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UNRECOGNIZED LUNG DISEASE IN CLASSIC RETT SYNDROME: A PHYSIOLOGIC AND HRCT STUDY

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Disclosure Statement

Dr. De Felice has no conflicts of interest to disclose.

Dr. Guazzi has no conflicts of interest to disclose.

Dr. Rossi has no conflicts of interest to disclose.

Dr. Ciccoli has no conflicts of interest to disclose.

Dr. Signorini has no conflicts of interest to disclose.

Dr. Leoncini has no conflicts of interest to disclose.

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Dr. Hayek has no conflicts of interest to disclose.

ABSTRACT

Background: Breathing disorders in Rett Syndrome (RS) have been generally attributed to severe autonomic and/or brain stem dysfunction and no information regarding lung morphology exists to date. The aim of the present study was to determine if there are RS associated pulmonary abnormalities.

Methods: A total of 27 girls (age: M±SD, 12.6±5.9 yr; age range: 3-32) with gene encoding methyl-CpG-binding protein 2 (*MeCP2*)-mutation confirmed RS underwent High-Resolution CT scans (HRCT) of the thorax. A volumetric acquisition was used, and isotropic datasets were acquired with thin collimation (<1-mm slice), scanning through the lungs and processing on a high spatial- resolution kernel (bony algorithm).

Results: Abnormal HRCT findings were observed in 15/27 (55.5%) of cases, consisting of centrilobular nodules (10/15=66.7%), thickening of the bronchial walls (8/15=53.33%), and patchy ground-glass opacities (4/15=26.7%), with upper lobe predominance. In addition, bronchiolectasis were found in 9/15=60% of the patients.

Conclusions: Pulmonary lesions, respiratory bronchiolitis associated interstitial lung disease (RB-ILD)-like lesions, are present on imaging studies in about half of typical RS patients. Further research is needed in order to clarify the epidemiology and the pathogenesis of these previously unrecognized pulmonary abnormalities.

Abbreviations

CTDI: Computed Tomography Dose Index

DAB: Diffuse aspiration bronchiolitis

DLP: Dose Length Product

DHA, docosahexaenoic acid

EPA, eicosapentaenoic acid

GERD, gastro-esophageal reflux disease

GGOs, Ground glass opacities

HRCT, High-resolution chest tomography

ILD, Interstitial lung disease

IGF-, insulin-like growth factor-1

omega-3 LC-PUFAs, Long-chain omega-3 fatty acids

MeCP2, Methyl-CpG-binding protein 2

O_2 [(A-a)PO2], Pulmonary gradient for O_2

PI, Perfusion Index

PVI, Pleth variability index

RB-ILD, Respiratory bronchiolitis associated interstitial lung disease

RS, Rett syndrome

V/Q, Ventilation/perfusion ratio

V_T , Tidal volume

INTRODUCTION

Rett Syndrome [(MIM 312750)] (RS) is a pervasive neurodevelopmental disorder affecting females and is considered to be the second most common cause of mental retardation in the female gender, with a reported frequency of ~1:10,000 to 1:15,000 females). Besides a classical type, characterized by a 4-stages of developmental regression following an apparently normal development for 6–18 months, several different clinical forms are currently known and include the congenital, "*forme fruste*", early seizures, and preserved speech variants.[1,2]

To date, no effective therapy to prevent the developmental regression, autonomous nervous system dysfunction, and loss of communication skills is available for these patients. The classic RS form is mainly caused by loss-of-function mutations in the gene encoding methyl-CpG-binding protein 2 (MeCP2),[3] a key transcription regulator gene.[4]

From a clinical point of view, disorders of respiratory control are a prominent feature of RS,[5] with a wide spectrum of reported breathing irregularities including breath holding, spontaneous Valsalva maneuvers, apnea, apneusis, hyperventilation, and rapid shallow breathing.

