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Forensic Efficiency Parameters for the 15 STR Loci in the Population of the Island of Cres (Croatia)

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A B S T R A C T

Forensic parameters based on 15 AmpFlSTR Identifiler short tandem repeat (STR) loci (D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, VWA, TPOX, D18S51, D5S818 and FGA) were evaluated in the sample of 122 unrelated, autochthonous, adult individuals from the Island of Cres (Croatia). PCR amplification was performed with the AmpFlSTR Identifiler PCR Amplification Kit and the amplified products were separated and detected using the ABI 3130 DNA genetic analyzer. The agreement with Hardy Weinberg Equilibrium (HWE) was confirmed for all loci ($p>0.05$). The combined power of discrimination (PD) and the combined power of exclusion (PE) for the 15 tested STR loci were 0.999999999999999997988728679 and 0.999997397, respectively. According to the presented data, D18S51 proved to be the most informative marker followed by markers D2S1338 and D21S11. Inter-population comparisons in allele frequencies with other East Adriatic Islands revealed significant differences for all analyzed population pairs ranging from 4 loci (Cres vs. *Hvar) to 1 locus (Cres* vs. *Krk). Furthermore, allele frequencies comparisons of Cres and Croatian mainland revealed the lack of statistically significant differences at all studied loci. The results of the current study indicate that the examined fifteen STR loci are useful genetic markers for individual identification and paternity testing in Croatian population from the Island of Cres.*

Key word: *STR_S, AmpFlSTR identifiler, forensic parameters, the Island of Cres, Croatia*

Introduction

Short tandem repeats (STRs) are loci with alleles composed of tandemly repeated short DNA sequences of 2–7 base pair in length[1]. There are hundreds of these STR regions in the human genome, but 10 to 15 regions are sufficient to give high levels of discrimination between individuals[2]. Numerous previous studies have demonstrated that STR_S have become the choice of loci for determination of biological and parentage relationship of individuals as well as for forensic analysis[2–10].

The island of Cres is the second largest island in the Croatian section of the Adriatic sea (Figure 1). The last official population census (2001) lists a total population of 2946 inhabitants living in 26 settlements. Cres is a hilly island, 66 km long and ranging in width from 2 to 12 km.

The current population structure of Cres was formed through several immigratory episodes of genetically distant populations. The earliest available archeological data show that this area was continuously inhabited since the middle Paleolithic. The oldest western Balkan population of a clearly Indo European-speaking Illyrians remained the basic population after which other popula-

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Fig. 1. Geographical position of the Island of Cres (Croatia).

tion followed such as Greeks (4th century B.C.), Romans (3rd to 6th centuries A.D.) and the Venetians (from 1409 to 1797)[11]. Apart from this, the current population structure is also shaped by a Slavic element, which was introduced through a first major immigration of Croats (people of Slavic origin) by the early 6th century $_{AD}$ in the region that is today's continental Croatia. In the 6th and 8th century A.D. Croats reached the Adriatic Sea coast where they became a predominant population. The second large immigration wave of Croats occurred during the Turkish wars between the 16th and 18th centuries, when population groups from the mountainous region of the Adriatic hinterland settled in the Adriatic islands[12].

In this study, we present the allele distribution and forensic parameters at fifteen highly polymorphic tetranucleotide STR loci included in the AmpF*l*STR Identifiler PCR amplification kit (D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, VWA, TPOX, D18S51, D5S818 and FGA) in a population sample of the island of Cres (Croatia).

Sample and Methods

Sample

One hundred and twenty two unrelated adult individuals from the Island of Cres (Croatia) participated in the study, after giving the informed consent. Whole blood samples were obtained by venipuncture, collected into EDTA tubes and stored at –20 °C.

Genomic DNA was extracted from whole blood samples using the NucleoSpin Blood kit (Macherey-Nagel, Germany) according to the manufacturer's instructions.

