SHORT COMMUNICATION

A YAC Contig in Xq22.3–q23, from *DXS287* to *DXS8088*, Spanning the Brain-Specific Genes *doublecortin* (*DCX*) and *PAK3*

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Although several genes for mental retardation and epilepsy, including double cortex/X-linked lissencephaly (*DC/XLIS*), have been localized to Xq21.3–q23, there has been no complete physical map of this region available. We constructed a YAC/STS contig map by initiating two yeast artificial chromosome (YAC) walks from the markers that flanked the *DC/XLIS* candidate gene region. We report an approximately 4-Mb contig extending from *DXS287* to *DXS8088*, encompassing *DXS1072* and *DXS1059*, and composed of 52 YACs identified with 15 previously published STSs and 19 novel YAC-end STSs. This contig also contains two brain-specific genes, *doublecortin* (HGMW-approved symbol *DCX*), responsible for *DC/XLIS*, and *PAK3*, which may be responsible for neurological diseases localized to this region. The new contig extends and incorporates several previously published contigs, providing a total overlapping contig extending approximately 34 Mb from *DXS441* in Xq13.1 to *DXS8088* in Xq23.

The Xq21–q24 region is interesting because several neurological disorders map to this region, including double cortex/X-linked lissencephaly (DC/XLIS) (6, 26), X-linked mental retardation limited to females (EFMR) (28), X-linked nonprogressive congenital cerebellar hypoplasia (15), and several nonspecific mental retardation disorders: MRX23 (13), MRX30 (9), MRX35 (14), and MRX47 (7). It is not known whether any of these disorders are allelic. Further mapping information across this region would therefore facilitate the identification of the genes responsible for these disorders.

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One of these disorders, DC/XLIS, also known as subcortical band heterotopia, shows an abnormal band of neurons in the white matter underlying an apparently normal cortex in affected females. Affected males show more severe lissencephaly with an abnormally thick cortex with decreased or absent surface convolutions (25). Affected double cortex patients have varying degrees of epilepsy and mental retardation, while lissencephaly patients have profound seizures and mental retardation (2, 8). Inheritance of double cortex in daughters and lissencephaly in sons of double cortex females suggested X-linked dominant inheritance (23). Linkage was previously established to Xq21.3–q24 within an approximately 12-cM candidate gene region defined by recombination events with the centromeric marker *DXS8020* and the telomeric marker *DXS1072* (6, 26). This region was further refined by analysis of a girl with classical lissencephaly and a *de novo*, apparently balanced X;2 (q22.3;p21) translocation within the candidate region (8). These results suggested that a single gene defect on the X chromosome was responsible for the abnormal neuronal migration, with the difference in phenotype between affected males and females presumably due to X-inactivation of the normal or mutant gene in the individual cells of carrier females.

As part of our effort to identify the DC/XLIS gene (12), we developed a yeast artificial chromosome (YAC) contig by conducting two YAC walks from the centromeric (*DXS1105* and *COL4A5)* and telomeric (*DXS1072)* markers flanking the Xq22.3–q23 candidate gene region (26). To begin building a contig, we first identified YACs at the limits of our candidate gene region by using *DXS1105* and *DXS1072* to query the Whitehead Institute/MIT Center for Genome Research (WI/MIT) and CEPH-Généthon (CEPH) databases. The general procedure for YAC walking was rescue and sequencing of YAC ends (17, 22) and generation of new STSs based on the YAC-end sequence (27). STSs generated from YAC ends (Table 1) as well as published STSs were used to identify subsequent YACs by

TABLE 1

Newly Generated YAC-End STSs

STS name	Forward primer	Reverse primer	Size (bp)
749F7-R	CAAATTCAGGCCATCTGGAT	CCCAAGTCTGTGCAGAGAAG	150
954D9-L	CGCCCATCAATTGTTGATTA	AGTTCCATCCATTTTGCTGC	109
148D1-R	CGGGCACACTTTATTCGG	CACTGCCACATCTGTCTCTCT	124
148D1-l	CCAGGGCAGTCAGGTAAGAG	TGCACACAACTTCAACTTTGC	175
7BC3-R	GTGTCCATCATCCAGAGT	CATTTTATAGGCCCTATG	100
749F7-L	CATCCCTGTGGGATTATTTAGC	TGGTTTAAGGCGCAAGACTT	159
17GH1-L	TCGCTTGAACCCAGGAAG	TGCTAGGGAAGCAGTGTGG	247
25DD1-L	GACTGGCCCTATGAAACAGC	ACAGCCAAAGCAAGGTTGAG	92
7BC3-L	AATGACCTCTGTGGTGAGGC	GCCATTGATGCTGTTTCCTT	162
15BB10-L	GGAGAAATGGGCCAACAGTA	ATTTGTCACTGCCACTGATCC	168
956C7-R	TAGCCCTGGGAACTCTCTCA	TTGACTTAGGAGCCCCCC	176
142A5-L	CTAAGGAAAAGAAGATTAAGTTCCTAT	GTGTATTGTAATGCTCTGTTATTAGG	174
956C7-L	TTGGCTTTGAGAAAGGCTGT	TTCACATGCTTATTTGCATTCC	208
963G9-L	CAGGGATGTCGGTGATTCTT	AGCAGCTGAGACCCACATTT	184
4Y9E4-L	TCGGGCAAATAAAGGGAAG	TTGTATAATGGGGCATGATGG	156
954D10-L	CCCGAAAAACCCCTTACAAG	GGTTAGGCGTGTGGGATAGA	272
142A5-R	TGGCTGGGCCATATAAGAAC	TGAAGATGGGACCCTCAAAG	197
4Y9E4-R	GGTCCAGCAACACCAAATG	GCAAATTCCTTTCTGTCCCA	201
15AC11-R	CACTACTGATCTCCCCTATGGC	GACAATAAAGCCCAACATCCA	165

