosis, lymphadenopathy, deafness, hyperpigmentation, hypertrichosis
		RBCK1	AR	Polyglucosan myopathy with immunodeficiency
		ACP5	AR	Spondyloenchondrodysplasia with immune dysregulation
		PLCG2	AD	PLAID/APLAID
		TNFRSF11A	AD	Familial osteolysis
		NLRP7	AR	Recurrent hydatidiform mole
		IFIH1	AD	Singleton-Merten (aorta calcification, dental abnormalities, psoriasis, glaucoma, infections)

N.B. Unless a single gene is selected, all genes in the panel will be sequenced.
Non-selected genes will still be available for future analysis.

Inclusion criteria

i) clinical picture consistent with an autoinflammatory syndrome with disease onset in the pediatric age

ii) clinical follow-up in our Center for at least 2 years

iii) negative or not conclusive genetic test at standard molecular analysis (Sanger) in the most probable genes

Patients and methods (II)

The phenotype-genotype correlation

- Possible pathogenic variants (PPV) *(in silico* pathogenic variant) if:
  - predicted damaging by 3 or > than 6 different software or
  - CLINVAR score >3

- Clearly/likely/unlikely related to the phenotype those PPVs that cause a disease resembling the patient's clinical picture

- Four groups of patients:
  A) with al least one PPV clearly related to the phenotype
  B) with al least one PPV likely related to the phenotype
  C) with al least one PPV unlikely related to the phenotype
  D) without PPV or variants

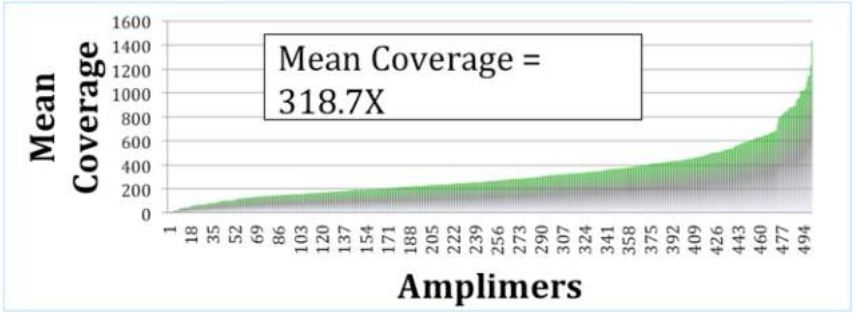
Results (I)

Cohort overview

Characteristics	Study cohort (N=50)
Demography	
Male:female	27:23
Aduts	7 (14%)
Caucasian	48 (96%)
Age at enrolment (mean, range; years)	12.5 (5-38)
Age at onset (mean, range; years)	3.7 (0-21)
Disease duration (mean, range; years)	7.9 (2-23)
Tested genes per patient (mean, range)	3 (1-7)
Phenotypes	
Periodic fever	43 (86%)
Prevalent skin/bone/joints involvement	5 (10%)
Prevalent intestinal involvement	1 (2%)
Suspected type 1 interferonopathies	7 (14%)
Genotypes	
Variants per patient	2 (0-6)
Variants in suspected subsets	29 (23%)
Variants related to phenotype	30 (23%)
Recurrent variants *	38 (30%)

* supplementary table.

Variant validation



Mean of variants for patient
173.17 (range 130-209)

Mean of variants in the coding and splice site regions for patient
59.71 (range 43-75)

Mean of missense and frameshift variants for patient
22.65 (range 15-34)

Mean of variants with frequency <3% in the general population for patient*
2.03 (range 0-6)

*at least one of the database available.

