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## Protocol – review article

# Where next for translational research in bronchopulmonary dysplasia? - insights from cellular pathology

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## Background

### Description of the condition in clinical practice

Preterm birth rates are increasing globally with approximately 15 million babies worldwide born before 37 weeks gestation.[1] Advances in neonatal care have meant survival for babies born preterm is improving. In England, results of the EPICure studies showed survival for babies born between 22-25 weeks gestation increased from 40% in 1995 to 53% in 2006. Survival for a live born infant at 26 weeks' gestation was 77%, equating to 448 babies.[2] In 2014 in the United States approximately 380,000 births were preterm (9.57% of all births) of which around 27,500 were extremely preterm births (<28 weeks' gestation)[3] with similar rates of survival.[4, 5]

However, despite increased survival, prematurity is still associated with increased morbidity both in childhood and adult life. Prematurity is associated with adverse neurodevelopmental outcomes, increased risk of hearing impairment, development of retinopathy of prematurity and bronchopulmonary dysplasia (BPD).[2] BPD is the most common Chronic Lung Disease (CLD) in children, primarily affecting the preterm neonate, defined as the need for supplementary oxygen at 28 days of life, or at 36 weeks post-conceptional age.[6, 7] Low birthweight and increasing prematurity both increase the risk of BPD development, and the incidence of BPD can vary widely between neonatal units. In addition, the proportion of extremely preterm babies surviving with BPD has remained similar for the last 30 years. In the UK, 68% of babies born at 22-26 weeks gestation will have BPD at discharge from hospital and in the USA 35% (18,000) of extremely preterm infants <28 weeks gestation will develop BPD.[2, 8]

There are both short term implications and longer term sequalae related to BPD. Up to 50% of patients will require readmission to hospital with a lower respiratory tract illness in the first 2 years of life. Children with BPD are at higher risk of airway hyper-responsiveness and asthma-like symptoms in addition to having abnormal lung function and reduced respiratory reserve as young adults.[9] There is also a greater risk of neurological and motor deficits in those preterm infants with BPD and increased pulmonary vascular resistance in this population group.[10, 11]

## Factors leading to the development of BPD

The pathogenesis of BPD development is complex and multifactorial in nature resulting from a combination of antenatal, perinatal and postnatal factors impacting on pulmonary vascular and alveolar development.[12] Antenatal risks include genetic factors, male sex, intrauterine growth restriction, maternal pre-eclampsia and chorioamnionitis. Perinatal and postnatal insults include preterm birth, mechanical ventilation, hyperoxia, infection and inflammation.[11]

BPD was first described in 1967 by Northway et al, who reported a severe form of CLD ("classical" BPD) in preterm infants identified on serial radiographs. This was characterised by progression from diffuse parenchymal lung disease with low lung volumes, to chronic changes of heterogenous infiltrations, cystic lesions, severe hyperinflation and cardiomegaly on x-ray. Post-mortem results on these infants showed hypertensive pulmonary vascular remodelling, large airway smooth muscle hyperplasia, heterogeneity of lung parenchyma with diffuse fibroproliferative changes.[12, 13] The most pathogenic attributing factors for classical BPD were oxidative stress and mechanical ventilation. The role of mechanical ventilation in the development of BPD is well described and demonstrated in animal studies occurring as a result of volutrauma and to a lesser extent barotrauma.[12] Northway and colleagues also demonstrated that 100% oxygenation in mice studies reproduced the same severe lung disease as seen in classical BPD.[11]

Through advances in neonatal care, such as exogenous surfactant administration, the use of antenatal steroids and changes in ventilation practices, the landscape of BPD has changed. A newer form of BPD has emerged likely related to the cessation of normal lung development secondary to extreme prematurity.[12] This is characterised by more homogenous lung parenchyma with a larger, simpler alveolar structure and mild airway muscle thickening and this has also been replicated in lamb and baboon animal studies.[12] X-ray changes appear less pronounced, mainly characterised by diffuse haziness, with occasional infiltrates and atelectasis.

