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SHORT COMMUNICATION

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Precise localization of the human thyroxine-binding globulin gene to chromosome Xq22.2 by fluorescence in situ hybridization

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Abstract The human thyroxine-binding globulin (TBG) gene has been localized to X chromosome (Xq22.2) by in situ hybridization using a biotinylated gDNA probe. This is consistent with previous mapping of the TBG gene to chromosome Xq21–q22.

Introduction

Thyroxine-binding globulin (TBG), a 54-kDa glycoprotein synthesized by the liver, is the principal thyroid hormone transport protein in human serum (Refetoff 1979). Cloning and sequencing of human TBG cDNA have revealed homology to serine protease inhibitors (Flink et al. 1986). The TBG gene has been localized to the X chromosome (Trent et al. 1987), and thus genetic abnormalities are transmitted in an X-linked fashion. To date, three complete and seven partial deficiencies have been analyzed, and a nucleotide deletion or substitution has been discovered in the coding region of the relevant TBG genes (Mori et al. 1989, 1990; Refetoff 1989). Here, we have precisely localized the human TBG gene by fluorescence in situ hybridization (FISH) with a previously cloned genomic TBG clone (Mori et al. 1989; Hayashi et al. 1993).

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Materials and methods

Chromosome preparation

Prometaphases were obtained from phytohemagglutinin-stimulated blood lymphocytes from a normal male and female, after thymidine synchronization and 5-bromodeoxyuridine incorporation during the last 7 h of culture, according to a method described previously (Viegas-Pequignot and Dutrillaux 1978; Takahashi et al. 1990, 1991).

DNA probe

The EMBL4 phage insert of the 14-kb *Eco*RI genomic fragment, which contained five exons and the 8-kb 5′ and 3-kb 3′ flanking regions of the human TBG gene, was isolated (Mori et al. 1989; Hayashi et al. 1993) and used as a probe.

In situ hybridization

Labeling and hybridization were performed according to the methods reported previously with minor modifications (Hori et al. 1990; Takahashi et al. 1990, 1991). Briefly, the DNA probe was labeled using a nick translation kit (BRL) with biotin-14-dATP. Hybridization was detected by incubation with fluorescein isothiocyanate (FITC)-avidin and the signal was amplified by further incubations with biotinylated anti-avidin followed by a second avidin-FITC. For R-banding, chromosomes were counterstained with propidium iodide and observed using a Nikon Microphoto epifluorescence microscope (B-2A filter).

Results and discussion

Twenty prometaphase chromosome cells from a male were analyzed. Sixteen of the cells had paired spots on the X chromosome (Xq22.2) (Fig. 1). Four showed a single spot in the same region or no signal on this chromosome. Similar results were obtained in cells from a female. Ten out of 20 cells had paired spots on both X chromosomes. No signal was observed as paired spots on other chromosomes in either the male or female. These experiments support the assignment of the TBG gene to Xq22.2. Thus,

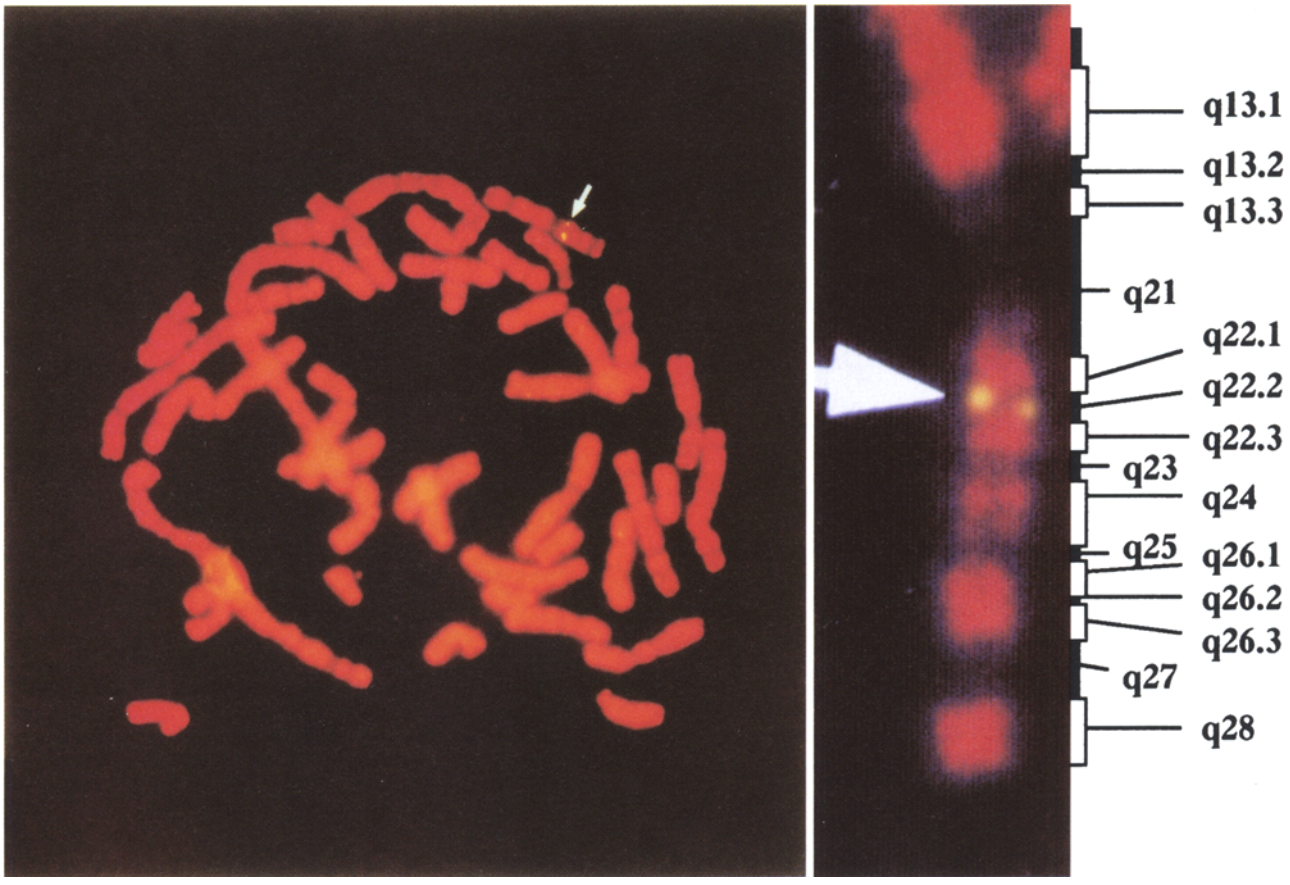

Fig. 1 Human male R-banded prometaphase after FISH with the human TBG gDNA. *Arrows* indicate signals on the X chromosome. The precise localization (Xq22.2) of the TBG locus is shown *right*

the locus for the human TBG, Xq21-q22, previously localized using an ^{3}H-labeled cDNA probe (Trent et al. 1987), has been precisely determined in the present study.

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