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High prevalence of the *GSTM3*A/B* polymorphism in sub-Sarahan African populations

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Abstract

A 3-bp insertion/deletion polymorphism in intron 6 of *GSTM3* (rs1799735, *GSTM3*A/*B*) affects the activity of the phase 2 xenobiotic metabolizing enzyme GSTM3 and has been associated with increased cancer risk. The *GSTM3*B* allele is rare or absent in Southeast Asians, occurs in 5-20% of Europeans but was detected in 80% of Bantu from South Africa. The wide genetic diversity among Africans led us to investigate whether the high frequency of *GSTM3*B* prevailed in other sub-Saharan African populations. In 168 healthy individuals from Angola, Mozambique and the São Tomé e Príncipe islands, the *GSTM3*B* allele was three times more frequent (0.74-0.78) than the *GSTM3*A* allele (0.22-0.26), with no significant differences in allele frequency across the three groups. We combined these data with previously published results to carry out a multidimensional scaling analysis, which provided a visualization of the worldwide population affinities based on the *GSTM3*A/*B* polymorphism.

Key words: African populations; *GSTM3* polymorphisms; Multidimensional scaling analysis; Pharmacogenetics; Population diversity

Introduction

Sistonen et al. (1,2) have recently described the worldwide patterns of distribution of functional polymorphisms in phase 1 drug-metabolizing enzymes of the cytochrome P450 (CYP) superfamily and emphasized the strong impact that population substructure may have on the variation seen in pharmacogenetic loci. The present communication extends these observations to GSTM3, a member of the glutathione S-transferase family of phase 2 enzymes, that catalyzes the conjugation of glutathione to a wide variety of xenobiotics. A 3-bp insertion/deletion polymorphism in intron 6 (rs1799735, *GSTM3*A/*B*) of the encoding *GSTM3* gene, which may affect the regulation and ultimately the amount and activity of GSTM3 (3), provides an extreme example of inter-population differentiation. The homozygous *GSTM3*B* genotype is ca. 20 times more frequent in an African Bantu group (frequency 0.64) than among Europeans (frequency <0.05) (4-6). The wide genetic diversity among African populations (7) led us to investigate whether the very high frequency of the *GSTM3*B* allele (0.80) in South African Bantu prevails in other sub-Saharan African populations. We screened population samples from Angola, Mozambique, São Tomé e Príncipe, and combined the new data with results from the literature to carry out a multidimensional scaling analysis, which provided a visualization of the worldwide population affinities based on the *GSTM3*A/B* polymorphism.

Material and Methods

A sample of 168 unrelated healthy, adult men and women from Cabinda (Angola, N = 73), Mozambique (Maputo, N = 66) and São Tomé e Príncipe (N = 29) was analyzed in the present study. The samples were collected by IPATIMUP collaborators according to research protocols approved by the ethic boards of all participating institutions in Africa. A single blood sample was drawn from each subject with

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informed consent. Genomic DNA was extracted using standard phenol-chloroform methodologies and genotyping for the *GSTM3*A/B* polymorphism was performed by restriction fragment length polymorphism (RFLP) as previously described (3).

The allelic frequencies were estimated by direct gene counting. Allele and genotype frequencies were compared using the χ^2 test, with the level of significance set at $P < 0.05$. The ARLEQUIN software, version 3.0 (http://cmpg.unibe.ch/software/arlequin3) was used to test for Hardy-Weinberg equilibrium and to produce pairwise fixation index (F_{ST}) genetic distances between populations. Multidimensional scaling (MDS) analysis based on F_{ST} values was conducted by the method included in the STATISTICA data analysis software system, version 8.0. (http://www.statsoft.com).

