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The population genetics of pharmacogenomics VIP variants in the Sherpa population

Li Wang [a, b, 1], Yongchao Ren [c, d, 1], Xugang shi [a, b], Dongya Yuan [a, b], Kai Liu [e], Tingting Geng [d], Gang Li [d], Longli Kang [a, b, *], Tian-bo Jin [a, b, c, d, **]

[a] Key Laboratory of High Altitude Environment and Genes Related to Diseases of Tibet Autonomous Region, School of Medicine, Xizang Minzu University, Xianyang, Shaanxi 712082, China
[b] Key Laboratory for Molecular Genetic Mechanisms and Intervention Research on High Altitude Disease of Tibet Autonomous Region, School of Medicine, Xizang Minzu University, Xianyang, Shaanxi 712082, China
[c] School of Life Sciences, Northwest University, Xi'an, Shaanxi 710069, China
[d] National Engineering Research Center for Miniaturized Detection Systems, Xi'an, Shaanxi 710069, China
[e] Tibet Vocational Technical College, Tibet Autonomous Region, Lasa 850000, China

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ABSTRACT

Polymorphic distributions of pharmacogenes among some ethnicities are under-represented in current pharmacogenetic research. Particularly, there is a paucity of pharmacogenetic information in the Sherpa population in Tibet. We used the Sequenom MassARRAY single nucleotide polymorphism (SNP) genotyping technology to detect 86 very important pharmacogene (VIP) variants in Sherpas and compared the genotypic frequencies of these variants with HapMap populations. Overall, 59 of the 60 previously reported variants in the HapMap populations were found in our study. We found minimal differences between populations of Sherpas and Chinese Han in Beijing (CHB), Chinese in Metropolitan Denver, Colorado (CHD), Japanese in Tokyo, Japan (JPT), and Mexicans in Los Angeles, California (MEX) after a strict Bonferroni correction. Only 8, 4, 5, 4 VIP genotypes, respectively, were different in these groups. Additionally, pairwise FST values and clustering analyses showed that the VIP variants in the Sherpa population exhibited a close genetic affinity with the CHB and JPT populations, but they were most similar to the CHD population. Our results contribute to a better understanding of the molecular basis underlying ethnic differences in drug response, which may potentially benefit the development of personalized medicine for the Sherpa population.

1. Introduction

Different individuals have different reactions to the same drug, and analyzing the safety and efficacy of a drug is complicated. These obstacles have led to difficulties in developing new treatments for major diseases. Inter–individual variability in drug response is determined by multiple factors, such as disease determinants, genetic and environmental factors (climate, smoking, alcohol consumption, etc.), and variability in either drug target response (pharmacodynamics) or idiosyncratic response [1,2]. These factors affect drug absorption, distribution, metabolism, excretion, and elimination [1,3]. The goal of pharmacogenomics research is to provide information for "personalized medicine"—providing a particular patient with the right medicine at the right dose for optimal treatment outcomes [4]. To date, the majority of pharmacogenomics studies have focused on candidate gene variants thought to be involved in the pharmacokinetics or pharmacodynamics of clinically relevant drugs [5]. These gene variants, which known as very important pharmacogene (VIP) variants [6], may determine an individual's response to specific medications. Some variants are listed in the Pharmacogenomics Knowledge Base (PharmGKB: http://www.pharmgkb.org).

Sherpa, a combination of two words in the Tibetan language, "Shyar" (east) and "Pa" (people), meaning "people who came from the east," refers to a minority ethnic population that has resided for almost 500 years in the Himalayan region. In China, most Sherpas

* Corresponding author.
** Corresponding author.
E-mail addresses: longlikang@126.com (L. Kang), tianbojinprofessor@163.com (T.-b. Jin).
[1] Joint first author.

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live in the border areas, such as Dingjie County and Zhangmu Town in Tibet, between the northern Tibetan Plateau and the southern mountains and plains in Nepal, where the elevation ranges from 1600 to 4000 m. To adapt to the low oxygen environment of the highlands, Sherpas are known for their extraordinary mountaineering ability and power of endurance [7–9]. In recent years, enormous efforts have been made to elucidate the genetic mechanism of highland adaptation. However, no report has addressed studies of pharmacogenetic information, especially for the VIP variants, which is of great importance for understanding the Sherpa population and further aiding the diagnosis, prevention, and treatment of specific diseases in this group.

This study investigated the genotypic frequencies of VIP variants in the Sherpa population and compared the differences between Sherpas and 11 major HapMap populations: Africans in southwest USA (ASW) (n = 83), northern and western Europeans (CEU) (n = 174), Chinese Han in Beijing (CHB) (n = 86), Chinese in Metropolitan Denver, Colorado (CHD) (n = 85), Gujarati Indians in Houston, Texas (GIH) (n = 88), Japanese in Tokyo, Japan (JPT) (n = 89), Luhya in Webuye, Kenya (LWK) (n = 90), Mexicans in Los Angeles, California (MEX) (n = 77), Maasai in Kinyawa, Kenya (MKK) (n = 171), Tuscans in Italy (TSI) (n = 88) and Yoruba in Ibadan, Nigeria (YRI) (n = 176). Finally, F_{ST} pairwise comparisons and Bayesian clustering analysis were used to analyze Sherpa population genetics.

