anti-inflammatory and a pro-inflammatory role, respectively, whereas galectin-9 exerts both sides mainly by interacting with eosinophils in the Th2-mediated eosinophilic allergic disorders.[2] Eosinophils are the most abundant cells in skin biopsy, however remain nearly unknown in the pathogenesis of CSU. Our findings open an avenue to evaluate the importance of the balance of the galectins and the contribution of eosinophils to CSU development regarding the galectins.

The limitation of this study is the small size of patients group. Rather than an elevation in all CSU patients, there were small elevations in a small subgroup of CSU compared to healthy controls, most CSU patients were comparable to healthy controls. Apart from the limitations, our current findings suggest that serum galectin-1, -3, and -9 are bystander molecules for CSU.

ACKNOWLEDGMENTS

This study was supported by the National Natural Science Foundation of China [grants 81872524, 81673063], Natural Science Foundation of Guangdong Province [grant numbers S2017A030313765, S2017A030313759,S2015A030313062].

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

Keywords
chronic spontaneous urticaria, galectin-1, galectin-3, galectin-9

Lin Zheng
Zhen-Rui Shi
Yan-Fang Han
Yi-Jin Luo
Zeng-Qi Tang
Guo-Zhen Tan
Liangchun Wang
Department of Dermatology, Sun Yat-sen Memorial Hospital, Sun Yat-sen University, Guangzhou, China

Correspondence
Guo-Zhen Tan, Liangchun Wang, Department of Dermatology, Sun Yat-sen Memorial Hospital, Sun Yat-sen University, 107 Yanjiang Rd W, Guangzhou, China 510120.
Emails: guozhentan1@hotmail.com (GT); wliangch@mail.sysu.edu.cn (LW)

ORCID

Liangchun Wang https://orcid.org/0000-0002-5169-2751

REFERENCES

1. Kolkhir P, Church MK, Weller K, Metz M, Schmetzer O, Maurer M. Autoimmune chronic spontaneous urticaria: what we know and what we do not know. *J Allergy Clin Immunol*. 2017;139(6):1772-1781.
2. Rao SP, Ge XN, Sriramarao P. Regulation of eosinophil recruitment and activation by galectins in allergic asthma. *Front Med (Lausanne)*. 2017;4:68.
3. Yang LT, Shu Q, Luo XQ, et al. Long-term effects: galectin-1 and specific immunotherapy for allergic responses in the intestine. *Allergy*. 2018;73(1):106-114.
4. Jauregui I, Ortiz de Frutos FJ, Ferrer M, et al. Assessment of severity and quality of life in chronic urticaria. *J Investig Allergol Clin Immunol*. 2014;24(2):80-86.
5. Sabroe RA, Fiebiger E, Francis DM, et al. Classification of anti-FcepsilonRI and anti-IgE autoantibodies in chronic idiopathic urticaria and correlation with disease severity. *J Allergy Clin Immunol*. 2002;110(3):492-499.
6. Saigusa R, Asano Y, Nakamura K, et al. Systemic sclerosis dermal fibroblasts suppress Th1 cytokine production via galectin-9 overproduction due to Fli1 deficiency. *J Invest Dermatol*. 2017;137(9):1850-1859.
7. Shi ZR, Tan GZ, Cao CX, et al. Decrease of galectin-3 in keratinocytes: a potential diagnostic marker and a critical contributor to the pathogenesis of psoriasis. *J Autoimmun*. 2018;89:30-40.
8. Shi Z, Meng Z, Han Y, Cao C, Tan G, Wang L. The involvement of galectin-3 in skin injury in systemic lupus erythematosus patients. *Lupus*. 2018;27(4):621-627.
9. Perugino CA, AlSalem SB, Mattoo H, et al. Identification of galectin-3 as an autoantigen in patients with IgG4-related disease. *J Allergy Clin Immunol*. 2019;143(2):736-745.

DOI: 10.1111/all.13836

Endothelial B2-receptor overexpression as an alternative animal model for hereditary angioedema

To the Editor

Hereditary angioedema (HAE) is a rare genetic disease mediated by bradykinin (BK) in most of the cases.[1] The existing HAE mouse models present only increased vascular permeability, but no spontaneous swelling episodes comparable to patients' characteristics.[2,3] Therefore, we aimed to develop and characterize a transgenic rat overexpressing the BK B2-receptor (B2R) in endothelial cells (*B2Rover*) as an alternative model for HAE and BK-mediated angioedema studies.