Respiratory dysfunction is known to be present during wakefulness as well as during sleep,[6] and is commonly attributed to brain-stem immaturity and central autonomic dysfunction,[6,7] or progressive neurochemical dysfunction in the respiratory network after birth.[8]

Recently we have reported chronic hypoxia, impaired gas exchanges, and increased oxidative stress in classic RS.[9]

However, no information regarding lung morphology in RS is available. The aim of the present study was to investigate a possible RS-associated pulmonary abnormality as evidenced by high-resolution chest tomography (HRCT).

METHODS

Patients

A total of 27 classic RS patients (age: M±SD, 12.6±5.9 yr; age range: 3-32) carrying a *MeCP2*-mutation underwent HRCT. During the enrollement period (approximately from March 2007-March 2009) the studied population was randomly selected (1 of 4 consecutive patients admitted for follow-up study to the Child Neuropsychiatry Unit) from a larger sample of patients with classic Rett syndrome who demonstrated the *MeCP2* gene mutation (n=125). Another entry criteria was the presence of at least a minimal respiratory dysfunction, as classified according to the Percy scale[10] of severity (i.e., +: minimal hyperventilation and/or apnoea; ++: intermittent hyperventilation and/or apnoea; +++: hyperventilation and/or apnoea with cyanosis). In particular, minimal respiratory dysfunction was arbitrarily defined on the basis of at least two of the following items: hyperventilation (i.e., respiratory rate between +2 and +3 SDS as compared to age-matched healthy girls), sporadic apnoea episodes without cyanosis, recurrent and unexplained fever episodes associated with lower respiratory tract infections, abnormal findings at standard chest X-ray, or diffuse fine crackles at physical chest examination. Information regarding history for gastro-oesophageal reflux, recurrent respiratory infections, abnormal air swallowing and aspiration pneumonias was obtained. No wheezing or asthma was observed. None of the patients had a clinically recognizable lung disease or were exposed to tobacco smoke, either active or passive.

Due to ethical reasons it was not possible to devise a specific control group, thus exposing healthy subjects to unneeded X-rays. As a possible alternative we examined a control group consisting in a population of 16 girls of age comparable to our examined RS patients population (age: M±SD, 12.5±6.1 yr; age range: 4-30), performing chest HRCT as a protocol for major head trauma with high impact (time period 2008-2009; sub-set of patients from all the car accident admissions to the Intensive Care Unit, University Hospital, Siena). As all subjects were to be considered to be healthy (at least as it concerns pulmonary pathology) prior to the trauma, we thought they could be used as a control group for our specific purpose (smokers were excluded).

Informed written consent from the parents for each patient and Institutional Review Board (IRB) approval were obtained (Clinical Responsibility: Joussef Hayek, MD).

High resolution Computed Tomography (HRCT) imaging of the Lung

We used a volumetric acquisition according to Klusmann & Owens.[11] Isotropic datasets were acquired with thin collimation (<1-mm slice), scanning through the lungs and processing on a high spatial- resolution kernel (bony algorithm).The benefits of volumetric scans are that the whole of the thorax can be scanned in either a single breath-hold in co-operative patients, or during spontaneous breathing in non-co-operative patients. An important side effect for this is that radiation dose is usually two to four times more, depending on the parameters used.[12] However, radiation doses were kept as low as possible, according to the "ALARA" (As Low As Reasonably Achievable) practice. HRCT was performed using a 64-slice CT Scanner (LightSpeed GE Medical Systems, Milwaukee, WI, USA) with the following acquisition parameters: 112 ± 10.95 KV; slice thickness 0.625 mm; pitch 0.890± 0.20 :1; average reconstruction interval 0.3 mm; noise index 18.78 ± 5.53; gantry rotation time 0.46 ± 0.05; average Computed Tomography Dose Index (CTDI) 10.98 ± 2.59 mGy; and Dose Length Product (DLP) 255.59 ± 81.73 mGy x cm. The axial images were transferred to a dedicated work station (Advantage Windows 4.3, GE Medical Systems, Milwaukee, WI, USA) and evaluated with the use of a high-resolution algorithm. It is to be considered that the impossibility of performing the imaging techniques with controlled ventilation or with inspiratory/expiratory views do to patients un-cooperativeness is a limitation of this study. However, this limitation was accepted in order to avoid sedation/anesthesia to the patients.

