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ORIGINAL RESEARCH

Genetic Variants in the Adhesive G Protein-Coupled Receptor ADGRG6 are Associated with Increased Susceptibility to COPD in the Elderly Han Chinese Population of Southern China

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Background: Mutations in ADGRG6 are associated with a variety of cancers and multiple types of diseases. However, the impact of genetic variations in ADGRG6 on chronic obstructive pulmonary disease (COPD) susceptibility has not yet been evaluated.

Methods: Considering the high prevalence of COPD among the elderly population in China, this study specifically targets the elderly Han population in Southern China as the study subject. Following the acquisition of participants' whole-genome DNA, genotyping was conducted using the Agena MassARRAY platform. The online tool 'SNPStats', which utilizes logistic regression, was employed to analyze and assess the correlation. Multi-factor dimensionality reduction was utilized to clarify the impact of "SNP-SNP" interactions on COPD risk. The False-Positive Report Probability (FPRP) was applied to determine whether significant results are noteworthy findings.

Results: The mutant allele "C" of rs11155242 was a protective genetic factor against COPD susceptibility (OR = 0.57, 95% CI = 0.36 to 0.91, p = 0.017). The heterozygous mutant genotype "CA" of rs11155242 was found to be significantly associated with reduced COPD risk (CA Vs AA: OR = 0.53, 95% CI = 0.32 to 0.90, p = 0.018). ADGRG6-rs11155242 was found to be strongly associated with a reduced risk of COPD in males, non-smokers, and subjects with a BMI below 24 kg/m^2 (OR < 1, p < 0.05). The FPRP analysis indicated that the positive results identified in this study are noteworthy new findings.

Conclusion: The mutant allele "C" and mutant genotype "CA" of rs11155242 act as protective genetic factors against COPD susceptibility. This study will provide a new research direction for the personalized prevention and treatment of COPD in the elderly Han population in southern China, and lay a potential scientific basis.

Keywords: COPD, ADGRG6, genetic variants, mutant allele

Introduction

Chronic Obstructive Pulmonary Disease (COPD) represents a diverse group of pulmonary disorders marked by ongoing breathing difficulties such as shortness of breath, coughing, and sputum production. These symptoms stem from lasting impairments in the bronchial tubes (bronchitis, bronchiolitis), air sacs (emphysema), and/or blood vessels of the lungs. A definitive diagnosis is made through spirometry, which measures airflow obstruction, and/or by other objective

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measures indicating lung structural or functional impairment.[1,2] As a major public health concern, COPD has emerged as the most common chronic respiratory disease worldwide.[3,4] In China, it represents a substantial threat to the health and well-being of residents. From 2012 to 2015, the overall prevalence of COPD in adults over 20 was 8.6%. Notably, the prevalence was only 2.1% among adults aged 20 to 39, but it rose sharply to 13.7% in those aged 40 and above.[5,6] These figures highlight a particularly high prevalence among the middle-aged and elderly populations in China, a trend closely linked to aging and prolonged exposure to risk factors. Despite advancements in medical care and increased public health awareness in recent years, the prevention and control of COPD remain critically important.

The development of COPD involves a complex interplay of multiple factors, including genetic contributions and environmental influences. Both environmental and genetic factors synergistically influence the pathogenesis and progression of COPD. Smoking and air pollution are recognized as primary risk factors for COPD,[7–9] while occupational and solid biofuel exposures are also acknowledged contributors to its onset and progression.[10,11] With industrialization and rapid economic growth, air pollution has become a serious problem in China. There are significant differences in air quality between the northern and southern regions of China, which leads to substantial differences in the prevalence and risk of COPD in the different areas of China.[12,13] In addition to the above environmental factors, the familial clustering of COPD highlights the importance of genetic determinants in its development.[14] Single nucleotide polymorphisms (SNPs), which are DNA sequence variations caused by changes in a single nucleotide, represent a common form of genetic variation. Beyond the well-established genetic risk factor of alpha-1 antitrypsin deficiency, polymorphisms in several other genes, including HIF1A, CDKN1A, IREB2, ADAM33, ERBB2, ATG16L1, and BAG3, have been implicated in COPD pathogenesis. These genetic variations can influence inflammatory responses, oxidative stress, and the pathological remodeling of lung tissue, thereby playing a crucial role in the emergence and development of COPD.[15–17] Although many SNPs related to COPD susceptibility have been identified, the specific pathogenesis of COPD remains unclear. Based on the above, it is necessary to identify genetic markers associated with COPD risk in specific populations.

