RFLP AND STR POLYMORPHISMS IN A SWISS MOUNTAIN POPULATION

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1. Introduction

In the Swiss Alps the inhabitants live to some extent in geographically separated regions and can form relatively isolated poulation groups. It has been assumed that such mountain populations would have different allele distributions from those observed in the general Swiss population. In order to determine if such differences exist approximately 200 unrelated individuals of a mountain region in the Rhone Vallely called Goms were examined. The 4 RFLP loci D12S11, D7S21, D7S22 and D2S44 and the 3 STR systems SE33, HUMTHO1 and D21S11 were analyzed and compared with a general Swiss Population sample.

2. Methods

High molecular DNA was isolated from 250 µl EDTA blood by Proteinase K / Phenol / Chloroform extraction.

a) RFLP: 1 - 2 µg DNA was digested with the restriction enzyme Hinf I. Electrophoretic separation was performed in 1% agarose gels (Sigma type II) in 1 x TBE buffer without ethidium bromide at room temperature for about 60 hours at 20 V. After Southern Blotting onto nylon membranes (Amersham Hybond N) hybridisation was done with the single locus probes MS43a, MS31, G3 and yNH24 using random primer oligolabelling [3] or the non-isotopic chemiluminescence detection described by Dimo-Simonin et al. [1]. Band lengths were measured with a BioImage Visage System of Millipore. Sizes of band fragments were calculated by the local reciprocal logarithmic approach of Elder and Southern [2].

b) STR systems SE33, HUMTHO1 and D21S11

The PCR-products of the 3 STR systems SE33 (5'->3' AAT CTG GGC GAC AAG AGT GA; ACA TCT CCC CTA CCG CTA TA) [6], HUMTHO1 (5'->3' GTG GGC TGA AAA GCT CCC GAT TAT; GTG ATT CCC ATT GGC CTG TTC CTC) [4] and D21S11 (5'->3' GTG AGT CAA TTC CCC AAG; GTT GTA TTA GTC AAT GTT CTC C) [7] were determined and detected on an ABI 373A automated DNA sequencer (ABI GeneScan 672). The Genescanner enabeled the simultaneous analysis of the 3 fluorescently labeled STR products and the automatically calculation of the allele length.

PCR reaction mixture: 10 ng template DNA, 5 µl 10x PCR buffer (GeneAMP, Perkin Elmer Cetus), 8 µl 1.25 mM dNTP's, 1 µl 12.5 µM of each primer (one primer labeled with a fluorescent dye), 1.5 U AmpliTaq DNA Polymerase (Perkin Elmer Cetus), 4 µl 0.2% BSA, sterile H_2O was added to a final volume of 50 µl; the reaction mixture was overlayed with a drop of oil.

Amplification conditions (Thermocycler: Biometra Triothermoblock):
SE33 and HUMTHO1: 94°C - 45 sec., 60°C - 30 sec., 72°C - 30 sec; 30 cycles.
D21S11: 94°C - 45 sec., 55°C - 30 sec., 72°C - 30 sec.; 30 cycles.
SE33 and HUMTHO1 were coamplified.

3. Results

a) RFLP loci D12S11, D7S21, D7S22 and D2S44

DNA samples of at least 500 unrelated Swiss individuals were investigated for the 4 RFLP loci D12S11, D7S21, D7S22 and D2S44. In collaboration with the Institute of Anthropology of the University of Zürich we could also examine a population sample of a relatively isolated mountain region in the Swiss Alps. The mountain region is called Goms and is part of the upper Rhone Valley of the Canton Valais. Approximately 200 unrelated individuals of this mountain region were analyzed; the frequency distributions obtained for the probes G3 and yNH24 are shown in figure 1 - 2. The

results exhibit the same kind of pattern of frequency distribution for the mountain population as for the large Swiss sample. Both population samples show an equally high degree of polymorphism and no reduction of the number of the alleles in the mountain population of Goms could be observed. The differences observed are not forensically relevant.

b) STR systems SE33, HUMTHO1 and D21S11
The mountain population of Goms was also investigated for the 3 STR systems SE33, HUMTHO1 and D21S11 and compared with a general Swiss population sample of at least 195 individuals. The observed allele frequencies are shown in figure 3 - 5. In contrast to the RFLP loci the STR systems showed some remarkable differences in the distribution of the allele frequencies. In the mountain population the most common alleles of SE33 are allele 7 and 16 while in the Swiss population the most common alleles are the alleles 6 and 17. The systems HUMTHO1 and D21S11 also show differences in the allele frequency of some common alleles. For all 3 examined STR systems the observed number of alleles is greater in the general Swiss population than in the mountain population. Almost all rare alleles obtained in the Swiss population could not be found in the mountain population of Goms.

4. Discussion

This study showed that for the 4 investigated RFLP it can be excluded that the degree of polymorphism is reduced in smaller and apparently isolated populations. The frequency distribution is also comparable to those reported for other Caucasian populations [5]. The 3 examined STR sytems show indeed some noticeable differences in the distribution of the allele frequencies but the differences are also of no practical forensic significance.

Acknowledgments: The authors thank R. Fatzer, E. Garbely, K. Schluep and M. Stadler for excellent technical assistance.

