Original Article
Genetic polymorphisms of pharmacogenomic VIP variants in the lhoba population of southwest China

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Abstract: Background: It is well-established that differences among ethnic groups in drug responses are primarily due to the genetic diversity of pharmacogenes. A number of genes or variants that play a crucial role in drug responses have been designated Very Important Pharmacogenes (VIP) by the PharmGKB database. Clarifying the polymorphic distribution of VIPs in different ethnic groups will aid in personalized medicine for specific populations. Methods: We sequenced 85 VIP variants in the Lhoba population based on the PharmGKB database. The polymorphic distribution of the 85 VIP variants in 100 Lhoba subjects was determined and compared with that of 11 major HapMap populations, including ASW, CEU, CHB, CHD, GIH, JPT, LWK, MEX, MKK, TSI, and YRI. We used χ^2 tests to identify significantly different loci between these populations. We downloaded SNP allele frequencies from the ALlele FREquency Database to observe the global genetic variation distribution for these specific loci. And then we used Structure software to perform the genetic structure analysis of 12 populations. Results: Based on comparisons of selected available loci, we found that 23, 28, 16, 10, 20, 16, 24, 19, 22, 21 and 36 of the selected VIP variant genotype frequencies in the Lhoba population differed from those of the ASW, CEU, CHB, CHD, GIH, JPT, LWK, MEX, MKK, TSI, and YRI populations, respectively. In addition, Pairwise FST values and clustering analyses also showed the VIP variants in Lhoba exhibited a close genetic affinity with CHD, CHB and JPT populations. Conclusion: Our results complement pharmacogenomic data on the Lhoba ethnic group and may be helpful in the diagnosis of certain diseases in minorities.

Keywords: Genetic polymorphism, pharmacogenomics, VIP variants, Lhoba

Introduction

The goals of personalized medicine are to maximize therapeutic efficacy and minimize the risk of drug toxicity for an individual patient. Pharmacogenomics plays a major role in personalized medicine. Pharmacogenetics is the study of how genetic variation influences the response to drugs. Race, ethnicity, and ancestry have a strong influence on pharmacogenetics and on our understanding of population-level differences in drug response [1, 2]. The primary goal of pharmacogenetics, however, is to identify the individual genetic determinants

of drug activity so that therapy can be tailored to the individual patient. The Pharmacogenetics and Pharmacogenomics Knowledge Base (PharmGKB: http://www.pharmgkb.org) was created in order to aggregate data from the primary research literature and distill and curate that information in multiple forms [3, 4].

A number of studies have highlighted the importance of ethnicity in influencing genetic variability. China is a multinational country, with 55 minority ethnicities, including the Lhoba ethnic minority. The 2,965 people of the Lhoba ethnic minority reside primarily in Mainling, Medog,

