RESEARCH ARTICLE

AMERICAN JOURNAL OF medical genetics PART A

# Respiratory System Involvement in Costello Syndrome

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Manuscript Received: 5 June 2015; Manuscript Accepted: 16 March 2016

**Costello syndrome (CS) is a multisystem disorder caused by heterozygous germline mutations in the *HRAS* proto-oncogene. Respiratory system complications have been reported in individuals with CS, but a comprehensive description of the full spectrum and incidence of respiratory symptoms in these patients is not available. Here, we report the clinical course of four CS patients with respiratory complications as a major cause of morbidity. Review of the literature identified 56 CS patients with descriptions of their neonatal course and 17 patients in childhood/adulthood. We found that in the neonatal period, respiratory complications are seen in approximately 78% of patients with transient respiratory distress reported in 45% of neonates. Other more specific respiratory diagnoses were reported in 62% of patients, the majority of which comprised disorders of the upper and lower respiratory tract. Symptoms of upper airway obstruction were reported in CS neonates but were more commonly diagnosed in childhood/adulthood (71%). Analysis of *HRAS* mutations and their respiratory phenotype revealed that the common p.Gly12Ser mutation is more often associated with transient respiratory distress and other respiratory diagnoses. Respiratory failure and dependence on mechanical ventilation occurs almost exclusively with rare mutations. In cases of prenatally diagnosed CS, the high incidence of respiratory complications in the neonatal period should prompt anticipatory guidance and development of a postnatal management plan. This may be important in cases involving rarer mutations. Furthermore, the high frequency of airway obstruction in CS patients suggests that otorhinolaryngological evaluation and sleep studies should be considered.** © 2016 Wiley Periodicals, Inc.

**Key words:** Costello syndrome; respiratory; pulmonary; upper airway; *HRAS*; obstructive sleep apnea

**How to Cite this Article:**
Gomez-Ospina N, Kuo C, Ananth AL, Myers A, Brennan M-L, Stevenson DA, Bernstein JA, Hudgins L. 2016. Respiratory system involvement in Costello syndrome.
Am J Med Genet Part A 170A:1849–1857.

## INTRODUCTION

Costello syndrome (CS) is one of the RASopathies, a group of conditions caused by germline mutations in genes that encode components of the Ras/mitogen-activated protein kinase (RAS/MAPK) pathway [Rauen, 2013]. Costello syndrome is caused by a heterozygous germline mutation in the *HRAS* proto-oncogene, a small guanosine nucleotide-bound GTPase with a central role in this pathway [Aoki et al., 2005]. Gain-of-function mutations in *HRAS* cause sustained activation of the HRAS protein, resulting in increased pathway activation. Because the RAS/MAPK pathway governs cell proliferation and differentiation, prolonged activation results in the abnormal development of multiple organ systems including the heart, brain, skin, and connective tissue.

Prenatal features of CS include prematurity, lymphatic dysplasia, macrosomia, and fetal arrhythmias [Myers et al., 2014]. In the neonatal period, severe feeding difficulties, hypotonia, craniofacial dysmorphism, and cardiac, musculoskeletal, and dermatological anomalies can be seen. The facial dysmorphism has been described as coarse facies with prominent epicanthal folds, full nasal tip, fleshy ear lobes, and a wide mouth with full lips. The cardiac abnormalities most commonly consist of supraventricular tachycardias, hypertrophy, and pulmonic valve stenosis [Lin et al., 2011]. The typical dermatologic findings include cutis laxa, curly hair, acanthosis nigricans, papillomas, and loose thick skin on the dorsum of the hands and feet [Nguyen et al., 2007]. Developmental delay and moderate to severe intellectual disability are prevalent.

Conflicts of interest: None.

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Article first published online in Wiley Online Library (wileyonlinelibrary.com): 22 April 2016
DOI 10.1002/ajmg.a.37655

Among patients with *HRAS* mutations, approximately 80% have a p.Gly12Ser substitution [Gripp et al., 2006; Kerr et al., 2006]. The clinical characteristics in CS patients with this mutation are thus considered the most typical presentation. A growing body of evidence suggests that less common mutations can result in attenuated or more severe phenotypes. Specifically, the p.Ser89Cys and p.Thr58Ile mutations have been linked to a milder phenotype [Gripp et al., 2012a,b] while the p.Gly12Cys, p.Gly12Asp, p.Gly12Glu, and p.Gly12Val mutations have been associated with an unusually severe course and early lethality [Lo et al., 2008; Kuniba et al., 2009; Burkitt-Wright et al., 2012; Lorenz et al., 2012; Weaver et al., 2014].

