

**A functional SNP upstream of the *ADRB2* gene is associated with COPD**

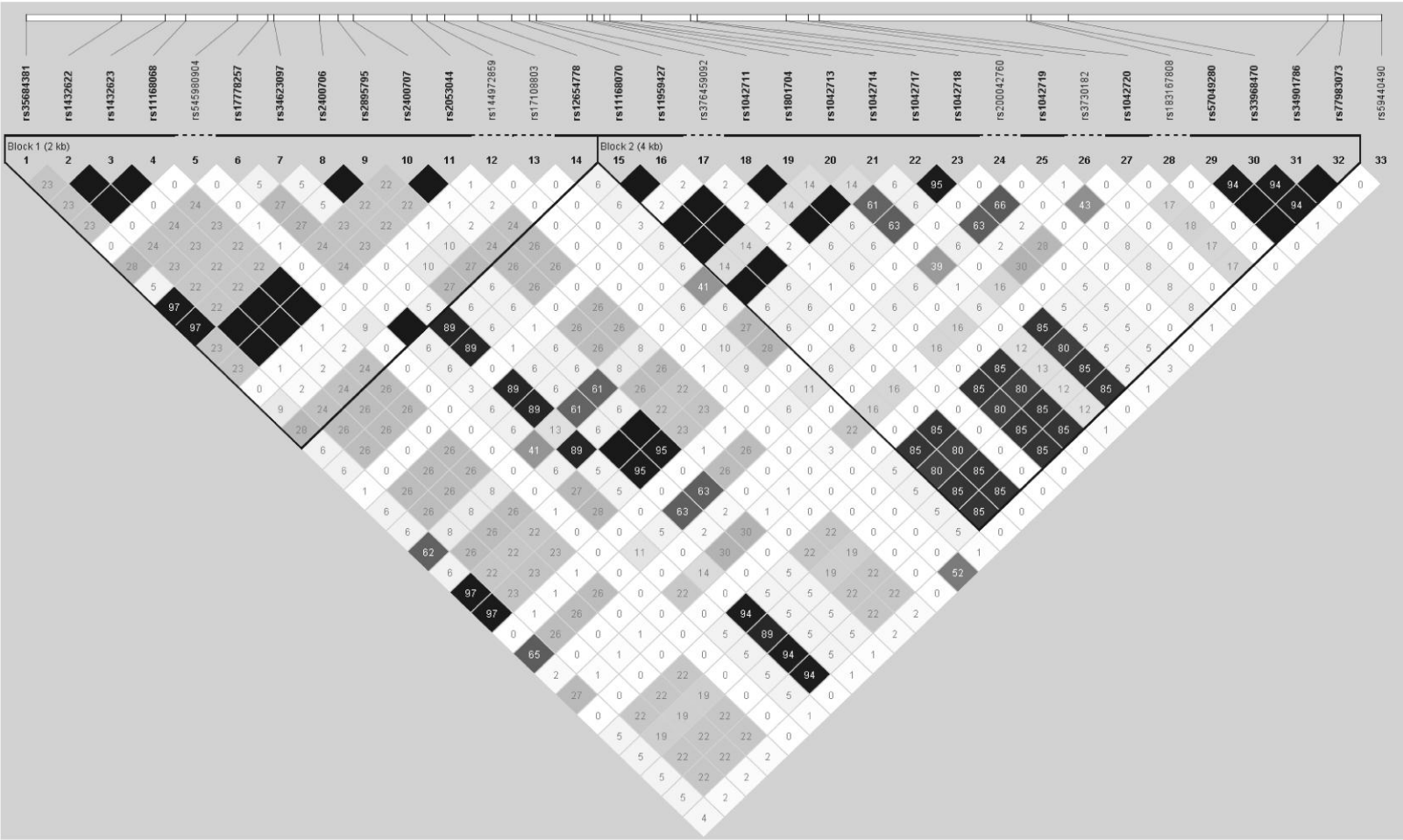
Jin-Xiu Li,<sup>1,2,\*</sup> Wei-Ping Fu,<sup>3,\*</sup> Jing Zhang,<sup>4</sup> Xiao-Hua Zhang,<sup>1,2</sup> Chang Sun,<sup>1,5</sup> Lu-Ming Dai,<sup>3</sup> Li Zhong,<sup>1,5,6</sup> Li Yu,<sup>1,2</sup> Ya-Ping Zhang,<sup>1,7</sup>

