SHORT COMMUNICATIONS

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Genome-wide association study of mitochondrial DNA in Chinese men identifies seven new susceptibility loci for high-altitude pulmonary oedema

Ispitivanjem udruženih genoma mitohondrijske DNK kod Kineza prepoznaje se sedam novih lokusa za visinski edem pluća

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Abstract

Background/Aim. High-altitude pulmonary oedema (HAPE), which normally occurs at altitudes higher than 3,000 m, is a potentially fatal disease due to hypoxia. The role of mitochondrial genomes in determining an individual's susceptibility to HAPE has not been determined yet. However, a number of genetic polymorphisms have recently been found to be overrepresented in HAPE patients. The majority of published genome-wide association studies have investigated only a small number of top-ranking single-nucleotide polymorphisms (SNPs)/genes by the overview of nuclear DNA and considered each of the identified SNPs/genes independently. Little research has been conducted on mitochondrial genomes in relapsing HAPE patients by genome-wide association studies. **Methods.** To identify biological pathways important to HAPE occurrence, we examined approximately 500,000 SNPs genome-wide from 10 unrelated cases of relapsing HAPE and we compared the SNPs in these cases with those in the Chinese in Beijing, China population (45 controls) to discover the association between genotypes and HAPE susceptibility among the mitochondrial function-related genes. We used the FUMA platform to expand those SNPs to selected candidate SNPs. **Results.** A total of 369 candidate SNPs, 4 lead SNPs, 4 genomic risk loci and 5 mapped genes were obtained. The 7 mapped genes were ADAMTS9-AS2, NEK1, CLCN3, C4orf27(HPF1), RP11-219J21.2, ANKRD26 and YME1L1. **Conclusion.** This study confirms the association of ADAMTS9-AS2, NEK1, CLCN3, C4orf27(HPF1), RP11-219J21.2, ANKRD26 and YME1L1 with HAPE, which may provide future targets for the treatment of this disease.

Key words:
genetic techniques; genome; hypoxia; polymorphism, genetic; pulmonary oedema; pulmonaty edema of mountaineers.

Apstrakt

Uvod/Cilj. Visinski edem pluća [*high-altitude pulmonary oedema* (HAPE)], koji se obično javlja na visinama većim od 3 000 m usled hipoksije, potencijalno je smrtonosna bolest. Uloga mitohondrijskih genoma u određivanju podložnosti pojedinca na HAPE nije određena. Međutim, nedavno je otkriveno da je veliki broj genetskih polimorfizama prekomerno zastupljen kod bolesnika sa HAPE. Većina objavljenih studija vezanih za genom istraživala je samo mali broj najčešćih jednonukleotidnih polimorfizama [*single-nucleotide polymorphisms* (SNPs)/gena] pregledom nuklearne DNK i razmotrila svaki od identifikovanih SNPs/gena nezavisno. Malo istraživanja je sprovedeno na mitohondrijskim genima kod bolesnika sa recidivantnim HAPE. **Metode.** Da bi se identifikovali biološki putevi važni za pojavu HAPE, ispitano je približno 500 000 SNPs genoma iz 10 nepovezanih slučajeva recidivantnih HAPE, i ti SNPs su upoređeni sa onima iz populacije Kineza, stanovnika Pekinga (n = 45; kontrolna grupa) kako bi se utvrdila povezanost između genotipova i osetljivost na HAPE među genima koji se odnose na funkciju mitohondrija. Korišćena je platforma FUMA u cilju proširenja lepeza SNPs na odabrane SNPs kandidate. **Rezultati.** Ukupno je dobijeno 369 SNPs kandidata, 4 vodeća SNPs, 4 lokusa genomskog rizika i 5 mapiranih gena. Mapirano je ukupno 7 gena: ADAMTS9-AS2, NEK1, CLCN3, C4orf27 (HPF1), RP11-219J21.2, ANKRD26 i IME1L1. **Zaključak.** Studija potvrđuje povezanost ADAMTS9-AS2, NEK1, CLCN3, C4orf27 (HPF1), RP11-219J21.2, ANKRD26 i IME1L1 sa HAPE, što obezbeđuje buduće ciljeve za lečenje te bolesti.

Ključne reči:
genetičke tehnike; genom; hipoksija; polimorfizam, genetički; plućni edem; plućni edem planinara.

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Introduction

High-altitude pulmonary oedema (HAPE) is a kind of pulmonary oedema that occurs primarily in the hypoxic environment at high altitude. HAPE occurs mostly among the residents of low-lying areas who enter the plateau for the first time or when the inhabitants of the plateau enter the higher-altitude areas. The incidence rate is 0.4~2%. Because HAPE has acute onset, rapid progress and causes considerable harm to the body, if the treatment is not timely, it can develop to coma or even death in a relatively short period of time, which seriously threatens life and health [1–4]. HAPE has an obvious susceptibility tendency.

Previous studies have shown that there are significant individual differences in susceptibility to HAPE in the same high-altitude hypoxia environment [5, 6]. Accumulated evidence has suggested that a large number of genetic factors are associated with genetic susceptibility to HAPE, including nitric oxide synthase 3 *(*NOS3), cytochrome b-245 (CYBA), angiotensin converting enzyme (ACE), surfactants A1 and A2, and hypoxia-inducible factor-1 (HIF-1) [5–8]. The genetic analysis of these studies was based on an overview of nuclear DNA. However, the role of mitochondria and their genomes is an area of genetic investigation that has been neglected.

Mitochondria are organelles that produce energy in aerobic cells and contain their own genome. Maintaining a sufficient quantity of mitochondrial DNA (mtDNA) in specific tissues is essential for cell viability. Therefore, many common human diseases, such as cancer [9, 10], cardiomyopathy [11] and liver disease [12], are associated with changing mtDNA levels. In a previous study, we sequenced the mtDNA of *Ochotona curzoniae* (Chinese red pika) and identified 15 novel mtDNA-encoded amino acid changes, including 3 in the subunits of cytochrome c oxidase. These amino acid substitutions may modulate mitochondrial complexes and electron transport efficiency during cold weather conditions and hypoxia adaptation [7]. In another study, we found that the sperm mtDNA copy number for those living at a high altitude (5,300 m) for one month was significantly higher than for those at the lower altitude (1,400 m) or in donors who had been living at the 5,300-m altitude for 1 year [13]. Anyway, the association between mitochondria and HAPE occurrence has not been confirmed.

In addition, with the emergence of genome-wide linkage disequilibrium (LD)–based marker panels and improvements in high-throughput genotyping technology, genome-wide association studies (GWAS) have become feasible [14]. GWAS can systematically survey the whole genome for causal genetic variants for complex traits/diseases and is a powerful tool for dissecting the genetic basis for HAPE. Combining the modest association signals in the GWAS data with the information on biological pathways and networks, the emerging pathway-based approaches can be designed to utilize the GWAS data to a greater extent and are likely to yield new insights into HAPE aetiology.

To identify the important aetiology mechanism of HAPE occurrence more systematically and comprehensively, we used a novel pathway-based GWAS to approximately 871,166 single-nucleotide polymorphisms (SNPs) from 10 unrelated reoccurrence HAPE, which is different from the other studies based on GWAS [15]. Those studies chose the patients appearing for only one time, which cannot demonstrate that these patients have HAPE susceptibility compared with the data of the Chinese in Beijing, China (CHB). Although these patients did not go to high-altitude areas, the incidence rate of HAPE is too low (0.4~2%) to affect CHB as a control group; therefore, we investigated the association between mtDNA function-related genes and HAPE susceptibility.

