Population analysis of pharmacogenetic polymorphisms related to acute lymphoblastic leukemia drug treatment

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Abstract. This study aimed to evaluate in the Brazilian population, the genotypes and population frequencies of pharmacogenetic polymorphisms involved in the response to drugs used in treatment of acute lymphoblastic leukemia (ALL), and to compare the data with data from the HapMap populations. There was significant differentiation between most population pairs, but few associations between genetic ancestry and SNPs in the Brazilian population were observed. AMOVA analysis comparing the Brazilian population to all other populations retrieved from HapMap pointed to a genetic proximity with the European population. These associations point to preclusion of the use of genetic ancestry as a proxy for predicting drug response. In this way, any study aiming to correlate genotype with drug response in the Brazilian population should be based on pharmacogenetic SNP genotypes.

Keywords: SNPs, Brazilian population, pharmacogenetic polymorphisms, HapMap

1. Introduction

Leukemia is a neoplastic disease derived from clonal expansion of blasts, which accumulate in the bone marrow preventing the formation of blood cells. Acute Lymphoblastic Leukemia (ALL) is the most common type of childhood cancer, corresponding to 25% of all cancers in children and 75% of leukemia cases [9,19, 22]. Progress in drug development and chemotherapy protocols has brought the cure rate for ALL to nearly 80% currently [21,24]. However, the response to treatment and the presence of side effects vary among patients due to factors such as age, sex and genetics [10].

For instance, the antifolate Methotrexate (MTX), an antineoplastic agent with immunosuppressive proper-

ties is used in the treatment of ALL and other diseases, such as rheumatoid arthritis [16,29]. MTX is transported to the inside of the cell by the reduced folate carrier (RFC), which is very important to MTX efficacy [12, 16]. The enzymes from folate pathways have single nucleotide polymorphisms (SNPs) that can influence the effects of MTX, and the study of these SNPs can help in the management of patient care [5,16,32], suggesting that population or inter-individual genetic differences may play an important role in pharmacogenetics responses [31]. The variability in the response to drugs occurs within and between populations and to individualize treatment it is necessary to characterize the genetic variations between individuals.

Admixed populations are prone to generating false negative/positive or spurious associations [28] due to the admixture stratification and different drug response phenotypes [26]. Thus, in such populations, studies may require the use of ancestry-informative markers (AIMs) to control for population stratification in or-

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Table 1
Pharmacogenetics polymorphisms and implicated drugs

Polymorphism	dbSNP ID	Implicated drugs (Known and Suspected)	Allele 1	Allele 2
MTHFR 677C>T	rs1801133	MTX	C	T
MTHFR 1298A>C	rs1801131	MTX	A	C
GSTP1 313A>G	rs1695	GC, MTX	A	G
RFC1 80G>A	rs1051266	MTX	G	A
GSTP1 A114V	rs1138272	GC, MTX	C	T
GGH 452C>T	rs11545078	MTX	C	T
MTHFD1 1958G>A	rs2236225	MTX	C	T
*TPMT*2*	rs1800462	MP	C	G
*TPMT*3B*	rs1800460	MP	A	G
*TPMT*3C*	rs1142345	MP	A	G
*TPMT*4*	rs1800584	MP	A	G
ABCC2	rs717620	MTX	A	G
SMARCA4	rs1122608	GC	G	T
ARID5B	rs10821936	MTX	C	T
ITPA IVS2+21A >C	rs7270101	MP	A	C
XDH A(1936) >G	rs17323225	MTX	C	T
XDH A(2107) >G	rs17011368	MTX	C	T
NR3C1	rs852977	GC	A	G
CYP3A	rs10264272	GC	C	T
CYP3A4	rs4986913	GC	C	T

(MTX = methotrexate, MP = mercaptopurine, GC = glucocorticoids).

der to avoid any association error [3,4]. Since the Brazilian population has a heterogeneous genetic background [18], studies have suggested that control for stratification using AIMs may be better for ensuring proper drug treatment according to the pharmacogenetics of the patient [26]. This is in preference to using a combination of the patients' physical traits, such as skin pigmentation combined with data extrapolated from other well-defined ethnic populations. In this study, we aimed to evaluate 20 different pharmacogenetic SNPs related to ALL drug response regarding population genetic differences between phase 3 HapMap populations [1] and the Brazilian population. In addition, we assessed correlation between SNP genotypes and genetic ancestry as inferred by the use of AIMs in the Brazilian population.

