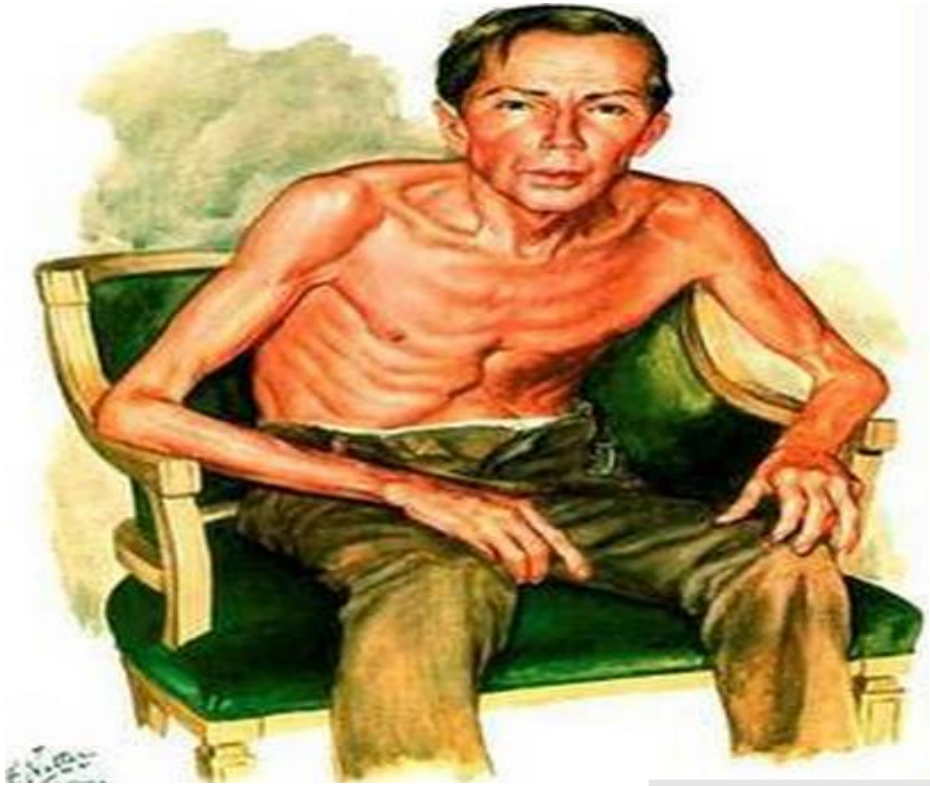
Abhd2 regulates alveolar type Ⅱ apoptosis and airway smooth muscle remodeling: a key target of COPD research

Shoude Jin

Harbin Medical University, China

Background

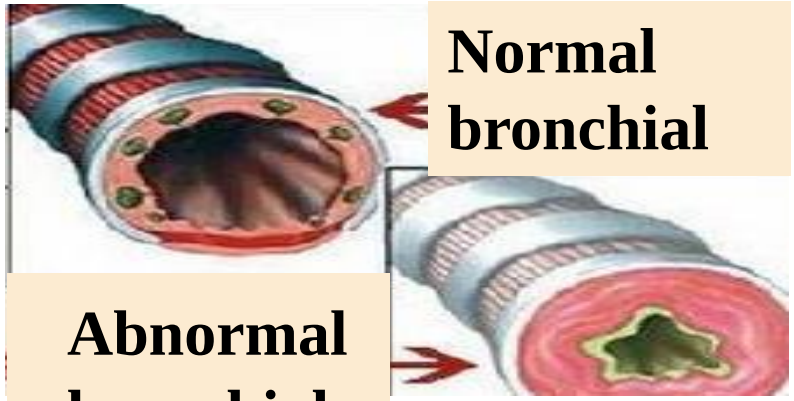
COPD ------ a silent killer



Insidious, diagnosed at late stages…

- **Third cause of death in the world**
- **Fifth of the world's disease burden**
- **Early diagnosis and early prevention are essential**

Pathophysiology



Normal bronchial

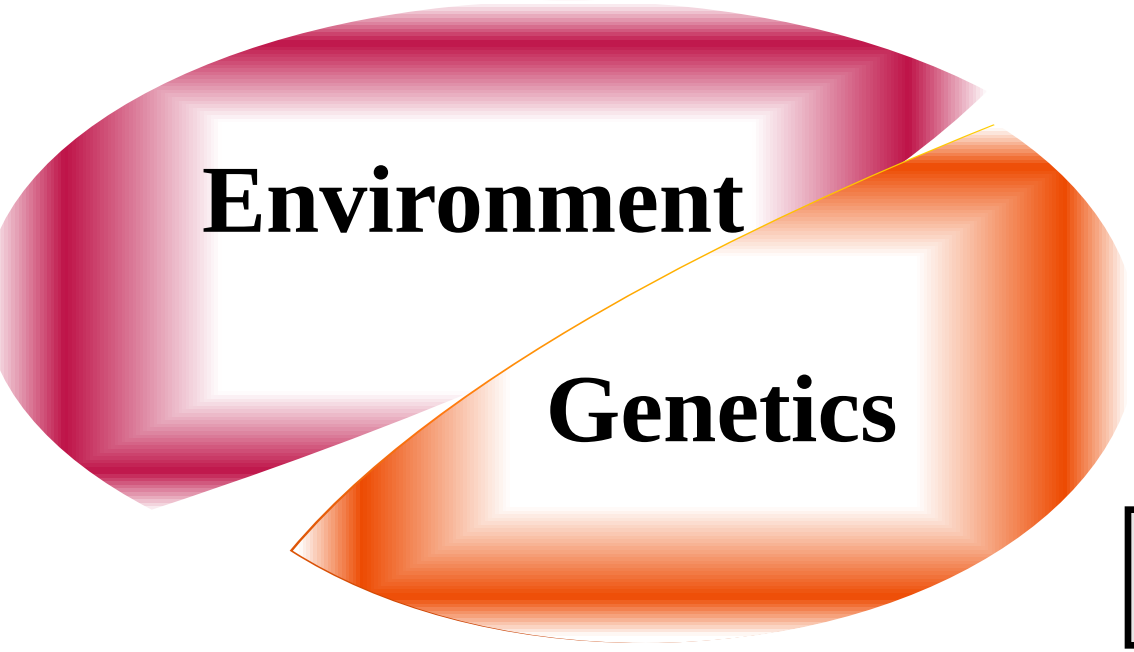
Abnormal bronchial

Airway remodeling

Normal vessel

Abormal vessel

Vessel remodeling



- Smoking is a major risk factor for COPD
- Only 10-20% of long-term heavy smokers develop into COPD
- Part COPD patients with no history of smoking

The importance of genetic susceptibility to COPD

Clinical characteristics and prevention direction of COPD：：

- Interaction of individual genetic susceptibility and environmental factors
- It is important of susceptible early screening and early intervention in the prevention of chronic obstructive pulmonary disease

Recent Advances and trends:

◆Genomic analyses to reveal genetic susceptibility to COPD

◆Regional and Ethnic differences:

α1-AT deficiency increase the risk of Caucasian COPD, it is a susceptibility gene for COPD

TNFα gene mutation associated with Asian COPD

such as EPHX1, IL4, IL-10 associated with COPD

◆In addition to α1-AT, it has not been determined that other genes are susceptibility gene for COPD

Our previous study

➢**IL-13 G + 2044A point mutations associated with Caucasian COPD, but no correlation with the Chinese COPD patients**

(2014, Molecular Biology Reports, Dr. Shoude Jin and colleagues)

➢**Key Findings: Abhd2 gene deletion in mice naturally develop into emphysema phenotype**

(2009, BBRC， Dr. Shoude Jin and colleagues)

Abhd2 protein is a lipid hydrolase

➢ **ABHD2 homologous gene mutations can increase the risk of COPD**

（2015, Plos one， Dr. Shoude Jin and colleagues）

PLOS ONE

RESEARCH ARTICLE

Associations of *ABHD2* Genetic Variations with Risks for Chronic Obstructive Pulmonary Disease in a Chinese Han Population

Li Liu[1☯], Xiangshun Li[2☯], Rui Yuan[1], Honghong Zhang[1], Lixia Qiang[2], Jingling Shen[1*], Shoude Jin[2*]

1 Department of Histology and Embryology, Harbin Medical University, Harbin, Heilongjiang Province, 150018, China, 2 Division of Respiratory Disease, The Fourth Hospital of Harbin Medical University, Harbin, Harbin, Heilongjiang Province, 150001, China

☯ These authors contributed equally to this work.
* jinshoude@163.com (SJ); shenjingling@rocketmail.com (JS)

Abstract

The human α/β hydrolase domain-containing protein 2 gene (*ABHD2*) plays a critical role in pulmonary emphysema, a major subset of the clinical entity known as chronic obstructive pulmonary disease (COPD). Here, we evaluated genetic variation in the *ABHD2* gene in a Chinese Han population of 286 COPD patients and 326 control subjects. The rs12442260 CT/CC genotype was associated with COPD ($P < 0.001$) under a dominant model. In the former-smoker group, the rs12442260 TT genotype was associated with a decreased risk of developing COPD after adjusting for age, gender and pack-years ($P = 0.012$). Rs12442260 was also associated with pre-FEV1 (the predicted bronchodilator forced expiratory volume in the first second) in controls ($P = 0.027$), but with FEV1/forced vital capacity (FVC) ratios only in COPD patients ($P = 0.012$) under a dominant model. Results from the current study suggest that *ABHD2* gene polymorphisms contribute to COPD susceptibility in the Chinese Han population.