Table 1. Basic characteristics of selected variants in the Lhoba

| SNP ID | Genes | Allele | | Categories | | | | Lhoba | | | HWE |
		A	B	Family	Phase	MAF	AA-Count	AB-Count	BB-Count	
rs10264272	CYP3A5	C	T	Cytochrome P450 superfamily	Phase I	0	100	0	0	/
rs1042713	ADRB2	G	A	Adrenergic receptors family	Others	0.485	22	53	25	0.542
rs1042714	ADRB2	G	C	Adrenergic receptors family	Others	0.145	2	25	73	0.934
rs1045642	ABCB1	A	G	ATP-binding cassette (ABC) transporters superfamiy	Others	0.475	20	55	25	0.304
rs1051266	SLC19A1	T	C	Solute carrier family	Others	0.332	44	43	11	0.919
rs1065776	P2RY1	T	C	G-protein coupled receptor family	Others	0.01	0	2	98	0.920
rs10929302	UGT1A10	G	A	UDP-glucuronosyltransferase family	Phase II	0.071	86	12	1	0.440
rs1128503	ABCB1	A	G	ATP-binding cassette (ABC) transporters superfamiy	Others	0.28	50	44	6	0.361
rs1142345	TPMT	T	C	Methyltransferase superfamily	Phase II	0.015	97	3	0	0.879
rs1229984	ADH1B	T	C	Alcohol dehydrogenase family	Phase I	0.06	1	10	89	0.256
rs12720441	KCNH2	G	A	Eag family	Others	0	100	0	0	/
rs12721634	CYP3A4	T	C	Cytochrome P450 superfamily	Phase I	0	100	0	0	/
rs1540339	VDR	C	T	Nuclear receptor famiy	Others	0.285	11	35	54	0.158
rs1544410	VDR	C	T	Nuclear receptor famiy	Others	0.07	87	12	1	0.433
rs1695	GSTP1	A	G	Glutathione S-transferase family	Phase II	0.105	80	19	1	0.913
rs17238540	HMGCR	T	C	HMGCR	Phase I	0	100	0	0	/
rs17244841	HMGCR	A	T	HMGCR	Phase I	0	100	0	0	/
rs1799853	CYP2C9	C	T	Cytochrome P450 superfamily	Phase I	0	100	0	0	/
rs1800460	TPMT	C	G	Methyltransferase superfamily	Phase II	0	0	0	100	/
rs1800497	DRD2	G	A	G-protein coupled receptor family	Others	0.24	57	38	5	0.677
rs1800888	ADRB2	C	T	Adrenergic receptors family	Others	0	100	0	0	/
rs1801030	SULT1A2	C	T	Sulfotransferase family	Phase II	0	100	0	0	/
rs1801131	MTHFR	T	G	Methylenetetrahydrofolate reductase family	Phase I	0.301	47	43	8	0.673
rs1801133	MTHFR	G	A	Methylenetetrahydrofolate reductase family	Phase I	0.227	58	37	4	0.524
rs1801252	ADRB1	A	G	Adrenergic receptors family	Others	0.135	75	23	2	0.879
rs1801253	ADRB1	G	C	Adrenergic receptors family	Others	0.211	6	29	62	0.310
rs1801272	CYP2A6	A	T	Cytochrome P450 superfamily	Phase I	0.015	0	3	97	0.879
rs1805124	SCN5A	T	C	Sodium channel gene family	Others	0.115	77	23	0	0.194
rs2032582	ABCB1	A	C	ATP-binding cassette (ABC) transporters superfamiy	Others	0.459	23	47	16	0.352
rs20417	PTGS2	C	G	PTGS2	Phase I	0.025	2	1	97	0.000
rs2046934	P2RY12	G	A	G-protein coupled receptor family	Others	0.21	4	34	62	0.805
rs2066702	ADH1B	G	A	Alcohol dehydrogenase family	Phase I	0	100	0	0	/
rs2066853	AHR	G	A	AHR	Others	0.235	57	39	4	0.397
rs2228570	VDR	T	C	Nuclear receptor famiy	Others	0.43	14	58	28	0.067
rs2239179	VDR	T	C	Nuclear receptor famiy	Others	0.18	69	26	5	0.233
rs2239185	VDR	G	A	Nuclear receptor famiy	Others	0.371	41	40	16	0.251
rs2740574	CYP3A4	A	T	Cytochrome P450 superfamily	Phase I	0	100	0	0	/
rs28371706	CYP2D6	C	G	Cytochrome P450 superfamily	Phase I	0	100	0	0	/
rs28371725	CYP2D6	G	A	Cytochrome P450 superfamily	Phase I	0.02	96	4	0	0.838
rs28399433	CYP2A6	G	T	Cytochrome P450 superfamily	Phase I	0.145	1	27	72	0.374
rs28399444	CYP2A6	A	T	Cytochrome P450 superfamily	Phase I	0	100	0	0	/
rs28399454	CYP2A6	C	T	Cytochrome P450 superfamily	Phase I	0	100	0	0	/
rs28399499	CYP2B6	T	C	Cytochrome P450 superfamily	Phase I	0	100	0	0	/
rs3211371	CYP2B6	C	T	Cytochrome P450 superfamily	Phase I	0.5	0	100	0	0.000
rs3745274	CYP2B6	G	T	Cytochrome P450 superfamily	Phase I	0.153	70	26	2	0.818
rs3782905	VDR	G	C	Nuclear receptor famiy	Others	0.1	1	18	81	1.000
rs3807375	KCNH2	C	T	Eag family	Others	0.275	6	43	51	0.433
rs3814055	NR1I2	C	T	Nuclear receptor famiy	Others	0.101	79	20	0	0.264
rs3815459	KCNH2	C	T	Eag family	Others	0.474	27	46	22	0.778
rs3846662	HMGCR	A	G	HMGCR	Phase I	0.485	22	53	25	0.542
rs3918290	DPYD	C	T	DPYD	Phase I	0	100	0	0	/
rs4124874	UGT1A10	T	G	UDP-glucuronosyltransferase family	Phase II	0.165	69	29	2	0.600

