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Supplementary information

Decoding variants of uncertain significance in systemic autoinflammatory diseases

In the format provided by the authors and unedited

Supplementary Information

Supplementary Table 1 | Percentage of VUS reported in variant databases Infevers and ClinVar for SAID. Interferonopathies and the associated genes are not presented.

Gene	Disease	Mode of Inheritance	Mechanism	%VUS (Infevers)	%VUS (ClinVar)	Reference(s)
ADA2	DADA2	Recessive	LoF	14/158 (8.9%)	262/551 (47.5%)	[1]
ADAM17	ADAM17 deficiency	Recessive	LoF	0/2	235/557 (42.2%)	[2]
ALPK1	ROSAH	Dominant	GoF	2/5 (40.0%)	483/798 (60.5%)	[3]
AP1S3	AP1S3 deficiency	Recessive	LoF	5/8 (62.5%)	7/30 (23.3%)	[4]
C2orf69	C2Orf69 deficiency	Recessive	LoF	NR	7/26 (26.9%)	[5,6]
CARD14	CAMPS (CARD14-mediated psoriasis)	Dominant	GoF	14/81 (17.3%)	716/1275 (56.2%)	[7,8]
CDC42	NOCARCH	Dominant	GoF	0/14	34/123 (27.6%)	[9,10]
CEBPE	C/EBPE-associated autoinflammation and immune impairment of neutrophils (CAIN)	Recessive	GoF	0/5	115/217 (53.0%)	[11]
DDP9	DDP9 deficiency/ HLH-like hyperinflammation	Recessive/ Dominant	LoF/ Dominant negative	NR	108/135 (80.0%)	[12,13]
DOCK11	DOCK11 deficiency	X-linked	LoF	NR	135/162 (83.3%)	[14]
ELF4	ELF4 deficiency	Recessive/ Dominant	LoF	0/11	52/70 (74.3%)	[15]
F12	FCAS-FXII	Dominant	GoF	0/1	115/209 (55.0%)	[16]
HAVCR2	T-cell lymphoma subcutaneous panniculitis-like (TIM3 deficiency)	Recessive	LoF	NR	40/59 (67.8%)	[17]