OPEN ACCESS

Citation: Liu L, Li X, Yuan R, Zhang H, Qiang L, Shen J, et al. (2015) Associations of *ABHD2* Genetic Variations with Risks for Chronic Obstructive Pulmonary Disease in a Chinese Han Population. PLoS ONE 10(4): e0123929. doi:10.1371/journal.pone.0123929

Academic Editor: Chunxue Bai, Zhongshan Hospital Fudan University, CHINA

Received: August 16, 2014

Accepted: March 9, 2015

Published: April 16, 2015

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Funding: This study was funded by the National Natural Science Foundation of China (30900413), Science Foundation of Heilongjiang Province of China (LC201024), the National Basic Science Research Program of China (973 Program) (grant no. 2012CBA01303).

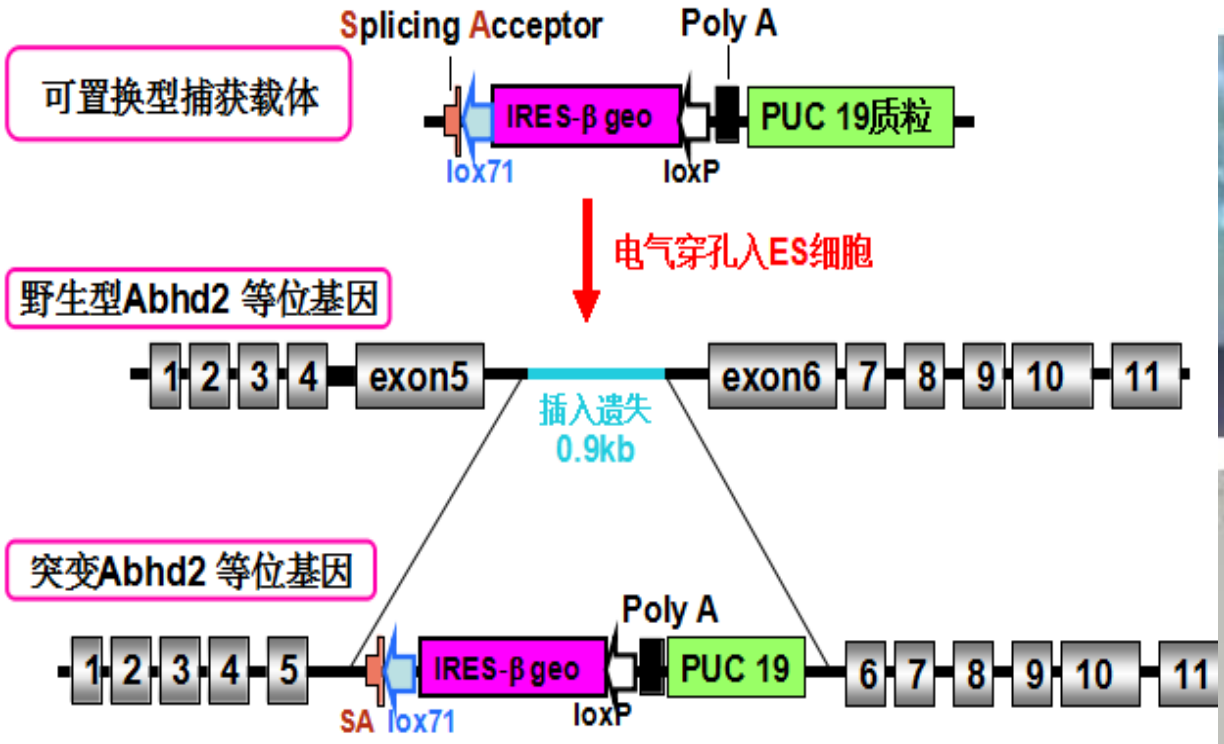
Competing Interests: The authors have declared that no competing interests exist.

Introduction

Chronic obstructive pulmonary disease (COPD), including chronic pulmonary emphysema and chronic bronchitis, is one of the leading causes of morbidity and mortality worldwide. It is characterized by a partially reversible airflow limitation, which is usually progressive and associated with abnormal inflammatory responses of the lungs when exposed to noxious particles or gases [1–4]. According to the World Health Organization, by 2020, COPD may become the fourth most common single cause of death and is expected to be the third-leading cause of death in developed countries [5–8]. The global incidence of COPD is approximately 9~10% in adults aged ≥40 years of age [9], and 8.2% in this age group in China [10]. Accounting for 80–90% of COPD cases, cigarette smoking is by far the major risk factor for COPD. Howeve 10~15% of smokers develop clinically significant COPD, and some former smokers and smokers can develop COPD [11–14]. Current evidence suggests that inherited risks influ

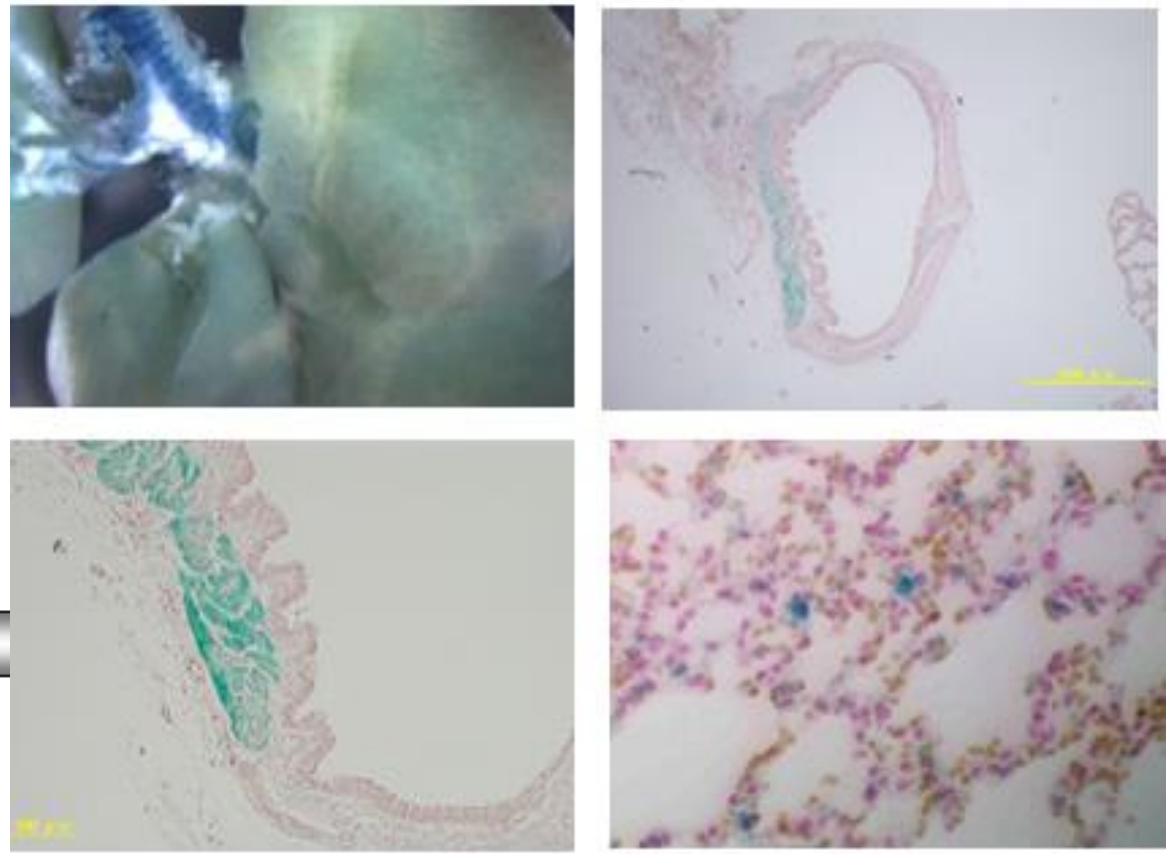
Yamamura team (our collaborators)
Kumamoto University, Japan

Trapping vector insertion point



IRES(Internal ribosome entrysite): IRES序列来自病毒, mRNA上有IRES序列时，核糖体与之结合，以非依赖帽子(cap)的形式进行蛋白翻译。

Abhd2 mouse gene expresses in alveolar type Ⅱ epithelial cells and airway smooth muscle cells



Our research team: using the Abhd2 knockout mice

Further to verify: Abhd2 expressed in alveolar type Ⅱ cells

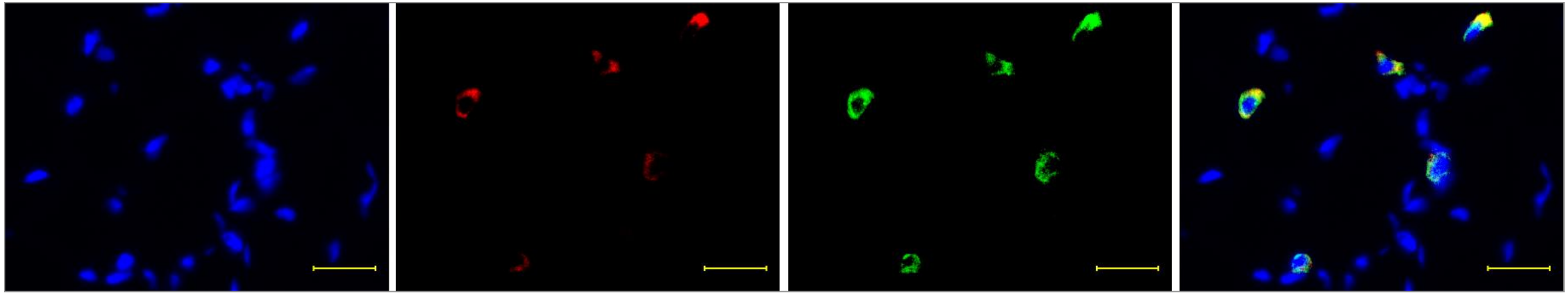


Figure. β-gal staining pattern P180 common immunofluorescence of 1.Abhd2 knockout mice lung tissue

