Cardiomyocytes of the Heart and Pulmonary Veins: Novel Contributors to Asthma?

Stephen Sai Folmsbee^{1,2} and Cara J. Gottardi^{1,3}

Departments of ¹Pulmonary and Critical Care Medicine, ²The Driskill Graduate Training Program in Life Sciences, and ³Cellular and Molecular Biology, Northwestern University Feinberg School of Medicine, Chicago, Illinois

ORCID ID: 0000-0003-0912-7617 (C.J.G.).

Abstract

Recent genome-wide association studies have implicated both cardiac and pulmonary vein–related genes in the pathogenesis of asthma. Since cardiac cells are not present in lung airways or viewed to affect the immune system, interpretation of these findings in the context of more well-established contributors to asthma has remained challenging. However, cardiomyocytes are present in the lung, specifically along pulmonary veins, and recent murine models suggest that cardiac cells lining the pulmonary veins may contribute to allergic airway disease. Notably, the cardiac cell-junction protein α T-catenin (α T-cat, *CTNNA3*), which is implicated in occupational and steroid-resistant asthma

by clinical genetic data, appears to play an important role in regulating inflammation around the cardiac cells of pulmonary veins. Beyond the potential contribution of pulmonary veins, clinical data directly examining cardiac function through echocardiography have found strong associations between asthmatic phenotypes and the mechanical properties of the heart. Together, these data suggest that targeting the function of cardiac cells in the pulmonary veins and/or heart may allow for novel and potentially efficacious therapies for asthma, particularly in challenging cases of steroid-resistant asthma.

Keywords: α -catenin; cell–cell junctions; cardiomyocytes; pulmonary veins; asthma

Over the past few years, an increasing number of genetic association studies have linked cardiac genes to the development of asthma, but the lack of a clear or plausible contribution to airway inflammation has perhaps limited appreciation of the full impact of these findings. Specifically, a genome-wide association study (GWAS) found that single-nucleotide polymorphisms (SNPs) in the cardiac intercellular junction protein α T-catenin (α T-cat; gene name: *CTNNA3*) were significantly correlated with the development of occupational asthma (1), and this was independently verified (2). Furthermore, in a study of atopic asthmatic children, an analysis of candidate genes previously linked to asthma revealed that an α T-cat haplotype associated with more severe and prevalent occupational asthma in the previous study also conferred a lack of

response to steroid treatment (3). The latter association between α T-cat and atopic asthma appears to be robust, as an independent GWAS found that α T-cat polymorphisms were also associated with an increased rate of asthmatic exacerbations in children (4). Lastly, in addition to asthmatic associations, α T-cat polymorphisms are associated with variability in lung mechanics, specifically the forced vital capacity (5). Thus, polymorphisms in α T-cat have been independently linked across divergent populations to both occupational and steroid-resistant forms of asthma, as well as lung function—a set of curious associations for a protein best known for its role in the heart.

α -Catenins are F-actin-binding proteins and critical subunits of the cadherin complex, which mediates strong intercellular adhesion by forming junctions

along neighboring cells (6) (Figure 1). α -Catenins are found in epithelial (E), neural (N), and testis/heart (T) forms (7): α E-cat is ubiquitously expressed and is essential for the cohesion and development of all tissues (8), whereas α N- and α T-cat serve neuronal (9) and cardiomyocyte (10) functions. Consistent with its role in cardiac function, α T-cat knockout (KO) mice develop an enlarged heart (cardiomyopathy) with decreased contractility and ejection fraction (11), and mutation of α T-cat has been implicated in the development of familial arrhythmogenic right ventricle cardiomyopathy (ARVC) (12). Since the number of families with nonsynonymous mutations in α T-cat associated with ARVC disease is small ($n = 2$), the degree to which patients with this disease also suffer from asthma, possibly

(Received in original form September 27, 2016; accepted in final form October 27, 2016)

S.S.F. was supported by the National Institutes of Health (T32GM008152, T32CA09560, and F30ES024622) and an American Heart Association predoctoral fellowship (15PRE21850010). C.J.G. was supported by the National Institutes of Health (GM076561 and HL094643) and Northwestern University Pulmonary Division funds (RHA2016-01-IPF to A. P. Lam and P01AG049665 to G. R. S. Budinger).

