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High-Resolution Mapping of the γ-Aminobutyric Acid Receptor Subunit β3 and α5 Gene Cluster on Chromosome 15q11-q13, and Localization of Breakpoints in Two Angelman Syndrome Patients

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Summary

The γ-aminobutyric acid ($GABA_A$) receptors are a family of ligand-gated chloride channels constituting the major inhibitory neurotransmitter receptors in the nervous system. In order to determine the genomic organization of the $GABA_A$ receptor β3 subunit gene (GABRB3) and α5 subunit gene (GABRA5) in chromosome 15q11-q13, we have constructed a high-resolution physical map using the combined techniques of field-inversion gel electrophoresis and phage genomic library screening. This map, which covers nearly 1.0 Mb, shows that GABRB3 and GABRA5 are separated by less than 100 kb and are arranged in a head-to-head configuration. GABRB3 encompasses approximately 250 kb, while GABRA5 is contained within 70 kb. This difference in size is due in large part to an intron of 150 kb within GABRB3. We have also identified seven putative CpG islands within a 600-kb interval. Chromosomal rearrangement breakpoints—in one Angelman syndrome (AS) patient with an unbalanced translocation and in another patient with a submicroscopic deletion—are located within the large GABRB3 intron. These findings will facilitate chromosomal walking strategies for cloning the regions disrupted by the DNA rearrangements in these AS patients and will be valuable for mapping new genes to the AS chromosomal region.

Introduction

The γ-aminobutyric acid ($GABA_A$) receptor is the major inhibitory neurotransmitter receptor in the mammalian central nervous system. The binding of GABA to these receptors causes an influx of chloride ions, which leads to hyperpolarization (and thus inhibition) of postsynaptic neurons (DeLorey and Olsen 1992). The $GABA_A$ receptor, together with glycine and acetylcholine receptors, is a member of a gene superfamily of ligand-gated hetero-oligomeric ion-channel receptors (Schofield et al. 1987; Betz 1990). The molecular cloning of $GABA_A$ receptor subunit cDNAs has resulted in the identification of a large diversity of $GABA_A$ receptor subunit genes (e.g., α1–6, β1–4, γ1–3, δ, and ρ1–2). These sequences are homologous, with about 60%–80% amino acid sequence identity between members of a subunit family and 20%–40% between the subunit classes (for review, see DeLorey and Olsen 1992). Additional diversity may arise from alternative RNA splicing (Whiting et al. 1990; Kofuji et al. 1991). A number of these subunit genes have been mapped and were found to cluster on different chromosomes (Buckle et al. 1989; Cutting et al. 1992; Wilcox et al. 1992).

The $GABA_A$ receptor β3 subunit gene (GABRB3) has been localized to chromosome 15q11-q13 (Wagstaff et al. 1991*b*), a region which is frequently deleted in two distinct genetic disorders, Angelman syndrome (AS) and Prader-Willi syndrome (PWS) (Ledbetter et al. 1981; Kaplan et al. 1987; Pembrey et al. 1989; Williams

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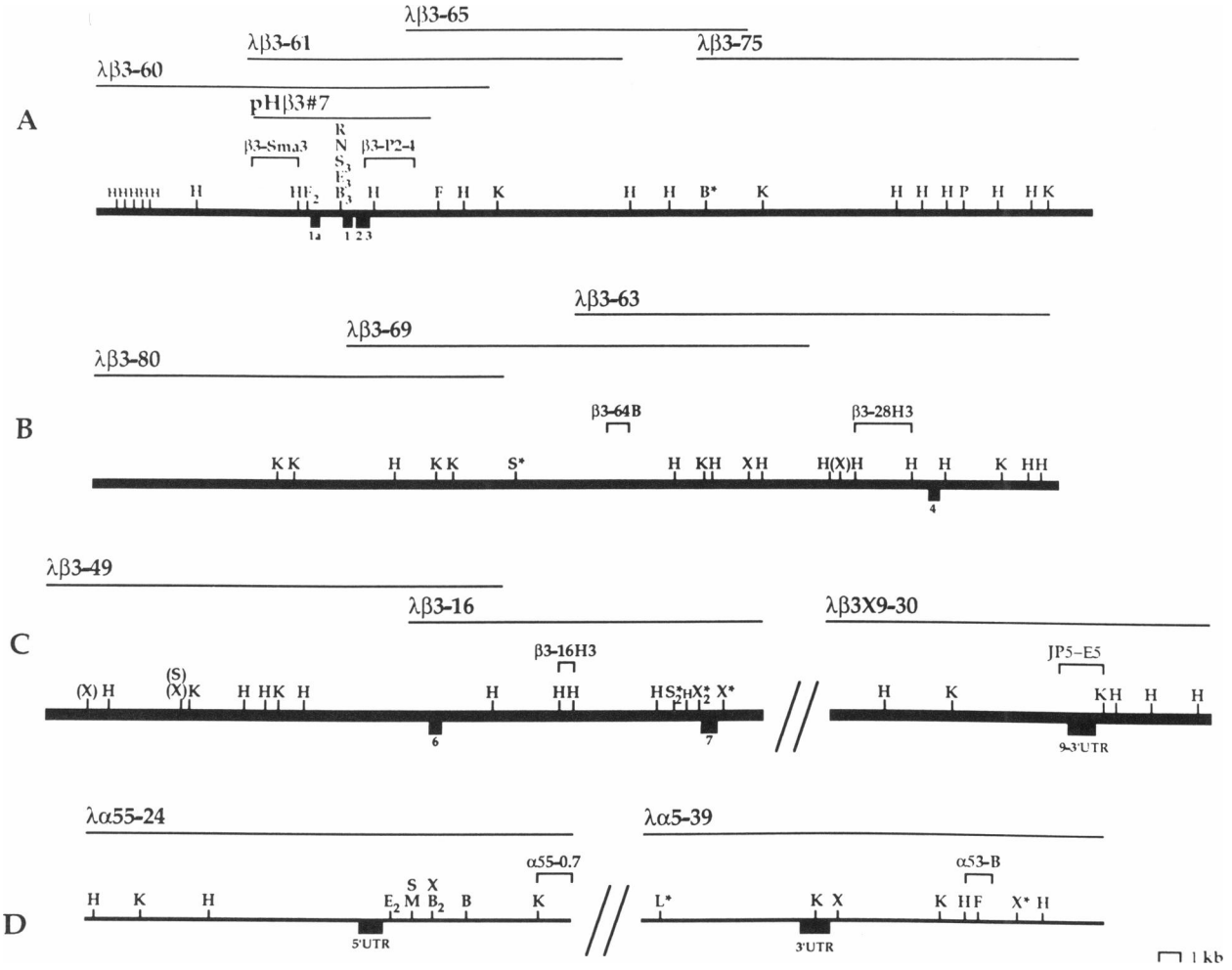


Figure 1 Restriction maps of human genomic subclones derived from GABRB3 and GABRA5 loci. A representative set of overlapping phages (indicated by thin lines) defining each region is given above the restriction map (indicated by thick lines). *A*, GABRB3 5′ end; *B*, GABRB3 exon 4; *C*, GABRB3 exons 6, 7, 9, and 3′UTR; and *D*, GABRA5 5′ and 3′UTR. The location of the DNA markers isolated from these genomic subclones is indicated by brackets. B = *Bss*HII; E = *Eag*I; F = *Sfi*I; H = *Hin*dIII; K = *Kpn*I; L = *Sal*I; M = *Mlu*I; N = *Not*I; P = *Pme*I; R = *Nru*I; S = *Sac*II; and X = *Xho*I. An asterisk (*) indicates a methylated site, and parentheses indicate an unknown methylation status in genomic DNA. The positions of exons and UTR are illustrated by black boxes below each restriction map.

et al. 1989; Butler 1990; Knoll et al. 1990). In AS, deletions occur on the maternally inherited chromosome 15 (Knoll et al. 1989; Magenis et al. 1990; Williams et al. 1990; Clayton-Smith et al. 1992), whereas in PWS the deletions are always of paternal origin (Butler and Palmer 1983; Hamabe et al. 1991*a;* Robinson et al. 1991). The other observed abnormalities of 15q11-q13 are uniparental maternal disomy in most nondeletion cases of PWS (Nicholls et al. 1989*a;* Hamabe et al. 1991*a;* Robinson et al. 1991) and uniparental paternal disomy in a few cases of AS (Malcom et al. 1991; Smeets et al. 1992). These observations are consistent with the occurrence of genomic imprinting whereby a defect in a gene(s) expressed on the 15q11-q13 region of maternal origin results in AS and abnormality of a gene(s) expressed on the paternally derived 15q11-q13 region causes PWS. GABRB3 is included in typical AS and PWS deletions (Wagstaff et al. 1991*b*). In addition, a deletion of the GABRB3 locus has been observed in two unusual chromosomal rearrangements associated with AS—a submicroscopic deletion involving D15S10 and GABRB3 (Hamabe et al. 1991*b;* Saitoh et al. 1992) and an unbalanced translocation t(13;15) (Greenberg and Ledbetter 1987; Wagstaff et al. 1991*b*). In the

