CASE RECORDS of the MASSACHUSETTS GENERAL HOSPITAL

Case 27-2014: A 10-Month-Old Boy with Microcephaly and Episodic Cyanosis

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PRESENTATION OF CASE

Dr. Kai-How Farh (Pediatrics): A 10.9-month-old boy with microcephaly and developmental delay was admitted to this hospital because of episodes of cyanosis.

The patient was born at another hospital after 39 weeks of gestation to a 36-year-old multigravida who had received prenatal care. Prenatal ultrasonography reportedly revealed mild hydronephrosis. Labor was induced because of maternal hypertension. Apgar scores were 8 and 9 at 1 and 5 minutes, respectively. On examination, microcephaly was noted; there were no dysmorphic features. The weight was 2.9 kg (13th percentile), the length 50.8 cm (60th percentile), and the head circumference 29.5 cm (<1st percentile) (Fig. 1 in the Supplementary Appendix, available with the full text of this article at NEJM.org). Results of the Massachusetts Newborn Screening Panel, which can identify more than 60 disorders, were normal.

On the patient's 18th day of life, radiographs of the head showed no abnormalities other than a small calvarium. Testing for toxoplasma and rubella was positive for IgG antibodies and negative for IgM antibodies; testing for cytomegalovirus was negative. At 3 weeks of age, he was evaluated by the genetics service at a second hospital because of microcephaly. His parents reported that he rolled his eyes when upset and had labored breathing after feeding but was otherwise well. On examination, there was microcephaly, the coronal ridges were palpable, and there were no other abnormalities. Array-comparative genomic hybridization testing revealed 16p11.2 duplication, which was thought to be benign. His infant formula was changed, and symptoms associated with feedings improved. Magnetic resonance imaging (MRI) that was performed when the infant was 6.5 weeks of age reportedly revealed a small brain, delayed myelination, and partial agenesis of the posterior corpus callosum.

At 5 months of age, the patient was unable to roll over, constipation had developed, and his mother noted poor visual tracking. One month later, neurologic examination revealed pupils that were equal in size and reactive to light, decreased visual fixation, and failure to track. There was decreased truncal control, axial hypotonia, and near-normal tone in the arms and legs, which the patient could move normally. Muscle bulk and deep-tendon reflexes were normal; plantar reflexes

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Table 1. Laboratory Data.*

Variable	Reference Range, Age-Adjusted†	17 Days before Admission, Other Hospital	On Admission	9th Day	16th Day	20th Day
Blood						
Hematocrit (%)	33.0–39.0	36.8	41.1	33.1	34.0	32.2
Hemoglobin (g/dl)	10.5–13.5	12.6	15.0	11.4	12.0	10.8
White-cell count (per mm^3)	6000–17,500	11,400	9300	11,200	14,300	9200
Differential count (%)						
Neutrophils	17–49	73.6 (ref 27.0–35.0)	47	74	54	51
Lymphocytes	67–77	18.1 (ref 57.0–65.0)	44	19	38	34
Monocytes	4–11	5.9	7	5	7	9
Eosinophils	0–8	2.0	1	1	1	5
Basophils	0–3	0.4	1	1	0	1
Platelet count (per mm^3)	150,000–400,000	422,000	551,000	381,000	118,000	169,000
Mean corpuscular volume (fl)	70–86	88.1	83	86	80	84
Creatinine (mg/dl)	0.30–1.00	0.22	0.28	<0.17	<0.17	<0.17
Globulin (g/dl)	2.3–4.1		1.9	1.3	1.7	
β-Alanine (nmol/ml)	0–7		8			10
Cystine (nmol/ml)	16–84		5			5
Lysine (nmol/ml)	52–196		202			180
Methionine (nmol/ml)	9–42		11			7
Homocysteine (μmol/liter)	0–12				159.5	
Activated partial-thromboplastin time (sec)	21.0–33.0			42.2	33.3	32.4
Prothrombin time (sec)	11.0–13.7			14.3	15.7	13.7
Prothrombin time–international normalized ratio				1.2	1.3	1.1
D-Dimer (ng/ml)	<350			336	1570	
Folic acid (ng/ml)	3.1–17.5				19.5	>20.0
Vitamin B_{12} (pg/ml)	>250				924	
Partial-thromboplastin time for lupus anticoagulant	Negative				Negative	
Antithrombin III (functional) (%)	80–130				96	
Protein C (functional) (%)	70–140				75	
Activated protein C resistance screening‡	>2.0				2.6	
Protein S (functional) (%)	70–140				151	
Venous blood gases and oximetry						
Fraction of inspired oxygen		Unspecified	Unspecified	1.00	0.30	0.35
Base excess (mmol/liter)		–8.0 (ref 0–3)	0.1	7.3	–2.6	0.8
pH	7.30–7.40	7.20	7.27	6.98	7.53	7.35
Partial pressure of carbon dioxide (mm Hg)	38–50	51 (ref 41–51)	64	189	23	51
Partial pressure of oxygen (mm Hg)	35–50	28 (ref 80–105)	62	62	47	40
Bicarbonate (mmol/liter)	22–27	20.0 (ref 23–28)		44	19	28
Oxygen saturation (%)	95–98	40.0				

Table 1. (Continued.)

