

The evolution of molecular ruler analysis for characterizing telomere imbalances: from fluorescence in situ hybridization to array comparative genomic hybridization

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Submicroscopic telomere imbalances are a significant cause of mental retardation with or without other phenotypic abnormalities. We previously developed a set of unique telomere clones that identify imbalances in 3% to 5% of children with unexplained mental retardation and a normal karyotype. This targeted screening approach, however, does not provide information about the size or gene content of the imbalance. To enable such comprehensive characterization, a “molecular ruler” clone panel, extending up to 5 Mb proximal to the first telomere clone for each chromosome arm, was developed. This panel of clones was successfully used to delineate the size of unbalanced telomere aberrations in a fluorescence in situ hybridization assay. However, the fluorescence in situ hybridization analysis was quite labor-intensive, and for many cases, the imbalance extended beyond our 5-Mb coverage. Therefore, to develop a more efficient and comprehensive method for characterizing telomere imbalances, we developed a custom oligonucleotide microarray consisting of high-density coverage of all telomere regions as well as a whole-genome backbone. Overall, 44 pathogenic imbalances studied by fluorescence in situ hybridization or oligonucleotide array showed a size range of 400 kb to 13.5 Mb. In four of these, the array detected additional interstitial imbalances adjacent to the telomere imbalance, demonstrating the usefulness of added probe coverage. In 10 cases with benign imbalances inherited from a normal parent, the size ranged from 170 kb to 1.6 Mb. These results demonstrate that array comparative genomic hybridization will aid in more efficient and precise characterization of telomere imbalances leading to the development of gene dosage maps at human telomere regions for genotype/phenotype correlations. **Genet Med 2007;9(9):566–573.**

Key Words: telomere imbalance, telomere, fluorescence in situ hybridization, array comparative genomic hybridization, molecular rulers

The telomeric regions of human chromosomes are frequently involved in chromosomal rearrangements due to their location at the terminus of each chromosome. Several of the first cytogenetically visible deletion syndromes described, including Wolf-Hirschhorn, cri du chat, and Miller-Dieker syndromes, were found to involve various telomeric regions. Based on these observations, it was hypothesized that smaller imbalances of these gene rich regions, which would be difficult to impossible to detect by routine G-banding analysis, could also contribute significantly to abnormal clinical pathology.¹ A

unique telomere clone set that could be used in molecular cytogenetic assays was envisioned for use in identifying these cryptic, or submicroscopic, imbalances.

As part of an international collaboration, a bacterial artificial chromosome (BAC) and/or P1-derived artificial chromosome (PAC) clone corresponding to each human telomere was identified, located just proximal to the subtelomeric repeat region.^{2,3} Each clone, therefore, represented the most distal unique DNA for each chromosome arm. Using this clone set, Knight et al.⁴ carried out the first large-scale study of 466 individuals with unexplained mental retardation and showed that 7.4% of individuals with moderate to severe mental retardation ($n = 284$) had a telomere imbalance compared with 0.5% of individuals with mild mental retardation ($n = 182$). Recently, our group reported the largest study to date to examine the frequency of telomere imbalances and demonstrate the clinical utility of this testing.⁵ Telomere fluorescence in situ hybridization (FISH) analysis of 11,688 patients with a developmental disability and a normal karyotype revealed the de-

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tection rate for clinically significant telomere abnormalities to be 2.5%, with an additional 0.5% detection of apparently benign familial variants.

Telomere FISH analysis, although extremely valuable for clinical diagnostics, proved to be very labor-intensive for routine laboratory testing. Therefore, alternative methods were investigated, including array comparative genomic hybridization (array CGH). Several groups have now reported the successful transition of telomere analysis from FISH to array CGH with similar yields for deletions, but improved detection rates for small duplications.^{6–10} However, most of these initial arrays only contained limited clone coverage for each telomere region.

Although a targeted screening approach is useful for the initial detection of a telomere imbalance, it does not provide any information about the size or gene content of the imbalance, which makes the determination of clinical significance and comparison with other published cases difficult. Therefore, to create genotype/phenotype correlations for each human telomere region, we developed a set of “molecular ruler” clones for each telomere region to not only measure the size of the imbalance, but to also aid in examining the gene content.