## Prevention and treatment of BPD

Given its clinical importance, understanding the underlying pathogenesis of the condition is essential in order to develop effective therapies in the prevention and treatment of BPD. Minimising BPD development is achieved through the use of maternal antenatal steroids, postnatal exogenous surfactant administration, caffeine citrate and glucocorticoid administration and using the minimal required mechanical ventilation.

For patients with established BPD, management strategies include non-invasive and invasive ventilatory support and supplemental oxygen. Treatment options include the use of diuretic

therapy, corticosteroids, and bronchodilators,[11, 14] however, despite their widespread use there is little evidence base for the use of either diuretics[15] or bronchodilators in the treatment of BPD. Bronchodilators have shown no benefit in their use to either prevent, or treat BPD, and are recommended for a sub-group of patients only with BPD and asthma type symptoms with lung function reversibility.[16, 17]

Emerging therapies include the use of mesenchymal stem cells[18] as well anti-inflammatory agents, such as inhaled corticosteroids which by acting locally, aims to avoiding the adverse effects of systemic corticosteroid administration.[19] By understanding the underlying mechanisms of lung development and disease, it can allow for new treatments to be targeted.

## Mechanotransduction pathways in the lung

Lung formation begins between 3-6 weeks gestation, with alveolar growth and development continued into the late teenage years. Normal lung development is the result of the balance of a number of cell signalling pathways. Heterotrimeric guanine nucleotide-binding protein (G protein) signalling links hundreds of G protein-coupled receptors with four G protein signalling pathways. One of which is the $G_{\alpha q/11}$ pathway. Previous animal studies in mice by our group has demonstrated that alveolar epithelial $G_{\alpha q/11}$ is required to maintain alveolar homeostasis, with knockout mice deficient in $G_{\alpha q/11}$ alveolar signalling having immature lung development and maturation phenotypically similar to BPD. Transforming growth factor beta (TGF-β) is thought to be implicated in this pathway through activation via G protein signalling and subsequent downstream pathways.

TGF-β is a profibrotic and anti-inflammatory cytokine, existing as three isoforms (TGF-β1, TGF-β2 and TGF-β3) with homeostasis required for normal lung development.[20] TGF-β1 is expressed by all cells within the body, and within the lungs it has been most studied in relation to lung development and disease. TGF-β1 overactivation promotes epithelial injury and fibrosis, demonstrated in both animal and patient studies of idiopathic pulmonary fibrosis.[21] In addition, overexpression of TGF-β has been implicated in BPD.[20] Studies have shown that one mechanism of TGF-β activation is through a force-dependent activation through cyclical stretch.[22] Given one of the main contributing factors identified in the development of BPD is the use of mechanical ventilation, an understanding of TGF-β activation through cyclical stretch is essential. At present, the role of the other isoforms TGF-β2 and TGF-β3 are less well understood in BPD but they may also be implicated.

## Objectives of this review

In this review we will provide a detailed overview of the underlying pathways in normal lung development and BPD. In particular we will primarily provide a comprehensive account of the

role of $G_{\alpha q/11}$ mediated TGF-β activation pathway in alveolar development from what is known so far in the literature on alveolar homeostasis. We will consider all TGF-β isoforms, and we will explore the involvement of TGF-β in the development of BPD. This will include a review of the role of pericytes and cyclical stretch in this pathway.

In addition, we will develop upon the work of a recent review article on BPD by Thébaud et al[11] which provided a detailed overview of the preventative strategies and treatment options for BPD. We will review these treatment strategies and give insights in the possible underlying pathways of mechanisms of action, in particular if TGF-β is implicated in this, in addition to recently discovered and emerging treatments for BPD not currently used in practice.

**Method:**

**Criteria for considering studies for this review**

**Types of studies**

We will include all cell biology studies presenting original data related to TGF-β in physiological lung development and BPD from the last 10 years, and in addition we will also specially detail the impact of $G_{\alpha q}$ and $G_{11}$ mediated TGF-β activation in the lung. We will consider all articles relating to cyclical or mechanical stretch in the lung.

