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## The role of E-cadherin/ $\beta$ -catenin signalling in the development of an asthmatic airway epithelial phenotype

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# Acknowledgements

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As my doctoral research, which began over 4.5 years ago is coming to a culmination, I would like to take a moment to convey my deepest appreciation to all the people who were a part of this extraordinary journey. Specially, I would like to sincerely thank my supervisors - Prof. Dr. Irene Heijink, Prof. Dr. Ir. Martijn Nawijn and Prof. Dr. Darryl Knight for providing me an opportunity to work on a fantastic project, which now resulted in this PhD thesis. I am extremely fortunate and privileged to have worked with three eminent researchers who had their own unique contribution to the success of my project. I would also like to thank University of Groningen and University of Newcastle for supporting my doctoral research.

Dear Irene, after my first unsuccessful attempt of being selected for the PhD position advertised by you, I was pleasantly surprised to receive an email from you after a few weeks regarding another PhD project, in collaboration with Martijn and Darryl. Thank you so much for having faith in me and selecting me to work on this ambitious project involving various *in vitro* and *in vivo* models, in addition to spending 2 years in Australia. You have been very supportive throughout my project and encouraged me to strive for excellence. I have been able to publish a letter, contribute to a review, write an editorial, apply and successfully obtain research grants and review few manuscripts under your guidance. Most importantly, I have learned the importance of time management from you, which has been immensely useful during my PhD. Even with your very busy schedule, you have always made time for me, quickly responded to my emails, and gave excellent feedback whenever needed. Thank you so much for being such a great supervisor.

Dear Martijn, during the early days of my PhD, I remember you giving me your personal copy of Janeway's Immunology after you found me struggling with immunology of asthma. Since then, I have gained adept knowledge in a lot of new areas under your supervision. With your support, I have come a long way from someone who has zero experience in animal studies to working with challenging experiments involving three different conditional knockout mouse models. It was a delight discussing different ideas and experimental plans with you. You made a lot of things easier for me with your calm mind and simplistic approach. You were always reassuring whenever things did not go as planned and when I

was stressed. Under your guidance, I was able to apply and successfully acquire grants and hope to publish my findings soon. It has been an absolute pleasure working with you.

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It is not an exaggeration when I say that it would have been impossible to have my animal experiments done without Laura Hesse. Dear Laura, from beginning to the end, you have been very kind and patient in training me, helping with the lab work, organising experiments and meetings, and managing the logistics. Even after I left Groningen, you have been very helpful in processing the samples and obtaining additional data for my thesis. You are an absolute powerhouse and always kept me on my toes. I used to dread waking up very early in the morning on section days and staying late nights to finish the flowcytometry, but I sometimes miss them now. With the project management skills I gained from working with you, I am now fully confident to take up any massive projects/experiments in the future. You were very understanding and encouraging during difficult and stressful moments. Thank you for everything Laura and I wish you the best for your future endeavours. I also want to thank Arjen Petersen for helping me with the animal experiments. It was a great experience working alongside you Arjen.

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some experiments and I really appreciate your contribution for my first publication. It's also been a lot of fun working with Harold and Ulke in the lab. I wish both of you are enjoying the new chapters in both your professional and personal lives. I also wish to specially thank Prof. Reinoud Gosens and his lab members Sophie Bos and Mariska van den Berg for their help in using their lab facilities for precision cut lung slices.

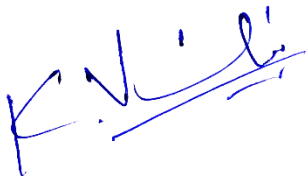
When I first started my PhD in Groningen, it was very fun sharing the office with Susana, Tamara, and Martin for the first few months. Thank you for making me feel welcome and for all the laughs. Hataitip, you were very kind, smart and hardworking and you were a great company both at work and outside. Sharing the office with Dennis, I often had the pleasure of having very interesting discussions and brainstorming ideas. I hope you find what you are looking for both personally and professionally. Most importantly, I would like to thank my best friend Mirjam for being an amazing person. I miss the banter and all the fun times we had at work. Mirjam – You are the best and I am very proud of you. I wish you all the success with your PhD and your future aspirations.

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connecting with other international students. Being a part of UNESN was a truly amazing experience filled with wonderful memories.