Our initial molecular ruler clone panel included a BAC/PAC contig for the most distal 1 Mb of each chromosome arm (starting at the unique telomere clone and moving proximal) and then a clone every 500 kb for the most distal 5 Mb.¹¹ These clones were used in pilot FISH studies to interrogate imbalances involving 1p, 6q, 10q, 16p, 17p, and 22q.^{8,11–13} Although these pilot studies only included a small number of cases per telomere, they demonstrated the utility of this high-density mapping for more accurate clinical interpretation of telomere imbalances. By determining the size of each telomere imbalance, pathogenic imbalances could be differentiated from those that were apparently benign variants, such as small deletions and duplications of 4q and 10q.^{8,11}

In this study, we have extended the use of FISH with our molecular ruler clone set to characterize a larger set of unbalanced telomere rearrangements. However, these analyses, as well as those of other investigators,^{14,15} quickly led to the realization that many submicroscopic telomere imbalances were larger than 5 Mb and that additional bioinformatics and clone validation work to identify sequential clones for FISH mapping was inefficient and time-consuming. Therefore, we used the mapping data from our well-characterized molecular ruler clone set to transition to array CGH using a custom designed oligonucleotide microarray with high-resolution coverage of each telomere region. Overall, 54 cases with 71 telomere imbalances were evaluated using either FISH or oligonucleotide array analysis.

MATERIALS AND METHODS

Patient samples

Individuals with developmental delay/mental retardation, a normal G-banded karyotype and an unbalanced telomere rearrangement, identified by telomere FISH or targeted microar-

ray analysis, were recruited from clinical diagnostic laboratories and genetics clinics for participation in this study. Informed consent was obtained from all patients, and samples were collected following approved human subjects protocols through the Institutional Review Boards at the University of Chicago and Emory University.

Genomic clone selection and FISH analysis

The previously characterized set of unique telomere clones, which corresponds to the most distal unique DNA for each chromosome arm, was used as the starting point for developing the molecular rulers for each telomere region.³ The BAC and PAC clones included in the molecular ruler assay were selected based on published physical maps and publicly available genome resources, such as the University of California Santa Cruz Human Genome Browser (<http://genome.ucsc.edu>)^{16,17} and the integrated STS/radiation hybrid maps (NCBI Map Viewer, <http://www.ncbi.nlm.nih.gov>).¹⁸ For the most distal 1 Mb, a complete contig of overlapping clones covering the region was developed to analyze smaller imbalances. Genomic clones were then selected at 500-kb intervals to cover the most distal 5 Mb of each chromosome of interest. FISH was used to verify a subset of clones for cytogenetic position and unique localization (i.e., no cross-hybridization signals to other chromosomes).

Bacterial stabs were obtained from the BACPAC Resource Center at Children’s Hospital Oakland Research Institute (Oakland, CA) or from Invitrogen (Carlsbad, CA) and streaked onto LB plates with the appropriate antibiotic. DNA from each genomic clone was isolated from overnight cultures using an automated DNA isolation system (AutoGen 740, Integrated Separation Systems, Natick, MA) and directly labeled with either Spectrum Orange-dUTP or Spectrum Green-dUTP (Abbott Molecular, Inc., Des Plaines, IL) using a standard nick translation reaction. Slide preparation, probe preparation, hybridization, and washing were carried out following previously described methods.¹¹

Array CGH analysis

Two different oligonucleotide arrays were used for these experiments: a commercially available array (Agilent Human Genome CGH Microarray Kit 44B, Agilent Technologies, Santa Clara, CA) and a custom designed 4 × 44 K oligonucleotide array (Agilent Technologies, manuscript in preparation). The oligonucleotides contained on both arrays were 60mers. Based on the genomic location of the first unique FISH clone for each telomere, we targeted coverage on the custom array and included at least 10 oligonucleotides to cover the most distal unique telomere clone, providing a resolution of 50 kb for these regions. An oligonucleotide was then placed every 75 kb across the length of each chromosome arm starting adjacent to the telomere clone coverage. For these regions, when mapping known telomere rearrangements, we set the minimum number of oligonucleotides included in a region of loss or gain at three oligonucleotides, which allows us to achieve a resolution of 200 to 300 kb.

