A novel form of pontocerebellar hypoplasia maps to chromosome 7q11-21

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Abstract—*Objective:* To describe a novel form of pontocerebellar hypoplasia (PCH) and map its genetic locus. *Background:* PCH is a heterogeneous group of disorders that are characterized by abnormally small cerebellum and brainstem. Autosomal recessive inheritance has been implied in many cases, but no genetic loci have been mapped to date. *Methods:* The authors studied a consanguineous family from the Sultanate of Oman with three siblings with a novel form of PCH. The authors performed clinical studies and linkage analysis of this pedigree. *Results:* The clinical features of the affected children include developmental delay, progressive microcephaly with brachycephaly, seizures during the first year of life, hypotonia with hyperreflexia, short stature, and optic atrophy. Imaging studies showed a small pons and cerebellum, prominent sulci and lateral ventricles, and decreased cerebral white matter volume. A lack of dyskinesias distinguishes this pedigree from PCH type 2. Genetic studies of this family revealed evidence of significant linkage to chromosome 7q11-21 (maximum multipoint lod score 3.23). *Conclusions:* This pedigree represents a novel form of autosomal recessive PCH, which the authors propose to call cerebellar atrophy with progressive microcephaly (CLAM). This disorder maps to chromosome 7q11-21, and this locus was named CLAM. This report represents the first identification of a genetic locus for PCH.

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Over the past several decades, a number of syndromes have been described that share a severe reduction in the size of cerebellum and brainstem.[1-7] In recent years, certain subgroups have emerged as distinct entities as a result of detailed clinical and pathologic studies of these patients.[8] Currently, it is generally accepted that pontocerebellar hypoplasia (PCH) is a heterogeneous disorder, with a genetic origin in many cases. Autosomal recessive inheritance has often been strongly implied; however, no genetic loci have been mapped to date. Here we describe a novel form of autosomal recessive PCH, which we named cerebellar atrophy with progressive microcephaly (CLAM), and demonstrate its linkage to chromosome 7q11–21.

Patients and methods. *Clinical studies.* We examined a consanguineous family from the Sultanate of Oman with three affected children with features of PCH. Two of the authors examined the children (A. Rajab and A. Riaz), and three reviewed the imaging studies independently (G.H.M., P.E.G., and C.A.W.).

Genotyping and genome-wide linkage screen. In an attempt to find the genetic locus for this condition, we studied this pedigree according to a study protocol approved by the institutional review board of Beth Israel Deaconess Medical Center. Blood samples from three affected children, two unaffected siblings, and the parents were collected after informed consent was obtained. DNA was extracted using standard protocols. A genome-wide linkage screen, using polymorphic microsatellite markers with an average spacing of 10 cM, was performed using the ABI PRISM Linkage Mapping Set version 2 (Applied Biosystems, Foster City, CA). Three affected children, one unaffected sibling, and the parents were included in the initial genome-wide screen. Multipoint lod scores for each chromosome were calculated from the genotype data in an attempt to identify potential candidate loci. Lod scores were calculated with GENEHUNTER version 1.2 program[9] assuming autosomal recessive inheritance with 100% penetrance, a disease allele frequency of 0.001, and initially assuming eight equal allele frequencies of 0.125.

Fine mapping of the candidate region. Once we identified the candidate region on chromosome 7q, we performed fine mapping of this region. A second unaffected sibling was included in this fine mapping. Both fluorescently labeled and nonlabeled microsatellite markers were used. The order and location of markers were determined according to the deCode genetic map.[10] For the markers that are not on that map, the genetic location was estimated based on their physical location on the November 2002 assembly of the University of California Santa Cruz Human Genome Browser (http://genome.ucsc.edu/) and local recombination rates according to the deCode map. For the purpose of fine mapping, both two-point and multipoint lod scores were calculated with the Allegro program,[11] assuming autosomal recessive inheritance with a penetrance of 0.99. The disease allele frequency was set as 0.001. Microsatellite marker allele frequencies for each marker were determined by genotyping 28 unrelated individuals from Oman, and these experimentally determined allele frequencies

