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COMMENTARY

Structural Abnormalities in the Brainstem and Cerebellum in Congenital Central Hypoventilation Syndrome

Commentary on the article by Kumar *et al.* on page 275

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Congenital central hypoventilation disorder (CCHS) is a clinically complex disorder characterized by ventilatory failure to respond to hypercapnia during sleep, autonomic dysfunction, and affective abnormalities (1,2). A major advance in our understanding of CCHS is the discovery that mutations in the PHOX2B gene, important in autonomic development, cause the majority of the cases (3). Yet, further advances in our understanding of CCHS and how the gene defect specifically results in respiratory, autonomic, and emotional dysfunction will depend on determination of the underlying neuropathology. This goal has been elusive in large part because of the rarity of clinically well-characterized cases that come to autopsy, and the lack of analysis of autopsied CCHS brains with modern quantitative and neurochemical techniques. In the few instances of examination of autopsied CCHS brains, abnormalities of the ventral medullary surface, *i.e.*, absence or hypoplasia of the arcuate nucleus, have been reported (4). The arcuate nucleus is postulated to contain neurons and/or glia which are the human homologue of the respiratory chemosensitive fields for carbon dioxide monitoring in cats and rodents (5,6). In addition, neuronal loss and gliosis in the reticular formation of the medulla oblongata has been described in autopsied CCHS infants (7). A major strategy to elucidate brain abnormalities in CCHS is the use of magnetic resonance imaging (MRI), but its standard (nonfunctional) application fails to demonstrate subtle or structural brainstem and/or forebrain differences between children with CCHS and controls (1). The application of hypoxic, ischemic, and cold pressor challenges in CCHS patients by Dr. Harper's group in the past, however, indicated *functional* MRI changes, notably within limbic, cerebellar, and midbrain regions, the latter broadly defined (8,9). T2 relaxometry procedures suggested damaged regions in limbic and cerebellar regions (8). The application of MRI techniques with homeostatic challenges was also an important step in the definition of CCHS neuropathology but it begged the question of why these methods failed to demonstrate putative long-standing pathology throughout the brainstem—a structure that the clinical presentation strongly suggested was at fault. Dr. Harper's group has repeatedly emphasized the crucial role of forebrain structures in the modulation of brainstem-mediated respiratory and autonomic control; nevertheless, the seemingly lack of structural pathology in brainstem sites critical to chemosensitivity to carbon dioxide, *e.g.*, ventral medullary surface (5,6,10), arousal to carbon dioxide, *e.g.*, rostral raphé (11,12), and autonomic regulation, *e.g.*, ventrolateral medulla, in CCHS was puzzling. Dr. Harper's group, however, was aware of the technical limitations in imaging the human brainstem with its well-recognized problems in spatial resolution and sensitivity (1). This group is to be applauded for keeping abreast of the technical advances in human brainstem neuroimaging and now providing us novel and important insight in the role of brainstem pathology in CCHS with the use of today's state-of-the-art technology, *i.e.*, diffusion tensor imaging (DTI) with comparison of axial and radial diffusivity maps (1).

In a study of 25 adolescent cases of CCHS (15.2 ± 2.4 y) compared with 26 controls (15.5 ± 2.4 y), Kumar *et al.* (1) report major structural abnormalities in the brainstem and cerebellum of the CCHS cases based on the superimposition of axial and radial diffusivity maps. Both of these maps depend on the directional diffusivity of water molecules, with their relationship to cellular structural injury established in experimental models, as reviewed in-depth by Kumar *et al.* (1). Axial diffusitivity, whereby water molecules diffuse parallel to fibers, is considered sensitive to axonal injury; radial diffusitivity, on the other hand, measures the diffusion of water molecules parallel to fibers, and is considered sensitivity to myelin injury (1). Of critical importance to understanding structural pathology in CCHS (and potentially multiple other neurologic disorders) is that DTI reveals abnormalities that are not apparent by conventional MRI. In CCHS, Kumar *et al.* now report with DTI a composite of neuropathologic brainstem changes that involve the lateral medulla, basis pontis, dorsal midbrain, oculomotor nuclei, periaqueductal gray, rostral raphé, and decussation of the superior cerebellar peduncle in the caudal midbrain (1). They also report injury in cerebellar structures, *i.e.*, cerebellar cortex and deep (roof) nuclei and superior and inferior cerebellar peduncles (1). Although this group has reported certain forebrain and limited brainstem regions involved in CCHS with the use of MRI in patients with hypoxic, hypercapnic, or cold pressor stressors (1,8,9), the sophisticated

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Abbreviations: CCHS, congenital central hypoventilation syndrome; **DTI,** diffusion tensor imaging

DTI analysis implicates previously unrecognized and specific structures with "chronic" injury, as opposed to dysfunction only, in the pathogenesis of CCHS.

