

## Beta-Amyloid Precursor Protein Staining in Nonhomicidal Pediatric Medicolegal Autopsies

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**Abstract.** Immunohistochemical staining for beta-amyloid precursor protein ( $\beta$ APP) has been validated as a marker for axonal injury in adults surviving  $\geq 2$  hours after white matter damage. The significance of  $\beta$ APP staining in pediatric brains and spinal cords is not as well established. We evaluated the white matter immunoreactivity for  $\beta$ APP from a variety of pediatric medicolegal autopsies: natural disease (non-Sudden Infant Death Syndrome [SIDS]), SIDS, motor vehicle accidents, drowning, near-drowning, overlay, carbon monoxide toxicity, miscellaneous trauma, and mechanical asphyxia. The cases of carbon monoxide toxicity, motor vehicle accidents (death at scene), drowning (with resuscitation), and a natural (non-SIDS) death had no significant white matter staining. The traumatic deaths with a significant survival interval, a variety of natural deaths, the near-drowning case, and surprisingly, all SIDS had detectable  $\beta$ APP white matter immunostaining. These results demonstrate that features other than traumatic axonal injury, such as metabolic insults and hypoxic-ischemic injury secondary to vascular compromise, must contribute to  $\beta$ APP immunostaining. In addition, we describe a variety of  $\beta$ APP-immunoreactive structures not previously reported in the pediatric population. This study illustrates that  $\beta$ APP immunostaining enhances detection of a variety of white matter changes, and provides a basis for interpretation of these results.

**Key Words:** Autopsy; Beta-amyloid precursor protein; Brain; Immunohistochemistry; Sudden infant death syndrome (SIDS); Pediatric.

### INTRODUCTION

A thorough neuropathological examination of pediatric medicolegal autopsies demands evaluation and interpretation of the findings within the context of each case. One must consider a spectrum of entities, including natural disease, accidental injury, nonaccidental injury, and the secondary manifestations of these processes. The clinical history provided by caretakers and investigators may be superficial, inaccurate, or conflicting. Thus, the forensic pathologist and neuropathology consultant are often in the position of confirming or refuting the validity of the stated history based upon the autopsy findings. Unfortunately, the morphological central nervous system (CNS) findings in natural disease, accidental, and nonaccidental head injury frequently overlap. The interpretation of findings may be further complicated by secondary changes such as “respirator brain.”

In adults, beta amyloid precursor protein ( $\beta$ APP) immunostaining has been used to enhance the detection of traumatic axonal injury (TAI), and a variety of nontraumatic disorders that affect white matter, for example, multiple sclerosis, human immunodeficiency virus (HIV) encephalitis, hypoxic-ischemic injury (1), and hypoglycemia (2).  $\beta$ APP, an integral membrane protein in neurons, is transported by fast anterograde axonal transport. Disruption of normal axonal transport mechanisms results

in its accumulation and enables its immunohistochemical detection. In adults it is clear that only a 2- to 3-hour (h) period of survival after axonal injury is required to detect  $\beta$ APP immunoreactivity (1).

There are relatively few descriptions of  $\beta$ APP immunostaining in nontraumatic pediatric autopsy material (3–5). In this study we have used routine hematoxylin and eosin (H&E) sections and  $\beta$ APP immunostaining to evaluate the brains and spinal cords from 29 infants and young children who died as a result of a variety of natural diseases, accidental injury, and SIDS. Our study establishes that significant  $\beta$ APP immunoreactivity is not normally present in white matter, demonstrates normal  $\beta$ APP-positive structures, and describes a variety of pathological white matter immunostaining patterns that may be observed.

### MATERIALS AND METHODS

#### Study Population

Twenty-nine cases were prospectively collected between July 2000 and April 2001. The study material consisted of consecutive brains and spinal cords of infant (0–12 months) and young children submitted from medical examiner autopsy cases for neuropathological consultation. The cases were typically selected by the medical examiner for documentation and interpretation of findings.

The classification of cause and manner of death in each autopsy was based upon review of a complete case file that included complete autopsy and investigative report, toxicology, medical records, as well as microbiological and metabolic evaluation, when appropriate. The cases were divided into 8 categories: 1) natural (non-Sudden Infant Death Syndrome [SIDS]): manner of death recorded by the autopsying medical examiner as natural, 9 cases; 2) SIDS, defined as “the sudden death of an infant under 1 year of age that remains unexplained after a

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TABLE 1  
Evaluation of Immunostaining

Microscopic appearance	Staining intensity
No staining	—
Mild staining: pinpoint, typically requiring high-power objective (×40)	+
Moderate staining: axonal swellings/bulbs, often in “patches”	++
Severe staining: extensive axonal staining, readily apparent with low-power objective (×2)	+++

thorough case investigation, including performance of a complete autopsy, examination of the death scene, and review of the clinical history” (6), 9 cases; 3) motor vehicle accident: based on investigative report, 4 cases, 3 of which were “dead at scene”; 4) drowning: based on investigative report, found floating in body of water with no evidence or suspicion of trauma, resuscitation attempted, 2 cases; 5) near-drowning: based on investigative report, “found floating in backyard pool,” with resuscitation and subsequent brain death, 1 case; 6) overlay: based on investigative report, clinical history of “found under sleeping mother in bed,” 1 case; 7) carbon monoxide (CO) toxicity: found dead with lethal blood levels of CO after house fire, 1 case; 8) mechanical asphyxia: investigative report, found hanging off the side of a bassinet, 1 case; and 9) miscellaneous trauma: fall after being punched by father while boxing, 1 case.

Many of the decedents in our study presented with at least minimal vital signs and underwent variable and often lengthy resuscitation. Due to the often-present, weak but definite vital signs and the possibility of continued CNS perfusion during resuscitation efforts, resuscitation time was included in the calculation of survival period.

