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Angelman Syndrome: Validation of Molecular Cytogenetic Analysis of Chromosome 15q11-q13 for Deletion Detection

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In a series of 18 individuals comprising parents of Angelman syndrome (AS) patients and AS patients with large deletions, microdeletions, and no deletions, we utilized fluorescence in situ hybridization (FISH) with genomic phage clones for loci D15S63 and GABRB3 for deletion detection of chromosome 15q11-q13. Utilization of probes at these loci allows detection of common large deletions and permits discrimination of less common small deletions. In all individuals the molecular cytogenetic data were concordant with the DNA deletion analyses. FISH provides an accurate method of deletion detection for chromosome 15q11-q13.

KEY WORDS: chromosome 15, Angelman Syndrome, FISH, deletion

INTRODUCTION

Angelman syndrome was first reported in 1965 by Dr. Angelman who described three children with happy demeanor, severe mental retardation, ataxic movements, absent speech, and seizures [Angleman, 1965]. A cytogenetic aberration involving the loss of chromosome 15q11-q13 was reported more than two decades later [Kaplan et al., 1987; Magenis et al., 1987]. Subsequently, cytogenetic analysis by GTG banding has shown deletions in approximately half of patients [Williams et al., 1989; Pembrey et al., 1989] and DNA analyses have documented deletions in more than 70% of patients [Zackowski et al., 1993; Beuten et al., 1993]. The lower deletion detection frequency by standard cytogenetic analysis is attributed to the proximity of the region to the centromere, differential condensation between the homologues, and the overall resolution of the testing. Since the recurrence risks to families with an AS child are different depending on the presence or absence of a deletion and the size of the deletion, it is important to determine accurately whether a deletion exists and if so to what extent. To evaluate the utility of FISH for deletion detection, we studied a series of AS patients and parents for which DNA analysis had been previously performed.

MATERIALS AND METHODS

Patients

EBV-transformed cell lines or fixed cell pellets of peripheral lymphocytes from 12 previously reported patients were utilized (Table I). Patients with differing sizes of deletion or no deletion were selected to test the utility of FISH. Fixed cells from six parents were also included in the study.

Cytogenetic preparations

Metaphase chromosomes were obtained from EBV-transformed lymphoblastoid lines by adding colcemid (final 10 μg/ml) for 1 hour, treating with 0.075 M KCl for 15 minutes, and fixing with 3:1 methanol: acetic acid. Slides of these fixed cell suspensions and those of lymphocyte fixed cell pellets that had been stored at 4°C for more than a year were prepared and stored at room temperature for several hours to several weeks prior to hybridization. All slide preparations were coded to preclude analysis bias.

DNA Probes

Two phage clones, JP3 (D15S63) and 16B3 (GABRB3) which have 15 kb and 16 kb inserts, respectively, were utilized for fluorescence in situ hybridization [Knoll et al., 1993]. Purified phage clones were labeled with digoxigenin 11-dUTP (Boehringer Mannheim, Indianapolis, IN) or with biotin 16-dUTP (Boehringer Mannheim) by nick translation and precipitated. The relative position of D15S63 and GABRB3 with respect to common AS deletions and other 15q11-q13 specific

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TABLE I. Comparison of Molecular Cytogenetic Data and DNA Analysis

	FISH analysis[a]		
	JP3 (D15S63)	16B3 (GABRB3)	DNA analysis
AS patients			
WJK1	+	+	Nondeletion
WJK4	+	+	Nondeletion
WJK14	+	+	Nondeletion
WJK15	+	+	Nondeletion
WJK18	−	−	Deletion
WJK24	−	−	Deletion
WJK36	−	−	Deletion
WJK43	−	−	Deletion
WJK48	−	−	Deletion
WJK67	−	−	Deletion
WJK70	−	−	Deletion
YS	+	−	Microdeletion
Parents			
KS (mother of YS)	+	−	Microdeletion
WJK20	+	+	Nondeletion
WJK25	+	+	Nondeletion
WJK30	+	+	Nondeletion
WJK68	+	+	Nondeletion
WJK71	+	+	Nondeletion

[a] +, no deletion; −, deletion. DNA results on the AS patients and KS were previously reported (WJK patients by Knoll et al. [1990]; YS, KS by Hamabe et al. [1991] and Saitoh et al. [1992]).

loci are shown in Figure 1. Our strategy for probe selection was to utilize a minimum number of sequences that would allow detection and discrimination of the common large deletions [Knoll et al., 1990] and the rare small deletions [Saitoh et al., 1992].

