

# Rapid prenatal diagnosis of the Lesch-Nyhan syndrome

D. HALLEY AND M. J. HEUKELS-DULLY

*From the Department of Cell Biology and Genetics, Erasmus University Rotterdam, Rotterdam, The Netherlands*

**SUMMARY** Autoradiographic demonstration of  $^3\text{H}$ -hypoxanthine incorporation in small numbers of amniotic fluid cells cultured on coverslips is a rapid and practical technique in the prenatal diagnosis of the Lesch-Nyhan mutation. An affected male fetus, a normal male fetus, and a heterozygous female fetus were identified within 14 days after amniocentesis in three pregnancies at risk for the Lesch-Nyhan syndrome.

The Lesch-Nyhan syndrome, an X-linked disorder of purine metabolism, can be diagnosed by autoradiographic analysis of a deficiency of hypoxanthine guanine phosphoribosyl transferase (HGPRT, E. C. 2.4.2.8.) or by the assay of HGPRT activity in cell extracts. In the prenatal diagnosis of this syndrome, autoradiography offers the advantages of short culture time for the relatively small number of cells sufficient to establish the diagnosis and the ability to show heterozygosity in clonally growing cultures. Boyle *et al.* (1970) and van Heeswijk *et al.* (1972) used autoradiographic techniques in prenatal diagnoses for the Lesch-Nyhan syndrome and found affected male fetuses in time for therapeutic abortion, and a heterozygous female fetus was identified by Fujimoto *et al.* (1968).

This report presents the results of 3 prenatal diagnoses in pregnancies at risk for the Lesch-Nyhan syndrome.

Transabdominal amniocenteses were performed in the 18th, 17th, and 16th week of pregnancy in Paris, Giessen, and Rotterdam, respectively. In the first case subcultured amniotic fluid cells were received from Paris. Amniotic fluid from the second case was sent from Giessen to Rotterdam by air.

## Methods

Amniotic fluid cells were cultured on coverslips as described earlier (Niermeijer *et al.*, 1973). Two days before incubation with  $^3\text{H}$ -hypoxanthine the cells were transferred to hypoxanthine-free F10 medium (Flow). Several clones were present after 7 days in culture in the 2 cases cultured in Rotterdam. Cells

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were labelled with 15  $\mu\text{Ci}$   $^3\text{H}$ -hypoxanthine/ml medium (specific activity 1.0 mCi/mmol, Radio-chemical Centre, Amersham) for 6 hours, washed twice with saline, and fixed with Bouin's fixative. Autoradiographs were made with Kodak AR-10 stripping film and were exposed for 2 days. The preparations were stained with haematoxylin and eosin. Chromosome analysis (Q banding) was performed after 14 days in culture. Control amniotic fluid cells and fibroblasts from the previous affected child were labelled simultaneously in each case.

## Case 1

Chromosome analysis performed in Paris revealed a 46,XY karyotype. The results of the autoradiographic analysis were available one week after the cultured amniotic fluid cells were received. Definite incorporation of  $^3\text{H}$ -hypoxanthine was seen (Fig. a), the level of which was comparable to that of control amniotic fluid cells and significantly greater than the labelling resulting from residual HGPRT activity in fibroblasts from the affected child in this family. The conclusion was that this fetus was not affected with the Lesch-Nyhan syndrome.

A boy was born in 1975 and clinical examination has been completely reassuring, since the child has a completely normal development.

## Case 2

Before prenatal diagnosis was requested in the second family, cultured fibroblasts from the son who showed clinical symptoms of the Lesch-Nyhan syndrome were examined for ability to incorporate  $^3\text{H}$ -hypoxanthine.