Gross Evaluation and Histological Sampling

A complete neuropathological examination of each brain and spinal cord was performed after fixation in 20% formalin for a minimum of 2 weeks. The minimum standard set of 3 × 2 × 0.5 cm histological sections included body of the corpus callosum with parasagittal white matter, corpus callosum at the level of the lateral geniculate body, fronto-parietal border zone cortex and white matter, hippocampus, posterior limb of the internal capsule with thalamus, rostral pons, medulla, spinal cord, and cerebellum. Histological samples were taken from both sides of each brain, although the samples were not bilaterally paired. Adjacent sections were stained with H&E and by βAPP immunohistochemistry. Brain swelling was defined as either a brain weight greater than 10% above expected for age, length, and weight, or gross, or microscopic evidence of herniation (subfalcine, uncal, or tonsillar).

Immunohistochemistry

Paraffin sections were cut at 3 μm on a rotary microtome, mounted on positively charged glass slides (POP100 capillary gap slides, Ventana Medical Systems, Tucson, AZ), and air-dried overnight in front of a cool fan. Sections were then deparaffinized in xylene and ethanol, and placed in 200 ml heat-induced epitope retrieval (HIER) Biobuffer® (BioPath Laboratories, Oklahoma City, OK), pH 6.8. The buffer was brought to a boil, after which 50 ml of deionized water was added. The buffer was again brought to a boil for 5 min, then

the slides were allowed to cool in buffer for 20 min, following which they were rinsed thoroughly in deionized water and then buffer. Sections were blocked with unlabeled avidin and biotin (Vector Laboratories, Burlingame, CA) for 15 min each, rinsing between each step with phosphate buffered saline, pH 7.4. The remaining steps were performed at 4°C except as noted. Staining was performed manually, with slides in a vertical position using capillary gap methodology. Buffers, blocking serum, secondary antibodies, avidin/biotin complex reagents, chromogen, and hematoxylin counterstain were used as supplied in the ChemMate® secondary detection kit (Ventana Medical Systems). Optimum primary antibody dilutions were predetermined using known positive control tissues. A known positive control section was included in each run to assure proper staining. Sections were incubated in unlabeled blocking serum for 1 h to block nonspecific binding of the secondary antibody. Sections were then incubated for 1 h with either primary antibody (mouse anti-APP, Chemicon, Temecula, CA) at a 1:400 dilution in buffer, or with buffer alone as a negative reagent control. Following washing in buffer, sections were incubated for 1 h with biotinylated polyvalent secondary antibody solution (containing goat antibodies to rabbit, mouse, and rat immunoglobulin). Sections were then incubated for 25 min with freshly prepared alkaline phosphatase-conjugated avidin-biotin complex. Sections were then washed in buffer, and incubated with 2 freshly prepared changes of BT Red chromogen (a new fuchsin-type chromogen), 3.5 min each, followed by washing in buffer and then water. Sections were then counterstained at room temperature with Mayer’s hematoxylin, dehydrated in a graded series of ethanols and xylene, and coverslipped. Slides were reviewed by light microscopy. Positive reactions with BT Red were identified as red reaction product. Sections were photographed using a CoolSNAP® digital camera (RS Photometrics, Tucson, AZ).

Assessment

Initially, all observers reviewed the cases “blind” to the diagnosis, including any clinical or autopsy data. The cases were then reviewed as a group with all available information and final categorization of each was recorded.

All histological sections were evaluated for the presence of hypoxic-ischemic injury (i.e. acute neuronal necrosis and neuronal karyorrhexis) using standard neuropathological criteria. White matter βAPP immunoreactivity was assessed and recorded by each observer and then as a group using a semi-quantitative scoring technique similar to that described by Gentleman et al (7). This type of analysis has been established as reproducible and used in several publications specifically evaluating βAPP immunostaining within axons (2, 8) (Table 1). The one

exception to the grading system was the occasional isolated, but well-formed, axonal bulb. These findings were noted but not considered as “significant” staining and therefore did not contribute to the score in a given case. When characteristic patterns of axonal immunoreactivity were present on a given slide this was noted. The observer then recorded a clinicopathological diagnosis of the type(s) of axonal injury for the entire case.

The following clinicopathological diagnostic categories were used: multifocal traumatic axonal injury (mTAI), diffuse traumatic axonal injury (dTAI), vascular axonal injury (VAI), metabolic axonal injury (MAI), and penumbral axonal injury (PAI).

**dTAI:** Diagnosed when there was a history of trauma and scattered and/or groups of  $\beta$ APP-immunoreactive axonal swellings/bulbs were present within the corpus callosum, cerebral hemispheric white matter, and brainstem that do not have a pattern consistent with VAI (see below). The immunoreactive axons of d/mTAI are typically scattered or in groups along the long axis of the axon.

**mTAI:** Diagnosed when there was a history of trauma and scattered and/or groups of  $\beta$ APP-immunoreactive axonal swellings/bulbs were present within the corpus callosum, cerebral hemispheric white matter, but not the brainstem, and did not have a pattern consistent with VAI (see below). Nonhuman primate experiments demonstrate that brainstem lesions do not occur in the absence of supratentorial axonal injury. Furthermore, TAI within the brainstem as a result of acceleration/deceleration injury does not occur in isolation (9).

**VAI:** A “zigzag” pattern of axonal immunoreactivity due to vascular compromise, typically due to internal herniation that results in secondary hypoxic-ischemic axonal injury (1). Therefore, VAI involves the parenchyma in characteristic vascular distributions and implies raised intracranial pressure, and both white and grey matter may be involved (Fig. 1G). Theoretically, vasospasm or other mechanisms of vessel compromise, such as compression of vessels by swollen hemispheric parenchyma, would result in similar patterns of immunoreactivity. The pattern of VAI is that of clusters of immunoreactive axons that are staining without regard for the course of the axon, i.e. there is a well-demarcated area of staining within a long white matter tract. In addition, “punched-out” islands of immunoreactivity were also considered VAI: a perpendicular perspective of the “zigzag” pattern (Fig. 1E). These staining patterns may be observed in cases without a history of trauma (unpublished data).

