Metabolic disturbances of the vitamin A pathway in human diaphragmatic hernia

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Submitted 23 April 2014; accepted in final form 27 October 2014

Metabolic disturbances of the vitamin A pathway in human diaphragmatic hernia. Am J Physiol Lung Cell Mol Physiol 308: L147–L157, 2015. First published November 21, 2014; doi:10.1152/ajplung.00108.2014.——Congenital diaphragmatic hernia (CDH) is a common life-threatening congenital anomaly resulting in high rates of perinatal death and neonatal respiratory distress. Some of the nonisolated forms are related to single-gene mutations or genomic rearrangements, but the genetics of the isolated forms (60% of cases) still remains a challenging issue. Retinoid signaling (RA) is critical for both diaphragm and lung development, and it has been hypothesized that subtle disruptions of this pathway could contribute to isolated CDH etiology. Here we used time series of normal and CDH lungs in humans, in nitrofen-exposed rats, and in surgically induced hernia in rabbits to perform a systematic transcriptional analysis of the RA pathway key components. The results point to CY26B1, CY26B1, and ALDH1A2 as deregulated RA signaling genes in human CDH. Furthermore, the expression profile comparisons suggest that ALDH1A2 overexpression is not a primary event, but rather a consequence of the CDH-induced lung injury. Taken together, these data show that RA signaling disruption is part of CDH pathogenesis, and also that dysregulation of this pathway should be considered organ specifically.

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be one of the megalin ligands, and megalin deficient patients have increased RBP urine spillage (14).

5) Finally, CDH has been demonstrated to be strongly associated with low retinol and RBP levels in newborns, independently of maternal retinol status (2, 22).

Even with all this attractive evidence of RA signaling involvement in nonisolated CDH, the role of this pathway in the isolated forms remains elusive. One possibility is that at least a subset of cases is promoted by low vitamin A intake. Retrospective questionnaires about maternal periconceptional nutrient intakes yields conflicting results (45). In the developing world, where vitamin A intake could be expected to be low, CDH is not reported to occur at higher rates (43). However, lack of birth defect registries may cause CDH incidence to be underestimated. Another possibility is that the RA pathway is intrinsically affected by subtle functional defects of one or more signaling components. Previously, a temporal and spatial expression study of the nuclear receptor genes RAR (α,β,γ) and RXR (α,β,γ) failed to demonstrate any difference between human CDH lungs, hypoplastic lungs due to other causes, and normal lungs (33). Here we extended this first line of analysis using transcriptional screening from the retinol binding protein receptor gene (STRA6) to the downstream actors of the RA pathway throughout the lung development. Comparing different conditions (human normal lungs, human CDH lungs, wild-type and nitrofen-exposed lungs in the rat, and wild-type and hernia-injured lungs in the rabbit), we show that RA signaling is affected by several transcriptional alterations in human CDH, of which ALDH1A2 overexpression is probably the most striking. The results from the rabbit CDH surgical model suggest that this dysregulation may be secondary to the lung injury process.

MATERIALS AND METHODS

Chemicals. All-trans retinol, all-trans RA (ATRA) and dimethylsulfoxide (DMSO) were purchased from Sigma-Aldrich (Saint-Quentin-Fallavier, France), culture medium and additives from Invitrogen (Cergy-Pontoise, France), and the transfection reagent GeneJammer from Stratagene (Amsterdam, The Netherlands).

Human tissue collection. With the approval of the Erasmus MC Medical Ethical Committee and the informed consent of the parents, human lung samples were obtained from the tissue bank of the Department of Pathology, Erasmus MC (Rotterdam). The selected lung tissues were collected after elective termination of pregnancy or at autopsy. The characteristics of the nine CDH patients are reported in Table 1. Lung tissues from 10 stage-matched fetuses without pulmonary abnormalities were used as controls (Table 1).
Table 1. Characteristics of the nine congenital diaphragmatic hernia patients

