Aicardi–Goutières syndrome

S. Orcesi*, R. La Piana, and E. Fazzi

IAGSA (International Aicardi Goutières Syndrome Association), Department of Child Neurology and Psychiatry, IRCCS ‘C. Mondino Institute of Neurology’ Foundation, Pavia, Italy

Introduction: Aicardi–Goutières syndrome (AGS) is an autosomal recessive encephalopathy characterized by acquired microcephaly, cerebral calcifications, leukodystrophy, cerebral atrophy and cerebrospinal fluid findings of chronic lymphocytosis and raised interferon-alpha (INF-alpha). The main extraneurological symptoms are chilblain-like skin lesions, usually on the fingers, toes and ears.

Sources of data: This review is based on a search of the published literature on AGS from 1984 onwards (particularly the most recent papers) and on knowledge and experience gained through the authors’ work with the International Aicardi-Goutières Syndrome Association (IAGSA).

Areas of agreement: It is accepted that AGS can be mistaken for a congenital infection and that the diagnostic significance of its cardinal signs (raised INF-alpha levels, basal ganglia calcifications) is different in different stages of the disease. Currently, we know of four genes that, if mutated, can give rise to AGS, but at least one other gene is believed to exist. These genes are involved in the DNA damage response, a defect of which could provoke an inappropriate innate immune response, triggering increased secretion of INF-alpha, ultimately responsible for the main features of the disease.

Areas of controversy: The natural history of AGS has not yet been definitively described given the lack of extensive, long-term neuroradiological follow-up studies. Furthermore, it is not yet clearly understood how the innate immune system is activated, what triggers the onset of the disease or why it tends to ‘burn out’ after several months. Immunosuppressive therapy in the active stage of the disease does not seem to produce any real change in the clinical course, but more data are needed.

Growing points and areas timely for developing research: Current studies aim to clarify the molecular mechanisms underlying the pathogenesis of AGS and to establish the exact pathway by which retained nucleic acids activate the immune system. This knowledge could allow the development of therapeutic strategies.

Keywords: basal ganglia calcifications/leukodystrophy/Aicardi–Goutières syndrome/TREX-1

Accepted: December 2, 2008

*Correspondence to:
Simona Orcesi
Department of Child Neurology and Psychiatry,
IRCCS ‘C. Mondino Institute of Neurology’
Foundation, Pavia, Italy
E-mail: simona.orcesi@mondino.it
Introduction

In 1984, Jean Aicardi and Françoise Goutières\textsuperscript{1} described eight children from five families presenting a severe early onset encephalopathy characterized by basal ganglia calcifications, white matter abnormalities and chronic cerebrospinal fluid (CSF) lymphocytosis.

The recurrence of this picture in siblings whose parents were consanguineous, together with the finding that both males and females could be affected, immediately suggested that this was a disease inherited as an autosomal recessive trait and at the same time highlighted the risk, from a clinical point of view, of mistaking this picture for the sequelae of congenital infection. Aicardi drew attention to cases previously described in the literature that may be the same clinical entity. A few years later, Lebon\textsuperscript{2} identified a further typical feature, helping to establish a more accurate diagnostic profile of the syndrome: the presence of raised levels of interferon-alpha (INF-alpha) in the CSF in the absence of demonstrable infections of the central nervous system (CNS). The eponym ‘Aicardi–Goutières syndrome’ (AGS) was used for the first time in 1992,\textsuperscript{3} and in the years that followed a number of new cases were added to the original series of eight children.\textsuperscript{4} By 2001, the year which saw the first International Meeting on AGS in Pavia, Italy, the number of known cases had risen to around 50,\textsuperscript{5,6} originating mainly from Europe and America, and there was a growing realization that there probably exist many more cases, as yet unrecognized, of this recessive autosomal encephalopathy, characterized fundamentally by the presence of microcephaly, bilateral basal ganglia calcifications, cerebral white matter abnormalities, cerebral atrophy, chronic CSF lymphocytosis and raised CSF levels of INF-alpha.

To date, thanks to the recent identification of four AGS-causing genes among a probably greater number responsible for the syndrome, and also to the emergence of a broader and more heterogeneous clinical spectrum of AGS, over 100 cases\textsuperscript{7} have been reported and the disease has started to show a wider geographical distribution.

Clinical onset

AGS typically has onset in the first year of life, usually at the age of around 4 months.\textsuperscript{6–8} It follows an uncomplicated pregnancy and delivery, and apparently normal early development.

