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Case report

Fatal infantile hypertrophic cardiomyopathy secondary to deficiency of heart specific phosphorylase b kinase

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Abstract. We describe here a male infant with a rare form of glycogenosis caused by deficiency of heart specific phosphorylase b kinase. The disease phenotype was characterized by severe glycogenosis restricted to the heart muscle with secondary rapidly progressive hypertrophic cardiomyopathy causing death at the age of 47 days.

Key words: Phosphorylase b kinase deficiency – Glycogen storage disease – Hypertrophic cardiomyopathy

Introduction

An oustanding feature of the genetic pathology of the phosphorylase enzyme complex, consisting of the phosphorylase itself (E.C. 2.4.1.1) and of its activator (E.C. 2.7.1.38), is a tendency to manifest in the form of an organ restricted deficiency. In phosphorylase activator (phosphorylase b kinase, PBK) deficiency, the following variants have been described; a generalized PBK deficiency (liver and muscle isoenzymes are deficient) with autosomal recessive mode of transmission (Bashan et al. 1981; Lederer et al. 1980; Lerner et al. 1982). Deficiency is also demonstrable in red blood cells (Lederer et al. 1975; Shin 1990). A liver PBK deficiency (isolated hepatic glycogenosis) exists with X-linked genetic transmission and with normal values of muscle isoenzyme activity (Huijing 1967; Huijing and Fernandez 1969; Besley 1987) and this also occurs in an autosomal recessive form, with muscle unaffected (Hug. et al. 1969; Sovik et al. 1982). The deficiency is demonstrable in both forms in red blood cells (Bashan et al. 1981; Besley 1987; Dahan et al. 1988; Lederer et al. 1975). An isolated skeletal muscle PKB deficiency (myopathic form) exists (Othani et al. 1982, Abarbanel et al. 1986) and myocardial PKB deficiency (cardiomyopathic form) has been described by Mizuta et al. (1984) and Servidei et al. (1988). A pathological report of Mizuta's patient was given by Eishi et al. (1985).

In this communication the third case of the rare isolated severe myocardial glycogenosis caused by PBK deficiency is described. This case differs from the two published cases by the short life span of the child.

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Case report

L.P., a male patient, was delivered at term (27. 09. 1991) with a birth weight of 2790 g after an uncomplicated pregnancy. A heart murmur, mild cardiomegaly and failure to thrive were noted at the age of 3 weeks. His general condition deteriorated progessively and at the age of 6 weeks he was admitted with severe heart failure. On admission he was hypotonic with tachycardia and dyspnoea. There was a systolic ejection murmur 3/6 along the left sternal edge and severe hepatomegaly.

The ECG (Fig. 1B) showed abnormally short P-R interval (0.06 s), right atrial enlargement, biventricular hypertrophy with subendocardial ischaemia and intraventricular conduction delay (QRS 0.11 s). A chest radiograph revealed severe cardiomegaly (Fig. 2A). Two-dimensional echocardiography showed severe intraventricular septal and left posterior wall hypertrophy, and small left ventricular cavity (Fig. 1A) with decreased diastolic function of the left ventricle. There was no left ventricular outflow tract gradient on doppler examination. A cardiac glycogenosis was suspected but the boy died on the second day of admission in intractable heart failure, aged 47 days.

The autopsy was performed 16 h after death. The dominating feature was excessive cardiomegaly (90 g against 21.7 g in controls) with hypetrophy of all compartments (Fig. 2B) and disproportional enlargement of the right auricle. The heart muscle was notably pale and tough. The ventricular cavities were slit like, the atria were slightly dilated. The thickness of individual walls was as follows: right atrium 3 mm, right auricle 4 mm, right ventricle at the inflow tract 8 mm, right ventricle at the outflow tract 3 mm, left atrium 3 mm, left auricle 2 mm, left ventricle 10 mm.

The rest of the autopsy protocol was unremarkable apart from generalized congestion, cerebral oedema and very slight fluid accumulation in the serous cavities.