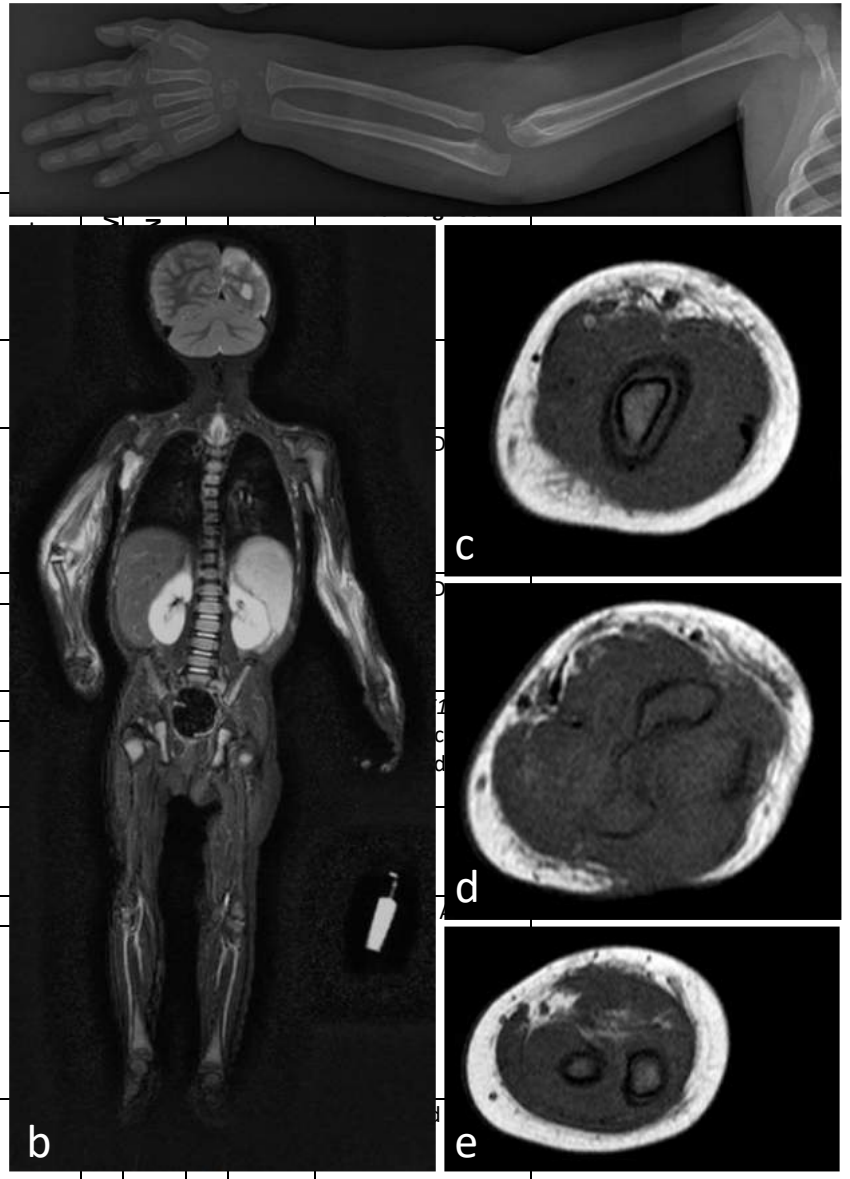
Results (II)

- Four groups of patients:
  - A) with al least one PPV **clearly** related to the phenotype: 3 pts
  - B) with al least one PPV **likely** related to the phenotype: 2 pts

  10 %
  - C) with al least one PPV **unlikely** related to the phenotype: 25 pts — 50 %
  - D) without PPV or negative: 20 pts — 40 %

Results (III)

N°	Required subset	Clinical features and response to treatments	Gene	Mutation	CLINVAR	ExAC	Eur 1000 Genomes	Mutation Taster
i) Patients with a confirmatory genotype								
1	C	Poliarthritis, periostitis, generalized lymphadenopathy, hepatosplenomegaly, dysmorphisms (epicanthus, frontal bossing, saddle nose), small cerebral hemisphere with pachygyria. Complete response to anakinra.	**MVK**	**Gly326Arg HOMO**	1	-	-	DC
2	A	Recurrent fever episodes since childhood with generalized lymphadenopathy, abdominal pain and erythematous rash. Complete response to steroids on-demand.	**MVK**	**Val377Ile**	5	1,60E-03	-	DC a
			MVK	**Ala147Thr**	-	4.06E-6	-	DC
3	A	Recurrent febrile episodes of 5-7 days, every month, with exudative pharyngitis, sometimes aphthosis or headache. Complete response to steroids on-demand.	CARD14	Ser200Asn	1	6,38E-03	2,00E-03	P
			TNFRSF11A	**Lys240Glu**	0	1,07E-03	1,00E-03	DC
			IFIH1	Glu627*	-	3,20E-03	8,90E-03	DC
ii) Patients with possibly pathogenic variant likely consistent with the clinical phenotype								
4	A	Recurrent fever episodes, every 8-10 days, with erythema nodosum and aphthosis. Lobar granulomatous panniculitis with interstitial and perivascular infiltrate of lymphocytes and histiocytes at skin biopsy. Complete response to steroids. No response to rapamycin, azathioprine and thalidomide. Partial response to anti-TNF treatment.	**PLCG2**	**Asn571Ser**	-	6,70E-03	1,39E-02	DC
			SH3BP2	Arg609Gly	-	1.966E-05	-	DC
5	B, C	Recurrent episodes of urticarial rash, aphthosis, exudative pharyngitis, cervical lymphadenopathy, abdominal pain, and arthromyalgia. Partial response to on-demand steroids. Infiltration of mast cells at skin biopsy.	PLCG2	Ala1130Ser	-	8,12E-06	-	DC



Estimated cost of the panel: 22.000 euros → estimated costs of the hospital admissions for pt 1: 28.000 euros

PLOS ONE

RESEARCH ARTICLE

Clinical impact of a targeted next-generation sequencing gene panel for autoinflammation and vasculitis

Ebun Omoyinmi[1]*, Ariane Standing[1], Annette Keylock[1], Fiona Price-Kuehne[1], Sonia Melo Gomes[1], Dorota Rowczenio[2], Sira Nanthapisal[1], Thomas Cullup[3], Rodney Nyanhete[3], Emma Ashton[3], Claire Murphy[1], Megan Clarke[1], Helena Ahlfors[3], Lucy Jenkins[3], Kimberly Gilmour[4], Despina Eleftheriou[1,5], Helen J. Lachmann[2], Philip N. Hawkins[2], Nigel Klein[1], Paul A. Brogan[1]

Table 1. Summary of disease groups and number of genes in the vasculitis and inflammation panel (VIP).