Methods

Patients and controls

Relapsing HAPE patients (n = 10) were recruited from the Han ethnic group in China. We compared the allele frequency of HAPEs with the CHB population (control = 45) to exclude 185,646 SNPs with minimum allele frequency (MAF) < 0.01. The SNPs with the last successful assay were 673,843. The recurrent HAPE patients consisted of 10 individuals (25.01 ± 10.70 years old) who had at least two episodes of HAPE, as determined by the standard diagnostic criteria [16], including cough and dyspnea at rest, with pulmonary rales, cyanosis, and patchy shadows detected using chest X-ray. Relapsing HAPE patients and controls were unrelated to each other and matched gender and age. This study was approved by the Ethics Committee of the Third Military Medical University in China.

Isolation of DNA

The samples of HAPE patients were collected before using drugs; the venous blood (2 mL) was collected from HAPE patients and healthy controls and placed in EDTA-anticoagulation tubes, which were stored at -80 °C prior to analysis. Genomic DNA was extracted from peripheral blood according to the introduction of Omega DNA extraction kits (Omega, USA). Genomic DNA was tested using gel electrophoresis on a 0.8% agarose gel stained with ethidium bromide.

Genotyping

Affymetrix Genome Wide SNP 6.0 arrays were used following the protocol supplied by the manufacturer (Affymetrix, Santa Clara, CA) at Capital Bio Corporation (Beijing, China). Briefly, 250 ng of genomic DNA was digested with Nsp and Sty enzymes, ligated with specific adaptors, and amplified by polymerase chain reaction (PCR) using the kit primers. The amplicons were purified and quantified. The products were fragmented and labeled, followed by hybridization to the array chips at 48 °C for 16–18 h. Excess unhybridized products were washed and

followed by scanning with a GeneChip Scanner 3000 (Affymetrix, Santa Clara, CA [19481479]). Genotypes were called using the Affymetrix BRLMM algorithm as implemented in the Genotyping Console software (Affymetrix, Santa Clara, CA). All samples had BRLMM call rates greater than the 95% cutoff. We used default parameters for the Birdseed algorithm (version 2) to determine genotypes for all samples (Affymetrix, Santa Clara, CA, USA). Genotypic data were analysed using the Affymetrix Genotyping Console 3.1 (Affymetrix) and included all autosomes but excluded the X and Y chromosomes and mitochondrial genome. Firstly, we performed principal components analysis based on genetic distances as previously described between HAPEs (n = 10) and controls (n = 45). We tested 871,166 SNPs, out of which 177,502 SNPs failed. Then, we compared the allele frequency of HAPEs with the CHB population to exclude 185,646 SNPs with MAF < 0.01. The SNP with the last successful assay was number 673,843.

Statistical analysis

Allele frequencies between the patient and control groups were compared using the χ^2 test. A stringent p value < 5×10–8 was considered significant for GWAS. We used Haploview 4.2 (http://www.broadinstitute.org/haploview) to create a Manhattan plot of p values from the GWAS study. A quantile-quantile (QQ) plot of p values from GWAS was created using R project (http://www.r-project.org). We used the FUMA platform (http://fuma.ctglab.nl/tutorial) to analyse GWAS results and selected SNPs of $p < 10^{-8}$, which was of the GWAS significance [17].

Results

In the GWAS, we genotyped a total of 871,166 SNPs, and 673,843 (77.35%) of SNPs were successfully genotyped. We ranked genotyped SNPs based on the strength of association using the allelic association test. Nominally significant results were detected for 1,558 SNPs ($p < 5 \times 10–8$) (Table 1). This analysis indicates that HAPE patients are genetically similar to the ones from the combined CHB population. HapMap populations provide context for the patterns of variation observed among these populations. Genotyping data yielded an average call rate of 96.6%, and apparent inheritance errors in trio samples were detected in < 0.2% of all SNPs. A Manhattan plot was generated for the SNPs in patients with recurrent HAPE in Figure 1. A quantile-quantile (QQ) plot for association results is provided in Figure 2 for all SNPs. The group of SNPs that slightly deviated from a diagonal straight line in the QQ plot are considered to reflect SNPs with weak genetic effects, and from the plot, it seems that there is not gross inflation of false-positive results derived from genotyping errors. We used the FUMA platform to expand those of SNP $p < 5 \times 10^{-8}$ to SNPs that included their linkage disequilibrium ($r^2 \geq 0.6$). Having imported the data into FUMA, we chose the East Asian population (EAS, consistent with the GWAS population), selected the SNP minimum allele frequency (MAF ≥ 0.01) and r^2 (minimum $r^2 \geq 0.6$). A total of 369 candidate SNPs, 4 lead SNPs, 4 genomic risk loci and 5 mapped genes were obtained. The 7 mapped genes were ADAMTS9-AS2, NEK1, CLCN3, C4orf27(HPF1), RP11-219J21.2, ANKRD26 and YME1L1 (Table 2).

Table 1

Significantly different SNPs between 10 recurrent HAPE cases and 45 Hapmap CHB subjects in the first stage

SNP ID	Chromosome	Position	Band	Allele A	Allele B	min_P_Chi	HWE	MAF
rs4353667	2	162025114	q24.2	A	G	4.099E-19	0.940	0.011
rs509193	13	101618897	q33.1	C	G	2.021E-16	0.572	0.078
rs890527	3	142257543	q23	A	T	3.287E-15	0.879	0.022
rs12593141	15	25878695	q13.1	C	T	6.838E-15	0.402	0.111
rs744306	3	186272442	q27.2	A	G	1.632E-14	0.693	0.056
rs9470449	6	37055364	p21.2	A	G	2.140E-14	0.939	0.012
rs4810414	20	42306337	q13.12	C	G	5.139E-14	0.122	0.133
rs10016530	4	184061978	q35.1	A	C	4.779E-13	0.940	0.011
rs8010479	14	80195033	q31.1	C	T	4.779E-13	0.940	0.011
rs2505465	10	26080532	p12.1	A	G	5.504E-13	0.693	0.056
rs12796975	11	132811275	q25	C	T	3.196E-12	0.755	0.044
rs7948049	11	98403015	q22.1	A	C	3.620E-12	0.701	0.189
rs2904699	8	17135169	p22	A	G	3.672E-12	0.362	0.100
rs7929194	11	62269326	q12.3	C	T	4.884E-12	0.318	0.159
rs10075708	5	35582672	p13.2	A	G	5.540E-12	0.940	0.011
rs9364178	6	168952425	q27	A	G	1.235E-11	0.693	0.056
rs3785499	17	17355942	p11.2	A	G	2.244E-11	0.879	0.022
rs7523787	1	94103203	p22.1	A	G	2.927E-11	0.456	0.100
rs6471504	8	96060736	q22.1	C	T	3.137E-11	0.502	0.222
rs1992305	7	41347571	p14.1	C	G	3.419E-11	0.000	0.022
rs9668938	12	9405128	p13.31	A	G	3.419E-11	0.000	0.022
rs8046088	16	77670982	q23.1	A	T	3.419E-11	0.000	0.500
rs1484545	3	641971	p26.3	A	G	3.819E-11	0.940	0.011
rs7199767	16	81560851	q23.3	C	G	3.950E-11	0.879	0.022

