

The cardiac protein α T-catenin contributes to chemical-induced asthma

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Folmsbee SS, Morales-Nebreda L, Van Hengel J, Tyberghein K, Van Roy F, Budinger GR, Bryce PJ, Gottardi CJ. The cardiac protein α T-catenin contributes to chemical-induced asthma. *Am J Physiol Lung Cell Mol Physiol* 308: L253–L258, 2015. First published December 5, 2014; doi:10.1152/ajplung.00331.2014.—Ten to 25% of adult asthma is occupational induced, a subtype caused by exposure to workplace chemicals. A recent genomewide association study identified single-nucleotide polymorphisms in the cardiac protein α T-catenin (α T-cat) that correlated with the incidence and severity of toluene diisocyanate (TDI) occupational asthma. α T-cat is a critical mediator of cell-cell adhesion and is predominantly expressed in cardiomyocytes, but its connection to asthma remains unknown. Therefore, we sought to determine the primary α T-cat-expressing cell type in the lung and its contribution to lung physiology in a murine model of TDI asthma. We show that α T-cat is expressed in lung within the cardiac sheath of pulmonary veins. Mechanically ventilated α T-cat knockout (KO) mice exhibit a significantly increased pressure-volume curve area compared with wild-type (WT) mice, suggesting that α T-cat loss affects lung hysteresis. Using a murine model of TDI asthma, we find that α T-cat KO mice show increased airway hyper-responsiveness to methacholine compared with WT mice. Bronchoalveolar lavage reveals only a mild macrophage-dominant inflammation that is not significantly different between WT and KO mice. These data suggest that α T-cat may contribute to asthma through a mechanism independent of inflammation and related to heart and pulmonary vein dysfunction.

α T-catenin; cell-cell adhesion; occupational asthma; lung hysteresis

OCCUPATIONAL ASTHMA OCCURS when workers are exposed to chemical irritants and is responsible for ~10–25% of adult onset asthma (4). Diisocyanate exposure is the most commonly identified source of occupational asthma, representing ~20% of cases (4). Recent data suggest that the intercellular adhesion protein α T-catenin (α T-cat) may contribute to its development since a genomewide association (GWA) study found that single-nucleotide polymorphisms (SNPs) within α T-cat were significantly correlated with both the severity and incidence of toluene diisocyanate (TDI) asthma in a Korean population (11), independently verified in a Caucasian population (2) (Fig. 1B, Table Summary). These odds ratios are among the highest described for occupational asthma (2), indicating a strong correlation. How α T-cat dysfunction affects occupational asthma has remained elusive.

α -Cat is an essential subunit of the cadherin adhesion complex, linking β -catenin (β -cat) to F-actin (Fig. 1A) mediating strong intercellular adhesion (20). It is found in epithelial (E), neural (N), and testis/heart (T) forms (9). α T-cat is primarily found in cardiomyocytes and is required for the coordination of hybrid junctions (area composita) in the heart (15), which are composed of cadherin junctions, gap junctions, and desmosomes. α T-cat may act as a bridge between these different junctions, since it can interact with both β -cat of the cadherin junction and the desmosome adhesion component plakophilin-2 (PKP2) (7). Interestingly, the α T-cat SNPs described above localize to intron 12 (Fig. 1A), which is between exons encoding the PKP2-binding domain (7). Whether these SNPs alter splicing, mRNA expression, or binding to PKP2 is not known. α T-cat knockout (KO) mice develop an age-related enlarged cardiomyopathy with decreased ejection fraction (15), but the connection between cardiomyocyte junction dysfunction and the development of occupational asthma is unclear.

α T-cat is also implicated in regulating overall lung function. Analysis of an African-American pediatric cohort identified two additional SNPs in α T-cat that were among the best correlated with changes in forced vital capacity (FVC) (21). Examination of FVC changes in Caucasian children also identified two SNPs near N-cadherin, the cadherin associated with α T-cat in cardiomyocytes (15). The extent to which α T-cat affects lung function is also unknown. Therefore, we sought to validate these GWA studies in a knockout mouse model to determine whether α T-cat loss contributes to both lung mechanics and the development of TDI asthma.

MATERIALS AND METHODS

Real-time quantitative PCR. RNA was isolated by RNeasy kit (Qiagen) purification. cDNA from heart tissue was provided by Konrad Sawicki and Dr. Hossein Ardehali. Real-time, quantitative PCR (RT-qPCR) was performed by using iQ SYBR Green Supermix (Bio-Rad, Hercules, CA) with the MyiQ Single Color Real-Time PCR by using Bio-Rad detection system and software (12) with the ribosomal gene RPL19 the normalizing control. Primers used were α T-cat, forward 5'-GGTTACTACCCTGGTGAATTGTCC-3', reverse 5'-CTCTTTTCGAACCTCTGGAGTGC-3'; RPL19, forward 5'-AGC-CTGTGACTGTCCATTC-3', reverse 5'-ATCCTCATCCTTCTCAT-CCAG-3'.

