Cree encephalitis is allelic with Aicardi-Goutières syndrome: implications for the pathogenesis of disorders of interferon alpha metabolism


Aicardi-Goutières syndrome (AGS) is an early onset, progressive encephalopathy characterised by calcification of the basal ganglia, white matter abnormalities, and a chronic cerebrospinal fluid (CSF) lymphocytosis. Cree encephalitis shows phenotypic overlap with AGS although the conditions have been considered distinct because of immunological abnormalities observed in Cree encephalitis. We report that levels of interferon alpha (IFN-α), a marker of AGS, are raised in Cree encephalitis. Moreover, linkage analysis indicates that the disorders are allelic and refines the AGS1 locus to a 3.47 cM critical interval. Our data show that a CSF lymphocytosis is not necessary for the diagnosis of AGS and strongly suggest that AGS and pseudo-TORCH syndrome are the same disorder. Recognition of immunological dysfunction as part of the AGS phenotype provides further evidence of a primary pathogenic role for abnormal IFN-α production in AGS.

In 1984, Jean Aicardi and Françoise Goutières described eight children with an early onset, progressive encephalopathy characterised by calcification of the basal ganglia, white matter abnormalities, and a chronic CSF lymphocytosis. Cree encephalitis is characterised by severe psychomotor retardation, progressive microcephaly, cerebral atrophy, white matter attenuation, intracerebral calcification, a CSF lymphocytosis, and systemic immune abnormalities. In 10 of 11 affected children described, premature death resulted at a median age of 20.6 months. Although these features were noted as reminiscent of AGS, the conditions were considered distinct in view of the observation of immunological abnormalities and an apparent susceptibility to infection in Cree encephalitis.

We recently reported localisation of a gene for AGS on chromosome 3p21 (AGS1). Our results suggested the existence of locus heterogeneity, with approximately 50% of families mapping to AGS1. In view of their phenotypic similarity, we hypothesised that AGS and Cree encephalitis might represent congenital infection which led to the measurement of IFN-α in children with the disease. A report of raised levels of CSF IFN-α in 14 of 15 patients with AGS suggests a close association between this parameter and the other clinical and laboratory features, such that an increase of CSF IFN-α in the absence of infection is currently considered a marker for the condition.

In 1988, Black et al described an early onset, progressive encephalopathy in an inbred Canadian aboriginal community. They termed this disease Cree encephalitis and distinguished it from another neurological condition, Cree leucoencephalopathy, occurring at high frequency in the same population. Cree encephalitis is characterised by severe psychomotor retardation, progressive microcephaly, cerebral atrophy, white matter attenuation, intracerebral calcification, a CSF lymphocytosis, and systemic immune abnormalities. In 10 of 11 affected children described, premature death resulted at a median age of 20.6 months. Although these features were noted as reminiscent of AGS, the conditions were considered distinct in view of the observation of immunological abnormalities and an apparent susceptibility to infection in Cree encephalitis.

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allelic disorders. Such a possibility was further strengthened by the observation of acrocyanosis, resulting in auto-amputation of the digits, in one child with Cree encephalitis. This description is reminiscent of the chilblain-like lesions seen in AGS. Because of the consistent association of raised levels of CSF IFN-α with AGS, we undertook to measure IFN-α in patients with Cree encephalitis. Additionally, using samples from families affected by Cree encephalitis, we performed a linkage analysis across the AGS1 interval. We report the results of these studies here.

SUBJECTS AND METHODS

Fourteen children are known to have been affected by Cree encephalitis since 1966 (fig 1). Blood or tissue samples from seven affected children, their parents, and four unaffected sibs were available for linkage analysis (fig 2). CSF and serum interferon alpha levels were measured in three of these affected subjects. The study was approved by the Leeds Health Authority/United Teaching Hospitals NHS Trust Research Ethics Committee, the Eeyou Awaash Foundation, and the Cree Board of Health and Social Services of James Bay, Canada.