Breathing and pulmonary gas exchange

Due to lack of co-operation, traditional testing (i.e., exercise tolerance, diffusion volumetry) is not feasible in patients with Rett syndrome. As a consequence, gas exchange, respiratory rate, and tidal volume were evaluated from direct measurements. Pulmonary exchange was assessed by using a portable commercially available gas analyzer (Hanky Hapy, version 1.2, Ambra Sistemi, Pianezza,

Turin, Italy), as previously reported.[9] Briefly, the system works essentially on a multicompartment model based on the West function. This methodology has been proven to be sufficiently simple, noninvasive, accurate, and precise in determining alveolar–arterial gradient lung exchange for O_2, and ventilation/perfusion (V/Q) ratio inequalities. Air gas sampling was obtained by applying a facial mask of appropriate size connected to the gas analyzer. Respiratory rate, total ventilation, and expired gas composition were measured during a 60-s period. All measures were carried out in duplicate and the average was used for data analysis. Arterial blood for gas analyses was sampled from either the humeral or the radial artery, and partial arterial pressures of oxygen (PaO_2) values were determined by a commercially available blood gas analyzer (ABL520 radiometer; Copenhagen, Denmark). Pulmonary gradients for O_2 [$(A\text{-}a)PO_2$]> 15 mm Hg were considered to be indicative for V/Q mismatch.

Pulse oximetry monitoring

Continuous and noninvasive measurements of Perfusion Index[13] (an objective indicator of skin perfusion, PI= AC/DC x 100%, where DC is the constant amount of light from the signal of the pulse oximeter absorbed by the skin, other tissues, and nonpulsatile blood, while AC is the variable amount of light absorbed by the pulsating arterial inflow), and Pleth variability index (PVI)[14] (a measure of the dynamic changes in the PI that occur during the respiratory cycle, PVI= PI max-PI min/PI max x 100%) were monitored. The pulse oximeter was connected to a Masimo Radical 7 monitor (Masimo SET; Masimo Corp.), and plethysmographic waveforms were recorded on a personal computer and analyzed by an observer blinded to CT data. Randomly measured, 1-h-long data were obtained in triplicate, and averaged values were used for data analysis.

Data Analysis

The MedCalc version 9.5.2.0 statistical software package, MedCalc Software, Mariakerke, Belgium) was used. A two tailed P-test <0.05 was considered to be statistically significant.

RESULTS

Lung HRCT

Abnormal HRCT findings were observed in 15 of the 27 (55.5%) patients and consisted of centrilobular nodules (10/15=66.7%), thickening of the bronchial walls (8/15=53.33%), and patchy ground-glass opacities (GGOs) (4/15=26.7%), (FIGURE, Panels A to E). In addition, bronchiolectasis (FIGURE, Panel F) was found in 9/15=60% of the patients with abnormal HRCT scans. The lesions were: (i) randomly distributed and there was prevalence in the upper pulmonary lobes; (ii) independent from patient positioning, and (iii) associated with a normal lung volume. The observed lung disease was usually not very extensive, although extension was found to be quite variable, ranging from cases with sporadic to those of massive bilateral abnormalities. Patients with pulmonary lesions were older than those with normal lung HRCT imaging (CT-positive: 14.6 ± 6.9 vs. CT-negative 7 ±3.74, p=0.0253). An age cut-off> 11 years significantly identified RS patients with abnormal lung HRCT-scan, with 80% sensitivity, 83.33% specificity, positive likelihood ratio: +LR 4.8, negative likelihood ratio: -LR:0.24, positive predictive value +PV:88.9%, negative predictive value: -PV:71.4% (AUC±SE: 0.858± 0.0947, 95% C.I.: 0.596-0.976, P=0.0002) (receiver-operating characteristic curve).