Western blotting. Cells and tissues were solubilized in 1% Triton X-100 lysis buffer containing protease inhibitor tablets (Roche Diagnostics); heart tissue was solubilized in the same buffer supplemented with 2% SDS. Samples were separated by SDS-PAGE, transferred to nitrocellulose membranes, and immunoblotted with 5B11 hybridoma

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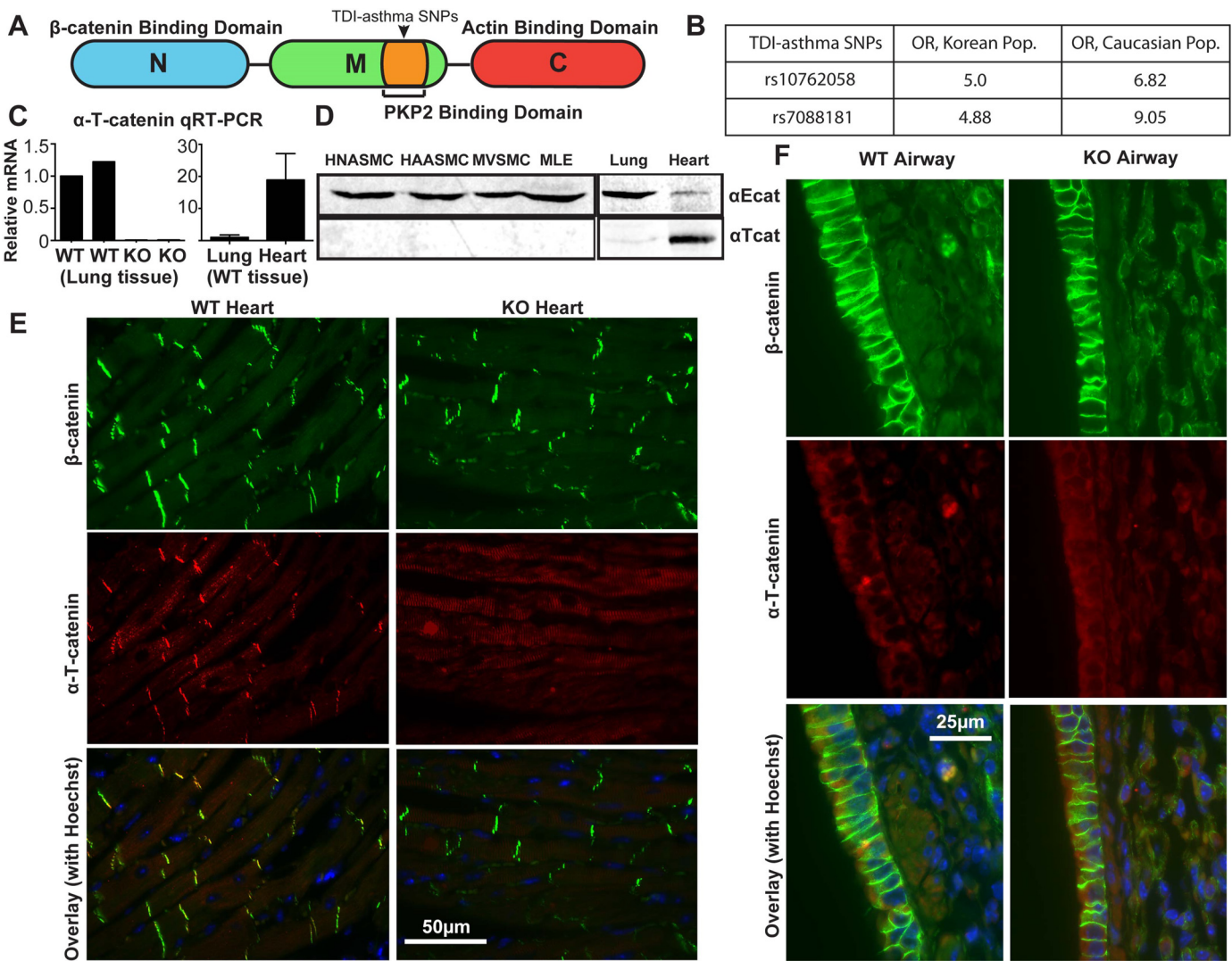