Endothelium-specific overexpression of B2R was achieved using the vascular endothelial cadherin promoter (VECDH—based on the

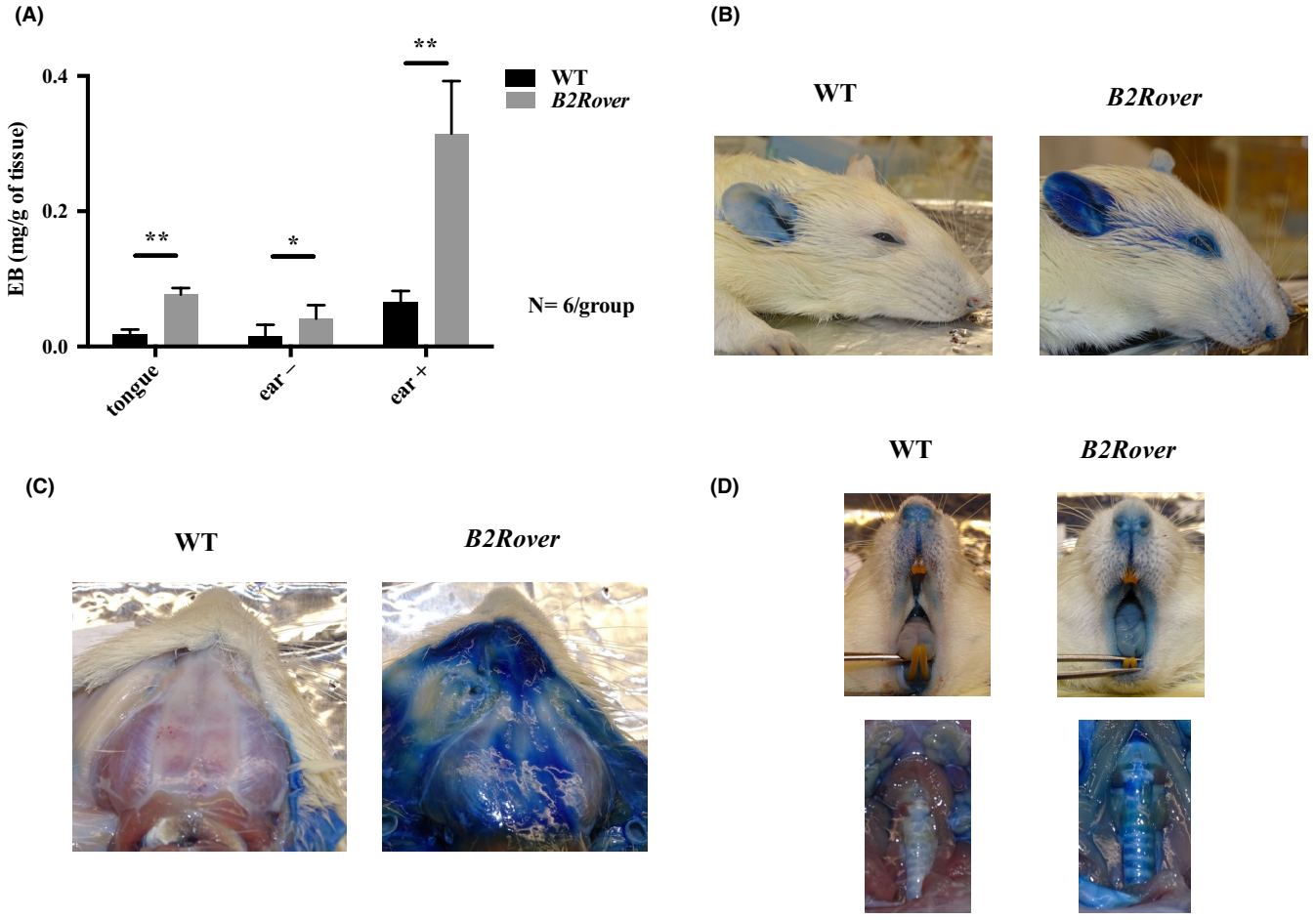


FIGURE 1 Evans blue extravasation after mustard oil challenge. A, The median Evans blue (EB) extravasation (mg/g of tissue) was significantly higher in the *B2Rover* ear where mustard oil was applied (ear+) (0.315 vs 0.065; $P = 0.0043$), but also in the other ear (ear-) (0.041 vs 0.015; $P = 0.0173$) and in the tongue (0.077 vs 0.018; $P = 0.0087$) compared to control rats. B, Ear, (C) head skin, (D) tongue, and trachea after mustard oil application. Error bars indicate interquartile range; $^*P < 0.05$; $^{**}P < 0.005$ (Mann-Whitney *U* test)

sequence of chromosome 18, NC_000074, and *Y10887 Mus musculus Cdh5 gene*), the rat B2R cDNA (*NM_001270713.2*), cloned in a pcDNA™3.1(−) vector (Thermo Scientific) (Figure S1). The transgenic rat was obtained by microinjection of DNA constructs into the pronuclei of one-cell *Sprague Dawley* embryos (wild type—WT). We identified one founder rat and its transgenic progeny by genotyping with transgene-specific primers (5′-TCACAGCTCCTCCGATGAGA-3′ and 5′-TCAGTGTCTGGGCAGTTGAC-3′). All animal experiments were performed according to the EU Directive 2010/63/EU for animal experiments and were approved by the Berlin State Office for Health and Social Affairs (*Landesamt für Gesundheit und Soziales*, G0124/17). Statistical analyses were performed by two-way ANOVA or Student's *t* test, or by Mann-Whitney *U* test when distribution was not normal. *P* value < 0.05 was considered significant.

