Title: Genetic interferonopathies: an overview

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The authors declare no conflict of interest

Dr Eleftheriou is supported by an ARUK grant (grant 20164).
Abstract

The interferonopathies comprise an expanding group of monogenic diseases characterised by disturbance of the homeostatic control of interferon (IFN)-mediated immune responses. Although differing in the degree of phenotypic expression and severity, the clinical presentation of these diseases show a considerable degree of overlap, reflecting their common pathogenetic mechanisms. Increased understanding of the molecular basis of these mendelian disorders has led to the identification of targeted therapies for these diseases, which could also be of potential relevance for non-genetic IFN-mediated diseases such as systemic lupus erythematosus and juvenile dermatomyositis. In this paper we summarise the current knowledge of the molecular basis, clinical features, and treatment of monogenic interferonopathies.

Key words: Interferonopathies, Aicardi Goutières syndrome, proteasome, CANDLE, SAVI.
Introduction

The interferons (IFN) are signalling proteins synthetized and released by immune host cells in response to the presence of several pathogens such as viruses, bacteria, parasites and tumour cells (1-5). The induction, transmission, and resolution of the IFN-mediated immune response is tightly regulated, and finely-tuned by opposing augmenting and suppressive signals induced by host factors (1-5). These signals rapidly mobilize an effective antimicrobial response against the invading pathogen, while restraining the magnitude of the response to avoid excessive inflammatory responses thus limiting host injury (1-5). The interferonopathies are an expanding group of complex genetic disorders characterised by disturbance of the homeostatic control of these IFN mediated immune responses (Figure 1) (1-5). Although differing in the degree of phenotypic expression and severity, the clinical presentation of these diseases shows a considerable degree of overlap reflecting their common pathogenetic mechanisms (1-5). This paper summarises the current knowledge of the molecular basis, clinical features and treatments available for the monogenic interferonopathies (Table 1).

Table 1: The genetic interferonopathies

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<th>Disease</th>
<th>Gene (s)</th>
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<td>Proteasome associated autoinflammatory syndromes</td>
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<td>Proteasome pathway: responsible for regulating proteolysis in eukaryotic cells</td>
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<td>Aicardi-Goutières syndromes (types 1-7)</td>
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<td>Disorder</td>
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<tr>
<td>Leukodystrophy (RVCL)</td>
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<td>Spondyloenchondrodysplasia (SPENCD)</td>
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<td>Lysosomal acid phosphatase activity/osteoclastic dysfunction</td>
</tr>
<tr>
<td>Singleton-Merten Syndrome</td>
<td>IFIHI/DDX58</td>
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<tr>
<td>ISG15 deficiency</td>
<td>ISG15</td>
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**Proteasome associated autoinflammatory syndromes (PRAAS)**

Autoinflammatory diseases resulting from dysfunctional proteasomes are termed “proteasome-associated autoinflammatory syndromes” (PRAAS) (6-11). PRAAS include the Japanese autoinflammatory syndrome with lipodystrophy (JASL), Nakajo-Nishimura syndrome (NNS), joint contractures, muscular atrophy, microcytic anaemia, panniculitis-associated lipodystrophy (JMP) syndrome, and chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature (CANDLE) syndrome (6-13). Despite the different nomenclature, they probably represent the same spectrum of disease rather than discrete disease entities; unsurprisingly therefore, they have many overlapping clinical features, all of which result from loss-of-function mutations in genes encoding proteasome components, and causing proteasome malfunction and proteostasis (proteome homeostasis: the process by which cells control the abundance and folding of the proteome) (6-11).

**Aetiology/pathogenesis**

**Proteasome, thymoproteasome and immunoproteasome**

The 26S proteasome complex is an evolutionarily conserved cylindrical organelle which plays an essential role in ubiquitin-tagged-protein degradation, and is expressed in all body cells (6-11). It comprises a single catalytic 20S proteasome with 19S regulatory components attached to the ends (6-11). The 20S proteasome is formed by 14 α subunits and the 14 β subunits, in which β1 (coded by the PSMB6 gene), β2 (coded by PSMB7 gene), and β5 (coded by PSMB5 gene) subunits possess protease activities (6-11). The standard constitutive
proteasome is present in most eukaryotic cells, and the thymoproteasome is a specific proteasome only found in the thymus (6-11). The immunoproteasome refers to a special type of proteasome, composed of β1i (coded by PSMB9), β2i (coded by PSMB10), and β5i (coded by PSMB8) subunits, instead of the β1, β2, and β5 subunits (respectively) of the standard proteasome (6-11). The immunoproteasome-specific β subunits are induced by IFN-γ stimulation (6-11). The β5i subunit possesses strong chymotrypsin-like activity, and β1i and β2i subunits have caspase and trypsin-like activities, respectively (14). The immunoproteasome efficiently generates peptides presented by MHC class I and degradation of oxidized proteins to maintain cellular homeostasis (8, 15, 16).

**Genetic mutations causing PRAAS**

NNS, JMP, and CANDLE syndromes are caused by mutations in the PSMB8 gene, although different regions of this gene are involved in these different subtypes (6-11). Agarwal et al. found a homozygous missense mutation, c.C224T, in the PSMB8 gene resulting in a p.T75M change in JMP patients (9). Arima et al. reported that the causal mutation of NNS is a p.G201V mutation in PSMB8 exon 5 (11); and Liu et al. described one patient with the CANDLE syndrome with a homozygous nonsense mutation at position 405 resulting in a C to A change with a protein truncation (8). Four other patients were homozygous and two others had a heterozygous missense mutation at c.C224T (8).