Fluorescence In Situ Hybridization

Cytogenetic preparations were denatured for 2 minutes in 70% formamide, 2 × SSC (pH 7) at 70°C, and dehydrated in an ethanol series. Then, 150 ng of biotinylated and/or digoxigenated DNA probe was lyopholized with 10 μg CotI DNA and mixed with 20 μl hybridization mix (50% formamide, 2 × SSC, 10% dextran sulphate). After denaturation at 70°C for 10 minutes and preannealing at 37°C for 30 minutes, the hybridization mixture was applied to each slide and sealed under a coverslip. Slides were placed in a 37°C moist chamber overnight, followed by three 30 minute washes in 50% formamide/2 × SSC at 37°C and 1 × SSC at room temperature. Detection of hybridized biotinylated probes and digoxigenin-labeled probes with FITC-conjugated avidin (7 μg/ml 1% BSA/4 × SSC) and rhodamine-conjugated digoxigenin antibodies (7 μg/ml 1% BSA/4 × SSC), respectively, was performed at 37°C. Slides were

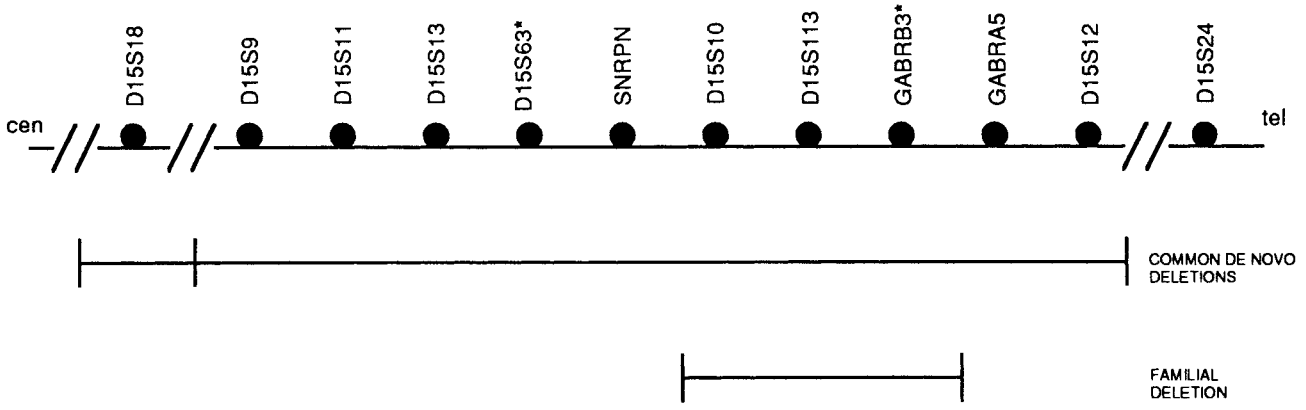

Fig. 1. Map of molecular deletions in AS. Large deletions, which span D15S18 and/or D15S9 through D15S12, are observed in most deletion positive patients [Knoll et al., 1990]. A smaller deletion, which includes D15S10 through GABRB3, was observed in three AS sibs who inherited the deletion from their mother [Hamabe et al., 1991; Saitoh et al., 1992]. * indicates the loci examined by FISH for deletion detection. D15S63 is contained within the large common deletion, while GABRB3 is within both the large de novo deletions and the smaller familial deletion. The order of 15q11-q13 loci was determined previously by FISH interphase cell gene ordering and deletion mapping [Kuwano et al., 1992; Knoll et al., 1993]. cen refers to centromere and tel to telomere.

washed 5 minutes each in 4 × SSC/0.1% Triton × and 4 × SSC. The chromosomes and nuclei were counterstained with DAPI (100 ng/ml in PBS) and mounted in antifade. DAPI counterstaining results in G-banding and permits chromosome identification. Each new batch of labeled probe was hybridized on control lymphoblast/lymphocyte slides in order to assess background fluorescence and ensure that the hybridization efficiency was greater than 90%. This FISH procedure is described in detail elsewhere [Knoll and Lichter, 1994].

