

CME Cerebrospinal fluid pterins and folates in Aicardi-Goutières syndrome

A new phenotype

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Abstract—Objective: To describe three unrelated children with a distinctive variant of Aicardi-Goutières syndrome (AGS) characterized by microcephaly, severe mental and motor retardation, dyskinesia or spasticity, and occasional seizures. **Results:** Neuroimaging showed bilateral calcification of basal ganglia and white matter. CSF glucose, protein, cell count, and interferon alpha were normal. Abnormal CSF findings included extremely high neopterin (293 to 814 nmol/L; normal 12 to 30 nmol/L) and biopterin (226 to 416 nmol/L; normal 15 to 40 nmol/L) combined with lowered 5-methyltetrahydrofolate (23 to 48 nmol/L; normal 64 to 182 nmol/L) concentrations in two patients. The absence of pleocytosis and normal CSF interferon alpha was a characteristic finding compared to the classic AGS syndrome. Genetic and enzymatic tests excluded disorders of tetrahydrobiopterin metabolism, including mutation analysis of GTP cyclohydrolase feed-back regulatory protein. CSF investigations in three patients with classic AGS also showed increased pterins and partially lowered folate levels. **Conclusions:** Intrathecal overproduction of pterins is the first biochemical abnormality identified in patients with AGS variants. Long-term substitution with folinic acid (2-4 mg/kg/day) resulted in substantial clinical recovery with normalization of CSF folates and pterins in one patient and clinical improvement in another. The underlying defect remains unknown.

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Aicardi-Goutières syndrome (AGS) is a heterogeneous autosomal recessive progressive encephalopathy, characterized by calcifications of basal ganglia that can extend into white matter, cerebellum, and rarely to the cortex.¹ Of diagnostic importance are chronic pleocytosis and elevated levels of interferon alpha (IFN- α) in the CSF.^{2,3} Clinical features include postnatal microcephaly, moderate to profound developmental delay, spastic quadriplegia, extrapyramidal dyskinesia, visual disturbance with abnormal eye movement, and refractory seizures. Diagnosis of AGS is based on the following criteria: onset in the first year of life; basal ganglia calcifications; chronic CSF lymphocytosis (>5 cells/mm³); negative HIV, Epstein-Barr, and TORCH (toxoplasmosis, rubella, cytomegalovirus, and herpes simplex); no evidence of metabolic disorders; and elevated INF- α in CSF (>30 U/mL).^{4,5} Despite extensive clinical investigations, no other laboratory results of interest were observed. Whereas CSF pleocytosis and IFN- α are detected in acute viral diseases, basal ganglia calcifications may be a part of severe encephalopathy described under the heading of Fahr syndrome or can be associated with metabolic disorders such as dihydropteridine reductase deficiency (DHPR; OMIM 261630)^{6,7} or hereditary folate malabsorption (OMIM 229050).⁸

We report three infants with infantile onset of the disease between 0.5 and 6 months, all born of nonconsanguineous parents. Neuroimaging showed symmetric calcifications of the striatum and periventricular regions and disturbed myelination. The clinical picture included microcephaly and psychomotor retardation with dyskinetic movement or spasticity, compatible with the clinical diagnosis of AGS. Cell counts, interferon, and interleukin levels in CSF were normal, whereas neopterin and biopterin were extremely high and folates were reduced. There was no evidence of metabolic, immune-mediated, endocrine, or infectious disorders. In order to characterize this new AGS-like syndrome, we investigated cultured fibroblasts from one of the patients for tetrahydrobiopterin (BH₄)-metabolizing enzymes, and completed DNA analysis of the GTP cyclohydrolase feed-back regulatory protein (GFRP).