The first symptoms are reminiscent of a systemic disorder: extreme irritability, disturbed sleep–wake patterns and feeding difficulties are all frequent early alarm signals.\textsuperscript{6–8} The occurrence of recurrent,
unexplained medium-grade fevers (38–38.5°C) is very commonly reported at clinical onset and can, in some cases, lead to erroneous diagnoses of meningitis or encephalitis. Instead, what these symptoms indicate is the presence of an encephalopathy in which a sub-acute onset is followed by the appearance of increasingly marked psychomotor delay and/or loss of early acquired skills, as well as signs of neurological impairment and a slowing of head growth. After this first phase, which usually lasts a few months, the clinical picture typically stabilizes and, according to most authors, no further progression of the disease is detectable. As Crow has rightly said ‘the stimulus for the disease onset is unknown, and why the disease tends to burn out after several months is also not understood’.

Although the picture just described is the most typical and frequent pattern of onset, AGS can also (in 20% of cases) manifest itself in the neonatal period. In these cases, the picture of neonatal neurological impairment, microcephaly and calcifications, sometimes even detected in utero, transitory hepatosplenomegaly with raised transaminases, thrombocytopenia and anaemia, becomes even more difficult to distinguish from those of congenital viral infections. It is worth noting that this haematological picture tends to resolve spontaneously within a few weeks. Rice et al. have also reported seizures in eight neonatal cases.

There have recently emerged reports of cases with an atypical age at onset (between the ages of 1 and 2 years), in which the disease appears after many months of normal psychomotor development.

**Neurological symptoms**

The onset phase is usually accompanied by the progressive manifestation of the neurological signs typical of AGS. Affected subjects generally develop tetraplegia, poor head control, trunk hypotonia, pyramidal and extrapyramidal signs, in particular persistence of archaic reflexes and dystonic postures and movements, and microcephaly, usually appearing in the course of the first year of life. Abnormal eye movements, nystagmus and poor visual performance are frequently associated and, in the presence of normal ocular structures, these features suggest that the visual impairment is of central origin. In particular, the absence of retinal abnormalities, combined with normal auditory function, can be useful in the differential diagnosis of AGS versus congenital infections.

Another interesting finding is the presence of the ‘startle reaction’ in response to sensory stimuli, even mild ones, in some cases in the absence of the habituation phenomenon; in most of the cases investigated, electroencephalographic recordings were able to exclude an
epileptic origin of this phenomenon, whereas in others its non-epileptic nature could not be established definitively.\(^7\) This diagnostic difficulty probably explains the differences in the reported frequency of epilepsy in AGS, which ranges from 25% of cases\(^6,8\) to 53% in the more recent literature.\(^7\)

The vast majority of affected subjects present severe motor and cognitive impairment: postural acquisitions are frequently limited to partial head control,\(^7\) while language is, in most cases, absent, even though some subjects show some awareness of their surroundings and a degree of understanding.\(^7,9\) As regards the severity of the clinical picture, published reports have revealed a certain heterogeneity of the syndrome, even within the same family.\(^7,15,16\) The literature contains a single report\(^17\) of a patient, affected by spastic diplegia, with a normal IQ and normal head circumference at the age of 19 years, while Rice\(^7\) reports six cases with some preserved intellectual function, good comprehension and at least some retained speech.

### Extraneurological symptoms

Extraneurological signs are frequent in AGS. The organ most typically affected is the skin, which can present chilblain-like skin lesions characterized by areas of inflammation and intermittent necrosis. These lesions, reported in around 40% of subjects\(^7\) at least once in their lifetime, are found mainly on the fingers, toes, pressure points and ears.\(^4,6,8\) Although more common in the winter, they can also show a more continuous presence throughout the year. Acrocyanosis is also very frequent, as is periungual erythema, which can be complicated by infections.\(^2,8\)

There exist other extraneurological manifestations, reported less frequently in sporadic cases of AGS: in addition to liver involvement (hepatosplenomegaly and raised transaminases) and transitory thrombocytopenia, found above all in cases with neonatal onset,\(^4,7,9\) there have been reports of congenital glaucoma,\(^18\) raised levels of autoantibodies, hypothyroidism, insulin-dependent diabetes mellitus, haemolytic anaemia, polygammaglobulinaemia, neonatal cardiomyopathy, demyelinating peripheral neuropathy, micropenis and transitory anti-diuretic hormone deficiency.\(^7,9\)

