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# An immunohistochemical study of human fetal liver in the Meckel–Gruber syndrome

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Summary

*Aims:* The ductal plate abnormality of the liver in fetuses with the Meckel–Gruber syndrome has been well characterised, but its aetiology remains unknown. We have analysed liver structure in six fetuses with this syndrome, using routine histology, immunocytochemistry, and electron microscopy.

*Methods:* Liver tissue from six fetuses of 11–27 weeks gestational age was examined by immunoperoxidase staining with antigens to cyokeratin (AE1/3) and polyclonal CEA. We also examined the ultrastructure of the syndromic fetal liver. The findings were compared with livers of control fetuses obtained from miscarriages, of similar size and gestational age but without dysmorphic features or developmental anomalies.

*Results:* The ductal plate abnormality was present in all the fetuses with the Meckel–Gruber syndrome. There were abnormalities of biliary excretion in all syndromic fetuses. Ultrastructural studies of the portal tract revealed abnormal collagen bundles in the Meckel–Gruber syndrome.

*Conclusions:* Our findings, in conjunction with other reports in the literature, suggest that the ductal plate abnormality may be caused by failure of anastomosis of the intra- and extrahepatic biliary systems, perhaps in association with abnormalities of the portal tract stroma and biliary excretion.

*Key words:* Meckel–Gruber syndrome, human fetus, liver, bile, cyst.

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## INTRODUCTION

The Meckel–Gruber syndrome is an uncommon autosomal recessive condition with a variable phenotype. The syndrome generally includes hepatic lesions, polydactyly, polycystic kidneys, and a central nervous system defect (most frequently occipital encephalocoele). Blankenberg *et al.*[1] studied a number of cases and argued that liver lesions are present consistently. The liver lesion has attracted much interest, especially with regard to its pathogenesis, which remains unknown despite much study. Hepatic cysts (mostly microscopic) are considered to occur as commonly as polycystic kidneys in this syndrome. Most investigations have concentrated on the hepatic ductal plate anomaly in the latter part of the second trimester or later. We have conducted an immunohistochemical study on the liver in six fetuses with this syndrome from 11 to 27 weeks gestation, to study in detail the development of the ductal plate and biliary excretion.

## METHODS

Pathological examination of six fetuses with Meckel–Gruber syndrome was conducted in the Department of Anatomical Pathology, South Western Area Pathology Service, Sydney, Australia, by one of the authors (CL) as part of the routine surgical pathology and autopsy service of the department. Macroscopic examination of fetuses and histology of fetal organs were performed according to standard protocols. Signed permission for the autopsy was obtained for Cases 5 and 6 and Control 6. The diagnosis of Meckel–Gruber syndrome was made at ultrasound examination and confirmed at autopsy. Chromosomal analysis on fetal tissue from Cases 3, 4, 5 and 6 showed a normal karyotype. Two fetuses were from the same woman (Cases 1 and 2), whose obstetric history included another fetus terminated for Meckel–Gruber syndrome and one normal child. Another pair of fetuses came from close relatives (Cases 4 and 5). Six control fetuses were chosen for similar gestational age and size and absence of dysmorphic features and developmental anomalies. These were studied over the same period as the Meckel–Gruber fetuses (1999–2003). Clinical and pathological findings from case and control fetuses are listed in Tables 1 and 2. Similar findings were obtained from another seven non-dysmorphic fetuses examined at this time with histology and various immunoperoxidase stains.

### *Immunohistochemistry*

At least one section was obtained from the liver, usually a sagittal section near the centre to include the gallbladder. Some sections also included the porta hepatis. The specimens were fixed in 10% formalin and processed to paraffin wax blocks for the preparation of routine H&E sections. One representative section of the liver from each fetus was immunostained with the following antigens: AE1/3 (1:100), polyclonal carcinoembryonic antigens (CEA; diluted 1:400). Both of the antigens were obtained from Dako (Denmark). The standard avidin–biotin immunoperoxidase technique was used.

Tissue sections were cut at 3 µm from the selected cases, together with positive controls. The sections were deparaffinised in xylol and hydrated through graded alcohols. Heat retrieval of antigen or enzyme digestion was carried out before immunostaining. Immunohistochemical staining was performed using the Dako immunoautostainer. The sections were placed in 3% hydrogen peroxide for 5 min to quench endogenous peroxidase. All slides were sequentially incubated with primary antibody, biotinylated secondary antibody (Dako) and peroxidase-labelled using streptavidin reagent (Dako). All incubations were performed at room temperature. Diaminobenzidine (Dako) was used as a colour chromogen and slides were counterstained with Harris' haematoxylin. In addition to positive controls, negative controls were treated identically with the exception that the slides were incubated with negative control reagent (Dako) instead of the primary antibody. In most cases, intestines were present in the slides containing liver tissue, providing a positive control for keratin stains. The case and control sections were stained in the same runs for each antigen. A representative section of the small intestine was stained with Perls and Fouchet van Gieson stains to test for bile.

