RHEUMATOLOGY

Rheumatology 2019;58:vi44–vi55
doi:10.1093/rheumatology/kez294

Current and future advances in genetic testing in systemic autoinflammatory diseases

Oskar Schnappauf [1] and Ivona Aksentijevich[1]

Abstract

Systemic autoinflammatory diseases (SAIDs) are a group of inflammatory disorders caused by dysregulation in the innate immune system that leads to enhanced immune responses. The clinical diagnosis of SAIDs can be difficult since individually these are rare diseases with considerable phenotypic overlap. Most SAIDs have a strong genetic background, but environmental and epigenetic influences can modulate the clinical phenotype. Molecular diagnosis has become essential for confirmation of clinical diagnosis. To date there are over 30 genes and a variety of modes of inheritance that have been associated with monogenic SAIDs. Mutations in the same gene can lead to very distinct phenotypes and can have different inheritance patterns. In addition, somatic mutations have been reported in several of these conditions. New genetic testing methods and databases are being developed to facilitate the molecular diagnosis of SAIDs, which is of major importance for treatment, prognosis and genetic counselling. The aim of this review is to summarize the latest advances in genetic testing for SAIDs and discuss potential obstacles that might arise during the molecular diagnosis of SAIDs.

Key words: autoinflammatory diseases, mosaicism, digenic inheritance, genetic testing, gene panel, NGS

SUPPLEMENT

Rheumatology key messages

- Diagnosis of systemic autoinflammatory diseases is hampered by paucity of clinical criteria and functional diagnostic tests.
- Genetic testing can greatly assist clinicians with diagnosis and selecting an appropriate treatment.
- About 60% of patients with suspected monogenic systemic autoinflammatory diseases are still molecularly undiagnosed.

Introduction

Autoinflammatory diseases are caused by defects in genes that regulate innate immunity leading to excessive production of proinflammatory cytokines such as IL-1, IL-6, TNF and type I interferons [1]. Typically, patients present with flares of inflammation manifesting with fever, elevated acute phase reactants, and with a range of tissue- and organ-specific manifestations. Although there are distinct clinical features that can help with the differential diagnosis of systemic autoinflammatory diseases (SAIDs), there is a considerable overlap in clinical presentations. The most predictive criteria include age of onset, duration of fever and disease flares, presence and type of skin rash, CNS involvement, positive family history, and patient's ancestry [2]. However, several problems exist regarding the applicability of clinical diagnoses for SAIDs. First, with the exception of prototypic autoinflammatory disorders, hereditary recurrent fevers (HRFs), clinical classification and/or clinical diagnostic criteria have not been developed for the majority of known disorders. Second, the proposed clinical criteria lacked accuracy, i.e. they were neither highly specific nor sensitive. This is comprehensible considering that mutations in different genes may lead to a similar disease (locus heterogeneity) as is the case in patients with familial cold autoinflammatory syndrome and Aicardi–Goutières syndrome, which have been linked to causal variants in three and eight different genes, respectively [3–6]. Mutations in the same gene can present with vast differences in disease severity and manifestation, as is the case with inflammasomopathies and pyrin-associated diseases (allelic heterogeneity). Differential diagnosis for SAIDs can also be challenging because patients with pathogenic

[1]Metabolic, Cardiovascular and Inflammatory Disease Genomics Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD, USA

Submitted 4 February 2019; accepted 22 May 2019

Correspondence to: Oskar Schnappauf, Metabolic, Cardiovascular and Inflammatory Disease Genomics Branch, National Human Genome Research Institute, National Institutes of Health, 10 Center Drive, Bethesda, MD 20892, USA. E-mail: oskar.schnappauf@nih.gov

mutations in different genes may present with similar clinical features (locus heterogeneity), as is the case in patients with familial cold autoinflammatory syndrome. Furthermore, disease expressivity is likely influenced by the presence of modifying genetic alleles, epigenetic modifications and environmental factors including therapies. New evidence-based classification criteria for four prototypic HRFs has been recently published and they include a combination of clinical features and genotypes and as such they have much higher specificity and sensitivity [7]. However, none of the existing clinical classification criteria have been developed or validated in genetically diverse populations. Thus, molecular diagnostics have become an integral part of clinical management and can assist clinicians in the process of choosing an appropriate treatment [8]. Biological therapies that target specific cytokines driving inflammation in SAIDs have been highly effective in suppressing disease activity in these oftentimes life-long and devastating conditions.

Monogenic autoinflammatory diseases and mode of inheritance

Autosomal dominant and autosomal recessive inheritance

Most monogenic SAIDs are inherited by one of the classical modes of inheritance, namely autosomal recessive or autosomal dominant (Table 1). An individual with an autosomal recessive genetic disorder must carry biallelic pathogenic variants in the same gene, either in a homozygous or in a compound heterozygous state. Each pathogenic mutation must be transmitted from each unaffected parent (*in trans*) unless one mutation arose as a *de novo* in the patient. Parental testing is highly recommended to confirm clinical diagnosis at the molecular level. The pathophysiology of recessively inherited diseases can usually be explained by a loss-of-function mechanism, which is when the loss of protein expression and/or function from both alleles causes the disease. These mutations are often found in genes that encode ubiquitously expressed enzymes and result in more global phenotypes that present early in life. The classic example of a recessive hereditary SAID is mevalonate kinase deficiency (MKD), caused by biallelic mutations in the *MVK* gene [9–11]. Other examples include deficiency of adenosine deaminase 2 (DADA2), caused by biallelic mutations in the *ADA2* gene, and sideroblastic anaemia with B cell immunodeficiency, periodic fevers and developmental delay, which is caused by biallelic mutations in the *TRNT1* gene [12–14].

Dominantly inherited disorders are caused by a single pathogenic variant that either arose *de novo* during gametogenesis or was inherited from an affected parent. However, there are examples of reduced penetrance in dominantly inherited traits whereby a causal mutation is inherited from an unaffected parent or is present in other unaffected family members. Typical examples of dominantly inherited SAIDs are inflammasomopathies, in which patients carry a heterozygous missense mutation that leads to gain in the protein function [15]. Another mechanism for dominantly inherited SAIDs is by a haploinsufficiency when the single functional copy of the gene is not enough to maintain the protein function. This example includes haploinsufficiency of A20 (HA20) that is caused by truncating mutations in the *TNFAIP3* gene [16]. Parental testing is necessary to confirm *de novo* mutations. Most dominantly inherited pathogenic variants are novel, but some are reported at a very low frequency in large public databases of human gene alleles.

Mosaicism

Mosaicism has been described in SAIDs and it is one mechanism that can lead to atypical or unexpected modes of inheritance. Mosaicism is caused by *de novo* mutations that occur post-zygotically, so called somatic mutations, which result in two genetically distinct cell populations within a single individual. Pathogenic somatic mutations in *NLRP3* were shown to cause neonatal onset multisystem inflammatory disease [also known as chronic infantile neurological cutaneous and articular syndrome (CINCA)], Muckle–Wells and Schnitzler syndrome and, depending on what cell types and tissues carry the altered genotype, disease manifestations and age of onset vary significantly [17–20]. Disease-causing somatic mutations are mainly seen in autosomal dominant inherited SAIDs and often only a small percentage of mutant cells, specifically myeloid lineage cells, is sufficient to initiate the inflammatory process [21].