Note. STSs are identified by the YAC address and an L or R, indicating the left or right YAC arm, respectively.

PCR-based screening of the CEPH-mega-YAC libraries (Research Genetics, Huntsville, AL) and the ICI and ICRF YAC libraries (HGMP, Cambridgeshire, UK). All isolated YAC clones were tested for all STSs in the region, and the STSs were ordered so as to minimize the number of YACs with a noncontiguous complement of STSs.

At the time we initiated our mapping, there was limited information available about the Xq21.3–q24 region. Several contigs were seeded in this region, and The Report of the Sixth International Workshop on X Chromosome Mapping presented the tentative alignment from centromere to telomere of six contigs, as well as five smaller unordered contigs (3, 32). The centromeric YAC walk was initiated with *DXS287* based on its tentative location distal to *DXS1105*. *DXS287* identified six YACs (Fig. 1A) including 749F7, 737H4, 810B1, 896D2, 860F12, and 744A12, which were part of a large (15 YAC) published contig, WCX.29 (WC-51), from the WI/MIT database. The most distal of the 16 STSs in WCX.29, *340za9*, was used for further walking. The orientation of subsequent walking steps was determined by analyzing the content of each new STS in the WCX.29 contig. The X chromosome-specific YAC end that was not present in WCX.29 was used for further screening of the YAC libraries.

DXS1072 was used to initiate the telomeric YAC walk toward *DXS287*. *DXS1072* identified YACs 956C7 and 963G9, which were part of the WI/MIT contig WCX.27, containing only two markers and two YACs. Unlike WCX.29, WCX.27 could not be used to determine orientation since the contig was unanchored. Thus the YAC walk proceeded bidirectionally using the ends of YAC 956C7.

After multiple rounds of YAC-end screening, the two contigs converged, thus closing the gap and completing the contig between *DXS287* and *DXS1072*, with at least twofold coverage. Furthermore, two markers, *DXS1059* and *DXS8110*, which were part of the WI/MIT contig WC-769, mapped within the presented contig centromeric to *DXS1072* (Fig. 1A). This corrects a discordance in the previous maps, which presented *DXS1059* telomeric to *DXS1072* (3, 32). We also incorporated several additional markers into the map and extended the contig beyond *DXS1072* and *DXS8088*.

The current YAC contig based on STS content is shown in Fig. 1A. More than 4 Mb of cloned coverage is estimated based on the size of several of the contained YACs. The contig consists of 52 overlapping YAC clones, identified with 15 previously published markers and 19 newly developed YAC-end STSs. This contig contains several YACs (810B1, 744A12, 148E8, 896D2, 141G2, 860F12, 749F7, and 737H4) that extend from the previously published contig in Xq22.3 at *DXS287* (16), and it incorporates three previously unconnected WI/MIT contigs, WCX.27, WCX.29 (WC-51), and WC-769. Thus, the presented YAC contig combined with the other published contigs in Xq (4, 10, 21, 29, 33) provides complete coverage from *DXS441* in Xq13.3 to *DXS8088* in Xq23 with a total size in excess of 34 Mb (Fig. 1B). YACs from the telomeric end of the contig do not yet overlap with the published contigs in Xq25 (18, 24). Thus, the region between Xq23 and Xq25 remains to be completely contiged.

