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Chondrodysplasia Punctata with a Mild Clinical Course

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Summary: We report a 7-year-old patient with chondrodysplasia punctata but without rhizomelia. He was born with typical clinical and radiological symptoms of this disease. He developed slowly with considerable psychomotor retardation but improved later, gaining some speech and psychosocial contacts. Joint contractures and bilateral cataracts are still major problems. *De novo* plasmalogen synthesis in fibroblasts was greatly reduced and DHAP-AT activity was at the lower limit of controls. Peroxisomal thiolase was present in its precursor form only. Membrane fluidity (measured by TMA-DPH fluorescence anisotropy) was increased in erythrocyte ghosts and in lymphocytes. Plasma phytanic acid concentration was elevated 5-fold. The patient represents a mild clinical course of chondrodysplasia punctata, resembling Conradi–Hünermann syndrome, but biochemically he has the typical peroxisomal dysfunction of rhizomelic chondrodysplasia punctata except for a high residual activity of DHAP-AT.

Classic rhizomelic chondrodysplasia punctata (RCDP) is an autosomal recessive disorder characterized by severe symmetrical proximal shortening of the limbs, bilateral cataracts, typical craniofacial dysmorphism, congenital joint contractures, severe mental retardation, typical radiological abnormalities, epiphyseal calcifications and failure to thrive (Spranger et al 1971). Biochemically RCDP is characterized by a tetrad of abnormalities including a partial deficiency of dihydroxyacetone phosphate synthase leading to an impairment in plasmalogen synthesis. Furthermore, phytanic acid α-oxidation is deficient and thiolase occurs in the immature 44 kDa form rather than the mature 41 kDa form (Hoefler et al 1988), although this is not associated with deficient peroxisomal β-oxidation. Very long-chain fatty acid (VLCFA) concentrations are normal in RCDP patients.

According to Spranger et al (1971) life expectancy of most affected children is reduced and the majority die during the first year of life. However, in recent years,

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an increasing number of older patients have been documented (Wardinsky et al 1990). Mild variants of RCDP have been described by Poll-Thé et al (1991) and by Pike et al (1990). One patient described by Smeitink et al (1992) exhibited all the biochemical abnormalities of RCDP but the phenotype was different. We present a patient with chondrodysplasia punctata without rhizomelia and with a mild course of the disease but otherwise with all clinical and biochemical critieria associated with this disorder.

CASE REPORT

The parents of this patient were third-degree cousins. He was born after an uneventful pregnancy with a normal delivery at term. Birth weight was 2610 g (10th centile), length 46 cm (3rd–10th centile), and head circumference 33 cm (3rd–10th centile). Physical examination at birth showed muscular hypertonia, limited hip abduction, abnormal Moro reflex, an immobile mass in the left upper arm, a slight valgus of the right hand and of the left foot, swollen knee joints with a small extension deficit, and an unimpressive systolic murmur in the 4th intercostal space. The left testicle was retained in the inguinal channel. The patient was transferred to the local hospital, where the radiological findings revealed chondrodysplasia punctata (Figure 1). Puncture of the mass yielded a sterile viscous liquid without cells; later the tumour

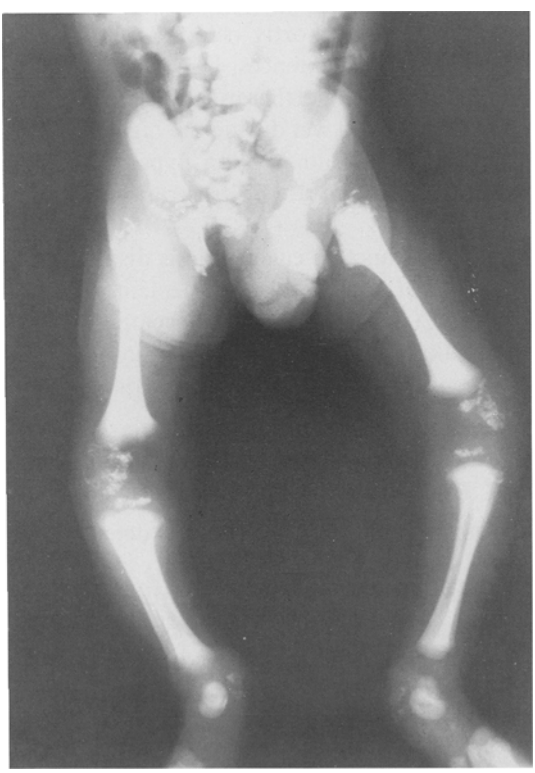

Figure 1 Radiograph of pelvis and legs of the patient at 4 days of age. Note the sy. of the chondral calcifications

disappeared spontaneously. Progressive bilateral cataracts were diagnosed at 9 months of age. Because of the psychomotor retardation, which became more and more apparent, he was admitted to the University Children's Hospital in Berne at 32 months. His speech was restricted to a few two-word sentences and his pronunciation was difficult to understand. At the time of admission he was hyperactive, easily upset and often bit himself.

