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Evaluating the Use of iPSC-Derived Models in Understanding the Pathogenesis of Childhood Interstitial Lung Disease

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ABSTRACT

Rationale: Genetic testing has significantly improved the diagnosis of childhood interstitial lung diseases (chILD), which have long challenged clinicians due to their heterogeneity and poor characterization. It is now imperative to study variants of unknown significance (VUS) to identify pathogenic mutations to optimize diagnosis and screening in patients. Furthermore, the limited treatment options for patients with chILD worsen patient outcomes. Induced pluripotent stem cell (iPSC)-derived models could be a tool to understand the effect of novel VUS and discover new therapeutic interventions.

Objective: This review seeks to evaluate the fidelity of iPSC-derived models to recapitulate the pathogenic processes of chILD and test therapeutic interventions.

Methods: This paper performs a systematic search over three databases to identify iPSC-derived models studying disease-causing mutations in pediatric patients with chILD and Hermansky-Pudlak Syndrome.

Results: Of the 1452 papers initially reviewed, eight papers met the inclusion criteria using iPSC-derived models to study genetic variants. The diseases covered included common manifestations of chILDs such as surfactant protein deficiencies and hereditary pulmonary alveolar proteinosis, as well as fibrotic disorders like Hermansky-Pudlak Syndrome. These models recapitulated patient histology and key pathogenic features reported in the literature, delivering mechanistic insights into these conditions. Some papers also explored the efficacy of novel treatments, such as gene therapy.

Conclusions: iPSC-derived models can mimic aspects of human lung responses to provide a platform for disease modeling and therapeutic testing in chILD. There are opportunities to develop more complex multi-cellular models and for the study of a wider range of variants using these tools.

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1 | Introduction

Childhood interstitial lung diseases (chILD) encompass a heterogeneous spectrum of respiratory conditions, primarily affecting children under 2 years. These conditions are associated with high mortality and morbidity from infancy [1]. chILD was initially thought to be very rare, however, more recent studies have reported a higher prevalence than prior studies [2, 3].

The pathogenesis of chILD is varied and often unknown. Recently it has been proposed that chILD cases can be classified into one of four causes: genetic, environmental, other known nongenetic, and unknown [4, 5]. Improvements in genetic testing have led to increased identification of the etiology of chILD cases, with recent studies suggesting genetic testing has supplanted lung biopsy as the key diagnostic test [6]. However, one issue with genetic testing is the identification of variants of unknown significance (VUS) where it is unclear if the identified mutation is responsible for the observed clinical phenotype.

Another issue with the management of children affected by chILD is the lack of effective treatments [7]. Currently many specific chILD cases lack an effective treatment, and some of the treatments used empirically are associated with significant iatrogenic harm (i.e., high dose systemic corticosteroids) [8]. As such there is a need to better understand the pathophysiology of specific chILD aetiologies to identify novel therapeutic approaches.

Induced pluripotent stem cell (iPSC) derived models of the lung have emerged as a potential tool to address the issues with determining the significance of VUS, better elucidating pathophysiology, and discovering potential therapies. These models can be created from easily collected biospecimens from patients (e.g., blood) and then can recapitulate cells in the lung interstitium. The genetic tractability of iPSCs means that isogenic patient lines can be created to precisely characterize the effect of VUS [9]. Further, these In Vitro models can be used to better assess disease pathophysiology to identify therapeutic targets, assess the efficacy of novel therapies or for high-throughput drug screening (Figure 1).

This paper aims to perform a scoping review on studies that have used iPSC-derived models to model genetic causes of chILD, with the aim of assessing whether these models can recapitulate the disease, if they have improved understanding of pathophysiology, and whether that have been used to test therapeutic interventions. In doing so, we hope to understand whether iPSC-based models can advance our current understanding and management of chILD.

2 | Methodology

2.1 | Identification of Relevant Studies

The search strategy used is reported in Appendix A. Languages other than English were included.

2.2 | Inclusion Criteria

Studies evaluating any genetic cause of chILD, as per prior classification systems were included [10]. Studies of chILD-affected patients who are children (under the age of 18) or adults who developed ILD as children were included. Studies without patient donors were also chosen if they investigated known mutations that cause chILD presentations. We included studies using iPSCs or embryonic stem cells (ESCs) to create In Vitro lung models, whether it be organoids or monocultures of a specific cell found in the alveolus.

2.3 | Exclusion Criteria

We excluded studies investigating adult-onset ILDs or idiopathic pulmonary fibrosis as this typically occurs in adults. Animal studies were excluded, including studies that generated iPSCs from animal somatic cells, even if they investigated human mutations. Studies that were not research articles and studies that only documented methodologies of

