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SHORT COMMUNICATION

Rhea V. Samonte · Robert A. Conte · Ram S. Verma

Localization of human midisatellite and macrosatellite DNA sequences on chromosomes 1 and X in the great apes

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Abstract The mechanism of speciation has remained largely unresolved, and hominoid evolutionary history based on chromosome rearrangements has been continuously challenged. The recent availability of the human-derived chromosome 1-specific midisatellite (D1Z2) and chromosome X-specific macrosatellite (DXZ4) DNA sequence probes has prompted us to hybridize the aforementioned to the members of the hominoid clade (chimpanzee, gorilla, and orangutan), using the fluorescence in-situ hybridization technique. Inconsistencies in the hybridization pattern for the D1Z2 DNA probe in the great ape species suggests that changes in this sequence have apparently taken place during the evolutionary process. No hybridization signal was observed in the orangutan chromosome 1, suggesting that a homologous D1Z2 DNA sequence may not be present in its genome, or that the sequence may be altered, rendering itself undetectable by human-derived DNA probes. Homology in the hybridization patterns for the DXZ4 probe in all three ape species illustrates that the sequence is apparently conserved. Such hybridization data provide some level of phylogenetic information on the recent ancestry of higher primates.

Key words Chimpanzee · Gorilla · Orangutan · Chromosomes 1 and X · Midisatellite and macrosatellite DNA

Numerous investigations into the evolutionary history of hominoid species have been performed over the past two decades. Whether or not higher primate speciation has resulted from chromosomal rearrangements remains unresolved. Chromosome banding pattern homologies have been reported among the chimpanzee, gorilla, and orangutan (Yunis and Prakash 1982). Molecular studies play an important role in the reconstruction of the hominoid phylogenetic tree (Ayala 1995; Archidiacono et al. 1995; Jauch et al. 1992; Wienberg et al. 1990). The recent availability of the human-derived chromosome 1 midisatellite and chromosome X macrosatellite DNA sequence probes has prompted us to hybridize the aforementioned to the members of the hominoid clade (chimpanzee, gorilla, and orangutan) to determine sequence and position concordance/discordance.

The distal portion of chromosome 1p, specifically known as band 1p36, is considered a critical region that contributes to the development of several malignancies in humans. This band contains a midisatellite (D1Z2) sequence, which is composed of highly repetitive tandemly arranged satellite DNA repeats of 40 bp, and the $p58^{clk\text{-}1}$ proto-oncogene, which has been implicated in several types of cancer (O'Connell et al. 1987). The exact location of the D1Z2 sequence is at 1p36.3 (Lamb et al. 1989), while the location of the $p58^{clk\text{-}1}$ proto-oncogene has been mapped to 1p36 (Eipers et al. 1991). The genomic organization of locus D1Z2 is similar to that of minisatellites (Jeffreys et al. 1985). Both sequences contain a variable number of short tandem repeats, which are responsible for the high degree of polymorphism for these loci (Buroker et al. 1987; Genuardi et al. 1989). As D1Z2 spans a considerably larger portion (250–500 kb) of DNA, the term "midisatellite" has been coined for this kind of chromosome-specific repeat (Nakamura et al. 1987; Magenis et al. 1987). Midisatellites contain a higher number of repeat units than do minisatellites, rendering them more liable to undergo nonhomologous pairing leading to deletion/duplication events (Vogt 1990).

The macrosatellite *DXZ4* locus, which has been mapped to Xq24, contains a major cluster of 50–100 tandemly repeated copies of a unique 3-kb sequence. This cluster, which has a base composition of 63% C + G, is hypomethylated on the inactive X chromosome and highly methylated on the active X. Since the *DXZ4* locus has the potential to contain more than 50 variable-length alleles, it can be a

R.V. Samonte · R.A. Conte · R.S. Verma (✉)
Division of Genetics, The Long Island College Hospital-SUNY Health Science Center, Brooklyn, NY 11201, USA
Tel. +1-718-745-0443; Fax +1-718-745-3966

Present address:
[1]Natural Sciences Research Institute, University of the Philippines, Diliman, Quezon City, Philippines 1101