Adhesion G protein-coupled receptors (aGPCRs) are a complex subgroup within the G protein-coupled receptors (GPCRs) family. aGPCRs are distinguished by their intricate structure, which incorporates a chimeric composition of various functional protein domains. This unique structure enables the integration of extracellular cell adhesion functions with intracellular GPCR signal transduction.[18] aGPCRs have a wide distribution and play pivotal roles in immune responses, tumorigenesis, and numerous developmental processes.[18,19] Specific aGPCRs, such as ADGRF5[20] and ADGRB3,[21] are linked to lung diseases. Gpr126/ADGRG6, an aGPCR of particular interest, is critical for Schwann cell (SC) myelination among other functions.[22,23] Research has revealed that mutations in ADGRG6 are associated with various cancers and diseases.[24–26] Notably, a study has identified a functional variant in ADGRG6, which plays a role in gas exchange and hypoxia.[27] This variant exhibits differential expression in the lung tissues of COPD patients and individuals with reduced diffusion capacity. While recent studies have underscored the biological significance of ADGRG6 in the development of various diseases, including lung diseases, the specific mechanisms by which this gene influences the onset and progression of COPD in the Chinese population require further exploration.

Focusing on the role of genetic factors in COPD predisposition, our investigation is directed towards examining the influence of genetic variations within the ADGRG6 gene on the susceptibility to COPD among Han Chinese individuals. This research endeavor will pioneer the exploration of the correlation between ADGRG6 genetic predisposition and COPD in the elderly Han population in southern China, offering novel insights for mitigating COPD incidence at the preventative stage. By doing so, the study aspires to contribute to the scientific groundwork necessary for a deeper understanding of ADGRG6's role in COPD pathogenesis and to inform personalized approaches to COPD prevention and treatment tailored to the elderly Han population in Southern China.

Materials and Methods

Recruitment of Participants

Between August 2020 and January 2023, a cohort of 270 COPD patients was identified from those attending and undergoing treatment at Hainan General Hospital, based on the study's eligibility requirements. The key diagnostic

criterion for COPD was established as a ratio of forced expiratory volume in one second to forced vital capacity (FEV1/FVC) below 70%.[28]

The inclusion criteria for the COPD case group included: (1) age over 60 years; (2) no prior hormone or immunomodulatory treatment within one month of study entry; (3) no mental health disorders and evidence of compliance; (4) consent to participate, as evidenced by a signed informed consent document.

The exclusion criteria for the COPD case group included: (1) cardiogenic asthma, pulmonary tumors, or allergic alveolitis; serious comorbidities in other organs; (2) a history of thoracic or abdominal surgery within the last three months; (3) non-adherence to the study protocol or withdrawal without authorization.

During the same period, 271 individuals without pulmonary diseases, as confirmed by medical assessment, and free from other organ diseases and recent respiratory infections, were recruited from the hospital's health check-up center to constitute the control group.

This study received approval from the hospital's Ethics Committee and was conducted under its supervision. We are committed that our study complies with the Declaration of Helsinki.

Selection of Candidate Genetic Variants

To identify the physical location of ADGRG6, we used it as a keyword in the Ensembl Genome Browser GRCh38.p14 version (http://asia.ensembl.org/Homo_sapiens/Gene/Summary?db=core;g=ENSG00000112414;r=6:142301854-142446266). This led to the download of two essential files, ".info" and ".ped", associated with ADGRG6, from the "VCF to PED Converter" function module on the Ensembl Tools website (http://asia.ensembl.org/info/docs/tools/index.html). These files were then imported into the Haploview software. Within Haploview, the tagSNPs of ADGRG6 were determined after setting specific parameters in the Tagger module (MAF > 0.05, $r^2 = 0.80$, Min Genotype > 75%, and HWE *P*-value > 0.01).[29] To refine our research focus, we selected six ADGRG6 variants from tagSNPs file (rs9399401,[30] rs116955726, rs35699755, rs11155242,[31] rs2143390,[32] and rs6937121[33]) as candidate genetic variants for this study, based on previous studies examining correlations in populations with varied genetic backgrounds.

DNA Extraction and Genotyping

Upon obtaining informed consent from all participants, we collected their peripheral blood for storage. Whole genome DNA was extracted using the DNA extraction and purification kit from GoldMag Nanobiotech Co., Ltd., Xi'an, China. The Agena MassARRAY platform (Agena Bioscience, San Diego, California, USA) was utilized for genotyping. The design of all primers was accomplished using the "MassARRAY Assay Design" online software (https://support.agenabio.com/s/online-tools), and the sequences of these primers are detailed in Supplemental Table 1.