Materials and methods. *Patients.* **Case 1.** After a normal pregnancy terminated by premature rupture of the amniotic membranes, this boy was admitted to the neonatal intensive care unit (NICU) for treatment of congenital pneumonia. His birth weight was 2550 g (P3), length 47 cm (P10), and head circumference 33 cm ($<P3$). The family history was unremarkable. After 10 days, he had fluctuations in muscle tone, marked excitability, and dyskinesias. His pupillary response to light was slow, associated with bilateral papillary hypoplasia and retinal atrophy. Tendon reflexes were markedly increased. Further investigation revealed

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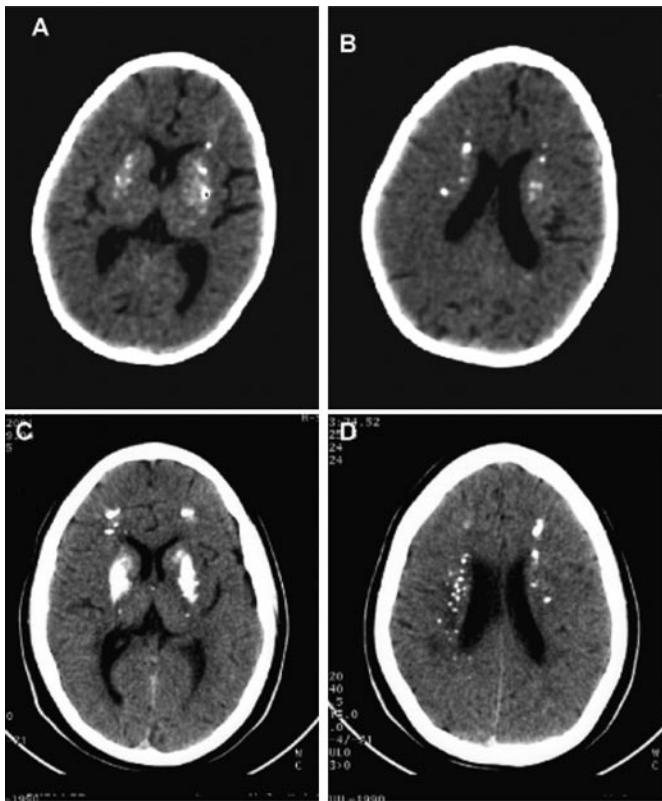


Figure 1. CT scan of Patient 2 at age 15 months (A and B) and 11 years (C and D) at the level of basal ganglia (A and C), showing calcification of the N. lentiformis, more pronounced in the putamen, and at the level of the cella media (B and D), showing periventricular calcification. In both locations, calcification is more marked at age 11 years.

progressive microcephaly, complete visual failure, profound psychomotor retardation, and development of tetraspasticity combined with dystonic movements. At 4 months of age, myoclonic seizures with electroencephalographic bursts of delta-activity and sharp wave discharges developed and he was started on primidone.

Neuroimaging showed marked white matter hypomyelination with bilaterally scattered calcifications, also involving the basal ganglia (data not shown).

Investigations ruled out hematologic, infectious, immune-mediated, endocrine, or metabolic causes. CSF repeatedly showed no pleocytosis. After finding lowered 5-methyltetrahydrofolate (5MTHF) in CSF, he was started on oral folic acid (2 to 4 mg/kg/day) and vitamin B12 (400 µg/day) from age 8 months.

Case 2. This is the only child of nonconsanguineous parents, born after a normal pregnancy without complications (Apgar 10/10, umbilical artery pH 7.38, weight 3820 g, length 52 cm, head circumference 36.5 cm). Development up to the age of 6 months was normal followed by loss of head control, truncal hypotonia, and squint. He then developed spasticity and cognitive development was severely delayed. Weight and length developed parallel to the percentiles, but head growth declined to the microcephalic range (<P3 at 15 months). At 12 years of age, he showed severe spastic tetraplegia with some dystonic features in hands and face; he had good head control but impaired truncal control, simple grasping, and rolling to supine; and he was severely mentally retarded with simple language perception and no active speech.