<table>
<thead>
<tr>
<th>Gestational Age (weeks)/Lung Developmental Stage</th>
<th>Sex</th>
<th>Lung Weight-to-Body Weight Ratio</th>
<th>Phenotype/Malformations</th>
<th>Karyotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal lungs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13.5 (pseudoglandular)</td>
<td>M</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>14 (pseudoglandular)</td>
<td>F</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>15 (pseudoglandular)</td>
<td>F</td>
<td>n.a.</td>
<td>normal ASD</td>
<td>n.a.</td>
</tr>
<tr>
<td>17 (pseudoglandular)</td>
<td>M</td>
<td>n.a.</td>
<td>normal RDS</td>
<td>n.a.</td>
</tr>
<tr>
<td>17.5 (canalicular)</td>
<td>M</td>
<td>n.a.</td>
<td>normal</td>
<td>n.a.</td>
</tr>
<tr>
<td>26 (canalicular)</td>
<td>F</td>
<td>normal</td>
<td>normal</td>
<td>n.a.</td>
</tr>
<tr>
<td>26 (canalicular)</td>
<td>M</td>
<td>normal</td>
<td>normal ASD</td>
<td>n.a.</td>
</tr>
<tr>
<td>28.5 (saccular)</td>
<td>M</td>
<td>normal</td>
<td>ASD, RDS</td>
<td>46, XY</td>
</tr>
<tr>
<td>31 (saccular)</td>
<td>F</td>
<td>normal</td>
<td>hydrothorax</td>
<td>46, XY</td>
</tr>
<tr>
<td>34 (saccular)</td>
<td>M</td>
<td>normal</td>
<td>EA, hydrocephalus</td>
<td>46, XY</td>
</tr>
<tr>
<td>CDH-injured lungs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21 (canalicular)</td>
<td>M</td>
<td>0.02</td>
<td>isolated CDH</td>
<td>46, XY</td>
</tr>
<tr>
<td>22 (canalicular)</td>
<td>M</td>
<td>n.a.</td>
<td>isolated CDH</td>
<td>46, XY</td>
</tr>
<tr>
<td>25 (canalicular)</td>
<td>M</td>
<td>0.012</td>
<td>normal</td>
<td>n.a.</td>
</tr>
<tr>
<td>34 (saccular)</td>
<td>M</td>
<td>0.005</td>
<td>PCH</td>
<td>46, XY</td>
</tr>
<tr>
<td>34 (saccular)</td>
<td>M</td>
<td>0.008</td>
<td>testes and kidney cysts</td>
<td>46, XY</td>
</tr>
<tr>
<td>37 (saccular)</td>
<td>M</td>
<td>0.005</td>
<td>isolated CDH</td>
<td>46, XY</td>
</tr>
<tr>
<td>38 (saccular-alveolar)</td>
<td>F</td>
<td>0.011</td>
<td>isolated CDH</td>
<td>n.a.</td>
</tr>
<tr>
<td>40 (alveolar)</td>
<td>M</td>
<td>&lt;0.001</td>
<td>Meckel's diverticulum</td>
<td>46, XY</td>
</tr>
<tr>
<td>41 (alveolar)</td>
<td>M</td>
<td>0.006</td>
<td>isolated CDH</td>
<td>46, XY</td>
</tr>
</tbody>
</table>

ASD, atrial septal defect; CDH, congenital diaphragmatic hernia; EA, esophagus atresia; HLHS, hypoplastic left heart syndrome; n.a., not available; PCH, pontocerebellar hypoplasia; RDS, respiratory distress syndrome.

ALDH1A2 immunohistochemistry protocol. Sections of normal human lungs, wild-type rabbit lungs, and rabbit CDH lungs were stained by incubation with anti-ALDH1A2 rabbit polyclonal primary antibody [ALDH1A2 (N-20): sc-22591; Santa Cruz Biotechnology] diluted 1:200 in PBS (Tebu, Le Perray-en-Yvelines, France). The samples were then examined, after Hoechst nuclear counterstaining, under a Zeiss Axioshot microscope.

ALDH1A2 Western blotting protocol. The extracts of total proteins were loaded (40 μg/lane) and separated on SDS-PAGE gel, and incubated overnight with goat anti-ALDH1A2 antibody (sc-22591, Santa Cruz Biotechnology) and anti-GAPDH antibody (sc-20357, Santa Cruz Biotechnology) diluted, respectively, 1:200 and 1:100. The blots were incubated with peroxidase conjugated secondary anti-IgG antibody (Abcam) diluted 1:5,000. Blots were stained using a chemiluminescence procedure (ECL Plus kit; Amersham). Band quantification was then performed using Scion software.

Statistical analysis. Results are expressed as means ± SD. Given the small size of the samples, Mann-Whitney U-test or alternatively Kruskal-Wallis one-way analysis of variance were performed to determine significance. A P value of <0.05 was considered statistically significant.