Correspondence and requests for reprints should be addressed to Cara J. Gottardi, Ph.D., Northwestern University, Lab Room: Mezzanine-530, McGaw Pavilion, M-323, 240 East Huron Street, Chicago, IL 60611. E-mail: c-gottardi@northwestern.edu

Am J Respir Cell Mol Biol Vol 57, Iss 5, pp 512–518, Nov 2017

Copyright © 2017 by the American Thoracic Society

DOI: 10.1165/rcmb.2016-0261TR

Internet address: www.atsjournals.org

Figure 1. Schematic of α T-catenin localization to hybrid junctions in cardiac cells. α E-catenin (light purple) interacts with β -catenin (green) and actin filaments (F-actin) in classical cadherin (blue)-based adherens junctions (left). In cardiac cells (right), hybrid junctions are reinforced between desmosomes and adherens junctions through α T-catenin (dark purple), which binds directly to plakophilin 2 (PKP2). The intermediate filament-binding protein, desmoplakin (DSP; orange), γ -catenin (also known as plakoglobin; light green), and p120-catenin (pink) constituents are also shown. cat, catenin.

secondary to their reduced cardiac function, is unclear.

Remarkably, titin (*TTN*), a large structural protein that allows for the contractility of striated muscle, was also identified in a GWAS of asthmatic exacerbations (13). Additionally, this GWAS identified SNPs in semaphorin 3D (*SEMA3D*), whose loss of function leads to perturbed development of the large pulmonary veins (PVs) adjacent to the heart (14). Although titin (15) and α T-catenin (16) encode some of the largest genes in the genome, increasing the probability of their association with disease (17), the identification of α T-catenin, titin, and semaphorin 3D in the same GWAS, and high odds-ratio associations (> 5.0) of α T-catenin with multiple independent genetic studies suggest a possible relationship between cardiac cell function and asthma phenotypes.

Cardiomyocyte-Containing PVs May Be an Understudied Contributor to Asthma

Although cardiac cells are absent from the lung airway, they can be readily found in the lung along the PVs. PVs are unique among

veins from other vasculature tissues because beneath the endothelium and basement membrane they are ensheathed by a layer of interconnected cardiomyocytes with characteristic striations and linear, intercalated, disk-like structures (18) (Figure 2). This layer extends to and is continuous with the cardiac cells of the left atrium of the heart (18). There are notable differences between rodents and humans in the gross-tissue structure of the PVs. In rodents, intrapulmonary veins appear to be surrounded by cardiac cells (18), whereas in humans the cardiac sheath appears to be limited to the largest PVs near the hilum of the thorax (18). It is important to point out, however, that the data on human PVs are limited to a small sample size in an autopsy series, and there was significant variability in the degree of PV ensheathment by cardiomyocytes among different samples (18).

Despite the fact that the PVs make up half of the vasculature of the lung, little is known about how these structures might contribute to lung pathology. There is some evidence to suggest that the PVs may play an underappreciated role in the pathogenesis of asthma. For example, PVs have been observed to become surrounded by cuffs of inflammatory cells in some mouse models of

asthma (19, 20). Other evidence from human (21) and equine (22) *ex vivo* models suggests that PVs have the capacity to respond to spasmogens, or agents that induce a specific contraction—a phenomenon that is not seen in the pulmonary arteries. However, the potential role of PVs in regulating airway inflammation, or how these contractive responses might influence overall lung function or asthma phenotypes, has remained largely unexplored. Perhaps one barrier to the study of PVs in airway disease is their unique developmental origins, as they form separately from the bundle containing arteries and airways of the lung (23). Since much of the PV network is not aligned with airways and pulmonary arteries, which are the major focus of asthma research, we speculate that their potential to affect airway-specific diseases may be underappreciated.