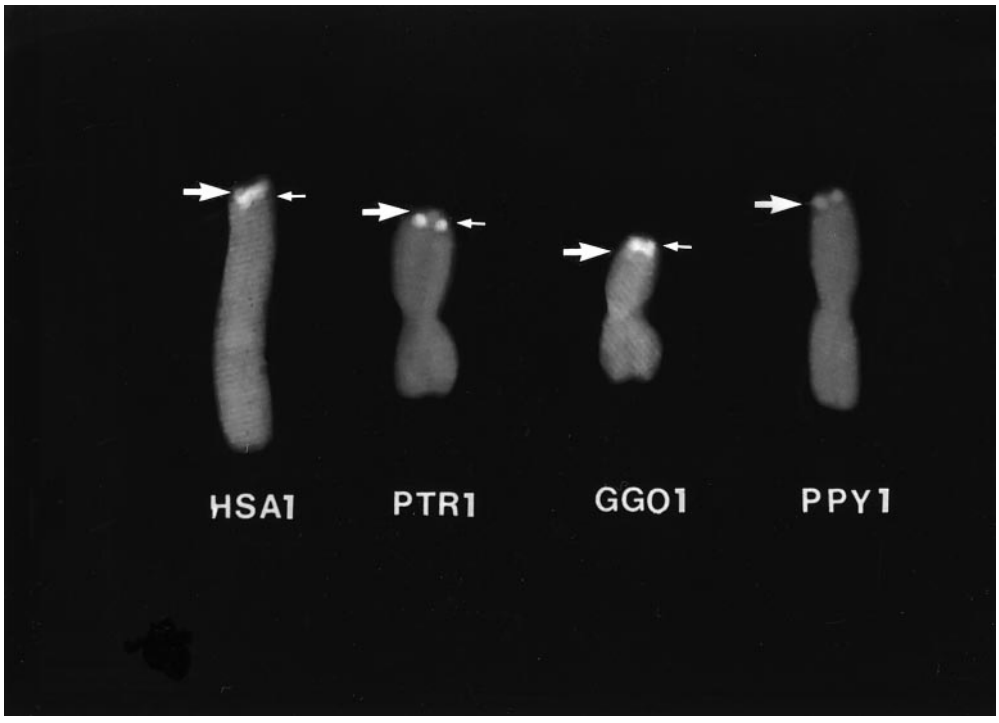

Fig. 1 Location of the chromosome 1-specific locus, D1Z2, in humans (HSA 1), the chimpanzee (PTR 1), and the gorilla (GGO 1), as shown by *small arrow* (*right*). No hybridization signal was observed in the orangutan (PPY 1). p58 is a control marker *large arrows* (*left*)

