© 1993 Oxford University Press Human Molecular Genetics, Vol. 2, No. 2 183–189

FISH ordering of reference markers and of the gene for the α5 subunit of the γ-aminobutyric acid receptor (GABRA5) within the Angelman and Prader–Willi syndrome chromosomal regions

Joan H.M.Knoll[1,2,3], Daniel Sinnett[1,3], Joseph Wagstaff[1,3], Karen Glatt[1], Andrea Schantz Wilcox[4], Paul M.Whiting[5], Peter Wingrove[5], James M.Sikela[4] and Marc Lalande[1,3,6]*

[1]Genetics Division, Children's Hospital, [2]Department of Pathology, Beth Israel Hospital, [3]Harvard Medical School, Boston, MA, [4]Department of Pharmacology, University of Colorado Health Sciences Center, Denver, CO, USA, [5]Merck Sharp and Dohme Research Laboratories, Harlow, Essex, UK and [6]Howard Hughes Medical Institute, Boston, MA, USA

Received August 19, 1992; Revised and Accepted November 25, 1992

ABSTRACT

We have established a probe order within the Angelman/Prader–Willi chromosomal regions by multicolor fluorescence *in situ* hybridization (FISH). The probe [locus] order extending distally from the centromere is 34[D15S9]–IR4-3R[D15S11]–189-1[D15S13]–PW71 [D15S63]–3-21[D15S10]–28β3-H3[GABRB3]–IR10-1 [D15S12]. This order agrees with that recently reported (1) with the exception of PW71 [D15S63]. In addition, a second γ-aminobutyric acid ($GABA_A$) receptor, the α5 subunit, has been localized within the reference map to between GABRB3 and D15S12. The locus order was further confirmed by DNA hybridization analysis of two patients, one with Angelman syndrome and one with Prader–Willi syndrome, with different unbalanced translocations and molecular extents of deletion. Our results provide a framework map of chromosome 15q11–q13 into which additional markers can be oriented and allow a further differentiation of the critical genetic regions of the two syndromes.

INTRODUCTION

The Angelman (AS) and Prader–Willi syndromes (PWS) are distinct genetic disorders in which the clinical phenotypes can be caused by a variety of chromosome 15q11–q13 abnormalities (2–5). Deletions of this region are of paternal origin in PWS (6) and of maternal origin in AS (7). Chromosome 15q11–q13 uniparental disomy of maternal origin is associated with PWS (8,9) while that of paternal origin results in AS (10). Other as yet undetermined genetic alterations are also observed in association with AS (11,12). A number of cloned markers—IR39d, IR4-3R, 3-21, 189-1, 34, IR10-1—have been mapped to the deletion region of PWS and AS (7, 13–15) but it has been difficult to establish the order of these markers. Deletion mapping has provided a very limited order of markers within 15q11–q13 as most AS and PWS patients have large interstitial cytogenetic deletions that differ only by the presence or absence of centromere proximal marker IR39d (D15S18) (16). Establishment of the probe order is crucial to the molecular genetic analysis of the PWS and AS chromosomal region and to defining the critical regions in the two disorders.

Fluorescence in situ hybridization (FISH) procedures that directly couple molecular and cytogenetic information have recently been utilized to establish probe order in chromosome Xq28 (17), within the Duchenne muscular dystrophy locus (18) and for the Y chromosome short arm (19). A series of YAC clones have been isolated from the PWS/AS region and used in FISH analysis of interphase nuclei from normal as well as AS and PWS deletion individuals (1). This has allowed an order to be established for the standard reference markers from the PWS/AS region. We have employed large insert lambda phage clones and multicolor FISH to confirm the order of most of the same reference markers and to assign a second $GABA_A$ receptor subunit gene (GABRA5) to the region.

RESULTS

Assignment of GABRA5 to the PWS/AS chromosomal region

The α53-B probe , a subclone of the 3′ end of GABRA5 (see materials and methods), was hybridized to the DNA from normal controls as well as PWS and AS deletion individuals (figure 1A). By quantitative hybridization relative to a chromosome 13 control probe, the AS and PWS deletion samples are hemizygous for a deletion of α53-B (figure 1A, lanes 4 and 5). The results of Figure 1A indicate that GABRA5 maps to the PWS/AS region of chromosome 15q11–q13. This localization to 15q11–q13 was confirmed by FISH in the probe ordering experiments (Table 1) and by FISH mapping to metaphase chromosomes (Table 2).

DNA analyses of translocation AS and PWS patients: partial ordering of probes

DNA analyses of PWS patient HS32.5 [45,XY,–9,15,+der(9),t(9;15)(q34.3;q11.2)] and AS patient WJK106 [45,XY,–13,15,+der(13),t(13;15) (p13;q13)mat; patient 2, ref 20] has provided grouping of markers within chromosome 15q11–q13 (21). HS32.5 is deleted for IR39d, 34 (figure 1B), IR4-3R, 189-1

* To whom correspondence should be addressed at: Division of Genetics, Children's Hospital, 300 Longwood Avenue, Boston, MA 02115, USA

