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American Journal of Medical Genetics Part A 146A:2898–2904 (2008)

Molecular and Clinical Characterization of a Recurrent Cryptic Unbalanced t(4q;18q) Resulting in an 18q Deletion and 4q Duplication

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Received 7 April 2008; Accepted 25 August 2008

Recurrent constitutional non-Robertsonian translocations are very rare. We present the third instance of cryptic, unbalanced translocation between 4q and 18q. This individual had an apparently normal karyotype; however, after subtelomere fluorescence in situ hybridization (FISH), he was found to have a cryptic unbalanced translocation between 4q and 18q [ish der(18)t(4;18)(q35;q23)(4qtel+, 18qtel−)]. Oligonucleotide array comparative genomic hybridization (aCGH) refined the breakpoints in this child and in the previously reported child and indicated that the breakpoints were within 20 kb of each other, suggesting that this translocation is, indeed, recurrent. A comparison of the clinical presentation of these individuals identified features that are characteristic of both 18q− and 4q+ as well as features that are not associated with either condition, such as a prominent metopic ridge, bitemporal narrowing, prominent, and thick eyebrows. Individuals with features suggestive of this 4q;18q translocation but a normal karyotype warrant aCGH or subtelomere studies.
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Key words: fluorescence in situ hybridization (FISH); cryptic translocation; array CGH; 18q deletion; chromosome 18; chromosome 4; recurrent translocation

How to cite this article: Horbinski C, Carter EM, Heard PL, Sathanoori M, Hu J, Vockley J, Gunn S, Hale DE, Surti U, Cody JD. 2008. Molecular and clinical characterization of a recurrent cryptic unbalanced t(4q;18q) resulting in an 18q deletion and 4q duplication. Am J Med Genet Part A 146A:2898–2904.

INTRODUCTION

Recurrent intrachromosome rearrangements are becoming increasingly appreciated as the source of multiple rare syndromes [Lupski and Stankiewicz, 2005]. These rearrangements are generated by mechanisms mediated by low copy number repeats in direct orientation up to 5 Mb apart on the same chromosome. This genomic architecture can yield interstitial deletions and more rarely interstitial duplications [Lupski, 2007].

On the other hand, interchromosome rearrangements involving different chromosomes are very common occurrences, occurring in an estimated 0.92% of births [Jacobs et al., 1992]. However, non-Robertsonian constitutional interchromosome translocations are rarely recurrent. Three examples are known: t(11;22)(q23;q11) [Ashley et al., 2006; Kurahashi et al., 2007], t(4:10)(q35;q26) [Van Overveld et al., 2000], and t(4;8)(p16;p23) [Giglio et al., 2002].

In order for a chromosome translocation to occur, several steps have to happen. Double stranded breaks must occur in two different chromosomes

Grant sponsor: National Center for Research Resources; Grant number: M01-RR-001346 for General Clinical Research Center, San Antonio, Texas; Grant sponsor: NIH/NICHD; Grant number: R01HD045907 to JDC.

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Published online 16 October 2008 in Wiley InterScience (www.interscience.wiley.com)
DOI 10.1002/ajmg.a.32557

that are in close proximity to each other in both time and space, presumably in germ cells. Studies on intranuclear chromosome localization show that within the interphase nucleus, specific chromosomes have a probabilistic territory that is cell type-dependent [Bickmore and Teague, 2002]. This regionalization of chromosomes may then create the temporal and spatial requirements necessary for a translocation to be recurrent [Bode et al., 2001; Meaburn et al., 2007].

Several factors may predispose DNA segments to translocation events. Sequence homology may be a primary factor. However, the local architecture of the individual chromosomes as influenced by the heterochromatin state and the three-dimensional sequence conformation can also be a factor in predisposing a locus for a translocation event. Non-B DNA conformations, such as palindromes or Z-DNA, can create regions of instability that can predispose a site for the double stranded break to occur [Bacolla et al., 2004; Kurahashi et al., 2006; Wang and Vasquez, 2006]. Additionally, chromatin structures such as scaffold-associated regions can influence DNA structural stability [Strick et al., 2006].

