Characterization of the Relationship between APOBEC3B Deletion and ACE Alu Insertion

Kang Wang¹, Yuanyuan Li¹, Chunyan Dai¹, Kaishi Wang¹, Jinghua Yu¹, Yiran Tan², Wenyan Zhang¹, Xiao-Fang Yu¹,²*, ¹Institute of Virology and AIDS Research, First Hospital of Jilin University, Changchun, Jilin, People’s Republic of China, ²Department of Molecular Microbiology and Immunology, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, United States of America

Abstract

The insertion/deletion (I/D) polymorphism of the angiotensin converting enzyme (ACE), commonly associated with many diseases, is believed to have affected human adaptation to environmental changes during the out-of-Africa expansion. APOBEC3B (A3B), a member of the cytidine deaminase family APOBEC3s, also exhibits a variable gene insertion/deletion polymorphism across world populations. Using data available from published reports, we examined the global geographic distribution of ACE and A3B genotypes. In tracking the modern human dispersal routes of these two genes, we found that the variation trends of the two I/D polymorphisms were directly correlated. We observed that the frequencies of ACE insertion and A3B deletion rose in parallel along the expansion route. To investigate the presence of a correlation between the two polymorphisms and the effect of their interaction on human health, we analyzed 1199 unrelated Chinese adults to determine their genotypes and other important clinical characteristics. We discovered a significant difference between the ACE genotype/allele distribution in the A3B DD and A3B II/ID groups (P = 0.045 and 0.015, respectively), indicating that the ACE Alu I allele frequency in the former group was higher than in the latter group. No specific clinical phenotype could be associated with the interaction between the ACE and A3B I/D polymorphisms. A3B has been identified as a powerful inhibitor of Alu retrotransposition, and primate A3 genes have undergone strong positive selection (and expansion) for restricting the mobility of endogenous retrotransposons during evolution. Based on these findings, we suggest that the ACE Alu insertion was enabled (facilitated) by the A3B deletion and that functional loss of A3B provided an opportunity for enhanced human adaptability and survival in response to the environmental and climate challenges arising during the migration from Africa.

Citation: Wang K, Li Y, Dai C, Wang K, Yu J, et al. (2013) Characterization of the Relationship between APOBEC3B Deletion and ACE Alu Insertion. PLoS ONE 8(5): e64809. doi:10.1371/journal.pone.0064809

Editor: Gil Ast, Tel Aviv University, Israel
Received: February 1, 2013; Accepted: April 18, 2013; Published: May 24, 2013
Copyright: © 2013 Wang et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
Funding: This work was supported in part by funding from the Chinese Ministry of Science and Technology (No. 2012CB911100 and No. 2013ZX0001-005), the Chinese Ministry of Education (No. IRT1016), and the Key Laboratory of Molecular Virology, Jilin Province (20102209), China. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: Xiao-Fang Yu currently serves as an editor of this journal. This does not alter the authors’ adherence to all the PLOS ONE policies on sharing data and materials.
* E-mail: xfyu@jhsph.edu

Introduction

Insertion/deletion (I/D) polymorphism of the angiotensin converting enzyme (ACE) gene is an important genetic marker that has been used in numerous studies [1–8]. ACE is a key enzyme of the renin-angiotensin system (RAS) and is widely distributed in human tissues, including the vascular endothelium, intestinal epithelium, kidney, lung, and testes [9,10]. The enzyme plays a vital role in the regulation of systemic blood pressure and renal electrolyte homeostasis by converting the inactive angiotensin I to the vasoconstrictor angiotensin II and inactivating the proinflammatory vasodilator bradykinin [9]. In Homo sapiens, the gene encoding ACE is located on the long arm of chromosome 17 and comprises 26 exons and 25 introns [11]. The insertion/deletion polymorphism is characterized by the presence (insertion) or absence (deletion) of a 287-base pair (bp) Alu repeat within intron 16, producing three genotypes: II, DD homozygote, and ID heterozygote [1]. The deletion allele was found to be associated with higher plasma ACE concentrations; that is, the average plasma ACE level of persons with the various genotypes follows the order DD>II>DD>II [1]. The I/D genotype distribution of ACE shows apparent variations among different ethnic populations throughout the world [8]. A number of research papers have reported a significant association between ACE I/D polymorphism and a series of diseases, including hypertension, type 2 diabetes, cardiovascular disease, kidney disease, cancer, and obesity [2–7].