A CT scan at 15 months revealed symmetric calcifications of the striatum and the periventricular region, as well as mild ventricular enlargement. A CT scan at 11 years showed some increase in calcifications, but no further evidence for brain atrophy (figure 1). MRI scan at 18 months revealed delayed myelination (corresponding to a 5-month-old child) and at 2 years and 9 months

there was progressive demyelination with additional loss of myelin in the periventricular region. MR spectroscopy of the occipital white matter showed a clearly abnormal lactate peak (at 18 months and at 2 years and 9 months). Extensive diagnostic investigations of blood and CSF samples were negative for TORCH infections and metabolic disorders (for CSF cells and INF-α, see table 1). Muscle biopsy showed no histopathologic abnormalities and mitochondrial enzyme activities were normal (Dr. Ruitenbeek, Nijmegen).

Owing to low folates in CSF, oral folic acid therapy was introduced at the age of 11.5 years.

Case 3. After a normal pregnancy of 39 weeks, this girl was born as the second child of healthy unrelated parents. Her Apgar score was 9/10/10, umbilical artery pH 7.23, birth weight 3250 g (P50), length 52 cm (P90), and head circumference 35.5 cm (P75). The neonatal period was normal. After breast-feeding had been started, it was noted that the child was quiet and had to be awakened for feeding. During the first months, she started to fix and follow, held her head in the upright position, and tried to turn over. However, after the first DPT, polio, and hemophilus influenza B vaccination at age 4 months, she developed unilateral strabismus, hyperexcitability, and hip muscle weakness. Two weeks following the second vaccination at age 5 months, she appeared ill with low-grade fever (38.2 °C), reduced consciousness, profuse sweating, and vomiting. On hospital admission, routine blood screen and CSF analysis had normal results. Following lumbar puncture, a generalized convulsion occurred. Thereafter, a slowly progressive neurologic deterioration was characterized by recurrent vomiting, decelerating head growth toward microcephaly, visual failure, generalized hypokinesia with dystonic limb movements, torticollis, and tonic eye deviation to the left in the presence of preserved tendon reflexes. Neuroimaging (CT, MRI) demonstrated bilateral calcifications of the basal ganglia and few scattered white matter calcifications (data not shown). EEG and electroneuromyographic studies had normal results. Acoustic evoked potentials showed bilateral prolonged latencies. Extensive diagnostic investigations of blood and CSF were negative.

Patients with AGS. Patients 1 through 3 were first observed at the ages of 6 months, 5 months, and 1 month. Cranial CT revealed the presence of multiple small, bilateral microcalcifications in the gray matter and partially at the border between gray and white matter. All three patients presented with feeding difficulties, irritability, and hypotonia/hypertonia/dystonia within the first month of life. TORCH was negative. Diagnosis was confirmed by chronic CSF pleocytosis and elevated INF-α.

Samples collection. Lumbar punctures were performed in the morning, and the first 0.5 mL of CSF was discarded or used for a cell count and glucose and protein levels. The next 1 to 2 mL of CSF were collected and frozen at -80 °C until analyzed. The procedures used were in accordance with the current revision of the Helsinki Declaration of 1975.

Pterins in CSF. Pterins in CSF were separated by RP-high-pressure liquid chromatography (HPLC) using column switching as described previously.⁹ Before HPLC, pterins in CSF were oxidized at acidic pH with manganese dioxide. Oxidized pterins are stable at room temperature and can be detected by their native blue fluorescence at 350/405 nm.

Folates in CSF. 5MTHF was measured using HPLC with electrochemical detection. CSF was centrifuged on the Ultrafree 10,000 filter unit (Millipore) at 2,000 g for 2 minutes. 5MTHF was separated on the 250 × 4.6 mm ODS1 column (Watters) using 50 mM sodium acetate with 22.5% (v/v) methanol and 6.5 µM EDTA as the mobile phase at a flow rate of 1.0 mL/min. Analytical cell 5011 (ESA) was controlled with the Coulochem Model 5100A detector (ESA) using -0.20 V potential and 2 sec response time.

