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Localization of the Gene Encoding the $GABA_A$ Receptor β3 Subunit to the Angelman/Prader-Willi Region of Human Chromosome 15

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Summary

Deletions of the proximal long arm of chromosome 15 (bands 15q11q13) are found in the majority of patients with two distinct genetic disorders, Angelman syndrome (AS) and Prader-Willi syndrome (PWS). The deleted regions in the two syndromes, defined cytogenetically and by using cloned DNA probes, are similar. However, deletions in AS occur on the maternally inherited chromosome 15, and deletions in PWS occur on the paternally derived chromosome 15. This observation has led to the suggestion that one or more genes in this region show differential expression dependent on parental origin (genetic imprinting). No genes of known function have previously been mapped to this region. We show here that the gene encoding the $GABA_A$ (γ-aminobutyric acid) receptor β3 subunit maps to the AS/PWS region. Deletion of this gene (GABRB3) was found in AS and PWS patients with interstitial cytogenetic deletions. Evidence of β3 gene deletion was also found in an AS patient with an unbalanced 13;15 translocation but not in a PWS patient with an unbalanced 9;15 translocation. The localization of this receptor gene to the AS/PWS region suggests a possible role of the inhibitory neurotransmitter GABA in the pathogenesis of one or both of these syndromes.

Introduction

The majority (approximately 60%) of patients with two distinct genetic disorders, Angelman syndrome (AS) and Prader-Willi syndrome (PWS), show cytogenetic deletions of the proximal long arm of chromosome 15 (15q11q13) (Ledbetter et al. 1981; Kaplan et al. 1987; Pembrey et al. 1989; Williams et al. 1989; Butler 1990). AS patients are characterized by developmental delay, seizures, inappropriate laughter, and ataxic movements (Angelman 1965). PWS patients display infantile hypotonia, childhood hyperphagia and obesity, developmental delay, and hypogonadism (Butler 1990). Cytogenetic and molecular studies have shown that the chromosome 15 deletions in AS occur on the maternally inherited chromosome 15 (Knoll et al. 1989; Magenis et al. 1990; Williams et al. 1990), whereas the deletions in PWS occur on the paternally derived chromosome 15 (Butler and Palmer 1983). The different phenotypes associated with different parental origin of deletions have been attributed to differential expression of one or more genes in this region on paternally versus maternally inherited chromosomes (genetic imprinting) (Knoll et al. 1989; Nicholls et al. 1989).

A number of cloned DNA probes obtained from flow-sorted chromosome 15 libraries (Donlon et al.

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1986) have been localized to the region frequently deleted in AS and PWS, but no genes of known function have previously been mapped to this region. In the present report, we show that the $GABA_A$ receptor β3 subunit gene (GABRB3), a recently characterized member of the GABA (γ-aminobutyric acid) receptor gene family (Lolait et al. 1989*b*; Ymer et al. 1989*b*), maps to the AS/PWS region of chromosome 15.

Material and Methods

Isolation of $GABA_A$ Receptor β3 Subunit Genomic Clones

A 1,761-bp *Bsm*I/*Bgl*II fragment of a cDNA clone encoding the bovine $GABA_A$ receptor β1 subunit (Schofield et al. 1987) was used to screen a human genomic library in vector λEMBL3 (Kirkness et al., in press). A number of λ clones were isolated and mapped with restriction endonucleases. Restriction fragments that hybridized with the β1 cDNA probe were subcloned into plasmid vectors and sequenced.

DNA Sequencing

Sequencing of both strands of exon-containing restriction fragments was performed on single-stranded templates by the dideoxy termination method, using a *Taq* polymerase sequencing system (Promega) on the 370A or 373A automated DNA sequencing systems (Applied Biosystems).

Fluorescence In Situ Hybridization

Hybridization and detection were performed as previously described (Lichter et al. 1990). β3 probes were biotinylated and detected with FITC-labeled avidin. Chromosomes were counterstained with propidium iodide. An R-banded chromosome fluorescence pattern was produced by hybridization with digoxigenin-labeled PCR products generated from human genomic DNA by using a single Alu oligonucleotide primer, as described (Baldini and Ward 1991).

