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Research Article

Fragility in the 14q21q translocation region

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Abstract

Aphidicolin (APC)-induced chromosomal breakage was analyzed for women representing three generations of a single family and carrying a Robertsonian translocation rob(14q21q). Fluorescence *in situ* hybridization (FISH) analysis confirmed the dicentric constitution of the derived chromosome and indicated the absence of β-satellite signal at the translocation region. Per-individual analysis of metaphases from APC-treated peripheral blood lymphocyte cultures identified significantly nonrandom chromosomal breakage at the translocation region in all three individuals examined. The APC-inducible fragility at the 14q21q translocation region suggests that this rearrangement was the result of chromosomal mutation at fragile site(s) in the progenitor chromosomes, or that this fragility was the result of the fusion of nonfragile progenitor chromosomes.

Key words: rob(14q21q), Robertsonian translocation, fragile sites, aphidicolin, heritability.

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Introduction

Robertsonian translocations are the most frequently observed structural rearrangement of human chromosomes. Occurring between acrocentric (and NOR-bearing) human chromosomes, Robertsonian rearrangements were historically thought to arise by centric fusion (Robertson, 1916). Recent investigations, however, indicate that more than 90% of human Robertsonian chromosomes are dicentric (one active and one latent centromere) with a small intercentromeric (translocation) region comprised of portions of p-arm material from each of the progenitor chromosomes and lack β-satellite and/or NOR sequence (Hurley and Pathak, 1977; Cheung *et al.,* 1990; Gravholt *et al.,* 1992; Wolff and Schwartz, 1992; Sullivan *et al.,* 1996; Page *et al*., 1996). The dicentric structure of these chromosomes is generally consistent with both recombination and breakage/reunion models for the origin of Robertsonian translocation. According to the recombination model, Robertsonian rearrangements result from meiotic association at NOR sequences and crossing-over between the satellite III regions (Therman *et al.,* 1989; Cheung *et al.,* 1990; Gravholt *et al.,* 1992; Sullivan *et al.,* 1996). The breakage/reunion model also invokes meiotic association of the acrocentric chromosomes, but postulates rearrangement resulting from breakage within the short arms, loss of the terminal p-arm material and fusion of the remaining segments (Hurley and Pathak, 1977; Stahl *et al.,* 1983).

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While there has been substantial progress in knowledge of the molecular basis and biomedical implications of rare (*e.g.*, folate-inducible) fragile sites, the common (*e.g.*, aphidicolin (APC)- inducible) fragile sites remain poorly understood. The potential for a disease relationship has been documented with certain rare fragile sites; FRAXA and FRAXE are associated with heritable mental retardation in humans, and FRA11B has been implicated in the genesis of chromosomal deletion syndrome (Jacobsen syndrome) sites (Fu *et al.,* 1991; Verkerk *et al.,* 1991; Knight *et al.,* 1993; Jones *et al.,* 1994; Nancarrow *et al.,* 1994; Parrish *et al.,* 1994; Ritchie *et al.,* 1994). Considerable interest in the common fragile sites has developed due to their potential role in cancer and cancer development. The cytogenetic locations of many of the common fragile sites map to regions that are frequently altered or rearranged during cancer development (Yunis and Soreng, 1984). There is also evidence to suggest that common fragile sites are preferred sites of sister chromatid exchange (Glover and Stein, 1987), chromosomal deletion and rearrangement (Glover and Stein, 1988; Wang *et al.*, 1993), gene amplification, (Coquelle *et al*., 1997), transfected plasmid DNA integration (Rassool *et al*., 1991) and viral integration

(Popescu and DiPaolo, 1990). Although common fragile sites have been hypothesized to experience significantly elevated levels of chromosomal mutation and, therefore, to be predisposed to chromosomal rearrangement (Hecht and Hecht 1984, Craig-Holmes *et al.,* 1987; Miró *et al.,* 1987; Yunis, 1987, 1990; McAllister and Greenbaum, 1997), there are scant data to support these hypotheses. In this paper we present and discuss evidence for the heritable occurrence of APC-inducibed fragility at the translocation region of a der (14; 21) (p10, p10) translocation chromosome in three related rob(14q21q) translocation carriers.