<table>
<thead>
<tr>
<th>Patient (see fig 1)</th>
<th>Age at presentation (months)</th>
<th>Birth OFC (gestation in weeks)</th>
<th>Postnatal OFC (age in months)</th>
<th>Brain calcification</th>
<th>White matter hypodensities</th>
<th>CSF WCC/mm³ (age in months)†</th>
<th>IFN-α IU/l in CSF/serum* (age in months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>XI.8</td>
<td>1</td>
<td>35 cm (40)</td>
<td>41 cm (27)</td>
<td>PV</td>
<td>Yes</td>
<td>9 (1)</td>
<td>100 / 20 (1)</td>
</tr>
<tr>
<td>XII.5</td>
<td>Birth</td>
<td>35.5 cm (40)</td>
<td>39.5 cm (8)</td>
<td>PV</td>
<td>Yes</td>
<td>9 (24)</td>
<td>NA</td>
</tr>
<tr>
<td>XII.7</td>
<td>1</td>
<td>33 cm (40)</td>
<td>&lt;2nd centile (2)</td>
<td>BG</td>
<td>Yes</td>
<td>47 (2)</td>
<td>NA</td>
</tr>
<tr>
<td>XII.9</td>
<td>Birth</td>
<td>NK</td>
<td>NK</td>
<td>BG, WM</td>
<td>Yes</td>
<td>25 (5)</td>
<td>NA</td>
</tr>
<tr>
<td>XII.12</td>
<td>9</td>
<td>35.5 cm (40)</td>
<td>46 cm (20)</td>
<td>BG, DN</td>
<td>Yes</td>
<td>5 (9)</td>
<td>12/50 (9)</td>
</tr>
<tr>
<td>XII.12</td>
<td>8</td>
<td>33 cm (40)</td>
<td>43 cm (48)</td>
<td>BG, WM, PV</td>
<td>Yes</td>
<td>11 (48)</td>
<td>NA</td>
</tr>
<tr>
<td>XIV.3</td>
<td>1</td>
<td>34.5 cm (39)</td>
<td>38 cm (3)</td>
<td>BG, WM, PV</td>
<td>Yes</td>
<td>4 (3)</td>
<td>50/NA (3)</td>
</tr>
</tbody>
</table>

OFC = occipitofrontal circumference. WCC = white cell count. BG = basal ganglia. WM = white matter. PV = periventricular. DN = dentate nuclei. NK = not known. NA = not analysed.

†Abnormal >5 cells/mm³.

*Normal levels <2 IU/l.
Information regarding marker order and genetic distance was obtained from the Marshfield Linkage Maps (http://research.marshfieldclinic.org/genetics) and the June 2002 freeze of the Human Genome Browser (http://genome.cse.ucsc.edu/). Two polymorphic markers, designated M2 and M3, were designed from available sequence data for BAC AC023910 (primer sequences available on request). A linkage analysis of Cree encephalitis patients was performed using polymorphic markers across the **AGS1** locus. After individually optimised PCR amplification, markers were analysed using previously described methods.\(^8\)

Autosomal recessive inheritance with full penetrance was assumed. Two cases of Cree encephalitis were identified between 1988 and 1999 among a population having 3400 live births recorded in the same period. The disease allele frequency was therefore estimated at 1 in 19. Marker allele frequencies were calculated by genotyping 29 unrelated subjects from the same population who had no close relative affected by Cree encephalitis. A minimum lower marker allele frequency was set at 0.1. Pedigree allele inconsistencies were identified using PedCheck.\(^10\)

Two point analysis of the simplified pedigree shown in fig 2 was performed using the LINKAGE analysis programs.\(^11\)

Interferon alpha assays were performed using a previously described method.\(^{12,13}\)

## RESULTS

All seven affected children included in this study showed progressive microcephaly, intracerebral calcification, and severe psychomotor delay. Only five of these seven exhibited raised numbers of white cells (>5 cells/mm\(^3\)) in the CSF. However, IFN-\(\alpha\) levels were raised in all three subjects examined, including the two children (XII.12 and XIV.3) with normal CSF white cell counts (table 1). In one case (XI.8), serum and CSF IFN-\(\alpha\) levels were consistently raised over a 12 month interval. A number of extra-neurological features were also noted. Specifically, abnormalities of liver function and derangement of haematological and immunological indices were observed frequently (table 2).