### Neuroimaging findings

Neuroradiologically, AGS is characterized by three cardinal features: cerebral calcifications, white matter abnormalities and cerebral
atrophy.6,8,9 These features are universally reported, but their natural history has not yet been definitively described or completely clarified,14 given the lack of extensive, long-term neuroradiological follow-up studies.19,20

The calcifications, typically bilateral and located in the basal ganglia, particularly the globus pallidus, putamen, caudate nucleus and often the cerebellar dentate nuclei,5,6,8 are best visualized on CT scan. In 50–70% of cases, the calcifications also extend to the white matter, particularly the periventricular areas (Fig. 1); they may be punctate or larger and more convergent, nevertheless, the number, size and pattern of the calcifications do not determine the severity of the neurological picture.8 Nearly always present at diagnosis of AGS, the calcifications tend to remain stable over time, even though they have shown a progression in a few cases6,10 and a reduction, following an initial progression, has been reported in one case.19

In the presence of a clinical picture suggestive of AGS, the cerebral calcifications constitute a very important sign; not easily identified on MRI, they should carefully be sought on CT scans in all cases of unexplained leukoencephalopathy.9 It is also worth bearing in mind that these calcifications need not necessarily be present from the onset of

Fig. 1 CT scan: axial brain CT scan: bilateral punctate calcifications are evident in the basal ganglia and in the white matter.
the disease, but can appear subsequently, as reported in two cases by Aicardi\cite{1,4,5} and in two recent descriptions of atypical cases.\cite{13,14} This means that, at onset at least, the presence of cerebral calcifications should not be regarded as indispensable for a diagnosis of AGS.\cite{7,9}

Another typical feature of the syndrome, reported in the earliest descriptions of AGS, is the presence of white matter abnormalities. Found in 75–100% of cases,\cite{5,6} these are detectable as hypodense areas on CT scans or, more clearly, as a hyperintense signal on T2-weighted brain MRI. Often showing a clearly leukodystrophic pattern, they are prevalent in the periventricular more than the subcortical\cite{19} white matter and can also be particularly prominent in frontal and temporal regions,\cite{5,6,18,20} sometimes showing cystic degeneration (Fig. 2).\cite{7,9}

Neuroradiological follow-up data, collected in an attempt to trace the natural history of AGS, are available in only a small number of cases.\cite{10,19} However, it appears that the leukodystrophy remains mainly stable over time. There is just one reported case\cite{13} of an evolution of the white matter involvement, from aspecific alterations at clinical onset to a picture of full-blown leukodystrophy, whereas in two cases,\cite{14,21} a partial regression of the white matter abnormalities was reported.

The third of the cardinal neuroradiological features of AGS, found in 94% of the cases reported in the literature,\cite{20} is cerebral atrophy, evident particularly in the periventricular region and in an enlargement of the cerebral sulci (Fig. 2).\cite{5,6,8} It tends to remain stable or show a progression,\cite{4–6,10,19} although there is also one report of a case showing remarkable reduction of brain atrophy.\cite{22}

Fig. 2 MRI 1.5 Tesla. (A) T2-weighted axial MRI of the brain shows symmetrical, bilateral hyperintensity of the white matter with areas of cystic degeneration in the frontal regions. (B) T1-weighted coronal MRI of the brain shows symmetrical, bilateral hypointensity of the white matter and supratentorial atrophy with enlargement of the ventricular system and sulci, while the cerebellum appears quite normal.
Cerebellar atrophy, brainstem atrophy and hypogenesis of the corpus callosum have also been reported as associated findings in few cases.\textsuperscript{18,20,23}

Magnetic resonance spectroscopy data in AGS are reported in just one study\textsuperscript{24} in which two cases showed reduced NAA/Cr, reflecting decreased neuronal/axonal density or viability, increased myo-inositol/Cr, reflecting gliosis or osmotic stress, and a persisting brain lactic alkalosis, similar to that seen in infants surviving perinatal hypoxia–ischaemia.

