

ADRB2 Haplotypes and Asthma Exacerbations in Children and Young Adults: An Individual Participant Data Meta-Analysis

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Supplemental Methods

Study Population

Data of ten independent studies participating in the Pharmacogenomics in Childhood of Asthma (PiCA) consortium¹ were analyzed. All the studies have been approved by their local medical ethics committees and parents and/or participants provided written consent.

BREATHE is an observational study that includes children and young adults (age: 3-22 years)² with physician-diagnosed asthma recruited from primary and secondary care units in Tayside, Scotland, and Brighton, United Kingdom. A detailed history including information on symptoms, treatment, asthma exacerbations, demographics, and anthropometric information was obtained from the participants. From 1,307 individuals, mouthwash samples were collected for DNA analysis and the patients were considered to be non-Hispanic whites. The Effectiveness and Safety of Treatment with Asthma Therapy in children (*ESTATE*), www.estate-studie.nl, is a case-control study that includes children and young adults (age: 4-19 years) with physician-diagnosed asthma recruited from primary care units in the Netherlands. Patients were selected from either the IPCI (Interdisciplinary Processing of Clinical Information) database or the PHARMO Database Network. Both databases contain the complete electronic medical records of more than one million patients throughout the Netherlands with detailed information on patient diagnosis, patient prescription (IPCI), or patient dispensing (PHARMO). During the study period (2000-2012), all children with asthma, 4 years or older and treated with asthma controller therapy were selected. Within this cohort, asthma exacerbation cases were defined as the use of systemic corticosteroids, asthma-related emergency department visits, or hospitalization. Each case was matched to four controls on age, sex, general practice (GP), and type of asthma controller therapy. Next, all potential cases and controls were invited to participate via their respective GP. If patients

agreed to participate, they completed a research questionnaire, including questions on asthma control, and provided a saliva sample (for DNA extraction).

The followMAGICS study is the follow-up study of the observational Multicenter Asthma Genetics in Childhood Study (MAGICS), which includes physician-diagnosed asthmatic children and young adults (age: 7-25 years)³ recruited from secondary and tertiary centers in Germany and Austria. The ethnicity of patients was non-Hispanic whites, which was validated through principal component analysis.

The Genes-Environment and Admixture in Latino Americans (GALA II) and the Study of African Americans, Asthma, Genes, and Environments (SAGE) studies are two independent case-control asthma cohorts that focus on two different racial/ethnic groups based on the self-identified ethnicity of the four grandparents of each subject: Hispanics/Latinos (GALA II) and African Americans (SAGE). Both studies recruited unrelated children and young adults (age: 8- 21 years) using the same protocol and questionnaires from different areas in the United States and Puerto Rico (Chicago, Illinois; New York City, New York; Houston, Texas; San Francisco, California; and San Juan, Puerto Rico) for GALA.SAGE only recruited participants from the San Francisco Bay Area and California.^{4,5} Cases were defined as subjects with physician-diagnosed asthma. Exclusion criteria were established as any of the following: ≥ 10 pack-years of smoking; any smoking within one year of the recruitment date; in the third trimester of pregnancy; or a history of any one of the following: sickle cell disease, cystic fibrosis, sarcoidosis, cerebral palsy, or heart or chest surgery. Exacerbations were defined as a patient-reported occurrence of at least one of the following asthma-related events during the last 12 months preceding study enrolment: emergency care, hospitalization, and oral corticosteroids use.

The Pharmacogenetics of Asthma Medication in Children Medication with Anti-inflammatory effects (PACMAN) study in the Netherlands,⁶ is an observational cohort study

that included children (age: 4-12 years) old with self-reported regular use of asthma medication recruited through community pharmacies. Children were selected from community pharmacies in the Netherlands that belonged to the Utrecht Pharmacy Practice Network for Education and Research (UPPER).⁷ During visits to community pharmacies, detailed information on exacerbations and medication over the last 12 months was collected. Dutch, Moroccan, and Turkish ethnicities were considered Caucasian.