Array CGH analysis was carried out following the manufacturer's protocol (Agilent Technologies). Briefly, genomic DNA (0.5–1.5 µg) was digested with *AluI* and *RsaI* (Promega Corporation, Madison, WI) for 2 hours. The DNA was labeled for 2 hours using random primers, Cy-3 and Cy-5-dUTP dyes, and Exo-Klenow fragment (Agilent Technologies). Patient DNA (labeled with Cy-3) was combined with a normal control DNA sample (labeled with Cy-5) of the opposite sex (Promega Corporation) and hybridized to the array in the presence of Cot-1 (Invitrogen). After a 24-hour hybridization at 65°C, the slides were washed and scanned using the GenePix Autoloader 4200AL (Molecular Devices, Sunnyvale, CA). Confirmatory FISH analyses of the breakpoints identified by oligonucleotide array were carried out using BAC clones from the molecular ruler panel, as described above.

BlueFuse microarray analysis software (BlueGnome Limited, Cambridge, UK) was used to examine the oligonucleotide array data. Normalization of the data was performed using Block Lowess. Regions of copy number alterations were detected using set thresholds for the channel ratios based on 2 or 3 SDs from the median of the autosomes. Channel 1 (Ch1) represents the patient sample and channel 2 (Ch2) represents the normal control DNA. The thresholds for the log₂ ratios were set at 0.26 for gains and -0.32 for deletions.

RESULTS

In this study, FISH and array CGH were employed to investigate telomere imbalances in 54 individuals with developmental delay/mental retardation who previously had a normal G-banded karyotype. Because some of these rearrangements were derivative chromosomes involving more than one telomere region, the total number of unbalanced segments characterized was 71. Although the majority of individuals examined carry a telomere imbalance that is pathogenic and related to their abnormal phenotypic findings, several cases were also examined where the imbalance was inherited from a phenotypically normal parent and are thus considered benign variants. Telomere mapping results are presented below in the context of these two categories.

Pathogenic telomere imbalances

Forty-four individuals with developmental delay/mental retardation were studied who carry a pathogenic telomere imbalance. Twenty-four cases had a pure de novo terminal deletion or duplication. As shown in Table 1, 23 de novo deletions, involving 14 different telomere regions, showed monosomy that ranged from 400 kb to 13.5 Mb. Figure 1, A shows oligonucleotide array analysis for Case 11 with a 5.9 Mb deletion of the 8p telomere. Only one pathogenic de novo duplication was analyzed in this study, which was shown to be 2.8 Mb in size (Case 24). Such small duplications are difficult to detect by FISH analysis. However, array analysis can accurately detect even small gains in copy number (≥100 kb).

Sixteen cases involved an unbalanced derivative chromosome resulting from a rearrangement involving more than one

Table 1
Pathogenic telomere imbalances sized by FISH and oligonucleotide aCGH

Case no.	Telomere	Size		Mapped by
		Deletion (Mb)	Duplication (Mb)	
Deletions (de novo)				
1	1p	4.0		FISH
2	1p	6.5–7.0		FISH
3	1q	7.9		aCGH
4	2q	1.9		aCGH
5	3q	1.5		aCGH
6	4p	5.6		aCGH
7	6q	12.9		aCGH
8 ^a	6q	400 kb		aCGH
9 ^b	6q	7.5–8.0		FISH
10	6q	10.5		aCGH
11	8p	5.9		aCGH
12	8p	10.0		aCGH
13	9p	1.5		FISH
14	9q	1.4		aCGH
15	9q	1.1		aCGH
16	12q	1.6		aCGH
17	12q	4.5		aCGH
18	14q	4.0		aCGH
19	16p	1.1–1.7		FISH
20	16p	1.8		aCGH
21 ^c	19p	1.2		aCGH
22	19p	1.1		FISH
23	20q	1.6		aCGH
Duplications (de novo)				
24	4q		2.8	aCGH
Derivatives				
25 ^d	1q;6q	3.5 (6q)	4.5 (1q)	aCGH
26	2q;22q	2.4 (2q)	8.2 (22q)	aCGH
27	3p;9p	3.5 (9p)	4.5 (3p)	aCGH
28	5q;10q	2.7 (10q)	6.8 (5q)	aCGH
29	6p;6q	13.5 (6p)	10.0 (6q)	aCGH
30 ^e	6q;21q	7.1 (6q)	5.3 (21q)	aCGH
31 ^f	7p;16p	None (7p)	4.5 (16p)	FISH
32 ^g	10p;10q	8.5 (10q)	10.4 (10p)	aCGH
33 ^h	10q;16p	None (10q)	10.0 (16p)	FISH
34 ⁱ	10q;16p	None (10q)	10.0 (16p)	FISH
35	10q;17q	1.4 (10q)	4.5 (17q)	aCGH
36	12p;17p	1.35 (12p)	5.5 (17p)	aCGH