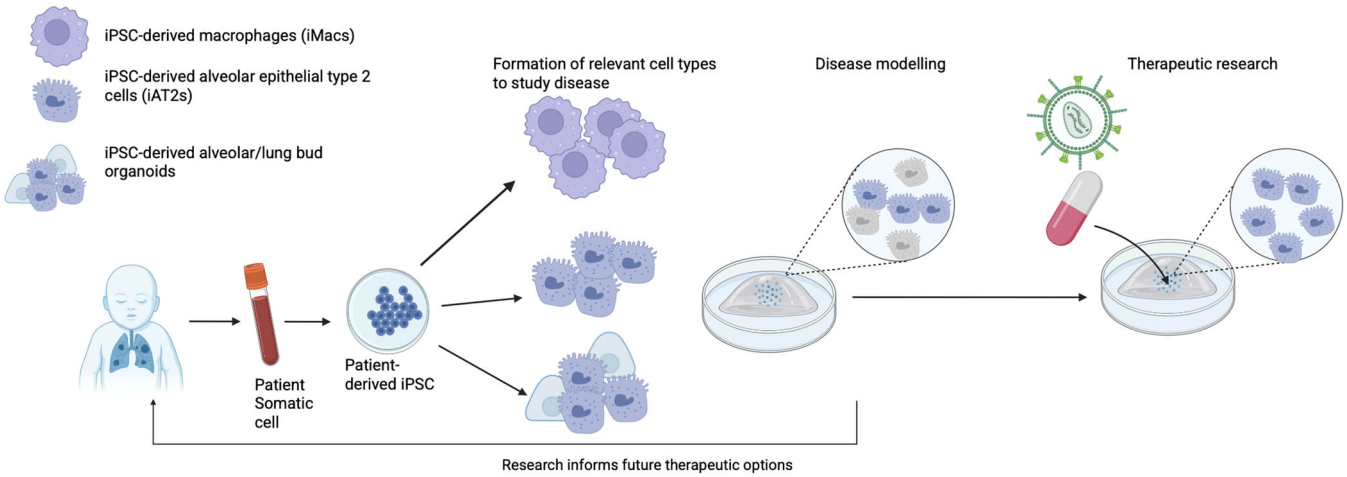

FIGURE 1 | Applications of patient derived-induced pluripotent stem cell (iPSC) organoids in disease modeling and therapeutic discovery. [Color figure can be viewed at wileyonlinelibrary.com]

making iPSC-derived cell types but did not perform disease modeling were also excluded.

2.4 | Planned Analysis of Included Papers

In this paper, we aim to narratively review the evidence from the selected studies to understand chILD pathogenesis and the utility of iPSC-derived models to achieve this goal.

3 | Results

The results of the literature search are summarized in Figure 2. A total of 1139 studies were initially reviewed with eight final studies included. These eight studies (summarized in Table 1) examined three conditions: surfactant protein deficiency (SPD) ($n = 4$), hereditary pulmonary alveolar proteinosis (hPAP) ($n = 2$), and Hermansky-Pudlak Syndrome (HPS) associated interstitial pneumonitis (HPSIP, $n = 2$).

3.1 | Surfactant Protein-B Deficiency

Pulmonary surfactant is composed of surfactant proteins (SP) A, B, C, D and lipids. Proteins B and C are encoded by the genes; *SFTPB* and *SFTPC* respectively [12]. Loss-of-function mutations in these genes are associated with chILD, often presenting with severe respiratory distress from birth [13–15].

Jacob and colleagues [16] developed a new differentiation protocol to create human iPSC-derived alveolar epithelial type 2 cells (iAT2s) and evaluated their capacity to mimic properties of primary AT2s and recapitulate surfactant protein B deficiency (SBD). They discovered that iAT2s could self-renew, possessed

