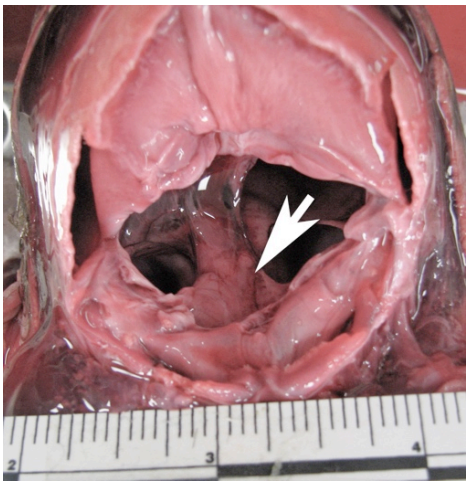
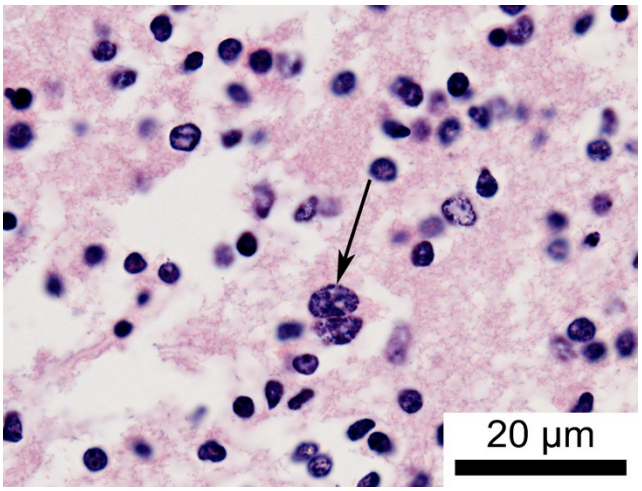
Supplement 2 (Frosk et al. 2016) – Detailed Pathology

Infant 303 (~30 weeks gestation estimated by dates)

Severely macerated stillborn male infant weighing 920 gm (30 ± 3 weeks) with a crown-heel length of 35 cm, a crown-rump length of 26 cm (30 ± 3 weeks), a foot length of 5.5 cm (29 ± 2 weeks), and head circumference of 24 cm (expected for weight 27 ± 1 cm). Limb anomalies included b hands, bilateral talipes equinovarus, flexion contracture of the left knee, fused right and left 4th and 5th toes and left 2nd and 3rd toes, shortened right 5th finger with fusion of the 4th and 5th finger metacarpals and only two ossified phalanges, and single transverse palmar creases. Facial dysmorphism is described in the main text. The placenta was slightly small (123g) and there was acute chorioamnionitis. There was prominent autolysis of most organs. Cardiac abnormalities included a small aortic valve and dilated left ventricle with widespread left ventricular wall fibrosis and dystrophic calcification. The kidneys were aplastic with hypoplastic bladder and oligohydramnios (Potter's) sequence including hypoplastic lungs. The intracranial contents were largely liquefied (Supplemental Figure 2.1), consisting of leptomeningeal membranes and fragments of soft tissue. Only in retrospect were large neurons with possible multiple nuclei recognized (Supplemental Figure 2.2).





Supplemental Figure 2.1 (left): Photograph showing posterior view of head with occipital bone removed. The head was appropriate size but largely filled with fluid. Only small fragments of brain were evident (arrow).

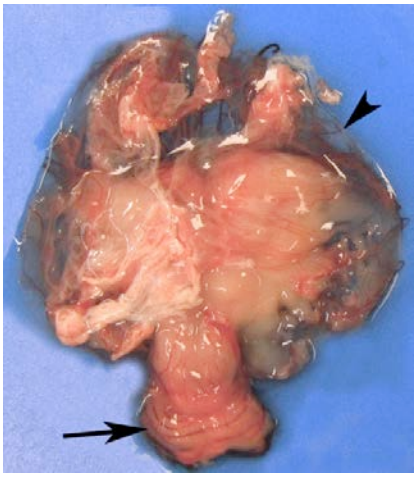
Supplemental Figure 2.2 (right): Photomicrograph showing highly fragmented brain tissue and a large neuron with closely apposed nuclei, suggestive of a binucleate cell (arrow) (hematoxylin and eosin stain; original magnification 1000x)