2. Materials and methods

2.1. Study participants

One hundred unrelated healthy Sherpa volunteers (50 males and 50 females) were recruited from southwestern Tibet. All patients gave a detailed medical history and underwent a physical examination prior to the study, including gynecological examinations and clinical laboratory tests. The subjects were carefully interviewed and considered to belong to the Sherpa population if they were born to a family with at least three generations from an identical nationality. Blood samples were obtained with signed informed consent from every participant enrolled. This research was approved by the Clinical Research Ethics of Xizang Minzu University and Northwest University and was in compliance with the Department of Health and Human Services (DHHS) regulations for the protection of human research subjects.

2.2. Variant selection and genotyping

For this study, we selected 86 genetic variants from public databases[2–5] according to available data on frequency, functionality, and linkage based on published research. Genomic DNA was extracted from peripheral whole blood with the TIANamp blood DNA extraction kit (GoldMag Ltd. Xi'an, China) according to the manufacturer's instructions; DNA concentrations were measured using a NanoDrop 2000C spectrophotometer (Thermo Scientific, Waltham, Massachusetts, USA).These measurements were then used to calculate total DNA concentrations. We designed polymerase chain reaction (PCR) and extension primers for these SNPs using the MassARRAY Assay Design 3.0 software (Sequenom) [10]. The genotyping of these SNPs was performed using the Sequenom MassARRAY RS1000 (San Diego, California, USA) following the manufacture's protocol. Sequenom Typer 4.0 software (San Diego, California, USA) was used to design multiplex reactions and analyze genotypes for 86 SNPs within these selected genes [11].

2.3. Data analysis

All analyses were carried out using Microsoft Excel 2010 (Microsoft Corporation, Redmond, Washington, USA) and IBM Statistical Package for the Social Sciences (SPSS) statistical software V.19.0 (SPSS, Inc., Chicago, Illinois, USA). For the controls of each study, Hardy–Winberg equilibrium (HWE) was evaluated using goodness of fit chi-square test and a $p < 0.05$ indicated disequilibrium of HWE. To reduce the false discovery rate of multiple testing, Bonferroni's multiple comparison adjustment was performed, and p value values less than 0.05/(83*11) was deemed statistically significant. The genotypic frequencies of the selected VIP variants in Sherpas and the other 11 HapMap populations (data from HapMap: http://hapmap.ncbi.nlm.nih.gov/) were calculated and compared using the chi-square test.

The program, Arlequin v3.5, was used to calculate global F_{ST} values together with pairwise F_{ST} values among all populations using loci that were polymorphic at the 5% level. Statistical significance was tested by comparing the observed F_{ST} value based on 1307 random permutations of individuals. Matrices of pairwise genetic distances [$F_{ST}/(1 - F_{ST})$], Slatkin linearized F_{ST} values, and co-ancestry coefficients were computed.

The model-based clustering method was carried out to infer the number of genetic groups (K) in the data set and to assign individuals among groups using a Bayesian statistical approach implemented in STRUCTURE v2.3.4 [12]. Simulations were run under the "admixture" model with correlated allelic frequencies. The following parameters were used: burn-in of 10,000 steps, run length of 10,000 steps, and five replicate simulations of each K-value (K = 2–8). $K = 3$ was selected, based on the values of mean estimated LnP (Data) and other recommendations of the STRUCTURE software manual.

3. Results

In this study, we selected 86 genetic variants based on previously published VIP variants from the PharmGKB database. The characteristics of the selected variants in the Sherpa population are listed in Table 1, including gene name, chromosome number and position, effect on protein, family to which it belongs, and allelic frequencies. Given that the genotype distribution of rs28399444, rs28399454 and rs28399499 was not in Hardy–Weinberg equilibrium in controls ($p < 0.05$), these polymorphisms were excluded from further analyses. Supplementary Table 1 lists the clinical/pharmacogenetic importance of the selected VIP variants.

Table 2 shows the genotypic frequencies in the Sherpa population compared to the other 11 HapMap populations (data from HapMap: http://hapmap.ncbi.nlm.nih.gov), which are listed in Supplementary Table 2. Of the 83 VIP variants, 59 variants identified in our study were also found in the HapMap populations. Additionally, one previously reported variant in the HapMap populations (rs10735810) was not detected in our study, two variants (rs12659 and rs34489327) were not detected in any populations, and 21 variants were detected only in Sherpa population. We also found that the Sherpa population differed from the ASW, CEU, GIH, LWK, MKK, TSI, and YRI populations in 12, 20, 14, 17, 20, 13 and 25 selected VIP genotypes, respectively, after a strict Bonferroni correction. However, minimal differences were observed between the Sherpa and CHB, CHD, JPT, and MEX populations, for which only 8, 4, 5, 4 VIP genotypes, respectively, were different. Furthermore, we also observed that the rs4680 *catechol-methyl-transferase*

[2] www.ncbi.nlm.nih.gov/snp.
[3] www.ncbi.nlm.nih.gov/omim.
[4] www.pharmgkb.org.
[5] www.phosphosite.org.

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