Table 1

Pulsed-Field DNA Fragments Detected by Nine DNA Markers around GABRB3 and GABRA5 Loci

Probe	Fragment Size (Kb)										
	*Bss*HII	*Eag*I	*Sfi*I	*Sal*I	*Mlu*I	*Not*I	*Pme*I	*Nru*I	*Sac*II	*Rsr*II	*Xho*I
JP5-E5	nd	nd	nd	nd	nd	LM	**220**	nd	220	nd	nd
β3-16H8	nd	**470** **290**	nd	nd	(**430**)	nd	**220**	nd	nd	nd	nd
β3-28H3	**850**	nd	(480) (470) (170) (120)	(**320**) 150	(900) (**430**)	LM	(480) **220**	nd	**180** **160**	nd	(**130**) (**110**) 50
β3-64B	**850**	**470** **290**	nd	(**320**) 150	(**430**)	LM	(480) **220**	**220** (50)	**180** **160**	LM	(**130**) (**110**) 12
β3-P2.4	**850**	110	**150** *60*	(**320**) *180* (17)	nd	LM (100)	*150*	(**220**) 120 (70)	150 (90)	nd	170
β3-Sma3	90	(*150*) 90	**150** *60*	*180*	nd	nd	(350) *150*	120 50	*170* 120	175 140	170
α55-0.7	30	(*150*) 110 30	(**150**) 90	*180*	nd	LM	nd	nd	210 *170* 45	**180** **145**	*120* (40)
α53-B	(*110*) *80*	210 (110) 90	(210) **150** 90	*180*	nd	LM	210	(220) (130) 90	210 170	**180** **145**	*120* (80)
MN47	(*110*) *80*	210 (110) 90	(210) 50	160	nd	nd	210	nd	210 170	**180** **145**	9

NOTE.—**Bold**, *italic*, underlined, and double-underlined numbers indicate physical linkage. nd = not determined; and LM = limiting mobility of fragments. Numbers in parentheses indicate weakly hybridizing bands.

former case, maternal transmission of the deletion resulted in AS, whereas paternal transmission did not result in an abnormal phenotype (Hamabe et al. 1991*b*; Saitoh et al. 1992). This result strengthened the previous hypothesis that the AS and PWS loci are distinct (Wagstaff et al. 1991*b*) and suggested that the minimal AS critical region was contained within the submicroscopic deletion encompassing D15S10 and GABRB3. The evidence for distinct AS and PWS loci is accumulating (Kuwano et al. 1992; Özçelik et al. 1992; Wagstaff et al. 1992; Knoll et al. 1993), and a gene encoding a small ribonucleoprotein-associated polypeptide (i.e., Snrpn), which is expressed only from the paternal chromosome in mouse (Leff et al. 1992), maps to the PWS chromosomal region in human (Özçelik et al. 1992). The murine $GABA_A$ receptor β3 subunit gene (gabrb3) is expressed in mice with paternal duplication/maternal nullisomy of the region containing this gene, indicating that gabrb3 may not be imprinted in mice. However, mice with paternal disomy of the region homologous to the human AS critical region, which includes gabrb3, do not appear to have a phenotype that resembles any manifestations of AS (Cattanach et al. 1992). This indicates that the AS gene may not be imprinted in mice and that the potential role for a defect of GABRB3 and/or surrounding genes in AS has not been excluded. The $GABA_A$ receptor α5 subunit gene (GABRA5) is also localized within 15q11-q13 and is distal to GABRB3 (Knoll et al. 1993).

In order to elucidate the genomic organization of GABRB3 and GABRA5 loci and their relation to the AS chromosomal region, the construction of a detailed physical map was initiated. In this study, we report the isolation of genomic phage clones containing various

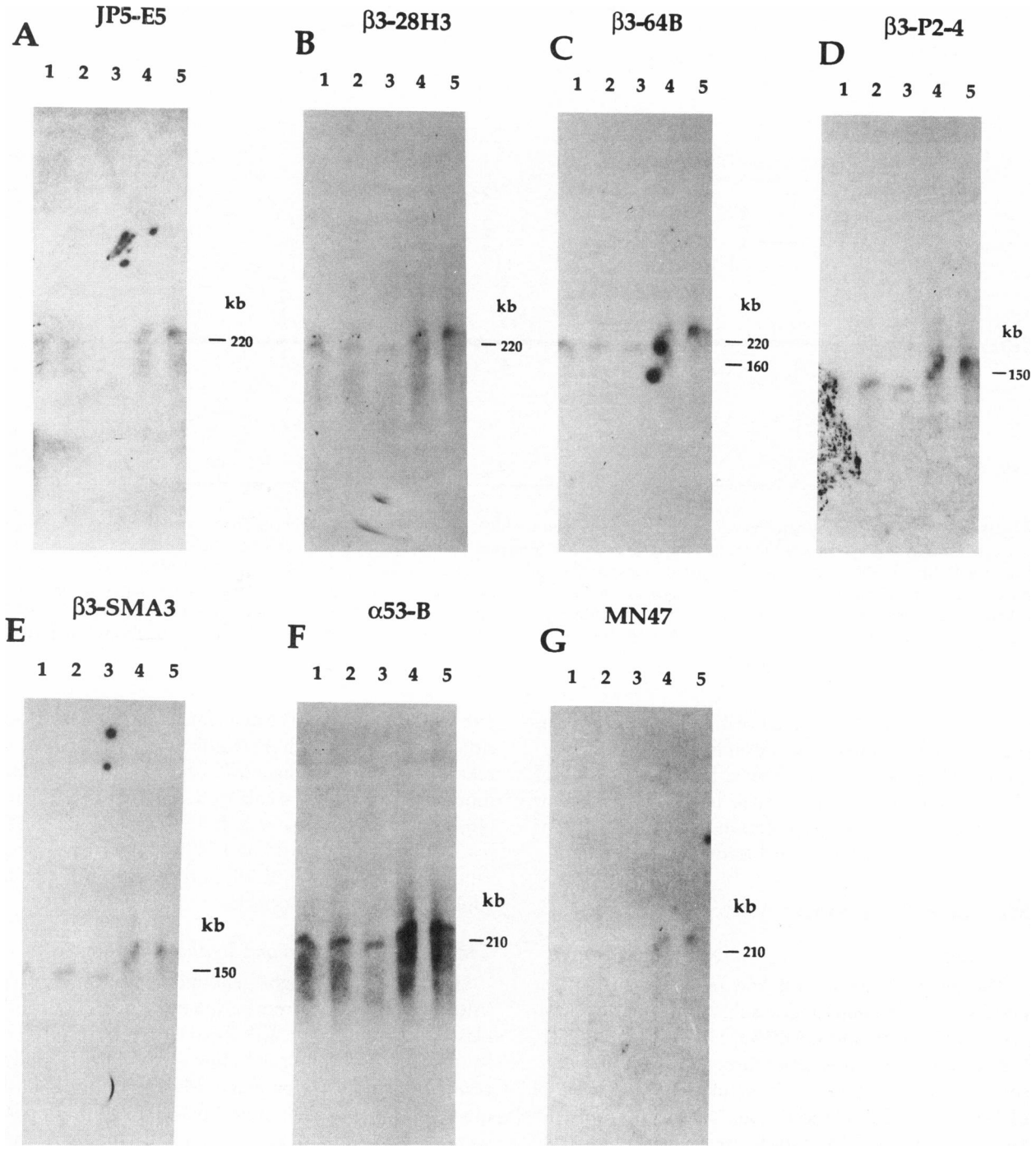


Figure 2 FIGE analysis of *Pme*I-digested DNA. Probes JP5-E5 (*A*), β3-28H3 (*B*), β3-64B (*C*), β3-P2.4 (*D*), β3-Sma3 (*E*), α53-B (*F*), and MN47 (*G*) were hybridized sequentially to a FIGE Southern blot containing *Pme*I DNA digests from two normal individuals (lanes 1 and 5) and WJK105 (mother of WJK106, lane 2), and AS patients WJK106 (lane 3) and DS176 (lane 4). The sizes of the restriction fragments detected by the probes are indicated in kilobases at the right and have been independently confirmed by separate Southern experiments done on normal individuals (not shown).