<sup>1</sup>State Key Laboratory for Conservation and Utilization of Bio-resource in Yunnan, Yunnan University, Kunming, China; <sup>2</sup>Key Laboratory for Animal Genetic Diversity and Evolution of High Education in Yunnan Province, School of Life Sciences, Yunnan University, Kunming, China; <sup>3</sup>Department of Respiratory Critical Care Medicine, the First Affiliated Hospital of Kunming Medical University, Kunming, China; <sup>4</sup>Department of thoracic surgery, the First Affiliated Hospital of Kunming Medical University, Kunming, China; <sup>5</sup>College of Life Sciences, Shaanxi Normal University, Xi'an, China; <sup>6</sup>Provincial Demonstration Center for Experimental Biology Education, Shaanxi Normal University, Xi'an, China; <sup>7</sup>State Key Laboratory of Genetic Resources and Evolution, and Yunnan Laboratory of Molecular Biology of Domestic Animals, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, China.

Correspondence: Ya-Ping Zhang, State Key Laboratory of Genetic Resources and Evolution, and Yunnan Laboratory of Molecular Biology of Domestic Animals, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming 650091, China. E-mail: [zhangyp@mail.kiz.ac.cn](mailto:zhangyp@mail.kiz.ac.cn), Tel: 0871-65130513, Li Zhong, State Key Laboratory for Conservation and Utilization of Bio-resource in Yunnan, Yunnan University, Kunming 650091, China. E-mail: [lizhong@snnu.edu.cn](mailto:lizhong@snnu.edu.cn), Tel: 029-85310469.

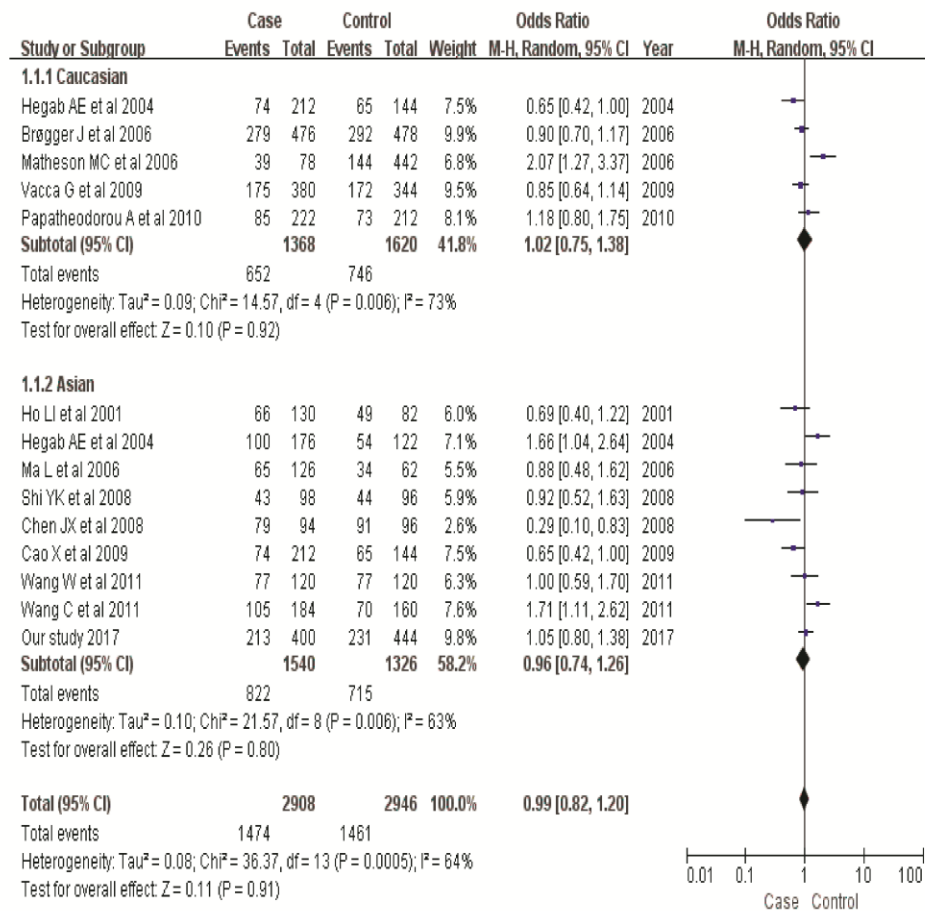
\*These authors contributed equally to this work.