Respiratory symptoms have been documented in individual cases, but respiratory involvement is not included as a typical or distinctive finding in CS. A recent study of the perinatal features of the RASopathies reported an estimated incidence of 63% of respiratory distress in CS newborns [Myers et al., 2014]. Here, we report clinical and molecular data of four unrelated patients with CS with prominent respiratory system involvement. We describe the clinical spectrum of respiratory symptoms and their frequency in a series of four patients and an additional 73 from the literature.

## MATERIALS AND METHODS

Approval was obtained from an institutional review board for a retrospective chart review. We reviewed the records of eight patients with a diagnosis of CS in the medical genetics database. These were evaluated at the authors' institution between 2001 and 2015. Two patients were excluded for lack of adequate documentation of the neonatal course. Two additional patients were excluded because of uncertainty of the diagnosis and absence of *HRAS* mutations. For the four remaining patients, we extracted information on the pre-, neo-, and postnatal courses including comorbidities and interventions.

We performed a PubMed search using the phrase "Costello syndrome" (Title/Abstract) limited to publications on humans and written in English, French, or Spanish. This search identified 254 articles published between 1991 and November 2014. All articles were reviewed for a description of clinical course in the neonatal period and descriptions of respiratory tract involvement at any age. For all institutional and literature patients, we recorded gestational age, type of respiratory symptoms, clinical course, mutations, autopsy, and pathology findings if available. We included articles prior to 2006 even if molecular diagnosis was not available.

## CLINICAL REPORTS

### Patient 1

Patient 1 was the second born male of two unrelated Mexican parents. The prenatal history was remarkable for polyhydramnios, mild ventriculomegaly, and soft tissue thickening. The mother was treated with betamethasone and received indomethacin and ampicillin for preterm labor. The proband was delivered at 32 +5/7 weeks via vaginal vertex delivery. Apgar scores were 7 at both 1 and 5 min for poor tone and color.

The patient's gestational age-adjusted birth measurements were weight 2,870 g (99th centile), length 46 cm (88th centile), and head circumference 35 cm (100th centile, $Z = 3.34$). Immediately after birth, he had persistent cyanosis with oxygen saturation of 70%, requiring supplemental oxygen and non-invasive positive pressure ventilation (NIPPV). Initial exam showed an edematous newborn with dysmorphic features (wide nasal bridge, full lips, hypoplastic nails, and wide upturned nose). Postnatal imaging studies confirmed mild ventriculomegaly and revealed hydronephrosis and mild pulmonary edema. Laboratory abnormalities were significant for hypoglycemia. Subsequent physical exam revealed loose skin and truncal hypotonia. Cardiac monitoring identified atrial ectopic beats, but echocardiogram showed normal cardiac anatomy. Together, the history and physical exam suggested a diagnosis of CS. *HRAS* sequencing showed the common p.Gly12Ser substitution.

The patient had a prolonged hospitalization after birth (53 days) due to frequent apneic spells and feeding difficulties. Beyond the neonatal period, he required multiple hospitalizations for respiratory distress with persistent stridor and demonstrated apnea with desaturations. Multiple laryngoscopies showed severe laryngomalacia with short aryepiglottic folds and bronchoscopies showed congenital subglottic stenosis, and an elliptical and narrow cricoid cartilage. Tracheomalacia was noted. He underwent laryngotracheal reconstruction, repeated supraglottoplasty procedures, and adenotonsillectomy. His stridor, apneic episodes, and sleep-disordered breathing improved with the surgeries but persisted. Multiple postsurgical polysomnography (PSG) studies were consistent with moderate-severe obstructive sleep apnea (OSA) (with Apnea Hypopnea Index (AHI) ranging from 9 to 12.7). He had documented hypoxemia during sleep even in the absence of documented apneas, confirming chronic respiratory insufficiency. A brain MRI at 2 years of age showed stable mild ventriculomegaly and Chiari I malformation. At 4 years, he continues to have moderate to severe snoring and exhibits obstructive symptoms.

### Patient 2

Patient 2 was the second born male of two unrelated Mexican parents. The prenatal history was remarkable for biventricular hypertrophy, short long bones, echogenic kidneys, and polyhydramnios. The mother received indomethacin for preterm labor. He was delivered via C-section for non-reassuring fetal heart tones and non-reactive stress test at 34 weeks gestation. At birth, he had respiratory distress requiring intubation.

Gestational age-adjusted birth weight was 2,688 g (86th centile), length 42 cm (14th centile), and head circumference 34.5 cm (94th centile). Initial exam revealed coarse facial features, redundant nuchal skin, skin edema, and a nevus sebaceous on the scalp. Postnatal ECHO confirmed the presence of mild concentric left ventricular hypertrophy in addition to redundant mitral valve leaflets but showed good biventricular function (LVEF 66%). An EKG showed ectopic atrial tachycardia. A kidney ultrasound showed left kidney hydronephrosis. Skeletal survey showed gracile bones with flexion contracture deformities in the hands. Given the combination of pre- and postnatal findings, a diagnosis of CS was considered. *HRAS* sequencing showed a c.35G>A pathogenic variant resulting in a p.Gly12Asp substitution.