If the somatic mutation is also found in other types of cells including gonadal tissue, it is called germline or gonadal mosaicism and the mutation has the potential to be passed on to the subsequent generation. Germline mosaicism has been reported in patients with Blau syndrome and with tumour necrosis factor receptor-1 (TNFR1)-associated periodic syndrome, caused by mutations in *NOD2* and *TNFRSF1A*, respectively [22, 23]. More recently, somatic mutations have been identified in keratinocytes of patients with STING-associated vasculopathy with onset in infancy (SAVI) and in a patient with an NLR family CARD domain-containing protein 4 (NLRC4)-associated haemophagocytic lymphohistiocytosis (HLH) disease [24, 25].

Digenic inheritance

Digenism is another mechanism that causes non-Mendelian inheritance. While monogenic traits, which follow Mendelian inheritance, are caused by one (autosomal dominant) or two (autosomal recessive) mutations in a single gene, digenic disease manifests if mutations occur simultaneously in two distinct genes. Given that next generation sequencing (NGS) allows the sequencing of many genes in parallel, the discovery of disorders that exhibit a digenic or oligogenic inheritance is greatly facilitated.

Identification of two or more pathogenic variants in different genes in a single patient is not a confirmation of

Table 1 Monogenic systemic autoinflammatory disorders

Disease acronym	Gene(s)	Disease name	Mode of inheritance	Disease mechanism	OMIM number[a]
ADAM17 deficiency	*ADAM17*	ADAM17 deficiency	AR	LoF	614328
AGS (Type 1-7)	*ADAR*, *DNASE2*, *IFIH1*, *RNASEH2A*, *RNASEH2B*, *RNASEH2C*, *SAMHD1*, *TREX1*	Aicardi–Goutières syndrome	AR (type 2-6) or AD/AR (type 1) or AD (type7)	LoF (type 1-6, DNase II deficiency), GoF (type 7)	615010, 615846, 610333, 610181, 610329, 612952, 225750
AIADK	*NLRP1*	Autoinflammation with arthritis and dyskeratosis	AD/AR	GoF	617388
AIFEC/NLRC4-MAS/FCAS4	*NLRC4*	Autoinflammation with infantile enterocolitis/NLRC4 macrophage activation syndrome/ familial cold autoinflammatory syndrome 4	AD	GoF	616115, 616050
AILJK	*COPA*	Autoimmune interstitial lung, joint, and kidney disease	AD	Dominant negative	616414
Blau syndrome	*NOD2*	Blau syndrome/early-onset sarcoidosis	AD	GoF	186580
CAPS	*NLRP3*	Cryopyrin-associated periodic syndromes (FCAS, MWS, NOMID/CINCA)	AD	GoF	120100, 191900, 607115
CAMPS/PSORS2	*CARD14*	CARD14-mediated psoriasis	AD	GoF	173200, 602723
Cherubism	*SH3BP2*	Cherubism	AD	GoF/dominant negative	118400
DADA2	*ADA2*	Deficiency of adenosine deaminase 2	AR	LoF	615688
DIRA	*IL1RN*	Deficiency of IL-1-receptor antagonist	AR	LoF	612852
DITRA	*IL36RN*	Deficiency of IL-36-receptor antagonist	AR	LoF	614204
EOIBD/IL-10 deficiency	*IL10*, *IL10RA*, *IL10RB*	Early-onset inflammatory bowel disease	AR	LoF	613148
FCAS2	*NLRP12*	Familial cold autoinflammatory syndrome 2	AD	LoF	611762
FMF	*MEFV*	Familial Mediterranean fever	AR	GoF	249100
PAAD/PAAND	*MEFV*	Pyrin-associated dominant diseases/pyrin-associated autoinflammation with neutrophilic dermatosis	AD	GoF	134610
AIADK/FKLC/MSPC	*NLRP1*	Familial keratosis lichenoides chronica/multiple self-healing palmoplantar carcinoma	AD	GoF	617388, 615225
H syndrome	*SLC29A3*	Histiocytosis lymph adenopathy plus syndrome	AR	LoF	602782
HA20	*TNFAIP3*	Haploinsufficiency of A20	AR	LoF	616744
HIDS/MKD	*MVK*	Mevalonate kinase deficiency/ hyperimmunoglobulinaemia D syndrome	AR	LoF	260920, 610377
HOIL1 deficiency	*RBCK1 (HOIL1)*	HOIL1 deficiency	AR	LoF	615895
HOIP deficiency	*RNF31 (HOIP)*	HOIP deficiency	AR	LoF	NA
LACC1-associted diseases	*LACC1*	LACC1-associated systemic JIA and early-onset IBD	AR	LoF	NA
LPIN2 deficiency/ Majeed	*LPIN2*	Majeed syndrome	AR	LoF	609628
OTULIN deficiency/ORAS	*OTULIN*	OTULIN-associated autoinflammatory syndrome	AR	LoF	617099
PAPA	*PSTPIP1*	Pyogenic arthritis, pyoderma gangrenosum and acne syndrome	AD	Not known	604416
PFIT	*WDR1*	Periodic fever, immunodeficiency, and thrombocytopenia	AR	LoF	NA

(continued)

Table 1 Continued

Disease acronym	Gene(s)	Disease name	Mode of inheritance	Disease mechanism	OMIM number[a]
PLAID/FCAS3/ APLAID	*PLCG2*	Familial cold autoinflammatory syndrome 3/autoinflammation, antibody deficiency, and immune dysregulation syndrome	AD	GoF	614878, 614468
PRAAS/CANDLE	*PSMB8*, *PSMB4*, *PSMB9*, *PSMA3*	Proteasome-associated autoinflammatory syndromes/chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature	AR	LoF	256040, 617591
Pustular psoriasis/ PSOR15	*AP1S3*	Generalized pustular psoriasis	AD	LoF	616106
SAVI	*TMEM173*	STING-associated vasculopathy with onset in infancy	AD	GoF	615934
SIFD	*TRNT1*	Sideroblastic anaemia with immunodeficiency, fevers and developmental delay	AR	LoF	616084
SPENCD	*ACP5*	Spondyloenchondrodysplasia with immune dysregulation	AR	LoF	607944
TRAPS	*TNFRSF1A*	TNFR1-associated periodic syndrome	AD	Not known	142680
TRAPS11	*TNFRSF11*	TNFR11-associted periodic syndrome	AD	Not known	603499
USP18 deficiency	*USP18*	USP18 deficiency	AR	LoF	617397

[a]No OMIM numbers available for DNASE2-associated AGS, HOIP deficiency, LACC1-associted diseases and PFIT. AD: autosomal dominant; AR: autosomal recessive; CARD14: caspase recruitment domain-containing protein 14; CINCA: chronic infantile neurological cutaneous and articular syndrome; FCAS: familial cold autoinflammatory syndrome; GoF: gain of function; HOIL1: heme-oxidized IRP2 ubiquitin ligase 1; HOIP: HOIL1-interacting protein; LACC1: laccase domain containing 1; LoF: loss of function; MWS: Muckle–Wells syndrome; NA: not applicable; NLRC4: NLR family CARD domain-containing protein 4; NOMID: neonatal-onset multisystemic inflammatory disease; OMIM: Online Mendelian Inheritance in Man (https://www.omim.org/); OTULIN: OTU deubiquitinase with linear linkage specificity; PLCγ2: phospholipase C-2; STING: stimulator of interferon genes; TNFR: tumour necrosis factor receptor; USP18: ubiquitin specific peptidase 18.

digenism unless it is demonstrated by a family-based segregation analysis.