Fig. 1. αT-catenin (αT-cat) is expressed in the lung but absent in the airway. **A**: protein diagram of αT-cat with β-catenin (β-cat), F-actin, and plakophilin-2 (PKP2) binding domains, the last of which may maintain hybrid junctions in the heart (7). TDI, toluene diisocyanate; N, NH₂-terminal domain; M, middle domain; C, COOH-terminal domain. **B**: table of previously published single-nucleotide polymorphisms (SNPs) in αT-cat with associated odds ratios (OR) for the development of TDI asthma in Korean (11) and Caucasian (2) populations. **C**: quantification of αT-cat mRNA in wild-type (WT) and knockout (KO) mouse lungs and heart by qRT-PCR (*n* = 3, error bars = SE). **D**: Western blot of αT-cat and αE-catenin (αE-cat) protein expression in various lung cell types. HAASMC/HNASMC, human asthmatic/nonasthmatic airway smooth muscle cells; MVSMC, mouse vascular smooth muscle cells; MLE, mouse lung epithelial cells. **E**: immunofluorescence detection of αT-cat in cell junctions of cardiac tissue, colocalizing with β-cat. **F**: αT-cat is not present in airway epithelial cell junctions.

mouse anti-αE-catenin (αE-cat) (5) and no. 952 rabbit-anti-αT-cat antibodies. Images were captured by use of the Odyssey Infrared Imaging System (LI-COR) and IRDye680 donkey anti-rabbit (926-68073) and IRDye800 donkey anti-mouse (926-32212) secondary antibodies.

Cell culture. Human airway smooth muscle cells were grown in F-12 Nutrient Mixture (Ham's) with L-glutamine, supplemented with 25 mM HEPES, 12 mM NaOH, 1.7 mM CaCl₂, 2 mM L-glutamine-200 mM, 1% penicillin-streptomycin, 10% fetal bovine serum, and 0.2% Primocin. Primary cells received at the first passage and maintaining an elongated, spindle morphology, were provided by Dr. Reynold Panettieri (University of Pennsylvania), grown in cell culture as described previously (17). To rule out αT-cat expression, cell junctions were identified by immunofluorescent staining with the antibodies against αE-cat, β-cat, and αT-cat described above, as well as the mouse monoclonal αT-cat antibody (Millipore MAB2087). Vascular smooth muscle cells were provided by Dr. Paul Schumacker

(Northwestern University) and grown in M199 supplemented with fetal bovine serum and penicillin-streptomycin. Mouse lung epithelial cells were grown in DMEM supplemented with fetal bovine serum and penicillin-streptomycin.

Histology. Mice were euthanized with 60 mg/kg sodium pentobarbital and tracheostomized with an angiocatheter. Mouse heart and lungs were removed together, perfused with saline, inflated through the catheter with 10% neutral buffered formalin, fixed in 10% neutral buffered formalin, and embedded in paraffin blocks (12). For immunofluorescence, tissue sections were deparaffinized and antigens were retrieved by boiling in citrate buffer for 30 min. Endogenous mouse IgG was blocked with goat anti-mouse Fab fragment (Jackson ImmunoResearch, 115-007-003). Fluorescent images were captured via a Zeiss Axioplan epifluorescence microscope. Primary antibodies used were the BD Biosciences mouse anti-αE-cat (no. 610193), BD Biosciences mouse anti-β-cat (no. 610154), and the no. 952 polyclonal rabbit-anti-αT-cat, and Sigma mouse anti-desmin (D1033). hematox-

ylin and eosin staining was performed by the Northwestern Pathology Core Facility.

Mice. α T-cat KO C57BL/6 mice were obtained from Dr. Glenn Radice (Thomas Jefferson University, Philadelphia, PA), which were bred with C57BL/6 wild-type (WT) mice to create heterozygote, C57BL/6 breeders. WT and α T-cat KO mice used for experimentation were littermates or descendants from littermates and were genotyped similarly as shown previously (14). α T-cat-null specific primers, which generate separate bands in KO and WT mice, were used to genotype by using PCR: forward 5'-TCTATTTTGGAGGCTGTCG-3'; reverse 5'-CAAACCTATGCGTGGTG-3'. α T-cat KO was confirmed by PCR, distinguishing from heterozygotes, showing absence of band generated by WT-specific primers: forward 5'-CCACCCCTGATATGACCTGTAG-3'; reverse 5'-TCCCCAGGAATCAAGTCGTT-3'.