\section*{Cerebrospinal fluid}

Analysis of the CSF is an important part of the diagnostic work up in AGS as it can reveal specific abnormalities: CSF lymphocytosis (\textgreater 5–100 cells/mm\textsuperscript{3}), recognized from the earliest reports as a fundamental diagnostic finding,\textsuperscript{1} and raised INF-alpha levels (\textgreater 2 UI/ml) in the absence of other signs of infectious processes, a finding first described by Lebon\textsuperscript{2,25} and confirmed by other authors as a specific marker of the disease.\textsuperscript{7} Increased levels of INF-alpha can also be found in the blood plasma, but, being less marked and less constant than the INF-alpha increases found in the CSF, they do not have the same diagnostic value.\textsuperscript{8} The increases found in the CSF appear very marked in children with a neonatal onset of the syndrome, and less so (although the levels are still high) in those with a clinical onset after 3 months of age.

The CSF lymphocytosis and increased INF-alpha concentrations are very evident at onset of the disease. Over time, these features tend to regress gradually to reach normal levels between the ages of 3 and 4 years;\textsuperscript{7,10} on the other hand, they may persist for many years in some subjects.\textsuperscript{2,17,26} Furthermore, they do not always co-exist: Crow and Rice\textsuperscript{7} reported subjects in an early stage of the disease with raised INF-alpha concentrations but normal lymphocyte counts in the CSF.

Other authors recently reported\textsuperscript{27} that another cytokine, CXCL10, is also markedly raised in the CSF of patients with AGS, suggesting that it may constitute an additional CSF marker of the disease. Given that this cytokine is a strong chemoattractant for activated lymphocytes, it can be hypothesized that it is responsible, at least in part, for the CSF lymphocytosis typical of AGS.

Other CSF parameters routinely investigated are normal in AGS, with the exception of the finding, in a few cases, of raised CSF protein without oligoclonal bands.\textsuperscript{8,10}

In 2003, Blau \textit{et al.} first described\textsuperscript{28} alterations in CSF pterins (increased) and folates (reduced) in AGS, and Rice\textsuperscript{7} confirmed that
many patients with molecularly proven AGS display neopterin and biopterin overproduction, in the presence of normal levels of the neurotransmitter metabolites. This profile may represent another good CSF marker of AGS and could be a further consequence of the primary overproduction of IFN-alpha.\textsuperscript{10}

**Neuropathology**

There are few studies\textsuperscript{3,29–31} that furnish data on the anatomopathological aspects of AGS. The main findings in this regard are the presence of microcephaly, of diffuse inhomogeneous demyelination with astrocystosis, and multiple microinfarctions in the neocortex and cerebellar cortex suggesting microangiopathy. Calcific deposits are present in the white matter, thalami, basal ganglia, dentate nuclei and also in the media, adventitia and perivascular spaces of small vessels.

In some subjects with high circulating levels of INF-alpha\textsuperscript{2,4} tubuloreticular inclusions in endothelian cells are observed.

A histological study of the skin lesions presented by a 2-year-old girl initially diagnosed with chilblain lupus and subsequently with AGS was described in a recent report:\textsuperscript{32} she presented epidermal necrosis with intraepidermal bulla formation, lymphocytic vasculitis with fibrinoid necrosis and thrombi formation.

**Genetics**

AGS has a genetic aetiology. This was suspected from as early as the first clinical description of the disease.\textsuperscript{1} With the identification of the first locus on chromosome 3, an autosomal recessive mode of transmission was established.\textsuperscript{33} It subsequently became clear that AGS is genetically heterogeneous, in other words that mutations in different genes can produce the phenotype typical of the syndrome.\textsuperscript{33} The second locus was identified on chromosome 13 in 2006.\textsuperscript{34} To date, we know of four genes that, if mutated, can give rise to AGS:\textsuperscript{35,36} they are TREX1 on chromosome 3, known as AGS1; RNaseH2B/FLJ11712 on chromosome 13, known as AGS2; RNaseH2C/AYP1 on chromosome 11, known as AGS3 and RNaseH2A on chromosome 19, known as AGS4.

The first of these genes, TREX1, codes for an enzyme (DNase III) involved in breaking down single strand DNA (ssDNA), while the RNaseH2A, B and C genes encode three separate proteins that function as a single enzyme complex called RNaseH2 which, instead, is involved in breaking down RNA; in particular, the RNaseH2 complex, at least
in yeast, has been implicated in the removal of Okazaki fragments (RNA primers on the lagging strand) during DNA synthesis and in cleaving ribonucleotides from DNA:DNA duplexes, an unusual event.\textsuperscript{35,36}

The inheritance of the disease is confirmed as autosomal recessive in the vast majority of cases, who are almost always homozygous or compound heterozygous for mutations within the same gene, even though there are reports of rare cases of AGS with heterozygous TREX1 mutations,\textsuperscript{9,37} suggesting that there may exist rare forms with a ‘de novo’ mutation and an autosomal dominant pattern of inheritance.