Variable	Reference Range, Age-Adjusted†‡	17 Days before Admission, Other Hospital	On Admission	9th Day	16th Day	20th Day
Cerebrospinal fluid						
Color	Colorless					Colorless
Turbidity	Clear					Clear
Xanthochromia	None					None
Red cells, tube 2 of 4 (per mm^3)	None					None
Total nucleated cells, tube 2 of 4 (per mm^3)	0–5					1
Monocytes (%)	Not defined					100
Total protein (mg/dl)	5–55					13
Glucose (mg/dl)	50–75					60
Lactate (mmol/liter)	0.5–2.2					2.1

* Ref denotes the reference range at the other hospital. To convert the values for creatinine to micromoles per liter, multiply by 88.4. To convert the values for calcium to millimoles per liter, multiply by 0.250.
† Reference values are affected by many variables, including the patient population and the laboratory methods used. The ranges used at Massachusetts General Hospital are age-adjusted for patients who are not pregnant and do not have medical conditions that could affect the results. They may therefore not be appropriate for all patients.
‡ The activated protein C resistance screening value is the ratio of the activated partial-thromboplastin time with the addition of activated protein C to the activated partial-thromboplastin time without the addition of activated protein C. The plasma sample is diluted beforehand with factor V–deficient plasma.

were flexor. Neuro-ophthalmologic examination did not reveal optic-nerve hypoplasia. A diagnosis of cortical blindness was made. Genetic testing for the primary microcephaly syndromes was suggested.

Twenty days before the current admission, episodes of head bobbing and perioral cyanosis occurred during feedings. Three days later, the patient was admitted to the second hospital, where he reportedly had episodes of respiratory distress with agitation or repositioning. On examination during an episode, he had near-agonal breathing with costal retractions and without tachypnea, an oxygen saturation between 80% and 89%, head bobbing, grunting, back arching, limb extension, increased muscle tone, cool and mottled arms and legs, perioral cyanosis, and a decreased heart rate (of approximately 75 beats per minute). The episodes occurred several times daily, resolved with oxygen administration, and when protracted, were followed by long naps. Growth measurements are shown in Figure 1 in the Supplementary Appendix, and laboratory test results are shown in Table 1. Computed tomography of the head reportedly revealed hypodensities in frontal and parietal white matter, mild prominence of the ventricles, and extra-axial spaces containing cerebrospinal fluid (possibly normal features for a patient of this age), with no acute hemorrhage, mass, or midline shift. Evaluation for gastroesophageal reflux revealed reflux below the level of the clavicles, without aspiration; an examination with a pH probe revealed no evidence of reflux during the episodes of cyanosis. An electroencephalogram showed slowing of the background rhythm, without evidence of seizure activity during an episode. Famotidine and lactulose were administered, and a nasojejunal feeding tube was placed.

The patient was discharged on the seventh hospital day but was readmitted 2 days later because of recurrent episodes of respiratory distress and cyanosis. Oxygen was administered through a nasal cannula. Chest radiographs reportedly showed mild bronchial-wall thickening and low lung volumes. Testing for respiratory viruses was negative. Evaluation of the respiratory and upper gastrointestinal tracts reportedly revealed small diaphragmatic excursions, mild esophagitis, and reflux below the level of the carina, with slow clearing. The transthoracic echocardiogram was normal. On the 10th hospital day, the patient was transferred to this hospital. Medications on transfer included lactulose and lansoprazole.