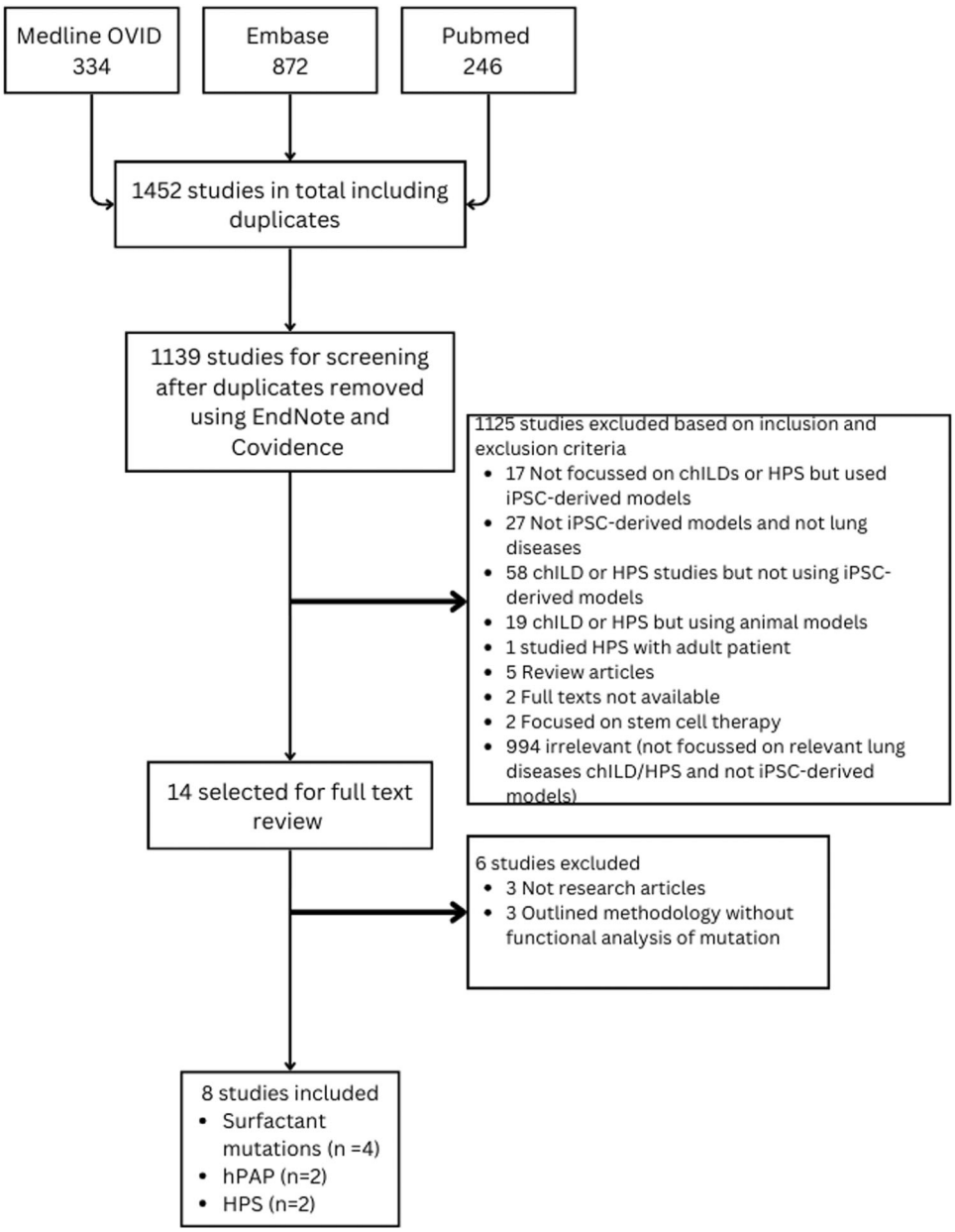

FIGURE 2 | Results of the literature search strategy.

TABLE 1 | Summary of the conditions and mutations studied in the included studies.

Title	Condition	Mutation loci and variant	iPSC-derived cell type	Control	Patient information	Patient histology
Jacob, A., Morley, M., Hawkins, F., Mccauley, K. B., Jean, J. C., Heins, H., Na, C.-L., Weaver, T. E., Vedaie, M., Hurley, K., Hinds, A., Russo, S. J., Kook, S., Zacharias, W., Ochs, M., Traber, K., Quinton, L. J., Crane, A., Davis, B. R.,...Kotton, D. N. (2017). Differentiation Of Human Pluripotent Stem Cells Into Functional Lung Alveolar Epithelial Cells. Cell Stem Cell, 21(4), 472-488.E410	Surfactant B deficiency (SBD)	Patient with SFTPB mutation ($SFTPB^{121ins2}$)	iPSC-derived Alveolar Epithelial Cells Type 2 (iAT2) monoculture	Isogenic, homozygous gene-edited corrected lines	Age of diagnosis 1 month, Homozygous for mutation, Treated with bilateral lung transplantation	Interstitial pneumonitis, AT2 hyperplasia, smooth muscle hypertrophy, abnormal small air space, alveolar proteinosis, vascular changes consistent with pulmonary hypertension
Leibel, S. L., Winquist, A., Tseu, I., Wang, J., Luo, D., Shojaie, S., Nathan, N., Snyder, E., & Post, M. (2019). Reversal Of Surfactant Protein B Deficiency In Patient-Specific Human Induced Pluripotent Stem Cell Derived Lung Organoids By Gene Therapy. Scientific Reports, 9(1), 13450. https://doi.org/10.1038/S41598-019-49696-8	Surfactant B deficiency (SBD)	Patient with SFTPB mutation (p. Pro133GlnfsTer95)	iPSC-derived epithelial cells and mesenchymal cell coculture	Allogeneic human embryonic stem cell line CA1 and iPSC line hi31616, or lentiviral rescue with wild-type SFTPB	Patient age and symptoms are not provided, implied a minor since parental consent is involved. Homozygous.	Not provided
Alysandratos, K.-D., Russo, S. J., Petcherski, A., Taddeo, E. P., Acín-Pérez, R., Villacorta-Martin, C., Jean, J., Mulugeta, S., Rodriguez,	Surfactant C deficiency (SCD)	Patients with SFTPC mutation (g.1286 T) > C (3 patients)	iPSC-derived Alveolar Epithelial Cells Type 2 (iAT2) monoculture	Isogenic, homozygous gene-edited corrected lines	Age at diagnosis infancy-late infancy, Heterozygous for the mutation, All patients were treated with corticosteroids +/- hydroxychloroquine, patient 1 underwent lung	Interstitial fibrosis, chronic inflammation, alveolar remodeling with AT2 hyperplasia and degenerating macrophages within

(Continues)

TABLE 1 | (Continued)

Title	Condition	Mutation loci and variant	iPSC-derived cell type	Control	Patient information	Patient histology
L. R., & Blum, B. C. (2021). Patient-Specific Ipscs Carrying An Sftpc Mutation Reveal The Intrinsic Alveolar Epithelial Dysfunction At The Inception Of Interstitial Lung Disease. Cell Reports, 36(9)					transplantation in early adolescence, patient 3 was listed for lung transplant but not transplanted	the remaining alveoli (patient 1)
Sun, Y. L., Hennessey, E. E., Heins, H., Yang, P., Villacorta-Martin, C., Kwan, J., Gopalan, K., James, M., Emili, A., Cole, F. S., Wambach, J. A., & Kotton, D. N. (2024). Human Pluripotent Stem Cell Modeling Of Alveolar Type 2 Cell Dysfunction Caused By Abca3 Mutations. The Journal Of Clinical Investigation, 134(2).	Surfactant protein deficiency caused by ABCA3 deficiency	Patients with ABCA3 mutation E690K (c.2068 G > A) or W308R (c.922 T > C)	iPSC-derived Alveolar Epithelial Cells Type 2 (iAT2) monoculture	Isogenic, homozygous gene-edited corrected lines	Both are homozygous mutations. Both patients underwent lung transplantation (at 5 or 21 months old).	Diffuse AT2 hyperplasia, alveolar septal thickening and neutrophilic and lymphoid infiltration. Ultrastructural examination of E690K mutant revealed AT2 dysmorphia and small dense lamellar bodies.
Suzuki, T., Mayhew, C., Sallese, A., Chalk, C., Carey, B. C., Malik, P., Wood, R. E., & Trapnell, B. C. (2014). Use Of Induced Pluripotent Stem Cells to Recapitulate Pulmonary Alveolar Proteinosis Pathogenesis. American Journal of Respiratory And Critical Care	Hereditary Pulmonary Alveolar Proteinosis (hPAP)	Patients with CSF2RA mutation (c.649 C.T; p. R217X) (2 patients)	iPSC-derived macrophage monoculture (iMacs)	Allogeneic ESCs or iPSCs generated from donors without lung disease	Age at diagnosis 2.5–4 years old [11] Homozygous mutations.	Foamy macrophages, glycoprotein and lipid accumulation in macrophages, accumulation of surfactant in alveoli, alveolar walls preserved [11]