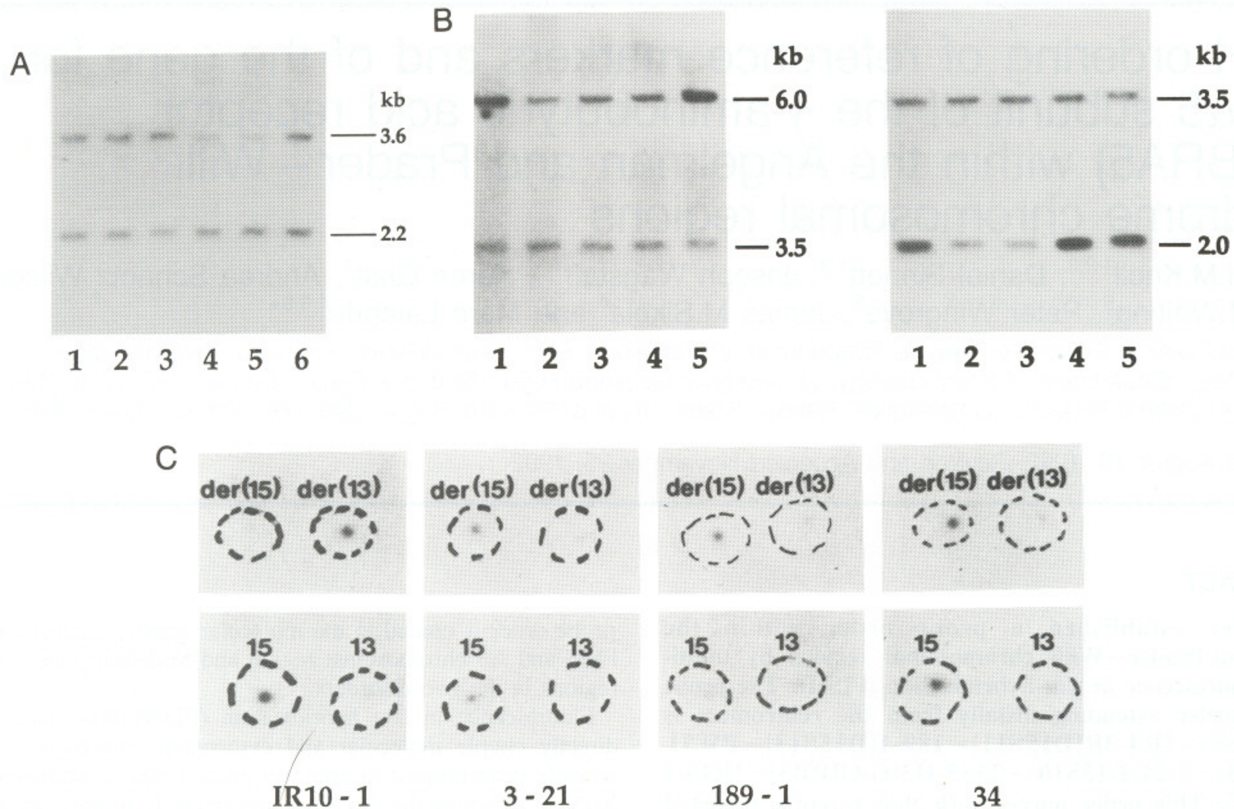

Figure 1. A) Quantitative Southern hybridization of the GABRA5 gene to DNAs from AS and PWS patients. Nylon filters were hybridized with probe α53-B and chromosome 13 specific probe, H2-42 (D13S25). α53-B and H2-42 recognize a 3.6 and 2.2 kb DNA fragment respectively on Hind III digested DNA. α53-B is deleted in AS and PWS patients with the usual interstitial cytogenetic deletions of proximal 15q (lanes 4 and 5; WJK107 and WJK122) but not in an unbalanced translocation AS patient [lane 3; WJK106−46,XY,−13,−15,+t(13;15)(p13;q13)] or in normal individuals (lanes 1, 2, 6). **B)** Quantitative DNA analyses of AS and PWS patients with chromosome 15q11−q13 specific markers 34 (left) and 3-21 (right). 34 and 3-21 recognize 6.0 and 2.0kb DNA fragments respectively on HindIII digested DNA. H2-26, a chromosome 13 specific probe, serves as a control two copy per diploid genome probe and recognizes a 3.5kb DNA fragment. Lane 1, DSB67, normal parent. Lane 2, HS24, interstitial deletion PWS patient. Lane 3, WJK24, interstitial deletion AS patient. Lane 4, HS32.5, translocation PWS patient. Lanes 5 and 6, DSB72 and HS30, nondeletion PWS patients. **C)** Hybridization of 15q11q13 specific DNA markers to flow sorted chromosomes of WJK105 (46,XX,−13,−15,+der(13),+der(15),t(13;15)(p13;q13). This translocation divided chromosome 15 at q13 with the pter to q13 region on the derivative 15 chromosome (der 15) and the q13 to qter region on the derivative 13 chromosome (der 13). IR10-1 is localized to der(13) and is therefore distal to the translocation breakpoint whereas the other sequences: 3-21, 189-1 and 34 are proximal to the breakpoint. These hybridized to the der(15). In addition, 189-1 is absent on the 'normal' chromosome 15.

and PW71 but intact for 3-21 (figure 1B), 28β3-H3, α53-B and IR10-1. Heterozygosity with a contribution from each parent was evident for IR10-1 on Sca I digested DNA (17.5 kb/16 kb). A single allele was observed for the other markers with only a maternal 14 kb allele evident with IR39d on Sac I digested DNA.

The deletion is larger in WJK106 (21) than in HS32.5 with markers 3-21 and 28β3-H3 also deleted. Markers α53-B (figure 1A, lane 3) and IR10-1 are also intact in WJK106. The 3-21 and 28β3-H3 markers are, therefore, distal to IR39d, 34, IR4-3R and 189-1 but proximal to α53-B and IR10-1. Hybridization of flow sorted chromosomes 13 and 15 and the derivative chromosomes from the mother of WJK106 confirmed that IR10-1 is distal to 3-21, 189-1 and 34 (figure 1C). Surprisingly, the 'normal' nontranslocated chromosome 15 in WJK105 was deleted for 189-1 (figure 1C), a finding confirmed by quantitative DNA hybridization analysis.