The digenic (double) pathogenic variants are usually identified in proteins that are part of the same multiprotein complex. A classic example of digenic SAIDs is the group of proteasome-associated autoinflammatory syndromes also known as chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature (CANDLE) syndrome. Most CANDLE syndrome patients have recessively inherited homozygous or compound heterozygous rare or novel mutations in the gene encoding the proteasome subunit β type 8 (PSMB8; β5i) [26–30]. However, in some patients with proteasome-associated autoinflammatory syndromes (PRAAS) only one mutation in *PSMB8* was found, which led to the hypothesis that these patients may carry a second pathogenic variant in any of the other genes encoding the subunits of the constitutive proteasome or the immune cell-specific immunoproteasome. Subsequently, a subset of these patients was found to carry heterozygous mutations in two different genes (*PSMB8*/*PSMA3* or *PSMB8*/*PSMB4* or *PSMB9*/*PSMB4*) [31]. In addition, a severe CANDLE phenotype with prominent neutrophilic dermatosis and immunodeficiency is linked to heterozygous mutations in proteasome maturation protein (POMP), which is essential for maturation of proteasomes [32].

Digenic and oligogenic inheritance has also been reported in other types of initially uncharacterized SAIDs [33–35]. The oligogenic type of inheritance is in the continuum between monogenic and polygenic inheritance, whereby a phenotype results from coinheritance of several low frequency variants (allele frequency 1–5%). The allele frequency is not low enough to explain association with rare diseases and these variants are often identified in unaffected parents. As the impact of these alleles on protein function is largely unknown, they are typically reported as variants of unknown clinical significance. These variants are often found in patients with non-specific and milder inflammatory phenotypes compared with patients who carry novel/rare variant(s) of the same gene [36]. They are thought to act by a synergistic effect to induce inflammation. Best known examples include p.R121Q (R92Q) and p.P75L (P46L) in *TNFRSF1A*, p.E148Q and p.P369S in *MEFV*, and p.V198M (V200M), p.R488K (R490K) and p.Q703K (Q705K) in *NLRP3*. Some of these variants have been identified at a higher frequency (also known as *burden of variants*) in patients with periodic fever, aphthous stomatitis, pharyngitis and adenitis syndrome [37–41].

Multifactorial inheritance and the contribution of environmental and epigenetic factors

Most known SAIDs are monogenic diseases, but there are also a number of multifactorial and complex SAIDs such as Behçet's disease, systemic juvenile idiopathic arthritis (sJIA), and Crohn's disease. The underlying hypothesis is that these common and genetically complex autoimmune/autoinflammatory disorders can be attributed to synergistic effects of common reduced-penetrance variants (population allele frequencies >5%) in the presence of appropriate environmental factors (e.g. pathogens). These susceptibility variants are identified by genome-wide association studies in large cohorts of unrelated patients and healthy controls. Known examples include risk alleles in *IL1A-IL1B*, *IL-10*, *IL23-IL12RB2*, *ERAP1* and HLA class I loci in patients with BD and in *AJAP1*, *COL11A1*, *HDAC9*, *ENC1* and HLA class II loci in patients with (sJIA) [42–44].

These complex genetic diseases are not suitable for genetic diagnosis and genetic counselling, as these risk variants or haplotypes are also found in asymptomatic people. However, without proper genetic testing of patients with presumably multifactorial diseases, a possible Mendelian genetic cause cannot be ruled out.

Over the past few years it has become apparent that the pathogenicity of SAIDs is not only determined by genetic factors, but that environmental and epigenetic factors additionally play a crucial role in disease expressivity. Epigenetic factors are defined as heritable changes in gene expression that do not affect the genomic DNA sequence. Because epigenetic changes are less stable and easier to modulate than DNA mutations, they serve to integrate environmental signals and to mediate interactions between environment and the resulting genomic output. The best understood epigenetic mechanisms comprise DNA methylation, histone modifications, remodelling of chromatin, and non-coding RNAs, and some of them have been associated with the pathogenesis of SAIDs [45–50].

Genetic testing methods for autoinflammatory diseases

Genetic testing for SAIDs is not absolutely required for classical and well-characterized diseases in certain populations (e.g. FMF and MKD in the Mediterranean and Dutch populations, respectively), but it might be important for predicting a disease course and for genetic counselling in a family. At the present time, universal recommendations for genetic testing in SAIDs do not exist except for prototypic HRFs: FMF, MKD, TNFR1-associated periodic syndrome and cryopyrin-associated periodic syndromes [51]. An appropriate genetic testing approach should be chosen depending on the phenotype of the patient and the suspected disease-causing gene [52]. Due to significant clinical overlap between different SAIDs, a differential diagnosis might require the sequential or simultaneous analysis of several genes. Prenatal diagnosis is not generally recommended, although this may change as new recessively inherited diseases with severe and potentially lethal phenotype are being identified, e.g. linear ubiquitin chain assembly complex deficiencies and Aicardi–Goutières syndrome [6, 53–55].

Sanger sequencing

Sanger sequencing still constitutes the mainstay of molecular testing for most SAIDs. This method is well-suited for low-throughput laboratories for which an NGS approach would not be profitable. It is recommended for patients with a clear clinical diagnosis (e.g. FMF, neonatal onset multisystem inflammatory disease/CINCA) or for testing mutational hotspots (e.g. exon 10 of the *MEFV* gene; exons 2–5 of the *TNFRSF1A* gene, exon 3 in the *NLRP3* gene) or to test for mutations in founder populations (e.g. FMF, MKD). Sanger sequencing can also assist in the confirmation of a biochemical diagnosis in patients with MKD or DADA2 and thereby contributes important information to genetic counselling. Furthermore, Sanger sequencing is also still indispensable in confirming variants that were identified via NGS methods. Nevertheless, performance and efficiency of Sanger sequencing in SAIDs is low, except for patients from founder populations with FMF and MKD.

NGS approaches

NGS panels are the current method of choice for patients who fulfil criteria for several possible diseases and/or present with ambiguous phenotypes. Initially, NGS was relatively expensive, but rapid advances in methodology and technology have turned it into a time- and cost-efficient sequencing technique. NGS is based on a new technology that generates and analyses millions of sequence-reads per run. Even though the different commercially available NGS platforms vary in their specific methodologies, the general sequencing process encompasses the same steps. First, the DNA samples are fragmented by either enzymatic digestion or sonication. The short fragments of DNA are then ligated to sample-specific adapters *in vitro*, followed by a so-called bridge-PCR step on a solid surface that is coated with complementary primers. Subsequently, the amplification products are sequenced by pyrosequencing, sequencing by ligation, or sequencing by synthesis. The resulting sequence-reads are then aligned to a reference genome, and finally variant-calling algorithms are applied to compare mapped reads to the reference genome and to identify potential variation [56].