**MAI:** Scattered axonal immunoreactivity identified without a clinical history or pathological evidence of trauma and not occurring in a pattern typical of VAI (Fig. 1A–D, F). The term “metabolic” is used broadly here and may represent the global effects of a specific disturbance, such as hypoglycemia (2) or global hypoxic-ischemic injury, although unusual (8). The underlying cause of MAI may not always be identifiable.

**PAI:** A descriptive phrase used to describe axonal immunoreactivity adjacent to isolated focal lesions, regardless of the etiology, such as lacunar infarcts, cortical lacerations, an abscess, etc. In other words, axonal damage around a laceration or infarct would be PAI, not TAI or VAI, respectively. The term PAI enabled observers to indicate the presence of an area of axonal immunostaining, often with a qualifier, without implying a more global process. For example a cortical laceration is present with PAI, but there is not m/dTAI. Multiple categories of axonal injury may be present in a single case.

## RESULTS

### General $\beta$ APP Findings

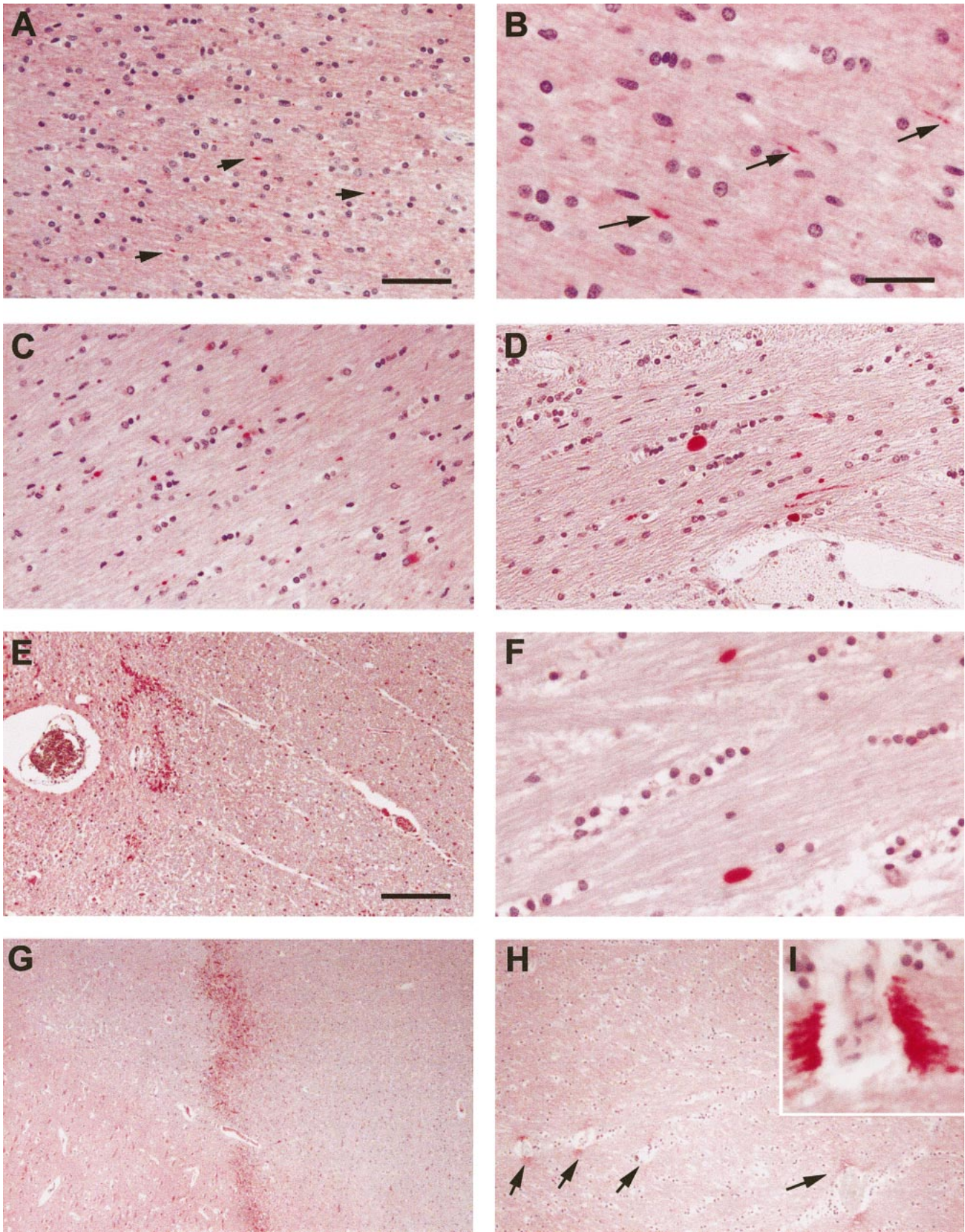
A variety of cells in the pediatric CNS showed  $\beta$ APP immunostaining, despite the presence or absence of white matter immunoreactivity and irrespective of the clinical history or post-event survival time. The cells routinely identified included neurons, blood vessels, meningotheelial cells, choroid plexus cells, ependymal cells, tanycytes, astrocytes, oligodendroglia, dorsal root ganglia neurons and satellite cells, peripheral myelin, and cells within the cerebellar internal granular layer (Fig. 2). A gamut of immunoreactivity patterns was identified, both within a case and between autopsies: immunostaining of a specific structure could be ubiquitous within the individual case, focal or multifocal. The presence of 1 immunoreactive structure did not correlate with immunoreactivity in the other listed nonwhite matter structures. The statistical significance of the presence or absence of individual immunoreactive cell types, including white matter immunoreactivity, was not determined.

