Do Cell Junction Protein Mutations Cause an Airway Phenotype in Mice or Humans?

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Cell junction proteins connect epithelial cells to each other and to the basement membrane. Genetic mutations of these proteins can cause alterations in some epithelia leading to varied phenotypes such as deafness, renal disease, skin disorders, and cancer. This review examines if genetic mutations in these proteins affect the function of lung airway epithelia. We review cell junction proteins with examples of disease mutation phenotypes in humans and in mouse knockout models. We also review which of these genes are expressed in airway epithelium by microarray expression profiling and immunocytochemistry. Last, we present a comprehensive literature review to find the lung phenotype when cell junction and adhesion genes are mutated or subject to targeted deletion. We found that in murine models, targeted deletion of cell junction and adhesion genes rarely result in a lung phenotype. Moreover, mutations in these genes in humans have no obvious lung phenotype. Our research suggests that simply because a cell junction or adhesion protein is expressed in an organ does not imply that it will exhibit a drastic phenotype when mutated. One explanation is that because a functioning lung is critical to survival, redundancy in the system is expected. Therefore mutations in a single gene might be compensated by a related function of a similar gene product. Further studies in human and animal models will help us understand the overlap in the function of cell junction gene products. Finally, it is possible that the human lung phenotype is subtle and has not yet been described.

Keywords: cell adhesion; airway epithelia; tight junction; adherens proteins; hemi-desmosomes

The conducting airway of the lung is lined with respiratory epithelia. These epithelial cells are connected to each other and to the extracellular matrix by cell junction proteins. Not only do cell junction proteins provide a physical connection between cells, but they also mediate transport between cells, segregate apical from basolateral membrane proteins, and assist in cell signaling. One way to investigate the contribution of a pathway for epithelial function is to examine how disrupting a gene functionally affects the phenotype. Classical examples in the lung include the study of mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene (1), aquaporins (2), epithelial

CLINICAL RELEVANCE

This review highlights the lack of an obvious airway phenotype when cell junction and adhesion genes are mutated in humans or deleted in murine models. This data will assist researchers in understanding the roles of compensatory cell junction proteins in the airway and help clinicians to search for a subtle airway phenotype.

sodium channels (*ENaC*) (3), and their effect on pulmonary function. This raises the question of how mutations in cell junction genes expressed in airway epithelia might affect the lung.

REVIEW OF CELL JUNCTION MOLECULES

Epithelia line all cavities and surfaces of the body and are composed of cells tightly bound to each other (cell–cell junction) and to the basement membrane (cell–matrix junction). The tight junction is the most apical protein complex of the cell junction proteins, thus the cell membrane above the tight junction is called the “apical membrane.” In the lung, the apical surface is exposed to an air–liquid interface. Several ion channels, water channels, receptors, and mucins are expressed specifically on the apical surface. Moreover, this apical surface varies between ciliated, goblet, and nonciliated cells. The remainder of the cell membrane below the tight junctions is the basolateral membrane. The lateral membrane connects neighboring cells via cell–cell junction molecules, such as tight junctions, adherens junctions, gap junctions, and desmosomes. The basal membrane attaches to the basement membrane of the extracellular matrix (basal lamina) via cell–matrix junction molecules, such as integrins, focal adhesions, and hemi-desmosomes (Figure 1). Several ion channels, water channels, pumps, and receptors are localized specifically to the basolateral membrane. We will briefly review the cell junction molecules and highlight human phenotypes associated with cell junction gene mutations.

TIGHT JUNCTIONS

Tight junctions are the most apical cell junction proteins and because of their location divide the cellular membrane into the apical membrane above the junction and the basolateral membrane below the tight junction. Tight junctions regulate both the “gate” and “fence” functions of the epithelial membrane. The gate function seals off the paracellular compartment from particles, cells, ions, and solutes (Figure 2A). Tight junctions can regulate the paracellular permeability of a membrane by allowing selective diffusion between the apical and basolateral compartments (4). Epithelia with tight junctions that are highly impermeable to ions are called “tight epithelia” such as in the cochlea; whereas epithelia with high permeability to ions are called “leaky

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Figure 1. Diagram of cell junction proteins and their location. Representation of cell–cell junction (abbreviated as jx) proteins (tight junctions, adherens junctions, desmosomal junctions, and gap junctions) and cell–matrix junction proteins (hemi-desmosomal junctions, focal adhesions, and integrins) between epithelia (ciliated and nonciliated).

epithelia” such as in the ascending part of Henle’s loop in the kidney (5). The fence function of the tight junction restricts diffusion of membrane proteins and lipids between the apical and basolateral surface, thus playing a role in maintaining polarity (Figure 2B). This fence prevents apical membrane proteins from traveling to the basolateral membrane and vice-versa.

The transmembrane tight junction proteins include claudins, occludins, and tricellulin. The cytoplasmic tight junction proteins include the zona-occludens, cingulin, and MAGI proteins that anchor the tight junction to the cellular actin cytoskeleton. The extended superfamily of claudins includes 24 different isoforms that are differentially expressed in specific tissues. Occludin and tricellulin are also transmembrane tight junction proteins that span the membrane four times with two extracellular loops. The occludin protein was the first tight junction protein to be identified and is found in nearly all tight junctions except for endothelial cells of nonneuronal tissue and the Sertoli cells of the testes (6, 7). Tricellulin has a similar structure as occludin but is found at cellular junctions where three epithelial cells are in contact with each other (8).

Tight junctions can act as a gate to maintain ionic gradients between fluid compartments. For example, the inner ear is separated into three different fluid compartments (scala vestibuli, scala media, and scala tympani) by the cochlear epithelia of Reissner’s membrane and the basilar membrane. The scala vestibuli and scala tympani are filled with perilymph, with a high sodium and low potassium concentration. The scala media is filled with endolymph, with a high potassium and low sodium concentration (Figure 3). Tight junctions prevent sodium and potassium transport between the perilymph and endolymph, allowing the scala media to maintain a high positive resting potential. If this epithelia is not “tight,” and the endolymph potential falls to zero, then this leads to hair cell death and subsequently deafness. Mutations in claudin 14 have been found

Figure 2. Representation of the two functions of the tight junction protein. (A) The tight junction can function as a gate by regulating paracellular transport between cells (demonstrated by red X). (B) The tight junction can also act as a fence, preventing apical cell membrane proteins from transporting to the basolateral surface and vice versa (demonstrated by red X).

to cause human autosomal recessive deafness (9). A mouse knockout model of claudin 14 found that deafness was secondary to the loss of the endocochlear potential and death of sensory hair cells (10).