RESULTS

Expression pattern of RA signaling genes during human lung development. Twenty-five critical RA signaling genes were screened by qualitative RT-PCR at the pseudoglandular, canalicular, and saccular stages in a series of 10 whole human lungs (Fig. 2A and Table 1). Most of the genes were expressed from the pseudoglandular to the saccular stages. This was the case in particular for genes encoding R, the main components of Rol uptake and intracellular transport (STRA6, CRBP1, and CRBP2), 2) the Rolβ-carotene-to-Ral converting enzymes (ADH3, ADH4, DHRS4, DHRS9, ROL10, and epimerase, BCM01, and BCO2), 3) the Rol-to-Rol aldoketoreductases (AKR1B1 and AKR1B10), 4) the Rol-to-RoA converting enzymes (ALDH1A1 and ALDH1A2), 5) the cellular retinoic acid binding proteins (CRABP1 and CRABP2), 6) the RA degrading enzyme (CYP26B1), and 7) the enzymes for storage and metabolism of Rol (DHRS1, DHRS5, DHRS6, DHRS7).
hydrolysis of retinyl esters (DGAT and REH). Consistent with animal models, this result suggests that RA pathways play a critical role throughout lung development in humans. By contrast, ALDH1A3 and ALDH5A1 expression was found to be restricted to the canalicular stage, whereas CYP26A1, CYP26C1, and LRAT were not expressed. Pooling the 10 human lung samples and matching the results with positive controls, we confirmed that CYP26A1, CYP26C1, and LRAT were not expressed. The relative expression of each gene of the panel was measured alternatively from nonisolated CDH (n = 3) compared (Fig. 3A). As described above for normal human lungs, LRA, CYP26A1, and CYP26C1 were not expressed in CDH lungs. None of the other RA signaling genes showed any statistically significant difference of expression between CDH and normal lungs, apart from three striking exceptions (Fig. 2B). Since the endodermal and mesodermal compartments were not discriminated by this whole lung screening, we also performed qualitative RT-PCR on the human alveolar epithelial cell line A549. The same expression pattern was found for all the genes (Fig. 2A). To further analyze the functional significance of this metabolic expression pattern, we performed an RA-dependent CAT gene reporter assay in A549 cells. After 24-h incubation with 1 μmol/l of retinol or RA, we observed, respectively, a 2.4-fold (P < 0.05) and 4.1-fold (P < 0.05) induction of CAT expression (Fig. 2C), demonstrating that the RA signaling gene expression pattern in this lung epithelial cell line is consistent with the metabolic activity of the pathway.

Changes in RA signaling gene expression in human CDH lungs. Next we examined the expression levels of the above RA signaling genes in human CDH-injured lungs. The 9 samples originated mainly from isolated CDH (n = 6) or alternatively from nonisolated CDH (n = 3), with a gestational age ranging from 21 to 41 wk (Table 1). The nonisolated CDH did not display classical RA signaling-related malformations. The relative expression of each gene of the panel was measured by quantitative PCR (qPCR), and the results are shown in Fig. 3B. Since the endodermal and mesodermal compartments were not discriminated by this whole lung screening, we also performed qualitative RT-PCR on the human alveolar epithelial cell line A549. The same expression pattern was found for all the genes (Fig. 2A). To further analyze the functional significance of this metabolic expression pattern, we performed an RA-dependent CAT gene reporter assay in A549 cells. After 24-h incubation with 1 μmol/l of retinol or RA, we observed, respectively, a 2.4-fold (P < 0.05) and 4.1-fold (P < 0.05) induction of CAT expression (Fig. 2C), demonstrating that the RA signaling gene expression pattern in this lung epithelial cell line is consistent with the metabolic activity of the pathway.
P = 0.03) with highest induction level (5.3-fold, P = 0.019) at the 24th hour (Fig. 3B). CRBP2 was also induced by RA treatment, but later (48 h) and to a lesser degree (2-fold, P = 0.03), with a progressive increase between 6 and 48 h of treatment (Fig. 3C). Together, these data were strongly suggestive of intracellular RA depletion in human CDH lungs. Because ALDH1A2 is mainly responsible for Ral-to-RA conversion, such loss of CRBP2 and CYP26B1 expression could result from a primitive decrease in ALDH1A2 transcription. On the contrary, we found that ALDH1A2 was strongly overexpressed (5.9-fold, P = 0.03) in human CDH lungs (Fig. 3A). Consistent with the ALDH1A2 mRNA overexpression, the respective protein level showed a 1.6-fold increase (P = 0.028) (Fig. 3, D and E).