The patient failed multiple extubation attempts secondary to severe apnea, desaturations, and hypercapnia. In addition, he was noted to have tracheomalacia and redundant tissue in nasopharynx. Postnatal chest radiographs showed diffuse granular bilateral pulmonary opacities. His condition remained tenuous during hospitalization for respiratory failure. He also had persistent hypoglycemia and severe feeding difficulties. Despite good ventricular function initially, his cardiac function worsened with time and gradually developed severe biventricular hypertrophy with impaired diastolic function, severe right ventricular hypertension, and pulmonary hypertension. He continued to deteriorate despite maximal support. He died at 2 months of age.

### Patient 3

Patient 3 was a Caucasian female first evaluated at our institution at 3 weeks of age for dysmorphic features and a history of failure to thrive. The pregnancy was reportedly uncomplicated. She was born at 38 +4/7 weeks gestational age. Her birth weight was 3,884 g (gestational age-adjusted 92nd centile). Apgar scores were 5 and 9 at 1 and 5 min, respectively. The patient was discharged from the hospital at age 3 days without mention of respiratory complications. At 3 weeks of age, the patient was hospitalized for feeding difficulties and failure to thrive. Examination showed redundant skin of the palms and soles and dysmorphic facial features raising the possibility of CS. *HRAS* sequencing was positive for the p.Gly12Ser mutation.

After the initial evaluation, the patient subsequently underwent gastrostomy tube placement and was diagnosed with chaotic atrial tachycardia, multiple dermatological disorders (xerosis, keratosis pilaris, ulerythema ophryogenes), and transient nystagmus. She was diagnosed with severe asthma at 4 years of age. A Chiari I malformation with syringohydromyelia in the upper cervical spine was discovered as part of an evaluation for new-onset left-sided dystonia when she was 5 years old. During a follow-up visit at 8 years of age, symptoms of abnormal breathing, snoring, and frequently night-time awakenings prompted a polysomnogram that showed an AHI of 23.8. This was consistent with severe obstructive sleep apnea syndrome and NIPPV was instituted. Her pulmonary evaluation revealed a history of chronic, daily productive cough, and mild centrilobular bronchiectasis was noted on chest CT that was not due to allergic bronchopulmonary aspergillosis. She was managed with daily chest physiotherapy and showed improvement.

### Patient 4

Patient 4 was a South Asian Indian female evaluated in our neonatal intensive care unit on day of life one. Prenatal history was significant for polyhydramnios. She was born at 36 +6/7 weeks gestational age by vaginal delivery. Apgar scores were 5 and 7 and NIPPV was placed in the delivery room due to poor respiratory effort. She was intubated upon admission to the neonatal intensive care unit for worsening respiratory distress. Gestational age-adjusted birth weight was 3.35 kg (60th centile), length 46 cm (5th centile), and head circumference 34.5 cm (59th centile).

The patient had a prolonged hospitalization of 78 days due to persistent respiratory and feeding difficulties. She required NIPPV during much of her hospitalization. Due to her prominent and severe respiratory symptoms, a primary respiratory disorder was considered. Pediatric pulmonology performed a nasal ciliary biopsy to evaluate for primary ciliary dyskinesia and was negative. Laryngoscopy showed laryngomalacia, subglottic stenosis, and prolapse of the ventricles of the larynx. At 6 weeks of age, she developed multifocal atrial tachycardia. She also had a G-tube placed due to poor feeding and ongoing respiratory concerns. A genetics examination showed dysmorphic features with coarse facial appearance, sparse hair frontally, and deep palmar creases all suspicious for CS. Sequencing of the *HRAS* gene identified the p.Gly12Ser mutation.

At her most recent follow-up at 3 months of age, she remained on room air, but required daily airway clearance therapy with nebulizer treatments and manual chest physiotherapy. She remained G-tube dependent due to poor management of her oral secretions and required frequent suctioning. She was planned to have a polysomnogram to evaluate for obstructive sleep apnea. Her arrhythmia was stable on propranolol and echocardiograms showed normal biventricular function.