Tight junctions can also be “leaky,” allowing the selective absorption and secretion of ions between the apical and basolateral compartments. Claudin 16 is a tight junction protein expressed in the ascending part of Henle’s loop in the kidney and regulates paracellular absorption of magnesium and calcium from the urine into the bloodstream (Figure 4). Mutations in claudin 16 can cause autosomal recessive familial hypomagnesemia with hypercalciuria and nephrocalcinosis, which is characterized by a failure to absorb magnesium and calcium in the urine, which can lead to progressive renal disease (11).

ADHERENS JUNCTIONS

Adherens junctions provide cell–cell adhesion through the interaction of transmembrane proteins that attach to the intracellular actin skeleton. The adherens junctions are composed of three different complexes: the cadherin-catenin, the nectin-afadin, and the junctional adhesion molecule complexes (JAM). The cadherins are homophilic Ca^{2+} -dependent adhesion transmembrane proteins that share a highly conserved intracellular armadillo-repeat family component. Although there are many cadherins, epithelial cadherin (E-cadherin) is most commonly expressed in epithelia. The associated intracellular catenin molecules include α -catenin, β -catenin, plakoglobin (γ -catenin) and p120 catenin that bind cadherins to the actin cytoskeleton. Similar to cadherins, nectins are Ig-like adhesion transmembrane

Figure 3. The tight junction regulating paracellular transport in health and disease. (A) In the cochlea, tight junctions of the cochlear membrane separate perilymph and endolymph, maintaining an endocochlear gradient necessary for hearing. The large font represents the ion in higher concentration, and the smaller font the ion in a smaller concentration. This is an example of a “tight” seal. (B) In the ascending loop of Henle in the kidney, the tight junction regulates the absorption of calcium and magnesium from the urine into the bloodstream. This is an example of a “leaky” seal. Mutations in claudin 16 prevent renal absorption of these ions, causing a buildup of calcium and magnesium in the urine that can lead to renal disease.

receptors that bind to other homophilic or heterophilic nectins. The intracellular nectin component has a highly conserved (PDZ)-binding domain. Afadin is an intracellular protein that binds to this PDZ domain and links nectin to the actin cytoskeleton (12).

Junctional adhesion molecules (JAMs) are cell junction proteins related to the Ig-like superfamily. This family also includes the coxsackie and adenovirus receptor (CAR), named for the virus receptor that allows entry and infection into the cell. The JAM family of proteins has been categorized as belonging to the adherens proteins because they are located inferior to the tight junctions (4). We have found that opening the tight junctions with EDTA greatly enhances adenovirus and reovirus interactions with CAR (13). Anchoring proteins, such as cingulin, link JAM proteins to the actin cytoskeleton via their PDZ-binding domain. JAM and CAR cell-junction proteins play key roles in cell signaling of airway epithelia in inflammation and disease (14).

Familial mutations of epithelial cadherin, or E-cadherin, have been linked to diffuse gastric cancer (15). The loss of the adherens junction in these tumor cells frees the confining restraints of contact inhibition that can lead to metastasis of the tumor cell. In the lung, *in vitro* and *in vivo* animal studies have shown changes in adherens junction protein expression levels in response to inflammation (16) and lung injury (17). There is extensive literature that investigates changes in cell adhesion molecules and lung metastasis (18). Although important, these specific changes in gene expression of cell adhesion molecules in inflammation, injury, and tumorigenesis are beyond the scope of this review article.

DESMOSOMAL JUNCTIONS

Desmosomal junctions are similar to adherens junctions, in that they are cell–cell adhesion molecules. They differ in that desmosomal junctions link the extracellular component of cell junctions to the intracellular intermediate filament cytoskele-

ton. Desmosomal junctions are composed of the extracellular component of desmogleins and desmocollins that belong to the cadherin family. Within the cell, these cadherins are linked to the intermediate filament cytoskeleton by the desmosomal plaque proteins desmoplakin, desmoglein, and plakoglobin (19).

Mutations in desmosomal junction proteins can lead to skin fragility and hyperkeratosis of the palms and soles. Mutations in desmoglein 1 are responsible for autosomal dominant striate palmoplantar keratoderma (PPK) and mutations in plakophilin 1 are responsible for autosomal recessive ectodermal dysplasia/skin fragility syndrome. Electron microscopy of the skin in these diseases shows widening of the intercellular spaces between the keratinocytes and loss of epithelial integrity (20, 21).

HEMIDESMOSOMAL JUNCTIONS AND FOCAL ADHESIONS

Hemidesmosomal junctions and focal adhesions anchor basal epithelial cells to the extracellular matrix of the basement membrane. Hemidesmosomal junctions, focal adhesions, and integrins belong to the family of cell-matrix adhesion molecules. The hemidesmosomal proteins BP230 and plectin bind to cytosolic keratin of the epithelial cell (22). The focal adhesion proteins talin, α -actinin, filamin, vinculin, and focal adhesion kinase bind to the actin cytoskeleton of the epithelial cell (23). Both hemidesmosomes and focal adhesions use cell adhesion integrins to span the transcellular space and bind with laminin in the extracellular matrix.

Mutations in hemidesmosomal junctions and focal adhesions can cause shearing and separation of epithelial cells to the basement membrane, particularly in organs with high mechanical stress or friction. The skin is constantly exposed to friction, and in junctional epidermolysis bullosa, the epidermis separates from the basement membrane causing an inherited blistering disease of the skin. Mutations in BP180, plectin, and laminin can cause junctional epidermolysis bullosa in both Herlitz (severe) and non-Herlitz (moderate) forms of epithelial separation (24).

