



## Febrile Seizures Research Is Really Heating Up!

### Epileptogenesis Provoked by Prolonged Experimental Febrile Seizures: Mechanisms and Biomarkers.

Dubé CM, Ravizza T, Hamamura M, Zha Q, Keebaugh A, Fok K, Andres AL, Nalcioğlu O, Obenaus A, Vezzani A, Baram TZ. *J Neurosci* 2010;30:7484–7494.

Whether long febrile seizures (FSs) can cause epilepsy in the absence of genetic or acquired predisposing factors is unclear. Having established causality between long FSs and limbic epilepsy in an animal model, we studied here if the duration of the inciting FSs influenced the probability of developing subsequent epilepsy and the severity of the spontaneous seizures. We evaluated if interictal epileptiform activity and/or elevation of hippocampal T2 signal on magnetic resonance image (MRI) provided predictive biomarkers for epileptogenesis, and if the inflammatory mediator interleukin-1 $\beta$  (IL-1 $\beta$ ), an intrinsic element of FS generation, contributed also to subsequent epileptogenesis. We found that febrile status epilepticus, lasting an average of 64 min, increased the severity and duration of subsequent spontaneous seizures compared with FSs averaging 24 min. Interictal activity in rats sustaining febrile status epilepticus was also significantly longer and more robust, and correlated with the presence of hippocampal T2 changes in individual rats. Neither T2 changes nor interictal activity predicted epileptogenesis. Hippocampal levels of IL-1 $\beta$  were significantly higher for >24 h after prolonged FSs. Chronically, IL-1 $\beta$  levels were elevated only in rats developing spontaneous limbic seizures after febrile status epilepticus, consistent with a role for this inflammatory mediator in epileptogenesis. Establishing seizure duration as an important determinant in epileptogenesis and defining the predictive roles of interictal activity, MRI, and inflammatory processes are of paramount importance to the clinical understanding of the outcome of FSs, the most common neurological insult in infants and children.

### Commentary

Febrile seizures are the most common neurologic disorder of childhood, affecting up to 5% of children under 5 years of age. One would think that with so many children affected, this disorder would be understood better in the year 2010. The cardinal clinical features of febrile seizures are well recognized. Some genetic markers of susceptibility to febrile seizures are established, and the proximate trigger of febrile seizures is obvious (fever!). Yet, it is still not really understood why certain children and not others are affected, how and why fever causes seizures to occur, the best way to prevent or treat febrile seizures, and biomarkers to determine which children are at greatest risk for later development of unprovoked seizures (i.e., epilepsy). Fortunately, most febrile seizures are “simple” in that they are brief, generalized, and not recurrent. However, febrile seizures that are prolonged (some even reaching status epilepticus), recurrent within a given fever, or that have a focal feature are more concerning. These “complicated” febrile seizures (or, “complex” febrile seizures, which is a less preferred term because it can be confused with complex partial seizures) carry a worse prognosis, higher risk of epilepsy, and greater chance of association with an underlying

structural lesion or other brain dysfunction. The potential relationship between complicated febrile seizures and temporal lobe epilepsy, via its structural analog (mesial temporal sclerosis), has remained a scientific enigma for decades. That is, whether febrile seizures predispose to temporal lobe epilepsy, one of the most refractory epilepsy syndromes in adulthood, has been a lingering and perplexing question with significant medical importance (1). Insights into this question are being pursued in the Consequences of Prolonged Febrile Seizures in Childhood (FEBSTAT) study, which is following over 100 children longitudinally with brain MRI scans and neuropsychological testing (2). Preliminary data are intriguing, suggesting that longer febrile seizures are more likely to lead to epilepsy than shorter febrile seizures.

Over the years, there have been many attempts to develop a realistic and clinically relevant animal model of febrile seizures to provide a substrate by which to investigate the questions posed above. On the surface, this would seem to be a straightforward task—since febrile seizures are provoked by fever, simply give an animal a fever and see if it experiences a seizure. But the experimental problem arises immediately—how to provoke a fever? Whereas fever is easily provoked in mature rodents, it is difficult if not impossible to evoke a fever in immature rodents during the developmental ages that correlate with human infancy and early childhood (3). Therefore, more complex approaches are required. In one model, rats are treated with the bacterial

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endotoxin lipopolysaccharide, which causes an immune response and augments core temperature in immature rodents by about 1°C (but only when rats are maintained at ambient temperature of 30°C); seizures are then evoked by a chemconvulsant (3). An alternative method has been to raise the core temperature by heating the animal. Over the years, a variety of heating methods has been used, including hot water, infrared heat lamp, and warmed air stream. In each case, the animal's core temperature rises (and in some cases, brain temperature also rises [4]), and this leads to "hyperthermic" seizures. Investigators acknowledge that hyperthermic seizures are not true febrile seizures, which can only be elicited when endogenous fever mediators are present (5). However, because similar mechanisms may be involved, critical information can be obtained from such studies regarding mechanisms and consequences of seizures resulting from elevated body temperature. The activation of immune mechanisms (increased inflammatory markers such as cytokines) in both fever-induced seizures and hyperthermia-induced seizures supports a link between these two models. Therefore, while true human febrile seizures are hard if not impossible to replicate in other species, animal studies can provide insights unobtainable in clinical trials.

