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Array-Based Comparative Genomic Hybridization Analysis of Recurrent Chromosome 15q Rearrangements

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Genomic rearrangements of chromosome 15q11-q13 cause diverse phenotypes including autism, Prader–Willi syndrome (PWS), and Angelman syndrome (AS). This region is subject to genomic imprinting and characterized by complex combinations of low copy repeat elements. Prader–Willi and Angelman syndrome are caused primarily by 15q11-13 deletions of paternal and maternal origin, respectively. Autism is seen with maternal, but not paternal, interstitial duplications. Isodicentric 15q, most often of maternal origin, is associated with a complex phenotype often including autistic features. Limitations of conventional cytogenetic tests preclude a detailed analysis in most patients with 15q rearrangements. We have developed a microarray for comparative genomic hybridization utilizing 106 genomic clones from chromosome 15q to characterize this region. The array accurately localized all breakpoints associated with gains or losses on 15q. The results confirmed the location of the common breakpoints associated with interstitial deletions and duplications. The majority of idic(15q) chromosomes are comprised of symmetrical arms with four copies of the breakpoint 1 to breakpoint 5 region. Patients with less common breakpoints that are not distinguished by routine cytogenetic methods were more accurately characterized by array analysis. This microarray provides a detailed characterization for chromosomal abnormalities involving 15q11-q14 and is useful for more precise genotype–phenotype correlations for autism, PWS, AS, and idic(15) syndrome.

KEY WORDS: Angelman syndrome; autism; chromosome 15; comparative genomic hybridization; isodicentric 15; Prader–Willi syndrome

INTRODUCTION

The chromosome 15q11-q13 interval is complex, having low copy repeat sequences, a high frequency of rearrangements, and the presence of imprinted genes. Phenotypes associated with rearrangements of 15q11-q13 include autism, Prader–Willi syndrome (PWS), and Angelman syndrome (AS). Autism is a neurodevelopmental disorder of early childhood characterized by social and communicative impairments and unusual patterns of stereotypical–repetitive behaviors [Bailey et al., 1996; Filipek et al., 2000]. Autism overlaps clinically with several neurodevelopmental disorders (e.g., AS, fragile X syndrome, Rett syndrome) [Barton and Volkmar, 1998; Folstein and Rosen-Sheidley, 2001; Peters et al., 2004; Trillingsgaard and Ostergaard, 2004; Veltman et al., 2004].

Numerous cytogenetic abnormalities have been detected in patients with autism, including segmental aneuploidies and translocations involving most chromosomes. The frequency of 15q11-q13 abnormalities is 0.5 to 3% making them the most common autosomal cytogenetic abnormality in autistic disorder [Browne et al., 1997; Cook et al., 1997; Schroer et al., 1998; Bolton et al., 2001; Borgatti et al., 2001; Roberts et al., 2002, 2003; Thomas et al., 2002]. Most of these abnormalities are of maternal origin suggesting that an imprinted gene in the region plays a role [Cook et al., 1997; Schroer et al., 1998; Bolton et al., 2001]. The 15q11-q13 aberrations in autism overlap the PWS and AS critical region, and both syndromes have phenotypic overlap with autism [Peters et al., 2004; Trillingsgaard and Ostergaard, 2004; Veltman et al., 2004]. Additionally, there are suggestions that the variable behavioral problems, including autism, in patients with AS/PWS, might be correlated with the nature and size of the 15q rearrangements [Butler et al., 2004; Varela et al., 2004]. Despite the high frequency of 15q11-q13 aberrations in autism, evidence for linkage to this region in multiplex autism families has been weak [Cook et al., 1998; Salmon et al., 1999; Liu et al., 2001; Nurmi et al., 2001; Shao et al., 2003]. The weak genetic linkage or association might be compatible with a major de novo component in the etiology as we have suggested in a mixed genetic and epigenetic model for autism [Jiang et al., 2004].

The existence of large low-copy-repeats (LCRs) in the vicinity of the three common PWS/AS chromosomal breakpoints (BP) and also telomeric to the PWS/AS critical region is suggested to be responsible for the recurrent deletion/duplication events involving 15q11-q13 [Christian et al., 1995, 1999; Fantes et al., 2002; Pujana et al., 2002; Ritchie et al., 1998]. Recent findings suggest that many chromosomal rearrangements, including translocations, inversions, isochromosomes, and small marker chromosomes, may involve susceptibility to

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rearrangement related to the presence of LCRs [Lupski, 1998; Ji et al., 2000; Stankiewicz and Lupski, 2002; Shaw and Lupski, 2004].

Microarray-based comparative genomic hybridization (CGH) using large-insert clones is useful for detecting deletions or duplications that are greater than about 10 kb but below the level of detection by karyotype analysis. Microarray CGH enables assessment of hundreds or even thousands of sites in a single hybridization thus providing a whole-genome scan for rearrangements [Pinkel et al., 1998; Pollack et al., 1999]. Array CGH has been used for the detection of constitutional chromosomal abnormalities [Shaw-Smith et al., 2004; Vissers et al., 2004]. Recent studies utilizing chromosome 15-specific bacterial artificial chromosome (BAC) microarrays of varying resolution have provided more detailed molecular characterization of chromosome 15 rearrangements [Locke et al., 2004; Wang et al., 2004].