Mutations in these four genes have been found in 83% of patients,\textsuperscript{7,38} in whom diagnosis of AGS is thus confirmed; this suggests that there must exist at least one other gene, still to be identified, responsible for the disease in the remaining 17% of cases.\textsuperscript{7,9} The frequency of mutation differs in the four known genes:\textsuperscript{9} mutations in the RNaseH2B (AGS2) gene, found in 40% of AGS patients known to have a mutation, are the most frequent, followed by mutations in TREX1 (AGS1) (25%), which is the gene most frequently mutated in Northern European families. The RNaseH2C (AGS3) gene is more rarely mutated (14%). The mutation most recurrent in this gene, restricted to cases belonging to families from Pakistan, seems to indicate a ‘founder effect’ in this population. Finally, mutations in RNaseH2A (AGS4) are very rare, found in less than 4% of patients with mutations.

Even though mutations in the different genes can produce very similar phenotypes, there may exist a certain genotype–phenotype correlation:\textsuperscript{7,9,38} in particular, the forms characterized by onset in the neonatal period, a more severe clinical picture and a higher childhood mortality rate (34%), are associated with mutations in the TREX1, RNaseH2A and RNaseH2C genes, whereas the later-onset forms (i.e. after the first months of life), in which the clinical picture is still severe but the life expectancy longer (mortality rate 8%), like the atypical pictures in which intellectual function is relatively preserved, are more frequently associated with mutations in RNaseH2B.

### Pathogenesis

Even though the enzymatic function of the TREX1 protein and RNASEH2 complex is not fully understood, there is no doubt that both are involved in the process of getting rid of the nucleic acids physiologically released into our own organisms as a result of normal cell death. The hypothesis is that in AGS a defect in the DNA damage response could lead to an accumulation of endogenous DNA or
DNA–RNA hybrids which, mistaken by the organism for DNA and RNA of viral origin, may trigger an INF-alpha-mediated immune response similar to that which occurs during viral infections.\textsuperscript{9,35–37} It seems quite clear that this is a response of the innate immune system, the ‘first-line’ mechanism of defence against pathogenic infection.\textsuperscript{39} Unlike the acquired immune system, which involves the production of immunoglobulin from T and B cells, the innate immune system depends on phagocytic cells, such as macrophages, that eliminate foreign substances, triggering a complex cascade of cell events leading to the synthesis of cytokines, such as interferons (INFs). The combined efforts of researchers working in the different fields—clinical, genetic, immunological, biochemical and biological—have produced numerous results that seem to confirm this pathogenetic hypothesis, particularly as regards the consequences at cell level of mutations in TREX1.\textsuperscript{40–42}

TREX1, which normally resides in the endoplasmic reticulum but relocates to the nucleus in response to oxidative stress,\textsuperscript{42,43} has a crystal structure made up of three sites of catalytic activity and a C-terminal domain that is crucial for the compartmentalization of TREX1 and for its relocation into the nucleus but has no role in its catalytic activity. Different mutations in TREX1 can lead to different phenotypes, depending on which TREX1 function is impaired (e.g. heterozygous mutations in the C-terminal region, that do not affect exonuclease activity but impact on TREX1 intracellular localization, have been identified in an autosomal dominant disease, retinal vasculopathy with cerebral leukodystrophy, RVCL).\textsuperscript{44}

In the animal model, TREX1(-/-) mice, deficient enzymatic activity of TREX1 leads to excessive accumulation of ssDNA in the cytoplasm of replicating cells: surprisingly, however, this mouse does not present an AGS-like phenotype, but rather a picture of inflammatory myocarditis with progressive dilatory cardiomyopathy, consistent with an autoimmune aetiology.\textsuperscript{45}

