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Postnatal decrease of cystic fibrosis transmembrane conductance regulator gene expression in nasal epithelium of healthy newborn infants

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Short title: Postnatal CFTR expression in humans

The fetal lung is filled with fluid as a result of the active ion transport towards the alveolar lumen, thereby promoting early lung development (1, 2). In late gestation and the perinatal period, the transition to extrauterine life requires a switch from fluid secretion to absorption. During gestation, the fetal airway epithelial cystic fibrosis transmembrane conductance regulator (CFTR) mRNA gradually declines, enabling epithelial sodium channel activity and lung fluid absorption to increase (2). Data on CFTR gene expression and chloride transport in the airways remain scarce during early postnatal adaptation and are primarily based on *in vitro* and animal studies (1, 2). Our aim was to examine changes in airway epithelial CFTR gene expression in nasal samples taken from human newborn infants during the critical transition to air breathing. We also assessed the association between nasal epithelial CFTR mRNA expression and lung compliance during the first postnatal hours.

We studied 48 healthy full-term newborn infants from normal pregnancies at Helsinki University Hospital from March 2011 to June 2015: 22 were born vaginally and 26 by elective Caesarean. The mean and standard deviation (SD) values were: gestational age (39.6 ±0.8), birth weight (3.639 ± 0.432 kg) and cord artery pH (7.28 ± 0.05). The median (range) one-minute Apgar score was nine (6–10). All the infants had an uncomplicated clinical course.

The local ethics committee approved the study and the parents provided written consent.

We gathered nasal epithelial samples as a surrogate for the epithelium of the distal respiratory tract, at a median and interquartile range (IQR) of two minutes (1–3 minutes) and 1.3 hours (0.9–1.7). CFTR mRNA was quantified in relation to the epithelial cell marker cytokeratin 18 mRNA level using real-time polymerase chain reaction, as previously described (3).

To assess lung elasticity, we measured static lung compliance in 26 infants at three hours (1.5–4.5) using the previously described double occlusion method (4). A paediatric pulmonologist, blinded to the patient history, analysed the pressure-volume curves.

Data are expressed as means and SDs and medians and IQRs, as appropriate, unless stated otherwise. Temporal changes in CFTR expression were calculated with the Wilcoxon signed-rank test and between-group comparisons with the Mann-Whitney U test. The association between CFTR expression and lung compliance was assessed using Pearson's correlation. All statistical analyses were performed using SPSS Statistics, version 23.0 (IMB Corp, New York, USA) and Prism 8.0 (GraphPad Software, California, USA).

The relative amount of CFTR mRNA in 37 infants was higher at birth than at one hour: 2.90 (1.39–4.41) versus 2.07 (1.28–2.86) ($p < 0.001$). The decrease in CFTR mRNA expression during the first postnatal hour was greater following Caesarean (ΔCFTR = 1.11 ± 0.87, $p = 0.001$) than vaginal birth (ΔCFTR = 0.43 ± 0.76, $p = 0.023$) (Figure 1). We found no difference in CFTR expression between birth methods at birth ($p = 0.967$) or at one hour postnatally ($p = 0.073$).

Mean lung compliance at three hours postnatally was 11.6 ml/kPa/kg (8.0–15.3) and negatively correlated with CFTR expression at birth (Pearson's $r = -0.424$, $p = 0.031$, $n = 26$). We found no correlation between CFTR expression at one hour and lung compliance at three hours (Pearson's $r = -0.068$, $p = 0.811$, $n = 15$).

Animal studies indicate that the role of CFTR expression changes during lung development. In sheep, the decrease in alveolar CFTR expression during the second trimester continues until birth (2). In rats, CFTR transcription in newborn lungs is twice as high as in an adult lung and declines during the first postnatal days (5). We also found a postnatal decrease in CFTR expression during the first hour of life.

In addition, we found that the decrease in CFTR expression was more pronounced in the Caesarean than vaginal group. This was somewhat unexpected, as glucocorticoids reduce CFTR expression *in vitro* and in rat lungs in a dose-dependent manner (1). Also, stress hormones' – cortisol and catecholamines – concentrations in human cord blood are markedly higher in vaginal than Caesarean births (3). The more pronounced decrease in CFTR expression in Caesarean births might be explained by a delayed surge of stress hormones compared with the surge in vaginal births.

Improved lung compliance has been suggested as a useful indicator of lung fluid clearance during the first hours of life (4). In our study, the association between higher lung compliance and a lower CFTR expression at birth may indicate a role for CFTR in the shift from lung fluid secretion to absorption, a prerequisite for the successful transition to extrauterine life. We found a correlation between lung compliance at three hours and CFTR expression at birth, but not at one hour postnatally. The smaller number of CFTR measurements at one hour precludes definitive conclusions, but a potential explanation may lie in the effect of the ion channel transport, which translates as a delay to end-organ function, as previously shown with epithelial sodium channel (4).

Our study had some limitations. We quantified no CFTR protein levels or channel activity, as early sampling and limited cell samples made combining additional parameters difficult. Also, noninvasive nasal epithelial samples were a surrogate for the epithelium of the respiratory tract, where ion transport processes may vary between alveolar epithelial cells and more proximal airway epithelial cells.

Our findings revealed a significant decrease in nasal epithelial CFTR expression during early postnatal life and a negative correlation between early postnatal nasal CFTR expression and static lung compliance. Whether this correlation can be explained by the link between the airway CFTR expression and transepithelial fluid transport requires further study.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

Abbreviations:

CFTR – cystic fibrosis transmembrane conductance regulator

IQR – interquartile range

mRNA – messenger ribonucleic acid

SD – standard deviation

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FIGURE LEGEND:

Figure 1. Temporal changes in the postnatal nasal epithelial CFTR expression according to the mode of delivery. CFTR expression decreases significantly between the two minutes and one hour time points (Wilcoxon signed-rank test). The box plots represent the median (IQR).