In this regard, little is known about the cellular or tissue properties of the cardiomyocytes of the PVs compared with those of the heart. Because the cardiomyocytes themselves appear to be nearly identical to those of the heart proper, with possibly only minor differences in troponin isoform expression (24), there are currently no Cre-recombinase-directed technologies to selectively target cardiomyocyte function between the PVs and the heart. In addition, little is known about their potential for electrical coordination, or whether the cardiac sheath of PVs retains the ability to beat in unison with the heart. Because PVs lack the dedicated electrical conduction system of the heart (25), it is unlikely that the PVs present deep in the lung would be able to contract as a unit. However, aberrant electrical signals in the veins are known to travel to the heart, which makes them a rare but significant cause of arrhythmias (26), and which is why ablation of the PV is a rare therapy for atrial fibrillation (26). Overall, we reason that the apparent molecular similarities between cardiomyocytes of the PVs and heart, together with evidence that cardiac cells along the PVs likely do not function synchronously (or in syncytium) with the heart, have limited research into the relative contribution of PVs and cardiac cells to lung pathophysiology.

The Cardiac Protein α T-catenin Regulates Cell Adhesion and PV Inflammation in a Murine Asthma Model

α T-catenin is the predominant α -catenin in the intercalated disks of cardiomyocytes (11). It

Figure 2. α T-cat localizes to cardiomyocytes along pulmonary veins in the lung. (A) Hematoxylin and eosin stain shows veins and a proximal airway. (B) Immunofluorescence of adjacent lung section. α T-cat (green) localizes to junctions of cardiomyocytes (red) stained for actinin-2. Nuclei are stained in blue. (C) Higher magnification of the boxed region in B. Arrowheads point to junctional α T-cat.

serves to physically bridge the cadherin/catenin complex, comprised of adherens junctions, with a component of the desmosome, an independent junction structure that links a distinct cadherin family of adhesion proteins to the intermediate filament cytoskeleton (27) (Figure 1). Specifically, α T-cat interacts directly with the cardiac desmosome component plakophilin-2 (PKP2) (28), which is notable because mutations in either α T-cat or PKP2 are associated with ARVC (29), and α T-cat germline KO mice develop an age-dependent enlarged heart with decreased contractility (11). Whether this heart-specific function of α T-cat underlies its asthmatic associations is presently unclear, and will be discussed below. Curiously, the SNPs described above that link α T-cat to occupational asthma are present in intron 12 (1, 2), which is located between the exons encoding the PKP2-binding domain (28). The extent to which these SNPs, or any others associated with asthma, alter splicing or mRNA expression is not well established. This is likely due to the restricted nature of α T-cat expression, as one would have to collect cardiomyocytes from humans to test the contribution of these SNPs. Although some labs have reported being able to acquire α T-cat mRNA from Epstein-Barr virus-transformed human-derived leukocytes (1, 2), the level of RNA in these cells is quite low or undetectable (30).

Recently, our group was able to validate the various clinical associations among α T-cat, lung function, and asthma

in mouse models of that disease (31, 32). As expected for a cardiac-restricted protein, we found that α T-cat protein was absent from airways (epithelial or smooth muscle), and instead was found exclusively within the cardiac cells of the PVs (31) (Figure 2C, green). In α T-cat KO mice, lung mechanics, specifically hysteresis, was altered, supporting the clinical data associating it with altered forced vital capacity (5). Importantly, α T-cat KO mice demonstrated an enhanced airway hyperresponsiveness to toluene diisocyanate-induced asthma (31), a typical cause of occupational asthma. Remarkably, the contribution of α T-cat to asthma was different in the dust-mite extract model of atopic asthma, where α T-cat KO mice showed decreased airway hyperresponsiveness and overall airway inflammation (20). In the latter study, the reduction in airway inflammation appeared to be related to the proximity of cardiomyocyte-containing PVs, which displayed strikingly less immune infiltrative cuffing in α T-cat KO mice relative to wild-type mice (20). How α T-cat may limit the development of chemical-induced asthma while playing a more permissive role in the development of house dust mite-induced asthma is presently puzzling, but the answer may be related to the distinct pathophysiology of these models, differing contributions of cardiac cells of the heart or PVs to asthma, or the methods of analysis used (e.g., plethysmography versus forced oscillation

