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# Laser microdissection allows detection of abnormal gene expression in cystic adenomatoid malformation of the lung

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**Abstract**

**Background/Purpose:** Congenital cystic adenomatoid malformation (CCAM) of the lung may result from a localized aberrant epithelial-mesenchymal interaction during lung development. We used laser microdissection (LMD) to isolate the epithelium and mesenchyme of CCAM, and studied candidate gene expression in these pure cell populations.

**Methods:** Congenital cystic adenomatoid malformation tissue was obtained from fetal (n = 5) and postnatal (n = 5) surgical specimens. Normal fetal lung (n = 10) was obtained from abortus material, and normal postnatal lung (n = 5) was identified from surgical specimens. Whole tissue was analyzed using immunohistochemistry and reverse transcriptase polymerase chain reaction (RT-PCR). Using LMD, columnar bronchiolar type epithelium and underlying mesenchyme were isolated. Multiplex nested RT-PCR was then used to detect message levels of candidate genes.

**Results:** Reverse transcriptase polymerase chain reaction performed on LMD-isolated tissue, but not whole tissue homogenate, revealed differences between CCAM and normal lung. In this report, we focus on the fibroblast growth factor (FGF) family. By RT-PCR, there was 4-fold more epithelial expression of FGF9 in fetal CCAM vs normal fetal lung ($P < .07$). This was qualitatively confirmed by immunohistochemistry. We also detected decreased FGF7 expression in CCAM mesenchyme ($P < .05$) but no significant differences in FGF10 or FGFR2.

**Conclusions:** LMD may be used to overcome the limitations of tissue heterogeneity in the study of CCAM. Abnormal growth factor expression may play a role in the etiology of this lesion.


Lung morphogenesis is an intricate process that is heavily dependent on the exchange of information between the developing cellular compartments, namely, epithelium, mesenchyme, and mesoderm, the primordial pleura. A local disruption of crosstalk between the epithelium and mesenchyme early in lung development could result in abnormal growth and differentiation of the developing lung. Depending on when during development this disruption occurs, epithelial differentiation might arrest at a more proximal or distal bronchiolar stage, or even at the early alveolar stage. In most types of congenital cystic adenomatoid malformations (CCAMs), the result is a focal preponderance of normal but proximal epithelium lining

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variably dilated tubular spaces, with abundant surrounding mesenchyme and a lack of well-developed distal alveoli.

When CCAM stromal growth or cyst expansion exceeds cardiopulmonary reserve in prenatal life, hydrops and intrauterine demise may result. Over the past 2 decades, fetal surgery has been performed in some of these severe cases, with specimens preserved from the procedures. Prenatal resection of CCAM as a treatment modality is rarely indicated because good outcomes are expected in most cases [1-5]. Thus, the supply of fetal CCAM specimens is extremely limited. Unfortunately, it is during fetal life that these lesions originate, grow, and often regress, and, therefore, the study of postnatal lesions alone is unlikely to yield etiologic information, because any inciting events occur early in lung development. In addition, no convincing animal model exists for this disease. Though studies have been performed on certain candidate factors with suggestive results, we theorized that a more controlled systematic analysis of fetal CCAM specimens might provide better insight into its origins [6-10].

Initially, we performed a microarray analysis of CCAM in our laboratory but detected very few significantly differentially expressed genes. Microarray is relatively insensitive to subtle differences in expression compared with reverse transcriptase polymerase chain reaction (RT-PCR) and can produce bias through nonlinear amplification of RNA. In addition, tissue heterogeneity is a confounding factor in the study of CCAM, and histology is highly variable from lesion to lesion [11]. Therefore, we used laser capture microdissection (LMD) to isolate the predominant epithelium in CCAM specimens, as well as its underlying mesenchyme, and performed careful analysis of gene expression on those cells.

Fibroblast growth factors (FGFs) are key regulators of lung development. Fibroblast growth factors 7 and 10 are both expressed in mesenchyme and signal to the epithelium to induce dilation and branching, respectively [12-14]. The pattern of FGF9 expression in humans is unknown, but in mice, it has been shown to be an essential epithelial and mesodermal regulator of mesenchymal proliferation [15-17]. Fetal epithelial (heterotopic) overexpression of FGF7 in transgenic mice produces cystic lungs that resemble macrocystic or Stocker type I CCAM [12], whereas FGF10 overexpression causes adenomatous malformations resembling type IV CCAM [13]. Fibroblast growth factor 9 epithelial (orthotopic) overexpression produces dramatic lung enlargement through mesenchymal proliferation, branching arrest, and luminal dilation [15]. This phenotype resembles a microcystic or type III CCAM.

In a previous study, FGF7 expression was similar in CCAM and normal lung (7). Fibroblast growth factors 9 and 10 expression in CCAM has not been reported, nor has the FGF7/9/10 receptor, FGFR2. We theorize that expression of these or other key growth factors could be abnormal in CCAM, and that failure in the past to detect any differences may have been caused by tissue heterogeneity and small sample size. Using LMD and pure cell populations, we aimed to overcome these limitations and detect aberrant gene expression in CCAM.

## 1. Materials and methods

### 1.1. Human tissue acquisition

Most of the tissue used in this study was part of the University of California at San Francisco (UCSF) Department of Pediatric Surgery and Fetal Treatment Center Fetal Tissue Bank, and approval for the generation and use of the tissue bank was granted by the UCSF Committee on Human Research (CHR, approval number H11258-25158-04). One fetal CCAM sample was the generous gift of Dr Portia Kreiger and Dr Phil Ballard of the Children's Hospital of Philadelphia.

Experimental and control groups and their characteristics are listed in Table 1. Fetal CCAM samples (n = 5) and postnatal CCAM samples (n = 5) as well as normal postnatal lung (n = 5) were obtained at the time of surgical resection. Age-matched mid-gestation (assessed by fetal foot length) fetal control samples (n = 10) were obtained immediately after evacuation. Written informed consent was obtained before the procedures. All samples for LMD and genetic studies were immediately flash frozen in liquid nitrogen and stored at −80°C. All samples for immunostaining were fixed in a solution of 4% paraformaldehyde in 0.1 mol/L phosphate buffer, submerged in a 30% sucrose solution overnight at 4°C, then frozen with freezing medium in liquid nitrogen.

**Table 1** Specimen characteristics

| Fetal CCAM (n = 5) | Fetal normal lung (n = 10) | Postnatal CCAM (n = 5) | Postnatal normal lung (n = 5) |
|---|---|---|---|
| Age (wk gestation) | Age (wk gestation) | Ages | Ages |
| 24.2 ± 3.2 | 23.1 ± 1.9 | 5 d | 1 mo |
| | | 3 wk | 4 mo |
| Sex | Sex | 4 mo | 7 mo |
| 3F, 2M | 5M, 5F | 7 mo | 17 mo |
| | | 7 y | 7 y |
| Age/sex (CCAM type) | Age/sex | | |
| 20.6M (III) | 20.6M | | |
| 21F (I) | 20M | | |
| 24.9M (II) | 21F | | |
| 27F (I) | 23.7F | | |
| 27.3F (II) | 25M | | |
| | 24F | | |
| | 24F | | |
| | 24F | | |
| | 24M | | |
| | 25M | | |