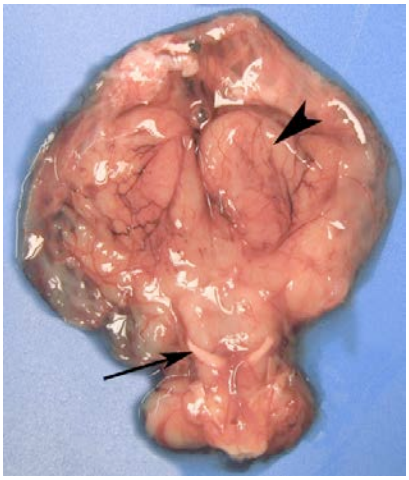
Infant 305 (~35 weeks gestation estimated by dates)

Male infant liveborn but was not resuscitated after birth. Autopsy was restricted to external examination with the exception of the brain. Weight was 1.559 kg, crown-rump length was 26 cm (33 ± 3 weeks), crown-heel length was 39 cm (33 ± 3 weeks), foot length was 6 cm (31 ± 2 weeks), and head circumference was 29 cm (expected for weight 30 ± 1 cm). Face anomalies consisted of micrognathia, low set ears, small palpebral fissures, bulbous pinched nose, and short thick neck. Limb anomalies included flexion contractures of the right and left elbows, contracture at the left knee, right talipes equinovarus, left talipes equinovalgus, broad wrinkled hands, single transverse crease of the left hand, right and left 5th finger shortening and clinodactyly, and fusion of right and left 2nd to 5th toes. Ultrasound examination showed no kidneys and postmortem examination of the head was performed (see main text).

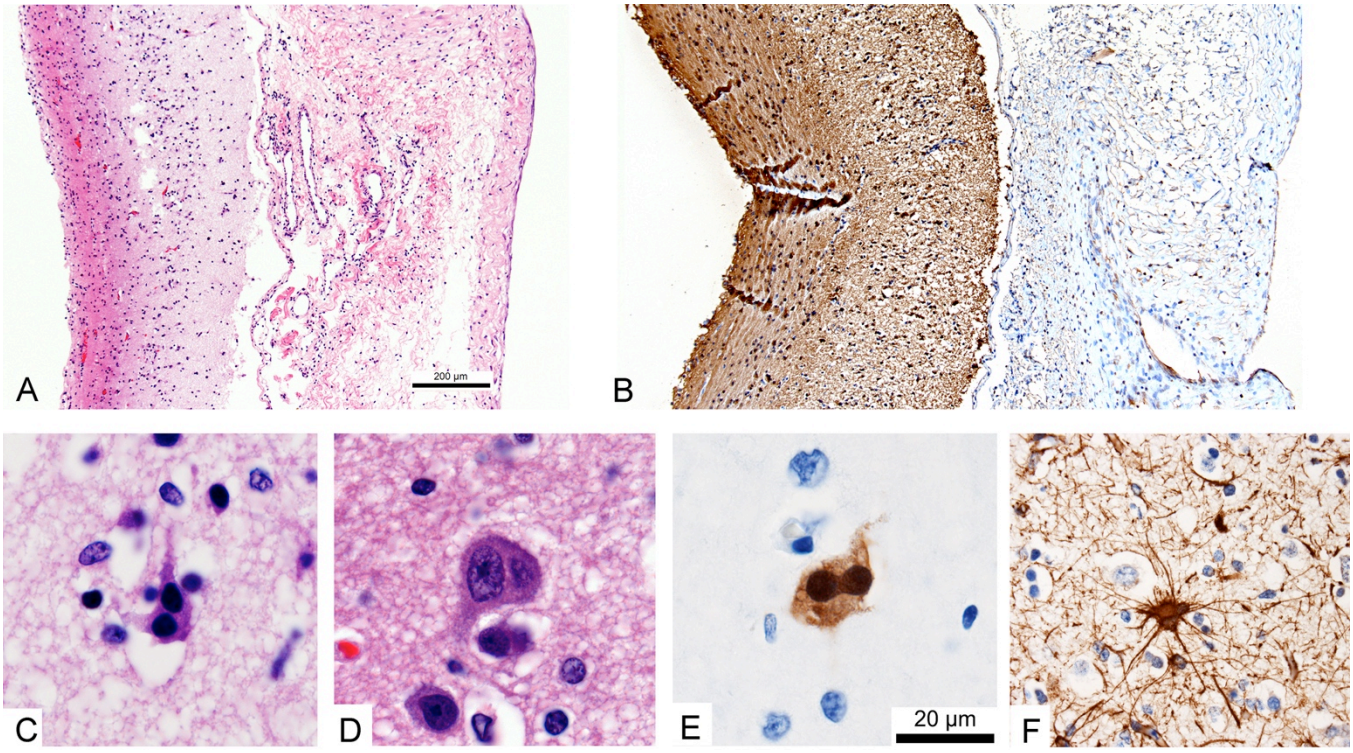
The occipital bone was excised to remove the brain. The dura was somewhat thickened with diffuse adhesion of the arachnoid. The cerebrum was reduced to a thin transparent membrane and the spaces were filled with slightly yellowish transparent fluid. Major cerebral arteries were identifiable within the leptomeninges There was partial preservation of the anterior tips of the both temporal lobes, basal nuclei, and thalami. The brainstem, cerebellum, and upper spinal cord were relatively preserved. With the exception of the olfactory nerves and tracts, which were not identified, the cranial nerves including the optic chiasm appear normal (Supplemental Figures 2.3 and 2.4).