Pulmonary RA pathway gene expression in teratogenic and surgical CDH models. To gain further insight into the RA pathway transcriptional signature of human CDH-affected lungs, we performed a second round of qRT-PCR in two CDH animal models.

First we screened the expression of RA signaling genes in the rat nitrofen-induced CDH model (7, 13, 31). This model has been extensively investigated over the past decade. Nitrofen toxicity is now thought to act through aldehyde dehydrogenase inhibition—mostly of ALDH1A2—which leads to a decrease in RA levels. Over the RA signaling gene panel we tested, the expression of Aldh1a1 and Aldh1a2 mRNA was not significantly affected in nitrofen-exposed lungs (Fig. 4A). Cyp26a1 expression appeared to be only slightly and not
Fig. 3. Diaphragmatic hernia disrupts RA signaling gene expression in human lung. A: relative mRNA expression of the RA signaling genes panel [congenital diaphragmatic hernia (CDH)/normal, respectively, \( n = 9 \) and \( n = 10 \)] as measured by qPCR. CYP26B1 and CRBP2 are not expressed in CDH-injured lungs. B and C: bar chart showing, respectively, CYP26B1 and CRBP2 relative induction in A549 cells as measured by qPCR (\( n = 4 \)) after treatment (6th, 24th, and 48th hours). White bar: no treatment (NT); gray bar: vehicle (DMSO); black bar (ATRA, 1 \( \mu \)mol/l). D: representative image of Western blotting with ALDH1A2 and GAPDH specific antibodies in one human CDH-injured lung and one normal human lung. According to the manufacturer’s technical information, the ALDH1A2 antibody (sc-22591) detects two ALDH1A2 isoforms around 55 kDa. E: relative ALDH1A2 protein levels in normal human lungs (\( n = 4 \)) and human CDH-injured lungs (\( n = 4 \)). Results are corrected for GAPDH protein levels. *\( P < 0.05. \)

statistically reduced, whereas Cyp26b1 and Lrat mRNA levels were halved (\( P < 0.05 \)). Furthermore, Dhrs4 mRNA level showed a 1.5-fold increase (\( P < 0.05 \)). Together, these data were consistent with a pattern of intracellular RA decrease. However, the transcription level of aldehyde dehydrogenase genes—especially Aldh1a2—remained unchanged, supporting the view, in parallel, that the ALDH1A2 overexpression in human CDH was not primarily promoted by a decrease in RA levels and positive feedback.

We thus went on to consider the rabbit CDH surgical model (10). In this model, diaphragmatic hernia results only from the surgical procedure on a wild-type background. Thus no genetic or toxic RA signaling disruption occurs, and any mRNA level variation in RA signaling genes is expected to be secondary to lung CDH-related injury. The hernia was created at day 23 (pseudoglandular stage), and the qRT-PCRs were performed at day 29 (saccular stage) on the ipsilateral lung. Crbp2 and Cyp26b1 expression levels remained unchanged. By contrast, we detected a dramatic 10.1-fold increase (\( P < 0.001 \)) in Aldh1a2 transcript level (Fig. 4B). This mRNA increase correlated with a 3.2-fold increase (\( P = 0.029 \)) in the ALDH1A2 protein level (Fig. 4, C and D). Because this induction could result from a putative systemic transregulatory factor, we compared Aldh1a2 mRNA expression in brains from CDH-affected and control rabbit fetuses. The surgical procedure did not affect the mRNA expression of Aldh1a2 in brain tissues (data not shown). During rabbit lung development, the retinol-aldehyde dehydrogenase ALDH1A2 was found to be specifically localized in the epithelial compartment from bronchi down to sacculles (Fig. 5, E–G) and later down to alveoli (Fig. 5, M–O). A similar pattern was evidenced in human fetal lungs at the saccular and alveolar stages (Fig. 5, A–D and I–K). The surgically induced CDH in the rabbit did not modify the site-specific ALDH1A2 localization in epithelial cells along the airways (Fig. 5, Q–T). Further quantitative analysis of Aldh1a2 mRNA level throughout the rabbit lung development showed two bursts of expression, respectively, at the pseudoglandular and saccular stages (Fig. 6A). Comparing this developmental sequence with ALDH1A2 expression in human fetal lung, we observed similar timing (Fig. 6B). In particular, Aldh1a2 mRNA levels appeared to be consistently high at the saccular stage, in both rabbits and
humans. Taken together, these data suggest that Aldh1a2 is strongly overexpressed at the saccular stage in surgically induced CDH and human CDH.