Transgene overexpression was determined by qPCR using SYBR Green PCR Master Mix (Invitrogen) with primers targeting the endogenous (5′-AGGAGAAGTTGGTGGGCTAC-3′, 5′-TCATCAGGGTGTCGACGAAA-3′) and the total B2R expression (transgene + endogenous) (5′- GGTGCTGAGGAACAACGAGA-3′, 5′-CCCAACACAGCACAAAGAGC-3′). The total B2R expression was significantly higher in all organs of *B2Rover* rats tested when compared to WT, while the expression of endogenous B2R was slightly reduced or not affected (Figure S1). *B2Rover* rats developed and bred normally.

Thoracic aorta relaxation was measured by in vitro "organ bath" technique as previously described.[4] BK induced a dose-dependent relaxation in isolated aortic rings of *B2Rover* rats, and this effect was abolished by preincubation of aortic rings with a B2R antagonist and a nitric oxide synthase inhibitor, confirming endothelial-specific transgene expression. Aorta of WT rats did not respond to BK confirming previous studies.[5] These data also confirmed that the phenotype of *B2Rover* rats depends on the endothelial overexpression of B2R and not on alterations in other genes at the transgene integration site. To investigate any possible up/down regulation of the kinin B1-receptor in the *B2Rover* model, a B1-receptor agonist was tested in aortic rings, but no significant effect was observed (Figure S2).

Vascular permeability was analyzed 30 minutes after Evans blue (EB) tail vein injections in 12-week-old conscious rats (30 mg/kg body-weight). The rats were perfused with PBS, pieces of organs were collected, weighed, and incubated in formamide for 48 hours

