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The neuropathology of stillbirth — Correlation with apolipoprotein genotype in a Scottish population based study

Jeanne Elisabeth Bell [a,*,1], Julie-Clare Becher [b], Jean Winifred Keeling [c], Neil McIntosh [b]

[a] Neuropathology, University of Edinburgh Division of Pathology, Western General Hospital, Crewe Road, Edinburgh EH4 2XU, UK
[b] Child Life and Health, University of Edinburgh, 20 Sylvan Place, Edinburgh EH9 1UW, UK
[c] Paediatric Pathology, Royal Hospital for Sick Children, 9 Sciennes Rd, Edinburgh, Midlothian EH9 1LF, UK

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ABSTRACT

Background: The neuropathology of stillbirths has been widely studied but rarely on a population basis. Whether foetal apolipoprotein E (APOE) genotype exerts any influence has been little investigated, despite well known effects in adult brains.
Aims: To establish the neuropathology of a population cohort of stillbirths and compare with the APOE genotype.
Study design and subjects: The brains of 191 stillbirths (≥24 weeks of gestation) were recruited from a Scottish population cohort and grouped by clinical history. APOE genotype was available for 97%.
Results and conclusions: One or more neuropathological features, most appearing relatively recent, were found in 54% of 157 antepartum singletons, 44% of 9 abruption-associated stillbirths, 85% of 13 in multiple pregnancies but in only 19% of 12 intrapartum stillbirths. White matter injury (WMI) occurred in 36% of preterm and 21% mature stillbirths. Fresh petechial haemorrhages were common in all groups (29%) but germinal matrix haemorrhage (GMH) (7%) and periventricular leucomalacia (1%) were confined to preterm. GMH was significantly associated with WMI ($p = 0.003$). Placental inflammation was common in intrapartum stillbirths (50%), compared with antepartum (15%), multiple pregnancy (23%) and abruption (0%). β-Amyloid precursor protein (βAPP) positive axons (36% stillbirths overall) correlated closely with WMI ($p < 0.0001$), justifying future routine inclusion in foetal neuropathological investigation.
This study highlights the paucity of brain damage in intrapartum stillbirths. While APOE2 was significantly overrepresented in stillbirths, there was no correlation between APOE genotype and neuropathological findings. We conclude that APOE does not influence neuropathological outcomes in stillbirths.

1. Introduction

The neuropathology of perinatal death has been described both in classic and recent studies [1–10], but most of these do not clearly separate live born from stillborn infants and relatively few are devoted solely to stillbirths [9,11–17]. However, there is sufficient evidence in the literature to show that most if not all of the lesions seen in neonates may also be found in the brains of stillborn foetuses [3,7,9,12]. In neonatal deaths it may be difficult to rule out the contribution of postpartum events to the pathogenesis of brain lesions. However there can be no dubiety regarding the antepartum origin of lesions seen in the brains of stillbirths.

Stillbirth is numerically a major cause of loss of life even in the developed world [18,19]. The cause of foetal death may be identified during obstetric care of the mother, or at postmortem examination of the foetus or placenta, or may remain obscure, provoking parallels with the sudden infant death syndrome [10,20,21]. The overall prevalence of brain damage in stillbirths is hard to establish in autopsy cohorts that are confined to specialist referral centres. Population based studies of stillbirth have been described recently [19,22,23] but these have rarely included pathological examination. As part of a two year Scottish Perinatal Neuropathology study that aimed to recruit all perinatal deaths occurring in Scotland, we examined the brains of 191 stillborn foetuses delivered between 24 and 40+ weeks of gestation in 22 obstetric units around the country [15]. The neuropathology findings in the corresponding 59 early neonatal deaths have been described previously [24,25]. To date, a pathological examination of the stillbirths in this study has been limited to an outline summary of recent, established or haemorrhagic brain injury in relation to the detailed epidemiology [15]. The present paper provides detailed data on 17 different parameters of brain pathology in stillbirths.