Recently however, it has become clear that CANDLE is a genetically heterogeneous recessive disease with new mutations in various combinations described in 8 patients (including a digenic disease model) involving: the PSMA3 gene encoding α7; PSMB4 encoding β7; PSMB9 encoding β1i; novel mutations in PSMB8; and in the proteasome maturation protein (POMP) gene (16). One patient had compound heterozygous PSMB4 gene mutations: c.G(-9)A. in the 5′ UTR; and a 9-bp in-frame deletion caused by p.D212_V214del (16). Other PSMB4 mutations were a monoallelic nonsense mutation c.C666C>A/p.Y222X found in an Irish patient; and heterozygous variants c.44-45insG/p.P16Sfs*45 in PSMB4 found in two Jamaican siblings also carriers of a variant c.494G>9/p.G165D in PSMB9 (digenic inheritance) (16). The mutations in PSMA3 found in two unrelated patients were heterozygous 3-bp in-frame deletions (c.696_698delAAG/p.R233del) and c.T(404+2)G/p.H111Ffs*10 at the splicing site (16). The mutation in PSMB9 was a missense substitution (c.G494A) and affected a conserved amino acid residue (p.G165D) (16).
newly identified PSMB8 mutation was a heterozygous missense mutation of c.A313C/p.K105Q in an Irish patient. A mutation in the POMP gene of a Palestinian patient was a heterozygous frameshift mutation, c.344_345insTTTGA/p.E115Dfs*20 (16). Described mutations (thus far) in proteasome genes causing PRAAS are shown in Figure 2. Other mutations causing CANDLE will likely be described, and are curated at: http://fmf.igh.cnrs.fr/ISSAID/infevers/.

Pathogenesis of PRAAS

The genetic loss of function mutations causing PRAAS cause either a decrease of the chymotrypsin-like catalytic activity of the immunoproteasome; or affect its assembly (6-11). The resultant loss of proteasome activity causes build-up of protein aggregates within the cytosol or endoplasmic reticulum (or both), inducing immune responses (6-11). Brehm et al. showed that proteasome gene mutations variably affect transcription, protein expression, protein folding, proteasome assembly, and, ultimately, proteasome activity (16). Moreover, defects in proteasome formation and function were recapitulated by siRNA-mediated knockdown of the respective subunits in primary fibroblasts from healthy controls (16). Patient-isolated haematopoietic and non-haematopoietic cells exhibited a strong IFN gene-expression signature, irrespective of genotype (16). Additionally, chemical proteasome inhibition or progressive depletion of proteasome subunit gene transcription with siRNA induced transcription of type I IFN genes in healthy control cells (16). These results provide further insight into CANDLE pathogenesis, and directly link global proteasome dysfunction to increased type I IFN production.

Similar to CANDLE, immortalised B-cells from JASL patients also show lower expression of PSMB8 at the mRNA and protein levels, and the activities of caspase-like, trypsin-like, and chymotrypsin-like proteases in cell extracts were decreased compared with healthy control samples (10). Immature proteasomes were increased in JASL immune cells, indicating that the immunoproteasome assembly was defective in the mutant cells (10). Consistent with these data, ubiquitinated proteins were increased in cell extracts of transformed cells and skin fibroblasts (10). Findings from cytokine profiling and analysis of the transcriptome were consistent with induction of inflammatory mediators such as IL-6 and the IFN pathway (10). In vitro studies using fibroblasts from NNS patients also showed accumulation of
ubiquitinated and oxidated proteins and subsequent hyperphosphorylation of p38, which may contribute to elevated IL-6 (11).

Of note, the mechanism underlying lipodystrophy in proteasome deficiencies remains unclear, but are probably also linked to loss of proteasome function. Downregulation of PSMB8 has been suggested to alter turnover of specific proteins that regulate adipocyte differentiation, but this requires further study (10).

Clinical features

Common clinical features of PRAAS include episodes of fever, elevated-acute phase reactants, skin eruptions (Figure 3), progressive lipodystrophy (Figure 4), muscular atrophy/myositis (Figure 5), failure to thrive and hepatosplenomegaly (6-11). Whilst there are subtle differences between the different named syndromes that make up PRAAS, it is likely that they represent a spectrum of one and the same overall disease caused by recessive loss of proteasome function.

CANDLE

CANDLE is not restricted to any particular ethnic group, with patients described from Jewish, Spanish, Jamaican, Caucasian, and Hispanic populations (6-8, 13, 16). Daily recurrent fevers begin usually in early infancy (6-8, 13, 16). Other typical features include delayed physical development and failure to thrive; hypochromic or normocytic anaemia; progressive lipodystrophy; arthritis and arthralgia; myositis; intracerebral calcification and increased acute phase reactants with variable other clinical features, including annular and purpuric skin lesions, oedematous eyelids, skin hyperpigmentation, spot alopecia, and polytrichia (6, 8, 16).

JMP

Patients with the JMP syndrome were described in families in Mexico and Portugal (6, 9). Features included hepatosplenomegaly; macrosomia; lipodystrophy affecting the face, arms,
and trunk; sclerodermatous skin disease with erythematous lesions; microcytic anaemia with higher serum levels of IFN-γ, IL-8, and IL-6; and hypergammaglobulinemia (6, 9).

NNS

NNS is a wasting disease seen early in life and it has been found (so far) only in the Japanese population (6, 11). Patients have: elongated, thickened, clubbed fingers; recurrent fevers; nodular erythema or pernio-like rash of the hands and feet; lipo-muscular atrophy; joint contractures; myositis; hepato-spleomegaly; basal ganglia calcification; and hypergammaglobulinemia (11). The disease is recessive therefore carriers display no clinical manifestations despite some degree of decreased proteasome function.

