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SHORT REPORT

Prenatal detection of Canavan disease by measurement of *N*-acetyl-L-aspartate in amniotic fluid

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The identification of aspartoacylase (EC 3.5.1.15) deficiency and increased urinary concentrations of *N*-acetyl-L-aspartate (NAA) as diagnostic abnormalities in Canavan disease (McKusick 271900) first suggested the possibility of prenatal diagnosis of this usually fatal disease by measurement of increased levels of NAA in amniotic fluid. Recently, Jakobs and colleagues (1992) reported two pregnancies at risk for Canavan disease in which pathologically increased levels of NAA were identified by isotope-dilution gas chromatography mass spectrometry (SID–GC/MS) in mid-trimester amniotic fluid. We report here our own experience with measurement of NAA amniotic fluid by SID–GC/MS and our finding of two additional cases of Canavan disease in which mid-trimester NAA levels were significantly increased compared with gestational age-matched control amniotic fluid specimens.

Amniotic fluids were recovered from freezer storage for two pregnancies that resulted in the birth of children with Canavan disease, as confirmed by urinary NAA quantification and assay of aspartoacylase in cultured fibroblasts. Control amniotic fluid specimens were from pregnancies not at risk for Canavan disease obtained between 14 and 27 post-conceptual weeks. The specimens were stored at −20°C from 1 week to 12 months until analysis by SID–GC/MS as described previously (Kelley and Stamas 1991).

The levels of NAA in 60 control amniotic fluids followed a trend of increasing value with gestational age. Example means and standard deviations were $1.14 \pm 0.14\,\mu$mol/L ($n = 7$) for specimens obtained between 14 and 15 weeks gestation and $1.83 \pm 0.26\,\mu$mol/L ($n = 11$) for specimens obtained between 19 and 20 weeks gestation. The levels of NAA in amniotic fluid from the two affected pregnancies were $4.76\,\mu$mol/L and $6.47\,\mu$mol/L, which were, respectively, 14.1 and 19.9 standard deviations above the mean for NAA in gestational age-matched control fluids (16 and 17 weeks, respectively). Consistent with the increased levels of NAA in amniotic fluid, both children were found at birth to have urinary NAA levels greater than 500 mmol/mol creatinine (normal ± SD = 12.7 ± 7.8 mmol/mol creatinine). There was no apparent effect of the length of freezing on the level of NAA in amniotic fluid based on quantification of NAA in several samples before and after storage at −20°C for 6 months.

The low absolute level of NAA even in pathological amniotic fluid and the poor extraction efficiency of NAA into organic solvents preclude accurate quantification of NAA in amniotic fluid by routine solvent extraction and gas chromatography.

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The much greater sensitivity and specificity of SID–GC/MS used in this study is required. Although there are as yet insufficient data to know the full range of normal values at all gestational ages or whether other pathological conditions cause abnormal increases in the level of NAA in amniotic fluid, our studies and the similar results of Jakobs et al (1992) suggest that measurement of NAA in amniotic fluid by SID–GC/MS may prove to be a more reliable method for prenatal diagnosis of Canavan disease than measurement of aspartoacylase activity in amniocytes (Matalon et al 1992; M. Gibson, personal communication). However, caution should be exercised in the application of this method to all pregnancies at risk for Canavan disease, because some patients with aspartoacylase deficiency excrete only mildly increased amounts of NAA (R. Kelley, unpublished). The trend of increasing levels of amniotic fluid NAA with gestational age also underscores the importance of using controls closely matched for gestational age when testing pregnancies at risk for Canavan disease.

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REFERENCES

Jakobs C, ten-Brink HJ, Divry P, Rolland MO (1992) Prenatal diagnosis of Canavan disease (letter). *Eur J Pediatr* **151**: 620.

Kelley RI, Stamas JN (1991) Quantification of *N*-acetyl-L-aspartic acid in urine by isotope-dilution gas chromatography/mass spectrometry. *J Inher Metab Dis* **15**: 97–104.

Matalon R, Michals K, Gashkoff P, Kaul R (1992) Prenatal diagnosis of Canavan disease. *J Inher Metab Dis* **15**: 392–394.