Neurotransmitter metabolites in CSF. Neurotransmitter metabolites in CSF were measured using HPLC with electrochemical detection.¹⁰

Pterins production and enzyme activities in fibroblasts. Cell culturing, neopterin and biopterin production in fibroblasts after stimulation with cytokines for 24 hours, measurement of GTP cyclohydrolase I activity in stimulated fibroblasts, and 6-pyruvoyl-tetrahydropterin synthase, dihydropteridine reductase, and sepiapterin reductase activities in nonstimulated fibroblasts was performed.¹¹

Amplification and direct sequencing of GFRP gene. Genomic DNA was extracted from cultured skin fibroblasts with the

Table 1 Total neopterin (Neo) and biopterin (Bio), neurotransmitter metabolites 5-hydroxyindoleacetic acid (5HIAA) and homovanillic acid (HVA), 5-methyltetrahydrofolic acid (5MTHF), and interferon alpha and cells in CSF of patients with a variant and the classical AGS

| Patients | Age | Neo, nmol/L | Bio, nmol/L | Neo/Bio | 5HIAA, nmol/L | HVA, nmol/L | 5MTHF, nmol/L | INF- α , U/mL | Cells, n |
|----------|-----------|-------------|-------------|---------|---------------|-------------|---------------|----------------------|----------|
| Case 1 | 1 mo | 548 | 100 | 5.5 | 416 | 609 | 23 | <1 | 0 |
| | 8 mo | 396 | 54 | 7.3 | 257 | 708 | 32 | ND | 0 |
| | 1 y | 413 | 135 | 3.1 | 280 | 387 | 121 | ND | 0 |
| | 1 y 4 mo | 427 | 133 | 3.2 | 218 | 496 | 89 | ND | 0 |
| | 2 y 4 mo | 27 | 33 | 0.8 | 288 | 613 | 87 | ND | 0 |
| | 2 y 7 mo | 31 | 30 | 1.0 | 265 | 557 | 82 | ND | 0 |
| Case 2 | 1 y 6 mo | ND | ND | — | ND | ND | ND | ND | 10 |
| | 2 y 8 mo | ND | ND | — | ND | ND | ND | 4 | 0 |
| | 3 y | 293 | 165 | 1.8 | 226 | 533 | 48 | <1 | 0 |
| | 11 y 2 mo | 136 | 91 | 1.5 | 180 | 394 | 34 | <1 | 0 |
| Case 3 | 10 mo | 814 | 186 | 4.4 | 336 | 528 | ND | <1 | 0 |
| | 11 mo | 502 | 164 | 3.1 | 281 | 492 | 104 | ND | 0 |
| AGS 1 | 7 mo | 397 | 116 | 3.4 | 262 | 560 | 38 | 150 | 60 |
| AGS 2 | 1 y 1 mo | 468 | 119 | 3.9 | 333 | 452 | 64 | 215 | 130 |
| AGS 3 | 1 y 3 mo | 327 | 100 | 3.3 | 198 | 317 | ND | 80 | 25 |
| Controls | <1 y | 12–35 | 15–70 | | 114–800 | 211–1100 | 64–182 | <2 | 0 |
| | >1 y | 9–20 | 10–30 | | 66–300 | 115–870 | 41–117 | <2 | 0 |

AGS = Aicardi-Goutières syndrome; ND = not done.

QIAamp DNA Blood Mini Kit (Qiagen). PCR amplification of *GFRP* gene was performed in a PE 9700 thermal cycler (Applied Biosystems) by using the *Taq* PCR Core Kit (Qiagen). A PCR program with an annealing temperature of 59 °C and 30 cycles was used to amplify all three exons.