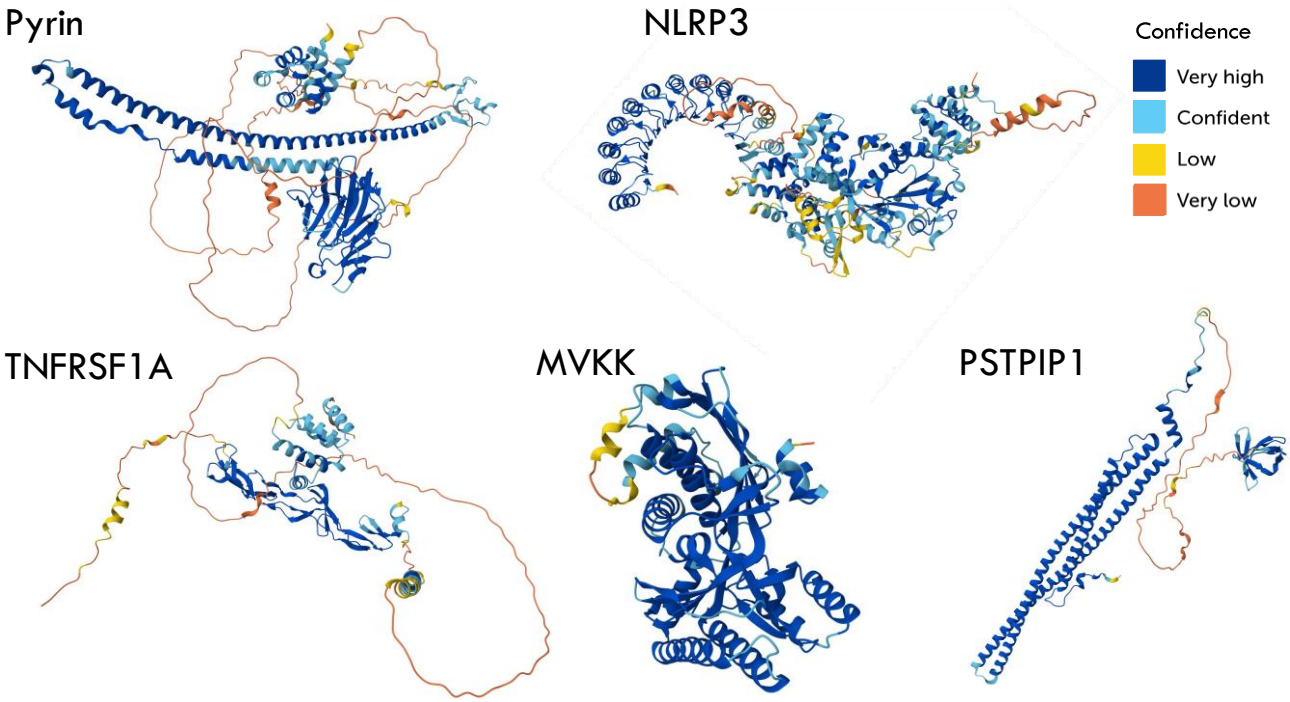
HCK	HCK GOF	Dominant	GoF	0/1	58/73 (79.5%)	[18]
IKBKG	NEMO exon 5 deletion	X-linked	GoF	0/12	79/201 (39.3%)	[19]
IL1R1	LIRSA	Dominant	LoF	0/1	25/36 (69.4%)	[20]
IL1RN	DIRA	Recessive	LoF	1/25 (4.0%)	110/236 (46.6%)	[21]
IL36RN	DITRA	Recessive	LoF	2/32 (6.3%)	114/199 (57.3%)	[22,23]
LACC1	Monogenic form of sJIA	Recessive	LoF	1/14 (7.2%)	44/59 (74.6%)	[24,]
LPIN2	Majeed syndrome	Recessive	LoF	12/46 (26.1%)	475/946 (50.2%)	[25]
LYN	LYN-associated SAID	Dominant	GoF	0/4	101/252 (40.1%)	[26,27]
MEFV	PAAD	Recessive/ Dominant	GoF	163/404 (40.4%)	773/1320 (58.6%)	[28-30]
MVK	MKD	Recessive	LoF	40/284 (14.1%)	311/819 (38.0%)	[31-34]
NCSTN	Acnea inversa	Dominant	LoF	9/35 (25.8%)	249/481 (51.8%)	[35]
NLRC4	NLRC4-AID/ NLRC4-MAS/ FCAS4	Dominant	GoF	13/46 (28.3%)	532/828 (64.3%)	[36-38]
NLRP1	MSPC/FKLC/NAIAD	Recessive/ Dominant	GoF	11/26 (42.4%)	657/1096 (59.9%)	[39,40]
NLRP12	FCAS2	Dominant	GoF	30/86 (34.9%)	862/1284 (67.1%)	[41]
NLRP3	NLRP3-AID/CAPS	Dominant	GoF	65/303 (21.5%)	648/1143 (56.7%)	[42,43]
NOD2	Blau syndrome	Dominant	GoF	65/192 (33.9%)	723/1142 (63.3%)	[44]
OTULIN	ORAS/Otulipenia/AIPDS	Recessive/ Dominant	LoF/ Dominant negative	2/18 (11.2%)	218/489 (44.6%)	[45]
PLCG1	PLCG1-associated immune dysregulation	Dominant	GoF	0/1	87/97 (89.7%)	[46]
PLCG2	PLAID/APLAID	Variable	GoF or LoF	17/41 (41.5%)	810/1650 (49.1%)	[47]
PMVK	PMVK deficiency	Recessive	LoF	NR	25/42 (59.5%)	[48,49]
PSTPIP1	PAPA syndrome	Dominant	GoF	44/77 (57.2%)	371/744 (49.9%)	[50]
RELA	RELA associated autoinflammatory disease	Dominant	LoF/ Dominant negative	0/13	203/449 (49.9%)	[51]
RIPK1	Cleavage-resistant RIPK1-induced autoinflammatory syndrome (CRIA)	Dominant	GoF	2/20 (10.0%)	284/492 (45.2%)	[52]