Table 1 (continued)

SNP ID	Chromosome	Position	Band	Allele A	Allele B	min_P_Chi	HWE	MAF
rs1536688	9	16119553	p22.3	A	G	4.179E-11	0.000	0.500
rs2132766	4	78019649	q21.1	C	T	5.684E-11	0.001	0.044
rs4707773	6	93740627	q16.1	A	C	6.125E-11	0.708	0.233
rs2253804	17	45710559	q21.33	A	G	6.770E-11	0.201	0.144
rs3780410	9	4588116	p24.2	C	G	6.838E-11	0.000	0.022
rs907425	8	57038845	q12.1	A	G	9.168E-11	0.675	0.239
rs6020381	20	48277755	q13.13	A	C	1.169E-10	0.578	0.244
rs13379947	15	59972093	q22.2	A	G	1.269E-10	0.996	0.211
rs4799715	18	29531002	q12.1	C	T	1.465E-10	0.701	0.189
rs803302	1	25328122	p36.11	A	G	1.880E-10	0.000	0.022
rs11577001	1	192870487	q31.3	C	T	1.880E-10	0.000	0.022
rs4428669	8	22951725	p21.3	A	T	1.880E-10	0.000	0.022
rs784814	14	47539712	q21.3	C	T	1.880E-10	0.000	0.022
rs16967738	17	37799793	q21.2	A	G	1.880E-10	0.000	0.022
rs7275393	21	40817980	q22.2	G	T	1.880E-10	0.000	0.022
rs11860414	16	13097760	p13.12	C	T	2.257E-10	0.000	0.023
rs6705908	2	238098704	q37.3	A	G	2.998E-10	0.227	0.151
rs17024521	1	120268277	p12	C	G	3.761E-10	0.000	0.033
rs9498354	6	149804544	q25.1	A	G	3.761E-10	0.000	0.033
rs13258727	8	16617623	p22	G	T	3.761E-10	0.000	0.033
rs497022	10	85442083	q23.1	C	T	3.761E-10	0.000	0.033
rs11051790	12	32132279	p11.21	C	G	3.761E-10	0.000	0.033
rs2941948	16	77117341	q23.1	C	G	3.761E-10	0.000	0.033
rs907661	1	117548617	p13.1	A	T	3.761E-10	0.940	0.011
rs2581409	1	112577867	p13.2	A	G	3.761E-10	0.940	0.011
rs10776807	1	109757679	p13.3	A	G	3.761E-10	0.940	0.011
rs12127734	1	102738259	p21.1	C	T	3.761E-10	0.940	0.011
rs1931256	1	95930004	p21.3	A	C	3.761E-10	0.940	0.011
rs6420974	1	86496645	p22.3	A	C	3.761E-10	0.940	0.011
rs6424623	1	79258910	p31.1	A	T	3.761E-10	0.940	0.011
rs12121720	1	75159525	p31.1	C	T	3.761E-10	0.940	0.011
rs10157120	1	52983476	p32.3	A	G	3.761E-10	0.940	0.011
rs7525612	1	47664398	p33	C	T	3.761E-10	0.940	0.011
rs41524944	1	44894612	p34.1	C	T	3.761E-10	0.940	0.011
rs2816602	1	43040557	p34.2	C	T	3.761E-10	0.940	0.011
rs2182111	1	29637387	p35.3	A	T	3.761E-10	0.940	0.011
rs2746535	1	17264939	p36.13	C	T	3.761E-10	0.940	0.011
rs16862547	1	19316539	p36.13	C	T	3.761E-10	0.940	0.011
rs6703014	1	151806944	q21.3	A	G	3.761E-10	0.940	0.011
rs10752607	1	152983427	q21.3	C	T	3.761E-10	0.940	0.011
rs6702567	1	157784484	q23.2	A	G	3.761E-10	0.940	0.011
rs1288913	1	161882823	q23.3	C	T	3.761E-10	0.940	0.011
rs4987357	1	167932764	q24.2	C	T	3.761E-10	0.940	0.011
rs12117954	1	170933444	q24.3	G	T	3.761E-10	0.940	0.011
rs539038	1	189048657	q31.2	A	G	3.761E-10	0.940	0.011
rs613232	1	209836516	q32.3	C	T	3.761E-10	0.940	0.011
rs714214	1	228825228	q42.2	C	T	3.761E-10	0.940	0.011
rs4658949	1	230014942	q42.2	A	C	3.761E-10	0.940	0.011
rs6665236	1	246060280	q44	A	G	3.761E-10	0.940	0.011
rs4852883	2	72708531	p13.2	C	T	3.761E-10	0.940	0.011
rs262501	2	63712161	p15	A	G	3.761E-10	0.940	0.011
rs6751340	2	54041121	p16.2	A	G	3.761E-10	0.940	0.011
rs17389310	2	42343095	p21	C	G	3.761E-10	0.940	0.011
rs13416119	2	42316434	p21	A	G	3.761E-10	0.940	0.011
rs17024325	2	39845266	p22.1	C	G	3.761E-10	0.940	0.011
rs4648234	2	37191174	p22.2	A	G	3.761E-10	0.940	0.011
rs12104627	2	35364483	p22.3	A	T	3.761E-10	0.940	0.011
rs11893869	2	106032330	q12.2	A	G	3.761E-10	0.940	0.011
rs260711	2	108923531	q13	C	T	3.761E-10	0.940	0.011
rs17783857	2	140102541	q22.1	C	G	3.761E-10	0.940	0.011
rs10185178	2	171064520	q31.1	A	G	3.761E-10	0.940	0.011
rs3914402	2	174296267	q31.1	C	G	3.761E-10	0.940	0.011

Table 1 (continued)