**DISCUSSION**

Multifactorial diseases result from multiple common variants with small effect sizes and environmental factors. Unlike syndromic CDH, isolated CDH etiology in humans is thought to arise from a complex inheritance (41). In this case, identifying small size variants or subtle transcriptional shifts requires huge cohorts of cases and controls, far more than is technically feasible in congenital defects. Therefore, by analyzing the expression of 25 genes of the candidate RA signaling pathway in 10 human normal lungs and 9 human CDH lungs, we expected to detect only significant variations of expression.

One striking feature of this stringent transcriptional screening was the overexpression of ALDH1A2 mRNA in human CDH lungs, subsequently confirmed at the protein level. ALDH1A2 is known to be the functionally most important aldehyde dehydrogenase during mammalian development (30). During human lung development, ALDH1A1 and ALDH1A2 transcripts were detected from the pseudoglandular to the saccular stages, whereas ALDH1A3 and ALDH8A1 were expressed only at the canalicular stage. The ALDH1A2 expression levels were higher at the pseudoglandular and saccular stages, with a maximum at the saccular stage. Together, these data strongly support the idea that ALDH1A2 expression is critical along these two distinct time windows to supply appropriate intracellular RA levels. This critical expression pattern of aldehyde dehydrogenases during human lung development raises the question of how ALDH1A2 is overexpressed in...
Fig. 5. Lung epithelial ALDH1A2 localization is not modified by rabbit surgically induced diaphragmatic hernia. Immunostained sections of human lung (A–D: canalicular stage; I–L: saccular stage), rabbit lung (E–H: saccular stage; M–P: alveolar stage), and CDH-injured lung in rabbit (Q–T: saccular stage) showing the temporal and site-specific expression of ALDH1A2 in the epithelial cells from bronchi down to alveoli. Q–T: CDH-injured lungs in rabbits showing increased ALDH1A2 staining in epithelial cells, without modification of localization. Right: higher magnifications. Counterstaining: Hoechst; b, bronchi; s, saccule; a, alveoli. Scale bar: 100 μm.
human CHD lungs. This transcriptional shift could reflect either a molecular signature of intracellular retinoid depletion or an intrinsic disruption of the pathway.

The former hypothesis was supported by the dramatic extinction of CRBP2 and CYP26B1 expression in the human CDH lungs. Yet both of these genes are known to be retinoid-induced in vivo and in vitro (29, 31, 34, 46). Furthermore, Lrat and Cyp26b1 were found to be expressed at low levels in the rat nitrofen model, whereas Cyp26a1 expression remains unaffected, as previously described (31). Conversely, Dhrs4 was upregulated. These transcriptional modifications can be interpreted as cellular metabolic adaptations to nitrofen-induced RA depletion. Retinol storage and retinoic acid catabolism decrease, whereas the retinol-to-retinal conversion increases to overcome the intracellular RA depletion. Consistent with this picture, Lrat and Cyp26b1 expression levels are also decreased in the mouse VAD model (34, 35). Together, these data support the idea that CRBP2 and CYP26B1 mRNA decreases in human CDH lungs are indirect marks of RA depletion. However, the transcription levels of DGAT, REH (the human retinyl ester storage enzyme), and DHR54 remains stable. These contrasting data suggest that, if present, the intracellular RA deficiency is not a primary event. Rather, the low retinol and RBP levels found in human newborns (2) probably induce chronic intracellular retinol deficiency and secondary RA depletion. Consistent with previous data (31), another finding from our screening in the rat nitrofen model is that Aldh1a2 expression is not sensitive to RA decrease. Thus ALDHA2 overexpression in human CDH lungs is unlikely to result from low intracellular RA levels.