JASL

Recently, another Japanese group described patients with findings similar to those of NNS patients, and named the disease in these patients JASL (6, 10). The three patients described were members of two different and unrelated consanguineous Japanese families and were homozygous for the c.602G>T, p.G201V mutation found also in NNS patients (6, 10). The clinical presentation is similar to those of the other diseases included in the PRAAS spectrum (6, 10). Patients develop lipodystrophy in their upper bodies, and their skin erupts with nodular erythema (6, 10). Patients also have hand deformities in addition to spiking fevers, and the related patients both had basal ganglia calcification (6, 10). All thus far reported JASL patients died from cardiac or respiratory failure (6, 10).

Laboratory/histologic and imaging findings

Patients with PRAAS commonly have elevated erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) and chronic anaemia (6-13). Mild leucocytosis can be seen with transient lymphopenia during disease flares (6-13). Hypergammaglobulinemia has been reported in most PRAAS, as has the presence of various (and usually transiently) positive autoantibodies (6-13). Patients may develop myositis (Figure 5) and therefore may have raised CPK and LDH (6-13). The common histological feature is a mixed perivascular and interstitial dense dermal infiltrate with predominantly CD 68+ mononuclear cells, histiocytes, eosinophils, and neutrophils (6-13). Additionally skin panniculitis has also been reported, as
has leukocytoclastic vasculitis (Figure 6). Basal ganglia calcification detected by computed tomography (CT) is another common feature of PRAAS, the mechanism of which remains unclear but probably related to dysregulated interferon production in the brain, as is the case for other interferonopathies (see below).

**Treatment**

Oral corticosteroids may reduce the frequency of fever episodes, lead to improvement of skin lesions, and normalisation of acute-phase reactants (6-13). However, in most patients, steroid response is not sustained, or systemic inflammation is corticosteroid dependant (6-13). A number of other treatments including the IL-1 receptor antagonist (anakinra), the IL-6 receptor antagonist (tocilizumab), and TNF-α inhibitors either have been shown to have no effect, or resulted in only transient clinical improvement in some patients with PRAAS (6-13). The most promising treatment to date is with oral Janus kinase inhibition (JAK inhibitors), that decrease STAT-1 phosphorylation thereby blocking IFN-signalling and IP-10 production (8, 16). In vitro experiments in patient-derived primary cells suggest that inhibition of this pathway could be a promising therapeutic strategy (8, 16). The favourable experience of use of baricitinib (which provides JAK 1/2 inhibition) in the context of a compassionate treatment programme run by the National Institutes of Health (NIH) for patients with PRAAS is soon to be published (https://clinicaltrials.gov/ct2/show/NCT01724580). Despite these so for encouraging advances, the outcome of the disease still remains quite poor with progressive lipodystrophy in PRAAS patients continuing regardless of treatment and death occurring due to respiratory, cardiac failure or infectious complications. In that context, opportunistic viral infection (such as BK virus) is likely to be an increasing concern with more widespread use of JAK inhibitors for interferonopathies.

**STING associated vasculitis with onset in infancy (SAVI)**

STING-associated vasculopathy with onset in infancy (SAVI) is an auto inflammatory disease characterized by neonatal-onset systemic inflammation with a severe cutaneous vasculopathy leading to extensive tissue loss and interstitial lung disease (17-19).

**Aetiology/pathogenesis**
**Causal mutations associated with SAVI**

SAVI is caused by gain-of-function mutations in \textit{TMEM173} which encodes the stimulator of interferon genes (STING), an adaptor molecule linking sensing of foreign (viral and bacterial) DNA to the production of type I IFNs as part of the innate immune response (17-19). These gain-of-function mutations lead to constitutive activation of STING and upregulated type I IFN production (17-19). STING is widely expressed in alveolar macrophages, bronchial epithelial cells, and alveolar pneumocytes, which may explain the extensive lung pathology seen in SAVI (17-19). In the initial description of the disease, Liu et al. performed whole exome sequencing of a single patient with early onset symptoms of systemic inflammation, cutaneous rash, and pulmonary manifestations and her unaffected parents and identify a \textit{de novo} mutation, p.N154S, in the \textit{TMEM173} gene (17). Further candidate gene screening in other patients with similar features identified mutations in five additional sporadic cases (17). In total, three \textit{de novo} deleterious missense mutations, (p.V147L, p.N154S, p.V155M,) were described in six patients with this severe vasculopathy (17). Subsequent studies suggest that the \textit{TMEM173} p.V155M mutation has highly variable clinical expression, and that it can be associated also with a phenotypically distinct phenotype (20). The p.V147L mutation, was suspected to be mosaic with a variable prevalence in different cell types, suggesting that SAVI joins the list of other autosomal dominant autoinflammatory diseases that can have disease caused by somatic mosaicism, with potentially important implications for methodologies used for genetic screening (17). Pathogenic variants are clustered in exon 5 of \textit{TMEM173}, and they reside close to the STING dimerization site. Of note, the patients reported in the study by Liu et al, are of diverse ancestry, which is consistent with \textit{de novo} origin of their causal variants (17). The study by Jeremiah et al, also importantly highlights the possibility of reduced penetrance in families with dominantly inherited traits (20). It is likely that germline and somatic mutations in \textit{TMEM173} may also be of relevance in other types of late onset idiopathic cutaneous vasculopathies and lupus like phenotype. Other \textit{TMEM173} mutations recently identified include: p.A284G, p.C206T and p.A281G (21). The reader is also referred to \url{http://fmf.igh.cnrs.fr/ISSAID/infevers/search.php?n=24} for regular updates on newly discovered \textit{TMEM173} variants and their associated phenotypes.