Disease group	Number of genes—VIP1	Number of genes—VIP2
Aortopathies	6	20
Associated with intestinal inflammation	31	44
Autoimmune lymphoproliferative syndrome (ALPS) and related disorders	6	7
Autoinflammatory	19	32
Complement and regulatory protein deficiencies	20	20
Vasculopathic Ehlers-Danlos syndrome	1	4
Haemophagocytic lymphohistiocytosis (HLH)	5	8
Hereditary amyloidosis	6	12
Paediatric stroke	6	6
SLE and Aicardi-Goutieres syndrome	10	10
Vasculitis/vasculopathy	3	3
TOTAL	**113**	**166**

VIP1: vasculitis and inflammation panel version 1; VIP2: vasculitis and inflammation panel version 2.

50 pts with undefined SAID

6/50 (12%) with clearly pathogenic variants
11/50 (22%) likely pathogenic variants

↓

Overall strong suspicion in 32%

PLOS ONE | https://doi.org/10.1371/journal.pone.0181874 July 27, 2017

Inborn errors of Immunity

(the classical view)

Clinical Immunologists

Primary immunodeficiencies
(WAS, CGD, CVID)

Rheumatologists

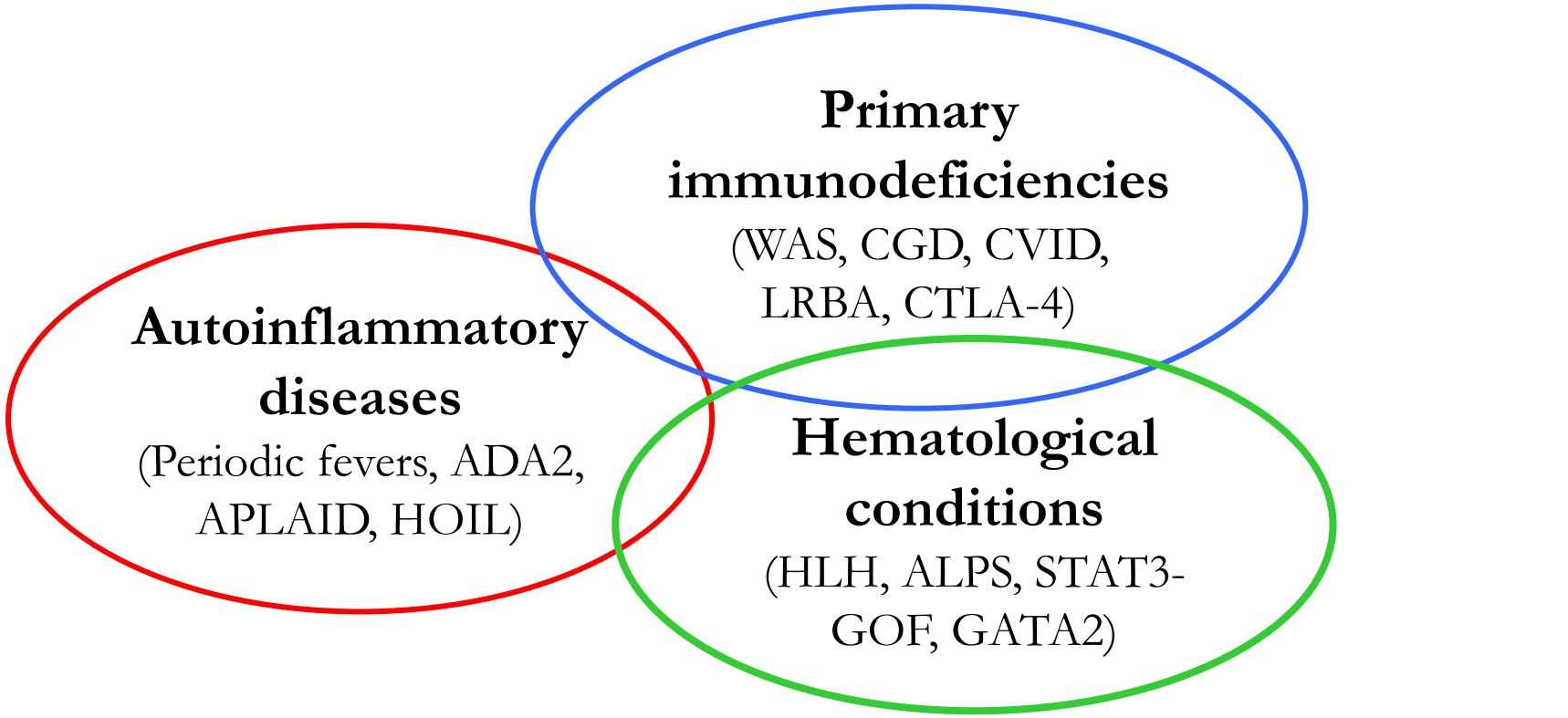
Autoinflammatory diseases
(Periodic fevers)