Statistical Analysis

The candidate variants were assessed for Hardy-Weinberg Equilibrium (HWE) by comparing the observed genotype frequencies against the expected frequencies in the control group. The online tool "SNPStats" (https://www.snpstats.net/start.htm?q=snpstats/start.htm) was utilized to evaluate the association between candidate ADGRG6 variants and COPD susceptibility. All statistical analyses were conducted using SPSS software version 22.0 (SPSS Inc., Chicago, IL, USA). Measurement data, tested for normal distribution, were presented as "mean ± standard deviation" when conforming to normal distribution. The independent sample *t*-test was employed for comparisons between two groups. Count data were expressed as "n (%)" and analyzed using the chi-square (χ^2) test. Furthermore, the False-Positive Report Probability (FPRP) test was applied to evaluate whether all significant results were noteworthy findings.[34] Additionally, Multi-factor Dimensionality Reduction (MDR) analysis was conducted to assess the impact of interactions between candidate genetic variants on COPD susceptibility. Results with a "p-value < 0.05" were considered statistically significant.

Results

Participant Characteristics

A total of 541 han participants of Southern China met the recruitment criteria, comprising 270 COPD cases (206 males and 64 females) and 271 healthy individuals (210 males and 61 females). The average age in the case group was 72.26 ± 10.40 years, while it was 69.21 ± 6.61 years in the control group. In the healthy control group, there were 136 (50.2%) smokers and 135 (49.8%) non-smokers; 133 (49.1%) drinkers, and 138 (50.9%) non-drinkers. In the case group, there were 128 (47.4%) smokers and 142 (52.6%) non-smokers; 126 (46.7%) drinkers, and 144 (53.3%) non-drinkers; with an average FEV1/FVC (%) of (58.56 ± 15.05) %. Detailed participant characteristics are presented in Table 1.

Genotyping of Candidate ADGRG6 Genetic Variants

The ADGRG6 gene is located at Chromosome 6: 142,301,854–142,446,266. There are 36704 variants for this gene. After screening, a total of 6 genetic variations were selected as candidates. Candidate genetic variants (rs9399401, rs116955726, rs35699755, rs11155242, rs2143390, and rs6937121) have been successfully genotyped. All candidate genetic variants conformed to Hardy-Weinberg equilibrium (HWE *P*-value > 0.01). The minor allele frequencies (MAF) of candidate SNPs in the participants and different genetic backgrounds (Han population in Shanghai/Beijing, China; African; and European) are summarized in Table 2. Notable differences in the MAF of candidate genetic variants among different populations were observed. This finding suggests that it is necessary to identify SNPs associated with COPD susceptibility in different populations.

Effect of Candidate ADGRG6 Genetic Variants on COPD Susceptibility

The overall analysis results, as shown in Table 3, indicate that the frequency of the mutant allele "C" of rs11155242 in COPD cases (7.74%) is significantly lower compared to the healthy control group (9.59%). This suggests that the mutant allele "C" of rs11155242 acts as a protective genetic factor against COPD susceptibility in the study subjects (OR = 0.57, 95% CI = 0.36 to 0.91, $p = 0.017$). Additionally, the heterozygous mutant genotype "CA" of rs11155242 was found to be significantly less frequent in COPD cases (10%) than in the healthy control group (16.2%), indicating a significant association with reduced COPD risk (CA Vs AA: OR = 0.53, 95% CI = 0.32 to 0.90, p = 0.018). Furthermore, under the

Table 1 Basic Characteristics of Participants (Cases with COPD and Healthy Individuals)

Characteristics		Cases	Control	*p*-value
		n = 270	n = 271	
Age (years)	Mean ± SD	72.26 ± 10.40	69.21 ± 6.61	< 0.0001[a]
	> 71 years old	162 (60.0%)	91(33.6%)	
	≤ 71 years old	108 (40.0%)	180 (66.4%)	
Gender	Male	206 (76.3%)	210 (77.5%)	0.742[b]
	Female	64 (23.7%)	61 (22.5%)	
Smoking status	Yes	128 (47.4%)	136 (50.2%)	0.518[b]
	No	142 (52.6%)	135 (49.8%)	
Drinking status	Yes	126 (46.7%)	133 (49.1%)	0.575[b]
	No	144 (53.3%)	138 (50.9%)	
BMI	≥ 24 kg/m^2	57 (21.1%)	65 (24.0%)	0.350[b]
	< 24 kg/m^2	213 (78.9%)	206 (76.0%)	
FVC (L)		1.98 ± 0.67		
FEV1 (L)		1.18 ± 0.54		
FEV1/FVC (%)		58.56 ± 15.05		

Note: [a] represents the *p*-value calculated by the *t*-test; [b] represents the *p*-value calculated by the chi-square test.

