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Brief Communication

Neutrality of miniSTR D22S1045 marker by Ewing's sarcoma phenotype

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ABSTRACT

Neutrality investigations of markers with forensic use are important to see if a phenotypic trait is being expressed in relation to the alleles of the marker. MiniSTR marker D22S1045 (locus 22q12.3) is localized near the breakpoint region of the *EWS* gene (22q12.2), which leads to the development of Ewing's Sarcoma. Analyzing allele frequencies and linkage disequilibrium in Ewing's sarcoma patients and non-affected populations, we found that the marker mD22S1045 was neutral when related to Ewing's Sarcoma.

1. Introduction

To recover information more effectively from degraded DNA samples, miniSTR typing systems have been developed to obtain smaller PCR products [1]. The amplicon sizes are reduced by moving the PCR primers closer to the short tandem repeat (STR) regions while retaining the same information [2]. The European Network of Forensic Science Institutes (ENFSI) and the European DNA Profiling Group (EDNAP) recommend that European laboratories adopt new miniSTR loci, including the marker mD22S1045, as additional loci to CODIS (Combined DNA Index System) for human identification using degraded DNA samples. However, one of the objective criteria to an eligible new locus is no known association with medical conditions or defects (refers to whether there is a reported association of the locus with a medical condition or disease status) [3,4]. If a locus is close to a gene or a specific chromosome region linked to a disease, it is important to determine if a particular forensic allele is associated or is in linkage disequilibrium with a disease state and hence subject to selection or to expose a phenotype [5]. The miniSTR marker D22S1045 (locus 22q12.3) is relatively close to the breakpoint region of the *EWS* gene (locus 22q12.2), which leads to the development of Ewing's Sarcoma (ES). Breakage hot spots around the *EWS* gene have been identified as being points to chromosome translocations responsible for this rare bone cancer [6]. Although the physical distance between mD22S1045 and *EWS* gene is ~6Mp, ES is the pathology most physically related to this miniSTR marker. We aimed to investigate if there was any linkage disequilibrium or any association between miniSTR D22S1045 marker alleles and Ewing's sarcoma phenotype.

2. Materials and methods

We enrolled in this study 24 Ewing's sarcoma patients (geographically matched with a control group) diagnosed at the Pediatric Oncology Unit of the UFRGS university hospital (HCPA), and 54 of their family members, including parents and healthy siblings. Our control population consisted of 296 DNA donors from Southern Brazil, which has mainly Portuguese, Italian, Spanish, and German descendants [7,8]. Informed consent was obtained from all participants. DNA was extracted from peripheral blood leukocytes following the protocol of Lahiri and Nurnberger [9] or purified from blood spots on Whatman FTA cards (Whatman

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Table 1
Allele frequencies of miniSTR marker D22S1045 in patients and different populations.

Alleles	ESP	RS	CA	AA	HI	IT	GE
8				0.010			
9							
10		0.003		0.043	0.018		
11	0.083	0.117	0.140	0.130	0.061	0.115	0.132
12	0.042	0.003	0.015	0.056	0.018	0.020	0.019
13		0.007	0.009	0.004	0.011		
14	0.021	0.025	0.058	0.080	0.025	0.045	0.035
15	0.396	0.373	0.332	0.259	0.454	0.415	0.403
16	0.417	0.353	0.326	0.187	0.311	0.300	0.298
17	0.021	0.11	0.079	0.210	0.096	0.100	0.093
18	0.021	0.008	0.005	0.016	0.007	0.005	0.016
19				0.006			0.004
N	24	296	265	257	140	100	133
H	0.791	0.73	0.785	0.817	0.721	0.680	0.721

ESP: Ewing's sarcoma patients; RS: Rio Grande do Sul (control group); CA: Caucasians from USA [17]; AA: African Americans [17]; HI: Hispanics from USA [17]; IT: Italians [18]; GE: Germans [19]; N: Sample size; H: observed heterozygosis.

Table 2
Comparison of allele frequencies between patients and different populations.