The build up of nucleic acids, particularly non-degraded ssDNA,\textsuperscript{46} occurring in the absence of TREX1 enzymatic activity, could activate the TLR9 toll-like receptors in the endoplasmic reticulum: these receptors work, like sensors, by detecting viral DNA and RNA and triggering a complex cascade of cellular events.\textsuperscript{39} Furthermore, recent work by Stetson\textsuperscript{47} indicates that in TREX1 null mice there exists a novel cytosolic cell-intrinsic mechanism, non-toll-like receptor dependent, that acts through the transcription factor INF regulatory factor 3 (IRF3) and activates a cell-intrinsic autoimmune response. In any case, multiple substrates\textsuperscript{47} of TREX1 might accumulate in absence of TREX1 and result is an increased genic expression and synthesis of cytokines, such as INFs, which have long been suggested to play a role in the pathogenesis of the lesions typical of AGS.\textsuperscript{40}
Even though patients with AGS who do not present mutations in TREX1 have not yet been shown in formal studies to present nucleic acid build ups, the mechanism associated with mutations in genes that encode the RNaseH2 enzyme complex may be very similar, except for the fact that, in this case, it would be other nucleic acids (like RNA or RNA–DNA hybrids) activating other receptors and, finally, the innate immune system. In any case, the outcome would still be inappropriate secretion of INF-alpha.40,41

The role of interferon-alpha

Even before the recent genetic discoveries, it was a widely held view among authors that INF-alpha may play a pathogenetic role in AGS:25 the vascular changes and calcification seen in AGS are very similar to those reported in the INF-alpha transgenic mouse,48 whose astrocytes are chronic producers of INF-alpha. Furthermore, patients receiving INF treatment (e.g. for cancer or hepatitis) present a syndrome characterized by fever, nausea, other influenza-like signs and also vasculitic lesions bearing similarities to the chilblains seen in AGS; they also have a greater risk of developing autoimmune diseases, including systemic lupus erythematosus (SLE).42 INF-alpha can also induce the presence of tubuloreticular inclusions,25 similar to those found in skin biopsies of patients with AGS2,4 and in subjects affected by SLE.

Further confirmation of the pathogenetic role of INF is provided by recent studies49 that have analysed CSF samples from patients with AGS using the cDNA-microarray technique: the emerging gene expression profile is characterized by upregulation of INF-related genes, supporting a role of INF-alpha, and by downregulation of angiogenesis-related genes, probably related to the INF-alpha increase in CSF, since it has been established that INF-alpha hampers angiogenesis.

Furthermore, the clinical evolution of AGS also ‘reflects’ the pattern of INF-alpha in the CSF: an initial ‘active’ stage of the disease, in which the level of INF-alpha in the CSF is probably responsible for many of the symptoms, is followed by a second phase in which the picture remains substantially stable, there are no INF-related symptoms and the concentration of INF-alpha in the CSF returns to normal.10 In the early stages of the disease, the high level of INF-alpha, also in humans produced by astrocytes,27 is capable, probably through a microangiopathic mechanism similar to that observed in the transgenic mouse,48 of giving rise to the disease’s characteristic lesions, in particular, the calcifications, white matter abnormalities and systemic symptoms.
Intriguingly, INF-alpha has been found to be increased in other autoimmune diseases, too; in particular, in SLE it is markedly raised in blood plasma and its level has been found to correlate with disease activity and severity. However, as recently underlined by Van Heteren, there are considerable differences between AGS and other autoimmune diseases such as SLE and Sjögren syndrome: for example, circulating autoantibodies are found present less frequently, and above all in lower concentrations, in patients with AGS than in those with autoimmune disorders; furthermore, in autoimmune disorders, they play an important pathogenetic role in the production of INF-alpha, whereas in AGS their presence is probably just a consequence of the INF increase and not its cause.

The recent identification of the genes responsible for AGS has led to the suggestion that there may exist other disease models deriving from genes involved in the DNA damage response, in which inappropriate activation of the innate immune system leads, ultimately, to increased secretion of INF-alpha.

**Diagnostic criteria**

In spite of the recent important advances in the genetics of AGS, it is nevertheless important, given that there is still a considerable proportion (around 17%) of cases in which genetic analysis is uninformative, to bear in mind the clinical and neuroradiological criteria fundamental for a diagnosis of the syndrome. It is to be recalled that some of these criteria are ‘age specific’ or rather ‘evolution specific’, in other words, that the significance of their presence or absence differs in different stages of the disease: for example, at onset of the symptoms, the presence of cerebral calcifications, while very frequent, is not an indispensable diagnostic criterion: they may be absent at onset and the patients should be monitored, over time, to see if they appear. Similarly, raised INF-alpha in the CSF is a very specific finding in the first stages of the disease, but its absence some years after the onset does not exclude a diagnosis of AGS.

