Environmental Toxicology and Pharmacology 46 (2016) 27–35

Contents lists available at ScienceDirect

Environmental Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/etap

Genetic polymorphisms study of pharmacogenomic VIP variants in Han ethnic of China's Shaanxi province

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ARTICLE INFO

Article history:
Received 5 February 2016
Received in revised form 21 April 2016
Accepted 26 June 2016
Available online 27 June 2016

Keywords:
Pharmacogenomics
Genetic polymorphisms
Han ethnic
VIP
Variant

ABSTRACT

Background: Multiple factors include genetic and non-genetic interactions induce to different drug response among different individuals. Lots of researches proved that different frequencies of genetic variants exists different ethnic groups. The aim of this study was to screen Han volunteers in Shaanxi for VIP gene polymorphisms.
Materials and methods: We genotyped 80 Very Important Pharmacogenes (VIP) (selected from the PharmGKB database) in 192 unrelated, healthy Han ethnic adults from Shaanxi, the northwest of China, and then analyzed genotyping data wtih Structure and F-statistics (Fst) analysis.
Results: We compared our data with 15 other populations (Deng, Kyrgyz, Tajik, Uygur and 11 HapMap populations), and found the frequency distribution of Han population in Shaanxi is most similar with CHB. Also, Structure and Fst showed that Shaanxi Han has a closest genetic background with CHB.
Conclusions: Our study have supplemented the Han Chinese data related to pharmacogenomics and illustrated differences in genotypic frequencies of selected VIP variants' among the Han population and 15 other populations.

1. Introduction

Amounts of researches proved that the frequencies of gene variants and the individual response to drugs have significant differences among different ethnic groups (Yunus et al., 2013; Janha et al., 2014; Suarez-Kurtz et al., 2014; Shi et al., 2015). For example, previous study compared the allele and genotype frequencies of the polymorphic cytochrome P450 genes (*CYP1A1*, *CYP3A4*, *CYP3A5*, *CYP2C9* and *CYP2C19*) among several populations (Caucasian, African-American and Asian) (Yousef et al., 2012), which powerfully proved the allele and genotype distributed frequencies vary from population to population.

Pharmacogenomics aim to optimize drug dose and recommend right medication for corresponding patient to reach the best treatment effect, and provide a theoretical basis for personalized medicine (Evans and Relling, 1999). Some online pharmacogenomics database such as Pharmacogenomics Knowledge Base (ParmGKB), PharmADME and PMT, have collected amounts of information about important drug-metabolizing enzyme genes and their variants which play important roles in the whole drug metabolism process (Sim et al., 2011). In PharmGKB database

Abbreviations: VIP, very important pharmacogenes; Fst, F-statistics; ASW, African ancestry in the southwestern USA; CEU, a northwestern European population; CHB, the Han Chinese in Beijing, China; CHD, a Chinese population of metropolitan Denver, Colorado, USA; GIH, the Gujarati Indians in Houston, Texas, USA; JPT, the Japanese population in Tokyo, Japan; LWK, the Luhya people in Webuye, Kenya; MEX, people of Mexican ancestry living in Los Angeles, California, USA; TSI, the Maasai people in Kinyawa, Kenya (MKK), the Tuscan people of Italy; YRI, the Yoruba in Ibadan, Nigeria; HWE, Hardy-Weinberg Equilibrium; ALFRED, ALlele FREquency Database.

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http://dx.doi.org/10.1016/j.etap.2016.06.026
1382-6689/

(http://www.pharmgkb.org), there is one kind of genes which called Very Important Pharmacogenes (VIP) genes. VIP genes thought to be involved in the pharmacokinetics or pharmacodynamics of clinically relevant drugs, which may determine an individual's response to specific medications. (Sangkuhl et al., 2008). The goal of VIP gene research is to understand the relationship between VIP gene variants and drug response, and to provide sufficient information for individual medicine.

The population of China consists of Han Chinese and 55 ethni minorities currently recognized by the People's Republic of China. The sixth population survey of China found that the Han ethnic is as many as 1.2–1.3 billion. They are widely distributed on the Chinese mainland, but it's interesting that the Han population from different regions of China can be genetically different. Previous pharmacogenomics study is always focus on one single gene or couples of genes in Han ethnic, little information is about the systematically screen pharmacogenes of Han ethnic, especially in northwest region of China.

We recruited 192 Han ethnic volunteers of Shaanxi province and genotyped 80 VIP variants selected from the PharmGKB database(Gabriel et al., 2009). Then, we compared the differences between our Han population and 15 other populations which include Deng, Kirgiz, Tajik and Uygur and 11 HapMap populations. We analyzed the genetic distance between the 15 populations to explore the simple evolution process of Shaanxi Han ethnic. The results will extend our knowledge about ethnic difference of pharmacogenomics and provide a solid theoretical foundation for promoting the development of Shaanxi Han population personalized treatment.