(Continues)

TABLE 1 | (Continued)

Title	Condition	Mutation loci and variant	iPSC-derived cell type	Control	Patient information	Patient histology
Medicine, 189(2), 183-193.						
Lachmann, N., Happle, C., Ackermann, M., Luttge, D., Wetzke, M., Merkert, S., Hetzel, M., Kensah, G., Jara-Avaca, M., Mucci, A., Skuljec, J., Dittrich, A. M., Pfaff, N., Brennig, S., Schambach, A., Steinemann, D., Gohring, G., Cantz, T., Martin, U.,...Moritz, T. (2014). Gene Correction of Human-Induced Pluripotent Stem Cells Repairs the Cellular Phenotype In Pulmonary Alveolar Proteinosis. American Journal Of Respiratory And Critical Care Medicine, 189(2), 167-182.	Hereditary Pulmonary Alveolar Proteinosis (hPAP)	Patient with CSF2RA mutation in exon 7 (Arg→TGA$^{(stop)}$)	iPSC–derived monocytes and macrophages culture (iMacs)	i) iPSCS generated from allogenic healthy donor ii)Lentiviral rescue with wild-type CSF2RA or allogeneic iPSCs	Age at diagnosis 3.5 years old. Homozygous mutation.	Proteinaceous macrophages
Korogi, Y., Gotoh, S., Ikeo, S., Yamamoto, Y., Sone, N., Tamai, K., Konishi, S., Nagasaki, T., Matsumoto, H., Ito, I., Chen-Yoshikawa, T. F., Date, H., Hagiwara, M., Asaka, I., Hotta, A., Mishima, M., & Hirai, T. (2019). In Vitro Disease Modeling Of Hermansky-Pudlak Syndrome Type 2 Using Human Induced	Hermanky-Pudlack Type 2 Syndrome (HPS2)	Patient with AP3B1 compound heterozygous nonsense mutations in exon 15 and 18	iPSC–derived Alveolar epithelial cells (type 1 and 2) in alveolar organoids (AOs)	Isogenic, gene-edited correction of exon 18 but not exon 15	Diagnosed at 20 months with nonspecific interstitial pneumonitis. Compound heterozygous. Information about treatment was not provided.	Not provided

(Continues)

TABLE 1 | (Continued)

Title	Condition	Mutation loci and variant	iPSC-derived cell type	Control	Patient information	Patient histology
Pluripotent Stem Cell-Derived Alveolar Organoids. Stem Cell Reports, 12(3), 431-440.						
Strikoudis, A., Cieslak, A., Loffredo, L., Chen, Y.-W., Patel, N., Saqi, A., Lederer, D. J., & Snoeck, H.-W. (2019). Modeling Of Fibrotic Lung Disease Using 3D Organoids Derived from Human Pluripotent Stem Cells. Cell Reports, 27(12), 3709-3723.E3705.	HPS- HPS-associated interstitial pneumonia (HPSIP)	Introduced frameshift mutations to create HPS1-/-, HPS2-/- and HPS4-/-	iPSC–derived lung bud organoids (alveolar epithelial cells type 1 and 2 and mesenchymal cells)	Wildtype and HSP8 -/- iPSC lines (which do not cause HPSIP)	Not applicable	Not applicable

lamellar bodies (LBs) and could therefore functionally process and secrete surfactant. LBs are intracellular organelles used for storing, post-Golgi processing and secreting surfactants [17]. iAT2s shared a transcriptomic profile more similar to fetal AT2s due to reduced expression of genes related to immune function [18], likely a consequence of In Vitro culturing. While the impact of these differences on disease modeling is unclear, this seemingly "immature" profile could be advantageous for modeling neonatal illnesses.

To investigate the pathogenesis of SBD the authors created iPSCs from a child with neonatal respiratory distress harboring a homozygous *SFTPB* mutation ($SFTPB^{121ins2}$). They used CRISPR to gene-correct this mutation In Vitro, then differentiated mutant and corrected iPSCs to create patient-specific iAT2s. Mutant iAT2s could not produce surfactant protein B (SP-B) protein, failed to form LBs, and accumulated misprocessed, premature *SFTPC* (pro-*SFTPC*). Importantly, this dysfunctional phenotype was absent in the gene-corrected iAT2s, confirming the *SFTPB* mutation was directly responsible.

Leibel et al. [19] studied SBD using "lung organoids," containing mesenchymal, proximal and distal epithelial cells by differentiating patient-specific iPSCs containing the known disease-causing p.Pro133GlnfsTer95 mutation in the *SFTPB* gene [20, 21]. To create control lines, they transduced patient cells with lentiviral vectors carrying the wild type *SFTPB* gene. Compared to the control organoids, mutant organoids showed limited differentiation to iPSC-derived AT2 cells and did not produce detectable levels of SP-B. Further analysis revealed that both surfactant protein genes (*SFTPB* and *SFTPC*) and genes related to LB development (*ABCA3* and *LAMP3*) [22, 23] were significantly downregulated in mutant organoids resulting in the absence of mature LBs and surfactant production. This could suggest that not only can *SFTPB* mutations impact surfactant production directly but could also influence genes involved in the surfactant secretory pathway. As LBs failed to develop, this could have inhibited the processing of pro-*SFTPC* and subsequent accumulation [24]. Of clinical importance was that exogenous delivery of the *SFTPB* gene by lentivirus rescued surfactant production, highlighting a potential therapeutic approach.