We present here the development of a high-resolution chromosome 15 array and its use in further characterizing cytogenetic abnormalities of 15q11-q13.

MATERIALS AND METHODS

Patient Samples

Samples were obtained from the South Carolina Autism Project (SCAP, Greenwood Genetics Center, Greenwood, SC), the Autism Genetic Resource Exchange (AGRE), the NIGMS Human Genetic Cell Repository (GM numbers, Coriell Institute, Camden, NJ), or Baylor College of Medicine (BCM) as indicated in Tables I and II. All patients including those from AGRE (http://www.agre.org/) and from the SCAP were diagnosed using the Autism Diagnostic Interview-Revised (ADI-R). The ADI-R is a semi-structured diagnostic interview for use with patients suspected of having a pervasive developmental disorder. It was developed for research purposes, but can also be used for clinical diagnoses. Samples were obtained at BCM and the Greenwood Genetic Center under protocols approved by the respective institutional review boards. The samples included well-characterized, cytogenetically abnormal patients, and some patients that were partially characterized by karyotype and/or FISH (Tables I and II). In familial patients, DNA from affected and unaffected siblings and both parents was analyzed when available. Cultured lymphoblasts were the source of DNA for most patients, and DNA was from blood leukocytes in others. Genomic DNA from whole blood and lymphoblast cell lines was made using the PureGene kit (Gentra Systems, Minneapolis, MN) as per manufacturer's protocol.

Array Design and Production

Chromosome 15 is ~100 Mb and can be covered by approximately 700 minimally overlapping BAC or PAC (P1 artificial chromosome) clones. Clone selection was based on the information from public databases (Fig. 1; http://genome.ucsc.edu; http://www.ensembl.org/Homo_sapiens/mapview) both of which use the July 2003 freeze of the draft genome sequence. The array included 106 clones with a variable distribution across 15q (Fig. 1; supplementary data). The highest density of clones is in the ~10-Mb 15q11-q14 interval encompassing the PWS/AS critical region and the common deletion/duplication breakpoints. A resolution of greater than one clone per megabase for 15q11-q13 and less than one clone per megabase for 15q14-qter was achieved. We included 38 clones specific for the subtelomeric region of chromosomes 1–22, 12 clones for chromosome X, and six clones for the Y chromosome. Chromosome- and locus-specificity of all selected clones was verified by FISH and end-sequencing. A few clones at the pericentromeric region of 15q cross-hybridize with other regions due to pseudogene sequences representing intra- and interchromosomal duplicons.

BAC/PAC DNA Preparation and Array Printing

Preparation of DNA from BACs/PACs and chemical cross-linking of DNA was performed as described previously with minor modifications [Cai et al., 2002; Yu et al., 2003].

Genomic DNA Labeling and Hybridization

Patient and reference genomic DNAs were labeled by random priming using a BioPrime DNA labeling kit (Invitrogen, Carlsbad, CA) as described [Cai et al., 2002; Yu et al., 2003]. All experiments included dye swap and two-array hybridizations.

Image Acquisition and Analysis

Hybridized arrays were scanned using a GenePix 4000B dual-laser scanner in combination with GenePix Pro 4.0 imaging and quantification software (Axon Instruments, Union City, CA). Simultaneous scans of each array were

TABLE I. CGH Analysis and FISH Validation of Patients With 15q Segmental Gains

			FISH verification				
Case number (phenotype)	CGH GAIN proximal clone	CGH GAIN distal clone	BP1–BP2 interval AC090764/ AC011767	BP2–BP3 interval AC087463/ AC124312	BP3–BP5 interval AC127522/ AC087455/ AC123768	BP5–Tel interval AC090877/ AC027559	Gain interval
013[a] (Autism)	AC116165	AC135348	Duplicated	Duplicated	Normal	Normal	BP1–BP3 interstitial
014[a] (Autism)	AC116165	AC135348	Duplicated	Duplicated	Normal	Normal	BP1–BP3 interstitial
012[a] (normal)	AC116165	AC135348	Duplicated	Duplicated	Normal	Normal	BP1–BP3 interstitial
016[b] (Autism)	AC116165	AC135348	Duplicated	Duplicated	Normal	Normal	BP1–BP3 interstitial
017[c] (Autism)	AC073446	AC135348	Normal	Duplicated	Normal	Normal	BP2–BP3 interstitial
011[c] (Autism)	AC126603	AC123768	Duplicated	Duplicated	Duplicated	Normal	Cen–BP5 idic(15)
015[d] (Autism)	AC126603	AC123768	Duplicated	Duplicated	Duplicated	Normal	Cen–BP5 idic(15)
019[b] (Autism)	AC126603	AC123768	Duplicated	Duplicated	Duplicated	Normal	Cen–BP5 idic(15)
020[b] (Autism)	AC126603	AC123768	Duplicated	Duplicated	Duplicated	Normal	Cen–BP5 idic(15)
018[c] (Autism)	AC126603	AC135348	Duplicated	Duplicated	Normal	Normal	Cen–BP3 marker(15)

[a]012, 013, and 014 are AGRE samples HI0118, HI0120, and HI0121 respectively; 012 is mother of 013 and 014.
[b]016, 019, and 020 are from Baylor College of Medicine.
[c]017, 011, and 018 are South Carolina Autism Project samples SC10545, SC14508, and SC12970 respectively.
[d]015 is NIGMS–Coriell repository line GM10183.