Genotyping across the **AGS1** locus identified a Cree ancestral haplotype homozygous in all seven affected subjects available for study (fig 2). Two point linkage analysis of markers across this Cree ancestral haplotype gave positive lod scores with a maximum score of 4.14 at D3S3629 (\(\theta=0\)) (table 3). The 3.47 cM interval consistent with linkage lies between markers D3S3559 and D3S1289 and falls within 1 lod unit support interval of the maximum **AGS1** HLOD score (fig 3).

### Table 2

Details of extra-neurological features noted in subjects affected by Cree encephalitis

<table>
<thead>
<tr>
<th>Patient (see fig 1)</th>
<th>Platelets ((\times 10^9/l))</th>
<th>Hepatosplenomegaly</th>
<th>Raised liver enzymes (AST/ALT)</th>
<th>IgG/IgM</th>
<th>Rheumatoid factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>XI.8</td>
<td>76. Purpura</td>
<td>Yes</td>
<td>Yes</td>
<td>5.15/1.06 Negative</td>
<td></td>
</tr>
<tr>
<td>XII.5</td>
<td>NK</td>
<td>No</td>
<td>No</td>
<td>1.7/0.21 Negative</td>
<td></td>
</tr>
<tr>
<td>XII.7</td>
<td>NK</td>
<td>No</td>
<td>No</td>
<td>1.8/0.47 NK</td>
<td></td>
</tr>
<tr>
<td>XII.9</td>
<td>Thrombocytopenia</td>
<td>Yes</td>
<td>NK</td>
<td>1.4/0.42 Positive</td>
<td></td>
</tr>
<tr>
<td>XII.12</td>
<td>Thrombocytopenia, purpura, and bruising</td>
<td>No</td>
<td>No</td>
<td>16.3/3.6 NK</td>
<td></td>
</tr>
<tr>
<td>XIII.8</td>
<td>NK</td>
<td>NK</td>
<td>CK 150</td>
<td>3.1/0.17 1:1800</td>
<td></td>
</tr>
<tr>
<td>XIV.3</td>
<td>20–35. Required several platelet transfusions</td>
<td>Yes</td>
<td>Yes</td>
<td>7.29/1.33 NK</td>
<td></td>
</tr>
</tbody>
</table>

NK = not known. Serum IgG normal range 0.55–1.8 g/l; IgM normal range 0.05–0.30 g/l.

### Table 3

Two point lod scores across the Cree ancestral haplotype using the simplified pedigree shown in fig 2

<table>
<thead>
<tr>
<th>Marker*</th>
<th>Maximum lod score at different values of theta ((\theta))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>D3S3559</td>
<td>0.11</td>
</tr>
<tr>
<td>D3S3582</td>
<td>2.41</td>
</tr>
<tr>
<td>D3S3629</td>
<td>4.14</td>
</tr>
<tr>
<td>D3S3729</td>
<td>2.32</td>
</tr>
<tr>
<td>D3S3640</td>
<td>3.41</td>
</tr>
<tr>
<td>M2</td>
<td>3.34</td>
</tr>
<tr>
<td>M3</td>
<td>2.90</td>
</tr>
<tr>
<td>D3S1289</td>
<td>2.57</td>
</tr>
<tr>
<td>D3S3721</td>
<td>-1.12</td>
</tr>
</tbody>
</table>

*Relative position determined according to June 2002 freeze of Human Genome Browser.

![Figure 3](http://img.bmj.com/)  
Schematic to show relative positions of **AGS1** critical interval and Cree ancestral haplotype.
DISCUSSION

In view of their phenotypic similarity, we hypothesised that AGS and Cree encephalitis might represent allelic disorders. The finding of raised levels of IFN-α in the children with Cree encephalitis specifically investigated is consistent with this hypothesis. Moreover, our genetic data confirm linkage of Cree encephalitis to the AGS1 locus on chromosome 3p21 and indicate that we have successfully refined the AGS1 critical interval to lie between D3S3559 and D3S1289 (fig 3). Because of probable genetic homogeneity in the Cree population, our data suggest that prenatal diagnosis of Cree encephalitis by linkage is now feasible. Locus heterogeneity precludes such a possibility in AGS at the present time.