A range of neuronal  $\beta$ APP immunostaining was consistently identified throughout the CNS. For example, anterior horn motor neurons of the spinal cord and thalamus

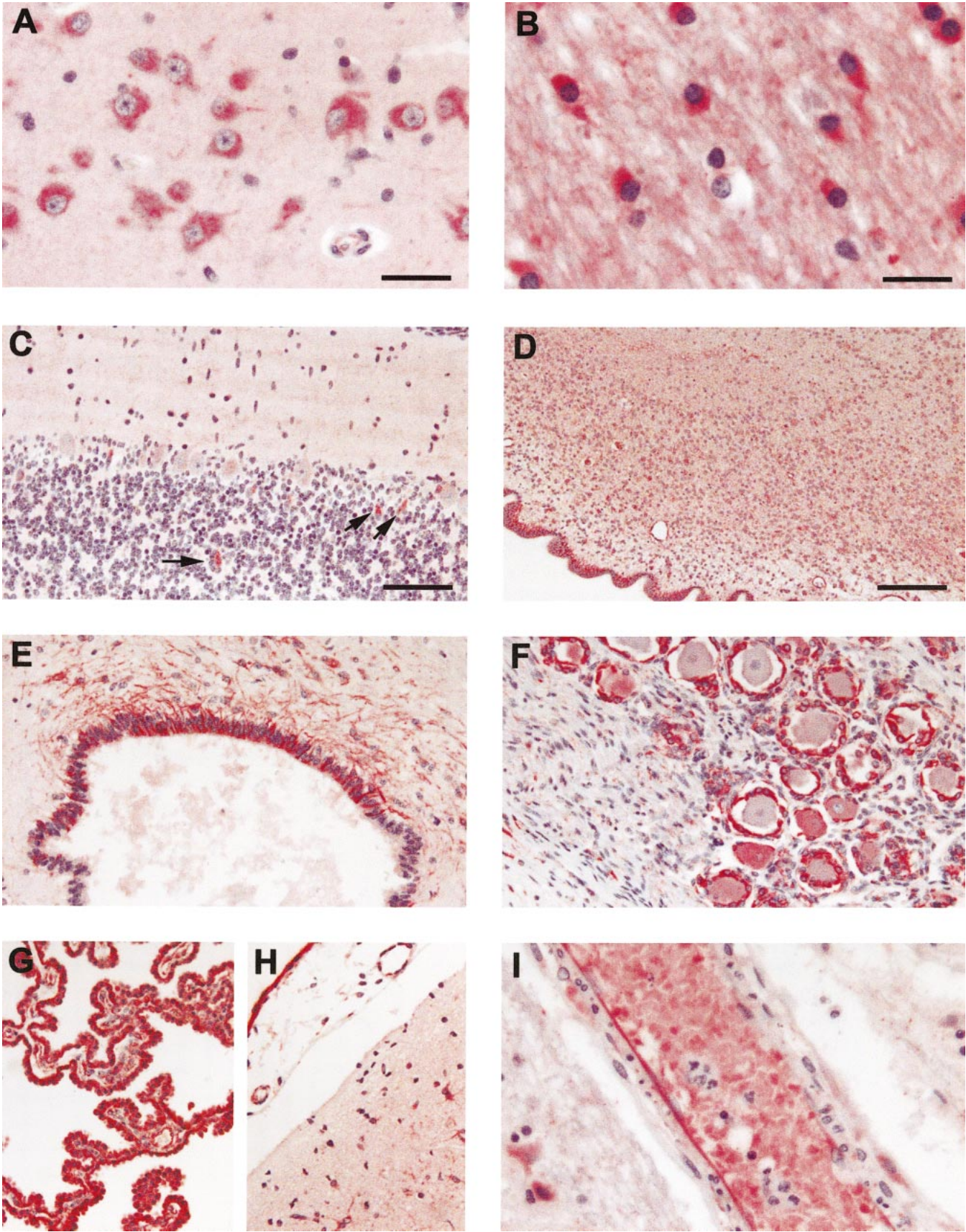
**Fig. 1.** Examples of abnormal  $\beta$ APP immunoreactivity. **A:** Corpus callosum, example of pinpoint (+) immunostaining pattern, MAI (Case 16). **B:** Corpus callosum, higher power view of axonal swellings, comparable to field in A (+), MAI (Case 18). **C:** Corpus callosum, well-developed axonal swellings and occasional axonal bulb (++), MAI (Case 7). **D:** Internal capsule, MAI (Case 7). **E:** Superior cerebellar peduncle, VAI (Case 21). **F:** Internal capsule, MAI (Case 21). **G:** Medial temporal lobe, VAI involving both grey and white matter, +++ (Case 28). **H:** Corpus callosum, perivascular immunostaining (Case 2). **I:** High power view of single vessel (Case 2). Scale bars: Case A, C, D = 80  $\mu$ m; B, F, I, = 40  $\mu$ m; E, G, H = 400  $\mu$ m.

**Fig. 2.** General  $\beta$ APP immunostaining. **A:** Thalamic neurons with vesicular staining pattern. **B:** Reactive glia filled with  $\beta$ APP. **C:** Immunoreactive cells within internal granular layer. **D:** Immunoreactive ependyma of lateral ventricle and endothelium. **E:** Immunoreactive ependyma and associated processes of central canal, note regional variability. **F:** Dorsal root ganglia with immunoreactive satellite cells; neurons with blush of immunostaining as well as nonreactive and peripheral myelin. **G:** Choroid plexus, note epithelial and endothelial immunoreactivity. **H:** Cerebral cortex, immunoreactive arachnoid and subpial stellate astrocytes. **I:** Immunoreactivity in circulating blood. Scale bars: A, E, I = 80  $\mu$ m; B = 40  $\mu$ m; C, F, G, H = 200  $\mu$ m; D = 400  $\mu$ m.











routinely exhibited  $\beta$ APP immunoreactivity in a vesicular distribution mirroring that of Nissl substance (Fig. 2A). At the other end of the spectrum were neurons with intense  $\beta$ APP immunoreactivity that filled the entire cell body. In addition, some neurons revealed a blush of cytoplasmic reactivity, intermediate between the vesicular and intense immunostaining patterns. The distribution of these neurons was both focal (admixed with negative cells) and geographic (involving entire regions). Quantitative analysis of involved neurons and their respective immunostaining patterns was not pursued.