**Pathogenesis of SAVI**
In vitro experiments suggest that gain of function \textit{TMEM173} missense mutations in HEK293T cells result in increased IFN\(\beta\) activity (17). Immune cells and fibroblasts derived from SAVI patients show constitutive, i.e., ligand-independent activation of the STING-IFN\(\beta\) pathway (17). Patients also exhibit a strong IFN response-gene expression signature in peripheral blood, elevated circulating levels of IFN-induced cytokines, and have constitutive phosphorylation of STAT1 in mutant cells (17). In addition skin biopsy samples obtained from patients with SAVI show widespread small-vessel vasculopathic changes, occlusions, and lymphocytic inflammation (17-20). STING is also expressed in endothelial cells and bronchial epithelium; thus, the pathological changes in the vascular wall and lung are considered also to be secondary to intrinsic defects in these cells, as well as up-regulation of type I interferons in immune cells (17, 19, 20). Stimulation of human primary endothelial cells with the STING ligand cGAMP resulted in increased expression of many genes that mediate inflammation and apoptosis (17). As a result, activated endothelial cells are more susceptible to apoptosis (17). Thus, these \textit{TMEM173} mutations are postulated to mediate chronic vascular inflammation, leading to the vasculitic rash and vaso-occlusive processes seen in SAVI (17). Lastly, higher rates of spontaneous cell death were also observed in patients’ monocytes and T cells, perhaps contributing further to the inflammatory phenotype (17, 19, 20).

**Clinical manifestations and laboratory findings**

Patients with SAVI have neonatal-onset systemic inflammation with an elevated ESR and elevated levels of CRP, a severe cutaneous vasculopathy leading to extensive tissue loss, and major interstitial lung disease (17-20). Liu et al. reported 6 unrelated children with SAVI. Four patients presented within the first 8 weeks of life with skin lesions on the extremities (Figure 7), including telangiectatic, pustular, or blistering rashes on the cheeks, nose, fingers, toes, and soles; 2 patients presented with tachypnoea in the perinatal period (17). All eventually developed severe skin lesions that extended to the pinnae of the ears and sites on the limbs (17). The acral skin lesions, which worsened in the winter, developed into painful, ulcerative lesions with eschar formation and tissue infarction, necessitating amputation of digits and causing scarring of the ear cartilage and perforation of the nasal septum (17). Other features included livedo reticularis, Raynaud phenomenon, nail bed capillary tortuosity,
failure to thrive and recurrent low-grade fevers (17-20). Patients in the initial and subsequent reports had radiographic evidence of interstitial lung disease and adenopathy with varying degrees of lung fibrosis (17-20) (Figure 8). Patients may also develop myositis and arthritis (17-20). Lesional skin biopsies show features of a dense neutrophilic inflammatory infiltrate with blood-vessel damage, and lung biopsies reveal a lymphocytic inflammatory infiltrate resulting in interstitial fibrosis and emphysematous changes (17-20). Other symptoms reported in subsequent reports of SAVI include necrotising fasciitis, significant pulmonary arterial hypertension, and polyarthritis with antinuclear and rheumatoid factor autoantibodies (22, 23). Interestingly, and in contrast to other interferonopathies, brain involvement has not been reported to date in patients with SAVI, and cognition is normal (24).

Treatment

Treatment options in SAVI remain limited (17-20, 24). There is usually partial response to glucocorticoids, and other disease-modifying antirheumatic drugs including biological therapies (17-20, 24). Intriguingly, Liu et al. were also able to show that incubation of lymphocytes from patients with SAVI with JAK inhibitors resulted in reduced levels of STAT1 phosphorylation and a reduction of IFN-β production in fibroblasts activated by cGAMP (17). These results suggested a possible avenue for treatment in patients with SAVI. In line with these observations, recent reports have suggested that there may be a therapeutic benefit from JAK 1/2 inhibition (ruxolitinib/baricitinib) to block type 1 IFN signalling, despite constitutively activated STING (25, 26). Treatment of patients with SAVI with JAK inhibition has resulted in normalisation of inflammatory markers, resolution of the cutaneous symptoms and gradual improvement of the lung disease in some, although data are scarce and largely anecdotal thus far (25, 26).

Aicardi-Goutières syndromes (AGS)

Aicardi-Goutières syndrome (AGS) was originally defined as an early onset progressive brain disease mimicking the sequelae of in utero viral infection (2, 3, 27, 28). Over time, as more genetic variants were identified associated with AGS, other features of the disease were also recognized including chilblains, raised intraocular pressure (glaucoma) and, in some cases, an overlap with systemic lupus erythematosus (SLE) clinical manifestations, thus expanding the disease phenotype (2, 3, 27, 28).
Aetiology/pathogenesis

Causal mutations associated with AGS

Mutations in the genes encoding any of the following proteins can cause disease consistent with AGS: DNA 3’ repair exonuclease 1 (TREX1), the three subunits of the ribonuclease H2 (RNase H2) endonuclease complex (RNase H2A, RNase H2B and RNase H2C), the deoxynucleoside triphosphate triphosphohydrolase and ribonuclease SAM domain and HD domain 1 (SAMHD1), adenosine deaminase acting on RNA (ADAR; also known as DRADA) or the double-stranded RNA (dsRNA) cytosolic sensor IFN-induced helicase C domain-containing protein 1 (IFIH1; also known as MDA5) (2, 3, 27-37). Table 2 summarises the spectrum of disease associated with mutations in AGS-related genes.