Abbreviations: COPD, chronic obstructive pulmonary disease; FVC, forced vital capacity; FEV1, forced the first second of expiratory volume;

Table 2 The Basic Information About the Candidate ADGRG6 Genetic Variants and HWE Analysis

SNP ID	Function	Chr: Position	Alleles (A/B)	MAF						HWE (*P* value)	Haploreg 4.1
				Cases	Controls	CHB	CHS	AFR	EUR		
rs9399401	intronic	6: 142347764	C/T	0.318	0.306	0.286	0.367	0.424	0.309	0.061	Enhancer histone marks; Selected eQTL hits
rs116955726	missense	6: 142367674	T/C	0.061	0.065	0.058	0.052	0.021	0.001	1.000	SiPhy cons; Promoter histone marks; Enhancer histone marks; DNAse; Proteins bound; Motifs changed
rs35699755	synonymous	6: 142367690	T/C	0.059	0.063	0.058	0.052	0.083	0.001	1.000	SiPhy cons; Promoter histone marks; Enhancer histone marks; DNAse; Proteins bound; Motifs changed
rs11155242	missense	6: 142370412	C/A	0.057	0.096	0.083	0.090	0.230	0.228	0.285	Enhancer histone marks; DNAse; Motifs changed; GRASP QTL hits
rs2143390	synonymous	6: 142382000	T/C	0.248	0.188	0.194	0.262	0.006	0.082	0.044	SiPhy cons; Promoter histone marks; Enhancer histone marks; DNAse
rs6937121	intronic	6: 142385996	G/T	0.374	0.367	0.350	0.419	0.939	0.320	0.694	Promoter histone marks; Enhancer histone marks; Motifs changed; Selected eQTL hits

Note: HWE *P* value > 0.01 indicates that the genotypes were in Hard-Weinberg Equilibrium.
Abbreviations: A: minor allele; B: wild-type allele; HWE: Hardy–Weinberg equilibrium; SNP: Single nucleotide polymorphisms; MAF: minor allele frequency.

Table 3 The Association Analysis Between Candidate ADGRG6 Genetic Variants and Susceptibility to COPD

SNP ID	Model	Genotype	control	case	OR (95% CI)	*p*-value
rs9399401	Allele	T	375 (69.44%)	367 (68.22%)	1	
		C	165 (30.56%)	171 (31.78%)	1.06 (0.82–1.37)	0.663
	Codominant	TT	137 (50.7%)	126 (46.8%)	1	
		TC	101 (37.4%)	115 (42.8%)	1.12 (0.77–1.62)	0.545
		CC	32 (11.8%)	28 (10.4%)	0.90 (0.51–1.60)	0.725
	Dominant	TT	137 (50.7%)	126 (46.8%)	1	
		TC-CC	133 (49.3%)	143 (53.2%)	1.07 (0.76–1.51)	0.710
	Recessive	TT-TC	238 (88.2%)	241 (89.6%)	1	
		CC	32 (11.8%)	28 (10.4%)	0.86 (0.50–1.48)	0.580
	Overdominant	TT-CC	169 (62.6%)	154 (57.2%)	1	
		TC	101 (37.4%)	115 (42.8%)	1.14 (0.80–1.63)	0.460
	Log-additive	-	-	-	1.00 (0.78–1.29)	0.990
rs116955726	Allele	C	507 (93.54%)	507 (93.89%)	1	
		T	35 (6.46%)	33 (6.11%)	0.94 (0.58–1.54)	0.814
	Codominant	CC	237 (87.5%)	237 (87.8%)	1	
		CT	33 (12.2%)	33 (12.2%)	0.99 (0.58–1.67)	0.961
		TT	1 (0.4%)	0 (0%)	/	/
	Dominant	CC	237 (87.5%)	237 (87.8%)	1	
		CT-TT	34 (12.6%)	33 (12.2%)	0.95 (0.57–1.60)	0.850
	Recessive	CC-CT	270 (99.6%)	270 (100%)	1	
		TT	1 (0.4%)	0 (0%)	/	/
	Overdominant	CC-TT	238 (87.8%)	237 (87.8%)	1	
		CT	33 (12.2%)	33 (12.2%)	0.99 (0.59–1.67)	0.970
	Log-additive	-	-	-	0.92 (0.55–1.53)	0.740
rs35699755	Allele	C	508 (93.73%)	508 (94.07%)	1	
		T	34 (6.27%)	32 (5.93%)	0.94 (0.57–1.55)	0.811
	Codominant	CC	238 (87.8%)	238 (88.2%)	1	
		TC	32 (11.8%)	32 (11.8%)	0.98 (0.58–1.67)	0.947
		TT	1 (0.4%)	0 (0%)	/	/
	Dominant	CC	238 (87.8%)	238 (88.2%)	1	

(*Continued*)

Table 3 (Continued).