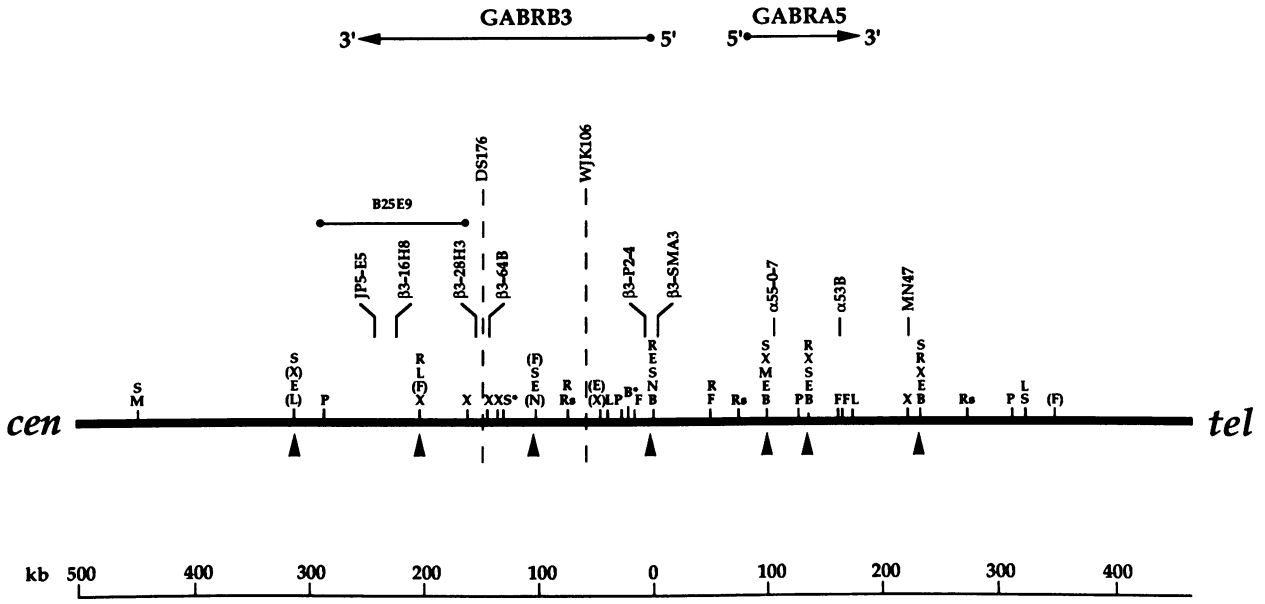


Figure 3 Long-range restriction map around GABRB3 and GABRA5 loci in a normal individual. The restriction map encompassing GABRB3 and GABRA5 genes was constructed using DNA isolated from two lymphoblastoid cell lines digested with the indicated enzymes and hybridized with the DNA markers shown above the map. Arrowheads indicate CpG islands revealed by the presence of a rare-cutting enzyme site cluster. Vertical broken lines indicate the localization of AS breakpoints. Horizontal arrows define the size and orientation of GABRB3 and GABRA5 transcriptional units. An asterisk (*) indicates a methylated site; parentheses indicate a tentative localization. B = *Bss*HII; E = *Eag*I; F = *Sfi*I; L = *Sal*I; M = *Mlu*I; N = *Not*I; P = *Pme*I; R = *Nru*I; S = *Sac*II; Rs = *Rsr*II; and X = *Xho*I. B25E9 is a GABRB3 YAC clone (Kuwano et al. 1992). cen = centromere; and tel = telomere.

portions of the GABRB3 and GABRA5 genes, as well as their use in long-range restriction mapping. The resulting 1.0-Mb physical map allowed further definition of chromosomal rearrangements in both the AS patient with the submicroscopic deletion and the AS patient bearing the unbalanced translocation.

Material and Methods

Cell Lines

The lymphoblastoid cell line from the AS patient DS176 who has inherited a submicroscopic deletion involving D15S10 and GABRB3 (Hamabe et al. 1991*b*; Saitoh et al. 1992) was generously provided by Professor Norio Niikawa of the Nagasaki University School of Medicine. The deletion in this patient 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 (Saitoh et al. 1992). The second 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 in Greenberg and Ledbetter 1987) and, in light of the association of maternal deletion of 15q11-q13 with AS (Knoll et al. 1989), has been reevaluated, with a diagnosis of AS confirmed. His mother, WJK105, is a balanced translocation carrier (46, XX, −13, −15, +der[13], +der[15], t[13;15] [p13;q13]). The WJK105 and WJK106 cell lines were established by Dr. Joan Knoll of the Genetics Division, Children's Hospital, Boston.

Genomic DNA Isolation and Southern Blotting

High-molecular-weight genomic DNA was isolated (Aldridge et al. 1984) from peripheral blood or lymphoblastoid cell lines from WJK106, DS176 (patient III-3 in Saitoh et al. 1992), normal individual GM0131, and a control deletion PWS patient (GM9133) for use in both quantitative and qualitative analyses. DNA samples were digested to completion with appropriate restriction endonuclease (New England Biolabs and Boehringer Mannheim), electrophoresed, transferred to nylon membranes (Hybond N+; Amersham), hybridized, washed, and autoradiographed as described elsewhere (Nicholls et al. 1989*b*).

For dosage-blotting analysis, fluorometric DNA quantification (Brunk et al. 1979) was done to assure a

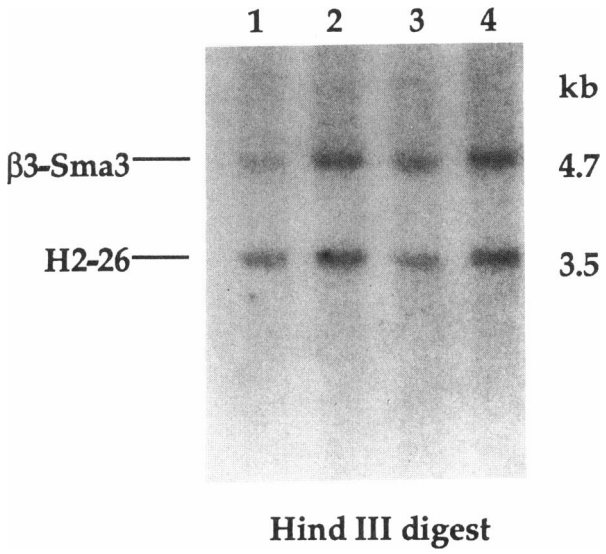


Figure 4 Quantitative hybridization analysis of the translocation and deletion AS patients with the DNA marker β3-Sma3. Filters were hybridized with β3-Sma3, derived from GABRB3 5′UTR, and with the control probe H2-26 (D13S28), from chromosome 13. β3-Sma3 and H2-26 recognize a 4.7- and 3.5-kb DNA fragment, respectively, on *Hin*dIII-digested DNA. β3-Sma3 is only deleted in the GM9133 cell line from a PWS patient with a large interstitial deletion of 15q11-q13 (lane 1) but not in the unbalanced translocation (lane 3) and deletion (lane 4) AS patients or the normal individual (lane 2). Lane 1, Interstitial deletion PWS patient (GM9133). Lane 2, Normal individual (GM0131). Lane 3, Unbalanced translocation AS patient (WJK106). Lane 4, AS deletion patient (DS176).

quantitative DNA loading per lane. Deletions in AS/PWS DNA samples were visualized as 50% reduction in the intensity of the hybridization band detected by the DNA probe (Tantravahi et al. 1989). For reference in hybridization quantification, blots were also hybridized with probes derived from chromosome 13 (see below).

Genomic Library Construction and Screening

Two large-insert phage libraries were used for screening experiments. The first library was derived from a partial *Mbo*I-digested human DNA cloned into the lambda EMBL3 vector (described by Monaco et al. [1986]). A second library was constructed from a *dam* methylase-controlled partial *Mbo*I digestion (Hoheisel et al. 1989) of flow-sorted inv dup (15) from the cell line GM06246 (47, XX, +dic[15][pter > q13::q13 > pter]), obtained from the NIGMS Human Genetic Cell Repository (Camden, NJ). The digestion products with an average size larger than 15 kb were treated with calf intestine alkaline phosphatase (Boehringer Mannheim) and ligated to *Bam*HI-digested vector lambda DASH II arms (Stratagene, La Jolla, CA). Packaging was carried out using a bacteriophage lambda in vitro packaging lysate (Gigapack II; Stratagene). Hybridization, purification of positive phage, and preparation of phage DNA were done according to standard procedures (Sambrook et al. 1989).