Like all ground-breaking studies, the study by Kumar *et al.* (1) raises more questions than it answers. First, what is the relationship of the delineated brainstem and cerebellar pathology in the adolescent CCHS cases to the PHOX2B gene? In this regard, a major limitation of this study is that genotyping was performed or technically successful in only 4/12 (30%) of CCHS cases (1). Moreover, it is uncertain in the report if the structural pathology occurs in the CCHS cases with the PHOX2B gene or if there are PHOX2B and non-PHOX2B subsets with different underlying pathologies. Moreover, knockout PHOX2B mice demonstrate in the central nervous system an underpopulation of noradrenergic neurons in the locus coeruleus and cellular anomalies of the nucleus of the solitary tract, *i.e.*, abnormalities which require microscopic examination, including with neurotransmitter-specific immunocytochemistry, to detect (13). Of note, the locus coeruleus and nucleus of the solitary tract are both regions known to be chemosensitive to carbon dioxide (14,15). The murine abnormalities in the locus coeruleus and nucleus of the solitary tract are potentially below the resolution of DTI and still may occur in the present CCHS cases, but autopsy confirmation is required. In addition, cerebellar abnormalities as extensive as that demonstrated by DTI in this series of CCHS patients have not been reported in the PHOX2B knockout mouse, necessitating return to microscopic examination of the knockout's cerebellum to ensure there are in fact no cerebellar abnormalities by quantitative, cellular, and/or molecular analysis. Clearly, the potential discrepancies between the findings in the CCHS cases here and that of mice with PHOX2B mutations need to be resolved to understand fully the role of the PHOX2B gene in brainstem and cerebellar pathology in CCHS.

The second question is what is the role(s) of the affected structures in the CCHS cases reported here in chemosensitivity to carbon dioxide, respiratory drive, autonomic control, and/or affective behavior? The authors highlight current knowledge about these structures that are relevant to the clinical constellation of abnormalities CCHS (1), but it is important to emphasize that several of these structures have never before been considered to play a role in CCHS, including the chemosensitive regions of the ventrolateral medulla (16), rostral raphé (11,12), and fastigial (roof) nucleus of the cerebellum (17), or the "defense" region of the periaqueductal gray (18). The involvement of multiple brainstem and cerebellar regions known to regulate responses to carbon dioxide suggest the possibility that CCHS involves a network of critically-related chemosensitive sites, rather than one specific site, which are likely to mediate different aspects of central chemosensitivity (*e.g.*, 10), and that linked together in a potential network, exert effects on the "defense" network of autonomic, respiratory, vocal, and affective (anxiety/fear) responses to threat that are coordinated by the periaqueductal gray and possibly rostral limbic and hypothalamic sites (18). Third, what is the neurochemical, cellular, and molecular pathology in the brainstem and cerebellar regions affected in the adolescent CCHS cases? Is it because of a lack of fetal development of these regions in which this development is altered in some way by one or more genes related to the cellular proliferation, migration, and/or different ion and/or axonal outgrowth of the affected sites which occurs or begins during gestation? Or is it because of acquired axonal/myelin degeneration from as yet undetermined insults to the affected neuronal populations? Fifth, how does the brainstem and cerebellar pathology interrelate in individual cases with forebrain abnormalities that reported by this group by MRI techniques (8,9)? The sixth but certainly not the final question is, at what age do the findings reported by Kumar *et al.* (1) in the adolescent CCHS brain arise in the course development? It is critical to determine whether these findings are present at birth or in early infancy, and if they become more severe with age, as suggested in the adolescent brain in this study, to establish the sequential development of the pathologic changes.

Because of the work of Harper's group, we now know where to focus our efforts in future brain research in CCHS. The present study raises new questions for testing in clinical trials, animal models, and autopsy studies: is, for example, CCHS the result of a defect in a network of multiple brainstem and cerebellar sites with different roles in central chemosensitivity (and autonomic function) that "funnel" their effects through the periaqueductal gray, the brainstem integrator of defense responses? The generation of new hypotheses are the very best that can come from state-of-the-art studies of CCHS such as the present study as the forthcoming questions will hopefully stimulate new approaches to drug and other treatments for this debilitating disorder.

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