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TABLE 1 Clinical findings in Meckel–Gruber and control fetuses

| Case* | Mat. age† | Gest. age‡ | Clinical and autopsy findings |
|---|---|---|---|
| 1 | 38 | 11 | D&E for Meckel–Gruber syndrome (encephalocoele); no polydactyly of hands and left foot (no right foot recovered) at pathological examination; kidneys not found. |
| 2 | 36 | 15 | TOP for Meckel–Gruber syndrome; female fetus; encephalocele; clinodactyly; renal cystic dysplasia. |
| 3 | 25 | 19 | TOP for gross fetal anomalies; no liquor; male fetus; meningocoele; contractures; bilateral cystic kidneys; heart defects (DORV, mitral and pulmonary atresia, VSD, patent left SVC); pulmonary hypoplasia; Dandy Walker malformation. |
| 4 | 34 | 19 | TOP for fetal anomalies; female fetus; postaxial polydactyly; posterior encephalocoele; bilateral cystic renal dysplasia; pulmonary hypoplasia. |
| 5 | 28 | (20) | TOP for fetal anomalies; IVF baby; postaxial polydactyly in feet and hands; syndactyly of fifth and sixth fingers and toes; posterior encephalocele; malformed brain with cerebellar hypoplasia; central cleft palate; bilateral polycystic kidneys; bicornuate uterus; small adrenal glands; enlarged thymus; pulmonary hypoplasia; fusion of tip of pancreas and spleen; mildly dilated ducts in pancreas; focal duplication of central canal of spinal cord. |
| 6 | 16 | 27 | TOP for fetal anomalies on ultrasound scan; male fetus; posterior encephalocoele; postaxial polydactyly; persistent left SVC; cardiac hypertrophy; accessory spleens; diffuse cystic renal dysplasia. |
| Control | | | |
| 1 | 32 | (11) | Inevitable miscarriage; female fetus with no developmental anomaly. |
| 2 | 25 | (11) | Threatened miscarriage. |
| 3 | 23 | (15) | Acute chorio-amnionitis; female fetus with no developmental anomaly. |
| 4 | 32 | 18 | 6-week history of PV bleed; placental haemorrhage; female fetus with no developmental anomaly. |
| 5 | 22 | (20) | Miscarriage; mild chorio-amnionitis; male fetus with no developmental anomaly. |
| 6 | 15 | 25 | Concealed pregnancy; live born female infant with no dysmorphic features; treatment withdrawn at 5 days of age; grade IV intraventricular haemorrhage; bronchopulmonary dysplasia and hyaline membrane disease; patent ductus arteriosus. |

*Cases 1 and 2 were from the same woman. Each parent from Case 4 was a sibling of one of the parents from Case 5.
†Maternal age (in years).
‡Gestational age in completed weeks, as determined by ultrasound or clinically (except for the figures in brackets, which were estimated from fetal measurements at pathological examination).
D&E, dilation and evacuation; TOP, termination of pregnancy; DORV, double-outlet right ventricle; VSD, ventricular septal defect; SVC, superior vena cava; IVF, *in vitro* fertilisation; PV, per vaginum.

TABLE 2 Autopsy measurements from Meckel–Gruber and control fetuses

| Case | Crown–rump length (mm) | Foot length (mm) | Fetal weight (g) | Liver weight (g) | Cut surface |
|---|---|---|---|---|---|
| 1 | 50* | 7 | Unknown (D&E) | Unknown | Fragmented |
| 2 | 110 | 20 | 78.5 | 3.3 | Normal |
| 3 | 185 | 28 | 367 | 17.2 | Normal |
| 4 | 140 | 32 | 241 | 19.3 | Polycystic |
| 5 | 140 | 32 | 274 | 15.0 | Normal |
| 6 | 265 | 48 | 1248 | 26.0 | Micronodular |
| Control | | | | | |
| 1 | 53 | 6 | 9.5 | 0.5 | Normal |
| 2 | 50 | 7 | 8.5 | 0.6 | Normal |
| 3 | 110 | 18 | 61 | 3.8 | Normal |
| 4 | 145 | 29 | 185 | 10.0 | Normal |
| 5 | 182 | 33 | 286 | 15.4 | Normal |
| 6 | 220 | 48 | 537 | 24.1 | Normal |

*Estimated by ultrasound scan before D&E.
D&E, dilation and evacuation.

*Electron microscopy*

Liver tissue from one fetus (Case 5) was obtained for ultrastructural studies. One portal tract with ductal plate malformation and a few central vein branches were present in the section. Liver tissue from a control fetus of 18 weeks gestational age (crown–rump length 150 mm, crown–heel length 218 mm, foot length 28 mm, 196 g) was obtained for comparison. The tissue was block-stained with osmium tetroxide and uranyl acetate. Ultrathin sections were stained with uranyl acetate and lead citrate. The sections were viewed with a Zeiss EM109T transmission electron microscope (Zeiss, Germany).

## RESULTS

The fetal crown–rump length, fetal weight, foot length, liver weight, and appearance of the cut surface of the liver are listed in Table 2. The macroscopic liver changes in the Meckel–Gruber syndrome resemble those of classical congenital hepatic fibrosis. The liver was usually enlarged, smooth-surfaced, and firm. Cysts were not visible macroscopically except in Case 4.

*Light microscopy: routine H&E sections*

Microscopy findings from the sections containing the porta hepatis were similar to those from sections containing the gallbladder. Portal tracts of varying sizes were found in all fetuses. In the smaller, peripheral portal tracts, the ductal plates in control fetuses were single layered with interspersed small tubules, usually seen in cross-section (Fig. 1, 2). Occasionally, plates and tubules appeared in longitudinal section around the portal tracts. In 13–20-week control fetuses, in the larger portal tracts where bile ducts were seen within fibrous tissue, the liver plate immediately adjacent to the portal tract usually did not exhibit typical