APOBEC3B (A3B) belongs to the apolipoprotein B mRNA editing catalytic polypeptide-like 3 (APOBEC3) protein family, whose members all possess cytidine deaminase activity and are therefore able to convert cytosine to uracil [12,13]. Most A3 proteins can restrict the replication of retroviruses or retrotransposition of endogenous elements to varying degrees [13–18]. A3B, in particular, has been shown to inhibit replication of HBV and HIV as well as the retrotransposition of Linel and Alu [13,15,16,17,19]. The human A3B gene is located on the long arm of chromosome 22, clustered with other A3 genes [12]. The gene is widely expressed at a low level in PBMCs, colon, lung, spleen, liver, ovary, and testis [20]. A common 29.5-kb gene deletion polymorphism that removes the entire A3B coding region...
has been identified and, like the ACE I/D polymorphism, its distribution varies significantly in diverse ethnic groups [21]. A3B I/D polymorphism has been suggested to influence host susceptibility to HBV infection and falciparum malaria [22,23].

The APOBEC3 gene family has undergone expansion during species evolution, increasing from a single copy in rodents to at least seven copies in primates [24]. It is believed that the APOBEC3 family has been subjected to strong and continuous selective pressure throughout primate evolution [25,26]. The A3B genotype distribution in world populations indicates a weak selection for the deletion [21]. In addition, the ACE D allele also presents a striking geographic distribution, showing evidence of “signatures of selection” that follows the out-of-Africa route [8].

The formation of A3B I/D polymorphism was caused by A3B deletion, while the formation of ACE I/D polymorphism was caused by the ACE Alu insertion. To investigate whether a correlation exists between the two I/D polymorphisms or between the A3B deletion and the ACE Alu insertion, we compared the genotype distributions of ACE and A3B across the world. The result of our analysis demonstrates that their change trends are almost completely opposite to each other along the modern human dispersal route. Based on this interesting discovery, we investigated their distributions and interaction effects on the clinical characteristics of a random sample of Chinese population. The combination of the ACE and A3B genotypes/alleles in the study population was not completely stochastic, but no interaction effects were embodied in the clinical characteristics.

Materials and Methods

Ethics Statement

The study protocol for the random population was approved by the ethics committee at the First Hospital of Jilin University. All participants or their parents gave written informed consent.

Publication selection and data extraction

A search was performed in the PubMed database to identify published studies reporting A3B or ACE genotype/allele distributions. The retrieved studies were manually screened to assess their appropriateness for inclusion. A3B genotype distributions in world populations have mainly been investigated and analyzed thus far by Kidd et al. [21]. A3B allele frequencies in various geographic regions were extracted and cited directly from the supplemental materials of this paper, except for Middle South Asia, because its data actually only included Pakistanis and few Uygur. The A3B deletion distribution in India has been reported in another study characterizing 25 ethnic populations [23]. Therefore, we extracted data from both studies to evaluate A3B deletion frequency in Middle South Asia in particular. Li et al. have compiled a worldwide spatial database on ACE I/D frequencies and genotypes. ACE genotype/allele distributions of some nations were extracted from the database to calculate ACE allele frequencies in Africa, Middle East, Europe, Middle South Asia, and East Asia, respectively, supplemented with related data for Pakistanis and Khoisan from the two original studies [27,28]. ACE genotype/allele distributions among the aboriginals of America and Australia were collected from several other papers [28–32].

We also extracted the data of the frequencies of other three Alu insertions (called TPA 25, PV92 and FXIIIB, respectively) among different populations from one study to calculate their geographic distributions [28].