INTEGRINS

Integrins are cell adhesion glycosylated heterodimer receptors composed of a pair of one α and one β subunit. There are 18 α and 8 β subunits, forming at least 24 different integrin combinations. Integrins are the principal adhesion molecules that anchor epithelial cells to the basement membrane in focal adhesions and hemidesmosomal junctions. Integrin expression is not limited to epithelial cells but is also expressed on leukocyte cells, which are critical for the cellular response in inflammation and injury.

Although integrins comprise the physical unit that joins epithelial cells to the basement membrane, they also interact with the extracellular matrix and are critical in cell signaling. Integrins in epithelial cells and leukocytes can mediate and influence cell shape, motility, growth, and progression through the cell cycle. Integrins can participate in “outside-in” signaling in that they can recruit cytoskeletal proteins and intracellular signaling cascades. Integrins can also participate in “inside-out” signaling, where the cell can modify the adhesive property of the extracellular integrin.

Genetic mutations in integrins can lead to immune deficiencies, bleeding disorders, skin disorders, and congenital muscular dystrophy. Integrin $\beta 2$ mutations, also known as CD18 mutations, can cause leukocyte adhesion deficiency (LAD) type 1 (25, 26). LAD is secondary to impaired neutrophil chemotaxis and adhesion leading to impaired immune deficiency and recurrent bacterial infections. Mutations in either the $\alpha 2b$ or the $\beta 3$ subunit can cause Glanzmann thrombasthenia, a bleeding disorder secondary to

Figure 4. Cell junction gene microarray analysis of *in vivo* and *in vitro* airway epithelia. (A) Tight junction proteins; (B) Adherens proteins; (C) Desmosomal, hemi-desmosomal, and focal adhesion proteins; (D) Integrin and gap junction proteins; *cell junction genes that exhibit a human phenotype when mutated

impaired platelet adhesion. Although there have been a few mouse lung phenotypes described in integrin knockout models related to neutrophil chemotaxis and adhesion (27, 28), there are no human lung diseases associated with integrin mutations.

GAP JUNCTIONS

Gap junctions are direct connections between cells that allow transport of ions and solutes from one cell to another. They are composed of connexin proteins that are ubiquitously expressed in the human body. There are 20 different connexins, which are labeled by their molecular mass and subgroup (α , β , or γ). Six connexins form one connexon, which is half of a gap junction. Gap junctions are important in cell-cell signaling, morphogenesis, and regulation of growth signals. Mutations in the human connexin family have been implicated in epithelial diseases of the eye, ear, skin, and the nervous system, highlighting their importance in a variety of different tissues and functions (29).

For example, gap junctions in neuron cells facilitate communication between the Schwann cell and axon. In X-linked Charcot Marie tooth disease, connexin mutations can block this communication and lead to progressive degeneration of peripheral nerves. Gap junctions in the lens of the eye regulate oxygenation and hydration. Connexin mutations can lead to cataract formation from cell death and subsequent lens opacification. Last, connexins are critical in maintaining the potassium/sodium balance of the endolymph of the inner ear. Mutations in the gap junction connexin 26 lead to the most common inherited form of deafness (30).

CELL JUNCTION MOLECULES EXPRESSED IN AIRWAY EPITHELIA

Review Methodology

A comprehensive list of cell junction genes in human epithelia was compiled by reviewing the literature. Global expression of

the cell junction protein was verified in normal tissue by utilizing the protein immunochemistry information provided by the Human Protein Atlas website (31). The Human Protein Atlas is a publicly available database containing immunohistochemistry staining of 48 different normal human tissues to many different antibodies. These antibodies have been validated through immunohistochemistry and Western blot analysis. Although there are limitations in the use of the Human Protein Atlas, it is an excellent resource for the purpose of this review and one method to support the presence of cell adhesion molecules in multiple organ systems. We investigated individual cell adhesion molecule staining in multiple organ systems including the gut, lung, kidney, hepatobiliary system, and the skin. In the lung, we focused on airway epithelia including tracheal and bronchial tissue. However, clinical phenotypes resulting from altered function in lung interstitium or the alveolar capillary bed would have been identified in either mice or humans. Tissues classified as staining strong or moderate were confirmed by reviewing the histology staining pattern on the slide. Genotype-phenotype correlations were recorded by reviewing original publications via the PubMed database for germline mutations in cell junction genes and their phenotypes in both human and animal models.

Pulmonary expression of cell adhesion proteins was quantified using microarray analysis of *in vivo* and *in vitro* lung tissue. Our group recently performed microarray analysis of *in vivo* lung tissue from the trachea and bronchus obtained from eight healthy subjects without lung disease (32). Airway epithelia gene expression *in vivo* can be confounded by non-epithelial cells so we also used an *in vitro* model free of inflammatory cells of fibroblast cells. Moreover, this model will allow for the investigation of subtle phenotypes. *In vitro* tracheal and bronchial epithelial tissue was cultured and grown from eight individual human donor lungs without primary lung disease, whose lungs were determined to be unsuitable for lung transplantation, in an air-liquid interface on millicells, as previously described (33). Cells were used after 2 weeks in culture, when cellular differentiation is reached and transepithelial resistance is 700–1,200 Ωcm^2 . Microarray expression was quantified on a logarithmic scale and via the Affymetrix algorithm to determine “present” and “absent” calls on the samples. To increase the specificity of the microarray, probes with present calls in less than 10 out of 16 samples in each group were excluded from the analysis.