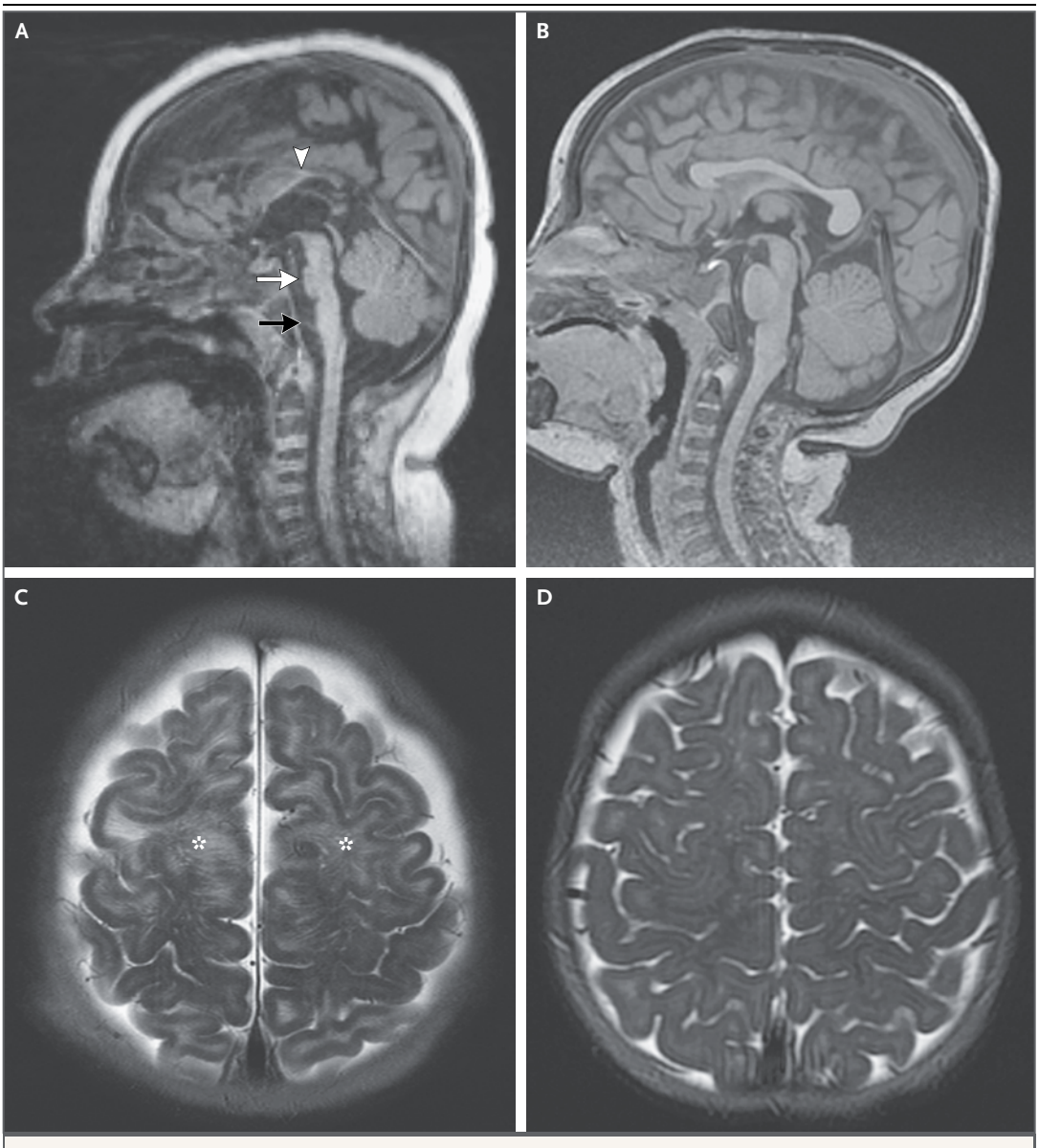

Figure 1. MRI Scans Obtained on Admission.

A sagittal T_1-weighted image (Panel A) shows hypoplasia of the pons (white arrow) and medulla (black arrow) and a diminutive corpus callosum (arrowhead), as compared with an image of an age-matched normal brain (Panel B). An axial T_2-weighted image (Panel C) shows a decrease in white-matter volume and hyperintense signal abnormality in the bilateral deep white matter (asterisks), as compared with an image of an age-matched normal brain (Panel D); these findings are suggestive of hypomyelination.

The parents reported that the patient was unable to roll over or sit up without assistance. He received infant formula orally. Immunizations were current, including one dose of seasonal influenza vaccine. He had no known allergies. He lived with his parents and older sibling and received early-intervention services. His parents were white, with no known history of consanguinity. His mother had had two spontaneous miscarriages; his parents and sibling were healthy, and the maternal and paternal head circumferences were normal. His maternal grandmother had a bifid uvula, but the three-generation family history was otherwise unremarkable.

On examination, the temperature was 36.2°C, the blood pressure 116/55 mm Hg, the pulse 120

beats per minute, the respiratory rate 50 breaths per minute (with deep subcostal retractions), and the oxygen saturation 99% while the patient was breathing oxygen at a rate of 1 liter per minute through a nasal cannula. The lungs were clear. On neurologic examination, the eyes were open when the patient was awake, with no tracking to visual stimuli, but the eyes closed in response to bright light and blinked in response to threat. The pupils contracted from 4 mm to 2 mm bilaterally, and a red reflex was seen. The face was symmetric, without facial diplegia. When the patient was in the supine position, the legs were abducted, the arms extended, and the hands fisted. There were spontaneous, symmetric, and antigravity limb movements. Head control and truncal tone were poor; he was unable to sit up without support and leaned toward the left. Patellar reflexes were brisk (3+), and there was sustained clonus at the left ankle and 5 beats of clonus at the right ankle; plantar grasp reflexes were present bilaterally. Blood levels of electrolytes, glucose, urea nitrogen, total protein, albumin, calcium, phosphorus, magnesium, creatine kinase, triglycerides, thyrotropin, copper, and carbohydrate-deficient transferrin were normal, as were the anion gap and results of liver-function tests; other test results are shown in Table 1. Lansoprazole, lactulose, lorazepam (as needed for agitation), and glycerin suppositories were administered.

On the first hospital day, the patient had two episodes of respiratory distress and cyanosis that were similar to previously reported episodes. The temperature was 35.3°C, the blood pressure 98/50 mm Hg, the pulse 75 to 130 beats per minute, the respiratory rate 50 breaths per minute, and the oxygen saturation 97% while he was breathing oxygen at a rate of 3 liters per minute through a nasal cannula. Venous blood gas measurements are shown in Table 1. The patient was transferred to the pediatric intensive care unit. Bilevel positive airway pressure was administered by face mask, with improvement. On the second day, apnea and cyanosis occurred intermittently. Otolaryngologic examination revealed incomplete vocal-cord adduction. Bronchoscopy revealed mild main-stem and lower-lobe bronchomalacia on the left side. A central venous catheter was placed in the right saphenous vein. Gabapentin was begun. Blood and urine amino acid profiles were reported to be normal (selected values are shown in Table 1).

Dr. Paul A. Caruso: MRI that was performed the next day, without the administration of contrast material, revealed pontomedullary hypoplasia, a diminutive corpus callosum, and a myelin disorder most consistent with hypomyelination. A sagittal T_1-weighted image shows a diminutive pons and medulla, which can be best visualized when compared with an image of an age-matched normal brain (Fig. 1). Although the diminutive brain stem may be in part explained by white-matter depletion, the findings raise concern for a component of malformation. The corpus callosum is diminutive; the anterior genu and body have formed but are abnormally thin, and the splenium appears deficient as it merges posteriorly with the hippocampal commissure and fornices.