DNA Isolation and Southern Blotting

DNA was isolated from lymphocytes or lymphoblasts (Aldridge et al. 1984) from AS and PWS patients and was digested with *Hin*dIII (Boehringer-Mannheim). Digested DNA fragments were separated by agarose gel electrophoresis, transferred to nylon filters (Hybond N or Hybond N-Plus; Amersham), and hybridized with radiolabeled DNA probes (Feinberg and Vogelstein 1983). Autoradiograms were scanned with an LKB laser scanning densitometer to permit estimation of gene copy number.

PCR Analysis of Flow-sorted Chromosomes

Flow sorting of chromosomes from unbalanced-translocation PWS patient HS32.5 and from deletion AS patient WJK18 was performed as previously described (Lalande et al. 1985). PCR reaction mixes (50-μl volume) contained DNA from approximately 5 × 10^3 flow-sorted chromosomes, 1 U *Taq* polymerase (Perkin Elmer–Cetus), 5 μl 10 × reaction buffer, 0.2 mM each dNTP, and 0.5 μM each primer. Primer sequences, from the rat $GABA_A$ receptor β3 subunit cDNA sequence (Lolait et al. 1989*b*; Ymer et al. 1989*b*), were 5′-GTTGGTGACACCAGGAATTCA-GC-3′ and 5′-GTACAGCCAGTAAACTAAGTTG-3′. Primers for the control β2-microglobulin sequence located in 15q21q22.2 were 5′-TGGGTTTCATC-AATCCGACAT-3′ and 5′-GGCAGGCATACTCA-TCTTTTT-3′ (Gussow et al. 1987). Amplification was performed in a Perkin Elmer–Cetus cycler, with 30 cycles of 1 min at 94°C, 1 min at 60°C, and 90 s at 72°C.

Results

A human genomic DNA library was screened with a cDNA probe encoding the bovine $GABA_A$ receptor β1 subunit (Schofield et al. 1987). In addition to a number of clones containing β1 exons that were isolated from this library (Kirkness et al., in press), two clones, λ28 and λ16, contained exons that were not derived from the β1 subunit gene. Open reading frames from these clones encoded amino acid sequences identical to sequences from the rat and bovine $GABA_A$ receptor β3 subunit (fig. 1). The β3 subunit exons from λ28 and λ16 correspond to exons 4 and 6 of the β1 subunit gene, respectively, and the intron/exon junctions are conserved precisely between the two genes (Kirkness et al., in press). The inferred β3 amino acid sequence differs from either the β1 or β2 sequence by five amino acids encoded by exon 4 and by 16 amino acids encoded by exon 6.

These genomic clones were used as probes for fluorescence in situ hybridization (fig. 2) (Lichter et al. 1990). Clone λ16 hybridized exclusively to the proximal long arm of chromosome 15. Clone λ28 hybridized predominantly to the proximal long arm of chromosome 15, although some hybridization to chromosome 14 was also detected (data not shown). Previous studies (Buckle et al. 1989) have localized the