We will consider all studies of both mothers or neonates which discuss the underlying pathways of preventative or treatment options for BPD and lab based and animal studies related to this. There will be no restrictions on the types of study design eligible for inclusion. We will include both published and ongoing studies which present original data: lab based, animal and human participant studies. We will check the reference lists of review articles for papers not identified by our primary search.

**Search methods**

Studies will be identified by searching PubMed, CINAHL, MEDLINE, EMBASE and the Cochrane library. The defined search strategies to be used are listed in appendix 2. In order to increase sensitivity, broad search criteria have been used. Searches will be conducted over the period 1/1/2020 – 1/4/2020, and articles published in English will be included. We will also perform searches in preprint servers which will include but will not be limited to MedRxiv, Biorxiv, Zenodo and SSRN.

In addition, searches will be completed of clinical trials registers over the same time period. Searches will be completed in EU clinical trials register, Australia and New Zealand clinical trials register, ISRCTN and Clinicaltrials.gov.

Search results will be downloaded into EndNote and checked for duplicates via the inbuilt duplicate finder followed by a manual check of remaining results. For studies identified through clinical trials registers or preprint servers these will be manually checked for duplicates and compared against results from the database searches and duplicates excluded. The results will be uploaded into Rayyan systematic review web application[23] for consideration of inclusion for the review.

## Data collection and analysis

### Selection of studies

To minimise selection bias, titles and abstracts will initially be scanned by two reviewers in Rayyan against the inclusion criteria (Appendix 1), and those not relevant will be excluded. Following this, full text articles will be sought for those articles which had met the inclusion criteria based on title or abstract and scanned again by the two reviewers for inclusion. For articles where either the full text article is not available, or is an abstract from a conference, the abstracts will be reviewed again. If any meaningful results can be gained from this which meet the inclusion criteria these will be included, otherwise they will be excluded. Any articles excluded at this stage will have the reason for exclusion recorded.

Where there is disagreement between the two reviewers at any stage of study selection, discrepancies will be discussed until an agreement is reached. If an agreement cannot be reached, we will ask a third reviewer to review the article and make the final decision.

### Data extraction and Analysis

The two reviewers will extract the data from original research publications using a pre-defined data extraction form created on google forms. This information will then be collated in excel for analysis. We will analyse the results in two parts.

Firstly, the physiology of normal lung development and the development in BPD. Included within this will be the role of TGF-β activation and cyclical stretch in the lung. Secondly, we will analyse articles related to the prevention and treatment of BPD including mechanism of action and underlying mechanotransduction pathways. Through doing this we aim to provide a detailed overview of the knowledge known so far and highlight gaps in the current evidence. This will then be complied into a state of the art review and submitted to a peer reviewed journal.

## References


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**Appendix 1.**

**Criteria for considering studies for review**

**Inclusion criteria**

- Any article related to the role of TGF-β in either lung development or its involvement in BPD
- Articles relating to the pathogenesis of BPD
- Any articles related to $G_{\alpha q}$ mediated TGF-β activation in the lungs
- Any articles related to $G_{a11}$ mediated TGF-β activation in the lungs
- Any articles related to cyclical or mechanical stretch in the lungs
- Any article with gives insights into the possible underlying pathways or mechanisms of action for prevention or treatment strategies used in BPD, or in other conditions related to the lung where the same treatments are also used in BPD.
- Types of studies: published and ongoing studies presenting original data which will include lab based, animal and human participant studies. Full text articles, abstracts and conference abstracts to be considered.
- Articles from 1/1/2010 – 1/4/2020