TABLE II. CGH Analysis and FISH Validation of Patients With 15q Segmental Losses

Patient number (phenotype)	CGH LOSS proximal clone	CGH LOSS distal clone	FISH verification				Deletion class
			BP1–BP2 interval AC090764/ AC011767	BP2–BP3 interval AC087463/ AC124312	BP3–BP4 interval AC127522AC087455/ AC123768	BP5–Tel interval AC090877/ AC027559	
001[a] (PWS)	AC116165	AC135348	Deleted	Deleted	Normal	Normal	Class I: BP1–BP3 interstitial
004[a] (AS)	AC116165	AC135348	Deleted	Deleted	Normal	Normal	Class I: BP1–BP3 interstitial
005[a] (AS)	AC116165	AC055876	Deleted	Deleted	Normal	Normal	Class I: BP1–BP3 interstitial
007[a] (AS)	AC116165	AC135348	Deleted	Deleted	Normal	Normal	Class I: BP1–BP3 interstitial
002[a] (PWS)	AC073446	AC135348	Normal	Deleted	Normal	Normal	Class II: BP2–BP3 interstitial
003[b] (PWS)	AC073446	AC135348	Normal	Deleted	Normal	Normal	Class II: BP2–BP3 interstitial
006[b] (PWS)	AC073446	AC135348	Normal	Deleted	Normal	Normal	Class II: BP2–BP3 interstitial
009[a] (AS)	AC073446	AC135348	Normal	Deleted	Normal	Normal	Class II: BP2–BP3 interstitial
021[a] (AS)	AC073446	AC087455	Normal	Deleted	Deleted to AC087455	Normal	Class IIA: BP2–BP4 interstitial
010[c] (DF, MR, CHD)	AC022407	AC092756	Normal	Normal	Normal	8-Mb deletion	del(15)(q21.2q22.1) interstitial

[a]Samples from Baylor College of Medicine; AS, Angelman syndrome; PWS, Prader–Willi syndrome.
[b]003 and 006 are NIGMS–Coriell repository lines GM9189 and GM 9133 respectively.
[c]DF, dysmorphic features (high arched palate, micrognathia, and ears with thickened helices); MR, mental retardation; CHD (juxtaductal aortic coarctation with near aortic arch interruption, severe septal hypertrophy, moderately depressed biventricular systolic function, biventricular hypertrophy, and bicommisural aortic valve).

obtained at 635 and 532 nm for the Cy5 and Cy3 dyes, respectively. For all the cases presented in this study, images quantified using the GenePix software were further analyzed and plotted using an analytical tool that was developed by us (Shaw et al. manuscript in preparation). After normalization of scanned arrays, inferences were made according to a clone-by-clone classification procedure to determine the gain/loss status of each spot (similar to the procedure published previously) [Yu et al., 2003; Shaw and Lupski, 2004]. The mean for the combined normalized mean $\log_2$ ratios across all clones for the gain state was $+0.22$, for the loss state -0.34, and for the no-change state -0.04 [Yu et al., 2003; Shaw and Lupski, 2004].

FISH Analysis for Chromosomal Abnormalities

Metaphase or interphase FISH was performed using PHA-stimulated peripheral leukocytes or transformed lymphoblast cell lines. The pericentromeric 15q α-satellite probe was used as a control for FISH (Cytocell, Cambridge, UK). Test probes included BACs from the arrays, as specified. Probes were labeled with digoxigenin-11-dUTP or biotin-14-dATP, and FISH was carried out using standard protocols [Trask, 1991]. A single subject was analyzed and scored for 30–100 interphase nuclei for duplications and at least 20 metaphase spreads for deletions. Duplications were scored as confirmed if $\geq$70% interphase nuclei were positive for three or more signals.

RESULTS

Array CGH Profiles for Normal Control Samples

A validation to assess the quality and variability of the array was carried out using DNA from a series of cytogenetically normal male–male, male–female (Fig. 1), and female–female pairs. Five clones localized to the pericentromeric region of 15q, showed high inter-individual variability and were excluded. However, eight pericentromeric clones, despite increased variability were included so as to enable a clearer definition of proximal breakpoint (BP1). The data for the chromosomes other than 15 represented normal by normal comparisons ($\log_2$ ratios close to 0), and the data for the clones showed $\log_2$ ratios close to 1 with good signal to noise ratios for the X chromosome in opposite sex hybridizations (Fig. 1). The combined normalized mean $\log_2$ ratio for autosomal clones using these normal versus normal samples was -0.04. As expected, variation in the $\log_2$ ratios was more pronounced for the clones proximal to (BP1) of the common 15q11.2-q13 PWS/AS deletions. Interestingly, the greatest variance for this proximal set of pericentromeric clones (with both negative and positive $\log_2$ normalized ratios) was observed in patients with rearrangements, although the biological significance of this is not clear.

