Localization of BRRN1, the Human Homologue of Drosophila barr, to 2q11.2

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During mitotic cell division genetic information is accurately transferred by the equal partitioning of a complete set of chromosomes from the mother to each of the two daughter cells. DNA replication is the initial step of cell division and is followed by cell progression to mitosis. Sister kinetochores attach each chromatid to spindle microtubules, bringing about chromosome alignment at the metaphase plate (8). Sister chromatids remain bound until all chromosomes are aligned and their cohesion is synchronously relieved at the anaphase transition (12).

Two mechanisms have been proposed to account for sister chromatid cohesion: (i) the existence of adhesion proteins that must be degraded at anaphase (6, 13) and (ii) the presence of DNA catenation sites subsequent to replication that are resolved by topoisomerase II (7).

Several genes have been implicated in the process of chromatid separation: pimples (15), three rows (3), aar (11), lodestar (5), and fizzi (4), but their human homologues have not been identified. The onset of anaphase also requires the anaphase-promoting complex, necessary for the ubiquitin-dependent degradation of B-type cyclins in budding yeast (9), Xenopus laevis (10), and human cells (16).

Recently Bhat et al. (2) have established in Drosophila melanogaster that the product of barr, barren, is necessary for sister chromatid separation and modulates topoisomerase II activity. Barr is an evolutionarily conserved gene, and sequence similarity searches identified a 2.7-kb human homologue of an uncharacterized function (HSORF007) (14). Here we report the genomic localization of this human member of the barr gene family: BRRN1.

We identified four EST clones encoding partial transcripts of BRRN1: 244767, 125761, 193222, and 112174. To generate a full cDNA clone of BRRN1, we used a unique Stu restriction site at bp 1363 to ligate overlapping clones 112174 (bp 1–1407) and 193222 (bp 1220–2721) in vector pT7T3(D)-Pac. The chromosomal localization of BRRN1 was established by Southern analysis on monochromosomal human–rodent hybrid cells. High-stringency hybridization with the full length cDNA probe confirmed that BRRN1 maps to chromosome 2 (Fig. 1A) (14).

To identify BRRN1-containing YAC clones, we screened the CEPH-Mark II YAC library by PCR. We used two oligonucleotide primer pairs designed to yield PCR products corresponding to positions 448–625 (primer set I) and 2229–2402 (primer set II) in the BRRN1 cDNA. Both sets of primers yielded unique PCR amplification products on human genomic DNA template. Seven of the PCR-positive clones also exhibited BRRN1 hybridization on Southern analysis. Clone 539D8 encompassed all restriction fragments identified by the genomic Southern blots (Fig. 1A). Alu-PCR products of YAC 539D8 were prepared as previously described (1) and used as probes for FISH, yielding a discrete hybridization signal at 2q11.2 (Fig. 1B).

To narrow the genomic position of BRRN1, primer set II was used to PCR-screen the radiation hybrid panel RH3 (Research Genetics). The resulting positive-signal matrix established that BRRN1 is linked to loci D2S113 and D2S2311 with lod scores of 11.1636 and 6.37, respectively. The map order in this region is SHGC 14343–D2S113–BRRN1–D2S2187–D2S2311 as defined by radiation hybrid panel positive-signal matrices. D2S113 is contained in YACs 847A1, 707E9, and 918H7, marking the most centromeric boundary of contig WC2.8. We were unable to detect BRRN1-specific PCR amplification products from these YACs, nor did we find D2S113 PCR products in BRRN1-containing YAC 539D8.

These results unequivocally demonstrate that BRRN1-containing YAC clone 539D8 maps to 2q11.2, consistent with the results from Southern hybridization on chromosome 2 monochromosomal hybrid DNA. Furthermore, these data clarify the position of D2S113 and anchor the centromere-proximal end of WC2.8 at 2q11.2. The map position assigned to BRRN1 as the result of this study does not correspond to any apparent rearrangement of amplification associated with human leukemia or other neoplastic disease.