2. Methods

2.1. Population sample

The Brazilian population sample (BRA) consisted of 200 healthy, unrelated individuals, randomly selected from investigation of paternity cases. Individuals had no cost and signed an informed consent enabling the use of their DNA for paternity testing and further anonymous population genetic research. The sample was subdivided based on geographical origin, into groups of 40 individuals from the five Brazilian geopolitical regions: Center-West (CW), Northeast (NE), North (N), Southeast (SE) and South (S) [18]. The 11 HapMap population genotypes of the 20 SNPs were obtained from the Phase 3 HapMap database. The population description and abbreviations in HapMap are as follows: African ancestry in Southwest USA (ASW); Utah residents with Northern and Western European ancestry from the CEPH collection (CEU); Han Chinese in Beijing, China (CHB); Chinese in Metropolitan Denver, Colorado (CHD); Gujarati Indians in Houston, Texas (GIH); Japanese in Tokyo, Japan (JPT); Luhya in Webuye, Kenya (LWK); Mexican ancestry in Los Angeles, California (MEX); Maasai in Kinyawa, Kenya (MKK); Toscans in Italy (TSI); Yoruba in Ibadan, Nigeria (YRI).

2.2. SNP selection and genotyping assay

SNPs related to drug response, focusing on those used in ALL treatment protocols, especially those related to methotrexate metabolism, were selected from the pharmacogenetics database (PharmgKB) and also from the literature [10,13,24]. We selected 20 SNPs mapping to 13 genes (Table 1). PCR was performed in multiplex panels using the Qiagen Multiplex PCR Kit. After PCR, excess deoxyribonucleotide (dNTP) and primers were enzymatically eliminated using Exonuclease I and shrimp alkaline phosphatase. Single-base extension was carried out using the SNaPshot Multi-

Table 2
Allelic frequencies of the 20 pharmacogenetic loci in 12 different populations (n/a = data not available)

Locus	dbSNP ID	Allele	Population											
			ASW	CEU	CHB	CHD	GIH	JPT	LWK	MEX	MKK	TSI	YRI	BRA
MTHFR 677C>T	rs1801133	C	0.877	0.690	0.524	0.659	0.830	0.640	0.911	0.580	0.916	0.540	0.907	0.655
MTHFR 1298A>C	rs1801131	A	0.811	0.659	0.780	0.788	0.608	0.814	0.817	0.790	0.734	0.682	0.885	0.723
GSTP1 313A>G	rs1695	A	0.547	0.593	0.810	0.794	0.670	0.907	0.494	0.450	0.643	0.693	0.606	0.658
RFC1 80G>A	rs1051266	A	0.472	0.438	0.500	0.465	0.392	0.558	0.739	0.330	0.773	0.449	0.690	0.480
GSTP1 A114V	rs1138272	C	0.972	0.903	1.000	n/a	0.909	1.000	0.989	0.920	0.976	0.938	1.000	0.963
GGH 452C>T	rs11545078	C	0.953	n/a	n/a	0.933	0.824	0.861	0.931	n/a	0.941	0.918	0.920	0.930
MTHFD1 1958G>A	rs2236225	C	0.792	0.584	0.795	0.813	0.523	0.733	0.767	0.410	0.629	0.591	0.810	0.578
*TPMT*2*	rs1800462	C	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	0.962
*TPMT*3B*	rs1800460	A	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	0.028
*TPMT*3C*	rs1142345	A	0.915	0.973	0.994	0.971	0.977	0.983	0.882	0.920	0.979	0.994	0.956	0.952
*TPMT*4*	rs1800584	A	n/a	0.009	n/a	n/a	n/a	n/a	n/a	n/a	n/a	0.006	n/a	0.023
ABCC2	rs717620	A	0.028	0.181	0.214	0.208	0.074	0.192	0.022	0.240	0.049	0.205	0.031	0.185
SMARCA4	rs1122608	G	0.981	0.743	0.911	0.929	0.761	0.907	0.972	0.840	0.948	0.750	1.000	0.802
ARID5B	rs10821936	C	0.160	0.310	0.321	0.387	0.608	0.320	0.178	0.370	0.199	0.398	0.200	0.374
ITPA IVS2+21A >C	rs7270101	A	n/a	0.872	1.000	n/a	0.972	1.000	0.878	0.940	0.843	0.898	0.894	0.851
XDH A(1936) >G	rs17323225	C	0.019	0.035	0.006	n/a	0.011	0.000	0.011	0.010	0.007	0.045	0.004	0.030
XDH A(2107) >G	rs17011368	C	0.142	0.040	0.000	n/a	0.011	0.000	0.156	0.010	0.164	0.040	0.119	0.053
NR3C1	rs852977	A	0.642	0.668	0.899	0.865	0.812	0.919	0.622	0.810	0.689	0.648	0.673	0.720
CYP3A	rs10264272	C	0.915	1.000	0.994	n/a	n/a	0.994	0.750	0.969	0.853	0.994	0.832	0.973
CYP3A4	rs4986913	C	n/a	1.000	0.994	n/a	n/a	1	n/a	0.990	n/a	n/a	1	1