to assess changes in airway hyperresponsiveness). Indeed, although loss of α T-cat in the heart, and the resulting effects on left ventricular function (11), would be predicted to exacerbate airway reactivity (i.e., elevated left-heart pressures are expected to result in airway wall edema, reduced airway caliber, and increased methacholine reactivity), evidence that α T-cat loss strongly attenuates house dust mite-induced asthma raises the possibility that cardiomyocyte-containing PVs play an underappreciated role in driving the inflammatory milieu proximal to bronchopulmonary vascular bundles in some asthma models (Figure 3). Nevertheless, given that the pulmonary vasculature is quite different between rodents and humans (18), and there is currently no way to distinguish potential differences in the contributions of cardiomyocytes from the PVs versus the heart, we cannot rule out the possibility that the GWASs linking cardiac genes to asthma suggest an underlying heart dysfunction in some forms of this disease.

Cardiac Function Correlates with Asthmatic Phenotypes

As most pulmonologists are well aware, any cardiac cell connection to asthma must extend beyond the PVs, because there is a substantial body of clinical evidence linking heart dysfunction itself to asthma. Although asthma is traditionally considered a disease of the lung, the physiologic and pathophysiologic contributions of the heart must be appreciated. Cardiac and pulmonary functions have a long history of interconnected and overlapping disease states. The best example of this is the condition known as cardiac asthma, wherein cardiac dysfunction, often in the form of left ventricular systolic heart failure, is associated with the symptoms of asthma, including shortness of breath, coughing, and even wheezing (33). This phenomenon is thought to be due to vascular congestion and perivascular edema of the bronchovascular bundle, leading to narrowing of the bronchi (34). In the presence of overt left ventricular systolic dysfunction on echocardiography, it is easy to eliminate a diagnosis of true asthma, as heart disease is evident upon

Figure 3. Cardiomyocyte junctions of pulmonary veins and heart in murine asthma. α T-cat expression in cardiomyocytes of pulmonary veins (upper right) permits the development of asthma in the house dust-mite model of asthma, possibly by affecting the recruitment or perdurance of immune cells proximal to airways (arrows pointing toward inflammatory milieu). Whether α T-cat dysfunction in cardiomyocytes of the heart (upper left) contributes to asthma differently is presently unclear.

further diagnostic tests and imaging. However, the role of a mild-to-moderate systolic or diastolic dysfunction in the pathophysiology of true, noncardiac asthma is not as clear.

Interestingly, atopic asthma has also been associated with changes in cardiac physiology. A recent clinical study used echocardiographic imaging of the heart to show that children with asthma manifest significant increased right ventricular mechanical delay (35). Furthermore, specific cardiac functional traits were used to segregate symptomatic asthma phenotypes. In these cases, asthmatic children who suffered primarily from shortness of breath, as opposed to wheezing, were more likely to show cardiac diastolic dysfunction (36). As these data are only associative, the direction and mechanistic origin of this physiological relationship remain unknown.

The conventional understanding of these studies is that the cardiac changes are reflective of chronic airway obstruction from asthma, which over time could lead to hypoxic-driven changes in cardiac structure and function (36). However, recent GWAS data linking cardiac proteins, such as α T-cat and titin, to asthma suggest the possibility that an underlying, mild cardiac dysfunction itself might be contributing to the outward pulmonary symptoms. Therefore, similar to the cardiac asthma observed in patients with heart failure, some population of asthmatics may have symptoms of asthma affected by an overlooked impairment of cardiac function. Alternatively, such cardiac changes may also simply be exacerbating the underlying airway disease. However, although the potential interactions among heart function, cardiogenic pulmonary edema, and allergic airway inflammation in asthma

may be important, they remain largely unknown.

Cardiac Implications for the Pathogenesis and Treatment of Asthma

Understanding the potential cardiac contribution to asthma is essential, as asthma is a disease that exists on a wide spectrum. Asthma is highly prevalent, affecting an estimated 300 million individuals worldwide and 8.2% of the United States' population (37). Asthma is also incredibly diverse, and its variability indicates that it should not be viewed through one mode of disease pathogenesis, but rather as a number of different underlying biological dysfunctions that culminate in a similar clinical phenotype. Supporting this notion, the diagnosis of

asthma does not rely on direct measurements of airway function. Instead, asthma is diagnosed through a clinical evaluation, with evidence of a chronic, reversible airway obstruction, and competing diagnoses are ruled out (38). This means that many competing underlying pathologies, which may have different etiologies and diverse cellular and tissue origins, may contribute to the common clinical disease we define as asthma.

