

Polymorphisms in the airway epithelium related genes *CDHR3* and *EMSY* are associated with asthma susceptibility

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Research

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Abstract

Background: As a main line of defense of the respiratory tract, the airway epithelium plays an important role in the pathogenesis of asthma. *CDHR3* and *EMSY* were reported to be expressed in the human airway epithelium. Although previous genome-wide association studies found that the two genes were associated with asthma susceptibility, similar observations have not been made in the Chinese Han population.

Methods: A total of 300 asthma patients and 418 healthy controls who were unrelated Chinese Han individuals were enrolled. Tag-single nucleotide polymorphisms (Tag-SNPs) were genotyped and the associations between SNPs and asthma risk were analyzed by binary logistic regression analysis.

Results: After adjusting for confounding factors, the A allele of rs3847076 in *CDHR3* was associated with increased susceptibility to asthma (OR = 1.407, 95% CI: 1.030-1.923). For the *EMSY* gene, the T alleles of both rs2508746 and rs12278256 were related with decreased susceptibility to asthma (additive model: OR = 0.718, 95% CI: 0.536-0.961; OR = 0.558, 95% CI: 0.332-0.937, respectively). In addition, the GG genotype of rs1892953 showed an association with increased asthma risk under the recessive model (OR = 1.667, 95% CI: 1.104-2.518) and the GATCTGAGT haplotype in *EMSY* was associated with reduced asthma risk ($P = 0.037$).

Conclusions: This study identified novel associations of rs3847076 in *CDHR3*, as well as rs1892953, rs2508746 and rs12278256 in *EMSY* with adult asthma susceptibility in the Chinese Han population. Our observations suggest that *CDHR3* and *EMSY* may play important roles in the pathogenesis of asthma in Chinese individuals. Further study with larger sample size is needed.

Trial Registration: Not applicable.

Introduction

Asthma is a chronic airway inflammatory disease that affects populations throughout the world. A World Health Organization report [1] predicted that the number of asthma patients would increase to 400 million by 2025 and 250,000 patients may die from this disease each year. A recent survey indicated that the prevalence of asthma among individuals aged > 14 years was 1.24% and there are approximately 30 million asthmatic patients in China [2]. The pathogenesis of asthma is still incompletely understood but it is known that genetic factors play a significant part in asthma susceptibility. The heritability of asthma was estimated to be 60–70% in an Australian twin study [3]. Genetic factors contributed to 90% of the variance in the susceptibility to asthma in a 5-year-old twin pair study [4].

As the first barrier between the human body and the environment, the airway epithelium has an important role in regulating the inflammation, immunity and tissue repair in the pathogenesis of asthma [5]. One genome-wide association study (GWAS) of a Danish population identified Cadherin related family member 3 (*CDHR3*), which is highly expressed in human airway epithelium, as a susceptibility locus for childhood asthma with severe exacerbations [6]. A GWAS in 2017 demonstrated that Chromosome 11 open reading frame 30 (*C11orf30*), also called *EMSY* or BRCA2-interacting transcriptional repressor, another gene expressed in airway epithelium, was a risk locus for food allergy in a Canadian population [7] and this gene has been shown to be involved in the epigenetic regulation of gene expression [8]. However, there have been few studies of these two genes in Chinese asthmatics. Therefore, this study aimed to investigate the association of common variants in *CDHR3* and *EMSY* with adult asthma in the Chinese population.

Materials And Methods

Study population

The asthmatic cases were diagnosed by at least three respiratory physicians from the West China Hospital according to the criteria of the Global Strategy for Asthma Management and Prevention [9]. The healthy controls were collected from the physical examination center in the same hospital. Subjects were excluded if any of the following conditions were present: chronic obstructive pulmonary disease, diabetes, tumors, any immune disease, and immune deficiency. Use of hormones or immunosuppressive drugs were also exclusion criteria. Cases and controls were unrelated Chinese Han individuals. After signing the informed consent, 3 ml of venous blood were drawn from every subject and stored in a -80°C freezer. All blood specimens were collected from September 2013 to September 2016. The study was approved by the ethical committee of the West China Hospital of Sichuan University (Protocol No. 23).