SUPPLEMENTAL FIGURES

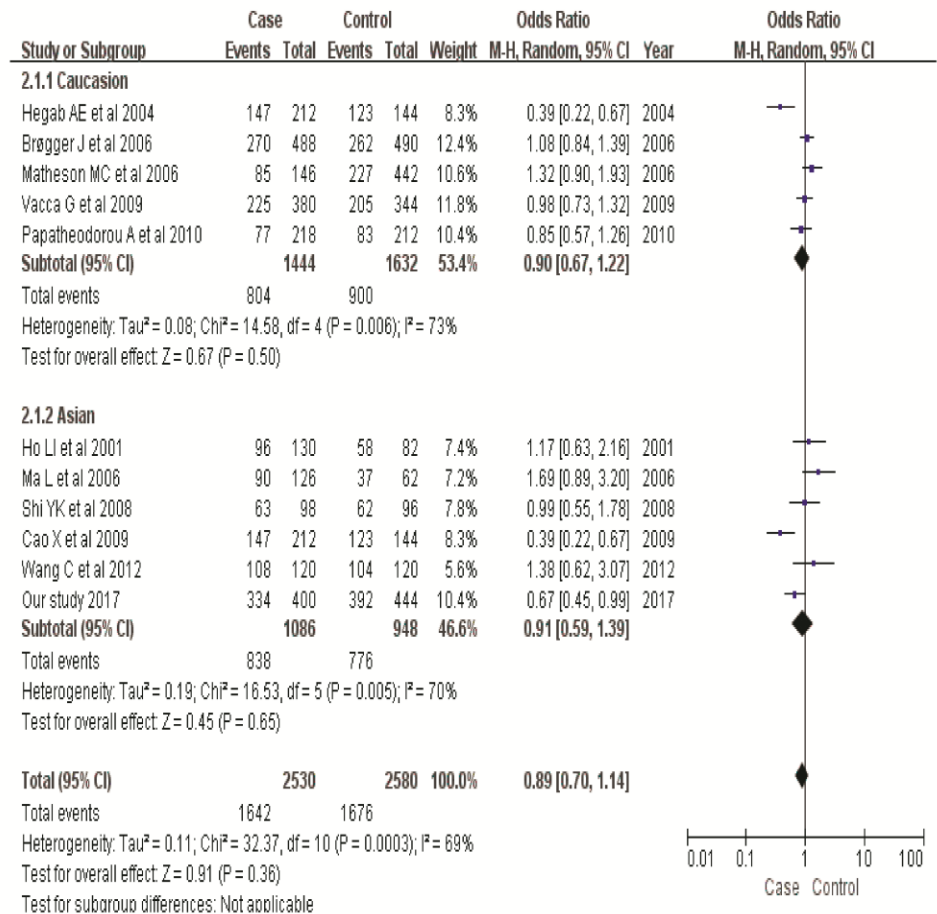


Supplementary Figure 1 The visual genotype of *ADRB2* in East Asian population from 1000 genomes project.

