Expression of Circulating Muscarinic Receptors in Infants With Severe Idiopathic Life-Threatening Events

About half of the apparent life-threatening events (ALTEs) that occur among infants remain unexplained and are called idiopathic. Yet there is no biomarker associated with idiopathic ALTEs. Although the connection between ALTEs and sudden infant death syndrome (SIDS) remains controversial, the cholinergic system has been investigated in the pathogenesis of both. We investigate the $M_2$ muscarinic receptors' expression in the blood samples of infants who experienced severe ALTEs.

Methods | We prospectively enrolled 17 consecutive, previously healthy infants (age, <1 year) who had a first episode of severe ALTE, which is defined as a sudden episode that is characterized by some combination of apnea, color change, marked change in muscle tone, choking, or gagging. Severity was defined as direct admission to an intensive care unit shortly after the event. Blood samples for $M_2$ analysis were collected at admission, and the diagnostic workup started after admission. Cases with no specific diagnosis were classified as idiopathic. After the diagnostic procedure was performed for all 17 infants, it was determined that 5 had idiopathic ALTEs and 12 had ALTEs of known etiology. A third group consisted of 9 healthy control infants who had no family history of ALTE/SIDS. The study was conducted in accordance with the national regulations on medical research, and written informed consent was obtained from the infants’ parents/guardians.

Whole-blood samples were collected in PaxGene Blood RNA Tubes (Qiagen/BD). Total RNA was extracted with the PaxGene RNA kit (Qiagen); 200 ng of total RNA were reverse transcribed into complementary DNA using the iScript cDNA Synthesis kit (Biorad). $M_2$ gene expression was measured by quantitative real-time polymerase chain reaction using a specific primer for the $M_2$ receptor gene CHRM2 (F: AAGACCCCGTTTCTCCAAGT; R: GAGGCAACAGCACTGACTGA). The rabbit 18S housekeeping gene (F: CCTGCGGCTTAATTTGACTC; R: ATGCCAGAGTCTCGTTGTT) was used for normalization, and $M_2$ levels are expressed as the $M_2$:18S ratio. The samples were analyzed following a blinded procedure. Statistical significance was set at $P = .05$.

Results | The baseline characteristics of the groups of infants and the specific diagnoses for ALTEs of known cause are summarized in the Table. The median $M_2$ expression was not significantly different between the 9 healthy control infants (0.19 [range, 0.02-1.30]) and the 12 infants who experienced ALTEs of known cause (0.13 [range, 0.01-0.65]). In contrast, $M_2$ expression was significantly higher in the 5 infants who experienced idiopathic ALTEs compared with other 2 groups of infants (11.73 [range, 3.32-16.35]) ($P < .001$) (Figure).

Discussion | In this study, the average $M_2$ expression in the 5 infants who experienced idiopathic ALTEs was 20 to 50 times higher than the other 2 groups of infants (ie, the 12 infants who experienced ALTEs of known etiology and the 9 healthy control infants), with no overlapping. This finding appears to be specific and is not likely to reflect an acute-phase effect because both the infants who experienced idiopathic ALTEs and the infants who experienced ALTEs of known etiology were severe cases. It also seems unlikely that the receptors' upregulation is driven by certain diseases or treatments because all the values of $M_2$ expression in the group of infants who experienced ALTEs of known cause were consistently low and were no different than the values found in the group of healthy control infants who did not have any pathologies or treatments. These preliminary observations imply that idiopathic ALTEs and ALTEs of known etiology may have different underlying mechanistic pathways.

Another study has reported alterations in cerebral muscarinic receptors in infants who died of SIDS. Although ALTEs are generally not considered to be related to SIDS, this is based on studies that have not necessarily distinguished groups by illness severity or lack of a diagnosis. The present data, together with our previous findings of cardiac muscarinic receptors in SIDS, suggest that parasympathetic overactivity may be a common vulnerability between SIDS and severe, idio-

| Table. Summary of Baseline Data* |
|-----------------------------|-----------------|-----------------|-----------------|
| Characteristic              | Healthy Controls | ALTEs of Known Cause | Idiopathic ALTEs |
|                            | (n = 9)          | (n = 12)         | (n = 5)         |
| Age, mo                     | 3.2 (1.2)        | 3.1 (1.1)        | 2.8 (1.4)       |
| Male sex, No.               | 6               | 7               | 3               |
| Birth weight, g             | 3192 (399)       | 2996 (468)       | 3542 (424)      |
| Gestational age, wk         | 38 (0.5)         | 37 (1.8)         | 38 (0.4)        |
| Apgar score at 5 min        | 9.5 (0.5)        | 9.5 (0.4)        | 9.6 (0.5)       |
| Preterm birth, No.          | 0               | 2               | 0               |

Abbreviation: ALTEs, apparent life-threatening events.

* The differences in the baseline parameters between the 3 groups of infants are not significant.

b Of the 12 infants who experienced ALTEs of known cause, 1 had a congenital airway defect, 1 had airway obstruction, 1 had pertussis infection, 4 had severe gastroesophageal reflux, 4 had seizures, and 1 had arrhythmia.
pathic ALTEs. This is also in line with recent evidence suggesting that both syndromes could be related.

A limitation of our study is the small sample size, but severe idiopathic ALTE is a rare condition. Larger studies are needed to assess the potential clinical implications of this novel, easily detectable, circulating marker, to understand the mechanisms and develop preventative strategies.

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Evaluation of Effectiveness of Mixed Rotavirus Vaccine Course for Rotavirus Gastroenteritis

Two rotavirus vaccines—RotaTeq (RV5; Merck and Company), a 3-dose series, and Rotarix (RV1; GlaxoSmithKline Biologicals), a 2-dose series—are licensed for use in US children. The US Advisory Committee for Immunization Practices (ACIP) recommends that a rotavirus vaccine series be completed with the same product whenever possible but allows for administering mixed vaccine types if a previous dose type is not available or is unknown. In such situations, the ACIP recommends, “If any dose in the series was RV5 or the vaccine product is unknown for any dose in the series, a total of 3 doses of rotavirus vaccine should be administered.” However, the effectiveness of a mixed rotavirus vaccine series remains unclear.

We evaluated the postlicensure vaccine effectiveness (VE) of a complete 3-dose course of mixed rotavirus vaccine types according to the ACIP definition and compared these results with published VE results for the same population and time.

Methods | We collected data on children enrolled in the New Vaccine Surveillance Network from pediatric hospitals and emergency departments in Nashville, Tennessee; Rochester, New York; Cincinnati, Ohio; Seattle, Washington; Houston, Texas; Kansas City, Missouri; and Oakland, California. Each child exhibited diarrhea (≥3 episodes within 24 hours) and/or vomiting (≥1 episode within 24 hours) from December 1, 2011, through November 30, 2013. Rotavirus infections were confirmed using enzyme immunoassays and reverse transcription- polymerase chain reactions. Approval was obtained from each institutional review board, and written informed consent was obtained from each patient’s parent or legal guardian. Further details of these methods were previously published.2

Verifications of rotavirus vaccination from primary care professionals were supplemented by regional immunization information systems. Among vaccine-eligible children who had reached the maximum ACIP-recommended age for completion of the vaccine series (ie, 8 months and older)2 and who had complete, valid vaccination data, we compared rotavirus test positivity from 715 children who were unvaccinated with 75 children who had received a mixed, 3-dose course of rota-