TDI model of asthma. TDI (Mondur 80 2,4-2,6) was provided by Bayer Pharmaceuticals. Female C57BL/6 mice at least 6 wk old underwent 5 days of intranasal injections of 20 μ l of 3% TDI in 1:4 ethyl acetate-olive oil while under isoflurane anesthesia (13). Four days after the last sensitization, they were exposed to 3 days of TDI

challenge, 1 h of 1% TDI in 1:4 ethyl acetate-olive oil by ultrasonic nebulization (Omron, NE-U17). The day after the final challenge, mice underwent plethysmography and bronchoalveolar lavage (BAL).

Lung mechanics. For lung mechanics and pressure-volume curve measurements, mice were continually anesthetized with inhaled isoflurane. After anesthesia, the mice were tracheostomized with an angiocatheter and paralyzed with an intraperitoneal injection of 50 μ l of 10 mg/ml rocuronium. After no independent breaths were recorded, pressure-volume curve protocols and measurements were collected. These were repeated three times for each subject as per FlexiVent manufacturer's protocol.

Plethysmography. Airway hyperresponsiveness (AHR) was assessed by use of the Buxco 8-chamber whole-body plethysmography system. Mice cohorts of eight at a time were placed in individual chambers and exposed to nebulized methacholine in PBS for 2 min, with 6 min of enhanced pause (P_{enh}) measurements directly following. Measurements were taken from 0, 1, 3.3, 10, 33, 100, and 333 mg/ml of methacholine. P_{enh} values reported were the mean of recorded values during the measurement after methacholine exposure.