$\beta$ APP immunoreactivity was identified in variety of other cell types. Immunoreactive granules were identified in all layers of large (meningeal) and small (capillary) blood vessel walls (Fig. 2D, G–I). Endothelial cytoplasm revealed the greatest frequency and intensity of immunostaining. A range of individual cell immunoreactivity was identified similar to that described for neurons. Leptomeningeal  $\beta$ APP immunoreactivity was regularly observed with varying intensity and distribution, both within and between cases (Fig. 2H). Histological sections of the dura were not routinely immunostained for  $\beta$ APP, but when present, they were noted to be immunoreactive.  $\beta$ APP immunostaining highlighted individual choroid plexus epithelial cells and the associated blood vessels, as described above (Fig. 2G). Ependymal and tanycyte  $\beta$ APP immunoreactivity was identified within all cerebral ventricular linings and the spinal central canal (Fig. 2D–E). There was immunoreactivity both within the perikaryal cytoplasm and associated radiating processes. In addition, some cases revealed regional variation of immunoreactivity (Fig. 2E). Based upon cell morphology there was both astrocytic and oligodendroglial  $\beta$ APP immunoreactivity, which was predominately perikaryal (Fig. 2B), although cellular processes were occasionally identified (Fig. 2H). The degree of glial immunoreactivity was highly variable; some cases were completely negative and others showed intense immunoreactivity throughout the CNS. Subjectively, the glial immunoreactivity of a particular case frequently correlated with the severity of the global insult and a greater length of post-event survival. Occasionally, a blush of dorsal root ganglia neuronal immunoreactivity was noted, with intense immunostaining of the satellite cells (Fig. 2F). Peripheral myelin was periodically immunoreactive. The immunopositivity was distinctly within the myelin sheath, not the axon proper (Fig. 2F). The internal granular layer of the cerebellum contained scattered small  $\beta$ APP-immunoreactive cells; the precise cell type was not further identified (Fig. 2C).  $\beta$ APP-immunoreactive vesicles were routinely identified within vascular lumens both admixed and adherent to circulating blood cells. Immunostaining of red blood cells was common. In contrast, leukocytes were negative (Fig. 2I), and immunohistochemistry for platelets with CD61 was consistently negative within

these vessels. The exact origin and nature of the intravascular  $\beta$ APP immunoreactivity is not clear. The statistical correlation of each immunoreactive cell type with another was not calculated.

### White Matter $\beta$ APP Findings

A summary of results is provided in Table 2. Eight of the 29 cases evaluated for this study did not reveal any  $\beta$ APP white matter immunoreactivity: 3 motor vehicular accidents, 2 drownings, 1 CO toxicity, 1 overlay, and 1 natural death. None of these cases had any underlying disease process (except the natural disease death), all survived less than 2 h after injury, and no cases demonstrated any evidence of global hypoxic-ischemic injury (GHII). Resuscitation was attempted unsuccessfully in the overlay case and in 1 drowning case.

A total of 21 cases demonstrated axonal  $\beta$ APP immunoreactivity. All 4 cases with a survival time of greater than 2 h, including resuscitation time, demonstrated some degree of  $\beta$ APP-positive white matter. In 8 natural (non-SIDS) cases there was  $\beta$ APP-immunoreactive white matter: 6 cases either found dead or with a short (<2 h) period of attempted resuscitation, and 2 cases with multiple in-hospital resuscitations. In addition, all cases of SIDS, including the near-SIDS (>24 h of survival), had at least mild  $\beta$ APP-positive white matter.

GHII was present in 5 cases: 1 natural (non-SIDS), 1 SIDS, 1 near-drowning, 1 miscellaneous trauma, and 1 motor vehicle accident. In all cases with a survival of at least 24 h there was evidence of brain swelling and  $\beta$ APP-positive white matter. Brain swelling was also diagnosed in 5 SIDS, 1 natural (non-SIDS), 1 overlay, and 1 mechanical asphyxia death. Based upon brain weight, 5 SIDS cases met a criteria used in this study to identify brain swelling; however, increased brain weight has been reported in SIDS and suggests megalencephaly (10) or may be a manifestation of cerebral edema or some other pathological process. Brain swelling did correlate with the presence of white matter immunoreactivity, but it manifested only as VAI in 3 cases: motor vehicle accident with survival, miscellaneous trauma, and a natural disease death (non-SIDS). The only case, excluding SIDS, without  $\beta$ APP immunoreactivity and brain swelling was the overlay.

The natural death (non-SIDS) cases revealed several different types of axonal immunoreactivity. In 6 cases there was PAI surrounding a focal lesion. For example, cases 3 and 9 (congenital heart disease) had multiple microscopic infarcts with surrounding  $\beta$ APP-immunoreactive axons (PAI). In addition,  $\beta$ APP-immunoreactive axons were adjacent to blood vessels in several cases, natural and SIDS (Fig. 1H).

MAI was present in 5 natural (non-SIDS) cases, 1 near-drowning, and all SIDS cases. There was a spectrum of

axonal immunoreactivity identified that ranged from subtle pinpoint immunostaining (+) to well-formed axonal swellings (+++). Case 12 (SIDS) revealed immunoreactive axons surrounding blood vessels and MAI. Evidence of GHII was present in the near-SIDS and the near-drowning, cases 10 and 21, respectively, both survived >24 h. The remaining cases with perivascular axonal immunoreactivity and/or MAI were either found dead or had attempted resuscitation only. Four cases (4, 7, 8, and 21) had antemortem documentation of an electrolyte abnormality, of which 3 revealed MAI. In addition, it is plausible that cases 2 and 5, both sepsis-related deaths, would likely have had antemortem electrolyte abnormalities.