## RESULTS

We found 31 articles that described 57 CS patients in the neonatal period and five articles reporting on respiratory symptoms later in life in 17 additional patients for a total of 74 literature patients [Say et al., 1993; Zampino et al., 1993; Torrelo et al., 1995; Fukao et al., 1996; Johnson et al., 1998; Kerr et al., 1998; Pratesi et al., 1998; Feingold, 1999; Flores-Nava et al., 2000; Boente et al., 2001; Van den Bosch et al., 2002; Katcher et al., 2003; Nasca et al., 2003; Dickson et al., 2004; Gregersen and Viljoen, 2004; Waldburg et al., 2004; Della Marca et al., 2006; Kerr et al., 2006; van Steensel et al., 2006; Denayer et al., 2008; Digilio et al., 2008; Gripp et al., 2008; Lo et al., 2008; O'Shea et al., 2008; Kuniba et al., 2009; Piccione et al., 2009; Smith et al., 2009; Girisha et al., 2010; Laux et al., 2011; Burkitt-Wright et al., 2012; Gripp et al., 2012a,b; Lorenz et al., 2012; Alfieri et al., 2014; Weaver et al., 2014]. The studies were all case reports or retrospective case series. The four institutional cases were included and a total of 78 patients were compiled. One literature case was eventually excluded for extreme prematurity leaving 77 patients, 60 for whom a clinical description of neonatal period was available and 17 with description of symptoms in childhood/adulthood (Fig. 1).

Preterm delivery prior to 37 weeks gestation is commonly observed in infants with CS. In our neonatal cohort, gestational age was reported for 53 patients of which 58% (n = 31) were born prematurely. Of these, 55% (n = 17) were late preterm (≥34 to <37 weeks), 29% (n = 9) were very preterm (≥28 to <32 weeks), and 3% (n = 1) was extremely preterm (<28 weeks). Because of the well-known short- and long-term respiratory complications associated with extreme prematurity, the latter case was excluded. Based on the remaining 60 patients, the prevalence of respiratory symptoms in the neonatal period in CS was found to be 78% (n = 47) (Fig. 1).

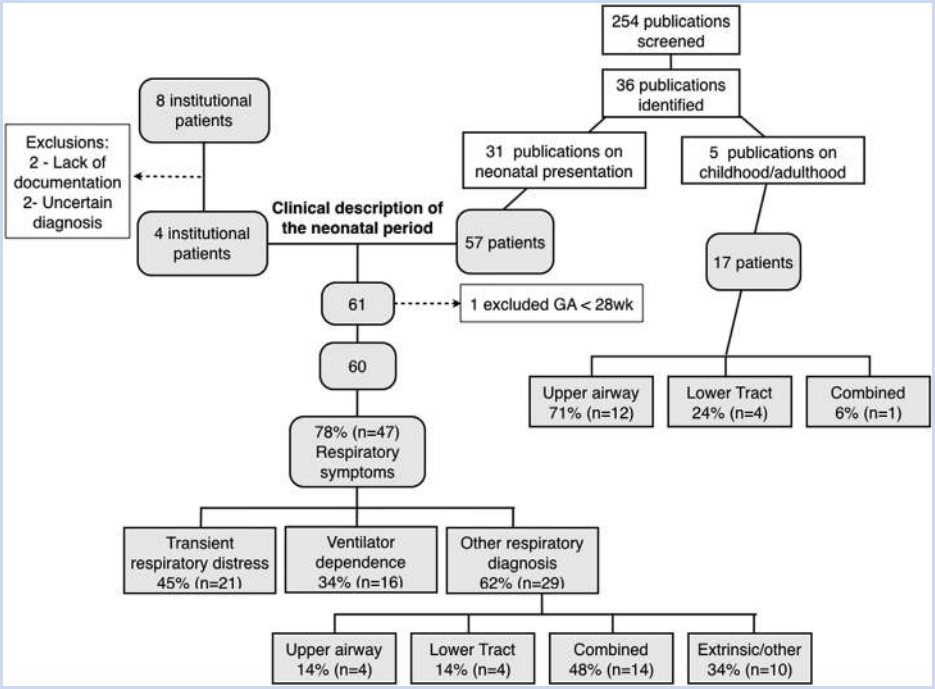


**FIG. 1. Flowchart that summarizes patient selection and the process and results of the literature review. GA, gestational age.**

Among the 47 patients who presented with respiratory symptoms approximately 45% (n = 21) presented with transient respiratory distress, 34% (n = 16) were dependent on mechanical ventilation and 62% (n = 29) had more specific respiratory diagnoses beyond transient respiratory distress or ventilator dependence (Fig. 1). The transient respiratory distress category was used for patients that required temporary respiratory support. In 17 out of 21 of these cases, the records only indicate "transient respiratory distress" in the neonatal period without further specifying etiology, duration, or intervention. The remaining four patients were intubated for 2, 4, 5, and 10 days. For the 16 patients reported to be dependent on mechanical ventilation, various suspected etiologies were reported in 10 of the cases and included pulmonary dysplasia/hypoplasia (n = 3), apnea (n = 2), poor respiratory effort (n = 2), fatigue (n = 1), pulmonary hypertension (n = 1), and severe upper airway obstruction (n = 1).