The current study by Dubé et al. seeks to discover biomarkers that can predict outcomes of febrile seizures. If such a marker was identified, it might become possible to intervene so as to prevent or ameliorate the rare but serious instances in which febrile seizures lead to refractory epilepsy. These investigators have developed a rat model in which seizures are induced by an external heat stream using a hair dryer (6). Seizures in this model reliably occur when core temperature reaches 39.5 to 41°C, usually within 3 minutes of hyperthermia, and involve fever mediators (5). The seizures are remarkably reproducible and exhibit characteristics that suggest limbic origin, involving behavioral arrest, staring, chewing and other facial automatisms, and forepaw clonus. Seizure duration can be controlled experimentally; previous studies demonstrated that a hyperthermic seizure duration of about 24 minutes, accompanied by rhythmic discharges on EEG, leads to spontaneous seizures (epilepsy) weeks to months later (7). Using this model, it has also been shown that rats experiencing experimental hyperthermic seizures have increased production of astrocyte-derived interleukin IL-1 $\beta$  (which is also increased in human febrile seizures), exhibit T2 signal changes transiently on MRI, and show a profusion of interictal discharges on EEG, suggesting hyperexcitability and hypersynchrony of neuronal circuits (5). No neuronal death was found to explain these epileptogenic changes (8).

In the current article, the authors were driven by emerging data from the FEBSTAT study to increase the mean febrile seizure duration and generate febrile status epilepticus, then examine subsequent measures such as the occurrence, frequency, and duration of spontaneous (i.e., nonfebrile) seizures, MRI changes, and alterations of inflammatory markers. Their previous work studied experimental febrile seizures with an average seizure duration of about 24 minutes; here, they found that increasing the seizure duration to 64 minutes significantly worsened the severity and duration of subsequent spontaneous seizures. Compared with spontaneous

seizures following 24 minutes of hyperthermia, rats that had 64 minutes of hyperthermia had more abundant interictal spikes, and the amount of interictal spiking correlated with MRI T2 signal changes in the hippocampal CA1 region. However, neither interictal spikes nor T2 signal changes correlated with spontaneous seizure occurrence, suggesting that these measures are not reliable biomarkers for epileptogenesis. Furthermore, increases of the proconvulsant inflammatory cytokine IL-1 $\beta$  followed a time-dependent course following experimental febrile seizures and could potentially be used as a biomarker, though these experiments could not directly assess this hypothesis since rats had to be killed to measure IL-1 $\beta$  levels.

It should be noted that in children, a febrile seizure of either 24 minutes or 64 minutes would be considered complicated, while the 64-minute seizures qualify as febrile status epilepticus. In the imaging studies of clinical febrile seizures (9), and in the FEBSTAT study led by Shinnar (2), it appears that a minimum duration of 55 minutes is required for a febrile seizure in a child to lead to MRI signal changes and potential epileptogenesis. Therefore, it was quite reasonable in the current study for Dubé et al. to increase the seizure duration in their animal model. It should also be noted that, unlike in the animal model, assessing the exact duration of a seizure, febrile or otherwise, is difficult in clinical practice, and estimates of seizure duration by both caretakers and emergency medical personnel are notoriously inaccurate (10). That being said, 64 minutes is almost three times longer than 24 minutes, so it is not surprising that rats have worse outcome from the longer seizure.

The results presented here do not define a biomarker to use in clinical practice to identify patients at risk for epilepsy after febrile seizures. They do provide important information on hyperthermia-induced neural excitability and may offer a window into possible mechanisms (e.g., IL-1 $\beta$ ). The results also clearly implicate the limbic system as a likely site of pathological consequences of febrile seizures with a possible link to mesial temporal sclerosis.

Debate continues to rage about several aspects of seizures in the immature brain, including the minimum duration and constituents of electrographic discharges necessary to define a seizure, whether death of neurons is a prerequisite for epilepsy to develop, and what comprises an appropriate developmentally specific treatment (11). The combination of comprehensive clinical investigations such as the FEBSTAT study plus well-designed laboratory investigations such as this one by Dubé et al. will hopefully provide some resolution to these uncertainties, to the benefit of patients with epilepsy.

by Carl E. Stafstrom, MD, PhD

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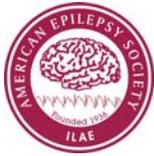
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