The Pediatric Asthma Gene Environment Study (*PAGES*) was a cross-sectional observational study designed to relate asthma outcomes to environmental and genetic factors. Children with physician-diagnosed asthma were recruited from primary care (age: 5-16 years) and secondary care (ages 2-16 years) centers across Scotland.⁸ In a single assessment, participants completed questionnaires with questions on demographic characteristics, asthma control, treatment, quality of life, and diet. Physiological testing was completed in a subgroup of participants and saliva was obtained for DNA extraction.⁸

The Pharmacogenetics of Adrenal Suppression Study (*PASS*) from the United Kingdom (age: 5-18 years) is a multicenter cohort of asthmatic children. The study initially aimed to explore the association between the use of corticosteroids and adrenal suppression. Children with asthma diagnosed by a secondary care pediatrician requiring inhaled corticosteroid therapy with clinical concern about adrenal suppression were included. The ethnicity of patients was self-reported as non-Hispanic whites.^{9,10}

The Singapore Cross Sectional Genetic Epidemiology Study (*SCSGES*) (age: 6-31 years)¹¹ is an ongoing cross-sectional genetic epidemiology study on allergic diseases among Singapore Chinese individuals. Volunteers were of Chinese ethnicity and resident in Singapore, and their DNA was extracted from mouthwash and blood. The ethnicity of subjects was self-reported Chinese and confirmed by principal component analysis between previous genome-wide genotyping data and Han Chinese (CHB) population from the HapMap project.^{11,12}

Asthma was defined as having a physician diagnosis of asthma symptoms prior to recruitment. The SLOVENIA study is a case-control cohort (age: 5-18) and includes asthmatic children and young adults recruited from tertiary health centers from Murska Sobota, Slovenia. Asthma was defined by physician diagnosis and hospital records. All patients were self-identified Caucasians of Slovenian origin.¹³

Supplemental Results

Table S1: Functional annotation of rs1042713 using the HaploRegv4.1¹⁴

Chr	pos (hg38)	LD (r ²)	LD (D')	variant	Ref	Alt	EUR freq	Enhancer histone marks	DNase	Motifs changed	Selected eQTL hits	GENCODE genes
5	148819704	0.9	0.95	rs35283004	A	G	0.38	BLD, MUS		GR,Maf	2 hits	6.9kb 5' of <i>ADRB2</i>
5	148820281	0.81	0.92	rs71582318	T	C	0.37	BLD, SKIN		Pou1f1,TATA		6.3kb 5' of <i>ADRB2</i>
5	148821442	0.94	0.97	rs12189018	T	C	0.38	BLD		RXRA	2 hits	5.2kb 5' of <i>ADRB2</i>
5	148822166	0.94	0.97	rs35019280	AG	A	0.38	BLD		CIZ,GATA,HNF1	2 hits	4.4kb 5' of <i>ADRB2</i>
5	148822926	0.93	0.97	rs33910799	AG	A	0.38	BLD	BD	CEBPB,DMRT2	1 hit	3.7kb 5' of <i>ADRB2</i>
5	148825014	0.97	0.99	rs17778257	A	T	0.38	9 tissues	SKIN	5 altered motifs	4 hits	1.6kb 5' of <i>ADRB2</i>
5	148826178	0.96	0.98	rs12654778	G	A	0.38		38 tissues	Foxp3,p53	4 hits	414bp 5' of <i>ADRB2</i>
5	148826877	1	1	rs1042713	G	A	0.38		28 tissues	4 altered motifs	3 hits	<i>ADRB2</i>

Pos; position, LD; Linkage disequilibrium, Ref; reference, Alt; alternative, EUR freq; European frequency, eQTL; expression quantitative trait loci