By further elucidating the cardiac contribution to asthma, we may obtain insights into novel treatments for this disease. Preliminary human trials have demonstrated that chronic, nonselective β -adrenergic blockers have a curious efficacy for improving lung function in asthmatics (39, 40), but the underlying mechanism has remained elusive. This is particularly interesting with regard to the cardiac connection to asthma, as chronic treatment with β -adrenergic blockers has become the standard of care for patients with heart failure. In such patients, although β -blockers will initially decrease cardiac output (41), over time, they can significantly improve heart function (41). This effect, at least symptomatically, is perfectly mirrored in the clinical trials of these drugs in asthma (39, 40), where patients initially show worsened lung function but then exhibit gradual improvement over time. If these β -blockers act in asthmatics through their established effects on cardiac function, it would illustrate the value of targeting cardiac function in the treatment of asthma. We further reason that these drugs might be best suited for individuals with steroid-resistant asthma, occupational asthma, or frequent exacerbations.

Future Clinical and Experimental Strategies

Before other cardiac-targeted treatments can be considered, additional clinical experiments connecting asthma and cardiac function seem to be necessary. First, echocardiographic imaging of individuals with occupational asthma, steroid-resistant asthma, and/or frequent asthmatic exacerbations would allow heart function to be analyzed in comparison with SNPs, the severity of asthma, and exposure to irritants or allergens. In addition, effects on cardiac

function could be analyzed in relation to steroid treatments, the frequency of exacerbations, and any potential physiologic changes that could result from both acute and chronic treatments with β -adrenergic agonists. Finally, all of these data should be correlated with overall lung function and the severity of other asthmatic symptoms, thus elucidating the potential contribution of cardiac function to asthma.

In terms of basic science, the independent associations between the cardiomyocyte-junction protein α T-cat and occupational and steroid-resistant asthma raise a number of outstanding questions. Are cardiomyocytes of the heart or PVs the cell type through which α T-cat contributes to asthma, or are there other rare or contextually activated cell types that express α T-cat protein that are less evident by immunodetection methods? Evidence from the human Genotype-Tissue Expression project reveals α T-cat mRNA expression in the brain and peripheral nervous system, as well as in the heart and skeletal muscle (<http://www.gtexportal.org/home/>). Although we recently confirmed α T-cat protein expression in the mouse brain (42), whether α T-cat expression in any of these neurological cell types contributes to asthma is unknown. However, the idea is admittedly intriguing, given recent interest in the parasympathetic

nervous system's control of airway hyperreactivity (43, 44) coupled with α T-cat's association with steroid-resistant asthma (3, 4).

Although cardiomyocytes lining the PVs are the most evident α T-cat-expressing cell type in the murine lung, important anatomical differences in the pulmonary venous systems of humans and rodents must also be considered in future studies. In rodents, cardiomyocytes line both large and small PVs, whereas in humans the available evidence suggests that cardiomyocytes only line the largest PVs, which would be in the vicinity of bronchovascular bundles outside of the lung parenchyma. So, if cardiomyocytes lining the PVs in mice contribute to the recruitment and/or perdurance of inflammatory cells proximal to the airways (Figure 3), for this model to apply to humans, the contribution of PVs to the airways would need to occur in the vicinity of only the largest airways (Figure 4). Given this conundrum, we reason that state-of-the-art molecular genetic tools (i.e., Cre drivers that remove α T-cat from cardiac cells or other cell types) may provide the most definitive answer. If a cardiac-cell contribution of α T-cat is unequivocally supported by targeted KO studies, we will be obliged to address the thornier issues of PV differences between rodents and

Figure 4. Cardiomyocyte-containing pulmonary veins may be largely restricted to only the largest veins in humans. Pulmonary arteries, veins, and bronchus are shown. The circle shows a large cardiomyocyte-ensheathed pulmonary vein (18) juxtaposed with a large airway. The degree to which this proximity occurs only outside of the lung or within the lung parenchyma across patients is unclear.

humans, and identify molecular differences between cardiomyocytes of the heart versus PVs to parse the contribution of each to asthma.