We first described a patient with a cytogenetically occult chromosome abnormality involving a 7 Mb deletion of 18q and a 7 Mb duplication of 4q [Gunn et al., 2003]. BAC clone array comparative genomic hybridization (aCGH) was used to define the breakpoints, which were between 68.9 and 69.5 Mb on chromosome 18q and between 182.2 and 185.2 Mb on chromosome 4. Since that publication there have been other reports in the literature of individuals similarly affected. Moncla et al. [2004] reported a patient with a similar rearrangement also detected using BAC clone aCGH. Their array used the same chromosome 4 flanking BAC clones (RP11-80P4 and RP11-90E7). One of their chromosome 18 BAC clones was identical to ours (RP11-25L3) and the other flanking clones (RP11-90L15) overlapped (RP11-125M20), thereby defining the same translocation region. Ravnan et al. [2006] reported the results of subtelomeric FISH analysis in 11,688 cases of developmental delay. They identified a total of 357 abnormal results, of which three had a chromosome 18q deletion and chromosome 4 duplication. As this methodology cannot determine exact translocation breakpoints, it is unknown whether any of these individuals had identical breakpoints on one or both chromosomes.

Here we report on another individual with a 4;18 translocation. In order to determine if the translocation breakpoints were the same in this person as in our previously reported individual, we used high-resolution oligonucleotide aCGH. In addition, we compared the clinical features of our two patients as well as the individual reported by Moncla et al. [2004].

METHODS

Participant Recruitment

The protocol to perform phenotypic and genotypic assessment of individuals with chromosome 18 abnormalities was approved by the Institutional Review Board of the University of Texas Health Science Center at San Antonio, the Research and Development Committee of the Audie L. Murphy VA Hospital, and the scientific advisory committee of the General Clinical Research Center. The process of informed consent was practiced throughout the study and documented for all participants.

Genotyping

Blood samples were obtained from the affected individual and the biological parents for chromosome preparations, DNA isolation, and creation of immortalized cell lines [Cody et al., 1997].

Microarray oligonucleotide CGH (aCGH) was performed using the Agilent Technologies system (Santa Clara, CA). Five hundred nanograms of genomic DNA was labeled using the Agilent Genomic Labeling Kit (#5188–5309) and hybridization procedures (CGH v4.0 protocol) per manufacturer's instructions. The arrays used contained 185,000 features across the entire genome for an average resolution of 20 kb.

Breakpoints were confirmed using quantitative real time PCR (QRT-PCR). This technique has been described for detecting both deletions and duplications of the *PMP22* gene [Aarskog and Vedeler, 2000]. We used the iCycler iQ Real-Time PCR Detection System (Bio-Rad, Hercules, CA) and designed TaqMan probe/primer sets from the genomic sequence data in the regions of interest.

Quantification of the amount of target sequence in unknown samples is accomplished by measuring the threshold cycle number (Ct value) using the standard curve and a housekeeping gene as an internal control reference. The fractional Ct values at which the amount of amplified target DNA reaches a fixed threshold is directly related to the amount of starting target DNA. A higher starting copy number of the genomic DNA target will result in an earlier and significant increase in fluorescence. DNA for the control samples for the QRT-PCR and the aCGH were purchased from Promega Corp. (Madison, WI) and are pooled samples from 10 individuals.

Fluorescence in situ hybridization (FISH) analysis was performed using a panel of 41 unique subtelomeric probes (ToTelVysion, Vysis, Des Plaines, IL) for the p and q arms of all of the autosomes and sex chromosomes (excluding the p arms of acrocentric chromosomes #13, 14, 15, 21, and 22). Hybridization and post-hybridization washes were performed according to the manufacturer's protocol and the signals were enumerated using a fluorescence microscope. Images were captured using CytoVision 3.6

software by Applied Imaging Corporation (Santa Clara, CA). FISH was also used to confirm breakpoints and localize chromosome segments.

