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Novel Alleles of the D16S752 Polymorphic Genetic Marker Linked to E-Cadherin Gene – A Potential Population Marker

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A B S T R A C T

Seven DNA variants that polymorphic genetic marker D16S752 reveals in Croatian population are reported in this paper. The marker is a GATA tetranucleotide repeat linked to human E-cadherin gene (CDH1). Prior studies involving this marker revealed only four DNA allele variants. The reported DNA variants contribute to the collection of hypervariable DNA polymorphisms data useful in the field of anthropological and population genetic and forensic medicine.

Introduction

Tetranucleotide repeat D16S752 (GATA-51G03) is a polymorphic marker linked to tumor suppressor gene E-cadherin (CDH1) (16q22.1)[1] which encodes a calcium-dependent cell adhesion molecule involved in epithelial development and maintenance[2].

Polymorphic markers have proved to be very useful in tumor allelotyping and detection of loss of heterozygosity (LOH) of putative tumor suppressor genes[3]. In this respect D16S752 marker has been used for LOH detection of CDH1 gene in carcinomas. Nevertheless, polymorphisms have found extensive application in anthropological and population genetic studies providing data for the genetic structure of different populations[4–5]. For this purpose it is very important to know all allelic variants this marker could reveal in normal tissue of certain population.

Here we report on novel allelic variants of D16S752 found in constitutive DNA from unrelated individuals.

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Materials and Methods

DNA was isolated from blood samples of unrelated individuals (54) from Croatia suffering from renal or colon carcinoma or phaeochromocytoma. The polymorphic region was PCR amplified (total volume – 25 μl, each primer (5'-AATTGACGGTATATCTATCTGTCTG-3'; and 5'-GATTGGAGGAGGGTGATTCT-3') 5 pmol, 200 ng DNA, 2.5 μl 10X buffer II, 1.5 mM $MgCl_2$, 2.5 mM of each dNTP, 0.25 U Taq polymerase; Eppendorf, Germany). PCR conditions: initial denaturation, 3 min/96 °C; denaturation, 30 s/96 °C; annealing, 35 s/55 °C; extension, 30+1 s/72 °C; final extension, 72 °C/10 min; 35 cycles.

Each polymorphism was analyzed both on 15% polyacrylamide gels run in 1X TBE (Tris/borate/EDTA, stained with silver) and on Spreadex EL 300 gels (Elchrom scientific, Cham, Switzerland). The electrophoresis conditions were: 0,75XTAE (Tris/acetate/EDTA), 120min/120V, 55 °C. Spreadex gels were Syber-Gold stained (Molecular Probes, Leiden, Netherlands).

All the procedures employed were approved by the appropriate review committee.

Results

In a random sample of constitutive DNA from unrelated individuals we found seven different allelic variants of polymorphic marker D16S752; three of them yet not described in the literature.

From 54 analyzed samples 48 were heterozygous which showed that D16S-752 polymorphism has mean observed and expected heterozygosity values of 89% and 92%, respectively.

Individual allele frequencies are shown in Table 1. The alleles were each assigned by numbers 1 to 7. The allele number 2 (106 bp) appeared most frequent, followed by alleles 3 (110 bp) and 4 (114 bp), 5 (118 bp), 1 (102 bp), 6 (122 bp) and 7 (126 bp).

Figure 1 demonstrates all seven allelic variants. By comparison to molecular marker we calculated that each allele differs by four nucleotides from the preceding one.

Discussion

In constitutive DNA samples from Croatia we noticed additional number of alleles in regard to D16S752 genetic marker. Sex and age of the subjects showed no correlation to the allele distribution.

TABLE 1
ALLELE FREQUENCIES AND THEIR COMBINATIONS IN HETEROZYGOUS INDIVIDUALS

Allele	Total allele frequency		Allele combination*	Number of individuals	Allele combination*	Number of individuals
	Heterozygous	Homozygous				
1	10	0	1, 2	5	3, 4	6
2	18	4	1, 3	2	3, 5	3
3	17	4	1, 5	3	3, 6	2
4	19	2	2, 2	2	4, 4	1
5	16	2	2, 3	4	4, 5	2
6	10	0	2, 4	3	4, 6	3
7	6	0	2, 5	4	4, 7	5
			2, 6	1	5, 5	1
			2, 7	1	5, 6	4
			3, 3	2		

*None of the samples tested had combinations 1/1, 1/4, 1/6, 1/7, 3/7,5/7, 6/6, 6/7, 7/7.

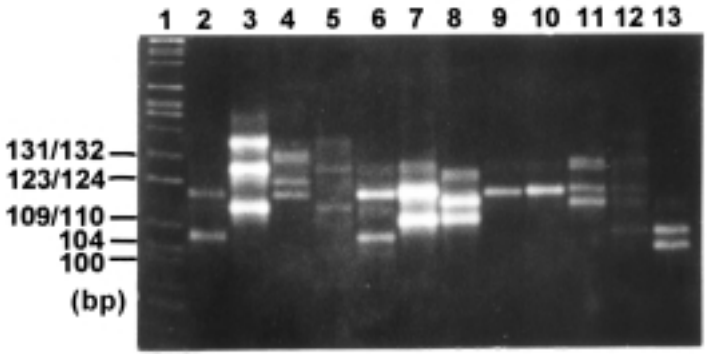

Fig. 1. Seven different D16S752 allele variants. Spreadex gel, stained with SyberGold (Molecular Probes). Lane 1– molecular marker M3 (Elchrom Scientific); lanes 2, 6 – alleles 2/5; lanes 3, 5 – alleles 4/7; lane 4 – alleles 5/6; lane 7 – alleles 3/5; lane 8 – alleles 3/4; lanes 9, 10 – homozygous samples, alleles 5/5; lanes 11, 12 – alleles 4/5; lane 13 – alleles 1/2.

According to the GenomeDataBase (http://www.gdb.org/) only four D16S752 allele variants were reported so far. Moreover, they were scored on northern European populations and very few people were tested (unpublished communication).

Additional number of alleles, in regard to D16S752, scored in samples obtained from Croats and as a consequence, high frequency of their heterozygotic combination may be helpful for further genetic studies on the role of CDH1 in tumor development and progression.

Measuring rates of genetic divergence among populations as well as establishing genetic markers characteristic for certain population is one of the promising uses of reported DNA variants in the field of anthropology and population genetic[6–7] as well as tumor allelotyping. Moreover, specific markers are useful for resolving genetic patterns of variability at regional levels[8]. The data presented in this paper broaden the genetic spectrum and contribute to our knowledge of the genetic nature of the Croatian population.

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NOVI ALELI POLIMORFNOG GENETIČKOG MARKERA D16S752: POTENCIJALNI POPULACIJSKI MARKER

S A Ž E T A K

Upotrebom polimorfnog genetičkog markera D16S752 otkriveno je sedam DNA varianti u populaciji Hrvatske. Marker je tetranukleotidno GATA ponavljanje vezano uz gen E-cadherin (CDH1) čovjeka. Prijašnja istraživanja ovim markerom pokazala su samo četiri različita alela. Prikazani polimorfizam vrijedan je podatak za prikupljanje DNA polimorfizama neophodnih u antropološkoj i populacijskoj genetici, te sudskoj medicini.