Hematological conditions
(HLH, ALPS)

Hemato-oncologists

Inborn errors of Immunity

(nowadays)



International Union Immunological Societies J Clin Immunol 2017

An evolving strategy

2000-14 Sanger sequencing

2014-15 10 genes panel for SAID

2016-17 41 genes panel for SAID

2017-18 250 genes panel for Inherited Immune mediated diseases (SAID, Immunodeficiencies, Autoimmune lymphoproliferative syndromes)

2019-20 Whole Exome Sequencing (WES)

in silico panels

Conclusions

- *Pros* and *cons* of NGS in the daily practice
- Role of the clinical characterization in NGS era
- Urgent need to combine clinical and genetic (and functional) data for a proper identification of inherited errors of Immunity
- A multidisciplinary approach!

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MP Sormani

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The Fantastic two!

S. Federici (Italy) and F. Vanoni (CH)

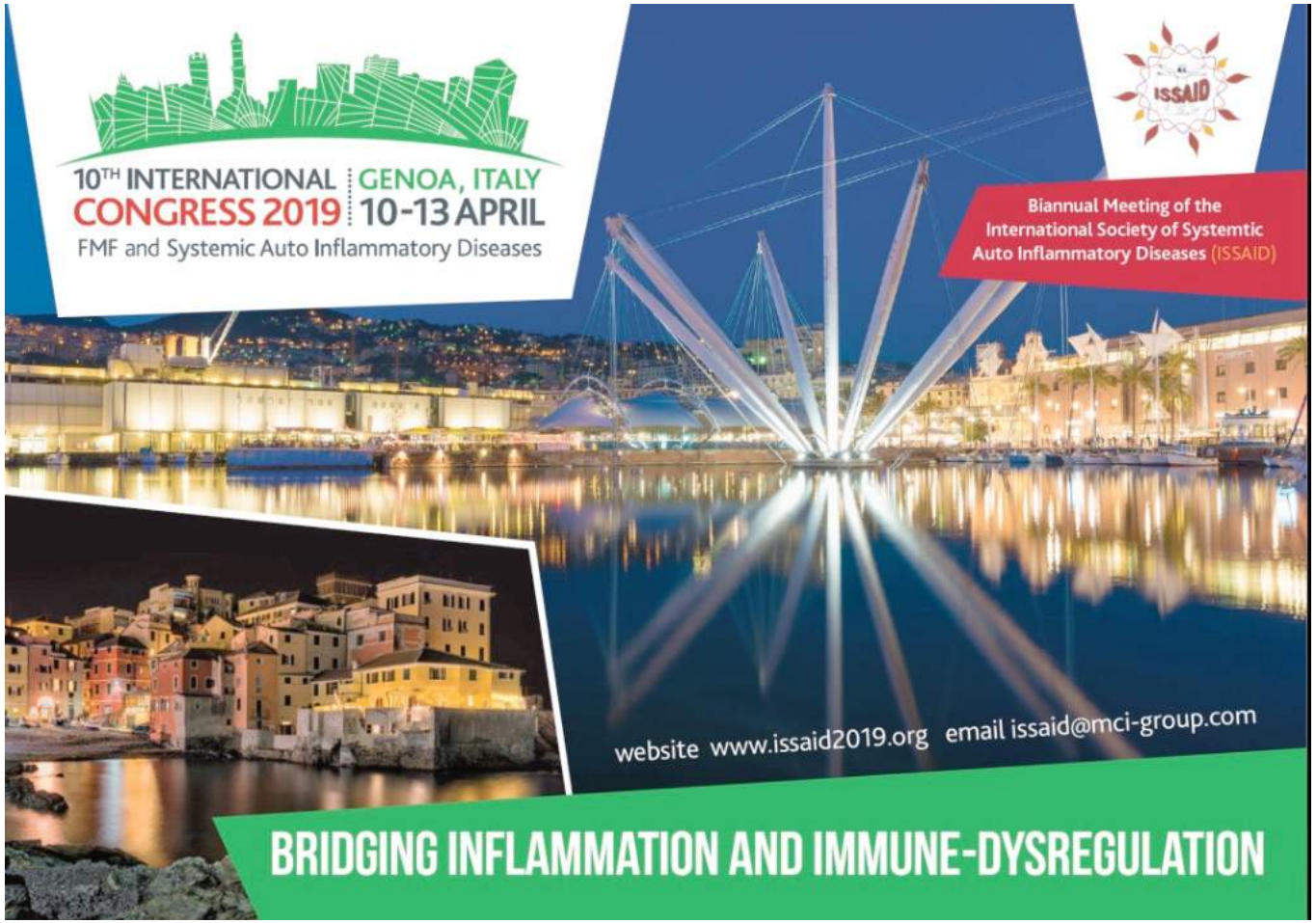
E-rare: INSAID project

Enrolling centres !!

PRINTO 's team (N. Ruperto, E. Mosci, L. Villa)

Novartis and SOBI (unrestricted grant)

Statisticians: MP Sormani and F. Bovis


10TH INTERNATIONAL CONGRESS 2019
GENOA, ITALY
10-13 APRIL
FMF and Systemic Auto Inflammatory Diseases
ISSAID
Biannual Meeting of the International Society of Systemtic Auto Inflammatory Diseases (ISSAID)
website www.issaid2019.org email issaid@mci-group.com
BRIDGING INFLAMMATION AND IMMUNE-DYSREGULATION

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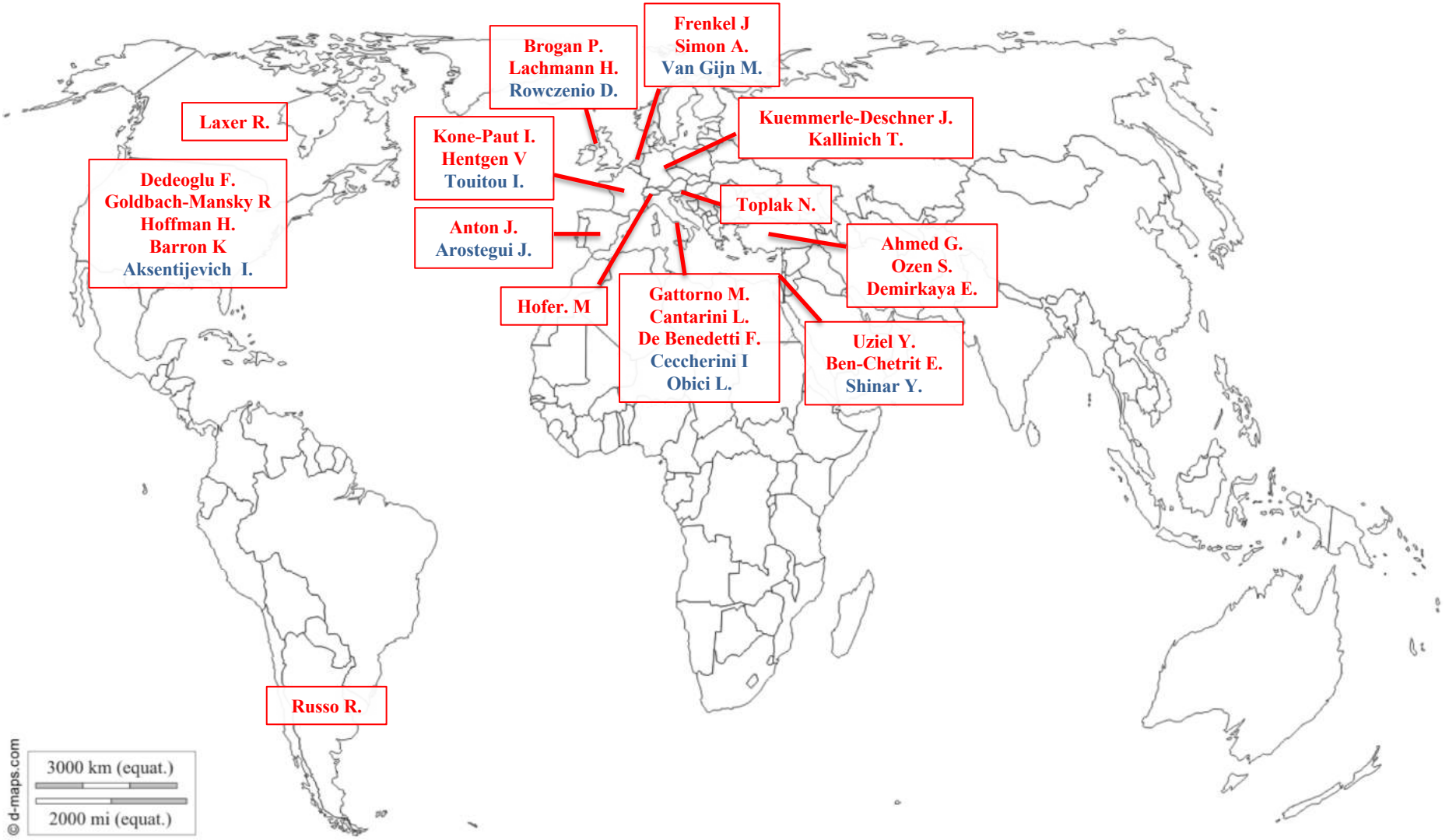
Panel of experts