The white-matter volume appears reduced as compared with an MRI scan from an age-matched developmentally normal child (Fig. 1). By 11 months of age, normal myelination usually results in low signal in parts of the supratentorial white matter on T_2-weighted imaging, but on this patient's T_2-weighted images, there was hyperintense signal abnormality (Fig. 1), which suggests an underlying delay in myelination or a genetic myelin disorder. Magnetic resonance spectroscopy showed no abnormalities.

Dr. Farh: On the ninth day, the patient's work of breathing increased, and there was associated bradycardia. Swelling at the site of the central catheter was noted. The catheter was removed and cultured, and broad-spectrum antimicrobial agents were administered. Test results are shown in Table 1. The trachea was intubated, and mechanical ventilation was begun. Cultures of the blood, urine, and catheter tip were negative, as were tests for respiratory viruses. Ultrasonography revealed thrombosis of the right common femoral vein. Low-molecular-weight heparin was administered subcutaneously.

Dr. Caruso: Because of concern for an underlying disorder of myelination, the patient underwent magnetic resonance spectroscopy of the white matter on the 16th day. During this study, the conventional MRI revealed that thrombus had formed in the sagittal and straight sinuses and that the vein of Galen had become engorged (Fig. 2).

Dr. Farh: The administration of betaine, folinic acid, riboflavin, hydroxocobalamin, pyridoxine, and carnitine was begun. On the 20th day, a lumbar puncture was performed. The results of cerebrospinal fluid analysis are shown in Table 1. The administration of low-molecular-weight hep-

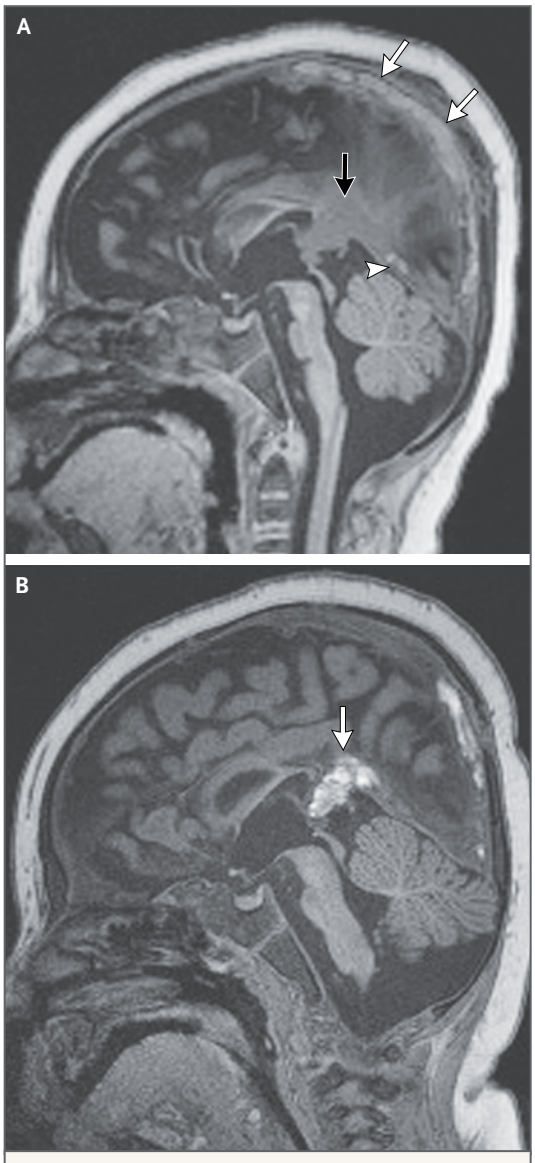

Figure 2. MRI Scans Obtained 2 Weeks and 3.5 Weeks after Admission.

A sagittal T_1-weighted image obtained 2 weeks after admission (Panel A) shows the development of thrombus, as reflected by hyperintense signal abnormality in the superior sagittal sinus (white arrows) and straight sinus (arrowhead), as well as engorgement of the vein of Galen (black arrow). An image obtained 10 days later (Panel B) shows that the thrombus has evolved in the vein of Galen (arrow).

arin at a dose of 200 U per kilogram of body weight twice daily was required to achieve a therapeutic level.

Diagnostic test results were received.

DIFFERENTIAL DIAGNOSIS

Dr. Ganeshwaran H. Mochida: All the discussants are aware of the diagnosis in this case. This child was born with microcephaly. Truncal hypotonia, global developmental delay, and visual deficits that were thought to be due to cortical blindness developed. MRI of the head revealed hypomyelination, partial agenesis of the corpus callosum, reduced white-matter volume, and a small pons but no malformation of the cerebral cortex. Subsequently, apnea and hypoventilation of central origin, deep venous thrombosis, and cerebral venous sinus thrombosis developed. Since microcephaly was the first sign, it would be helpful to review the differential diagnosis of microcephaly (Table 2).

Microcephaly literally means "small head." It is diagnosed when the head circumference is more than 2 SD below the mean for age and sex, although sometimes a stricter cutoff of 3 SD below the mean is used.[1] The underlying causes of microcephaly can be divided into nongenetic and genetic causes.

NONGENETIC CAUSES OF MICROCEPHALY

Common nongenetic causes of microcephaly include intrauterine infections and in utero exposure to drugs, toxins, and ionizing radiation. In this case, the normal prenatal and perinatal history and negative serologic and microbiologic tests make such causes unlikely. Other nongenetic causes, such as disruptive brain injuries and systemic disorders, are also ruled out by the history, laboratory test results, and imaging of the head. Therefore, a genetic cause of microcephaly appears much more likely.