The *GFRP* gene was amplified in three fragments containing the whole coding region of the three exons and intron-exon boundaries (exon number, length of amplicon in base pairs, primer forward and reverse): exon 1, 639 bp, GFRP-5-F (5'-TCCTCCCCGACTCCACCTT-3'), GFRP-6-R (5'-GCCGACAGGTAGGACAGGAG-3'); exon 2, 800 bp, GFRP-7-F (5'-TCAAACCTCCACCTCAA-3'), GFRP-8-R (5'-CTCCCTGCTCCCTGCTTCTA-3'); exon 3, 590 bp, GFRP-9-F (5'-CTGGGACGCTGGGGAACCTG-3'), GFRP-10-R (5'-GCCCTCTCCACTGCTTGAC-3'). Genomic DNA-specific primers were designed on the published genomic sequence (GenBank accession number U78190). After purification of PCR products through QIAquick columns (QIAquick Gel Extraction Kit, Qiagen), *GFRP* amplicons were sequenced in both directions using the same primers as for amplification.

The ABI Prism BigDyeTerminator Ready Reaction version 1.1 kit and protocol (Applied Biosystems) were used for sequencing in an ABI Prism 310 Genetic Analyzer.

Results. The most prominent clinical findings are summarized in table 2.

CSF investigations. Concentrations of total neopterin and biopterin were significantly elevated in CSF of all three patients compared with healthy controls (see table 1). The ratio between neopterin and biopterin was initially increased (1.8 to 5.5; controls <1) and normalized in one patient (no. 1) after oral treatment with folinic acid (Leucovorin). The three patients with AGS showed similar neopterin and biopterin concentrations in CSF and similar neopterin/biopterin ratios. Elevation of neopterin in our patients was similar to that seen in CSF from patients with inflammatory neurologic diseases such as meningitis (293 to 814 nmol/L vs 80 to 580 nmol/L), but the concentrations of biopterin were much higher (226 to 416 nmol/L vs 15 to 73 nmol/L). Furthermore, the ratio of neopterin/

biopterin was much lower than in patients with meningitis (1.8 to 5.5 vs ~15), indicating that intrathecal overproduction of pterins may be related to the activation of the immune system. Pterins were normal in all urine and plasma samples from all patients (data not shown).

Neurotransmitter metabolites 5HIAA and HVA were in the normal range both in our patients and in the patients with AGS (see table 1). 5MTHF concentrations were significantly decreased in Patients 1 and 2 as well as in one of the patients with AGS. In Patient 3, 5MTHF was normal, and in one patient with AGS, 5MTHF measurement was not possible. In Patient 1, 5MTHF normalized within 2 months after initiation of therapy with folinic acid (see table 1).

In contrast to patients with AGS, IFN- α was marginally

Table 2 Main clinical symptoms in three patients with excessive intrathecal pterins synthesis

| Symptoms | Case 1 | Case 2 | Case 3 |
|------------------------------|--------|--------|--------|
| Basal ganglia calcifications | + | + | + |
| Dysmyelination | + | + | + |
| Hypotonia | – | + | + |
| Hypertonia | + | + | – |
| Irritability | + | – | + |
| Dystonia | + | + | + |
| Athetotic movements | + | – | + |
| Microcephaly | + | + | + |
| Retardation | + | + | + |
| Reduced or absent vision | + | – | + |
| Seizures | + | – | +/- |