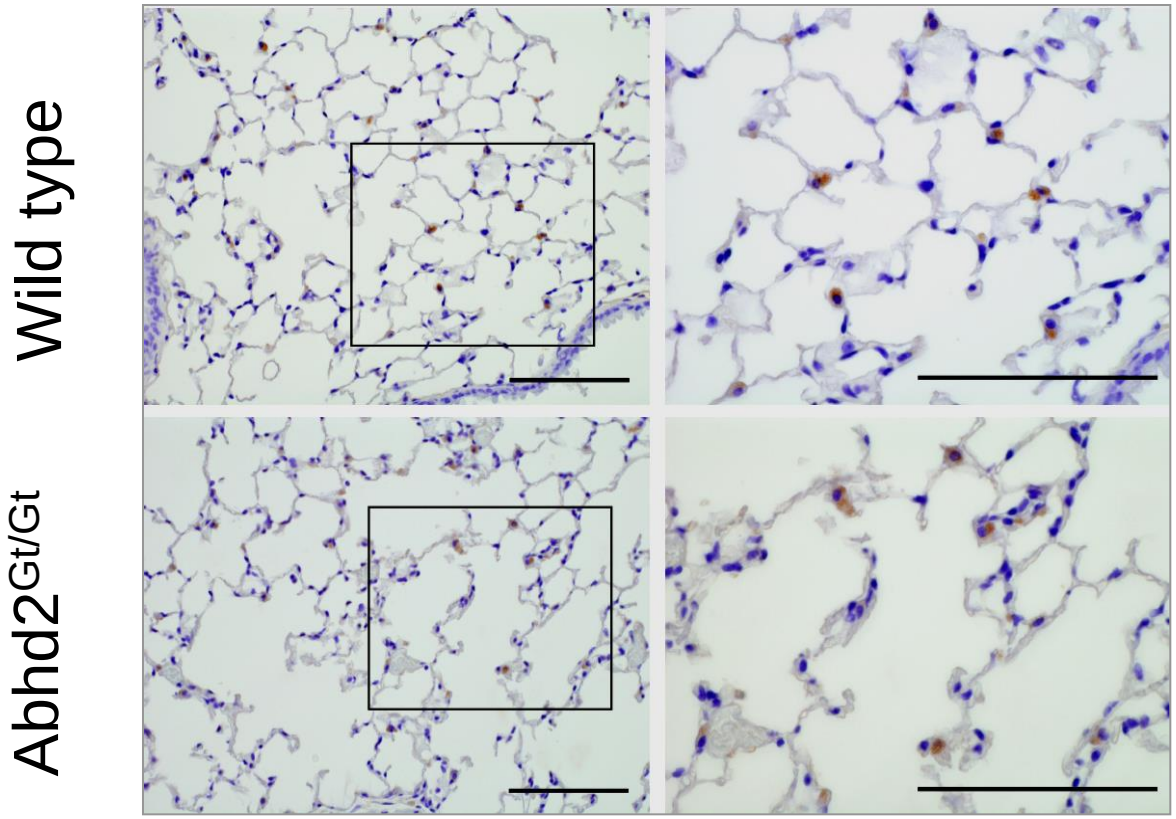
Phospholipid metabolism in lavage fluid

Table. the content and relatively of PC, DSPC and protein in lavage fluid and lung tissue homogenates

Age	Phospholipid / protein	Lavage		Lung homogenates	
		Wild type	Abhd2Gt/Gt	Wild type	Abhd2Gt/Gt
2 months	PC (μg)	331.85±19.76	266.76±9.40**	669.56±65.63	568.89±65.63
	DSPC (μg)	215.74±19.45	150.92±20.57*	275.42±62.95	236.51±15.23
	Protein (μg)	278.44±42.16	272.67±14.81	7236.55±542.80	6856.15±424.56
		(n=4)	(n=4)	(n=4)	(n=4)
4 months	PC (μg)	239.55±13.08	255.53±60.91	481.46±45.16	622.16±104.75
	DSPC (μg)	140.06±15.56	174.42±59.54	229.47±100.20	210.46±88.71
	Protein (μg)	339.78±32.10	174.42±59.54	6935.79±5 33.20	7329.65±493.72
		(n=5)	(n=5)	(n=5)	(n=5)
6 months	PC (μg)	302.46±12.73	146.52±19.09**	715.19±80.78	603.28±66.20*
	DSPC (μg)	233.84±31.45	193.39±31.16	297.97±40.31	268.44±21.12
	Protein (μg)	344.48±35.92	286.48±22.67*	6756.56±160.32	6044.77±298.96*
		(n=5)	(n=6)	(n=5)	(n=6)
12 months	PC (μg)	309.55±32.52	265.56±19.24*	6680.6±107.49	781.61±141.55
	DSPC (μg)	244.87±34.89	220.62±19.37	292.27±34.84	324.83±98.40
	Protein (μg)	341.59±28.94	325.59±38.94	7363.39±1140.19	8157.91±601.80
		(n=5)	(n=4)	(n=5)	(n=4)

Values are mean ± standard deviation. Two groups were compared using t-test. *$p < 0.05$, ** $p < 0.01$

Increased apoptosis of alveolar type Ⅱ



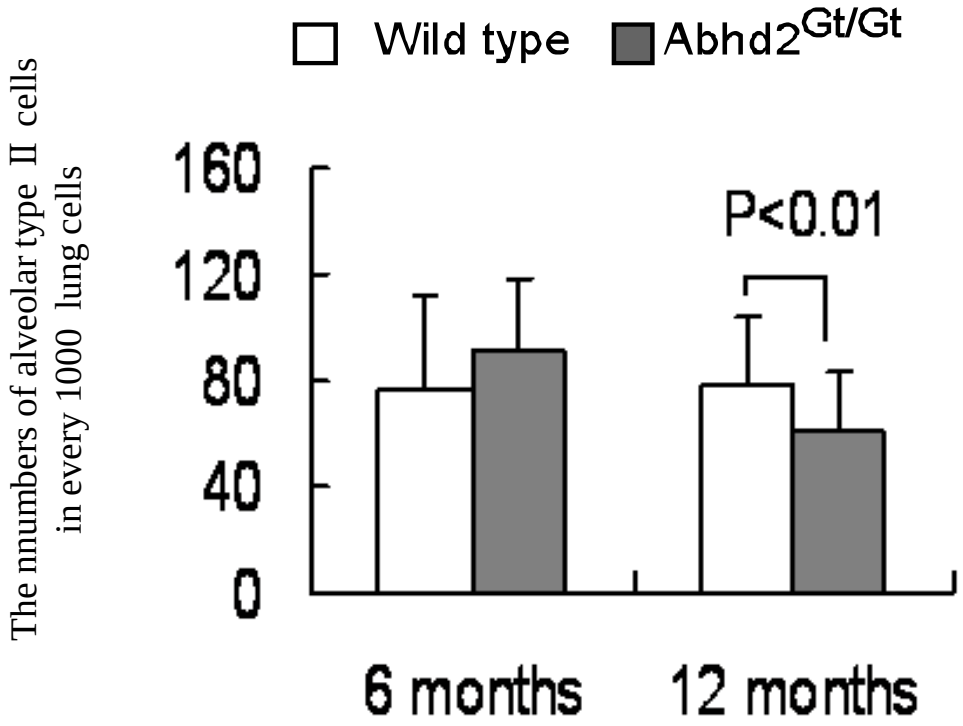
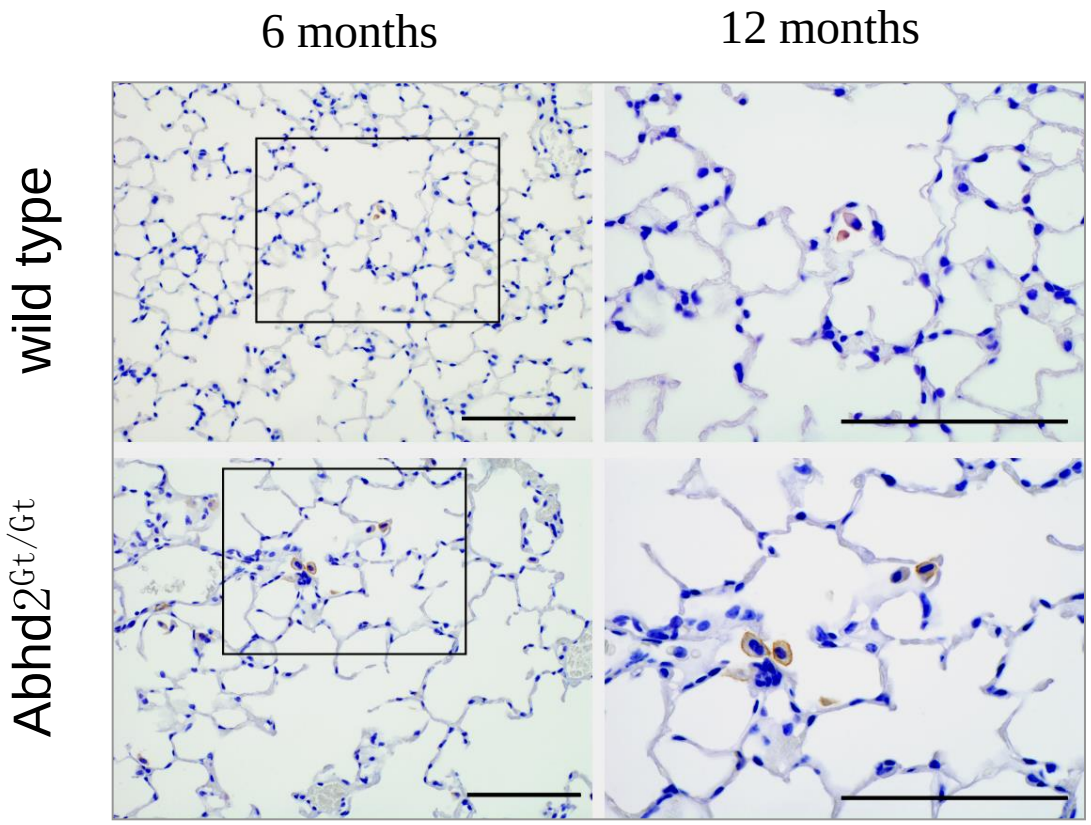


