Aicardi-Goutières syndrome (AGS) is a rare encephalopathy, arising during the first year of life, characterized by cerebral atrophy, leukodystrophy, basal ganglia calcification, raised interferon (IFN)-alpha in the CSF, CSF lymphocytosis, and negative serologic findings for infections. Identification of AGS pathogenesis may provide insights into the neurodegenerative mechanism resulting from exposure of the developing human brain to IFN-alpha.

The aim of this study is to provide an insight into global gene expression of CSF cells in AGS using microarray technology.

Methods. CSF aliquots have been collected from patients with AGS and controls for diagnostic purposes. The study was approved by the Ethical Committee of the IRCSS Mondino, University of Pavia, Italy.

Patients with AGS (n = 20), 12 boys and 8 girls (age 4.5 ± 4.4 years), have been identified by the presence of an appropriate clinical picture in presence of CSF lymphocytosis (>5 cells/mm³), IFN-alpha in CSF ≥2 IU/mL, in absence of any infections.

Controls, 11 boys and 9 girls, matched to patients with AGS by gender, age (age 4.4 ± 4.3 years), and number (n = 20), devoid of any alteration in the CSF, included 13 subjects with congenital hydrocephalus, 3 with endocranial tumor, 2 with myelo-meningocele with hydrocephalus, 1 with choroid plexus papilloma, and 1 with upper sella turcica cyst.

Lymphocyte was the main cell type detected (>80%) in the CSF of all recruited subjects, the cell count being 40.8 ± 8.66 cells/mm³ in patients with AGS and 2.0 ± 1.13 cells/mm³ in controls (mean ± SE). IFN-alpha in CSF was <2 IU/mL in controls and 52.95 ± 12.15 IU/mL (mean ± SE) in patients with AGS.

RNA, as purified from CSF, underwent reverse transcription and amplification using real-time PCR obtaining cDNA sequences complementary to mRNA. An aliquot (20 μL) of each sample was used to determine the pre-plateau amplification cycle, the remaining sample (80 μL) being amplified at this cycle. Fluorescent probes complementary to cDNAs were synthesized using amino-allyl modified uridine nucleotides, DNA-polymerase, and Cy3/Cy5 labeling. A standardized amount of labeled oligonucleotides (20 pmol) was used for microarray hybridization. Used microarrays (Microarray Department of the University of Amsterdam, The Netherlands), spotted in duplicate with the expressed sequence tags of 19,000 genes, were hybridized and underwent laser scanning and data analysis by GeneSpring software (Silicogenetics, Billerica, CA).

Five genes (CathepsinD, IFRG28, BAI2, VEGFB, and GAPDH) were analyzed for their expression also by QPCR to substantiate microarray results.

Results. Results of patients with AGS as compared to controls are reported in the figure, A. Differences in gene expression accounted for a different location of patients with AGS and controls in a three-dimensional analysis-plot of the principal components of variance (figure, B). By applying the supervised k nearest neighbor algorithm, AGS cases and controls were correctly classified obtaining 38 correct predictions of the disease status, 0 incorrect prediction, while two AGS cases were not classified in any category. By using the unsupervised hierarchical cluster analysis (figure, C) all patients with AGS were located separately from controls in the hierarchical tree. However, the two patients with AGS unclassified by the k nearest algorithm were located in the hierarchical tree near to controls, thus indicating that the gene expression pattern of these two samples was similar between AGS patients and controls. These two patients were the oldest among those tested, being 7.6 and 11.8 years old.

One additional subject raising a diagnostic problem was included in the analysis (2-month-old girl bearing bilateral basal-ganglia calcification, normal neurologic examination in absence of any infection). This subject was classified as a control when her CSF gene-expression profile was inserted as unknown dis-
cases status in the k-nearest neighbor algorithm and after 2-years follow-up did not develop AGS.

As far as concerns gene alterations detected in all patients with AGS tested, 198 genes varied ($p < 0.05$ as tested by analysis of variance) their expression more than twofold in patients with AGS as compared to controls (figure, D). AGS predictor genes included upregulated genes, involved in interferon-dependent lymphocyte activation, and downregulated genes, involved in angiogenesis and cell cycle suppression.

**Discussion.** Obtained results provide evidence that it is feasible to apply gene expression analysis to CSF cells distinguishing between patients with AGS and unaffected controls. Diagnostic performance was good in subjects younger than 7 years, while patients with AGS older than 7 years cannot be identified.

Functional classification of genes undergoing altered expression in AGS provides information on disease pathogenesis. Upregulation of interferon-related genes supports the role of interferon in AGS. Downregulation of angiogenesis-related genes in AGS is likely to be a consequence of IFN-alpha increase in CSF, being established that IFN-alpha hampers angiogenesis.\(^5\)

Results of the current study are consistent with the hypothesis that AGS is an interferon-induced microangiopathy, which occurs early in life. We propose that brain injury results from insufficient vessel development, paralleled by lymphocyte activation in CSF.

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