Array CGH Profiles for Patients With Segmental Gains or Losses in 15q

Preliminary validation of the array was performed using 20 individual cases with known 15q segmental deletions and duplications. Each sample had been shown to contain a rearrangement by conventional FISH or G-banding techniques. We first carried out array-CGH, and potential breakpoints were predicted depending on the gain or loss in the CGH profile; FISH analysis was then carried out using clones flanking and within rearranged segments. Southern blotting, microsatellite marker analysis, and pulsed-field gel electrophoresis (PFGE) were performed in a few patients for additional confirmation as previously reported [Jiang et al., 2004].

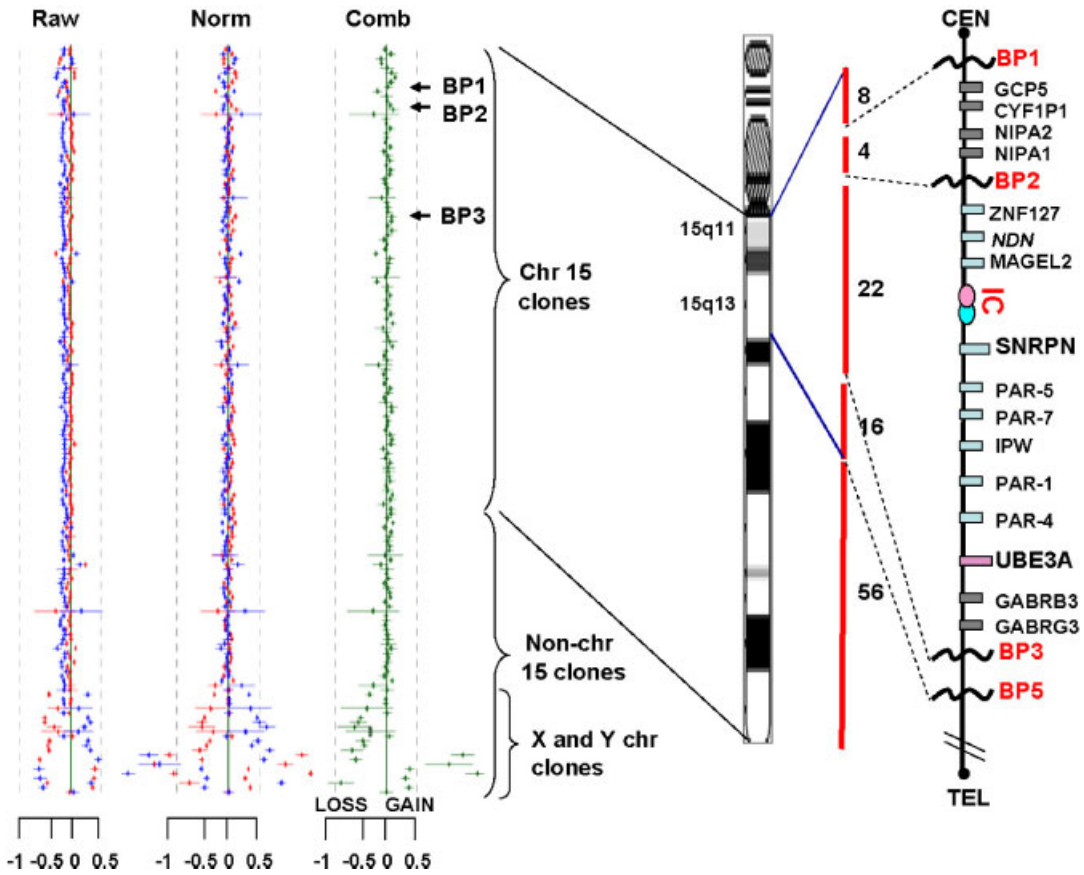

Fig. 1. A normal male to normal female comparison on the C15 array. On the right is a map of human chromosome 15 highlighting the 15q11-q13 segment encompassing the PWS/AS imprinted domain. The common deletion breakpoints are indicated as BP1, BP2, and BP3. BP5 refers to the common distal breakpoint involved in idic(15q). The number of clones in each interval separated by gaps is indicated beside the red vertical line. The raw (Raw), normalized (Norm), and combined (Comb) $\log_2$ ratio plots of array hybridization are shown. The combined ratio plot provides a final estimate of a gain, loss, and no-change distribution for each clone. The dashed lines on the scale for the logarithmic plot (X axis) indicate the position of −1.0 and +0.5, which are the theoretical values for single copy loss or gain, respectively. The plot is in clone-by-clone order starting from 15 centromere to 15q telomere followed by chromosomes 1 to Y (Y axis: top to bottom). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.].

Ten patients with interstitial duplications or idic(15) were analyzed in detail to evaluate the ability of the array to identify segmental gains and define breakpoints (Fig. 2). In all ten patients, the boundaries of the segmental gain were accurately predicted to within one to two clone resolution as confirmed by FISH analysis (Table I). The patients with interstitial duplications involved the segment between the previously described BP1 and BP3 (Fig. 2 and Table I). It is noteworthy that there are gaps in the genome assembly at the BP1, BP2, and BP3 boundaries. This may reflect difficulties in assembling repetitive regions. The location of clones relative to breakpoints is included in the legend to Figure 2 and depicted in Figure 4. The associated breakpoints generally agree with published data [Wang et al., 2004]. The centromeric boundary of BP3 occasionally varied within a ~400-kb interval, but never extended telomeric to clone AC055876. The occurrence of two potential breakpoints for BP3 (see legend to Fig. 2) have been reported earlier as BP3A and BP3B [Wang et al., 2004]. The distal boundary for the larger isodicentric 15q chromosomes (BP5) is consistent and was contained within two overlapping clones. This confirms published data regarding the boundaries and the sequence surrounding duplication breakpoints [Roberts et al., 2003; Locke et al., 2004; Wang et al., 2004]. These interstitial duplications and idic(15) result in trisomy, tetrasomy, or even hexasomy [Wang et al., 2004] for the genomic segment between BP1 to BP3 or BP4 and consequently an increased dosage for the genes contained within the interval.