Table 2. Range of diseases caused by mutations in Aicardi Goutieres Syndrome (AGS)-related genes.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Inheritance</th>
<th>Phenotype</th>
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</thead>
<tbody>
<tr>
<td>TREX1 (AGS type 1)</td>
<td>Autosomal recessive or autosomal dominant</td>
<td>AGS Familial chilblain, SLE, and retinal vasculopathy with cerebral leukodystrophy</td>
</tr>
<tr>
<td>RNASEH2B (AGS type 2)</td>
<td>Autosomal recessive</td>
<td>AGS and spastic paraparesis</td>
</tr>
<tr>
<td>RNASEH2C (AGS type 3)</td>
<td>Autosomal recessive</td>
<td>AGS</td>
</tr>
<tr>
<td>RNASEH2A (AGS type 4)</td>
<td>Autosomal recessive</td>
<td>AGS</td>
</tr>
<tr>
<td>SAMHD1 (AGS type 5)</td>
<td>Autosomal recessive</td>
<td>AGS Familial chilblain lupus chronic lymphocytic leukaemia</td>
</tr>
</tbody>
</table>
**Pathogenesis of AGS**

The identification of the genetic basis of AGS has highlighted the fundamental role of nucleic acid signalling in the induction of type I IFNs (2, 3, 27-37). For patients with TREX1 and the RNASEH2 gene mutations and AGS, the hypothesis is that dysfunction of the proteins encoded by these genes might result in the accumulation of endogenous nucleic acid products, which are then sensed as non-self by the innate immune machinery (2, 3, 28). In TREX1-deficient mice, activation of a TLR-independent cytosolic pathway by DNA has been shown to lead to a type I IFN response as a consequence of signalling through cGAS, STING, IFN regulatory factor 3 (IRF3) and serine/threonine-protein kinase TBK1 (38, 39). By contrast, knock-out of the RNASEH2b gene in mice is embryonically lethal, but is not associated with type I IFN induction (40, 41). Instead, this mouse model has demonstrated an essential role of the RNaseH2 complex in removing the ribonucleotides that are incorporated into DNA during DNA replication (40, 41). More recent reports have established an additional knock-in mouse model with an RNaseH2 AGS mutation in a highly conserved residue of the catalytic subunit, *Rnaseh2a*<sup>G37S/G37S</sup> (G37S) (42). Importantly aG37S homozygotes are perinatal lethal, in contrast to the early embryonic lethality previously reported for *RNASEH2b* or *RNASEH2c* -null mice described above (42). Pokatayev et al. showed in this study that the G37S mutation led to increased expression of IFN stimulated genes dependent on the cGAS–STING signaling pathway (42). These studies overall suggest that by-products of defective DNA replication trigger an IFN-mediated immune response. It is possible however that RNaseH2 complex may have additional completely distinct activity that remains to be established.

| ADAR1  
(AGS type 6) | Autosomal recessive or autosomal dominant | AGS dyschromatosis symmetrica hereditaria, bilateral striatal necrosis and spastic paraparesis, complete non penetrance |
|---|---|---|
| IFIH1 
(AGS type 7) | Autosomal dominant | Various neuroimmunological and non-neurological phenotypes, including AGS, spastic paraparesis, complete non penetrance and Singleton Merten syndrome |
The subsequent identification of AGS-associated mutations in SAMHD1, ADAR and IFIH1 added weight to the hypothesis that disturbance of endogenous nucleic acid pathways triggers an innate immune response normally induced by exogenous nucleic acids (35-37, 43-45). SAMHD1 has been shown to have a role in regulating the dNTP pool, and this is necessary for DNA synthesis in the context of HIV-1 infection (45). Notably, SAMHD1-deficient mice and ADAR deficient mice also have an upregulated IFN signature (46, 47). Finally, mice with an N-ethyl-N-nitrosourea (ENU)-induced missense mutation in IFIH1 have upregulation of IFN-induced signalling and develop an autoimmune phenotype (48).

Clinical features

AGS is often considered in the context of an early-onset encephalopathy with basal ganglia calcification (Figure 9) and cerebral white matter abnormalities (2, 3, 27-37). An estimated 20% of patients with AGS develop severe neurological dysfunction diagnosed soon after birth, manifesting as spasticity, dystonia, seizures, cortical blindness and progressive microcephaly (2, 3, 27-37). In general, this early-onset neonatal form of AGS is most frequently seen in association with biallelic pathogenic variants in RNASEH2A, RNASEH2C, or TREX1(2, 27-29, 49). The patients may develop fevers with no clear causal infection and severe irritability in the first few months of the disease process (2, 27-29, 49). Most children deteriorate neurologically and end up with no purposeful gross motor, hand or communication function (2, 27-29, 49). Some patients with TREX1-related AGS may also develop other symptoms mimicking congenital infection such as thrombocytopenia, hepatosplenomegaly and transaminitis (29). Chilblain-like lesions are observed in 1/3 of TREX1 patients (Figure 10) while some may have more severe skin disease (29, 50). Some cases have been described with prominent lupus like features and a range of autoantibodies detected (29). A full blown picture of systemic lupus erythematosus (SLE) is very unusual but has been reported (29).

Late-onset presentation of AGS, sometimes occurring after some months of apparently normal child development, as also described (2, 3, 27-37). The first symptoms can be very non-specific such as extreme irritability, disturbed sleep, feeding difficulties, and low grade
pyrexias which may be the first signs of the onset of sub-acute encephalopathy (2, 3, 27-37). This may be followed by psychomotor delay and/or loss of acquired skills and poor head growth. This encephalopathic phase usually lasts a few months, beyond which time the clinical picture typically stabilizes (2, 3, 27-37). A later-onset presentation, than can sometimes occur after several months of normal development and is occasionally associated with remarkably preserved neurologic function, is most frequently seen in association with biallelic pathogenic variants in RNASEH2B, SAMHD1, or ADAR; but may also be seen in individuals who have an autosomal dominant heterozygous pathogenic variants in ADAR or IFIH1 (2, 3, 27-37). Of note, some individuals with biallelic pathogenic variants in RNASEH2B have relatively preserved intellectual function, with a few having completely normal intellectual development and head circumference (29).