NGS gene panels

NGS is now increasingly used for the diagnosis of autoinflammatory disorders in the clinical setting, mainly in the context of targeted gene panels. The complete coding sequence of a selected set of genes is specifically enriched and sequenced in these gene panels (Table 2). The SAID gene panels used by commercial and research diagnostic laboratories vary in their exact composition but usually coincide in the inclusion of the classical autoinflammatory genes (*ELANE*, *LPIN2*, *MEFV*, *MVK*, *NLRP12*, *NLRP3*, *NOD2*, *PSTPIP1* and *TNFRSF1A*) [57–60]. An overview of the available SAID gene panels can be found in the Genetic Testing Registry database

Table 2 Comparison of commonly used sequencing applications

Category	Sanger	Gene panel	WES	WGS
Number of genes	1–10	10–300	~20 000	Whole genome
Covered regions	Single region per run; up to 500 bp	Targeted regions in genome, i.e. coding regions of set of genes	Most coding regions, flanking non-coding sequences	Whole genome but some regions are missing, i.e. repetitive sequences
Detectable variants	SNVs and small deletions and insertions	SNVs and deletions and insertions (exonic/whole gene)	SNVs and deletions and insertions (exonic/whole gene)	All variants including large deletions and insertions and CNVs
Typical coverage	NA	200–1000×	30–100×	30–60×
Data size (GB)	0.01	<1	5–10	50–200
Estimated raw sequencing costs per sample (US$)[a]	10–20	200–500	800–1000	1500–2500

[a]Analysis costs not included. bp: base pair; CNV: copy number variant; GB: gigabyte; NA: not applicable; Sanger: Sanger sequencing; SNV: single nucleotide variant; WES: whole exome sequencing; WGS: whole genome sequencing.

(https://www.ncbi.nlm.nih.gov/gtr/). Diagnostic yield of a comprehensive gene testing panel that may include a couple of hundreds of immune-related genes is not higher than 30% [57, 58].

An NGS gene panel is also recommended if the presenting phenotype can be explained by somatic mutations. Those include, but are not limited to, *NLRP3*, *NOD2*, *TNFRSF1A*, *TNFAIP3* and *TMEM173*. Using a targeted gene panel as a genetic testing approach for an individual with an uncharacterized autoinflammatory syndrome also increases the probability of diagnosing SAIDs with digenic or oligogenic inheritance.

Whole exome sequencing

Depending on the specific capture kit for target enrichment, whole exome sequencing (WES) can be used to sequence a greater number of genes, or even almost the entire coding region of the human genome. While several studies demonstrated its increased diagnostic yield compared with NGS gene panels, WES is not yet used in routine diagnostics [61]. Hindrances for its clinical implementation include the higher demand in computational infrastructure, the still higher costs and the reduced read depth compared with gene panels (Table 2). It is also important to bear in mind that copy number variants (CNVs), depending on their size, can be easily missed by this technology. Another limiting factor is the mere presence of thousands of rare single variants without an obvious pathogenic effect in every individual's whole exome data [62]. This makes the interpretation of genetic variation in WES a complex and time-consuming process.

Nevertheless, WES should be considered in undiagnosed cases and, if possible, in the context of a family-based trio approach. Emerging data demonstrate that the WES leads to improved diagnostic yield and reduced costs compared with standard diagnostic tests if performed early during the diagnostic process [63, 64]. Furthermore, from a research perspective, WES encompasses the capability to discover novel variants in genes that were already known to cause disease, and even novel genes not yet associated to human disease may be uncovered.

Many genetic testing providers also offer a so-called exome-slice technology. This approach still captures and sequences the whole exome, but the analysis is informatically limited to a specific gene list. This method is best suited for individuals with a clearly defined phenotype and/or where a comprehensive gene panel is not available. Since the analytic pipeline will only report data on genes included in the predefined list the burden of false-positive variants will be reduced.

Whole genome sequencing

Compared with WES, whole genome sequencing (WGS) is intended to capture the complete human genome in an unbiased manner (Table 2). In addition to the detection of all disease-causing variants found by conventional approaches, WGS also has the potential to identify deep intronic variants and other cryptic mutations in non-coding regulatory regions and therefore increases the diagnostic yield [65–67]. Moreover, the genome-wide uniformly distributed coverage allows a much more reliable identification of CNVs as compared with WES. An additional important advantage of WGS is the opportunity to periodically reanalyse the genome-wide data for recently discovered or re-classified variants [68]. Despite the emerging evidence on the effectiveness and benefit of WGS in clinical diagnosis for a variety of genetic disorders, it is still mainly used for the discovery of novel disease genes in a research setting [69–71].

Classification criteria for genetic variation

As the number of variants detected by NGS approaches is much higher compared with Sanger sequencing, the interpretation and comprehension of sequence variation and causality have become much more complex. The

correct estimation of the clinical significance of genetic variation therefore necessitates clear and thorough guidelines in order to standardize the interpretation process. This need led to the development of a more structured workflow for variant interpretation by the American College of Medical Genetics and Genomics and the Association for Molecular Pathology in 2015 [72]. Their guidelines recommend an assessment of the different pieces of evidence for or against pathogenicity for each variant. The different criteria are then combined according to a scoring scheme in order to apply a final variant classification from a five-tier classification system (benign, likely benign, variants of unknown clinical significance, likely pathogenic, or pathogenic). Specifically, for HRFs the International Study Group for Systemic Autoinflammatory Diseases recently applied a new workflow for the classification of genetic variants in four genes (*MEFV*, *TNFRSF1A*, *NLRP3* and *MVK*) [73].

The most common pathogenic variants seen in the genes associated with prototypic HRF syndromes are non-synonymous nucleotide changes, and except for *MVK*, structural mutations (deletions, duplications, rearrangement) are infrequent. More recently, protein truncating mutations were reported in *IL1RN*, *IL36RN* and *TNFAIP3* genes associated with deficiency of IL-1 receptor antagonist, deficiency of IL-36 receptor antagonist (DITRA) and HA20. Rare large genomic deletions were identified in the *IL1RN* [74], *ADA2* [75] and *TNFAIP3* gene loci [76]. A comprehensive list of classified variants associated with autoinflammatory diseases can be found in the online database Infevers (https://infevers.umai-montpellier.fr/web/index.php) [77].

Evidence based on computational predictions

Most of the American College of Medical Genetics and Genomics /Association for Molecular Pathology variant interpretation criteria rely on *in silico* prediction of the effect of a specific variant on protein function. In general, these criteria use current knowledge on functional and clinical impact of similar variants to predict the pathogenicity of the variant in question. Clearly, the predictive value of this category increases with more knowledge on the molecular disease mechanism as well as the functional domains of a specific gene or protein. For instance, the very strong criteria for pathogenicity can be applied for protein-truncating variants (i.e. nonsense, frameshift, canonical splice site, single-exon or multiexon deletion) only if the underlying mechanism of pathogenesis is known to be loss of function. Other criteria within this category rely on the existing knowledge of variants within important functional regions of a protein.

Evidence based on population data

Population data are an important line of evidence to support the pathogenicity of identified variants. The allele frequency of severe disease-causing alleles is expected to be rare in the general population and a frequency of $>5\%$ in any population is considered a 'stand-alone' benign classification (exceptions exist for well-known founder alleles including several variants in the *MEFV* gene).

Population-wide variation resources include the Exome Aggregation Consortium (http://exac.broadinstitute.org), the Genome Aggregation Database (http://gnomad.broadinstitute.org), and the 1000 Genomes Project (http://www.internationalgenome.org) [78, 79]. However, when applying these criteria, it is essential to recognize factors that can affect allelic population frequency such as disease prevalence, expressivity, penetrance and genetic heterogeneity [80].