A. λ28

```
D  Y  T  L  T  M  Y  F  Q  Q  S  W  K  D  K  R  L  S  Y   RAT BETA1
D  Y  T  L  T  M  Y  F  Q  Q  S  W  K  D  K  R  L  S  Y   BOV BETA1
D  Y  T  L  T  M  Y  F  Q  Q  A  W  R  D  K  R  L  S  Y   RAT BETA2
D  Y  T  L  T  M  Y  F  Q  Q  A  W  R  D  K  R  L  S  Y   BOV BETA2
D  Y  T  L  T  M  Y  F  Q  Q  Y  W  R  D  K  R  L  A  Y   RAT BETA3
D  Y  T  L  T  M  Y  F  Q  Q  Y  W  R  D  K  R  L  A  Y   BOV BETA3

D  Y  T  L  T  M  Y  F  Q  Q  Y  W  R  D  K  R  L  A  Y   λ28
                              *     *              *

GAT TAT ACC TTA ACC ATG TAT TTT CAA CAA TAT TGG AGA GAT AAA AGG CTC GCC TAT


S  G  I  P  L  N  L  T  L  D  N  R  V  A  D  Q  L  W  V   RAT BETA1
S  G  I  P  L  N  L  T  L  D  N  R  V  A  D  Q  L  W  V   BOV BETA1
N  V  I  P  L  N  L  T  L  D  N  R  V  A  D  Q  L  W  V   RAT BETA2
N  V  I  P  L  N  L  T  L  D  N  R  V  A  D  Q  L  W  V   BOV BETA2
S  G  I  P  L  N  L  T  L  D  N  R  V  A  D  Q  L  W  V   RAT BETA3
S  G  I  P  L  N  L  T  L  D  N  R  V  A  D  Q  L  W  V   BOV BETA3

S  G  I  P  L  N  L  T  L  D  N  R  V  A  D  Q  L  W  V    λ28
*  *
TCT GGG ATC CCT CTC AAC CTC ACG CTT GAC AAT CGA GTG GCT GAC CAG CTA TGG GTG


P  D  T  Y  F  L  N  D  K  K  S  F  V  H  G  V  T  V  K   RAT BETA1
P  D  T  Y  F  L  N  D  K  K  S  F  V  H  G  V  T  V  K   BOV BETA1
P  D  T  Y  F  L  N  D  K  K  S  F  V  H  G  V  T  V  K   RAT BETA2
P  D  T  Y  F  L  N  D  K  K  S  F  V  H  G  V  T  V  K   BOV BETA2
P  D  T  Y  F  L  N  D  K  K  S  F  V  H  G  V  T  V  K   RAT BETA3
P  D  T  Y  F  L  N  D  K  K  S  F  V  H  G  V  T  V  K   BOV BETA3

P  D  T  Y  F  L  N  D  K  K  S  F  V  H  G  V  T  V  K   λ28

CCC GAC ACA TAT TTC TTA AAT GAC AAA AAG TCA TTT GTG CAT GGA GTG ACA GTG AAA


N  R  M  I  R  L  H  P  D  G  T  V  L  Y  G  L              RAT BETA1
N  R  M  I  R  L  H  P  D  G  T  V  L  Y  G  L              BOV BETA1
N  R  M  I  R  L  H  P  D  G  T  V  L  Y  G  L              RAT BETA2
N  R  M  I  R  L  H  P  D  G  T  V  L  Y  G  L              BOV BETA2
N  R  M  I  R  L  H  P  D  G  T  V  L  Y  G  L              RAT BETA3