Familial chilblain lupus, often associated with dominant Asp18Asn mutation in TREG-1, may be the only manifestation in some patients (50). Lesions are characterized by cold-induced, bluish-red lesions on the hands, feet and ears that may ulcerate, occasionally leading to significant tissue loss (50, 51). The skin lesions are similar to those seen in patients with AGS with neurological involvement, and in some patients with non-genetic forms of SLE.

Mutations in ADAR have been associated with acute bilateral striatal necrosis (35, 36). Such patients present with encephalopathy with changes on brain imaging (Figure 11) which include symmetric signal changes in the caudate and putamen, often associated with swelling and later shrinkage in the context of an acute or subacute onset of refractory four-limb dystonia (35, 36). These symptoms may develop in early childhood on the background of a completely normal development, and might be initially confused with a viral aetiology (35, 36).

Intracerebral vasculopathy, including intracranial stenosis and aneurysms, is observed more frequently in individuals who have biallelic pathogenic variants in SAMHD1(43, 44). These patients may also develop chilblains and glaucoma (43, 44).

Mortality rates are overall high for patients with AGS (29, 52). Rice et al, reported on a large cohort of patients with AGS, and demonstrated that mortality was correlated with genotype: 34% of individuals with RNASEH2A, RNASEH2C, and TREGI pathogenic variants were known to have died the majority (81%) by age 10 years old compared to only 8% with RNASEH2B pathogenic variants (p=0.001)(52).
Laboratory/imaging findings

Typical neuroimaging features of AGS include intracranial calcification, leukodystrophy, and cerebral atrophy (2, 3, 27-37). The distribution and extent of the calcification can be variable, with periventricular distribution in some cases (2, 3, 27-37). It is important to emphasise that intracranial calcification is not always recognized on MRI. Thus AGS should be considered in the differential diagnosis of any unexplained leukoencephalopathy, and CT scanning is often required to establish the presence of calcification, particularly early on in the pathogenesis. Some patients have frontotemporal white-matter involvement with cyst formation (2, 3, 27-37). CSF analysis shows chronic leucocytosis predominantly lymphocytes (> 5–100 cells/mm³); elevated neopterin (often highest in the early stages of the disease) that may normalize over time; and elevated IFN-α activity, which again, declines with increasing age (2, 3, 27-37).

In addition to elevated IFN-α that has been long known to be detected in the CSF of patients with AGS, an increased level of expression of IFN-stimulated genes (ISGs) in peripheral blood — an 'IFN signature' — has been reported to be present at any age in almost 100% of patients with mutations in TREX1, RNASEH2A, RNASEH2C, SAMHD1, ADAR or IFIH1 (53). Approximately 30% of patients with RNASEH2B mutations had no such upregulation of ISGs, however (53). More recently Rodero et al. reported their experience of using very high sensitivity single-molecule array (Simoa) digital ELISA technology coupled with a very high affinity antibody against IFNα derived from patients with genetically confirmed autoimmune polyendocrinopathy (APECED), that enabled detection of differences in IFNα at the attomolar level in healthy donors, viral infection, and complex and monogenic interferonopathies (54). These IFNα levels correlated well with interferon gene expression scores. Thus, the ability to now detect very low concentrations of IFNα concentrations by digital ELISA and high affinity antibodies will undoubtedly enhance our understanding of IFN biology in health and disease, and could lead to improvement in diagnostic strategies, and also to track therapeutic efficacy (for example with JAK inhibitors or other targeted treatments) in individual patients with AGS, and other interferonopathies.

Treatment
Generally the management of AGS is supportive, with no cure available. Anti-epileptic treatments may be required for those with seizures (2, 3, 27-37). Some patients may derive relief from dystonia from botulinum toxin injections, or levodopa (2, 3, 27-37). Corticosteroids have been shown to lower the CSF concentration of IFNα, and may be beneficial for the cutaneous manifestations of AGS, but do not reverse the neurological phenotype (2, 3, 27-37). The occlusive and aneurysmal arteriopathies described in association with SAMHD1 could be amenable to revascularization procedures for select cases, but is limited to centres with particular expertise. Novel strategies to block IFN-signalling through use of JAK inhibition are also emerging, and may be beneficial in AGS as well as the other interferonopathies discussed above (https://clinicaltrials.gov/ct2/show/NCT01724580). In addition, given the possible role of endogenous retroviruses in the activation of nucleic acid receptors in AGS, a phase 2 trial with reverse transcriptase inhibitors has been developed (https://clinicaltrials.gov/ct2/show/NCT3304717). Some patients have been treated empirically with reverse transcriptase inhibitors although preliminary results have not yet been reported.

