BRIEF REPORTS

The Oxytocin Receptor Gene (OXTR) Localizes to Human Chromosome 3p25 by Fluorescence *in Situ* Hybridization and PCR Analysis of Somatic Cell Hybrids

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The human oxytocin receptor regulates parturition and myometrial contractility (2), breast milk let-down (8), and reproductive behaviors in the mammalian central nervous system (3). Kimura *et al.* (5) recently identified a human oxytocin receptor cDNA by means of expression cloning from a human myometrial cDNA library.

To elucidate further the molecular mechanisms that regulate oxytocin receptor gene expression and to define the expected Mendelian inheritance of possible human disease states, we must determine the number of genes, their localization, and their organization and structure. We summarize below our data indicating that the human oxytocin receptor gene is localized to 3p25 and exists as a single copy in the haploid genome.

Initial studies indicated that a single gene (OXTR) encodes the human oxytocin receptor (data not shown). To localize the OXTR gene, we performed polymerase chain reaction (PCR) analysis of rodent–human somatic cell hybrids. Partial sequencing of mouse and human genomic clones (Simmons, unpublished data) allowed design of common and unique oligonucleotide primers for the mouse and human oxytocin receptor genes. Polymerase chain reaction of DNA from NIGMS rodent–human monochromosomal hybrids with common oxytocin receptor primers revealed a 189-bp amplified product. In contrast, a 150-bp amplification product of a specific 5′ human oxytocin receptor oligonucleotide primer and common 3′ primer revealed the presence of human oxytocin receptor sequence only in cell hybrids that contained human chromosome 3 (Fig. 1). [NIGMS Panel, Human Genetic Mutant Cell Repository, Coriell Institute for Medical Research, Camden, NJ; 1, X (NA 07299); 2 (NA 10826B); 3 (NA 10253); 4 (NA 10115); 5 (NA 10114); 6 (NA 10629); 7 (NA 10791); 8 (NA 10156B); 9 (NA 10611); 10 (NA 10926B); 11 (NA 10927A); 12 (NA 10868); 13 (NA 10898); 14 (NA 10479); 15 (NA 11418); 16 (NA 10567); 17 (NA 10498); 18 (NA 11010); 19 (NA 10449); 20, 4 (NA 10478); 21 (NA 10323); 22 (NA 10888); X (NA 06318B); Y (NA 06317).]

To sublocalize the human oxytocin receptor gene, we performed fluorescence *in situ* hybridization on human metaphase chromosomes from a normal male (6). Twelve metaphases with single or double chromatid hybridizations were examined. All demonstrated hybridization to chromosome 3p25 (Fig. 2).

These data demonstrate that the human oxytocin receptor is encoded by a single gene that resides on chromosome 3p25. Of note, the RAF-1 oncogene, interleukin-5 receptor gene, and the Von Hippel–Lindau gene, VHL, map to 3p25 (1, 4, 7). In addition, many uterine and breast neoplasms exhibit cytogenetic abnormalities in this area, including translocation and deletion breakpoints in the region 3p25 (9). The possible contribution of mutations in the oxytocin receptor gene to the molecular mechanisms of oncogenesis in these tissues remains to be determined. Furthermore, knowledge of the localization of the human oxytocin receptor gene to 3p25 will assist in the development of a physical map of chromosome 3p.

These results will facilitate efforts to define the molecular events that regulate pregnancy duration in humans. We have demonstrated that the oxytocin receptor gene exhibits striking transcriptional regulation in myometrium in the last 5% of murine gestation (Simmons, unpublished data). The role of transcriptional regulation of human oxytocin receptor gene expression remains to be evaluated.

Finally, the present study suggests that inherited mutations in the human oxytocin receptor gene should exhibit an autosomal pattern of inheritance. This mode of inheritance contrasts with that of X-linked nephrogenic diabetes insipidus, a disorder caused by mutation of the closely related V_2 receptor for antidiuretic hormone, located on the X chromosome. The evolution of these genes from a putative common ancestral gene may have involved duplication, divergence, and migration of genes to disparate chromosomes.

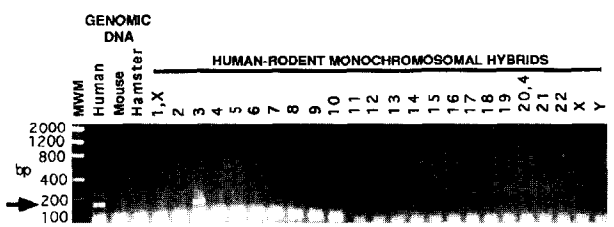

FIG. 1. Ethidium bromide-stained 2% agarose gel of polymerase chain reaction amplification products from genomic DNA and the NIGMS panel. Target DNA was subjected to 40 cycles of amplification with a human-specific 5′ primer (5′ GCT TCC TGT GCT GCT CCG CCA 3′) and a common 3′ primer (5′ TCA CGC CGT GGA TGG CTG GGA GCA GCT 3′). Only human genomic DNA and the hybrid cell line containing human chromosome 3 revealed a specific 150-bp amplification product.