Ordering of Probes by FISH

For interphase FISH ordering, two- and three-color, three-probe hybridizations were performed. Three chromosome 15q11−q13 specific probes were hybridized simultaneously and detected by red and green fluorescence or by red, green and yellow fluorescence. The detection and labelling systems were often varied for the triple hybridizations so that different color sequences were observed figure 2A−2C. Results of the three way ordering experiments are presented in Table 1. From the combined results the following probe (locus) order is deduced: 34-10(D15S9)−JP2(D15S11)−189-41(D15S13)−JP3 (D15S63) −3-21-12(D15S10)−16β3(GABRB3)−α5-39(GABRA5)−DN10(D15S12). Three-color fluorescence (figure 2C) was very useful in orienting JP2 (D15S11) relative to 34-10 (D15S9) (experiment 6, Table 1). Two-color fluorescence yielded ambiguous results in about one quarter of nuclei (experiments 4 and 5, Table 1). In this case, marker 189-41 was distal to JP2 in most nuclei but about one third of nuclei appeared to have 189-41 between JP2 and 34-10 (experiment 7, Table 1). This ambiguity may be a reflection of the probe itself, the proximity of markers for loci D15S9, D15S11 and D15S13, replication differences and/or chromatin instability/conformation.

DNA probes greater than 1000 kb apart can be resolved on normal metaphase chromosomes. Examples of two-color/two-probe hybridizations in which probes can or cannot be resolved

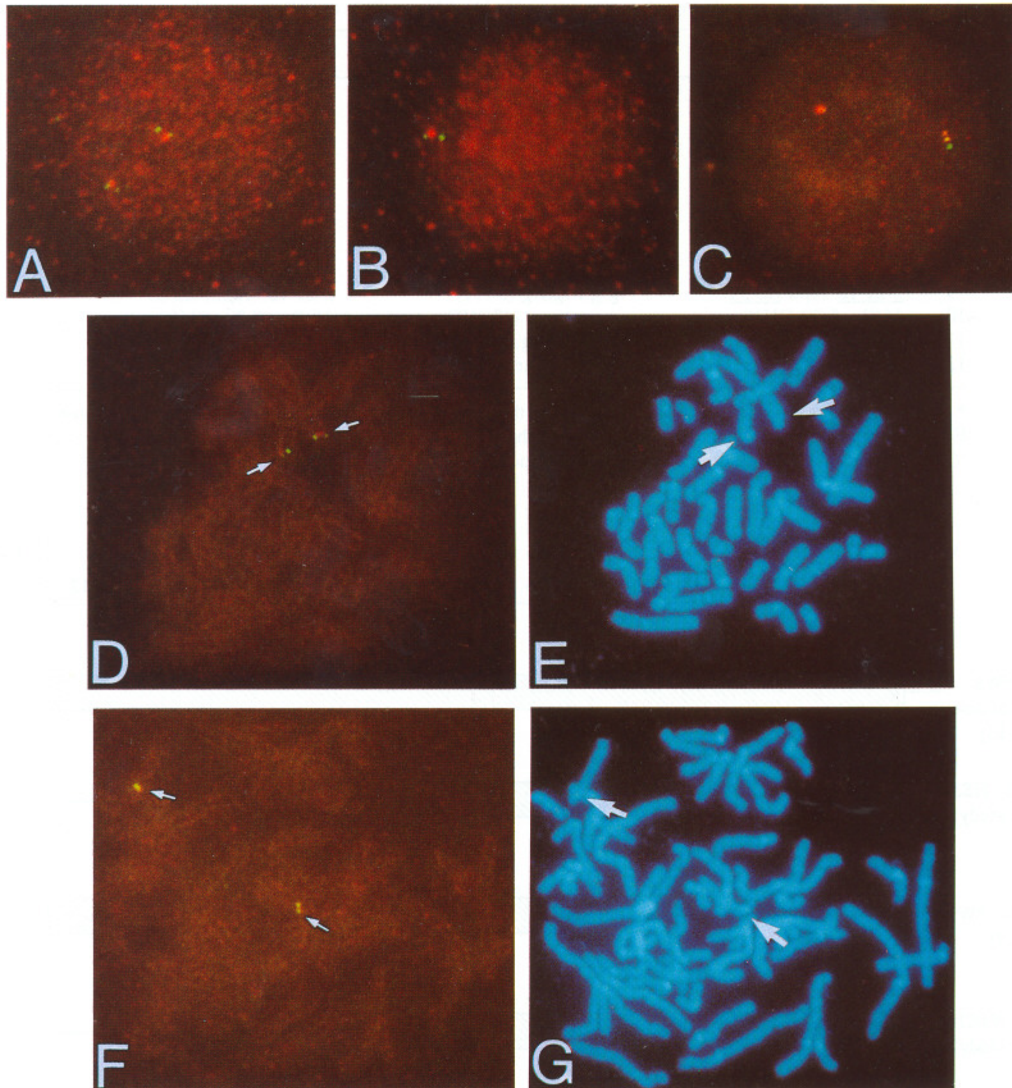

Figure 2. Two and three color fluorescence in situ hybridization with chromosome 15q11−q13 specific probes on interphase (A,B,C) and metaphase cells (D,E,F,G). Green/red/green sequence ordering on interphase cells from a normal individual and a deletion-bearing AS patient (A, B, respectively, experiment 5, Table 1). Red/yellow/green or 'traffic light ordering' of three sequences in an AS deletion patient (C, experiment 6, Table 1). The sequence order is immediately apparent with three colors. Panel D, metaphase ordering of 34-10 (green) and 3-21-12 (red). The green hybridization signals (from both chromatids) are proximal to the red signals indicating that the order is centromere, 34-10, 3-21-12. Panel F, sequences 3-21-12 and 16β3 are too close to be ordered on metaphase chromosomes and appear as a single yellow signal . Arrows in panels E and G indicate chromosome 15 centromeres in DAPI images which correspond to hybridizations in D and F. Nuclei and chromosomes are delineated by incomplete suppression of repetitive DNA. Panels D and F are overexposed to permit the outline of the DAPI-stained chromosomes to show through the double band pass filter set. Red, green and yellow fluorescence are viewed through a double band-pass filter without filter change.