Materials and Methods

Peripheral whole blood samples were obtained from three women (aged 25, 49 and 69 years) previously ascertained to have 45,XX,t(14;21) chromosomal constitution. Previous chromosomal examination was conducted as they are the sister (N. 6611), mother (N. 6612), and maternal grandmother (N. 6613) of a woman (not included in this study) with 46,XX,der(14;21)(q10;q10),+21 Down syndrome. Whole blood samples were also obtained from 20 normal adults (10 males and 10 females), one of who is the male sibling (C_1, age 22) of individual 6611. The use of human subjects for this project was approved (#E96-199) by the Institutional Review Board of Texas A&M University.

For each subject, Two lymphocyte cultures were established for each subject, each from 1 ml of peripheral whole blood and 9 mls of RPMI 1640 media (Sigma #R8758), supplemented with fetal bovine serum, penicillin, streptomycin, and phytohemagglutinin. Cultures were incubated at 37 °C for 72 h. Twenty hours prior to harvest, one culture from each subject was inoculated with 0.2 mL of a 0.2 μM aphidicolin solution; the other culture served as an untreated control. Cell harvest and metaphase preparations followed routine cytogenetic laboratory techniques.

For the translocation carriers, confirmation of G-banded (Verma and Babu, 1995) karyotypes, detection of NOR expression by silver staining; (Howell and Black, 1980), and fluorescence *in situ* hybridizations (FISH) were conducted on metaphases from control cultures. *In situ* hybridization of the β-satellite acrocentric chromosome-specific probe (D13F39S1,S2/D14F39S3,S4/D15F39S6, S7/D21F39S7,S8/D22F39S9,S10; Oncor), and of the 13/21 and 14/22 α-satellite probes (D14Z1/D22Z1 and D13Z1/ D21Z1; Oncor) followed minor modifications of the manufacturer's protocols. Photomicroscopy was performed using a Zeiss Axioplan 2 fluorescence microscope and the MacProbe 3.4 program of the PowerGene computer-imaging system (Perceptive Scientific Instruments, Inc.).

For all individuals, chromosomal-breakage data were scored separately for the control and APC-treated cultures. Breakage was initially scored from 100 well-spread and directly Giemsa-stained metaphases with a complete complement of chromosomes. These metaphases were digitized, downloaded to image files, recorded, enhanced, printed and archived using the MacKtype program of the PowerGene chromosome-imaging system. The chromosomal preparations were then destained and G-banded. For optimally G-banded metaphases, the location of each break was determined by comparison of the directly Giemsa-stained and G-banded images of the same metaphase and mapped (at the 400 band level) to specific G-band location according to the International System for Human Cytogenetic Nomenclature (Mitelman 1995).

Fragile sites were identified using the FSM statistical program version 697 (Böhm *et al.,* 1995; Greenbaum and Dahm, 1995). The FSM model separately analyzes data from individuals and identifies fragile sites as loci at which chromosomal breakage is nonrandom with respect to the distribution of total per-individual breakage. The standardized X^2 (X^2_s) test statistic ($\alpha = 0.05$) was used for all fragile-site identifications.