To better characterize the etiology of respiratory system impairment or failure, we classified the specified respiratory abnormalities into four categories by anatomic site: (i) upper airway; (ii) lower respiratory tract; (iii) combined upper airway and lower respiratory tract; and (iv) extrinsic/other. The upper airway was defined as the nasal cavity, paranasal sinuses, oropharynx, supraglottic larynx, and glottis. The lower respiratory tract was defined as the subglottic larynx, trachea, bronchi, and lung parenchyma. The extrinsic/other category includes pathologies such as pleural effusion, pneumothorax, chylothorax, and "poor respiratory effort," which are extrinsic to the lungs but cause respiratory insufficiency. Diseases of the pulmonary vasculature were also included in this category. Patients commonly presented with symptoms in more than one category. We found that during the neonatal period, among the patients with a more specific diagnosis (n = 29), approximately 48% (n = 14) had evidence of combined upper and lower respiratory tract involvement, 14% (n = 4) had symptoms of isolated upper airway obstruction or lower respiratory tract involvement, and 34% (n = 10) had extrinsic/other factors reported. The descriptions of respiratory involvement in childhood and adulthood were limited. Most of the reported symptoms of the 17 patients were of upper airway obstruction (n = 12, 71%), with fewer cases of lower respiratory tract (n = 4, 24%) and combined involvement (n = 1, 6%) (Fig. 1).

Table I lists all reported respiratory abnormalities classified by anatomic site in neonates and older individuals with CS. Abnormal findings in the upper airway included choanal abnormalities, nasal papillomata, redundant nasal tissue, adenoid hypertrophy, tonsillar enlargement, macroglossia, retrognathia, retropalatal or retrolingual upper airway narrowing, laryngomalacia, laryngeal papillomata, laryngeal cleft, vocal cord abnormalities, hypopharynx wall collapse, non-specified upper airway obstruction requiring tracheostomy, and OSA. In the neonatal period, the most commonly diagnosed abnormalities were laryngomalacia (n = 5), and non-specified severe upper airway obstruction requiring tracheostomy (n = 5). Tonsillar enlargement, OSA, nasal papillomata, macroglossia, upper airway narrowing, and retrognathia were reported more commonly in older individuals (Table I). Abnormal findings involving the lower respiratory tract included subglottic stenosis, tracheobronchial anomalies, pulmonary infiltrates, bronchopulmonary dysplasia, lung hypoplasia, and lung fibrosis. Among these, tracheomalacia (n = 6) was the most common

**TABLE I. Respiratory System Abnormalities Classified by Anatomic Site in 77 Individuals With CS**

| | Neonatal (N) | Later (N) |
|---|---|---|
| **Upper airway** | | |
| Nasal/nasopharyngeal/oropharyngeal | | |
| Choanal abnormalities[a] | 2 | 1 |
| Nasal papillomata | 0 | 9 |
| Redundant nasal tissue | 1 | 0 |
| Adenoid hypertrophy | 1 | 5 |
| Tonsillar enlargement | 1 | 11 |
| Macroglossia | 1 | 8 |
| Retrognathia | 1 | 7 |
| Upper airway narrowing[b] | 1 | 7 |
| Supraglottic/glottic larynx | | |
| Laryngomalacia | 5 | 2 |
| Laryngeal papillomata | 1 | 1 |
| Laryngeal cleft | 0 | 1 |
| Vocal cord abnormalities | 2 | 1 |
| Hypopharynx wall collapse | 1 | 3 |
| Upper airway obstruction NOS | | |
| Non-specified obstruction requiring obstruction requiring tracheostomy | 5 | 2 |
| Obstructive sleep apnea (OSA) | 3 | 10 |
| **Lower tract** | | |
| Subglottic larynx | | |
| Subglottic stenosis | 4 | 1 |
| Tracheobronchial | | |
| Tracheomalacia | 6 | 2 |
| Bronchomalacia | 3 | 0 |
| Bronchial narrowing | 1 | 0 |
| Bronchiectasis | 0 | 1 |
| Lung parenchyma | | |
| Pulmonary infiltrates | 1 | 1 |
| Bronchopulmonary dysplasia | 1 | 0 |
| Lung hypoplasia | 1 | 0 |
| Lung fibrosis | 0 | 1 |
| **Extrinsic/other** | | |
| Pleural effusions | 7 | 0 |
| Chylothorax | 1 | 0 |
| Pneumothorax | 1 | 0 |
| Poor respiratory effort | 3 | 0 |
| Pulmonary hypertension | 2 | 0 |

NOS, not otherwise specified.
[a]Stenosis, hypoplasia, atresia.
[b]Retropalatal or retrolingual.

reported finding in the neonatal period, followed by subglottic stenosis (n = 4). Among extrinsic/other causes of respiratory compromise, pleural effusions were also commonly reported in the neonatal period (n = 7).