on metaphase chromosomes are shown in figures 2D and 2F, respectively. Overlapping signals from hybridization of probes detected by rhodamine and fluorescein result in a yellow fluorescence signal. Probes 34-10, JP2 and 189-41 could be resolved from 3-21-12, 16β3, α5-39 and DN10 but could not be resolved from each other. Similarly, 3-21-12, 16β3 and α5-39 could not be resolved from one another on metaphase chromosomes.

FISH to metaphase chromosomes of PWS and AS cell lines bearing structural aberrations of chromosome 15q11−q13 was performed using the 5′ and 3′ probes from GABRA5 (Table 2). These results indicate that GABRA5 is distal to the breakpoints in both the translocation carrier WJK105 (mother of AS patient WJK106) and PWS translocation patient HS32.5 and are consistent with the quantitative hybridization (figure 1A, lane 3). The results of Table 2 also demonstrate that GABRA5 is not deleted in an AS sib bearing a submicroscopic deletion of 15q11−q13 and suggest that the gene is distal of the AS and PWS critical deletion regions.

DISCUSSION

The large consistent size of deletions in most Angelman and Prader−Willi syndrome patients does not readily permit ordering of most of the chromosome 15q11−q13 specific markers by deletion mapping (15). This difficulty has led to discrepancies in the reported orders for some of the commonly used markers and has at best permitted grouping of the others (21−24). In order to resolve these discrepancies, multicolor FISH has been employed as a powerful approach for establishing the order of

Table 1. Three sequence ordering of chromosome 15q11−q13 markers on interphase cells

Expt.	*Probe hybridization	Color sequence (#nuclei)	**Deduced probe order
1	3-21-12 34-10 16β3	g.g.r//g.r.g (228//17)	34-10, 3-21-12/16β3
2	3-21-12 34-10 16β3	g.r.r//r.g.r (192//16)	34-10, 3-21-12/16β3
3	3-21-12 34-10 16β3	r.r.g//r.g.r (12//109)	34-10, 3-21-12, 16β3
4	JP2 34-10 3-21-12	g.g.r//g.r.g (176//31)	34-10, JP2/3-21-12
5	JP2 34-10 3-21-12	r.g.g//g.r.g (27//102)	34-10, JP2, 3-21-12
6	**JP2** 34-10 16β3	r.y.g//other (52//4)	34-10, JP2, 16β3
7	JP2 189-41 34-10	g.g.r//g.r.g (119//46)	34-10/JP-2, 189-41
8	JP2 189-41 3-21-12	g.g.r//g.r.g (117//9)	JP2/189-41, 3-21-12
9	3-21-12 16β3 α5-39	g.g.r//g.r.g (5//91)	3-21-12, 16β3, α5-39
10	16β3 α5-39 DN10	r.r.g//r.g.r (13//107)	16β3, α5-39, DN10
11	189-41 JP3 3-21-12	r.g.r.//r.r.g. (95//14)	189-41, JP3, 3-21-12
12	189-41 JP3 3-21-12	g.r.g.//g.g.r. (87//7)	189-41, JP3, 3-21-12

* probes underlined are those detected by FITC and therefore green (g); **bold** denotes probe detected by both FITC and rhodamine and therefore yellow (y); all others detected by rhodamine and therefore red (r); ** and '/' refer to pairs of probes that cannot be unambiguously oriented within a given experiment.