Figure . P180 antibody immunohistochemistry of 12-month-old mouse lung slices, the mouse type II alveolar cell count of 6 months and 12 months

Increased macrophage infiltration in Lung tissue



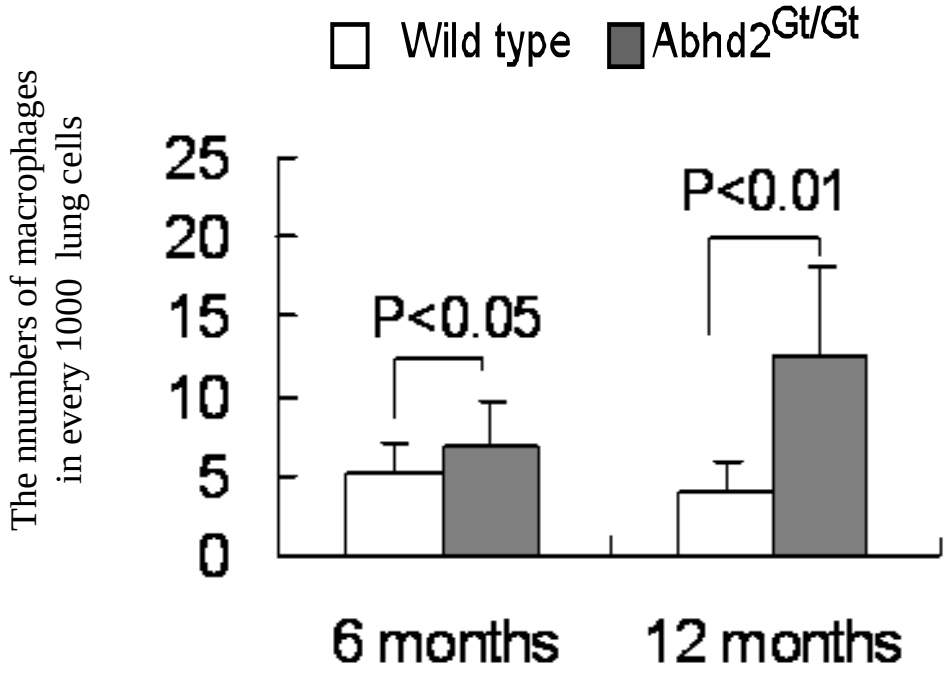


Figure. F4 / 80 antibody immunohistochemistry and lung macrophages count comparison in Abhd2Gt / Gt and wild type lung tissue sections

Elastic fibrin reduction

Wild type **Abhd2$^{Gt/Gt}$**

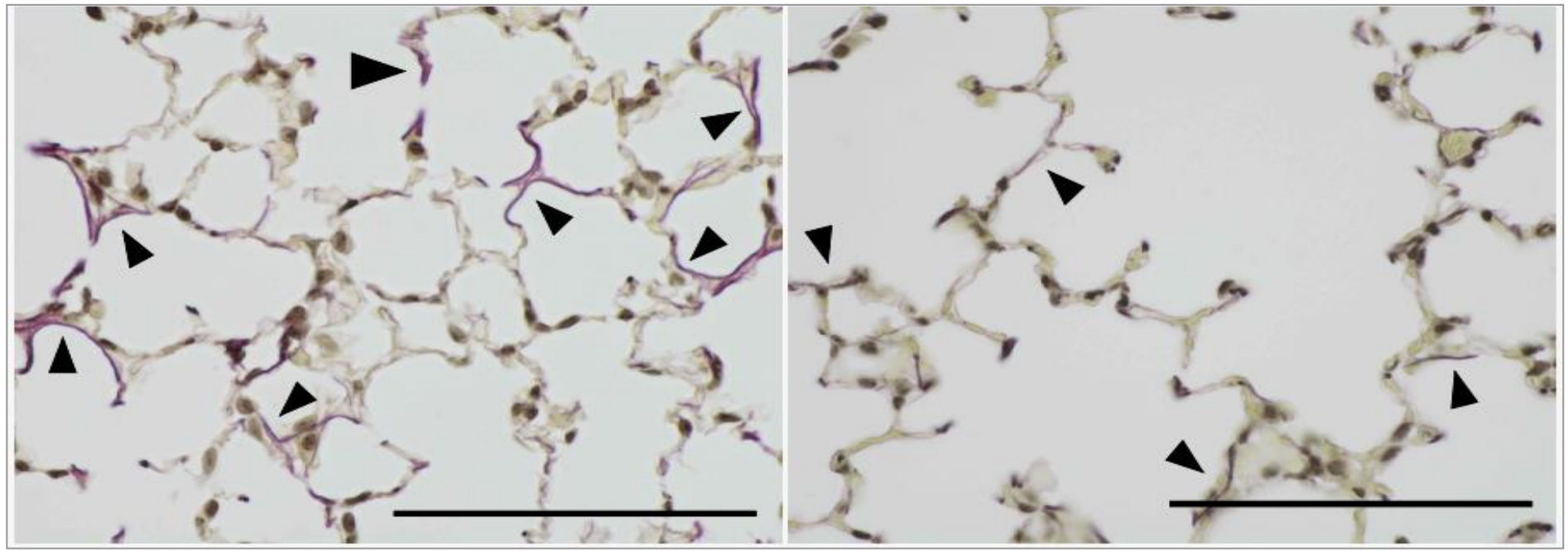


Figure. The staining of elastic fiber

Alveolar destruction, expanding and spontaneously formed emphysema phenotype

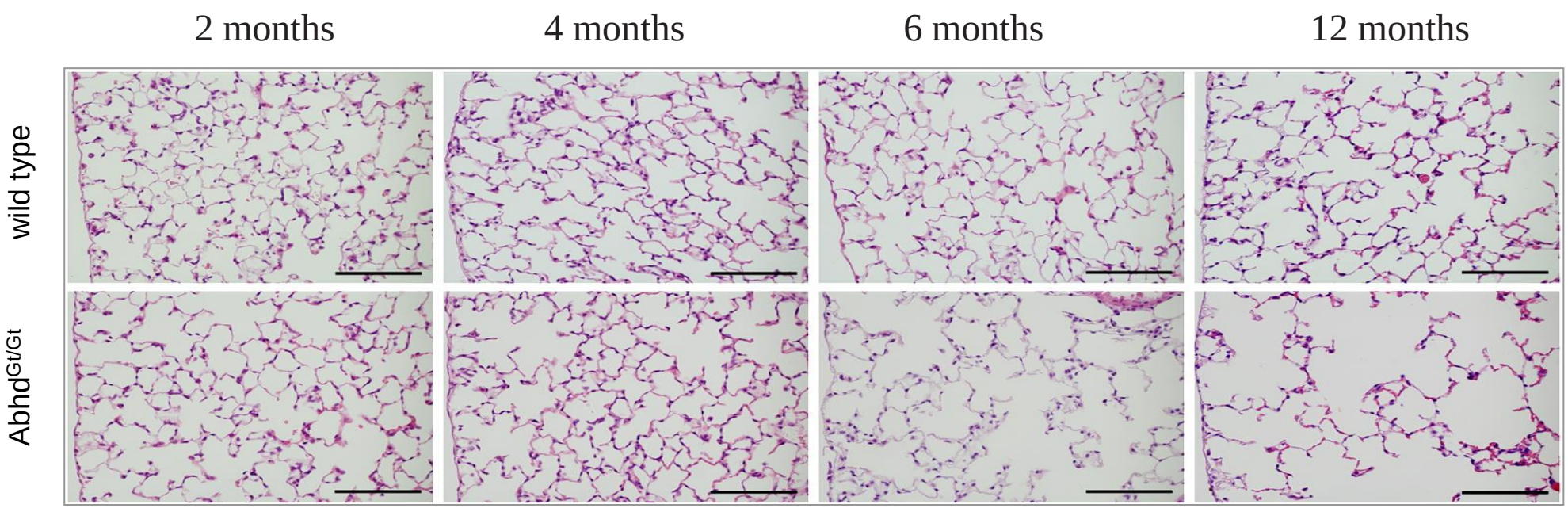
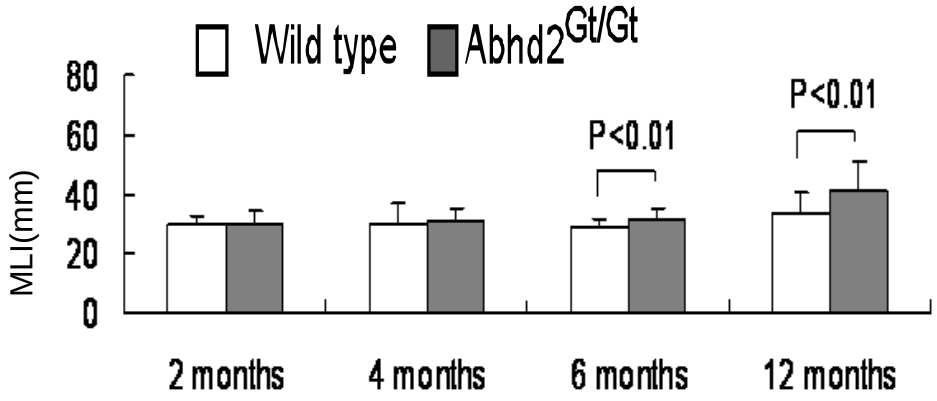