SNP ID	Model	Genotype	control	case	OR (95% CI)	*p*-value
		TC-TT	33 (12.2%)	32 (11.8%)	0.95 (0.56–1.60)	0.840
	Recessive	CC-TC	270 (99.6%)	270 (100%)	1	
		TT	1 (0.4%)	0 (0%)	/	/
	Overdominant	CC-TT	239 (88.2%)	238 (88.2%)	1	
		TC	32 (11.8%)	32 (11.8%)	0.99 (0.58–1.68)	0.960
	Log-additive	-	-	-	0.91 (0.55–1.53)	0.730
rs11155242	Allele	A	490 (90.41%)	509 (94.26%)	1	
		C	52 (9.59%)	31 (7.74%)	0.57 (0.36–0.91)	**0.017**
	Codominant	AA	223 (82.3%)	241 (89.3%)	1	
		CA	44 (16.2%)	27 (10%)	0.53 (0.32–0.9)	**0.018**
		CC	4 (1.5%)	2 (0.7%)	0.47 (0.08–2.6)	0.385
	Dominant	AA	223 (82.3%)	241 (89.3%)	1	
		CA-CC	48 (17.7%)	29 (10.7%)	0.53 (0.32–0.87)	**0.011**
	Recessive	AA-CA	267 (98.5%)	268 (99.3%)	1	
		CC	4 (1.5%)	2 (0.7%)	0.51 (0.09–2.82)	0.420
	Overdominant	AA-CC	227 (83.8%)	243 (90%)	1	
		CA	44 (16.2%)	27 (10%)	0.54 (0.32–0.90)	**0.018**
	Log-additive	-	-	-	0.57 (0.36–0.90)	**0.013**
rs6937121	Allele	T	342 (63.33%)	338 (62.59%)	1	
		G	198 (36.67%)	202 (37.41%)	1.03 (0.81–1.32)	0.801
	Codominant	TT	110 (40.7%)	107 (39.6%)	1	
		GT	122 (45.2%)	124 (45.9%)	0.95 (0.65–1.38)	0.781
		GG	38 (14.1%)	39 (14.4%)	0.98 (0.58–1.67)	0.941
	Dominant	TT	110 (40.7%)	107 (39.6%)	1	
		GT-GG	160 (59.3%)	163 (60.4%)	0.96 (0.67–1.36)	0.800
	Recessive	TT-GT	232 (85.9%)	231 (85.6%)	1	
		GG	38 (14.1%)	39 (14.4%)	1.01 (0.62–1.65)	0.970
	Overdominant	TT-GG	148 (54.8%)	146 (54.1%)	1	
		GT	122 (45.2%)	124 (45.9%)	0.95 (0.67–1.35)	0.790
	Log-additive	-	-	-	0.98 (0.76–1.26)	0.870

Note: "-" indicates Log-additive model, "/" indicates missing data. *p*-value < 0.05' and bold text represent statistical significance.
Abbreviations: COPD, chronic obstructive pulmonary disease; SNP: Single nucleotide polymorphism; OR, Odds ratio; CI, Confidence interval.

dominant (OR = 0.53, p = 0.011), overdominant (OR = 0.54, p = 0.018), and log-additive (OR = 0.57, p = 0.013) genetic models, rs11155242 demonstrated a significant association with a reduced risk of COPD among the subjects.

The other genetic variants, rs9399401, rs116955726, rs35699755, rs2143390, and rs6937121, were not found to be associated with COPD susceptibility in the study subjects.

FPRP results showed that the positive results found in the overall analysis were all noteworthy findings at a probability level of 0.25 and FPRP cut-off value of 0.2 (Supplemental Table 2).

Stratified Analysis of Subgroups

We performed stratified analyses to assess the impact of potential influencing factors, including gender, age, smoking/drinking status, and BMI, on COPD susceptibility. The positive findings from these analyses are depicted in a forest plot, as illustrated in Figure 1.

Notably, rs11155242 was found to be strongly associated with a reduced risk of COPD. This association was particularly significant in males (dominant: OR = 0.53, p = 0.032; log-additive: OR = 0.55, p = 0.025), subjects aged 71 years or younger (codominant: OR = 0.35, p = 0.032; overdominant: OR = 0.35, p = 0.020), non-smokers (allele: OR = 0.46, p = 0.014; codominant: OR = 0.44, p = 0.030; dominant: OR = 0.42, p = 0.014; log-additive: OR = 0.46, p =