References:

1. Dimo-Simonin N, Brandt-Casadevall C, Gujer HR (1992): Chemiluminescent DNA probes: evaluation and usefulness in forensic cases. Forensic Sci Int 57: 119-127
2. Elder JK, Southern EM (1987): Computer-aided analysis of one-dimensional restriction fragment gels. In: Bishop MJ, Rawlings CJ (eds) Nucleic acid and protein sequence analysis. IRL Press, Oxford, pp 165-172
3. Feinberg AP, Vogelstein B (1983): A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 132: 6-13
4. Gill P, Kimpton C, D'Aloja E, Anderson J, Bär W, Brinkmann B, Holgersson S, Johnsson V, Kloostermann AD, Lareu MV, Nellemann L, Pfitzinger H, Phillips CP, Schmitter H, Schneider PM, Stenersen M (1993): Report of the European DNA Profiling Group (EDNAP) - towards standardisation of short tandem repeat (STR) loci. in press
5. Gill P, Woodroffe S., Lygo JE, Millican ES (1991): Population genetics of four hypervariable loci. Int J Med 104: 221-227
6. Polymeropoulos MH, Rath DS, Xiao H, Merril CR (1992): Tetranucleotide repeat polymorphism at the human beta-actin related pseudogene H-beta-Ac-psi-2 (ACTBP2). Nucleic Acids Res 20, 1432
7. Sharma V and Litt M (1991): Tetranucleotide repeat polymorphism at the D21S11 locus. Human Molecular Genetics 1, 67

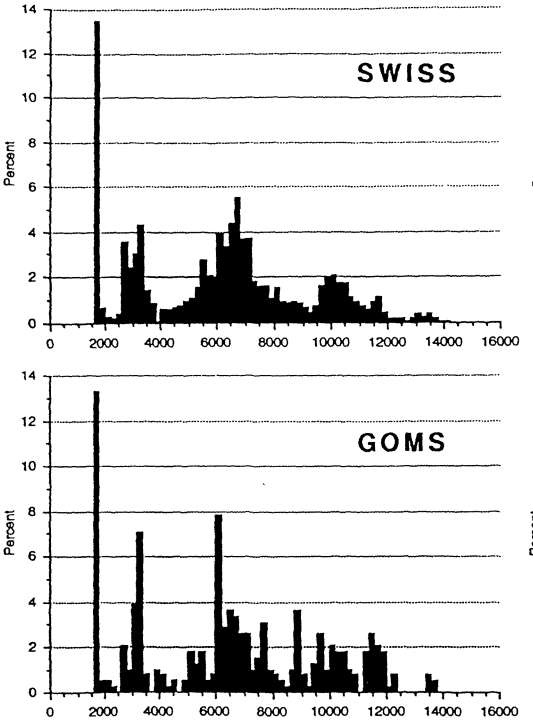

Fig. 1: Frequency distribution of probe G3

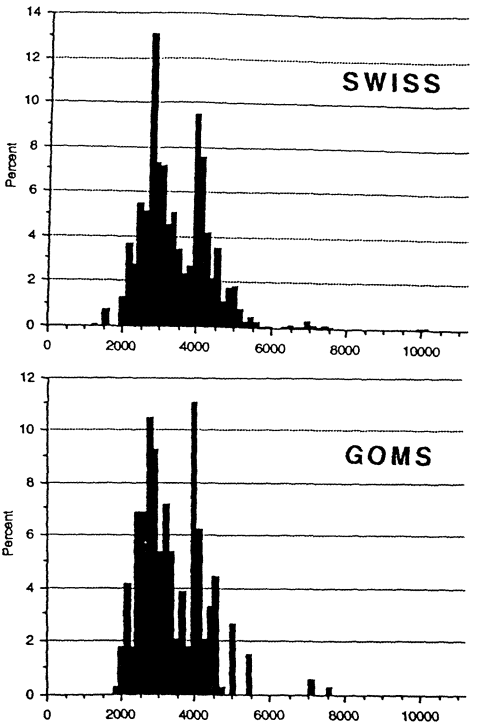

Fig. 2: Frequency distribution of probe yNH24

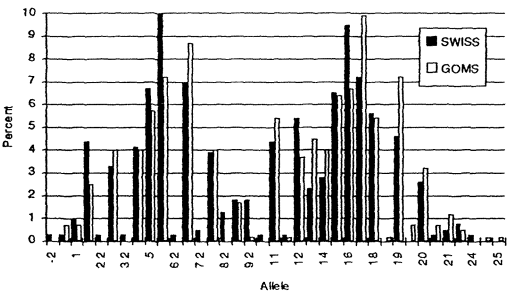

Fig. 3: Distribution of the SE33 alleles

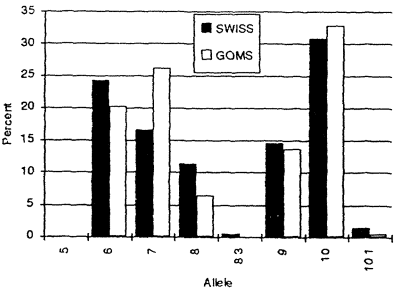

Fig. 4: Distribution of the HUMTHO1 alleles

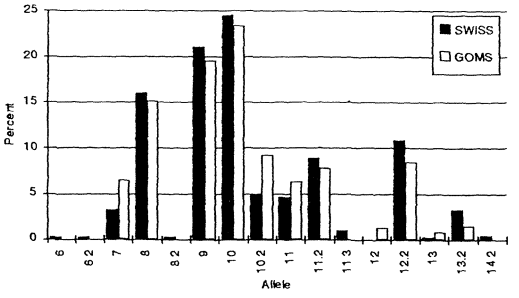

Fig. 5: Distribution of the D21S11 alleles