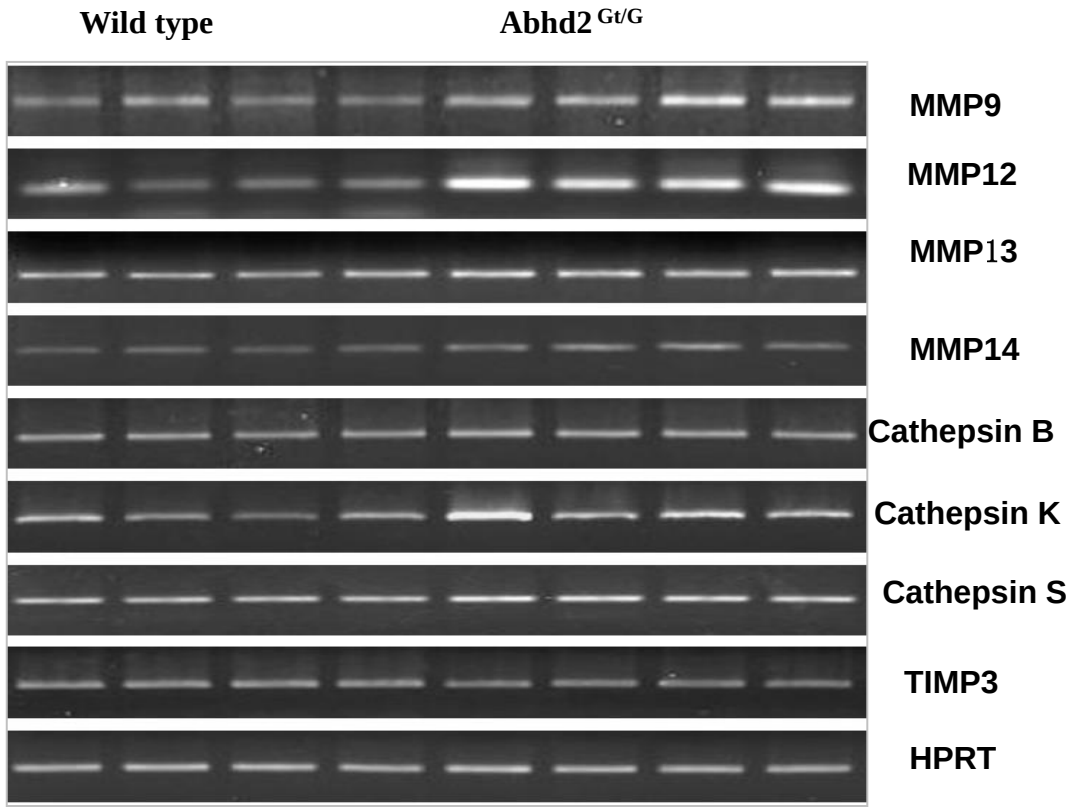
Figure. the stained with HE of lung tissue



Mean linear intercept(MLI)

Figure. The mean linear intercept measurements of enlarged terminal alveolar space different age groups Abhd2 knockout mice to compare to wild-type

Protease /anti-protease imbalance



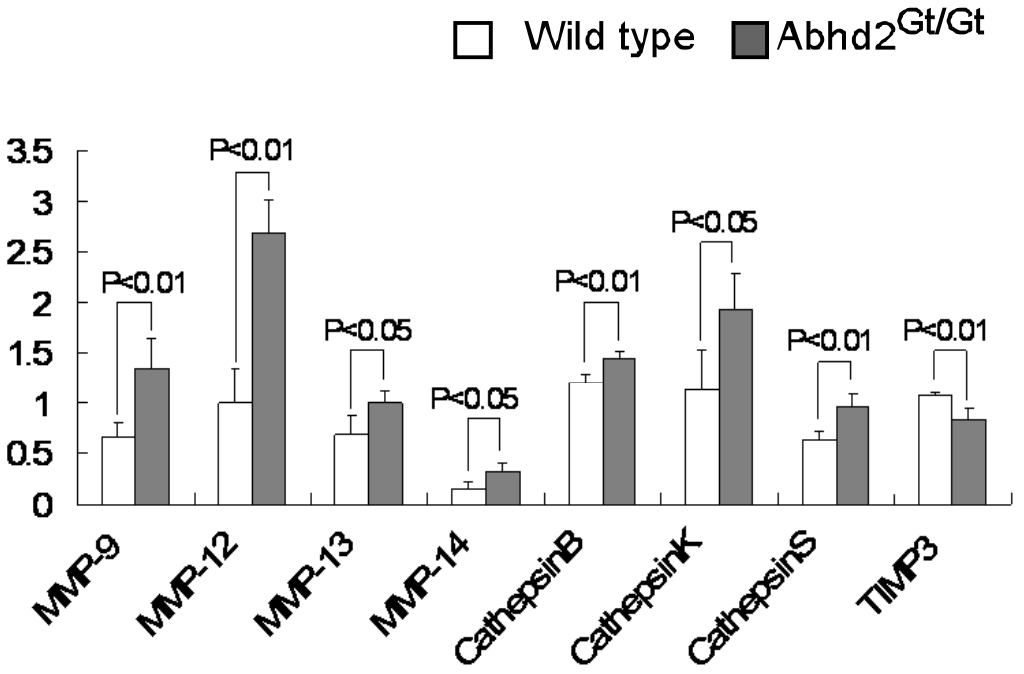
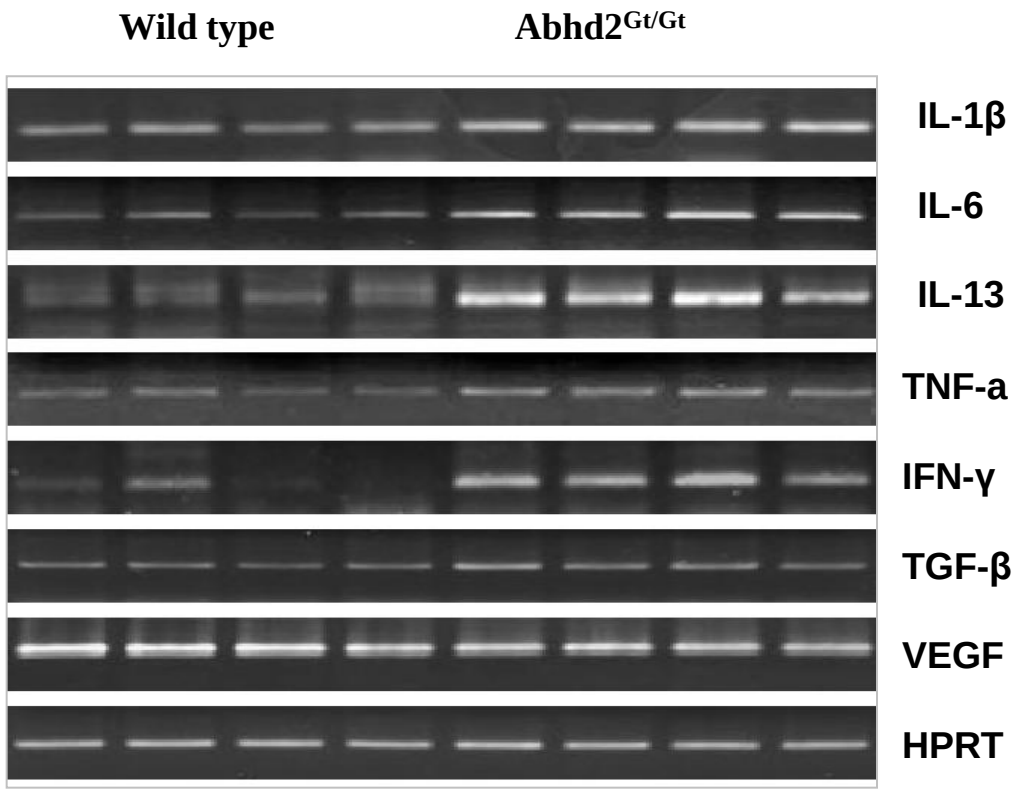


