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Early-Infantile Galactosialidosis: Clinical, Biochemical, and Molecular Observations in a New Patient

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Few patients with the early-infantile form of galactosialidosis have been described to date. Presented here is the first Italian case. Fetal hydrops was detected by ultrasound at week 24 of gestation. At birth, the infant presented with hypotonia, massive edema, a flattened coarse facies, telangiectasias, and hepatosplenomegaly, but no dysostosis multiplex. The patient died 72 days postpartum. Excessive sialyloligosaccharides in urine, as well as vacuolation of lymphocytes and eosinophilic granulocytes in peripheral blood, were indicative of a lysosomal storage disease. In the patient's fibroblasts, both α-neuraminidase and β-galactosidase activities were severely reduced, and cathepsin A activity was <1% of control levels, confirming the biochemical diagnosis of galactosialidosis. However, in contrast to previously reported early-infantile cases, a normal amount of protective protein/cathepsin A mRNA was detected on Northern blots. This mutant transcript was translated into a precursor protein that was not processed into the mature enzyme and lacked both protective and catalytic activities.

KEY WORDS: galactosialidosis, cathepsin A, protective protein, lysosomal storage disease

INTRODUCTION

Galactosialidosis [d'Azzo et al., 1995] is an autosomal-recessive lysosomal storage disease caused by a primary defect of protective protein/cathepsin A (PPCA) [Wenger et al., 1978; d'Azzo et al., 1982; Galjart et al., 1988]. This enzyme has a specific serine carboxypeptidase/deamidase activity and, in addition, associates with lysosomal β-galactosidase and N-acetyl-α-neuraminidase, thereby regulating the intralysosomal stability and activity of the two glycosidases [reviewed in d'Azzo et al., 1995]. Deficiency of PPCA severely alters the activities of both β-galactosidase and neuraminidase, and this combined deficiency has been the hallmark of galactosialidosis. As in most other lysosomal storage diseases, galactosialidosis patients are clinically heterogeneous, having either a very severe early-onset form of the disease, mostly fatal at birth, or mild and slowly progressive late-onset types [d'Azzo et al., 1995]. Intermediate clinical manifestations of early- and late-infantile variants have also been observed in 2 patients [Sewell et al., 1987; Okada et al., 1983]. The spectrum of clinical manifestations correlates in part with differences in the expression level of PPCA mRNA [Galjart et al., 1988], as well as with the amount and quality of immunoprecipitated polypeptide [d'Azzo et al., 1982; Palmeri et al., 1986; Strisciuglio et al., 1988]. The isolation and characterization of human PPCA cDNA [Galjart et al., 1988] has enabled the identification of mutations associated with different clinical phenotypes [Zhou et al., 1991; Shimmoto et al., 1993; Suzuki et al., 1993].

Galactosialidosis is a rare lysosomal disorder. Thus far, most reported cases are juvenile/adult patients of Japanese origin [Suzuki et al., 1988; Takano et al., 1991]. Only a small number of Caucasian patients with the early-infantile form of the disease have been described [Kleijer et al., 1981; Gravel et al., 1979; Lowden et al., 1981; Carton et al., 1989]. Here, we report on the clinical, biochemical, and molecular characterization of the first Italian case.

CLINICAL REPORT

The male patient was born to healthy, nonconsanguineous Italian parents (Fig. 1A). The first pregnancy