Finally, I would like to thank my parents and my family for believing in me and encouraging me to pursue my dreams. Without their support, it is hard to imagine that an ordinary kid from a humble background like me, was able to achieve the things I have, and I owe every bit of success to them.

A handwritten signature in blue ink, appearing to read 'K. N. S.', written in a cursive style with a horizontal line underneath.

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Virinchi Naga Sarma Kuchibhotla

# Curriculum vitae

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The author of this thesis was born on the 12<sup>th</sup> of July 1992 in Hyderabad, India. He completed his Bachelor's degree (B.Tech) in Biotechnology at the National Institute of Technology, Warangal (NITW), India, and graduated in first division with distinction in 2013. He then finished his Masters' degree (MSc.) in Biomedical Science from Cardiff Metropolitan University, United Kingdom and graduated with a distinction in 2015. He also received the prestigious Erasmus Mundus scholarship which supported his Master's studies. Later, he joined the group of 'Cellular Biomechanics' as a research intern, where he investigated the cell signalling pathways of mechanical compression-induced osteogenic differentiation of mesenchymal stromal cells (MSCs) under the supervision of Dr. Ansgar Petersen at the Julius Wolff Institute for Biomechanics and Musculoskeletal Regeneration, Berlin, Germany. After that, he commenced his doctoral studies in 2016 at the University of Groningen, Netherlands in with collaboration with University of Newcastle, Australia, under the supervision of Prof. Dr. Irene H. Heijink, Prof. Dr. Ir. Martijn Nawijn and Prof. Dr. Darryl Knight. Here, he worked on investigating the role of E-cadherin/ $\beta$ -catenin signalling in the development of an asthmatic airway epithelial phenotype.

# List of publications

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\***Kuchibhotla VNS**, \*Starkey MR, Reid AT, Heijink IH, Nawijn MC, Hansbro PM\*, Knight DA\*. Inhibition of  $\beta$ -catenin/CREB binding protein signaling attenuates house dust mite-induced goblet cell metaplasia in mice. Submitted to Front Physiol 2021

Heijink IH, **Kuchibhotla VNS**, Roffel MP, Maes T, Knight DA, Sayers I, Nawijn MC. Epithelial cell dysfunction, a major driver of asthma development. Allergy. 2020 Aug;75(8):1902-1917

**Kuchibhotla VNS**, Jonker MR, de Bruin HG, Noordhoek JA, Knight DA, Nawijn MC, Heijink IH. Inhibition of  $\beta$ -catenin/CBP signalling improves airway epithelial barrier function and suppresses CCL20 release. Allergy. 2020 Jul;75(7):1786-1789

**Kuchibhotla VNS**, Heijink IH. Join or Leave the Club: Jagged1 and Notch2 Dictate the Fate of Airway Epithelial Cells. Am J Respir Cell Mol Biol. 2020 Jul;63(1):4-6

Schreivogel S, **Kuchibhotla V**, Knaus P, Duda GN, Petersen A. Load-induced osteogenic differentiation of mesenchymal stromal cells is caused by mechano-regulated autocrine signaling. J Tissue Eng Regen Med. 2019 Nov;13(11):1992-2008

Post S, Heijink IH, Hesse L, Koo HK, Shaheen F, Fouadi M, **Kuchibhotla VNS**, Lambrecht BN, Van Oosterhout AJM, Hackett TL, Nawijn MC. Characterization of a lung epithelium specific E-cadherin knock-out model: Implications for obstructive lung pathology. Sci Rep. 2018 Sep 5;8(1):13275

# Abbreviations

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AB	Alcian blue
ADAM10	A disintegrin and metalloproteinase domain-containing protein 10
AHR	Airway hyperresponsiveness
AJ	Adherens junctions
ALI	Air-liquid interface
AM	Alveolar macrophage
APC	Adenomatous polyposis coli
A-SMA	alpha-smooth muscle actin
ATI	Alveolar type 1
ATII	Alveolar type 2
ATP	Adenosine triphosphate
AUC	Area under the curve
BAL	Bronchoalveolar lavage
BALF	Bronchoalveolar lavage fluid
BHR	Broncho hyperreactivity
Ca <sup>2+</sup>	Calcium
cAMP	Cyclic adenosine monophosphate
CBF1	Centromere-binding protein 1
CBP	CREB binding protein
CCL	Chemokine (C-C motif) ligand
CCND1	Cyclin D1
CCSP	Club cell secretory protein
CD	Cytochalasin D
cDNA	complementary DNA
CHX	Cycloheximide
CK-1	Casein kinase-1
CLR	C-type lectin receptor
CRE	Cre recombinase
CREB	cAMP Response Element-Binding Protein