rs4148323	UGT1A10	G	A	UDP-glucuronosyltransferase family	Phase II	0.39	41	40	19	0.111
rs4149056	SLCO1B1	T	C	Solute carrier family	Others	0.045	91	9	0	0.637
rs4244285	CYP2C19	G	A	Cytochrome P450 superfamily	Phase I	0.28	51	42	7	0.677
rs4986893	CYP2C19	G	A	Cytochrome P450 superfamily	Phase I	0.02	96	4	0	0.838
rs4986910	CYP3A4	A	G	Cytochrome P450 superfamily	Phase I	0	99	0	0	/
rs4986913	CYP3A4	G	A	Cytochrome P450 superfamily	Phase I	0	100	0	0	/
rs5030656	CYP2D6	AAG	/	Cytochrome P450 superfamily	Phase I	0	100	0	0	/
rs5219	KCNJ11	C	T	Inward-rectifier potassium channel family	Others	0.434	28	55	15	0.158
rs59421388	CYP2D6	C	G	Cytochrome P450 superfamily	Phase I	0.01	98	2	0	0.920
rs6025	F5	T	C	F5	Others	0	0	0	100	/
rs61736512	CYP2D6	C	G	Cytochrome P450 superfamily	Phase I	0	100	0	0	/
rs6277	DRD2	G	A	G-protein coupled receptor family	Others	0	100	0	0	/
rs6791924	SCN5A	G	A	Sodium channel gene family	Others	0.005	99	1	0	0.960
rs689466	PTGS2	T	C	PTGS2	Phase I	0.44	31	50	19	0.884
rs698	ADH1C	T	C	Alcohol dehydrogenase family	Phase I	0.116	78	19	2	0.516
rs701265	P2RY1	A	G	G-protein coupled receptor family	Others	0.135	74	25	1	0.481
rs7294	VKORC1	C	T	VKORC1	Phase I	0.055	90	9	1	0.180
rs7626962	SCN5A	G	T	Sodium channel gene family	Others	0	100	0	0	/
rs776746	CYP3A5	C	T	Cytochrome P450 superfamily	Phase I	0.19	66	30	4	0.800
rs7975232	VDR	C	A	Nuclear receptor famiy	Others	0.38	40	44	16	0.508
rs975833	ADH1A	G	C	Alcohol dehydrogenase family	Phase I	0.42	32	45	17	0.865
rs9934438	VKORC1	G	A	VKORC1	Phase I	0.055	1	9	90	0.180

Lhunze, and Nangxian counties in southeastern Tibet. Additionally, a small number live in Luoyu in southern Tibet. Because of differences in genetics, physiology, pathology, eating habits, living environment, and nutritional status, the same drug dosage regimen for every ethnic group may be inappropriate [5]. Genetic variations, such as single nucleotide polymorphisms (SNPs), play an important role clinically [6]. SNPs have been shown to alter enzyme activity, resulting in abnormally increased or decreased metabolism of drugs [7]. For instance, it has been estimated that CYP2C9 is responsible for the metabolic clearance of up to 15-20% of all drugs undergoing phase I metabolism [8]. Significant differences are found in *CYP2C9*2* allele frequencies in different populations. For example, the *CYP2C9*2* allele frequency in Spain is 26.7% but zero in east Asia [9]. Genotyping all patients for relevant genetic variants before beginning a drug treatment is not feasible. As an alternative, population allele frequency data may be able to substitute for individual genotyping. However, minimal relevant data are available for minorities. The goal of this study was to address the lack of data regarding the allele frequencies of clinically relevant SNPs within the Lhoba population. Our results complement current pharmacogenomic data on the Lhoba ethnic group. Further genotyping studies of specific populations are necessary to provide the best medical care to all individuals.