RESULTS

We previously reported an individual who had a karyotypically occult translocation, which we determined to be the loss of terminal 18q substituted with a duplication of terminal 4q [Gunn et al., 2003]. In the present study we identified another individual with an identical rearrangement. This individual was first assessed using conventional cytogenetics at 725 G-band level of resolution and revealed a 46,XY normal male karyotype (Fig. 1C). An all-telomere FISH assay detected a rearrangement involving chromosome 18. One 18q subtelomere signal was absent; instead a signal for the 4q subtelomere was present on the abnormal chromosome 18. The other chromosome 18 and both homologues of the chromosome 4 showed normal hybridization signals (Fig. 2). The patient was therefore diagnosed with a cryptic unbalanced terminal rearrangement between chromosomes 4 and 18 resulting in partial trisomy 4q and partial monosomy 18q. His karyotype was 46,XY.ish der(18)t(4;18)(q35;q23)(4qtel+,18qtel−). His parent's karyotypes were normal.

The DNA from this new case as well our previously reported case was then assessed using 185 K oligo aCGH. The aCGH data are shown in Figure 2. Both the chromosome 4 breakpoints and chromosome 18 breakpoints were determined to be between the same consecutive array features. Since these features are approximately 20 kb apart, the breakpoints in the two individuals are the same to within 20 kb. The chromosome 18 breakpoint was between 69,132,637 and 69,153,087 bp and the chromosome 4 breakpoint was between 184,105,384 and 184,125,584 Mb (build hg18).

In order to delineate the clinical characteristics associated with this unbalanced translocation, we compared the features of these two individuals and the patient reported by Moncla et al. [2004]. The results are presented in Table I.

The current patient was a boy born at term with no perinatal complications. Shortly after birth bilateral congenital atresia of the external auditory canals were noted. His birth weight was 3.6 kg. On physical exam at age 5 his height was 111 cm (50–75th centile), weight was 18 kg (50th percentile), and head circumference was 49 cm (3rd centile). A prominent metopic ridge was noted, as well as bitemporal narrowing. Other features included prominent, thick eyebrows, hair on his eyelids, broad nasal bridge, bilateral epicanthal folds, hypoplastic ala nasi; and downslanting palpebral fissures. Further examination found a high arched palate; low set ears; mild pectus carinatum; fifth finger clinodactyly; and only ulnar loops on his finger pads. No abnormalities of his feet