GENETIC CAUSES OF MICROCEPHALY

Genetic causes of microcephaly are diverse; more than 200 genes are associated with microcephaly in the Online Mendelian Inheritance in Man database. MRI of the head can be helpful in guiding genetic testing, because many genetic disorders that occur in patients with microcephaly are associated with characteristic structural abnormalities.[2]

Structural abnormalities that are associated with microcephaly include abnormal brain patterns (e.g., holoprosencephaly), abnormal cortical gyral patterns (e.g., lissencephaly and polymi-

Table 2. Differential Diagnosis of Microcephaly.

Nongenetic causes of microcephaly
Infectious diseases
TORCH (toxoplasmosis, other [syphilis, varicella–zoster, parvovirus B19], rubella, cytomegalovirus, and herpes) infections
Other prenatal, perinatal, and early postnatal infections of the central nervous system
Intrauterine exposure to teratogenic agents
Ionizing radiation
Alcohol (fetal alcohol syndrome)
Medications and recreational drugs
Hypoxic–ischemic injury
Head trauma and intracranial hemorrhage
Metabolic disturbances and systemic diseases (e.g., severe hypoglycemia and renal failure)
Severe malnutrition
Genetic causes of microcephaly
Causes associated with characteristic brain imaging abnormalities
Disorders with abnormal brain patterns
Holoprosencephaly
Disorders with abnormal cortical gyral patterns
Lissencephaly or microlissencephaly (e.g., *LIS1*, *DCX*, *ARX*, and *NDE1* mutations)
Cobblestone dysplasia (e.g., congenital muscular dystrophies)
Polymicrogyria associated with microcephaly (e.g., *TUBB2B* mutations)
Disorders with neuronal heterotopia
Disorders with abnormal hindbrain structures
Pontocerebellar hypoplasia (e.g., *TSEN54*, *CASK*, and *CHMP1A* mutations)
PEHO (progressive encephalopathy with edema, hypsarrhythmia, and optic atrophy) syndrome
Causes associated with normal or mildly simplified gyral pattern and no syndromic features
Autosomal recessive primary microcephaly (microcephaly vera) (e.g., *ASPM* and *WDR62* mutations)
Early infantile epileptic encephalopathy 10 (microcephaly, seizures, and developmental delay) (*PNKP* mutations)
Others (most likely highly heterogeneous) (e.g., *TRAPPC9* mutations)
Causes associated with syndromic features (only select examples are listed)
Rubinstein–Taybi syndrome (*CREBBP* and *EP300* mutations)
Cornelia de Lange's syndrome (e.g., *NIPBL* and *SMC1A* mutations)
Seckel's syndrome (e.g., *ATR* mutations)
Rett's syndrome (*MECP2* mutations)
Angelman's syndrome (deletion 15q11.2-q13 [maternal], uniparental disomy 15q11.2-q13 [paternal], and *UBE3A* mutations)
Chromosomal aberrations
Craniosynostosis
Neurodegenerative disorders (early onset)
Inborn errors of metabolism

crogyria), and neuronal heterotopia. None of these were seen in this case. Microcephaly can be associated with abnormalities of the posterior fossa (e.g., pontocerebellar hypoplasia); these abnormalities are worth considering in this case because the child had a small pons, but the absence of severe cerebellar hypoplasia or atrophy on MRI makes this group of disorders unlikely.

Some disorders associated with microcephaly are not characterized by major structural abnormalities (other than a possible mildly simplified gyral pattern) or syndromic features. The prototype of this group of disorders is autosomal recessive primary microcephaly (also known as microcephaly vera). To date, at least nine genes (*MCPH1*, *WDR62*, *CDK5RAP2*, *CASC5*, *ASPM*, *CENPJ*, *STIL*, *CEP135*, and *CEP152*) have been associated with this disorder, and *ASPM* is the most commonly mutated gene among them.[3] Persons with microcephaly vera present with congenital microcephaly, and the adult head circumference typically falls between 4 SD and 12 SD below the mean.[4] However, this child's marked truncal hypotonia, cortical blindness, and hypomyelination, as well as the later development of apnea and thrombosis, suggest that microcephaly vera is not the diagnosis.

Microcephaly is a feature of various syndromes, such as the Rubinstein–Taybi syndrome (which is characterized by intellectual disability, postnatal growth retardation, broad thumbs and halluces, and dysmorphic facial features) and the Cornelia de Lange syndrome (which is characterized by unique facial features, prenatal and postnatal growth retardation, and intellectual disability and often by upper-limb anomalies); however, no clear syndromic features were noted in this child. Congenital disorders of glycosylation (CDG) (e.g., CDG type Ia) are an important consideration, since they can be associated with thrombotic tendencies, but negative testing for carbohydrate-deficient transferrin in this child rules out this diagnosis. Pathologic chromosomal aberration is also unlikely on the basis of the results of a chromosomal microarray. In rare cases, neurodegenerative disorders, such as neuronal ceroid lipofuscinosis 10, can manifest prenatally and the child is born with congenital microcephaly, but such neonates are much more ill at birth than this patient was.

INBORN ERRORS OF METABOLISM

Inborn errors of metabolism are important to consider. This child did not have respiratory difficulties initially, but episodes of central apnea and hypoventilation developed at approximately 10 months of age. This time course is consistent with a progressive condition, such as an inborn error of metabolism. Furthermore, hypomyelination, as seen in this child, is frequently reported in persons with inborn errors of metabolism,[5] including 3-phosphoglycerate dehydrogenase deficiency,[6] glycine encephalopathy,[7] and 5,10-methylenetetrahydrofolate reductase (MTHFR) deficiency[8]; anomalies of the corpus callosum have been reported in persons with oxidative phosphorylation disorders.[9] Thus, inborn errors of metabolism are a strong consideration in this child.