Study Population

The subjects were selected by a simple random sampling approach from persons who visited the general health check-up center of the First Hospital of Jilin University in 2010. Subjects who were pregnant or were suffering from serious illnesses were excluded from the investigation to ensure the validity of results. A group of 1199 adults (683 males, 516 females) from the participants were genotyped for ACE and A3B I/D polymorphisms, in addition to being measured for some specific clinical characteristics according to their individual willingness to undergo testing. Another 1301 participants (756 males, 525 females) were also randomly chosen to supplement the number of A3B deletion homozygotes. These persons were initially genotyped for A3B polymorphism, and only those identified as A3B deletion homozygotes were then genotyped for ACE polymorphism. All participants were unrelated Han Chinese and local residents of Jilin Province in northeast China.

Measurement of clinical characteristics

All measurements were performed by skilled technicians in the early morning after participants had fasted overnight. Body weight and height were measured twice in light indoor clothing without shoes, and body mass index (BMI) was calculated as body weight divided by the square of height (kg/m²). Heart rate was determined from the resting electrocardiogram recorded by full automatic electrocardiograph (Nihon Kohden ECG-9130P, Tokyo, Japan). Blood pressure was measured at least twice with an electronic sphygmomanometer (Omron BP-203RVIIIIC, Kyoto, Japan) from the right arm of subjects in the seated position after 5 minutes of rest according to a standard protocol. These anthropometric and physiological parameters were measured at the general health check-up center of the First Hospital of Jilin University.

Fasting blood samples were drawn from the antecubital vein by nurses, while urine samples were collected by participants themselves according to the instructions of nurses. Blood and urine biochemistry were measured in the Department of Laboratory Medicine, First Hospital of Jilin University. The concentrations of serum lipids, plasma glucose, blood urea nitrogen and hepatic function indexes were assayed by standard enzymatic methods in fresh blood samples using commercial reagent kits (Kehua Bioengineering, Shanghai, China) with an autoanalyzer (Hitachi 7600, Tokyo, Japan). Urinary protein and occult blood were tested in fresh urine samples using urinalysis strips with an automated urine analyzer (DIRUI H-800, Changchun, China). Urinary protein test results were categorized based on semi-quantitative values as: (–) for 0.3 g/l; (±) for 1.0 g/l; and (+) for 5.0 g/L. Urine occult blood results were categorized based on semi-quantitative values as: (–) for 10 erythrocytes/µl; (±) for 25 erythrocytes/µl; (±) for 80 erythrocytes/µl; and (+) for 200 erythrocytes/µl.

Genotyping

Genomic DNA was extracted from EDTA blood with Blood DNA Mini Kits (SIMGEN Biotech, Hangzhou, China). Genotyping was performed on the extracted DNA by polymerase chain reaction (PCR) with specific oligonucleotide primers.

Genotypes of ACE I/D polymorphism were detected according to the methods of Rigat et al. and Lindpaintner et al. [33,34,35]. To guarantee the reliability of the genotyping results, all samples were amplified with both primer pairs (5’-CTGGGAG CCACCTCCATCCATTTCTTCT-3’ and 5’-GATGTGGCCATCA- CATTTCGTCAGAT-3’) and 5’-TGAGCCGCAAGCGCCGCG- CACCTAG-3’ and 5’-TGCCGGACGCTCCCTGATGC CATAA-
SD, with a range of 18 to 84 years. The average age of the
ultraviolet light to judge the genotype.
agarose gel mixed with ethidium bromide and visualized under
PCR products were separated by electrophoresis on a 1.5%
temperature parameters as described previously [21,33,34,35].