Results and Discussion

The allele frequency and genotype distribution of the *GSTM3*A/*B* polymorphism in the three sub-Saharan population samples are shown in Table 1. The genotype frequencies in the three populations did not deviate from expected Hardy-Weinberg proportions. The *GSTM3*B* allele was three times more frequent (0.74-0.78) than the *GSTM3*A* allele (0.22-0.26) in each group. There were no significant differences in allele frequencies across the three populations. These results are in excellent agreement with previously published data for a Bantu group recruited at Durban, South Africa, where the frequency of *GSTM3*B* was 0.8 (5). Bantu people comprise the majority of the population from Mozambique and Angola, and were as well the main African contributors to the settlement of São Tomé e Príncipe in the 15th century. Thus, collectively our data indicate that the *GSTM3*B* variant is 3-4 times more frequent than the wild-type *GSTM3*A* in sub-Saharan populations of Bantu influence, spread out over a large geographical area. Within these populations, allele frequencies for the tested *GSTM3* polymorphism are, however, very different from the ranges typically found either in populations of European descent, where the *GSTM3*B* allele usually does not add more than 20% (4-6), or in Asian populations, where the same variant is virtually absent (5). Accordingly, when F_{ST} distances were calculated between each of the African populations and different European or Asian populations, all values were found to be highly statistically significant (results not shown). The resulting F_{ST} distances were submitted to MDS analysis and the corresponding plot (Figure 1) clearly demonstrates the ability of this unique polymorphism to discriminate among populations of African, Asian or European ancestry. Not surprisingly, therefore, populations such as the Creoles from Mauritius Island, U.S. African-Americans and Brazilians self-identified as White, Intermediate or Black (8) displaying variable degrees of genetic admixture of continental ancestral populations, all occupy intermediate positions in the MDS representation. Despite the limitation of our MDS analysis, based on F_{ST} distances from a single gene, *GSTM3*, it does not seem that major distortions have been produced since the representation portraits reasonably well the known historical relationships between the populations under study.

The plot demonstrates how heterogeneous the populations referred to as African-Americans can be, as illustrated by the relative position of the two samples considered here (Af-Am1 and Af-Am2 in Figure 1). These two groups, which in fact are from U.S. states quite distant geographically - one from California, on the West Coast (9) and the other from Pennsylvania and New York, on the East Coast (10) - show very distinct levels of admixture between European and African ancestry regarding *GSTM3*. As a matter of fact, the demographic history varied enough among different African-American populations to explain the diverse genetic structure of contemporary African-Americans (11).

Concerning the highly heterogeneous Brazilian population, the MDS representation is consistent with our previous demonstration that the frequency of the *GSTM3*B* allele increases from self-reported White, to Intermediate and to Black individuals, in parallel to the increase in the average proportion of African ancestry across these groups, although at the individual level, the frequency of the *GSTM3*B* allele increases continuously as the individual proportion of African ancestry increases, irrespective of self-reported "color/race" (8).

Table 1. Allele frequencies and observed genotype distributions of *GSTM3*A/B* in African populations.

Population	*GSTM3* genotypes				*GSTM3* alleles	
	AA	*AB*	*BB*	P HWE[a]	**A*	**B*
Angola (N = 73)	0.080 (0.019-0.145)	0.356 (0.246-0.466)	0.562 (0.448-0.675)	0.544	0.260 (0.189-0.332)	0.740 (0.669-0.811)
Mozambique (N = 66)	0.061 (0.030-0.118)	0.318 (0.206-0.431)	0.621 (0.504-0.738)	0.491	0.220 (0.149-0.290)	0.780 (0.710-0.851)
São Tomé (N = 29)	0.069 (0-0.161)	0.345 (0.171-0.518)	0.586 (0.407-0.766)	0.645	0.241 (0.131-0.362)	0.759 (0.648-0.869)
P value[b]		0.96				0.73

Data are reported as means and 95%CI. N = number of individuals. [a]Probability values for tests of departure from Hardy-Weinberg expectations (HWE). [b]χ^2 tests for comparison across the three population groups.