plex kit reaction mix (Applied Biosystems). The products were analyzed on the ABI 3130 Genetic Analyzer (Applied Biosystems) using POP-6 polymer [17]. Genotypes were called using the GeneMapper version 4.0 software (Applied Biosystems).

The ancestry-informative markers were genotyped using SNaPshot Multiplex panels totaling 28 autosomal AIMs as described elsewhere [18], following basically the same aforementioned procedure. The selected AIMs were reported as having large allele frequency differences among European, West African, and Amerindian populations [18].

2.3. Statistical analysis

Estimates of allelic frequencies, tests of Hardy-Weinberg Equilibrium (HWE), AMOVA, F-statistics and pairwise Fst were carried out using algorithms implemented through the Arlequin 3.0 software [11]. The Whap software [23] was used to calculate single-marker regression association between the pharmacogenetic SNPs and individual ancestry estimates used as quantitative traits in the BRA population.

3. Results

The allele frequencies of the 20 SNPs in the 12 different populations are displayed in Table 2. At present there are no genetic data for two SNPs (rs1800462

Table 3
Pairwise Fst estimates for the 20 pharmacogenetic SNPs between pairs of regional populations of Brazil. The inferior diagonal shows Fst values and the superior diagonal shows p-value

	CW	NE	N	SE	S
CW	–	0.081	0.306	0.414	0.243
NE	0.0092	–	0.567	0.135	0.540
N	0.0025	0.0005	–	0.342	0.288
SE	0.0006	0.0058	0.0026	–	0.189
S	0.0029	0.0021	0.0027	0.0039	–

and rs1800460) for any of the HapMap populations and therefore they were excluded from the population analysis. The SNP rs1138272 was monomorphic in three HapMap populations (CHB, JPT and YRI); the SNPs rs1122608 (*SMARCA4*), rs17323225 (*XDH*) and rs10264272 (*CYP3A5*) were monomorphic only in the YRI, JPT and CEU populations respectively; the SNPs rs7270101 (*ITPA*) and rs17011368 (*XDH*) were monomorphic in two populations (CHB and JPT); and the SNP rs4986913 (*CYP3A4*) monomorphic in four populations (CEU, JPT, YRI and BRA).

In the Brazilian population (BRA) there was one SNP (rs4986913, *CYP3A4*) that was monomorphic (for the C allele). The T allele was present only in the CHB and MEX populations, and then at a very low frequency. For this SNP there are no data available for the remaining populations. All of the SNPs in the BRA population were in accordance with HWE expectations, indicating the absence of inbreeding or population stratification [30].

Table 4
Pairwise Fst estimates for 18 pharmacogenetic SNPs between pairs of populations

	BRA	ASW	CEU	CHB	CHD	GIH	JPT	LWK	MEX	MKK	TSI	YRI
BRA	–	< 0.01	0.36	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.054	< 0.01
ASW	0.044	–	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
CEU	< 0.001	0.043	–	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
CHB	0.035	0.098	0.055	–	0.14	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
CHD	0.029	0.065	0.044	0.002	–	< 0.01	0.05	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
GIH	0.021	0.061	0.023	0.083	0.061	–	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
JPT	0.039	0.103	0.066	0.007	0.005	0.077	–	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
LWK	0.076	0.023	0.080	0.145	0.114	0.104	0.140	–	< 0.01	< 0.01	< 0.01	0.06
MEX	0.032	0.100	0.033	0.095	0.094	0.061	0.116	0.148	–	< 0.01	< 0.01	< 0.01
MKK	0.059	0.052	0.069	0.126	0.101	0.071	0.104	0.021	0.142	–	< 0.01	< 0.01
TSI	0.004	0.068	0.004	0.036	0.038	0.041	0.051	0.109	0.040	0.093	–	< 0.01
YRI	0.065	0.016	0.079	0.118	0.083	0.101	0.107	0.004	0.157	0.021	0.103	–

