CLINICAL REPORT

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Amish Microcephaly: Long-Term Survival and Biochemical Characterization

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Amish microcephaly (MCPHA, OMIM #607196) is a metabolic disorder that has been previously characterized by severe infantile lethal congenital microcephaly and alpha-ketoglutaric aciduria. All reported patients have been from the Pennsylvania Amish community and homozygous for a p.Gly177Ala mutation in *SLC25A19*. We present a further male patient with MCPHA born to distantly consanguineous parents in Ontario, Canada with Amish ancestors. Microcephaly was evident at 21 weeks gestation on ultrasound. At birth, the facial appearance and brain MRI scan were characteristic of MCPHA, with the additional features of partial agenesis of the corpus callosum and a closed spinal dysraphic state. Urine levels of alpha-ketoglutaric acid were normal at birth and during metabolic crisis, but were markedly elevated during a time of metabolic stability. A severe lactic acidosis was present during metabolic crises and responded to treatment with a high fat diet. At age 7 years, the child is healthy but has severe microcephaly and profound developmental delay. SLC25A19 has been described as a mitochondria inner membrane transporter for both deoxynucleotides and thiamine pyrophosphate (TPP). The biochemical phenotype of MCPHA may be attributable to decreased activity of the three mitochondrial enzymes that require TPP as a cofactor: pyruvate dehydrogenase, alpha-ketoglutarate dehydrogenase, and branched chain amino acid dehydrogenase. We confirm that alpha-ketoglutaric aciduria is not a constant finding in MCPHA and suggest that a persistent lactic acidemia may be more common. The diagnosis should be considered in patients with severe congenital microcephaly, especially in association with lissencephaly, dysgenesis of the corpus callosum, or a spinal dysraphic state.

Key words: Amish; microcephaly; MCPHA; alpha-ketoglutaric acid; lactic acidosis; mitochondrial disease; thiamine pyrophosphate; agenesis of corpus callosum

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INTRODUCTION

Amish lethal microcephaly (MCPHA, OMIM #607196) was described in the Amish of Lancaster County, Pennsylvania [Kelley et al., 2002] as an extreme congenital microcephaly with infantile lethality. Elevated excretion of alpha-ketoglutaric acid was present in the urine of all five infants tested. Affected individuals were homozygous for a mutation in *SLC25A19*, the gene coding for mitochondrial deoxynucleotide carrier (DNC) [Rosenberg et al., 2002]. We report the first confirmed patient with MCPHA born outside of the Lancaster county Amish population and describe further biochemical and MRI findings and the natural history of this disorder.

In southwestern Ontario Canada there are two main Old Order Amish groups. The larger group traces its origins to several families who migrated to the area in the 1820s from Alsace-Lorraine, the Palatinate, and Bavaria [Nolt, 1992]. Genetic disorders in this group are generally distinct from those seen in the United States, probably due to different founder effects. However, some Ontario Amish are descendants of families that migrated from the United States to Ontario in the 1830s [Nolt, 1992]. Most surnames and genetic disorders seen in this latter group are the same as those seen in the Lancaster County Amish.

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CLINICAL REPORT

The male proband is the first child born to a 23-year-old TPAL0010 mother and 26-year-old father who are fifth cousins, related through Amish/Mennonite ancestors, some of whom were from the United States. The surname of father's paternal grandmother is a likely a derivation of a surname commonly observed in previously reported families with MCPHA [Kelley et al., 2002]. At 14 weeks gestation, obstetrical ultrasound measurements of femur length and biparietal diameter were normal. By 21 weeks gestation, microcephaly was apparent with the biparietal diameter and OFC corresponding to 18 weeks gestation (−3 SD). The occipital bone in particular appeared less densely ossified than other skull bones, while the facial profile appeared to show a sloping forehead. A Dandy–Walker variant and spinal dysraphism were suspected. Amniotic fluid volume was normal and amniocentesis showed a normal 46,XY karyotype. The mother consumed routine maternal vitamins during the pregnancy and did not take megavitamin doses, thiamine, or health food supplements.