Other interferonopathies

Retinal vasculopathy with cerebral leukodystrophy (RVCL)

Retinal vasculopathy with cerebral leukodystrophy is usually an adult-onset autosomal dominant disorder involving the microvessels of the brain, with CNS degeneration and progressive loss of vision, stroke, motor impairment, and cognitive decline (55, 56). Recent genetic analyses have demonstrated that RVCL is caused by heterozygous frameshift mutations in TREX1 in the C-terminus required for ER localisation (55, 56). RVCL encompasses three conditions: Cereboretinal Vasculopathy (CRV), Hereditary Vascular Retinopathy (HRV); and Hereditary Endotheliopathy with Retinopathy, Nephropathy and Stroke (HERNS), which have previously been regarded as distinct clinical entities, but are now known to be caused by mutations in the same gene (55, 56). Death occurs in most patients 5 to 10 years after onset. A subset of affected individuals have other symptoms such as Raynaud's phenomenon, micronodular cirrhosis, or glomerular dysfunction (55, 56).
Spondyloenchondrodysplasia with immune dysregulation (SPENCDI)

Spondyloenchondrodysplasia with immune dysregulation (SPENCDI) is an immuno-osseous dysplasia combining the typical metaphyseal and vertebral bone lesions of spondyloenchondrodysplasia (SPENCD) with immune dysfunction and neurologic involvement. SPENCDI is a recessive genetic disease caused by homozygous or compound heterozygous mutation in the ACP5 gene on chromosome 19p13 (57-59). The skeletal dysplasia is characterized by radiolucent and irregular spondylar and metaphyseal lesions that represent islands of chondroid tissue within bone (57-59). The vertebral bodies show dorsally accentuated platyspondyly with disturbance of ossification (57-59). Clinical abnormalities such as short stature, rhizomelic micromelia, increased lumbar lordosis, barrel chest, facial anomalies, and clumsy movements may be present (57-59). Central nervous system involvement includes spasticity, mental retardation, and cerebral calcifications. Immune dysregulation ranges from autoimmunity to immunodeficiency (57-59). Neurologic and autoimmune manifestations have been observed in different combinations (57-59). Briggs et al. also noted variability in skeletal, neurologic, and immune phenotypes, which was sometimes marked even between members of the same family (57, 59).

Singleton-Merten Syndrome

Singleton-Merten syndrome (SGMRT) is a rare dominant disorder caused by heterozygous mutation in the IFIHI1 gene (60). The disease is characterized by abnormalities of blood vessels, teeth, and bone (60). Calcifications of the aorta and aortic and mitral valves occur in childhood or puberty and can lead to early death (60). Dental findings include delayed primary tooth exfoliation and permanent tooth eruption, truncated tooth root formation, early-onset periodontal disease, and severe root and alveolar bone resorption associated with dysregulated mineralization, leading to tooth loss (60). Osseous features consist of osteoporosis, either generalized or limited to distal extremities, distal limb osteolysis, widened medullary cavities, and easy tearing of tendons from bone (60). Less common features are mild facial dysmorphism (high anterior hair line, broad forehead, smooth philtrum, thin upper vermilion border), generalized muscle weakness, psoriasis, early-onset glaucoma, and recurrent infections (60). An atypical form of Singleton-Merten syndrome (SGMRT2) characterised by glaucoma, aortic calcification, and skeletal anomalies is caused by mutations in the DDX58 gene (61).
**USP18 deficiency (pseudo-TORCH syndrome)**

Loss-of-function recessive mutations of Ubiquitin-specific peptidase 18 (USP18), a key negative regulator of type I IFN signalling were recently identified as the cause of a type I interferonopathy leading to severe pseudo-TORCH syndrome (PTS), characterized by microcephaly, enlarged cerebral ventricles, cerebral calcification, and, occasionally, by systemic features at birth resembling the sequelae of congenital infection but in the absence of an infectious agent (62).

**ISG15 deficiency**

A less severe phenotype than that associated with AGS has been described in patients presenting with idiopathic basal ganglia calcification, seizures and autoantibodies, and harboring mutations in the ISG15 gene (63). Intracellular ISG15 is an interferon (IFN)-α/β-inducible ubiquitin-like modifier which can covalently bind other proteins (63). Absence of intracellular ISG15 prevents the accumulation of USP18, a potent negative regulator of IFN-α/β signalling, resulting in the enhancement and amplification of IFN-α/β responses (63). Patients with ISG15 deficiency are prone to mycobacterial disease and also display cellular, immunological and clinical signs of enhanced IFN-α/β immunity, similar to other interferonopathies (63).

**Trichohepatoenteric syndrome 2**

Trichohepatoenteric syndrome-2 is caused by mutation in the SKIV2L gene (64). Typical characteristic features of THES include intrauterine growth retardation, woolly hair, facial dysmorphism, intractable diarrhoea in infancy requiring total parenteral nutrition, and immunodepression (64). Hepatic involvement contributes to the poor prognosis of affected patients (64).

**Conclusion/summary**

The interferonopathies are a relatively new class of inherited disorders associated with an inborn elevated IFN response leading to overlapping disease phenotypes (1-5). The study of patients with these rare genetic diseases has revealed a central role of abnormal nucleic acid recognition and type I IFN pathway activation in human diseases characterized by autoinflammation and autoimmunity (1-5). These conditions often present as complex clinical cases and remain undiagnosed for years. Considering the complexity of the IFN
response and given the advent of next generation genetic sequencing techniques, the identification of further monogenic diseases belonging to this disease grouping seems likely. Development of biomarkers such as the transcriptomic signature of IFN-stimulated genes and IFNα measurement by digital ELISA even at very low concentrations will undoubtedly improve the diagnosis and stratification of diseases associated with IFN dysregulation (53, 54). Lastly, several non-Mendelian disorders, particularly SLE and dermatomyositis, are also characterized by an up-regulation of type I IFN signalling (65, 66). Therefore the insights derived from these monogenic diseases with respects to novel targets for therapy could have relevance for the management of these sporadic conditions.