Array CGH profiles for patients with interstitial deletions involving proximal 15q also shared the major breakpoints (BP1, BP2, and BP3). Representative results are presented in Figure 3 and Table II. During the course of this study we identified a few unusual rearrangements involving 15q. Patient 021 had a deletion of ~7 Mb between BP2 and BP4; this is ~1.8 Mb larger at the telomeric end than the usual class II deletion (Fig. 3C); in patient 010, there was an interstitial deletion of ~8.2 Mb encompassing the 15q21.2-q22.1 segment. We estimate that the smaller class II deletions encompass a segment of ~5 Mb and the larger class I deletions a segment of ~6 Mb. These estimates are larger than previous estimates of ~4 Mb [Christian et al., 1995]. A conservative estimate of the size of the 15q segment that is tetrasomic in the largest idic(15q) is ~11 Mb (Fig. 4). The locations of the common and recurrent breakpoints in 15q11-q14 relative to the arrayed contig is displayed in Figure 4. Some uncertainty persists in the ordering of clones across the most proximal segment of the PWS/AS critical region, mostly between BP1 and BP2. Our interpretation is that the order of the clones within the BP1–BP2 interval is cen-BP1-AC116165-AC090764-AC011767-AC091565-BP2-tel.

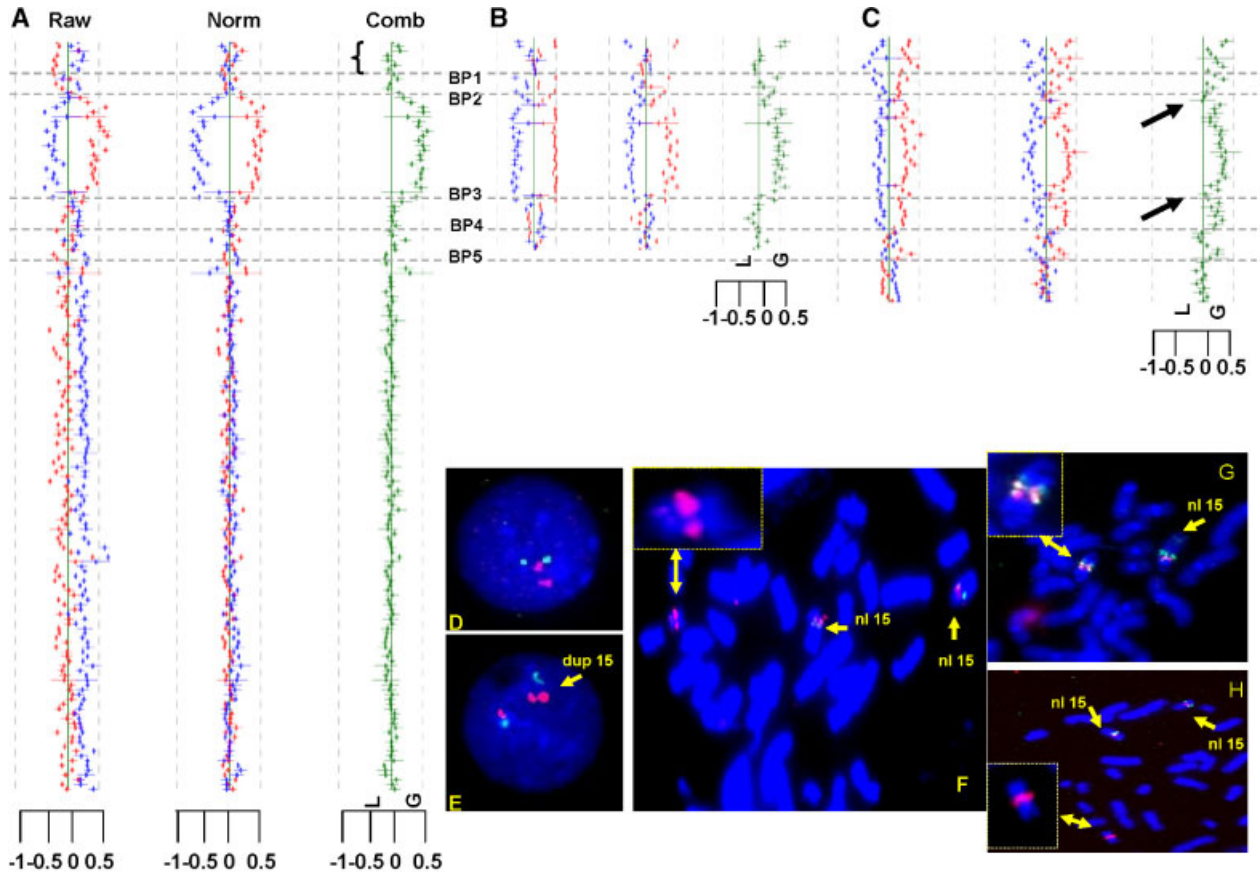

Fig. 2. Examples of chromosome 15 gains from three unrelated patients with autism. Dotted horizontal lines indicate the approximate positions for the common breakpoints. The complete data plot is shown only for patient 017 in **panel A** and provides an example of clone variance across the array. Panel A is from patient 017 with an interstitial duplication for the BP2–BP3 segment with a gain for a series of clones across 15q11-q13. Interphase FISH for patient 017 with probe AC061965 (red) distal to BP3 shows a single signal for each chromosome 15 (**Panel D**; not duplicated). Interphase FISH for the same patient 017 using probe AC124312 (red) within the BP2–BP3 interval shows two signals on one chromosome 15 indicative of duplication (**Panel E**: arrow). **Panel B** is case 018 showing partial plots of the $\log_2$ ratios for an extra marker 15 with gain for clones within Cen-BP3 segment. Metaphase FISH for patient 018 with probe AC090764 (red) within the BP1–BP2 interval shows that the clone is present in the extra marker 15 (**Panel F**; insert; double-headed arrow); probe AC127522 (green) distal to BP3 is absent on marker, thus confirming that the gain does not extend distal to BP3. There are two normal chromosome 15s in panel F (single headed arrows). **Panel C** (patient 015) with a large idic(15) shows gain for the majority of clones from