Hybridization signals were viewed with an epifluorescence microscope equipped with a dual band (FITC/Texas Red; Omega Optical, Brattleboro, VT) and triple band pass filter set (FITC/Texas Red/DAPI; Omega Optical). The counterstain was viewed through a standard single band pass filter set for DAPI (Zeiss, Germany). Cells were photographed with color film (Kodak Ektar 1000 film). Hybridization signals on 10 to 20 metaphases were examined and/or 20 interphase cells were examined.

RESULTS

There was complete concordance between the molecular cytogenetic data and the DNA data. The results are presented in Table I. All DNA deletions were detectable by FISH including the two individuals, YS and KS, who had smaller deletions that included 16B3 but not JP3. All patients and parents without deletions documented no detectable deletions by FISH. Parental specimens with the exception of KS served as nondeletion controls. In all deletion and nondeletion individuals, more than 90% of metaphase spreads and interphase cells disclosed 1 or 2 chromosome hybridizations, respectively. Figure 2 provides examples of metaphase hybridizations in a deletion and nondeletion individual. Interphase analysis was very useful for detecting deletions. Two probe-2 color hybridizations with both phage clones were utilized to unequivocally identify the chromosome 15s on cell preparations with some background fluorescence.

DISCUSSION

Angelman syndrome, a well-recognized syndrome with an estimated incidence of 1 in 20,000, results from the absence of a maternal contribution of chromosome 15q11-q13 sequences in most cases [Knoll et al., 1989; Malcolm et al., 1991]. Most deletions are de novo and span a large molecular region but a smaller familial deletion in a three-generation family has been reported