The *GSTM3*A/*B* polymorphism has been investigated as a risk factor for cancer, with contradictory results. For example, the original observation in a North-American cohort of predominantly white individuals that the *GSTM3*B/*B* genotype was associated with increased risk of glioma and meningioma compared to the *GSTM3*A/*A* genotype (12) was not subsequently verified in residents of Sweden, England, Denmark, and Finland (13). The small number of cancer patients with the *GSTM3*B/*B* genotype in these studies, carried out in White populations, may account for the inconsistent association of the *GSTM3*A/*B* polymorphism with cancer risk. It might be anticipated that the same caveat applies to association studies of the *GSTM3*A/*B* polymorphism in sub-Saharan Africans, because of the very high frequency of the *GSTM3*B* allele. Along this reasoning, the range of *GSTM3** allele frequencies among admixed groups such as Brazilians (0.32-0.64) (Ref. 8) or African-Americans (0.60-0.68) (Refs. 10,11) may prove advantageous for exploring the association of the *GSTM3*A/*B* polymorphism with cancer risk. This notion is consistent with suggestions that the genetic structure of admixed populations could be exploited to identify variants that underlie ethnic variation in diseases or traits of interest, in the broader context of admixture mapping (14).

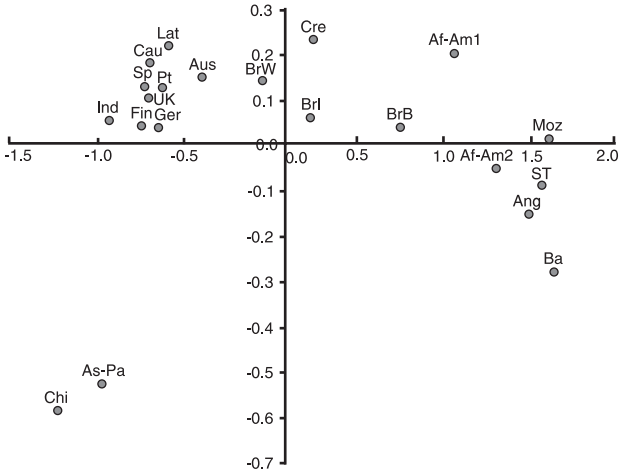

Figure 1. Multidimensional scaling (MDS) analysis plot of populations based on FST distances. In the MDS plot, distances between points approximately reproduce the original pairwise FST distances between populations. Of note, the populations of European, African and Asian descent are distinctly positioned in the upper left, lower right and lower left quadrant of the plot, respectively. Admixed populations such as Brazilians or African-Americans occupy the upper right quadrant of the plot, which is an intermediate position between African and European populations. The numbers within parentheses indicate reference number. Af-Am1 = African-American, Cau = Caucasian, Lat = Latino, As-Pa = Asian-Pacific (9); Af-Am2 = African-American (10); Cre = Creole, Aus = Australia, Chi = China, Ba = Bantu (5); Sp = Spain (15); BrW = Brazilian White, BrI = Brazilian Intermediate (self-identified as "Pardo", in Portuguese), BrB = Brazilian Black (8); Ind = Indian (16); UK = United Kingdom (17); Ge = Germany (18); Pt = Portugal (19); Fin = Finland (20); Moz = Mozambique, Ang = Angola, ST = São Tomé e Príncipe (present study).

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References

1. Sistonen J, Sajantila A, Lao O, Corander J, Barbujani G, Fuselli S. CYP2D6 worldwide genetic variation shows high frequency of altered activity variants and no continental structure. *Pharmacogenet Genomics* 2007; 17: 93-101.
2. Sistonen J, Fuselli S, Palo JU, Chauhan N, Padh H, Sajantila A. Pharmacogenetic variation at CYP2C9, CYP2C19, and CYP2D6 at global and microgeographic scales. *Pharmacogenet Genomics* 2009; 19: 170-179.
3. Inskip A, Elexperu-Camiruaga J, Buxton N, Dias PS, MacIntosh J, Campbell D, et al. Identification of polymorphism at the glutathione S-transferase, GSTM3 locus: evidence for linkage with GSTM1*A. *Biochem J* 1995; 312 (Part 3): 713-716.
4. Garte S, Gaspari L, Alexandrie AK, Ambrosone C, Autrup H, Autrup JL, et al. Metabolic gene polymorphism frequencies in control populations. *Cancer Epidemiol Biomarkers Prev* 2001; 10: 1239-1248.
5. Tetlow N, Robinson A, Mantle T, Board P. Polymorphism of human mu class glutathione transferases. *Pharmacogenetics* 2004; 14: 359-368.
6. Ye Z, Song H, Higgins JP, Pharoah P, Danesh J. Five glutathione s-transferase gene variants in 23,452 cases of lung cancer and 30,397 controls: meta-analysis of 130 studies. *PLoS Med* 2006; 3: e91.
7. Campbell MC, Tishkoff SA. African genetic diversity: implications for human demographic history, modern human origins, and complex disease mapping. *Annu Rev Genomics Hum Genet* 2008; 9: 403-433.
8. Suarez-Kurtz G, Vargens DD, Struchiner CJ, Bastos-Rodrigues L, Pena SD. Self-reported skin color, genomic ancestry and the distribution of GST polymorphisms. *Pharmacogenet Genomics* 2007; 17: 765-771.
9. Cortessis V, Siegmund K, Chen Q, Zhou N, Diep A, Frankl H, et al. A case-control study of microsomal epoxide hydrolase, smoking, meat consumption, glutathione S-transferase M3, and risk of colorectal adenomas. *Cancer Res* 2001; 61: 2381-2385.
10. Park LY, Muscat JE, Kaur T, Schantz SP, Stern JC, Richie JP Jr, et al. Comparison of GSTM polymorphisms and risk for oral cancer between African-Americans and Caucasians. *Pharmacogenetics* 2000; 10: 123-131.
11. Reiner AP, Carlson CS, Ziv E, Iribarren C, Jaquish CE,