Labor was induced at 40 weeks followed by a vaginal vertex delivery. The baby was active with birth weight 3.31 kg (~25th centile), birth length 50 cm (~25th centile) and head circumference 29.5 cm (<3rd centile, 50th centile for $31^1/_2$ weeks gestation). Severe microcephaly, a sloping forehead, extremely small anterior fontanel, and closed posterior fontanel were present. Marked ridging was noted over the confluence of the sagittal and lambdoid sutures. The microcephaly and sloping forehead are evident in the photograph taken at age 1 year (Fig. 1). Neurological examination showed truncal hypotonia with hypertonia of the extremities and spontaneous myoclonic jerks. There was no cutaneous evidence of a spinal dysraphic state. Extreme irritability with inconsolable crying was noted in the first week of life, requiring occasional sedation. He had a weak suck with poor weight gain and by 5 months, was admitted to a hospital for failure-to-thrive. His head circumference was 31.5 cm (50th centile for 32 weeks gestation) with all sutures fused and the anterior fontanel closed. At admission he was stable with normal blood gases, slightly elevated ammonia of 47 μmol/L (reference range 11–35 μmol/L), and elevated lactate of 6.7 mmol/L (reference range 0.5–2.2 mmol/L) on a regular infant formula diet. After a few days in the hospital, he became tachypneic and comatose with a metabolic lactic acidosis (pH 7.1, bicarbonate 6 mmol/L, and lactate 16.2 mmol/L). Ammonia was elevated at 113 μmol/L. Liver enzymes were modestly elevated with ALT 173 U/L (normal 10–42 U/L) and AST 104 (normal 10–40 U/L). He required intubation and ventilation, and was started on intravenous sodium bicarbonate, a low protein diet and an "empiric mitochondrial cocktail" (thiamine 50 mg bid, coenzyme Q 10 mg bid, carnitine 200 mg bid, vitamin E 400 IU daily, vitamin K 1 mg daily, riboflavin 100 mg bid, and vitamin C 125 mg bid). Biotin (1 mg daily) and dichloroacetate (5 mg/kg/d) were added 1 week later. His level of consciousness improved transiently despite persistence of lactic acidosis (6.4–13.9 mmol/L).

A high fat diet was then introduced with 70% fat, 4% protein (1.5 g/kg/day), and 26% carbohydrates (Product 80056 protein free®, Microlipids®, and infant formula) via nasogastric tube. After 3 days of the high fat diet, lactic acid levels had dropped to about 4 mmol/L. He was extubated, gradually resumed full bottle feeds with the high fat diet and vitamin "cocktail" and gained weight well. Gastrostomy feeds were started at 27 months to prevent aspiration.

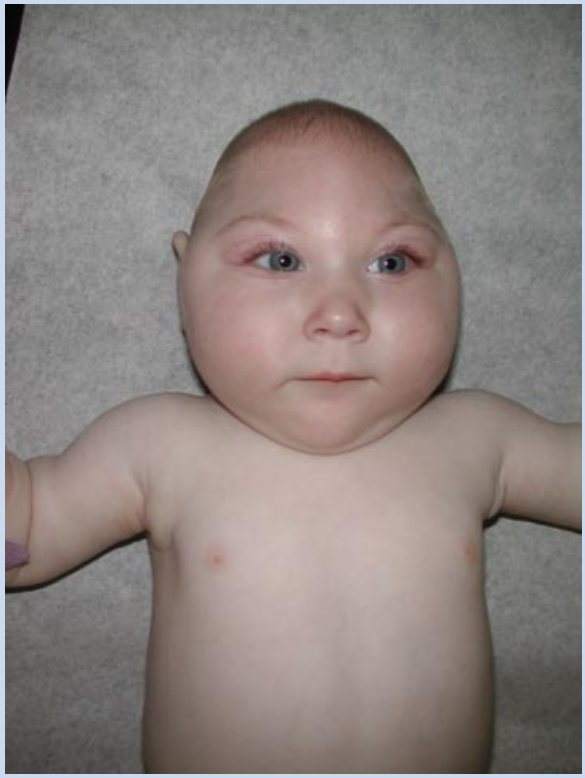

FIG. 1. Patient at age 1 year with microcephaly and sloping forehead.