Inborn errors of metabolism are typically associated with a postnatal onset of microcephaly; however, in three notable disorders, microcephaly is often present at birth[10] — maternal phenylketonuria, Amish-type microcephaly, and 3-phosphoglycerate dehydrogenase deficiency — and therefore these conditions are worth further consideration. Maternal phenylketonuria is unlikely on the basis of the history, and the absence of metabolic acidosis and Amish ancestry makes Amish-type microcephaly unlikely. A deficiency of 3-phosphoglycerate dehydrogenase is a possibility because it is also associated with hypomyelination. However, thrombosis is not a known complication of any of these three disorders.

Inborn errors of metabolism that are associated with hyperhomocysteinemia can confer a predisposition to thrombosis, and among such disorders, the constellation of microcephaly, hypomyelination, and apnea may be seen in persons with severe 5,10-MTHFR deficiency.[11] Although prenatal onset of microcephaly and pontine hypoplasia are not usually seen in persons with this condition, it seems to best explain this child's overall presentation and clinical progression. Before the onset of thrombosis, however, it would be difficult to single out this condition, and a broader workup for inborn errors of metabolism is indicated.

CEREBRAL SINUS VENOUS THROMBOSIS IN A CHILD

Dr. Eric F. Grabowski: This patient had clinically significant cerebral sinus venous thrombosis, which is uncommon in pediatric critical care, with a reported incidence of no more than 1 case per 100,000 patients per year.[12]

The differential diagnosis of cerebral sinus venous thrombosis in a child includes an infec-

tion of the head, neck, or both (which was not seen in this case), a structural or anatomical irregularity of the cerebral vasculature (which was not seen in this case), acute systemic illness (this patient had an acute respiratory illness), a chronic systemic disease, and a thrombophilic risk factor. There are many congenital thrombophilic risk factors for venous thrombosis in children, including an elevated homocysteine level (which was seen in this case).

In this patient, cerebral sinus venous thrombosis involved the left transverse, superior sagittal, and straight sinuses, with thrombus present in the torcula. The torcula (torcular Herophili) is a confluence of the sagittal and straight sinuses, from which drainage occurs through the transverse sinuses. Involvement of the torcula indicates high risk for substantial occlusion of venous outflow from the brain.

This patient also had a catheter-related deep venous thrombosis 1 week before discovery of the cerebral sinus venous thrombosis. Catheter-related deep venous thrombosis is relatively common in the intensive care setting; one report shows that it occurs in 18% of pediatric patients in the intensive care unit.[13] Thus, the deep venous thrombosis was probably unrelated to the cerebral sinus venous thrombosis, except insofar as the underlying hyperhomocysteinemia contributed to both.

The management of cerebral sinus venous thrombosis in this patient was performed by the Pediatric Stroke Service. We immediately started treatment with standard unfractionated heparin while we completed a diagnostic workup for thrombophilia; the dose of heparin was determined according to body mass and age and was adjusted not according to the partial-thromboplastin time but instead according to heparin (anti–factor Xa) levels. Such levels often provide a more accurate assessment of the degree of anticoagulation, especially in a critical care situation, since the partial-thromboplastin time can be falsely high in the presence of a lupus anticoagulant, falsely low in the presence of elevated levels of clotting factor VIII, and inaccurate owing to heparin binding by acute-phase plasma proteins. Standard unfractionated heparin is the preferred treatment option in many acute illnesses, because if unexpected bleeding occurs, a heparin infusion can simply be stopped (since heparin has a short half-life of 60 minutes) and a specific antidote (protamine sulfate) can be administered.

When we transitioned to low-molecular-weight heparin, we measured the patient's heparin levels and found that the dose required for therapeutic heparin levels was at least twice the expected dose for the patient's age and weight. A reexamination of the metabolic principles underlying this method indicated the presence of a metabolic abnormality. The plasma level of a renally excreted drug that is administered on a per-kilogram basis increases with increasing body mass.[14,15] In this case, however, the entire dose–response curve appeared to be shifted upward. This shift is consistent with enhanced renal excretion, which in turn suggests an augmented metabolic rate and the presence of an underlying metabolic disorder.

CLINICAL DIAGNOSIS

Severe methylenetetrahydrofolate reductase deficiency.

ADDITIONAL DIAGNOSTIC TESTING AND MANAGEMENT

Dr. Inderneel Sahai: The majority of the metabolic workup, including analyses of amino acids and organic acids, was normal. However, there was an elevated level of plasma homocysteine.

Homocysteine is a sulfur-containing nonprotein amino acid that is synthesized from methionine and subsequently metabolized through two biochemical pathways (Fig. 3). It can be converted to cysteine by transsulfuration or remethylated to regenerate methionine. An elevation in the homocysteine level (homocystinuria) can occur with a block at either branch. Defects in transsulfuration lead to an elevation in the methionine level, whereas defects in remethylation result in low methionine levels. A closer look at the amino acid levels in this patient revealed that methionine was at the lower limit of the normal range (11 μM; normal range, 9 to 42), suggesting a remethylation defect.

