that the left hemisphere was more severely affected by epileptic activities because the right extremities were flaccid, similar to Todd's paralysis, and the EEG showed generalized activity at that time. Shortly after status epilepticus was controlled with pyridoxine, an EEG showed left centroparietal spikes (see Figure 2). Furthermore, she showed persistent left hand preference with mild weakness of the right upper extremity months after her seizures were controlled with pyridoxine, and her language skills were delayed before 48 months old. These findings suggest more involvement of the left hemisphere in our patient. The reason for more excitability of the left hemisphere is unclear. One can attribute it to a small structural abnormality, which was beyond the resolution in her MRI performed at 7 months old. A high prevalence of structural abnormalities has been reported in pyridoxine-dependent epilepsy. These changes include callosal thining,[10] periventricular hyperintensity and cortical atrophy,[11] and longitudinal structural changes.[12] In our case, there were no obvious MRI abnormalities, such as a cortical lesion, to explain the focal onset seizures, although her young age at the time could limit the interpretation. No follow-up MRI has been obtained since her seizures were controlled. Another possibility is that the dominant hemisphere might be more susceptible to excitability.

We suggest that pyridoxine-dependent seizures should be considered in the differential diagnosis of recurrent complex febrile seizures or refractory focal seizures that might appear as potential candidates for epilepsy surgery, such as focal resection.

Akira Yoshii, MD
Pediatric Neurology Unit
Department of Neurology
Massachusetts General Hospital
Boston, Massachusetts
Masanori Takeoka, MD
Division of Epilepsy and Neurophysiology
Department of Neurology
Boston Children's Hospital
Harvard Medical School
Boston, Massachusetts
Peter J. Kelly, MB, MRCPI
Kalpathy S. Krishnamoorthy, MD
Pediatric Neurology Unit
Department of Neurology
Massachusetts General Hospital
Boston, Massachusetts

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Address correspondence to Dr Kalpathy K. Krishnamoorthy, VBK731, Massachusetts General Hospital, 55 Fruit Street, Boston, MA 02114. Tel: 617-724-7866; fax: 617-724-7860; e-mail: address:kkrishnamoorthy@partners.org.

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Multivoxel Magnetic Resonance Spectroscopy in a Rhizomelic Chondrodysplasia Punctata Case

ABSTRACT

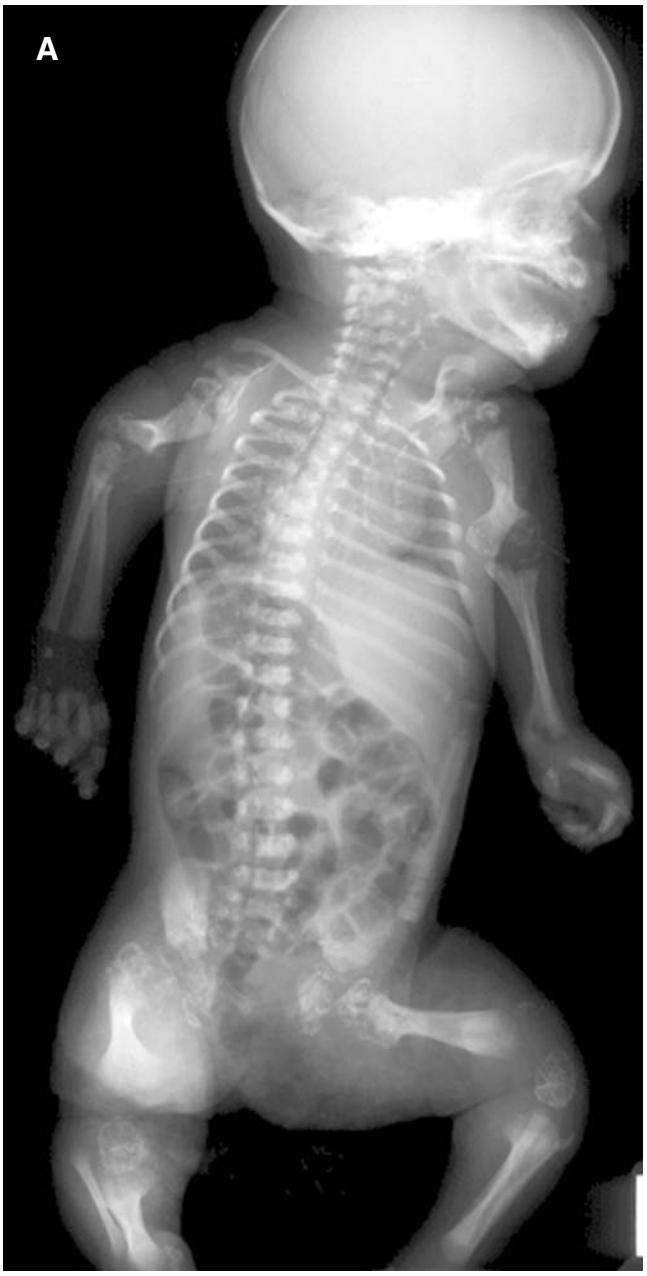
A case of a 5-day-old newborn with rhizomelic chondrodysplasia punctata was investigated with multivoxel magnetic resonance spectroscopy, including chemical shift imaging maps, which disclosed a decrease in the choline peak and the choline signal intensity, respectively, in the right cerebral hemisphere. This is the second report of multivoxel magnetic resonance spectroscopy examination of the brain associated with rhizomelic chondrodysplasia punctata in the literature. Multivoxel magnetic resonance spectroscopy with chemical shift imaging maps has the advantage of obtaining more information in a short period of time, which shortens the duration of anesthesia and its associated risks and complications. We suggest that future efforts be directed to evaluating such patients with multivoxel magnetic resonance spectroscopy instead of single-voxel magnetic resonance spectroscopy. (*J Child Neurol* 2005;20:698–701).