None of the control girls were found to have lung abnormalities comparable to those found in RS patients (RS patients vs. control group: 15/27 vs. 0/16, p=0.000151). As it concerns HRCT individual features, differences were statistically significant for centrilobular micronodules (RS patients vs. control group: 10/27 vs. 0/16, p=0.006921), bronchial wall thickening (8/27 vs. 0/16, p=0.017529) and bronchiolectasis (9/27 vs. 0/16, p=0.017529), whereas the occurrence of patchy GGOs between the two groups was not statistically significant (4/27 vs. 0/16, p=0.279475). As a consequence, it seems unlikely that the observed HRCT pulmonary abnormalities in patients with classic RS could be ascribed to chance.

Breathing and pulmonary gas exchange

Clinical findings and results of pulmonary gas exchange in CT-positive patients are shown in the Table. In CT-positive patients minimal respiratory dysfunction, as defined in the methods section, was present in 5/15 (33.3%), moderate in 3/15 (20%), and severe in 7/15(46.7%); PaO_2 levels were below the normal range (mean ± SD, 84.57 ± 17.31 mmHg) despite a higher than normal V_T (7.36 ± 2.61 mL/kg b.w./min); tachypnea was present in 7/15 (46.7%), and pulmonary V/Q mismatch in 12/15 (80%) of the cases.

Pulse oximetry

Significantly lower PI (median, 0.52 %, 25^{th}-75^{th} percentiles: 0.48-0.75 *vs*. 1.78%,25^{th}-75^{th} percentiles:0.75-2.25, $p<0.001$) and higher PVI (median, 54.5 %, 25^{th}-75^{th} percentiles: 42-69 vs. 36.28 %,25^{th}-75^{th} percentiles: 26-42, $p<0.001$) values were observed in the patients with evidence of pulmonary lesions, as compared to those without HRCT abnormalities.

DISCUSSION

These findings indicate for the first time the presence of a previously unrecognized interstitial lung disease in patients with classic RS carrying *MeCP2* gene mutations, and suggest that at least a decade is necessary for the lung abnormalities to develop.

The observed pulmonary abnormalities share features in common (centrilobular nodules, thickening of the bronchial walls, GGOs with upper lobe predominance) with respiratory bronchiolitis associated interstitial lung disease (RB-ILD),[15] a well-established smoking-related disease. While obliterative bronchiolitis is known occur in some chronic disease (rheumatoid arthritis), or as expression of graft versus host disease, in lung transplantation, RB-ILD is associated 99% to tobacco smoke and with interstitial lung disease (ILD). HRCT and histology patterns are known to be non-specific and may somehow overlap to those of desquamative interstitial pneumonia (DIP).[16] RB-ILD is usually an incidental finding in asymptomatic smokers. While chest x ray may be normal or showing reticulo-nodular patterns or GGOs, RB-ILD is characterized at HRCT by centrilobular nodules reported in about 38%, GGOs in 50%, and fine reticular pattern (possibly to be ascribed to bronchiolar walls or inter-alveolar septa thickening) in 25% of the cases, while DIP shows GGOs in 100%, with 63% of reticulo-nodular pattern.[17] These data are consistent with the concept a spectrum of disease ranging from brionchiolocentric RB-ILD to more diffuse involvement of the secondary lobule in DIP. Unlike RB-ILD, the GGOs areas observed in RS patients appear to be larger and not necessarily in a centrilobular distribution, while bronchiolectasis and air trapping is more prominent. In addition, if we compare observed vs. predicted findings in RS-associated ILD vs. RB-ILD, the occurrence of centrilobular nodules (observed vs. predicted, p=0.8374) and bronchiolar wall thickening (observed vs. predicted, p values ranging from to p=0.7562 to 1.0) are compatible with RB-ILD, while the frequency of GGOs is significantly lower than that predicted in RB-ILD (p value ranging from 0.017694 to 0.008412).