DNA analysis

AmpF*l*STR Identifiler PCR Amplification Kit (Applied Biosystems, Foster City, CA, USA) was applied to simultaneously amplify 15 STR loci. The PCR amplification was carried out in AB Gene Amp PCR System 9700 Thermal Cycler (Applied Biosystems) according to the manufacturer's recommended protocol. DNA typing was performed on the ABI PRISM 3130 Genetic Analyzer (Applied Biosystems) with accessory software – ABI Data Collection Software and GeneMapper^{TM} 3.2 (Applied Biosystems).

Data analysis

Allele frequencies were computed by the gene counting method using the software package SPSS 7.5 for Windows. The agreement with the Hardy-Weinberg expectations (HWE) of genotype frequencies was determined by the chi-square test based on the number of observed and expected heterozygotes and the exact test based on the number of observed and expected genotypes[13]. Forensic efficiency parameters such as Heterozygosity (H), Polymorphism Information Content (PIC), Power of Discrimination (PD), Probability of Match (PM) and Power of Exclusion (PE) were calculated by PowerStats v 1.2 software package[14]. The exact tests of population differentiation were computed according to the methods implemented in Arlequin version 2.000 software adapted for STR loci (Schneider 2000)[15].

Results and Discussion

Forensic statistical parameters for forensic testing at the 15 AmpF*l*STR Identifiler Loci in the Cres population are presented in Table 1. According to the measures of heterozygosity and the polymorphism information content (PIC), as measures of informativeness, D18S51, D2S1338 and D21S11 loci are the most variable and the most informative markers for population genetic analyses and forensic testing showing the highest level of observed (0.844–0.877) and expected (0.841–0.871) heterozygosity and polymorphism information content (0.82–0.86). On the other hand, loci TPOX and D16S539 proved to be the least variable according to the lowest observed (0.590 and 0.672, respectively) and expected (0.626 and 0.744, respectively) heterozygosity, and the lowest value of polymorphism information content (0.57 and 0.70 respectively).

The probability that two randomly chosen persons have the same unspecified genotype at a locus is designated as probability of match (PM) or individualization potential of a locus[2], and is used to calculate the power of discrimination (PD) of a locus, 1-PM. The probability of match (PM) for the 15 studied loci ranged from 0.035 (D18S51) to 0.195 (TPOX). Even relatively reduced levels of genetic diversity at some of the loci provide discrimination power between 80.5% (TPOX) and 96.5% (D18S51) (average of 91.5%). The combined power of discrimination (PD) for the 15 studied loci was 0.99999999999999997988728679, which means when used together these fifteen loci can distinguish samples from different individuals with a probability of 99.99%.

Forensic parameter, namely, the power of exclusion (PE) is used to evaluate the strength of a locus to exclude faulty accused individuals. It represents the percentage of individuals in the relevant population who would not

TABLE 1
FORENSIC STATISTICAL PARAMETERS AT THE 15 STR LOCI IN THE POPULATION FROM THE ISLAND OF CRES (CROATIA)

Allele	D8S1179	D21S11	D7S820	CSF1PO	D3S1358	TH01	D13S317	D16S539	D2S1338	D19S433	VWA	TPOX	D18S51	D5S818	FGA
H_{obs}[a]	0.795	0.844	0.786	0.721	0.819	0.762	0.795	0.672	0.868	0.745	0.819	0.59	0.877	0.713	0.795
H_{exp}[b]	0.808	0.841	0.817	0.7	0.779	0.758	0.796	0.744	0.863	0.78	0.81	0.626	0.871	0.737	0.836
χ^2-test	0.064	0.001	0.565	0.17	0.917	0	0.005	2.985	0.002	0.67	0.02	0.556	0.003	0.267	1.218
Exact test (p)	0.524	0.592	0.107	0.274	0.45	0.647	0.099	0.06	0.352	0.29	0.579	0.348	0.662	0.073	0.723
PM[c]	0.071	0.048	0.068	0.145	0.089	0.098	0.079	0.108	0.040	0.076	0.071	0.195	0.035	0.105	0.049
PD[d]	0.929	0.952	0.932	0.855	0.911	0.902	0.921	0.892	0.960	0.924	0.929	0.805	0.965	0.895	0.951
PE[e]	0.590	0.684	0.575	0.462	0.636	0.531	0.590	0.386	0.732	0.503	0.636	0.279	0.749	0.449	0.590
PIC[f]	0.78	0.82	0.79	0.65	0.75	0.72	0.77	0.70	0.85	0.75	0.78	0.57	0.86	0.70	0.82