SNP Selection and Genotyping

Single nucleotide polymorphisms (SNPs) with minor allele frequency (MAF) ≥ 0.05 and $r^2 \geq 0.64$, located in the region 3000 base pairs upstream to 300 base pairs downstream of *CDHR3* were downloaded from the Han Chinese in Beijing database of the Genome Variation Server 147 (<http://gvs.gs.washington.edu/GVS/>), which is an online resource based on dbSNP. The final selections were 23 tag-SNPs including rs3887998, rs12155008, rs41267, rs3892893, rs10270308, rs34426483, rs193795, rs2526978, rs381188, rs10241452, rs3847076, rs11981655, rs10808147, rs193806, rs2528883, rs41269, rs2526979, rs2526976, rs41262, rs41266, rs6967330, rs41270 and rs448024. The selection of SNPs in *EMSY* was based on the tag-SNP strategy and literature review [10-13]. The tag-SNP selection strategy was the same as above except for $r^2 \geq 0.80$. The 17 SNPs selected were rs3753051, rs7125744, rs7926009, rs4945087, rs2508740, rs1939469, rs7115331, rs1044265, rs12278256, rs2513513, rs2508755, rs2155219, rs2513525, rs2508746, rs1892953, rs7130588 and rs10899234.

Genomic DNA was extracted from the blood samples using a genomic DNA

purification kit (Axygen Scientific Inc, Union City, CA, USA). SNPs were genotyped by Genesky Bio-Tech Co., Ltd (<http://geneskybiotech.com/index.html>) using the SNPscanTM multiplex SNP genotyping technique based on double ligation and multiplex fluorescence polymerase Chain Reaction (PCR) [14]. As a quality

control measure, 5% of random samples were repeat genotyped with a concordance rate of 100%.

Data Analyses

Statistical tests were performed using the Statistical Package for the Social Sciences (SPSS, SPSS Inc., Chicago, IL, USA), version 21.0. A p value <0.05 was considered to be statistically significant. Continuous and categorical variables were analyzed by student's t -test and chi-square test, respectively. Genotype distributions under additive, dominant and recessive models were calculated by binary logistic regression analysis. Hardy-Weinberg equilibrium (HWE) among the controls was tested using plink software. Haploview and SHEsis software (<http://analysis.bio-x.cn>) were combined to perform linkage disequilibrium (LD) and haplotype analysis. Statistically significant SNPs were predicted by the software RegulomeDB (<http://www.regulomedb.org/>) and Haploreg v4 (<http://compbio.mit.edu/HaploReg>).

Results

Subject characteristics

A total of 300 asthma patients and 418 healthy controls were enrolled. The average ages of asthma patients and controls were 43.6 ± 13.48 and 44.09 ± 13.75 years, respectively. No significant differences in sex, body mass index (BMI) and smoking history were observed between case and control groups (Table 1). Late-onset asthma (age of asthma onset ≥ 18 years) accounted for 74.3% in the case group.

Association analyses between *CDHR3*, *EMSY* SNPs and asthma susceptibility

The characteristics of the selected SNPs are listed in Table S1 and S2. Rs10899234 in *EMSY* and rs6967330 in *CDHR3* were excluded due to their deviation from HWE in the control subjects ($P < 0.05$). The genotyping assays failed for rs12155008, rs41270 and rs448024 in *CDHR3*.

After adjusting for confounding factors including age, sex, BMI and smoking history, four SNPs were found to be associated with asthma susceptibility (Table 2). The A allele of rs3847076 in *CDHR3* was associated with increased susceptibility to asthma under the additive model ($P = 0.032$, OR = 1.407, 95% CI: 1.030-1.923). For *EMSY*, both the TC/TT genotype and T allele of rs2508746 were associated with decreased risk of asthma (dominant model: $P = 0.019$, OR = 0.660, 95% CI: 0.465-0.935; additive model: $P = 0.026$, OR = 0.718, 95% CI: 0.536-0.961). The TG/TT genotype and T allele of rs12278256 were associated with reduced asthma risk (dominant model: $P = 0.033$, OR = 0.563, 95% CI: 0.332-1.953; additive model: $P = 0.027$, OR = 0.558, 95% CI: 0.332-0.937). Finally, the GG genotype of rs1892953 showed an association with increased asthma risk under the recessive model ($P = 0.015$, OR = 1.667, 95% CI: 1.104-2.518).