Rhizomelic chondrodysplasia punctata is an autosomal recessive peroxisomal disorder characterized by a symmetric shortening of the proximal limbs, punctate calcifications of the cartilage, contractures of joints, vertebral clefts, cataracts, a characteristic facial appearance, severe growth deficiency, and mental retardation.[1] In the liver and fibroblasts of patients with rhizomelic chondrodysplasia punctata, there is a deficiency in the biosynthesis of plasmalogens owing to the reduced or absent activity of the precursor enzymes acyl coenzyme A (CoA) hydroxyacetone phosphate, alkyl dihydroxyacetone phosphate synthase, 3-ketoacyl-CoA thiolase, and phytanol-CoA hydroxylase.[2,3]

Previous magnetic resonance imaging (MRI) studies have demonstrated increased signal intensity in the periventricular white matter and centrum semiovale; furthermore, delayed myelination, especially in the occipital region, was reported.[4–6] Single-voxel[7] and multivoxel proton magnetic resonance spectroscopy[8] findings of rhizomelic chondrodysplasia punctata were reported in two cases. To our knowledge, in the literature, this is the second case reporting multivoxel magnetic resonance spectroscopy findings of rhizomelic chondrodysplasia punctata.[8]

Case Report

Our patient was the eighth child of healthy and unrelated parents. His seven siblings had no symptoms or signs of rhizomelic chondrodysplasia punctata. Following the development of preeclampsia in the mother, the



