
Peer Review File

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Reviewer Comments

The aim of this study, according to the authors, was to expand current knowledge of clinical and genetic features of neonates with CCHS and provide data on the genotype-phenotype correlation. In order to achieve this goal the authors collected data on 14 CCHS cases from their institution and also reviewed previously published neonatal onset cases. Clinical and genetic data were analyzed.

This paper may have some important additional information especially by enlarging the experience with CCHS and providing data from China, albeit, restricted to a specific area in this huge country. Nevertheless, their revision of already published data is of very limited additional value. I suggest that they concentrate only on their population, add information from other area in China, if available and omit their analysis of cases they retrieved from the already published cases in the literature. In the present form, the paper is not ready for publication.

The manuscript is poorly written and requires major revisions in order to meet basic standards for a scientific paper. I also suggest that a professional whose native language is English will correct language.

Reply: Thank you for your constructive comments in this round of review. CCHS is a rare condition with an estimated incidence of 1 in 148,000 to 200,000 live births. In this study, we conduct a retrospective analysis of neonates with genetic confirmed CCHS evaluated at Children's Hospital of Fudan University, a national children's medical center and a major tertiary referral center for critical infants in China. More than 8000 cases of critically ill newborns are treated per year in our hospital, including patients from other area in China (Fujian Province, Yunnan Province, Shandong Province, Xinjiang Province etc.). In 2016, the "China neonatal genomes project" was launched by our center. A total of 98 units across mainland China participated in the plan, and formed a multi-center research network targeting rare neonatal diseases. By 2020, a total of 12,560 critically ill newborns had been tested for genetic sequencing and 1,486 cases of rare genetic diseases had been diagnosed. Accordingly, we believe that the population in this study is representative to some extent.

To date, neonatal cases of CCHS with both detailed clinical and genetic information are relatively rare, so we retrieve them from the existing literature and carry out the literature review to get a better understanding of genotype-phenotype correlation.

We are so sorry for our poor English and the manuscript has been carefully revised.

Introduction

The authors state that "hypoventilation and the absence of a chemoreflex to carbon dioxide and is frequently accompanied by Hirschsprung disease (HSCR), neuroblastoma and dysregulation of the autonomic nervous system (3)".

Obviously a chemoreflex is not "absent" and "frequently" is a vague term. The rate of HSCR depends on the mutation, so are other manifestations such as sinus pauses etc. This should be clearly presented with numbers and references. Similarly, the meaning of "frequently accompanied by dysregulation of the autonomic nervous system" is vague and does not provide the reader with any idea what stands behind this statement.

Reply: Thanks for your suggestion. Based on the extensive review of the literature, we have given a more accurate and detailed description. (see Page 5, line 9-13)

Line 94: However, there are a limited number of studies detailing clinical and genetic findings in neonates.

I disagree. There are many papers, reviews and conferences reports "detailing clinical and genetic findings". Many do relate to the early presentation. Most cases manifest clinically during the first days or weeks of life and other reports and reviews approach this presentation. For example: Basu SM, Chung FF, AbdelHakim SF, Wong J. *Anesth Analg.* 2017 Jan;124(1):169-178, Sandoval RL, Zaconeta CM, Margotto PR, Cardoso MT, França EM, Medina CT, Canó TM, Faria AS. *Rev Paul Pediatr.* 2016 Sep;34(3):374-8, Marion TL, Bradshaw WT. *Neonatal Netw.* 2011 Nov-Dec;30(6):397-401, Patwari PP, Carroll MS, Rand CM, Kumar R, Harper R, Weese-Mayer DE. *Respir Physiol Neurobiol.* 2010 Oct 31;173(3):322-35, Weese-Mayer DE, Rand CM, Berry-Kravis EM, Jennings LJ, Loghmanee DA, Patwari PP, Ceccherini I. *Pediatr Pulmonol.* 2009 Jun;44(6):521-35 and more. See also Line 188 in this manuscript.

Reply: Thanks for your carefully reading. As you mention, there are papers reporting early presentation, however, cases with detailed clinical (including laboratory tests findings) and genetic information are relatively rare, so we provide a review of the

literature in the manuscript.