Overall, *SFTPB* mutations seemed to exert widespread effects along the surfactant secretion pathway which could explain the severe presentation often seen in SBD [25].

3.2 | Surfactant Protein-C Deficiency

To characterize the pathological effects of surfactant protein C deficiency (SCD), Alysandratos et al. [26] utilized Jacob et al.'s [16] established protocol to create isogenic mutant and gene-corrected iAT2s from iPSCs derived from three donors heterozygous for the $SFTPC^{I73T}$ mutation. In parallel, the authors created $SFTPC^{I73T}$ knock-in mice to observe In Vivo manifestations of the mutation. Mutant iAT2s had impaired proliferative capacity without significant differences in apoptosis. However, in $SFTPC^{I73T}$ mice, AT2 proliferative rates were normal, suggesting the lung environment In Vivo may compensate for the mutation.

In the iAT2 model, surfactant protein C (SP-C) production was initially similar between the corrected and mutant lines; however, at later time points, surfactant protein genes and genes related to autophagy, lysosome formation and protein secretion were enriched, indicating accumulating cell stress. The disease phenotype manifested over time, reflecting how SCD typically presents later than SBD [25]. Morphologically, mutant iAT2 alveolospheres appeared shrivelled, with numerous LBs and fragmented mitochondria, which likely influenced metabolic functioning related to surfactant processing and bioenergetics of the iAT2 cells. These morphological abnormalities were similarly observed in the *SFTPC*^{I73T} mice and donor lung explant histology. Lastly, the authors used iAT2s as a preclinical tool to test compounds. Treatment of *SFTPC*^{I73T} mutant iAT2s with hydroxychloroquine exacerbated autophagic dysfunction, raising doubts on the benefit of this medication for chILD treatment.

3.3 | ABCA3 Protein Deficiency

ATP binding cassette transporter member A3 (ABCA3), is found on LB membranes and it is involved in mediating the transport of surfactant components across the LB membrane [27, 28]

Sun and colleagues [29] studied the effects of two homozygous mutations in the *ABCA3* gene; the W308R (c.922 T > C) variant and the E690K (c.2068 G > A) variant. Using the protocol developed by Jacob et al. [16], here the authors generated iPSCs and gene-corrected, isogenic controls to create iAT2s to study *ABCA3* deficiency. Both mutations caused ABCA3 mistrafficking, leading to smaller LBs which resulted in impaired secretion of surfactant and lipids. Interestingly, iAT2s from the E690K variant, but not the W308R variant, had impaired self-renewing capacity and more differentially expressed genes, reflecting different effects of the variants. Despite this, both variants triggered pro-inflammatory signaling in iAT2s.

As an adjunct experiment, the authors used A549 lung adenocarcinoma cells, which likely originated from AT2s [30] to study *ABCA3* mutations. Although A549s lack key AT2 functions (e.g., surfactant protein expression) [31, 32], mutant ABCA3 overexpression replicated protein trafficking defects seen in iAT2s.

3.4 | Hereditary Pulmonary Alveolar Proteinosis

Hereditary pulmonary alveolar proteinosis (hPAP) is commonly associated with mutations of the *CSF2RA* gene [11, 33, 34] which causes dysfunction in the granulocyte-macrophage colony-stimulating factor (GM-CSF) signaling pathway used by macrophages to differentiate and clear surfactant [35]. This results in the accumulation of surfactant within macrophages and alveolar lumens and leads to inflammation-mediated damage of the lung interstitium [36].

To model the consequences of impaired GM-CSF-signaling on macrophage function, Suzuki et al. [37] generated iPSC-derived macrophages (iMacs) from patients who harbored a homozygous recessive *CSF2RA*$^{649C>T}$ mutation. While iMac differentiation and phagocytosing capabilities were unaffected, mutant iMacs were less able to clear surfactant from phagosomes compared to the iMacs generated from allogenic healthy patients. This led to intracellular surfactant accumulation, a known feature of hPAP [34, 38, 39]. Reduced *ABCG1* expression, likely due to impaired GM-CSF signaling, suggested that impaired cholesterol transport could be a contributing mechanism to surfactant accumulation within macrophages rather than solely a dysfunction in phagocytosis. Additionally, pro-inflammatory signaling was dampened in mutant iMacs, even after exposure to endotoxin, indicating broad impairment of immune responses.

Lachmann et al. [40] also investigated the effect of *CSF2RA* mutations using iMacs derived from an hPAP patient with a homozygous *CSF2RA*^{R199X} nonsense mutation (Arg to TGA$^{(stop)}$ at exon 7). This resulted in truncated, nonfunctional GM-CSF receptor α chains, evident in mutant iMacs by reduced receptor expression and impaired GM-CSF receptor-mediated signaling, compared to iMacs from allogeneic healthy donors. In contrast to the *CSFRA*$^{649C>T}$ mutation above, this nonsense mutation significantly impaired GM-CSF-mediated functions such as phagocytosis, which perturbed surfactant clearance.

Both studies explored the delivery of wild-type *CSF2RA* gene using lentiviral transduction, showing this therapy restored GM-CSF-mediated functions in the mutant iMacs. This highlighted the potential efficacy of autologous gene-corrected alveolar macrophage transplantation therapy for hPAP.

3.5 | Hermansky-Pudlak Syndrome

Hermansky-Pudlak Syndrome (HPS) is a multisystemic disease involving pulmonary fibrosis in subtypes 1, 2, and 4 [41]. The pulmonary manifestation often occurs in the later decades of life; however, HPS can result in a childhood onset lung disease [42].