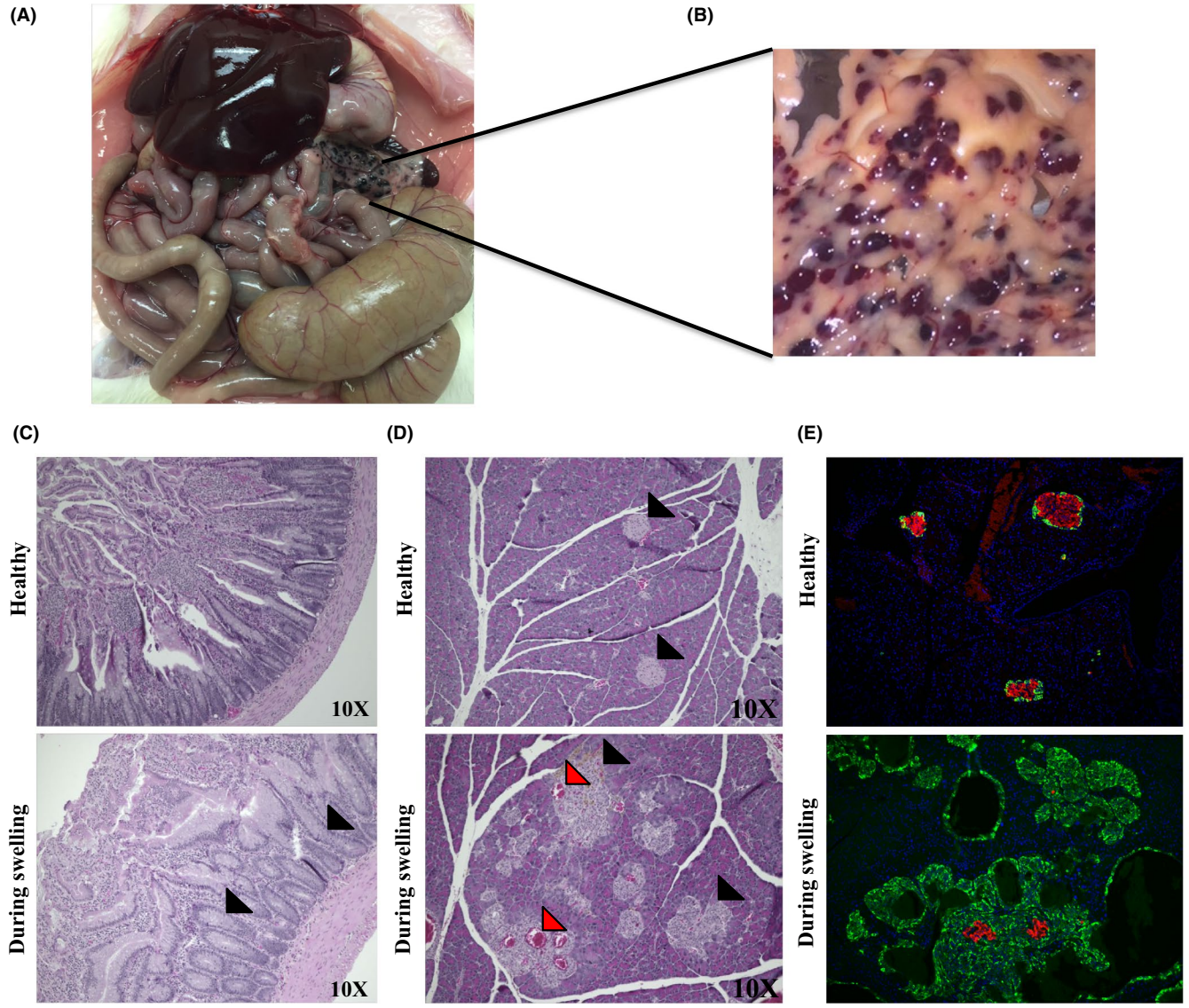


FIGURE 2 Spontaneous angioedema affecting intestine and pancreas. *B2Rover* presented sporadic spontaneous episodes of angioedema affecting intestine (A) and pancreas (B). Hematoxylin-eosin stained ileum (C) and pancreas (D) sections of healthy *B2Rover* rats (upper panels) and *B2Rover* rats during abdominal swellings (lower panels). Thickened villi can be observed during abdominal swellings (C, black arrow heads). *Langerhans* islets (D, black arrow heads) are larger during swelling episodes, and contain thrombi (red arrow heads). E, Anti-insulin (red) and anti-glucagon (green) staining revealed a decrease in β-cells and an increase in α-cells, respectively

at 55°C to extract the dye. EB extravasation was measured by spectrophotometry at 620 nm and quantified according to a standard curve. Acute angioedema attacks were induced by two topical applications of mustard oil (5% in mineral oil) on the dorsal surface of the right ear 1 minute after EB injection and again 15 minutes after EB injection. In the ear where mustard oil was applied, EB extravasation was more than 4-fold higher in *B2Rover* rats when compared to WT, and also the skin surrounding the region where mustard oil was applied showed higher vascular permeability (Figure 1). In HAE patients, attacks can simultaneously appear in multiple and anatomically different places,[1] as well as a subcutaneous attack can evolve to a submucosal swelling. Therefore, we also evaluated internal tissues to observe any possible systemic effect. After mustard oil challenge, EB extravasation in male *B2Rover* rats was significantly higher only in kidney ($P = 0.0108$) (Figure S3A). We assume that this increased vascular permeability remote from the application site was independent of mustard oil treatment but we did not check male *B2rover* rats at baseline. Accordingly, 12-week-old female transgenic rats presented increased vascular permeability under basal conditions in bowel, uterus, liver, and kidney when compared to age-matched WT females (Figure S3B). This exacerbated vascular permeability in females compared to males supports the potential of the transgenic rat model as a tool for the study of sexual hormones' influence on the regulation of the BK-release pathway and HAE. However, further experiments comparing male and female *B2Rover* rats are necessary to explain this apparent gender difference.

Although no skin swelling was observed, 12% percent of *B2Rover* rats (10/84) presented intestinal swellings independent of sex (5/49 male vs 5/35 female) (Figure 2A,B). This phenotype was not observed in age-matched WT rats analyzed (42 male, 18 female). Neither EB nor mustard oil was determinant for the development of this phenotype (4/10 rats underwent no procedure), indicating spontaneous and sporadic occurrence of abdominal angioedema. Histological analysis of intestine evidenced increased thickness of submucosal tissue and an apparent thickening of villi compared to healthy *B2Rover* rats (Figure 2C). Interestingly, the expression of B2R in swollen ileum was not higher than in nonswollen tissues (Figure S1). This indicates that the amount of B2R expressed in intestine is not significantly changed during angioedema attacks in the *B2Rover* rats. However, it does not exclude the possibility that B2R is augmented in HAE patients during attacks, since this animal model already has a constantly increased expression of the B2R under the control of a foreign promoter.

Pancreatic lesions characterized by blood clots spread throughout the tissue were observed in *B2Rover* rats with intestinal swellings. Comparing *B2Rover* pancreas with and without hemorrhagic clots (Figure 2B), an increase in the size and amount of *Langerhans* islets was observed (Figure 2D). Immunohistochemical analysis indicated a large increase in α-cells and decrease in β-cells when compared to nonaffected *B2Rover* rats (Figure 2E). The pancreatic lesions observed can be a consequence of pancreatic edema or represent a secondary event to intestinal swelling, which can induce partial or complete ductal obstruction resulting in pancreatitis.[6] The precise relationship between HAE and pancreatitis is not yet fully established. Since the first descriptions of HAE patients developing idiopathic pancreatitis more than 20 years ago, a few independent cases have been reported.[6,7] The hemorrhagic injuries found in our model could correspond to necrotizing hemorrhagic pancreatitis. Hemorrhage can occur in patients with severe necrotizing pancreatitis or as a result of pancreatic pseudoaneurysm rupture, representing a life-threatening emergency in humans. However, none of our rats showed signs of pancreatitis, such as pain or suffering; also, we did not notice any sudden death among these rats. Despite that we never observed the pancreatic lesions in the absence of intestinal swelling, we cannot exclude a direct effect of excessive B2R activation causing pancreatic abnormalities. Some reports showed a protective effect of B2R antagonists in different rodent models of induced pancreatitis,[8,9] suggesting that B2R is involved in the onset of pancreatic edema and inflammation, encouraging further studies on the role of B2R in pancreatic physiology.