The child has remained clinically stable since 6 months of age, tolerating intercurrent illnesses. At 6 years of age, his length was 85 cm (50th centile for 2 years), weight 13.4 kg (50th centile for 2 years), and head circumference 36.5 cm (50th centile for 1 month). Brainstem auditory evoked responses were normal. Ophthalmology assessment showed symmetrical bilateral optic nerve atrophy with foveal hypoplasia and cortical visual impairment.

Developmental Progress

From birth, he tended to be difficult to settle. At 6 weeks of age, he was cooing and smiling spontaneously. At $4^1/_2$ months of age, he had developed hand regard and could bring his hands to his mouth. After institution of the high fat diet, he was more content, settling to voices, and more visually aware of his environment. At 7 months of age, he was cooing responsively, making laughing sounds, and batting at toys. He did not track objects. Deep tendon reflexes were 2–3 plus bilaterally. When held in a sitting position, he could hold his head but he could not lift up his head when placed in prone position. He rarely showed a startle reflex. He kicked his legs in supine position. At nearly 6 years of age, he has made no further developmental progress. He has mild gaze-evoked nystagmoid jerks of his eyes. Leg spasticity was initially managed with baclofen

(GABA derivative), but he reacted adversely with prolonged periods of encephalopathy. Botulinum toxin injections have been used effectively.

Laboratory Results and Pathology

Semi-quantitative urine organic acids were normal at 3 days of age. Alpha-ketoglutarate was normal. At 5 months during a metabolic crisis prior to the introduction of mitochondrial "cocktail" and the high fat diet there was a massive increase in lactic aciduria with lesser increases in pyruvic acid and 2-hydroxyisovaleric acid. Alpha -ketoglutarate continued to be normal. The profile was felt to be suggestive of pyruvate dehydrogenase deficiency and prompted introduction of the modified high fat diet. During the acute episode, lactate concentrations ranged from 6.7 to 16.7 mmol/L (Fig. 2). At 8 months when metabolically stable, organic acid analysis identified an elevated alpha-ketoglutarate (>3,700 mg/g creatinine, reference range <200) with small increases in 2-hydroxyglutaric and 2-ketoadipic acids similar to the pattern observed in other patients with MCPHA (Clinical Mass Spectrometry Laboratory, Kennedy Kreiger Institute, Baltimore MD). This pattern of organic aciduria has persisted. In the 6 years since the introduction of the high fat diet, plasma lactate concentrations averaged 4.6 mmol/L (29 determinations). Serum lactate to pyruvate ratio and pyruvate dehydrogenase activity in fibroblasts were normal. The activities of quadriceps muscle mitochondrial respiratory chain enzymes complexes I, II, III, and IV were normal at 6 months of age and there was no evidence of mitochondrial DNA depletion in cultured fibroblasts (data not shown). Light and electron microscopy of the muscle biopsy did not show significant morphological changes in mitochondria. The diagnosis of MCPHA was suspected and sequencing of all nine exons and intron/exon boundaries of the *SLC25A19* determined the patient to be homozygous for the c.530C>G mutation, which predicts p.G177A as reported by Rosenberg et al. [2002] for patients with MCPHA. Both parents were heterozygous. As well, the patient was homozygous for two SNPs (rs7213318 and rs4789164).