Evidence based on functional studies

Molecular and cellular biology data on the effect of a specific variant on protein function is taken into account in this category of evidence. Not all functional experiments are useful in predicting the pathogenicity of a specific variant and it is extremely important to question the biological relevance of a specific assay to disease mechanism and manifestation. For example, although many autoinflammatory diseases are mediated by the cytokine IL-1β, pathogenicity of novel NACHT, LRR and PYD domains-containing protein 3 (NLRP3) variants should not be assessed by measuring the cytokine production in patients' serum samples as the serum levels of IL-1β are functionally irrelevant. A more reliable functional assay is to measure the IL-1β production in supernatants of stimulated peripheral blood cells. The appropriate functional IL-1β test should be consistently used in research and clinical testing. In addition, the subjective nature and variability of functional experiments can cause significant interlaboratory differences in the interpretation of clinical relevance of candidate variants and substantial efforts are made to increase concordance in variant prediction [81]. It is therefore essential that the functional tests have been validated in a clinical diagnostic laboratory setting to achieve high clinical validity (accuracy) and utility. Specific functional tests for SAID causal genes are still scarce with the exception of diseases caused by enzyme deficiencies: MKD, DADA2 and phospholipase Cγ2 (PLCγ2)-associated diseases [82, 83].

Evidence based on mode of inheritance and segregation

Because monogenic disorders are expected to be inherited in a Mendelian fashion, the evidence of segregation of a specific variant in a family can also be used for the interpretation of its clinical significance. For instance, the occurrence of a variant *de novo* is considered strong evidence of pathogenicity if several criteria are met: confirmation of maternity and paternity; presence in a gene associated to a disease that is consistent with the phenotype; parents must definitely be unaffected. For a dominantly inherited disorder, the co-segregation of disease in affected family members can be used as supporting evidence of pathogenicity. In contrast, inheritance of a variant from an unaffected parent is considered strong evidence that the variant is benign. The application of the described criteria relies heavily on an accurate and

correct phenotypic evaluation of all included family members and can therefore be influenced by factors such as penetrance, expressivity and age of disease onset.

Confirmation of the diagnosis

As for any Mendelian conditions, the definitive genetic diagnosis of SAIDs is based on the finding of unambiguous mutations in the causative genes. Theoretically, finding two biallelic clearly pathogenic mutations (assessed by studying the parental alleles) in recessive diseases or one mutation in dominant diseases would confirm the diagnosis. Heterozygous mutations must be either already known to cause the disease or, if they are novel and/or *de novo* variants, laboratories should carefully evaluate the clinical relevance of these variants. In all other cases, patient care should be based on clinical grounds and should not prevent initiation of therapies.

Limitations of molecular diagnosis

Limited sensitivity of molecular test

Limitations in sensitivity can occur if only a subset of the known disease-causing mutations are tested by a given method. For example, a sequencing approach that focuses on the coding regions of a specific gene might miss deep intronic variants or variants in *cis* regulatory elements that can affect protein translation and expression. However, to date, there are no deep intronic variants or variants in regulatory elements described for SAIDs.

In some cases, the second cryptic (non-coding) mutation may not be easily identified and require additional analyses. For DADA2, for instance, several pathogenic deletions and duplications comprising one or several exons of the *ADA2* gene have been described [12, 84–86]. Depending on the size of those CNVs, Sanger sequencing alone would not be sufficient for their detection and more elaborate laboratory techniques such as multiplex ligation-dependent probe amplification, digital droplet PCR (ddPCR) or NGS approaches are needed.

Another explanation for limited testing sensitivity is somatic mutations that are below the detection threshold of a specific genetic testing method. Even though Sanger sequencing is a reliable and robust experimental verifying method, it is not sensitive enough to detect somatic mutations below a frequency of ~20% [87, 88].

Other factors contributing to an inconclusive genetic test

Other factors that may contribute to an apparently negative genetic test can come from misinterpretation of the results. The best example to illustrate is the *MEFV* gene that causes FMF. Based on segregation analyses, FMF has long been considered a recessive disorder. As the frequency of pathogenic variants in *MEFV* varies significantly between different populations, the estimated sensitivity for finding biallelic mutations ranges from 70% to 95% depending on the ethnic background of the patient [89, 90]. However, in some FMF cases, only a single known pathogenic mutation in *MEFV* is identified [91–94]. Furthermore, heterozygous mutations in other domains of pyrin have been found in families with clearly dominantly inherited pyrin-associated diseases [95, 96]. One of them is pyrin-associated autoinflammation with neutrophilic dermatosis (PAAND), a dominantly inherited disease caused by amino acid substitution at position 242 (S242R) or 244 (E244K), which is critical for pyrin inhibition [97, 98]. The phenotype of PAAND patients is very similar to pyogenic arthritis, pyoderma gangrenosum and acne (PAPA) syndrome that is caused by heterozygous mutations in the *PSTPIP1* gene. Interestingly, proline–serine–threonine phosphatase-interacting protein 1 (PSTPIP1) and pyrin are proteins known to interact, and they associate with the cytoskeleton [99–101]. Similar to *MEFV*, a complex pattern of inheritance has been described for the *NLRP1* gene-associated diseases [102, 103].

Finally, a negative genetic test result in a patient with an apparent SAID can simply be due to the fact that the disease-causing gene has not yet been identified.

Conclusion

The number of molecularly characterized SAIDs has grown rapidly over the past 20 years and has been fuelled by the development of new sequencing technologies. Despite the major progress in the identification of novel genes and pathways associated with SAIDs, many more disease genes are still unknown and over 50% of patients are mutation-negative [58, 104]. A periodic re-evaluation and re-analysis of the genetic testing opportunities is therefore recommended for undiagnosed patients. New NGS technologies and algorithms are being developed to help with detection and classification of all genetic variants. Novel concepts regarding modes of inheritance and interpretation of variant pathogenicity will require a systematic review and update of genetic diagnostic strategies and new guidance on standardization and reporting of genetic tests [51].

Funding: This paper was published as part of a supplement funded by an educational grant from Sobi. The authors were entirely independent, and received no remuneration for their work.

Disclosure statement: The authors have declared no conflicts of interest.

References

1 Manthiram K, Zhou Q, Aksentijevich I, Kastner DL. The monogenic autoinflammatory diseases define new pathways in human innate immunity and inflammation. Nat Immunol 2017;18:832–42.

2 Federici S, Sormani MP, Ozen S *et al*. Evidence-based provisional clinical classification criteria for autoinflammatory periodic fevers. Ann Rheum Dis 2015;74:799–805.

3 Hoffman HM, Mueller JL, Broide DH, Wanderer AA, Kolodner RD. Mutation of a new gene encoding a putative pyrin-like protein causes familial cold autoinflammatory

syndrome and Muckle-Wells syndrome. Nat Genet 2001;29:301–5.

4 Jeru I, Le Borgne G, Cochet E *et al*. Identification and functional consequences of a recurrent NLRP12 missense mutation in periodic fever syndromes. Arthritis Rheum 2011;63:1459–64.

5 Kitamura A, Sasaki Y, Abe T, Kano H, Yasutomo K. An inherited mutation in NLRC4 causes autoinflammation in human and mice. J Exp Med 2014;211:2385–96.