In conclusion, the *B2Rover* rat is a promising model for the study of BK-mediated angioedema. Such studies can tackle the influence of the contact system on vasculature and fibrinolysis through a possible interaction between B2R activation and the function of new molecules recently associated to HAE with normal C1 inhibitor, angiopoietin 1, and plasminogen. Furthermore, this model will allow us to perform studies on the mechanisms involved in spontaneous abdominal BK-mediated angioedema, its time-course and duration, as well as in vivo screening of new specific drugs for HAE treatment.

ACKNOWLEDGMENTS

We thank Susanne da Costa Gonçalves and Cathrin Gerhard for the technical support; and Laura Rosenzweig, Annegret Dahlke, and Monique Bergemann from the animal care facility, MDC, Germany. This work was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP – grants No. 2013/23117-1, 2014/27198-8 and 2016/23304-3).

CONFLICT OF INTEREST

CLV received grants from Shire International GmbH. The other authors declare no conflict of interest.

Keywords

abdominal swelling, animal disease model, B2-receptor, bradykinin, Hereditary angioedema

Camila Lopes Veronez[1,2]
Sara Maghsodi[1,3]
Mihail Todiras[1,4]
Elena Popova[1]
André Felipe Rodrigues[1]
Fatimunnisa Qadri[1]
João Bosco Pesquero[2]
Michael Bader[1,3,5,6,7]

[1]*Max-Delbrück-Center for Molecular Medicine (MDC), Berlin, Germany*
[2]*Department of Biophysics, Universidade Federal de São Paulo, São Paulo, Brazil*
[3]*German Center for Cardiovascular Research (DZHK), Berlin, Germany*
[4]*Nicolae Testemițanu State University of Medicine and Pharmacy, Chișinău, Moldova*
[5]*Berlin Institute of Health (BIH), Berlin, Germany*
[6]*Charite – University Medicine, Berlin, Germany*
[7]*Institute for Biology, University of Lübeck, Lübeck, Germany*

Correspondence
Michael Bader, Max-Delbrück-Center for Molecular Medicine, Robert-Rössle-Strasse 10, 13125, Berlin, Germany.
Email: mbader@mdc-berlin.de

Camila Lopes Veronez and Sara Maghsodi should be considered joint first author.

ORCID

Camila Lopes Veronez https://orcid.org/0000-0001-9565-1911

REFERENCES

1. De Maat S, Hofman ZLM, Maas C. Hereditary angioedema: the plasma contact system out of control. *J Thromb Haemost*. 2018;16(11):2349-2351. https://doi.org/10.1111/jth.14209

2. Han ED, MacFarlane RC, Mulligan AN, Scafidi J, Davis AE 3rd. Increased vascular permeability in C1 inhibitor-deficient mice mediated by the bradykinin type 2 receptor. *J Clin Invest*. 2002;109(8):1057-1063.
3. Qiu T, Chiuchiolo MJ, Whaley AS, et al. Gene therapy for C1 esterase inhibitor deficiency in a murine model of hereditary angioedema. *Allergy*. 2018. [Epub ahead of print]. https://doi.org/10.1111/all.13582
4. Santos RA, Simoes e Silva AC, Maric C, et al. Angiotensin-(1-7) is an endogenous ligand for the G protein-coupled receptor Mas. *Proc Natl Acad Sci USA*. 2003;100(14):8258-8263.
5. Siltari A, Korpela R, Vapaatalo H. Bradykinin -induced vasodilatation: role of age, ACE1-inhibitory peptide, mas- and bradykinin receptors. *Peptides*. 2016;85:46-55.
6. Czaller I, Molnár K, Csuka D, Varga L, Farkas H. Successful outcome using C1-inhibitor concentrate in acute pancreatitis caused by hereditary angioedema. *Gastroenterol Nurs*. 2011;34(1):60-63.
7. Brickman CM, Tsokos GC, Balow JE, et al. Immunoregulatory disorders associated with hereditary angioedema. I. Clinical manifestations of autoimmune disease. *J Allergy Clin Immunol*. 1986;77(5):749-757.
8. Chen Q, Vera-Portocarrero LP, Ossipov MH, Vardanyan M, Lai J, Porreca F. Attenuation of persistent experimental pancreatitis pain by a bradykinin b2 receptor antagonist. *Pancreas*. 2010;39(8):1220-1225.
9. Hirata M, Hayashi I, Yoshimura K, et al. Blockade of bradykinin B(2) receptor suppresses acute pancreatitis induced by obstruction of the pancreaticobiliary duct in rats. *Br J Pharmacol*. 2002;135(1):29-36.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