VAI was identified in a total of 4 cases. Case 8 had a witnessed cardiac arrest in the emergency department and was resuscitated for 1 h and 30 min before declaration of death. Resuscitation was attempted on case 4. In cases 22 and 28 there was a history of trauma; however, only in case 22 was it possible to definitively diagnose dTAI and VAI.

A distinctive pattern of perivascular  $\beta$ APP-immunoreactive axons was identified in 5 nontraumatic cases (Fig. 1H–I).

## DISCUSSION

The human  $\beta$ APP gene and its product have been identified, using a variety of techniques, in virtually all cell types (11). Immunohistochemistry has been used to identify  $\beta$ APP within neurons, blood vessels, meninges, ependyma, and choroid plexus from adult autopsy brains (12). In addition,  $\beta$ APP has been identified within human dorsal root ganglia satellite cells (13) and neurons (14).  $\beta$ APP is also associated with and released by platelets (11, 15). Although  $\beta$ APP expression has been reported in a variety of adult human cells, developmental studies and other cell types expressing  $\beta$ APP have been described primarily in experimental animals. Studies have demonstrated  $\beta$ APP immunoreactivity in fetal and early postnatal mouse radial glia as well as in mature mouse neuron cell bodies and dendrites (16). Similar results were demonstrated by immunohistochemical analysis of the developing rat CNS. In addition,  $\beta$ APP immunoreactivity is present within rat spinal anterior horn cells, ependyma, and choroid plexus epithelium (17). In situ hybridization has been used to demonstrate  $\beta$ APP mRNA in rat neurons, including Purkinje cells and cerebellar granular cells, and in choroid plexus, ependyma, astrocytes, oligodendroglia, and microglia (18).  $\beta$ APP immunoreactivity has also been detected in myelin sheaths in the rat central nervous system (19). In a model of axonal injury it has been demonstrated that  $\beta$ APP is moved from neuronal cell bodies towards dystrophic nerve endings via rapid anterograde axonal transport

(20). Immunohistochemistry of canine brains has revealed  $\beta$ APP within neurons, damaged axons, glia, and blood vessels: endothelium, smooth muscle, and transmurally within capillaries (21). Since the  $\beta$ APP gene and its product are highly conserved between species (11), these animal studies provide insight toward interpreting the results of this study.

The presence of  $\beta$ APP immunoreactivity within human pediatric neurons, glia, leptomeninges, blood vessels, dorsal root ganglia, tanycytes, choroid plexus, ependyma, and blood elements, as described in our study, has many implications. First, it may play a role in normal development and persist postnatally. Secondly, some cells normally express  $\beta$ APP in amounts detectable by routine immunohistochemistry. Thirdly,  $\beta$ APP expression may vary between individuals. Fourthly,  $\beta$ APP may be expressed or upregulated in response to injury or stress. Finally, and most importantly, it is crucial not to misinterpret immunoreactive structures such as endothelium, glia, peripheral myelin, or circulating  $\beta$ APP as axonal immunostaining and hence axonal injury. When such nonwhite matter immunoreactive structures are present they may be used as an internal positive control. However, the converse does not necessarily apply, as some cases may have immunoreactive injured axons even though other normal structures are negative due to intrinsic biological variability in staining.

In addition to the above-mentioned factors, there are a number of other possible explanations for the variability in  $\beta$ APP immunoreactivity observed. The post-event survival interval is known to affect detection of  $\beta$ APP. The intensity of staining generally increases in injured axons as survival time increases up to several days and will then eventually decrease and disappear with prolonged survival. However, immunoreactive axons have been reported in adults as long as 99 days after mild head injury (22). The ultimate size of a particular  $\beta$ APP axonal swelling or bulb reflects many factors (23) and therefore seems unlikely to accurately reflect length of survival. Furthermore, it is often difficult to determine and thoroughly characterize the true CNS survival interval in medicolegal cases because of vague clinical history, varying cerebral perfusion pressure/brain death, and length of ventilator support. Also, it has been proposed that  $\beta$ APP is an acute phase reactant and neuronal production is increased after central nervous system insults (24). Expression of  $\beta$ APP has been demonstrated in reactive astrocytes after neuronal damage (25) and at the periphery of infarcts (26). A reactive multifactorial process is currently the best explanation for the variation in both neuronal and glial immunoreactivity. Each case represents a variety of pathophysiological processes and the variability of  $\beta$ APP immunoreactivity mirrors this complexity.