DNA Markers

Chromosome 15q11-q13–specific probes for quantitative and qualitative Southern blot hybridizations were MN47 (Buiting et al. 1990) and β3-28H3 and β3-16H8, genomic clones near exons 4 and 6, respectively, of GABRB3 (Wagstaff et al. 1991*b*). Chromosome 13–specific probes H2-26 (D13S28) and H2-42 (D13S25) (Lalande et al. 1984) served as control probes for quantitative hybridizations (Tantravahi et al. 1989).

In addition, single-copy genomic probes were derived from the phages isolated during the screening experiments described later in this paper: β3-Sma3, a 2.0-kb *Hin*dIII-*Bgl*II fragment, and β3-P2.4, a 2.4-kb *Pst*I fragment, were isolated from the GABRB3 subclones lambda β3-60 (fig. 1*A*). These fragments flank the GABRB3-associated CpG island, characterized by a cluster of rare-cutter sites. β3-64B is a 1.3-kb *Eco*RI fragment isolated from the GABRB3 genomic clone lambda β3-63 (fig. 1*B*). JP5-E5 is a 2.1-kb *Eco*RI fragment obtained from the phage β3X9-30 encompassing

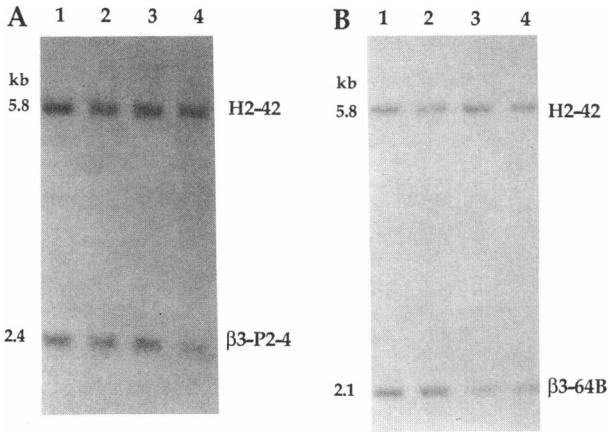


Figure 5 Dosage analysis with GABRB3 intron 3 DNA markers. Nylon filters were hybridized with probes β3-P2.4 (*A*) and β3-64B (*B*) and the chromosome 13 control probe H2-42 (D13S25). H2-42, β3-P2.4, and β3-64B recognize a 5.8-, 2.4-, and 2.1-kb DNA fragments, respectively, on *Pst*I-digested DNA. Lane 1, Normal individual (GM0131). Lane 2, AS deletion patient (DS176). Lane 3, Unbalanced translocation AS patient (WJK106). Lane 4, Interstitial deletion PWS patient (GM9133).

Table 2

Junction Fragments Detected by FIGE Analysis in AS Patients with Deletion and Translocation Breakpoints

Probe and Individual	Enzyme	Normal Fragment	Junction Fragment
β3-Sma3:			
WJK105/WJK106	*Xho*I	170	190
	*Rsr*II	175/145	200
	*Sfi*I	(150)/60	180
DS176	*Nru*I	120/50	400/360
β3-P2.4:			
WJK105/WJK106	*Not*I	LM	50
	*Mlu*I	LM	200
	*Bss*HII	LM	100/170
	*Nru*I	120/70	80
	*Rsr*II	175/145	200
	*Eag*I	100	50
DS176	*Not*I	LM	300
	*Mlu*I	LM	110
β3-64B:			
DS176	*Pme*I	220	160
	*Bgl*II	50	10
	*Rsr*II	LM	190/210
β3-28H3:			
WJK105	*Sfi*I	440	340

NOTE.—Restriction fragment sizes are given in kilobases. LM = limiting mobility of fragments. Numbers in parentheses indicate weakly hybridizing bands. WJK105 is the mother of the unbalanced translocation AS patient WJK106; and DS176 is the submicroscopic deletion AS patient.

the exon 9 and the 3′-untranslated region (UTR) of GABRB3 (fig. 1*C*). α53-B is a 1.1-kb *Hin*dIII-*Eco*RI fragment derived from the phage α5-39, corresponding to the 3′UTR of GABRA5 (fig. 1*D*). α55-0.7 is a 0.7-kb *Bam*HI-*Eco*RI fragment isolated from lambda α55-24 that contains the CpG island associated with the 5′UTR of GABRA5, as illustrated by the cluster of rare-cutter sites (fig. 1*D*).

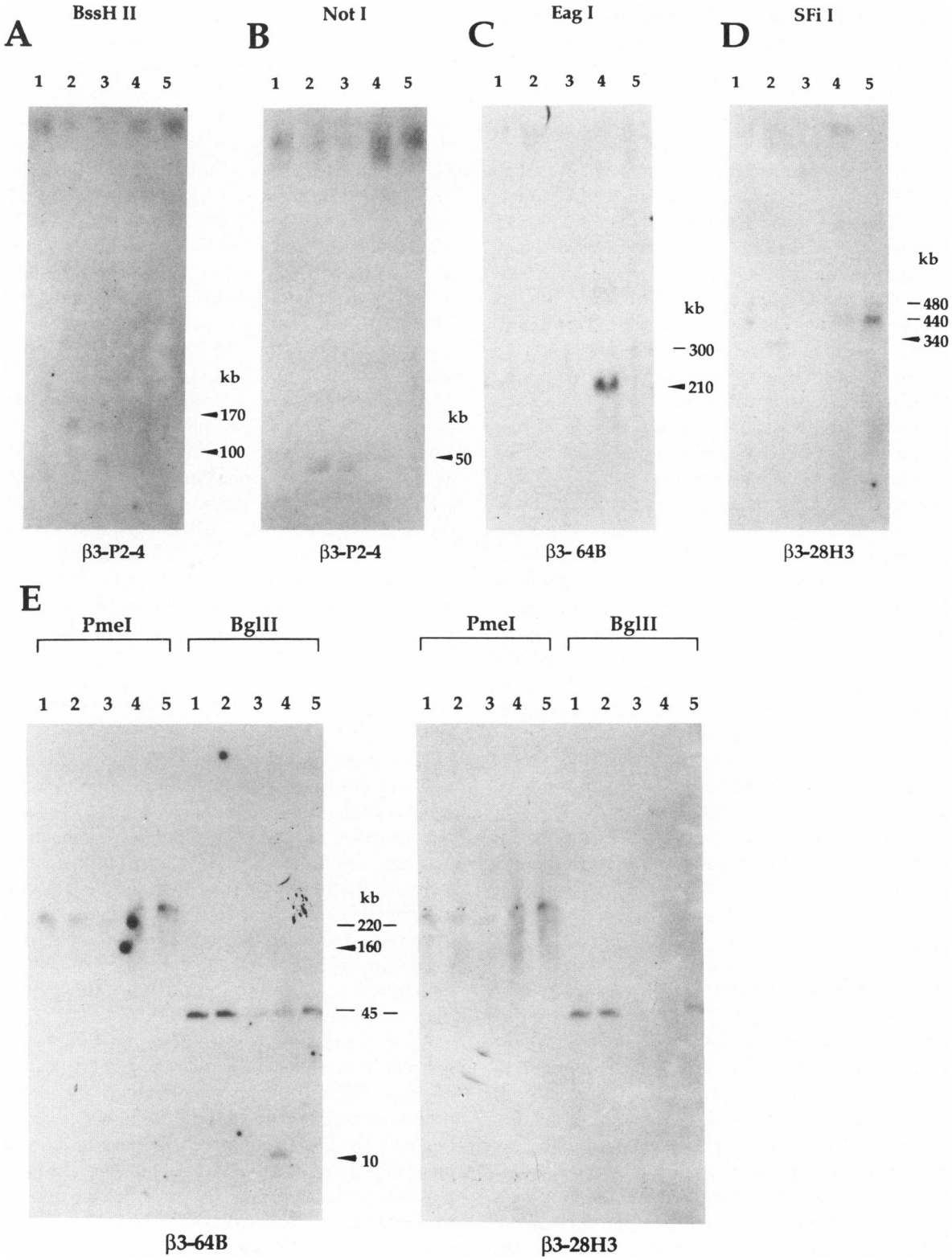
Each DNA marker was (*a*) tested to ensure that it was specific to chromosome 15 by Southern analyses using somatic cell hybrids containing either one human chromosome 15 (CHO hybrid 15A) or only the 15pter-q14 portion of chromosome 15 (CHO hybrid CP22) and (*b*) assigned to 15q11-q13 by quantitative hybridization analysis using AS and PWS deletion patients (data not shown). DNA probes were purified by agarose gel electrophoresis and radiolabeled by the random priming method of Feinberg and Vogelstein (1983).