Cell Junction and Adhesion Proteins Are Expressed in Multiple Organs, Including the Lungs

We reviewed a total of 122 cell junction and adhesion genes (Table 1). Although this list is not exhaustive, we believe it is a comprehensive effort to characterize many genes from different cell junction and adhesion classes. We investigated specific pulmonary expressions of cell junction proteins by microarray analysis of *in vivo* and *in vitro* lung tissue. Fifty-four cell junction and adhesion genes were present using our microarray algorithm in either *in vivo* or *in vitro* lung tissue (Figure 4). Because microarray analysis examines mRNA expression, we also looked at immunochemistry staining in the lungs. We were able to obtain protein expression profiles for 71 of the cell junction and adhesion genes from the Human Protein Atlas website. Nearly all of these adhesion proteins (70/71) stained positive in the lungs. Cell junction and adhesion proteins were ubiquitously present in the gut, kidney, and lungs with a majority also present in the hepatobiliary system, heart, and skin (Figure 5). Based on these data we would expect that mutations in some of these proteins would result in alterations of the epithelia that may predispose, cause, or modify the course of lung diseases.

Figure 5. Cell junction protein expression by organ system. Number of proteins expressed by immunochemistry based on Human Protein Atlas data (31). Nearly all of the proteins were expressed ubiquitously throughout multiple organ systems.

Human and Mouse Mutations of Cell Junction and Adhesion Genes

One-third (44/122) of the cell junction and adhesion genes had human mutations as reported in the published literature. Table 1 presents a list of cell junction genes with a human mutation, a resulting human phenotype, and a knockout mouse phenotype. A complete list of cell junction genes with additional information on organ expression, microarray data, and inheritance pattern is available on the online supplement. Of those mutations that had an inheritance pattern, the majority (64%) were autosomal dominant (AD) versus 36% that were autosomal recessive (AR). Mutations were classified by type: missense (45%), nonsense (29%), splice (14%), and insertion/deletions (12%). The prevalence of autosomal dominant mutations is suggestive of gain of function mutations. These kinds of mutations could result in a phenotype, even if there are redundant proteins with similar function.

Cell Junction and Adhesion Gene Mutations are Reported to Have Phenotypes in Multiple Organ Systems, but None Are Reported to Involve the Lung in Humans

Human mutations in cell junction and adhesion genes expressed various phenotypes in multiple organ systems (Figure 6). Some mutations result in phenotypes that encompass multiple organ systems. For example, several genetic syndromes result in deafness and skin disorders. Surprisingly, none affect the lung. Those genes expressing phenotypes in the skin (20), sensory organs (11), and heart (10) predominated. These organs may be preferentially affected due to a high mitotic turnover rate, an obvious phenotype, or subject to repetitive stress. Surprisingly, even though many of the genes mutated were expressed in the lung, the review of the literature did not identify any lung phenotype. These results have several implications for airway epithelia cell biology, function, and disease in the lungs.

Targeted Deletions of Most Adhesion Molecules Do Not Result in a Lung Phenotype in Mice

Independently, both Hynes (34) and Sheppard (27) have reviewed the phenotype of cell junction and adhesion knockout models in mice. Interestingly, not all knockout mice had drastic phenotypes in the organs in which they were expressed. The phenotype in the lungs can present as defects in changes in development and morphogenesis, altered immune response to infectious challenges, and structural abnormalities.

TABLE 1. GENOTYPE-PHENOTYPE OF CELL JUNCTION GENE MUTATIONS

Cell junction Protein	Gene Symbol	Human Mutation	Micro-Array Present in Lung	Human Protein Atlas Staining in Lung	Human Phenotype	Mouse Phenotype
Tight junction proteins						
Claudin-1	<i>CLDN1</i>	200-201TT (44), 358delG (45)	Y	Y	Neonatal ichthyosis-sclerosing cholangitis syndrome. Inflammation and obliterative fibrosis of the intra and extrahepatic bile ducts; ichthyosis and sclerosing cholangitis affecting skin and bile ducts	Perinatal lethality: epidermal barrier affected; animals die within 1 d of birth; severe dehydration due to excessive water losses (46)
Claudin-2	<i>CLDN2</i>		N	Y	Up-regulated in Crohn's disease	
Claudin-3	<i>CLDN3</i>	Large single allele deletion	Y	Y	Williams-Beuren syndrome: developmental defects, hypercalcemia, constipation	
Claudin-4	<i>CLDN4</i>	Large single allele deletion	N	Y	Williams-Beuren syndrome	
Claudin-5	<i>CLDN5</i>	Large single allele deletion	N	Y	VCFS, 22q11	Perinatal lethality: increased permeability of blood brain barrier (47)
Claudin-9	<i>CLDN9</i>		N/A	Y		Deafness in mice (48)
Claudin-11	<i>CLDN11</i>		N	Y	Associated with autoimmunity of MS	Loss of endocochlear potential, CNS myelin defect, male sterility, hind limb weakness, deafness (49)
Claudin-14	<i>CLDN14</i>	398delT, VAL85ASP (9), GLY101ARG (50)	N	N/A	Autosomal recessive deafness	Phenocopy of human deafness; normal endocochlear potential but progressive degeneration of hair cells (10)
Claudin-16	<i>CLDN16</i>	Multiple (11), (51), (52)	N	Y	Familial hypomagnesemia w/hypercalciuria and nephrocalcinosis: patients present with UTI, polyuria, hematuria, hypomagnesemia then ESRD	Claudin 16 knockdown (53): stunted growth, altered electrolytes
Claudin-19	<i>CLDN19</i>	G20D, Q57E, L90P (54)	N	N/A	HOMGO, renal hypomagnesemia renal with ocular abnormalities	Schwann cell barrier defect; behavior defect (55)
Occludins	<i>OCLN</i>		Y	Y		Viable with complex phenotype; no paracellular defects, no gastric parietal cells; postnatal growth retardation, abnormalities in testis leading to male infertility, inability of females to suckle young, salivary gland abnormalities, thinning of compact bone, brain calcium deposits, chronic gastritis, hyperplasia of gastric epithelium (7)
Tricellulin	<i>MARVELD2</i>	IVS3-1G > A; IVS4+2delTGAG; IVS4+2T > C; 1498C > T (56); IVS4DS(57)	Y	N/A	Autosomal recessive deafness	
zo-2 (TJP2)	<i>TJP2</i>	VAL48ALA(58)	Y	Y	Familial hypercholanemia: elevated serum bile acid concentrations, itching, and fat malabsorption; missense mutation associated with defective bile secretion	Neonatal lethal: arrest in early gastrulation (59)