Figure. Change of protease and anti-protease Factor

Enhanced expression of inflammatory and apoptosis-related factors



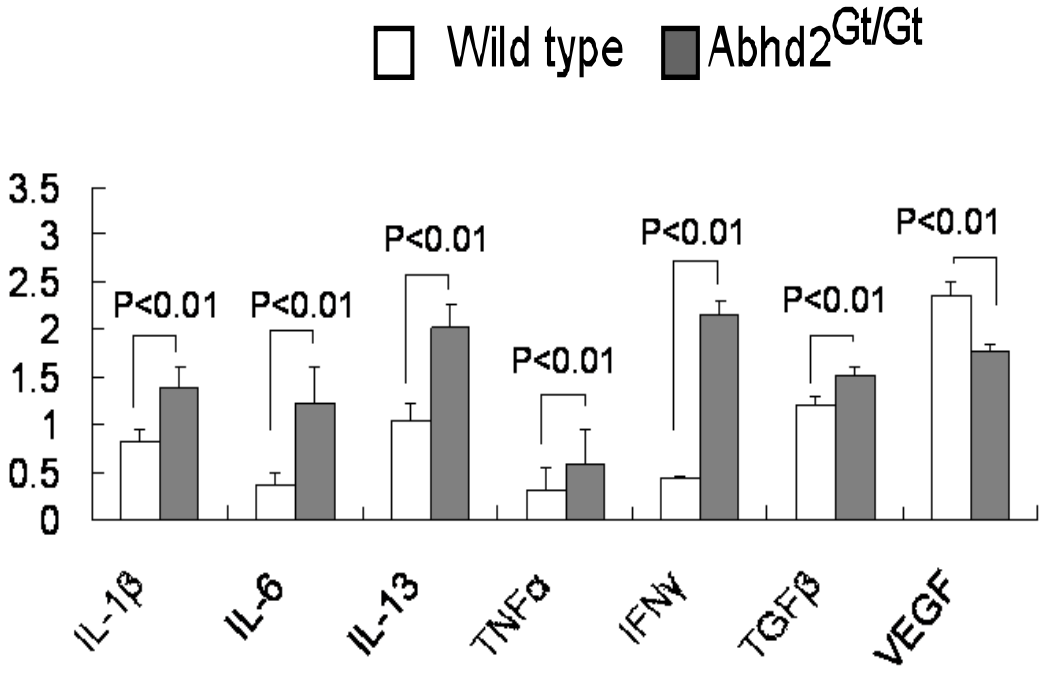
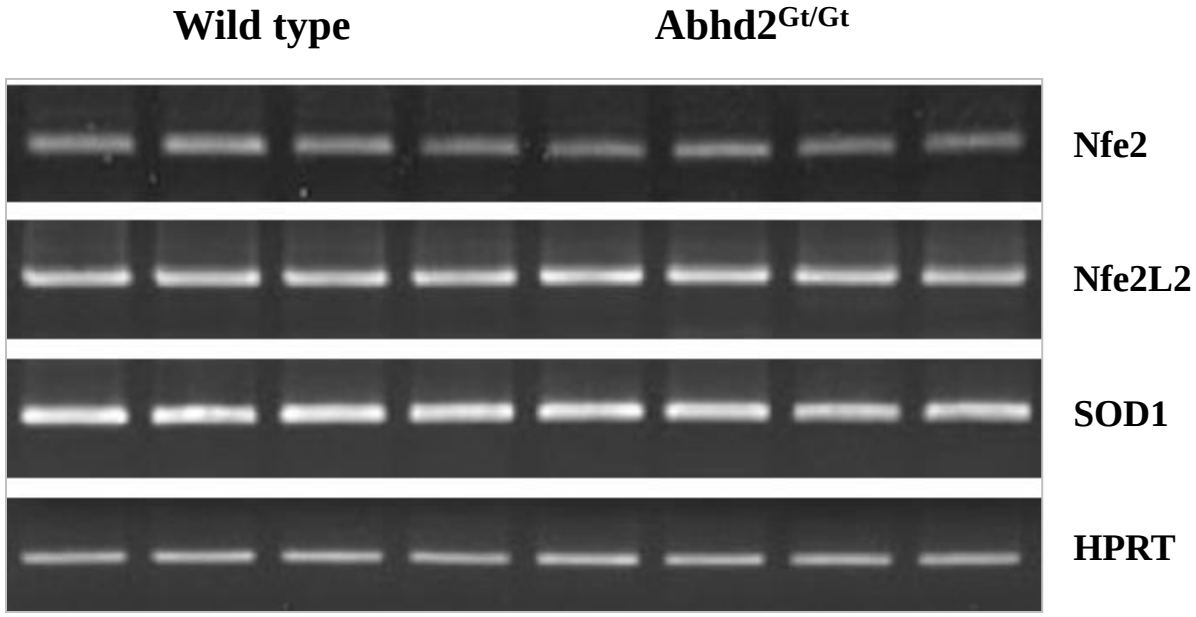


Figure. The expression of proinflammatory cytokines and apoptosis related genes at the mRNA level

Oxidation and antioxidant factors indifference



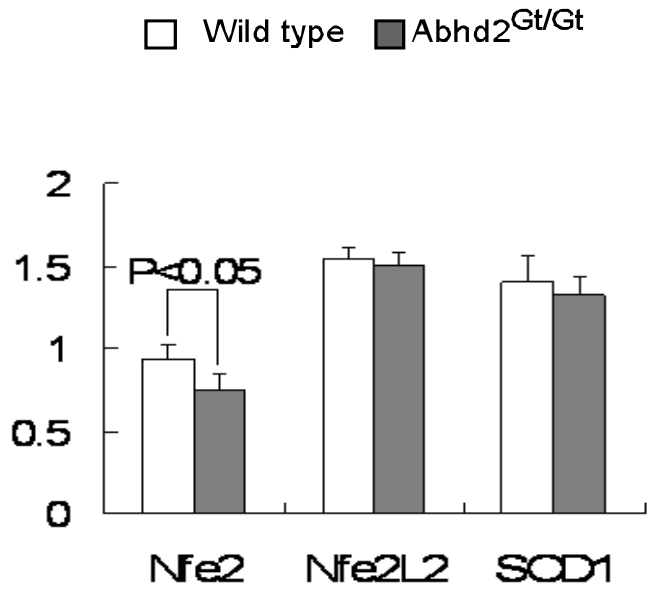


Figure. The expression levels of oxidation and antioxidant factors

- **Mouse Abhd2 playes an important role in emphysema**

- **But the function of hABHD2 was unclear**

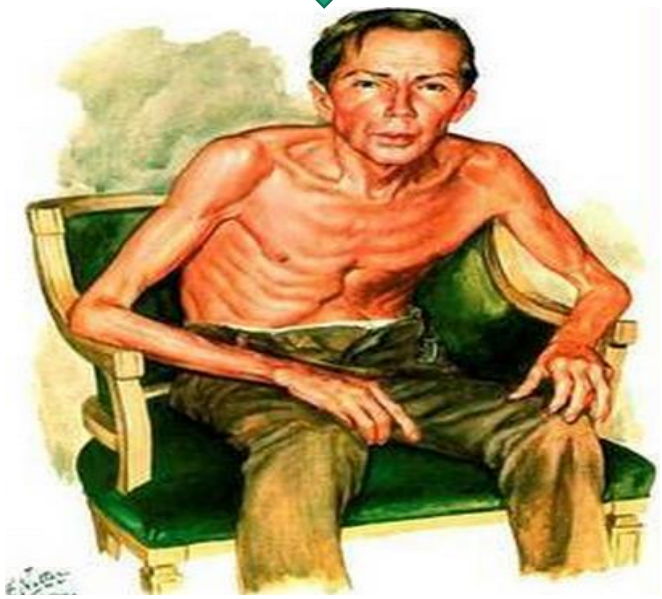
- **The exact mechanism was unclear**

◆ Clinical manifestations of COPD：

- lung: airway remodeling, emphysema,
- vascular remodeling: smooth muscle cell proliferation, migration
- lung outside: peripheral muscle atrophy and dysfunction and other symptoms

◆ Abhd2 features：

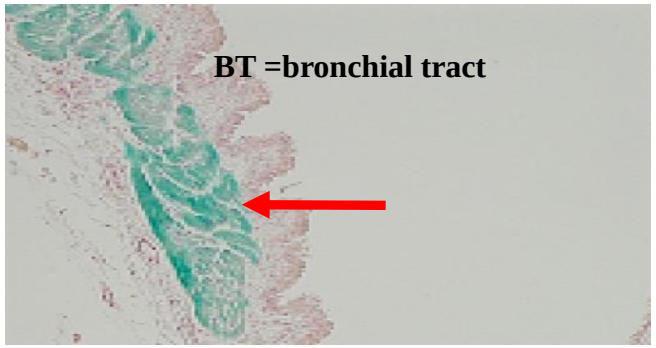
- Abhd2 deficient mice spontaneously emphysema
- Abhd2 expressed in airway smooth muscle and vascular smooth muscle cells.
- Abhd2 deletion induced vascular smooth muscle cell migration and intimal hyperplasia

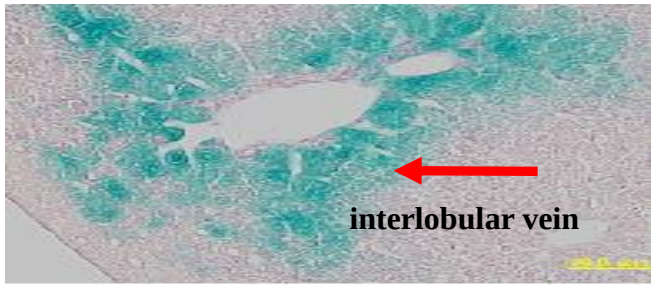


We speculate:

ABHD2 associated with

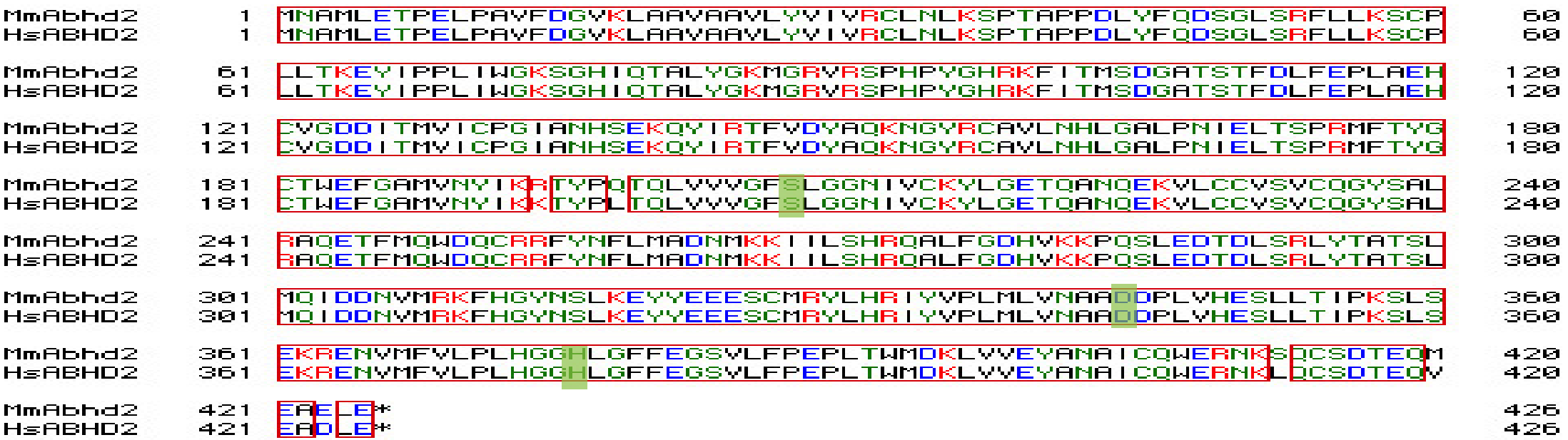
COPD





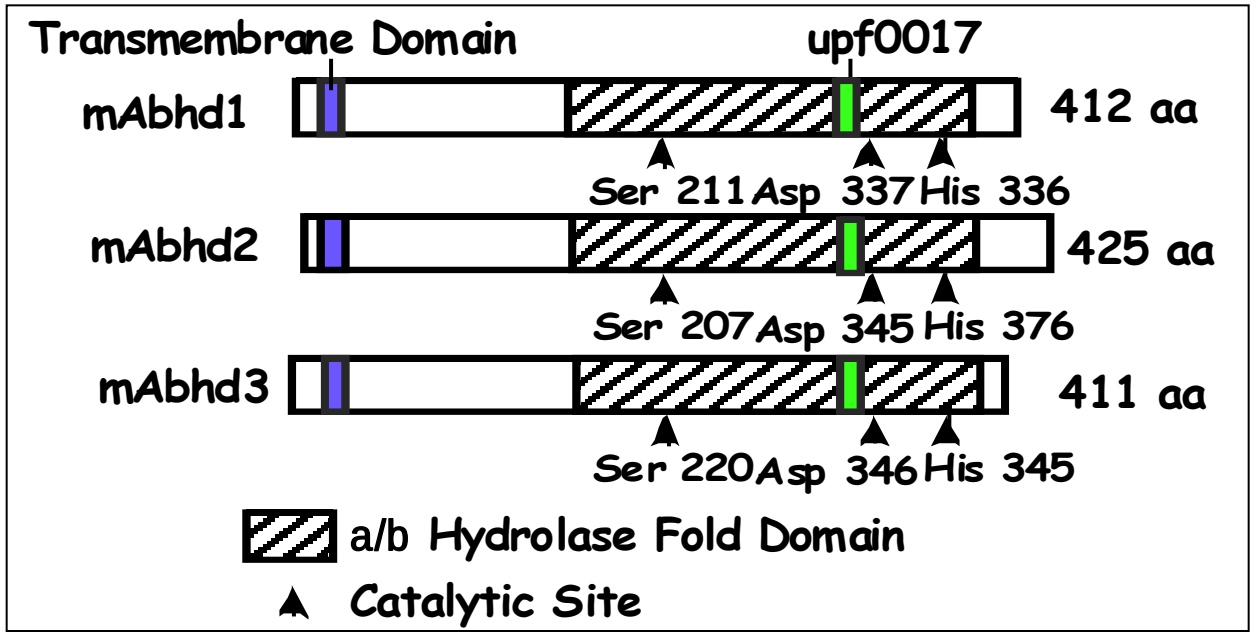
mAbhd2 and hABHD2 Homology

Protein Similarity 98.59%/425aa (from NCBI)



Abhd family and Abhd2 features:

1. **Origin:** Edgar and colleagues cloned α/β hydrolase family encoding a protein from emphysema model mouse lung cDNA library in 2002 : α/β hydrolase gene 1 (Abhd1), Abhd2 and Abhd3
2. **Structural domains:** Abhd1, Abhd2 and Abhd3 may play an important role in gene function with the same structural domain

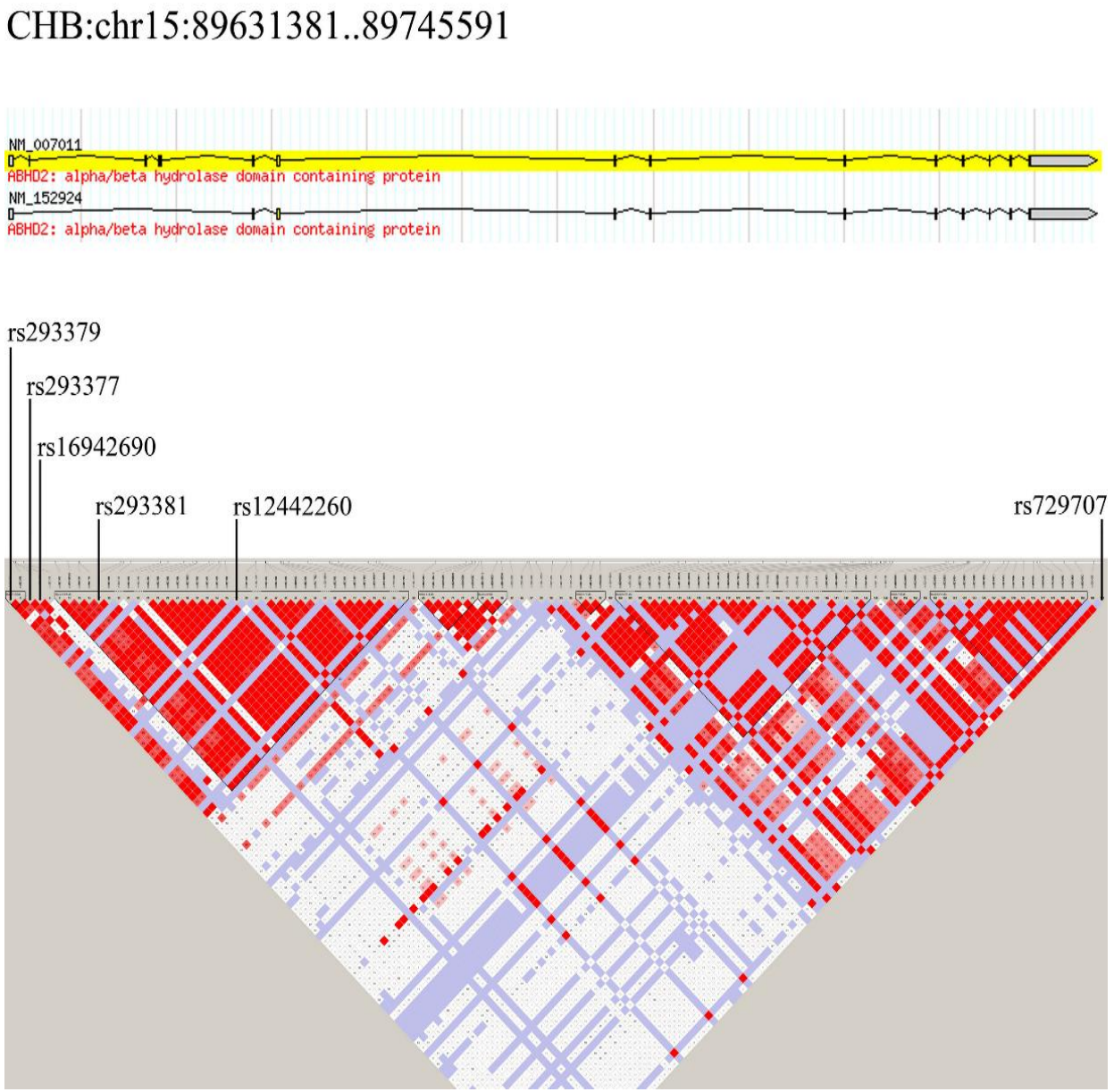


Alasdair J. Edgar and Julia M. Polak. Cloning and Tissue Distribution of Three Murine α/β Hydrolase Fold Protein cDNAs. *Biochemical and Biophysical Research Communications 292*, 617–625 (2002)

Study:

Associations of ABHD2 Genetic variations with Risks for Chronic Obstructive Pulmonary Disease in a Chinese Han Population

1. Extraction and comparative analysis genomic DNA of COPD patients and normal population
2. Screening gene SNP point of ABHD2 : from shared domain of Abhd2

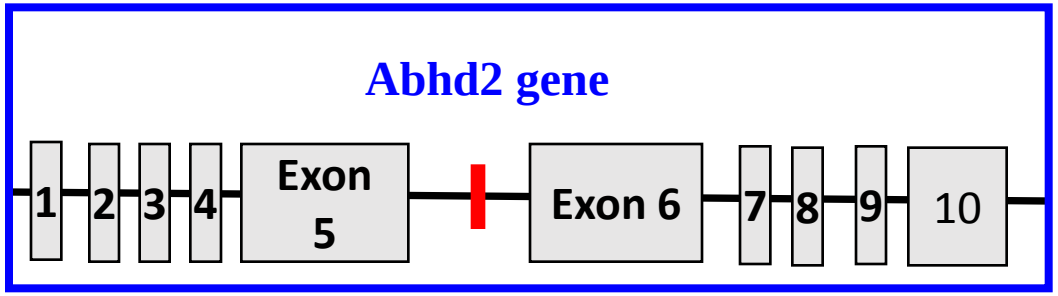


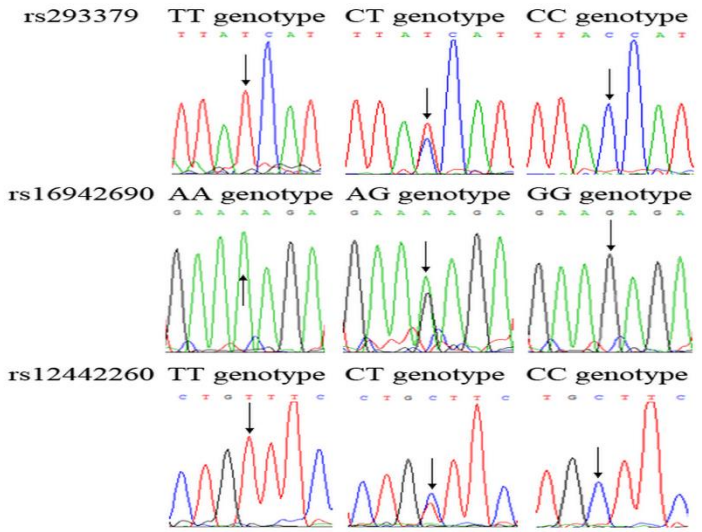
The study found: Rs12442260 mutants (CT / CC), upstream of the translation initiation site in the fifth ABHD2 gene intron from the sixth exon upstream 489bp, increased the risk of COPD

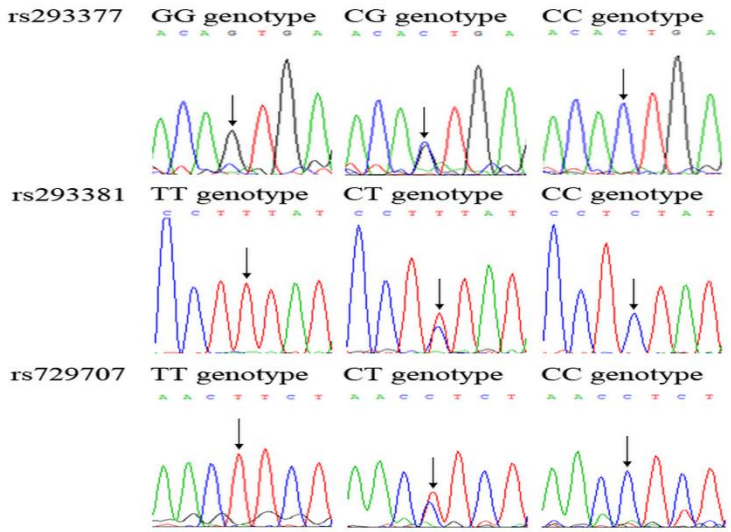
Conclusion: **ABHD2 gene polymorphism was associated with COPD risk**

Supplementary Table. Markers genotyped in the current study.