Stratified analysis results by gender, smoking status, BMI status and onset age of asthma were shown in Table 3. Allele A of rs3847076 was associated with increased susceptibility to asthma in male subgroup, smoking subgroup, non-overweight subgroup and late onset asthma subgroup ($P = 0.023$, OR = 1.869; $P = 0.009$, OR = 2.168; $P = 0.005$, OR = 1.835 and $P = 0.023$, OR = 1.457, respectively). Similarly, rs2508746 TC+TT was related with decreased asthma susceptibility in the non-smoking subgroup, non-overweight subgroup, and late-onset asthma subgroup in dominant model ($P = 0.014$, OR = 0.618; $P = 0.027$, OR = 0.612 and $P = 0.016$, OR = 0.637, respectively). Meanwhile, rs1892953 GG shown increased risk of asthma in the female subgroup, non-smoking subgroup, non-overweight subgroup, and late onset asthma subgroup in recessive model ($P = 0.038$, OR = 1.738; $P = 0.04$, OR = 1.615; $P = 0.017$, OR = 1.910 and $P = 0.017$, OR = 1.680, respectively). Rs12278256 T was still associated with decreased asthma susceptibility in female subgroups, non-smoking subgroups, and non-overweight subgroups in additive model ($P = 0.032$, OR = 0.465; $P = 0.02$, OR = 0.508 and $P = 0.028$, OR = 0.481, respectively).

Haplotype and LD analysis

The LD between SNPs of *CDHR3* and *EMSY* was low and those SNPs were divided into eight haplotype blocks with Haploview software (Figures 1 and 2). Only the haplotype consisting of GATCTGAGT in block 1 of *EMSY* was associated with decreased risk of asthma ($P = 0.037$, OR = 0.615, 95% CI: 0.388-0.975) (Table 4).

Functional prediction results

Four statistically significant SNPs were predicted using the software RegulomeDB and Haploreg v4 (Table S3). Rs144934374 is strongly linked to rs12278256 and its RegulomeDB scores is lower than that of rs12278256, suggesting that it may be the functional site represented by rs12278256. Acting as promoter histone marks or enhancer histone marks, or affecting DNase is suggested to be associated with chromatin status, and binding proteins or altering regulatory motifs in ChIP-Seq suggest that transcription levels may be affected. It seems that these four SNPs may have certain effects on chromatin status and transcription level. Rs1892953 appears as an expression quantitative trait loci (eQTL) SNP in thyroid tissue [12].

Discussion

In this group of Chinese Han adults, the relationship between two airway epithelial-related genes *EMSY* and *CDHR3* and risk of asthma were investigated, and four polymorphisms related to asthma susceptibility were obtained, which were rs3847076 of *CDHR3* and rs2508746, rs1892953 and rs12278256 of *EMSY*. A further subgroup analysis of these four variants revealed that their association with asthma was present in the subgroup.

CDHR3, located on chromosome 7, is specifically expressed in ciliated airway epithelial cells which are the targets of Rhinovirus C (RV-C) infection, and its expression was positively associated with RV-C binding, replication and entry into the host cells [15,16]. There are only a few studies describing the relationship between *CDHR3* polymorphisms and asthma, and the results were inconsistent in different populations. The A allele of rs6967330 in *CDHR3* increased the risk of wheezing illnesses and hospitalizations for childhood asthma in a Danish study [6]. Rs17152490, in LD with rs6967330 was reported to affect asthma risk through *cis*-regulation of its gene expression in cells from human bronchial epithelial biopsy [17]. However, rs6967330 was only related to

early-onset asthma in a Japanese population [18] and no association between rs6967330 and asthma was found in Chinese children [19]. In the present study, rs6967330 was not in HWE and our data suggest that rs3847076 may increase the risk of asthma in adults, which were inconsistent with the previous studies. The potential reasons for this discrepancy are as follows: Firstly, the susceptibility to asthma may differ in different populations, and secondly, late-onset asthma patients accounted for the majority of the case group in this study, in contrast to the above Japanese study which reported the positive relationship between rs6967330 and early-onset asthma in children. A future study of different asthma phenotypes would be beneficial to the accurate prevention and treatment of asthma.