Materials and methods

Routine histology was performed on tissues fixed in 10% formaldehyde. Selected samples of myocardium, diaphragm, skeletal mus-

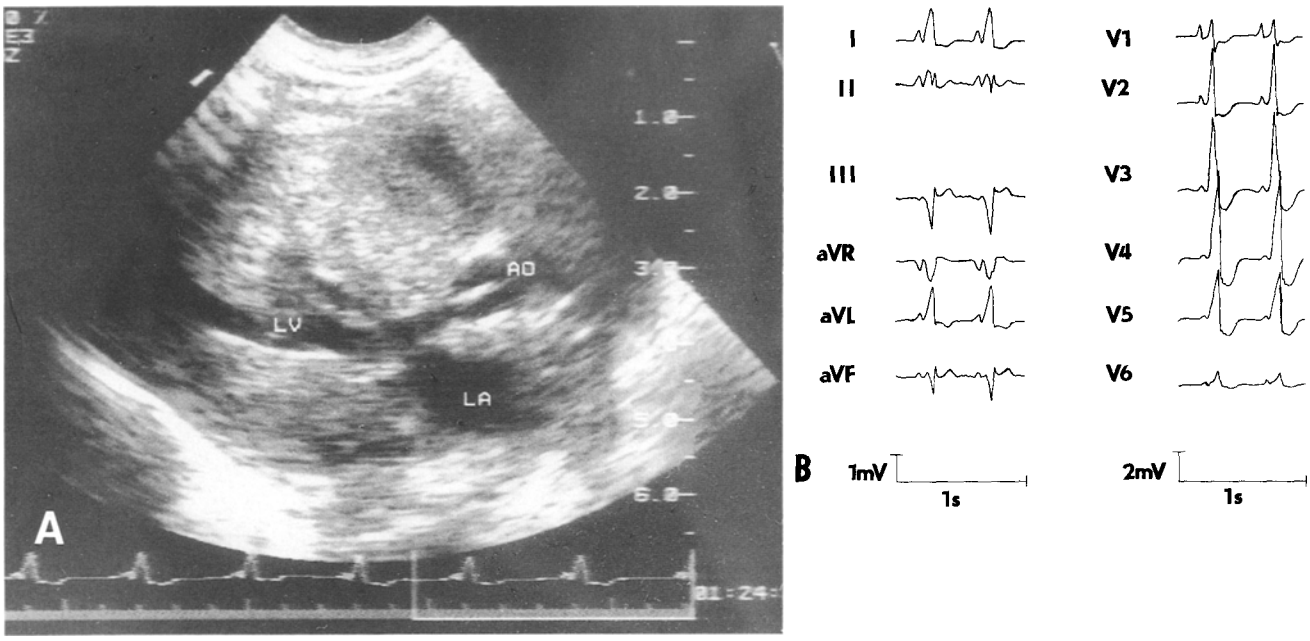

Fig. 1. **A** Two-dimmensional echocardiography. Precordial long-axis view showing severe thickening of the interventricular septum and left ventricular posterior wall and a small left ventricular cavity. **B** 12-lead electrocardiogram show abnormally short PR interval (0.06 s), right atrial enlargement, biventricular hypertrophy with subendocardial ischaemia and intraventricular conduction delay (QRS duration of 0.11 s)

cle and liver were fixed in absolute ethanol and embedded in paraffin. Sections of formaldehyde or ethanol-fixed, paraffin-embedded blocks were stained with HE, PAS (with or without diastase predigestion), and with Alcian blue. Ultrastructure was studied in samples fixed with 10% buffered paraformaldehyde, dehydrated in ethanol and embedded in Araldite-Epon mixture. Thin sections were stained with uranyl acetate and lead citrate. Histochemical enzyme activities (acid phosphatase using azocoupling method, and phosphorylase) were followed in unfixed cryostat sections from samples quenched in petrol ether cooled with acetone-dry ice mixture (for details see Elleder et al. 1984).

Glycogen content, PBK and alpha-glucosidase activities were examined in frozen samples according to published procedures (Shin 1990).