EUROFEVER PROJECT



Delphi survey → selection of the most relevant varibles to be included in the classification criteria

Go to real patients

Diagnosis (in medicine)

- The act or process of **identifying or determining the nature and cause of a disease** or injury **through the evaluaiton of patient history, clinical examination, and review of laboratory data**.
- It is the opinion derived from such evaluations

Medical dictionary

Diagnostic criteria

- Tend to focus on listing or determining the **combination of findings that need to be present in order to be certain that a particular disease is present**
- Manifestations that are not unique to the disease but are found in related conditions **are likely to be excluded**
- Usually rely on **pathognomic findings** (eg genetic analysis, glicemia for diabetes etc)

ACR vasculitis criteria. A&R 1990

Classification criteria

- Include manifestations that are **characteristics of the disease in question and occur with less frequency** or are absent in other conditions.
- Symptoms or findings that might by typical or common but may also be present in other diseases **tend to be excluded**
- to identify a set of clinical findings (criteria) that recognise a high proportion of patients with the particular disease **(sensitivity)** and exclude a high proportion of patients with other diseases **(specificity)**

ACR vasculitis criteria. A&R 1990

Active enrolling centre

Anuela Kondi, Albania
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Susan Nielsen, Denmark
Troels Herlin, Denmark
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Anna Kozlova, Russian Federation
Irina Nikishina, Russian Federation
Wafaa Mohammed Saad Sewairi, Saudi Arabia
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Peter Ciznar, Slovakia
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Consuelo Modesto, Spain
Rosa Merino, Spain
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Carlos Rose, USA

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MEDICINE® 81: 349-68, 2002

Vol. 81, No. 5
Printed in U.S.A.

The TNF Receptor-Associated Periodic Syndrome (TRAPS)

Emerging Concepts of an Autoinflammatory Disorder

KEITH M. HULL, ELIZABETH DREWE, IVONA AKSENTIJEVICH, HARJOT K. SINGH, KONDI WONG, ELIZABETH M. MCDERMOTT, JANE DEAN, RICHARD J. POWELL, AND DANIEL L. KASTNER

TABLE 5. Diagnostic indicators of TRAPS

1. Recurrent episodes of inflammatory symptoms spanning a period of >6 mo duration (several symptoms generally will occur simultaneously)
   - 1.1 Fever
   - 1.2 Abdominal pain
   - 1.3 Myalgia (migratory)
   - 1.4 Rash (erythematous macular rash occurs with myalgia)
   - 1.5 Conjunctivitis/periorbital edema
   - 1.6 Chest pain
   - 1.7 Arthralgia or monoarticular synovitis.
2. Episodes last >5 days on average (although variable).
3. Responsive to glucocorticosteroids but not colchicine.
4. Affected family members (although may not always be present).
5. Any ethnicity may be affected.

Long-Term Follow-Up, Clinical Features, and Quality of Life in a Series of 103 Patients With Hyperimmunoglobulinemia D Syndrome

*Jeroen C. H. van der Hilst, Evelien J. Bodar, Karyl S. Barron, Joost Frenkel, Joost P. H. Drenth, Jos W. M. van der Meer, Anna Simon, and the International HIDS Study Group**

TABLE 8. Clinical Guideline for When to Consider Testing for HIDS

Consider testing for HIDS if patient has

Recurrent fever episodes lasting 3–7 days persisting more than 6 months

AND 1 or more of the following:

1. Sibling with genetically confirmed HIDS
2. Elevated serum IgD (>100 IU/L)
3. First attack after childhood vaccination
4. Three or more of the following symptoms during attacks:
   - Cervical lymphadenopathy
   - Abdominal pain
   - Vomiting or diarrhea
   - Arthralgia or arthritis of large peripheral joints
   - Aphthous ulcers
   - Skin lesions