In the process of constructing this map, several YACs (749F7, 148D1, and 17GH1) were identified, by PCR and FISH analysis, that span the X;2 translocation in the girl with classical lissencephaly (12; and data not shown). Several other YACs (25DD1, 49F7, 15BB10, 32GA1, 10HE10, 18AE3, 18DE3, 110B12, 954D9, 737H4, and 810B1) may span the X;2 translo-

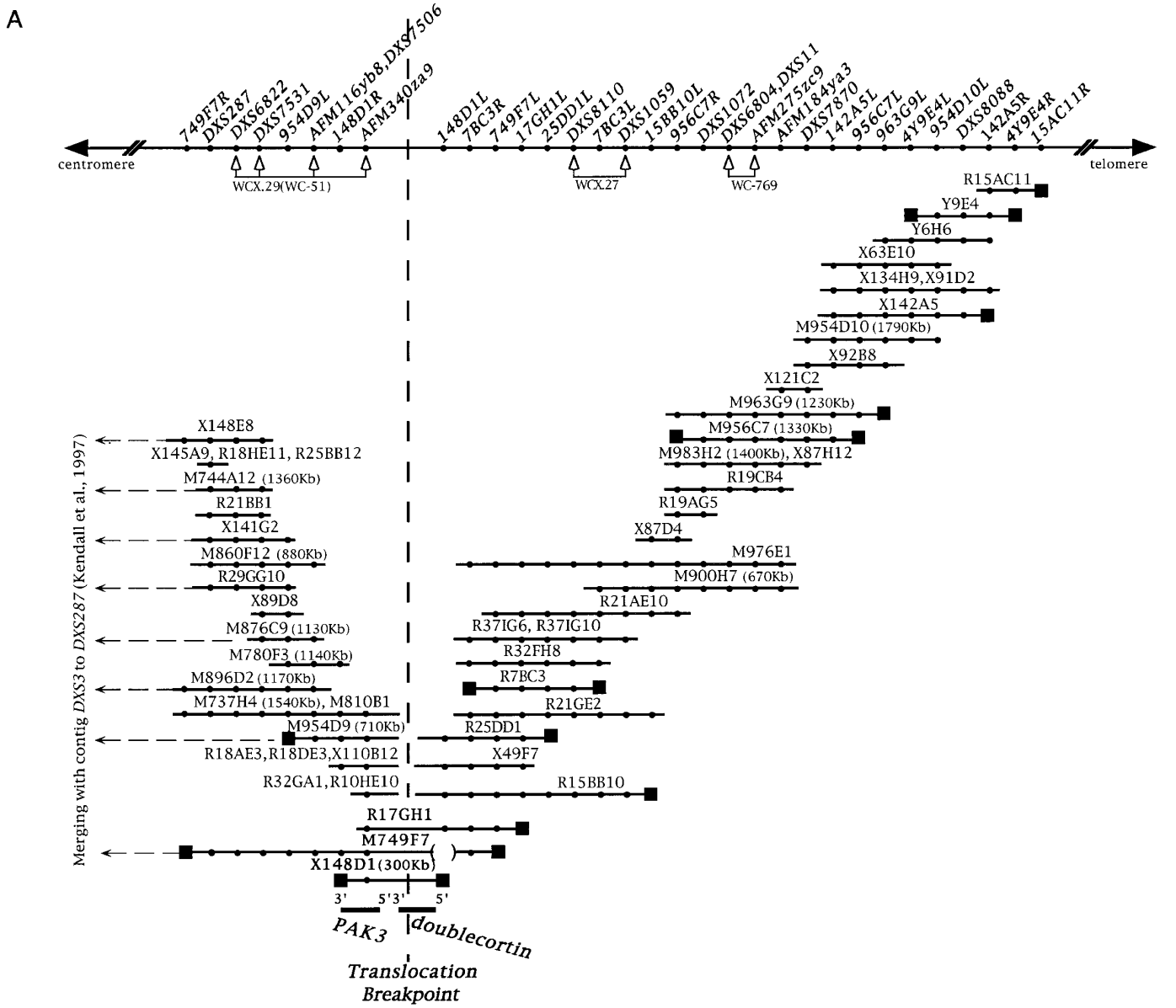

FIG. 1. (A) Overlapping YAC contig and STS map representing the segment of human X chromosome from the published markers *DXS287* to *DXS8088*. The upper line indicates the X chromosome with the centromere to the left and the telomere to the right. YACs were tested for the presence or absence of 34 STSs, indicated along the top. YACs are depicted as horizontal bars, whose length represents their STS content rather than their physical length. The published size of individual YACs is indicated in parentheses next to the YAC name (WI/MIT). STSs that mapped to the same YACs and whose order could not be resolved are listed together. STS content is indicated by a filled square, indicating the origin of an STS from a YAC insert end, or by a filled circle, indicating the presence of the STS. Parentheses indicate that the STS gave no PCR product in the YAC. The letter preceding the YAC address indicates the library source: M, CEPH; R, ICI; and X or Y, ICRF. YACs with the same STS content are shown on the same YAC bar. Below the chromosome are arrows indicating those markers present in contigs reported by the WI/MIT database. Centromeric arrows indicate that these YACs connect with a centromeric contig (16). The X;2 translocation breakpoint identified in the female patient with classical lissencephaly is indicated by a vertical dotted line. The position of the two genes, *doublecortin* and *PAK3*, is indicated. The location of several anonymous ESTs is also indicated. (B) Merged contigs across Xq13–q23. Contigs are aligned and anchored by overlapping STSs at the ends. Across the top is a schematic representation of the chromosome bands. Each horizontal line represents a contig between the STSs indicated, with the approximate contig size and reference shown.

cation, but were not confirmed by PCR or FISH analysis. The spanning YAC 148D1 was used to characterize more precisely the translocation and to identify two genes, *doublecortin*[3] and *PAK3*.

