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ORIGINAL ARTICLE

Hypergonadotropic hypogonadism in a patient with inv ins (2;4)

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Summary

We report on a 30-year-old man with azoospermia, primary hypogonadism and minor dysmorphic features who carried a balanced insertional chromosome translocation inv ins (2p24;4q28.3q31.22)*de novo*. Molecular cytogenetic analyses of the chromosome breakpoints revealed the localization of the breakpoint in 4q28.3 between BACs RP11-143E9 and RP11-285A15, an interval that harbours the *PCDH10* gene. In 4q31.22, a breakpoint-spanning clone (RP11-6L6) was identified which contains the genes *LSM6* and *SLC10A7*. On chromosome 2, BACs RP11-531P14 and RP11-360O18 flank the breakpoint in 2p24, a region void of known genes. In conclusion, the chromosome aberration of this patient suggests a gene locus for primary hypogonadism in 2p24, 4q28.3 or 4q31.2, and three possible candidate genes (*LSM6*, *SLC10A7* and *PCDH10*) were identified by breakpoint analyses.

Introduction

Hypergonadotropic hypogonadism in males reflects primary testicular dysfunction and is characterized by elevated serum luteinizing hormone (LH), follicle-stimulating hormone (FSH) and low serum testosterone levels. It is associated with impaired spermatogenesis (usually azoospermia) and can be caused by extrinsic (e.g. infections, trauma) or genetic factors. The most frequent genetic cause of hypergonadotropic hypogonadism is Klinefelter's syndrome (typically 47,XXY) (Lanfranco *et al.*, 2004; Bojesen & Gravholt, 2007). Little is known, however, about other underlying genetic causes. The elucidation of causative gene defects is hindered by the sporadic occurrence of most patients. Because of infertility, large families are not available which impedes mapping of genes by linkage analysis.

Disease-associated balanced chromosome aberrations have led to the identification of numerous genes involved in genetic disorders (Bugge *et al.*, 2000). Chromosome breakpoints either disrupt genes directly, or they can influence the expression of neighbouring genes by position effects (Velagaleti *et al.*, 2005).

Here, we report on the molecular cytogenetic characterization of the chromosome breakpoints of a patient with azoospermia and hypergonadotropic hypogonadism who carries a balanced insertional chromosome translocation involving chromosome arms 2p and 4q.

Clinical report

The proband was a 30-year-old man with primary infertility for more than 5 years. His medical history included circumcision because of phimosis at the age of 20 years, and surgical correction of a unilateral inguinal hernia in childhood. Age of onset of puberty was reported to be 12 years. No major infections or other diseases had been reported. Physical examination at the age of 30 years revealed a small penis (6 cm) and low testicular volume (3 mL, normal range >12 mL). He had short stature [height 163 cm (<3rd percentile, father 167 cm, mother 165 cm)]. Other clinical features presented were myopia, prognathia, a fissured tongue, thin and sparse scalp hair, and brachydactyly. Psychomotor development was slightly retarded, but within the normal range. He attended a

general-education, secondary school and worked as a baker for many years.

Semen analysis repeatedly showed complete azoospermia. Endocrinological tests revealed hypergonadotropic hypogonadism: elevated serum FSH (42.7 mE/mL, normal range 0.70 – 11.10 mE/mL), elevated serum LH (17.7 mU/mL, normal range 0.80–7.60 mU/mL), low free serum testosterone (193 ng/dL, normal range 270–1730 ng/dL), normal serum dehydroepiandrosterone sulphate (95.5 μg/dL, normal range 80–560 μg/dL) and normal serum dihydrotestosterone (389 pg/mL, normal range 250–995 pg/mL).

Material and methods

Karyotyping, multi-colour banding and fluorescence in situ hybridization

Cytogenetic investigations (GTG banding) on 25 metaphases obtained from phytohaemagglutinin -stimulated peripheral lymphocytes were performed according to standard protocols.