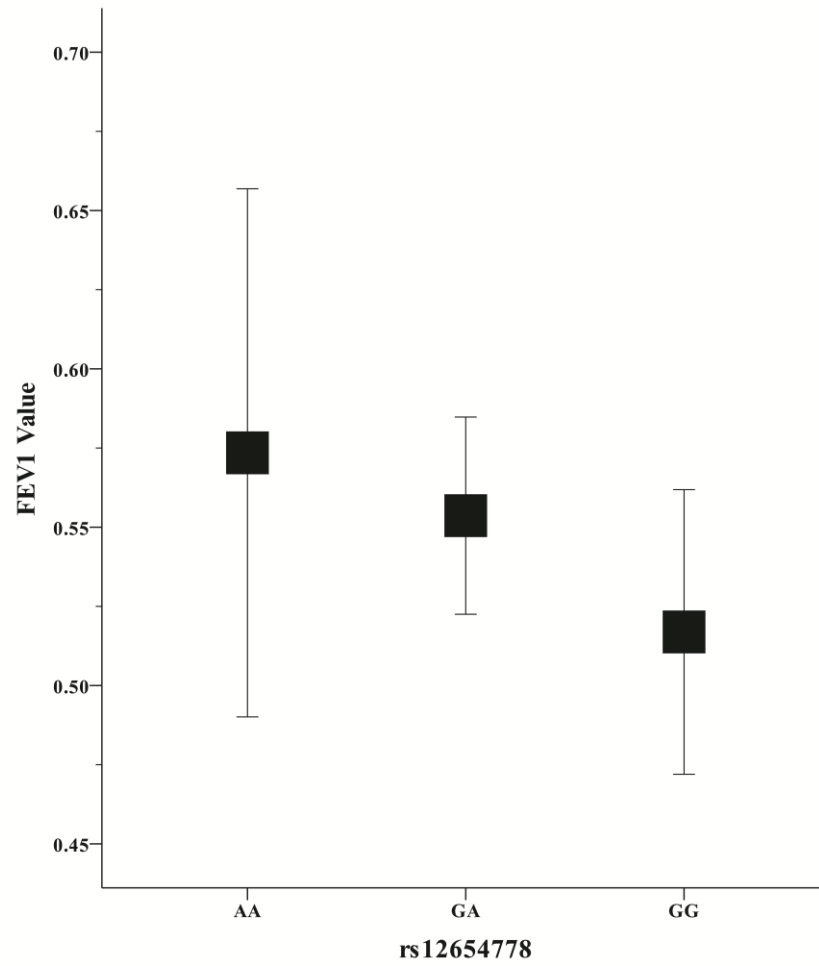
### 1 rs1042713-A forest plot under allele model