EMSY, located on chromosome 11q13.5, is expressed in the human airway epithelium and encoded by the *EMSY* protein. GWAS studies showed that *EMSY* was involved in allergic diseases including atopic dermatitis and food allergy [20,21]. Several SNPs, rs7130588, rs10899234, rs6592657, as well as we studied SNPs rs2508746 and rs1892953 were associated with total serum immunoglobulin E (IgE) levels in non-Hispanic Caucasian asthmatic patients [10]. In an eQTL analysis, Li et al [17] reported that rs2508740, rs2513525, rs4300410 (in complete LD with rs7926009), rs10793169 (in complete LD with rs7926009), rs2513513 and rs4245443 were significantly correlated with mRNA expression levels of *EMSY* in human bronchial alveolar lavage. Another GWAS study reported that rs7130588 in *EMSY* was associated with asthma [22]. A meta-analysis demonstrated that rs2155219 in *EMSY* increased the risk of allergic sensitization [11]. In the present study, three SNPs (rs2508746, rs1892953 and rs12278256) were related to asthma susceptibility in the Chinese Han population, of which rs12278256 has not been reported in previous studies. As a variant located in the upstream region of *EMSY*, rs12278256 might affect the regulatory motifs and chromatin status of this gene and further study is needed to verify this hypothesis.

Studies in the twin population have shown that susceptibility to asthma can be attributed to genetic factors [3,4]. Although current genome-wide association studies have identified numerous polymorphisms associated with asthma susceptibility, the odds ratio (OR) is around 1.2, and only a small percentage of asthma prevalence can be contributed to them. Some experts have proposed to study the interaction between genes and environment [23,24]. It is well known that environmental factors such as smoking and obesity are susceptibility factors for asthma, but the specific mechanism is not clear. A number of studies have shown that smoking is associated with increased risk of asthma, reduced efficacy of inhaled corticosteroids treatment, acute exacerbations, and airway remodeling in asthma [25-29]. Mechanisms of asthma in the obese may include mechanical factors and inflammatory immunity [30]. Studies have shown that the SNPs at 17q21.2 is associated with BMI levels in asthmatic patients [31]. In our study, further analysis of the interaction between rs3847076 and environmental factors (smoking, BMI) revealed negative (Table S4). Functional prediction suggests that rs3847076 may affect the motif TCF4, and further investigation is needed.

Recently, genetic studies have detected a lot of susceptibility genes for asthma. This study was the first attempt to investigate the association between *CDHR3*/*EMSY* and adult asthma susceptibility in the Chinese Han population. We found rs3847076 in *CDHR3*, rs2508746, rs1892953 and rs12278256 in *EMSY* were associated with the risk of adult asthma. However, there were some limitations to this study. Adjustment was not performed to correct the results for multiple testing, due to the weak effect of each single polymorphism on asthma susceptibility. In addition, the allergic phenotypes of the asthma patients were not clear and serum IgE levels were not analyzed in the study. Lastly, *CDHR3* is a huge gene spanning over 159kb and the strategy of tag-SNPs selection with $r^2 > 0.64$ in this study may have missed some SNPs associated with the disease.

Conclusions

In conclusion, this study is the first to identify that the airway epithelium related genes *EMSY* and *CDHR3* were associated with adult asthma susceptibility in the Chinese Han population. The *CDHR3* rs3847076 allele A and *EMSY* rs1892953 genotype GG may increase the risk of asthma. The *EMSY* rs2508746 and rs12278256 allele T may decrease asthma risk. A population with a larger sample size is needed for further exploration of the association.