Results

The glycogen storage was expressed only in the heart muscle which was loaded with glycogen granules and transformed to give a lacy appearance in routine HE stains. Glycogen storage was reliably demonstrated using PAS stain in sections from ethanol-fixed samples (Fig. 3A, B). It was present partly in cardiomyocytes and partly extracellularly. Amylase digestion abolished the staining entirely. In formaldehyde-fixed samples the fibres were PAS negative. Activity of acid phosphatase was barely detectable. Phosphorylase activity was very low against controls, but strong in the skeletal muscle and in the liver.

Electron microscopy showed extensive lucent spaces in heart muscle fibre cytosol with moderate amount of glycogen particles which were mostly of monoparticulate type. Alpha aggregates were seen only occasionally (Fig. 3C).

Biochemical examination showed both elevated glycogen content and significantly diminished PKB activity restricted to the cardiac muscle (see Table 1).

Discussion

Myocardial PKB deficiency is now well-defined in terms of its clinical pathology and biochemistry. The clinical phenotype seems to be remarkably uniform. The life span has not exceeded 5 months. The heart weight may be increased six-fold (see Table 2). The clinical picture is that of rapidly progressing increase in heart size and heart failure. The cardiomyocyte seems to be the sole cell type affected and the excess of glycogen is deposited in the cytosol in the form of beta type granules (Eishi et al. 1985). Some minor admixture of glycogen alpha particles was observed in our case. This alpha particle participation, though of minor degree, is atypical since muscle is generally considered to harbour only the monoparticulate form, but exceptions have been described (Ghadially 1982). Minor glycogen accumulation was described in the heart mitochondrial matrix (Eishi et al. 1985), but this was regarded as a secondary phenomenon described under various situations (Ghadially 1982). The heart glycogen accumulation range is 1.87%–6.2% wet tissue weight which may represent 300% increase (Mizuta et al. 1984).

The enzyme deficiency is confined to the myocardium, other tissues examined to date (muscle, liver, kidney, spleen) displayed normal enzyme activities. Slightly decreased enzyme activity in the liver of our case (see

