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SHORT COMMUNICATION

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Allele frequencies of three STRs of the human von Willebrand factor gene (vWF) in a Brazilian population sample

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Abstract Allele frequencies were calculated for three tetrameric short tandem repeats (STRs) located in intron 40 of the human von Willebrand factor (vWA, vWF1 and vWF2) in 352 white individuals sampled from an urban population from the northeastern region of the State of São Paulo, Brazil. The exact test did not indicate any significant deviation from HWE for any of the three investigated loci. The allele frequencies of vWA and vWF1 showed unimodal and bimodal distributions, respectively, and the frequencies of vWF2 in our sample exhibited bimodal or even trimodal patterns. These differing patterns could reflect the differential action of one selective factor or of the distribution of mutations in these STRs, although the STRs are very close to one another and belong to the same gene. The frequency of paternity exclusions observed for each of these three loci conform to the theorectical expectations. The lack of difficulties regarding the methodology of typing and the forensic value of statistical parameters confirm the usefulness of these systems to study Brazilian populations.

Key words STRs · Brazil · von Willebrand factor · vWA · vWF1 · vWF2

Introduction

Short tandem repeats (STRs) exhibit high levels of heterozygosity and discrimination power. Thus, the typing of these genetic markers with the polymerase chain reaction (PCR) has been applied throughout the world in forensic laboratories as the method of choice for the identification of human DNA [13]. Intron 40 of the von Willebrand factor gene in humans contains a region with three STRs, HUMvWA31A (vWA), vWF1 and vWF2, formed by imperfect tetranucleotide short tandem TCTA repeats with 11, 10 and 8 alleles, respectively [2, 9, 14, 16]. This allows the possibility of hundreds of different haplotypes, thus constituting a quick and efficient method for the study of segregation in families with the von Willebrand disease [5] in addition to its use in forensic and paternity tests in which genealogical investigations are performed. The aim of this study was to characterize the allele frequency distributions and the values of various statistical parameters with forensic relevance for these three polymorphisms in a sample from a Brazilian population for which vWA frequencies have not yet been described.

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Material and methods

The population sample was selected from individuals involved in disputed paternity cases investigated by the Department of Genetics of the University of São Paulo Hospital at Ribeirão Preto, São Paulo between January 1996 and May 1997. The subjects were white, originating from the surrounding area, and they included 157 mother-child-alleged father trios, 3 mother-2 children-alleged father cases, 7 pairs of genitor-child and 25 independent individuals.

Routinely, each case was submitted to a series of six or more STRs until an exclusion was obtained at two loci, or a 99.99% probability of paternity was achieved.

DNA extracted from whole blood was amplified by PCR and amplification products were separated by denaturing polyacrylamide gel electrophoresis followed by silver staining [2, 9, 14]. Alleles were designated with numerals in accordance with the number of repeats. The allele frequency estimates and the calculations of Hardy-Weinberg equilibrium (HWE) were based on the test results from 352 genetically non-related individuals (it was not possible to analyze 7 of these for vWA, 4 for vWF1 and 4 for vWF2). The calculations were determined with the exact test using the GENEPOP program [17]. The GENIOC program [3] was used for the comparison of frequencies between populations.

Results and discussion

The allele frequencies from the sample are presented in Table 1. The exact test did not indicate any deviations

Table 1 Allele distributions and forensic values for vWA (n = 345), vWF1 (n = 348) and vWF2 (n = 348) in a sample of 352 Brazilians

Alleles	System VWA	System VWF1	System VWF2
5		0.1365	
6		0.4770	
7		0.0244	
8		0.0144	
9		0.0776	0.0057
10		0.1494	0.0963
11	0.0043	0.1034	0.0560
12	0.0029	0.0158	0.3405
13	0.0159	0.0014	0.3420
14	0.0783		0.0632
15	0.1493		0.0862
16	0.2232		0.0101
17	0.2493		
18	0.1493		
19	0.0899		
20	0.0333		
21	0.0043		

System	H[a]	PD[b]	PIC[c]	PE[d]	EO[e]
vWA	0.8638	0.9482	0.8057	0.6587	22 (P = 0.1377)
vWF1	0.7241	0.8889	0.6845	0.5102	19 (P = 0.6580)
vWF2	0.6897	0.8953	0.7045	0.5253	23 (P = 0.7629)

[a] Observed heterozygosity
[b] PD indicates power of discrimination calculated using the formula PD = 1-Σ(Pi)^2, where Pi is the frequency of each genotype
[c] PIC- indicates polymorphism information content
[d] PE indicates the expected probability of exclusion
[e] Exclusions observed among the 41 cases in which there was exclusion of paternity

from expected genotype frequencies for any of the three loci investigated assuming HWE (P = 0.0723, 0.0549 and 0.2811, respectively, for vWA, vWF1 and vWF2). The allele frequencies of vWA and vWF1 have unimodal and bimodal distributions, respectively. The frequencies of vWF2 in our sample and for the Caucasoid mean exhibit an antimode corresponding to allele 11 and, in our sample, possibly another antimode corresponding to allele 14. These various patterns could reflect the differential action of a selective factor or of the distribution of mutations in these STRs, although they are very close to one another and belong to the same gene [1].

The allele frequency distribution of the locus vWA in our sample consistently had values between the African [2, 15] and Caucasoid [2, 8, 11, 15] ones. In addition, we observed three heterozygotes in our sample, carriers of allele 11 (0.43%), found in Africans with polymorphic frequencies (~ 2%), but not reported in any of the more than 6000 European Caucasoids analyzed up to the present. This finding is consistent with earlier references that there is a significant African-derived component within the white Brazilian population [1, 12].

The significant difference (P < 0.001) between the frequencies of the vWF1 alleles from our sample and the Caucasoid mean [6, 9, 18] disappears (P = 0.07) when one removes the data related to the Italian sample described by Trabetti et al. [18]. Moreover, there is no significant difference when comparing the data from our sample with other urban Brazilian samples (blood donors from a northeastern Brazilian city [1], whites from the region of Belo Horizonte, Minas Gerais [14] and blacks and whites from the region of Ribeirão Preto [20]). There was a significant difference (P < < 0.001) only in the comparison with genetically isolated black populations of the northeastern Brazil [1]. There are no reports of vWF1 and vWF2 frequencies in African populations.

Of the only two urban populations studied for the vWF2 system in Brazil [1, 14], totaling 274 individuals, only the first is significantly different from ours (P < 0.0001). This may be due to the disequilibrium observed there. Our data were also not statistically different from that reported in the few studies completed up to now among Caucasoids [5, 6, 16, 19]. The instability cited by other authors for the vWF1 and vWF2 systems [4, 7, 10] was not observed in our sample and we did not encounter any maternal exclusions or any case in which the paternity was excluded by a single locus.

In 41 confirmed cases of paternity exclusion, the rate of observed exclusions for each locus was within the expected ranges (Table 1). The simple methodological typing procedures and the forensic value of several statistical parameters demonstrate the utility of these loci for general applications in population genetics, paternity tests and linkage analysis.

Although these systems can be used individually for such purposes, the combined use in haplotypes (detailed data in http://rgm.fmrp.usp.br/alsimoes) must take into account the possible linkage disequilibrium, a topic which will be discussed in future work.

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