6 Uggenti C, Lepelley A, Crow YJ. Self-awareness: nucleic acid-driven inflammation and the type I interferonopathies. Annu Rev Immunol 2019;37:247.

7 Gattorno M, Hofer M, Federici S *et al*. Classification criteria for autoinflammatory recurrent fevers. Ann Rheum Dis 2019 (in press) doi: 10.1136/annrheumdis-2019-215048.

8 Federici S, Vanoni F, Ben-Chetrit E *et al*. An international Delphi survey for the definition of new classification criteria for familial Mediterranean fever, mevalonate kinase deficiency, TNF receptor-associated periodic fever syndromes, and cryopyrin-associated periodic syndrome. J Rheumatol 2019;46:429–36.

9 Drenth JP, Cuisset L, Grateau G *et al*. Mutations in the gene encoding mevalonate kinase cause hyper-IgD and periodic fever syndrome. Nat Genet 1999;22:178–81.

10 Stoffels M, Simon A. Hyper-IgD syndrome or mevalonate kinase deficiency. Curr Opin Rheumatol 2011;23:419–23.

11 Houten SM, Romeijn GJ, Koster J *et al*. Identification and characterization of three novel missense mutations in mevalonate kinase cDNA causing mevalonic aciduria, a disorder of isoprene biosynthesis. Hum Mol Genet 1999;8:1523–8.

12 Zhou Q, Yang D, Ombrello AK *et al*. Early-onset stroke and vasculopathy associated with mutations in ADA2. New Engl J Med 2014;370:911–20.

13 Navon Elkan P, Pierce SB, Segel R *et al*. Mutant adenosine deaminase 2 in a polyarteritis nodosa vasculopathy. New Engl J Med 2014;370:921–31.

14 Chakraborty PK, Schmitz-Abe K, Kennedy EK *et al*. Mutations in TRNT1 cause congenital sideroblastic anemia with immunodeficiency, fevers, and developmental delay (SIFD). Blood 2014;124:2867–71.

15 de Torre-Minguela C, Mesa Del Castillo P, Pelegrin P. The NLRP3 and pyrin inflammasomes: implications in the pathophysiology of autoinflammatory diseases. Front Immunol 2017;8:43.

16 Zhou Q, Wang H, Schwartz DM *et al*. Loss-of-function mutations in TNFAIP3 leading to A20 haploinsufficiency cause an early-onset autoinflammatory disease. Nat Genet 2016;48:67–73.

17 Saito M, Nishikomori R, Kambe N *et al*. Disease-associated CIAS1 mutations induce monocyte death, revealing low-level mosaicism in mutation-negative cryopyrin-associated periodic syndrome patients. Blood 2008;111:2132–41.

18 Lasiglie D, Mensa-Vilaro A, Ferrera D *et al*. Cryopyrin-associated periodic syndromes in Italian patients: evaluation of the rate of somatic NLRP3 mosaicism and phenotypic characterization. J Rheumatol 2017;44:1667–73.

19 Zhou Q, Aksentijevich I, Wood GM *et al*. Brief Report: Cryopyrin-associated periodic syndrome caused by a myeloid-restricted somatic NLRP3 mutation. Arthritis Rheumatol (Hoboken, NJ) 2015;67:2482–6.

20 Labrousse M, Kevorkian-Verguet C, Boursier G *et al*. Mosaicism in autoinflammatory diseases: cryopyrin-associated periodic syndromes (CAPS) and beyond. A systematic review. Crit Rev Clin Lab Sci 2018;55:432–42.

21 de Koning HD, van Gijn ME, Stoffels M *et al*. Myeloid lineage-restricted somatic mosaicism of NLRP3 mutations in patients with variant Schnitzler syndrome. J Allergy Clin Immunol 2015;135:561–4.e4.

22 Mensa-Vilaro A, Cham WT, Tang SP *et al*. Brief Report: First identification of intrafamilial recurrence of blau syndrome due to gonosomal NOD2 mosaicism. Arthritis Rheumatol (Hoboken, NJ) 2016;68:1039–44.

23 Rowczenio DM, Trojer H, Omoyinmi E *et al*. Brief Report: Association of tumor necrosis factor receptor-associated periodic syndrome with gonosomal mosaicism of a novel 24-nucleotide TNFRSF1A deletion. Arthritis Rheumatol (Hoboken, NJ) 2016;68:2044–9.

24 Liu Y, Jesus AA, Marrero B *et al*. Activated STING in a vascular and pulmonary syndrome. New Engl J Med 2014;371:507–18.

25 Liang J, Alfano DN, Squires JE *et al*. Novel NLRC4 mutation causes a syndrome of perinatal autoinflammation with hemophagocytic lymphohistiocytosis, hepatosplenomegaly, fetal thrombotic vasculopathy, and congenital anemia and ascites. Pediatr Dev Pathol 2017;20:498–505.

26 Agarwal AK, Xing C, DeMartino GN *et al*. PSMB8 encoding the beta5i proteasome subunit is mutated in joint contractures, muscle atrophy, microcytic anemia, and panniculitis-induced lipodystrophy syndrome. Am J Hum Genet 2010;87:866–72.

27 Kitamura A, Maekawa Y, Uehara H *et al*. A mutation in the immunoproteasome subunit PSMB8 causes autoinflammation and lipodystrophy in humans. J Clin Invest 2011;121:4150–60.

28 Arima K, Kinoshita A, Mishima H *et al*. Proteasome assembly defect due to a proteasome subunit beta type 8 (PSMB8) mutation causes the autoinflammatory disorder, Nakajo-Nishimura syndrome. Proc Natl Acad Sci USA 2011;108:14914–9.

29 Liu Y, Ramot Y, Torrelo A *et al*. Mutations in proteasome subunit beta type 8 cause chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature with evidence of genetic and phenotypic heterogeneity. Arthritis Rheum 2012;64:895–907.

30 de Jesus AA, Brehm A, VanTries R *et al*. Novel proteasome assembly chaperone mutations in PSMG2/PAC2 cause the autoinflammatory interferonopathy CANDLE/PRAAS4. J Allergy Clin Immunol 2019;143:1939.

31 Brehm A, Liu Y, Sheikh A *et al*. Additive loss-of-function proteasome subunit mutations in CANDLE/PRAAS patients promote type I IFN production. J Clin Invest 2015;125:4196–211.

32 Poli MC, Ebstein F, Nicholas SK *et al*. Heterozygous truncating variants in POMP escape nonsense-mediated decay and cause a unique immune dysregulatory syndrome. Am J Hum Genet 2018;102:1126–42.

33 Touitou I, Perez C, Dumont B, Federici L, Jorgensen C. Refractory auto-inflammatory syndrome associated with digenic transmission of low-penetrance tumour necrosis factor receptor-associated periodic syndrome and cryopyrin-associated periodic syndrome mutations. Ann Rheum Dis 2006;65:1530–1.

34 Murarasu A, Dode C, Sarrabay G *et al*. Digenic MEFV/TNFRSF1A autoinflammatory syndrome with relapsing aseptic neutrophilic meningitis and chronic myelitis. Clin Exp Rheumatol 2017;108(Suppl 35):128–9.

35 Neocleous V, Byrou S, Toumba M *et al*. Evidence of digenic inheritance in autoinflammation-associated genes. J Genet 2016;95:761–6.