Field-Inversion Gel Electrophoresis (FIGE)

Lymphoblast cells from normal individuals and AS patients were embedded in agarose (5×10^6 cells/ml) by standard techniques (Gardiner et al. 1986). The agarose plugs were incubated in 0.5 M EDTA, 1% sarcosyl containing proteinase K (2 mg/ml) for 24 h at 50°C and then treated with 0.04 mg phenylmethylsulfonyl fluoride/ml for 1 h, and rinsed in 1 × TE. DNA in agarose plugs was digested with different restriction enzymes, following the manufacturer's instructions (Boehringer Mannheim and New England Biolabs).

DNA samples were subjected to asymmetric FIGE as described elsewhere (Higgins et al. 1989), with the fol-

Figure 6 FIGE detection of breakpoint junction fragments in two AS patients. Hybridization is of β3-P2.4 (*A* and *B*), β3-64B (*C* and *E*), and β3-28H3 (*D* and *E*) probes to FIGE Southern blots containing genomic DNA from two normal individuals (lanes 1 and 5), WJK105 (mother of WJK106, lane 2), WJK106 (lane 3), and DS176 (lane 4) AS patients. The DNA was digested with the indicated endonucleases. Arrowheads indicate junction fragments, and the sizes (in kb) of restriction fragments detected by the probes are indicated to the right.


A
BssH II
1 2 3 4 5
kb
170
100
β3-P2-4
B
Not I
1 2 3 4 5
kb
50
β3-P2-4
C
Eag I
1 2 3 4 5
kb
300
210
β3- 64B
D
SFi I
1 2 3 4 5
kb
480
440
340
β3-28H3
E
PmeI
BglII
PmeI
BglII
1 2 3 4 5 1 2 3 4 5
1 2 3 4 5 1 2 3 4 5
kb
220
160
45
10
β3-64B
β3-28H3

lowing modifications to the pulse time and field intensity: the forward electric field, E+, of +3 V/cm was applied for a period, T+, varying from 50–100 s, followed by a second field of inverse polarity, E−, of −1.5 V/cm for an interval, T−, varying from 20–40 s over a duration of 48 h at 16°C. These pulse conditions were optimal for resolving restriction fragments in the range of 10–800 kb. A high-molecular-weight DNA ladder, 8–48 kb (BRL), and *Saccharomyces cerevisiae* strain YP148 chromosomes in agarose plugs were used as molecular size markers. In YP148, chromosome XV is broken in two fragments of 92 and 1,025 kb, each containing sequences hybridizing to plasmid DNA and therefore providing convenient reference points for alignment of autoradiograms and molecular weight determination (Forus and Myklebost 1992). The electrophoresed DNA was stained in the gel for 60 min with 1 μg ethidium bromide/ml and was visualized using an ultraviolet transilluminator (UVP).

Prior to the transfer to nylon membranes (Hybond N+; Amersham), ethidium bromide–stained gels were irradiated for 30 s on a UV light box (UVP transilluminator). Blotting, hybridization, and posthybridization washes were performed according to the manufacturer's recommendations. Membranes were exposed to X-ray film (Kodak XAR) at −70°C with intensifying screens (Dupont Cronex Lightning-Plus) for 1–9 d. In order to detect physical linkage, the same membranes were used repeatedly, and autoradiograms were superimposed for comparison of fragment sizes. For sequential hybridization with different probes, filters were stripped in 0.05 × SSC/0.1% SDS at 80°C for 1 h. Autoradiography was performed to be certain that no radioactivity remained before hybridizing with the second probe.

Results

Isolation and Characterization of GABRB3 and GABRA5 Genomic Clones

In order to study the genomic organization of GABRB3 and GABRA5 loci, two human genomic libraries were screened with probes derived from both loci (fig. 1). Six phage clones (β3-61, β3-63, β3-69, β3-75, β3-80, and α55-24) were isolated from the flow sorted inv dup (15) genomic library in vector lambda DASH II, and five phage clones (β3-49, β3-60, β3-65, β3X9-30, and α5-39) were obtained from the large-insert genomic library cloned in EMBL3. These phages were restriction mapped with a combination of *Kpn*I, *Hin*dIII, *Sal*I, *Eco*RI, and/or *Bgl*II (only *Kpn*I and *Hin*dIII sites are shown in fig. 1). In addition, all phages were digested with the rare-cutter enzymes used in the long-range mapping analysis. Contigs, restriction maps, and the locations of DNA markers generated during the chromosome walk are shown in figure 1. The phage-contig assembly of 45 kb, encompassing the 5′UTR of GABRB3, was initiated with the probe β3-P2.4 isolated from the subclone pHb3#7 derived from the 5′ end of the gene (Kirkness and Fraser 1993) (fig. 1*A*). A second contig of 43 kb was built 5′ from exon 4 of GABRB3 (fig. 1*B*) and contains the DNA marker, β3-28H3, described elsewhere (Wagstaff et al. 1991*b*). These two contigs do not overlap and are separated by a gap of approximately 75 kb, as suggested by FIGE analysis. Exons 6 and 7 of GABRB3 are defined by a 32-kb contig (fig. 1*C*) that includes the phage β3-16, described by Wagstaff et al. (1991*b*). The genomic clone β3X9-30 was obtained by screening the EMBL3 library with a DNA probe derived from the GABRB3 cDNA (Wagstaff et al. 1991*a*) and contains exon 9 and the 3′UTR (fig. 1*C*). The GABRA5 genomic clones α5-39 and α55-24 (fig. 1*D*) were isolated by using a PCR product, derived from the 3′UTR, and a 450-bp *Xho*II fragment, encompassing the 5′UTR and exon 1, derived from the GABRA5 cDNA, respectively (Knoll et al. 1993).

Long-Range Restriction Mapping around GABRB3 and GABRA5 Loci

To map more precisely the GABRB3 and GABRA5 loci, we performed Southern hybridization to high-molecular-weight DNA fractionated by FIGE. The sizes of the genomic fragments identified by nine DNA markers, after digestion with rare-cutting enzymes, were analyzed by FIGE, and these results are summarized in table 1. Only the restriction sites that have been observed are indicated in table 1; there may be additional sites for some of the enzymes that have been undetected (e.g., due to methylation) using the long-range mapping strategy.

Figure 2 presents representative results of Southern hybridization obtained by the sequential hybridization of *Pme*I-digested DNA samples with the indicated probes such that the resulting autoradiograms could be overlaid for direct comparison of the restriction fragment detected, allowing physical linkage. The endonuclease *Pme*I recognizes an 8-bp AT-rich site insensitive to methylation. Probes JP5-E5, β3-28H3, and β3-64B share a common 220-kb *Pme*I fragment (fig. 2*A*, *B*, and *C*). One *Pme*I restriction site was found within intron 3 at 28 kb from the GABRB3 CpG island in phage lambda

β3-75 (fig. 1*A*), and another was found near exon 9 in the GABRB3 yeast artificial chromosome (YAC) clone B25E9 (fig. 3), suggesting that the maximal distance between exon 3 and the 3′UTR of GABRB3 gene is less than 220 kb. β3-P2.4 and β3-Sma3 recognize the same 150-kb *Pme*I fragment (fig. 2*D* and *E*). A 210-kb *Pme*I fragment is detected with markers α53-B and MN47 (fig. 2*F* and *G*).

The data in table 1 were used to construct a physical map spanning 1 Mb around GABRB3 and GABRA5 loci (fig. 3). This map shows the positions for each of the nine probes linked together by FIGE analysis. The centromere/telomere orientation of this linkage group was determined previously by fluorescence in situ hybridization analysis using deletion/translocation patients (Knoll et al. 1993). The physical relation of the two $GABA_A$ receptor genes was investigated by hybridizing genomic probes specific to GABRB3 and GABRA5 5′ and 3′ ends to the same filters. These two genes lie in a head-to-head configuration, separated by less than 100 kb. The size of the GABRA5 gene is around 75 kb, and the GABRB3 is about 250 kb mainly because of the large 150-kb intron 3.