Results

Chromosomal analyses of metaphases from the control cultures for the translocation carriers confirmed a 45,XX,der(14;21) chromosomal constitution and failed to detect NOR expression in the rob(14q21q) chromosome. Fluorescence *in situ* hybridization identified the translocated chromosome as containing α-satellite sequences for both chromosomes 14 and 21 (Figure 1) with an intercentromeric region absent of β-satellite signal (suggesting

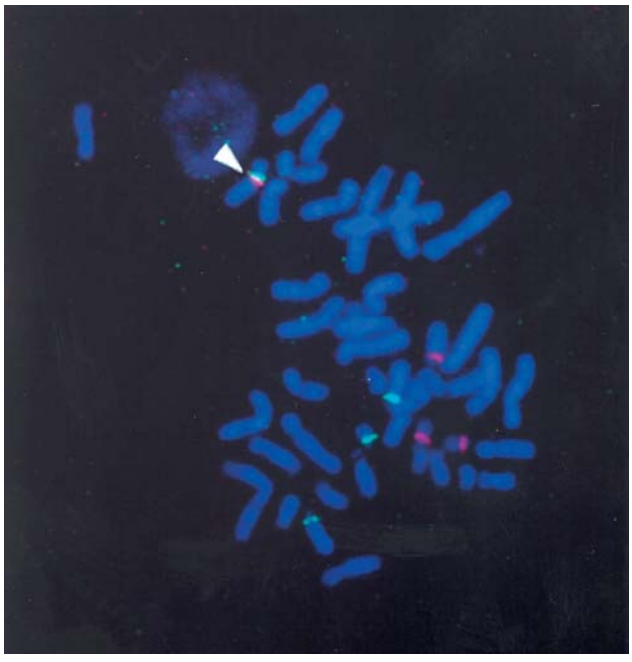

Figure 1 - FISH using 13/21 and 14/22 α-satellite probes (D14Z1/D22Z1 and D13Z1/D21Z1; Oncor) on a metaphase from the control culture for individual 6612. The dicentric structure of the rob(14q21q) chromosome is seen at the arrow. Detection of the 13/21 and 14/22 α-satellite probes was performed using FITC (green) and Cy3 (red), respectively.

the lack of NOR sequence); therefore, the subjects' karyotypes are 45,XX,dic(14;21)(p11.2;p11.2). For these individuals, analysis of 300 metaphases from the control cultures yielded a total of 25 chromosomal breaks at 16 different bands; one of these breaks (in subject 6611) mapped to the rob(14q21q) translocation region. Breakage data from the control cultures were not subjected to FSM analysis as such sparse data have been shown to be unreliable for statistical identification of fragile sites (Greenbaum *et al.,* 1997).

Fragile-site analysis of the translocation-carrying subjects (Table I) was based on a total of 959 APC-induced breaks from 257 metaphases mapping to 241 different G-bands. The FSM-determined minimum number of breaks necessary for any particular site to be declared fragile (C_α) was four for the data from individuals 6611 and 6612 and three for the data from individual 6613. Among these subjects, FSM analysis identified a total of 27 different APC-induced fragile sites including one at or near the 14q21q translocation region; we refer to this fragile site as 14/21tr (Figure 2; Table II).

Table I - Chromosomal-breakage data from APC-treated metaphases obtained for the three translocation-carrying subjects (6611, 6612, and 6613) and for each of the 20 (C_1 - C_{20}) karyotypically-normal control individuals. FS_T is the total number of sites identified as fragile in that individual.

Individual	Metaphases	Breaks	Breaks per metaphase	FS_T
6611	81	355	4.38	15
6612	86	392	4.56	23
6613	90	212	2.36	15
C_1	60	199	3.32	14
C_2	123	213	1.73	21
C_3	94	253	2.69	16
C_4	102	202	1.98	9
C_5	82	244	2.98	19
C_6	107	238	2.22	16
C_7	74	183	2.47	9
C_8	100	237	2.37	13
C_9	94	237	2.52	13
C_{10}	78	293	3.76	18
C_{11}	75	201	2.68	12
C_{12}	101	209	2.07	12
C_{13}	97	207	2.13	10
C_{14}	90	214	2.38	21
C_{15}	89	236	2.65	21
C_{16}	81	218	2.69	15
C_{17}	72	259	3.60	17
C_{18}	101	227	2.28	22
C_{19}	79	213	2.70	15
C_{20}	101	217	2.15	18
Total	1990	5459	2.74	50[1]

[1]Total number of different fragile sites identified for the 23 individuals.