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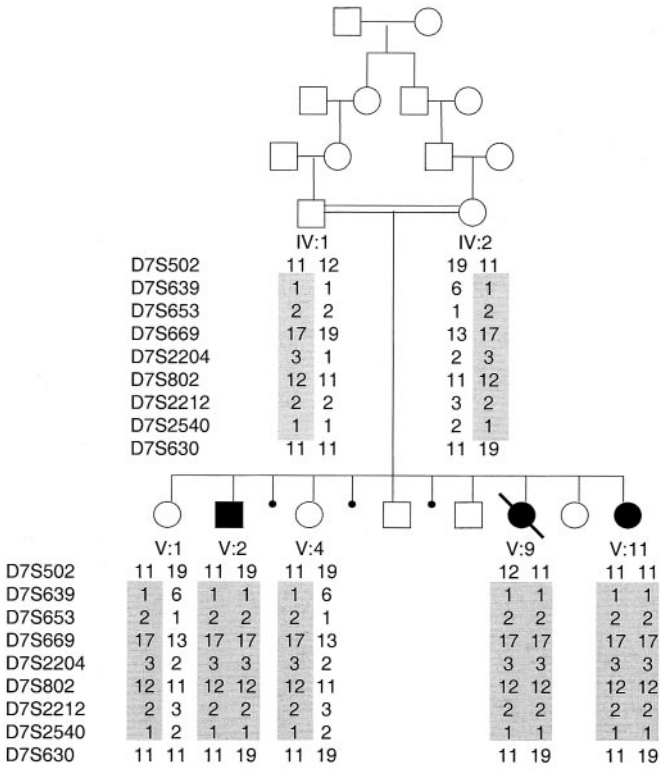

Figure 1. Pedigree structure of the family and genotypes of the individuals tested on chromosome 7q11–21. Nine markers tested in the region of chromosome 7q11–21 are indicated. The marker order is cen-D7S502-D7S630-tel. Haplotypes were manually reconstructed. The disease-associated haplotype is indicated by shading.

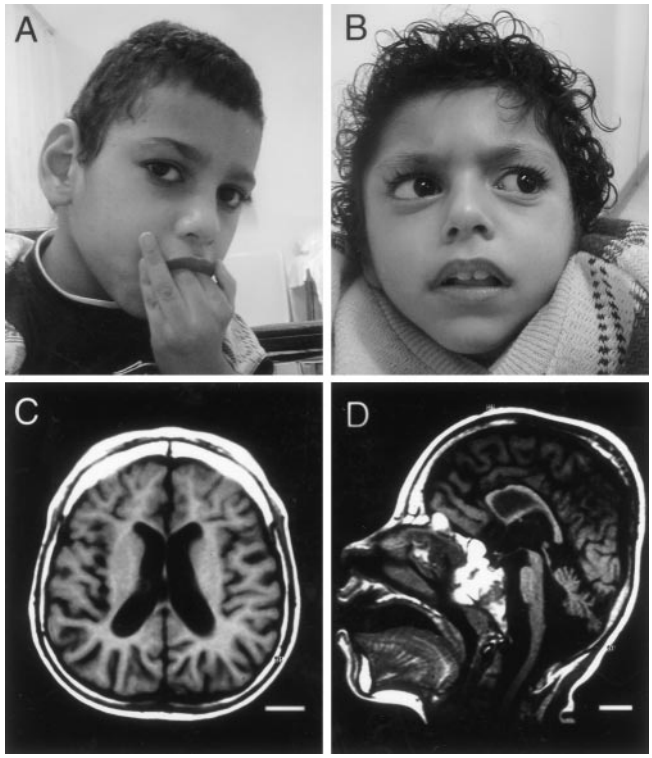

Figure 2. Facial features and MRI of the patients with cerebellar atrophy with progressive microcephaly. Patient 1 (A) and Patient 3 (B) share features of microcephaly with brachycephaly, prominent eyes, and low-set ears. T1-weighted axial (C) and sagittal (D) MR images of Patient 1 show atrophy of the cerebellum, brainstem, and cerebrum. Scale bar = 2 cm.

were used for the two-point and multipoint lod scores reported here.