Materials and methods

Study participants

We recruited a random sample of 100 unrelated Lhoba adults from the Xinjiang Region of China. In general, all control subjects were healthy, without chronic diseases or disorders related to the vital organs. To ensure that controls were cancer-free, they were tested for the presence of plasma carcinoembryonic antigen and alpha-fetoprotein. In addition, we selected individuals who had exclusively Lhoba ancestry for at least the previous three generations. All participants signed informed consent forms, after which 5 ml of peripheral blood was drawn from each subject. The study protocol was approved by the Clinical Research Ethics Committees of Northwest University.

Variant selection and genotyping

We selected genetic variants from published polymorphisms associated with VIP variants from the PharmGKB database and excluded loci that could not be designed. A total of 86 genetic variant loci were selected. Genomic DNA was extracted from whole blood using a GoldMag-Mini Whole Blood Genomic DNA

Table 2. Lhoba compared with the11 HapMap populations after Bonferroni's multiple adjustment

SNP	ASW	CEU	CHB	CHD	GIH	JPT	LWK	MEX	MKK	TSI	YRI
rs10264272	/	/	/	/	/	/	1.445E-12	/	8.823E-08	/	8.95E-09
rs1042713	0.6380937	0.0044716	0.5270236	0.444125	0.1707646	0.2674132	0.772293	0.5414963	0.811307	0.0016608	0.3529788
rs1042714	/	6.805E-08	0.6277193	/	/	0.1785214	/	/	/	/	0.5692257
rs1045642	1.001E-05	0.1065589	0.0577659	0.0802092	0.0280032	0.9051566	/	0.9568834	5.145E-13	0.6065329	5.413E-15
rs1051266	0.0043283	6.895E-06	0.0063808	0.0003241	7.061E-07	0.1000146	0.2811009	8.062E-07	0.042643	0.000215	0.4522463
rs1065776	/	/	/	/	/	/	/	/	/	/	/
rs10929302	/	6.513E-06	0.5364754	/	/	0.5128563	/	/	/	/	3.074E-08
rs1128503	6.812E-16	6.961E-08	0.6064446	0.6460303	0.0325144	0.0187387	3.476E-25	4.099E-05	6.934E-28	1.293E-07	3.563E-27
rs1142345	/	/	/	/	/	/	0.0004143	0.0312395	/	/	0.2549644
rs1229984	/	0.0289084	7.297E-23	/	/	2.618E-22	/	/	/	/	0.0306175
rs12720441	/	/	/	/	/	/	/	/	/	/	/
rs12721634	/	/	/	/	/	/	/	/	/	/	/
rs1540339	1.703E-11	4.77E-10	0.6820561	0.4519886	8.313E-10	0.8053552	3.919E-21	4.041E-06	3.041E-23	1.172E-09	3.378E-19
rs1544410	5.43E-05	2.932E-14	0.4616421	0.4491027	2.135E-14	0.060301	8.076E-06	0.0001279	4.388E-12	6.733E-13	1.573E-07
rs1695	4.163E-10	7.76E-12	0.0563114	0.0184164	6.755E-07	0.886209	3.546E-16	1.97E-12	5.743E-09	6.537E-06	3.281E-10
rs17238540	/	/	/	/	/	/	/	/	/	/	/
rs17244841	/	/	/	/	/	/	/	/	/	/	/
rs1799853	/	/	/	/	/	/	/	/	/	/	/
rs1800460	/	/	/	/	/	/	/	/	/	/	/
rs1800497	0.0082188	0.3206538	0.0010863	0.0002928	0.3810381	0.002664	0.0272732	0.0074558	0.0215364	0.6832066	0.0009302
rs1800888	/	/	/	/	/	/	/	/	/	/	/
rs1801030	/	/	/	/	/	/	/	/	/	/	/
rs1801131	0.0910937	0.6534614	0.1965392	0.0670376	0.1433041	0.0333442	0.0275723	0.2363873	0.4899573	0.3715165	1.416E-05
rs1801133	0.0010973	0.1472415	6.751E-06	0.0451049	0.3713918	0.0159539	0.0009765	0.002124	4.855E-05	2.086E-05	0.0004851
rs1801252	/	/	0.0011173	/	/	0.001287	/	/	/	/	0.0006274
rs1801253	/	0.1442617	0.4732605	/	/	0.4736514	/	/	/	/	0.0008045
rs1801272	/	9.854E-34	5.38E-32	/	/	9.095E-31	/	/	/	/	1.805E-35
rs1805124	0.0032287	0.1268706	/	/	0.0849444	/	0.0002433	0.3269952	1.05E-07	0.0057815	9.724E-06
rs2032582	/	/	/	/	/	/	/	/	/	/	/
rs20417	/	1.167E-29	2.058E-29	/	/	3.392E-29	/	/	/	/	3.207E-24
rs2046934	/	0.8499027	0.9614884	/	/	0.8712248	/	/	/	/	0.6454923
rs2066702	1.065E-10	/	/	/	/	/	4.081E-07	/	/	/	5.646E-15
rs2066853	0.0141483	0.0004519	0.0011956	0.0048595	0.0023018	2.2E-05	1.45E-06	0.0472198	0.0013065	0.0003466	3.642E-05
rs2228570	/	/	/	/	/	/	/	/	/	/	/