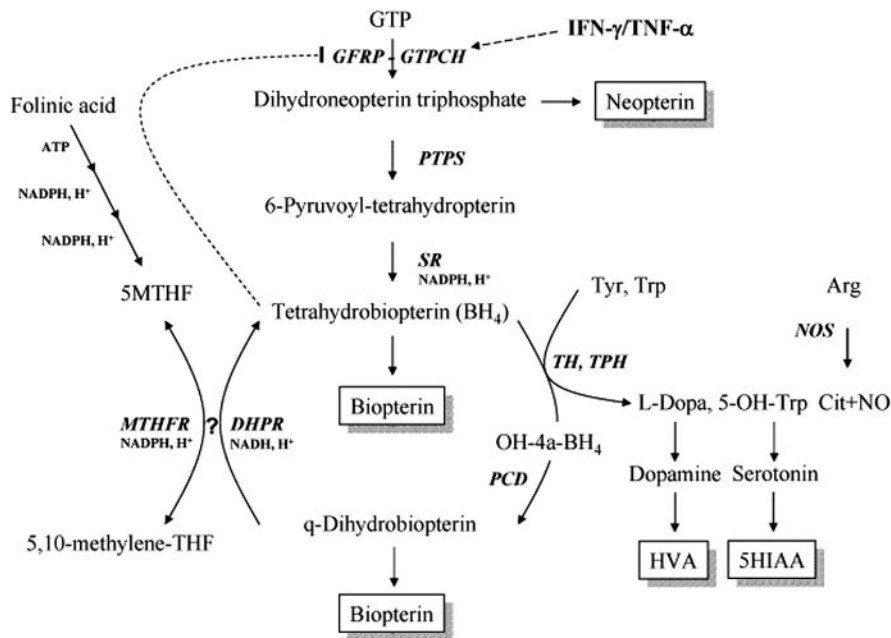


Figure 2. Tetrahydrobiopterin (BH_4) pathway and functions in the brain and proposed connection to the folates. Tetrahydrobiopterin is synthesized from GTP by the enzymes GTP cyclohydrolase I (GTPCH), 6-pyruvoyl-tetrahydropterin synthase (PTPS), and sepiapterin reductase (SR). During the hydroxylation of tyrosine (Tyr) to L-dopa by tyrosine hydroxylase (TH) and tryptophan (Trp) to 5-hydroxytryptophan (5-OH-Trp) BH_4 is oxidized to OH-4a- BH_4 and subsequently regenerated by the enzymes pterin-4a-carbinolamine dehydratase (PCD) and dihydropteridine reductase (DHPR). BH_4 is also essential for the nitric oxide synthase (NOS), which produces free radical NO and citrulline (Cit) from arginine (Arg). Biosynthesis of BH_4 is regulated by the GTPCH feed-back regulatory protein (GFRP) or by cytokines such as interferon gamma

($INF-\gamma$) and tumor necrosis factor alpha ($TNF\alpha$). Homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5HIAA) are the end metabolites and biochemical markers in the catecholamine and serotonin pathways. Folic acid (5-formyltetrahydrofolate) is converted to 5-methyltetrahydrofolate (5MTHF) by three enzymes: 5-formyltetrahydrofolate cyclohydrolase, 5,10-methylenetetrahydrofolate dehydrogenase, and 5,10-methylenetetrahydrofolate reductase (MTHFR).

elevated only in Patient 2 at the age of 2 years and 8 months; however, without any pleocytosis. Mild pleocytosis (10 cells/mm³) had been noted in the same patient about 1 year earlier, but at that time $INF-\alpha$ was not investigated. CSF from Patient 3 was investigated in more detail at the age of 8 months. In her CSF different cytokines such as tumor necrosis factor- α ($TNF-\alpha$), $INF-\gamma$, GM-CSF, interleukin (IL)-1, and IL-6 were all in the normal range.

Pterins production and enzyme activities in fibroblasts. In order to investigate the abnormally high intrathecal pterins production and to locate the possible enzyme defect, fibroblasts from Patient 1 were stimulated with $INF-\gamma$ and $TNF-\alpha$ for 24 hours. This test indicates possible enzyme defects in the BH_4 pathway.¹¹ Both neopterin (37 pmol/mg prot; controls 18 to 98) and biopterin (123 pmol/mg prot; controls 154 to 303) production were normal in stimulated cells. In contrast to control cells, fibroblasts of this patient produced relative high amounts of pterin (146 pmol/mg prot; control <10). Pterin is a breakdown product of biopterin and is formed, probably nonenzymatically, by the side-chain cleavage of BH_4 .

The enzyme activities of GTP cyclohydrolase I in stimulated fibroblasts (3.0 uU/mg prot; controls 1.6 to 6.5) and 6-pyruvoyl-tetrahydropterin synthase (1.0 uU/mg prot; controls 0.40 to 1.60), sepiapterin reductase (151 uU/mg prot; controls 99 to 185), and dihydropteridine reductase (8.0 mU/mg prot; controls 4.5 to 8.3) in nonstimulated cells were all in the normal range.