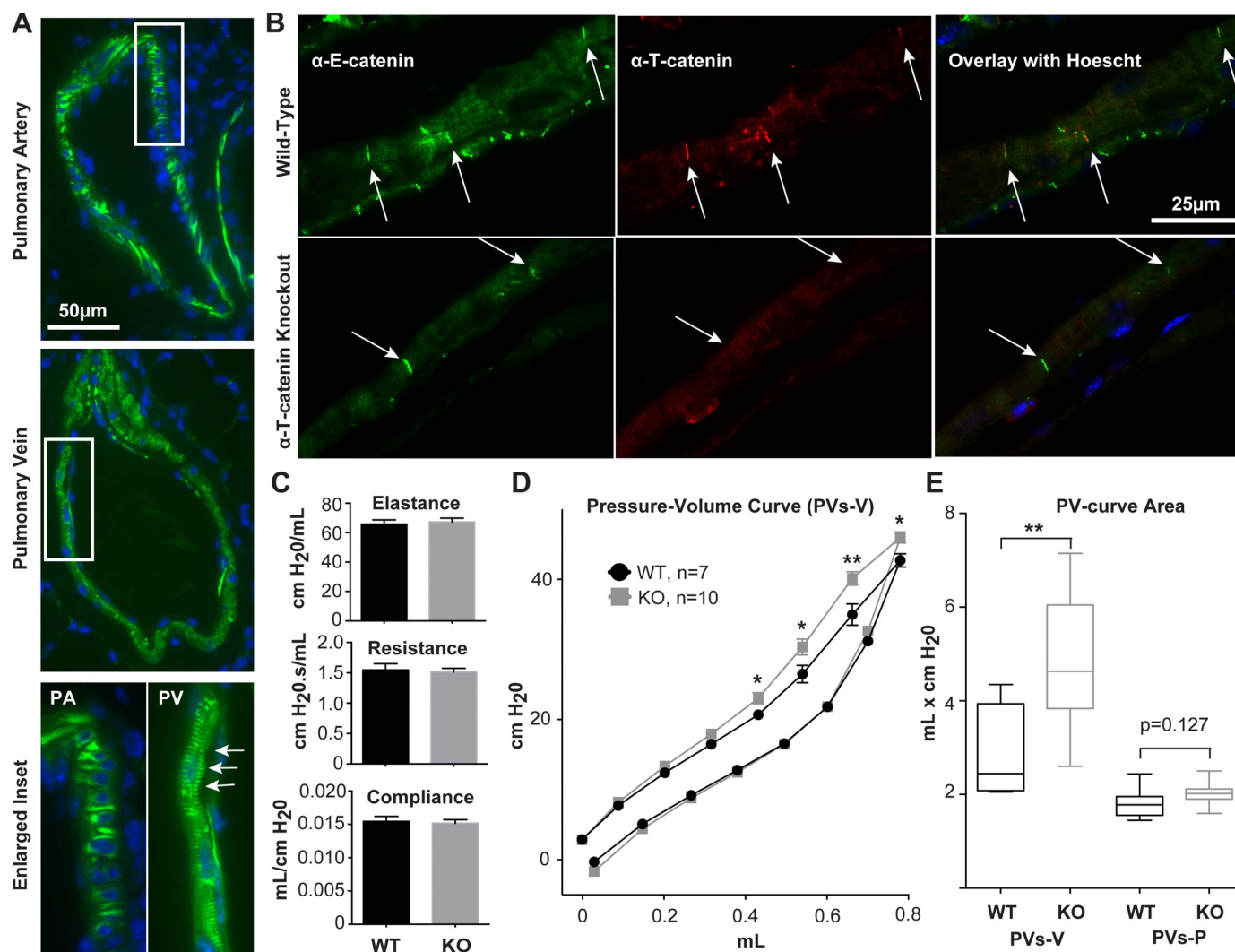


Fig. 2. α T-cat localizes to cardiomyocyte junctions along pulmonary veins and contributes to lung mechanics. **A:** immunofluorescence of murine pulmonary arteries (PA), surrounded by smooth muscle, and the pulmonary veins (PV), surrounded by striated cardiomyocytes (arrows), as visualized by staining with antibody to the intermediate filament desmin (shown in green); nuclei (blue, Hoechst). **B:** immunofluorescence double labeling of PV of WT and α T-cat KO mice with antibodies to α T-cat and α E-cat. α T-cat KO veins only express α E-cat at cell-cell junctions (arrows). **C:** quantification of lung compliance, resistance, and elastance by ventilation. **D:** pressure-volume curves for WT and α T-cat KO mice generated with constant volumes (PVs-V). **E:** calculation of the area under the pressure-volume curve for WT and α T-cat KO mice for those generated with constant volume (PVs-V) and constant pressure (PVs-P). $^{*}P < 0.05$, $^{**}P < 0.01$, by Student's *t*-test. Error bars represent SE.

Bronchoalveolar lavage. Saline fluid (0.8 ml) was lavaged then aspirated from the lungs of mice through the tracheal angiocatheter. Cell counts were measured by Trypan blue exclusion and the automated Countess system. To measure the cell differential, 200 μ l of BAL fluid was placed in Cytospin funnels and spun 2,000 rpm 5 min onto a glass slide. Cells were stained by Wright-Giemsa and manually counted and identified.

Statistics. Statistical analysis was performed by Student's *t*-test, with a *P* value of less than 0.05 considered significant. Calculations were performed with GraphPad Prism software (Graph Pad Software, La Jolla, CA).

Study approval. All experiments were approved by the Northwestern University IACUC.

RESULTS AND DISCUSSION

α T-cat is expressed in the lung, but not in the airway. To understand the genetic links between α T-cat dysfunction and

asthma, we sought to identify the primary α T-cat-expressing cells in the lung using an α T-cat total KO mouse (15). We showed that α T-cat mRNA was indeed present in WT lungs but not KO mice by qRT-PCR and was $\sim$ 20 times less abundant than in the heart (Fig. 1C). α T-cat protein was only barely detected in whole mouse lung lysate compared with that in the heart (Fig. 1D). Instead, the dominant α -cat in the lung was α E-cat. We also could not detect α T-cat in various lung cell lines, including human airway smooth muscle cells acquired from both asthmatic and nonasthmatic patients, mouse vascular smooth muscle cells, and mouse lung epithelial cells, which instead all expressed α E-cat (Fig. 1D). We next used immunofluorescence to show α T-cat was present in the WT cardiomyocyte junctions of the heart, colocalizing with β -cat, but absent in KO mice (Fig. 1E). In the airway of the lung, no α T-cat was detected with β -cat (Fig. 1F).