[a]Hobs – observed heterozygosity
[b]H_{exp} – expected heterozygosity
[c]PM – probability of match
[d]PD – power of discrimination
[e]PE – power of exclusion
[f]PIC – polymorphism information content

share the same DNA profile presented in a paternity case[16]. Single locus PE values ranged from 0.279 (TPOX) to 0.749 (D18S51), whereas combined value using all fifteen loci has increased the forensic utility to 0.999997397 (99.99%).

The agreement with Hardy-Weinberg equilibrium (Table 1), tested by the χ^2-test based on the number of observed and expected heterozygotes and the exact test based on the number of observed and expected genotypes, is confirmed for all tested loci ($p>0.05$).

In this study we also compared allele frequencies of fifteen loci of the Cres population with the same number of loci obtained from Croatian mainland[17], whereas nine available loci were compared with East Adriatic Islands[18–22]. These exact tests of population differentiation[23] show statistically significant differences (significance level was 0.05) in allele frequencies between Cres and Hvar at 4 loci (D7S820, CSF, TPOX and D5S818), followed by Cres and Korčula at 3 loci (D7S820, CSF and D3S1358), Cres and Vis at 3 loci (D3S1358, D13S317 and FGA), Cres and Brač at 2 loci (TH01 and FGA). There are significant differences at 1 locus (D7S820) only between two geographically closest islands Cres and Krk. Significant differences in allele frequencies between the Island of Cres and Croatian mainland were not observed at any of the studied loci.

In conclusion, the analysis of fifteen AmpFlSTR Identifiler short tandem repeat (STR) loci yielded reliable forensic parameters and was highly differentiating in studied isolated rural populations from Eastern Adriatic islands, thus proving the effectiveness of multiple STR locus profiles even in small rural populations.

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UČINKOVITOST FORENZIČKIH PARAMETARA 15 STR LOKUSA U POPULACIJI OTOKA CRESA (HRVATSKA)

S A Ž E T A K

Forenzički parametri određeni su na uzorku od 122 nesrodne, odrasle osobe podrijetlom sa otoka Cresa, na temelju 15 AmpF*l*STR Identifiler (D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, VWA, TPOX, D18S51, D5S818 and FGA) kratkih uzastopno ponavljajućih sljedova DNK (STR). Lančana reakcija polimerazom provedena je upotrebom AmpF*l*STR Identifiler PCR Amplification Kit sustava. Umnoženi produkti razdvojeni su i određeni pomoću instrumenta ABI 3130 DNK *Genetic Analyzer*. Slaganje s Hardy-Weinbergovom ravnotežom (HWE) potvrđeno je za sve analizirane lokuse ($p>0,05$). Zajednička snaga diskriminacije (PD) i zajednička snaga isključivanja (PE) za 15 analiziranih lokusa iznosila je 0,99999999999999997988728679 odnosno 0,999997397. Prema dobivenim rezultatima, lokus D18S51 je najinformativniji, a zatim slijede lokusi D2S1338 i D21S11. Na temelju usporedbe učestalosti alela između populacije otoka Cresa i ostalih istraživanih otoka istočnog Jadrana, utvrđene su značajne razlike između svih analiziranih otočnih parova. Najveća razlika utvrđena je između otoka Cresa i Hvara (na 4 lokusa), dok je najmanja razlika utvrđena između otoka Cresa i Krka (na 1 lokusu). Nadalje, usporedba otoka Cresa i kontinentalne Hrvatske nije pokazala statistički značajnu razliku niti na jednom od analiziranih lokusa. Rezultati provedenog istraživanja ukazali su na značajnu učinkovitost 15 analiziranih STR lokusa kao važnih genetičkih biljega za utvrđivanje identiteta osoba i dokazivanje očinstva u populaciji otoka Cresa.