GFRP mutation analysis. In liver and brain GFRP mediates feed-forward activation of GTP cyclohydrolase I activity by enhancing GTP binding in the presence of phenylalanine while inducing feedback inhibition of enzyme activity in the presence of BH_4 . Thus, changes in the GFRP gene structure may influence pterins production in some regions of the brain, which may in turn explain high

CSF neopterin and biopterin concentrations. However, we found no mutations within the coding region of the GFRP gene.

Effect of folic acid substitution. In Patient 1, combined substitution with folic acid (2 to 4 mg/kg/day) and vitamin B12 (400 μ g/day) during a period of 2 years has resulted in normalization of folate and pterin values in CSF (see table 1), as well as partial clinical improvement. The child appeared completely blind after birth. After therapy for 4 months, he started to smile and turn his head to bright visual objects. Further visual improvement continued so that from the age of 1.5 years he could discriminate big objects at 0.5 m distance. After treatment during the following 4 months the extreme hyperexcitability and dyskinesias present after birth diminished while the dystonic upper and lower limb movements became less marked. However, at the age of 4 years, his arms and neck still tend to dystonic extensor spasms upon attempted grasping. Introduction of folic acid (Leucovorin, 2 to 4 mg/kg/day) and vitamin B12 (400 μ g/day) resulted in dramatic improvement of seizure control and EEG readings. However, during febrile infectious episodes, seizures relapsed. Follow-up neuroimaging (CT) documented no further cortex or white matter destruction compared to the first scan at the age of 1 month. However, as shown by MRI, hypomyelination of the frontal white matter persisted, but the further periventricular regions and corpus callosum began to show some myelin deposits (data not shown).

In Patient 2, treatment with folic acid (Leucovorin, 0.5 mg/kg) resulted in improvement of language perception, alertness, and nonverbal interaction as noticed 6 to 8 weeks after treatment onset by parents, teachers, and family doctor independently.

In Patient 3 with normal CSF folates, no therapy was introduced.

Discussion. Calcifications of basal ganglia may originate from a necrosis of neural tissue caused by various toxic, infectious, or physical agents with secondary calcium depositions.² However, they may also be observed in neurometabolic disorders such as autosomal recessive inherited DHPR deficiency,¹² type 1 GM1 gangliosidosis,¹³ in rare cases of mitochondrial encephalopathy,¹⁴ and in hereditary folate malabsorption.⁸ In patients with DHPR deficiency, an enzyme defect in the regeneration of BH₄, there is a strong link between the BH₄ and folates pathways and basal ganglia calcifications.^{6,7,15-17} These patients are characterized by persistent hyperphenylalaninemia, severe neurotransmitters deficiency, and cerebral folate depletion.¹² Combined therapy with L-dopa/carbidopa, 5-hydroxytryptophan, and folinic acid (Leucovorin) in combination with a low phenylalanine diet prevents both neurologic symptoms and calcification of the basal ganglia in most patients with DHPR deficiency.^{16,18-20}