(Continued)

TABLE 1. (CONTINUED)

Cell junction Protein	Gene Symbol	Human Mutation	Micro-Array Present in Lung	Human Protein Atlas Staining in Lung	Human Phenotype	Mouse Phenotype
Adherens junctions						
Junctional adhesion molecule-1	<i>F11R</i>		Y	Y		Increased trafficking of dendritic cells to lymph nodes, activation of specific immunity (60)
Junctional adhesion molecule-3	<i>JAM3</i>		Y	Y		Growth retardation, pneumonia, poor survival, mega-esophagus, altered airway responsiveness, increasing granulocytes (38)
CAR: Coxsackie virus and adenovirus receptor	<i>CXADR</i>		Y	Y		Embryonic lethality with focal cardiomyocyte apoptosis and extensive thoracic hemorrhaging (61)
Afadin	<i>MLLT4</i>		Y	N		Homozygous mice display embryonic lethality, abnormal ectoderm including disrupted cell jx, absence of somites, notochord, allantois, and neural folds (62)
Nectin-1	<i>PVRL1</i>	W185X, 1-BPDEL, 1-BP DUP (63)	N/A	Y	Cleft-lip palate, ectodermal dysplasia	
E-cadherin	<i>CDH1</i>	Multiple (15), (64), (65), (18), (66), (67), (68)	Y	Y	Familial gastric CA with or without CL/P; somatic mutations associated with many CA	Neonatal lethal (69)
N-cadherin	<i>CDH2</i>		N/A	Y		Perinatal lethality: myocardium dissociates, somites and neural tube poorly organized (70)
P-cadherin	<i>CDH3</i>	1-BP DEL 981G (71), ARG503HIS (72), ASN322ILE, 1-BP DEL 829G (73)	Y	Y	Hypotrichosis with juvenile macular dystrophy: early hair loss followed by progressive degeneration of the central retina leading to blindness	Normal, fertile, except for precocious mammary gland development (74)
P120-catenin	<i>CTNND1</i>		Y	Y		Develop inflammatory skin lesions with age (75)
Alpha-catenin	<i>CTNNA1</i>		Y	Y		Embryonic lethal; conditional knockout: blockage of hair follicle development, defects in adherens junction formation, intercellular adhesion, and epithelial polarity(76)
Beta-catenin	<i>CTNNB1</i>	Somatic mutations in tissues	Y	Y	Mutations cause (via activation Wnt pathway): endometrial, hepatocellular, endometrioid type ovarian, anaplastic thyroid, colorectal, squamous cell, prostate, pancreatic, melanoma, hepatoblastoma, desmoid tumor, pilomatricoma, medulloblastoma (77)	Unable to form dorsal structures and die before gastrulation due to impaired Wnt pathway signaling(78)
Desmosomal junctions						
Desmocollin 1	<i>DSC1</i>		N/A	Y		Flaky skin, punctate epidermal barrier defect (79)
Desmocollin 2	<i>DSC2</i>	1-bp DEL, 1430C, 2-BP INS, 2687GA (80); IVS5AS, A-G,-2 (81)	Y	Y	Can cause (arrhythmogenic right ventricular cardiomyopathy/dysplasia)	
Desmocollin 3	<i>DSC3</i>		Y	N/A		Embryonic lethal (82)

(Continued)

TABLE 1. (CONTINUED)

Cell junction Protein	Gene Symbol	Human Mutation	Micro-Array Present in Lung	Human Protein Atlas Staining in Lung	Human Phenotype	Mouse Phenotype
Desmoglein 1	<i>DSG1</i>	IVS2AS (21)(Rickman)	N/A	Y	Striate palmoplantar keratoderma; pemphigus foliaceus; bullous Impetigo; staphylococcal scalded skin syndrome	
Desmoglein 2	<i>DSG2</i>	Multiple (83), (84); (85)	N	Y	Arrhythmogenic right ventricular cardiomyopathy with one case of skin ulcerations	Embryonic lethal (86)
Desmoglein 3	<i>DSG3</i>		N	N	Pemphigus vulgaris antigen	Oral lesions, low weight, crusting of skin and suprabasilar acantholysis, hair loss (87)
Desmoglein 4	<i>DSG4</i>	EX5-8DEL (88)	N/A	N/A	Localized recessive hypotrichosis and recessive monilethrix	Abnormal short hair and thickened skin (88)
Desmoplakin	<i>DSP</i>	Multiple (89), (90); (91); (92); (93); (94) (95)	Y	N/A	AD: Striate palmoplantar keratoderma AR:(1) wooly hair, keratoderma, cardiomyopathy; (2) RV cardiomyopathy, arrhythmia; (3) striate palmoplantar keratoderma; (4) lethal acantholytic epidermolysis bullosa: fatal skin fragility, alopecia, nail loss, neonatal teeth	Knockout mice die E6.5 from extra-embryonic tissue defects. Rescued embryos die E9.5 of heart, epidermal, vascular and neuroepithelial defects (96, 97)
Plakophilin 1	<i>PKP1</i>	GLN304TER, 28-BP DUP, NT1132 (20), IVS6,A-T,-2 (98)	N	N/A	Ectodermal dysplasia: skin fragility, ectodermal dysplasia syndrome	
Plakophilin 2	<i>PKP2</i>	ARG79TER, ARG735TER, IVS10,G-C,-1, IVS12,G-A,+1 (99)	Y	Y	RV cardiomyopathy, arrhythmia	Embryonic lethal: lethal alterations in heart morphogenesis and stability at midgestation (100)
Plakoglobin (gamma-catenin)	<i>JUP</i>	2-BP Del, 2157TG Deletion (102); 3-BP INS, 117 GCA(102)	Y	Y	Naxos disease:heart, skin, and hair abnormalities	Lethal and die at E10.5-12.5 due to severe heart defects from impaired desmosome formation(78)
Hemi-desmosomal junctions						
BP180	<i>COL17A1</i>	Multiple (103), (104), (105), (106), (107), (108), (109), (110)	N	N/A	Autoimmune target for bullous pemphigoid, cicatrial pemphigoid, linear immunoglobulin A dermatosis, pemphigoid gestationis; genetic target of junctional EBnon-Herlitz; generalized atrophic epidermolysis bullosa.	
Plectin	<i>PLEC1</i>	Multiple (111), (112), (113), (114), (115), (116), (117)	Y	Y	Autoimmune target for bullous pemphigoid and cicatrial pemphigoid; genetic target of epidermolysis bullosa with muscular dystrophy	Die 2-3 d after birth. Phenotype of skin blistering, degeneration of keratinocytes. Abnormalities of myopathies in skeletal muscle and disintegration of intercalated discs in heart (118).
BP230	<i>BPAG1</i>		N	N/A	Autoimmune target for bullous pemphigoid	
CD151	<i>CD151</i>	1-BP INS, G383 (119)	Y	Y	Nephropathy with pretibial epidermolysis bullosa and deafness	Phenotypically normal, normal hemidesmosomes, but minor bleeding abnormalities (120)
Laminin alpha 3	<i>LAMA3</i>	1-bp del, 300g (121), ARG650TER (122), GLN1368TER (24), 1-BP INS, 151G (123)	N	Y	Herlitz or non-Herlitz JEB, laryngoonychocutaneous syndrome	Neonatal lethality: junctional blisters in the skin w separation of dermal/epidermal jx (124)
Laminin beta 3	<i>LAMB3</i>	Multiple (125), (126), (127), (122), (128), (129), (130), (131)	Y	Y	Junctional epidermolysis bullosa (Herlitz and non-Herlitz)	