Pharmacogenomics VIP polymorphisms

rs2239179	0.0019673	3.597E-07	0.2087307	0.6354083	6.432E-09	0.6468278	0.0010861	0.0869567	9.07E-08	3.708E-06	0.0159804
rs2239185	/	/	0.4685249	/	/	0.2766129	/	/	/	/	0.0172396
rs2740574	/	/	/	/	/	/	/	/	/	/	/
rs28371706	/	/	/	/	/	/	/	/	/	/	/
rs28371725	/	/	/	/	/	/	/	/	/	/	/
rs28399433	/	/	/	/	/	/	/	/	/	/	/
rs28399444	/	/	/	/	/	/	/	/	/	/	/
rs28399454	/	/	/	/	/	/	/	/	/	/	/
rs28399499	0.0003479	/	/	/	/	/	/	/	0.1678005	/	2.037E-06
rs3211371	/	/	/	/	/	/	/	/	/	/	/
rs3745274	0.0210484	0.0147724	0.8048011	0.9542118	7.743E-07	0.603502	0.0012926	0.0102078	1.156E-06	0.0094913	6.105E-08
rs3782905	/	2.278E-19	2.913E-21	/	/	7.343E-25	/	/	/	/	1.07E-22
rs3807375	0.7813935	1.863E-11	0.7706129	0.9413499	4.484E-10	0.081591	0.0162642	0.0206186	0.9907614	1.658E-11	0.2726303
rs3814055	2.4E-05	1.382E-07	5.46E-05	0.0006412	3.023E-11	0.0001352	2.845E-05	6.538E-05	0.0015888	2.053E-09	2.662E-05
rs3815459	/	/	0.0007393	/	/	1.5E-06	/	/	/	/	0.1291254
rs3846662	5.804E-09	0.1248809	0.825542	0.6531327	0.0221528	0.8459821	2.521E-20	0.0480714	5.189E-13	0.3144964	4.179E-22
rs3918290	/	/	/	/	/	/	/	/	/	/	/
rs4124874	3.298E-19	1.126E-08	0.0017157	0.0003381	2.688E-16	0.0010809	5.565E-30	2.673E-08	5.62E-33	2.929E-07	7.398E-35
rs4148323	/	6.646E-13	0.0003685	0.0001079	2.382E-14	1.467E-06	/	7.694E-09	/	/	6.646E-13
rs4149056	0.3727746	0.000974	0.002254	0.0263308	/	0.0638509	/	/	0.0257473	3.261E-06	/
rs4244285	/	0.0151862	0.1531883	/	/	0.9912602	/	/	/	/	0.0136854
rs4986893	/	/	/	/	/	/	/	/	/	/	/
rs4986910	/	/	/	/	/	/	/	/	/	/	/
rs4986913	/	/	/	/	/	/	/	/	/	/	/
rs5030656	/	/	/	/	/	/	/	/	/	/	/
rs5219	/	/	/	/	/	/	/	/	/	/	/
rs59421388	/	/	/	/	/	/	/	/	/	/	/
rs6025	/	0.1646743	/	/	/	/	/	/	/	/	/
rs61736512	/	/	/	/	/	/	/	/	/	/	/
rs6277	/	1.54E-26	/	/	/	/	/	/	/	/	/
rs6791924	/	/	/	/	/	/	/	/	/	/	/
rs689466	3.389E-08	1.522E-08	0.534223	0.6954382	1.963E-08	0.214661	1.783E-17	0.005605	4.43E-24	4.283E-07	7.182E-14
rs698	0.2472551	3.218E-13	0.1091256	0.3145579	0.0027601	0.2195671	0.4639062	0.0115411	0.0084764	0.0001312	0.1139771
rs701265	6.476E-16	0.301935	0.0005385	0.0151595	0.0445677	0.0055455	6.36E-27	0.34792	1.494E-30	0.3207243	1.784E-29
rs7294	1.998E-16	2.767E-13	0.612706	0.3582965	1.542E-31	0.0691227	5.411E-17	6.888E-09	5.207E-21	8.073E-12	2.356E-21