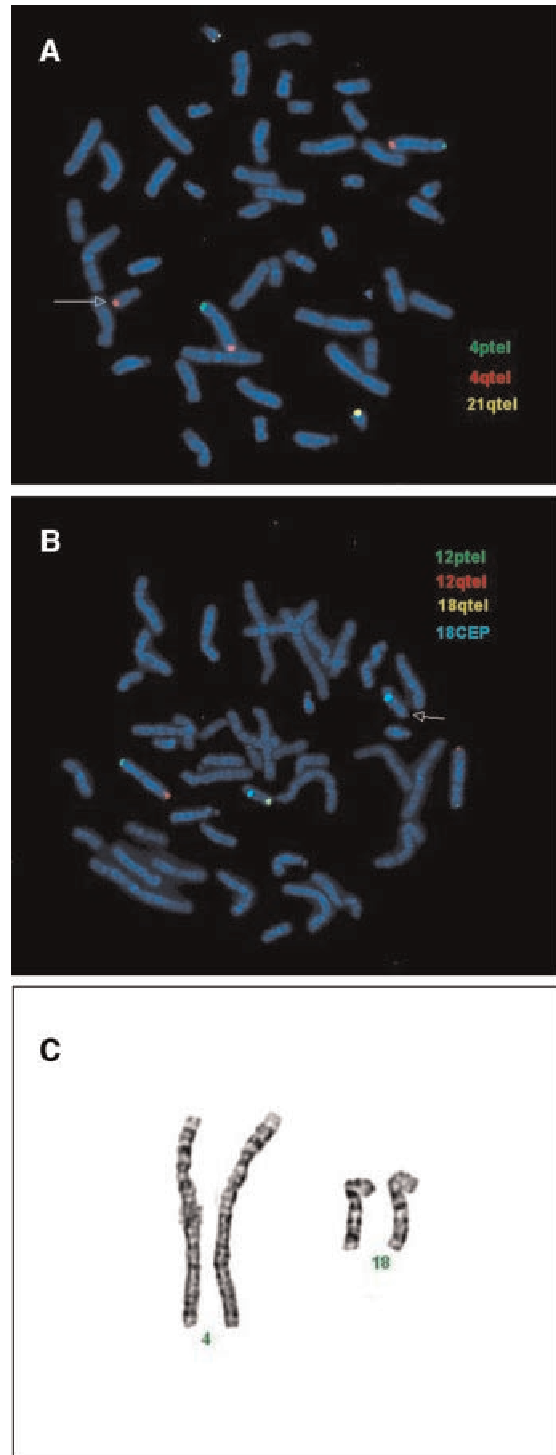

FIG. 1. Subtelomere FISH assay using ToTel Vysion,(Vysis). **A**: Metaphase spread showing 4p tel and 4q tel signals on both #4 chromosomes and an additional signal for 4q tel on another chromosome presumably #18 (arrow). **B**: Metaphase spread showing 18q tel on the normal #18 chromosome and missing on the other #18 chromosome (arrow). The #18 chromosome specific centromere probe is used as an internal control. **C**: High-resolution G-banded chromosomes of the patient revealing an apparently normal chromosome 4 and 18. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