Abbreviations

Tag-SNPs, tag-single nucleotide polymorphisms

GWAS, genome-wide association study

CDHR3, cadherin related family member 3

C11orf30, chromosome 11 open reading frame 30

SNPs, single nucleotide polymorphisms

MAF, minor allele frequency

PCR, polymerase chain reaction

SPSS, statistical package for the social sciences

HWE, Hardy-Weinberg equilibrium

LD, linkage disequilibrium

BMI, body mass index

RV-C, rhinovirus C

IgE, immunoglobulin E

eQTL, expression quantitative trait loci

OR, odds ratio

Declarations

Ethics approval and consent to participate

All protocols for this study were reviewed and approved by the Institutional Review Board of the West China Hospital of Sichuan University (Protocol No. 23). Written informed consent was obtained from all the study participants.

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Author Contributions

Conceptualization, Jianqing He; Data curation, Yu Wang and Shou-Quan Wu; Formal analysis, Yu Wang and Shou-Quan Wu; Project administration, Miao-miao Zhang, Guo Chen and Jianqing He; Supervision, Jianqing He; Writing – original draft, Miao-miao Zhang and Guo Chen; Writing – review & editing, Andrew J Sandford.

Competing interests

The Authors declare that there is no conflict of interest.

Consent for publication

Not applicable.

Availability of data and materials

Some or all data, models, or code generated or used during the study are available from the corresponding author by request.

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Tables

Table 1 Characteristics of cases and controls

Characteristic	Control n(%)	Case n(%)	P value
Gender			0.876
Male	162 (38.76)	118 (39.33)	
Female	256 (61.24)	182 (60.67)	
Age (mean±SD,years)	44.09 ± 13.75	43.6 ± 13.48	0.64
Smoking status			
Current and ex-smokers	55 (13.16)	49 (16.33)	0.179
Non-smoking	207 (49.52)	247 (82.33)	
Smoking status unclear	156(37.32)	4(1.33)	
BMI(mean±SD)	22.94 ± 3.34	23.11 ± 3.28	0.517
BMI<24	227(54.31)	197(65.67)	
BMI≥24	121(29.67)	103(34.33)	
Asthma onset time			
Early-onset asthma(<18 years old)		42 (14.00)	
Late-onset asthma(≥18 years old)		223 (74.33)	
Onset time unclear		35(11.67)	
Asthma with pulmonary function test			
FEV1% predicted (mean±SD)		83.61 ± 19.97	
FEV1/FVC(mean±SD)		72.37 ± 13.63	

Values are means ± standard deviation (SD) and absolute numbers (percentages). BMI, body mass index; Early-onset asthma, age of asthma onset <18years; Late-onset asthma, age of asthma onset ≥18years; FEV1, forced expiratory volume in one second; FVC, forced vital capacity.

Table 2 The four SNPs associated with asthma

Genes	SNPs	Genetic models	Genotypes	Control n(%)	Case n(%)	P*	OR 95%CI*
<i>CDHR3</i>	rs3847076	Dom	CC	285(68.2)	185(61.7)	0.081	1.378(0.962-1.973)
			CA+AA	133(31.8)	115(38.3)		
		Rec	CC+CA	408(97.6)	285(95.0)	0.060	2.689(0.958-7.545)
			AA	10(2.4)	15(5.0)		
		Add	CC/CA/AA			0.032*	1.407(1.030-1.923)*
<i>EMSY</i>	rs2508746	Dom	CC	244(58.4)	197(65.7)	0.019*	0.660(0.465-0.935)*
			TC+TT	174(41.6)	103(34.3)		
		Rec	CC+TC	396(94.7)	288(96.0)	0.445	0.733(0.331-1.626)
			TT	22(5.3)	12(4.0)		
		Add	CC/TC/TT			0.026*	0.718(0.536-0.961)*
<i>EMSY</i>	rs1892953	Dom	AA	115(27.5)	76(25.3)	0.647	1.094(0.745-1.605)
			GA+GG	303(72.5)	224(74.7)		
		Rec	AA+GA	319(76.3)	219(73.0)	0.015*	1.667(1.104-2.518)*
			GG	99(23.7)	81(27.0)		
		Add	AA/GA/GG			0.081	1.240(0.974-1.579)
<i>EMSY</i>	rs12278256	Dom	GG	357(85.4)	272(90.7)	0.033*	0.563(0.332-0.953)*
			TG+TT	61(14.6)	28(9.3)		
		Rec	GG+TG	417(99.8)	300(100)	1	-
			TT	1(0.2)	0(0)		
		Add	GG/TG/TT			0.027*	0.558(0.332-0.937)*

* Adjusted for sex, age, body mass index and smoking history with logistic regression, P<0.05. Add: additive model; Dom: dominant model; Rec: recessive model.