TABLE 2  
Case Data

Case	COD	MOD	Age	LOS	SDH	SAH	BS	GHII	BZ	CC-B	CC-LGB	IC/Thal
1	Cong. Heart	N	17 m	N	—	—	—	—	—	—	—	—
2	Sepsis	N	35 m	R	—	—	—	—	+	++	++	+
3	Cong. Heart	N	2 m	N	—	—	—	—	++	+	++	++
4	Hyperkalemia	N	2 m	R	—	—	—	—	+++	+++	+++	++
5	Suspected Sepsis	N	2 wk	R	—	—	—	—	—	—	—	+
6	Comp. Prem.	N	47 d	R-M	—	—	—	—	+	++	+	+++
7	Dehydration	N	9 m	R	—	—	—	—	++	++	NE	+++
8	Myocarditis	N	11 m	R-100 m	—	+	+	—	++	++	+++	++
9	Cong. Heart	N	8 m	R-M	—	—	—	+	—	—	—	+++
10	Near-SIDS	N	9 m	>24 h	—	—	+	+	+	+	—	—
11	SIDS	N	4 m	N	—	—	+	—	++	++	+++	++
12	SIDS	N	40 d	R	—	—	—	—	+	+	+	+
13	SIDS	N	1 m	N	—	—	—	—	—	+	+	++
14	SIDS	N	7 m	N	—	—	—	—	+	+	—	+
15	SIDS	N	3 m	R	—	—	—	—	+	+	++	++
16	SIDS	N	2 m	R	—	—	+	—	+	+	+	+
17	SIDS	N	2 m	R	—	—	+	—	+	+	+	+
18	SIDS	N	3 m	R	—	—	+	—	++	+	++	+
19	Drowning	A	3 yr	N	—	—	—	—	—	—	—	—
20	Drowning	A	11 m	R-100 m	—	—	—	—	—	—	—	—
21	Near-drowning	A	21 m	36 h	—	—	+	+	+	+	+	++
22	MBFI	A	12 m	>24 h	+	+	+	+	+++	+++	+++	+++
23	MBFI	A	16 m	N	+	+	—	—	—	—	—	—
24	BFHNI	A	1 yr	N	+	+	—	—	—	—	—	—
25	BFHI	A	1 yr	N	+	+	—	—	—	—	—	—
26	Mech. Asphyxia	A	10 m	N	—	—	+	—	—	—	++	+
27	Overlay	A	5 wk	R-90 m	—	—	+	—	—	—	—	—
28	BFHI	A	8 yr	>24 h	+	+	+	+	+	++	+++	+++
29	CO (housefire)	A	1 yr	N	—	—	—	—	—	—	—	—

Abbreviations: COD, cause of death; MOD, manner of death; SDH, subdural hemorrhage; SAH, subarachnoid hemorrhage; BS, brain swelling; GHII, global hypoxic-ischemic injury; BZ, border zone (fronto-parietal); CC-B, corpus callosum body; CC-LGB, corpus callosum at level of lateral geniculate body; IC/Thal, internal capsule and thalamus; Hippo, hippocampus; SC, spinal cord; dTAI, diffuse traumatic axonal injury; VAI, vascular axonal injury; MAI, metabolic axonal injury; PAI, penumbral axonal injury; Extra-CNS, extra-central nervous system injury; Skull Fx., skull fracture; Lac./Cont., laceration and/or contusion; Cong. Heart, complications of congenital heart disease; MBFI, multiple blunt force injuries; BFHNI, blunt force head and neck injuries; BFHI, blunt force head injury; N, natural; A, accident; m, months; wk, weeks; d, days; yr, years; h, hours; LOS, length of survival; N, none; R, resuscitation only ( $\pm$ 1 h); R-M, resuscitation multiple times.

In our study we demonstrate that white matter  $\beta$ APP immunoreactivity is not normally detectable in the pediatric CNS using standard immunohistochemical techniques. Specifically, the lack of significant white matter immunoreactivity in the motor vehicle accident fatalities (dead at scene), carbon monoxide toxicity (house fire), drownings, overlay, and 1 natural (non-SIDS) death establishes that diffuse  $\beta$ APP positivity is not a normal finding in infants and young children.

This study reveals examples of various types of white matter axonal  $\beta$ APP immunoreactivity. All SIDS autopsies, 1 near-drowning, and 5 natural (non-SIDS) cases revealed MAI. The findings in these cases ranged from scattered pinpoint accumulations of  $\beta$ APP (+) to diffuse well-formed axonal swellings and bulbs (+++) (Fig. 1). The mild immunostaining typified by the SIDS cases

likely reflects a generalized increased production, accumulation, and thus detection of  $\beta$ APP secondary to global CNS stress. In contrast, in the cases with moderate to severe axonal staining there may be metabolic insults that lead to axonal injury. For example, antemortem blood work revealed electrolyte disturbances in case 7 (hyperkalemia) and case 21 (hyponatremia, diabetes insipidus). Perhaps in these types of cases the electrolyte abnormalities actually resulted in axonal injury in a manner similar to that proposed for hypoglycemia, which has been reported as a cause of axonal injury (2). Furthermore, areas of our cases, when viewed in isolation, mimicked TAI. Whether the range of axonal immunoreactivity reflects a continuum of injury or distinct processes is unclear.

Another potential global stress that might cause axonal injury is GHII. While, in the adult population it has been



TABLE 2  
Extended

Hippo	Pons	Medulla	SC	Cerebellum	dTAI	VAI	MAI	PAI	Extra-CNS	Skull Fx.	Lac./Cont
-	-	-	-	-	-	-	-	-	-	-	-
+	-	-	-	-	-	-	+	+	-	-	-
-	+	++	+	-	-	-	-	+	-	-	-
+++	+	-	-	+	-	+	+	-	-	-	-
+	-	+	+	-	-	-	+	+	-	-	-
+++	+	+	++	++	-	-	+	-	-	-	-
++	-	-	-	-	-	-	+	-	-	-	-
+	+	-	NE	-	-	+	-	+	-	-	-
-	+++	+	NE	-	-	-	-	+	-	-	-
+	-	-	-	-	-	-	+	+	-	-	-
++	++	-	+	-	-	-	-	-	-	-	-
-	+	+	+	+	-	-	-	-	-	-	-
+	+	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-
+	+	+	++	-	-	-	-	-	-	-	-
+	+	+	+	-	-	-	-	-	-	-	-
+	+	+	NE	+	-	-	-	-	-	-	-
++	+	-	-	+	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-
-	++	+++	-	-	-	+	+	-	-	-	-
+++	+++	+++	++	+++	+	+	-	-	+	-	+
-	-	-	-	-	-	-	-	-	+	+	+
-	-	-	-	-	-	-	-	-	-	+	+
-	++	+++	+	-	-	-	+	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-
+++	+++	+++	+++	+++	-	+	-	-	-	-	+
-	-	-	-	-	-	-	-	-	-	-	-

reported that GHII is an uncommon cause of axonal injury (8), 3 cases with MAI also revealed evidence of GHII; however, the survival time of the other cases of MAI was insufficient for detection of corresponding light microscopic changes of GHII. Our current understanding of GHII and other metabolic disturbances does not allow us to discriminate the exact etiology of MAI.