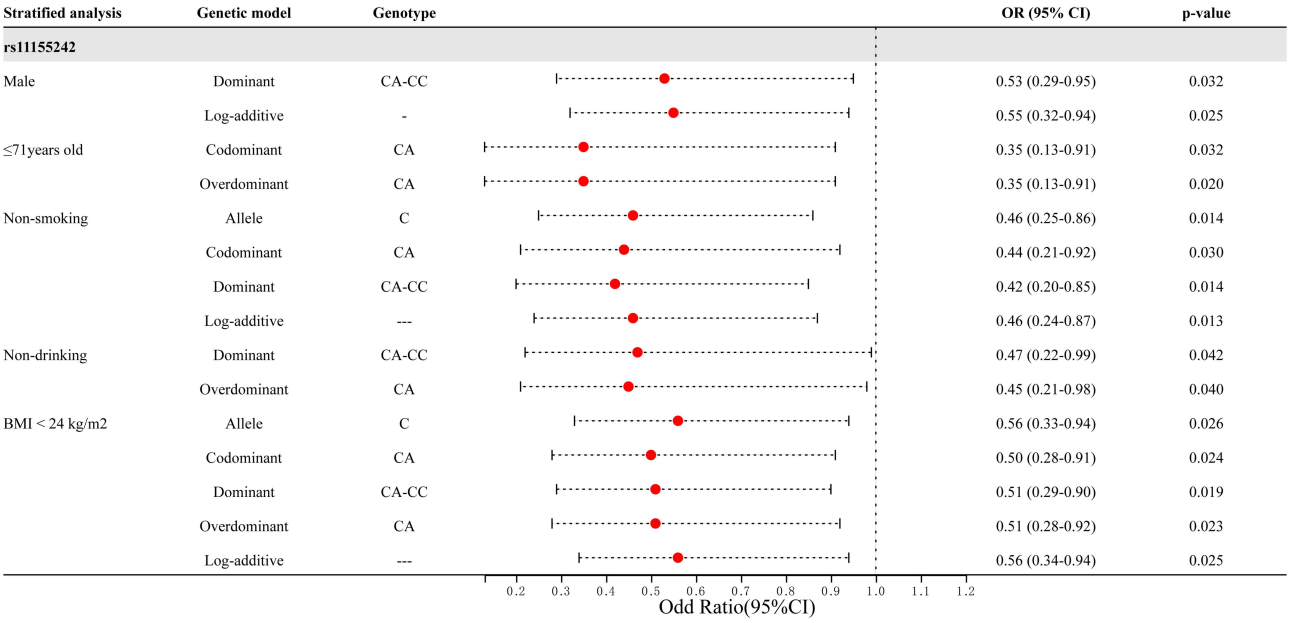

Figure 1 Forest map: significant association between *ADGRG6* genetic variants and chronic obstructive pulmonary disease (COPD) susceptibility in stratified analysis.

0.013), non-drinkers (dominant: OR = 0.47, $p = 0.042$; overdominant: OR = 0.45, $p = 0.040$), and subjects with a BMI below 24 kg/m^2 (allele: OR = 0.56, $p = 0.026$; codominant: OR = 0.50, $p = 0.024$; dominant: OR = 0.51, $p = 0.019$; overdominant: OR = 0.51, $p = 0.023$; log-additive: OR = 0.56, $p = 0.025$).

FPRP analysis, as shown in Supplemental Table 2, indicates that the positive results observed in males, non-smokers, and subjects with a BMI of less than 24 kg/m² are all considered notable findings. However, the observed prior probabilities of positive results in subjects aged 71 years or younger and non-drinkers exceed 0.2 (with a prior probability level set at 0.25 and an FPRP threshold of 0.2). This suggests that these positive results might not be particularly noteworthy.

MDR Analysis

The MDR analysis, as detailed in Table 4, reveals that of all the genetic models, the three-site model comprising rs116955726, rs11155242, and rs6937121 demonstrates the highest testing balanced accuracy (0.5195) and perfect cross-validation consistency (10/10). This indicates that this specific three-site model is the most effective for predicting COPD susceptibility in the study subjects, with a significant *p*-value of 0.0072. Figure 2 showed a dendrogram and a Fruchterman-Reingold, both illustrating the interactions among pairwise genetic variants across the five candidate variants. Figure 2A illustrates that rs11155242 and rs6937121 have a strong synergistic effect in predicting COPD susceptibility. In addition, Figure 2B showed that the information gain (IG) between rs11155242和rs6937121 was the highest.

Table 4 The Influence of Interaction Between ADGRG6 SNP-SNP on the Susceptibility to COPD Analyzed by MDR

Model	Training Bal. Acc	Testing Bal. Acc	OR (95% CI)	*p*-value	CVC
rs9399401	0.5258	0.4935	1.23 (0.87–1.74)	**0.2379**	8/10
rs116955726, rs11155242	0.5425	0.4881	1.56 (1.04–2.36)	**0.0319**	6/10
rs9399401, rs11155242, rs6937121	0.5570	0.5195	1.64 (1.14–2.36)	**0.0072**	10/10
rs9399401, rs116955726, rs11155242, rs6937121	0.5578	0.5158	1.64 (1.14–2.36)	**0.0072**	10/10
rs9399401, rs116955726, rs35699755, rs11155242, rs6937121	0.5578	0.5158	1.64 (1.14–2.36)	**0.0072**	10/10

Note: *p* values were calculated using χ^2 tests; "p-value < 0.05" and "bold text" represent statistical significance.
Abbreviations: COPD, chronic obstructive pulmonary disease; MDR, multifactor dimensionality reduction; Bal. Acc., balanced accuracy; CVC, cross-validation consistency; OR, odds ratio; 95% CI, 95% confidence interval.