Results. The pedigree is shown in figure 1. The parents are of Omani origin, and are second cousins. In this pedigree, the parents are consanguineous and children of both sexes are affected. This strongly suggested autosomal recessive inheritance.

Case histories. Patient 1. The proband (V-2) is a 12-year-old boy who was born after an uncomplicated pregnancy via a normal spontaneous vertex delivery. The birth occipito-frontal circumference (OFC) was 33 cm (−1.0 SD), length was 47 cm (tenth centile), and weight was 3 kg (tenth centile). There were no perinatal complications. He was floppy as an infant and did not show interest in his surroundings. He did not achieve head control, did not roll over, and did not attempt to sit. The parents reported frequent upper respiratory tract infections in infancy; however, there were no hospitalizations or major illnesses during the first year of life. At 8 months, the anterior fontanelle was almost closed, and pale optic disks were noted on funduscopic examination. At 1 year, he had a febrile seizure. Later he had afebrile generalized tonic-clonic seizures, which were controlled by sodium valproate. At 18 months, OFC was 40 cm (−6.1 SD), length was 75 cm (< third centile), and weight was 9.4 kg (< third centile). At 12 years, OFC was 45 cm (−6.3 SD), length was 116 cm (< third centile), and weight was 21 kg (< third centile). He was markedly irritable and could not speak, but showed dissatisfaction when disturbed. He could follow light and react to loud noises. He was unable to crawl, sit unsupported, or walk, and was able to sit in a wheelchair only for a short period of time. Craniofacial features included marked brachycephaly, prominent eyes, and low-set ears with some degree of uplift of the earlobe (figure 2A). Spindle-shaped fingers were noted. Cardiac examination was unremarkable. There was a poorly developed scrotum and testicular volume was 1.5 mL. Sexual development was Tanner stage 1. Muscle bulk and power appeared normal. Exaggerated deep tendon reflexes and truncal hypotonia were noted. There were no joint contractures. Abdominal ultrasonography and a skeletal X-ray survey had normal results. Results on laboratory tests including hematologic parameters, serum biochemical parameters, thyroid hormone, serum amino acids, urinary organic acids, and chromosomal analysis were normal. Brain MRI showed a small brainstem as well as a small vermis and small cerebellar hemispheres with prominent fissures (figure 2, C and D). The visualized portions of the spinal cord appeared normal in size. White matter volume was diminished, resulting in prominent lateral ventricles. The corpus callosum was thin, but fully formed.

Patient 2. This patient (V-9) is a younger sister of the proband, and was born normally at term with birth OFC of 32 cm (−1.3 SD), length of 46 cm (fifth centile), and weight of 3.2 kg (25th centile). She was noted to be floppy since birth. Examination at 6 months showed OFC of 39 cm (−2.6 SD) with an almost closed anterior fontanelle. At 8 months, she had a prolonged febrile seizure lasting 24 hours and thereafter she developed chronic epilepsy. The parents noted frequent respiratory illnesses and bouts of diarrhea. Examination at 6 years of age revealed OFC of 42 cm (−6.8 SD), height of 90 cm (< third centile), and weight of 7.4 kg (< third centile). She was a thin, malnourished child with an open-mouthed appearance. She had thoracic scoliosis, contractures of knees and elbows, and clubfoot on the left. Dysmorphic features included brachycephaly, prominent eyes, low-set ears, and gum hypertrophy. Neurologic examination showed truncal hypotonia and spasticity of the limbs. There were exaggerated deep tendon reflexes. There was optic atrophy and intermittent horizontal nystagmus. An EEG showed sharp discharges from the temporal regions bilaterally. Laboratory studies including hematologic parameters, thyroid hormone, serum ammonia and lactate, serum amino acids, urine organic acids, and chromosomal analysis had normal results. A skeletal X-ray survey showed thin osteopenic long bones and a delayed bone age. She died from an acute respiratory illness at 6 years of age.