Cen–BP5. Metaphase FISH for patient 015 using probe AC127511 (red) proximal to BP3 shows presence on the idic(15) as two copies (**Panel G** insert; double-headed arrow); probe AC123768 between BP3 and BP5 (green) is present on the idic(15), (probably two copies). Metaphase FISH for patient 015 with probe AC103745 (red), within BP2–BP3 shows presence on the idic(15) (**Panel H**; insert; double-headed arrow); probe AC090877 (green), immediately distal to BP5 is absent on the idic(15), confirming the extent of the distal boundary to BP5. The positions of all clones within the 15 centromere-q13 interval are depicted in Figure 4. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.].

Variability in Fluorescence Intensity Ratios for Pericentromeric Clones

The fluorescence intensity values for clones proximal to BP1 were seen to vary more than for other regions, for both normal individuals and those with segmental gains or losses of adjacent regions. This variability for the proximal pericentromeric clones (clones 1 to 8 bracketed in panel A of Fig. 2) was greater in patients with deletions or duplications than in normal individuals. We also observed suppression of the gain or loss signals for clones flanking the breakpoints. This is illustrated in the plots for patient C in Figure 2, who was tetrasomic for proximal 15q as a result of a idic(15) (q11-q13.3). The combined normalized $\log_2$ ratio values for clones adjacent to the breakpoints are skewed towards the baseline even in the presence of the overall gain for the entire segment. As has been proposed earlier, multiple copies of pseudogenes or inter- and intrachromosomal duplicons (e.g., the presence of blocks of the *HERC2* transcript) are the likely cause for this bias [Pinkel et al., 1998; Albertson et al., 2000; Vissers et al., 2004]. The smoothing parameters incorporated into the spatial data normalization protocol may contribute to the behavior of clones that flank the known breakpoints, but the effect persisted with analysis under a variety of parameters. We conclude that sensitivities of the pericentromeric clones proximal to BP1 are affected by rearrangements in the neighboring segments. Repeats within clones can suppress apparent gain or loss when gain or loss is in fact present, and they can cause artifactual evidence of gain or loss when the region is not involved in any change.

DISCUSSION

We have developed a chromosome 15 CGH array and tested its ability to detect proximal 15q rearrangements. This high-density 15q-specific array allowed us to map breakpoints for recurrent rearrangements and for less common deletions and duplications of 15q. A higher density of clones was used for the