SNP ID	Chromosome	Position	Band	Allele A	Allele B	min_P_Chi	HWE	MAF
rs12989588	2	194838617	q32.3	A	G	3.761E-10	0.940	0.011
rs16842071	2	201639975	q33.1	A	G	3.761E-10	0.940	0.011
rs11902586	2	213683899	q34	C	G	3.761E-10	0.940	0.011
rs11898042	2	220596890	q35	A	G	3.761E-10	0.940	0.011
rs6431283	2	233888576	q37.1	C	T	3.761E-10	0.940	0.011
rs10175460	2	231048405	q37.1	A	G	3.761E-10	0.940	0.011
rs10933609	2	241092142	q37.3	A	G	3.761E-10	0.940	0.011
rs6548631	3	79729007	p12.3	C	G	3.761E-10	0.940	0.011
rs9847658	3	70073539	p14.1	A	C	3.761E-10	0.940	0.011
rs755358	3	62509509	p14.2	C	T	3.761E-10	0.940	0.011
rs9830403	3	27938612	p24.1	C	G	3.761E-10	0.940	0.011
rs778044	3	10255233	p25.3	C	T	3.761E-10	0.940	0.011
rs352748	3	6615700	p26.1	C	G	3.761E-10	0.940	0.011
rs1144107	3	101924406	q12.2	C	T	3.761E-10	0.940	0.011
rs2056534	3	115966848	q13.31	A	G	3.761E-10	0.940	0.011
rs13326852	3	121649170	q13.33	C	T	3.761E-10	0.940	0.011
rs6769033	3	137066778	q22.2	C	T	3.761E-10	0.940	0.011
rs344076	3	158035479	q25.31	C	T	3.761E-10	0.940	0.011
rs2566339	3	159791569	q25.32	C	T	3.761E-10	0.940	0.011
rs16846456	3	174240032	q26.31	C	T	3.761E-10	0.940	0.011
rs6788878	3	178926662	q26.32	G	T	3.761E-10	0.940	0.011
rs10002498	4	47623342	p12	C	G	3.761E-10	0.940	0.011
rs5743591	4	38479523	p14	C	G	3.761E-10	0.940	0.011
rs13105862	4	36976442	p14	C	T	3.761E-10	0.940	0.011
rs41339448	4	19206250	p15.31	A	G	3.761E-10	0.940	0.011
rs13148734	4	63013453	q13.1	A	G	3.761E-10	0.940	0.011
rs313139	4	127754207	q28.1	C	G	3.761E-10	0.940	0.011
rs1201202	4	152060202	q31.3	A	G	3.761E-10	0.940	0.011
rs1594869	4	158681812	q32.1	A	G	3.761E-10	0.940	0.011
rs17628308	4	171106945	q33	A	G	3.761E-10	0.940	0.011
rs2173826	4	170922763	q33	A	G	3.761E-10	0.940	0.011
rs17057309	4	172849798	q34.1	C	T	3.761E-10	0.940	0.011
rs17074536	4	184417378	q35.1	C	T	3.761E-10	0.940	0.011
rs4862023	4	183246608	q35.1	A	C	3.761E-10	0.940	0.011
rs6879532	5	23092333	p14.3	A	G	3.761E-10	0.940	0.011
rs17295893	5	14125258	p15.2	C	T	3.761E-10	0.940	0.011
rs10472006	5	56791259	q11.2	C	T	3.761E-10	0.940	0.011
rs158342	5	55661090	q11.2	A	C	3.761E-10	0.940	0.011
rs10057147	5	53473290	q11.2	A	G	3.761E-10	0.940	0.011
rs255233	5	56633746	q11.2	C	T	3.761E-10	0.940	0.011
rs6896756	5	66947893	q13.1	C	T	3.761E-10	0.940	0.011
rs11959381	5	75724016	q13.3	C	T	3.761E-10	0.940	0.011
rs16902631	5	86679983	q14.3	A	T	3.761E-10	0.940	0.011
rs2963029	5	108782510	q21.3	C	G	3.761E-10	0.940	0.011
rs4272129	5	124365847	q23.2	C	T	3.761E-10	0.940	0.011
rs7707878	5	126011942	q23.2	A	C	3.761E-10	0.940	0.011
rs3861854	5	141280553	q31.3	C	T	3.761E-10	0.940	0.011
rs1432672	5	143945814	q32	C	T	3.761E-10	0.940	0.011
rs10037531	5	156738482	q33.3	A	G	3.761E-10	0.940	0.011
rs4868935	5	164941974	q34	A	G	3.761E-10	0.940	0.011
rs10462997	5	169942958	q35.1	C	T	3.761E-10	0.940	0.011
rs10067345	5	171183175	q35.1	A	G	3.761E-10	0.940	0.011
rs10039715	5	173603095	q35.2	C	T	3.761E-10	0.940	0.011
rs3129704	6	30342679	p21.33	C	T	3.761E-10	0.940	0.011
rs7767176	6	28033346	p22.1	C	T	3.761E-10	0.940	0.011
rs10484632	6	20755639	p22.3	A	C	3.761E-10	0.940	0.011
rs13206084	6	16653930	p22.3	A	G	3.761E-10	0.940	0.011
rs11969660	6	14503352	p23	A	G	3.761E-10	0.940	0.011
rs6919114	6	10780583	p24.2	A	G	3.761E-10	0.940	0.011
rs3804481	6	6577398	p25.1	A	G	3.761E-10	0.940	0.011
rs2110903	6	107679904	q21	G	T	3.761E-10	0.940	0.011
rs3757302	6	108478901	q21	C	G	3.761E-10	0.940	0.011

Table 1 (continued)

SNP ID	Chromosome	Position	Band	Allele A	Allele B	min_P_Chi	HWE	MAF
rs6913809	6	113957665	q22.1	A	C	3.761E-10	0.940	0.011
rs6569290	6	123195382	q22.31	A	G	3.761E-10	0.940	0.011
rs12110924	6	118674618	q22.31	C	G	3.761E-10	0.940	0.011
rs12205922	6	128127367	q22.33	A	G	3.761E-10	0.940	0.011
rs9480356	6	156948860	q25.3	A	G	3.761E-10	0.940	0.011
rs10486806	7	40468520	p14.1	A	G	3.761E-10	0.940	0.011
rs12536300	7	33159362	p14.3	A	G	3.761E-10	0.940	0.011
rs17675986	7	29077382	p15.1	A	T	3.761E-10	0.940	0.011
rs10251505	7	7221014	p21.3	A	G	3.761E-10	0.940	0.011
rs1207867	7	78239513	q21.11	A	G	3.761E-10	0.940	0.011
rs7802018	7	94898249	q21.3	A	G	3.761E-10	0.940	0.011
rs1558005	7	100936342	q22.1	A	G	3.761E-10	0.940	0.011
rs10252737	7	101486484	q22.1	A	C	3.761E-10	0.940	0.011
rs13231181	7	103979084	q22.1	C	T	3.761E-10	0.940	0.011
rs10261618	7	136853662	q33	A	C	3.761E-10	0.940	0.011
rs4335058	7	132550141	q33	A	C	3.761E-10	0.940	0.011
rs851734	7	146993038	q35	C	G	3.761E-10	0.940	0.011
rs6967282	7	150538127	q36.1	A	G	3.761E-10	0.940	0.011
rs2101138	8	26186805	p21.2	C	G	3.761E-10	0.940	0.011
rs2410675	8	20915740	p21.3	G	T	3.761E-10	0.940	0.011
rs369240	8	55686306	q12.1	C	T	3.761E-10	0.940	0.011
rs35711827	8	76793565	q21.11	G	T	3.761E-10	0.940	0.011
rs1448676	8	92396335	q21.3	A	C	3.761E-10	0.940	0.011
rs16870588	8	104706458	q22.3	C	G	3.761E-10	0.940	0.011
rs3018507	8	103347864	q22.3	C	G	3.761E-10	0.940	0.011
rs7826950	8	134980387	q24.22	A	C	3.761E-10	0.940	0.011
rs10088738	8	139205255	q24.23	A	G	3.761E-10	0.940	0.011
rs17247766	9	33098605	p13.3	G	T	3.761E-10	0.940	0.011
rs1885170	9	17554267	p22.2	C	T	3.761E-10	0.940	0.011
rs13285034	9	74559353	q21.13	A	T	3.761E-10	0.940	0.011
rs10993086	9	95990540	q22.32	G	T	3.761E-10	0.940	0.011
rs10441773	9	107233498	q31.2	C	T	3.761E-10	0.940	0.011
rs12553905	9	121402295	q33.1	C	T	3.761E-10	0.940	0.011
rs16929767	9	129113684	q33.3	A	T	3.761E-10	0.940	0.011
rs3011286	9	134883811	q34.13	C	T	3.761E-10	0.940	0.011
rs2643955	10	29197524	p11.23	G	T	3.761E-10	0.940	0.011
rs11015156	10	26863974	p12.1	G	T	3.761E-10	0.940	0.011
rs661882	10	27808089	p12.1	A	G	3.761E-10	0.940	0.011
rs17465850	10	17812128	p12.33	A	G	3.761E-10	0.940	0.011
rs12358414	10	3707846	p15.2	C	T	3.761E-10	0.940	0.011
rs17501883	10	44506780	q11.21	A	C	3.761E-10	0.940	0.011
rs11001982	10	78468130	q22.3	A	G	3.761E-10	0.940	0.011
rs17334741	10	90168436	q23.31	A	C	3.761E-10	0.940	0.011
rs11597377	10	121733856	q26.12	C	T	3.761E-10	0.940	0.011
rs17594946	10	122702917	q26.12	A	C	3.761E-10	0.940	0.011
rs12412522	10	122789916	q26.12	C	T	3.761E-10	0.940	0.011
rs2818393	10	133792619	q26.3	A	G	3.761E-10	0.940	0.011
rs4755364	11	34249101	p13	A	G	3.761E-10	0.940	0.011
rs1482734	11	23211390	p14.3	A	T	3.761E-10	0.940	0.011
rs7939809	11	13862425	p15.2	C	G	3.761E-10	0.940	0.011
rs12807017	11	9635721	p15.4	A	G	3.761E-10	0.940	0.011
rs17704641	11	60939964	q12.2	C	T	3.761E-10	0.940	0.011
rs3017605	11	61017594	q12.2	A	C	3.761E-10	0.940	0.011
rs632280	11	78178911	q14.1	G	T	3.761E-10	0.940	0.011
rs7121003	11	86964252	q14.2	A	C	3.761E-10	0.940	0.011
rs4512880	11	86955572	q14.2	A	G	3.761E-10	0.940	0.011
rs655922	11	100153283	q22.1	G	T	3.761E-10	0.940	0.011
rs522819	11	100460929	q22.1	A	G	3.761E-10	0.940	0.011
rs7113906	11	101758880	q22.2	C	T	3.761E-10	0.940	0.011
rs1375423	11	104601723	q22.3	A	T	3.761E-10	0.940	0.011
rs1902238	11	106468971	q22.3	C	T	3.761E-10	0.940	0.011
rs7122110	11	120527150	q23.3	A	G	3.761E-10	0.940	0.011