NCBI rs number	Position	Gene redion	Genotype	F-PCR primer	R-PCR primer
rs293379	89633940	Intron	C/T	TGCCTATTTGTCAGACCCAC	TCCCTTGTACTTGCCATC
rs293377	89634414	Intron	G/C	TTCAGCCCTCCTCCCAAGC	ACCGAAATTCAGAATCAACTCA
rs16942690	89634442	Intron	A/G	TTCAGCCCTCCTCCCAAGC	ACCGAAATTCAGAATCAACTCA
rs293381	89644938	Intron	C/T	GCCCAATGTAATAATCTG	AAGCATTTACTTGGCTAC
rs12442260	89656467	Intron	T/C	ATGGTGATTAAGAGGAGGAT	TCCAGAAATGCCTAACAG
rs729707	89743043	Exon	A/G	TTCCTCAAGTGGCCTGTA	GAAAGCTCTACCCACATACA







- **ABHD2 may be susceptibility gene of COPD**
- **But it is not clear that whether the specific site mutations affect gene function**

- **Outlook**
  - **Analysis and screen specific mutations sites of ABHD2 in COPD patients**
  - **Construct ABHD2 specific mutations knock humanized mouse by use of genetic manipulation techniques**
  - **Determine causality of people ABHD2 specific locus mutation occurs with COPD**
  - **Determine COPD susceptible early screening biomarkers**

- **ACOS is the main reason for the late COPD patients died. COPD patients often appear ACOS phenotype with age and progression of the disease in COPD population over the age of 55 may be as high as 55%.**
- **Therefore, to effectively prevent the development of ACOS and treatment is essential for COPD**

- **Abhd2 gene express in bronchial smooth muscle cells and vascular smooth muscle cells**
- **The study found: Abhd2 gene deletions may promote vascular smooth muscle cells Remodeling**
- **We hypothesized that: Abhd2 genes may be involved in airway smooth muscle remodeling ACOS**

Our previous study:

Abhd2 knockout mice was sensitized by ovalbumin to airway mucus hypersecretion and eosinophil Granulocyte infiltration, increased smooth muscle layer thickening, increased α- myosin expression and other pathological features of asthma

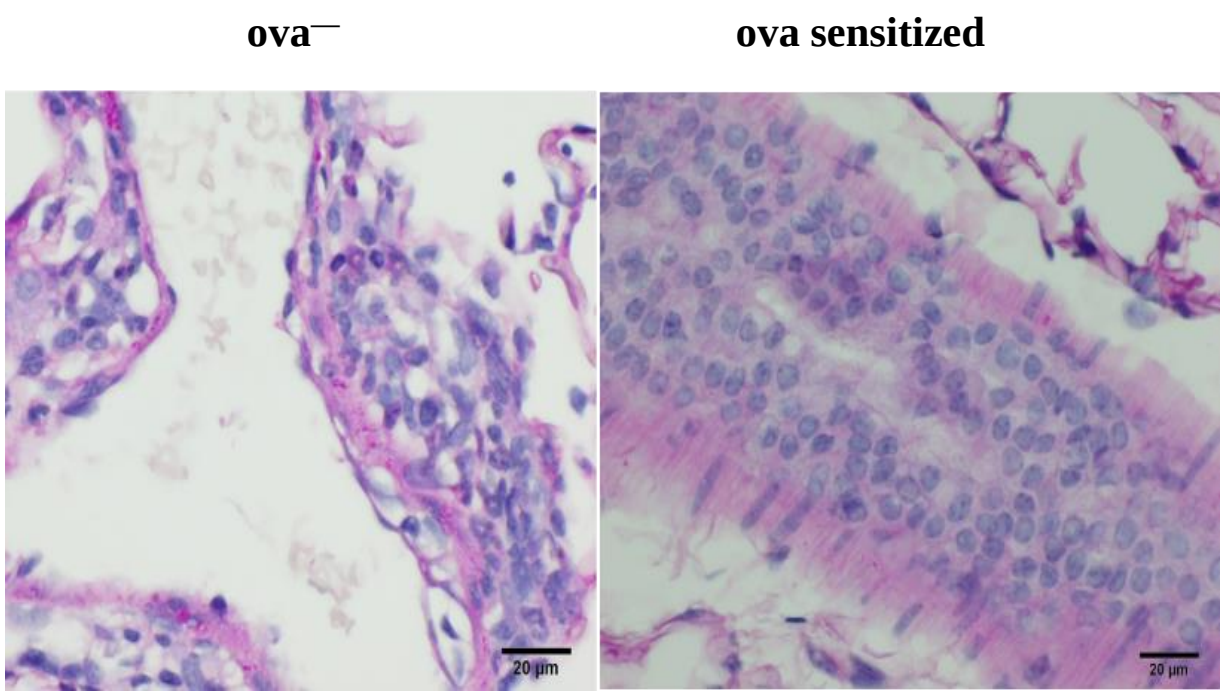


Figure . Observate inflammatory cell infiltration of Abhd2 knockout mice before and after lung ovalbumin sensitized

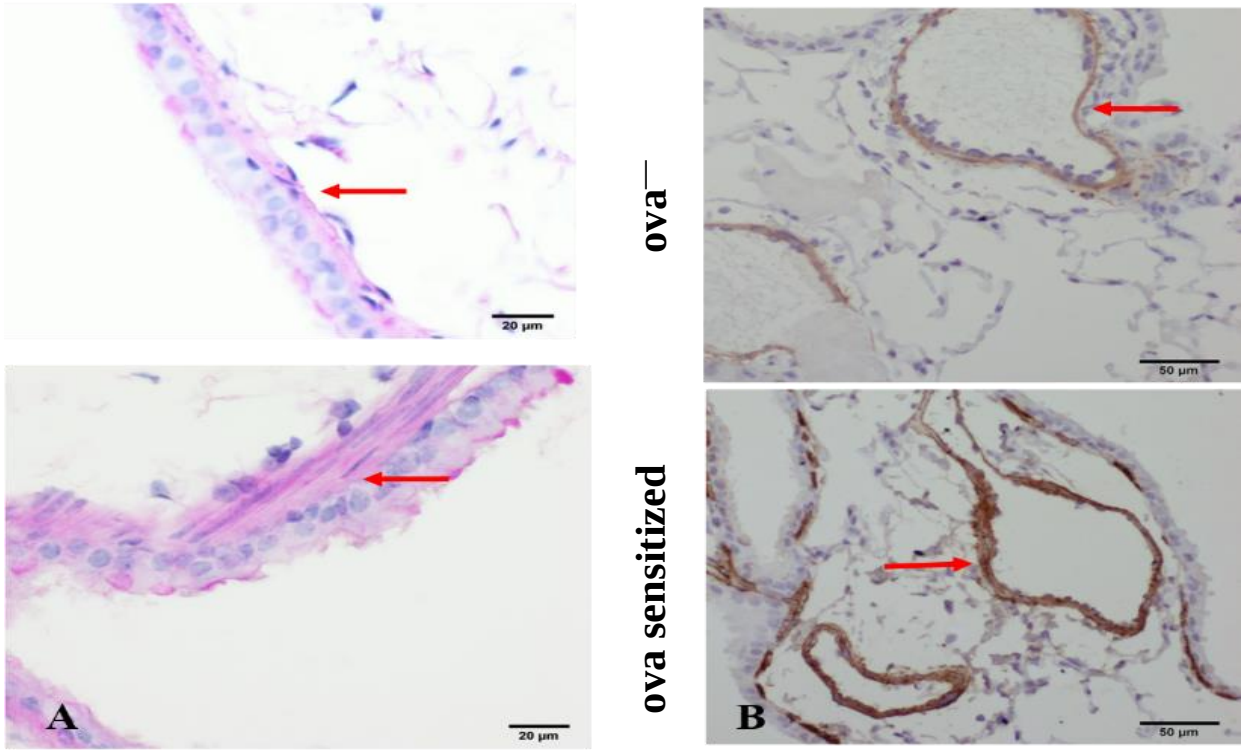


Figure . Observate smooth muscle hyperplasia in Abhd2 knockout mice before and after the degree of airway by ovalbumin sensitized

- **We have constructed ACOS multivariate dangerous animal model**
- **We will further verify: Abhd2 may be involved in airway smooth muscle cells remodeling, and the possible relationship between the occurrence of ACOS**

Outlook:

1. **Explore the possible relationship between Abhd2 gene polymorphism and asthma - COPD overlap syndrome-associated risk**
2. **Further to constructe humanized animal models of ABHD2 and to verify**

Thank You ！