36 Kuemmerle-Deschner JB, Verma D, Endres T *et al*. Clinical and molecular phenotypes of low-penetrance variants of NLRP3: diagnostic and therapeutic challenges. Arthritis Rheumatol (Hoboken, NJ) 2017;69:2233–40.

37 Grandemange S, Cabasson S, Sarrabay G *et al*. Clinical dose effect and functional consequences of R92Q in two families presenting with a TRAPS/PFAPA-like phenotype. Mol Genet Genomic Med 2017;5:110–6.

38 Dagan E, Gershoni-Baruch R, Khatib I, Mori A, Brik R. MEFV, TNF1rA, CARD15 and NLRP3 mutation analysis in PFAPA. Rheumatol Int 2010;30:633–6.

39 Kolly L, Busso N, von Scheven-Gete A *et al*. Periodic fever, aphthous stomatitis, pharyngitis, cervical adenitis syndrome is linked to dysregulated monocyte IL-1beta production. J Allergy Clin Immunol 2013;131:1635–43.

40 Di Gioia SA, Bedoni N, von Scheven-Gete A *et al*. Analysis of the genetic basis of periodic fever with aphthous stomatitis, pharyngitis, and cervical adenitis (PFAPA) syndrome. Sci Rep 2015;5:10200.

41 Yamagami K, Nakamura T, Nakamura R *et al*. Familial Mediterranean fever with P369S/R408Q exon3 variant in pyrin presenting as symptoms of PFAPA. Mod Rheumatol 2017;27:356–9.

42 Takeuchi M, Kastner DL, Remmers EF. The immunogenetics of Behçet's disease: A comprehensive review. J Autoimmun 2015;64:137–48.

43 Takeuchi M, Mizuki N, Meguro A *et al*. Dense genotyping of immune-related loci implicates host responses to microbial exposure in Behçet's disease susceptibility. Nat Genet 2017;49:438–43.

44 Ombrello MJ, Arthur VL, Remmers EF *et al*. Genetic architecture distinguishes systemic juvenile idiopathic arthritis from other forms of juvenile idiopathic arthritis: clinical and therapeutic implications. Ann Rheum Dis 2017;76:906.

45 Kirectepe AK, Kasapcopur O, Arisoy N *et al*. Analysis of MEFV exon methylation and expression patterns in familial Mediterranean fever. BMC Med Genet 2011;12:105.

46 Akkaya-Ulum YZ, Balci-Peynircioglu B, Karadag O *et al*. Alteration of the microRNA expression profile in familial Mediterranean fever patients. Clin Exp Rheumatol 2017;108(Suppl 35):90–4.

47 Aubert P, Suarez-Farinas M, Mitsui H *et al*. Homeostatic tissue responses in skin biopsies from NOMID patients with constitutive overproduction of IL-1beta. PLoS One 2012;7:e49408.

48 Latsoudis H, Mashreghi MF, Grun JR *et al*. Differential expression of miR-4520a associated with pyrin mutations in Familial Mediterranean Fever (FMF). J Cell Physiol 2017;232:1326–36.

49 Hortu HO, Karaca E, Sozeri B *et al*. Evaluation of the effects of miRNAs in familial Mediterranean fever. Clin Rheumatol 2018;38:635–43.

50 Wada T, Toma T, Matsuda Y *et al*. Microarray analysis of circulating microRNAs in familial Mediterranean fever. Mod Rheumatol 2017;27:1040–6.

51 Shinar Y, Obici L, Aksentijevich I *et al*. Guidelines for the genetic diagnosis of hereditary recurrent fevers. Ann Rheum Dis 2012;71:1599–605.

52 Rowczenio DM, Lachmann HJ. How to prescribe a genetic test for the diagnosis of autoinflammatory diseases? Presse Med 2019;48:e49–59.

53 Boisson B, Laplantine E, Dobbs K *et al*. Human HOIP and LUBAC deficiency underlies autoinflammation, immunodeficiency, amylopectinosis, and lymphangiectasia. J Exp Med 2015;212:939–51.

54 Boisson B, Laplantine E, Prando C *et al*. Immunodeficiency, autoinflammation and amylopectinosis in humans with inherited HOIL-1 and LUBAC deficiency. Nat Immunol 2012;13:1178–86.

55 Giannelou A, Zhou Q, Stoffels M *et al*. Immune dysregulation in patients with TRNT1 deficiency. Pediatr Rheumatol Online J 2015;13(Suppl 1): O88.

56 Goodwin S, McPherson JD, McCombie WR. Coming of age: ten years of next-generation sequencing technologies. Nat Rev Genet 2016;17:333.

57 Omoyinmi E, Standing A, Keylock A *et al*. A targeted next-generation sequencing gene panel for autoinflammation. Ann Rheum Dis 2016;75:667.2.

58 Omoyinmi E, Standing A, Keylock A *et al*. Clinical impact of a targeted next-generation sequencing gene panel for autoinflammation and vasculitis. PLoS One 2017;12:e0181874.

59 Nakayama M, Oda H, Nakagawa K *et al*. Accurate clinical genetic testing for autoinflammatory diseases using the next-generation sequencing platform MiSeq. Biochem Biophys Rep 2017;9:146–52.

60 Rusmini M, Federici S, Caroli F *et al*. Next-generation sequencing and its initial applications for molecular diagnosis of systemic auto-inflammatory diseases. Ann Rheum Dis 2016;75:1550–7.

61 Dillon OJ, Lunke S, Stark Z *et al*. Exome sequencing has higher diagnostic yield compared to simulated disease-specific panels in children with suspected monogenic disorders. Eur J Hum Genet 2018;26:644–51.

62 Vears DF, Senecal K, Borry P. Reporting practices for variants of uncertain significance from next generation sequencing technologies. Eur J Med Genet 2017;60:553–8.

63 Stark Z, Schofield D, Alam K *et al*. Prospective comparison of the cost-effectiveness of clinical whole-exome sequencing with that of usual care overwhelmingly supports early use and reimbursement. Genet Med 2017;19:867–74.

64 Stark Z, Lunke S, Brett GR *et al*. Meeting the challenges of implementing rapid genomic testing in acute pediatric care. Genet Med 2018;20:1554.

65 Lionel AC, Costain G, Monfared N *et al*. Improved diagnostic yield compared with targeted gene sequencing panels suggests a role for whole-genome sequencing as a first-tier genetic test. Genet Med 2018;20:435.

66 Belkadi A, Bolze A, Itan Y *et al*. Whole-genome sequencing is more powerful than whole-exome sequencing for detecting exome variants. Proc Natl Acad Sci USA 2015;112:5473–8.

67 Meynert AM, Ansari M, FitzPatrick DR, Taylor MS. Variant detection sensitivity and biases in whole genome and exome sequencing. BMC Bioinform 2014;15:247.

68 Costain G, Jobling R, Walker S *et al*. Periodic reanalysis of whole-genome sequencing data enhances the diagnostic advantage over standard clinical genetic testing. Eur J Hum Genet 2018;26:740–4.

69 Willig LK, Petrikin JE, Smith LD *et al*. Whole-genome sequencing for identification of Mendelian disorders in critically ill infants: a retrospective analysis of diagnostic and clinical findings. Lancet Respir Med 2015;3:377–87.