Medicine • Volume 87, Number 6, November 2008

Demographic characteristics of 360 patients

	N° of PTS	Gender		Age years median (range)	Age disease onset median (range)	Disease duration median (range)	Episodes duration median (range)	Number episodes/yr median (range)
		M	F					
FMF	60	30	30	10,5 (7,0-15,5)	3,4 (1,2-6,4)	5,6 (2,7-10,2)	3,0 (2,0-4,0)	12,0 (10,0-20,0)
CAPS	60	32	28	16,0 (8,9-31,6)	3,0 (0,5-11,2)	9,0 (4,6-19,1)	2,0 (0,8-5,0)	12,0 (6,0-25,0)
MKD	60	26	34	16,2 (9,1-23,0)	0,4 (0,2-0,9)	14,2 (7,9-20,8)	5,0 (4,0-7,0)	12,0 (10,0-16,0)
TRAPS	60	35	25	21,9 (10,5-41,1)	3,4 0,8-10,6)	13,3 (6,8-23,2)	8,0 (5,0-18,0)	6,0 (4,0-12,0)
PFAPA	60	32	28	6,6 (3,8-9,5)	1,5 (0,7-3,0)	3,9 (2,3-6,8)	4,0 (3,0-5,0)	12,0 (12,0-18,0)
UNDEFINED	60	32	28	13,5 (8,2-26,4)	5,9 (2,0-19,1)	4,8 (3,0-8,2)	4,0 (3,0-7,0)	12,0 (5,0-13,0)

Scoring of genotypes

On the basis of the results of the evaluation process done by the experts, we assign to each genotype of the 360 patients a score from 5 to 1 to "grade" the results of genetic analysis.

This process was done to be able to include this item in the statistical analysis in order to create definitions combining clinical and genetic data.

Scoring of genotypes

To be able to include genetic data in the statistical analysis, we assign to each genotype of the 360 patients a score from 5 to 1.

This scoring was indirectly derived from the results of the evaluation process of the patients done by the experts.

Validation of pediatric FMF criteria in multi-ethnic population

339 FMF patients
- 211 from Eastern Mediterranean region
- 54 from European countries (Italy, Spain and Greece).
- 74 with Arab, Turkish, Jewish or Armenian origin were living in Western European countries.

377 control group
- 53 TRAPS
- 45 MKD
- 32 CAPS,
- 160 PFAPA
- 87 undefined periodic fevers.

E. Demirkaya

Patients and Methods (gold standard selection)

MKD: 2 *MVK* gene mutations

TRAPS: (**not chronic disease course**): 1 *TNFRSF1A* *gene* (low penetrance mutation/polymorphysm excluded) mutation

CAPS: (**not chronic disease course**) : 1 *NLRP3* gene mutation (low penetrance mutation/polymorphysm excluded)

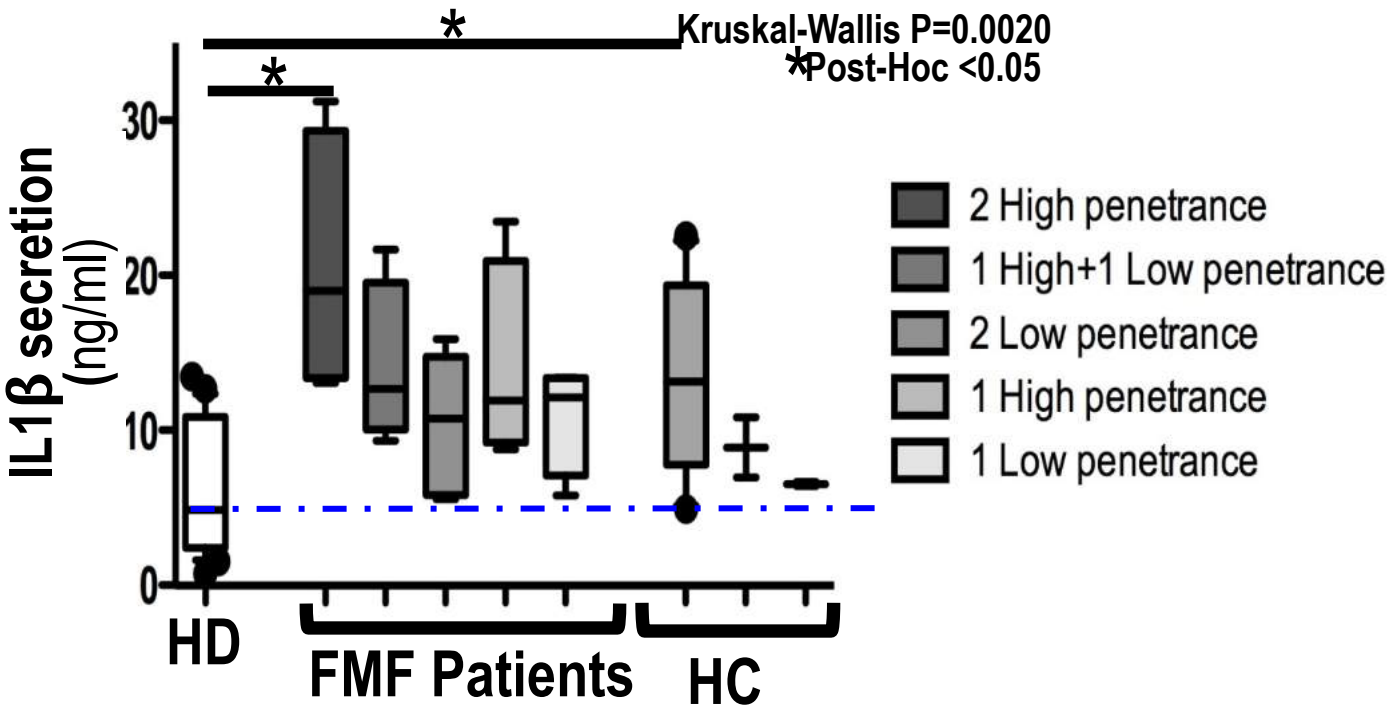
FMF: 2 *MEFV* mutations (at least one on exon 10)