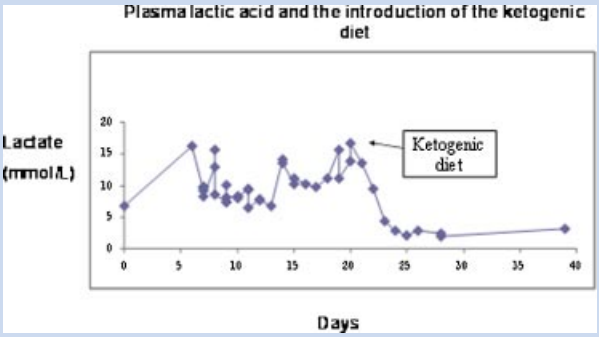

FIG. 2. Lactic acid concentrations at age 5 months from the beginning (day 0) to the introduction of a high fat diet (day 20) and the end of the metabolic lactic acidosis crisis (day 40). Data were obtained from arterial, venous, and capillary specimens.

Radiologic Investigations

The small anterior fontanel precluded a head ultrasound at birth. A CT scan of the head at 2 days of age showed partial agenesis of the corpus callosum and large cisterna magna communicating with the fourth ventricle, enlarged lateral ventricles, hypoplastic cerebellar vermis, and few gyri with a flat surface of brain compatible with lissencephaly, most marked in the frontal lobes. A cranial MRI was similar to images in the original report of MCPHA [Kelley et al., 2002], with the additional feature of corpus callosum dysgenesis (Fig. 3). Ultrasound and MRI of the lumbosacral spine demonstrated splaying of the laminae and absent spinous processes at L4 and L5, compatible with a closed spinal dysraphism. X-rays of the pelvis at 4 years showed diffuse osteopenia, bilateral coxa valga, and mild lateral subluxation of the proximal femurs.

DISCUSSION

The only previous cases of MCPHA [Kelley et al., 2002] manifested severe congenital microcephaly, early lethality, and alpha-ketoglutaric aciduria. The diagnosis of MCPHA in the present patient was suspected because of the clinical presentation, the distant Amish/Mennonite background, and the similarity of the MRI findings. Normal urinary alpha-ketoglutarate excretion at birth and at 5 months did not support the diagnosis of MCPHA, but the diagnosis was again suspected when marked alpha-ketoglutaricaciduria was noted when the infant was well. The diagnosis was confirmed with the finding of homozygosity for the p.G177A mutation in *SLC25A19*. Thus, we confirm that increased urine alpha-ketoglutaric acid is not a constant feature of MCPHA. This also suggests that alpha-ketoglutaric acid may not be elevated in the amniotic fluid in pregnancies with a fetus affected with MCPHA.

Microcephaly was apparent by the second trimester of pregnancy, suggesting that the diagnosis may be suspected prenatally in at-risk pregnancies. Postnatal MRI of the head was similar to the previously published cranial MRI [Kelley et al., 2002]. The additional findings of partial agenesis of the corpus callosum and a spinal dysraphic state have not been reported. Previous patients with MCPHA died within a year of age, presumably of a metabolic decompensation. The present patient is alive at 7 years and presents as a static microcephaly with the metabolic lactic acidosis controlled by a high fat diet.

SLC25A19 was initially considered to transport cytoplasmic deoxynucleotides into mitochondria for mitochondrial DNA synthesis [Dolce et al., 2001]. Disorders such as deoxyguanosine kinase deficiency (OMIM #601465) and thymidine kinase II deficiency (OMIM #609560) that perturb mitochondrial deoxynucleotide pools result in mitochondrial DNA depletion syndromes. In agreement with the studies performed by Lindhurst et al. [2006] on *SLC25A19*$^{-/-}$ mice, we found no evidence for mitochondrial DNA depletion in patient fibroblasts (data not shown). This may indicate tissue specificity that excludes fibroblasts or, more likely, that the primary function of SLC25A19 is not as a deoxynucleotide transporter into mitochondria. Indeed, observations in the *SLC25A19*$^{-/-}$ mouse and fibroblasts from an MCPHA patient suggest that the primary function of SLC25A19 is to