CSL	CBF1–Suppressor of Hairless–LAG1
D	Day
DAMP	Damage associated molecular patterns
DC	Dendritic cell
Der f	<i>Dermatophagoides farinae</i>
Der p	<i>Dermatophagoides pteronyssinus</i>
DLL	Delta like protein
DNA	Deoxyribonucleic acid
ECIS	Electric Cell-substrate Impedance Sensing
EDA	Extra Domain A
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
ELISA	Enzyme-linked immunosorbent assay
EMT	Epithelial-mesenchymal transition
EO	Eosinophil
EWAS	(epi)genome-wide analyses
qQTL	Expression quantitative trait loci
ER	Endoplasmic reticulum
ERK	Extracellular signal-regulated kinase
EV	Extracellular vesicle
FOXA2	Forkhead box protein A2
FOXJ1	Forkhead box protein J1
GM-CSF	Granulocyte-macrophage colony-stimulating factor
GSK-3 $\beta$	Glycogen synthase kinase-3 $\beta$
GWAS	Genome-wide association study
HBEC	Human bronchial epithelial cell
HDAC	Histone deacetylase
HDM	House dust mite
H & E	Hematoxylin and eosin
HES1	Hairy and enhancer of split 1
ICS	Inhaled corticosteroids

IFN	Interferon
IgE	Immunoglobulin E
IL	Interleukin
ILC	Innate lymphoid cell
IM	Interstitial macrophage
i.n	Intranasal
i.p	Intraperitoneal
JAG	Jagged
JAM	Junctional adhesion molecules
KLRG1	Killer cell lectin-like receptor G1
KO	Knockout
LABA	Long acting $\beta$ 2-agonists
LAG1	Longevity-assurance gene 1
LAMA	Long-acting muscarinic antagonists
LEF	Lymphoid enhancer factor
loxP	locus of x-over, P1
LPS	Lipopolysaccharide
MAML1	Mastermind-like transcriptional co-activator 1
MAPK	Mitogen-activate protein kinase
miRNA	Micro RNA
mRNA	messenger RNA
NK	Natural killer
MC	Mast cell
MUC	Mucin
NCID	Notch intracellular domain
NF- $\kappa$ B	Nuclear factor-kappa B
NHBE	Normal human bronchial epithelial cells
NLR	NOD-like receptors
NOD	Nucleotide-binding oligomerisation domain
OVA	Ovalbumin
PAMP	Pathogen-associated molecular patterns

PAR	Protease activated receptor
PAS	Periodic acid–Schiff
PAEC	Primary airway epithelial cell
PBEC	Primary bronchial epithelial cell
PBS	Phosphate buffered saline
PC2	Physical Containment 2
PCR	Polymerase chain reaction
PI3K	Phosphoinositide 3-kinases
PRR	Pattern recognition receptor
RANTES	Regulated on activation, normal T cell expressed and secreted
RBPJ	Recombination Signal Binding Protein For Immunoglobulin Kappa J Region (also known as CSL)
RIG	Retinoic acid-inducible gene
RLR	RIG-I-like receptor
RNA	Ribonucleic acid
ROS	Reactive oxygen species
RSV	Respiratory syncytial virus
rtTA	reverse tetracycline transactivator
RV	Rhinovirus
scRNA-Seq	single-cell RNA sequencing
SERCA	Sarco-endoplasmic reticulum Ca <sup>2+</sup> ATPase
siRNA	small interfering RNA
SNP	Single nucleotide polymorphism
SP-C	Surfactant protein C
SPF	Specific pathogen-free
TAGC	Trans-National Asthma Genetic Consortium
TCF	T-cell factor
TDI	Toluene diisocyanate
TEER	Trans-epithelial electric resistance
tetO	Tet operator
TGF-β	Transforming growth factor-β

TRPM8	Transient receptor potential melastatin 8
Th	T helper
TJ	Tight junction
TLR	Toll-like receptor
Treg cell	Regulator T cell
TSLP	Thymic stromal lymphopoietin
tTA	tetracycline transactivator
VEGF	Vascular endothelial growth factor
Wnt	Wingless-related integration site
WT	Wildtype
ZO	Zona occludens