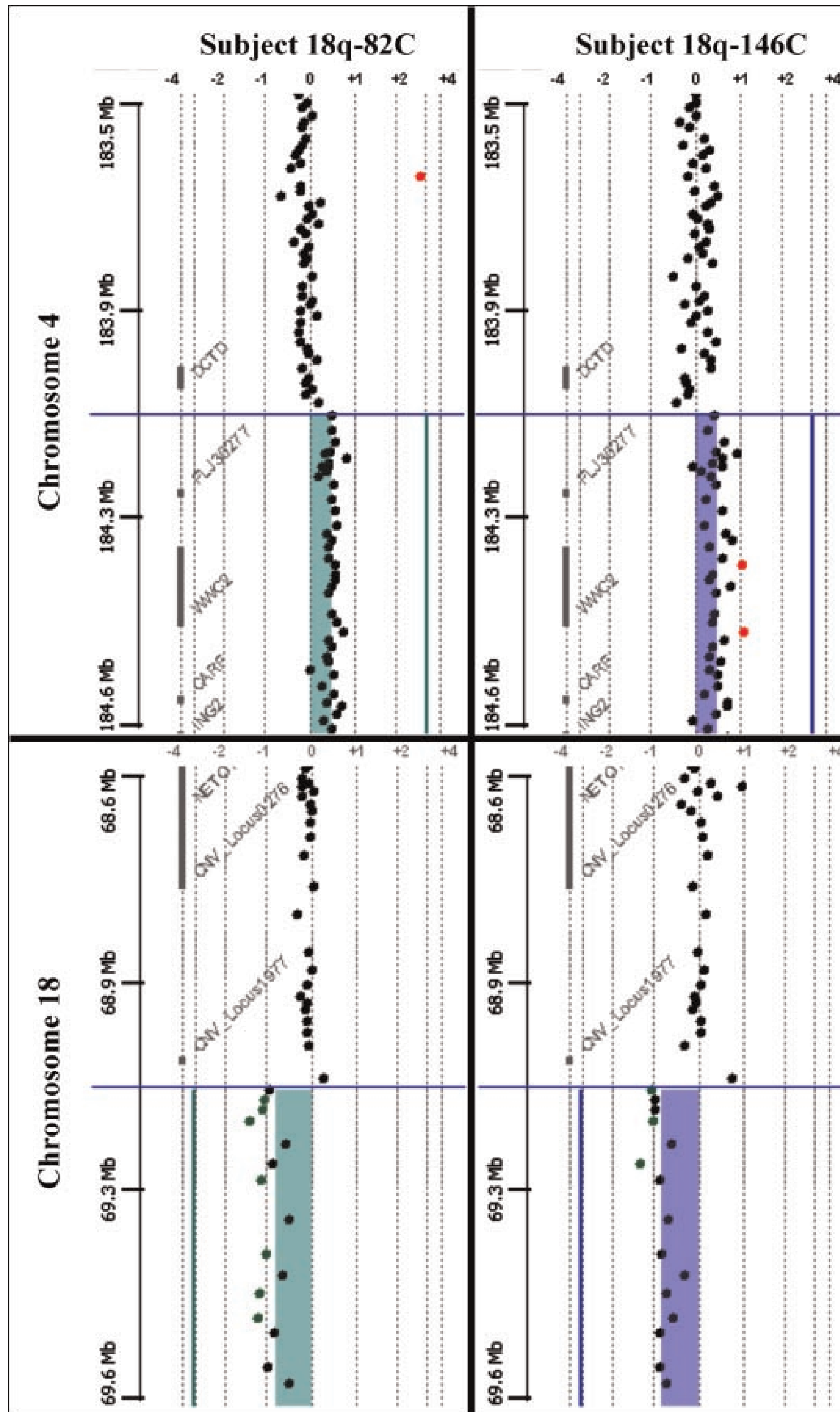

Fig. 2. aCGH analysis. Loss of 18q22.3-qter in conjunction with a gain of 4q35.1-qter is depicted in a zoomed-in gene view of chromosome 4 in the top panels and chromosome 18 in the bottom panels. The data from participant 18q-82C is in the left panels and 18q-146C is in the right panels. The regions of net copy number change are indicated by the wide bars (green for subject 18q-82C and blue for 18q-146C) within which the data points are significantly different on the $\log^2$ scale. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

were seen. He was Tanner stage 1 with fully descended testes. He had a slight cardiac flow murmur. Deep tendon reflexes were normal, but overall muscle tone was low. He had strabismus, poor vision, gait and balance problems, encopresis, and mild motor delays. At 5 years of age, his speech was at a 2-year-level. Other relevant past medical history included tibia, fibula, and wrist fractures from falls related to his

TABLE I. Comparison of Clinical Findings Between the Three Reported Cryptic Unbalanced t(4q;18q) Patients With the Characteristic Features of 18q− and 4q+

	18q− [Cody et al., 1999]	4q+ [Schinzel, 2001]	Gunn et al. [2003]	Moncla et al. [2004]	Current case
Height (centile)	51% < −2SD	Growth retardation	10th	NA	50th–75th
Weight (centile)	49% < −2SD	Low birth weight	10th	NA	50th
Head circumference (centile)	51% < −2SD	Microcephaly	2nd	NA	< 3rd
Vision	NA	NA	Poor	NA	Poor, strabismus
Auditory canal atresia	47%	NA	+, bilateral	+, bilateral	+, bilateral
Palate abnormality	43%	-	NA	NA	High, arched
Facial features:					
Prominent metopic ridge	−	−	+	+	+
Bitemporal narrowing	−	NA	+	+	+
Broad nasal bridge	−	+	+	+	+
Midfacial hypoplasia	48%	NA	+	+	+
Prominent thick eyebrows,	−	NA	+	+	+
Retromicrognathia	−	+	−	NA	−
Hands	Low implantation of thumbs, 65%	Single palmer crease	NA	Low implantation of thumbs	Fifth finger clinodactyly, ulnar loops
Lower limb abnormalities	Tapering legs	Hip dislocation	Hypoplastic calves, proximal tibia widening	NA	NA
Foot deformity	81%	Club foot	+	Bilateral vertical talus	Normal
Genitourinary abnormalities	50%	Hypoplastic genitalia	Small testes, penile chordae	NA	Normal
Cognitive and language development	Delayed	Delayed	Delayed	normal	Delayed
Motor development	Delayed	Delayed	Delayed	NA	Delayed, hypotonia
Brain MRI finding of hyperintense white matter	97%	NA	+	+	NA
Karyotype	del(18)(q21 > qter)	dup(4)(q31 > qter)	Normal	Normal	Normal

poor balance. His diet was unremarkable, and he was taking no medications. No MRI or photographic imaging had been completed. Family history was negative for any significant congenital problems, including ear anomalies or microcephaly.

