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Characteristics of D5S818 mutations revealed through study of a flanking marker

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Abstract. A cytosine/thymosine (C/T) polymorphism was discovered 13 nucleotides upstream from the 5′ end of the tandem array of the D5S818 locus (− 13 SNP). The − 13 SNP coincidently creates a restriction site polymorphism for the restriction endonuclease SnaB1 that is tightly linked to the D5S818 tandem array. RFLP mapping of the − 13 SNP was used to characterize 40 D5S818 addition/deletion mutations encountered in routine parentage testing. Most mutations showed paternal bias and single repeat changes to the tandem array. In 19 instances, it was possible to identify the parental allele that underwent the mutation. Alleles 13 and 14 were especially prone to mutation whereas allele #11 was unexpectedly resistant. © 2003 Elsevier B.V. All rights reserved.

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1. Introduction

Occasionally, in parentage investigations using DNA typing, results inconsistent with parentage are encountered with the true parent of a child. The explanation for such inconsistencies are mutations that either add or delete repeats from the tandem array [1, 2].

The D5S818 locus represents a typical VNTR consisting of a 4-basepair repeat element of homogeneous AGAT sequence [4]. In preliminary studies, an RFLP for the enzyme SnaB1 was discovered upstream from the tandem array due to a C/T polymorphism (hereafter called the − 13 SNP). The existence of a flanking SNP marker provided an opportunity to examine the molecular details of 40 D5S818 mutations.

2. Materials and methods

Mutations used for this study were identified from routine parentage testing performed by Orchid-GeneScreen (Dayton, OH) or the H.A. Chapman Institute of Medical Genetics

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Table 1
Observed versus expected mutations at the D5S818 locus

Progenitor allele	Observed	Expected[a]
< 11	0	2.57
11	2	6.71
12	7	6.384
13	6	3.192
14	4	0.247
>14	0	0.076

$p \leq 0.001$[b].

[a] Predicted from allele frequency (or combined allele frequency) averaged over the Caucasian, Black and Hispanic population.

[b] Chi-square statistic considering significance of deviation from expectations. Calculated with 5 degrees of freedom.

(Tulsa, OK). DNA was extracted from buccal swabs or blood samples using standard inorganic extraction methods and amplified using a monoplex kit for the D5S818 locus (Promega Madison, WI). Amplicons were digested with SnaB1 and visualized using a fluorescent scanner (FMBIO, Hitachi) following electrophoresis in 6% polyacrylamide gels.

3. Results

The − 13 SNP exhibits two genotypes in the population. When the C allele is present at the SNP site (~ 80% frequency), D5S818 allele size is reduced by SnaB1 digestion by 27 basepairs from the 5′ end of the amplicon. Amplified alleles of the T genotype (~ 20% frequency) are unaffected by SnaB1 digestion.

The RFLP analysis was applied to 40 D5S818 mutations accumulated from approximately 20,000 paternity cases representing almost 40,000 meioses. The majority of mutations occurred within the paternal lineage (97%) and involved changes of a single repeat (92%), consistent with published data for the D5S818 locus [5]. The number of additions was roughly equal to the number of deletions of repeats from the tandem array (54% versus 46%, respectively). Finally, the C/T genotype at the − 13 SNP site among mutations was consistent with their population frequency, suggesting no correlation of − 13 SNP genotype with susceptibility of a D5S818 allele to undergo mutation.

For 19 mutations, it was possible to determine progenitor alleles in the parent producing the mutant allele in the child. The results of this analysis are shown in Table 1. Allele 14 was identified as the progenitor in 4 of 19 mutations (~ 21%), yet this allele has an average population frequency of only slightly above 1%. In contrast, allele 11 was the progenitor for 2 of 19 mutations (~ 11%), but exhibits a population frequency of about 35%.

4. Discussion

Mutations in our panel were analyzed assuming all trios represent true families, an assumption strongly supported by the extended testing performed in each case. It was also assumed that the addition or deletion to the tandem array did not also alter the genotype of

the flanking − 13 SNP. Although "null alleles" are known to exist for STR loci due to single nucleotide variations in the primer binding regions (5, 6), they generally do not affect the integrity of the tandem array [6].

A direct relationship between the number of repeats in the tandem array and the instability of the allele was suggested [2,3,7]. All 40 of our mutations involved progenitor alleles belonging to the 11, 12, 13 or 14 allele classes. Allele 14 (one of the larger alleles at the locus), which accounts for only about 1% of D5S818 alleles, was the progenitor in over 20% of informative mutations. Two additional alleles at the D5S818 locus, 15 and 16 were not involved in any of the mutations in our panel and yet exhibit a combined population frequency somewhat similar to allele 14. If allele instability is directly proportional to the number of repeats, the involvement of either allele 15 or 16 in at least one mutation might have been expected. In contrast, allele 11 (about 35% frequency) was the progenitor in only about 11% of the mutations suggesting an enhanced stability. Taken together, these results suggest that allele length alone is not responsible for instability and that a more complex relationship exists between the allele length and stability from additions and deletions.

The results also suggest that accurate weighting of evidence inconsistent with parentage at the D5S818 locus should incorporate the mutation rate for the particular D5S818 allele rather than an average mutation rate for the locus.

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