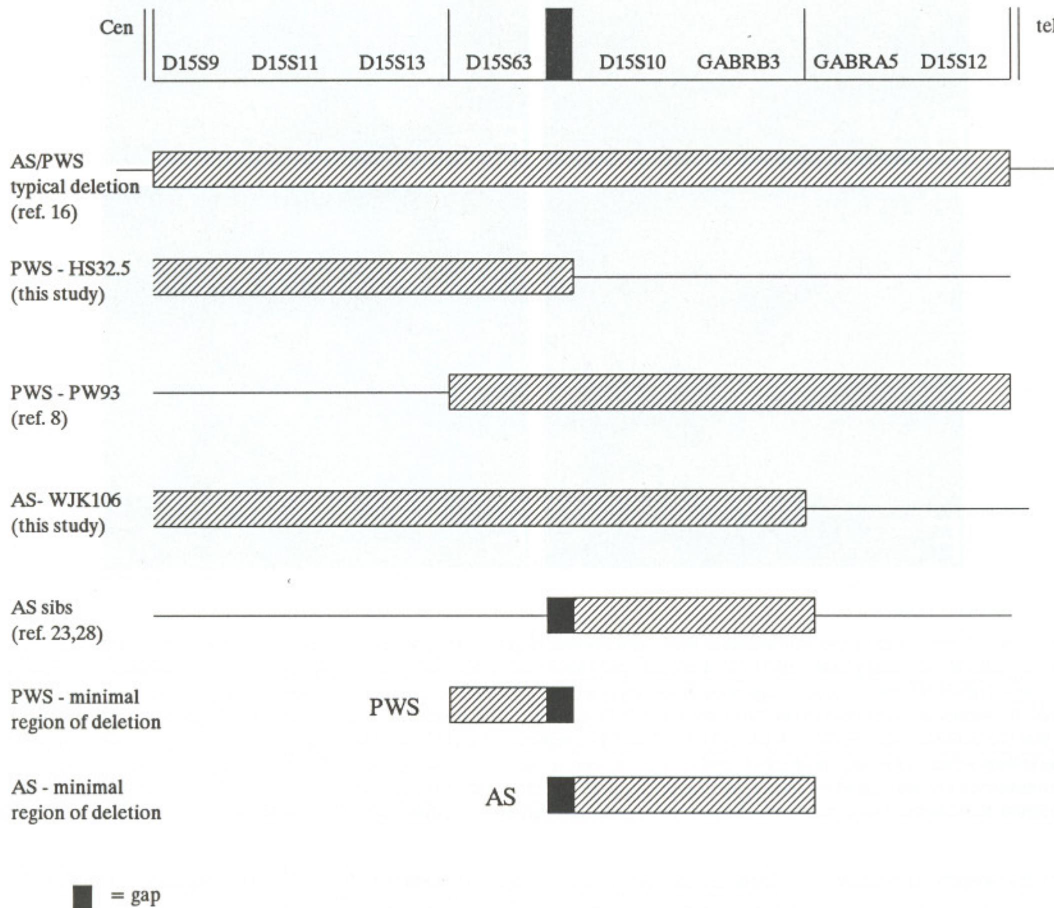

Figure 3. Locus order and minimal regions of deletion in the Angelman (AS) and Prader−Willi syndromes (PWS). The crosshatched areas correspond to deleted regions. There are no well characterized DNA markers within the gap region.

reference markers in 15q11−q13 (ref 1 and this study). FISH in combination with YAC contig construction allowed the following locus order to be established: cen-D15S18 −D15S9−D15S11−D15S63−D15S13−D15S113−D15S10−GABRB3−D15S12−D15S24-tel (1). The order determined in the present study (Figure 3) agrees with that of Kuwano et al (1) with the exception that D15S63 is distal of D15S13 rather than proximal. In fact, the only probe combination to yield an ambiguous order in the latter study was cen-D15S11−D15S63−D15S13 (1). The reason for this ambiguity was that the FISH signals from YACS for these three markers could not reliably be distinguished (1). The order (cen-D15S11−D15S13−D15S63) which was derived in the present study using FISH analysis with phage clones was unambiguous