DOI: 10.1111/all.13837

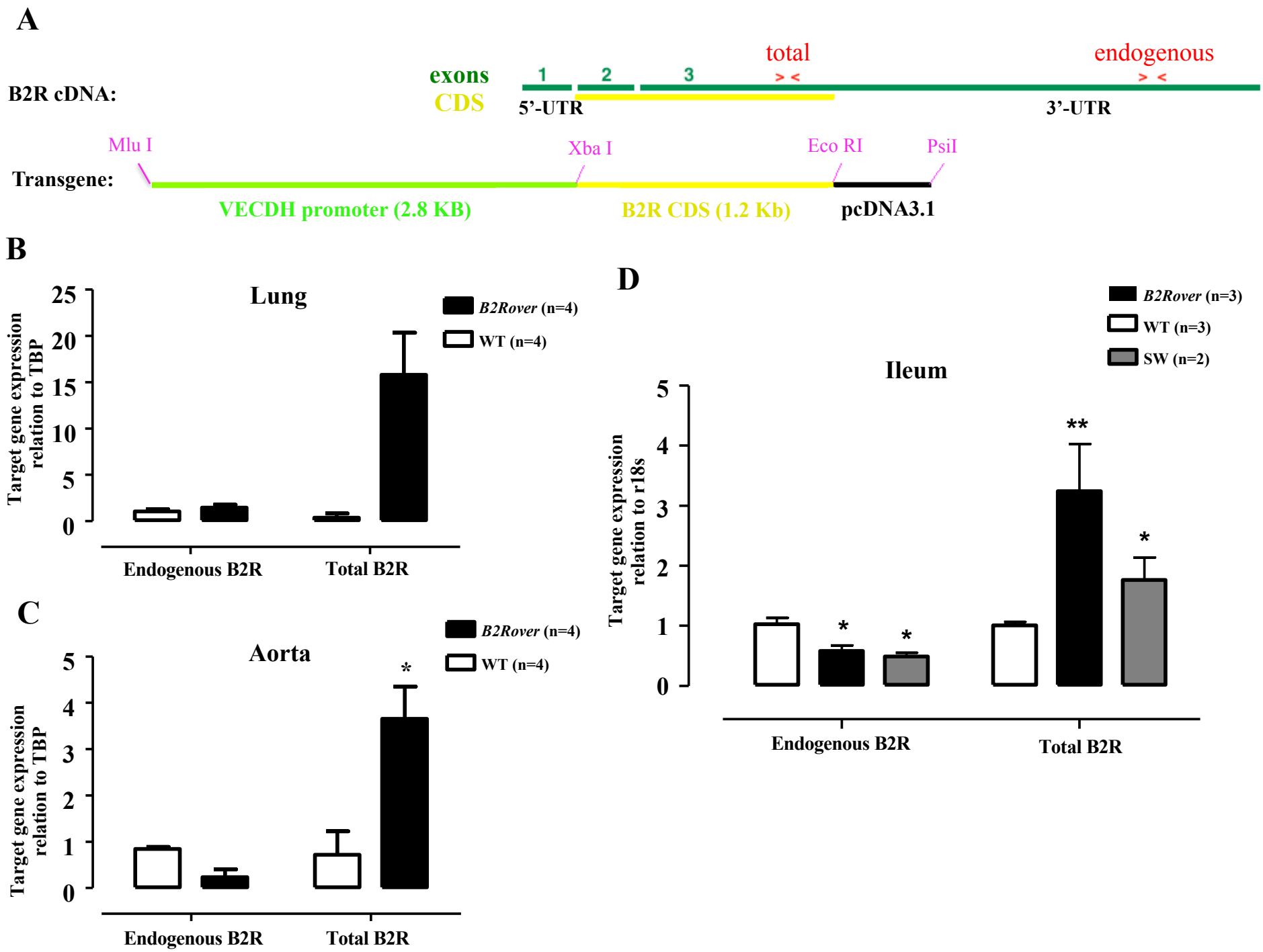
Longitudinal trends of serum IgE and *IL5RA* expression throughout childhood are associated with asthma but not with persistent wheeze