Data analysis
ACE allele frequency in a geographic region was calculated as the
average value for several representative countries (File S1). If there was more than one published source reporting the ACE
genotype/allele distribution of the same country, then several
main publications were selected together to calculate the
calculate the frequency in the country by the gene-counting method. Two
African ethnic groups, Pygmy and Khoisan, were included
together with the relevant countries in order to calculate the
ACE insertion frequency in Africa. To evaluate the A3B deletion
deletion frequency among
Indians was first computed as the mean value for 25 ethnic
populations. Then, the Indian deletion value and the deletion
value in Middle South Asia reported by Kidd et al. (mainly as
frequency in Middle South Asia, the deletion frequency among
ACE insertion frequency in Africa. The chi-square test was used to assess whether A3B and ACE
ACE and A3B genotype/allele distributions in the study population

Results
Comparison of the global distributions of ACE and A3B I/ D polymorphisms
Li et al. have discovered that ACE deletion shows an obvious
decreasing geographic genetic cline following the route of out-of-
Africa expansion from East Africa [8]. In the report of Kidd et al., A3B deletion was shown to be almost null in Africans, rare in
Middle East populations and Europeans, slightly more frequent in
Central South Asians, common in East Asians and American
Indians, and almost fixed in Oceanic populations [21]. The A3B
deletion also displayed an evident increasing trend following the
out-of-Africa expansion route in the 51 different ethnic populations.
Taken together, these data indicate that ACE and A3B I/D
d polymorphisms show opposite geographic distributions along the
modern human dispersal route.

Because the sample sizes of some ethnic populations in the
report of Kidd et al. were too small to guarantee the precision of the
A3B allele/genotype frequencies estimated, we chose to
calculate the frequencies of the ACE I and A3B D alleles in
different geographic regions to give a concrete and intuitive
impression. ACE insertion frequencies were calculated to be 34.1, 32.6, 46.7, 55.7, 63.3, 73.8, and 83.5 percent in Africa, the Middle
East, Europe, Central South Asia, East Asia, America, and
Oceania, respectively (Table S1). A3B deletion frequencies were
cited or calculated to be 0.9, 7.7, 6.5, 17.8, 36.9, 47.7 and 92.9 percent, respectively, in those same geographic regions (Table S1).
The results clearly confirmed the opposite variation trends of ACE
and A3B I/D polymorphisms along the human dispersal route.
Variation curves for ACE insertion and A3B deletion among the
genographic regions were plotted based on the results. Amazingly,
their variation curves were observed to be almost parallel, both
rising continuously along the out-of-Africa expansion route (Fig. 1).
The distributions of three additional Alu insertions in different
genealographic regions presented variation trends similar to that of the
ACE Alu insertion (Table S1).

ACE and A3B genotype/allele distributions in the study population
The 1199 adults in the study were aged 41.6±12.3 years (mean ± SD), with a range of 18 to 84 years. The average age of the
other 1581 subjects was 42.5±14.5 years, with a range of 15 to 86 years. Most of the participants were genotyped successfully and
accurately, with the exception of three whose DNA failed to
amplify. No contradictory ACE genotyping results were found for
the PCR of the two pairs of primers.
The ACE genotype/allele distribution in the 1199 adults and
A3B genotype/allele distribution in all participants are listed in
Table 1. The frequencies of the ACE I and D alleles were 0.674 and 0.326, respectively, consistent with previous reports (File S1). The
genotype distribution of ACE was almost completely identical to
the expected Hardy-Weinberg frequency (P = 0.962). The A3B
genotype/allele frequencies did not differ between the 1199 adults
and the other 1581 subjects or between males and females
(Table 1, Table S2). The A3B D allele frequency was 0.353, which is
very close to the frequency among the Han Chinese population and
East Asians reported by Kidd et al. [21]. Even so, the A3B
genotype distribution deviated significantly from HWE (P<0.001).
Thus, we did not pursue the analysis of the A3B genotype/allele

and BMI as covariates to adjust the P-value. All statistical analyses
were performed with SPSS Version 19.0 software (SPSS Inc.,
Chicago, USA). A two-tailed P-value of <0.05 was considered
statistically significant.
frequencies or values for clinical characteristics in the different A3B genotype groups in this study.