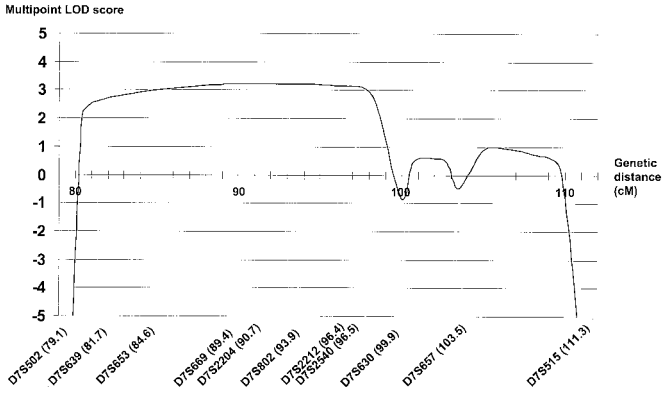

Figure 3. Multipoint lod score analysis of the cerebellar atrophy with progressive microcephaly pedigree in chromosome 7q11–21. The x-axis indicates the centimorgan distance from the p-terminus. Genetic location of each marker based on the deCode genetic map[10] is shown in parenthesis. Multipoint lod score is shown on the y-axis.

Patient 3. This patient (V-11) is a younger sister of Patients 1 and 2. She was born by cesarean section because of cord prolapse. Apgar scores were 7 at 1 minute and 10 at 5 minutes. There were no perinatal complications. Measurements at birth showed OFC of 34 cm (−0.4 SD), length of 50 cm (fifth centile), and weight of 3.2 kg (25th centile). The mother noted absence of head control at 5 months. Examination at 6 months showed OFC of 38.5 cm (−3.0 SD), length of 65 cm (50th centile), and weight of 6 kg (tenth centile). She did not show head control and did not roll over or bear weight. She could grasp objects placed onto her palm and she could bring her hand to her mouth. Facial features were similar to Patients 1 and 2 with brachycephaly, prominent eyes, and low-set ears (see figure 2B). She had febrile convulsions and a history of frequent hospital admissions because of respiratory infections and constipation. Examination at 1 year showed OFC of 40 cm (−4.1 SD), height of 71 cm (25th centile), and weight of 6 kg (< third centile). Neurologic examination revealed central hypotonia with occasional generalized rigidity (spastic episodes). Muscle bulk was normal. Deep tendon reflexes were increased, whereas the plantar responses were downgoing and there was no clonus and no fisting. There appeared to be normal sensation. The fundi could not be visualized, but she followed the light. She failed a hearing test. Hematologic parameters including white blood cell count and morphology; serum biochemical parameters; immunoglobulin (Ig)G, IgA, and IgM values; thyroid hormone; chromosomal analysis; and lysosomal enzymes were normal. Isoelectric focusing of serum transferrin was not suggestive of carbohydrate deficient glycoprotein syndrome. CT of the head revealed wide sulci and sylvian fissures, enlargement of the lateral ventricles, and evidence of thin corpus callosum. The cisterna magna was enlarged, indicative of atrophy of the cerebellum. None of these three patients had a history of edema of the limbs.

Genetic studies. An initial genome-wide linkage screen revealed that D7S669 was the only marker with homozygosity in all three affected children and heterozygosity in both parents and one unaffected sibling tested. When multipoint lod scores were calculated using the results of the genome-wide screen, a maximum lod score of 2.63 was obtained near marker D7S669 on chromosome 7. This was the only region that gave a lod score greater than 1.5 from the genome scan.

Further microsatellite marker analysis was performed in this region, and six further markers tested within a 20.9 cM interval between D7S502 and D7S630 were all found to be homozygous in all three affected children and heterozygous in unaffected siblings, suggesting identity by descent (see figure 1). The candidate region was identified as the interval between these two markers, which covers 21.5 Mb of physical distance according to the November 2002 assembly of the University of California Santa Cruz Human Genome Browser (http://genome.ucsc.edu/). The maximum two-point lod score was 2.43 at D7S2204. The maximum multipoint lod score was 3.23 near markers D7S669 and D7S2204 (figure 3).