RNF31	HOIP deficiency	Recessive	LoF	NR	415/769 (54.0%)	[53]
SH3BP2	Cherubism	Dominant	GoF	4/23 (17.4%)	427/833 (51.3%)	[54]
SHARPIN	SHARPIN deficiency	Recessive	LoF	NR	51/65 (78.5%)	[55]
SLC29A3	H syndrome	Recessive	LoF	9/54 (16.7%)	257/516 (49.8%)	[56]
STAT4	Disabling pansclerotic morphea	Dominant	GoF	NR	199/434 (45.9%)	[57]
SYK	SYK GOF	Dominant	GoF	NR	37/67 (55.2%)	[58]
TBK1	TBK1 deficiency	Recessive	LoF	NR	252/538 (46.8%)	[59]
TNFAIP3	HA20	Dominant	LoF	19/90 (21.2%)	333/625 (53.3%)	[60]
TNFRSF1A	TRAPS	Dominant	LoF	24/184 (13.1%)	280/595 (47.1%)	[61]
TRNT1	SIFD	Recessive	LoF	5/48 (10.5%)	303/630 (48.1%)	[62]
UBA1	VEXAS	Somatic	LoF	6/22 (27.3%)	299/662 (45.2%)	[63]
WDR1	PFIT	Recessive	LoF	0/9	148/514 (28.8%)	[64]

For the Infevers registry (https://infevers.umai-montpellier.fr/web/), variants of uncertain significance (VUS) and unsolved cases were reported relative to the total number of entries. For the ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/), VUS and conflicting classifications were reported relative to the total number of short (<50bps) classified variants.