He was incontinent during day and night, and hypersalivated. Although he was able to hold a spoon correctly, he was unable to eat by himself and had to be fed. At the time of admission his weight was 12.6 kg (10th centile), length 92 cm (50th centile) and head circumference 49 cm (10th–25th centile). He had a dysmorphic facial appearance with epicanthus and a deep, broad nasal bridge. The systolic murmur was still present. He had a genital hypoplasia with a very small scrotum and testicles. His muscular tone was still slightly elevated, and abduction of the legs on both sides was limited.

Clinical course: Although spontaneous mobility at birth was reduced by severe contractures, he was able, with intensive physiotherapy, to walk at the age of 2 years. He started feeding himself after the age of 3 years but at present (7 years of age) he still has problems with chewing. Verbalization started at 1 year with single words, progressed to two word sentences shortly before 3 years. At the age of 5 years he could master three- to four-word sentences. His speech was difficult to understand and he therefore received logopaedic therapy. He became bladder-controlled at 5 years during the daytime, but is still wet during the night. He presently attends special educational classes but his behaviour is often hyperactive. Furthermore, he easily becomes angry but cannot communicate his frustration. In this situation he starts to bite himself.

Most of the characteristic punctate calcifications disappeared during the first year of life. At the age of 1 year ossification of the femoral head was reduced with metaphysial irregularities. A severe coxa vara developed by the age of 4 years, due to deficient femur head calcification. The congenital joint contractures improved but are still limiting movements in shoulders and hips. Because of bilateral cataracts his visual acuity is progressively impaired.

BIOCHEMICAL STUDIES

De novo synthesis of plasmalogens in fibroblasts was determined by a double-labelling method according to Schrakamp et al (1985). Activity of dihydroxyacetone phosphate acyltransferase (DHAP-AT) was measured according to Schutgens et al (1986). Latency of catalase in fibroblasts was measured as described by Wanders et al (1986). Very long-chain fatty acids in fibroblasts were quantified according to Moser et al (1984). Analysis of plasmalogens in fibroblasts was performed according to Björkhem et al (1986).

Skin fibroblasts were cultured by standard methods in MEM containing 10% fetal calf serum. All analyses were performed after 10–15 passages. Membrane fluidity was analysed in erythrocyte ghosts and in isolated lymphocytes as fluorescence anisotropy

using trimethylammonium diphenylhexatriene (TMA-DPH) as a marker for superficial layers of the membranes, essentially as described by Toplak et al (1990). Immunoblot of peroxisomal β-oxidation enzymes was performed as described by Wanders et al (1991). Determination of phytanic acid in plasma was performed by gas chromatography–mass spectrometry.

RESULTS

The activity of fibroblast DHAP-AT was at the lower limit of controls. Latency of catalase was 60%, suggesting intact peroxisomes. The proportion of C_{16} and C_{18} plasmalogens was greatly reduced as was the *de novo* synthesis of plasmalogens. According to the immunoblot studies, acyl-CoA oxidase and the bifunctional protein were normally processed and of normal quantity, while the peroxisomal thiolase was present in its precursor form (44 kDa) only (Table 1).

Fluorescence anisotropy with TMA-DPH as a marker was reduced in the patient's erythrocyte ghosts and in lymphocytes, suggesting an increase in membrane fluidity (Table 2). At the age of 5 years the concentration of plasma phytanic acid was 14.3 μg/ml compared to 1.9 $\pm$ 1.5 μg/ml (mean $\pm$ SD) for controls.

Table 1 Results of specific biochemical analyses in fibroblasts of the patient in comparison with controls, classical chondrodysplasia (RCDP) and Zellweger patients

Parameter	*Patient*	*RCDP*[a]	*Zellweger*[a]	*Controls*[a]
		5–95% (range)	5–95% (range)	5–95% (range)
DHAP-AT activity[b] (nmol/2h per mg protein)	3.7	0.8–2.4 (8)	0.1–1.2 (42)	3.0–11.0 (37)
VLCFA				
$C_{26:0}$ (μg/mg protein)	0.03	0.03–0.11 (8)	0.21–1.21 (66)	0.02–0.10 (57)
($C_{26:0}/C_{22:0}$) ratio	0.04	0.02–0.06 (6)	0.21–1.08 (66)	0.02–0.05 (57)
Plasmalogens (% of controls)				
$C_{16:0}$	29	20–39 (15)	23–59 (31)	100
$C_{18:0}$	8	0–12 (15)	9–61 (31)	100
De novo plasmalogen biosynthesis[c]				
% pPE in PE	9	0.9–10.3 (13)	4.6–55.4 (46)	83–92 (59)
% pPC in PC	0.4	0.3–0.7	0.4–1.2 (46)	3.3–13.6 (59)
$^3H/^{14}C$ ratio in				
Alkenyl	30	42–400	6.4–63.1 (15)	0.4–1.5 (25)
Alkenyl	5	3.5–10.7	3.1–10.9 (15)	0.3–1.0 (25)
Catalase (particle-bound, %)	60	> 60	< 5	> 60
Immunoblot bands (kDa)				
Acyl-CoA-oxidase	70,50,20	70,50,20	70	70,50,20
Thiolase (kDa)	44	44	44 (trace)	41