Unfortunately, due to lack of cooperation in this Autism Spectrum Disorder, it was not possible to perform the traditional pulmonary function tests. Fiberoptic bronchoscopy and swallowing studies

were not performed due to the lack of specific clinical indications, although breathing dysfunction and pulmonary gas exchange were evaluated in all patients. Evaluations such as swallowing studies and bronchoscopy were not felt clinically-indicated and were not performed as part of this research study. While aspiration cannot be definitely excluded as a contributor to the radiographic abnormalities, the clinical history and character and geographic distribution of the radiographic abnormalities suggest that aspiration is not the likely etiology. Correctly diagnosing diffuse aspiration bronchiolitis (DAB), a clinico-pathological pathological entity firstly described in the elderly,[18] can be difficult.[19]

The lack of a true gold standard for the clinical diagnosis of aspiration adds further difficulty to the issue, although video fluoroscopic and endoscopic methods of swallow assessment are the most well-studied and widely used diagnostic tools for diagnosing oropharyngeal dysphagia.[20]

Conditions known to predispose patients to DAB include neurologic impairment (i.e., stroke, neuromuscular diseases), esophageal disorders (i.e., achalasia, tracheoesophageal fistula, gastro-oesophageal reflux disease) with consequent dysphagia,[18,21] obesity, and altered consciousness,[22,23] although some studies suggest that half of a healthy volunteers population may aspirate oropharyngeal contents during sleep.[24,25]

Nevertheless, in the lack of a histologic documentation, at least five lines of reasoning seem to differentiate the observed pulmonary abnormalities noted in RS patients from those reported in DAB: (1) none of our patients with pulmonary abnormalities documented at the chest HRCT had obesity, or proven esophageal disorder with documented dysphagia, with the single exception of gastro-oesophageal reflux in 3 patients, which was unrelated to the presence of pulmonary abnormalities (see Table legend); (2) although mental retardation is an essential feature of classic RS, none of the patients had altered consciousness, or a neuromuscular disease; (3) although at least 3 patients with documented dysphagia and DAB in the first two decades of life have been reported,[21,26] the mean age of the RS patients here described with pulmonary abnormalities is approximately 5 to 8 times lower than that of typical patients with DAB;[18,19] (4) although

bronchiolar aspiration can be asymptomatic,[24,25] DAB should be suspected in younger patients with esophageal achalasia if respiratory symptoms (i.e., recurrent bronchorrhea, bronchospasm and dyspnea), are related to the assumption of meal.[21] This was obviously not the case in our RS patients population with pulmonary abnormalities evidenced at the chest HRCT; (5) HRCT scanning is known to be a sensitive method for the detection and characterization of diffuse infiltrative lung diseases, and primary centrilobular nodules are known to be found in patients with several pulmonary diseases, including DAB.[27]

However, centrilobular nodules with a "tree-in-bud" appearance (mainly representing lymphocytic infiltration distributed along respiratory bronchioles, or bronchiolar luminal impaction with mucous or pus) potentially suggestive of DAB,[19,27] were not evidenced in our RS patients with pulmonary abnormalities.

The clinical implications of our findings in RS remain unknown but of potential significance in light of patient respiratory symptoms and the age-dependency of findings. Since CT scans were not done in this study in an age-matched cohort of RS patients without respiratory dysfunction, it is unknown whether or not the hyperventilation/tachypnea/apnea symptoms are or are not related to the radiographic abnormalities. This would be of particular interest for future studies, as perhaps the pulmonary parenchymal abnormalities provide the explanation for why RS patients have control of breathing issues when awake, but not particularly with sleep. In other words, perhaps our data suggest that the symptoms are due to a primary pulmonary problem, rather than a neurologic one. A comparison of our findings with the lung HRCT results in known interstitial lung diseases would suggest that the observations in RS are more likely reactive responses and less likely related to *MeCP2* gene defect.