PAI was detected in 6 cases of natural deaths. In all cases it was likely a result of focal hypoxic-ischemic injury. In cases 3 and 9, both with congenital heart abnormalities, emboli would be the most probable etiology of the white matter injury. Periventricular leukomalacia should also be considered as a cause of PAI in infants evaluated with  $\beta$ APP immunostains when the timing is appropriate.

Perivascular  $\beta$ APP-immunoreactive axons were identified in 5 nontraumatic cases (Fig. 1H-I). This pattern of staining most likely results from secondary changes that are not specific to the underlying disease process. The pathogenesis is not clear, but may reflect alterations in vascular autoregulation and/or perfusion effects on adjacent axonal processes. These findings indicate that in infants and young children the pattern of perivascular axonal immunoreactivity should not be interpreted as definitive evidence of trauma.

The current literature indicates that a minimum of 2 h of survival time is required in adults for the immunohistochemical detection of  $\beta$ APP at the site of axonal injury. The length of survival needed for immunohistochemical detection of  $\beta$ APP is not as well established in children. In an attempt to determine the shortest survival time necessary to detect  $\beta$ APP immunoreactivity, we felt that it was important to consider the resuscitation interval as a period of survival. Case 8 was resuscitated for 1 h and 30 min and revealed white matter immunostaining, suggesting that reactive  $\beta$ APP expression may follow a time course similar to that in adults. In contrast, the SIDS cases revealed white matter immunoreactivity regardless of resuscitation. Of course SIDS is a diagnosis of exclusion and may reflect a variety of antemortem pathophysiological processes.

Several researchers have reported the results of  $\beta$ APP immunohistochemistry on a variety of nonhomicide pediatric cases (3-5). Gleckman et al (5) described suffocation (2 cases), SIDS (1 case), and drowning (1 case) with no survival interval as negative for  $\beta$ APP. Our study confirms the absence of  $\beta$ APP-positive axons in non-SIDS controls (traumatic and nontraumatic) with no survival except resuscitation. Shannon et al (4) reported

$\beta$ APP-positive axons in “6 of 7 children dying of non-traumatic hypoxic ischemic encephalopathy.” We found similar results, although they do not comment on the pattern of axonal immunoreactivity in their cases. Geddes et al (3) reported 14 cases of infants dying from causes other than homicidal (nonaccidental) head injury, including SIDS (7), respiratory infection (5), perinatal asphyxia (1), and gastroenteritis (1). All SIDS infants were found dead, 1 case of respiratory infection survived less than 30 min, and the remaining cases survived >4 h. Only 2 of these cases, the gastroenteritis and perinatal asphyxia, revealed  $\beta$ APP immunoreactivity, both of VAI pattern. Our findings are similar except that we observed at least mild axonal immunostaining in all SIDS autopsy brains.

The SIDS cases were included within this study because of the thorough autopsy and investigation of each case in the absence of trauma, not to specifically investigate the pathophysiology of SIDS. Clearly a much larger data set would be needed to establish more definitive findings within the SIDS population. However, the discrepancy between the immunoreactivity in SIDS cases in this study and those of other researchers is significant and has several possible explanations. Other observers may have regarded pinpoint or mild axonal immunoreactivity as insignificant. However, we have demonstrated that healthy children do not have a significant number of  $\beta$ APP-immunoreactive axons. To address differences in immunohistochemical techniques we repeated staining on representative sections from several cases using horseradish peroxidase/diaminobenzidine and found similar results. Cerebral white matter gliosis and periventricular leukomalacia has been reported in SIDS (27), suggesting that  $\beta$ APP immunostaining as noted in our study may be detecting “early” or ongoing white matter injury. The possibility that staining represented postmortem artifact was considered; however, our understanding is that  $\beta$ APP immunostaining within axons is possible because of “accumulation” of  $\beta$ APP due to disruption of normal fast anterograde axonal transport. In addition, it is believed that  $\beta$ APP production may be upregulated under a generalized stress and is an energy-requiring process and therefore increased staining as a postmortem artifact would seem unlikely. In our opinion, the immunoreactivity within the white matter in SIDS cases most likely represents a metabolic disturbance: whether it reflects a specific biochemical alteration, agonal GHII, or another process, will require additional research.

The intention and design of this study was not to provide a detailed analysis of developmental  $\beta$ APP expression. In the authors’ opinion there is an inherent age bias in the SIDS cases because they are by definition less than 1 year of age, frequently less than 6 months old, and likely reflect a mixture of underlying processes. All SIDS cases demonstrated some axonal immunoreactivity. In contrast, there were 7 cases <17 months of age with no

$\beta$ APP axonal staining. Most importantly several of the cases without significant white matter immunostaining were normal healthy children with a very specific and rapid cause of death. Therefore it seems that using the  $\beta$ APP axonal staining predominately seen in SIDS cases as an example of possible “modeling” could be misleading. Although the findings are intriguing, the study was not designed as a developmental study, and due to the patient population the data necessary to calculate information such as postconceptional age was not available.

In summary, this study confirms that a variety of processes may lead to the emergence of  $\beta$ APP-immunoreactive axons. Specifically, pediatric white matter does not normally have significant  $\beta$ APP immunoreactivity and therefore its presence likely reflects a pathological process. In addition, we illustrate that a number of nonaxonal structures may show  $\beta$ APP immunoreactivity. The practical importance of the observation within the context of this study is to prevent misinterpretation of glial immunoreactivity as axonal staining. The exact pathophysiologic significance of these results remains to be elucidated, but the findings should aid the pathologist in distinguishing between normal expression of  $\beta$ APP and pathological changes.

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