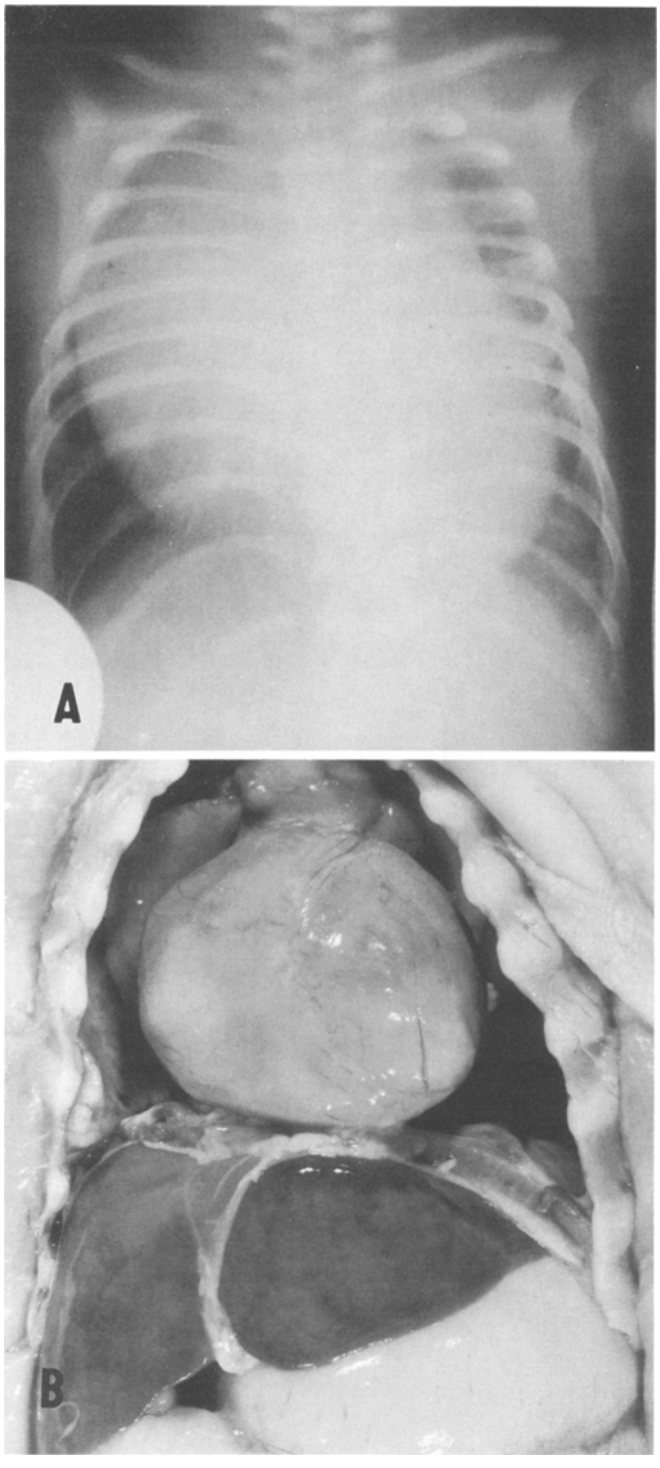

Fig. 2A, B. Severe cardiomegaly in (**A**) chest X-ray (cardiothoracic index 0.81) and (**B**) in autopsy

Table 1) may have been caused by autolysis. We were not able to analyse red blood cells. These were reported as suitable for the diagnosis of the "liver type" isoenzyme deficiency (Bashan et al. 1987) and are most probably unsuitable for diagnosis of the missing heart PKB isoenzyme activity.

Table 1. Results of biochemical examination in frozen samples

	Myocardium	Liver	Diaphragm	Kidney
Glycogen (g/100 g wet weight)				
Patient	3.1	0.89	1.5	N.D.
Controls	0.4–1.5	2.0–6.0	0.5–2.0	
PBK (μmol/min/mg protein)				
Patient	0.75–0.81	0.56	1.3	2.0
Controls	3.0–30.0	1.0–10.0	1.0–10.0	1.0–10.0
Alpha-glucosidase (nmol/min/mg protein)				
Patient	1.0	2.7	1.7	N.D.
Controls	0.5–3.0	1.0–5.0	0.5–3.0	N.D.

PBK, Phosphorylase b kinase; N.D., not done

Table 2. Comparison of basic clinico-pathological data of the so far published cases of heart restricted PKB deficiencies. (The values in parentheses represent the heart weight of controls)

	Sex	Age at death	Heart weight
Mizuta et al. (1984)	M	5 m	160 g (40 g)[a]
Servidei et al. (1988)	F	4 m	190 g (30 g)
Present case	M	1 m 18 d	90 g (21.7 g)

[a] Details of the pathology are published elsewhere (Eishi et al. 1985)

The only explanation for the phenomenon of PBK deficiency confined to a single organ must be the existence of organ specific enzyme isoforms. All the so far known isoforms of the PBK consist of four subunits (alpha, beta, gamma, delta), only some of which are catalytic; the others are regulatory in nature. Differences in composition, functional properties, and immunological differences have been described between some of them (Bender and Emerson 1987; Killilea and Ky 1983; Podskarbi et al. 1991; Taira et al. 1982; Yoshikawa et al. 1983) suggesting protein sequence differences coded by different DNA loci. All this has important practical implications as the heart tissue sample seems to be the sole source for histopathological or biochemical diagnosis of this form of glycogenosis. This practically excludes the possibility of prenatal diagnosis of the disorder at present. A further advance in enzyme isoform analysis and the molecular genetics may open new diagnostic possibilities using peripheral blood.

Three cases published during the 8 year period represent a sufficiently weighty argument for taking this new form of isolated heart PKB deficiency as an important entity in the differential diagnosis of infantile hypertrophic cardiomyopathy.