Table 1 (continued)

SNP ID	Chromosome	Position	Band	Allele A	Allele B	min_P_Chi	HWE	MAF
rs11216478	11	117016434	q23.3	A	G	3.761E-10	0.940	0.011
rs41507249	11	122112574	q24.1	C	T	3.761E-10	0.940	0.011
rs583194	11	125456998	q24.2	C	T	3.761E-10	0.940	0.011
rs10894844	11	133952614	q25	C	T	3.761E-10	0.940	0.011
rs17472165	12	26494853	p11.23	C	T	3.761E-10	0.940	0.011
rs3863355	12	25850114	p12.1	C	T	3.761E-10	0.940	0.011
rs4350408	12	22043980	p12.1	G	T	3.761E-10	0.940	0.011
rs11045107	12	20220645	p12.2	G	T	3.761E-10	0.940	0.011
rs6487064	12	20226964	p12.2	G	T	3.761E-10	0.940	0.011
rs16915116	12	19186252	p12.3	A	T	3.761E-10	0.940	0.011
rs12307636	12	9512800	p13.31	C	T	3.761E-10	0.940	0.011
rs1805731	12	8986493	p13.31	A	G	3.761E-10	0.940	0.011
rs7312896	12	662066	p13.33	C	T	3.761E-10	0.940	0.011
rs9325199	12	70273227	q21.1	A	C	3.761E-10	0.940	0.011
rs310836	12	76001666	q21.2	C	T	3.761E-10	0.940	0.011
rs4143188	12	81326916	q21.31	A	C	3.761E-10	0.940	0.011
rs10777572	12	92977940	q22	A	T	3.761E-10	0.940	0.011
rs9669774	12	113260569	q24.21	C	G	3.761E-10	0.940	0.011
rs17441172	12	117352644	q24.23	C	T	3.761E-10	0.940	0.011
rs7298854	12	125553390	q24.32	A	C	3.761E-10	0.940	0.011
rs10847172	12	125560866	q24.32	A	G	3.761E-10	0.940	0.011
rs9314935	13	28583729	q12.3	A	G	3.761E-10	0.940	0.011
rs9548515	13	38338848	q13.3	A	C	3.761E-10	0.940	0.011
rs2503454	13	46987969	q14.2	A	G	3.761E-10	0.940	0.011
rs12429341	13	47347285	q14.2	A	G	3.761E-10	0.940	0.011
rs17060868	13	61588183	q21.31	A	C	3.761E-10	0.940	0.011
rs9516058	13	91762201	q31.3	A	G	3.761E-10	0.940	0.011
rs9514865	13	107995471	q33.3	C	T	3.761E-10	0.940	0.011
rs6650482	13	111970835	q34	A	G	3.761E-10	0.940	0.011
rs7160516	14	43848866	q21.3	A	G	3.761E-10	0.940	0.011
rs10484082	14	51162516	q22.1	C	T	3.761E-10	0.940	0.011
rs17107847	14	78091511	q24.3	G	T	3.761E-10	0.940	0.011
rs6574673	14	81183387	q31.1	G	T	3.761E-10	0.940	0.011
rs6574612	14	80473827	q31.1	C	T	3.761E-10	0.940	0.011
rs4905612	14	97248380	q32.2	A	G	3.761E-10	0.940	0.011
rs4924188	15	35766234	q14	A	G	3.761E-10	0.940	0.011
rs8041819	15	50401611	q21.2	A	G	3.761E-10	0.940	0.011
rs11858794	15	57498627	q22.2	A	G	3.761E-10	0.940	0.011
rs1912049	15	61942659	q22.31	G	T	3.761E-10	0.940	0.011
rs7171610	15	63227145	q22.31	A	T	3.761E-10	0.940	0.011
rs11630776	15	76234845	q25.1	C	G	3.761E-10	0.940	0.011
rs9944345	16	49976666	q12.1	C	G	3.761E-10	0.940	0.011
rs2058673	16	45580279	q12.1	A	G	3.761E-10	0.940	0.011
rs12597729	16	49769655	q12.1	C	G	3.761E-10	0.940	0.011
rs13332434	16	58632433	q21	A	C	3.761E-10	0.940	0.011
rs16957304	16	65892470	q22.1	C	T	3.761E-10	0.940	0.011
rs9935976	16	85593861	q24.1	C	T	3.761E-10	0.940	0.011
rs6540041	16	85961876	q24.2	A	T	3.761E-10	0.940	0.011
rs1015218	17	20673956	p11.2	C	G	3.761E-10	0.940	0.011
rs7503902	17	59833749	q23.3	A	G	3.761E-10	0.940	0.011
rs12150174	17	62856936	q24.2	C	T	3.761E-10	0.940	0.011
rs6501586	17	68456801	q25.1	A	T	3.761E-10	0.940	0.011
rs4006794	17	69999430	q25.1	C	T	3.761E-10	0.940	0.011
rs610541	18	11960809	p11.21	C	T	3.761E-10	0.940	0.011
rs566559	18	5960704	p11.31	C	G	3.761E-10	0.940	0.011
rs9646461	18	4075990	p11.31	A	G	3.761E-10	0.940	0.011
rs2846834	18	861268	p11.32	C	T	3.761E-10	0.940	0.011
rs1623716	18	30665290	q12.1	A	C	3.761E-10	0.940	0.011
rs1790534	18	30663569	q12.1	A	G	3.761E-10	0.940	0.011
rs11873775	18	24417919	q12.1	C	T	3.761E-10	0.940	0.011
rs654975	18	58418480	q21.33	G	T	3.761E-10	0.940	0.011
rs1704816	18	62280193	q22.1	C	T	3.761E-10	0.940	0.011