* Corresponding author at: Neuropathology, University of Edinburgh, Division of Pathology, Western General Hospital, Crewe Road, Edinburgh EH4 2XU, UK. Tel.: +44 131 552 1545.
E-mail addresses: Jeanne.Bell@ed.ac.uk (J.E. Bell), Julie-Clare.Becher@luht.scot.nhs.uk (J.-C. Becher), JeanWKeeling@aol.com (J.W. Keeling), neil.mcintosh@ed.ac.uk (N. McIntosh).
[1] Present address: 29 Starbank Road, Edinburgh EH5 3BY, UK.

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The important role of the placenta in relation to foetal brain injury has been stressed in a number of studies of stillbirths [8,10,13,16] and examination of the placenta is included in the present study. In addition, genetic and environmental factors are increasingly recognised as likely contributors to the risk of stillbirth and of foetal brain injury [26]. Apolipoprotein E (APOE) is one gene of possible interest since the different APOE alleles (APOE 2, APOE 3 and APOE 4) are known to affect the outcome in a variety of adult brain disorders including Alzheimer's disease [27], amyloid angiopathy [28] and head injury [29]. We have shown previously that the distribution of APOE alleles in infants who die in the perinatal period differs from that found both in healthy live born and in adults, with significant over-representation of APOE 2 amongst stillbirths [30]. However the relationship, if any, between APOE genotype and human foetal neuropathology has not been explored previously to our knowledge.

Here we present a comprehensive review of the different forms of brain damage in the brains of these stillbirths, including a search for expression of beta amyloid precursor protein (βAPP) as a possible marker of tissue damage, and for any deposits of beta amyloid (βA4), derived from βAPP and characteristically present in a number of neurodegenerative conditions. We also investigated whether the cellular expression of ApoE (expressed from the corresponding APOE alleles), correlates with areas of brain injury. Nothing is known of the expression of ApoE in developing human brain tissues and whether this might be influenced by the APOE genotype or by the onset of prenatal hypoxic challenge.

2. Materials and methods

The brains of 191 stillborn foetuses were available from the Scottish Perinatal Neuropathology Study, as described previously [15,24,25]. This Scottish population based study aimed to examine the brains of all foetuses of 24 weeks of gestation and more who had died before birth (as well as those dying in the first week of life), during a two year study period in Scotland. The total number of stillbirths in this period was 524. Stillborn foetuses with neural defects, cardiac malformations and chromosomal abnormalities ($n = 21$) were excluded from this study. Also excluded were foetuses that were too macerated for detailed examination i.e. those with liquefying organs ($n = 118$) or that were not consented for autopsy or research examination ($n = 162$) [24,25]. There were 32 failures of notification to the study. Clinical data, including the results of investigations relating to pregnancy, labour and delivery, was obtained from detailed questionnaires completed in each centre by specially trained midwives. The local research ethics committee in each participating centre provided approval for the study.

Up to 20 paraffin blocks were prepared from each brain according to a standard protocol, including representative samples from the cerebrum and hippocampus, basal ganglia and thalami, midbrain, pons, medulla, vermis and cerebellar hemispheres. Sections were stained with haematoxylin and eosin (HE), Luxol fast blue (LFB) and antibodies to glial fibrillary acidic protein (GFAP: Dako, diluted 1:1000, with trypsin pretreatment at 37 °C for 20 min) and CD68 (a macrophage marker: Dako, diluted 1:200, with microwave antigen retrieval). Perls method for haemosiderin was used in cases that showed evidence of significant haemorrhage. Routinely stained sections (HE, LFB and Perls) were assessed independently by two observers.