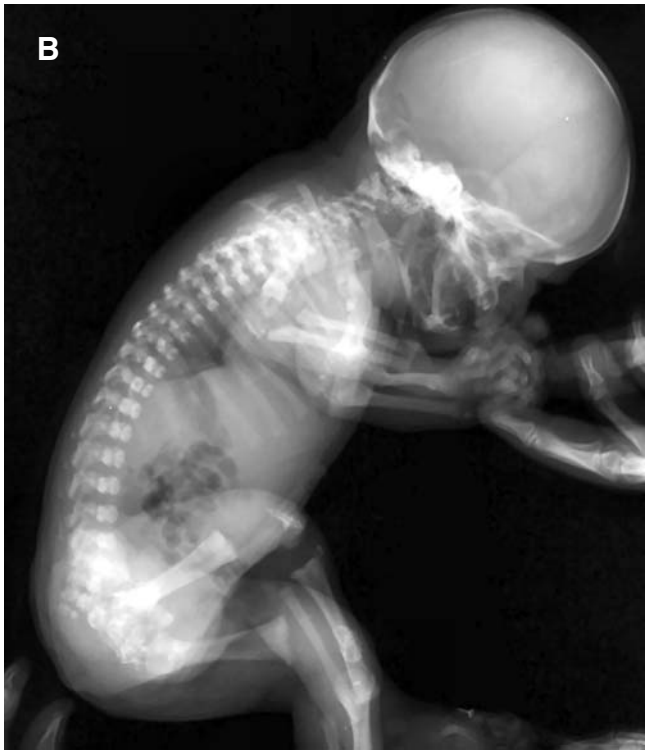


Figure 1. Plain radiographs: *A* shows symmetric bilateral shortening and metaphyseal widening of the humerus and femur with punctate calcifications of epiphysis of the long bones and the pubis, and *B* shows coronal clefts in the vertebral bodies.

patient was born at the thirty-eighth week of gestation by cesarean section. At birth, his Apgar scores were 5.5 and 7 at 1 and 5 minutes, respectively. His mother had no history of drug use or evidence of any infection during pregnancy. The patient's weight at birth was 1860 g (< 3rd percentile), his height was 42 cm (< 3rd percentile), and his head circumference was 29 cm (< 3rd percentile). He was hypoactive and hypotonic and had feeding difficulties. Physical examination revealed typical facial dysmorphisim consisting of a broad nasal bridge; hypertelorism; a short neck; a wide forehead; symmetric shortening of the proximal limbs in the extremities; flexion contractures of the knee, elbow, and especially the hip joints; and bilateral cataracts with vitreous hemorrhage. The only laboratory finding was thrombocytopenia (76,000/μL), which increased to 217,000/μL during follow-up. Plain radiographs (Figure 1) showed symmetric bilateral shortening and metaphyseal widening of the humerus and femur, punctate calcific stippling of the epiphysis of the long bones and pubis, and coronal clefts in the vertebral bodies. Clinical symptoms and radiologic findings were highly suggestive of rhizomelic chondrodysplasia punctata.

When the patient was aged 5 days, MRI and multivoxel magnetic resonance spectroscopy of the brain were performed on a 1.5-Tesla system (Philips, Gyroscan Intera Master, Best, the Netherlands). Prior to this procedure, the patient's parents were informed about MRI and the possible risks of anesthesia and their oral and written consent was received. The patient was sedated with chloral hydrate (dose 50 mg/kg of body weight). Axial and sagittal T_1-weighted images (repetition time 560 milliseconds, echo time 15 milliseconds) and axial and coronal T_2-weighted images (repetition time 4530 milliseconds, echo time 100 milliseconds) with 5 mm slice thickness were obtained. The MRI study showed that overall myelination of the brain was consistent with the gestational age of the neonate.

Multivoxel magnetic resonance spectroscopy data sets were acquired by using point-resolved spectroscopy with acquisition parameters of 1500/136/1 (echo time 136 milliseconds, long echo time) and a transverse field of view of 230 mm with a 16×16 rectangular sampling array. The three orthogonal base images were obtained by automatic shimming of the magnetic field, and a 30 mm–thick volume of interest was identified. The volume of interest was placed to cover the thalamus and the parieto-occipital white matter. Multivoxel magnetic resonance spectroscopy data were accumulated after the optimal water signal was suppressed by the chemical shift–selective technique. Total study time, including MRI and multivoxel magnetic resonance spectroscopy, averaged about 25 minutes.

Multivoxel magnetic resonance spectroscopy disclosed a relative decrease in the choline peak in the right parieto-occipital white matter and the right thalamus (Table 1) that appeared on chemical shift imaging of the choline map as decreased signal intensity (Figures 2 and 3).