To this regard prior evidence suggests an oxidative stress/mediated pathogenesis in pulmonary smoking-related diseases.[28] This would suggest that some common pathogenetic pathways, such as increased oxidative stress, might be shared by RB-ILD and RS-associated lung disease.[9] As cyclic changes in blood pressure and the pulse oximeter pleth waveform occur during airway obstruction,

with the magnitude of the cyclic waveform correlating with the severity of obstruction,[29] the high PVI here observed in RS-associated lung disease may reflect increased intrathoracic pressure potentially related to subclinical airway obstruction. Likewise, lower PI values, possibly reflecting a higher degree of skin vasoconstriction in patients with lung lesions, suggest a higher clinical severity. It seems then that physiologic changes are occurring even in the presence of only mild radiographic abnormalities. Thus, it is possible that CT is a less sensitive technique for recognizing the early pulmonary physiologic dysfunction in RS. At this stage of the research, the authors have not had opportunity to evaluate the histology of the pulmonary lesions in RS, as lung biopsy is not clinically indicated in these relatively asymptomatic patients. To the best of our knowledge, lungs have not been previously examined post-mortem (or either findings not reported) in patients with RS. Nevertheless, our observation adds a new element to the natural history of classic RS.

Unfortunately, no therapy is to date available for RS. In the recent past, however, several molecules (e.g., desipramine, ampakine, recombinant insulin-like growth factor-1 (IGF-1), recombinant MeCP2 proteins) or strategies (e.g., gene therapy) have been proposed or suggested to either improve or partially reverse clinical symptoms in RS. However, to date, therapeutical trials are either still in progress (i.e., desipramine or IGF-1), currently limited to experimental settings (e.g., ampakine, MeCP2 gene activation, recombinant MeCP2 proteins) or simply foreseeable (gene therapy). Ongoing studies from our group would suggest that RS is not limited to a primary brain disorder, but appears to be a multi-system disease sharing chronic hypoxia and a pro-oxidant/antioxidant balance shifted towards the pro-oxidant arm.[9] In particular, we have proposed a possible use of long-chain omega-3 fatty acids (omega-3 LC-PUFAs) supplementation (De Felice et al, unpublished). Our preliminary data indicate that a daily supplementation with docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA) for 6-12 months is able to partially reverse oxidative stress imbalance, respiratory disorders, and lung gas exchanges in patients with classic RS. A possible clinical and/or biochemical improvement in the patients obtained by supplementation with omega-3 LC-PUFAs -i.e., widely available substances with very low toxicity-would pave the way

in the near future for a systematic dietary supplementation starting at an early clinical stage (i.e., classic RS, stages I-II) aiming at potentially changing the clinical course of the disease, including the observed pulmonary abnormalities here reported.

Future research is needed in patients and *MeCP*-2 null mouse models to better understand the pathology and pathogenetic mechanisms for the observed lung disease.

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Single Authors' contributions to the present manuscript.

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Contributions	**CDF**	**GG**	**MR**	**LC**	**CS**	**SL**	**GT**	**GL**	**GV**	**JH**
Conception & Design	+	+	+						+	+
Data acquisition	+	+								+
Data Analysis	+			+	+		+			
Data interpretation	+	+	+	+	+	+	+	+	+	+
MS Drafting	+			+	+		+		+	
Critical revision for important intellectual content				+	+	+	+	+		
Final Approval of the version to be published	+	+	+	+	+	+	+	+	+	+

Legend:

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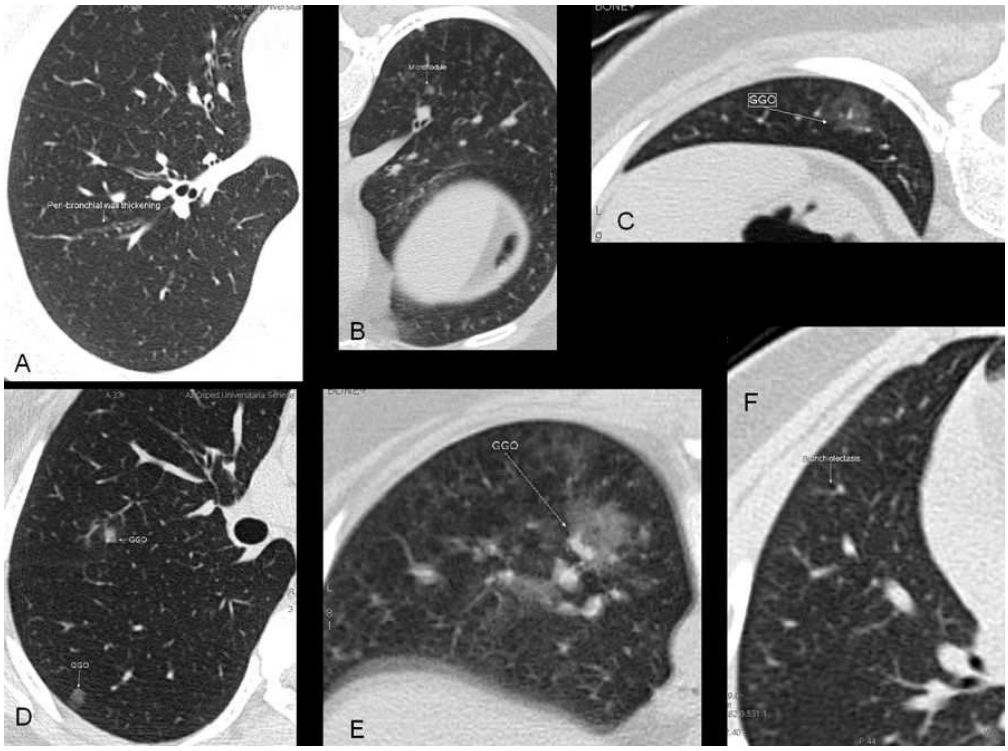
Figure Legend: Lung HRCT features of RB-ILD in patients with Rett syndrome. Peri- bronchial wall thickening (A), centrilobular nodules (B), ground glass opacities (GGOs) (C-D-E), and bronchiolectasis (F) are shown.



316x234mm (72 x 72 DPI)

Table. Relevant demographical, clinical and functional characteristics of Rett syndrome girls with abnormal pulmonary features at High Resolution Computed Tomography.

Case No.	Age (years)	Tachypnea	Respiratory Dysfunction	Other clinical features	V_T	PaO_2 mmHg	V/Q mismatch	HRCT Features			
								GGOs	Nodules	BW-Thick.	Bronchiolectasis
1	11	-	++		5.20	88.5	+	+	+	+	+
2	18	+	+++	Air-S	12.8	64.7	+	-	-	+	+
3	8	+	++		8.8	100	+	-	+	+	-
4	15	-	+		6.6	71.4	+	-	+	+	+
5	18	-	+++	Air-S	6.8	100	+	+	-	+	+
8	22	-	+++		5.08	53.8	+	-	+	+	+
10	11	+	+		8.36	97	-	-	+	-	-
11	12	+	+		8.91	94.3	-	+	+	-	+
14	20	+	++		6.94	100	+	-	-	-	+
19	3	+	+++		11.02	52	+	-	-	-	+
20	10	-	+++		4.23	89.5	+	-	+	-	-
21	9	-	+		6.32	100	+	+	+	-	-
24	17	-	+		6.24	84.8	+	-	+	+	-
26	32	+	+++	Air-S	9.99	100	-	-	-	-	-
27	14	-	+++	GERD, RRIs	3.21	72.6	+	-	+	+	+

Legends & Notes:
HRCT: High Resolution Computed Tomography of the lungs; GGOs: Ground-glass opacities; BW-Thick: bronchial wall thickening; V/Q: ventilation/perfusion; V_T: pulmonary tidal volume, mL/kg b.w./min; Tachypnea was objectively defined as a respiratory rate > 1.8 times (i.e., above the upper quartile) of expected respiratory rate for age and gender. Respiratory dysfunction was categorized based on the corresponding Percy scale item (see reference#10) (+: minimal hyperventilation and/or apnoea; ++: intermittent hyperventilation and/or apnoea; +++: hyperventilation and/or apnoea with cyanosis). Positive history for gastro-esophageal reflux disease (GERD) was also present (and treated) in cases 9 and 13, (data not shown). A positive history for recurrent respiratory infections (RRIs) was in a single patient, as shown. History for abnormal air swallowing (Air-S) was present for three cases as shown. None of the patients exhibited a history or clinical signs suggestive for aspiration pneumonias.

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