N  R  M  I  R  L  H  P  D  G  T  V  L  Y  G  L              BOV BETA3

N  R  M  I  R  L  H  P  D  G  T  V  L  Y  G  L              λ28

AAC CGC ATG ATC CGT CTT CAC CCT GAT GGG ACA GTG CTG TAT GGG CTC
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B. λ16

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G  Y  T  T  D  D  I  E  F  Y  W  N  G  G  E  G  A  V  T   RAT BETA1
G  Y  T  T  D  D  I  E  F  Y  W  N  G  G  E  G  A  V  T   BOV BETA1
G  Y  T  T  D  D  I  E  F  Y  W  R  G  D  D  N  A  V  T   RAT BETA2
G  Y  T  T  D  D  I  E  F  Y  W  R  G  D  D  N  A  V  T   BOV BETA2
G  Y  T  T  D  D  I  E  F  Y  W  R  G  G  D  K  A  V  T   RAT BETA3
G  Y  T  T  D  D  I  E  F  Y  W  R  G  G  D  K  A  V  T   BOV BETA3

G  Y  T  T  D  D  I  E  F  Y  W  R  G  G  D  K  A  V  T   λ16
                              *     *  *  *
GGC TAC ACC ACG GAT GAC ATT GAG TTT TAC TGG CGA GGC GGG GAC AAG GCT GTT ACC


G  V  N  K  I  E  L  P  Q  F  S  I  V  D  Y  K  M  V  S   RAT BETA1
G  V  N  K  I  E  L  P  Q  F  S  I  V  D  Y  K  M  V  S   BOV BETA1
G  V  T  K  I  E  L  P  Q  F  S  I  V  D  Y  K  L  I  T   RAT BETA2
G  V  T  K  I  E  L  P  Q  F  S  I  V  D  Y  K  L  I  T   BOV BETA2
G  V  E  R  I  E  L  P  Q  F  S  I  V  E  H  R  L  V  S   RAT BETA3
G  V  E  R  I  E  L  P  Q  F  S  I  V  E  H  R  L  V  S   BOV BETA3

G  V  E  R  I  E  L  P  Q  F  S  I  V  E  H  R  L  V  S   λ16
      *  *                          *  *  *  *  *  *
GGA GTG GAA AGG ATT GAG CTC CCG CAG TTC TCC ATC GTG GAG CAC CGT CTG GTC TCG


K  K  V  E  F  T  T                                        RAT BETA1
K  K  V  E  F  T  T                                        BOV BETA1
K  K  V  V  F  S  T                                        RAT BETA2
K  K  V  V  F  S  T                                        BOV BETA2
R  N  V  V  F  A  T                                        RAT BETA3
R  N  V  V  F  A  T                                        BOV BETA3

R  N  V  V  F  A  T                                        λ16
*  *     *     *
AGG AAT GTT GTC TTC GCC ACA
```