(Continued)

Table 1
Continued

Case no.	Telomere	Size		Mapped by
		Deletion (Mb)	Duplication (Mb)	
37 ^l	14p; 16p	None (14p)	10.0 (16p)	FISH
38 ^k	16p; 17p	4-5 (17p)	3.5 (16p)	FISH
39	Xp; 16p	2.5 (Xp)	2.9 (16p)	aCGH
40	Xp; Xq	4.6 (Xp)	3.6 (Xq)	aCGH
Complex				
41	2q	3.0	4.8	aCGH
42	6q	1.0	102 kb	aCGH
43	22q	320 kb	5.2	aCGH
44	9p; 20p	10.4 (9p)	1.2 (9p) 4.3 (20p)	aCGH

FISH, fluorescence in situ hybridization; aCGH, array comparative genomic hybridization.

^aCase 1.¹³

^bCase 2.¹³

^cArcher et al.¹⁹

^dCase 3.¹³

^eCases 4 and 5.¹³

^fCase 2.¹¹

^gCase 3.¹¹

^hCase 4.¹¹

ⁱCase 5.¹¹

^jCase 5.¹¹

^kCase 1.¹¹

chromosomal segment. Although the majority of these were unbalanced translocations that were inherited from a normal parent who carried the balanced form of the translocation, a few cases were de novo. In this category, because more than one chromosomal region was involved in the rearrangement, 28 imbalances were characterized. For four cases, however, although two chromosomes were involved, only one telomere region had an abnormal copy number of unique sequence DNA, i.e., the derivative chromosome contained only a duplication of a telomere region translocated distal to an intact telomere. Figure 1, C shows an example of array results from an unbalanced telomere translocation between the long arms of chromosomes 5 and 10 (Case 28) resulting in trisomy for a 6.8 Mb region of 5q and monosomy for a 2.7-Mb region of 10q.

The size of the monosomic segments associated with the derivative chromosomes ranged from 1.35 to 10 Mb. For the trisomic segments, sizes ranged from 2.9 to 10.4 Mb. As shown in Table 1, for many of the derivative chromosomes that were characterized, the size of the deleted segment was similar to the size of the duplicated segment. This observation explains why many of these large imbalances are still cryptic by G-banding because one region is deleted and replaced with another region of similar size and G-banding pattern.

Complex chromosome rearrangements, also listed in Table 1, were discovered in four cases that were originally referred to our study as pure telomere deletions (Cases 41, 42, and 43) or

an unbalanced telomere translocation (Case 44). In each of these cases, molecular ruler mapping by array CGH revealed an additional imbalance that was not detected by FISH analysis with a single telomere clone. Each case contained an additional interstitial duplication that was located adjacent to the originally identified deleted region. For example, Case 43 was referred to our study because of a 22q telomere deletion identified by FISH analysis. As shown in Figure 2, array analysis revealed a 5 Mb duplication of the 22q telomere region in addition to the 320 kb deletion in this individual. By telomere FISH analysis, Case 44 was shown to have an unbalanced telomere translocation between the short arms of chromosomes 9 and 20. Array analysis determined the size of the 9p deletion to be 10.4 Mb and the size of the 20p duplication to be 4.3 Mb, but also identified a 1.2-Mb duplication of 9p just proximal to the deleted region.

Inherited benign variants

Ten individuals with developmental delay/mental retardation examined in this study carried deletions or duplications that were inherited from a phenotypically normal parent. These cases are analogous to previous reports where the imbalance is assumed to be a benign variant because it is inherited from a normal parent.²⁰⁻²³ In fact, five of 10 benign telomere variants analyzed in this study have been previously reported, including deletions of 4q, 10q, and Yq and duplications of 4q.⁵ By the same logic, the additional inherited imbalances described in this report, including deletions of 9p and 21q and duplications of 3p and 15q, are most likely benign variants.

As shown in Table 2, six cases, involving five different chromosomes, were pure terminal deletions with sizes ranging from 300 kb to 1.6 Mb. Three cases were pure terminal duplications with sizes ranging from 170 kb to 1.5 Mb. One benign variant was caused by an unbalanced translocation (Case 54) resulting in a derivative 12 with 538 kb of the telomeric region of 15q on the short arm of 12 with no associated loss of the 12p telomere region. Figure 1, B shows array results from Case 54, which accurately detected and sized this small duplication.