Microscopic examination showed arachnoid adherent to foci of extremely atrophic brain tissue. There was no hemosiderin or inflammation. In the small plaques of cortical tissue, immunohistochemical stains for GFAP and S100 showed small clusters of glial tissue and NeuN showed a small quantity of neurons. HLA-DR immunostain showed an extensive network of reactive microglia. The residual temporal lobes were severely disorganized with many binucleate neurons and fewer multinucleated glial cells (Supplemental Figure 2.5). The thalamus and striatum had relative preservation of the normal anatomical organization, but there were many multinucleate cells. The midbrain had small substantia nigra and a tiny, non-patent cerebral aqueduct. The inferior olivary nuclei and pontine tegmentum were normal, but there is no obvious basis pontis. The cerebellum, although small, had a normal layered pattern. The choroid plexus, pituitary and pineal were unremarkable.





Supplemental Figure 2.3 (left): Photograph showing dorsal view of brain with empty leptomeningeal sac (arrowhead) and small cerebellum (arrow).
Supplemental Figure 2.4 (right): Photograph showing ventral view of brain with relatively preserved temporal lobes (arrowhead) and flat pons above the intact trigeminal nerves (arrow).



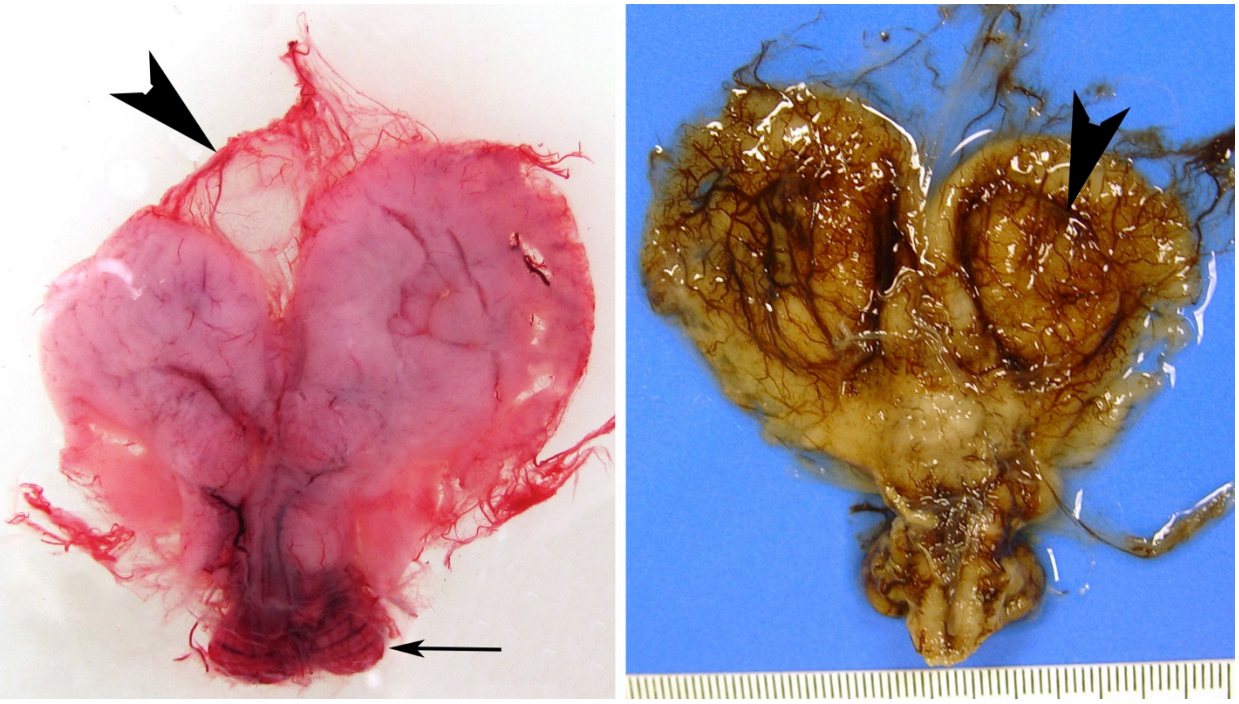
Supplemental Figure 2.5: (A) Photomicrograph showing narrow plaque of brain tissue adherent to leptomeninges (hematoxylin and eosin; original magnification 40x). (B) Immunostain for glial fibrillary acidic protein (GFAP) shows the delineation more clearly (original magnification 40x). Within the frontal and temporal cortex were multinucleated neurons (C, D), which were immunoreactive for NeuN (Rbfox3)(E), as well as rare multinucleate astrocytes, which were immunoreactive for GFAP (F) (all original magnification 1000x).