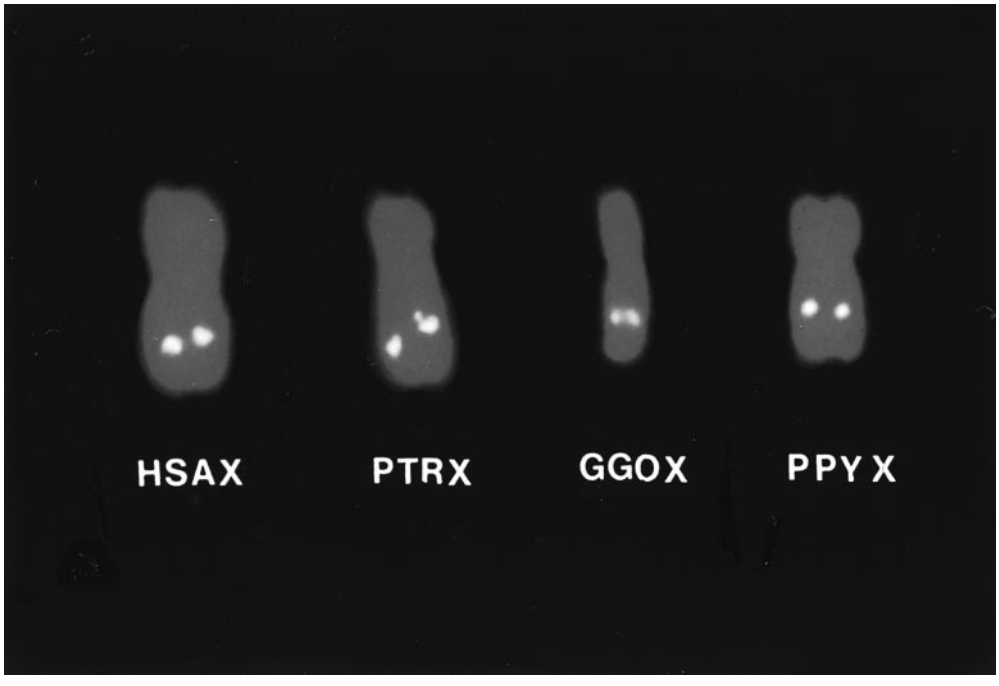

Fig. 2 Location of the DXZ4 DNA region in humans (HSA X), the chimpanzee (PTR X), the gorilla (GGO X), and the orangutan (PPY X)

useful marker for individual analysis and typing (Giacalone et al. 1992).

Ape chromosomes were obtained from fibroblast cell lines (Coriell Cell Repositories, Camden, NJ, USA) of chimpanzee (AGO 3450; *Pan troglodytes*, PTR), gorilla (AGO 5351; *Gorilla gorilla*, GGO), and orangutan (AGO 4742 and AGO 6213; *Pongo pygmaeus*, PPY) using standard procedures. Human chromosomes (*Homo sapiens*, HSA) were prepared from phytohemagglutinin-stimulated blood lymphocytes obtained from normal individuals (Verma and Babu 1995). The fluorescence in-situ hybridization technique of Lichter et al. (1995) was employed, with minor modifications. For localizing the chromosome 1-specific midisatellite DNA region, we used the human-

derived biotin-labeled chromosome 1 midisatellite (D1Z2) DNA probe specific for locus 1p36.3 (Oncor, Gaithersburg, MD, USA). The digoxigenin-labeled $p58^{clk-1}$ DNA probe specific for locus 1p36 was used as a control to confirm the 1p36 band region (Oncor). For localizing the chromosome X-specific macrosatellite DNA region, we used the biotin-labeled chromosome X macrosatellite (DXZ4) DNA probe specific for locus Xq24 (Oncor).

Chromosome- and loci-specific DNA probes have recently been used to determine the specificity of such probes in the great ape species (Samonte et al. 1997). Hybridization of these human-derived sequences suggests conservation of specific genomic sequence material. The FISH technique provides insights into genome evolution by pinpointing exact gene loci in each species. The chromosome 1-specific midisatellite (D1Z2) DNA region has been localized to band 1p36.3 in humans (HSA 1), and to the corresponding equivalent band locations in the two species of higher primates: band 1p36 in the chimpanzee (PTR 1) and the gorilla (GGO 1) (Fig. 1). No hybridization signals were observed for the D1Z2 DNA probe on the orangutan equivalent chromosome (PPY 1) in either cell line. The control probe, $p58^{clk-1}$, has been localized to band 1p36 in humans (HSA 1), and to the corresponding equivalent band locations in the three great ape species: band 1p36 in the chimpanzee (PTR 1), gorilla (GGO 1), and the orangutan (PPY 1). The chromosome X-specific macrosatellite (DXZ4) DNA region has been localized to band Xq24 in humans (HSA X), and to the corresponding equivalent band locations in the great apes: band q24 in the chimpanzee (PTR X), orangutan (PPY X), and gorilla (GGO X) (Fig. 2).

The hybridization data presented here show that both conservation and divergence of highly repetitive DNA sequences may have occurred during the evolutionary process. Detection of the DXZ4 DNA sequences in the three great ape species suggests a common ancestry. The detection of D1Z2 DNA sequences in only two species, chimpanzee and gorilla, reflects ancestral loci polymorphism in related primate species. Loss or retention of such sequences may produce a different phylogeny without data from other adjacent loci. All loci in the genome must not necessarily show the same pattern of inter-species relationship (Wu 1991). Individual gene trees are all equally valid, since these reflect molecular similarity, but they need not necessarily reflect the overall pattern of genetic similarity in the entire genome (Rogers 1993). Such findings do provide some level of phylogenetic information on higher primate ancestry.

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