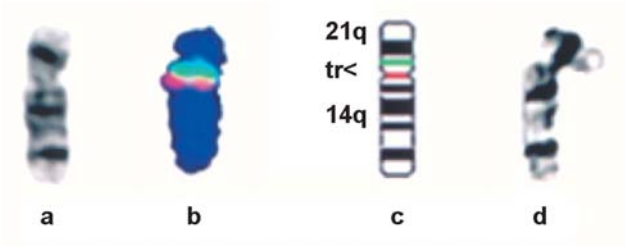

Figure 2 - (a) G-banded chromosome showing rob(14q21q), (b) FISH image of a dicentric rob(14q21q) chromosome (green = chr 21 centromere, red = chr 14 centromere), (c) ideogrammatic representation of the dicentric rob(14q21q) indicating the 14/21 translocation region (tr) and, (d) a dicentric rob(14q21q) chromosome (from an APC-treated metaphase) with a break at or near 14/21tr.

Table II - Observed chromosomal gaps/breaks at sites determined to be fragile (FSM, X^2_s test α=.05) in one or more of the rob(14q21q) carriers examined in this study. The sites are arranged by frequency of occurrence. The critical value (C_α) is the minimum number of breaks necessary for FSM to declare a site fragile from the data for that individual. An asterisk (*) designates the per-individual data indicating fragility according to FSM. 14/21tr refers to the fragile site at or near the 14q21q translocation region. The number of individuals fragile at each site and the number of sites identified as fragile in each individual are designated by n and N, respectively. Sites identified as fragile in one or more of the rob(14q21q) carriers but not identified as fragile in the control individuals are in bold.

	Individual			
Band	6611	6612	6613	n
3p14	17*	13*	33*	3
4p15	5*	7*	4*	3
6q26	5*	5*	7*	3
7q11	7*	5*	3*	3
14/21tr	10*	13*	6*	3
16q23	9*	22*	16*	3
1p22	2	8*	7*	2
2p24	5*	7*	2	2
2p13	5*	7*	1	2
2q33	1	14*	11*	2
5p14	6*	8*	2	2
7q22	3	5*	4*	2
7q32	2	15*	11*	2
8p11-q11	19*	5*	1	2
8q24.3	6*	4*	1	2
10q11	16*	1	3*	2
14q23	0	4*	3*	2
17q23	14*	9*	1	2
Xp22.3	1	9*	3*	2
1p11-q11	3	2	8*	1
1q44	0	4*	0	1
2p11-q11	8*	3	2	1
2q37	0	4*	1	1
6p25	0	7*	2	1
6p22	1	9*	1	1
9q22	1	2	4*	1
10q22	1	5*	1	1
16p11-q11	10*	3	0	1
C_α	4	4	3	
N	15	23	15	

G-band analysis of metaphase chromosomes obtained from the control cultures of the control subjects confirmed that each of the 20 individuals was karyotypically normal. For the 20 karyotypically-normal individuals, the total 4561 APC-induced breaks mapped to 333 different G-bands from 1816 metaphases (Table I); the C_α value for the FSM analyses of the data from these individuals was 3 or 4. In the control population, FSM identified a total of 45 different fragile sites in the control population, 43 of which (95.6%) have been previously identified as common fragile sites in humans (Hecht *et al.,* 1988, and 1990). Neither APC-induced nor spontaneous chromosomal fragility was observed on the p-arms of chromosomes 14 or 21 in any of the control individuals.