Pharmacogenomics VIP polymorphisms

rs7626962	/	/	/	/	/	/	/	/	/	/	0.0012409
rs776746	3.516E-12	5.209E-06	0.0524418	0.5040015	0.4270217	0.1157965	7.21E-27	0.2287979	3.231E-11	0.0003761	4.227E-29
rs7975232	0.0002258	0.0009613	0.3768935	0.2806556	0.0085925	0.6861881	1.387E-09	0.0949649	3.44E-09	0.0003739	4.224E-06
rs975833	/	0.0756969	1.535E-09	/	/	6.723E-08	/	/	/	/	0.0474908
rs9934438	8.943E-27	3.766E-24	0.612706	0.4507347	1.051E-33	0.0738706	8.196E-37	2.08E-16	2.051E-43	6.33E-20	2.593E-43

p value <0.05/(74×11) indicates statistical significance.

Table 3. Distribution of pairwise Fst distances among the among the 12 populations

	Lhoba	ASW	CEU	CHB	CHD	GIH	JPT	LWK	MEX	MKK	TSI	YRI
Lhoba	0											
ASW	0.26016	0										
CEU	0.1452	0.14514	0									
CHB	0.03002	0.21775	0.14398	0								
CHD	0.02218	0.21406	0.13922	-0.00152	0							
GIH	0.19204	0.09749	0.03622	0.17875	0.17232	0						
JPT	0.02671	0.20065	0.1332	0.00419	0.00331	0.16642	0					
LWK	0.33588	0.02041	0.223	0.29502	0.29455	0.17418	0.27481	0				
MEX	0.11532	0.10756	0.02912	0.08894	0.08553	0.05365	0.08526	0.18942	0			
MKK	0.26376	0.02066	0.143	0.23816	0.23644	0.10624	0.21391	0.02616	0.1355	0		
TSI	0.13181	0.14758	0.00249	0.1233	0.12296	0.04433	0.11399	0.22728	0.0279	0.14895	0	
YRI	0.33185	0.01407	0.22394	0.29007	0.2897	0.16559	0.26752	0.00487	0.1878	0.02375	0.22898	0
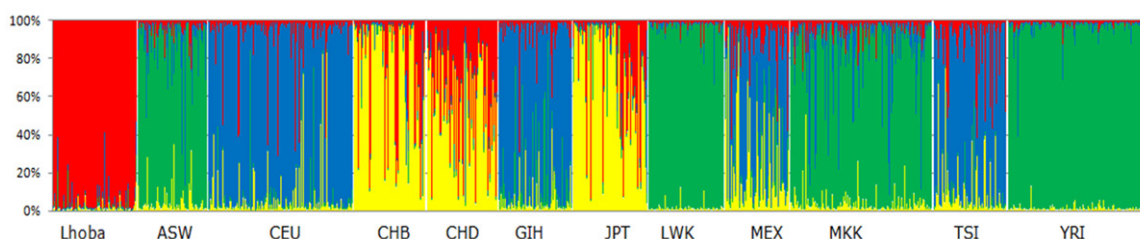
Figure 1. Results of STRUCTURE clustering analysis (K = 4) for Lhoba and Hapmap populations. ASW: African ancestry in southwest USA; CEU: Utah residents with Northern and Western European ancestry from the CEPH collection; CHB: Han Chinese in Beijing, China; CHD: Chinese in Metropolitan Denver, Colorado; GIH, Gujarati Indians in Houston, Texas; JPT: Japanese in Tokyo, Japan; LWK: Luhya in Webuye, Kenya; MEX: Mexican ancestry in Los Angeles, California; MKK: Maasai in Kinyawa, Kenya; TSI: Toscans in Italy; YRI: Yoruba in Ibadan, Niger.