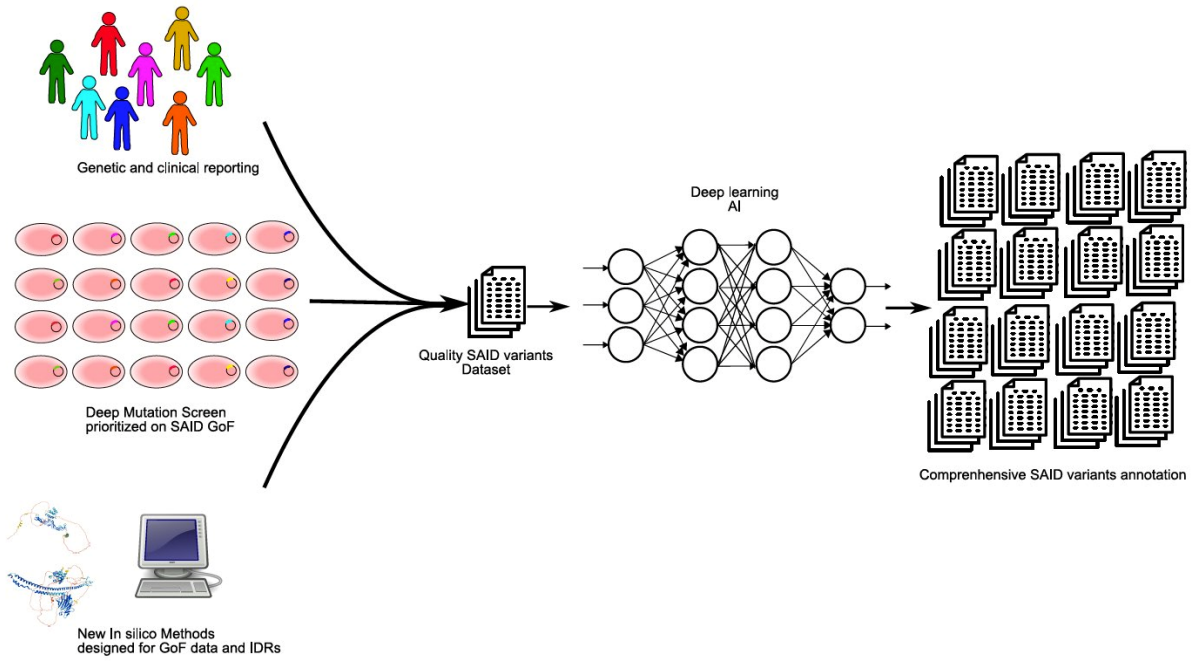
Abbreviations: NR, not reported; AID, Autoinflammatory Disease; AIPDS, Autoinflammation with Periodic Fever, Dermatitis and Splenomegaly; APLAID, Autoinflammation and PLCγ2-associated Antibody Deficiency; CAIN, C/EBPE-associated Autoinflammation and Immune impairment of Neutrophils; CAMPS, CARD14-Mediated Psoriasis; CAPS, Cryopyrin-Associated Periodic Syndrome; CRIA, Cleavage-resistant RIPK1-induced Autoinflammatory Syndrome; DADA2, Deficiency of Adenosine Deaminase 2; DIRA, Deficiency of the Interleukin-1 Receptor Antagonist; DITRA, Deficiency of the Interleukin-36 Receptor Antagonist; GoF, Gain of Function; HLH, Hemophagocytic Lymphohistiocytosis; LoF, Loss of Function; LIRSA, IL-1 Receptor Antagonist Syndrome (autosomal dominant form); MAS, Macrophage Activation Syndrome; MKD, Mevalonate Kinase Deficiency; MSPC, Multiple Self-healing Palmoplantar Carcinoma; NAIAD, NLRP1-associated Autoinflammation with Arthritis and Dyskeratosis; NEMO, NF-κB Essential Modulator; NOCARCH, Neonatal-Onset Cytopenia with Dyshaematopoiesis, Autoinflammation, Rash, and HLH; ORAS, OTULIN-Related Autoinflammatory Syndrome; PAAD, Pyrin-Associated Autoinflammation with Disease; PFIT, Periodic Fevers with Immunodeficiency and Thrombocytopenia; PLAID, PLCγ2-associated Antibody Deficiency and Immune Dysregulation; ROSAH, Retinal dystrophy, Optic nerve oedema, Splenomegaly, Anhidrosis, Headache; SAID, Systemic Autoinflammatory Disease; SIFD, Sideroblastic Anaemia with Immunodeficiency, Fevers, and Developmental delay; sJIA, systemic Juvenile Idiopathic Arthritis; TIM3, T-cell Immunoglobulin and Mucin-domain containing-3; TRAPS, Tumor necrosis factor Receptor-Associated Periodic Syndrome; VEXAS, Vacuoles, E1 enzyme, X-linked, Autoinflammatory, Somatic.

Supplementary Figure 1 | AlphaFold-predicted structures of proteins implicated in SAID and IDR



Structural models for five key proteins associated with systemic autoinflammatory diseases (SAIDs) are shown: Pyrin, NLRP3, TNFRSF1A, MVK (mevalonate kinase), and PSTPIP1. Many of these proteins contain intrinsically disordered regions (IDR), which appear as low-confidence segments (orange) in AlphaFold predictions, highlighting regions of structural flexibility possibly relevant to disease mechanisms while poorly amenable to in silico predictions using current computational methods. The structures have been extracted from the AlphaFold database [64], under a Creative Commons Attribution 4.0 (CC-BY 4.0) licence terms.

Supplementary Figure 2 | Roadmap to VUS eradication



Large scale mutagenesis screens should focus on systemic autoinflammatory disease (SAID) genes with Gain-of-function (GoF) variants and be combined to novel in silico methods specifically adressing the challenges of GoF and intrinsically-disorder regions (IDR)-containing proteins. Finally, sharing of genomic and clinical data on SAID patients will be key to generate a dataset of SAID variants with high quality annotation. This dataset could be then used by Aritificial intelligence (AI) and deep-learning models to generate a comprehensive dataset of all possible human SAID variants.

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