An important feature of the nitrofen model is that aldehyde dehydrogenase inhibition impairs lung development, diaphragm development, or both (15, 19). RA signaling is involved in both lung and diaphragm development, and it is usually difficult to parse out the respective effects of the hernia and the RA signal disruption on lung development. This fact is further illustrated by the Matthew-Wood syndrome (MIM no. 601186), where lung hypoplasia can occur independently of the diaphragmatic hernia (11). Thus a critical issue in understanding the cause of ALDHA2 overexpression in human CDH lungs was to study the transcriptional consequences of diaphragmatic hernia on a wild-type background. In the light of this concern, we took advantage of the rabbit model to investigate this last hypothesis. The temporal ALDHA2 and spatial ALDHA2 expression patterns were very similar during rabbit and human lung development. In particular, ALDHA2 showed a burst of expression at the saccular stage both in humans and rabbits. It has been previously proposed that this specific sequence plays a critical role in RA-dependent alveolization (21). But most importantly, just as in human CDH, we found that both Aldh1a2 mRNA and ALDHA2 protein levels were strongly increased in rabbit hernia-injured lungs. Therefore, we concluded that ALDHA2 overexpression in human CDH is likely to be a mechanistic side effect of the hernia rather than a constitutive disruption of the RA pathway. The retinoid pathway regulates a wide range of cellular behaviors during embryonic development and adult homeostasis (6, 38). Of note, RA signaling is involved in tissue regeneration and repair. Consistent with our findings in the rabbit CDH surgical model, ALDHA2 increases in the first week after rat spinal cord injury, suggesting that retinoid signaling is not involved in the early phase of inflammation in vivo, but rather promotes tissue regeneration in subsequent phases of the inflammatory reaction (16). Furthermore, in situ hybridization experiments showed that Aldh1a2 is strongly expressed around the wound at day 2 during caudal fin regeneration in zebrafish (25). Thus we consider that the Aldh1a2/ALDHA2 overexpression in rabbit and human CDH could result from the hernia-mediated lung injury. The herniated organs not only reduce the lung expansion space in the chest, but also compress lung tissues. How tissue compression intrinsically impairs the lung developmental program is still unclear. Moreover, lung volume measurements in human fetuses with CDH demonstrate that lung volume can vary dramatically and is of major interest for

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**Fig. 6.** Aldh1a2 is strongly expressed at the saccular stage, in both rabbit and human lungs. Relative Aldh1a2 mRNA expression in rabbit (A) and human lung (B), as measured by qPCR from the pseudoglandular to the alveolar or alternatively saccular stage (pseudoglandular stage: n = 4; the following stages: n = 3). *P < 0.05.
the fetal prognosis and postnatal outcome (18). Beside the volume of the hernia and the intensity of the compression, the timing of the compression could also modulate the tissue injury. In the rabbit CDH surgical model, the homolateral lung experiences acute compression. Conversely, in human CDH, tissue compression is expected to be more progressive, and lung hypoplasia occurs or is aggravated concomitant to the hernia. Although the timing and the extent of the hernia are controlled in our surgical model, the human CDH samples we analyzed may represent heterogeneous conditions, especially in hernia severity. Therefore, determining the etiology of the lung developmental changes in CDH remains a critical issue. Comparisons can be made only if appropriate precautions are taken, and we can only suppose from the above discussion that rabbit Aldh1a2 and human ALDH1A2 overexpression is related to lung tissue injury.

In conclusion, although metabolic retinoid signaling disruption does probably not recapitulate the complete pathogenesis of CDH, our systematic screening in a human CDH cohort identified a molecular signature for the retinoid pathway disruption. On the one hand, CYP26B1 and CRBP2 downregulation suggests that intracellular fetal lung levels of RA are low, consistent with low retinol and RBP levels found in cord fetal blood. On the other hand, ALDH1A2 upregulation is not expected to be secondary to low RA levels. Rather the CDH rabbit model we used suggests it could be due to lung tissue injury per se. Thus this study completes the overall CDH mechanistic picture where the retinoid signaling can be primarily disrupted by single-gene mutations or large genomic alterations in the nonisolated forms, or only be affected by subtle primary or secondary modifications in the isolated forms. In the second case, how these modifications contribute to lung damage requires further investigation.

ACKNOWLEDGMENTS

The authors thank Prof. R. R. de Krijger (pathologist) for support in collecting post mortem lung tissue, Dr. I. Shuter for support in collecting rat lung tissue, and A. Herbet for qPCR experiment adjustment on human and rabbit samples.

GRANTS

K. Coste was supported by the French Pediatric Pulmology and Allergology Society (SP2A).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

K.C. and L.W.B. performed experiments; K.C. and V.S. analyzed data; K.C., L.W.B., P.B., D.G., A.D., L.B., D.T., A.L., R.J.R., and V.S. approved the final version of manuscript; D.T. and V.S. conceived and designed of research.

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