According to your suggestion, we reviewed the papers mentioned above.

Basu SM, Chung FF, AbdelHakim SF, Wong J. *Anesth Analg*. 2017 Jan;124(1):169-178,

In this paper, a newborn with a brief clinical information was presented, and we could not find the genetic information in the reference paper “Epidural analgesia in a newborn with Hirschsprung’s disease, associated with congenital central hypoventilation syndrome”.

Author, Year	Gender	Age (y)	Clinical Presentation	Diagnosis	Surgical Procedures	Anesthetic Technique	Clinical Outcome
Coccagna et al 1984 ¹⁵	M	8	Bronchopneumonia associated with severe respiratory insufficiency	CCHS based on PSG findings	Cardiac catheterization and later cerebral angiography	Ketamine sedation, TIVA for cerebral angiography	Serious respiratory depression followed ketamine sedation
Wiesel and Fox 1990 ¹⁶	M	59	Polycythemia. Pulmonary hypertension. Cor pulmonale.	Clinical diagnosis of CCHS	Anterior resection of carcinoma of bowel	Awake lumbar epidural + GA: thiopental, succinylcholine, isoflurane, N ₂ O, O ₂ , vecuronium, sufentanil. Epidural morphine	Repeated reintubations for drowsiness, confusion, and agitation. Epidural with bupivacaine, without opiates. Discharged 27 days postoperatively.
Brouwers et al 2000 ¹⁷	M	Neonate	Admitted to neonatal ICU for mechanical ventilation due to respiratory failure	Haddad syndrome	Explorative laparotomy d2 of life for HD. Stoma revision d10, at 6 wk re-exploration, colectomy at 8 mo	GA with sevoflurane/N ₂ O/O ₂	First 2 surgeries: postoperative continuous IV morphine infusion. Severe postoperative respiratory depression. Subsequent surgeries postoperative epidural bupivacaine only. No postoperative ventilation required.

Sandoval RL, Zaconeta CM, Margotto PR, Cardoso MT, França EM, Medina CT, Canó TM, Faria AS. *Rev Paul Pediatr*. 2016 Sep;34(3):374-8,

In this paper, a newborn diagnosed with CCHS associated with Hirschsprung’s disease was reported, however, we are failed to find the molecular diagnosis. The genetic information is described as follow:

The presence of a mutated allele in the *PHOX2B* gene was demonstrated by the molecular technique of polymerase chain reaction, but the technique used does not quantify the number of polyalanine expansions, as it only identifies the presence of above than normal expansions. However, it seems to be the most severe phenotype, due to the onset of symptoms in the neonatal period and the association with Hirschsprung’s disease. Approximately 87–100% of the

Marion TL, Bradshaw WT. *Neonatal Netw*. 2011 Nov-Dec;30(6):397-401,

This paper provides an overview of CCHS and discuss the implementation and value of genetic screening to assist in the diagnosis. We are unable to find any clinical and genetic information of a specific patient.

Patwari PP, Carroll MS, Rand CM, Kumar R, Harper R, Weese-Mayer DE. *Respir Physiol Neurobiol*. 2010 Oct 31;173(3):322-35,

This paper mainly discusses control of breathing within the complex physiological network of the autonomic nervous system. We are unable to find any clinical and genetic information of a specific patient.

Weese-Mayer DE, Rand CM, Berry-Kravis EM, Jennings LJ, Loghmanee DA, Patwari PP, Ceccherini I. *Pediatr Pulmonol*. 2009 Jun;44(6):521-35

This paper is a review article and we are unable to find any clinical and genetic information of a specific patient.

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Methods

Line 109: study. Inclusion criteria consisted of: (1) patients with a diagnosis of CCHS based on clinical symptoms in keeping with the Statement of American Thoracic Society and molecular analysis of the *PHOX2B* gene (5); (2) patients with symptoms suggestive of hypoventilation and identification of pathogenic variants in *PHOX2B* gene.

I fail to understand the difference between criteria 1 and 2. Both are molecular diagnosis due to clinical presentation. Criteria 1 mentions ATS statement which is not part of criteria 2. The authors should clarify the criteria and be very specific.