Remethylation defects can be caused by decreased activity of methionine synthase (apoenzyme or cofactors) or 5,10-MTHFR. Deficiency of functional methionine synthase traps folates in the 5-methyltetrahydrofolate (5-MTHF) form, consequently causing a deficiency of other active forms

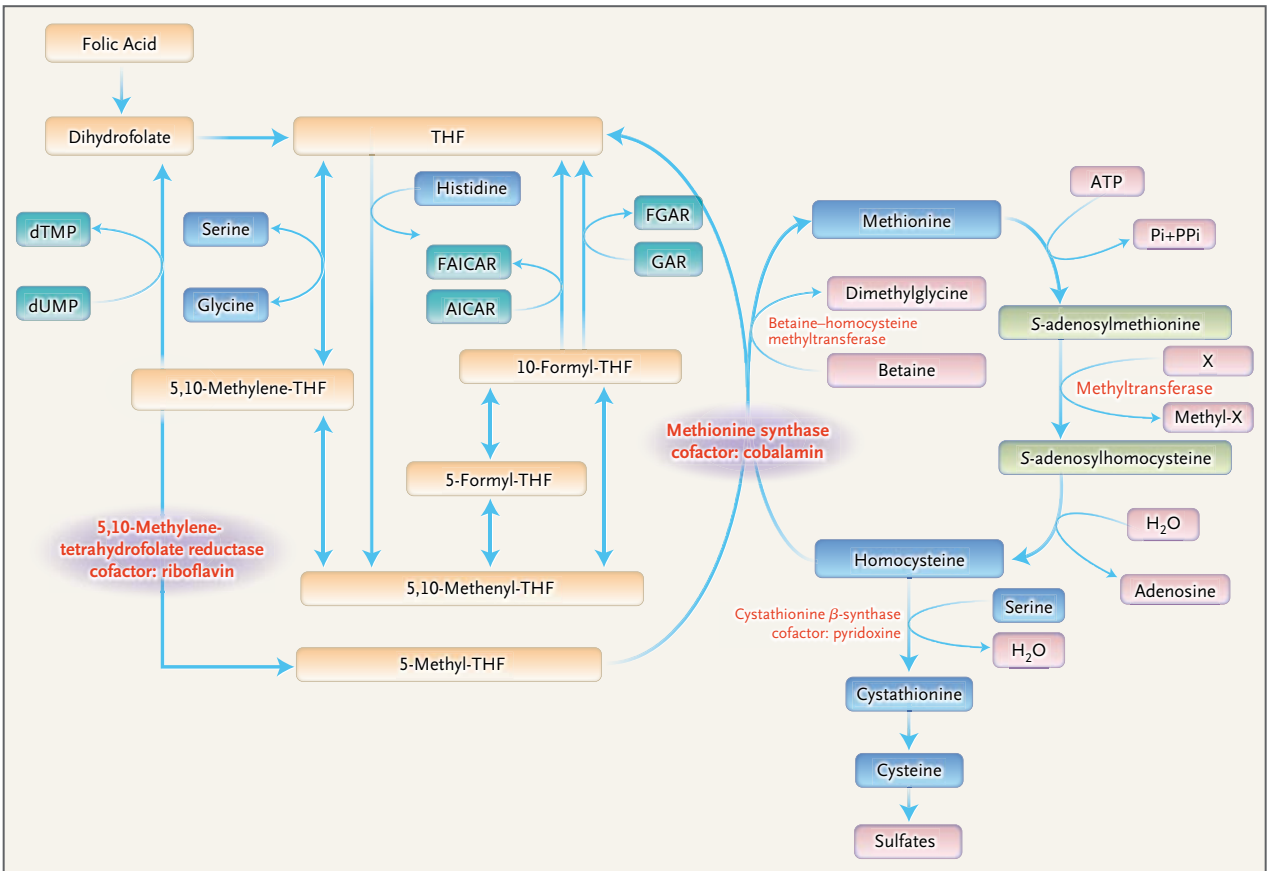

Figure 3. The Enzymes and Cofactors of the Metabolic Pathways Involved in Homocystinuria.

Homocysteine, which is synthesized from methionine, is metabolized through two biochemical pathways. One pathway is transsulfuration, wherein homocysteine condenses with serine to form cystathionine in a reaction catalyzed by cystathionine β-synthase that requires pyridoxine as a cofactor. Cystathionine is further hydrolyzed to form cysteine, and excess cysteine is oxidized to form taurine or inorganic sulfates. The other pathway is remethylation, wherein homocysteine acquires a methyl group from 5-methyltetrahydrofolate in a reaction catalyzed by cobalamin-dependent methionine synthase to regenerate methionine; defects of methionine synthase (CblG) and methionine synthase reductase (CblE) disrupt normal activity. A minor pathway catalyzed by betaine–homocysteine methyltransferase also exists, in which the methyl group acquired from betaine is used in the remethylation of homocysteine. FAICAR and FGAR are intermediates in purine synthesis. AICAR denotes aminoimidazole carboxamide ribonucleotide, dTMP deoxythymidine monophosphate, dUMP deoxyuridine monophosphate, FAICAR formyl-AICAR, FGAR formylglycinamide ribonucleotide, GAR glycinamide ribonucleotide, Pi+PPi phosphate and pyrophosphate, and THF tetrahydrofolate. The letter X represents any methyl acceptor.

of folate (including tetrahydrofolate [THF], 5,10-methenyl-THF, 5,10-methylene-THF, and 10-formyl-THF) and thereby resulting in megaloblastic anemia, which was not seen in this patient. In the severe form of MTHFR deficiency, the 5-MTHF level is reduced but anemia is not seen, because the 5,10-methylene-THF and other active forms of THF (including 10-formyl-THF) that are required for purine and pyrimidine synthesis are not affected.

The combination of homocysteinemia, low methionine levels, and absence of anemia indicated a diagnosis of severe MTHFR deficiency. Confirmatory studies, including measurement of cerebrospinal fluid folate levels and *MTHFR* gene sequencing, were performed.