We attempted to identify genomic features that might account for this recurrent translocation in unrelated individuals. We used the UC Santa Cruz Genome Browser to search for repetitive elements within 200 kb of each breakpoint and found no similar repetitive elements. In addition there were no regions of sequence homology between chromosome 4 and 18 in the breakpoint regions. Next we analyzed the regional sequences for palindromes as described by Ashley et al. [2006], and no palindromes were identified. We then sought to determine if these two chromosome regions were in close proximity to each other in the interphase nucleus. We performed FISH analysis using BAC clones that hybridize at the breakpoint region for each chromosome. We used RP11-80P4 from chromosome 4 and RP11-382C11 from chromosome 18. This analysis revealed that these two probes were not co-localized in the 50 interphase nuclei observed from immortalized lymphoblast cell lines derived from one of the parents. We were therefore unable to identify any contributory factors for this particular translocation.

DISCUSSION

We report here on a second recurrent constitutional non-Robertsonian translocation. Importantly, the t(4:18) constitutional translocation has relevance to the clinician as it cannot be identified using routine cytogenetics. The 4q duplication/18q deletion can only be detected using subtelomere FISH or array CGH.

In this report, we have sought to clinically characterize the 4q+/18q− syndrome and to understand the molecular mechanism by which it arose. As would be expected, at least some patients had several physical features that are common in individuals with 18q− deletions, including auditory canal atresia, delayed CNS myelination, midfacial hypoplasia, and foot deformities [Cody et al., 1999]. In additional they had several features found in individuals with duplications of 4q, including microcephaly and broad nasal bridge [Schinzel, 2001].

However, several features set these individuals apart from those with pure 18q deletions or 4q duplications. These features include a prominent metopic ridge, bitemporal narrowing, prominent thick eyebrows, and vision problems. Thus, patients presenting with these features in addition to any

features of 18q− or 4q+ could have the clinical diagnosis confirmed using genome wide aCGH or sub telomere FISH or MLPA.

We have investigated several potential molecular mechanisms for the etiology of the 4;18 translocation that might explain the de novo recurrence in three unrelated families. Possible explanations for the recurrent nature of these translocations include:

- Homologous recombination resulting from sequence homology between 4 and 18 at or near the translocation breakpoint.
- Genomic instability caused by non-B DNA conformations such as palindromic sequences on each chromosome [Bacolla et al., 2004; Kurahashi et al., 2006].

Chromosome co-localization in the interphase nucleus is required regardless of the molecular mechanism [Ashley et al., 2006]. We could find no evidence to support any one of these mechanisms. However, since interphase nucleus chromosome territories are cell type-specific, and we did not investigate co-localization of the breakpoints in germ cells, this is an area that warrants further study.

If this translocation is mediated by some genomic architecture feature, then we would expect to find other derivates of this recombination event, such as der(4)t(4q;18q), or a balanced t(4q;18q). However, to our knowledge no such cases have been reported. Thus it appears that this translocation may be mediated by nongenomic features.

The recurrent nature of this translocation event is even more intriguing considering our analysis of over 180 individuals with 18q− deletions using aCGH with an average resolution of 2 kb; we have not identified any two unrelated individuals, other than the individuals reported here, who have the same chromosome 18 breakpoint. We therefore speculate that these findings will provide insight into a novel mechanism of chromosome rearrangement.

ACKNOWLEDGMENTS

The authors would like to thank the families for their help and cooperation in the evaluation of their children for the purpose of advancing clinical care. The authors would also like to thank the technical staff of Pittsburgh Cytogenetics Laboratory for the cytogenetic and FISH analysis as well as Courtney Sebold, MS for helpful editorial comments regarding the manuscript. This work was supported by the National Center for Research Resources grant M01-RR-001346 for the Frederic C. Bartter General Clinical Research Center, San Antonio, Texas; NIH/NICHD grant R01HD045907 to JDC, The Chromosome 18 Registry and Research Society and the MacDonald family.

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