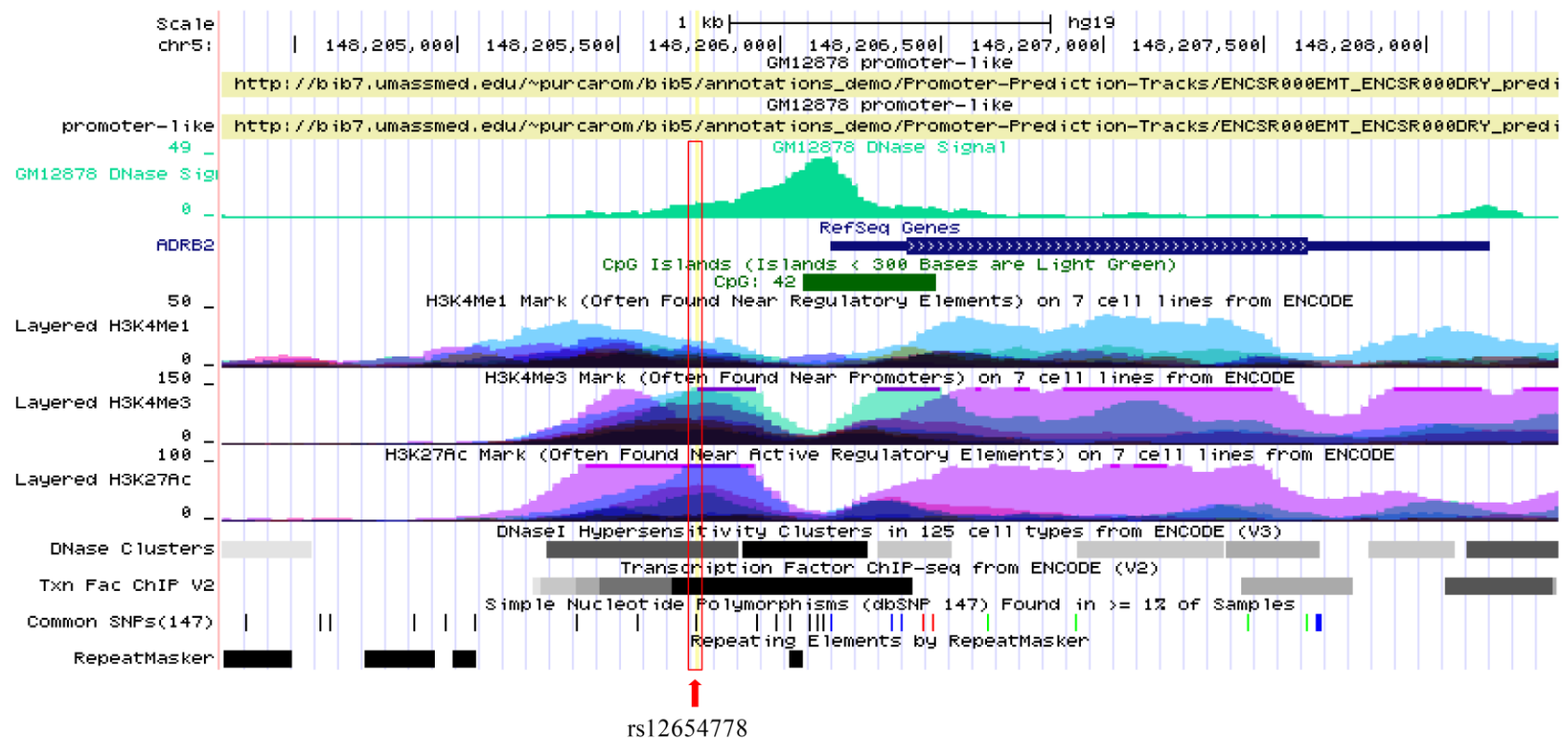
### 2 rs1042714-C forest plot under allele model



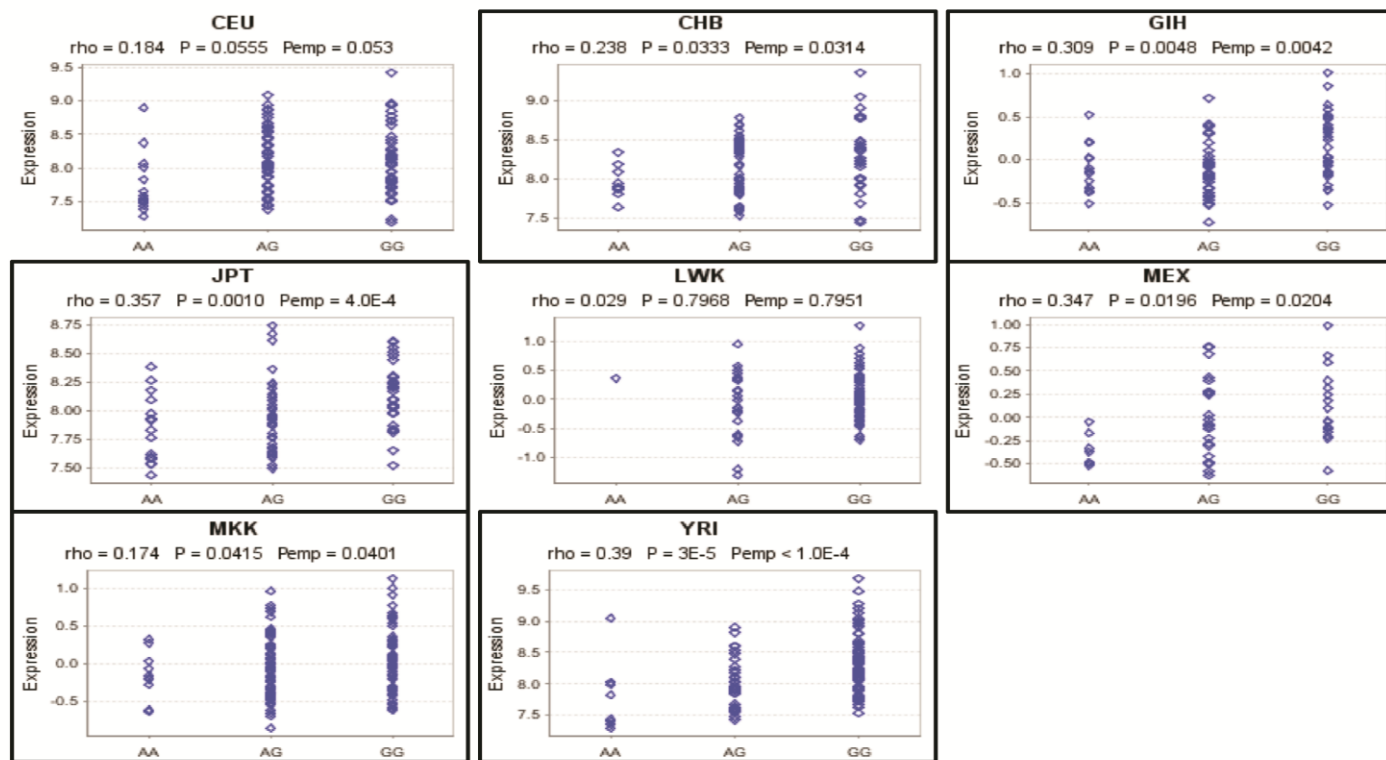
**Supplementary Figure 2** The meta-analysis of rs1041713-A and rs1042714-C with COPD in allele frequency.