Table 1 (continued)

SNP ID	Chromosome	Position	Band	Allele A	Allele B	min_P_Chi	HWE	MAF
rs12962239	18	73493166	q23	A	G	3.761E-10	0.940	0.011
rs12981996	19	20342025	p12	A	T	3.761E-10	0.940	0.011
rs16996008	19	19226400	p13.11	A	G	3.761E-10	0.940	0.011
rs6511939	19	14545425	p13.12	A	G	3.761E-10	0.940	0.011
rs11672838	19	14948335	p13.12	C	T	3.761E-10	0.940	0.011
rs7003	19	14486790	p13.12	C	T	3.761E-10	0.940	0.011
rs12983312	19	10190245	p13.2	C	T	3.761E-10	0.940	0.011
rs407743	19	6593417	p13.3	C	G	3.761E-10	0.940	0.011
rs1558133	19	1253965	p13.3	C	T	3.761E-10	0.940	0.011
rs8112607	19	38162646	q13.11	C	G	3.761E-10	0.940	0.011
rs1661906	19	58201490	q13.41	A	T	3.761E-10	0.940	0.011
rs6510101	19	62999086	q13.43	G	T	3.761E-10	0.940	0.011
rs6042568	20	1418343	p13	C	T	3.761E-10	0.940	0.011
rs13041282	20	29836903	q11.21	G	T	3.761E-10	0.940	0.011
rs2868093	20	42397212	q13.12	C	T	3.761E-10	0.940	0.011
rs6073310	20	42139597	q13.12	A	G	3.761E-10	0.940	0.011
rs928072	20	48368185	q13.13	A	G	3.761E-10	0.940	0.011
rs6020818	20	48926335	q13.13	C	G	3.761E-10	0.940	0.011
rs1980424	21	15164448	q11.2	A	G	3.761E-10	0.940	0.011
rs13048221	21	14381307	q11.2	A	G	3.761E-10	0.940	0.011
rs551680	21	39876578	q22.2	A	G	3.761E-10	0.940	0.011
rs2535708	22	16564169	q11.21	A	G	3.761E-10	0.940	0.011
rs7293008	22	27772666	q12.1	C	T	3.761E-10	0.940	0.011
rs3730114	22	24421306	q12.1	C	T	3.761E-10	0.940	0.011
rs17834914	22	45605985	q13.31	A	G	3.761E-10	0.940	0.011
rs8137937	22	45846062	q13.31	C	G	3.761E-10	0.940	0.011
rs243505	7	148066272	q36.1	A	G	4.008E-10	0.360	0.267
rs7119096	11	127453448	q24.3	C	T	4.429E-10	0.649	0.122
rs7872136	9	85091738	q21.32	A	G	4.597E-10	0.939	0.011
rs4584989	2	108686189	q13	C	T	4.597E-10	0.939	0.011
rs4378452	12	109988416	q24.11	A	G	4.597E-10	0.939	0.011
rs8130198	21	42503393	q22.3	C	T	4.597E-10	0.939	0.011
rs7909124	10	97709510	q23.33	C	G	5.641E-10	0.939	0.012
rs17261573	2	80528623	p12	C	G	5.868E-10	0.996	0.211
rs6762195	3	126740626	q21.2	C	T	7.214E-10	0.726	0.278
rs11199331	10	122174433	q26.12	A	T	8.142E-10	0.290	0.244
rs6854931	4	6828065	p16.1	A	G	1.034E-09	0.940	0.011
rs6720335	2	233540064	q37.1	A	G	1.128E-09	0.000	0.044
rs41453247	14	54982693	q22.3	A	G	1.128E-09	0.000	0.500
rs581459	1	36147697	p34.3	C	T	1.129E-09	0.848	0.222
rs250238	5	50302287	q11.1	A	C	1.129E-09	0.502	0.222
rs16992471	19	4591295	p13.3	A	C	1.129E-09	0.502	0.222
rs241301	1	227029050	q42.13	C	T	1.276E-09	0.103	0.289
rs2078330	16	73137556	q22.3	C	T	1.276E-09	0.859	0.289
rs8100750	19	55775407	q13.33	C	T	1.462E-09	0.667	0.178
rs7221423	17	78551921	q25.3	C	T	1.561E-09	0.130	0.078
rs8118315	20	4109500	p13	C	T	1.573E-09	0.000	0.489
rs17483466	2	111513929	q13	A	G	1.880E-09	0.940	0.011
rs9878562	3	53864028	p21.1	C	T	1.880E-09	0.000	0.033
rs31745	5	140400408	q31.3	A	G	1.880E-09	0.000	0.033
rs1778894	9	125595350	q33.2	A	C	1.880E-09	0.940	0.011
rs10501627	11	86029148	q14.2	A	C	1.880E-09	0.000	0.033
rs568739	11	127565639	q24.3	A	G	1.880E-09	0.940	0.011
rs7142084	14	91892784	q32.12	C	T	1.880E-09	0.000	0.033
rs11854845	15	69688499	q23	A	G	1.880E-09	0.940	0.011
rs11806573	1	62591934	p31.3	A	C	1.880E-09	0.940	0.011
rs473223	1	54896976	p32.3	A	G	1.880E-09	0.940	0.011
rs12066062	1	149925647	q21.3	C	T	1.880E-09	0.940	0.011
rs11583867	1	183984337	q25.3	A	G	1.880E-09	0.940	0.011
rs2867890	1	203736379	q32.1	A	G	1.880E-09	0.940	0.011
rs12731771	1	202027279	q32.1	C	T	1.880E-09	0.940	0.011
rs11118935	1	206171611	q32.2	A	G	1.880E-09	0.940	0.011

Table 1 (continued)