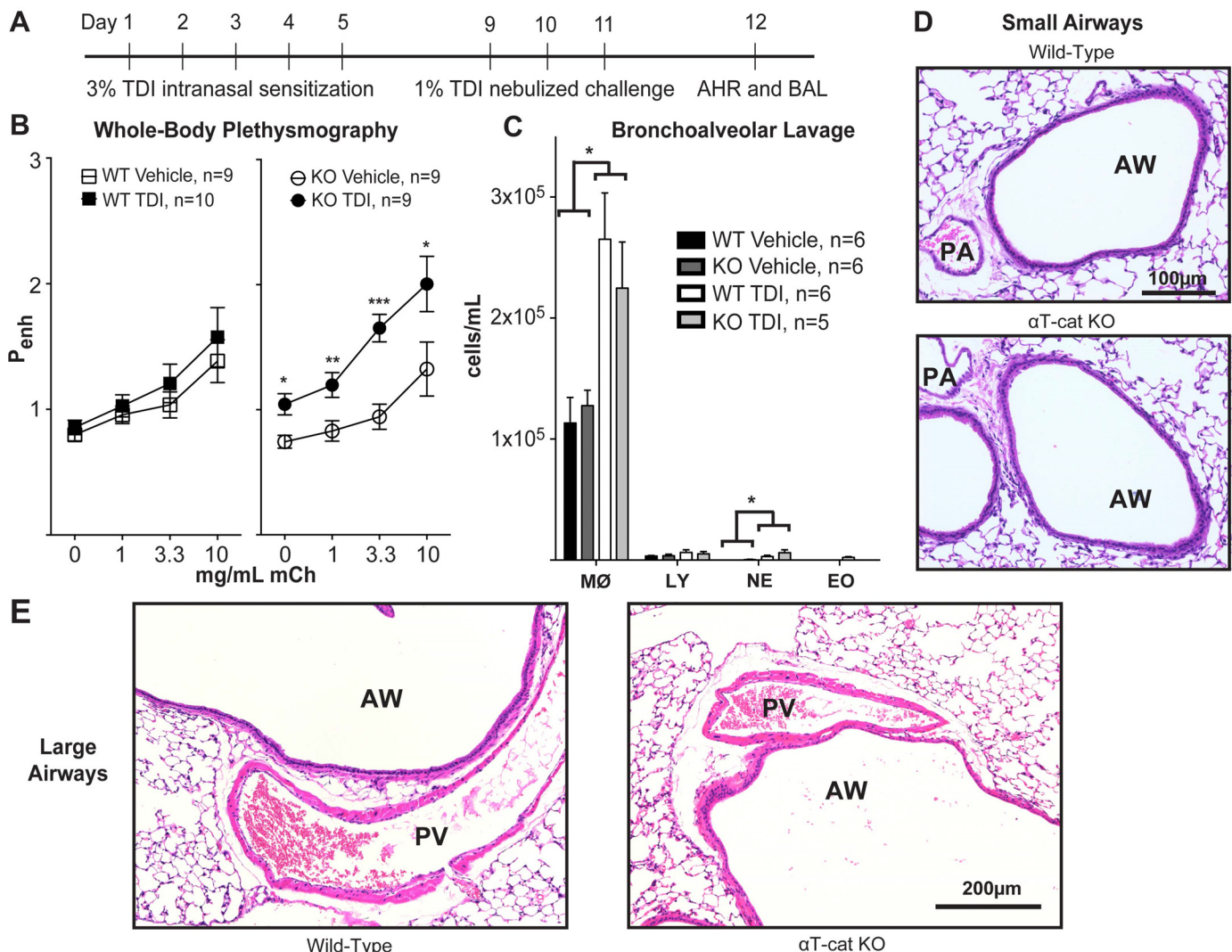


Fig. 3. α T-cat loss enhances the severity of TDI asthma. **A**: murine model for TDI asthma with sensitization by intranasal injection, challenge by nebulization and assessment by airway hyperresponsiveness (AHR) and bronchoalveolar lavage (BAL). **B**: whole-body plethysmography assessment of AHR. WT mice showed no increase in enhanced pause (P_{enh}) AHR when exposed to TDI, whereas KO mice demonstrated a significant increase in P_{enh} after TDI exposure. **D**: analysis of airway inflammation by BAL. TDI exposure increases macrophage and neutrophil infiltration similarly in WT and KO mice. M ϕ , macrophage, LY, lymphocyte, NE, neutrophil, EO, eosinophil. **E**: by hematoxylin and eosin staining, the small airways (AW) and pulmonary arteries (PA) of WT and KO mice exposed to TDI show no significant differences. **F**: in larger AW, WT and KO mice show adjacent pulmonary veins (PV). * P < 0.05, ** P < 0.01, *** P < 0.001, by Student's *t*-test. Error bars represent SE.

α T-cat is expressed in the cardiomyocyte sheath of the pulmonary veins. The pulmonary arteries (PA) and veins (PV) have a unique tissue patterning and cellular architecture. The PA track to the airways and are surrounded by smooth muscle cells, whose cytoplasm stains uniformly positive for the intermediate filament marker desmin (Fig. 2A). In contrast, PV do not follow the branching airways and are sheathed in cardiomyocytes, identified by desmin-positive sarcomere striations (Fig. 2A, inset). These cardiomyocytes have been shown to extend throughout the entirety of the PV in rodents and in the large PV of humans (19). We detected α T-cat in the PV cardiomyocytes of WT mice, colocalizing with α E-cat, whereas it was absent in KO mice (Fig. 2B).