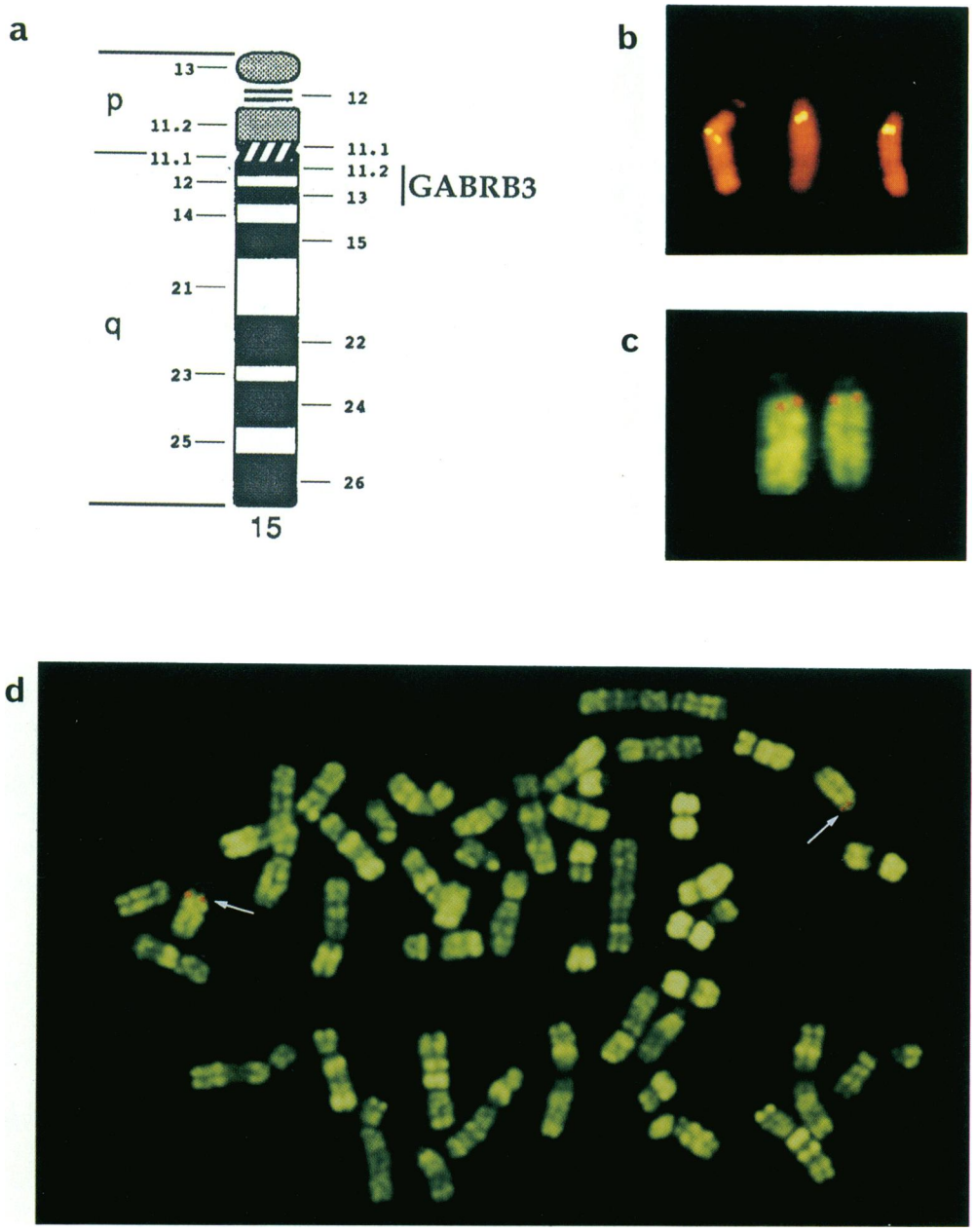

Figure 2 Chromosomal localization of $GABA_A$ receptor β3 subunit clone, λ16, established by fluorescence in situ hybridization. *a*, R-banded ideogram of chromosome 15, depicting region of hybridization with clone λ16 (bands 15q11q13). The location of the β3 gene is indicated by the locus designation GABRB3. *b*, Montage of three chromosome 15s after hybridization with biotinylated λ16 probe. *c*, Closeup view of two chromosome 15s from panel *d*. *d*, Metaphase chromosome spread cohybridized with biotinylated λ16 probe and digoxigenin-labeled PCR products generated from human genomic DNA by using single Alu oligonucleotide primer. The hybridization signal of the β3 probe (red) is indicated by arrows. The R-banded chromosome fluorescence pattern is produced by the Alu PCR probe set. Note that this does not label the centromeric regions or the short arm of chromosome 15.

Figure 1 Nucleotide sequences of open reading frames from clones λ28 (*A*) and λ16 (*B*), and deduced amino acid sequences. Amino acid sequences of corresponding portions of the rat and bovine $GABA_A$ receptor β1, β2, and β3 subunit genes (positions 56–128 and positions 158–202 in fig. 2 of Ymer et al. 1989*b*) are shown for comparison. Residues that distinguish β3 subunit sequences from β1 or β2 subunit sequences are designated by asterisks (*).

$GABA_A$ receptor β1 subunit gene to chromosome 4, so this cross-hybridization is likely to be due to sequence similarity between the β3 subunit gene and an as yet unmapped β-related gene.

DNA from AS patients and PWS patients with cytogenetic and molecular deletions (Tantravahi et al. 1989; Knoll et al. 1990) was analyzed by Southern hybridization for evidence of deletion of β3 subunit gene sequences. The two probes used were 16β3-H8, a 0.4-kb *Hin*dIII subclone of λ16, and 28β3-H3, a 2.5-kb *Hin*dIII subclone of λ28. Of eight AS patients and six PWS patients with interstitial deletions of chromosome 15q11q13, all were heterozygous for a deletion of 28β3-H3 (fig. 3) and 16β3-H8 (data not shown).

