First Trimester Prenatal Diagnosis of Metachromatic Leukodystrophy on Chorionic Villi by ‘Immunoprecipitation–electrophoresis’

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Summary: Prenatal diagnosis of metachromatic leukodystrophy (MLD) due to arylsulphatase A (ASA) deficiency can be performed by amniocentesis with the disadvantage of a late pregnancy termination.

Whether chorionic villi (CV) obtained by trophoblast biopsy during the first trimester of pregnancy can be useful for diagnosis depends on the reliability of results. The complexity of arylsulphatase expression in CV and the existence of several isozymes make diagnosis difficult. However, the use of an anti-ASA antiserum enabled us to discriminate between ASA and a co-migrating contaminant isozyme, and the antigen–antibody (Ag–Ab) complex gave better evidence of the presence or absence of ASA after enhancement of activity with 4-methylumbelliferyl sulphate (4-MUS).

We propose that immunoprecipitation followed by electrophoresis could be a valuable method of MLD prenatal diagnosis on chorionic villi.

Metachromatic leukodystrophy (MLD) [McKusick 25010] comprises a group of inherited degenerative diseases of the nervous system caused by a deficiency of arylsulphatase A (ASA, EC 3.1.6.1) that can be demonstrated by synthetic chromogenic, p-nitrocatechol sulphate (PNCS) or fluorogenic, 4-methylumbelliferyl sulphate (4-MUS) substrates (Austin et al., 1965; Rattazzi et al., 1973).

The severe clinical manifestations and the absence of an effective therapy make this group of sulphatide-lipidoses a major indication for prenatal diagnosis. Prenatal diagnosis of MLD is possible by ASA determination on cultured amniotic cells obtained by amniocentesis (Booth et al., 1972; Leroy et al., 1973; Van Der Hagen, 1973; Rattazzi and Davidson, 1977) or, in some late-presenting pregnancies, on fetal blood collected at fetoscopy (Rodeck et al., 1983). The main disadvantage of these methods is the late pregnancy termination in the case of an affected fetus.

The possibility of obtaining chorionic villi (CV) by trophoblast biopsy between 8 and 12 weeks of gestation has permitted the diagnosis of several metabolic

MS received 30.6.87  Accepted 7.10.87
disorders in the first trimester of pregnancy (Poenaru et al., 1984a; Besançon et al., 1984a; Grabowski et al., 1984; Gibbs et al., 1984).

ASA determination in normal chorionic villi shows a high activity suggesting that diagnosis of MLD should be possible in this tissue (Poenaru et al., 1984b). ASA deficiency in MLD is rarely total when assayed using PNCS in the presence of sodium chloride as inhibitor of arylsulphatase B (ASB, EC 3.1.6.1) (Schapira and Nadler, 1975): 10 to 15% of normal levels is generally found. In some cases, and because of the wide range of normal values, it becomes difficult to distinguish between a deficiency and a heterozygote state. To facilitate interpretation we usually complete the assay with electrophoresis visualized with 4-MUS which shows a total absence of ASA in some tissues in classical MLD (Rattazzi et al., 1973). In MLD fresh chorionic villi extract, the residual activity of arylsulphatase A is marked and supplementary bands of activity are revealed by electrophoresis. This makes diagnosis difficult and obliged us to search for the ideal assay conditions.

We propose immunoprecipitation followed by electrophoresis as a reliable method for prenatal diagnosis of classical MLD on chorionic villi.

MATERIAL AND METHODS

Family B

In the index case MLD was determined by ASA assay on leukocytes and the diagnosis confirmed on fibroblasts.

In this family at risk for MLD, a first prenatal diagnosis was performed on amniotic cells and a normal fetus was found. We present in this paper the second prenatal diagnosis (propositus B) made in the first trimester of pregnancy on trophoblast biopsy material obtained at ten weeks gestation.

Tissue extraction

Tissues were extracted in two ways: (1) in water containing 0.1% (w/v) Triton X-100. The homogenates, prepared to contain a constant concentration of protein, were frozen and thawed three times and immediately submitted to assay and electrophoresis for arylsulphatases A and B; (2) in sodium acetate buffer, 0.1 mol L⁻¹, pH 5, the homogenates were frozen and thawed three times and centrifuged for 30 min at 16,400 g at 4°C. The supernatants were immediately submitted to electrophoresis.

Quantitative assay

The quantitative assay of ASA with the artificial substrate p-nitrocatechol was based on the principle that ASB, also active towards the same substrate, is selectively inhibited by 1% (w/v) sodium chloride. The complete procedure has been described by Chang et al. (1981).

Arylsulphatase electrophoresis

Electrophoresis on cellulose acetate (Cellogel-Chemetron, Italy) was carried out according to Rattazzi et al. (1973) with a veronal, barbiturate, acetate buffer.