Doublecortin is a novel brain-specific gene (GenBank Accession No. AF034634) that is disrupted by the X;2 translocation and is mutated in patients with double cortex and X-linked lissencephaly (12). *Doublecortin* was also identified by mapping and characterization of an anonymous EST (SGC 34529) to a partial YAC contig of this region (5). Doublecortin contains potential MAP kinase family phosphorylation sites and is

[3] The HGMW-approved symbol for the gene described in this paper is *DCX*.

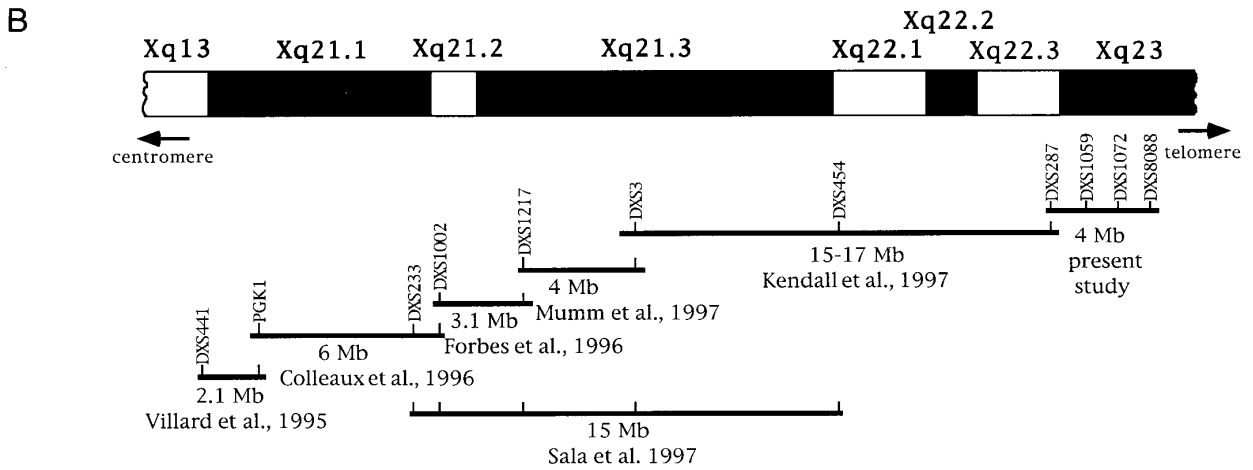

FIG. 1—*Continued*

likely phosphorylated by Abl, suggesting that doublecortin functions as an intracellular signaling molecule critical for the migration of developing neurons.

Analysis of YAC 148D1 also identified a second gene, *PAK3*, adjacent to *doublecortin*. *PAK3* is homologous to the rat and mouse p21-activated kinase (*PAK*) genes, *β-PAK* and *mPAK-3*, respectively (1, 20). Like its rodent homologs, *PAK3* is highly expressed only in fetal brain (J. G. Gleeson and K. M. Allen, unpublished results). The PAK genes encode serine threonine kinases that act as signal transduction molecules that influence cell shape and motility as well as actin organization (19, 31). While this gene has been characterized biochemically, its role in brain development remains unclear.

Several anonymous ESTs have also been mapped within the presented contig. EST417033 was identified within an intron of *doublecortin* (12). Additional ESTs, including WI/MIT 8307, WI/MIT, 16868, SGC 34377, SGC 32977, WI/MIT 14094, SGC 16527, and U 00944, which were localized to Xq22–q24 on radiation hybrid panels (30), were fine-mapped by PCR amplification and hybridization to a YAC contig within this region (5). Other ESTs that have been mapped to Xq22–q23 have not been mapped in our contig (11, 30).

Currently, the list of potential susceptibility loci that may fall in or near the region contiged includes several neurological disorders, including mental retardation, epilepsy, and cerebellar hypoplasia. As both *doublecortin* and *PAK3* are brain-specific and map to Xq22.3–q23, we are currently examining the known diseases with CNS phenotypes that link to this region for mutations in these genes. The identification of other candidate genes for disorders that map to this region will be facilitated by the YAC contig and STS map presented here.

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