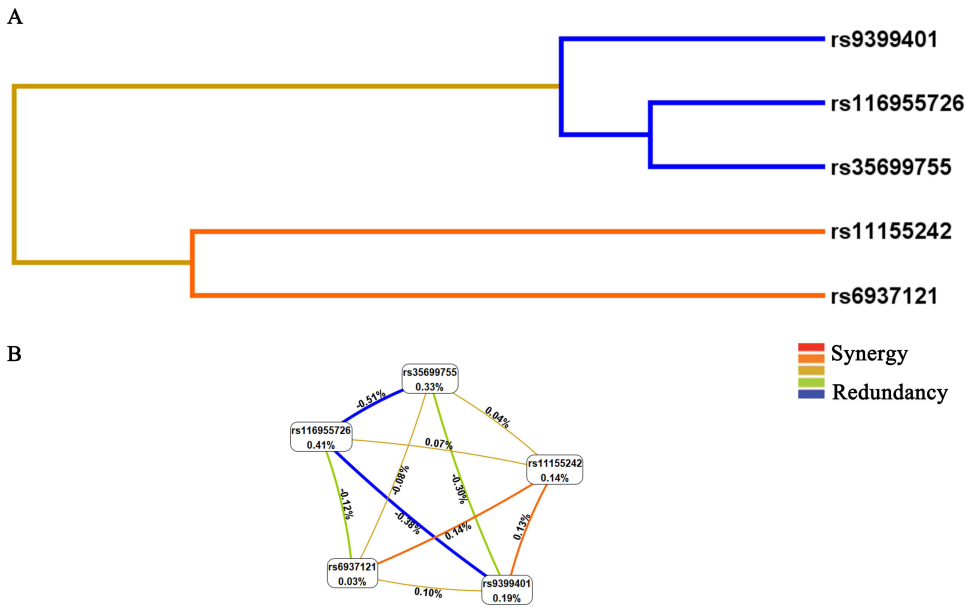

Figure 2 MDR analysis of the interaction between *ADGRG6* SNP-SNP. (**A**) SNP-SNP Interaction Dendrogram: the color represents the degree of redundancy or synergy between SNP-SNP; the closer the color is to red, the more synergy, and the closer to blue, the more redundancy. (**B**) Fruchterman-Reingold: values in nodes represent the IGs of individual attributes (main effects). Values between nodes are IGs of each pair of attributes (interaction effects).

Discussion

An analysis of online databases revealed six candidate ADGRG6 variants exhibiting significant variations in minor allele frequencies across populations with diverse genetic backgrounds. This underscores the importance of investigating ADGRG6's role in COPD susceptibility among individuals with varying genetic backgrounds. We examined the relationship between these candidate ADGRG6 variants and COPD susceptibility in the elderly Han Chinese population of Southern China. Our findings indicate that the rs11155242 variant is significantly associated with a decreased risk of developing COPD: the mutant allele "C" and the heterozygous mutant genotype "CA" of rs11155242 acted as protective genetic factors against COPD susceptibility in the study subjects. Rs11155242 was found to be strongly associated with a reduced risk of COPD in males, non-smokers, and subjects with a BMI below 24 kg/m^2. More importantly, the FPRP analysis indicated that the above positive results identified in this study are noteworthy new findings.

In a meta-analysis encompassing genome-wide association studies with individuals of European descent, the rs11155242 variant was significantly correlated with the pulmonary function metric Forced Expiratory Volume in one second (FEV1), a pivotal physiological biomarker for COPD.[28,35] This association suggests a potential role for rs11155242 in the pathogenesis of COPD. Our investigation has substantiated this correlation, revealing a robust association between rs11155242 and decreased COPD susceptibility among the elderly Han Chinese population in southern China. It is postulated that the "C" allele and the heterozygous genotype "CA" of rs11155242 may mitigate COPD risk by modulating the FEV1 index; however, this hypothesis requires validation through further functional studies to ascertain the variant's effect on FEV1 and, consequently, COPD susceptibility. Despite this, our findings present a novel perspective for COPD diagnostics within a distinct demographic. Discrepancies in the literature have been noted. Young, R. P.et al, reported no association between rs11155242 and COPD in their GWAS study concentrating on lung cancer susceptibility.[36] Additionally, a predictive model analysis within a Shanghai cohort detected no significant allele frequency disparities in rs11155242 between COPD patients and healthy controls.[37] These inconsistencies may arise from differences in study population selection criteria. Genetic background and age are significant modifiers of COPD susceptibility. To the best of our knowledge, this study is the inaugural investigation to identify a genetic variant (rs11155242) associated with COPD susceptibility in the elderly Han population of southern China, thus paving the way for novel diagnostics research in targeted demographics.