Table 5
Regression beta coefficients of association between SNPs and genetic ancestry in the Brazilian sample

Locus	Allele	Ancestry		
		EUR	AFR	AMR
rs1801133	T	0.029	−0.028	−0.002
rs1801131	C	0.013	−0.004	−0.009
rs1695	G	−0.044*	0.036*	0.009
rs1051266	A	−0.043*	0.016	0.027*
rs1138272	T	0.057	−0.111*	0.023
rs11545078	T	0.047	−0.024	−0.024
rs2236225	A	0.055*	−0.05**	−0.005
rs1800462	G	0.082	−0.128**	0.018
rs1800460	A	−0.009	0.025	−0.021
rs1142345	G	−0.022	0.03	−0.009
rs1800584	A	0.004	0.026	−0.055
rs717620	T	0.015	−0.006	−0.009
rs1122608	T	0.049	−0.022	−0.029
rs10821936	C	−0.013	0.012	0.001
rs7270101	C	0.032	−0.014	−0.019
rs17323225	G	0.03	0.004	−0.051
rs17011368	G	−0.03	0.026	0.005
rs852977	G	−0.004	0.013	−0.009
rs10264272	T	−0.179**	0.067	0.082**
rs4986913	C	monomorphic		

*p-value < 0,05 and **p-value < 0,01.

As it has a continental size, the Brazilian sample was divided into five equal subsamples (corresponding to the country's geopolitical regions), according to the subject's birth place. The pairwise Fst estimates based on the 20 SNPs in the BRA subsamples are displayed in Table 3. There were no significant values of Fst, and therefore the subsamples could be grouped into one BRA sample. Table 4 shows Fst estimates for all pairwise population comparisons at 18 loci. The p-value was significant for most of the population pairs, with the exception of the following pairs: BRA-CEU, BRA-TSI, CHB-CHD, CHD-JPT, and LWK-YRI. The lowest Fst value was between the BRA and CEU populations, and the highest was between MEX and YRI.

Thereafter, regression analysis between individual genetic ancestry in the BRA sample and the 19 polymorphic SNPs was performed. Six SNPs (rs1695 – GSTP1, rs1051266 – RFC1, rs1138272 – GSTP1, rs2236225 – MTHFD1, rs1800462 – TPMT*2, rs10264272 – CYP3A5) showed significant correlation between allelic frequency and ancestry estimates (Table 5).

4. Discussion

The present study described the allelic frequencies of 20 pharmacogenetic polymorphisms in the Brazilian population, their comparison with HapMap populations and their correlation with genetic ancestry.

Few studies have investigated any of these 20 pharmacogenetic SNPs in other Brazilian samples. Of those that have, some showed similar frequency distribution when compared to our data. The frequency of the G allele of rs1051266 (in the RFC1 gene) in a sample of 172 individuals was 0.500 [6] and in the present study, we found a comparable frequency of 0.520. The A allele from SNP rs1695 (GSTP1 gene) showed a frequency of 0.687 when genotyped in 592 volunteers [25]. Its frequency in our sample was 0.658, very similar to the other studies.

The pairwise Fst calculations among the Brazilian regions showed no significant p-value, indicating no differences between the five regions of Brazil regarding the studied SNPs. As such all Brazilian regions could be grouped and considered as one non-structured population with regard to these pharmacogenetic polymorphisms. This knowledge is very important for planning future association studies using samples from all Brazilian regions. It minimizes concerns about potentially spurious associations due to population structure.

