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SHORT COMMUNICATION

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Population genetic data of the STR HumD3S1358 in two regions of Germany

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Abstract This report gives the results of two population studies on HumD3S1358 from a northern and a southern region of Germany. The numbers of unrelated individuals were 326 and 666, respectively and seven main alleles, three rare allelic variants and 29 different genotypes were encountered. No significant statistical differences were seen between the northern and southern populations. The HumD3S1358 allele distributions were in agreement with Hardy-Weinberg expectations and two mutations were found in 780 meiotic events.

Key words STR · HumD3S1358 · Population genetics · Mutation · Paternity testing

Introduction

The HumD3S1358 locus displays a variable tetranucleotide short tandem repeat (STR) [5].

This report summarises the results of two studies on the allele and genotype distributions in two geographically separated regions of Germany. Unrelated individuals from a northern region (n = 328) and a southern region (n = 666) were investigated. Mendelian inheritance was analysed from 390 female and 390 male meiotic events.

Materials and methods

DNA was prepared in cases of paternity testing from parents and their presumptive children where the parents were considered as being unrelated individuals. The number of meioses was established from triplets if non paternity exclusion was seen in 12 classical systems and up to 9 well established STR systems. In all these cases the probability of paternity was computed to be 99.98% or more. DNA was prepared from fresh blood using the proteinase K/phenol-chloroform extraction method [1]. The PCR mixture contained 10–20 ng of template DNA, 0.1 10 × PCR-buffer (Eurogenetec), 0.2 mM each dNTP, 1.5 mM $MgCl_2$ 1.5 pmol of each primer and 0.5 U Goldstar polymerase (Eurogenetec) and was made up to a total volume of 25 µl with double distilled

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Table 1 Repeat region of random selected HumD3S1358 alleles and their corresponding allelic designation

Allele size (base pairs)	Repeat region	Allelic designation	Alleles sequenced
101	TCTA $(TCTG)_2$ $(TCTA)_{11}$	14	2
105	TCTA $(TCTG)_3$ $(TCTA)_{11}$	15	1
105	TCTA $(TCTG)_2$ $(TCTA)_{12}$	15	1
109	TCTA $(TCTG)_3$ $(TCTA)_{12}$	16	2
113	TCTA $(TCTG)_3$ $(TCTA)_{13}$	17	2
117	TCTA $(TCTG)_3$ $(TCTA)_{14}$	18	2

Table 2 Allele distribution of the STR Hum D3S1358 in two regions in North and South Germany

Alleles	North Germany n: 656	South Germany 1332
	f ± s. e.	f ± s. e.
13	0.011 ± 0.004	0.007 ± 0.002
14	0.140 ± 0.010	0.102 ± 0.006
15	0.230 ± 0.012	0.249 ± 0.008
16	0.215 ± 0.004	0.233 ± 0.008
17	0.245 ± 0.012	0.218 ± 0.008
18	0.149 ± 0.010	0.171 ± 0.007
19	0.006 ± 0.002	0.017 ± 0.003
var.	0.003 ± 0.002	0.003 ± 0.001
PIC:	0.78	0.78
HET:	0.80	0.80
MEC:	0.59	0.58

Table 3 Genotype frequencies (%) of the STR HumD31358 in two regions in South and North Germany. Underlined type corresponds to the South German sample ($\underline{n = 666}$) and italic type to the North German sample ($n = 328$). The Hardy-Weinberg equilibrium was tested for with Pearsons χ^2-method

Alleles	13	14	15	16	17	18	19	var.
13	0							
	0							
14	0.45	1.20						
	0.30	*0.91*						
15	0.15	4.80	6.61					
	0.61	*8.54*	*2.74*					
16	0	4.50	11.41	4.95				
	0	*9.76*	*10.06*	*4.00*				
17	0.15	5.26	10.81	11.11	4.05			
	1.22	*5.18*	*11.59*	*9.15*	*7.32*			
18	0.75	2.70	8.41	8.71	7.36	2.70		
	0	*1.83*	*9.45*	*5.49*	*7.32*	*2.74*		
19	0	0.30	0.90	0.75	0.60	0.75	0	
	0	*0.30*	*0.30*	*0.30*	*0*	*0.30*	*0*	
var.	0	0	0.15	0.15	0.15	0.15	0	0
	0.30	*0*	*0.30*	*0*	*0*	*0*	*0*	*0*

$\underline{\chi^2 = 13.06, P = 0.99}$;
$\chi^2 = 17.98, P = 0.99$

water. The set of primers used was as described by Li et al. 1994 [5]: 5′ ACTGCAGTCCCAATCTGGGT-3′(F); 5′ ATGAAATCAACAGAGGCTTG-3′(R). Cycle conditions (PTC-100, MJ Research, Inc.): 94° C – 3 min soak, 94° C – 1 min, 58° C – 1 min, 72° C – 1 min, 30 cycles, 72° C – 2 min. Amplified DNA fragments were separated by horizontal electrophoresis through 0.7 mm-thick native gels according to Möller et al. 1994 [6]. The polyacrylamide gels (7.5%T, 3% C) contained piperazine diacrylamide as cross-linker a 28 mM CHES (Cyclohexylaminomethane sulfonic acid) and 81 mM formate (pH 9.0). Bands were visualised by silver staining [2] and alleles were named according to the number of repeats as recommended [3]. Typing was performed by side-to-side comparison with an allelic ladder, containing alleles 13–19. Following electrophoresis of the amplified PCR products, the target alleles were excised from PAG and extracted by elution. The DNA was cleaned and concentrated in Millipore Ultrafree MC Filters 30.000 NMGG. The same primers were used for sequencing performed using the Ready Reaction Dyedeoxy Terminator Sequencing Kit and the 373 sequencer (Perkin-Elmer-ABI).

Results and discussion

PCR amplifications under the described conditions were successful and remarkably robust. Both populations investigated displayed the 7 common alleles 13–19, which ranged from 97 bp to 121 bp in length. In addition, the rare variants (var.) observed were alleles 11 ($n = 2$), 12 ($n = 2$) and 20 ($n = 2$). Two samples of each of the common alleles were sequenced. The sequence structure of the repeat region, fragment lengths and allele designations are given in Table 1. Due to the small number of sequenced samples the frequency of a sequence variability within alleles of equal length, i.e. a common phenomenon in STRs [4, 7] could not be estimated. Allele frequencies for both German populations are presented in Table 2. No significant differences were seen between the populations investigated. The number of observed genotypes is given in Table 3. Statistical tests were carried out separately for each sample and in both studies the allele distribution met the Hardy-Weinberg equilibrium. In total, two mutations (1 male and 1 female) were found in a total of 780 meioses. Our findings and those given in the literature [5, 8] show that the allele distribution of HumD3S1358 is quite similar when geographically isolated Caucasian populations are compared.

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