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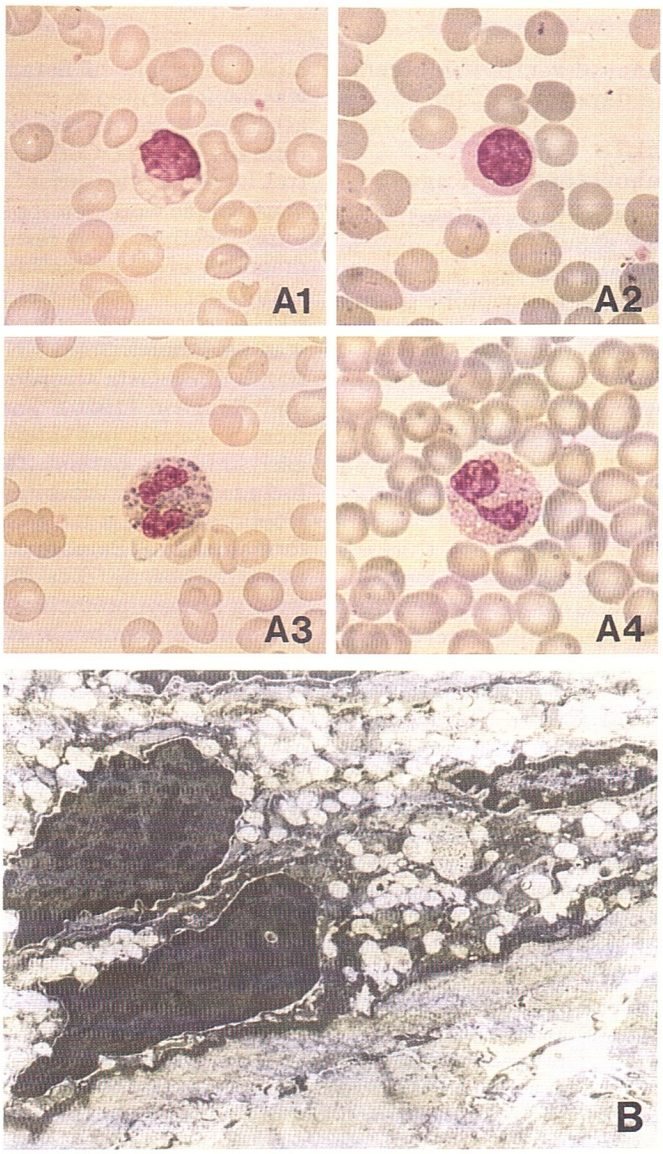

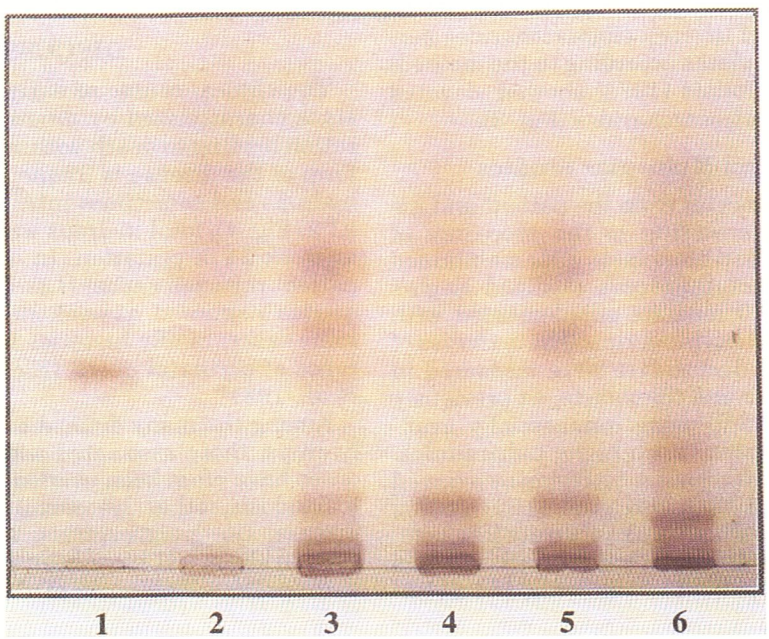

Figs. 2 and 3

TABLE I. Enzyme Activities in Leukocytes and Fibroblasts From the Patient and His Parents*

	Leukocytes				Fibroblasts			
Enzyme	Patient	Mother	Father	N.V.	Patient	Mother	Father	N.V.
β-galactosidase	3.42	137	140	100–270	53	430	579	427–995
Neuraminidase			1.5	0.59–1.1	2.2	63	49	42–117
Cathepsin A					0.5	173	181	243–486

* β-galactosidase and α-neuraminidase activities are expressed in nmol/hr/mg protein; cathepsin A is expressed in nmol/min/mg protein. N.V., normal values.

logical manifestation in early-infantile galactosialidosis patients, as well as in other patients with lysosomal storage diseases, may be more common than previously suspected, and the diagnosis may be missed because of the frequent occurrence of intrauterine or early postnatal death. Establishing the diagnosis by enzymatic assay is essential, not only for accurate genetic counseling, but also for prenatal diagnosis in subsequent pregnancies.