The Brazilian population is considered to be one of the most heterogeneous in the world, resulting from the admixture between Europeans, Amerindians and Africans over a period of just over 500 years. In previous studies, the ancestry of the Brazilian population, estimated based on mtDNA which informs regarding the maternal lineage, indicated 33% Amerindian contribution and 28% African contribution in white Brazilians [2]. The paternal lineage, determined by chromosome Y, in white Brazilians demonstrated a majority of European ancestry [8]. These results indicated that the Brazilian population was formed by European men and African and Amerindian women, in accordance with the developmental history of the Brazil population [8, 20]. The samples used in the study showed autosomal ancestry of 77.1% European, 14.3% African and 8.5% Amerindian [18]. Some SNPs in our population had allelic frequencies more similar to African populations, while others were more similar to Europeans, reinforcing the hypothesis that the admixture should have led to the inheritance and segregation of linkage disequilibrium blocks during the historical formation of the current Brazilian population.

The comparisons of allelic frequencies of 18 SNPs showed greatest similarity levels for genetically closer populations; for example, the pairs BRA-CEU, BRA-TSI, CHB-CHD, CHD-JPT, LWK-YRI, had higher similarity estimates as demonstrated by Fst pairwise calculations. The Brazilian population had a higher similarity for these polymorphisms with European populations (CEU and TSI), especially with the CEU sample. The greatest level of population divergence for BRA was with the African sample, followed by the Asian.

For some loci it was not possible to group different populations from HapMap according to geographic region, because the populations showed significant Fst values between them (Table 4). This result shows that geographically proximate populations may not always be grouped together because they may present significant differences amongst their genetic loci. Thus, results for one population cannot be extrapolated to others in these cases. Interestingly, if allelic frequencies are different, then, drug response in the nearest populations should also be investigated to establish and validate the variability regarding drug response and side effects between and within groups, and between individuals of different ethnicities [15,31].

We found six loci that were correlated with ancestry, though none of the loci presented high correlation values (Table 5). The low correlation observed between pharmacogenetic SNP genotype and ancestry indicates that in the Brazilian population, even at loci with different frequencies among populations, geographic ancestry has low influence on the allele frequency. This is due to the intensive admixture of the population. In association studies, population homogeneity is important mostly in the case of admixed populations, such as the Brazilian population. Understanding the genetic diversity in case-control or cross-sectional studies of disease association and drug response is necessary in order to avoid bias or spurious results, and there exists a consensus that this understanding should be achieved using ancestry informative markers [3,4,7,26,28]. However the present dataset does not support this consensus as for these polymorphisms in the Brazilian population, ancestry has no association with SNP genotypes suggesting that it is not useful to consider AIMs in this case.

Results found with CYP2C9 shows how ancestry and population specific alleles can be poorly correlated in an admixed population [14,27]. This gene is responsible for the inactivation of important drugs and is important in pharmacogenetic studies concerning populations differences, as one of its alleles, *CYP2C9*5*, was reported only in populations of African origin [14, 27]. This polymorphism was found in a Brazilian subject self-declared as white that had individual genetic ancestry estimated as 92% European, 7.5% Amerindian, and 0.5% African. The analysis of the siblings and parents of this individual revealed that the African contribution was of maternal origin, as was the allele *CYP2C9*5* [27]. This exemplifies that even small proportions of admixture can lead to adverse analytical risk in genetic research and drug response studies. Although studies show correlation between ancestry and response to treatment of ALL [5,32], the causes of these differences are not clear. To individualize the treatment it is necessary to study the genetic differences between individuals and not only between populations.

In conclusion, the present work reported a population study of pharmacogenetic polymorphisms related to the response to drugs used in treatment of ALL. The correlation between the genotypes at these loci and ancestry is low, and their frequencies differ between HapMap populations, even within the same geographic region. Genetic ancestry can influence the drug response in treatment of ALL [32], however the pharmacogenetic polymorphisms studied do not show a strong association with ancestry in an admixed population. These findings indicate that at least in the general Brazilian population, drug response phenotypes cannot

be inferred by observing biogeographical ancestry, as it has been in other ALL studies [32]. It is necessary to go straight to DNA variation in loci that have been identified as influencing drug response. We would like to point out the importance of incorporating SNPs related to drug response in future treatment protocols in the Brazilian population. In such studies ancestry estimates may also be incorporated as a wider measure of genetic influence on drug response. In this study it was not possible to evaluate the importance of the pharmacogenetic polymorphisms in the response to treatment in ALL, however our group is studying the same SNPs in Brazilian patients with ALL under treatment.

Acknowledgments

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