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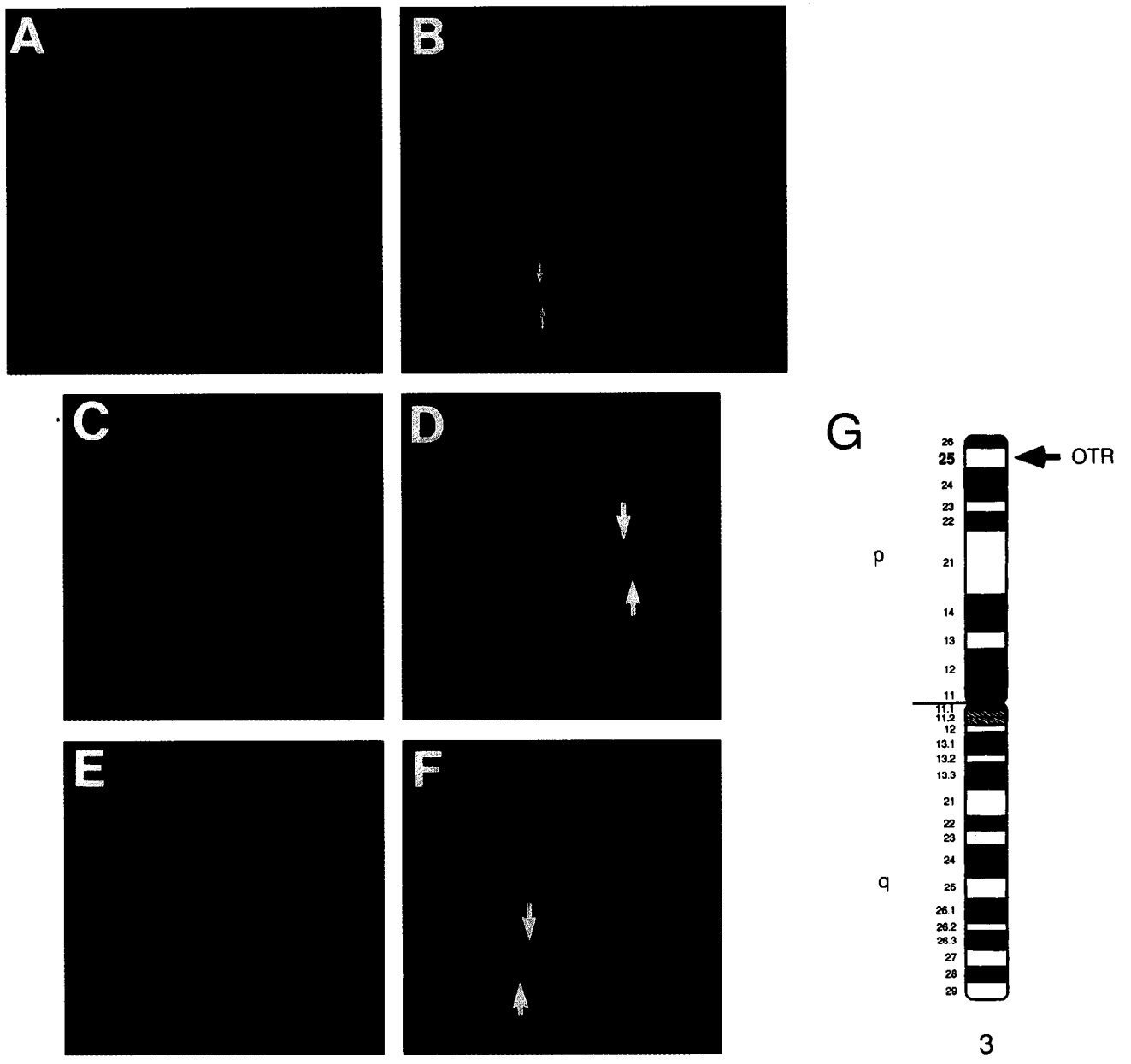