Strikoudis et al. [43] introduced key mutations causing different pulmonary fibrosis-causing HPS subtypes into relevant genes (*HPS1, HPS2*, and *HPS4)* using CRISPR-Cas9 gene-editing in ESCs. They then used established differentiation protocols [44] to create organoids, termed “lung bud organoids” (LBOs), containing progenitor epithelial cells, and mesenchymal cells. They discovered that the LBOs with HPS 1, 2, or 4 mutations exhibited phenotypic abnormalities including compacted morphologies, increased collagen and fibronectin deposition in the extracellular matrix and enhanced mesenchymal marker expression, suggestive of fibrosis. One of the insights into pathogenesis which emerged from this study was that cellular injury was the initiator of fibrosis, not a consequence. Interleukin-11 (IL-11) expression, which was primarily derived by AT2s within the mutant organoids, was upregulated after exposure to injury implicating it as an inducing factor for fibrosis.

Using iPSCs derived from a HPS2 patient harboring heterozygous nonsense mutations in exons 15 and 18 of the *AP3B1* gene, Korogi et al. [45] employed a different differentiation approach to create alveolar organoids (AOs) containing alveolar epithelial

(type 1 and type 2) cells after coculture with human fetal lung fibroblasts. Their control AOs were generated using CRISPR/Cas9 gene-correction of exon 18. The *AP3BI* gene encodes for the Adaptor Protein 3 complex (AP-3) which directs proteins to enable the proper formation of LBs and regulate surfactant homeostasis [46]. Mutations within this gene have been documented to cause HPS2 [47–49]. Surfactant lipid secretion was significantly impaired in the HSP2 AOs, compared with gene-corrected controls. Live cell imaging revealed that LBs in mutant AOs were abnormally large and distributed randomly across the cytoplasm rather than close to the plasma membrane, in a configuration less favorable for surfactant secretion. One theory suggests that "giant LB" formation and the resulting surfactant secretion impairments stem from AP-3 complex dysfunction, while other studies argue that "giant LBs" arise due to an inability to secrete surfactant [50]. Korogi et al. argue that AP-3 dysfunction, rather than the size of the LBs, impaired surfactant secretion.

Overall, both studies support that epithelial cells are intrinsically impaired in HPS. These features have been shown to underpin inflammation which leads to fibrosis in HPS [50–52].

4 | Discussion

We identified eight studies which have used iPSCs to model a range of chILD conditions. In general they were able to recapitulate chILD related pathology, identified novel pathophysiological mechanism, and were able to assess the effect of therapeutic intervention.

Since the emergence of organoid modeling, two critical questions have been put forward, challenging their reliability. Firstly, to what extent can they faithfully reproduce physiological responses to pathological conditions? The organoids used in these studies effectively mimicked primary human cell phenotypes and functions. For example, iAT2s produced and secreted surfactant via LBs [16, 26, 29]. One study suggested that the transcriptomic profile of iAT2s was more "immature" [16]; however, the iAT2s in their study were assessed on day 35 of the differentiation, with subsequent studies demonstrating maturity increases in a time dependent manner in iPSC-derived lung organoids [53]. Mutation corrected organoids modeling hPAP demonstrated typical macrophage functions like phagocytosis and immune sensing [37, 40, 43, 45]. These features ensure that iPSC-derived lung cells can model aspects of normal human responses.

The second question involves interrogating how accurately organoids can recapitulate disease processes and manifestations. Pathognomonic features of surfactant protein disease, hPAP, and HPS were observed in mutant organoids, such as surfactant mistrafficking and/or accumulation, and cell intrinsic stress. Interestingly, one of the studies examining *SFTPC* mutations compared their findings directly to a mouse model and discovered that diseased mice AT2s did not recapitulate the proliferation impairment present in mutant iAT2s [26]. This could be due to natural differences between species, or may suggest that the absence of the In Vivo microenvironment could, in part, limit understanding of pathological processes. Since current organoids are relatively simplistic, often incorporating only a few cell types, the complexities that result from multicellular interactions in the lung milieu will not be captured in iPSC models. Despite these limitations, we did find that the current organoid models mirrored aspects of disease processes successfully.

Resultingly, many insights into the pathogenic process of chILD were unveiled. For SP-B deficiency, the possibility that *SFTPB* mutations could induce more drastic malfunctions to surfactant processing, affecting SP-C production as well [16], aligned with the earlier onset and severity of symptoms, compared to *SFTPC* mutations which required accumulating cell stress [26]. For *CSF2RA* mutations, inhibiting GM-CSF signaling affected many macrophage functions from proliferation to immune activation [37, 40]. Significantly, the findings from HPS organoids answered a key debate in pulmonary fibrosis research by suggesting that cellular injury must precede fibrosis, not vice versa. Additionally, these studies gave insight into the fibrosis pathway by suggesting a previously unknown role for AT2s as an inducer of the fibrosis cascade by secreting IL-11 [43].

The goal of disease modeling is to find therapeutic opportunities. Many of these studies managed to test treatment options using organoids. *In Vitro* lentiviral-mediated gene delivery was assessed in three studies, and was able to successfully restore function in all of them [19, 37, 40]. However, this approach may not be successful if mutations result in a toxic gain of function phenotype (e.g., $SFTPC^{I73T}$), suggesting that precise gene-editing techniques, like CRISPR/Cas9, would be necessary as a therapeutic strategy. Additionally, iPSC-derived lung organoids enabled preclinical drug efficacy testing, demonstrating that hydroxychloroquine may be deleterious in surfactant protein C deficiency [26]. This is of interest given this treatment is frequently used empirically in chILD patients with only modest evidence to support its use [54].