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   1 GAATTCCCCCCTTGCAGGCCGAGCCGGGGCCCTGCGCCCTCCCCCTCCGCCCAGCTCGGCCAAGGGCGC   69
  70 ATTTGCTGAGCGTCTGGCGGCCTCTACCGGAGCACCTCTGCAGAGGGCCGATCCTCCAGCCCAGAGACG  138
 139 ACATGTGGCGCTCGGGCGAGTGCCTTGCAGAGAGAGGAGTAGCTTGCTGGCTTTGAACGCGTGGCGTGG  207
 208 CAGATATTTCAGAAAGCTTCAAGAACAAGCTGGAGAAGGGAAGAGTTATTCCTCCATATTCACCTGCTT  276
 277 CAACTACTATTCTTATTGGGAATGGACAATGGAATGTTCTCTGGTTTTATCATGATCAAAAACCTCCTT  345
 -31                       M  D  N  G  M  F  S  G  F  I  M  I  K  N  L  L  -16
 346 CTCTTTTGTATTTCCATGAACTTATCCAGTCACTTTGGCTTTTCACAGATGCCAACCAGTTCAGTGAAA  414
 -15  L  F  C  I  S  M  N  L  S  S  H  F  G  F  S  Q  M  P  T  S  S  V  K    8
 415 GATGAGACCAATGACAACATCACGATATTTACCAGGATCTTGGATGGGCTCTTGGATGGCTACGACAAC  483
   9  D  E  T  N  D  N  I  T  I  F  T  R  I  L  D  G  L  L  D  G  Y  D  N   31
 484 AGACTTCGGCCCGGGCTGGGAGAGCGCATCACTCAGGTGAGGACCGACATCTACGTCACCAGCTTCGGC  552
  32  R  L  R  P  G  L  G  E  R  I  T  Q  V  R  T  D  I  Y  V  T  S  F  G   54
 553 CCGGTGTCCGACACGGAAATGGAGTACACCATAGACGTGTTTTTCCGACAAAGCTGGAAAGATGAAAGG  621
  55  P  V  S  D  T  E  M  E  Y  T  I  D  V  F  F  R  Q  S  W  K  D  E  R   77
 622 CTTCGGTTTAAGGGGCCCATGCAGCGCCTCCCTCTCAACAACCTCCTTGCCAGCAAGATCTGGACCCCA  690
  78  L  R  F  K  G  P  M  Q  R  L  P  L  N  N  L  L  A  S  K  I  W  T  P  100
 691 GACACGTTCTTCCACAACGGGAAGAAGTCCATCGCTCACAACATGACCACGCCCAACAAGCTGCTGCGG  759
 101  D  T  F  F  H  N  G  K  K  S  I  A  H  N  M  T  T  P  N  K  L  L  R  123
 760 CTGGAGGACGACGGCACCCTGCTCTACACCATGCGCTTGACCATCTCTGCAGAGTGCCCCATGCAGCTT  828
 124  L  E  D  D  G  T  L  L  Y  T  M  R  L  T  I  S  A  E  C  P  M  Q  L  146
 829 GAGGACTTCCCGATGGATGCGCACGCTTGCCCTCTGAAATTTGGCAGCTATGCGTACCCTAATTCTGAA  897
 147  E  D  F  P  M  D  A  H  A  C  P  L  K  F  G  S  Y  A  Y  P  N  S  E  169
 898 GTCGTTTACGTCTGGACCAACGGCTCCACCAAGTCGGTGGTGGTGGCGGAAGATGGCTCCAGACTGAAC  966
 170  V  V  Y  V  W  T  N  G  S  T  K  S  V  V  V  A  E  D  G  S  R  L  N  192
 967 CAGTACCACCTGATGGGGCAGACGGTGGGCACTGAGAACATCAGCACCAGCACAGGCGAATACACAATC 1035
 193  Q  Y  H  L  M  G  Q  T  V  G  T  E  N  I  S  T  S  T  G  E  Y  T  I  215
1036 ATGACAGCTCACTTCCACCTGAAAAGGAAGATTGGCTACTTTGTCATCCAGACCTACCTTCCCTGCATA 1104
 216  M  T  A  H  F  H  L  K  R  K  I  G  Y  F  V  I  Q  T  Y  L  P  C  I  238
1105 ATGACCGTGATCTTATCACAGGTGTCCTTTTGGCTGAACCGGGAATCAGTCCCAGCCAGGACAGTTTTT 1173
 239  M  T  V  I  L  S  Q  V  S  F  W  L  N  R  E  S  V  P  A  R  T  V  F  261
1174 GGGGTCACCACGGTGCTGACCATGACGACCCTCAGCATCAGCGCCAGGAACTCTCTGCCCAAAGTGGCC 1242
 262  G  V  T  T  V  L  T  M  T  T  L  S  I  S  A  R  N  S  L  P  K  V  A  284
1243 TACGCCACCGCCATGGACTGGTTCATAGCTGTGTGCTATGCCTTCGTCTTCTCGGCGCTGATAGAGTTT 1311
 285  Y  A  T  A  M  D  W  F  I  A  V  C  Y  A  F  V  F  S  A  L  I  E  F  307
1312 GCCACGGTCAATTACTTTACCAAGAGAGGCTGGGCCTGGGATGGCAAAAAAGCCTTGGAAGCAGCCAAG 1380
 308  A  T  V  N  Y  F  T  K  R  G  W  A  W  D  G  K  K  A  L  E  A  A  K  330
1381 ATCAAGAAAAAGCGTGAAGTCATACTAAATAAGTCAACAAACGCTTTTACAACTGGGAAGATGTCTCAC 1449
 331  I  K  K  K  R  E  V  I  L  N  K  S  T  N  A  F  T  T  G  K  M  S  H  353
1450 CCCCCAAACATTCCGAAGGAACAGACCCCAGCAGGgACGTCGAATACAACCTCAGTCTCAGTAAAACCC 1518
 354  P  P  N  I  P  K  E  Q  T  P  A  G  T  S  N  T  T  S  V  S  V  K  P  376
1519 TCTGAAGAGAAGACTTCTGAAAGCAAAAAGACTTACAACAGTATCAGCAAAATTGACAAAATGTCCCGA 1587
 377  S  E  E  K  T  S  E  S  K  K  T  Y  N  S  I  S  K  I  D  K  M  S  R  399
1588 ATCGTATTCCCAGTCTTGTTCGGCACTTTCAACTTAGTTTACTGGGCAACGTATTTGAATAGGGAGCCG 1656
 400  I  V  F  P  V  L  F  G  T  F  N  L  V  Y  W  A  T  Y  L  N  R  E  P  422
1657 GTGATAAAAGGAGCCGCCTCTCCAAAATAACCGGCCACACTCCCAAACTCCAAGACAGCCATACTTCCA 1725
 423  V  I  K  G  A  A  S  P  K                                            431
1726 GCGAAATGGTACCAAGGAGAGGTTTTGCTCACAGGGACTCTCCATATGTGAGCACTATCTTTCAGGAAA 1794
1795 TTTTTGCATGTTTAATAATATGTACAAATAATATTGCCTTGATGTTTCTATATGTAACTTCAGATGTTT 1863
1864 CCAAGATGTCCCATTGATAATTCGAGCAAACAACTTTCTGGAAAAACAGGATACGATGACTGACACTCA 1932
1933 GATGCCCAGTATCATACGTTGATAGTTTACAAACAAGATACGTATATTTTTAACTGCTTCAAGTGTTAC 2001
2002 CTAACAATGTTTTTTATACTTCAAATGTCATTTCATACAAATTTTCCCAGTGAATAAATATTTTAGGAA 2070
2071 ACTCTCCATGATTATTAGAAGACCAACTATATTGCGAGAAACAGAGATCATAAAGAGCACGTTTTCCAT 2139
2140 TATGAGGAAACTTGGACATTTATGTACAAAATGAATTGCCTTTGATAATTCTTACTGTTCTGAAATTAG 2208
2209 GAAAGTACTTGCATGATCTTACACGAAGAAATAGAATAGGCAAACTTTTATGTAGGCAGATTAATAACA 2277
2278 GAAATACATCATATGTTAGATACACAAAATATT 2310
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Figure 4. Nucleotide and predicted amino acid sequence of the $GABA_A$ receptor $\alpha 5$ subunit gene. The closed triangle indicates the position of the putative signal sequence cleavage site. The four transmembrane regions are overlined. The primers in the 3′ UTR used for amplifying genomic DNA by PCR are indicated by the open triangles.