In this study, approximately 58% of CS individuals were born prematurely and prematurity itself may be associated with short-term respiratory complications such as apnea, respiratory distress syndrome (RDS), transient tachypnea of the newborn (TTN), and long-term complications such as bronchopulmonary dysplasia (BPD) in a subset of premature infants. To investigate the possibility that the observed respiratory morbidity is not explained fully by prematurity, we stratified the patients by degree of prematurity based on gestational age and compared their rates of respiratory morbidity, ventilator use, and mortality (<1 year) to published numbers for premature infants (Table II). Despite the great variability in the prevalence and severity of respiratory distress associated with premature birth across centers and years, for all gestational ages (term, late [≥34 to <37], moderate [≥32 to <34], and very preterm [≥28 to <32]) CS infants had much higher prevalence of overall respiratory morbidity and mortality by 1 year of age. The prevalence of ventilator use was also higher in moderate, late and term gestational ages. The differences were most striking in the term and late preterm cohorts were CS infants had an incidence of respiratory symptoms between 68% and 80% compared to the highest reported rates available 0.84% (term) and 10.5% (late preterm) [Hibbard et al., 2010; Celik et al., 2013; Natile et al., 2014; Prefumo et al., 2015] suggesting that the respiratory phenotype in term and late preterm CS infants cannot be explained by respiratory complications due to prematurity. CS patients had higher mortality by the first year of life: 18% (term) and 31% (late preterm) compared to 0.2% and 1.75% (term and later preterm, respectively). The high incidence of anatomic upper and lower respiratory tract abnormalities not commonly described in preterm infants suggests a CS respiratory phenotype distinct of prematurity (Table II).

Approximately 80% of CS patients share a common *HRAS* p.Gly12Ser mutation, but less common mutations have been reported that affect the severity of the phenotype. Specifically, the p.Gly12Cys, p.Gly12Asp, p.Gly12Glu, and p.Gly12Val mutations have been found in CS patients with an unusually severe course and early lethality. To examine for genotype and respiratory phenotype associations, we looked at the incidence of transient respiratory distress, ventilator dependence, upper or lower respiratory tract involvement, and absence of respiratory issues for the reported *HRAS* pathologic allelic variants (Table III). The common p.Gly12Ser mutation was associated with highest incidence of lower respiratory tract involvement, upper airway, and transient respiratory issues as exemplified by patients 1, 3, and 4 described here. Other mutations with high incidence of transient symptoms were p.Ser89Cys, p.Gly12Ser mosaic, p.Gly13Cys, p.Gly13Asp, p.Glu37dup, and p.Lys117Arg. None or minimal respiratory complications were reported in seven patients with p.Gly12Ala, p.Thr58Ile, and p.Ala146Val, consistent with these mutations having a more attenuated phenotype. As previously suggested, p.Gly12Cys, p.Gly12Asp, p.Gly12Glu, and p.Gly12Val mutations presented most commonly with respiratory failure, ventilator dependence, and lower respiratory tract involvement.

It is likely that there are multiple mechanisms underlying the complex spectrum of respiratory symptoms in CS individuals. To look for common histologic abnormalities in the respiratory tract of CS patients, we collected information on autopsy and histopathologic findings reported in the literature. Table IV represents a compilation of histopathologic findings in the respiratory tract of CS patients. A variety of pulmonary malformations have been described including vascular dysplasia, bronchopulmonary dysplasia, congenital alveolar dysplasia, congenital pulmonary lymphangiectasia, alveolar-capillary dysplasia, as well as

TABLE II. Respiratory Complications and Mortality (<1 year) in CS Patients Stratified by Degree of Prematurity

| GA | Mortality <1 year | Published rate | Respiratory morbidity | Published rate | Ventilator | Published rate | Other respiratory diagnosis |
|---|---|---|---|---|---|---|---|
| ≥39 (10) | 10 (1) | 0.21 [Mathews and MacDorman, 2012] | 80 (8) | RDS 0.3 TTN 0.3 all 0.84 [Hibbard et al., 2010] | 0 (0) | 0.1–0.3 [Hibbard et al., 2010; Teune et al., 2011] | 20 (2) |
| ≥37 (22) | 18 (4) | 0.74 [Mathews and MacDorman, 2012] | 68 (16) | RDS 0.4 TTN 0.44 all 8.3 [Hibbard et al., 2010] | 5 (1) | 1.2–1.6 [Hibbard et al., 2010; Teune et al., 2011] | 27 (6) |
| ≥34 to <37 (17) | 31 (5) | 1.75 [Mathews and MacDorman, 2012] | 76 (13) | RDS 10.5 TTN 6.4 [Hibbard et al., 2010] | 31 (5) | 2.5 [Hibbard et al., 2010; Teune et al., 2011] | 82 (14) |
| ≥32 to <34 (4) | 25 (1) | 14–17.5 [Mathews and MacDorman, 2012; Chow et al., 2015] | 100 (4) | 46–17 [Gouyon et al., 2012] | 25 (1) | 40–15 [Gouyon et al., 2012] | 75 (3) |
| ≥28 to <32 (9) | 78 (7) | 10–26 [Chow et al., 2015] | 100 (9) | a | 78 (7) | 29–75[a] [Finer, 2006] | 67 (6) |