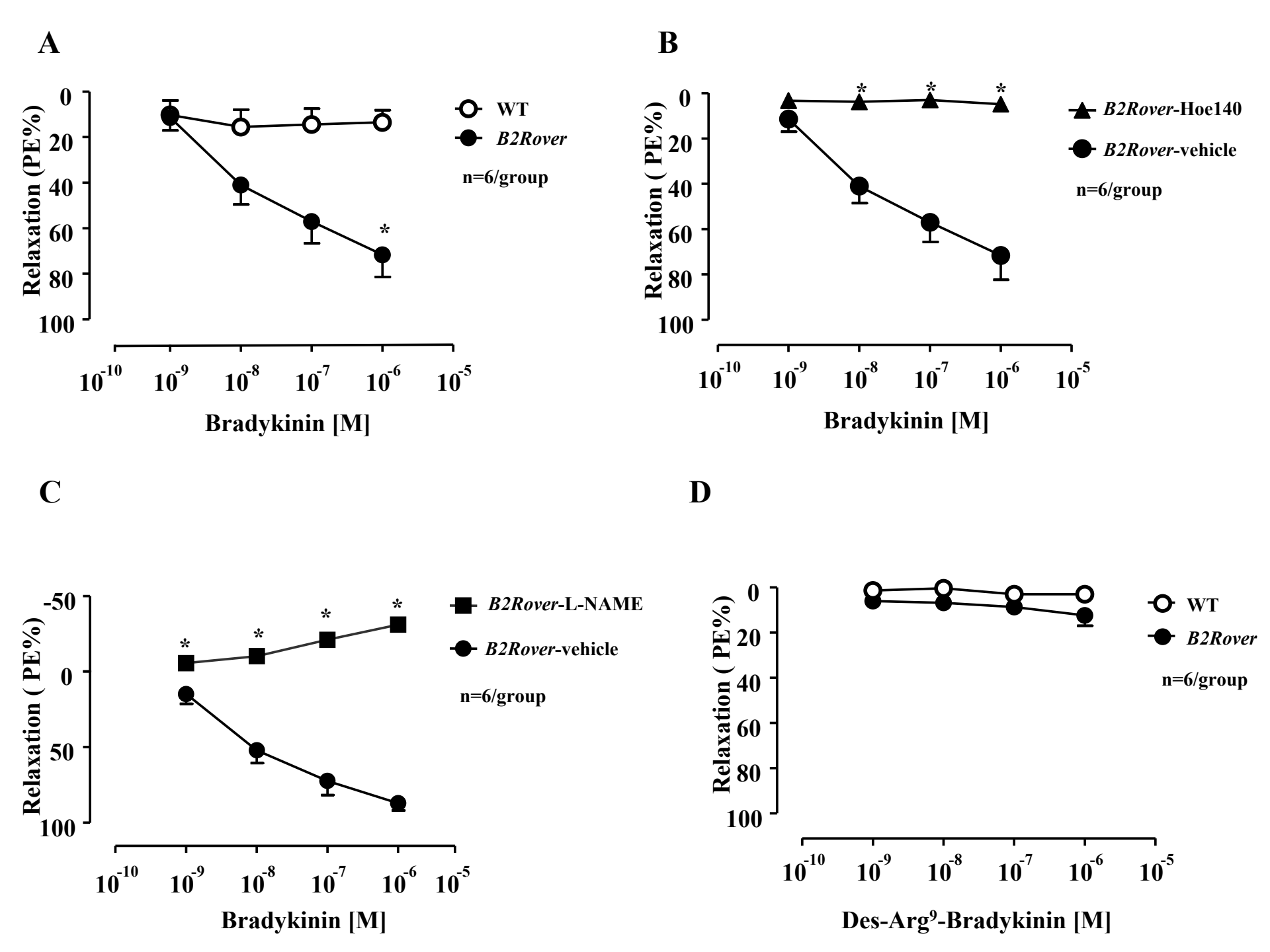
To the Editor,

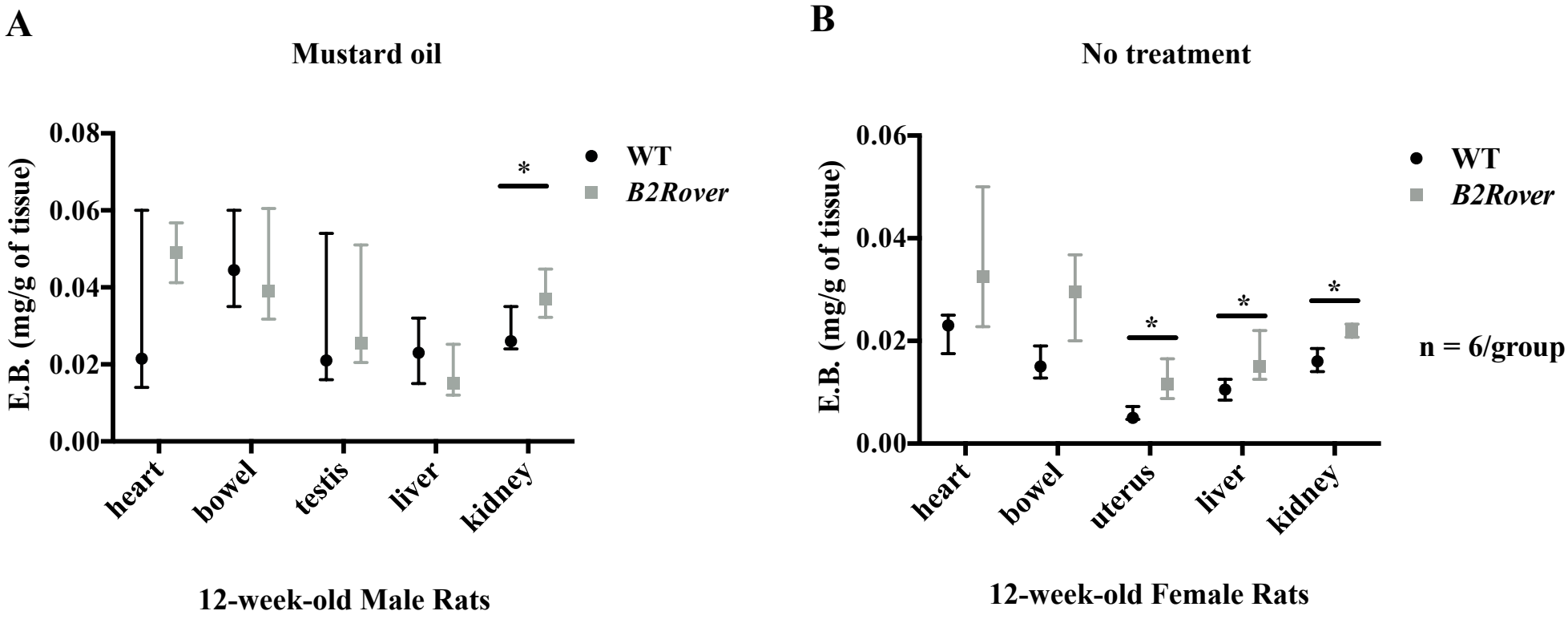
Wheezing episodes are common in young infants affecting every third child before the age of 3.[1,2] While wheezing episodes in early life are mostly triggered by viral lower respiratory tract infection, the development of asthma and airway hyperresponsiveness seems to be promoted by immunoglobulin E (IgE)–mediated lung inflammation.[3-5] Although the development of an asthma phenotype does not necessarily require an atopic status, early childhood asthma is mostly associated with atopy while nonatopic, intrinsic asthma is a rather rare condition.[3,4] Considering the critical role of IgE in the development of impaired lung function in early childhood, several studies tried to differentiate children with transient wheezing episodes from those with persistent wheeze based on early childhood serum IgE levels providing evidence that persistently wheezing children seem to have elevated IgE levels already very early in life.[6] Notably, although not every child with persistent or late-onset wheeze will be diagnosed with asthma, none of the earlier studies focusing on IgE, wheeze, and asthma distinguished between persistent wheeze and asthma (eg, [4,6]). Thus, one major aim of the present study was to evaluate the longitudinal association between serum IgE levels, wheeze, and asthma considering persistent wheeze and asthma as independent endotypes. In addition to serum IgE, we included interleukin-5 receptor-α (*IL5RA*) measured by qPCR from whole-blood RNA in our analyses as *IL5RA* has been closely linked to both IgE and asthma development.[7]