Purification Kit (GoldMag Co. Ltd., Xi'an City, China). The DNA concentration was measured using a NanoDrop 2000 (Thermo Scientific, Waltham, Massachusetts, USA). Sequenom MassARRAY Assay Design 3.0 software (Sequenom, San Diego, California, USA) was used to design a Multiplexed SNP MassEXTEND assay (Sequenom) [10]. A Sequenom Mass ARRAY RS1000 was used to genotype SNPs using the standard protocol recommended by the manufacturer [10]. Finally, Sequenom Typer 4.0 software was used for data management and analysis [10, 11]. Laboratory personnel were blinded to genotyping results.

Statistical analyses

Microsoft Excel and SPSS 19.0 statistical software (SPSS, Chicago, IL) were used to perform Hardy-Weinberg equilibrium (HWE) and chi-square tests. All p values in this study were two-sided and a p value of ≤ 0.05 after Bonferroni correction was considered the threshold for statistical significance. The Bonferroni correc-

tion was applied to determine the p value threshold of significance: 0.05/(74*4). Validation of the frequency of each variant in the Lhoba population was tested for departure from the HWE using an exact test. We calculated and compared the genotype frequencies in the Lhoba population with those of 11 other populations (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). (data from HapMap: http://hapmap.ncbi.nlm.nih.gov) separately using a chi-square test. The program Arlequin v3.5 was used to calculate global FST together with pairwise FST values among all of the populations using loci that

were polymorphic. The Structure (version 2.3.4) software were used to analysis the genetic structure within a hypothetical K number in 12 populations.

Results

Basic information about the selected VIP loci of the Lhoba population listed in **Table 1**. Twelve loci were disregarded in our study because genotype data were lacking or the call rate was < 90%. In addition, 27 loci were removed from the analyses of VIP variants for lack of genotype results in the HapMap database, and therefore, data for comparisons were unavailable.

Table 2 lists the genotypic frequencies of tested variants in the Lhoba population and illustrates the variants with frequencies in the Lhoba population that were significantly different from the 11 HapMap populations. Based on comparisons of selected available loci, we found that 23, 28, 16, 10, 20, 16, 24, 19, 22, 21 and 36 of the selected VIP variant genotype frequencies in the Lhoba population differed from those of the ASW, CEU, CHB, CHD, GIH, JPT, LWK, MEX, MKK, TSI, and YRI populations, respectively ($P < 0.05$). However, after validation using the Bonferroni correction ($P < 0.05/(74 \times 11)$), the differences in frequency distribution of the variants in the Lhoba population versus the 11 populations were 14, 21, 7, 0, 13, 8, 16, 8, 18, 13 and 26 respectively. The rs3814055 (*NR1I2*), rs4124874 (*UGT1A1*), and rs2066853 (AHR) locus showed the greatest number of significant differences between Lhoba and 11 HapMap populations.

In **Table 3** we listed the Pairwise F_{ST} values between Lhoba and other 11 populations. F_{ST} value is less than 0.15 represent there is no genetic differentiation between the two populations. populations. Comparing other populations, the lower level of differentiation were found between Lhoba and CHD ($F_{ST} = 0.022$), JPT ($F_{ST} = 0.027$) and CHB ($F_{ST} = 0.03$) populations, while the greater divergence were observed in YRI ($F_{ST} = 0.331$), MKK ($F_{ST} = 0.264$), and GIH ($F_{ST} = 0.192$).

STRUCTURE analysis provided complementary methods for visualizing patterns of genetic similarity and differentiation between Lhoba and other eleven populations. Best model at K = 4,

where the proportion of each ancestral component in a single individual is represented by a vertical bar divided into four colors. In **Figure 1**, the bar plot revealed that the individuals sampled in Lhoba were near to cluster of CHD population.