Reply: Thanks for your suggestion. According to the Statement of ATS in 2010, diagnosis of CCHS is established based on: (1) clinical findings of alveolar hypoventilation and autonomic nervous system dysregulation in the absence of primary pulmonary, cardiac, or neuromuscular disease, or a causative brain stem lesion that can account for the entire phenotype and (2) identification of a pathogenic variant in *PHOX2B*. However, diagnosis is confounded in a critically ill neonate without typical periodic alveolar hypoventilation or when the patient is complicated with other conditions predispose to respiratory insufficiency (like MAS, HIE), especially in premature newborns. So our inclusion criteria consisted of 2 groups: (1) patients with typical clinical findings and identification of a pathogenic variant in *PHOX2B*, (2) patients with suspected clinical findings and identification of a pathogenic variant in *PHOX2B*. To avoid ambiguity, we have modified our expression. (see Page 6, line 21 to 25)

It is important to know where in China is the Children's Hospital of Fudan University located,

what is the population, what is the size of population that this hospital serves as a referral center, were other CCHS cases diagnosed in neighbor hospitals?

Reply: Thanks for your suggestion. Children's Hospital of Fudan University, is a national children's medical center and a major tertiary referral center in east of China. More than 8000 cases of critically ill newborns are treated per year in our hospital, and among them about 1000 patients were transferred from other area in China (Fujian Province, Yunnan Province, Shandong Province, Xinjiang Province etc.). In 2016, the "China neonatal genomes project" was launched by our center. A total of 98 units across mainland China participated in the plan, and formed a multi-center research network targeting rare neonatal diseases. By 2020, a total of 12,560 critically ill newborns had been tested for genetic sequencing and 1,486 cases of rare genetic diseases had been diagnosed. In this study, a few CCHS cases were diagnosed in other units. We have added related information (see Page 7, line 4 to 6)

The authors "classified HSCR cases as either long-segment HSCR (aganglionosis extended to above the splenic flexure or total colonic form) or short-segment HSCR (left-colonic or rectosigmoid forms)".

They should be aware that while in "classical" HSCR that is not associated with CCHS, the lack of ganglions in the colon defines the disease and diagnosis, this is not the case with HSCR in CCHS. In CCHS case, HSCR may clinically occur in spite the finding of ganglions in biopsies. The clinical presentation of HSR in CCHS results not only from the absence of ganglions, but also from their dysfunction, i.e. from functional deficiency. So HSCR may very well present without negative biopsy (presence of ganglions) – "clinical HSCR". Moreover, the functional deficiency may be manifested as gut dysmotility that may involve long and large segments of the entire gut from the esophagus to the sigmoid section and the presence of ganglions does not rule out HSCR. Also surgical resection of a non-ganglionic colon may not resolve clinical HSCR due to the functional deficit.

Reply: Thanks for your carefully reading. We classified HSCR cases according to the classification method in a previous study published in *Journal of Pediatric Surgery* (Broch A, Trang H, Montalva L, et al. Congenital central hypoventilation syndrome and Hirschsprung disease: A retrospective review of the French National Registry Center on 33 cases. *J Pediatr Surg.* 2019;54(11):2325-30). And we find similar classification method in other literature (PMID: 31791203). On the other hand,

according to the latest review in GeneReviews, patients with CCHS may have neurocristopathy, including HSCR, severe constipation and esophageal dysmotility/dysphagia. Severe constipation is a hallmark of the gastrointestinal phenotype among individuals with CCHS who do not have Hirschsprung disease. The prevailing theory is that ganglion cells are present but their function may not be entirely normal (variant Hirschsprung disease). In our study, we focused on patients with a diagnosis of Hirschsprung disease, and cases with variant HSCR were included to see if a correlation existed between the genotype and the extent of HSCR.

Results

Line 167. All the patients underwent chest radiograph and seven (50.0%) of them showed abnormal.

A rate of 50% abnormal findings in chest radiography requires that the authors provide details. The reader does not understand from this statements what were the findings and whether they were related to CCHS.

Reply: Thanks for your suggestion. Detailed chest radiography findings were provided in Table 1. This data showed that CCHS patients may be complicated by other conditions predispose to respiratory insufficiency that make the diagnosis confused and molecular diagnosis is necessary.