Table 3 Results of stratification analysis based on gender, smoking status, BMI status, and onset age of asthma

SNPs	Genetic models		Stratified by gender			Stratified by smoking status			Stratified by BMI status			Stratified by of asthma	
			P	OR 95%CI		P	OR 95%CI		P	OR 95%CI		P	
rs3847076	Dom	male	0.048*	1.834(1.005-3.347)	smoking	0.018*	2.252(1.149-4.413)	BMI \geq 24	0.004*	1.925(1.233-3.007)	late onset asthma	0.063	1
	Rec		0.115	5.656(0.654-48.882)		0.09	4.222(0.799-22.320)		0.12	2.835(0.761-10.559)		0.049*	2
	Add		0.023*	1.869(1.091-3.202)		0.009*	2.168(1.212-3.872)		0.005*	1.835(1.234-2.726)		0.023*	3
rs2508746	Dom	female	-	-	non-smoking	0.014*	0.618(0.420-0.908)	BMI \geq 24	0.027*	0.612(0.396-0.946)	late onset asthma	0.016*	0
	Rec		-	-		0.498	0.737(0.304-1.782)		0.862	0.920(0.361-2.347)		0.419	1
	Add		-	-		0.022*	0.685(0.495-0.947)		0.06	0.710(0.496-1.015)		0.021*	2
rs1892953	Dom	female	0.548	1.159(0.717-1.873)	non-smoking	0.456	0.174(0.770-1.790)	BMI \geq 24	0.702	1.097(0.682-1.766)	late onset asthma	0.692	1
	Rec		0.038*	1.738(1.031-2.927)		0.04*	1.615(1.021-2.553)		0.017*	1.910(1.123-3.250)		0.017*	2
	Add		0.108	1.282(0.947-1.737)		0.091	1.259(0.964-1.644)		0.096	1.297(0.955-1.761)		0.094	3
rs12278256	Dom	female	0.037*	0.468(0.229-0.955)	non-smoking	0.023*	0.512(0.287-0.913)	BMI \geq 24	0.033*	0.485(0.249-0.944)	late onset asthma	-	-
	Rec		1			1			1			-	
	Add		0.032*	0.465(0.231-0.936)		0.02*	0.508(0.288-0.897)		0.028*	0.481(0.250-0.923)		-	-

* Adjusted for sex, age, body mass index and smoking history with logistic regression, P<0.05. Add: additive model; Dom: dominant model; Rec: recessive model.

Table 4 The association between *EMSY* haplotypes in block 1 and asthma susceptibility

Haplotype	Case N (%)	Control N (%)	Chi ²	Pearson's p	OR (95% CI)
AAGTTAAAT	207.00(0.345)	285.58(0.342)	0	0.999	1.000 (0.801-1.248)
GAGCGGAGC	43.00(0.072)	57.59(0.069)	0.023	0.878	1.033 (0.685-1.556)
GAGCTAAAT	44.00(0.073)	58.04(0.069)	0.054	0.815	1.050 (0.699-1.577)
GAGCTGAGC	35.00(0.058)	52.18(0.062)	0.134	0.714	0.921 (0.592-1.432)
GAGTTAGGT	178.00(0.297)	230.22(0.275)	0.589	0.443	1.095 (0.868-1.382)
GATCTGAGT	28.00(0.047)	61.00(0.073)	4.346	0.037*	0.615 (0.388-0.975)*
GGGCTAAAT	60.00(0.100)	76.32(0.091)	0.246	0.62	1.094 (0.766-1.562)
Global result	600	836	4.912565	0.555	

For each haplotype, alleles were arranged in order of rs4945087, rs7125744, rs12278256, rs7926009, rs7115331, rs2508740, rs1939469, rs2508755, rs3753051.