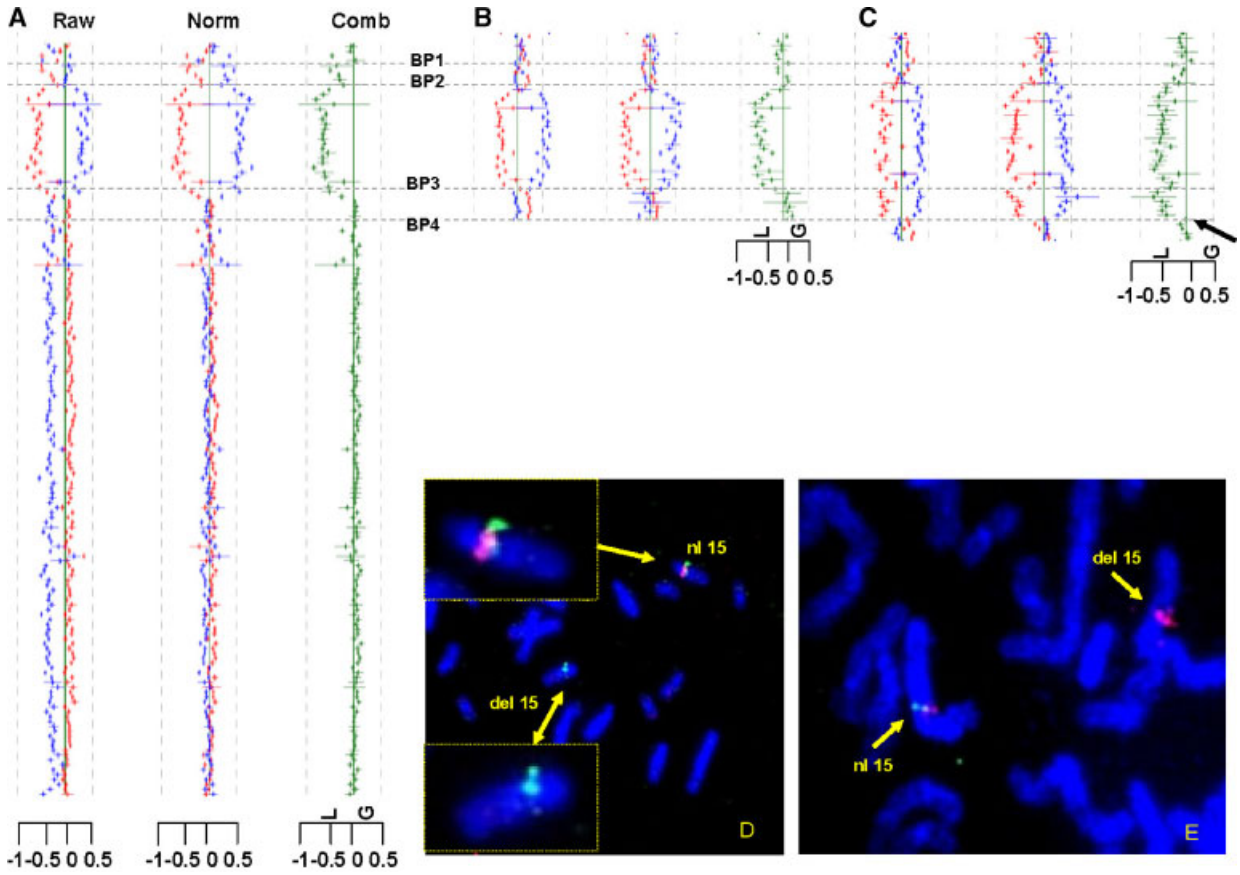

Fig. 3. Examples of chromosome 15 deletions. The plot of the hybridization results and FISH analyses are shown for representative patients (PWS and AS) with segmental losses within 15q. **Panels A** (patient 004) and **B** (patient 003) represent plots for typical class I (BP1–BP3) and class II deletions (BP2–BP3) respectively. **Panel C** (patient 021) shows the plot for an unusual deletion extending from BP2 to BP4. The complete data plot is shown only for panel A and provides an example of clone variance across the entire array. **Panel D** is metaphase FISH for patient 004 with a BP1–BP3 deletion; the probe AC090764 (red) within BP1–BP2 interval is deleted on one chromosome 15 (insert; double-headed arrow) but not the normal 15 (insert; single-headed arrow); probe AC127522 (green) which is just distal to BP3 is present on both chromosomes 15. **Panel E** is metaphase FISH analysis for patient 003 utilizing probe AC090764 (red) between BP1 and BP2 that is not deleted on either chromosome 15 (arrows); probe AC124312 (green) within BP2–BP3 interval shows loss on one chromosome 15 (right arrow). Metaphase FISH with a set of clones distal to BP3 confirmed the unusual larger deletion in patient 021 in panel C (data not shown). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.].

proximal segment of 15q as this region is involved in recurrent deletions and duplications, and a specific delineation of these rearrangements is necessary for optimal genotype–phenotype correlations. This study shows that array-CGH can detect dosage differences across 15q even in the presence of a complex genomic architecture for the region. Array CGH of 15q11-q13 is useful for detecting duplications causing autism, because these duplications would often be missed using metaphase FISH using the widely available probes for PWS and AS. Additionally, array CGH allows analysis of post-mortem samples, where conventional cytogenetics is limited by the inability to culture cells. We have also obtained good results using cell pellets stored in cytogenetic labs (data not shown).

The rearrangement breakpoints were accurately determined by array-CGH in all 20 analyses that were used for validation (Tables I and II). FISH validation was carried out for all the patients presented, and there was complete concordance of the CGH breakpoints to those confirmed by FISH to within two flanking clones. The common distal breakpoint (BP3) commonly involved in interstitial deletions and duplications showed variability across a ~400-kb segment. One atypical deletion extended to BP4, while the idic(15) chromosomes uniformly involved BP5. To our knowledge, involvement of BP4 in interstitial deletions has been reported only once [Locke et al., 2004], but this breakpoint has been determined to be a preferential recombination site in the formation of idic(15); we did not detect involvement of BP4 in any of the three families with interstitial gains. It is interesting that interstitial duplications involve primarily the same recombination sites as deletions whereas the idic(15) marker chromosomes more often involve the more distal sites, indicating that the mechanisms for recurrence of the two types of rearrangements is complex.