Discussion

Rhizomelic chondrodysplasia punctata is a genetically heterogeneous, autosomal recessive disorder of peroxisomal metabolism that is associated with mutations of the *PEX7* gene and characterized by a deficiency in the

Table 1. Comparison of Choline-to-Creatine Ratios from Multivoxel Magnetic Resonance Spectroscopy* Findings in the Right and Left Sides of the Parieto-occipital White Matter and Thalamus

	Parieto-occipital White Matter		*Thalamus*	
Metabolite Ratio	*Right*	*Left*	*Right*	*Left*
Choline to creatine	1.26	1.38	1.77	1.86

*Echo time 136 milliseconds.

biosynthesis of plasmalogens.[3,9] Several variants of the disease owing to deficiencies in the activity of phytanoyl-CoA hydroxylase, dihydroxyacetone phosphate acyltransferase, alkyl dihydroxyacetone phosphate synthetase and 3-ketoacyl-CoA thiolase have been described.[2,10] Plasmalogens are major constituents of myelin phospholipids, and it was reported that abnormal formation of myelin is probably related to the inadequacy of plasmalogen biosynthesis.[5]

Information on neuronal or axonal viability and cellular energetic and cellular membrane status could be obtained by magnetic resonance spectroscopy.[11] Most of the pathologic conditions demonstrate the combination of processes such as demyelination, neuronal dysfunction, and anaerobic glycolysis, all of which could be demonstrated by magnetic resonance spectroscopy. Single-voxel and multivoxel magnetic resonance spectroscopy examinations are the methods that reveal the aforementioned processes. The rationale behind multivoxel magnetic resonance spectroscopy is to assess chemical shift imaging maps, which show the areas of metabolic changes in the brain. Multivoxel magnetic resonance spectroscopy with chemical shift imaging has the advantage of obtaining multiple spectra simultaneously during a single measurement; therefore, more information can be obtained in a short period of time compared with single-voxel magnetic resonance spectroscopy, which, in turn, will reduce the duration of the patient's sedation and its associated risks and complications.

The proton magnetic resonance spectra show various peaks, all of which have a different origin and significance. *N*-Acetylaspartate is accepted as a neuronal marker. Choline is a component of the phospholipid metabolism of cell membranes, especially myelin sheaths, and reflects membrane turnover. Major components of the choline resonance are choline-containing compounds with a small molecular weight, such as phosphorylcholine and glycerophosphorylcholine, which form a pool involved in the membrane synthesis and degradation. Choline is the dominant peak in the newborn. During brain maturation, an increase in the size of the large *N*-acetylaspartate peak (at chemical shift 2.01 ppm) relative to the choline (at 3.21 ppm) and the creatine phosphocreatine (at 3.03 ppm) occurs. Creatine plays an important role in the cellular energy metabolism. The creatine peak tends to remain relatively unchanged if there is no evidence of trauma, stroke, tumor, or creatine deficiency syndromes. Therefore, creatine is often used as a putative internal standard against which the other metabolites can be compared.[12,13] During autopsies, it has been observed that the brain of the patients with rhizomelic chondrodysplasia punctata has a reduced number of neurons in the cortex.[8] In our case, the *N*-acetylaspartate peaks were normal. Myelination of the brain begins with the myelination of the cranial nerves on the fifth fetal month and progresses from caudal to cephalad, from dorsal to ventral, and from functional systems that are used in early life to ones that are not used until older childhood.[13] In our case, MRIs revealed a normal myelination pattern, which was consistent with the neonate's gestational age. However, the choline-to-creatine ratios obtained from multivoxel magnetic resonance spectroscopy (echo time 136 milliseconds) findings showed a reduced choline peak in the right parieto-occipital white matter and the right thalamus (see Table 1), and chemical shift imaging maps of the choline showed low signal intensity in the same regions (see Figures 2 and 3). These findings are presumably the sign of hypomyelination owing to the deficiency of plasmalogens for myelin synthesis, which has been mentioned in previous studies,[7,8] in the right cerebral hemisphere of our case.

To document myelination more properly, in our case, we have planned new multivoxel magnetic resonance spectroscopy at the sixth month.