Figures

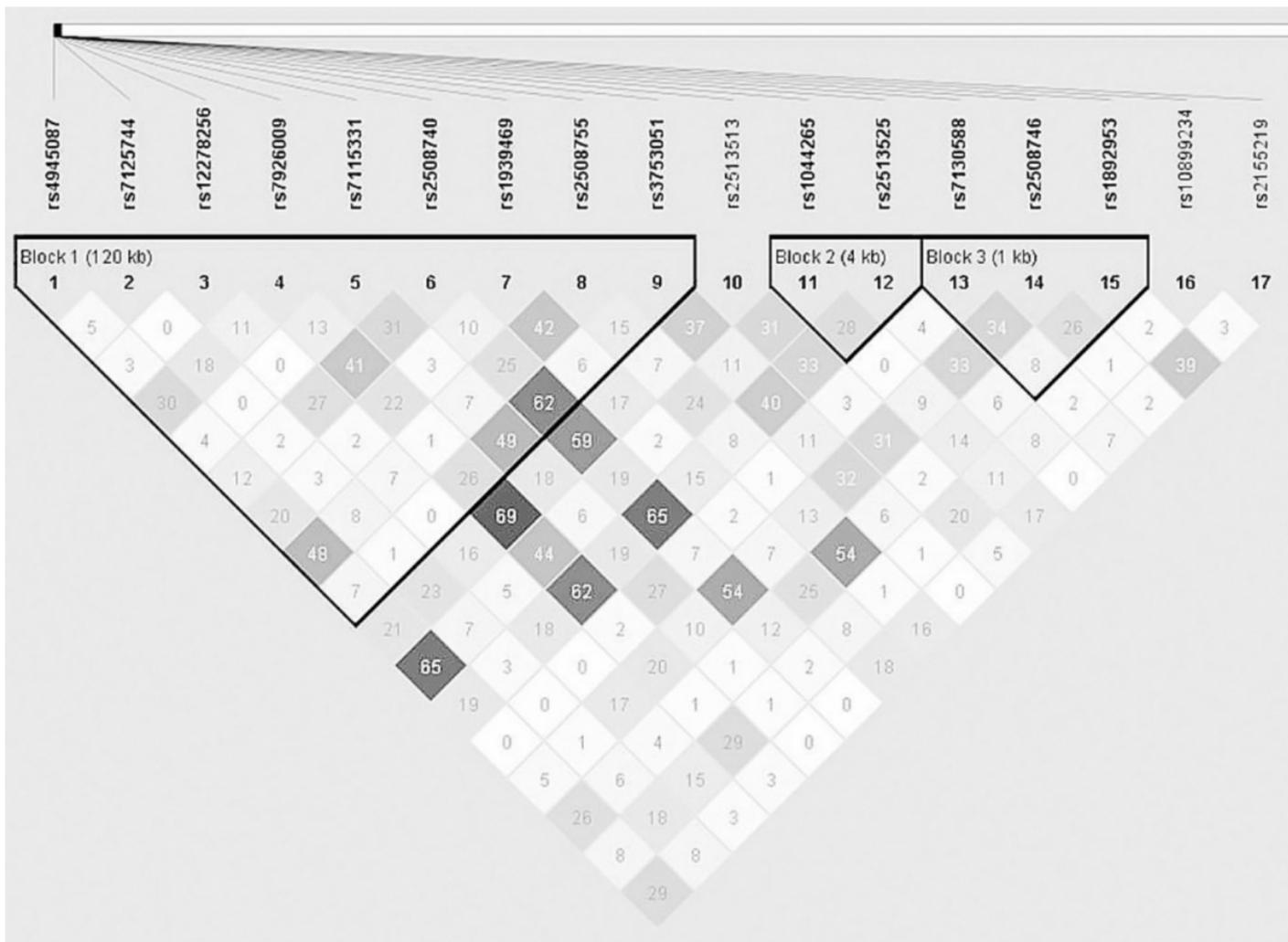


Figure 1

Analysis of linkage disequilibrium of SNPs in EMSY Note: Each square represents the linkage disequilibrium of two corresponding SNPs, which is displayed as $r^2 \times 100$. The larger the darkness of the square, the larger the value of $r^2 \times 100$.