(Continued)

TABLE 1. (CONTINUED)

Cell junction Protein	Gene Symbol	Human Mutation	Micro-Array Present in Lung	Human Protein Atlas Staining in Lung	Human Phenotype	Mouse Phenotype
Laminin gamma-2	<i>LAMC2</i>	Multiple (132), (133), (134), (135), (24), (135)	Y	Y	JEB (Herlitz and non-Herlitz)	Skin blistering and neonatal death (137); widened tracheal hemidesmosomes but normal lung differentiation and morphology (43)
Focal adhesions vinculin	<i>VCL</i>	3-bp Del, 2862GTT, ARG975TRP (138)	Y	Y	Dilated cardiomyopathy	
Integrins itga1	<i>ITGA1</i>		N/A	N/A		Increase in skin thickness, higher rate of collagen synthesis (139)
itga2	<i>ITGA2</i>		Y	Y	Polymorphisms: Neonatal alloimmune thrombocytopenia	Morphologically normal, clinically without bleeding anomalies, but less adhesion to collagens in vitro (140)
itga2b	<i>ITGA2B</i>	Multiple (141), (142), (143), (144), (145), (146), (147), (148), (149)	N	N	Glanzmann thrombasthenia	Mice with bleeding disorders similar to Glanzmann (150)
itga3	<i>ITGA3</i>		Y	N/A		Defects of kidney and SMG, decreased branching of lungs, skin blistering at dermal-epidermal jx, abnormal layering of cerebral cortex, perinatal lethality (37). Double knockout with <i>ITGA6</i> can cause stunted lung development (35)
itga4	<i>ITGA4</i>		N	N/A		Embryonic lethality: cardiac abnormalities (151)
itga5	<i>ITGA5</i>		N	Y		Intracerebral hemorrhages at midgestation and death shortly after birth. Embryonic mesodermal defects (152).
itga6	<i>ITGA6</i>	1-BP DEL 791C (153)	Y	Y	Epidermolysis bullosa with pyoric atresia	Normal development before birth. Phenotype reminiscent of human epidermolysis bullosa shortly after birth with death. Defects in cerebral cortex and retina (154) Double knockout with <i>ITGA3</i> can cause stunted lung development (35)
itga7	<i>ITGA7</i>	21-BP INS, 98-BP DEL, 1-BP DEL 1,204 (155)	N	Y	Congenital muscular dystrophy	Muscular dystrophy (155)
itga8	<i>ITGA8</i>		N	N		Majority die perinatal. Those that survive have defective kidney morphogenesis and ciliogenesis (156)
itga9	<i>ITGA9</i>		N	N/A		Born normally, but develop respiratory failure and die perinatally. Caused by accumulation of large volumes of pleural fluid; possible congenital chylothorax (42)
itga10	<i>ITGA10</i>		Y	N/A		Growth retardation of long bones (157)
itga11	<i>ITGA11</i>		N	N/A		Dwarfism, increased mortality with age, defective incisors (158)
itgad	<i>ITGAD</i>		N/A	N/A		Reduced staph enterotoxin-induced T cell response (159)

(Continued)

TABLE 1. (CONTINUED)