Infant 306 (~32 weeks gestation estimated by dates)

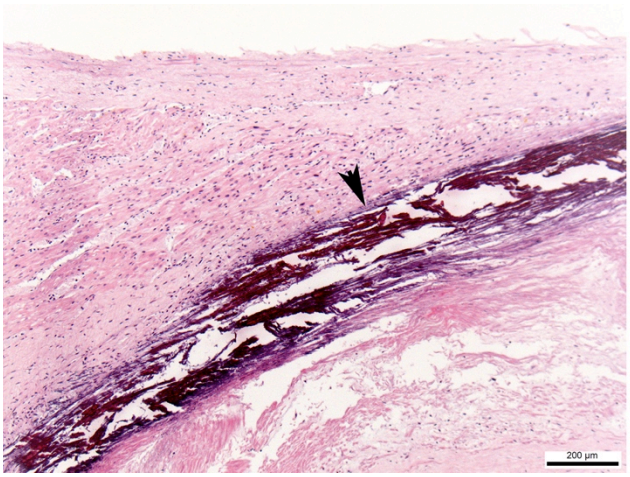
Stillborn male infant with early maceration. Weight was 1025 g (31 ± 2 weeks gestation), crown-heel length of 33 cm, crown-rump length of 21 cm, foot length of 4.8 cm, and head circumference 26 cm (expected for weight 28 ± 1 cm). Head and face abnormalities included low set (but normally shaped) ears, small wide-spaced palpebral fissures, bulbous pinched nose, small round mouth, tethered tongue tip, mandibular hypoplasia, and short neck. Skeletal and limb anomalies included thoracic scoliosis, possible right mid thoracic vertebral bar, complete right single palmar crease and left transitional crease, bilateral 5th finger shortening, short 4th finger middle phalanges on X ray, and bilateral soft tissue syndactyly of the 2nd to 5th toes.

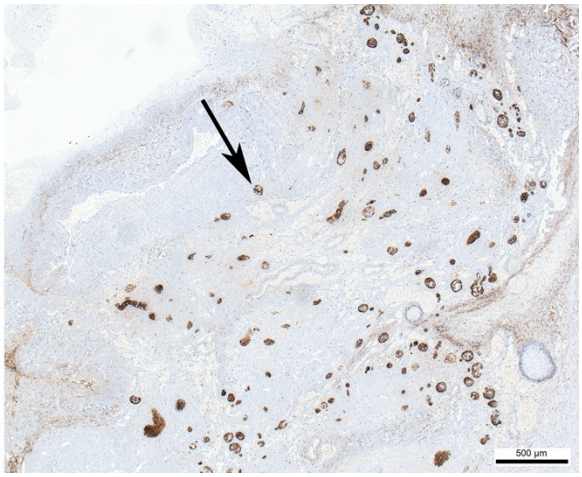
Complete autopsy was performed. The heart had a normal external appearance but the left ventricle was slightly dilated and the aortic valve cusps were thickened. The lungs were very small (oligohydramnios sequence). Fatty tissue containing small nodules of solid tissue were seen in place of kidneys, no ureters were identified, and the bladder was hypoplastic. The placenta was slightly small. The gross appearance of the brain was almost identical to that of the other two fetuses; the empty dorsal leptomeningeal sac had prominent arteries, the ventral portions of the cerebrum were partially preserved, and the cerebellum was small (Supplemental Figures 2.6 and 2.7).

Microscopic examination showed focal fibrosis and calcification in the myocardium (Supplemental Figure 2.8) and rare glomerulus-like structures in the retroperitoneal tissue where the kidneys would normally be located (Supplemental Figure 2.9). Multinucleate cells were identified in the partially autolyzed liver, autonomic ganglia in the retroperitoneum, frontal and temporal cortex, striatum, spinal cord, and retina (Supplemental Figure 2.10).