Nickerson DA. Genetic ancestry, population sub-structure, and cardiovascular disease-related traits among African-American participants in the CARDIA Study. *Hum Genet* 2007; 121: 565-575.
12. De Roos AJ, Rothman N, Inskip PD, Linet MS, Shapiro WR, Selker RG, et al. Genetic polymorphisms in GSTM1, -P1, -T1, and CYP2E1 and the risk of adult brain tumors. *Cancer Epidemiol Biomarkers Prev* 2003; 12: 14-22.
13. Schwartzbaum JA, Ahlbom A, Lonn S, Malmer B, Wigertz A, Auvinen A, et al. An international case-control study of interleukin-4Ralpha, interleukin-13, and cyclooxygenase-2 polymorphisms and glioblastoma risk. *Cancer Epidemiol Biomarkers Prev* 2007; 16: 2448-2454.
14. McKeigue PM. Prospects for admixture mapping of complex traits. *Am J Hum Genet* 2005; 76: 1-7.
15. Ladero JM, Martinez C, Fernandez JM, Martin F, Garcia-Martin E, Ropero P, et al. Glutathione S-transferases pi 1, alpha 1 and M3 genetic polymorphisms and the risk of hepatocellular carcinoma in humans. *Pharmacogenomics* 2007; 8: 895-899.
16. Buch SC, Notani PN, Bhisey RA. Polymorphism at GSTM1, GSTM3 and GSTT1 gene loci and susceptibility to oral cancer in an Indian population. *Carcinogenesis* 2002; 23: 803-807.
17. Loktionov A, Watson MA, Gunter M, Stebbings WS, Speakman CT, Bingham SA. Glutathione-S-transferase gene polymorphisms in colorectal cancer patients: interaction between GSTM1 and GSTM3 allele variants as a risk-modulating factor. *Carcinogenesis* 2001; 22: 1053-1060.
18. Risch A, Wikman H, Thiel S, Schmezer P, Edler L, Drings P, et al. Glutathione-S-transferase M1, M3, T1 and P1 polymorphisms and susceptibility to non-small-cell lung cancer subtypes and hamartomas. *Pharmacogenetics* 2001; 11: 757-764.
19. Medeiros R, Vasconcelos A, Costa S, Pinto D, Ferreira P, Lobo F, et al. Metabolic susceptibility genes and prostate cancer risk in a southern European population: the role of glutathione S-transferases GSTM1, GSTM3, and GSTT1 genetic polymorphisms. *Prostate* 2004; 58: 414-420.
20. Mitrunen K, Jourenkova N, Kataja V, Eskelinen M, Kosma VM, Benhamou S, et al. Glutathione S-transferase M1, M3, P1, and T1 genetic polymorphisms and susceptibility to breast cancer. *Cancer Epidemiol Biomarkers Prev* 2001; 10: 229-236.