α T-cat loss impacts respiratory function. To investigate how α T-cat regulates lung function, we used the FlexiVent ventilation system to measure mechanical properties of the lung. No differences in elastance, resistance, or compliance were found due to α T-cat KO (Fig. 2C). However, compared with WT mice, α T-cat KO mice displayed a significant increase in lung pressures within the pressure-volume curve generated with constant volumes (PVs-V) (Fig. 2D). To quantify this difference, we measured the area of the PV curves and found that the KO mice PVs-V curve area was significantly greater than that of WT (Fig. 2E). The difference between PV-curve areas was not statistically significant when the PV curve was generated with constant pressure (PVs-P) (Fig. 2E). Increases in PV curve area have been seen in other models of heart dysfunction (16), which suggests that α T-cat loss affects lung hysteresis.

α T-cat loss increases susceptibility to TDI asthma. To determine the role of α T-cat in TDI asthma, WT and KO mice were sensitized by intranasal injection and challenged by nebulized TDI and assessed by measuring AHR and BAL (Fig. 3A). Because forced oscillation has been shown to be ineffective in TDI asthma models of C57BL/6 mice (6), we instead used whole-body plethysmography to measure the P_{enh} , an established measure of AHR in TDI asthma models (13), particularly in C57BL/6 mice (10, 18). After TDI exposure, WT mice showed no significant changes to AHR, but KO mice showed a significant increase in P_{enh} along each methacholine dose, as well as at baseline (Fig. 3B), consistent with previous work with this model of TDI asthma (13). This indicates that α T-cat loss increases sensitivity to TDI. Both WT and KO mice showed a significant and similar increase in macrophage and neutrophil cells after TDI exposure (Fig. 3C), consistent with other models of TDI asthma in C57BL/6 mice (6). We observed no obvious differences in airway architecture between the WT and KO mice by histology (Fig. 3D), where PV were found adjacent to the large airways (Fig. 3E).

Role of cardiac function in asthma. We show that the cardiac protein α T-cat contributes to the development of TDI asthma, validating prior human GWA studies. Since no inflammatory differences between WT and KO mice are observed in the TDI asthma model, our data suggest a novel mechanism for the pathogenesis of occupational asthma. Since the pulmonary veins can be found near the large airways, we speculate that cardiomyocyte adhesion dysfunction in the heart and pulmonary veins may lead to interstitial airway edema. Lung edema is a common consequence of heart dysfunction (1) frequently seen in patients with heart failure, in which presentations of shortness of breath and wheezing are referred to as “cardiac asthma” (3). Supporting this edematous connection to asthma,

other GWA studies performed in populations of atopic asthmatic subjects found that those with certain SNPs in α T-cat showed no response to corticosteroid therapy (22), consistent with our proposed noninflammatory connection between cardiac dysfunction and asthma. Furthermore, recent preliminary clinical trials demonstrate the surprising efficacy of β -adrenergic receptor blockers in treating patients with asthma (8), but its mechanism remains elusive. If these drugs are acting through their known cardiac effects, heart cell function may be a novel therapeutic target for asthma. Future studies are required to determine whether α T-cat KO mice are more susceptible to models of atopic asthma, which also may be better suited to discerning a molecular mechanism owing to their relative robustness compared with TDI asthma models. Overall, our study has validated the genetic associations of α T-cat with TDI asthma and lung mechanics (2, 11, 21, 22), suggesting the potential importance of cardiac cell junction function in the development of occupational asthma.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

S.S.F., F.V.R., and C.J.G. conception and design of research; S.S.F. and L.M.-N. performed experiments; S.S.F., F.V.R., and C.J.G. analyzed data; S.S.F., J.V.H., K.T., G.S.B., P.J.B., and C.J.G. interpreted results of experiments; S.S.F. prepared figures; S.S.F. drafted manuscript; S.S.F., J.V.H., K.T., F.V.R., G.S.B., P.J.B., and C.J.G. edited and revised manuscript; S.S.F., L.M.-N., J.V.H., K.T., F.V.R., G.S.B., P.J.B., and C.J.G. approved final version of manuscript.