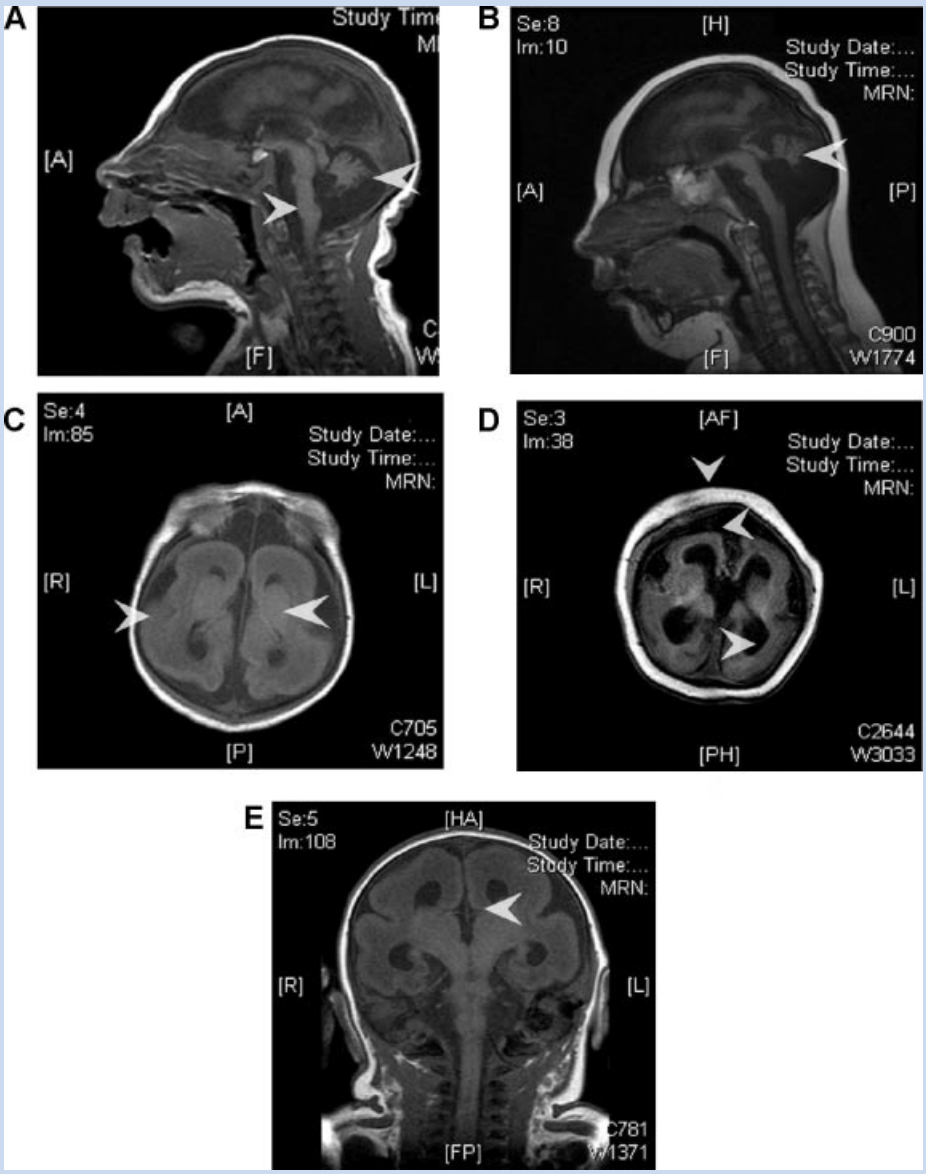

FIG. 3. Selected MRI images from patient (after birth A,B,E) and during follow up (C,D). Sagittal T1 (A) weighted sequences show severe microcephaly (frontal sloping), cerebellar atrophy, and a Dandy–Walker cyst (<arrowhead), and abnormal brain stem morphology (arrowhead>). Axial T1 (B) weighted sequences show the smooth pachygyric cortex (lissencephaly—>arrowhead) and poorly differentiated deep gray nuclei (<arrowhead). The coronal T1 weighted sequence (E) shows a thinned corpus callosum (arrowhead). Follow-up studies (C) show progression of cerebellar atrophy and posterior fossa abnormalities noted earlier (arrowhead). Axial T1 weighted sequences (D) show the thickening of the calvarium (arrowhead down), cerebral atrophy (<arrowhead), and ventriculomegaly (>arrowhead) indicating continued loss of gray-white matter thickness and ex vacuo ventricular enlargement.