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FIG. 1. (A) Southern blot hybridization of BRRN1 cDNA to genomic DNA isolated from human chromosome 2-containing human–rodent monochromosomal hybrids, human peripheral blood lymphocytes, and YAC clone 539D8. (B) FISH of BRRN1-containing YAC clone 539D8. (C) BRRN1 maps to chromosome 2q11.2.

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Localization of the Human HIP1 Gene Close to the Elastin (ELN) Locus on 7q11.23

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Williams-Beuren syndrome (WBS) is a genetic disorder with a variable phenotype including mental retardation, infantile hypercalcaemia, dysmorphic features, and various types of congenital heart defects, notably supravalvular aortic stenosis (SVAS) (10). WBS is generally sporadic with an incidence of 1 in 20,000–50,000 live births (5). Nevertheless, a few familial cases have been described with apparent autosomal dominant inheritance (2). The disorder is in most cases associated with a deletion of contiguous genes at 7q11.23. In particular it has been shown that in 94% of WBS individuals, the elastin gene (ELN), which was previously mapped to 7q11.2 (11), is deleted. ELN disruption is likely to be responsible for the SVAS phenotype (1). Recently, Frangiskakis and colleagues (3) have demonstrated that deletions of the LIM kinase 1 (LIMK1) gene, which also maps to the WBS critical region, are associated with impaired visuospatial constructive cognition. However, the full phenotype, including characteristic facial features, hypercalcaemia, renal anomalies, distinct personality traits, mental retardation, and developmental delay, cannot be easily explained by the deletion of these two genes (9). Therefore, several groups started positional cloning approaches to isolate new candidate genes in the region surrounding ELN and LIMK1. Other genes mapped recently into the deletion include the gene for replication factor C subunit 2 (RFC2) (13) and FZD3, a human homolog of the Drosophila tissue polarity gene frizzled, which functions as a transmembrane receptor for the wnt protein (17).

We have evaluated HIP1 as a candidate gene for the neurobehavioral features of WBS. The HIP1 gene encodes a huntingtin interacting protein, which was isolated by two independent groups using the yeast two-hybrid system (8, 18). The gene is ubiquitously expressed in human tissues at low levels. Using an affinity-purified polyclonal antibody raised against recombinant HIP1, a 116-kDa protein was detected in brain and peripheral tissues by Western blot analysis (18). In contrast, Kalchman et al. (8) showed that the protein was exclusively present in the central nervous system. However, in both studies it was conclusively shown that HIP1 and huntingtin interact with each other in vivo and in vitro and that they were both present in the membrane-containing fractions isolated from human brain cells. There is evidence that in individuals with Huntington disease the normal interaction between HIP1 and huntingtin is disrupted by an elongated polyglutamine expansion in the huntingtin protein (8). However, additional experiments will be necessary to prove this assumption.

Kalchman et al. (8) mapped HIP1 to human chromosome band 7q11.2 by fluorescence in situ hybridization of HIP1 probes to metaphase chromosomes. We decided to map the gene more precisely by using the GeneBridge 4 radiation hybrid (RH) panel (Research Genetics, Huntsville, AL). PCR was performed with primers 5’-GCATCCTCTTGAATAGGAAGATCG-3’ and 5’-CCATCTAGAAGGAAAAGTGCTG-3’, designed from the 3’-untranslated region of the HIP1 cDNA sequence (Accession No. U79734), that amplify a 465-bp fragment. Linkage analysis was carried out using the program provided by the server http://www-genome.wi.mit.edu/cgi-bin/contig/rmapper.pl, to interpret the results from the 93 (radiation hybrid) cell lines. The statistical evaluation showed that HIP1 maps 6.40 cR distal to the marker D7S489 (LOD > 3) (Fig. 1). Two groups have reported that D7S489 detects at least three microsatellite loci that are spread over a distance of at least 1 cm (14, 16). Only one locus, however, called either D7S489B (14) or D7S489U (16), falls within the common deletion. To confirm the localization of HIP1 in the WBS chromosome region, we also mapped ELN in the RH