4.1 | Future Directions

As iPSC-derived organoids recapitulated major features of diseases, they should be used in the future to model and characterize additional genetic mutations causing chILD. This could be used by physicians to develop standardized screening and diagnostic pathways which could reduce disease burden. Studies could also be performed to assess the clinical utility of organoids in characterizing and modeling the effects of VUS in patients to aid their diagnosis. Additionally, modeling additional chILD manifestations would be vital to understanding their pathophysiology since there is very limited data on these. Similarly, since our understanding of therapeutic effectiveness for chILD disorders are poor, these iPSC models could be used to test the efficacy of drugs and/or identify novel drug targets. A limitation from many of the studies examined was the simplicity of the organoids employed. Therefore, future studies should aim to develop protocols for the development of more complex, multicellular organoids to understand the effects of mutations on cellular interactions [55]. Lastly, few papers chose to introduce stressors to their organoids [37]. As the lung is an environment prone to many infections and inorganic hazards, studying the mutation effect under these conditions, particularly regarding immune functions of cells, would be informative.

4.2 | Limitations

While we attempted to use a broad and comprehensive search strategy, as papers may have used specific condition names rather than "chILD" or "childhood interstitial lung disease," some may have been missed. Including all individual condition names (over 200) was impractical.

5 | Conclusion

The papers discussed in this review examined the effect of genetic mutations associated with chILD on the lung interstitium using organoid models. The organoids were largely successful at recapitulating hallmark pathogenic features of these illnesses as they would occur In Vivo, underscoring their potential use as effective proxies for studying human tissue. The self-renewability, In Vitro stability and genetic customisability of organoid models, will enable rapid and easy experimentation to study the effect of mutations on both the development and functionality of human lung cells. Novel genetic perturbations associated with chILD will likely continue to be discovered, and we contend that the use of iPSC-derived organoid modeling will be tantamount to disentangling their pathogenic effects as well as exploring therapeutic options.

Author Contributions

Swetha Jinson: methodology, writing – original draft, investigation, formal analysis. **Shivanthan Shanthikumar:** conceptualization, writing – review and editing, funding acquisition, supervision. **Rhiannon B Werder:** conceptualization, writing – review and editing, funding acquisition, supervision. All authors critically reviewed and approved the final version of the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The authors have nothing to report.

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Appendix A

See Table A1

TABLE A1 | Outline of the search strategies on Medline Ovid, Embase and PubMed.

Database: Medline Ovid (1995–2024)	Search strategy	Results from 9 Aug 2024
1	*models, biological/or *models, genetic/or *patient-specific modeling/	140,685
2	*adult germline stem cells/or *induced pluripotent stem cells/	15,535
3	*Alveolar Epithelial Cells/	1,497
4	exp *Pluripotent Stem Cells/	38,101
5	exp *Organoids/	6,177
6	(model* or pluripotent-stem-cell* or alveolar-epithelial-cell* or organoid* or iPSC or iPSCs or PSC or PSCs or AT2 or AT2s or iAT2 or iAT2s).tw,kf.	4,141,736
7	1 or 2 or 3 or 4 or 5 or 6	4,197,139
8	exp *Lung Diseases, Interstitial/or lung diseases/or *Pulmonary Alveolar Proteinosis/or *Hermanski-Pudlak Syndrome/	146,136
9	(diffuse-parenchymal-lung-disease* or interstitial-lung-disease* or interstitial-pneumonia* or interstitial-pneumonitide* or interstitial-pneumonitis or ILD or alveolar-proteinosis or alveolo-pulmonary-proteinosis or alveolus-proteinosis or lung-alveolar-proteinosis or lung-proteinosis or pulmonary-alveolus-proteinosis or pulmonary-proteinos or albinism-with-hemorrhagic-diathesis or albinism-with-hemorrhagic-diathesis).tw,kf.	31,243
10	(hermansky-pudlak-syndrome or hermanski-pudlak-syndrome).tw,kf.	3
11	8 or 9 or 10	159,232
12	exp *ATP-Binding Cassette Transporters/	34,959
13	*pulmonary surfactant-associated protein b/or *pulmonary surfactant-associated protein c/	625
14	exp *Mutation/	318,439
15	(ATP-binding-cassette-transporter* or pulmonary-surfactant-associated-protein* or mutation* or mutant* or SFTPB* or SFTPC* or NHLRC2* or ABCA3* or Surfactant-Protein-C* or Surfactant-Protein-B* or NHL-Repeat-Containing-2*).tw,kf.	1,133,430
16	12 or 13 or 14 or 15	1,288,685
17	7 and 11 and 16	528
18	limit 17 to (comment or editorial or guideline or letter or practice guideline or preprint)	7
19	(exp animals/or (rat or rats or mouse or mice or rodent* or swine or porcine or murine or sheep or lamb or lambs or pig or pigs or piglet or piglets or rabbit or rabbits or cat or cats or dog or dogs or cattle or bovine or monkey or monkeys or trout or marmoset or marmosets).ti.) not human*.sh.	5,408,216
20	17 not (18 or 19)	334
Database: Embase (1995-2024)	**Search strategy**	**Results from 9 Aug 2024**
1	biological model/or exp organotypic model/	212,879
2	adult stem cell/	6,474
3	pluripotent stem cell/or embryonic germ cell/or induced pluripotent stem cell/	66,368
4	exp lung alveolus epithelium cell/	10,758
5	exp Organoid/	20,549
6	(model* or pluripotent-stem-cell* or alveolar-epithelial-cell* or organoid* or iPSC or iPSCs or PSC or PSCs or AT2 or AT2s or iAT2 or iAT2s).tw,kf,dq.	5,278,798
7	1 or 2 or 3 or 4 or 5 or 6	5,412,065
8	exp interstitial Lung Disease/or lung disease/or lung alveolus proteinosis/or ocular albinism/	260,674

(Continues)

TABLE A1 | (Continued)