We relied on a combination of long-range restriction mapping in genomic DNA and phage restriction mapping to identify CpG islands. A cluster of recognition sites for rare-cutter enzymes is suggestive of a CpG island associated with coding sequences (Bird 1987; Lindsay and Bird 1987). We identified seven CpG islands within a 600-kb region, as shown in figure 3. Besides the CpG islands associated with GABRB3, GABRA5, and MN47 loci, two new islands mapped within the GABRB3 gene, and another was localized proximal to this gene. A second island 3′ of the GABRA5 transcriptional unit was identified. Such genes with additional CpG island 3′ of the transcription start have been described elsewhere (Larsen et al. 1992), but at this point we cannot exclude the possibility that this island covers the start of an unidentified transcript. The genomic and phage restriction maps are concordant. When an unmethylated rare-cutter site was identified in genomic DNA by long-range restriction mapping, the same site was found to be present within the corresponding phage clone (figs. 1 and 3).

Restriction mapping of the GABRB3 YAC clone B25E9 (Kuwano et al. 1992) was performed with rare cutters and was probed sequentially with DNA markers β3-16H8, JP5-E5, and β3-28H3 after FIGE (data not shown). This allowed the construction of a long-range restriction map of the YAC in which exon 4 is not present, as indicated by the absence of hybridization with the probe β3-28H3 (data not shown). Using the internal *Sal*I and *Pme*I sites as anchors, we have localized the GABRB3 YAC on the physical map (fig. 3).

Assignment of the Translocation and Deletion Breakpoints within the GABRB3 Locus by Quantitative Hybridization Analysis

The DNA marker β3-Sma3, derived from the GABRB3 5′UTR, was hybridized to *Hin*dIII DNA digests from normal control, deletion individual, and both AS patients (fig. 4). By quantitative hybridization, only the sample from a PWS patient with a large interstitial deletion of 15q11-q13 is hemizygous for a deletion of β3-Sma3 (fig. 4, compare lane 1 to lanes 2–4). This result indicates that the GABRB3 5′UTR is intact in both AS patients but is hemizygously deleted in the control deletion patient. Previously, we have shown that the 3′ and 5′ ends of the GABRA5 gene were intact in the AS translocation (WJK106), as well as in the deletion (DS176) patients (Knoll et al. 1993), suggesting that GABRA5 was distal to the AS chromosomal region. Moreover, it has been shown that β3-28H3 is deleted in these two AS patients (Wagstaff et al. 1991*b*; Saitoh et al. 1992). When taken together, these results indicate that both breakpoints involve the region contained between DNA markers β3-Sma3 and β3-28H3 on the deleted side.

Further dosage analyses were performed on *Pst*I-digested DNA with intron 3 probes β3-P2.4 and β3-64B (fig. 5). β3-P2.4 hybridizes with two copies per genome intensity in both AS patients (fig. 5*A*), while β3-64B is intact in DS176 (fig. 5*B*, lane 2) but deleted in the patient WJK106 (fig. 5*B*, lane 3). These data indicate that the two breakpoints are located at different positions within intron 3. The DS176 breakpoint is located within the region delimited by markers β3-64B and β3-28H3, and the WJK106 breakpoint is located closer to exon 3, between β3-P2.4 and β3-64B.

Localization of Rearrangement Breakpoints by FIGE Analysis

The localization of both AS breakpoints within the GABRB3 gene (figs. 4 and 5), with β3-28H3 on the deleted side, strongly suggests that rearranged DNA fragments (junction fragments) should be found by using probes within the large intron 3. Table 2 summarizes the sizes of the aberrant restriction fragments identified by DNA markers β3-Sma3, β3-P2.4, β3-64B, and β3-28H3, after FIGE analysis. Figure 6 shows representative results of Southern hybridization of the deletion (DS176) and the unbalanced translocation

(WJK106) AS patients with probes β3-28H3, β3-P2.4, and β3-64B, after FIGE analysis. β3-P2.4 detects two aberrant *Bss*HII fragments of 170 and 100 kb, as well as a 50-kb *Not*I fragment unique to WJK105 and WJK106 (fig. 6*A* and *B*), whereas β3-28H3 reveals a 340-kb *Sfi*I junction fragment only in WJK105, that is generated from the der(15) absent in WJK106 (fig. 6*D*). β3-64B identifies additional fragments in *Eag*I, *Pme*I, and *Bgl*II digests of DNA from the patient DS176 (fig. 6*C* and *E*). As shown in figure 6*E*, probes β3-28H3 and β3-64B share a small 50-kb *Bgl*II fragment disrupted by the deletion in DS176, indicating that the breakpoint must lie between these two markers, with β3-28H3 on the deleted side. This result confirms the quantitative hybridization analysis (figs. 4 and 5). These data allowed the localization of the WJK106 breakpoint at a maximal distance of 50 kb from exon 3 of GABRB3 gene and the DS176 breakpoint at ~10 kb distal to the marker β3-64B (fig. 3).

Discussion

In order to delineate the physical relationship between the GABRB3 and GABRA5 loci, a long-range restriction-mapping strategy using FIGE allowed the construction of a physical map covering approximately 1.0 Mb around these two genes (fig. 3). DNA markers corresponding to the 3′ and 5′ ends of GABRB3 and GABRA5 genes were used to establish the orientation of both transcriptional units as centromere/3′GABRB3 5′/5′GABRA5 3′/telomere. These two $GABA_A$ receptor subunits separated by less than 100 kb form a tight cluster, with GABRA5 spanning ~75 kb and GABRB3 ~250 kb. The large size of the β3 subunit is mainly due to its 150-kb intron 3, which makes GABRB3 the largest member of the $GABA_A$ receptor gene family yet described. By comparison, the sizes for the mouse δ subunit gene and the chicken β4 subunit gene were reported as approximately 13 kb and 65 kb, respectively (Sommer et al. 1990; Lasham et al. 1991).

Other $GABA_A$ receptor genes also appear to be organized in clusters (Wilcox et al. 1992), with the α2 and β1 subunits localized to chromosome 4p1-p13 (Buckle et al. 1989), the α1 and γ2 subunits localized to chromosome 5q34-q35 (Buckle et al. 1989; Wilcox et al. 1992), and the ρ1 and ρ2 subunits localized to chromosome 6q14-q21 (Cutting et al. 1992). The significance of the apparent clustering of these $GABA_A$ receptor subunit genes is not known. Other members of the ligand-gated ion-channel receptor superfamily are found clustered in the genome. The genes coding for the δ and γ subunits of the chicken nicotinic acetylcholine receptor (nAChR) are separated by only 740 bp (Nef et al. 1984). In addition, the α3, α5, and β4 genes of the rat nAChR have been shown to be tightly clustered, with nAChR β4 and α3 genes being transcribed in the same direction, while α5 transcripts have opposing polarity (Boulter et al. 1990). In such cases it is suggested that a gene duplication event has led to the differentiation of one subunit type into two. The $GABA_A$ receptor–encoded proteins are members of a multigene family that appears to be conserved through evolution. The similarities in both sequence and genomic organization are strong indications that members of the $GABA_A$ receptor subunits gene family evolved through tandem duplication of a common ancestor. Members of such a gene family may remain tightly clustered or, by a process of translocation, be dispersed to distant loci.

The physical relation between such duplicated sequences seems to be variable. The head-to-head configuration of GABRB3 and GABRA5 was also reported in collagen and histone genes (Hentschell and Birnstiel 1981; Poschl et al. 1988). Whether genomic organization is related to the possibility that their expression might be coordinately regulated, as suggested for GABRA1 and GABRG2, is not known (Wilcox et al. 1992).