**Supplementary Figure 3** Lung function level (FEV<sub>1</sub> value) of COPD patients with different genotype of rs12654778. The data was displayed by means  $\pm$  SD.



**Supplementary Figure 4** Regulatory motifs within the rs12654778 locus. The rs12654778 locus is shown in yellow vertical bar with red box, annotated with Refseq genes, histone marks (H3K4Me1, H3K4Me3, H3K27Ac), DNase hypersensitivity, transcription factor binding and CpG islands (UCSC Genome Browser (<http://genome.ucsc.edu/>)) on the Human Feb 2009 (GRCh37/hg19) assembly.



**Supplementary Figure 5** The association between rs12654778 and *ADRB2* expression in LCL in HapMap3 populations. Black frame represented rs12654778 in LCL in HapMap3 populations associated with a significant *ADRB2* gene expression ( $P < 0.05$ ).

## SUPPLEMENTAL TABLES

**Supplementary Table 1** The primers used in SNaPshot genotyping

SNP ID	PCR primers(5'–3')	Single base extension primer (5'–3')
rs17108803	GAGTACCCAGATGGAGACATCC AGAAACAAACACAGAAGCACC	CCTCCAAGCCAGCGTGTGTTTACTT
rs12654778	TGGGAGGGTGTGTCTCAG AGGCACGCACATACAGGC	TGACTTATGGCTGTGGTTCGGTATAAGTCT
rs1042713	TCACCTGCCAGACTGCGC ACACCTCGTCCCTTTTCCT	ACTGACTGACTGACTCAGCGCCTTCTTGCTGGCACCCCAAT
rs1432623	AGAAGACTAACAACATATTCTAAACCACTA ATAGTAAGAAATATGAAAATGCTTTTGC	GACTGACTGACTGACTGACTTTTATGTAAACTTCGCTTACAAACTA
rs1042720	ATAACATTGATTCACAAGGGAGG TTTAGTGTTCTGTTGGGGGG	CTGACTGACTGACTGACTGACTGACTGACTATTGTAGTACAAATGACTCACTGCT
rs1042714	TTCTTGCTGGCACCCAAT AGACATGACGATGCCCAT	ACTGACTGACTGACTGACTGACTGACTGACTGACTTGCGCCGGACCACGACGTCACGCAG
rs1042717	TCACCAACTACTTCATCACTTCAC AAGTTGCCAAAAGTCCACATT	GACTGACTGACTGACTGACTGACTGACTGACTGACTGACTCCTGTGCTGATCTGGTCATGGGCCT
rs1042719	TTCCAGGAGCTTCTGTGC ATATCCACTCTGCTCCCCTG	TGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGCAGGTCTTCTTTGAAGGCCTATGG