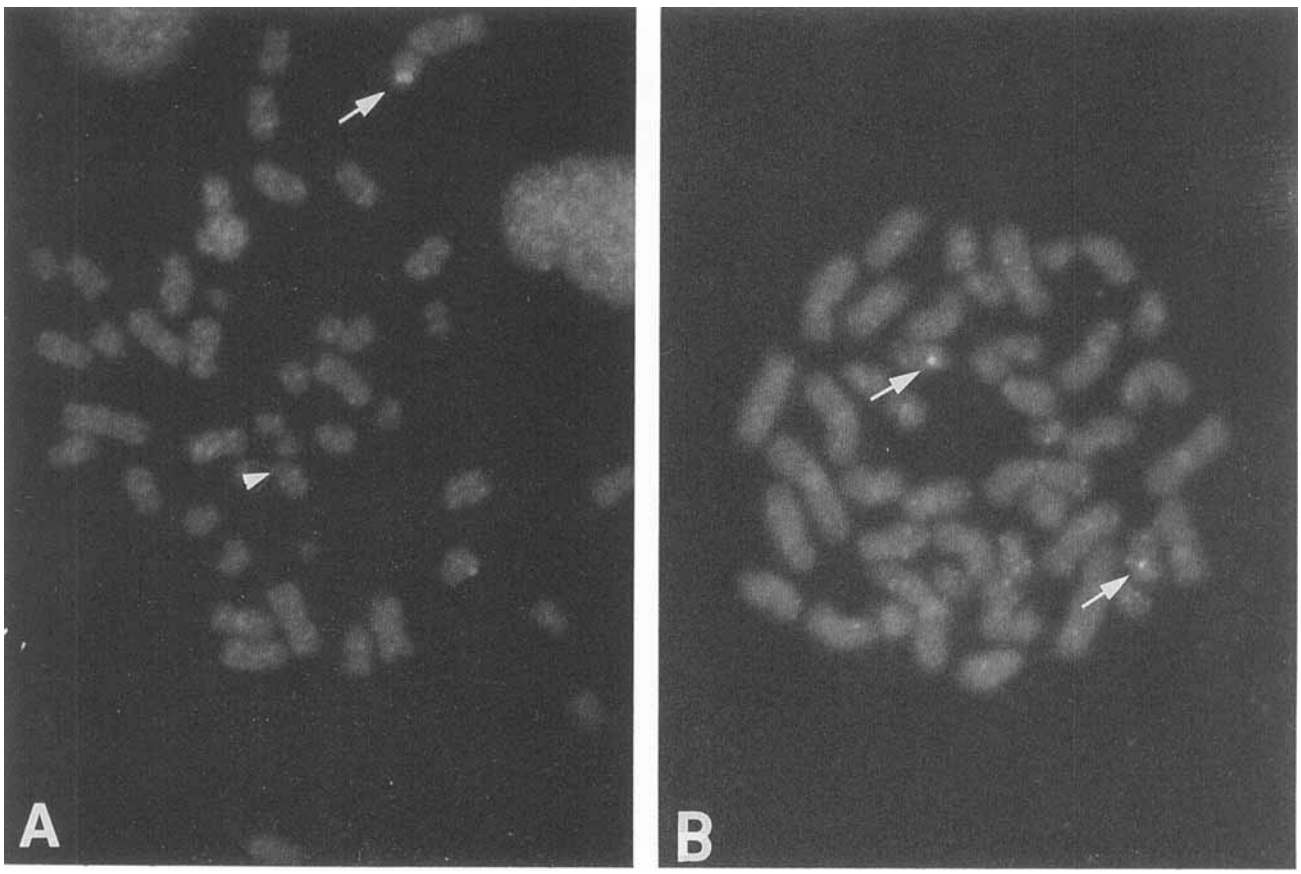

Fig. 2. FISH analysis of chromosome 15s from WJK24 and WJK20. **A**: Hybridization of probe JP3 (D15S63) on metaphase chromosomes from deletion positive patient WJK24. One chromosome 15 is hybridized (arrow), the other is not (arrowhead). **B**: Hybridization of probe 16B3 (GABRB3) on metaphase chromosomes from deletion negative parent WJK20. Both chromosomes 15 show hybridization (arrows).

(Fig. 1). As the recurrence risks to a family range from as low as that of the general population (common large deletions) to as high as 50% (small familial deletions and nondisomic nondeletions), it is necessary to detect differences in deletion size or the absence of a deletion.

In this study, we utilized molecular cytogenetics on cells of AS patients and their parents to assess the presence or absence of deletions and to determine the size of the deletion. Probes were selected so as to provide a way of discriminating large molecular deletions from absent or smaller deletions. GABRB3 is included within the smallest familial deletion and D15S63 is proximal to that region (Fig. 1). Probes for both loci are deleted in large deletions. The FISH interpretations were consistent with the previously identified molecular classification in all individuals examined (Table I). The smaller deletions in YS and KS were discriminated from the larger deletions or no deletions. These results provide validation of molecular cytogenetics for diagnostic purposes.

For those patients in which small deletions/rearrangements or no deletions are detected, additional testing is recommended. In the case of small deletions/rearrangements, additional testing with adjacent 15q11-q13 sequences should be performed to determine the extent of the alternation and parental specimens should be analyzed to determine if the alteration is de novo or familial. For apparent nondeletion cases additional testing to exclude uniparental disomy [Malcolm et al., 1991], locus specific methylation aberrations [Dittrich et al., 1992; Driscoll et al., 1992; Glenn et al., 1993; Reis et al., 1994], or biparental inheritance of chromosome 15q11-q13 with no known aberration [Wagstaff et al., 1992] should be performed. This additional testing, with the possible exception of uniparental disomy testing which can be determined by examining the level of replication asynchrony of 15q11-q13 sequences on hybridized interphase nuclei [unpublished data; Kitsberg et al., 1993; Knoll et al., 1994], requires DNA analysis of the proband and parents.

While this study examined only AS patients, probes for D15S63 and GABRB3 are also useful for detecting deletions in most Prader-Willi syndrome (PWS) patients since they have the same large deletions as AS individuals. FISH at these loci provides an accurate and rapid means for determining deletions in most patients. It is advantageous over quantitative DNA analysis in that it permits detection of chromosome rearrangements that do not result in a deletion (i.e., translocations and inversions). As the gene(s) causing AS or PWS are elucidated, the strategy of probes utilized for FISH will be defined further.

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NOTE ADDED IN PROOF

Since submission of this manuscript, Weyerts et al. [1994] reported on an AS patient with a deletion that resulted from an unbalanced segregation product of a maternal balanced cryptic translocation involving the pericentromeric regions of chromosomes 14 and 15. This finding suggests that the location of the centromeric region of chromosome 15 be examined on deletion positive patients as the recurrence risks will be greater if a familial cryptic translocation is found.

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