Cell junction Protein	Gene Symbol	Human Mutation	Micro-Array Present in Lung	Human Protein Atlas Staining in Lung	Human Phenotype	Mouse Phenotype
itgae	<i>ITGAE</i>		Y	N/A		Reduced numbers of intraepithelial lymphocytes (160)
itgal	<i>ITGAL</i>	Mutations occur in itgb2	Y	N	Binds to itgb2. Mutations in itgalb2 cause LFA1 immunodeficiency with defects in adhesion-dependent granulocyte, monocyte and B/T lymphocyte function.	Decreased leukocyte adhesion (40)
itgam	<i>ITGAM</i>		N/A	N		Reduced staph enterotoxin-induced T cell response (161)
itgav	<i>ITGAV</i>		Y	Y		80% lethality. Intracerebral and intestinal hemorrhages and cleft palate. Exaggerated inflammation and protection from pulmonary fibrosis (161)
itgaw	<i>ITGAW</i>		N/A	N/A		
itgax	<i>ITGAX</i>		N	N		Alterations in T cells and their response (40)
itgb1	<i>ITGB1</i>		Y	Y	Susceptibility to Hirschprung disease	Homozygous null mutants are lethal (162)
itgb2	<i>ITGB2</i>	ARG593CYS, LYS196THR (25), LEU149PRO, GLY169ARG (26), Initiation mutation (163)	N	Y	LAD1- Leukocyte adhesion deficiency with defects in adhesion-dependent granulocyte, monocyte and B/T lymphocyte function.	Homozygotes are subject to granulocytosis, impaired inflammatory and immune responses, chronic dermatitis. Increased circulating neutrophils, defective response to chemical peritonitis, delayed transplantation rejection (164).
itgb3	<i>ITGB3</i>	Multiple (165), (166), (167), (168), (169), (170), (171), (172), (141), (173);	N	N	Glanzmann thrombasthenia	Homozygotes exhibit platelet defects, extended bleeding, systemic bleeding (174)
itgb4	<i>ITGB4</i>	Multiple (175), (176), (177), (178), (179), (180), (181), (182)	N	Y	Epidermolysis bullosa with pyloric atresia. Epidermolysis bullosa, generalized atrophic benign. Epidermolysis bullosa of hands and feet	Perinatal lethality with extensive detachment of epidermis and other squamous epithelia. Stratified tissues lack hemidesmosomes and simple epithelia are defective in adherence (183)
itgb5	<i>ITGB5</i>		Y	Y		Phenotypically normal (184)
itgb6	<i>ITGB6</i>		N	N/A		Homozygotes exhibit baldness with macrophage infiltration of skin, exaggerated pulmonary inflammation, impaired mucosal mast cell response (39)
itgb7	<i>ITGB7</i>		N	N/A		Hypoplasia of gut-associated lymph tissue due to defects in lymphocyte migration
itgb8	<i>itgb8</i>		N	N/A		Embryonic or perinatal lethality with profound defects in vascular development (185)
Gap junctions						
Cx26	<i>gjb2</i>	Multiple (186), (187), (188), (189), (190), (191), (192, ASP66HIS {Maestrini, 1,999 #52} (193), (194), (195), (196), (197), (198), (199), (200), (201), (202), (203), (204), (205), (206, 207), (208),	Y	Y	Deafness, skin disease	KO perinatal death with developmental retardation and impaired transplacental nutrient/glucose uptake. Conditional ear KO with deafness (209)

(Continued)

TABLE 1. (CONTINUED)

Cell junction Protein	Gene Symbol	Human Mutation	Micro-Array Present in Lung	Human Protein Atlas Staining in Lung	Human Phenotype	Mouse Phenotype
Cx30	<i>Gjb6</i>	THRSMET (210), GLY11ARG (211), VAL37GLU (212), 309-kb DEL, 232-kb DEL (213)	N/A	Y	Deafness, ectodermal dysplasia	Mutants display progressive HL (214)
Cx30.3	<i>Gjb4</i>	PHE137LEU (215), PHE137LEU, THR85PRO, GLY12ASP, ARG22HIS, PHE189TYR (216, 217)	N/A	N/A	Erythrokeratoderma variabilis	Mutants are normal, but altered sense of smell (218)
Cx31	<i>Gjb3</i>	Multiple (216, 217), (219), (220), (221), (222)	N	N/A	Deafness, EKV	Partial lethality and transient placental dysmorphogenesis but no impairment in hearing or skin differentiation (223)
Cx31.1	<i>Gjb5</i>		Y	N/A		KO with some embryonic lethality, reduced weight, reduced placental development (224)
Cx32	<i>Gjb1</i>	ARG142TRP, PRO172SER, VAL139MET (225), (226), (227), (228), (229), (230), (231), (232), (233), (234), (235), (236), (237)	Y	Y	X-linked CMTD	KO have decrease in body weight, enhanced neuronal sensitivity to ischemic insults, increased susceptibility to liver tumors (238)
Cx36	<i>Gjd2/gja9</i>		N/A	Y		KO mice have decreased electrical synapses in neocortex, decreased vision (239, 240)
Cx37	<i>Gja4</i>		N	N	Atherosclerosis, MI (Polymorphisms in gene associated with atherosclerosis and MI)	KO mice develop more atherosclerotic lesions (241)
Cx40	<i>Gja5</i>		N	Y	Idiopathic A-fib (allelic variants)	KO mice have higher incidence of cardiac abnormalities and conduction deficiencies (242, 243)
Cx43	<i>Gja1</i>	Multiple (245), (246), (247), (248), (249), a(250), (251)	Y	Y	Oculodentodigital dysplasia, hypoplastic left heart syndrome, deafness	KO mice die postnatal due to failure of pulmonary gas exchange from blockage of RV outflow track of heart (251)
Cx46	<i>Gja3</i>	ASN63SER, 1137C INS (253), PRO187LEU (254), ARG76HIS (255)	N/A	N/A	Cataract, zonular pulverulent	Targeted disruption; homozygous mice developed nuclear cataracts (255)
Cx47	<i>Gjc2/gja12</i>	MET286THR, PRO90SER, 1-BP DEL, 989C, ARG240TER, TYR272ASP (256), 34-BP DEL, NT914 (257), 1-bp INS, 695G (258)	N/A	N/A	Pelizaeus-Merzbacher-like disease; hypomyelinating leukodystrophy	KO mice w/o phenotype (259)
Cx50	<i>Gja8</i>	Multiple (260), (261), (262), (263), (264), (265)	N/A	N/A	Cataract, zonular pulverulent	Microphthalmia and nuclear cataracts (266)

Definition of abbreviations: AD, autosomal dominant; Afib, Atrial fibrillation; AR, autosomal recessive; B/T, B-cell/T-cell; CA, cancer; CL/P, cleft lip/palate; CMTD, Charcot Marie Tooth Disease; CNS, Central Nervous System; E, Epithelial; EB, Epidermolysis Bullosa; ESRD, End Stage Renal Disease; EKV, erythrokeratoderma variabilis; FHHN, familial hypomagnesemia with hypercalciuria and nephrocalcinosis; HL, Hearing loss; HOMGO, hypomagnesemia renal with ocular involvement; JEB, junctional epidermolysis bullosa; jx, cell-cell junction; KO, knockout; LAD1, Leukocyte Adhesion Disorder 1; LFA1, Lymphocyte Function-associated Antigen 1; MI, Myocardial infarction; MS, Multiple Sclerosis; RV, Right Ventricle; SMG, submucous gland; UTI, urinary tract infection; VCFS, Velo-Cardio Facial Syndrome; Wnt, catenation of Wg (wingless) and Int.