GA, gestational age in weeks; RDS, respiratory distress syndrome; TTN, transient tachypnea of the newborn. Data are expressed as % (No) of infants.
[a]Respiratory impairment is almost universal before 32 weeks gestation and management with intubation was standard in the past. Numbers can vary by center and year of study due to differences in clinical practice.

pulmonary infiltrates with macrophages. Abnormal elastic fibers in the tongue, pharynx, and larynx were described in a 6-month-old patient with CS and abnormal elastogenesis primarily affecting the pulmonary vasculature was reported in one additional patient. Two reports of CS adults showed lung fibrosis and pulmonary infiltrates with atypical collagen fibers and abnormal elastin fibers in the alveolar walls. In the few reported cases where lung histology was available [Burkitt-Wright et al., 2012], the degree of alveolar underdevelopment is out of proportion to the gestational age at birth, which supports the idea that intrinsic pulmonary or respiratory tract abnormalities in CS patients result in respiratory insufficiency or failure not accounted for by prematurity alone.

## DISCUSSION

Herein, we describe the respiratory complications seen in patients with CS, incorporating information from four patients and a comprehensive literature review. The cases presented here illustrate two *HRAS* mutations with distinct respiratory phenotypes, morbidity, and mortality. The first, third, and fourth patients were affected with the most common p.Gly12Ser mutation. Patients 1 and 4 presented in the neonatal period with transient respiratory distress and combined upper and lower airway abnormalities while patient 3 was diagnosed with airway anomalies in the form of severe OSA in late infancy. The second patient had a p.Gly12Asp mutation and presented with intractable respiratory failure and died at 2 months of cardiorespiratory failure. Review of the literature revealed that 78% of CS patients experience respiratory complications as newborns. Transient respiratory distress and combined upper and lower airway anomalies were typical neonatal presentations especially in the common p.Gly12Ser mutation. The causes of respiratory airway obstruction vary, but patients with CS often present with combined involvement of the upper and lower respiratory tract with airway malacia. Respiratory failure and dependence on mechanical ventilation occurred almost exclusively in patients with the p.Gly12Cys, p.Gly12Asp, p.Gly12Glu, and p.Gly12Val mutations. Although cardiac involvement was also common in the severe cases, respiratory failure in the absence of clinically significant cardiomyopathy as well as the abnormal histological findings strongly suggest that, at least early in the presentation, the respiratory failure could be due to underlying congenital lung and airway anomalies (Case 2, [Lo et al., 2008; Burkitt-Wright et al., 2012; Lorenz et al., 2012; Weaver et al., 2014]).

Multiple mechanisms likely underlie the wide spectrum of respiratory symptoms in CS. Pulmonary edema, pleural effusions, and hypotonia may contribute to the transient respiratory distress in the newborns. Della Marca et al. [2006] reported on common upper airway findings in 10 patients with CS ages 3–29 years. Including many of the findings described in Table I, they noted that all patients presented with sites of upper airway narrowing and had a characteristic soft tissue hyperlaxity of the pharyngeal wall. Early studies into the pathological abnormalities in CS revealed abnormal elastic tissue in the upper airway (tongue, pharynx, larynx) [Mori et al., 1996]. This could explain the hyperlaxity and the frequently noted airway malacia, and suggests a possible common mechanism. A review of published histopathology of the

TABLE III. HRAS Mutations and Respiratory Symptoms in the Neonatal Period

| | | Respiratory symptoms | | | | |
|---|---|---|---|---|---|---|
| **Mutation** | **n** | **None** | **Transient** | **Upper airway** | **Lower airway** | **Ventilator dependence** |
| p.Gly12Ser | 12 | | 6 | 6 | 10 | 1 |
| p.Ser89Cys | 2 | | 2 | 1 | | |
| p.Gly12Ser mosaic | 1 | | 1 | | | |
| p.Gly13Cys | 1 | | 1 | | | |
| p.Gly13Asp | 1 | | 1 | | | |
| p.Glu37dup | 1 | | 1 | | | |
| p.Lys117Arg | 1 | | | 1 | 1 | |
| p.Gly12Asp | 6 | | | | 3 | 6 |
| p.Gly12Val | 4 | | | | 3 | 4 |
| p.Gly12Cys | 2 | | | 1 | 1 | 2 |
| p.Gly12Glu | 2 | | | | 2 | 2 |
| p.Gly12Ala | 3 | 2 | | 1 | 1 | |
| p.Thr58Ile | 3 | 3 | | | | |
| p.Ala146Val | 1 | 1 | | | | |