ACE genotype/allele distributions in different A3B genotype groups were compared, and significant differences were discovered between the A3B DD group and the II/ID group (P = 0.045 and 0.015, respectively; Table 2). The distribution of the ACE allele in the A3B DD group of 1199 adults was approximately the same as that of the other 1581 subjects. The frequency of the ACE D allele in the A3B DD group (0.272) was lower than that in the A3B II/ID group (0.33). The ACE ID and DD genotype frequencies in the A3B DD group (0.417 and 0.064, respectively) were both lower than in the A3B II/ID group (0.444 and 0.108, respectively). No significant difference was found in the ACE genotype/allele distribution of the A3B II and A3B ID groups (data not shown).

Interaction effects between ACE and A3B I/D polymorphisms on clinical characteristics

No significant differences were found between the clinical characteristics of the carriers of both the ACE D and A3B I alleles and the remainder among the males or females of the 1199 adults, although there was a strong trend for aspartate aminotransferase in females (P = 0.051; Table 3 and Table 4). The same results were obtained when the subjects were grouped into ACE/A3B D allele carriers and others (Table S3 and Table S4). As a control, the ACE genotype itself was only observed to be significantly related to blood urea nitrogen values among the male subjects (P = 0.031). This is a finding that, to our knowledge, has not been previously reported (Table S5 and Table S6).

Discussion

In the present study, we have observed that the ACE Alu insertion genotype is not randomly distributed among individuals with different A3B deletion genotypes. People who are homozygous for A3B deletions (A3B DD) have higher rate of ACE Alu insertion alleles (72.8%) than do people who are A3B II or ID (67%) (P = 0.015) (Table 2). Interestingly, the frequency of the ACE Alu insertion alleles was also higher among people who carry the higher A3B deletion alleles in geographically different locations (Fig. 1). The cause for this association between the A3B deletion and ACE Alu insertion is not clear. However, there are several possible explanations: (1) The ACE Alu insertion and the A3B deletion occurred as independent events. Selection pressure resulted in a non-random distribution of these two genetic alterations. (2) The two I/D polymorphisms have interactive

Table 1. A3B and ACE genotype/allele distributions in the study population.

| Gene   | Population       | Genotype counts (HWE expected) | Allele frequency |
|--------|------------------|--------------------------------|------------------|
|        |                  | II | ID  | DD  | I   | D  | HWE P-value |
| ACE    | 1199 adults      | 542(545) | 533(527) | 124(127) | 0.674 | 0.326 | 0.962 |
| A3B    | 1199 adults      | 470 (514) | 630 (542) | 99 (143) | 0.655 | 0.345 | <0.001 |
| A3B    | 1581 subjects    | 624 (676) | 818 (714) | 136 (188) | 0.655 | 0.345 | <0.001 |
| A3B    | Total            | 1094 (1190) | 1448 (1256) | 235 (331) | 0.655 | 0.345 | <0.001 |

Figure 1. Variation curves of ACE insertion and A3B deletion distributions among different geographic regions. ‘C/S Asia’ corresponds to Central South Asia. America refers to Amerindians; Oceania refers to Oceania aboriginals.

doi:10.1371/journal.pone.0064809.g001
effects on the survival of humanity. (3) Cause-and-effect relationship exists between A3B deletion and ACE Alu insertion. The selection pressure for the existence of the A3B deletion is unknown. DNA mutations induced by elevated expression of A3B have been reported in liver cancer and breast cancer [36,37]. Thus, the A3B deletion may offer a reduced risk for the

Table 2. ACE genotype/allele distributions in different A3B genotype groups.