In summary, this Commentary highlights recent GWASs that link an understudied cell–cell junction component (α T-cat) to the development of asthma. What distinguishes this GWAS target from the many others that have been linked to asthma is its remarkably limited

cell/tissue distribution, which implicates a potentially noncanonical cell type (e.g., cardiomyocytes, brain, and/or peripheral nerve) in the pathogenesis of asthma. Although we have highlighted the mouse model and clinical evidence supporting a cardiomyocyte connection to asthma, we reason that more definitive molecular genetic approaches (e.g., Cre-Lox) will be required to support or refute the intriguing possibility of a

cardiomyocyte–junction contribution to asthma. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

Acknowledgments: The authors thank Anna P. Lam, G. R. Scott Budinger, Sergio E. Chiarella, and Ravi Kalhan (Pulmonary Division, Northwestern University, Chicago, IL) for valuable discussions, and Jacqueline Schaffer for designing the illustration in Figures 3 and 4.

References

- Kim SH, Cho BY, Park CS, Shin ES, Cho EY, Yang EM, Kim CW, Hong CS, Lee JE, Park HS. Alpha-T-catenin (CTNNA3) gene was identified as a risk variant for toluene diisocyanate-induced asthma by genome-wide association analysis. *Clin Exp Allergy* 2009;39:203–212.
- Bernstein DI, Khashan M, Lummus ZL, Johnson VJ, Fluharty K, Gautrin D, Malo JL, Cartier A, Boulet LP, Sastre J, et al. CTNNA3 (α -catenin) gene variants are associated with diisocyanate asthma: a replication study in a Caucasian worker population. *Toxicol Sci* 2013;131:242–246.
- Perin P, Potočník U. Polymorphisms in recent GWA identified asthma genes CA10, SGK493, and CTNNA3 are associated with disease severity and treatment response in childhood asthma. *Immunogenetics* 2014;66:143–151.
- McGeachie MJ, Wu AC, Tse SM, Clemmer GL, Sordillo J, Himes BE, Lasky-Su J, Chase RP, Martinez FD, Weeke P, et al. CTNNA3 and SEMA3D: promising loci for asthma exacerbation identified through multiple genome-wide association studies. *J Allergy Clin Immunol* 2015;136:1503–1510.
- Ong BA, Li J, McDonough JM, Wei Z, Kim C, Chiavacci R, Mentch F, Caboot JB, Spergel J, Allen JL, et al. Gene network analysis in a pediatric cohort identifies novel lung function genes. *PLoS One* 2013;8:e72899.
- Nagafuchi A, Takeichi M, Tsukita S. The 102 kd cadherin-associated protein: similarity to vinculin and posttranscriptional regulation of expression. *Cell* 1991;65:849–857.
- Janssens B, Goossens S, Staes K, Gilbert B, van Hengel J, Colpaert C, Bruyneel E, Mareel M, van Roy F. α T-catenin: a novel tissue-specific β -catenin-binding protein mediating strong cell–cell adhesion. *J Cell Sci* 2001;114:3177–3188.
- Dietrich JE, Panavai L, Gunther S, Wennekamp S, Groner AC, Pigge A, Salvenmoser S, Trono D, Hufnagel L, Hiragi T. Venus trap in the mouse embryo reveals distinct molecular dynamics underlying specification of first embryonic lineages. *EMBO Rep* 2015;16:1005–1021.
- Park C, Falls W, Finger JH, Longo-Guess CM, Ackerman SL. Deletion in Catna2, encoding α N-catenin, causes cerebellar and hippocampal lamination defects and impaired startle modulation. *Nat Genet* 2002;31:279–284.
- Li J, Gao E, Vite A, Yi R, Gomez L, Goossens S, van Roy F, Radice GL. Alpha-catenins control cardiomyocyte proliferation by regulating Yap activity. *Circ Res* 2015;116:70–79.
- Li J, Goossens S, van Hengel J, Gao E, Cheng L, Tyberghein K, Shang X, De Rycke R, van Roy F, Radice GL. Loss of α T-catenin alters the hybrid adhering junctions in the heart and leads to dilated cardiomyopathy and ventricular arrhythmia following acute ischemia. *J Cell Sci* 2012;125:1058–1067.