There are certain methodological aspects that can contribute to a delay in diagnosis: for example, CSF INF-alpha is a very reliable marker in the early stages of the disease but few centres seem able to measure it. Similarly, cerebral calcifications are not readily identified on MRI, even though this technique is routinely used in the diagnostic work up. It is, therefore, important to perform brain CT scans in all cases of unexplained early onset leukoencephalopathy.

These considerations apart, the main criteria for a diagnosis of AGS are:
1. Early onset encephalopathy with psychomotor delay, spasticity, extrapyramidal signs and microcephaly, the latter appearing in the course of the first year of life.

2. Calcifications particularly visible at basal ganglia level (putamen, pallidus and thalamus), but also extending to the periventricular white matter.

3. Cerebral white matter abnormalities.

4. Cerebral atrophy.

5. Exclusion of pre-/perinatal infections, in particular the TORCH complex (toxoplasmosis, rubella, cytomegalovirus, herpes simplex virus).

6. Chronic lymphocytosis (>5 cells/mm³) on CSF examination, not accompanied by any other sign of an infectious process.

7. Raised INF-alpha in the CSF (>2 IU/ml).

8. Elevated neopterins and biopterins in CSF, sometimes associated with decreased folates.

9. Important systemic symptoms in the early stages of the disease include irritability, feeding and sleeping difficulties, unexplained fevers and the appearance of chilblain-like skin lesions on the fingers, toes and ears.

10. Genetic screening for mutations in the four genes known to cause AGS allows definitive confirmation of the diagnosis in the majority (83%) of cases.

**Prenatal diagnosis**

Since AGS is, with rare exceptions, inherited as an autosomal recessive trait, most couples with an affected child have a 25% risk of the disease recurring in each future pregnancy. The rare cases in which it is inherited as an autosomal dominant trait (heterozygous TREX1 mutation) appear to be sporadic and attributable to de novo mutations. The first prenatal diagnosis of AGS, reported in 2005, was based on the evidence of increased INF-alpha levels in foetal blood taken from a 28-week-old foetus which had presented cerebral calcifications on the 26-week ultrasound scan. The following year, Desanges reported a second diagnosis, based on a similar increase.

Today, genetic tests allow us to confirm the AGS diagnosis in a large proportion of cases. However, it is important to note that diagnostic prenatal testing is possible only in families that already have an affected child, in whom the disease-causing gene has been identified. If the affected child’s mutation is known, then the DNA of foetal cells obtained by chorionic villus sampling (at 10–12 weeks of gestation) or by amniocentesis (15–18 weeks of gestation) can be examined for the presence of the same mutation.
**TREX1-related disorders**

In addition to AGS, other known diseases have been linked to mutations in the TREX1 gene.

*Cree encephalitis* is a familial encephalopathy present in Indian Cree children in Northern Quebec, and it shows many similarities with the most severe forms of AGS (progressive microcephaly, cerebral atrophy, calcifications and white matter abnormalities, CSF lymphocytosis and raised levels of INF-alpha, systemic immune abnormalities). As long ago as 2002, it was suggested that the two disorders are allelic and this was subsequently confirmed by molecular genetic analysis: all children with Cree encephalitis are homozygous for the same TREX1 mutation.

*Familial chilblain lupus* (FCL) is a rare, autosomal dominant cutaneous form of lupus in which patients, in childhood, display painful bluish-red inflammatory skin lesions on fingers, toes, ears and nose; the lesions worsen with cold or wet exposure, sometimes leading to skin ulcers, atrophy and sometimes necrosis affecting the extremities. Circulating immunocomplexes are sometimes present, but, unlike the non-FCL variant, no risk of developing SLE is reported. Furthermore, there are no associated neurological symptoms. Both Rice and Lee-Kirsch (2007) have identified heterozygous TREX1 mutations in these subjects.

*RVCL* is an adult-onset genetic disease inherited as an autosomal dominant trait. Although essentially affecting the CNS and retina, it has a broad spectrum of symptoms. The presence of progressive neurological impairment means that the prognosis is often poor. Microvascular obliterative endotheliopathy leads to loss of central vision and causes migraine, stroke, dementia and Raynaud’s phenomenon. Affected subjects sometimes present abnormal kidney, liver and gastrointestinal function parameters. Richards identified heterozygous mutations in the C-terminal domain of TREX1, which do not affect exonuclease activity, but impact on TREX1 intracellular localization.