2. Materials and methods

2.1. Study participants

We randomly recruited 192 unrelated, healthy Han ethnic adults from Shaanxi province of North China to join the present study (96 males and 96 females in the age range of 30–55 years old) between October and December 2014 using concrete recruitment and exclusion criteria. These people had exclusively Han ethnic ancestry for at least the last three generations.

2.2. Ethnic statement

All volunteers signed informed consent documents and provided blood samples. Both verbally and in writing of the purpose and procedures of the research were informed by all participants. The Clinical Research Ethnics of Xizang Minzu University and Northwest University approved the clinical protocol which is compliance with Department of Health and Human Services (DHHA) regulations for protection of human research subject.

2.3. VIP loci selection and genotyping

We selected genetic variants from PharmGKB database. We excluded loci that could not to be designed and finally designed 80 VIP variants to carry the experiment. The GoldMag- Mini Whole Blood Genomic DNA Purification Kit (GoldMag Ltd. Xi'an, China) was used to extract genomic DNA from whole blood in accordance with the manufacturer's protocol. Then we measured the DNA concentration with a NanoDrop 2000C spectrophotometer (Thermo Scientific, Waltham, MA, USA). The Sequenom MassARRAY Assay Deaign 3.0 software (San Diego, CA) was used to design the Mutiplexed SNP MassEXTEND assays (Gabriel et al., 2009). We used Sequenom MassARRAY RS1000 (San Diego, CA, USA) according to the standard protocol recommended by the manufacturer to perform SNP genotyping analysis. Managed and analyzed the SNP genotyping data was carried by Sequenom Typer 4.0 software as previous studies (Yunus et al., 2013; Shi et al., 2015; Zhang et al., 2014; Wang et al., 2015).

2.4. Genotype data

The genotype data of individuals from fifteen populations include eleven populations which were downloaded from the International HapMap Project web site (HapMap_release127) at http://hapmap.ncbi.nlm.nih.gov/biomart/martview/ (Gibbs et al., 2003) and four populations from our researches (Deng people, Kirgiz, Tajik, Uygur) (Yunus et al., 2013; Shi et al., 2015; Zhang et al., 2014; Wang et al., 2015). The eleven HapMap populations are: a population of African ancestry in the southwestern USA (ASW); a northwestern European population (CEU); the Han Chinese in Beijing, China (CHB); a Chinese population of metropolitan Denver, Colorado, USA (CHD); the Gujarati Indians in Houston, Texas, USA (GIH); the Japanese population in Tokyo, Japan (JPT); the Luhya people in Webuye, Kenya (LWK); people of Mexican ancestry living in Los Angeles, California, USA (MEX); the Maasai people in Kinyawa, Kenya (MKK); the Tuscan people of Italy (TSI); and the Yoruba in Ibadan, Nigeria (YRI).

2.5. Statistical analyses

Microsoft Excel and SPSS 19.0 statistical packages (SPSS, Chicago, IL) were used to perform Hardy-Weinberg Equilibrium (HWE) analysis and χ^2 test. Pearson's $\chi 2$ test was used to test the validation of each variant frequency in the Han ethnic which departure from HWE. We calculated and compared the genotype frequencies of the loci in Shaanxi Han ethnic with those in the fifteen populations respectively using χ^2 test which p values obtained were two sided. The Bonferroni's correction as the multiple hypothesis testing performed with the significant level ($p < [(0.05/80 \times 15)]$). The testing aimed to find out the sites with significant differences. And then, from the ALlele FREquency Database (http://alfred.med.yale.edu, ALFRED) we downloaded the SNP allele frequencies of global populations and observed the distribution of genetic variants at specific site.

2.6. Population genetic structures analysis

Previous study have point out that population genetic structure is crucial to the study of human origins, DNA forensics and complex disease (Elhaik, 2012). So we performed the common genetic structure analysis include STRUCTURE and Fst (F-statistics) in our study. STRUCTURE ver.2.3.4 (Pritchard Lab, Stanford University, USA) (http://pritchardlab.stanford.edu/software/structure_v.2.3.4.html) which based on the Bayesian clustering algorithm was used to assign the samples within a hypothetical K number of populations to analyze the genetic structure (Pritchard et al., 2000). We carried the analyses using the ancestry model with correlated allele frequencies. The MCMC analyses for each structure analysis (from $K = 2$ to $K = 8$) was run for 10,000 steps after an initial burn-in period of 10,000 steps. We calculated $\triangle K$ in accordance with the method of Evanno (Evanno et al., 2005) to assess the most likely number of clusters. Combined with the results, we using the drawing software constructed bar charts summarizing the results. F-statistics measure a subpopulation differentiation relative to the total population and provide the differentiation process among populations which is directly related to the variance in allele frequency between subpopulation (Holsinger and Weir, 2009). Arlequin ver 3.5.1.3 software (Institute of Ecology and Evolution, University of Bern, Switzerland) using genotype data was used to calculate the value of Fst to infer the pairwise distance between populations (Excoffier and Lischer, 2010). The Fst value is small when the allele frequencies within