(Table 1, Expt 11 and 12) and is consistent with that from a PWS deletion patient, PW93 (9, 25), which is not deleted for D15S9, D15S11 and D15S13 but displays a hemizygous deletion of the distal markers D15S63, D15S10, GABRB3 and D15S12. Our results suggest that FISH ordering of tightly linked markers may be more readily accomplished with phage clones than with YAC clones. This difference is due to the smaller hybridization signals produced by phage clones which are less likely to co-localize with neighboring signals on interphase chromatin. The probe order obtained by FISH is consistent with the grouping observed by DNA analyses in the two unbalanced translocation patients in this study. By DNA analyses, the markers for D15S9, D15S11 and D15S13 are centromere proximal, followed by those for D15S10 and GABRB3 and then those for GABRA5 and D15S12.

A second γ-aminobutyric acid receptor gene, the $\alpha 5$ subunit (GABRA5), has been placed in the 15q11−q13 reference map. GABRA5 is distal and tightly linked to GABRB3. Long range restriction mapping (D.S. et al., in preparation) suggests that the two genes are less than 100 kb apart. Other $GABA_A$ receptor genes also appear to be organized in clusters (26) with the $\alpha 2$ and $\beta 1$ subunits localized to chromosome 4p1p13 (27); the $\alpha 1$ and $\gamma 2$ subunits to 5q34−q35 (26,27) and the $\varrho 1$ and $\varrho 2$ subunits to chromosome 6q14−q21 (28). The significance of the apparent clustering of these $GABA_A$ receptor subunit genes is not known.

Table 2. Metaphase chromosome hybridization for delineating the extent of deletion in different cell lines

Cell line	Chromosome	*Hybridization of probe			
		JP3	3-21-12	α55-24	α5-39
1) WJK105	15	+	+	+	+
	der(13)	ND	−	+	+
	der(15)	ND	+	−	−
2) HS32.5	15	+	+	+	ND
	der(9)	−	+	+	ND
3) AS Sib	15/del(15)	+/+	+/−	+/+	+/+

* at least 10 metaphases were examined for each hybridization; signal detected (+), not detected (−) or hybridization not done (ND) for the indicated chromosome. The AS sib bears a submicroscopic deletion (ref 23) and the 15/del(15) could not be differentiated in metaphase spreads. Of the probes hybridized, only 3-21-12 was hemizygously deleted in the AS sib.

Probes from the 3′ and 5′ end of GABRA5 are distal of the WJK105 translocation breakpoint (Figure 1A and Table 2). In addition, both the 5′ and 3′ ends of GABRA5 were intact (Table 2) in an AS sibling with a submicroscopic deletion involving only D15S10 and GABRB3 (23,29). This deletion in the three AS sibs currently defines the minimal AS critical region since maternal transmission of this deletion results in AS whereas paternal transmission is associated with a normal phenotype(23,29). These data strongly suggest that GABRA5 is distal of the AS critical region (Figure 3).

On metaphase chromosomes, the markers could be subdivided into two groups: markers for D15S9, D15S11 and D15S13 co-localized; and markers for D15S10, GABRB3, GABRA5 and D15S12 co-localized. The ability to order markers between the groups suggest that there is a large gap between these two groups (≥1 Mb). The HS32.5 PWS translocation breakpoint is located within the gap since it displays a hemizygous deletion for the proximal but not the distal set of markers (Figure 3). By combining our data with those of Robinson et al. (9), all loci proximal to and including D15S13 (patient PW93, ref 9) and all loci distal to and including D15S10 (patient HS32.5 in this study) have been excluded in PWS (Figure 3). These results suggest that the critical region for PWS is in the 'gap' between D15S13 and D15S10 and includes locus D15S63. In AS, the critical region of deletion encompasses both D15S10 and GABRB3 (23,29) but excludes the more distal GABRA5 and D15S12 loci. Any overlap of the AS and PWS critical regions would be in the gap region. Isolation of additional markers in the gap region will aid in determining if the critical regions in the two syndromes overlap. With the current map and refinement of the critical regions of AS and PWS, we can now select appropriate probes from each side of the gap for reliable deletion detection by molecular cytogenetic techniques.

MATERIALS AND METHODS

Patient samples

Heparinised peripheral blood samples were obtained from two patients with unbalanced chromosomal translocations and their parents. AS patient, WJK106 is the result of malsegregation of a maternal balanced 13;15 translocation [45,XY,−13,−15,+der(13)t(13;15)(p13;q13)mat]. WJK106 has been previously described (patient 2, ref. 20) and, in light of the association of maternal deletion of 15q11−q13 with AS (7), has recently been re-evaluated and a diagnosis of AS confirmed. His mother, WJK105 is a balanced translocation carrier (46,XX, −13, −15, +t(13;15)(p13;q13). The second patient, HS32.5 has PWS and carries a de novo unbalanced 9;15 translocation [45,XY,−9,−15,+der(9)t(9;15)(q34.3;q11.2)]. His clinical features include neonatal hypotonia and failure to thrive, developmental delay, mild mental retardation, hypogonadism, hyperphagia, scoliosis, short stature, small hands and feet and normal pigmentation. Epstein-Barr virus transformed lymphoblastoid lines were established from each patient.