Systemic lupus erythematosus: Many genes are involved in the pathogenesis of SLE, considered the prototype of autoimmune diseases. Heterozygous TREX1 mutations were reported in 3% of the population with SLE analysed by Lee-Kirsch. The literature also contains reports of patients in whom clinical pictures of AGS and SLE overlap.

These findings raise the possibility that heterozygous parents of children with AGS caused by TREX1 mutations may be at risk of developing RVCL and SLE.
Differential diagnosis

Once congenital infections belonging to the TORCH spectrum have been ruled out, the first step in the differential diagnosis of AGS is usually to exclude other conditions in which basal ganglia calcifications are associated with early onset encephalopathy.

It is worth pointing out that some cases described in the literature as independent clinical entities seem to fit the picture of the early onset neonatal forms of AGS, for example, many of the cases designated MICS (microcephaly–intracranial calcification syndrome) or ‘pseudo-TORCH syndrome’, and also the cases Dale referred to as ‘familial systemic lupus erythematosus’.

The main forms to be taken into consideration in the differential diagnosis of AGS are Cockayne syndrome, the mitochondrial diseases, haemophagocytic lymphohistiocytosis and metabolic encephalopathies with basal ganglia calcifications such as, for example, parathormone metabolism disorders, biotinidase deficiency, 3-hydroxyisobutyric aciduria, Hoyeraal–Hreidarsson syndrome and cerebroretinal microangiopathy with calcifications and cysts (CRMCC).

It is also important to consider the possibility of AGS in cases of unexplained early onset leukodystrophy, both those with predominant fronto-temporal white matter involvement with cyst formation—resembling Alexander disease or vanishing white matter disease—and those with more diffuse and non-specific white matter involvement, especially when CT is not performed.

Management and therapy

Treatment of AGS is currently only symptomatic and includes the use of drugs to control epilepsy, the prevention of complications and postural abnormalities, respiratory physiotherapy to treat lung infections and dietary monitoring to ensure adequate caloric intake. It is particularly important to screen patients regularly for the symptoms that can be treated, such as glaucoma or endocrine problems (e.g. diabetes or hypothyroidism). As regards the chilblain lesions, neither vasodilators nor immunosuppressors have shown any real therapeutic efficacy in AGS, and treatment of these symptoms is limited to protecting the vulnerable parts from the cold and preventing infections that could complicate the situation.

In accordance with the suggestions advanced by Blau in 2003, oral treatment with folinic acid might produce a general improvement of the clinical conditions in AGS patients presenting reduced folates in the CSF, but there are as yet no data in the literature confirming this hypothesis.
Given the involvement of immune system activation as a pathogenetic mechanism in AGS, it has been suggested that high-dose corticosteroid therapy might alter the course of the disease. Kuijpers, treating three cases, found a reduction in the levels of INF-alpha in the CSF, but no change in the clinical picture. Similarly, recent reports of genetically confirmed cases treated with high doses of steroids or i.v. immunoglobulin in the active stage of the disease do not seem to show any real change in the clinical course.

Conclusion

AGS is a genetic disease with a severe clinical picture. It can be misdiagnosed as a congenital infection or, unless a brain CT scan is performed, as a leukoencephalopathy of unknown origin. Recent advances in the genetics of AGS have revealed a phenotype that is broader and more heterogeneous than the classical description of the disease and there is no doubt that this syndrome, albeit rare, is currently under-diagnosed.

Current studies aiming to clarify the molecular mechanisms underlying the pathogenesis of AGS could lead to the development of new therapeutic strategies. These treatments may, for example, act on the pathway between the undigested nucleic acids and activation of the innate immune system, be based on drugs targeting the cells responsible for the production of cytokines, or block INF-alpha activity directly.

Acknowledgements

Our sincere thanks go to Dr C. Uggetti, for providing the neuroradiological images, and to Dr Y. Crow (Leeds) and Prof. P. Lebon (Paris) for their continuous and helpful collaboration in our clinical and scientific work. We also thank Catherine Wrenn for her valuable help in translating the manuscript.

Special thanks to IAGSA (International Aicardi-Goutières Syndrome Association) and in particular to Mrs Fiammetta Boni Longo, President of IAGSA, and to the affected children and their families for their support and encouragement in our daily work.

References