Figure 1, D shows array results for chromosome 4 from Case 45 with a 1 Mb benign 4q deletion using two different oligonucleotide array platforms, our custom designed oligonucleotide array and an off-the-shelf oligonucleotide array. Only our custom array, with enhanced coverage of the telomere regions, detected this 1-Mb deletion. The off-the-shelf commercial array failed to detect this imbalance due to poor genomic coverage at this telomere region.

Pathogenic versus benign telomere imbalances

Although our molecular ruler clone set was originally designed with tiling path coverage of the most distal 1 Mb because we hypothesized that most telomere imbalances would be small, only three of 61 (5%) of the pathogenic imbalances that were delineated were <1 Mb in size. Thirty-nine of 61 (64%) were <5 Mb in size. However, surprisingly, 22 of 61 (36%) of the pathogenic imbalances were >5 Mb, which extended beyond the initial upper limit for our molecular ruler coverage. In fact, the largest pathogenic imbalance was a 13.5 Mb

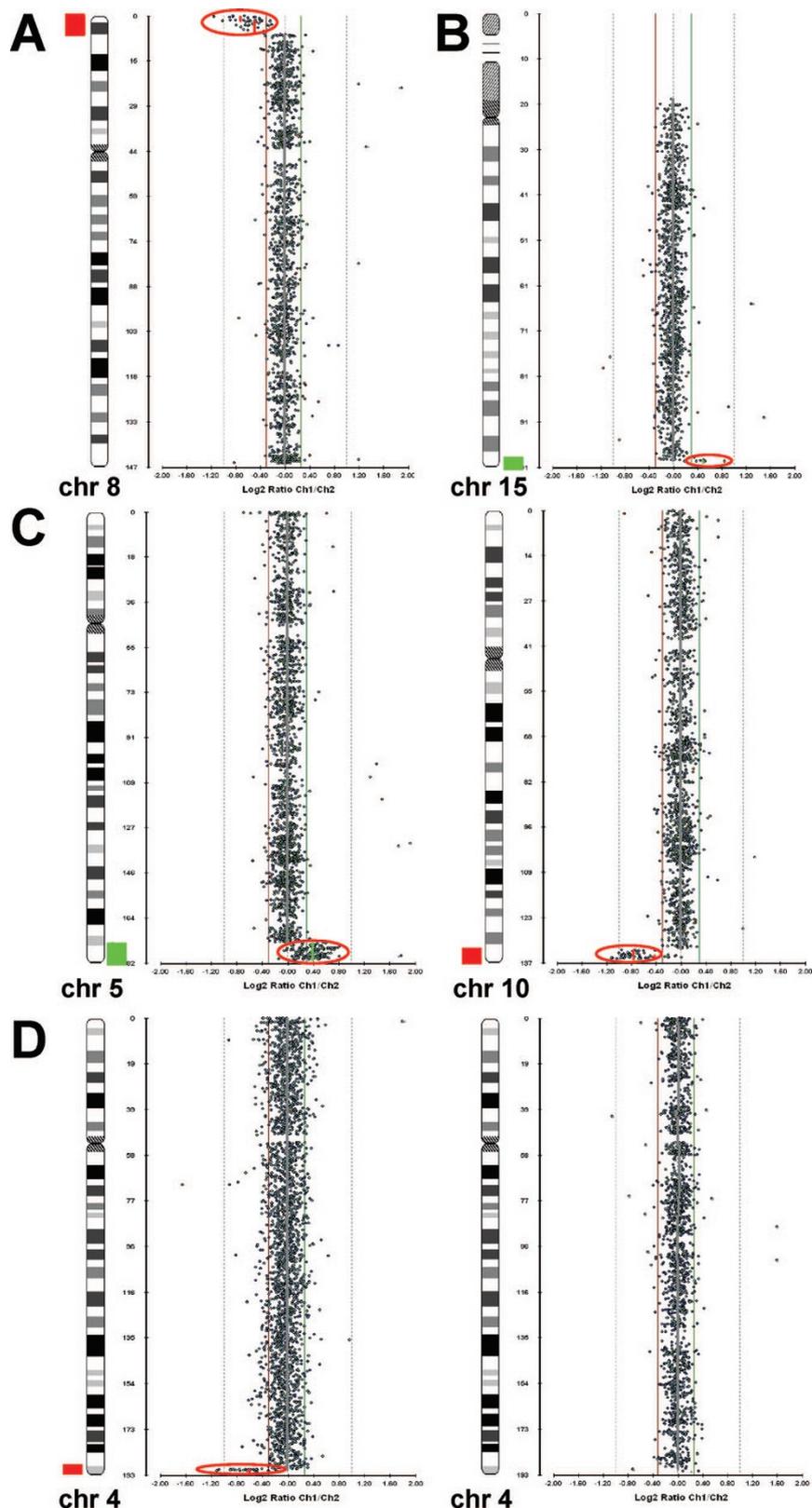


Fig. 1. Representative results from oligonucleotide array comparative genomic hybridization. Each spot represents a single oligonucleotide probe on the array. The spots shifted to the left showed a decreased C_γ3/C_γ5 signal intensity ratio, indicating reduced copy number in the patient versus the control. The red line (to the left of the ideogram) and green line (to the right of the ideogram) represent