DNA probes

A rat $GABA_A$ receptor α5 subunit cDNA probe, containing the entire coding region, was isolated by PCR amplification of rat brain cDNA using oligonucleotide primers derived from the published sequence (30) and used as a probe to screen a human hippocampal cDNA library (31). One of the isolated clones contained the entire coding region (31). The nucleotide and predicted amino acid sequence of this cDNA clone is presented in Figure 4. PCR primers were derived from the 3′ untranslated region (5′-ACTCCAAGACAGCCATACTTCC and 5′-GGCATCTGAGTGTCAGTCATCG, respectively) and were used to assign the GABRA5 gene to chromosome 15 by PCR analysis of a panel of somatic hybrid DNAs using the approach described previously (32). The same human PCR product was used to screen a genomic library and an EMBL3 phage clone, α5-39, was obtained. The α53-B probe is a 1.1 kb HindIII/EcoRI fragment sucloned from the 19.5 kb α5-39 phage insert. α55-24 is a phage clone containing a 20 kb insert from the 5′ end of GABRA5. It was isolated by screening a recombinant lambda DASH II library constructed from flow sorted chromosome 15 (D.S. et al., manuscript in preparation) with a 450 bp probe containing the 5′UTR and first exon of a human α5 subunit cDNA isolated from a human fetal brain library (kindly provided by Dr. L.Kunkel). The other chromosome 15q11−q13 specific probes used for quantitative and qualitative Southern blot hybridizations were as follows: 34 (D15S9); 3-21 (D15S10); IR4-3R (D15S11); IR10-1 (D15S12); 189-1 (D15S13); IR39d (a SacI/HindIII subfragment of IR39 [D15S18]) (13,14); PW71 (D15S63) (33); 28β3-H3, a genomic clone for the $GABA_A$ receptor B3 subunit gene (GABRB3) (21). Chromosome 13 specific probes, H2-26 (D13S28) and H2-42 (D13S25) served as control probes for quantitative hybridizations (34).

Large insert phage clones 34-10, 3-21-12, 189-41, JP2, JP3, 16β3, α55-24 and α5-39 as well as one cDNA probe, DN10 which was isolated by screening a cDNA library with probe IR10-1 (13), were utilized for FISH. Clones 34-10, 3-21-12, 189-41, JP2 and JP3 were obtained by screening a genomic library, derived from a partial MboI digested human DNA cloned into the lambda EMBL3 vector, with the DNA markers 34, 3-21, 189-1, IR4-3R (13) and PW71 (33), respectively. These EMBL3 clones contain 19.6, 17.8, 16.7, 15 and 15 kb genomic inserts, respectively.

Fluorescent *in situ* hybridization (FISH)

For metaphase mapping and ordering, PHA stimulated lymphocytes and lymphoblasts from chromosomally normal donors or AS and PWS donors with a deletion or translocation of 15q11−q13 were synchronized by a methotrexate block and subsequent bromodeoxyuridine or thymidine release (35,36). For interphase ordering, PHA-stimulated G1 peripheral lymphocytes and unstimulated G1 peripheral lymphocytes were utilized. Unstimulated peripheral lymphocytes were isolated from heparinised whole blood by Ficoll-paque separation (Pharmacia), washed in Hanks balanced salt solution, treated with .075M KCl at 37°C for 15 minutes, fixed in three changes of 3:1 methanol:actic acid and dropped onto precleaned microscope slides. Slides were stored at −20°C with desiccant until hybridized.

Purified phage and plasmid DNA were labelled via nick translation with either biotin-16-dUTP (Boehringer-Mannheim) or digoxigenin-11-dUTP (Boehringer-Mannheim). Hybridization and detection conditions were as described elsewhere (18). Biotinylated probes were visualized in green and digoxigenated probes in red. A mixture of biotinylated and digoxigenated probe was visualized in yellow. Interphase probe ordering was accomplished by hybridizing three sequences simultaneously onto fixed cell preparations and scoring nuclei that showed two hybridization sites in one color and a third in the second color or three hybridization sites in three colors. Metaphase probe ordering was accomplished by hybridizing two alternately labelled probes simultaneously and scoring chromosome 15s that showed both red and green fluorescent labels. Probes were hybridized individually before being combined in order to assess background fluorescence and insure that the efficiency of hybridization of each probe was at least 80%. Cell preparations were counterstained with 4′-6-diamidino-2-phenylindole (DAPI) to produce a Q band-like pattern for chromosome identification (37). Fluorescein and rhodamine were simultaneously viewed through a double band-pass filter (Omega, Brattleboro, VT) and DAPI through a second filter on a Zeiss Axiophot epifluorescence microscope with a Zeiss Plan Neofluar oil immersion objective (100X;1.3 NA). Cells were photographed on Kodak Ektar 1000 color film.

Southern blot analysis

DNA was extracted from lymphoblastoid cell lines of WJK106, HS32.5, normal individuals including the parents of WJK106 and HS32.5 and from control deletion AS and PWS patients for use in both quantitative and qualitative analyses. DNA

samples were digested to completion with appropriate restriction enzymes (New England Biolabs), electrophoresed, transferred to nylon membranes (HN+, Amersham), hybridized, washed and autoradiographed as described elsewhere (14).

Chromosome sorting and spot blot analysis

Chromosomes were isolated from lymphoblasts of WJK105 (46, XX, −13, −15, +t(13;15)(p13;q13), stained with Hoechst 33258 and chromomycin A3 and sorted by using a Becton−Dickinson dual-beam fluorescence-activated chromosome sorter (FACS IV) (38). Thirty thousand chromosomes each of 15, derivative 15, 13 and derivative 13 were sorted onto nylon membranes (HN, Amersham) (39). The membranes were hybridized and washed in the same way as for routine Southern filters (14).

ACKNOWLEDGEMENTS

We gratefully acknowledge Dr Jeanne Lawrence and members of her laboratory for suggestions and assistance in the in situ hybridization technique, Dr Bernhard Horsthemke for supplying probe PW71, Dr Norio Niikawa for providing the cell lines of the AS siblings and Alan Flint for flow sorting of the chromosomes. This research was supported by NIH grant HD18658 (ML) and USPHS grants NS27322 and MH44212 and the Veterans Administration Medical Research Service (JMS). DS was supported by a fellowship from the Medical Research Council of Canada and ASW was supported in part by a predoctoral fellowship from the National Institute for Drug Abuse (DA05480).

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