**Supplementary Table 2** The LD blokes of 32 SNPs in *ADRB2* gene and Tag-SNPs selection

Bin	Total number of sites	Average Minor Allele Frequency	SNPs	Location in <i>ADRB2</i> gene region <sup>a</sup>	Tag-SNP
1	5	31%	rs1432622	5' intergenic	rs1432623
			rs1432623	5' intergenic	
			rs11168068	5' intergenic	
			rs2400707	5' UTR	
			rs2053044	5' UTR	
2	1	5%	rs17108803	5' UTR	rs17108803
3	2	36%	rs17778257	5' UTR	rs12654778
			rs12654778	5' UTR	
4	1	44%	rs1042713	missense	rs1042713
5	10	10%	rs34623097	5' UTR	rs1042714
			rs11168070	5' intergenic	
			rs11959427	5' intergenic	
			rs1042711	5' UTR	
			rs1801704	5' UTR	
			rs1042714	missense	
			rs57049280	3' intergenic	
			rs33968470	3' intergenic	
			rs34901786	3' intergenic	
			rs77983073	3' intergenic	
			rs35684381	5' intergenic	
			rs2400706	5' UTR	
			rs2895795	5' UTR	
6	5	33%	rs1042717	synonymous	rs1042717
			rs1042718	synonymous	
			rs1042719	synonymous	
			rs1042720	synonymous	
			rs59440490	3' intergenic	
7	1	43%	rs1042719	synonymous	rs1042719
8	1	38%	rs1042720	synonymous	rs1042720
9	1	4%	rs59440490	3' intergenic	
10	1	2%	rs182759770	5' UTR	
11	1	1%	rs3730182	synonymous	
12	1	1%	rs144972859	5' UTR	
13	1	1%	rs200042760	missense	
14	1	1%	rs183167808	3' intergenic	

**Note:** <sup>a</sup>: Location in *ADRB2* gene region as described in SNP database.



**Supplementary Table 3** Main characteristics of the studies included in the meta-analysis

First author(Ref.)	Year	Ethnicity/country	rs1042713-A				rs1042714-C				Source of controls	Smoking status	HWE	Genotyping methods
			Case		Control		Case		Control					
			Allele	Total	Allele	Total	Allele	Total	Allele	Total				
Hegab AE <sup>1</sup>	2004	European/Egypt	74	212	65	144	147	212	123	144	Healthy smokers	Smokers	Yes	TaqMan allelic discrimination
Brogger J <sup>2</sup>	2006	European/Norway	197	476	189	478	270	488	262	490	Healthy smokers	Smokers	Yes	TaqMan PCR
Matheson MC <sup>3</sup>	2006	European/Australia	39	78	144	442	85	146	227	442	General population	Mixed	Yes	ARMS-PCR
Vacca G <sup>4</sup>	2009	European/Germany	175	380	172	344	225	380	205	344	Healthy volunteers	Smokers	Yes	Allele-specific PCR
Papatheodorou A <sup>5</sup>	2010	European/Greece	85	222	73	212	77	218	83	212	Healthy smokers	Smokers	Yes	Nanogen NanoChip®400
Ho LI <sup>6</sup>	2001	Asian/China	66	130	49	82	96	130	58	82	Healthy population	Not mentioned	Yes	Allele-specific PCR
Hegab AE <sup>1</sup>	2004	Asian/Japan	100	176	54	122	—	—	--	--	Healthy smokers	Smokers	Yes	TaqMan allelic discrimination
Ma L <sup>7</sup>	2006	Asian/China	65	126	34	62	90	126	37	62	Healthy population	Not mentioned	No	Allele-specific PCR
Shi YK <sup>8</sup>	2008	Asian/China	43	98	44	96	63	98	62	96	Healthy smokers	Smokers	Yes	PCR direct sequencing
Chen JX <sup>9</sup>	2008	Asian/China	79	94	91	96	--	--	--	--	Healthy population	Not mentioned	No	PCR direct sequencing
Cao X <sup>10</sup>	2009	Asian/China	74	212	65	144	147	212	123	144	Healthy smokers	Smokers	Yes	PCR-RFLP
Wang C <sup>11</sup>	2011	Asian/China	77	120	77	120	--	--	--	--	Healthy smokers	Smokers	Yes	Allele-specific PCR
Wang W <sup>12</sup>	2011	Asian/China	105	184	70	160	--	--	--	--	Healthy smokers	Smokers	Yes	Allele-specific PCR
Wang C <sup>13</sup>	2012	Asian/China	--	--	--	--	108	120	104	120	Healthy smokers	Smokers	Yes	Allele-specific PCR
Our study	2017	Asian/China	213	400	231	444	334	400	392	444	Healthy smokers	Smokers	Yes	SNaPshot