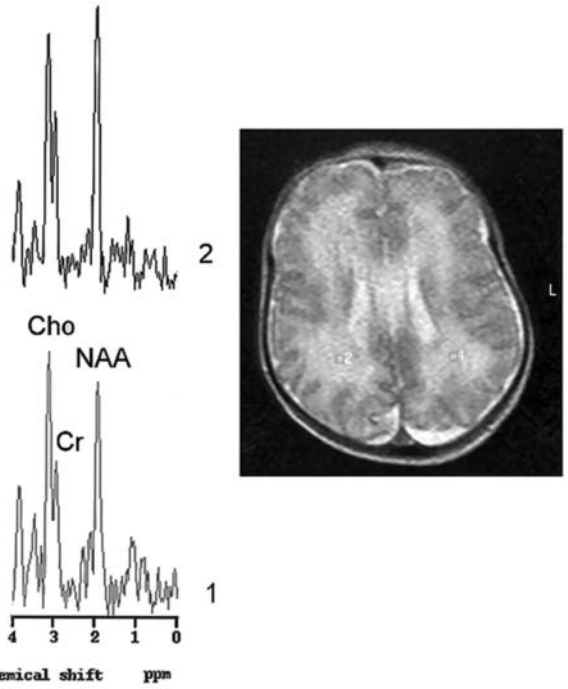


Figure 2. Axial T_2-weighted localizer image (repetition time 3000 milliseconds, echo time 120 milliseconds) for multivoxel magnetic resonance spectroscopy shows periventricular hyperintensities indicating myelinization areas in the white matter that match the gestational age of the patient. Magnetic resonance spectrum (repetition time 1500 milliseconds, echo time 136 milliseconds) (2) reveals a decrease in the choline peak in the right parieto-occipital white matter.The choline peak appears (1) normal in the left side. Cho = choline; Cr = creatine; NAA = *N*-acetylaspartate.

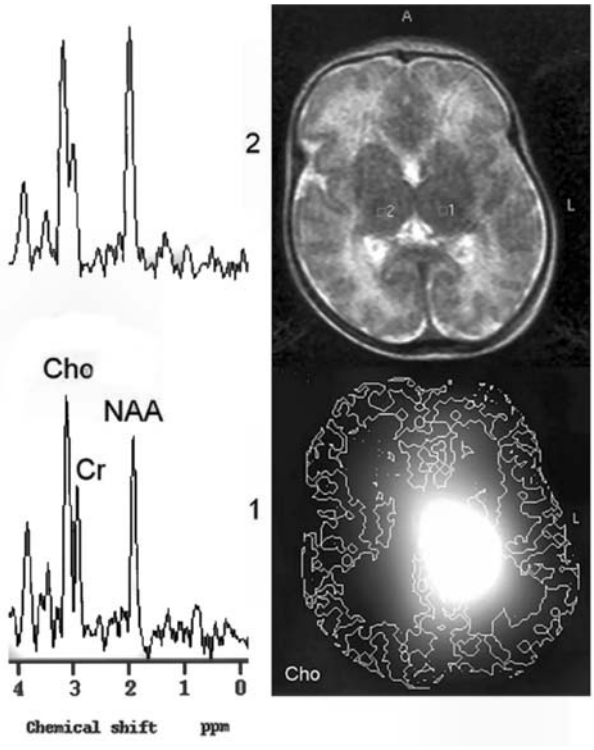


Figure 3. Magnetic resonance spectrum obtained from the thalamus (repetition time 1500 milliseconds, echo time 136 milliseconds) shows a decrease in the choline peak in the right side (2). The choline peak appears (1) normal in the left side. Cho = choline; Cr = creatine; NAA = *N*-acetylaspartate.

However, the patient died from severe respiratory problems at the third month; most patients with rhizomelic chondrodysplasia punctata do not survive beyond the first year.[14]

In conclusion, in our case of rhizomelic chondrodysplasia punctata, although the MRIs revealed a normal myelination pattern that was consistent with the neonate's gestational age, multivoxel magnetic resonance spectroscopy with chemical shift imaging maps disclosed a relative decrease in the choline peak, which is presumably a sign of hypomyelination owing to the lack of plasmalogens for myelin synthesis. We recommend that future efforts be directed to evaluating such patients with multivoxel magnetic resonance spectroscopy instead of single-voxel magnetic resonance spectroscopy to achieve more information in a short period of time, which will significantly decrease the duration of the patient's sedation and its associated risks and complications.