[a]Number of measurements in parenthesis
[b]DHAP-AT = acyl-CoA: dihydroxyacetone phosphate acyltransferase
[c]pPE = plasmalogen phosphatidylethanolamine; PE = total phosphatidylethanolamine; pPC = plasmalogen phosphatidylcholine; PC = total phosphatidylcholine

Table 2 Fluorescence anisotropy (TMA-DPH)[a]

	Erythrocytes $r\ (G)^b$	*Lymphocytes* $r\ (G)^b$
	(mean ± SD)	
Patient	0.209 ± 0.007*	0.216 ± 0.115**
Controls	0.216 ± 0.006	0.236 ± 0.012

[a]Toplak et al (1990
[b]$r(G)$: Fluorescence anisotropy; G = correction factor for the optical system
*$p < 0.05$ (t-test)
**$p < 0.01$ (t-test)

DISCUSSION

Heymans et al (1985, 1986) and Hoefler et al (1988) described the following peroxisomal abnormalities in patients with classical rhizomelic chondrodysplasia punctata: (1) defect in plasmalogen synthesis; (2) impairment of phytanic acid oxidation; and (3) presence of a precursor protein of the peroxisomal 3-oxoacyl-CoA thiolase with an almost normal overall activity of peroxisomal oxidation system. There is a deficiency of alkyl DHAP synthase activity, the second step in plasmalogen biosynthesis, and there is also a partial reduction in the activity of DHAP-AT, the first enzyme in plasmalogen biosynthesis. Reduced plasmalogen synthesis results in lowered plasmalogen content in cellular membranes similar to that in Zellweger syndrome. Reduced plasmalogen content in Zellweger fibroblasts correlates with higher membrane fluidity (Hermetter et al 1989). Reduced membrane fluorescence anisotropy, which is inversely related to fluidity, was observed in erythrocytes and in lymphocytes of our patient, suggesting a lowered plasmalogen content in those cells also. All other peroxisomal functions were found to be normal. Catalase activity was sedimentable, indicating that peroxisomal structures were intact.

Recently several patients have been found who presented clinically with classical RCDP but with the biochemical defect restricted to a DHAP-AT deficiency (Wanders et al 1992; Barr et al 1993). This suggests that the defect in plasmalogen synthesis is directly related to the phenotype.

Our patient has a mild clinical course of chondrodysplasia punctata with minimal shortening of his upper arms; skeletal proportions were otherwise normal. Clinically our patient resembled the Conradi–Hünermann syndrome, which presents with a wide range of radiological abnormalities, with or without symmetric limb shortening (Spranger et al 1971). Most of the patients are of normal intelligence. Except for one patient reported by Holmes et al (1987) and another reported by Clayton et al (1989), no peroxisomal dysfunction could be found in larger series by Schutgens et al (1988) and Moser et al (personal communication). In our patient, Conradi–Hünermann syndrome (Conradi 1914) is excluded because of the typical involvement of the long bones and the chondrodysplasia punctata at birth. Although some typical radiological findings were lacking (vertebral body abnormalities) and mental retardation was considerably less severe than in the other patients reported, other findings provide strong support for the diagnosis. The mild form of this disorder and its clinical course may be related to the high residual activity of DHAP-AT in cultured fibroblasts from

our patient. Interestingly, the two other biochemical abnormalities, defect in phytanic acid oxidation and maturation defect of the 3-oxoacyl-CoA thiolase, were comparable to the findings in fibroblasts of the severe forms of rhizomelic chondrodysplasia punctata. This, together with the fact that the plasmalogen content of erythrocyte membranes was abnormal, with membrane fluidities increased as reported in cultured fibroblasts of severely affected RCDP and Zellweger patients (Hermetter et al 1989), may yield some further insight towards the pathogenesis of this disorder.

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ERRATUM

Goebel-Schreiner B, Schreiner R (1993) Identification of a new missense mutation in Japanese phenylketonuric patients. J Inher Metab Dis ***16****:950–956.*

The sequencing gel in Figure 1 (page 952) in this paper was inadvertently printed upside down. A correct version of Figure 1 is shown below. We apologize for this mistake.