In the patient presented here, we suspected a lysosomal storage disease because of the presence of fetal hydrops, hepatosplenomegaly, coarse facies, and telangiectasias. Skeletal X-rays showed no dysostosis multiplex, but a coarse trabecular pattern and thinning of the osseous cortex were present. These findings have been reported in patients with neonatal ascites due to lysosomal storage diseases [Daneman et al., 1983], and they must be considered when the cause of hydrops or neonatal ascites is uncertain.

The presence of vacuolated lymphocytes in peripheral blood and foamy cells in the bone marrow is suggestive of a lysosomal storage disease. However, the numerous abnormal eosinophils detected in blood smears of the patient are an unusual feature that has been previously reported only in G_{M1}-gangliosidosis [Gitzelmann et al., 1985; Hansen and Graucob, 1985]. It is possible that this anomaly is specifically related to a primary or secondary deficiency of β-galactosidase [Donati et al., 1988]. If so, it would represent an additional diagnostic marker.

Histopathological examination of the patient's tissues demonstrated extensive cytoplasmic vacuolation of both epithelial cells and mesenchymal cells in all organs, although to variable degrees. The mechanism leading to the development of fetal hydrops is, however, unclear, although vacuolated cells were clearly evident in liver, kidney, and heart tissues.

In contrast to other cases of early-infantile galactosialidosis [Galjart et al., 1988], molecular studies in this patient showed a surprisingly normal level of PPCA mRNA, which directs the synthesis of a normal amount and size of the protective protein precursor. At this time, it is impossible to correlate the molecular data with the severe clinical phenotype. Further studies on a larger number of patients may clarify the biochemical and molecular bases of the severe early-infantile forms of galactosialidosis. The identification of the genetic lesion in the index patient may help to explain the effect of the mutation on the specific functions of PPCA which, in turn, may lead to a better understanding of the physiological role of this lysosomal enzyme in normal individuals.

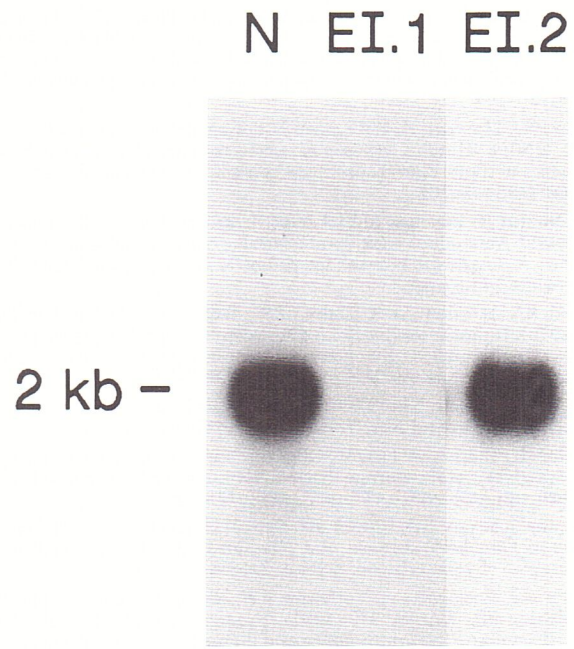

Fig. 4. Northern blot analysis of fibroblast total RNA. N, normal control; EI.1, early-infantile galactosialidosis patient without mRNA; EI.2, our early-infantile patient with normal amount of mRNA. Samples were fractionated on a formaldehyde agarose gel and probed with full-length PPCA cDNA. Size of PPCA transcript is indicated.

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