the deleted and duplicated regions, respectively. (A) Case 11 with a 5.9 Mb pathogenic deletion of the 8p telomere. (B) Case 54 with a 538 kb duplication of 15q that was inherited as a benign variant. (C) An unbalanced telomere translocation in Case 28 resulting in trisomy for a 6.8 Mb region of 5q and monosomy for a 2.7 Mb region of 10q. (D) Array results for chromosome 4 for Case 45 using two different microarrays: the results on the left are from the molecular ruler telomere array, which detected a 1 Mb deletion, whereas the results on the right show results from an off-the-shelf commercial array that failed to detect the deletion due to incomplete telomeric coverage.

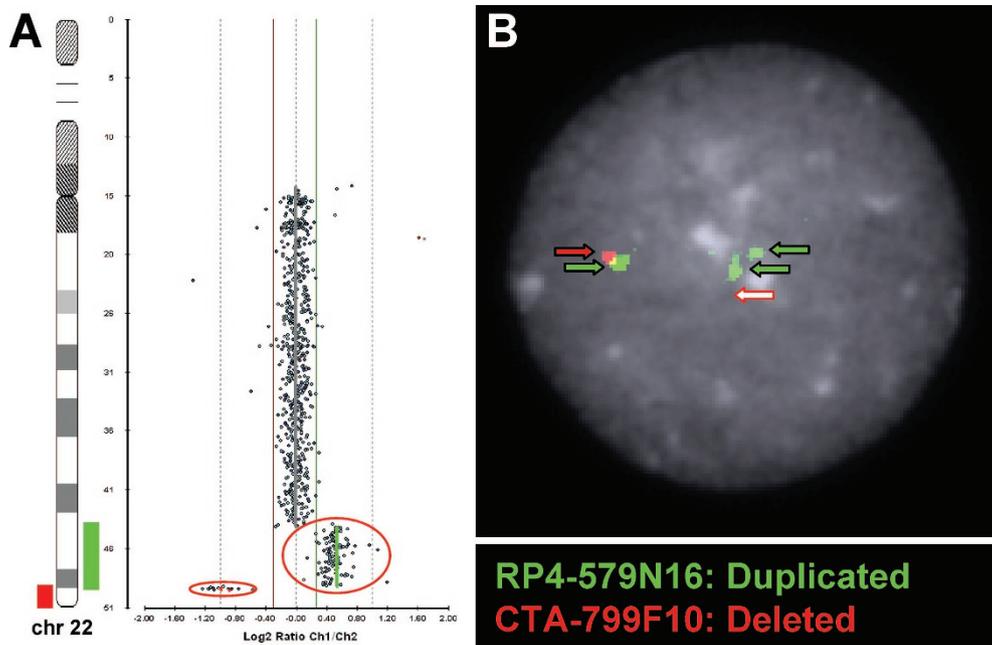


Fig. 2. Microarray and interphase FISH results for Case 43 with a complex rearrangement of the telomeric region of 22q. (A) Microarray array analysis detected a 5 Mb interstitial duplication adjacent to the 320 kb terminal telomere deletion. (B) Interphase fluorescence in situ hybridization analysis using a clone from the deleted region (CTA-799F10, red) and a clone from the duplicated region (RP4-579N16, green) verified the imbalances detected by array analysis. The single red and single green arrows on the left side of the nucleus represent the normal 22 homolog. The arrows on the right side of the nucleus are showing the abnormal hybridization signals: the two green arrows show the duplicated signal, and the white arrow depicts the deleted signal.