REFERENCES

1. Apostolo A, Giusti G, Gargiulo P, Bussotti M, Agostoni P. Lungs in heart failure. *Pulm Med* 2012; 952741, 2012.
2. Bernstein DI, Kashon M, Lummus ZL, Johnson VJ, Fluharty K, Gautrin D, Malo JL, Cartier A, Boulet LP, Sastre J, Quirce S, Germolec D, Tarlo SM, Cruz MJ, Munoz X, Luster MI, Yucesoy B. CTNNA3 (α -catenin) gene variants are associated with diisocyanate asthma: a replication study in a Caucasian worker population. *Toxicol Sci* 131: 242–246, 2013.
3. Buckner K. Cardiac asthma. *Immunol Allergy Clin North Am* 33: 35–44, 2013.
4. Chan-Yeung M, Malo JL. Occupational asthma: definitions, epidemiology, causes, and risk factors. *UpToDate*, 2014. <http://www.uptodate.com/contents/occupational-asthma-definitions-epidemiology-causes-and-risk-factors>
5. Daugherty RL, Serebryanny L, Yemelyanov A, Flozak AS, Yu HJ, Kosak ST, deLanerolle P, Gottardi CJ. α -Catenin is an inhibitor of transcription. *Proc Natl Acad Sci USA* 111: 5260–5265, 2014.
6. De Vooght V, Vanoirbeek JA, Luyts K, Haenen S, Nemery B, Hoet PH. Choice of mouse strain influences the outcome in a mouse model of chemical-induced asthma. *PLoS One* 5: e12581, 2010.
7. Goossens S, Janssens B, Bonne 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* 120: 2126–2136, 2007.
8. Hanania NA, Singh S, El-Wali R, Flashner M, Franklin AE, Garner WJ, Dickey BF, Parra S, Ruoss S, Shardonofsky F, O'Connor BJ, Page C, Bond RA. The safety and effects of the beta-blocker, nadolol, in mild asthma: an open-label pilot study. *Pulm Pharmacol Ther* 21: 134–141, 2008.
 9. 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* 114: 3177–3188, 2001.
 10. Johnson VJ, Yucesoy B, Luster MI. Prevention of IL-1 signaling attenuates airway hyperresponsiveness and inflammation in a murine model of toluene diisocyanate-induced asthma. *J Allergy Clin Immunol* 116: 851–858, 2005.
 11. 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* 39: 203–212, 2009.
 12. Lam AP, Herazo-Maya JD, Sennello JA, Flozak AS, Russell S, Mutlu GM, Budinger GR, DasGupta R, Varga J, Kaminski N, Gottardi CJ. Wnt coreceptor Lrp5 is a driver of idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 190: 185–195, 2014.
 13. Lee SH, Jang AS, Kwon JH, Park SK, Won JH, Park CS. Mesenchymal stem cell transfer suppresses airway remodeling in a toluene diisocyanate-induced murine asthma model. *Allergy Asthma Immunol Res* 3: 205–211, 2011.
 14. Li J, Gao E, Vite A, Yi R, Gomez L, Goossens S, van Roy F, Radice G. Alpha-catenins control cardiomyocyte proliferation by regulating Yap activity. *Circ Res*, 2014. Oct 10. pii: [CIRCRESAHA.114.304472](https://doi.org/10.1161/CIRCRESAHA.114.304472). [Epub ahead of print]
 15. 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* 125: 1058–1067, 2012.
 16. Lowe K, Alvarez DF, King JA, Stevens T. Perivascular fluid cuffs decrease lung compliance by increasing tissue resistance. *Crit Care Med* 38: 1458–1466, 2010.
 17. Malavia NK, Raub CB, Mahon SB, Brenner M, Panettieri RA Jr, George SC. Airway epithelium stimulates smooth muscle proliferation. *Am J Respir Cell Mol Biol* 41: 297–304, 2009.
 18. Matheson JM, Johnson VJ, Vallyathan V, Luster MI. Exposure and immunological determinants in a murine model for toluene diisocyanate (TDI) asthma. *Toxicol Sci* 84: 88–98, 2005.
 19. Mueller-Hoecker J, Beiting F, Fernandez B, Bahlmann O, Assmann G, Troidl C, Dimomeletis I, Käb S, Deindl E. Of rodents and humans: a light microscopic and ultrastructural study on cardiomyocytes in pulmonary veins. *Int J Med Sci* 5: 152–158, 2008.
 20. Nagafuchi A, Takeichi M, Tsukita S. The 102 kd cadherin-associated protein: similarity to vinculin and posttranscriptional regulation of expression. *Cell* 65: 849–857, 1991.
 21. Ong BA, Li J, McDonough JM, Wei Z, Kim C, Chiavacci R, Mentch F, Caboot JB, Spergel J, Allen JL, Sleiman PM, Hakonarson H. Gene network analysis in a pediatric cohort identifies novel lung function genes. *PLoS One* 8: e72899, 2013.
 22. Perin P, Potocnik U. Polymorphisms in recent GWA identified asthma genes CA10, SGK493, and CTNNA3 are associated with disease severity and treatment response in childhood asthma. *Immunogenetics* 66: 143–151, 2014.

