Sudden Infant Death Syndrome Is Not Associated with the Mutation of PHOX2B Gene, a Major Causative Gene of Congenital Central Hypoventilation Syndrome

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KIJIMA, K., SASAKI, A., NIKI, T., UMETSU, K., OSAWA, M., MATOBA, R. and HAYASAKA, K. Sudden Infant Death Syndrome Is Not Associated with the Mutation of PHOX2B Gene, a Major Causative Gene of Congenital Central Hypoventilation Syndrome. Tohoku J. Exp. Med., 2004, 203 (1), 68-68 —— Sudden infant death syndrome (SIDS) is a major cause of infant death, but its etiology is unknown. There are several independent risk factors for SIDS, and prone sleeping is a major risk factor. SIDS is probably based on a compromise in arousal response to breathing or blood pressure during sleep. Congenital central hypoventilation syndrome (CCHS or Ondine’s curse) is a disorder characterized by an idiopathic failure of the autonomic control of breathing and has been regarded as one of the compromised conditions in SIDS. Recently, mutations of the PHOX2B gene have been detected in half to two-thirds of CCHS patients. We therefore analyzed the PHOX2B gene in 23 cases of SIDS and did not find any mutations, except for three polymorphic nucleotidic substitutions. The mutation of PHOX2B is thus not likely associated with SIDS. ——— sudden infant death syndrome (SIDS); congenital central hypoventilation syndrome; PHOX2B
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Sudden infant death syndrome (SIDS) is the third leading cause of infant death in Japan and its incidence is estimated as 0.3-0.5/1000 live births. SIDS is defined as the sudden death of any infant or young child that is unexpected by history and unexplained by a thorough postmortem examination, which includes a complete autopsy, investigation of the scene of death, and review of the medical history (Hunt and Hauck 2003).

The independent risk factors for SIDS are prone sleeping, maternal smoking during pregnancy, overheating, and abnormal arousal or ven-
tilatory response to hypoxemia and hypercapnia. Prone sleeping has been recognized as a major risk factor and avoidance of prone sleeping has led to a significant decrease in the SIDS rate around the world (Hunt and Hauck 2003).

SIDS is probably based on a compromise in response to blood pressure or breathing challenges during sleep. SIDS infants may have a congenital defect of arousal or cardiorespiratory control. Polymorphisms in the serotonin transporter gene (Narita et al. 2001) and mutations of the sodium-channel (Ackerman et al. 2001) or potassium-channel (Schwartz et al. 2001) gene have been reported in association with SIDS. Congenital central hypoventilation syndrome (CCHS or Ondine’s curse) is a disorder characterized by an idiopathic failure of the autonomic control of breathing and has been regarded as one of the compromised conditions of SIDS. A higher incidence of SIDS was reported among relatives of CCHS patients with accompanying Hirschsprung disease or chronic constipation (Weese-Mayer et al. 1993). Postmortem findings of SIDS infants are consistent with pre-existing chronic asphyxia. In addition, rebreathing of expired hypercapnic gases has been hypothesized as one of the major pathophysiological mechanisms underlying the risk due to prone position. SIDS and near-miss SIDS infants are considered to have a defect in hypoxic and hypercapnic response similar to CCHS (Shannon et al. 1977; Hunt et al. 1981).

Recently, it was reported that half to two-thirds of CCHS patients had mutation of the PHOX2B gene (Amiel et al. 2003; Sasaki et al. 2003). In the present study, we investigated whether the mutation of PHOX2B is associated with SIDS.