Whole chromosome paint (WCP) analysis was performed with wcp2 and wcp4 (QBiogene, Irvine, CA, USA) according to the manufacturer's recommendations.

High-resolution multicolour-banding (MCB) based on microdissection-derived region-specific libraries for chromosomes 2 and 4 were performed as described before (Liehr *et al.*, 2002). A total of 20 metaphase spreads were analysed, each using a fluorescence microscope (Axioplan 2 mot; Carl Zeiss, Oberkochen, Germany) equipped with appropriate filter sets to discriminate between a maximum of five fluorochromes and the counterstain DAPI (4′6′-diamino-2-phenyl-indole). Image capturing and processing were carried out using the ISIS mFISH imaging system (MetaSystems, Altlussheim, Germany).

For fluorescence in situ hybridization (FISH) experiments, a permanent lymphoblastoid cell line of the patient was established by Epstein-Barr virus (EBV) transformation according to standard protocols after obtaining informed consent. Single-clone FISH was performed using YAC clones selected from the Whitehead Institute and BAC/PAC clones from the Human '32k' BAC Re-Array set (http://bacpac.chori.org/pHumanMinSet.htm; kindly provided by Pieter de Jong, Children's Hospital Oakland Research Institute) (Table 1). DNA samples were prepared according to standard protocols and were labelled by nick translation with either biotin-16-dUTP or digoxigenin-11-dUTP. Immunocytochemical detection of probes was performed as described elsewhere (Wirth *et al.*, 1999).

Array CGH

A single hybridization was performed on a '32k' BAC array as described previously (Erdogan *et al.*, 2006). Aberrations were only considered if at least three adjacent clones were involved unless they coincided with published DNA copy number variants as listed in the Database of Genomic Variants (http://projects.tcag.ca/variation/). Detailed step-by-step protocols are provided on our website (http://www.molgen.mpg.de/~abt_rop/molecular_cytogenetics/).

Table 1 FISH results for CEPH YAC clones and BAC clones mapping to chromosomes 2 and 4

Probe, clone ID	Sequence information, marker	Position in cM (YACs)	Signal
Breakpoint in 2p24.1			
851g7	D2S2373	44 cM	Distal
969f9	D2S2170	48 cM	Proximal
RP11-79O8	AC013400.8		Distal
RP11-531P14	AC011752.2		Overlap with inserted BAC clone
RP11-368O18	AC096570.1		Overlap with inserted BAC clone
RP11-169L20	AC013459		Proximal
Chromosome 4q			
892f3	D4S1615	127 cM	4, der (4) (proximal)
801b7	D4S1576	135 cM	4, der (2) (inserted into chr. 2p)
771f7	AFM282ze5	147 cM	4, der (2) (inserted into chr. 2p)
871f2	D4S3008	152 cM	4, der (4) (distal)
RP11-143E9	AC021203.5		4, der (4) (proximal)
RP11-285A15	AC105252		4, der (2) (inserted into chr. 2p)
RP11-294H08	No accession number		4, der (2) (inserted into chr. 2p)
RP11-6L6	AC097372		4, der (4), der (2) (overlapping)
RP11-552I10	AC093863.3		4, der (4) (distal)

Results

Karyotyping

Chromosome analysis of the proband revealed an apparently balanced, but non-reciprocal insertional translocation of chromosomal segment 4q28.3q31.22 into chromosome band 2p24 (Fig. 1f). WCP analysis using paints for chromosome 2 and 4 confirmed this finding (Fig. 1a,b), and MCB FISH showed an inverted orientation of the inserted segment (Fig. 1c,d). The insertional translocation was not present in the parents or in the two healthy siblings of the proband. His mother and brother carried a small pericentric inversion of one homologue of chromosome 2 [inv (2) (p11.2q13)], which is a common structural variant without known phenotypic effect (Fickelscher *et al.*, 2007).

The proband's karyotype was determined as 46, XY, inv ins (2;4) (2pter → 2p24::4q28.3 → 4q31.2::2p24 → 2 qter; 4pter → 4q28.3::4q31.2 → 4qter) *de novo*.