SNP ID	Chromosome	Position	Band	Allele A	Allele B	min_P_Chi	HWE	MAF
rs12074002	1	209897308	q32.3	C	G	1.880E-09	0.940	0.011
rs2965012	1	216853172	q41	G	T	1.880E-09	0.940	0.011
rs6696165	1	242834795	q44	C	T	1.880E-09	0.940	0.011
rs11125521	2	54205862	p16.2	A	T	1.880E-09	0.940	0.011
rs1403450	2	45696779	p21	C	T	1.880E-09	0.940	0.011
rs908679	2	22283114	p24.1	A	G	1.880E-09	0.940	0.011
rs1983376	2	17289515	p24.2	A	C	1.880E-09	0.940	0.011
rs11889931	2	106141807	q12.2	C	T	1.880E-09	0.940	0.011
rs13021341	2	144247607	q22.2	C	T	1.880E-09	0.940	0.011
rs1113988	2	168059681	q24.3	A	C	1.880E-09	0.940	0.011
rs3914752	2	170833364	q31.1	A	C	1.880E-09	0.940	0.011
rs10179515	2	212255007	q34	C	G	1.880E-09	0.940	0.011
rs1082901	3	77834657	p12.3	A	G	1.880E-09	0.940	0.011
rs1502616	3	59505361	p14.2	C	T	1.880E-09	0.940	0.011
rs9845785	3	31504110	p23	C	G	1.880E-09	0.940	0.011
rs17015506	3	24956816	p24.2	A	G	1.880E-09	0.940	0.011
rs17036852	3	12518475	p25.1	A	G	1.880E-09	0.940	0.011
rs9864656	3	137126228	q22.2	C	T	1.880E-09	0.940	0.011
rs7639012	3	155697801	q25.2	G	T	1.880E-09	0.940	0.011
rs16832690	3	183003503	q26.33	A	T	1.880E-09	0.940	0.011
rs17513709	4	40496876	p14	G	T	1.880E-09	0.940	0.011
rs6831500	4	17810438	p15.32	C	T	1.880E-09	0.940	0.011
rs17592868	4	68897521	q13.2	C	T	1.880E-09	0.940	0.011
rs3792662	4	95689234	q22.3	C	G	1.880E-09	0.940	0.011
rs10517681	4	159059047	q32.1	A	C	1.880E-09	0.940	0.011
rs11723043	4	189744112	q35.2	C	T	1.880E-09	0.940	0.011
rs16901423	5	31715101	p13.3	A	G	1.880E-09	0.940	0.011
rs13362111	5	33328915	p13.3	C	G	1.880E-09	0.940	0.011
rs7734697	5	7469304	p15.31	A	T	1.880E-09	0.940	0.011
rs2897554	5	81311997	q14.2	C	T	1.880E-09	0.940	0.011
rs41459348	5	94239098	q15	C	T	1.880E-09	0.940	0.011
rs10477915	5	107955270	q21.3	C	T	1.880E-09	0.940	0.011
rs10042652	5	141636901	q31.3	G	T	1.880E-09	0.940	0.011
rs10072565	5	166242667	q34	A	G	1.880E-09	0.940	0.011
rs9313568	5	171344886	q35.1	A	C	1.880E-09	0.940	0.011
rs6867969	5	172157416	q35.1	C	T	1.880E-09	0.940	0.011
rs13156607	5	168832565	q35.1	C	T	1.880E-09	0.940	0.011
rs9475536	6	56008167	p12.1	C	G	1.880E-09	0.940	0.011
rs513248	6	53546485	p12.1	A	G	1.880E-09	0.940	0.011
rs7766333	6	25070202	p22.2	A	G	1.880E-09	0.940	0.011
rs6900027	6	10760336	p24.2	A	G	1.880E-09	0.940	0.011
rs10455706	6	71345716	q13	C	T	1.880E-09	0.940	0.011
rs10944336	6	88718737	q15	C	T	1.880E-09	0.940	0.011
rs9489754	6	98342750	q16.1	A	C	1.880E-09	0.940	0.011
rs4377817	6	115194976	q22.1	C	T	1.880E-09	0.940	0.011
rs17250161	6	153849770	q25.2	C	G	1.880E-09	0.940	0.011
rs1737317	6	163709828	q26	A	G	1.880E-09	0.940	0.011
rs856588	7	46703840	p12.3	C	T	1.880E-09	0.940	0.011
rs11979904	7	38684422	p14.1	A	C	1.880E-09	0.940	0.011
rs10257031	7	35907991	p14.2	A	C	1.880E-09	0.940	0.011
rs2098273	7	36484536	p14.2	C	T	1.880E-09	0.940	0.011
rs17457143	7	20559116	p15.3	C	G	1.880E-09	0.940	0.011
rs3807573	7	5636086	p22.1	C	T	1.880E-09	0.940	0.011
rs6463483	7	5497369	p22.1	C	T	1.880E-09	0.940	0.011
rs6460734	7	71597254	q11.22	C	G	1.880E-09	0.940	0.011
rs4730058	7	104347376	q22.1	C	T	1.880E-09	0.940	0.011
rs706561	7	136925970	q33	C	G	1.880E-09	0.940	0.011
rs17667159	7	156988826	q36.3	A	C	1.880E-09	0.940	0.011
rs17595134	8	40076812	p11.21	C	G	1.880E-09	0.940	0.011
rs7822050	8	72730829	q13.3	C	T	1.880E-09	0.940	0.011
rs16938568	8	74209396	q21.11	C	T	1.880E-09	0.940	0.011
rs16874193	8	107268534	q23.1	A	C	1.880E-09	0.940	0.011

Table 1 (continued)

SNP ID	Chromosome	Position	Band	Allele A	Allele B	min_P_Chi	HWE	MAF
rs2799753	9	38475256	p13.1	A	T	1.880E-09	0.940	0.011
rs7021837	9	13844176	p23	A	G	1.880E-09	0.940	0.011
rs10959547	9	11110180	p23	C	G	1.880E-09	0.940	0.011
rs35613585	9	74634393	q21.13	C	G	1.880E-09	0.940	0.011
rs1330288	9	74626903	q21.13	A	G	1.880E-09	0.940	0.011
rs12686427	9	88530367	q21.33	C	T	1.880E-09	0.940	0.011
rs4314720	9	112411728	q31.3	C	T	1.880E-09	0.940	0.011
rs41407147	9	121795392	q33.1	G	T	1.880E-09	0.940	0.011
rs12554146	9	133317958	q34.13	A	T	1.880E-09	0.940	0.011
rs2797468	10	29197311	p11.23	A	C	1.880E-09	0.940	0.011
rs16926660	10	26523271	p12.1	C	T	1.880E-09	0.940	0.011
rs11256585	10	10468085	p14	C	T	1.880E-09	0.940	0.011
rs1005907	10	4863106	p15.1	A	G	1.880E-09	0.940	0.011
rs4881163	10	3395755	p15.2	C	G	1.880E-09	0.940	0.011
rs12242220	10	49698112	q11.22	C	T	1.880E-09	0.940	0.011
rs17500631	10	52297578	q11.23	G	T	1.880E-09	0.940	0.011

SNPs – single-nucleotide polymorphisms; HAPE – high-altitude pulmonary oedema; CHB – Chinese in Beijing, China; HWE – Hardy-Weinberg Equilibrium; MAF – minimum allele frequency.

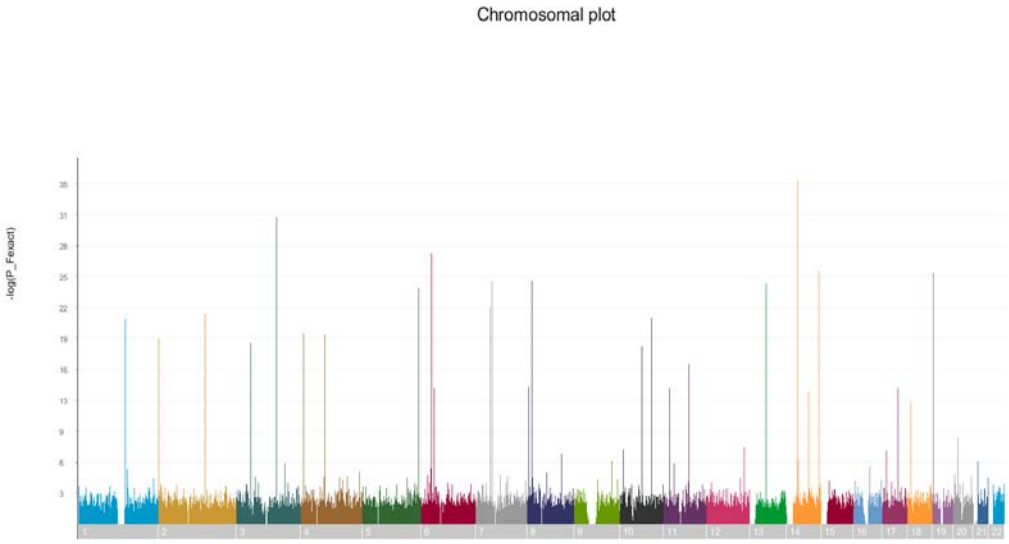

Fig. 1 – Manhattan plot for the whole single-nucleotide polymorphisms (SNPs) in recurrent high-altitude pulmonary oedema (HAPE) subjects of Chinese Han descent. Demonstrating the distribution of *p* values of the Fisher's exact test in the whole genome under four genetic models of allele, genotype, recessive and dominant. The horizontal axis is the physical position of each SNP, and the vertical axis is the negative logarithm of the *p* value.