Table S2: Functional annotation of rs1042714 using the HaploRegv4.1¹⁴

chr	pos (hg38)	LD (r ²)	LD (D')	variant	Ref	Alt	EUR freq	Enhancer histone marks	DNase	Motifs changed	Selected eQTL hits	GENCODE genes
5	148819436	0.88	0.94	rs4705059	C	T	0.59	BLD, HRT, MUS	HRT	5 altered motifs		7.2kb 5' of <i>ADRB2</i>
5	148819441	0.88	0.94	rs4705060	G	A	0.59	BLD, MUS		4 altered motifs		7.2kb 5' of <i>ADRB2</i>
5	148819679	0.9	0.96	rs10078004	G	A	0.60			Mrg,NRSF		6.9kb 5' of <i>ADRB2</i>
5	148819882	0.9	0.96	rs67339154	A	G	0.60	BLD		Brachyury,TBX5		6.7kb 5' of <i>ADRB2</i>
5	148820448	0.94	0.97	rs56330463	T	C	0.59	BLD, SKIN		PPAR		6.1kb 5' of <i>ADRB2</i>
5	148820990	0.94	0.98	rs2082382	G	A	0.60	BLD	38 tissues	Foxo,Rad21	2 hits	5.6kb 5' of <i>ADRB2</i>
5	148821037	0.97	0.99	rs2082395	A	G	0.59	BLD	25 tissues	5 altered motifs	2 hits	5.6kb 5' of <i>ADRB2</i>
5	148821395	0.95	0.99	rs9325120	C	A	0.58	BLD		4 altered motifs		5.2kb 5' of <i>ADRB2</i>
5	148821692	0.97	0.99	rs11168066	C	A	0.59	BLD		Dmbx1,Otx2	2 hits	4.9kb 5' of <i>ADRB2</i>
5	148821753	0.96	0.99	rs11959615	T	A	0.59	BLD			2 hits	4.8kb 5' of <i>ADRB2</i>
5	148821910	0.97	0.99	rs35875547	AT	A	0.59	BLD, BRN		10 altered motifs		4.7kb 5' of <i>ADRB2</i>
5	148821922	0.97	0.99	rs11958940	A	T	0.59	BLD, BRN		NRSF,Zbtb3		4.7kb 5' of <i>ADRB2</i>
5	148822006	0.97	0.99	rs34064454	A	G	0.59	BLD, BRN		AIRE,Pax-4		4.6kb 5' of <i>ADRB2</i>
5	148823105	0.97	0.99	rs11746634	C	G	0.59	ESC, BLD		LUN-1,RORalpha1		3.5kb 5' of <i>ADRB2</i>
5	148823238	0.97	0.99	rs11168067	A	G	0.59	BLD		NRSF,Pitx2,SETDB1		3.4kb 5' of <i>ADRB2</i>
5	148823373	0.95	0.99	rs9325122	C	T	0.60	BLD		HDAC2,Pou2f2,Pou3f3		3.2kb 5' of <i>ADRB2</i>
5	148824199	0.97	0.99	rs1432622	T	C	0.59	BLD		7 altered motifs	2 hits	2.4kb 5' of <i>ADRB2</i>
5	148824445	0.97	0.99	rs1432623	C	T	0.59	BLD, SKIN		Nkx2		2.1kb 5' of <i>ADRB2</i>
5	148824558	0.97	0.99	rs11168068	C	T	0.59	BLD, SKIN		8 altered motifs		2kb 5' of <i>ADRB2</i>
5	148825489	0.97	0.99	rs2400707	A	G	0.59	12 tissues	SKIN,SKIN	HLF	2 hits	1.1kb 5' of <i>ADRB2</i>
5	148825809	0.97	0.99	rs2053044	A	G	0.59	5 tissues	35 tissues	8 altered motifs		783bp 5' of <i>ADRB2</i>
5	148826364	0.99	0.99	rs11168070	G	C	0.59		51 tissues	GR		228bp 5' of <i>ADRB2</i>
5	148826465	0.99	1	rs11959427	C	T	0.59	BRN	52 tissues	11 altered motifs		127bp 5' of <i>ADRB2</i>
5	148826785	0.98	1	rs1042711	C	T	0.59		35 tissues	6 altered motifs		5'-UTR of <i>ADRB2</i>
5	148826812	0.98	1	rs1801704	C	T	0.59	BRN	37 tissues	E2A,Sin3Ak-20,ZEB1		5'-UTR of <i>ADRB2</i>
5	148826910	1	1	rs1042714	G	C	0.59		21 tissues	GATA,PU.1		<i>ADRB2</i>

Pos; position, LD; Linkage disequilibrium, Ref; reference, Alt; alternative, EUR freq; European frequency, eQTL; expression quantitative trait loci

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