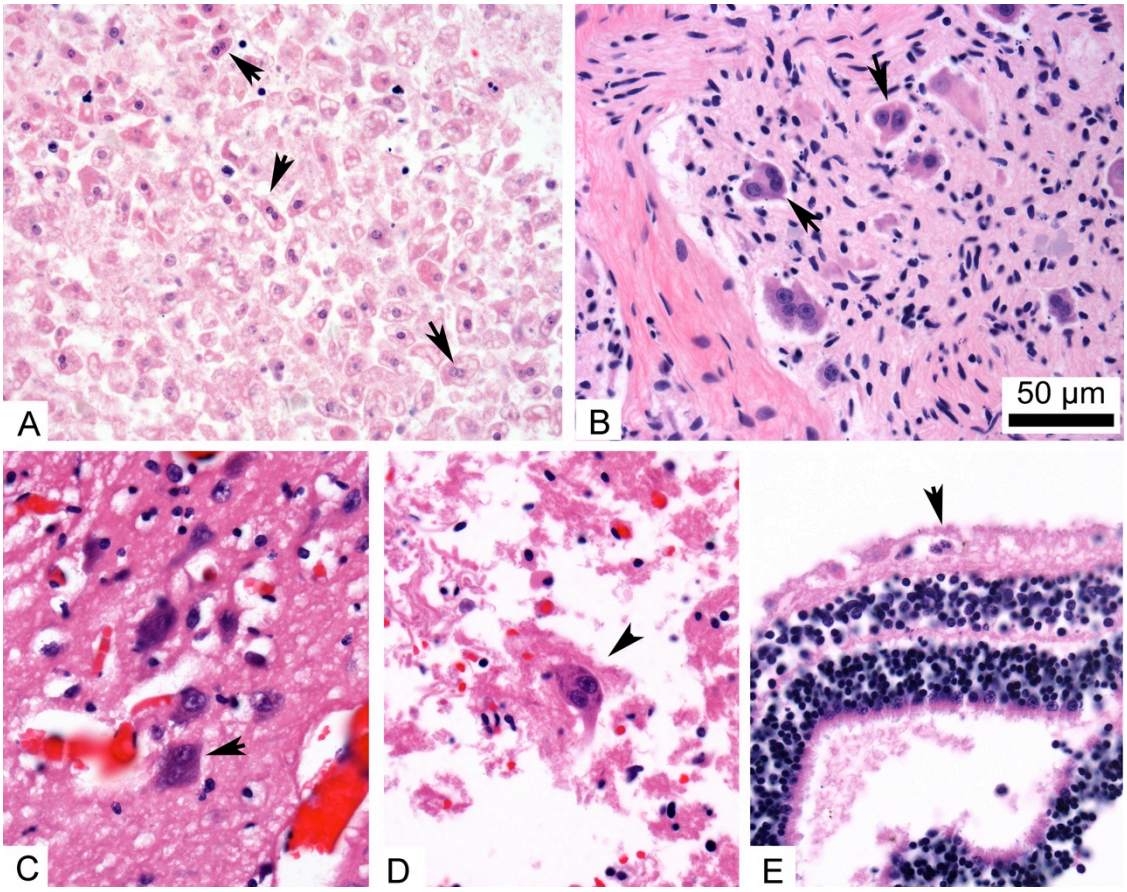


Supplemental Figure 2.6 (left): Photograph showing dorsal view of unfixed brain with empty leptomeningeal sac (arrowhead) and small cerebellum (arrow).
Supplemental Figure 2.7 (right): Photograph showing ventral view of fixed brain with relatively preserved temporal lobes (arrowhead).





Supplemental Figure 2.8 (left): Photomicrograph showing myocardium with focal calcification (arrow) (hematoxylin and eosin; original magnification 100x).
Supplemental Figure 2.9 (right): Photomicrograph showing epithelial membrane antigen (EMA) immunoreactivity in glomerulus-like structures (arrow) within the retroperitoneum (original magnification 40x).



Supplemental Figure 2.10: Photomicrographs showing (A) multinucleate hepatocytes in the liver, (B) multinucleate neurons in the parasympathetic ganglia of the retroperitoneum, (C) multinucleate neurons in the frontal cortex, (D) multinucleate neurons in the anterior horn of the (autolytic) spinal cord, and (E) multinucleate neurons in the ganglion cell layer of the retina (all hematoxylin and eosin; original magnification 600x).