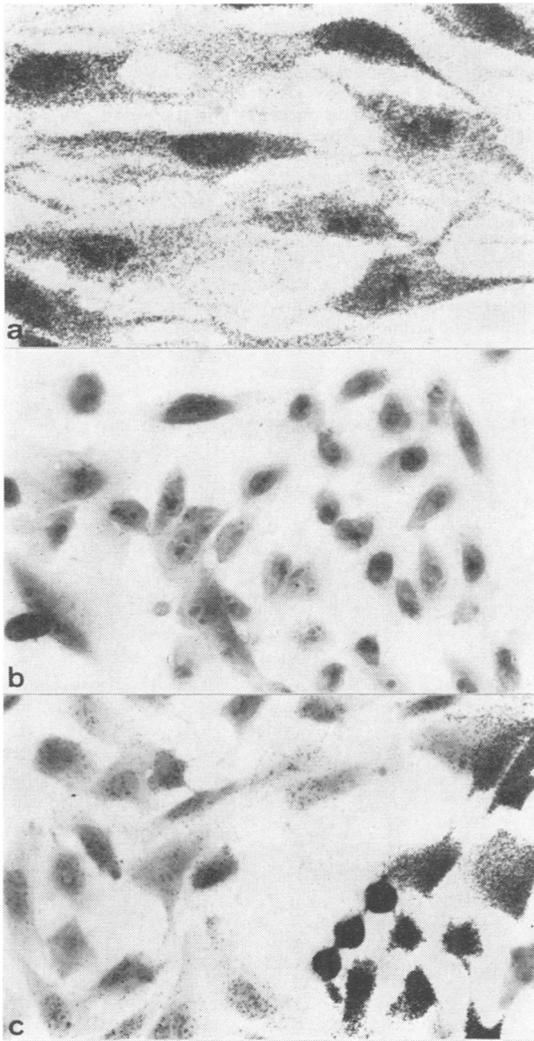


Fig. (a) Cultured amniotic fluid cells, Case 1, normal fetus, showing uptake of  $^3\text{H}$ -hypoxanthine; (b) Case 2, Lesch-Nyhan fetus; (c) Case 3, heterozygote for Lesch-Nyhan syndrome.

These fibroblasts and fibroblasts from a known Lesch-Nyhan patient had equally low levels of labelling, clearly distinguished from control fibroblasts.

Results of the prenatal diagnosis were obtained 14 days after amniocentesis. A 46,XY karyotype and an almost complete absence of label in the cultured amniotic fluid cells were found (Fig. b), comparable to that seen in fibroblasts from the affected child. It was concluded that this fetus was affected with the Lesch-Nyhan syndrome, and confirmation of the

diagnosis was provided by autoradiography on fibroblasts grown from fetal skin obtained at abortion.

### Case 3

Two cell populations in fibroblasts from the mother in this family had earlier been shown, and fibroblasts from the previous affected child were available in the cell bank, Erasmus University, Rotterdam, and had proved to be clearly distinguishable from control cells by autoradiography. Results of the prenatal diagnosis were again available 14 days after amniocentesis. Of 10 clones of amniotic fluid cells, 3 were clearly labelled and 7 were not (Fig. c) and the karyotype was 46,XX, indicating that this female fetus was heterozygous for the Lesch-Nyhan syndrome. The baby girl was found to be healthy.

### Conclusions

An unaffected male fetus, an affected male fetus, and a heterozygous female fetus were identified in three pregnancies at risk for the Lesch-Nyhan syndrome. All three diagnoses were completed within 2 weeks after amniocentesis or receipt of the cultured cells in our laboratory. The availability of cultured fibroblasts from the affected children in these families was essential in evaluating residual activity of HGPRT since a high residual activity in a patient could theoretically be difficult to distinguish from a control by autoradiography.

HGPRT activity can be assayed by the ratio of  $^{14}\text{C}$ -hypoxanthine:  $^3\text{H}$ -adenine uptake in amniotic fluid cell colonies of 2000 to 8000 cells by means of differential scintillation spectrometry (Richardson and Cox, 1973) and by  $^{14}\text{C}$ -hypoxanthine uptake in fibroblast extracts measured by thin-layer chromatography (Willers *et al.*, 1975). These methods require the availability of equipment for liquid scintillation counting or thin-layer chromatography and the diagnosis of heterozygosity is not possible.

Biochemical assay of HGPRT is indicated if quantitative studies of HGPRT levels are needed, but the qualitative result obtained by autoradiography is generally sufficient for prenatal diagnosis. The presence of a mosaic pattern in the heterozygote can also be shown by autoradiography. The reliability and rapidity of this method make prenatal diagnosis more acceptable to women heterozygous for this severe X-linked disease, since the result may be available in 2 weeks after amniocentesis in the 16th week of pregnancy.

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#### References

- Boyle, J. A., Raivio, K. O., Astrin, K. H., Schulman, J. D., Graf, M. L., Seegmiller, J. E., and Jacobsen, C. B. (1970). Lesch-Nyhan syndrome: preventive control by prenatal diagnosis. *Science*, **169**, 688-689.
- Fujimoto, W. Y., Seegmiller, J. E., Uhlendorf, B. W., and Jacobson, C. B. (1968). Biochemical diagnosis of an X-linked disease in utero. *Lancet*, **2**, 511-512.
- Niermeijer, M. F., Halley, D., Sachs, E. S., Tichelaar-Klepper, C., and Garver K. L. (1973) Transport and storage of amniotic fluid samples for prenatal diagnosis of metabolic diseases. *Humangenetik*, **20**, 175-178.
- Richardson, B. I. and Cox, D. M. (1973). Rapid tissue culture and microbiological methods for analyzing colonially grown fibroblasts from normal, Lesch-Nyhan and Tay-Sachs patients and amniotic fluid cells. *Clinical Genetics*, **4**, 376-380.
- van Heeswijk, P. J., Blank, C. H., Seegmiller, J. E., and Jacobsen, C. B. (1972). Preventive control of the Lesch-Nyhan syndrome. *Obstetrics and Gynecology*, **40**, 109-113.
- Willers, I., Agarwal, D. P., Singh, S., Schloot, W., and Goedde, H. W. (1975). Rapid determination of hypoxanthine-guanine-phosphoribosyl transferase in human fibroblasts and amniotic cells. *Humangenetik*, **27**, 323-328.