Although most PWS and AS patients with cytogenetic abnormalities have interstitial deletions of 15q11q13, some patients with these disorders lack 15q11q13 as a result of unbalanced chromosomal translocations. One such AS patient, WJK106, who has an unbalanced 13;15 translocation resulting from aberrant segregation of a maternal reciprocal translocation (Greenberg and Ledbetter 1987), was deleted for 28β3-H3 (fig. 4) and 16β3-H8 (data not shown), as were AS patients with interstitial deletions. HS32.5, a patient with PWS due to an unbalanced 9;15 translocation, displayed a heterozygous deletion of proximal probes 34 (locus D15S9), 189-1 (D15S13), and IR4-3R (D15S11) and showed deletion of a paternal *Sac*I allele detected with probe IR39d (D15S18) (J. H. M. Knoll and M. Lalande, unpublished results). This patient was not deleted for either 28β3-H3 (fig. 4), 16β3-H8, or the more distal marker IR10-1 (locus D15S12) (J. H. M. Knoll and M. Lalande, unpublished results). When DNA from flow-sorted translocation chromosomes (der(9)) from patient HS32.5 was analyzed by the PCR using primers specific for exon 9 of the β3 gene, there was no evidence for deletion of β3 sequences. (fig. 5). By contrast, PCR analysis of flow-sorted chromosome 15s from an AS patient with an interstitial deletion of chromosome 15q11q13 showed absence of exon 9 sequences on one of the two chromosome 15s (fig. 5).

Approximately 40% of AS and PWS patients show no cytogenetic or molecular evidence of deletion (Pembrey et al. 1989; Williams et al. 1989; Butler 1990). In PWS, most of these patients have two maternal chromosome 15s (maternal uniparental disomy) (Nicholls et al. 1989), supporting the inference that PWS results from absence of a paternal copy of one or more imprinted genes. In AS, most nondeletion patients have both a maternal and a paternal chromosome 15 (Knoll et al. 1991), and the reason for their AS phenotype is not known. Two nondeletion AS patients showed no evidence for β3 subunit gene deletion when probes 28β3-H3 (fig. 4) and 16β3-H8 (data not shown) were used. This result rules out submicroscopic deletion of the entire β3 gene in these patients,

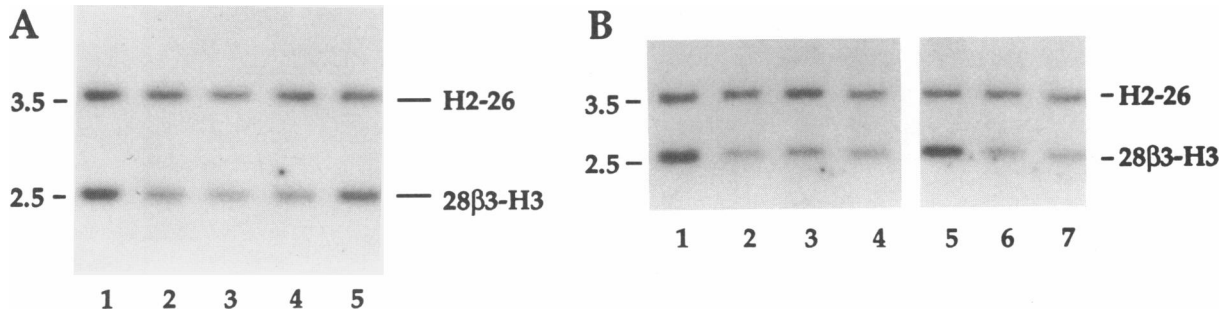

Figure 3 Dosage analysis of $GABA_A$ receptor β3 subunit probe 28β3-H3 in PWS and AS patients with interstitial deletions of chromosome 15q11q13. Filters were hybridized with probe 28β3-H3 and with a control probe, H2-26 (D13S28), from chromosome 13 (Lalande et al. 1984). Autoradiograms were scanned with an LKB laser scanning densitometer to permit estimation of gene copy number. *A,* Analysis of PWS patients. Lane 1, WJK76, control female. Lane 2, DS21, deletion PWS patient. Lane 3, DS40, deletion PWS patient. Lane 4, HS24, deletion PWS patient. Lane 5, HS30, nondeletion PWS patient. Gene copy numbers detected with 28β3-H3 and based on two copies per genome in WJK76 were DS21, 1.3; DS40, 1.0; HS24, 1.0; and HS30, 1.8. Probe 28β3-H3 detects deletions in DS21, DS40, and HS24. *B,* Analysis of AS patients. Lane 1, GM7000, control male. Lane 2, WJK116, deletion AS patient. Lane 3, WJK119, deletion AS patient. Lane 4, WJK53, deletion AS patient. Lane 5, JK, control female. Lane 6, WJK48, deletion AS patient. Lane 7, WJK67, deletion AS patient. All seven lanes are from the same autoradiogram. Gene copy numbers detected with 28β3-H3 and based on two copies per genome in GM7000 and JK are WJK116, 1.1; WJK119, 1.2; WJK53, 0.7; WJK48, 0.8; and WJK67, 0.9. 28β3-H3 detects deletions in WJK116, WJK119, WJK53, WJK48, and WJK67.