We have previously drawn attention to the phenotypic overlap of AGS with pseudo-TORCH syndrome (MIM 251290). 8,14 Both conditions show basal ganglia calcification, a leucoencephalopathy, and brain atrophy. However, they are said to differ by the presence in pseudo-TORCH syndrome of an early onset microcephaly, neonatal disturbance of liver function with thrombocytopenia, and a normal CSF white cell count. 5 Importantly, the majority of children described here showed significant thrombocytopenia and abnormalities of liver function. Moreover, while most affected subjects showed a CSF leukocytosis, two children had raised levels of CSF IFN-α despite normal CSF white cell counts. This latter observation confirms earlier reports suggesting that a CSF pleocytosis should not be considered mandatory for the diagnosis of AGS and highlights the importance of IFN-α measurement in this condition. 16 On the basis of these findings, we suggest that AGS and pseudo-TORCH syndrome are probably the same disorder.

Dale et al 2 recently reported a congenital infection-like syndrome comprising intracranial calcification, hepatitis, thrombocytopenia, and immunological abnormalities including hypocomplementaemia, progressive autoantibody activation, and raised levels of IgG and IgM. So striking were the immunological abnormalities that these authors described this disorder as “familial systemic lupus erythematosus”. However, an accompanying editorial highlighted the similarity of this condition to AGS and suggested that immune system dysfunction might form part of the AGS phenotype. 18 Our present findings support this suggestion. Other similarities between AGS and systemic lupus erythematosus (SLE) are of interest. Thus, the skin lesions seen in AGS 9 and Cree encephalitis 16 bear clinical and pathological similarity 17 to those observed in SLE. 11 Additionally, intracranial calcification, with a predilection for the basal ganglia, is well recognised in SLE, 5,19 occurring in up to 30% of patients with cerebral lupus. 20

There is evidence to suggest that AGS results from a primary genetic defect of IFN-α metabolism. Briefly, CSF IFN-α levels are consistently raised in the early stages of the disease, 3,10 treatment with IFN-α in a number of clinical settings 21 can induce skin lesions similar to those seen in AGS, 22,23 and of particular importance, astrocyte specific chronic overproduction of IFN-α in transgenic mice recapitulates the neuropathological findings observed in AGS 24 and Cree encephalitis. 16 Notably, as in AGS, serum levels of IFN-α are raised in SLE 25–27 and therapy with IFN-α can actually induce the clinical and immunological features of the latter condition. 28–30 Raised levels of IFN-α are not seen in other autoimmune disorders. 31–33 These observations suggest that IFN-α plays a fundamental role in the pathogenesis of SLE. 34

It was the resemblance of the clinical phenotype to congenital infection which first led to the measurement of IFN-α levels in children with AGS. 5 The effects of congenital human immunodeficiency virus (HIV) infection also include intracranial calcification and white matter abnormalities. These features are quite distinct from those resulting from HIV infection acquired postnatally, 35–37 suggesting a specific susceptibility of the developing brain to intrauterine HIV exposure. Interestingly, high levels of IFN-α are observed in neonates showing evidence of prenatal HIV infection. 38

We hypothesise that the phenotypic similarities shared by AGS, SLE, congenital rubella and in utero HIV infection result from the abnormal regulation of IFN-α. In the case of prenatally acquired rubella and HIV infections, IFN-α production is presumably triggered by viral exposure, with the pathological consequences determined by the developmental stage at which exposure occurs. The mechanism for stimulation of IFN-α production in SLE is unknown. 39 However, evidence exists for the presence of specific IFN-α inducing activity in the serum of SLE patients 40 and induction of dendritic cells by IFN-α possibly driving the autoimmune response. 41 In the case of AGS/Cree encephalitis/pseudo-TORCH syndrome, excess IFN-α production might result from an inborn error of IFN-α metabolism. Identification and characterisation of the AGS1 gene may therefore provide new insights into the control of IFN-α production and its role in congenital infection and particular autoimmune diseases.

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