70 Taylor JC, Martin HC, Lise S *et al*. Factors influencing success of clinical genome sequencing across a broad spectrum of disorders. Nat Genet 2015;47:717–26.

71 Stavropoulos DJ, Merico D, Jobling R *et al*. Whole genome sequencing expands diagnostic utility and improves clinical management in pediatric medicine. NPJ Genom Med 2016;1:15012.

72 Richards S, Aziz N, Bale S *et al*. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015;17:405–24.

73 Van Gijn ME, Ceccherini I, Shinar Y *et al*. New workflow for classification of genetic variants' pathogenicity applied to hereditary recurrent fevers by the International Study Group for Systemic Autoinflammatory Diseases (INSAID). J Med Genet 2018;55:530–7.

74 Aksentijevich I, Masters SL, Ferguson PJ *et al*. An autoinflammatory disease with deficiency of the interleukin-1-receptor antagonist. N Engl J Med 2009;360:2426–37.

75 Zhou Q, Yang D, Ombrello AK *et al*. Early-onset stroke and vasculopathy associated with mutations in ADA2. N Engl J Med 2014;370:911–20.

76 Oda H, Kastner DL. Genomics, biology, and human illness: advances in the monogenic autoinflammatory diseases. Rheum Dis Clin North Am 2017;43:327–45.

77 Milhavet F, Cuisset L, Hoffman HM *et al*. The infevers autoinflammatory mutation online registry: update with new genes and functions. Hum Mutat 2008;29:803–8.

78 Lek M, Karczewski KJ, Minikel EV *et al*. Analysis of protein-coding genetic variation in 60,706 humans. Nature 2016;536:285.

79 The 1000 Genomes Project Consortium. A global reference for human genetic variation. Nature 2015;526:68.

80 Tarailo-Graovac M, Zhu JYA, Matthews A, van Karnebeek CDM, Wasserman WW. Assessment of the ExAC data set for the presence of individuals with pathogenic genotypes implicated in severe Mendelian pediatric disorders. Genet Med 2017;19:1300.

81 Harrison SM, Dolinsky JS, Knight Johnson AE *et al*. Clinical laboratories collaborate to resolve differences in variant interpretations submitted to ClinVar. Genet Med 2017;19:1096.

82 Neves JF, Doffinger R, Barcena-Morales G *et al*. Novel PLCG2 Mutation in a Patient With APLAID and Cutis Laxa. Front Immunol 2018;9:2863.

83 Zhou Q, Lee GS, Brady J *et al*. A hypermorphic missense mutation in PLCG2, encoding phospholipase Cgamma2, causes a dominantly inherited autoinflammatory disease with immunodeficiency. Am J Hum Genet 2012;91:713–20.

84 Uettwiller F, Sarrabay G, Rodero MP *et al*. ADA2 deficiency: case report of a new phenotype and novel mutation in two sisters. RMD Open 2016;2:e000236.

85 Fellmann F, Angelini F, Wassenberg J *et al*. IL-17 receptor A and adenosine deaminase 2 deficiency in siblings with recurrent infections and chronic inflammation. J Allergy Clin Immunol 2016;137:1189–96.e2.

86 Claassen D, Boals M, Bowling KM *et al*. Complexities of genetic diagnosis illustrated by an atypical case of congenital hypoplastic anemia. Cold Spring Harbor Mol Case Stud 2018;4,a003384.

87 Giardina T, Robinson C, Grieu-Iacopetta F *et al*. Implementation of next generation sequencing technology for somatic mutation detection in routine laboratory practice. Pathology 2018;50:389–401.

88 Rohlin A, Wernersson J, Engwall Y *et al*. Parallel sequencing used in detection of mosaic mutations: comparison with four diagnostic DNA screening techniques. Hum Mutat 2009;30:1012–20.

89 Yepiskoposyan L, Harutyunyan A. Population genetics of familial Mediterranean fever: a review. Eur J Hum Genet 2007;15:911.

90 Mikula M, Buller A, Sun W, Strom CM. Prevalence of known mutations in the familial Mediterranean fever gene (MEFV) in various carrier screening populations. Genet Med 2008;10:349–52.

91 Booty MG, Chae JJ, Masters SL *et al*. Familial Mediterranean fever with a single MEFV mutation: where is the second hit? Arthritis Rheum 2009;60:1851–61.

92 Marek-Yagel D, Berkun Y, Padeh S *et al*. Clinical disease among patients heterozygous for familial Mediterranean fever. Arthritis Rheum 2009;60:1862–6.

93 Kone-Paut I, Hentgen V, Guillaume-Czitrom S *et al*. The clinical spectrum of 94 patients carrying a single mutated MEFV allele. Rheumatology (Oxford) 2009;48:840–2.

94 Booth DR, Gillmore JD, Lachmann HJ *et al*. The genetic basis of autosomal dominant familial Mediterranean fever. QJM 2000;93:217–21.

95 Aldea A, Campistol JM, Arostegui JI *et al*. A severe autosomal-dominant periodic inflammatory disorder with renal AA amyloidosis and colchicine resistance associated to the MEFV H478Y variant in a Spanish kindred: an unusual familial Mediterranean fever phenotype or another MEFV-associated periodic inflammatory disorder? Am J Med Genet Part A 2004;124a:67–73.

96 Stoffels M, Szperl A, Simon A *et al*. MEFV mutations affecting pyrin amino acid 577 cause autosomal dominant autoinflammatory disease. Ann Rheum Dis 2014;73:455–61.

97 Masters SL, Lagou V, Jeru I *et al*. Familial autoinflammation with neutrophilic dermatosis reveals a regulatory mechanism of pyrin activation. Sci Transl Med 2016;8:332ra45.

98 Moghaddas F, Llamas R, De Nardo D *et al*. A novel Pyrin-Associated Autoinflammation with Neutrophilic Dermatosis mutation further defines 14-3-3 binding of pyrin and distinction to Familial Mediterranean Fever. Ann Rheum Dis 2017;76:2085–94.

99 Shoham NG, Centola M, Mansfield E *et al*. Pyrin binds the PSTPIP1/CD2BP1 protein, defining familial Mediterranean fever and PAPA syndrome as disorders in the same pathway. Proc Natl Acad Sci USA 2003;100:13501–6.

100 Akkaya-Ulum YZ, Balci-Peynircioglu B, Purali N, Yilmaz E. Pyrin-PSTPIP1 colocalises at the leading edge during cell migration. Cell Biol Int 2015;39:1384–94.

101 Waite AL, Schaner P, Richards N *et al*. Pyrin modulates the intracellular distribution of PSTPIP1. PLoS One 2009;4:e6147.

102 Grandemange S, Sanchez E, Louis-Plence P *et al*. A new autoinflammatory and autoimmune syndrome associated with NLRP1 mutations: nAIAD (NLRP1-associated autoinflammation with arthritis and dyskeratosis). Ann Rheum Dis 2017;76:1191–8.

103 Zhong FL, Mamai O, Sborgi L *et al*. Germline NLRP1 mutations cause skin inflammatory and cancer susceptibility syndromes via inflammasome activation. Cell 2016;167:187–202.e17.

104 Karacan İ, Balamir A, Uğurlu S *et al*. Diagnostic utility of a targeted next-generation sequencing gene panel in the clinical suspicion of systemic autoinflammatory diseases: a multi-center study. Rheumatol Int 2019;39:911–9.