Table 2

Benign telomere imbalances sized by FISH and oligonucleotide aCGH

Case no.	Telomere	Size		Mapped by
		Deletion (Mb)	Duplication (Mb)	
Deletions				
45	4q	1.0		aCGH
46	9p	300 kb		aCGH
47	10q	1.3		aCGH
48	10q	100 kb		FISH
49	21q	1.6		aCGH
50	Yq	317 kb		aCGH
Duplications				
51	3p		1.5	aCGH
52	4q		1.2	aCGH
53	10q		170 kb	aCGH
Derivative				
54	12p; 15q	None (12p)	538 kb (15q)	aCGH

FISH, fluorescence in situ hybridization; aCGH, array comparative genomic hybridization.

deletion. In contrast, all the benign variants were <2 Mb in size. Figure 3 summarizes the size distribution of pathogenic and benign telomere imbalances and illustrates the size distinction between these two categories.

DISCUSSION

This study used molecular ruler analysis with FISH or array CGH analysis to characterize the size of telomere imbalance in 54 individuals with developmental delay/mental retardation who previously had normal G-banding analysis. The telomere imbalances examined included pure deletions and duplications as well as derivative chromosomes involving more than one rearrangement. Although these studies were initiated using FISH analysis as a primary method, it quickly became apparent that more efficient methods were necessary for high-resolution mapping of telomere imbalances.

Therefore, we transitioned our molecular ruler analysis for telomeres from FISH to array CGH by creating a custom oligonucleotide array with high-density coverage of each telomere region. FISH analysis is labor-intensive for fine mapping studies because multiple hybridizations are necessary for each case, which increases the analysis time and is subject to an individual’s interpretation. In addition, duplications are hard to identify and delineate using FISH analysis. Unlike FISH analysis, array CGH provides an objective method for the efficient and accurate characterization of telomere imbalances in a single experiment. In addition, because of the ease of adding increased genome coverage with oligonucleotide-based arrays, additional imbalances can be accurately detected that could be missed with FISH or arrays containing lower resolution telomere coverage. However, as demonstrated by Case 45 (Fig. 1, D), careful evaluation of the telomeric content of microarrays is needed because pathogenic imbalances could be missed