The long-range restriction map was used to determine the presence of potential additional genes in the region. This is possible because restriction sites for the rare-cutter enzymes used in pulsed-field mapping often cluster in unmethylated CpG dinucleotides, known as "CpG islands," which are usually associated with the 5′ region of expressed DNA sequences (Bird 1987; Lindsay and Bird 1987; Larsen et al. 1992). It has been predicted that 89% of unmethylated *Not*I sites and 74% of the *Eag*I, *Bss*HII, and *Sac*II sites present in mammalian DNA are located within such CpG islands (Lindsay and Bird 1987). Thus, we can estimate the number of genes mapping to a region on the basis of the number of CpG islands identified within that interval. In our study, restriction analysis of genomic DNA after FIGE allowed the identification of seven clusters of rare-cutter sites within 600 kb. Four of these islands are associated with known sequences: both $GABA_A$ receptor genes have CpG islands associated with their 5′ ends, whereas an additional island is found 3′ of GABRA5. Another CpG island is coincident with the locus MN47 known to be evolutionarily conserved and potentially expressed (Buiting et al. 1990). One CpG island was found proximal to the GABRB3 locus, and the other two islands map within the GABRB3 gene. There is no established case in which an island is not associated with a gene

(Larsen et al. 1992), suggesting that the CpG islands observed 3′ to the GABRB3 transcription start site are associated with the start of unidentified transcripts. There are some examples of such nested genes found in mammalian introns (Williams and Fried 1986; Adelman et al. 1987; Miyajima et al. 1989; Levinson et al. 1992). We are currently searching for expressed sequences associated with these putative CpG islands.

The large size of the deletions in AS and PWS patients makes refinement of the critical region for these disorders difficult. The construction of a long-range map around the GABRB3 and GABRA5 loci allowed the analysis of chromosomal rearrangements in two AS patients. Both breakpoints were mapped at different locations within the large intron 3 of the GABRB3. The breakpoint in the unbalanced translocation patient WJK106 lies at less than 50 kb from exon 3, whereas the deletion breakpoint in patient DS176 is located within 20 kb from exon 4. This deletion presents in three AS sibs and 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 (Hamabe et al. 1991*b*; Saitoh et al. 1992). These results suggest that the AS critical region excludes the chromosomal region distal to GABRB3, although there is a possibility that the DNA rearrangements result in abnormal regulation of genes distal to the breakpoints. In this regard, a deletion upstream of the human β-globin locus has been shown to inactivate the genes of the globin cluster and result in thalassemia (Forrester et al. 1990). The same phenomenon could be involved in an AS patient with a translocation breakpoint located between the GABRB3 locus and D15S10 (Reis et al. 1993), affecting the expression of the more distal genes. The physical map of this gene cluster provides a starting point for cloning the rearranged DNA of such AS patients.

In summary, we have used the combined techniques of FIGE and lambda-phage genomic library screening to construct a physical map around the GABRB3 and GABRA5 gene cluster. This map covers 1.0 Mb and should be valuable for further mapping of genes localized to this chromosomal region. The long-range restriction analysis has allowed the localization of breakpoints, in two AS patients, within GABRB3 and a further refinement of the AS critical region.

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References

Adelman JP, Bond CT, Douglass J, Herbert E (1987) Two mammalian genes transcribed from opposite strands of the same DNA locus. Science 235:1514–1517

Aldridge J, Kunkel L, Bruns G, Tantravahi U, Lalande M, Brewster T, Moreau E, et al (1984) A strategy to reveal high-frequency RFLPs along the human X chromosome. Am J Hum Genet 36:546–564

Betz H (1990) Ligand-gated ion channels in the brain: the amino acid receptor superfamily. Neuron 5:383–392

Bird PA (1987) CpG islands as gene markers in the vertebrate nucleus. Trends Genet 3:324–347

Boulter J, O'Shea-Greenfield A, Duvoisin RM, Connolly JG, Wada E, Jensen A, Gardner PD, et al (1990) α3, α5, and β4: three members of the rat neuronal nicotinic acetylcholine receptor-related gene family form a gene cluster. J Biol Chem 265:4472–4482

Brunk C, Jones K, James T (1979) Assay for nanogram quantities of DNA in cellular homogenates. Anal Biochem 92:497–500

Buckle VJ, Fujita N, Ryder-Cook AS, Derry JMJ, Bernard PJ, Lebo RV, Schofield PR, et al (1989) Chromosomal localization of $GABA_A$ receptor subunit genes: relationship to human genetic disease. Neuron 3:647–654

Buiting K, Neumann M, Ludecke H-J, Senger G, Claussen U, Antich J, Passarge E, et al (1990) Microdissection of the Prader-Willi syndrome chromosome region and identification of potential gene sequences. Genomics 6:521–527

Butler MG (1990) Prader-Willi syndrome: current understanding of cause and diagnosis. Am J Med Genet 35:319–332

Butler MG, Palmer CG (1983) Parental origin of chromosome 15 deletion in Prader-Willi syndrome. Lancet 1:1285–1286

Cattanach BM, Barr JA, Evans EP, Burtenshaw M, Beechey CV, Leff SE, Brannan CI, et al (1992) A candidate mouse model for Prader-Willi syndrome which shows an absence of Snrpn expression. Nature Genet 2:270–282

Clayton-Smith J, Webb T, Pembrey ME, Nichols M, Malcom S (1992) Maternal origin of deletion 15q11-13 in 25/25 cases of Angelman syndrome. Hum Genet 88:376–378

Cutting GR, Curristin S, Zoghbi H, O'Hara B, Seldin MF, Uhl GR (1992) Identification of a putative γ-aminobutyric acid (GABA) receptor subunit rho2 cDNA and colocalization of the genes encoding rho2 (GABRR2) and rho1 (GABRR1) to human chromosome 6q14-q21 and mouse chromosome 4. Genomics 12:801–806

DeLorey TM, Olsen RW (1992) γ-aminobutyric $acid_A$ receptor structure and function. J Biol Chem 267:16747–16750

Feinberg AP, Vogelstein B (1983) A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 132:6–13

Forrester WC, Epner E, Driscoll MC, Enver T, Brice M, Papayannopoulou T, Groudine M (1990) A deletion of the human β-globin locus activation region causes a major alteration in chromatin structure and replication across the entire β-globin locus. Genes Dev 4:1637–1649

Forus A, Myklebost O (1992) A physical map of a 1.3-Mb region on the long arm of chromosome 12, spanning the GLI and LRP loci. Genomics 14:117–120

Gardiner K, Laas W, Patterson D (1986) Fractionation of large mammalian DNA restriction fragments using vertical pulsed-field gradient gel electrophoresis. Somat Cell Mol Genet 12:185–195

Greenberg F, Ledbetter DH (1987) Deletions of proximal 15q without Prader-Willi syndrome. Am J Med Genet 28:813–820

Hamabe J, Fukusima Y, Harada N, Kyohdo A, Matsuo N, Nagai T, Yoshioka A, et al (1991*a*) Molecular study of the Prader-Willi syndrome: deletions, RFLP and phenotype analyses of 50 patients. Am J Med Genet 41:54–63

Hamabe J, Kuroki Y, Imaisumi K, Sugimoto T, Fukushima Y, Yamaguchi A, Izumikawa Y, et al (1991*b*) DNA deletion and its parental origin in Angelman syndrome patients. Am J Med Genet 41:64–68

Hentschell CC, Birnstiel ML (1981) The organization and expression of histone gene families. Cell 25:301–313

Higgins MJ, Hansen MF, Cavenee WK, Lalande M (1989) Molecular detection of chromosomal translocations that disrupt the putative retinoblastoma susceptibility locus. Mol Cell Biol 9:1–5

Hoheisel JD, Nizetic D, Lehrach H (1989) Control of partial digestion combining the enzymes dam methylase and MboI. Nucleic Acids Res 17:8571–8582

Kaplan LC, Wharton R, Elias E, Mandell F, Donlon T, Latt SA (1987) Clinical heterogeneity associated with deletions in the long arm of chromosome 15. Am J Med Genet 28:45–53

Kirkness EF, Fraser CM (1993) A strong promoter element is located between alternative exons of a gene encoding the human $GABA_A$ receptor β3 subunit (GABRB3). J Biol Chem 268:4420–4428

Knoll JHM, Nicholls RD, Magenis RE, Glatt K, Graham JM Jr, Kaplan L, Lalande M (1990) Angelman syndrome: three molecular classes identified with chromosome 15q11q13-specific DNA markers. Am J Hum Genet 47:149–154

Knoll JHM, Nicholls RD, Magenis RE, Graham JM, Lalande M, Latt SA (1989) Angelman and Prader-Willi syndromes share a common chromosome 15 deletion but differ in parental origin of the deletion. Am J Hum Genet 32:285–290

Knoll JHM, Sinnett D, Wagstaff J, Glatt K, Wilcox AS, Whiting PM, Wingrove P, et al (1993) FISH ordering of reference markers and of the gene for the α5 subunit of the γ-aminobutyric receptor within the Angelman and the Prader-Willi syndrome chromosomal regions. Hum Mol Genet 2:183–189