These data complement and extend array-CGH data for 15q rearrangements from other groups [Locke et al., 2004; Wang et al., 2004]. This is particularly significant for the distinction of class I and class II deletions causing AS or PWS. A clearer definition of the two classes of recurrent deletions is important for optimal genotype–phenotype relationships. The class I deletions cause haploinsufficiency for at least four genes (*TUBGCP5*, *NIPA1*, *NIPA2*, *CYFIP1*), while these genes would not be haploinsufficient in class II deletions. Given the consistent agreement of array CGH and FISH, either may be adequate as a single assay. This array may be useful for detecting small rearrangements in patients with PWS-like or AS phenotypes with no identifiable molecular defect.

In all clinical studies using array CGH, there is a need to try to distinguish benign variation in copy number from variation

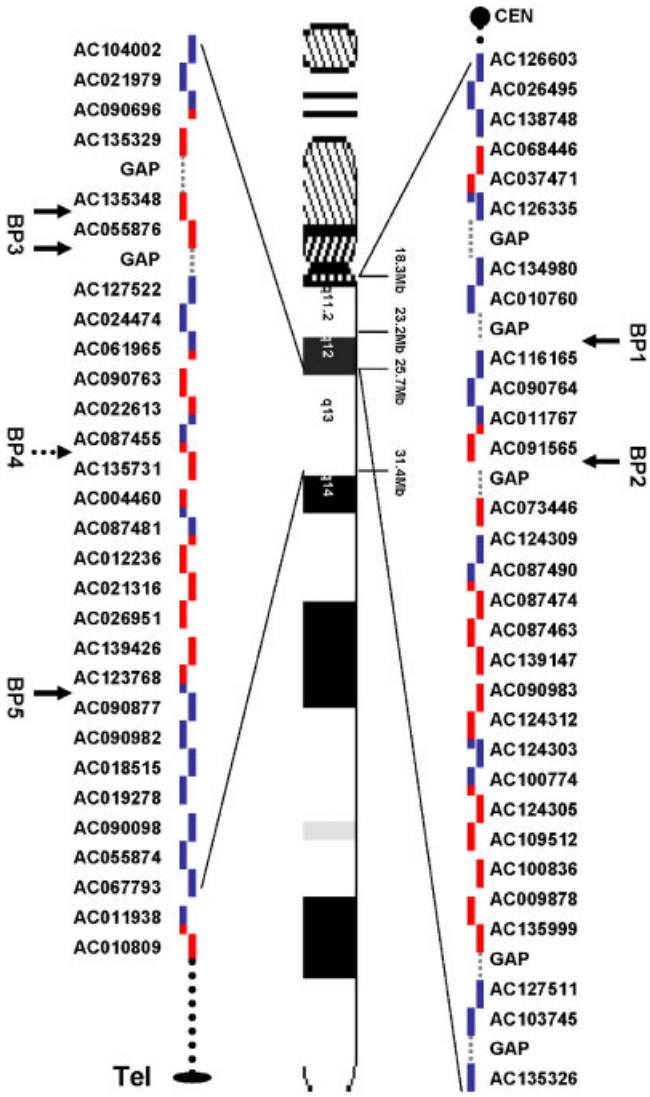

Fig. 4. Tiling path for the clones across the 15q11-q13 segment included in the array. The clone coverage for the rest of chromosome 15 and all other chromosomes is available as supplemental data (see the online portion of Fig. 4 at http://www.interscience.wiley.com/jpages/1552-4825/suppmat/index.html). Blue bars indicate overlap with a neighboring clone, and red bars indicate that the clone is adjacent to but does not overlap with neighboring clone. Sequence gaps in the chromosome 15 contig are indicated by dotted lines in gray. Breakpoints located between or within clones are indicated by solid arrows; dotted arrow for BP4 indicates position for the unusual distal deletion breakpoint in one patient. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.].

that affects phenotype. The region of 15q proximal to BP1 is highly variable in the normal population. This is consistent with the known heterogeneity for copy number of pseudogenes proximal to BP1 [Christian et al., 1995; Ritchie et al., 1998; Ji et al., 2000; Fantes et al., 2002; Locke et al., 2004]. There may be great potential for position effects involving this region, because it is known that the PWS/AS imprinting center affects expression over megabase distances, both centromeric and telomeric. The presence of LCRs is common in many deletion/duplication rearrangements, and the LCRs act as recombination substrates. Recombination between non-allelic LCR copies can cause deletion or duplication of the intervening segment. In many microdeletion/duplication syndromes, accurate delineation of breakpoints has revealed the presence of LCRs, AT-rich palindromes, and pericentromeric repeats at such rearrangement breakpoints [Lupski, 1998; Pujana et al., 2002; Stankiewicz and Lupski, 2002; Shaw and Lupski, 2004].

Interstitial duplications causing autism may be missed using metaphase FISH with standard probes. In addition, we have found interphase FISH to be difficult to interpret in a series of autism patients (Sahoo et al., unpublished) making array CGH the preferred method for diagnosis of interstitial 15q11-q13 duplications causing autism. This array allows for better definition of genotype and thus better potential for genotype/phenotype correlations regarding autism, AS, PWS, and isodicentric 15q syndrome.

ELECTRONIC-DATABASE INFORMATION

The URLs for data presented herein is as follows:

http://www.ncbi.nlm.nih.gov/mapview/>
http://genome.ucsc.edu
http://www.ensembl.org/Homo_sapiens/mapview?chr=15

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