Analysis of cell junction gene mutations, protein expression via microarray and immunocytochemistry and the human and mouse phenotypes when mutated. A full table of all cell junction genes investigated, with additional information on expression is included as supplementary data.

Integrin α 3 (*ITGA3*) knockout mice, the integrin α 3 α 6 double knockout mice (*ITGA3/ITGA6*) (35), and the integrin 8 knockout mice (*ITGA8*) (36) have stunted lung development that can lead to decreased secondary branching of the lungs or the fusion of lung lobes (37). Integrins may be important in morphogenesis and development of the lung; however, there

are no postdevelopmental lung consequences similar to the skin phenotype of epidermolysis bullosa in humans. Knockout models of junctional adhesion molecules and integrins can affect the immune response in mice, predisposing them to infection or protecting them from lung injury. Junctional adhesion molecule 3 (*JAM3*) knockout mice are predisposed to pneumonia sec-

Figure 6. Cell junction mutation phenotype in human organ systems. Cell junction mutations can exhibit a wide variety of phenotypes in multiple organ systems. Phenotypes in the skin, sensory organs, and cardiac system predominate. Surprisingly, there was no human lung phenotype found.

ondary to an altered immune response in the airway (38). Interestingly, this enhanced inflammatory response in integrin α v (*ITGAV*) and integrin β 6 (*ITGB6*) knockout mice can lead to possible protection from pulmonary fibrosis (39–41). Both integrin and junctional adhesion molecules are important in cell signaling of epithelia in inflammation and disease. These molecules are also expressed on inflammatory cells, and the lung phenotype may actually be secondary to an impaired inflammatory response rather than an epithelial defect. Knockout models of integrins and laminins can also cause structural abnormalities leading to disease. Integrin α 9 (*ITGA9*) knockout mice have defective ciliogenesis and develop respiratory failure and accumulate large volumes of pleural fluid leading to perinatal death (42). Laminin C2 (*LAMC2*) knockout mice have altered tracheal hemidesmosomes but normal lung morphology and differentiation (43). Understanding the pathogenesis of how these knockout models produce a pulmonary phenotype in mice may translate to further testing and diagnosis in humans.

DISCUSSION

Whereas several phenotypes have been described in humans for genetic mutations of cell adhesion molecules, none appear to affect the lung. This is surprising and led us to explore three different explanations.

The Lung Phenotype Is Missed Because of Embryonic Lethality

Many cell junction and adhesion genes are critical for life and when mutated can cause embryonic or neonatal lethality. Mutations that affect the pulmonary system alone are unlikely to be missed, because lung viability is not necessary in utero and would be realized only when the first breath occurs. But mutations that affect multiple organ systems critical for intrauterine development such as the brain, skin, or heart may cause embryonic or neonatal lethality and therefore mask any pulmonary phenotype that could be present.

The Lung Phenotype Is Subtle and Therefore Missed

Many of the phenotypes affected by cell junction and adhesion gene mutations affect sensory organs or manifest as obvious physical findings. Even a casual observer can recognize mutations causing deafness, blindness, or skin blistering. But phenotypes

involving the lung can be subtle and difficult to characterize. After reviewing the functions of cell junction proteins, we would expect a lung phenotype to present as changes in paracellular permeability, abnormal epithelial cell differentiation, impaired response to inflammation or injury, altered ion selectivity, and malignancy. Possible pulmonary phenotypes secondary to tight junction mutations could include changes in airway surface liquid composition from altered paracellular transport. Airway surface liquid is a thin layer of fluid bathing the apical surface of airway epithelia and its depth is measured in micrometers, making testing of the fluid very difficult. Adherens mutations could increase susceptibility to pulmonary neoplasms, as epithelia may metastasize and lose contact inhibition with neighboring cells. Desmosomal mutations could lead to damage and shearing of lung epithelia from the chronic stress of respiration or acute trauma that may be missed because the lungs are not visible. Integrin mutations could lead to increased pulmonary inflammation and infection that may not cause mortality, but may increase morbidity and go unrecognized. Finally, a pulmonary phenotype may not manifest unless the pulmonary system is challenged by infection or inflammation.

Compensatory Cell Junction and Adhesion Proteins in the Lungs

Because the lungs are critical for life, multiple cell junction genes with overlapping functions are likely expressed. Individual cell adhesion genes are therefore dispensable, and mutations in a single gene can be compensated by function of a similar cell adhesion gene. Other organs that are not fundamental to life, such as the ear, may not express compensatory mechanisms and therefore have a phenotype when single genes are mutated. However, this contrasts with the high prevalence of autosomal dominant diseases in other organs.

Do Cell Junction Protein Mutations Cause an Airway Phenotype in Mice or Humans?

We have presented an argument that mutations in cell junction and adhesion genes rarely produce a pulmonary phenotype in mouse knockout models and do not produce an obvious pulmonary phenotype in humans through a comprehensive review of human genotype-phenotype and expression data. It would be presumptive to assume that because a pulmonary phenotype is not obvious in humans, cell junction and adhesion molecules are not important in the airway. In fact, the mouse knockout literature highlights that the pulmonary phenotype may be subtle and require challenges to the lung to manifest itself.

Our research suggests that simply because a cell junction or adhesion protein is expressed in an organ does not imply that it will exhibit a drastic phenotype when mutated. One explanation is that because a functioning lung is critical to survival, redundancy in the system is expected. Therefore, mutations in a single gene might be compensated by a related function of a similar gene product. Further studies in human and animal models will help us understand the pathogenesis and overlap in the function of cell junction and adhesion gene products. Finally, it is possible that the human lung phenotype is subtle and has not yet been described. We encourage clinicians and researchers to become aware of the breadth of the field of cell adhesion, encourage exploratory study, and develop a keen eye to identify possible pulmonary phenotypes so we can improve the care of our patients.

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