Thus, investigation of CSF metabolites seems to be a valuable tool in the diagnosis and management of neurometabolic disorders presenting with basal ganglia calcifications. Although patients with AGS present with a number of clinical symptoms common to disorders of biogenic amine neurotransmitters, routine CSF investigations performed in the three patients presented in this article as well as in patients with the classic form of AGS revealed normal catecholamine and serotonin homeostasis. To our knowledge, measurement of CSF biopterin and folate metabolites has not been reported in AGS or related disorders. The main biochemical abnormality we found in both variants of AGS was high concentrations of neopterin and biopterin in CSF. Neopterin and biopterin are the two main metabolites in the BH₄ pathway. BH₄ is synthesized de novo by the enzymes GTP cyclohydrolase I, 6-pyruvoyl-tetrahydropterin synthase, and sepiapterin reductase (figure 2) and required for the hydroxylation of aromatic amino acids as well as for nitric oxide synthase.²¹ Biosynthesis of BH₄ is regulated via GFRP at the level of GTP cyclohydrolase I by the end product BH₄ and by cytokines. Inflammatory cytokines such as IFN- γ and TNF- α induce GTP cyclohydrolase I expression²² while lipopolysaccharides (LPS) suppress the expression of GFRP,²³ resulting in an increased GTP cyclohydrolase I activity. In addition, particular combinations of cytokines, including interleukin 1 β (IL-1 β), stimulate the second enzyme in the BH₄ pathway, 6-pyruvoyl-tetrahydropterin synthase.²⁴ BH₄ itself acts as a feed-back inhibitor of GTP cyclohydrolase I. Thus, depending on the cell type and the type of stimuli, only neopterin (originating from dihydroneopterin triphosphate, see figure 2) or both neopterin and biopterin (originating from BH₄ via q-dihydrobiopterin, see figure 2) may accumulate. However, this is generally a short-time activation of the BH₄ pathway and neopterin and biopterin production usually normalizes with the normalization of cytokine levels. We investigated CSF levels of different cytokines, including IFN- α ,

IFN- γ , GM-CSF, IL-1, and IL-6, in CSF from one of our patients (no. 3) and found them to be in the normal range. In the other two patients (nos. 1 and 2), only IFN- α was investigated and, except for the mild elevation in Patient 2 at the age of 2 years and 8 months, it was always within normal range. The elevation of neopterin and biopterin in CSF of our patients was persistent over months to years, suggesting activation of the BH₄ pathway by an unknown agent or mechanism. A loss of GFRP function is unlikely as no nucleotide changes could be found by sequencing of the whole coding region of *GFRP* gene in one patient. In patients with classic AGS, pterins elevation is most probably directly associated with the increased IFN- α levels in the CNS, which usually occurs over a long period. Thus, in contrast to our patients with the AGS variant, the neuropathologic findings and neopterin and biopterin overproduction in classic AGS may represent a result of a primary genetic interferonopathy with overproduction of IFN- α .⁵ Interestingly, neopterin and biopterin levels in CSF normalized in Patient 1 after substitution with folinic acid during a 2-year period.

Although multiple studies provide indirect evidence for a connection between the BH₄ and folate pathways, the exact intersection point is not clear. There is evidence that a defect in the reduction of 5,10-methylenetetrahydrofolate to 5MTHF also affects the synthesis of BH₄, at least in CSF.²⁵ It has also been shown that patients with DHPR deficiency have low 5MTHF levels in CSF, for which Kaufman²⁶ proposed a "methyl trap" mechanism as a possible cause. BH₄ and the folate coenzymes can reciprocally interact in ways that would be useful to the metabolic pathways subserved by both of these coenzymes. Thus, through one of the reactions catalyzed by 5,10-methylenetetrahydrofolate reductase, 5MTHF can regenerate BH₄ from q-dihydrobiopterin (see figure 2), and q-dihydrobiopterin can provide an escape from the methyl trap. 5MTHF levels were reduced in two of our patients (nos. 1 and 2), as well as in one patient with classic AGS. Oral substitution with folinic acid (Leucovorin) was beneficial in both patients and normalized CSF 5MTHF levels in Patient 1. It would be interesting to investigate more patients with classic AGS in more detail and to consider folinic acid substitution in those with low CSF 5MTHF levels. Because lowered 5MTHF levels are not a consistent finding in either classic or variant forms of AGS, a primary disturbance of metabolism is unlikely. Secondary phenomena due to a depletion of the folate pool or another derangement of folate metabolism in the brain are possible causes.

The etiology of increased intrathecal production of neopterin and biopterin needs further investigation in AGS and its variants. Levels of these two pterins in addition to 5MTHF should be used in the differential diagnosis of conditions associated with basal ganglia calcifications.

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