Discussion. The patients reported here appear to represent a novel form of PCH. They are characterized by atrophy of cerebellum, brainstem, and cerebrum (causing progressive microcephaly), severe developmental delay, short stature, truncal hypotonia with increased reflexes in the limbs, seizures during the first year of life, and optic atrophy. These patients appear to have substantial similarities to previously reported patients with PCH. A commonly used classification distinguishes two types of PCH: type 1 and type 2.[8,12] In addition to a small pons and cerebellum, PCH type 1 is characterized by spinal anterior horn degeneration and type 2 is characterized by the presence of extrapyramidal dyskinesias and normal spinal cord findings.[8,12] Many observed features of the patients reported here resemble autosomal recessive PCH type 2. The MRI patterns are similar, as is the presence of progressive microcephaly, seizures, and severe developmental delay. However, extrapyramidal involvement with obvious dyskinesias is a prominent feature of PCH type 2, whereas it is absent in the patients described here. Also, optic atrophy is not seen in PCH type 2, whereas Patient 2 had this and Patient 1 was noted to have pale optic disks. Therefore, if the disorder we describe is allelic to PCH type 2, one must find an explanation for these differences. Progressive encephalopathy with edema, hypsarrhythmia, and optic atrophy syndrome bears some similarities to the patients described here, but lack of progressive encephalopathy, infantile spasms, and edema makes this diagnosis unlikely.[13–16]

There are many similarities between the syndrome described here and autosomal recessive cerebellar ataxia described in a large Lebanese pedigree.[17] These patients were also characterized by spastic ataxia, short stature, and cerebellar and cerebral atrophy. Subsequently this syndrome was mapped to chromosome 15q24–26.[18] Because this region has been excluded in the pedigree we describe, allelism of these two pedigrees is highly unlikely. Patients similar to the ones we describe are also found in another report.[19] Two of the patients reported are sisters, suggesting a genetic etiology. These patients also resembled PCH type 2; however, the lack of dyskinesias and the presence of optic atrophy in one of these patients are more similar to CLAM than to PCH type 2. This raises a possibility that this condition might be allelic to CLAM. Among known metabolic disorders, the carbohydrate deficient glycoprotein syndrome is an important differential diagnosis in PCH, and this has been excluded in one of our patients by isoelectric focusing of serum transferrin.

This report describes the first identification of a genetic locus for PCH. Although the two-point lod score in our pedigree was less than 3 because not all members of the consanguineous loop were genotyped, the maximum multipoint lod score was 3.23, and genome-wide screening did not identify any other areas of likely linkage. These findings support linkage of this locus to chromosome 7q and are a

starting point for positional cloning of the gene mutated in this disorder.

The biologic basis for PCH is not yet known. It probably encompasses both static, nonprogressive disorders and degenerative, progressive disorders. In our pedigree, the clinical picture was not indicative of a progressive condition, although the MRI showed enlargement of sulci and fissures, which is generally more suggestive of atrophy than true hypoplasia. Therefore, we have named this condition cerebellar atrophy with progressive microcephaly rather than using the more commonly applied term, hypoplasia. Understanding the biologic nature of the abnormalities, however, awaits identification of the *CLAM* gene. The critical interval contains more than 80 known and predicted genes, according to the November 2002 assembly of the University of California Santa Cruz Human Genome Browser (http://genome.ucsc.edu/). Several of these have a known or potential role in the CNS, and are potential candidate genes. Calneuron 1 (*CALN1*) is a brain-specific gene, which belongs to the calmodulin superfamily.[20] The orthologous gene in mouse (*Caln1*) shows increased expression postnatally, and a high level of expression is seen in the cerebellum, hippocampus, and cortex.[20] This temporal and spatial pattern of expression coincides with the progressive microcephaly with cerebral and cerebellar atrophy in the patients described here. Frizzled homolog 9 (*FZD9*) is a homolog of the *Drosophila melanogaster* tissue polarity gene, *frizzled*, and is potentially important in transmitting Wnt signals, which are involved in early development of the CNS.[21] Identification of the *CLAM* gene will likely help us understand not only the pathogenetic mechanisms of PCH, but also genetic mechanisms of normal brain development.

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