Database: Embase (1995-2024)	Search strategy	Results from 9 Aug 2024
9	(diffuse-parenchymal-lung-disease* or interstitial-lung-disease* or interstitial-pneumonia* or interstitial-pneumonitide* or interstitial-pneumonitis or ILD or alveolar-proteinosis or alveolo-pulmonary-proteinosis or alveolus-proteinosis or lung-alveolar-proteinosis or lung-proteinosis or pulmonary-alveolus-proteinosis or pulmonary-proteinos or albinism-with-hemorrhagic-diathesis or albinism-with-hemorrhagic-diathesis).tw,kf,dq.	55,871
10	(hermansky-pudlak-syndrome or hermanski-pudlak-syndrome).tw,kf,dq.	12
11	8 or 9 or 10	269,694
12	exp ABC transporter/	22,324
13	surfactant associated protein/	746
14	exp Mutation/	1,489,992
15	(ATP-binding-cassette-transporter* or pulmonary-surfactant-associated-protein* or mutation* or mutant* or SFTPB* or SFTPC* or NHLRC2* or ABCA3* or Surfactant-Protein-C* or Surfactant-Protein-B* or NHL-Repeat-Containing-2*).tw,kf,dq.	1,500,971
16	12 or 13 or 14 or 15	2,016,707
17	7 and 11 and 16	2,285
18	limit 17 to (conference abstract or conference paper or "conference review" or editorial or letter or "preprint (unpublished, non-peer reviewed)")	1,148
19	(rat or rats or mouse or mice or rodent* or swine or porcine or murine or sheep or lamb or lambs or pig or pigs or piglet or piglets or rabbit or rabbits or cat or cats or dog or dogs or cattle or bovine or monkey or monkeys or trout or marmoset or marmosets).ti. and animal experiment/	1,300,426
20	Animal experiment/not (human experiment/or human/)	2,664,254
21	17 not (18 or 19 or 20)	872

PubMed (1995–2024)

Date Searched: 09/08/24

((("model*"[Title/Abstract] OR "pluripotent-stem-cell*"[Title/Abstract] OR "alveolar-epithelial-cell*"[Title/Abstract] OR "organoid*"[Title/Abstract] OR "iPSC"[Title/Abstract] OR "iPSCs"[Title/Abstract] OR "PSC"[Title/Abstract] OR "PSCs"[Title/Abstract] OR "AT2"[Title/Abstract] OR "AT2s"[Title/Abstract] OR "iAT2"[Title/Abstract] OR "iAT2s"[Title/Abstract] OR "germline-stem-cell*"[Title/Abstract]) AND ("diffuse-parenchymal-lung-disease*"[Title/Abstract] OR "lung-disease*"[Title/Abstract] OR "interstitial-pneumonia*"[Title/Abstract] OR "interstitial-pneumonitide*"[Title/Abstract] OR "interstitial-pneumonitis"[Title/Abstract] OR "ILD"[Title/Abstract] OR "alveolar-proteinosis"[Title/Abstract] OR "alveolo-pulmonary-proteinosis"[Title/Abstract] OR "alveolus-proteinosis"[Title/Abstract] OR "lung-alveolar-proteinosis"[Title/Abstract] OR "lung-proteinosis"[Title/Abstract] OR "pulmonary-alveolus-proteinosis"[Title/Abstract] OR "pulmonary-proteinos"[Title/Abstract] OR "albinism-with-hemorrhagic-diathesis"[Title/Abstract] OR "albinism-with-hemorrhagic-diathesis"[Title/Abstract] OR "hermansky-pudlak-syndrome"[Title/Abstract] OR "hermanski-pudlak-syndrome"[Title/Abstract]) AND ("ATP-binding-cassette-transporter*"[Title/Abstract] OR "pulmonary-surfactant-associated-protein*"[Title/Abstract] OR "mutation*"[Title/Abstract] OR "mutant*"[Title/Abstract] OR "SFTPB*"[Title/Abstract] OR "SFTPC*"[Title/Abstract] OR "NHLRC2*"[Title/Abstract] OR "ABCA3*"[Title/Abstract] OR "Surfactant-Protein-C*"[Title/Abstract] OR "Surfactant-Protein-B*"[Title/Abstract] OR "NHL-Repeat-Containing-2*"[Title/Abstract]) AND (NOTNLM OR publisher[sb] OR inprocess[sb] OR pubmednotmedline[sb] OR indatareview[sb] OR pubstatusaheadofprint)) NOT (("Animal" OR "animals" OR "rat" OR "rats" OR "mouse" OR "mice" OR "rodent*" OR "swine" OR "porcine" OR "murine" OR "sheep" OR "lamb" OR "lambs" OR "pig" OR "pigs" OR "piglet" OR "piglets" OR "rabbit" OR "rabbits" OR "cat" OR "cats" OR "dog" OR "dogs" OR "cattle" OR "bovine" OR "monkey" OR "monkeys" OR "trout" OR "marmoset" OR "marmosets") NOT ("human" OR "humans" OR "patient" OR "patients" OR "newborn*" OR "baby" OR "babies" OR "neonat*" OR "infan*" OR "toddler*" OR "pre-schooler*" OR "preschooler*" OR "kindergarten" OR "boy" OR "boys" OR "girl" OR "girls" OR "child" OR "children" OR "childhood" OR "adolescen*" OR "pediatric*" OR "pediatric*" OR "youth*" OR "teen" OR "teens" OR "teenage*" OR "school-aged*" OR "school-child*" OR "school-girl*" OR "school-boy*" OR "schoolgirl*" OR "schoolboy*" OR "man" OR "men" OR "woman" OR "women" OR "adult" OR "adults" OR "middle-age*" OR "elderly")))

Results = 246.