The level of 5-MTHF in the cerebrospinal fluid was below the lower limit of the reportable range. (Such low values can be found in patients with MTHFR deficiency and in patients with defects affecting folate receptor α.) Sequencing revealed two novel mutations of the *MTHFR* gene (c.1539_1542delAG, p.E514VfsX31 and c.968T→C, p.L323P) in a compound heterozygous state, confirming the diagnosis. In addition, we believe that in utero exposure to elevated levels of ho-

mocysteine (the maternal homocysteine level, measured after the diagnosis had been established, was 28 μM) and the 16p11.2 duplication probably contributed to his phenotype.

Severe MTHFR deficiency is extremely rare but is the most common disorder of folate metabolism. Approximately 100 cases have been reported, and fewer than 15 have manifested before 6 weeks of age. The clinical presentation is nonspecific, with some age-related variations. In the newborn period and during early infancy, seizures and acute neurologic deterioration are the common initial manifestations, whereas in older infants and children, nonspecific psychomotor deterioration, acquired microcephaly, and abrupt deterioration (e.g., respiratory failure) can be seen. In older patients, isolated stroke, arterial or venous thromboembolic disease, and combined degeneration of the spinal cord have been reported as initial manifestations.

Treatment of the remethylation defects is directed toward reducing homocysteine levels (thereby lowering the risk of thromboembolism) and normalizing methionine levels (and normalizing the availability of methionine in the central nervous system). These are achieved through betaine supplementation, which remethylates homocysteine to form methionine through an alternative pathway present in the liver and kidney. Additional treatment involves folate and cofactor supplementation for enzymes in the pathway; hydroxocobalamin (a cofactor for methionine synthase) and riboflavin (a cofactor for MTHFR) are administered to enhance residual activities and improve remethylation, and pyridoxine (a cofactor for cystathionine β-synthase) is administered to enhance degradation of homocysteine through the degradation pathway.

Once MTHFR deficiency was confirmed in this patient, 5-MTHF was administered, folinic acid was continued, and the administration of hydroxocobalamin was changed from an intramuscular to an oral route. The apneic episodes resolved, homocysteine levels decreased (mean level before treatment, 163 μM; mean level after treatment, 70 μM), and methionine levels normalized (mean level before treatment, 9 μM; mean level after treatment, 22 μM) within a few days after the start of treatment. At the patient's follow-up visit a year later, his global development was delayed for his age but was progressing well.

Dr. Caruso: MRI that was performed at the 18-month follow-up revealed no evidence of cerebral venous sinus thrombosis. There was increased myelination, most notably in the bilateral superior, middle temporal, and bilateral supramarginal gyri. The deficiency of the corpus callosum was still present.

Dr. Sahai: The patient's mother is here today, and I would like to invite her to comment.

The Patient's Mother: Thank you for the opportunity to come and hear the presentation. Having seen this case from the perspective of the patient and family, I am fascinated to see it from the perspective of the doctors. I would like to thank the staff at Massachusetts General Hospital for saving our baby for us. I don't know that we would have him if we hadn't been able to come here and to work with this incredible team of people. This period was not without its troubles — he was in the hospital for 75 days. But he is doing remarkably well, and he is a completely different baby from when he was here.

Dr. Nancy Lee Harris (Pathology): Are there any questions for any of our participants?

A Physician: Will the pons enlarge again?

Dr. Caruso: I think there is some chance that the pons will enlarge, since the diminutive pons might be due to white-matter depletion.

Dr. Harris: Are all the neurologic deficits attributable to a defect in myelination?

Dr. Mochida: A large number of the neurologic symptoms and signs might be attributable to hypomyelination, since clinical improvement after initiation of treatment was accompanied by improvement in myelination in this child. Episodes of apnea, for example, might be explained by hypomyelination of the neurons of the respiratory center.

Dr. Harris: How do the homocysteine pathways play into myelination?

Dr. Sahai: The primary pathophysiological consequences on myelination appear to be due to a deficiency of *S*-adenosylmethionine (SAM) that results from decreased methionine. SAM serves as a methyl donor of numerous methylation reactions. Transmethylation reactions are critical steps in the formation of many compounds and in the stabilization of many proteins. A deficiency is expected to lead to defects in proteins required for myelin synthesis.

Dr. Ronald L. Kleinman (Pediatrics): Dr. Grabowski, could you remind us of how excessive homocysteine encourages thrombosis?

Dr. Grabowski: That mechanism is not fully worked out. The accepted view is that elevated homocysteine levels, and perhaps also elevated methionine levels, cause direct injury to the vessel wall, resulting in increased risk of both arterial and venous thrombosis. This direct injury may also disrupt some of the normal antithrombotic functions of the endothelium.

Dr. Sahai: Homocysteinemia results in generation of abnormal thioesters during protein synthesis (by selection of homocysteine instead of methionine) and *N*-homocysteinylation of lysine in proteins such as fibrinogen, thereby affecting their function. It can also interfere with cysteine disulfide bridges and hydroxylysine amino acid residues in proteins (e.g., collagen, elastin, and the proteoglycans) and alter their function. These are important components of the blood vessels, and these changes affect vasculature and increase susceptibility to damage and thrombosis.

FINAL DIAGNOSIS

Severe methylenetetrahydrofolate reductase deficiency.

This case was presented at Pediatric Grand Rounds.

Dr. Mochida reports receiving lecture fees from UCB Japan and Otsuka and grant support from Roche. No other potential conflict of interest relevant to this article was reported.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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