**Practice points.**

1. The interferonopathies are monogenetic disorders that usually present early in life, and may mimic congenital infection or be mistaken for sporadic autoimmune diseases such as SLE or JDM.
2. Although differing in the degree of phenotypic expression and severity with some patients developing some and not all typical symptoms, the clinical presentation of these diseases shows a considerable degree of overlap reflecting their common pathogenetic mechanisms.
3. The proteasome associated autoinflammatory syndromes are caused by loss-of-function mutations in genes encoding proteasome components thus leading to proteasome malfunction, disruption of proteostasis, and dysregulation of interferon signalling.
4. STING-associated vasculopathy with onset in infancy (SAVI) is characterized by neonatal-onset systemic inflammation with a severe cutaneous vasculopathy, extensive tissue loss, and interstitial lung disease.
5. Aicardi-Goutières syndrome (AGS) was originally defined as an early onset progressive brain disease mimicking the sequelae of in utero viral infection, but is now known to have an expanded phenotype that includes chilblains, other lupus like symptoms, and glaucoma.
6. A diagnosis of a monogenic interferonopathy should still be explored in patients who have some of the described and not all clinical features as
7. Treatments targeting the interferon pathway are beginning to be used in small numbers of patients with interferonopathies. In particular, the JAK inhibitors are emerging as a potentially important therapeutic strategy for interferonopathies.

**Research agenda**

1. An increased level of expression of IFN-stimulated genes (ISGs) in peripheral blood, and IFNα measurement by digital ELISA may be useful for diagnostic screening for interferonopathies, and may allow therapeutic stratification and/or monitoring therapeutic responses.

2. Considering the advent of next generation genetic sequencing techniques, the identification of further monogenic interferonopathies seems likely.

3. The insights derived from these monogenic interferonopathies with respects to novel targets for therapy could have relevance for the management of other IFN mediated sporadic conditions such as SLE and dermatomyositis.

**Figure legends:**

**Figure 1.** Schematic representation of pathways affected in genetic interferonopathies. Coloured in purple are some of the proteins mutated in type I interferonopathies. STING: stimulator of interferon genes, SAMHD1: deoxynucleoside triphosphate triphosphohydrolase SAM domain and HD domain 1, TREX1: DNA 3’ repair exonuclease 1, ISG15: interferon-stimulated gene 15, MAVS: mitochondrial antiviral-signalling protein, RIG-I: retinoic acid-inducible gene 1, TBK1: TANK-binding kinase 1, USP18: ubiquitin-specific protease 18, RNASHEH2: Ribonuclease H domain 2, IFIH1: IFN-induced helicase C domain-containing protein 1, IRF3: Interferon regulatory factor 3, IRF7: Interferon regulatory factor 7, IRF9: Interferon regulatory factor 9, cGAMP: cyclic di-GMP-AMP, cGAS: cyclic GMP-AMP synthase, ER: endothelial reticulum, ERGIC: endothelial reticulum-Golgi intermediate compartment, IFNAR: interferon-α receptor, ISGF3, the transcriptional activator induced by
interferon alpha, ISRE: Interferon-sensitive response element, GASs: candidate interferon activated sites; JAK1: Janus kinase 1; TYK2: tyrosine kinase 2. P indicates phosphorylation.

Figure 2. Summary of reported proteasome gene mutations causing proteasome associated autoinflammatory diseases.

Figure 3. Typical cutaneous eruption in CANDLE (PSMB4/PSMB9 digenic form)

Figure 4. Figure 4 A shows lipodystrophy (right arm); and B same infant demonstrating abdominal adiposity.

Figure 5. Magnetic resonance imaging of lower limbs demonstrating myositis in a patient with CANDLE.

Figure 6. Skin biopsy from a patient with CANDLE: a mixed dermal inflammatory infiltrate, with the subcutis and fat involved to a lesser extent; leucocytoclasis with prominent vessel wall endothelial cells but no fibrinoid necrosis.

Figure 7: Cutaneous lesions in STING associated vasculitis with onset in infancy (SAVI).

Figure 8: CT thorax, bilateral lower lobe fibrosis in an 18 month old infant with STING associated vasculitis with onset in infancy (SAVI).

Figure 9: Magnetic resonance imaging of brain of a patient with Aicardi Goutieres syndrome (homozygous TREX-1 mutation). Figure 9A shows widespread intracerebral calcification (which appears as areas of low intensity on this T2 weighted image); Figure 9B shows reduced white matter bulk and dilatation of the ventricles in the same patient.

Figure 10: Typical chilblain lesions of Aicardi Goutieres syndrome (Heterozygous TREX-1 mutation).
**Figure 11:** Magnetic resonance imaging brain from a child with Aicardi Goutieres syndrome caused by homozygous mutations in ADAR1. Figure 11A shows bilateral basal ganglia changes; Figure 11 B shows widespread small areas of classification indicated as multiple areas of low signal intensity.

**References:**


RNase H2 removes ribonucleotides from DNA to maintain genome integrity. Journal of 
removal of ribonucleotides from DNA is essential for mammalian genome integrity and 
catalytic core Aicardi-Goutières syndrome–related mutant invokes cGAS–STING innate 
large artery disease in Aicardi–Goutières syndrome implicates SAMHD1 in vascular 
44. Ravenscroft JC, Suri M, Rice GI, Szynkiewicz M, Crow YJ. Autosomal dominant 
inheritance of a heterozygous mutation in SAMHD1 causing familial chilblain lupus. 
involved in Aicardi-Goutieres syndrome implicate SAMHD1 as regulator of the innate 
al. Mouse SAMHD1 has antiretroviral activity and suppresses a spontaneous cell-intrinsic 
47. Hartner JC, Walkley CR, Lu J, Orkin SH. ADAR1 is essential for the maintenance of 
15.
49. Rice GI, Rodero MP, Crow YJ. Human disease phenotypes associated with mutations 