**Abbreviations:** COPD: chronic obstructive pulmonary disease; HWE: Hardy-Weinberg equilibrium; PCR: polymerase chain reaction; RFLP: restriction fragment length polymorphism.

## References

1. Hegab AE, Sakamoto T, Saitoh W, et al. Polymorphisms of IL4, IL13, and ADRB2 genes in COPD. *Chest*. 2004;126(6):1832-1839.
2. Brogger J, Steen VM, Eiken HG, et al. Genetic association between COPD and polymorphisms in TNF, ADRB2 and EPHX1. *Eur Respir J*. 2006;27(4):682-688.
3. Matheson MC, Ellis JA, Raven J, et al. Beta2-adrenergic receptor polymorphisms are associated with asthma and COPD in adults. *J Hum Genet*. 2006;51(11):943-951.
4. Vacca G, Schwabe K, Duck R, et al. Polymorphisms of the beta2 adrenoreceptor gene in chronic obstructive pulmonary disease. *Ther Adv Respir Dis*. 2009;3(1):3-10.
5. Papatheodorou A, Makrythanasis P, Kaliakatsos M, et al. Development of novel microarray methodology for the study of mutations in the SERPINA1 and ADRB2 genes--their association with Obstructive Pulmonary Disease and Disseminated Bronchiectasis in Greek patients. *Clin Biochem*. 2010;43(1-2):43-50.
6. Ho LI, Harn HJ, Chen CJ, et al. Polymorphism of the beta(2)-adrenoceptor in COPD in Chinese subjects. *Chest*. 2001;120(5):1493-1499.
7. Ma L, Feng DX, Zhang XY, et al. Association between the genetic polymorphisms of  $\beta$ 2-adrenergic receptor and the chronic obstructive pulmonary disease. *Chinese J Pract Intern Med*. 2006;26(4):267-269.
8. Shi YK, Ma J, Yuan ZJ, et al. Investigation on the relation between polymorphisms of  $\beta$ 2 adrenergic receptor and the chronic obstructive pulmonary disease. *Shandong Med*. 2008;48(13):9-11.
9. Chen JX, Shi YK, Li SL, et al. The Study of  $\beta$ 2-AR Polymorphisms and Chronic Obstructive Pulmonary Disease on Relations. *Acta Acad Med Weifang*. 2008;30(1):63-65.
10. Cao X, Li QQ, Chen GZ, et al. Polymorphism of IL-4, IL-13, and ADR Beta 2 Genes in Patients with Chronic Obstructive Pulmonary Disease. *Med J Wuhan Univ*. 2009;30(2):219-223.
11. Wang C, Tan F, Yang AL, et al. Association between the Arg16Gly  $\beta$ 2-adrenoceptor polymorphisms and the older-aged chronic obstructive pulmonary disease with hypertension(in Chinese). *J Pract Med*. 2011;28(7):4442-4444.
12. Wang W, Yu YJ, Qian R, et al. Association between the susceptibility of COPD and IL-13, IL-4, polymorphisms of beta2-adrenergic receptor. *J Clin Intern Med*. 2011;28(5):332-334.
13. Wang C, Yang AL, Li H, et al. Association between the Gln27Glu  $\beta$ 2-adrenoceptor

polymorphisms and the older-aged chronic obstructive pulmonary disease with hypertension(in Chinese). *J Pract Med*. 2012;28(7):1100-1103.