| A3B genotype group | ACE genotype counts (frequencies) | Added up | P-value | Allele counts (frequencies) | P-value |
|--------------------|----------------------------------|----------|---------|-----------------------------|---------|
| II in 1199 adults  | II = 222 (0.472)                 |          |         |                             |         |
|                    | I = 199 (0.423)                  |          |         |                             |         |
|                    | DD = 49 (0.058)                  |          |         |                             |         |
| II in 1199 adults  | II = 271 (0.430)                 |          |         |                             |         |
|                    | I = 248 (0.411)                  |          |         |                             |         |
|                    | DD = 70 (0.111)                  |          |         |                             |         |
| DD in 1199 adults  | II = 49 (0.495)                  | 0.045    | 0.015   |                             |         |
|                    | I = 45 (0.454)                   |          |         |                             |         |
|                    | DD = 5 (0.051)                   |          |         |                             |         |
| DD in 1581 subjects| II = 73 (0.537)                  |          |         |                             |         |
|                    | I = 98 (0.417)                   |          |         |                             |         |
|                    | DD = 15 (0.064)                  |          |         |                             |         |
| DD in 1581 subjects| DD = 10 (0.073)                  |          |         |                             |         |


doi:10.1371/journal.pone.0064809.t002

Table 3. Characteristics of male subjects grouped by ACE and A3B genotypes.

| Characteristic                  | Mean ± SD (No. of subjects measured) | P-value |
|--------------------------------|-------------------------------------|---------|
|                                | ACE D/A3B I carriers | others |         |
| Age (years)                    | 41.1±11.9 (340)       | 41.5±12.4 (343) | 0.692   |
| BMI (kg/m²)                    | 26.2±3.2 (296)        | 25.8±3.3 (297) | 0.220   |
| Heart rate (beats/min)         | 76.7±10.6 (305)       | 76.3±10.7 (305) | 0.637   |
| Blood pressure (mm Hg)         | 135.9±18.6 (305)      | 133.7±17.9 (305) | 0.201   |
| Systolic                       | 83.8±11.9 (305)       | 83.1±11.4 (305) | 0.490   |
| Diastolic                      | 5.81±1.91 (304)       | 5.58±1.14 (312) | 0.629   |
| Plasma glucose (mmol/L)        | 4.92±0.87 (304)       | 4.83±0.92 (313) | 0.153   |
| Triglycerides                  | 1.94±1.41 (304)       | 1.80±1.36 (313) | 0.098   |
| HDL-cholesterol                | 1.54±0.54 (253)       | 1.55±0.57 (261) | 0.943   |
| LDL-cholesterol                | 3.11±0.81 (253)       | 3.05±0.76 (261) | 0.513   |
| HDL-C/LDL-C ratio              | 0.52±0.21 (253)       | 0.53±0.23 (261) | 0.701   |
| Renal function indexes         | 5.27±1.29 (305)       | 5.40±1.35 (314) | 0.222   |
| Urinary protein                | —— a (319)            | —— a (328) | 0.956   |
| Urinary occult blood           | —— a (319)            | —— a (328) | 0.155   |
| Liver function indexes (U/L)   | 32.9±25.0 (323)       | 31.0±19.2 (332) | 0.915   |
| ALT                            | 45.2±45.6 (323)       | 43.5±43.4 (332) | 0.735   |
| γ–GT                           | 26.0±12.4 (323)       | 24.8±9.3 (333) | 0.784   |
| AST                            | 1.95±1.45 (284)       | 1.77±1.36 (291) | 0.103b  |

*belong to categorical variables.

aP-value from analysis of covariance including subjects whose BMI data were available.

Abbreviations: BMI, body mass index; HDL, high density lipoprotein; LDL, low density lipoprotein; BUN, blood urea nitrogen; ALT, alanine aminotransferase; γ–GT, gamma-glutamyl transpeptidase; AST, aspartate aminotransferase.

doi:10.1371/journal.pone.0064809.t003
development of certain cancers. Links between the lack of the ACE Alu insertion and hypertension, as well as other diseases, have been reported [2–7]. It is therefore also possible that A3B deletion is detrimental to human survival, but this outcome could be partially offset by the ACE Alu insertion, leading to the apparent association of these two genetic alterations.