PFAPA (Centre confirmation): negative controls

Federici et al ARD 2015

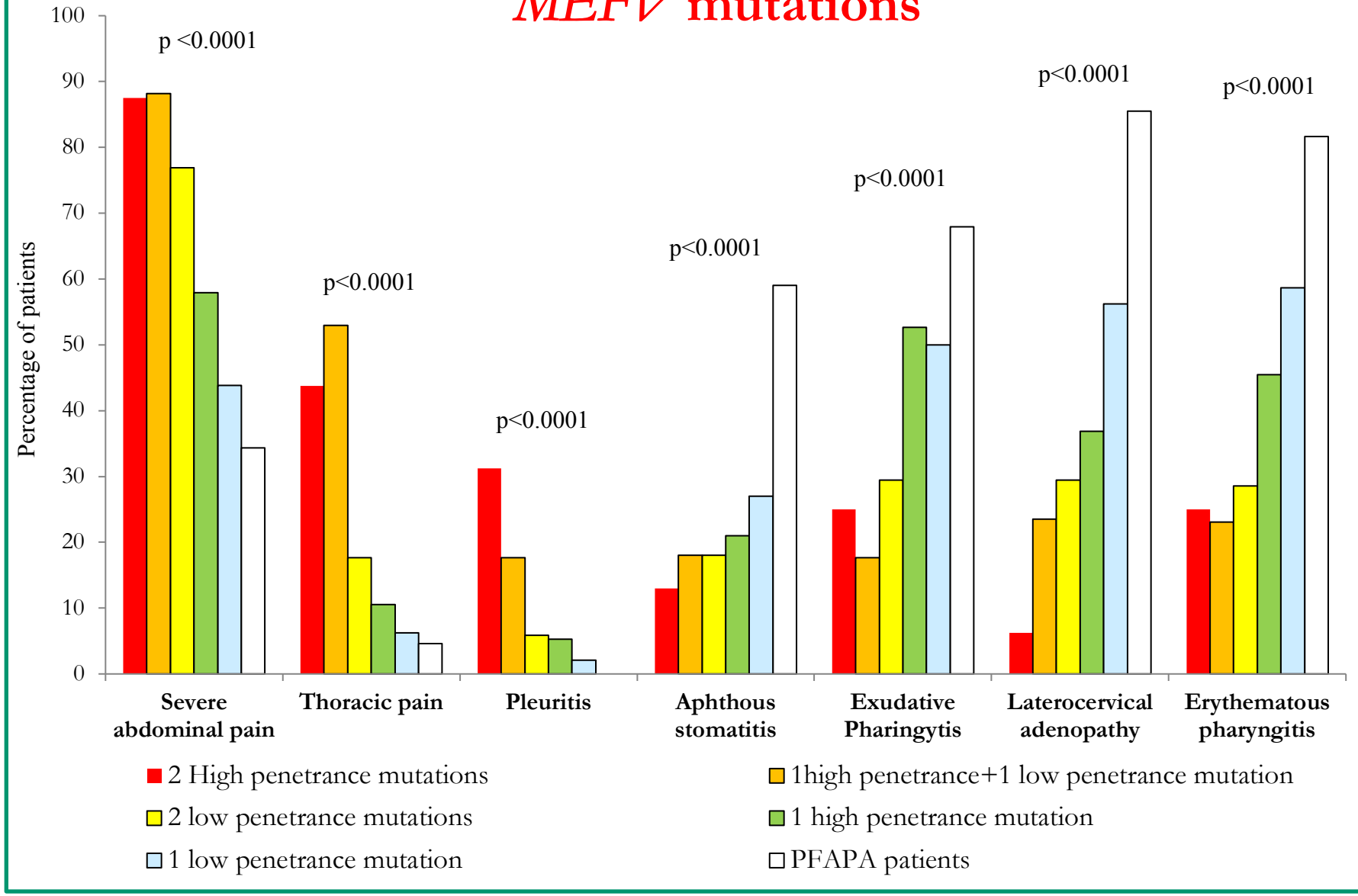
Can we postulate a dose-effect for genes associated to AID?

Levels of IL-1 secretion correlate with the number and penetrance of MEFV mutations



A. Omenetti et al, ARD 2013

MEFV mutations



Federici et al ARD 2012