Data and samples from two population-based German birth cohorts, LINA[8] and LISA,[9] were used for this study. While the majority of birth cohorts dispose of only few blood sampling time points, the LINA study with annual clinical visits and blood sampling until the age of 8 offers the opportunity to study the timing of sensitization, wheezing symptoms, and asthma onset in a tightly time-resolved manner. The LISA study with available blood samples at ages 2, 6, 10, and 15 was used as a replication cohort. For the current analyses, only children with longitudinal blood samples (LINA age 1-8, n = 98; LISA age 2, 6, 10, and 15, n = 453) and available RNA (LINA age 1, 4, and 8; LISA age 15) were included (for the description of the study population, see Figure S1 and Table S1, Supporting Information Methods). Wheezing children without an asthma diagnosis were grouped according to their wheezing endotypes: transient (reported wheezing up to the age of 3 but not thereafter), late-onset (wheezing reported after age 3), and persistent wheeze (wheezing during the first 3 years of life and at least one time thereafter). These children were compared to apparently healthy controls and children diagnosed with asthma, respectively. The asthma outcome was defined according to the question asked at each follow-up (see Figure S1): "Has a physician diagnosed your child with asthma during the past 12 months?" Within the studied LINA and LISA subpopulations, 10.2% (10/98) and 10.8% (49/453) of the children were ever diagnosed with asthma, respectively.

As expected, in both cohorts the specific IgE concentrations (sIgE) (Figure 1A) and sensitization percentages (Figure S2) against aeroallergens (sx1/rx1) were significantly different between groups over time with highest levels in asthmatic children (one-way repeated-measurement ANOVA; Figure 1A). Compared to healthy controls, LINA children diagnosed with asthma later in life showed significantly higher sIgE levels already starting at age 4 (post hoc


A
B2R cDNA:
exons
CDS
1
2
3
total
endogenous
5'-UTR
3'-UTR
Transgene:
Mlu I
Xba I
Eco RI
PsiI
VECDH promoter (2.8 KB)
B2R CDS (1.2 Kb)
pcDNA3.1
B
Lung
B2Rover (n=4)
WT (n=4)
Target gene expression relation to TBP
Endogenous B2R
Total B2R
C
Aorta
B2Rover (n=4)
WT (n=4)
Target gene expression relation to TBP
Endogenous B2R
Total B2R
D
Ileum
B2Rover (n=3)
WT (n=3)
SW (n=2)
Target gene expression relation to r18s
Endogenous B2R
Total B2R


A
Relaxation (PE%)
0
20
40
60
80
100
WT
B2Rover
n=6/group
*
10-10
10-9
10-8
10-7
10-6
10-5
Bradykinin [M]
B
Relaxation (PE%)
B2Rover-Hoe140
B2Rover-vehicle
n=6/group
*
*
*
Bradykinin [M]
C
Relaxation (PE%)
-50
0
50
100
B2Rover-L-NAME
B2Rover-vehicle
n=6/group
*
*
*
*
Bradykinin [M]
D
Relaxation (PE%)
WT
B2Rover
n=6/group
Des-Arg9-Bradykinin [M]


A
Mustard oil
E.B. (mg/g of tissue)
0.08
0.06
0.04
0.02
0.00
WT
B2Rover
*
heart
bowel
testis
liver
kidney
12-week-old Male Rats
B
No treatment
E.B. (mg/g of tissue)
0.06
0.04
0.02
0.00
WT
B2Rover
*
*
*
n = 6/group
heart
bowel
uterus
liver
kidney
12-week-old Female Rats