- van Hengel J, Calore M, Baue B, Dazzo E, Mazzotti E, De Bortoli M, Lorenzon A, Li Mura IE, Beffagna G, Rigato I, et al. Mutations in the area composita protein α T-catenin are associated with arrhythmogenic right ventricular cardiomyopathy. *Eur Heart J* 2013;34:201–210.
- Gerull B. The rapidly evolving role of titin in cardiac physiology and cardiomyopathy. *Can J Cardiol* 2015;31:1351–1359.
- Degenhardt K, Singh MK, Aghajanian H, Massera D, Wang Q, Li J, Li L, Choi C, Yzaguirre AD, Francey LJ, et al. Semaphorin 3d signaling defects are associated with anomalous pulmonary venous connections. *Nat Med* 2013;19:760–765.
- Chauveau C, Rowell J, Ferreira A. A rising titan: TTN review and mutation update. *Hum Mutat* 2014;35:1046–1059.
- Smarr CB, Hsu CL, Byrne AJ, Miller SD, Bryce PJ. Antigen-fixed leukocytes tolerate Th2 responses in mouse models of allergy. *J Immunol* 2011;187:5090–5098.
- Smuda C, Wechsler JB, Bryce PJ. TLR-induced activation of neutrophils promotes histamine production via a PI3 kinase dependent mechanism. *Immunol Lett* 2011;141:102–108.
- Mueller-Hoecker J, Beiting F, Fernandez B, Bahlmann O, Assmann G, Troidl C, Dimomeletis I, Käab S, Deindl E. Of rodents and humans: a light microscopic and ultrastructural study on cardiomyocytes in pulmonary veins. *Int J Med Sci* 2008;5:152–158.
- Wachtel MS, Shome G, Sutherland M, McGlone JJ. Derivation and validation of murine histologic alterations resembling asthma, with two proposed histologic grade parameters. *BMC Immunol* 2009;10:58.
- Folmsbee SS, Budinger GR, Bryce PJ, Gottardi CJ. The cardiomyocyte protein α T-catenin contributes to asthma through regulating pulmonary vein inflammation. *J Allergy Clin Immunol* 2016;138:123–129.e2.
- Schellenberg RR, Foster A. In vitro responses of human asthmatic airway and pulmonary vascular smooth muscle. *Int Arch Allergy Appl Immunol* 1984;75:237–241.
- Hanna CJ, Eyre P. Pharmacological studies on the pulmonary vein of the horse. I. Effects of selected spasmogens. *Can J Physiol Pharmacol* 1978;56:812–817.
- Peng T, Tian Y, Boogerd CJ, Lu MM, Kadzik RS, Stewart KM, Evans SM, Morrissey EE. Coordination of heart and lung co-development by a multipotent cardiopulmonary progenitor. *Nature* 2013;500:589–592.
- Kracklauer MP, Feng HZ, Jiang W, Lin JL, Lin JJ, Jin JP. Discontinuous thoracic venous cardiomyocytes and heart exhibit synchronized developmental switch of troponin isoforms. *FEBS J* 2013;280:880–891.
- Sedmera D, Gourdie RG. Why do we have Purkinje fibers deep in our heart? *Physiol Res* 2014;63(Suppl 1):S9–18.
- Takahara A, Hagiwara M, Namekata I, Tanaka H. Pulmonary vein myocardium as a possible pharmacological target for the treatment of atrial fibrillation. *J Pharmacol Sci* 2014;126:1–7.
- Patel DM, Green KJ. Desmosomes in the heart: a review of clinical and mechanistic analyses. *Cell Commun Adhes* 2014;21:109–128.
- Goossens S, Janssens B, Bonnè S, De Rycke R, Braet F, van Hengel J, van Roy F. A unique and specific interaction between α T-catenin and plakophilin-2 in the area composita, the mixed-type junctional structure of cardiac intercalated discs. *J Cell Sci* 2007;120:2126–2136.
- Li Mura IE, Baue B, Nava A, Fanciulli M, Vazza G, Mazzotti E, Rigato I, De Bortoli M, Beffagna G, Lorenzon A, et al. Identification of a pkp2 gene deletion in a family with arrhythmogenic right ventricular cardiomyopathy. *Eur J Hum Genet* 2013;21:1226–1231.