To mitigate the impact of confounding factors on genetic associations, we conducted a stratified analysis focusing on potential risk factors for COPD. This analysis reinforced the genetic association of the ADGRG6-rs11155242 variant with increased susceptibility to COPD. Notably, a pronounced association was observed between rs11155242 and COPD susceptibility in men, non-smokers, and individuals with a Body Mass Index (BMI) below 24 kg/m^2. Emerging evidence suggests a slower increase in COPD prevalence among men compared to women.[38] Given that COPD is primarily caused by the inhalation of toxic particles, particularly tobacco smoke and environmental pollutants, the incidence of COPD tends to be lower in non-smokers compared to smokers.[39] Building on the findings of this study, we propose that the ADGRG6-rs11155242 variant is linked to a reduced risk of Chronic Obstructive Pulmonary Disease (COPD). Additionally, this genetic association appears to be more pronounced in men and non-smokers. This supports the notion that COPD results from a combination of environmental and genetic factors. Moreover, existing research indicates that low BMI and weight loss can increase COPD mortality in men,[40] which seems to contradict our findings. Consequently, we hypothesize that the ADGRG6-rs11155242 variant influences COPD susceptibility independently of the participants' BMI. In the elderly Han population of southern China, the genetic variation rs11155242 seems to have a more substantial impact on COPD susceptibility compared to BMI.

In this study, we investigated six candidate ADGRG6 genetic variants; however, only ADGRG6-RS11155242 demonstrated an association with COPD susceptibility. Previous research has identified a link between rs9399401 and COPD risk in Northern Swedish populations,[41] while rs6937121 has shown a significant genome-wide association with FEV1/FVC levels in European ancestry populations.[42] Additionally, a notable correlation was found between rs2143390 and COPD susceptibility in a Northern Swedish cohort.[32] In contrast, these genetic variants did not exhibit a similar association with COPD susceptibility in the elderly Han population of Southern China. These findings suggest that the impact of ADGRG6 genetic variations on COPD susceptibility varies across different genetic backgrounds, highlighting the importance of exploring the relationship between ADGRG6 variants and COPD risk in specific populations.

ADGRG6, a well-studied member of the adhesion G protein-coupled receptor (aGPCR) family, plays a crucial role in peripheral nerve development, angiogenesis, and inner ear formation.[22,43–47] Although there has been some progress in understanding the function of ADGRG6 genetic variants in various diseases,[48–51] their mechanisms related to lung function remain less explored. It is reported that ADGRG6 expression varies throughout human lung development, indicating its significance in lung function.[52] Studies have also demonstrated that individuals carrying protective alleles exhibit reduced GPR126 expression, leading to decreased IL33 production.[53] In COPD patients, serum IL33 levels have been found to be elevated compared to control groups.[54] Integrating findings from previous research and this study, we hypothesize that individuals in our study population carrying the mutant allele "C" of rs11155242 may have a reduced risk of COPD. This could be attributed to the genetic variant's potential to lower ADGRG6 expression, subsequently affecting the expression of certain inflammatory factors. However, this hypothesis is speculative, and further functional validation experiments are essential to confirm these findings.

In summary, our study demonstrates that ADGRG6 genetic variants are associated with the reduced risk of COPD in elderly Han Chinese populations in Southern China. This research contributes a novel perspective to the personalized prevention and treatment of COPD among the elderly Han Chinese. However, the limitations of our study should not be overlooked. The genetic background of our sample is relatively simple, underscoring the importance of expanding the sample size in subsequent validation studies. Including other minority groups in Hainan in future studies to expand the sample size will help to enrich our research. In addition, considering the important association between ADGRG6 and the pathogenesis of COPD, it is necessary to further explore the correlation between the ADGRG6-rs11155242 locus and clinical indicators in COPD patients. At the same time, it is imperative to design functional verification experiments for rs11155242. These experiments will lay a solid theoretical foundation for elucidating the role of ADGRG6 in the pathogenesis and progression of COPD.

Conclusion

The "C" allele and "CA" genotype of the rs11155242 variant in ADGRG6 are identified as in ADGRG6 are identified as genetic factors that confer protection against the development protection against the development of COPD. Our research

marks a pioneering effort to establish a robust correlation between a robust correlation between the ADGRG6-rs11155242 polymorphism and COPD risk. In the clinic, this study is expected to provide significant insights for the development of strategies for COPD risk screening, diagnosis, classification, assessment, and stratification in the elderly Han Chinese population in southern China who possess these protective genetic markers.

Data Sharing Statement

All data generated or analyzed during this study are included in this manuscript.

Ethics Approval Statement

This study was conducted under the standard approved by the Biomedical Ethics Committee of Hainan General Hospital.

Patient Consent Statement

All participants signed informed consent forms before participating in this study.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis, and interpretation, or in all these areas; took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no conflict of interest.

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