Line 169. one had variant HSCR.

Please explain "variant HSCR"

Reply: Thanks for your question. "variant Hirschsprung disease" is a collective term for several different pathological disorders with Hirschsprung disease-like symptom but do not meet the histological diagnostic criteria for HSCR. In these conditions, ganglion cells are mostly present while abnormal in number and distribution. (PMID: 27988850). We have added the definition (see Page 9, line 3).

Line 170. Three patients (21.4%) had neurological complications of seizure.

What were the causes of seizures? Were these secondary to brain hypoxia or primary presentations? This is of major importance since delayed diagnosis and treatment have been related brain damage and seizure disorders (see reports from Japan in cases where invasive ventilation was delayed).

Reply: Thanks for your suggestion. This topic has been talked about in the discussion part. Seizures have been observed in other cases from literature, and it is unclear whether this is due to hypoxemia or a direct result of the primary neurologic problem associated with CCHS, which needs more research.

Line 173. No abnormalities in heart rate variability were reported.

Please specify how evaluated and confirmed since this is not mentioned in the Methods section.

Reply: Thanks for your question. In our NICU, patients are monitored by ECG 24 hours per day and abnormal findings will be recorded. Patients with abnormalities in heart rate will be provided further 24-hour Holter.

Line 177. Thirteen guardians (92.9%) elected withdrawal of treatment. Detailed clinical and laboratory tests findings of the 14 cases can be found in Table 1.

This is most amazing, disturbing and intriguing information that needs major explanation and discussion. Many questions should be addressed. Why? Have parents been informed on the outcome of CCHS patients? Were treatment options been offered? Is home ventilation provided by public health organization? Is there a cost? Is the hospital situated in an area where only one child per family is allowed? Are decision to withhold life-saving treatment in China are the priority of parents? Do doctors have to follow parents' decisions even when they disagree? Since all cases were ventilated, did withdrawal of treatment resulted in babies just dying of failure to adequately breath and suffocation? The large number of cases where information is marked as NA (not available) requires explanation since outcome is most important under this extraordinary situation.

Reply: Thanks for your carefully reading. This topic has been talked about in the discussion part (page 16, line 7 to page 17, line 4). As many guardians elected withdrawal of treatment and left hospital, there were obstacles for us to get patients' outcome. But we speculate that most of them would be died as cases with neonatal-onset CCHS are in such a critical condition.

Line 185. Parents were analyzed in 6/14 cases and none PHOX2B pathogenic variants were identified.

Were the parents of the siblings (4&12) studied for being mosaics?

Reply: No, we are so sorry that the parents refused further investigations.

Line 192. with our cohort of 11 patients.

14 or 11? Please explain 11.

Reply: Thank you for pointing this out. We are sorry for the mistake and we have corrected it in the manuscript (see Page 9, line 24).

There is interesting information in this manuscript. I suggest that the authors rewrite this paper concentrating on their 14 cases, add missing important information both for the cases and their outcome and in relation to Chinese population (including socio-economic, geographic, health and public health facilities. Medico-legal data etc.). I suggest to omit all analysis of the literature from the study but use this information to compare their cohort to other Chinese experiences (as long as available) and to the information from the literature, i.e. to use already published data as reference and to highlight their special and extraordinary findings in relation to other places. They may do this using a table in the Discussion. This may provide important additional information on CCHS worldwide.

Hence, I am not going into details of the Discussion section. The Discussion should not be just a summary of CCHS issues or repeat of findings mentioned in the Results section, but a critical comparison with explanations and suggestions for possible differences from previous reports and reviews. If the authors accept my suggestion, then comparison between their cohort and other relevant publication may indeed be a part of the Discussion emphasizing the similar and differences between their findings and information from other areas in China and other countries. This is the only way I see that the paper might be of significant addition to the present knowledge.

Reply: Thanks for your excellent comments. As CCHS is an extremely rare disease, much work will be done in the future. We will try to establish a national CCHS center to aggregate the rare disease patients and do continuous investigation to enhance knowledge about clinical characteristics, genotype, management, and outcome of this disease and improve patient care and prognosis finally.