Populations	ESP	RS	CA	AA	HI	IT	GE
ESP	–	–	–	–	–	–	–
RS	$p = 0.119$	–	–	–	–	–	–
CA	$p = 0.264$	$p = 0.267$	–	–	–	–	–
AA	$p = 0.002$*	$p < 0.001$*	$p < 0.001$*	–	–	–	–
HI	$p = 0.527$	$p = 0.138$	$p = 0.030$*	$p < 0.001$*	–	–	–
IT	$p = 0.463$	$p = 0.706$	$p = 0.833$	$p = 0.004$*	$p = 0.575$	–	–
GE	$p = 0.433$	$p = 0.523$	$p = 0.616$	$p < 0.001$*	$p = 0.285$	$p = 0.992$	–

ESP: Ewing's sarcoma patients; RS: Rio Grande do Sul (control group); CA: Caucasians from USA [17]; AA: African Americans [17]; HI: Hispanics from USA [17]; IT: Italians [18]; GE: Germans [19].
* Statistically significant, Chi-Square Test.

Bioscience, Cambridge, UK). Amplification was performed following the parameters outlined by Coble and Butler [10] for the non-CODIS 01 miniplex. Electrophoresis of the amplified fragments was performed in an ABI PRISM® 3100-*Avant* Genetic Analyzer using the separation medium performance optimized polymer (POP) 4 and 47 cm capillaries (Applied Biosystems, Foster City, USA). Allelic designation was determined using Applied Biosystems GeneMapper®*ID-X* Software v1.2. Using Fisher's or Pearson's Chi-Square Test, we compared allele frequencies from ES patients with those obtained from non-affected populations from Rio Grande do Sul (Brazil), Italy, Germany and USA (Caucasian, African American, and Hispanic). Transmission Disequilibrium Test (TDT) was performed to determine if there was a presence of genetic linkage between allelic inheritance and Ewing's sarcoma phenotype. A p-value <0.05 was assumed as significant in all tests conducted.

3. Results and discussion

Allele frequencies are presented in Table 1. Association study in a complex disease can be difficult, and we detected seven alleles from our 24 patients. Even though our patient sample number may seem low, it is in accordance with the number of cases of ES expected to occur in Rio Grande do Sul (ES frequency is 2–3 per million in Caucasian populations in Western countries [11] and Rio Grande do Sul has a population of 10.5 million people (IBGE Census 2010 – http://www.censo2010.ibge.gov.br/dados_divulgados/index.php?uf=43). No differences in allele frequencies were found between patients and the other populations with the same genetic background, but we found significant differences between the African American population and all other groups (Table 2). These results indicate a genetic background effect but no association between ES and any D22S1045 allele. We believe this comparison strategy is appropriate for our purpose, since a case-control study would be very difficult with ES patients.

Randall et al. [12] found an incidence of Ewing's sarcoma in siblings [13–15], evidencing a familial predisposition and a noticeable genetics contribution to Ewing's sarcoma. To examine if any allele could have been preferentially inherited by affected individuals but not by healthy siblings, we performed a Transmission Disequilibrium Test comparing data from ES patients and their relatives. No statistically significant result was found ($p > 0.05$).

Although loss of heterozygosis (LOH) and microsatellite instability (MSI) usually occur when loci containing microsatellite repeated regions are amplified in patients with any type of cancer [16], these alterations were not found in our sample because DNA was extracted from non-tumor leukocytes. LOH and MSI are most likely to be found in cells from the bone tumor. When analyzing the families pedigree, allele frequencies in the first generation are similar to those in the second generation (Chi Square Test; $p > 0.05$). We can notice that all alleles found in patients are also present in their parents, showing a classic mendelian heritage and absence of LOH and MSI.

4. Conclusion

Analyzing allele frequencies and linkage disequilibrium, we concluded that the miniSTR D22S1045 was neutral when related to Ewing's Sarcoma trait. This result is a contribution to neutrality studies of non-CODIS miniSTR D22S1045 forensic marker.

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