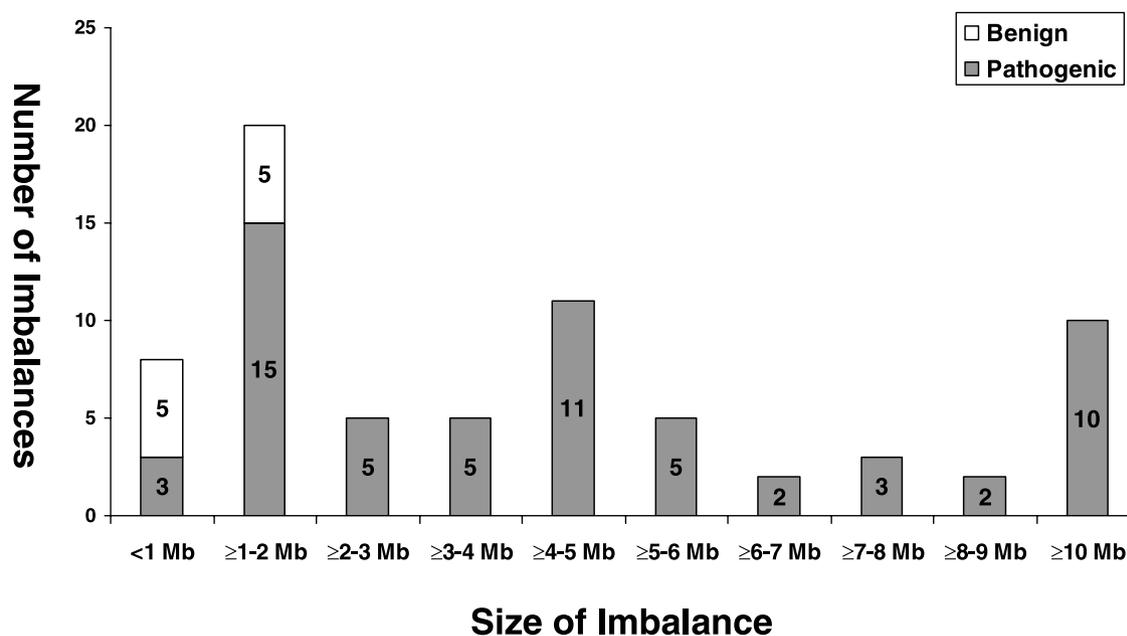


Fig. 3. The size distribution of pathogenic and benign telomere imbalances mapped by molecular ruler analysis. All the benign variants were <2 Mb in size. The pathogenic imbalances ranged from <1 Mb to >10 Mb.

without adequate coverage that extends to the most distal unique DNA for each telomere region.

High-resolution mapping of telomere imbalances that are either causative of a clinical phenotype or represent benign variants is useful for developing gene dosage maps. Such detailed characterization of the genes that are sensitive to dosage changes (haploinsufficient or triplosensitive) versus those that are tolerant will be instrumental in the elucidation of genotype/phenotype correlations. As more individuals are studied with telomere imbalances involving each telomere region, additional information can be gained that will aid in providing better prognostic information related to each unique imbalance. For example, in this study, the size of pathogenic imbalances ranged from 400 kb to 13.5 Mb, whereas all the benign variants examined were <1.6 Mb in size. The smallest de novo deletion was 400 kb of the 6q telomere region in a male with developmental delay, short stature, and congenital anomalies. The deleted segment in this individual only includes three genes (*PSMB1*, *PDCD2*, and *TBP*); however, this small deletion results in a severe clinical phenotype. In contrast, a terminal deletion of 1.6 Mb of 21q was shown to be an inherited benign variant that includes ~15 known genes.

In the current study, we identified and characterized 10 cases with benign familial variants that had deletions or duplications of the telomeric regions of chromosomes 3p, 4q, 9p, 10q, 15q, 21q, and Yq. Imbalances in the telomeric regions have previously been reported in normal subjects without any apparent phenotype.^{5,24–26} The absence of phenotypic effects is most likely related to the size and gene content of the genomic region involved in the imbalance. From our data and those of others, benign variants >2 Mb in size are very rare (Database of Genomic Variants, <http://projects.tcag.ca/variation/>). In

addition, many genes in the genome are likely not dosage sensitive (e.g., genes for autosomal recessive conditions); therefore, copy number changes of these regions would be predicted to not cause an abnormal clinical phenotype. Additional detailed characterization of telomeric imbalances with and without phenotypic effects will aid in the definition of how much monosomy or trisomy can be tolerated without phenotypic effects.

We originally predicted that most cryptic telomere imbalances that were identified in individuals with developmental delay/mental retardation and a normal karyotype would be <1 Mb in size.⁶ However, our detailed mapping results presented here and in previous publications^{11,13} and those of Ballif et al.¹⁴ have demonstrated that imbalances of this size actually account for the minority of cases. In fact, 36% of our cases and 44% of those of Ballif et al. had imbalances that extended beyond 5 Mb. Thus, many telomere imbalances are quite large and still not detectable by G-banding analysis, which has been deemed the gold standard for detecting chromosomal gains or losses.

Furthermore, using high-definition telomere coverage with oligonucleotide arrays, we identified four cases with additional imbalances that were not detected using a single genomic clone for telomere analysis. Each of these cases had additional duplications of genomic material that were located adjacent to the deleted telomere region. This phenomenon has previously been reported by high-resolution characterization of specific cases involving terminal rearrangements, including deletions and ring chromosomes.^{27–30} Additional imbalances such as these are important to identify because they may modify the clinical presentation of an individual. Microarray analysis will

facilitate such detailed studies to become part of routine clinical practice.

Given these results and those of Ballif et al.,¹⁴ the sensitivity and reliability of the cytogenetic gold standard are being challenged by results from new molecular cytogenetic methods. Detailed characterization of telomere imbalances alone has demonstrated that the resolution of G-banding analysis is not as high as previously predicted and clearly the lower limit of G-banding detection is well above the threshold for significant phenotypic effects. This information for the telomere regions is likely reflective of imbalances across the whole genome. Therefore, higher resolution methods, such as microarray analysis, are necessary for a more sensitive methodology to detect clinically relevant chromosomal imbalances.

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