30. Bacchelli E, Ceroni F, Pinto D, Lomartire S, Giannandrea M, D'Adamo P, Bonora E, Parchi P, Tancredi R, Battaglia A, *et al.* A CTNNA3 compound heterozygous deletion implicates a role for α T-catenin in susceptibility to autism spectrum disorder. *J Neurodev Disord* 2014;6:17.
31. Tai PC, Sun L, Spry CJ. Effects of IL-5, granulocyte/macrophage colony-stimulating factor (GM-CSF) and IL-3 on the survival of human blood eosinophils in vitro. *Clin Exp Immunol* 1991;85: 312–316.
32. McCoy-Simandle K, Stewart CR, Dao J, DebRoy S, Rossier O, Bryce PJ, Cianciotto NP. Legionella pneumophila type II secretion dampens the cytokine response of infected macrophages and epithelia. *Infect Immun* 2011;79:1984–1997.
33. Tanabe T, Rozycki HJ, Kanoh S, Rubin BK. Cardiac asthma: new insights into an old disease. *Expert Rev Respir Med* 2012;6:705–714.
34. Apostolo A, Giusti G, Gargiulo P, Bussotti M, Agostoni P. Lungs in heart failure. *Pulm Med* 2012;2012:952741.
35. Ciftel M, Yilmaz O, Kardelen F, Kahveci H. Assessment of atrial electromechanical delay using tissue Doppler echocardiography in children with asthma. *Pediatr Cardiol* 2014;35:857–862.
36. Zedan M, Alsawah GA, El-Assmy MM, Hasaneen B, Zedan MM, Nasef NA. Clinical asthma phenotypes: is there an impact on myocardial performance? *Echocardiography* 2012;29:528–534.
37. Akinbami LJ, Moorman JE, Liu X. Asthma prevalence, health care use, and mortality: United States, 2005–2009. *Natl Health Stat Report* 2011;12:1–14.
38. Agarwal R, Dhooria S, Aggarwal AN, Maturu VN, Sehgal IS, Muthu V, Prasad KT, Yenge LB, Singh N, Behera D, *et al.* Guidelines for diagnosis and management of bronchial asthma: joint ICS/NCCP (I) recommendations. *Lung India* 2015;32:S3–S42.
39. Moore VC, Burge PS. Occupational asthma to solder wire containing an adipic acid flux. *Eur Respir J* 2010;36:962–963.
40. Hanania NA, Singh S, El-Wali R, Flashner M, Franklin AE, Garner WJ, Dickey BF, Parra S, Ruoss S, Shardonofsky F, *et al.* The safety and effects of the β -blocker, nadolol, in mild asthma: an open-label pilot study. *Pulm Pharmacol Ther* 2008;21:134–141.
41. Barrese V, Taglialatela M. New advances in beta-blocker therapy in heart failure. *Front Physiol* 2013;4:323.
42. Folmsbee SS, Wilcox DR, Tyberghein K, De Bleser P, Tourtellotte WG, van Hengel J, van Roy F, Gottardi CJ. α T-catenin in restricted brain cell types and its potential connection to autism. *J Mol Psychiatry* 2016;4:2.
43. Lewis MJ, Short AL, Lewis KE. Autonomic nervous system control of the cardiovascular and respiratory systems in asthma. *Respir Med* 2006;100:1688–1705.
44. McGovern AE, Mazzone SB. Neural regulation of inflammation in the airways and lungs. *Auton Neurosci* 2014;182:95–101.