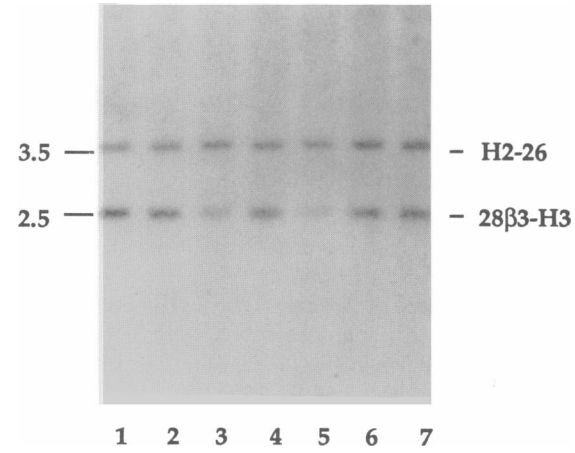

Figure 4 Dosage analysis of $GABA_A$ receptor β3 subunit probe 28β3-H3 in translocation and nondeletion AS and PWS patients. Filters were hybridized with probe 28β-H3 and with a control probe, H2-26 (D13S28), from chromosome 13. Autoradiograms were scanned with an LKB laser scanning densitometer to permit estimation of gene copy number. Lane 1, WJK104, normal father of AS patient WJK106. Lane 2, WJK105, balanced carrier of 13;15 translocation, mother of WJK106. Lane 3, WJK106, unbalanced translocation AS patient, 45,XY, − 13, − 15, + der(13)t(13;15) (p13;q13) mat. Lane 4, HS32.5, unbalanced translocation PWS patient, 45,XY, − 9, − 15, + der(9)t(9;15) (q34.3;q11.2). Lane 5, WJK107, interstitial deletion AS patient. Lane 6, WJK113, nondeletion AS patient. Lane 7, WJK121, nondeletion AS patient. Gene copy numbers detected with probe 28β23-H3 and based on two copies per genome in WJK104 and in two other control individuals on the same autoradiogram (not shown) are WJK105, 2.4; WJK106; 1.1; HS32.5, 2.0; WJK107, 0.6; WJK113, 1.7; and WJK121, 2.1. Probe 28β3-H3 detects a deletion in WJK106 and WJK107 but not in PWS patient HS32.5 or in nondeletion AS patients WJK113 and WJK121.