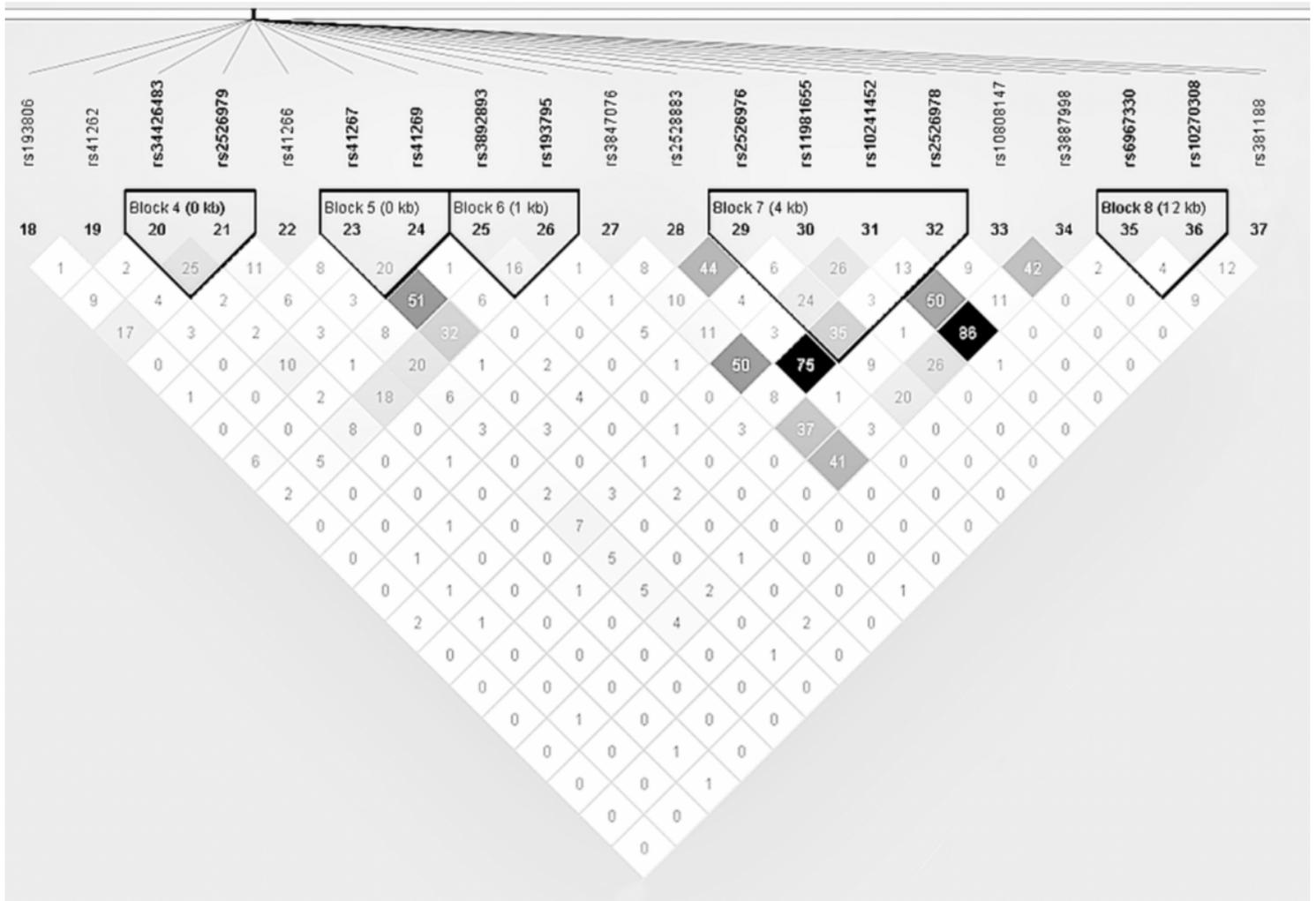


Figure 2

Analysis of linkage disequilibrium of tag-SNPs in CDHR3 Note: Each square represents the linkage disequilibrium of two corresponding SNPs, which is displayed as $r^2 \times 100$. The larger the darkness of the square, the larger the value of $r^2 \times 100$.

Supplementary Files

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- [SupplementaryMaterials.doc](#)