It is intriguing to consider that the A3B deletion may be the cause of the ACE Alu insertion. Members of the APOBEC3 (A3) family were initially discovered as host restriction factors for exogenous retroviruses [13,14]. Their roles in controlling the retrotransposition of endogenous retroelements have also attracted much attention in recent years [38,39]. The sequence variability of primate A3 genes indicates that this gene cluster has been subjected to strong positive selective pressure for more than 30 million years, clearly preceding the appearance of primate lentiviruses [26]. By contrast, there is a striking evolutionary coincidence between the expansion of the A3 gene cluster and the abrupt drop in retrotransposon activity that took place in primates 35–50 million years ago [40]. Moreover, A3 proteins are expressed at relatively high levels in germ cells and embryonic stem cells, where retrotransposition occurs actively and can be inherited by the offspring [16,20]. A3B is a powerful inhibitor of Alu retrotransposition. It is the only member of the A3 family that is consistently localized to the nucleus, where Alu is reverse-transcribed and inserted into other loci [17]. Endogenous A3B has been detected in embryonic stem cells, where it modulates LINE-1 retrotransposition [16]. If the A3B deletion is indeed the cause of the ACE Alu insertion, then the occurrence of the A3B deletion has great significance for the history of modern humanity, because the ACE Alu insertion is believed to have greatly promoted human adaptability and human survival rates during the out-of-Africa expansion [8]. It should be noted that the A3B deletion may have enabled more than one Alu insertion event, so its geographic distribution may also have been influenced by other Alu I/D polymorphisms. In particular, some other Alu I/D polymorphisms present geographic distributions analogous to that of the ACE I/D polymorphism (Table S1) [28]. This is the first report of an association between A3B deletion and Alu insertion. Further study is required to establish what selection pressures may have contributed to this association.

### Supporting Information

**File S1** Calculation for the geographic distributions of A3B deletion, ACE Alu insertion and other three Alu insertions. (XLS)

**Table S1** Geographic distributions of A3B deletion, ACE Alu insertion and other three Alu insertions. (DOC)

**Table S2** A3B genotype/allele distributions in subjects stratified by sex. (DOC)
Table S3 Characteristics of male subjects grouped by ACE and A3B genotypes.

(DOC)

Table S4 Characteristics of female subjects grouped by ACE and A3B genotypes.

(DOC)

Table S5 Characteristics of male subjects grouped by ACE genotypes.

(DOC)

Table S6 Characteristics of female subjects grouped by ACE genotypes.

(DOC)

References

1. Rigat B, Hubert C, Allerence F, Cambien F, Corvol P, et al. (1990) An insertion/deletion polymorphism in the angiotensin-I-converting enzyme gene accounting for half of the variance of serum enzyme levels. J Clin Invest 86: 1343–1346.

2. Di Pasquale P, Cannizzaro S, Paterna S (2004) Does angiotensin-converting enzyme gene polymorphism affect blood pressure? Findings after 6 years of follow-up in healthy subjects. Eur J Heart Fail 6: 11–16.

3. Feng Y, Tian T, Xu X, Chen C, Li Q, et al. (2002) Insertion/deletion polymorphism of the ACE gene is associated with type 2 diabetes. Diabetes 51: 1896–1898.

4. Schunkert H, Heine HW, Holmer SR, Stender M, Perez S, et al. (1994) Association between a deletion polymorphism of the angiotensin-converting enzyme gene and left ventricular hypertrophy. N Engl J Med 330: 1634–1638.

5. Hardin PN, Geddes C, Rowe FA, McIvor JH, Boulou-Jones M, et al. (1993) Polymorphisms in angiotensin-converting enzyme gene and progression of IgA nephropathy. Lancet 343: 1540–1542.

6. Medeiros R, Vasconcelos A, Costa S, Pinto D, Lobo F, et al. (2004) Linkage of angiotensin I-converting enzyme gene insertion/deletion polymorphism to the progression of human prostate cancer. J Pathol 202: 330–335.