Breakpoint analysis by FISH

The insertional breakpoint on chromosome 2p24 was narrowed to a 1.3 Mb interval flanked by BAC clones RP11-531P14 (AC011752) and RP11-360O18 (AC096570). The proximal breakpoint on chromosome 4 (4q28.3) was narrowed to a 2.4 Mb interval flanked by BAC clones RP11-143E9 (AC021203) and RP11-285A15 (AC105252). For the distal breakpoint on chromosome 4 (4q31.22), a breakpoint-spanning clone was identified: RP11-6L6 (AC097372) (Fig. 1e). Thus, the segment of chromosome 4q inserted into 2p24 has a size of approximately 15 Mb.

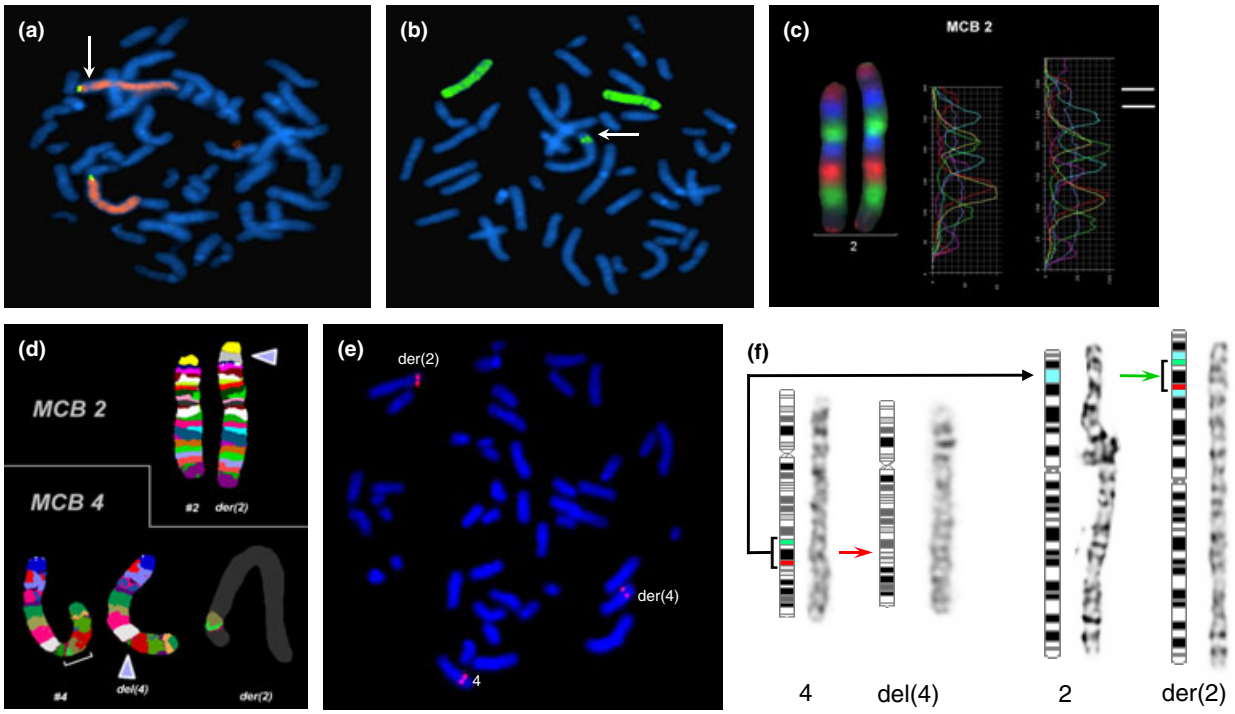

Figure 1 (a) FISH with subtelomere probe 2p (green) and whole chromosome paint 2 (red), indicating the presence of a non-2 fragment (arrow). (b) FISH with whole chromosome paint 4 (green), showing the insertion of a chromosome 4 segment (arrow) in der (2). (c) Multi-colour banding of chromosome 2 (MCB 2): overlay of three colour channels (SpectrumOrange, Diethylaminocoumarine and SpectrumGreen) and the corresponding fluorochrome profiles of normal chromosome 2 (left) and der (2) (right). The unstained gap of der (2) is marked with white bars in the fluorochrome profiles. (d) FISH in pseudocolour depiction after application of the MCB 2 and MCB 4 probe sets. In MCB 2, a small region remained unstained on der (2) (grey colour marker with arrowhead in the upper part of the figure). In MCB 4, the region marked by a bracket in normal chromosome 4 (no. 4) lacks in der (4) (arrowhead); the latter region is inserted in der (2). The insertion of chromosome 4 material appears inverted compared with the original orientation in chromosome 4. (e) Hybridization signals (red) of the breakpoint-spanning BAC RP11-6L6 on normal chromosome 4, derivative chromosome 4 [der (4)] and derivative chromosome 2 [der (2)]. (f) Diagram of normal and aberrant chromosomes 2 and 4. The bands 4q28.1 and 4q31.1 of the translocated segment of chromosome 4 are depicted in green and red respectively. The recipient band 2p24 is shown in turquoise. Red arrow indicates loss of chromosomal material and the area of refusion. Green arrow indicates the area of insertion of chromosomal material.

A list of all clones used for breakpoint mapping is given in Table 1.

Array CGH

Array CGH analysis revealed no copy number changes that were no known polymorphisms.

Discussion