Kofuji P, Wang JB, Moss SJ, Huganir RL, Burt DR (1991) Generation of two forms of the γ-aminobutyric $acid_A$ receptor γ2-subunit in mice by alternative splicing. J Neurochem 56:713–715

Kuwano A, Mutirangura A, Dittrich B, Buiting K, Horsthemke B, Saitoh S, Niikawa N, et al (1992) Molecular dissection of the Prader-Willi/Angelman syndrome region (15q11-13) by YAC cloning and FISH analysis. Hum Mol Genet 1:417–425

Lalande M, Dryja TP, Schreck RR, Shipley J, Flint A, Latt SA (1984) Isolation of human chromosome 13-specific DNA sequences cloned from flow sorted chromosomes and potentially linked to the retinoblastoma locus. Cancer Genet Cytogenet 13:283–295

Larsen F, Gundersen G, Lopez R, Prydz H (1992) CpG islands as gene markers in the human genome. Genomics 13:1095–1107

Lasham A, Vreugdenhil E, Bateson AN, Barnard EA, Darlison MG (1991) Conserved organization of γ-aminobutyric $acid_A$ receptor genes: cloning and analysis of the chicken β4-subunit gene. J Neurochem 57:352–355

Ledbetter DH, Riccardi VM, Airhart SD, Strobel RJ, Keenan BS, Crawford JD (1981) Deletions of chromosome 15 as a cause of the Prader-Willi syndrome. N Engl J Med 304:325–329

Leff SE, Brannan CI, Reed ML, Özçelik T, Francke U, Copeland NG, Jenkins NA (1992) Maternal imprinting of the mouse Snrpn gene and conserved linkage homology with the human Prader-Willi syndrome. Nature Genet 2:259–264

Levinson B, Kenwrick S, Gamel P, Fisher K, Gitschier J (1992) Evidence for a third transcript from the human factor VIII gene. Genomics 14:585–589

Lindsay S, Bird AP (1987) Use of restriction enzymes to detect potential gene sequences in mammalian DNA. Nature 337:336–338

Magenis RE, Toth-Fejel S, Allen LJ, Black M, Brown MG, Budden S, Cohen R, et al (1990) Comparison of the 15q deletions in Prader-Willi and Angelman syndromes: specific regions, extent of deletions, parental origin, and clinical consequences. Am J Med Genet 35:333–349

Malcom S, Clayton-Smith J, Nichols M, Robb S, Webb T, Armour JAL, Jeffreys AJ, et al (1991) Uniparental paternal disomy in Angelman's syndrome. Lancet 337:694–697

Miyajima N, Horiuchi R, Shibuya Y, Fukushige S-I, Matsubara K-I, Toyoshima K, Yamamoto T (1989) Two erbA homologs encoding proteins with different T3 binding capacities are transcribed from opposite DNA strands of the same genetic locus. Cell 57:31–39

Monaco AP, Neve RL, Colletti-Feener C, Bertelson CJ, Kur-

nitt DM, Kunkel LM (1986) Isolation of candidate cDNAs for portions of the Duchenne muscular dystrophy gene. Nature 323:646–650

Nef P, Mauron A, Stalder R, Alliod C, Ballivet M (1984) Structure, linkage, and sequence of the two genes encoding the δ and γ subunits of the nicotinic acetylcholine receptor. Proc Natl Acad Sci USA 81:7975–7979

Nicholls RD, Knoll JHM, Butler MG, Karam S, Lalande M (1989*a*) Genetic imprinting suggested by maternal heterodisomy in nondeletion Prader-Willi syndrome. Nature 342:281–285

Nicholls RD, Knoll JHM, Glatt K, Hersh JH, Brewster TD, Graham JM, Wurster-Hill D, et al (1989*b*) Restriction fragment length polymorphisms within proximal 15q and their use in molecular cytogenetics and the Prader-Willi syndrome. Am J Med Genet 33:66–77

Özçelik T, Leff S, Robinson W, Donlon T, Lalande M, Sanjines E, Schinzel A, et al (1992) Small nuclear ribonucleoprotein polypeptide N (SNRPN), an expressed gene in the Prader-Willi syndrome critical region. Nature Genet 2:265–269

Pembrey M, Fennell SJ, van den Berghe J, Fitchett M, Summers D, Butler L, Clarke C, et al (1989) The association of Angelman's syndrome with deletions within 15q11-13. J Med Genet 26:73–77

Poschl JE, Pollner R, Kuhn K (1988) The genes for the alpha1(IV) and alpha 2(IV) chains of human basement membrane collagen type IV are arranged head-to-head and separated by a bidirectional promoter of unique structure. EMBO J 7:2687–2695

Reis A, Kunze J, Ladanyi L (1993) Exclusion of the $GABA_A$-receptor β3 subunit gene as the Angelman syndrome gene. Lancet 341:122–123

Robinson WP, Bottani A, Yagang X, Balakrishman J, Binkert F, Mächler M, Prader A, et al (1991) Molecular, cytogenetic, and clinical investigations of Prader-Willi syndrome patients. Am J Hum Genet 49:1219–1234

Saitoh S, Kubota T, Ohta T, Hinno Y, Niikawa N, Sugimoto T, Wagstaff J, et al (1992) Familial Angelman syndrome caused by imprinted submicroscopic deletion encompassing $GABA_A$ receptor β3-subunit gene. Lancet 338:366–367

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

Schofield PR, Darlison MG, Fujita N, Burt DR, Stephenson FA, Rodriguez H, Rhee LM, et al (1987) Sequence and functional expression of the GABA-A receptor shows a ligand-gated receptor superfamily. Nature 328:221–227

Smeets DFCM, Hamel BCJ, Marcel MD, Nelen MR, Smeets HJM, Bollen JHM, Smits APT, et al (1992) Prader-Willi syndrome and Angelman syndrome in cousins from a family with a translocation between chromosomes 6 and 15. N Engl J Med 326:807–811

Sommer B, Poustka A, Spurr NK, Seeburg PH (1990) The murine $GABA_A$ receptor δ subunit gene: structure and assignment to human chromosome 1. DNA Cell Biol 9:561–568

Tantravahi U, Nicholls RD, Stroh H, Ringer S, Neve RL, Kaplan L, Wharton R, et al (1989) Quantitative calibration and use of DNA probes for investigating chromosome abnormalities in the Prader-Willi syndrome. Am J Med Genet 33:78–87

Wagstaff J, Chaillet JR, Lalande M (1991*a*) The $GABA_A$ receptor β3 subunit gene: characterization of a human cDNA from chromosome 15q11q13 and mapping to a region of conserved synteny on mouse chromosome 7. Genomics 11:1071–1078

Wagstaff J, Knoll JHM, Fleming J, Kirkness EF, Martin-Gallardo A, Greenberg F, Graham JM Jr, et al (1991*b*) Localization of the gene encoding the $GABA_A$ receptor β3 subunit to the Angelman/Prader-Willi region of human chromosome 15. Am J Hum Genet 49:330–337

Wagstaff J, Knoll JHM, Glatt KA, Shugart YY, Sommer A, Lalande M (1992) Maternal but not paternal transmission of 15q11-13-linked nondeletion Angelman syndrome leads to phenotypic expression. Nature Genet 1:291–294

Whiting P, McKernan RM, Iversen LL (1990) Another mechanism for creating diversity in γ-aminobutyric type A receptor: RNA slicing directs expression of two forms of γ2 subunit, one of which contains a protein kinase C phosphorylation site. Proc Natl Acad Sci USA 87:9966–9970

Wilcox AS, Warrington JA, Gardiner K, Berger R, Whiting P, Altherr MR, Wasmuth JJ, et al (1992) Human chromosomal localization of genes encoding the γ1 and γ2 subunits of the γ-aminobutyric acid receptor indicates that members of this gene family are often clustered in the genome. Proc Natl Acad Sci USA 89:5857–5861

Williams CA, Gray BA, Hendrickson JE, Stone JW, Cantu ES (1989) Incidence of 15q deletion in the Angelman syndrome: a survey of twelve affected persons. Am J Med Genet 32:339–345

Williams CA, Zori RT, Stone JW, Gray BA, Cantu ES, Ostrer H (1990) Maternal origin of 15q11q13 deletions in Angelman syndrome suggests a role for genomic imprinting. Am J Med Genet 35:350–353

Williams T, Fried M (1986) A mouse locus at which transcription from both DNA strands produces mRNAs complementary at their 3′ ends. Nature 322:275–279