7. Eisenmann JC, Sarzyński MA, Gleen K, Rothschild M, Heelan KA (2009) ACE I/D genotype, adiposity, and blood pressure in children. Cardiocratives Diabetol 8: 314–319.

8. Li X, Sun X, Jin L, Xue F (2011) Worldwide spatial genetic structure of angiotensin-converting enzyme gene: a new evolutionary ecological evidence for the thrifty genotype hypothesis. Eur J Hum Genet 19: 1002–1008.

9. Ehlers MR, Rissed JR (1989) Angiotensin-converting enzyme: new concepts concerning its biological role. Biochemistry 28: 5311–5318.

10. Lieberman J, Sastre A (1983) Angiotensin-converting enzyme activity in postmortem human tissues. Lab Invest 48: 711–717.

11. Hubert C, Houston AM, Corvol P, Souhrier F (1991) Structure of the angiotensin I-converting enzyme gene. Two alternate promoters correspond to evolutionary steps of a duplicated gene. J Biol Chem 266: 15377–15383.

12. Jarmuz A, Chester A, Bayliss J, Gusbrough J, Dunham I, et al. (2002) An anthropoid-specific locus of orphan C to U RNA-editing enzymes on chromosome 22. Genomics 79: 255–296.

13. Bishop KN, Holmes RK, Sheehy AM, Davidson NO, Cho SJ, et al. (2004) Cytidine deamination of retroviral DNA by diverse APOBEC proteins. Curr Biol 14: 1392–1396.

14. Dang Y, Wang X, Esselman WJ, Zheng YH (2006) Identification of APOBEC3DE as another antiretroviral factor from the human APOBEC family. J Virol 80: 10322–10333.

15. Stenglein MD, Harris RS (2006) APOBEC3B and APOBEC3F inhibit LTR retrotransposition by a DNA deamination-independent mechanism. J Biol Chem 281: 10837–10841.

16. Wissing S, Montano M, Garcia-Gonzalez JL, Morvan J, Greene WC (2011) Endogenous APOBEC3 restricts LINE-1 retrotransposition in transformed cells and human embryonic stem cells. J Biol Chem 286: 36427–36437.

17. Roger HP, Wiegand HL, Hulme AE, Garcia-Perez JL, O’Shea KS, et al. (2006) Cellular inhibitors of long interspersed element 1 and Alu retrotransposition. Proc Natl Acad Sci U S A 103: 8780–8785.

18. Tan L, Sarkis PT, Wang T, Tian C, Yu XF (2009) Sole copy of Z2-type human cytidine deaminase APOBEC3B has inhibitory activity against retrotransposons and HIV-1. FASEB J 23: 279–287.

19. Zhang W, Zhang X, Tian C, Wang T, Sarkis PT, et al. (2008) Cytidine deaminase APOBEC3B interacts with heterogeneous nuclear ribonucleoprotein K and suppresses hepatitis B virus expression. Cell Microbiol 10: 112–121.

20. Redfield EW, Stenglein MD, Shindo K, Albin JS, Brown WL, et al. (2010) Quantitative profiling of the full APOBEC3 miRNA repertoire in lymphocytes and tissues: implications for HIV-1 restriction. Nucleic Acids Res 38: 4274–4294.

Acknowledgments

We thank the general health check-up center and the Department of Laboratory Medicine, First Hospital of Jilin University for technical assistance on the measurements of clinical characteristics. We thank the Division of Clinical Epidemiology, First Hospital of Jilin University for guidance on statistical analysis and Drs. Richard Markham and Deborah McCellian for discussion and editorial assistance.

Author Contributions

Conceived and designed the experiments: X-FY Kang Wang WZ. Performed the experiments: Kang Wang YL CD Kaishi Wang JY. Analyzed the data: Kang Wang X-FY YT. Contributed reagents/materials/analysis tools: WZ YL CD. Wrote the paper: Kang Wang X-FY YT. Collection and arranging of the clinical data: YL CD Kaishi Wang.