The 191 stillborn foetuses were divided into 8 groups (Table 1) using the following parameters. The singleton stillborn foetuses who died before the onset of labour (*antepartum stillbirths*, Groups 1–4) were grouped according to gestational age (mature, 37 weeks and greater, and preterm, 24–36 weeks), and by the presence or absence of growth restriction, defined as <3rd centile for gestational age at birth, corrected for gender but not for ethnicity. Only 4 foetuses in toto were postterm stillbirths at ≥ 42 weeks of gestation. Foetuses dying during labour (*intrapartum stillbirths*, Groups 5 and 6), were similarly divided into mature and preterm stillbirths. Intrapartum stillbirths were defined as those which were known to have been alive at the onset of labour, as documented by auscultation or observation of a beating foetal heart in labour. Further small groups of stillborn foetuses were assessed separately because they were associated with *placental abruption* (Group 7) or with *multiple* pregnancies (Group 8).

Based on the routine diagnostic neuropathology assessment of all the stillbirths, a representative subset of cases to include brains with and without evidence of brain damage was selected from each of Groups 1–4 and from Groups 7 and 8, (Table 2). Immunostaining was undertaken in these subsets for βAPP (Chemicon monoclonal, clone 22C11, diluted 1:100, with microwave pretreatment), for β amyloid (Dako monoclonal, diluted 1:100, with microwave pretreatment) and for ApoE (Chemicon monoclonal, diluted 1: 6000, with formic acid and microwave pretreatment). Stained slides were coded for subsequent blinded examination. The localisation and patterns of βAPP, β-amyloid and ApoE immunopositivity were recorded and after decoding were correlated with gestation, foetal growth and clinical history, as well as evidence of neuronal and white matter status assessed on HE, GFAP and CD68 staining throughout the brain.

Information on placental growth and infection/inflammation status was available for 183 stillborn babies in the study (96%). If chorioamnionitis was present, it was graded + to +++. The APOE genotypes for 186 (97%) of this cohort were available from a previous study [30].

Statistical analyses were performed using the χ^2 test (Fisher's exact test where sample size was <20). A value of $p < 0.05$ was considered significant.

3. Results

The brains of 191 stillborn babies were examined where consent had been given for research neuropathological examination and in whom no other cause for exclusion was present. These infants represented 36% of the total number of stillbirths in the Scottish study, and were unselected save for the criteria defined in Materials and methods section. 157 singleton babies had died before the onset of labour (*antepartum stillbirths*). Of these, 58 were mature (47 normally grown, Group 1, and 11 growth restricted, Group 2); 99 were preterm (54 normally grown, Group 3, and 45 growth restricted, Group 4). There were 12 *intrapartum stillbirths*; 8 of these were mature (Group 5) and 4 were preterm (Group 6). Placental abruption led to 9 stillbirths (Group 7) and there were 13 stillbirths occurring in multiple pregnancies (Group 8). Of the four post term stillbirths, three were in Group 1 and one was in Group 5 and none showed significant brain pathology.

Brain histology was sufficiently well preserved to exclude the possible confounding effects of maceration in that neuronal changes proved to be selective, with eosinphilic or karyorrhectic neurons juxtaposed with neurons or grey matter areas of normal appearance. Table 1 shows the distribution of neuropathological features in the different groups, together with evidence of placental status. Overall, a significant difference was found in the pattern and prevalence of brain damage between babies dying antepartum (Groups 1–4) and those dying intrapartum (Groups 5 & 6) ($p = 0.02$ for white matter injury (WMI) and $p = 0.04$ for neuronal injury). Histological evidence of brain damage was almost entirely confined to the former groups, with the latter displaying only focal neuronal eosinophilia in three cases of twelve, scattered petechial haemorrhages in four, and gliosis within the grey matter of the olive (Fig. 1a) in one. Conversely, placental inflammation was present more frequently in the intrapartum stillbirths and was more commonly severe in this group ($p = 0.03$).

Examining these brains in detail, neuronal eosinophilia (Fig. 1b) signifying recent hypoxic injury, and neuronal karyorrhexes (Fig. 1c) indicating more established neuronal injury, were found to a variable degree in different brains, in the cortex, deep grey matter (basal ganglia, thalami), brain stem and cerebellum. Evidence of recent hypoxia was