**Exclusion criteria**

- Any article related to the explanation of normal foetal lung development via non-TGF-β signally pathways
- Any article related to the development of BPD including its effect on lung development structure and lung remodelling where the above mentioned pathways are not discussed
- TGF-β where its involvement is related to another organ or disease process i.e. outside normal lung development or disease affecting the lung, or cancer related pathways involving TGF-β
- Mechanotransduction processes in BPD unrelated to TGF-β
- Articles related to the preventive therapies or treatments of BPD, which do not offer explanation of the underlying pathways in BPD.
- Review articles which do not present their own original data
- Articles published outside the designated time period above

**Appendix 2.**

**Search terms**

Searches to be completed using the filter "Title/Abstract" or similar, and "published 1/1/2010 – 1/4/2020"

**Pubmed**

1) Bronchopulmonary dysplasia
2) Chronic lung disease AND (neonatal OR neonate)
3) 1 OR 2
4) Transforming growth factor beta OR TGFβ OR TGF-β
5) Gaq OR Gq OR Ga11 OR G11
6) Myofibroblast OR pericytes
7) Caffeine citrate OR Vitamin A OR corticosteroid OR bronchodilator OR diuretic OR mesenchymal stem cell
8) Cyclical stretch OR mechanical stretch
9) Alveologenesis OR lung development OR alveolar development
10) Pathogenesis
11) 3 AND 10
12) 3 AND 4
13) 3 AND 7
14) 4 AND 5
15) 4 AND 9
16) 4 AND 8
17) 3 AND 4 AND 6
18) 3 AND 4 AND 7
19) **11 OR 12 OR 12 OR 14 OR 15 OR 16 OR 17 OR 18**

**EMBASE and Medline**

1) bronchopulmonary dysplasia.mp. or *lung dysplasia/
2) chronic lung disease.mp. or *chronic lung disease/
3) prematurity/ or preterm.mp.
4) (1 AND 3) OR (2 AND 3)
5) transforming growth factor beta/
6) pathogenesis/
7) cyclical stretch.mp.
8) mechanical stretch.mp.
9) caffeine citrate/
10) corticosteroid/
11) bronchodilator.mp.
12) diuretic.mp.
13) mesenchymal stem cell/
14) vitamin/

15) GaQ.m_titl.
16) G11.m_titl.
17) pericyte/
18) myofibroblast/
19) *lung development/ or alveologenesis.mp.
20) Drug mechanism
21) 9 OR 10 OR 11 OR 12 OR 13 OR 14
22) 4 AND 21
23) 4 AND 5
24) 4 AND 6
25) 6 AND 22
26) 5 AND (7 OR 8)
27) 5 AND (17 OR 18)
28) 5 AND 19
29) 20 AND 21
**30) 15 OR 16 22 OR 23 OR 24 OR 25 OR 26 OR 27 OR 28 OR 29**

**CINAHL**

1) AB bronchopulmonary dysplasia
2) AB chronic lung disease of prematurity
3) AB premature
4) 3 AND (1 OR 2)
5) AB caffeine citrate OR AB bronchodilator OR AB corticosteroid OR AB Vitamin A OR AB diuretic OR AB mesenchymal stem cells
6) AB pericyte OR AB myofibroblast
7) AB Gaq OR AB Gq OR AB Ga11 OR AB G11
8) AB pathogenesis
9) AB Alveologenesis OR AB Lung development
10) AB cyclical stretch OR AB mechanical stretch
11) AB transforming growth factor beta OR AB TGF-β OR AB TGFβ OR AB TGF beta
12) 4 AND 11
13) 7 AND 11
14) 4 AND 5
15) 4 AND 8
16) 4 AND 9
17) 6 AND 11
18) 10 AND 11
19) 7 AND 9
20) **12 OR 13 OR 14 OR 15 OR 16 OR 17 OR 18 OR 19**

**Cochrane reviews, trials and protocols**

1) Bronchopulmonary dysplasia OR BPD
2) Transforming growth factor beta OR TGF-β OR TGFβ
3) 1 AND 2

4) caffeine citrate OR bronchodilator OR corticosteroid OR Vitamin A OR diuretic OR mesenchymal stem cells OR treatments
5) 1 AND 4