respiratory tract in CS patients showed a range of abnormalities. Congenital anomalies have been described, such as dysplasia of the pulmonary vasculature, lymphatics, airways, and alveoli. Other histologic findings reported include fibromuscular dysplasia of arteries, lung fibrosis, and various pulmonary infiltrates. Several studies described abnormal connective tissue in pleura, septal connective tissue, vessel, and alveolar walls. Studies are needed to define the structural abnormalities in these patients and possible genotype–phenotype correlations.

There are several limitations to our study. Estimation of the incidence of symptoms during the neonatal period based on published reports is likely imprecise due to incomplete documentation and a bias in the literature for more unusual or severe phenotypes. This reporting bias and/or testing bias is notable in our figures where the distribution of mutations in our cohort does not agree with larger epidemiological studies. In our series of 77 patients, 40 had a known *HRAS* mutation and of these 32% (n = 13) had the common p.Gly12Ser substitution, 35% (n = 14) had severe rare mutations, 18% (n = 7) were known mild rare mutations, and the remaining 15% (n = 6) had other rare mutations. In the literature, the term "transient respiratory distress" was often not further specified with etiology, duration, or intervention. Estimations of the frequency of airway obstruction can be imprecise due to the lack of complete otorhinolaryngological evaluations and a reporting bias toward abnormal findings. The histopathology studies were mostly of severe cases with rare mutations and early lethality. Finally, CS individuals are often born prematurely. Although prematurity likely contributes to the severity of clinical

TABLE IV. Histopathological Findings in the Respiratory Tract in CS

| **Findings** | **Death (age)** | **Mutation** | **Reference** |
|---|---|---|---|
| Pulmonary vascular dysplasia with abnormal elastin distribution | 3 months | G12E | Weaver et al. [2014] |
| Bronchopneumonia and bronchopulmonary dysplasia | 42 days | G12V | Burkitt-Wright et al. [2012] |
| Congenital alveolar dysplasia | 36 days | G12V | Burkitt-Wright et al. [2012] |
| Congenital pulmonary lymphangiectasia and alveolar-capillary dysplasia | 3 months | G12D | Lo et al. [2008] |
| Lung fibrosis | 25 years | G12A | Kerr et al. [2006] |
| Inflammatory subglottic tracheal polyp and fibromuscular dysplasia of coronary arteries | 37 days | G12E | Kerr et al. [2006] |
| Pulmonary infiltrates: macrophages and pulmonary hemorrhages | 3 months | ND | Dickson et al. [2004] |
| Pulmonary infiltrates: intra-alveolar foamy macrophages. Atypical collagen fibers in pleura, septal connective tissue adventitia of medium sized pulmonary vessels. Abnormal elastin fibers in alveolar walls | 37 years | ND | Waldburg et al. [2004] |
| Fine and disrupted elastin fibers in tongue, pharynx, larynx. Fine but relatively preserved elastin fibers in bronchi and alveoli | 6 months | ND | Mori et al. [1996] |

ND, not determined.

presentation in CS as evidenced by the increasing morbidity and mortality with decreased gestational age at birth, several lines of evidence suggest that prematurity does not fully account for the incidence and degree of respiratory impairment. The analysis after stratification based on gestational age showed substantially higher incidences of respiratory morbidity and mortality in CS patients regardless of gestational age. Furthermore, the high incidence of anatomic respiratory tract abnormalities and the specific histological abnormalities found in CS suggest a different pathophysiology.

The prenatal findings for CS have recently been reviewed [Myers et al., 2014]. The presence of these findings can increase the suspicion for CS and improve our ability for prenatal diagnosis. The high incidence of respiratory complications should be considered and anticipated in prenatally diagnosed CS. A prenatal diagnosis of CS with the p.Gly12Cys, p.Gly12Asp, p.Gly12Glu, and p.Gly12Val mutations should prompt a counseling and management plan for a more severe cardiac and respiratory phenotype. The high incidence of upper and lower respiratory tract involvement due to various mechanisms supports the recommendation for complete otorhinolaryngological and pulmonology evaluations, especially when surgery, general anesthesia, and intubation are considered. Pulmonary histology should be examined in future studies to better elucidate which of constituent lung cell types (alveolar epithelial, fibroblasts, endothelial, smooth muscle) are affected by *HRAS* mutations during development.

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