Discussion

This study assessed the polymorphic distribution of 86 pharmacogenomic VIP variants from the PharmGKB database in the Lhoba population as compared with four major HapMap populations. We found that the allelic frequencies in the Lhoba population are generally similar to those in the CHD, CHB and JPT populations but differ significantly from those in the MKK and YRI populations. The differences are particularly pronounced in the YRI population. Pairwise F_{ST} values and clustering analyses also showed the VIP variants in Lhoba exhibited a close genetic affinity with most similar to CHD population. These results suggest that genotype data from one group or subgroup (i.e., nation or ethnicity) should not be overly generalized and applied to genetically distinct groups.

In our study, we found that the genotype frequency of rs3814055 in *NR1I2* differed significantly among the 12 selected populations. The pregnane X receptor (PXR), also known as nuclear receptor subfamily 1, group I, member 2 (NR1I2), pregnane-activated receptor (PAR), and steroid and xenobiotic receptor (SXR), is a transcription factor belonging to the nuclear hormone receptor superfamily [12, 13]. PXR/NR1I2 is a key regulator of the expression of genes involved in all stages of drug metabolism and transport [14, 15]. Phase I drug-metabolizing enzymes regulated by PXR/NR1I2 include several CYPs (e.g., CYP3A4, CYP3A5, CYP2B6, and CYP2C8), carboxylesterases, and dehydrogenases [16-19]. The most common clinical reason for the activation of PXR/NR1I2 is the occurrence of drug-drug interactions mediated by up-regulated CYP3A4 expression. Surprisingly, the level of CYP3A4 expression can vary by up to 100-fold between individuals [20, 21]. It is well known that ethnicity is an important factor contributing to the large inter-individual variability in drug metabolism, therapeutic response, and toxicity [22, 23]. The frequency of rs3814055 in the *NR1I2* gene varies in different ethnic groups. In Han Chinese, the frequency of rs3814055 (-25385T), a variant that leads to enhanced CYP3A4 metabolic activity

[24], is lower than in Caucasians, Europeans, and African Americans and similar to that of Asians and African Americans. The variant occurs at a frequency of 0.218 in Han Chinese [25], 0.21 in Asians [26], 0.39 in Caucasians [24], 0.50 in Europeans [26], 0.36 in the Dutch [27], and 0.34 in African Americans [26]. In our study, the frequency of the rs3814055 SNP variant was 0.101 in the Lhoba population.

The allelic frequencies in the Lhoba population were generally similar to those in the CHD population. However, the frequency of rs1801133 in the *MTHFR* gene differed in the two populations. The allelic frequency was 0.227 in the Lhoba population and 0.341 in the CHB population. The enzyme 5,10-methylenetetrahydrofolate reductase (MTHFR) catalyzes the conversion of 5,10-methylenetetrahydrofolate (5,10-methylene-THF) into 5-methyltetrahydrofolate (5-methyl-THF), a major circulating form of folate that provides a methyl group for homocysteine methylation [28, 29]. MTHFR plays a key role in folate metabolism by channeling one-carbon units between nucleotide synthesis and methylation reactions. Reduced 5-methyl-THF levels may decrease methylation of homocysteine to methionine, resulting in hyperhomocysteinemia and DNA hypomethylation [30]. On the other hand, reduced levels of 5,10-methylene-THF, which is required for thymidylate synthesis, could lead to misincorporation of uracil into DNA, increasing the frequency of chromosome damage [30], an effect that facilitates the action of certain chemotherapeutics, including MTX. MTHFR deficiency is the most common inherited folate metabolism disorder. More attention should also be paid to capecitabine, cisplatin, pemetrexed, cyanocobalamin, and related agents in the Lhoba population.

Conclusions

Our results demonstrate that national populations in similar geographic regions, such as the Lhoba, may have widely varying genetic allele frequencies for clinically relevant SNPs. The genotype frequencies of VIP variants significantly affect a population's response to a given drug. Determination of the genotype distribution and frequencies of VIP variants in different populations would provide a theoretical foundation for safer drug administration and enhanced curative effects. Our results complement current data contained in the pharma-

cogenomics database as it pertains to the Lhoba ethnic group. Additional genotyping studies of specific populations are necessary to provide the best medical care to all individuals. However, the sample size of the Lhoba population in our study was relative small, and further investigation involving a larger cohort of Lhoba individuals is necessary to determine the generalizability of our results to these and other conditions in the Lhoba population.

Disclosure of conflict of interest

None.

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