Intercalation of a part of one chromosome into another (interchromosomal insertional translocation, IT) is a very rare event (estimated incidence 1: 80 000 live births) (Van Hemel & Eussen, 2000). The patient reported here presented azoospermia and hypergonadotropic hypogonadism and had a *de novo* balanced IT of a 15 Mb fragment from 4q into 2p24. To our knowledge, this is the first report of a *de novo* IT associated with primary hypogonadism. In general, balanced chromosome aberrations are relatively frequent findings in patients with oligozoospermia/azoospermia and normal sex hormones (Mau-Holzmann, 2005), but they are very rare in patients with primary hypogonadism (Hughes *et al.*, 1993; Zahed *et al.*, 2004). Assuming a possible causative association between the chromosome aberration and the clinical features, we analysed the chromosome breakpoints in detail.

On chromosome 4, the BAC clone which spans the distal breakpoint (RP11-6L6) contains two genes: *LSM6* and *SLC10A7*. The proximal breakpoint region on chromosome 4 (defined by BACs RP11-143E9 and RP11-285A15) contains the gene *PCDH10*, which encodes for a protocadherin that is expressed – among many other tissues – also in the testis (Nagase *et al.*, 2000). The breakpoint region on chromosome 2p24 (the point of insertion of the 4q fragment) harbours no known genes that could have been directly disrupted. No disease associations have been reported for any of the three possibly disrupted genes *LSM6*, *SLC10A7* and *PCDH10* to date. Assumptions about a causative role for the patient's phenotype of these or other genes in the vicinity of the breakpoints must remain speculative unless supported by additional data in future, e.g. information on the function of the gene products or the identification of chromosome aberrations involving these regions in other patients with similar clinical problems.

In summary, the chromosome aberration of this patient suggests the localization of a gene for primary hypogonadism in either 2p24 or 4q28-31. This case illustrates the importance of chromosome analyses in patients with hypergonadotropic hypogonadism without a known aetiology. Apart from confirming or excluding the diagnosis of Klinefelter's syndrome, such analyses can identify patients with rare chromosome aberrations that can pave the way for the elucidation of novel disease genes. Detection of balanced aberrations is particularly important for patients who plan to use artificial reproductive technologies such as in vitro fertilization or intracytoplasmic sperm injection because they may have lower fertilization rates and elevated risks for abortions or children with unbalanced chromosome aberrations.

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