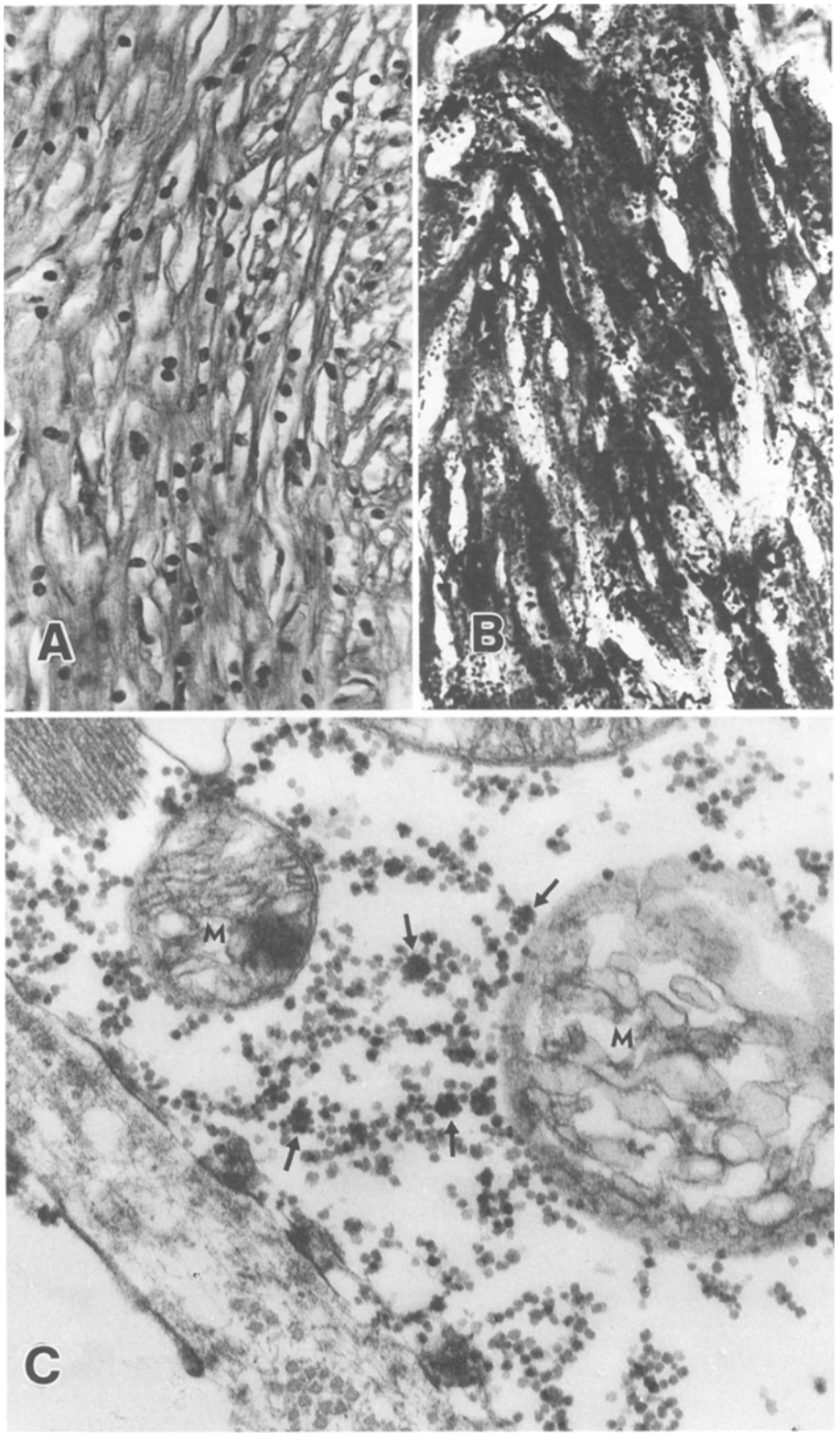

Fig. 3A–C. Histological appearance of myocardial fibres and glycogen detectability in combined PAS and HE staining. In the formaldehyde-fixed sample (**A**) there is PAS negative extensive storage compartment in the cardiocytes and glycogen is detectable only in the ethanol fixed sample (**B**), ×300. **C** Ultrastructure of the cytosol storage compartment with persisting glycogen alpha rosettes some of which are large *(arrows)*. *M* mitochondria altered by autolysis. ×52 000

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