Distribution of variable number of tandem repeat (VNTR) DNA polymorphism at D2S44 locus in Tuscany (Italy)

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INTRODUCTION

RFLP (restriction fragment-lenght polymorphism) at the D2S44 locus, detected by the highly polymorphic probe YNH24 (Nakamura et al. 1987), can be used as an efficient tool in stain and paternity testing, as long as reliable fragment size frequencies are available (see van Eede et al. 1991, Gill et al. 1991, Budowle et al. 1991).

Pascali et al. (1990) and Gasparini et al. (1990) previously described distribution of allelic frequencies in some Italian populations (roman with YNH24/PstI, venetian with YNH24/MspI, respectively).

We report here RFLP frequencies for YNH24/HinfI in a population sample from Pisa, Tuscany.

MATERIALS AND METHODS

Samples (5 ml) from 100 unrelated healthy blood donors, born in Pisa province, were collected at the Transfusion Center of S. Chiara Hospital, Pisa, Italy.

DNA was purified from 500μl buffy coats after haemolysis of erytrocytes in 1xSSC (twice): pellets were resuspended in NaAc 0.2M containing Proteinase K (0.5 mg/ml) and SDS (0.6%) 56°C 1hr. Extraction was carried out in phenol/chloroform 1:1 and subsequent precipitation by addition of 2 volumes of ETOH (twice). Estimation of DNA concentration was carried out by direct comparison to undigested Lambda DNA loaded in 0.4% agarose gels at different concentrations. 5 μg DNA restriction enzime digestion was carried out at 37°C overnight with HinfI (3U/μg): fragments were run in 0.8% agarose gel (15x25) in TBE at constant voltage (30V) for approximately 48 hr until the 4.36 Kb fragment of the Lambda marker had migrated approximately 9 cm. Gel pretreatment, blotting and baking was according to the conditions recommended from the blotting paper supplier (Schleicher & Schuell). YNH24 probe was random primed labelled with P^{32} d(CTP) and purified on Sephadex G50. Prehybridization, hybridization, washing and autoradiography were carried out as previously described (Ceccherini-Nelli et al., 1987). The typing results were recorded manually by the "ruler and pencil" method. The size of the fragments detected was calculated by comparison of the known Lamba Hind III digested fragments used as external marker (Southern 1979).

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RESULTS AND DISCUSSION

YNH24/HinfI DNA VNTR polymorphism was examined in 100 unrelated samples; Fig.1 shows some of the results obtained.

Fig.1

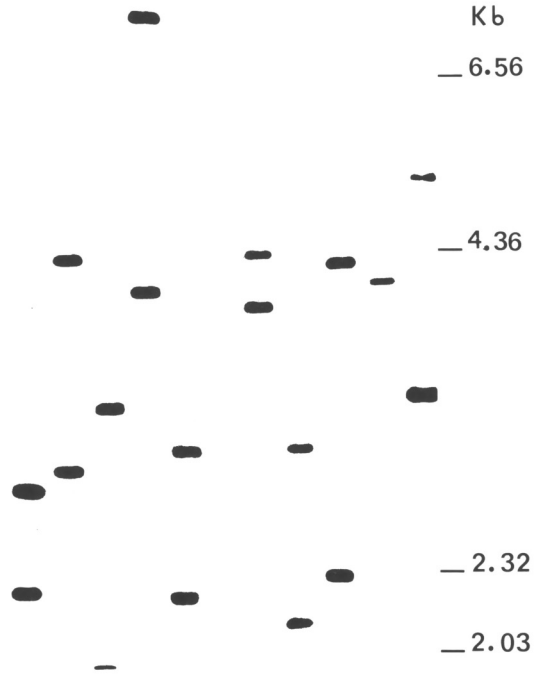
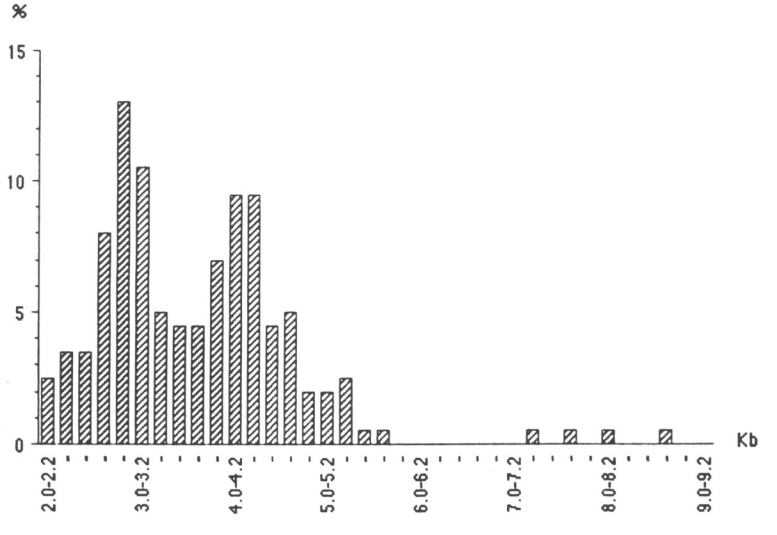


Fig.2



Five individuals showed only one fragment and therefore they were regarded as homozygous (heterozygosity, 95%). Fragment size distribution was continuous in the 2.0-5.5 Kb range. The average measurement error was estimated about 2% . After a conservative approach (see Budowle et al. 1991, van Eede et al. 1991), fragments were grouped at 0.2 Kb intervals. The histogram in Fig.2 illustrates the data and shows a bimodal distribution (in accordance to what reported in the literature) at 2.8-3.0 Kb and 4.0-4.4 Kb. The most common bin frequency estimate for Tuscany population was 13% (this figure was comparable with what shown from Budowle et al. 1991 and van Eede et al. 1991).

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