Ahmet Sığırcı, MD
Alpay Alkan, MD
Ramazan Kutlu, MD
Department of Radiology
Hande Gülcan, MD
Department of Radiology
Inonu University School of Medicine
Malatya, Turkey

Received

Address correspondence to Dr Ahmet Sığırcı, Department of Radiology, Inonu University School of Medicine, 44069 Malatya, Turkey. Tel: +90-422-3410660; fax: +90-422-3410834; e-mail: ahmets@inonu.edu.tr.

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The Human Secretin Gene in Children With Autistic Spectrum Disorder: Screening for Polymorphisms and Mutations

ABSTRACT

We screened 29 children with autism for mutation in the human secretin gene using single-strand conformation polymorphism. No mutation was detected in exon 2, 3, or 4. Polymerase chain reaction and DNA sequence of 5′ variable number of tandem repeats showed two polymorphisms with deletion or duplication of a repeat unit that failed to show any gene expression with transient transfection assay. We did not find evidence of a relationship between human secretin gene mutation and autism. (*J Child Neurol* 2005;20:701–704).

Autism is an increasingly common neurodevelopmental disorder.[1] The cause of autism is still unknown, although evidence has shown that it involves a complex interaction of environmental and genetic factors.[2,3] The fact that autism is more prevalent in boys than girls (male-to-female ratio about 3:1) suggests that at least one of the genes might be X-linked.[4] Various genome-wide screens have reported genetic linkage for other chromosomes as well.[5–10] Therefore, it is likely that a cluster of genes will interact for the clinical presentation with autistic traits in the autism spectrum disorder.

Several anecdotal reports have shown that secretin, a peptide hormone that stimulates the secretion of pancreatic juice, is effective in improving the symptoms of autistic children.[11–13] Recently, our laboratory demonstrated that secretin acts as a neuropeptide to modulate γ-aminobutyric acid (GABA) release in the cerebellum,[14] a region that has been closely linked with the pathogenesis of autism.[15] These studies have led to the postulation that secretin, as a brain-gut peptide, might normalize gastrointestinal and brain functions in children with autism. However, initial clinical trials have failed to demonstrate the efficacy of secretin infusion in the majority of autistic patients.[16–20] With the heterogeneity of the pathogenesis of autism, these contradictory reports suggest that most children with autism might not benefit from secretin or that only a specific subgroup of autistic children, who might harbor mutations in the secretin gene, might respond to secretin.

In this study, we tried to determine whether the human secretin gene is a potential susceptibility gene for mutations in children with autistic spectrum disorder. In all 29 children (26 boys, 3 girls) examined, the secretin gene was not mutated. Polymorphisms were identified in the 5′ flanking sequence, but they had no effect on secretin gene expression. Thus, we did not find any evidence of a relationship between mutation in the human secretin gene and autism.

Materials and Method

A cohort of 29 autistic children (26 boys, 3 girls), aged 2 to 18 years, was recruited for this study. Diagnosis was made by the Autism Diagnostic Interview-Revised (ADI-R).[21] These children were regularly followed up in the Autism Research Clinic of the Department of Paediatrics and Adolescent Medicine of The University of Hong Kong. At the time when blood samples were collected from these children, none of them had been treated with intravenous secretin because this is not available locally.

Genomic DNAs were extracted from the blood samples collected using the QIAamp DNA Blood Mini kit (Qiagen). Genomic polymerase chain reaction (PCR) was performed using intronic primers flanking individual exons of the human secretin gene. The sequences of the primers are HSEx1F1 (5′ TGACCTTCCC GGGATCGCTG GGCGC 3′), HSEx1R1 (5′ GGCGGGCCTG GCGGGCGGCT CACCT 3′), HSEx2F1 (5′ TGCGCCCTGA CCCCGACCCC CGACC 3′), HSEx2R1 (5′ ACCACGCCAG GACCCCCCAC CCCAC 3′), HSEx3F1 (5′ AGCAGCAACG CGCGACCCCC CAGCT 3′), HSEx3R1 (5′ ATTGGGCCTC CAGTGGCCAC CAGCG 3′), HSEx4F1 (5′GCC-