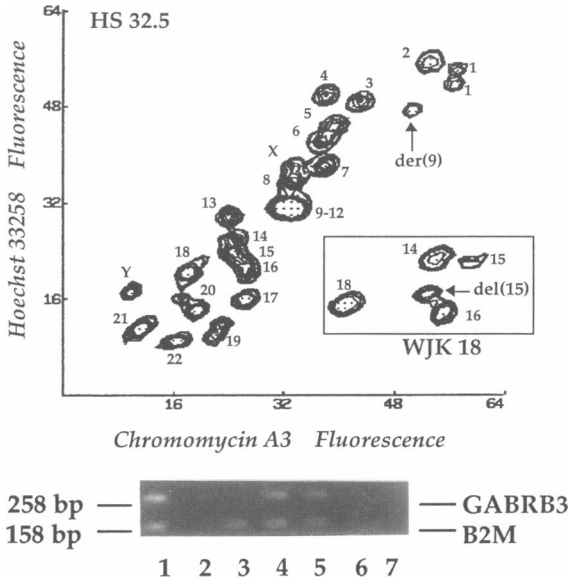

Figure 5 PCR analysis of β3 exon 9 in flow-sorted chromosomes from AS and PWS patients. *Top,* Flow karyotype of unbalanced translocation patient HS32.5, showing normal chromosome 15 and 9;15 translocation chromosome (der(9)) deleted for proximal 15q and also showing (*inset*) partial flow karyotype of deletion AS patient WJK18, showing resolution of deleted chromosome 15 (del15)) from normal chromosome 15. *Bottom,* PCR analysis of DNA from flow-sorted chromosomes. Primer sequences from the rat β3 cDNA sequence amplify a 258-bp fragment, corresponding to a portion of β3 exon 9, from both rat and human genomic DNA (designated GABRB3). Primer sequences from the human β2 microglobulin gene in 15q21q22.2 (designated B2M) amplify a 158-bp fragment. Lane 1, Total human genomic DNA. Lane 2, chromosome 13. Lane 3, Deleted chromosome 15 from WJK18. Lane 4, Intact chromosome 15 from WJK18. Lane 5, der(9) chromosome from HS32.5. Lane 6, chromosome 17. Lane 7, no DNA.

although they may have more subtle β3 gene mutations.

Discussion

GABA is the major inhibitory neurotransmitter in the mammalian brain, where it acts at $GABA_A$ receptors, which are ligand-gated chloride channels. Chloride conductance of these channels can be modulated by agents such as benzodiazepines that bind to the $GABA_A$ receptor (Olsen and Venter 1986). Recent molecular analysis of $GABA_A$ receptors has revealed a previously unsuspected multiplicity of receptor subunit types, with at least 13 distinct subunits (α1–α6, β1–β3, γ1, γ2, δ, and ρ1) (Schofield et al. 1987; Levitan et al. 1988; Khrestchatisky et al. 1989; Lolait et al. 1989*b;* Shivers et al. 1989; Ymer et al. 1989*a,* 1989*b;* Luddens et al. 1990; Ymer et al. 1990; Cutting et al. 1991). The stoichiometry of association of these subunits in individual GABA receptors in vivo is unknown. Among numerous possible physiologic roles for GABA, evidence from both in vitro (Taylor 1988) and in vivo (Meldrum 1989) studies indicates a role for GABA in the suppression of seizure activity. Genetic analysis, either by implication of specific receptor subunits in human diseases or by gene manipulation in experimental systems, will provide the most powerful approach for assigning specific functional roles to the members of this multigene family.

We have found deletions of GABRB3 in AS patients with cytogenetic deletions and in an AS patient with an unbalanced translocation. Although most cytogenetically abnormal PWS patients also were deleted for

probes from the β3 subunit gene, one unbalanced-translocation PWS patient in our study was not deleted for these probes, raising the possibility that deletion of this gene is not necessary for the PWS phenotype. Previous studies have localized β3 subunit gene expression to rat cerebral cortex, caudate, hippocampus, periventricular thalamic nucleus, and cerebellum (Lolait et al. 1989*a*). Defects in GABA receptor function in these regions might be expected to produce seizures, uncontrolled behaviors, and disorders of movement, all of which are aspects of the AS phenotype. The observed effect of parental origin on the phenotypes produced by 15q11q13 deletions suggests that, if this receptor subunit gene plays a role in the pathogenesis of AS, the gene may be expressed preferentially on the maternal chromosome 15. We have not seen evidence of submicroscopic GABRB3 deletion in AS patients without cytogenetic deletions. If the β3 subunit gene plays a role in AS, inactivation of the gene in nondeletion AS patients could be due to either point mutations or epigenetic mechanisms. Further studies will be required to determine whether the structure and function of this gene are completely normal in the translocation PWS patient reported here, whether some nondeletion AS patients have point mutations in this gene, and whether deletion of GABRB3 is responsible for some aspects of the phenotypes in AS or PWS.

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