FIG. 2. Chromosome mapping by FISH. Human metaphase chromosomes were prepared from PHA-stimulated lymphocytes of a normal male by standard techniques (6). The oxytocin receptor cDNA, pOTR, was labeled by nick-translation with digoxigenin-11–dUTP. Hybridization and detection were performed with 100 ng of labeled probe as previously described (6). Hybridization signals were detected with rhodamine-conjugated digoxigenin antibody (Boehringer Mannheim 5 μg/ml) and localized to 4,6-diamidino-2-phenylindole (DAPI)-banded chromosomes (0.1 μg/ml). Rhodamine and DAPI fluorescence were viewed through a FITC/Texas red/DAPI triple band-pass filter set (Chroma Technology), and DAPI through a single band-pass filter (Zeiss), respectively, on an epifluorescence microscope (Zeiss). Cells were photographed on color film (Kodak Ektar ASA 1000). (**A**) Partial metaphase of human chromosomes, DAPI counterstained. (**B**) FISH localization of pOTR to chromosome 3p25 (double chromatid hybridization) (arrows). (**C**) Chromosome 3, enlarged from A, DAPI counterstained. (**D**) FISH localization of pOTR to chromosome 3p25 (double chromatid hybridization) (arrows). (**E**) Chromosome 3, independent metaphase, DAPI counterstained. (**F**) FISH localization of pOTR to chromosome 3p25 (double chromatid hybridization) (arrows). (**G**) Idiogram of human chromosome demonstrating FISH localization of OTR in 12 metaphases (400 band level resolution).

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Assignment of the Human Hippocampal Inward Rectifier Potassium Channel (HIR) Gene to 22q13.1

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The HIR gene encodes a small-conductance inward rectifier potassium channel (HIR or $K_{IR}2.3$) that is found in heart and brain (8–10). Inward rectifiers are a specialized class of potassium channels that produce large inward currents at potentials negative to the potassium equilibrium potential and only small outward currents at more positive potentials (11). This asymmetry in K^+ conductance plays a key role in the excitability of muscle cells and neurons. Inward rectifier potassium channels are the major contributors to the basal potassium conductance in cardiac muscle, where their role is to modulate cell excitability and heart beat frequency, maintain the resting potential, and terminate the long-duration cardiac action potentials (11). In the central nervous system, inward rectifiers are involved in similar aspects of the modulation of cell excitability (8–11). The central role of inward rectifiers in cardiac and neuronal function suggest that they might be involved in the etiology of human cardiovascular and neurological diseases.

The chromosomal assignment of the HIR gene was determined by PCR using a somatic cell hybrid panel of 20 human/hamster and human/mouse cell lines (BIOS Lab.) that contain each of the human chromosomes in one or more hybrid lines (Fig. 1A). The subchromosomal localization of the HIR gene was determined by Southern blot hybridization using a somatic cell hybrid panel that subdivides chromosome 22 into 25 distinct regions. The complete chromosome 22 somatic cell hybrid panel, which consists of 26 cell lines, will be described elsewhere (Budarf *et al.*, submitted). Fourteen of these cell lines were used to sublocalize the HIR gene (Fig. 1B): GM10888 is a human/hamster hybrid containing a normal chromosome 22 as its only intact human chromosome (7); Rad-110a is a radiation reduced human/hamster hybrid that contains a large portion of chromosome 22 (5); Cl 1-1/TW is a human × hamster hybrid made from a human cell line with a Ewing sarcoma associated t(11;22)(q24;q12), containing a der(11) as the only relevant human chromosome (2); D6S5 is a cell line derived from a normal human × mouse fusion that spontaneously lost the distal long arm of chromosome 22, retaining Cλ and all of the BCR related genes, but being negative for PDGFB (3); Cl-3/5878, Cl-1/5878, Cl-2/5878, and Cl-8/5878 are human × hamster hybrids that each contain a deleted chromosome 22 (Budarf *et al.*, submitted), due to the selection of adenylosuccinate lyase subsequent to fusion of GM05878 with Ade-I Cl-15-1/PB is a human × hamster hybrid that contains the der(22) of a t(7;22)(q36;q13), and Cl 21-2/PB contains the der(7) of the same hybrid fusion (Budarf *et al.*, submitted); 1/22AM-6 (GM11224C) is a human × hamster hybrid that contains the der(1) of a constitutional t(1;22)(q42;q13), and 1/22AM-27 (GM11223C) contains the der(22) of the aforementioned constitutional translocation (6). Cl-2/DIBA is a human × hamster hybrid that contains the der(12) of a constitutional t(12;22)(q13;q13.31) (Budarf *et al.*, submitted); APR 8.5 is a human × hamster hybrid that contains a der(22) of a constitutional t(11;22)(p11;q13.2) translocation (4).

The STSs for HIR were generated from the published cDNA sequence (9). Two primer pairs that bracket the translational start and stop, respectively, were used for chromosomal assignment (primer pair 5′-AAACTTGGCCCTGCGTC-3′ and 5′-AGGTTGGCGAAGTACAC-3′ from −46 to +104 bp of the HIR cDNA relative to the translational start, and primer pair 5′-AAGGAGGAGGCGGGCAT-3′ and 5′-TGGAGTCAGGAGGAAGG-3′ from 1222 to 1501 bp). For both pairs of PCR primers, the size of the PCR product obtained with genomic DNA as template was identical to that predicted from the

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