St 14 (DX S52) VNTR polymorphism in the Indian population and its application in carrier detection and prenatal diagnosis of haemophilia A families

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Abstract—The frequency of different polymorphic variants of the multi-allelic locus DXS52 (St14) of the human X chromosome, adjacent to the factor VIII gene, was evaluated by means of PCR for the heterogeneous population of India. It was shown that the heterozygosity index of this polymorphism in the studied population of 282 unrelated subjects was much higher (88%) than reported elsewhere. Two new alleles (1750 bp and 1420 bp) were detected during this study. Out of 65 families studied using this polymorphism for carrier detection and antenatal diagnosis, 58 were informative with this polymorphism, thus indicating that this polymorphism can serve as an important marker in the carrier detection and prenatal diagnosis of haemophilia A families.

Key words: Haemophilia A; St 14 allele frequency; polymorphism.

INTRODUCTION

The cloning and sequencing of the factor VIII gene [1], DNA polymorphisms from various ethnic groups have been reported and have proved to be extremely useful in the detection of carriers and in prenatal diagnosis in haemophilia A families [2]. The chromosomal band Xq 28 has been a focus of interest in human genetics because more than 20 hereditary diseases have been mapped to this region. The region around the polymorphic DXS52 locus (St14) within Xq 28 detects several polymorphisms including a highly polymorphic variable number of tandem repeats (VNTR), which is about 3 CM from the factor VIII gene. Analysis of this VNTR by Southern blot has revealed 8 alleles ranging in size from 3.4 kb to 6.6 kb [3]. However recently the polymerase chain reaction (PCR) has been applied for the analysis of this polymorphism [4]. We established the allele frequencies of the St 14...
VNTR in the Indian population by PCR and subsequently assessed its usefulness in carrier detection and the prenatal diagnosis of haemophilia A families.

MATERIAL AND METHODS

The heterozygosity frequency was studied in 282 unrelated X chromosomes (84 females, 114 males), belonging to haemophilia A families and a normal control group (190 belonging to haemophilia A families, 92 from normal control group).

The carrier detection was performed in 52 haemophilia A families, 48 severe and 4 moderate. Of these forty-two families had a positive family history and the remaining ten had a negative family history with only one affected person in the family. Thirty-three females were obligatory carriers whose carrier status could be given by family pedigree analysis alone. The remaining sixty-eight females were prospective carriers whose carrier status was to be given by DNA analysis. The pre-natal diagnosis was done in 13 families, all severe haemophilia A. To all the carriers and the patients who came for prenatal diagnosis, relevant genetic counselling was given. They were also appraised of the mis-classification rate of St14 marker being approximately 3%. Chorionic villus sampling was done trans-abdominally under ultrasound guidance at 9.5–12 weeks of gestation using a 20 gauge needle (89 mm long). Prior to the procedure, local anaesthesia (2% xylocaine) was given. The villi were collected in RPMI (1640) medium and were cleaned under a dissecting microscope (SMZ-2B; Nikon, Japan) to remove any maternal decidual tissue contamination.

DNA was extracted from the peripheral blood leukocytes and from the villi, after proteinase K digestion and phenol-chloroform extraction [5]. About 500 ng of the DNA was amplified by PCR using specific primers (Cybersyn, USA). Amplified reaction was performed by the standard protocol with 26 cycles consisting of 94°C for 1 h, 63°C for 50 min, 55°C for 1 h [4].

RESULTS

The agarose gel pattern of the various alleles of the St14 (VNTR) polymorphic system is shown in Fig. 1 and summarised in Table 1. The allele frequencies were more or less uniform in their distribution. This finding was important because India is a vast country with many differences in the various ethnic groups. However, Mumbai which has a population of only 12 million from which most of our samples were randomly drawn, represents the true microcosm of Indian diaspora. The observed heterozygosity rate was slightly less than the expected heterozygosity rate. This difference, however was not statistically significant ($\chi^2 = 0.9390; P > 0.3325$). Two new alleles were also reported in our population.

In the families investigated for the detection of carriers, out of 52 families, 48 were informative. One of these families was mis-classified with St14 and with