**MATERIALS AND METHODS**

This study was approved by the Ethics Committee of the Yamagata University School of Medicine. We studied 23 cases of SIDS or undetermined infant death identified by the Departments of Forensic Medicine of Osaka University and Yamagata University School of Medicine from 1999 to 2003. The main characteristics of the 23 cases were: male-to-female ratio, 11:12; age 4.5±3.7 (mean±s.d.) months. Genomic DNA was extracted from frozen tissues and subjected to PHOX2B mutational analyses. DNA specimens were also collected as controls from 50 Japanese co-workers who agreed to the study protocol. All coding regions of the PHOX2B gene including exon-intron boundaries were amplified by PCR with each set of primers: PR1F: 5’-GGATCAGGGAGAATCGTCAC-3’ and PR1R: 5’-TGTGACTAGGATGTGTAACC-3’ for exon 1; PR2F: 5’-TATGACCTGACCTTGGAGTC-3’ and PR2R: 5’-TTGCATCGCTCATCCAGTC-3’ for exon 2; PR3F: 5’-TGCTTCACCGTCTCTCACC-3’ and PR3R: 5’-GCCTCCGCGCCAGGATTCCAGTCACA-3’ for exon 3 (Sasaki et al. 2003). After introducing heteroduplexes, mutations were screened by denaturing high performance liquid chromatography (DHPLC) analysis using the WAVE DNA-fragment analysis system (Transgenomic). Exons with abnormal elution profiles on DHPLC were sequenced by dye-primer chemistry using an ABI 310 automated sequence analyzer (Applied Biosystems).

**RESULTS**

We did not detect any mutation other than three polymorphic nucleotidic substitutions: C1103T (leading to G90G innocent mutation), IVS3-36A>T and IVS3-101A>G. The gene frequencies of these polymorphic nucleotidic substitutions were 0.01, 0.01 and 0.09, respectively.

**DISCUSSION**

To investigate the relationship between SIDS and CCHS, we analyzed the PHOX2B gene, a major causative gene of CCHS, in 23 cases of SIDS. However, we did not find any mutations other than three polymorphic nucleotidic substitutions.

SIDS infants may have a congenital defect of arousal or cardiorespiratory control. The prominence of neuroepithelial bodies in the lung of the
SIDS victims suggested an impairment of central respiratory control rather than a defect in airway chemoreceptors (Gillan et al. 1989). Data are accumulating that specific genotypes link to impaired brainstem regulation of breathing or other autonomic control as a risk factor for SIDS. Several reports have described near-miss SIDS infants as having a defect in hypercarbic and hypoxic ventilatory arousal responses similar to CCHS patients (Hunt et al. 1981; McCulloch et al. 1982). The receptor tyrosine kinase RET (rearranged during transfection) proto-oncogene (RET) and the glial cell line-derived neurotrophic factor (GDNF) signaling pathways play significant roles in the development of the respiratory center. The expression of RET is probably controlled by the HASH-1, PHOX2A and PHOX2B genes, and PHOX2B also has a key role in the ontogeny of the autonomic nervous system (Brunet and Pattyn 2002). Expression of PHOX2B was observed in cranial sensory ganglia of the VIIth, IXth, Xth nerves, terminal rhombomeres 4-8 and the presumptive carotid body (Amiel et al. 2003).

Amiel et al. (2003) analyzed the PHOX2B gene in 29 patients and found that sixteen patients had heterozygous 5-9 alanine expansions within a 20-residue polyalanine tract and two patients had heterozygous frameshift mutations. Nearly all of the cases of CCHS were sporadic and could be explained by de novo mutations. Few patients showed dominant inheritance from their affected mothers. Amiel et al. (2003) speculated that a polyalanine expansion and a polyalanine contraction might occur due to unequal crossing-over during meiosis.

In summary, we analyzed the PHOX2B gene as a candidate of SIDS but did not find any mutation. It is therefore conceivable that the mutation of PHOX2B is likely not associated with SIDS. One-third to half of CCHS patients do not carry the mutation of the PHOX2B gene (Amiel et al. 2003; Sasaki et al. 2003). We cannot completely deny the possibility that SIDS subjects might have defects in hypoxic and hypercapneic response similar to those in CCHS. The genes involved in the formation of the respiratory center should be studied as candidates for SIDS.

References


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