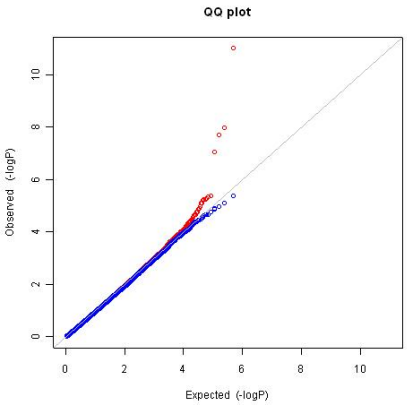

Fig. 2 – Quantile-quantile (QQ) plot for association results of the first-stage analysis (red plots are the cases for all loci, and blue plots are the cases after removing the significant locus).

Table 2

Main effects of tested SNPs on HAPE risk by FUMA

Symbol gene	Chromosome	Start	End	Strand	Type
ADAMTS9-AS2	3	64670585	64997143	1	antisense
NEK1	4	170314426	170533780	-1	protein-coding
CLCN3	4	170533784	170644824	1	protein-coding
C4orf27	4	170650616	170679104	-1	protein-coding
RP11-219J21.2	8	25634195	25634972	1	lncRNA
ANKRD26	10	27280843	27389421	-1	protein-coding
YME1L1	10	27399383	27444195	-1	protein-coding

SNPs – single-nucleotide polymorphisms; HAPE – high-altitude pulmonary oedema.

Discussion

We performed a GWAS to identify susceptibility genes and risk variants for HAPE in Chinese populations. Seven novel candidate genes have emerged from our staged association analyses. Specifically, NEK1, CLCN3, C4orf27, ANKRD26 and YME1L1 are protein-coding genes, and ADAMTS9-AS2 and YME1L1 are RNA genes.

ADAMTS9-AS2 (ADAMTS9 antisense RNA 2) is located at the positive strand of chromosome 3 (chr3: 64, 684, 935-65, 053, 439) with a length of 2.258 kb and is classified as an lncRNA. ADAMTS9-AS2 is an antisense transcription of ADAMTS9. ADAMTS plays important roles in connective tissue organization, coagulation, inflammation, arthritis, and angiogenesis and is regulated by the tissue inhibitor of metalloproteinase 3 gene (TIMP3) [18, 19]. Moreover, it has been shown that in the Japanese population TIMP3 was associated with HAPE susceptibility [20–22]. TIMP plays a key role in the physiological turnover of the extracellular matrix (ECM) by closely regulating the activity of matrix metalloproteinase (MMP). TIMP3 is the only TIMP closely integrated with ECM. The balance between MMP and TIMP plays an important role in maintaining the integrity of healthy tissues. The disturbance of the TIMP/MMP system is related to various pathological conditions of the lungs, including pulmonary inflammation, oedema, emphysema and fibrosis, among which the loss of ECM integrity is the main feature [23]. Our results, together with those of previous studies, suggest that the balance between MMPs and TIMPs plays an important role in the pathogenesis of HAPE.

Chloride voltage-gated channel 3 (ClC-3) is a protein-coding gene. Among its related pathways are ion channel transport and transport of glucose and other sugars, bile salts and organic acids, metal ions and amine compounds [24]. This protein plays a role in both acidification and transmitter loading of GABAergic synaptic vesicles and in smooth muscle cell activation and neointima formation [25]. This protein is required for lysophosphatidic acid (LPA)-activated Cl^- current activity and fibroblast-to-myofibroblast differentiation. Dai et al. [26] observe that ClC-3 in rat hypertensive lung and heart is a novel upregulation. These researchers also suggest that upregulation of ClC-3 is an adaptive response of the inflamed pulmonary artery. ClC-3 may be associated with the adaptability of the pulmonary artery to the plateau environment in HAPE.

Ankyrin repeat domain 26 (ANKRD26) is a protein-coding gene. Diseases associated with ANKRD26 include thrombocytopenia 2 and platelet disorder, familial, with associated myeloid malignancy. There is a case reporting that ANKRD26-related thrombocytopenia resulting in lower-limb deep vein thrombosis was complicated by pulmonary embolism [27]. NIMA-related kinase 1 (NEK1) is a protein-coding gene. Diseases associated with NEK1 include short-rib thoracic dysplasia 6 with or without polydactyly and amyotrophic lateral sclerosis. NEK1 is involved in DNA damage checkpoint control and proper DNA damage repair [28]. In response to injury that includes DNA damage, NEK1 phosphorylates VDAC1 to limit mitochondrial cell death [28]. YME1L1 (YME1-like 1 ATPase) is a protein-coding gene. Diseases associated with YME1L1 are spastic paraplegia 7, autosomal recessive and include optic atrophy 11 [29]. Gene Ontology (GO) annotations related to this gene include metalloendopeptidase activity. This protein is localized in the mitochondria and can functionally complement a YME1 disruptant yeast strain. It is proposed that this gene plays a role in mitochondrial protein metabolism and could be involved in mitochondrial pathologies [30]. ATP-dependent metalloprotease, which catalyses the degradation of folded and unfolded proteins with a suitable degron sequence in the mitochondrial intermembrane region [31], takes a big part in regulating mitochondrial morphology and function by cleaving OPA1 at position S2, giving rise to a form of OPA1 that promotes maintenance of normal mitochondrial structure and mitochondrial protein metabolism [31–33]. C4orf27 (also known as histone PARylation factor 1) (HPF1) is a protein-coding gene [34]. C4orf27 acts as a cofactor for serine ADP-ribosylation by conferring serine specificity on PARP1 and PARP2: this protein interacts with PARP1 and PARP1 and is able to change amino acid specificity towards serine [35]. However, ANKRD26, NEK1, YME1L1 and C4orf27 in HAPE remain unknown and require additional studies.

This study has several limitations. The small size of this study does not provide sufficient power for a conclusive analysis of the association. We hope that collaboration with other researchers with access to more HAPE patients will lead to the identification of gene(s) responsible for HAPE. Controls are not known to have traveled to high-altitude regions. We believe that only 0.5–2% of the population experienced HAPE after ascending to high-altitude regions. Considering the rarity of HAPE, we deem that all of these people can be used as healthy controls.

Conclusion

In summary, we provide evidence for the contribution of ADAMTS9-AS2, NEK1, CLCN3, C4orf27 (HPF1), RP11-219J21.2, ANKRD26 and YME1L1 to the pathogenesis of HAPE in Chinese populations. This prioritized gene deserves further evaluation to improve the understanding of HAPE genetics.

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Conflict of interest

The authors declare that they have no competing interests.

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