transport thiamine pyrophosphate (TPP) from the cytoplasm into mitochondria [Lindhurst et al., 2006]. The murine *SLC25A19*$^{-/-}$ genotype is uniformly lethal by embryonic day 12 and shows severe neural tube defects with exencephaly and other CNS anomalies primarily affecting forebrain structures [Lindhurst et al., 2006]. The finding of splayed lamina and absent spinous processes in our patient supports the hypothesis that altered SLC25A19 function affects neural tube closure.

TPP is a cofactor for pyruvate dehydrogenase, alpha-ketoglutaric acid dehydrogenase, branch chain ketoacid dehydrogenase, and transketolase. In the present patient, lactic acidemia was a constant feature, alpha-ketoglutaric aciduria was present when the patient was metabolically stable and 3-hydroxyisovaleric aciduria has not been observed. These biochemical characteristics can be explained by deficient activities of two (pyruvate dehydrogenase and alpha-ketoglutaric acid dehydrogenase) of the three mitochondrial

TPP-requiring enzymes. The in vitro activity of fibroblast pyruvate dehydrogenase was normal because the assay is not dependent on TPP transport. Transketolase is cytoplasmic and should not be dependent on the activity of SLC25A19.

The high fat diet and supplemental thiamine present in the ''mitochondrial cocktail'' did not normalize the lactic acidemia but reduced lactic acid concentrations in a manner similar to that seen in patients with pyruvate dehydrogenase deficiency [Falk et al., 1976]. During the initial acute metabolic lactic acidosis episode when lactic acid was 16.7 mmol/L, the present patient did not excrete alpha-ketoglutaric acid. The inverse relationship between the production of lactic acid and alpha-ketoglutaric acid can be explained by reduced pyruvate dehydrogenase activity during a metabolic crisis caused by functional thiamine deficiency. We speculate that this results in lactic acidosis and a decline of acetyl -CoA entering the tricarboxylic acid cycle, with a consequent decreased production of alpha-ketoglutaric acid. In this model, the high fat diet provided energy in mitochondria primarily through fatty acid β-oxidation that produced acetyl-CoA, bypassing pyruvate dehydrogenase to directly enter the tricarboxylic acid cycle. The subsequent accumulation of alpha-ketoglutarate may occur because thiamine deficiency reduces alpha-ketoglutaric acid dehydrogenase activity. The metabolic similarity of MCPHA to pyruvate dehydrogenase deficiency suggests that glucose should be administered with caution to patients with MCPHA to avoid a risk of exacerbating the lactic academia.

All reported patients with MCPHA including the current case have been homozygous for the same p.G177A mutation and have had Amish origins, therefore it seems likely that they may all share a common ancestor. It remains to be determined whether other mutations in *SLC25A19* are associated with a similar phenotype. The p.G177A mutation phenotype in humans is less severe than the embryonic lethal phenotype of the *SLC25A19*$^{-/-}$ mouse [Lindhurst et al., 2006]. Phenotypes associated with mutations in *SLC25A19* were recently expanded to include recurrent episodes of flaccid paralysis and encephalopathy associated with bilateral striatal necrosis and chronic progressive polyneuropathy [Spiegel et al., 2009].

Most mitochondrial disorders are associated with neurodegenerative processes and a subset exhibit specific CNS malformations [Brown, 2005]. Dobyns [2002] suggested assay of urine organic acids and blood lactate levels to screen for MCPHA in patients with microcephaly and simplified gyral pattern and brainstem/cerebellar hypoplasia. This report adds dysgenesis of the corpus callosum and neural tube defects to the spectrum of CNS malformations associated with MCPHA and supports the importance of screening for lactic acidosis.

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