Discussion

Experiments aimed at testing the biological and biomedical implications of common fragile sites have been hindered by analytical difficulties associated with identifying which sites are fragile in single individuals. The FSM methodology (Böhm *et al.,* 1995), however, accounts for the relative paucity of chromosomal breaks compared to the number of chromosomal loci and allows the per-individual identification of fragile sites as loci at which breakage occurred at significantly nonrandom frequencies. FSM employs a stepwise procedure of fitting a Poisson distribution to the per-individual breakage data and tests the assumption that all sites have homogeneous breakage probabilities (*i.e.*, are nonfragile). FSM initially tests the entire data set for breakage homogeneity. If the hypothesis is rejected, the chromosomal locus with the highest observed breakage is removed and the remaining loci are tested for homogeneity. The iterative phase of the breakage analysis continues until the subset of the breakage data does not reject the assumption of homogenous breakage probabilities. For each individual data set analyzed, FSM provides a "critical value" (C_α) corresponding to the per-individual minimum number of breaks necessary for any particular site to be declared fragile. Any locus with observed breakage greater than C_α, is identified as fragile. The derivation, proofs and tests of the FSM statistical model are contained in Böhm *et al.* (1995).

In this study, FSM analysis of APC-induced chromosomal breakage in the three rob(14q21q) translocation carriers identified significant fragility at 27 different loci (Table II). Twenty-two of these sites were also identified by FSM as fragile in the control population. Of the 50 different sites that were identified as fragile in the 23 individuals sampled, 49 have been previously reported as fragile in humans (Hecht *et al.,* 1988 and 1990). The previously unreported fragility occurred at or near the 14q21q-translocation region (14/21tr). The 14/21tr locus was one of six sites identified as fragile in all three translocation carriers (Tables II and III). As these women represent successive generations of a single family, this 14/21tr fragile site appears to be both constitutional and heritable.

Table III - Percentage of total breakage at each of the six sites identified as fragile in all three translocation-carrying individuals. 14/21tr refers to the fragile site at or near the 14q21q translocation region.

	Individuals		
Fragile site	6611	6612	6613
3p14	4.8	3.3	15.6
4p15	1.4	1.8	1.9
6q26	1.4	1.3	3.3
7q11	2.0	1.3	1.4
14/21tr	2.8	3.3	2.8
16q23	2.5	5.6	7.5

The occurrence of APC-inducible fragility at or near the 14q21q translocation region has one of two potential explanations. The APC-inducible fragility at 14/21tr was either *de novo* (the result of fusion of nonfragile regions of the progenitor chromosomes), or was inherited from fragility in the p-arm(s) of one or both of the progenitor chromosomes 14 and 21. Although there are no reports of APC-inducible fragility on the p-arms of chromosomes 14 or 21 (Craig-Holmes *et al.,* 1987; Hecht *et al.* 1988 and 1990; this report), few individuals have been assayed for such fragility. For any particular locus, documentation of fragility in relatively low populational frequency will require per-individual analysis of appropriately large populations of individuals or analyses of individuals expected to carry such fragility. If analyses of additional 14q21q translocation carriers establish APC fragility as generally characteristic of the 14/21tr region, cytogenetic analyses of the chromosomally normal parents of *de novo* 14q21q translocation carriers should allow determination of whether this fragility is the *de novo* result of fusion of nonfragile regions or represents the maintenance of fragility present in one or both of the progenitor chromosomes.

Although Robertsonian translocations involving all the possible combinations of the five different acrocentric chromosomes have been observed, exchanges between chromosomes 13 and 14 and between chromosomes 14 and 21 together constitute ≥80% of recovered Robertsonian translocations (Therman *et al.* 1989). The recombination model has become the widely accepted explanation for the nonrandom participation of chromosomes 13, 14 and 21 in Robertsonian translocations (Choo *et al.* 1988, 1989; Therman *et al.* 1989; Choo 1990). Studies have identified homologous repetitive sequences in the pericentromeric regions of chromosomes 13 and 21, which is inverted in chromosome 14, and suggested that these sequences lead to preferential pairing and frequent ectopic recombination events resulting in the preferential formation of rob(13q14q) and rob(14q21q) translocations (Choo *et al.* 1988, 1989; Therman *et al.* 1989; Choo 1990). The infre-

quent observance of other documented nonhomologous and homologous Robertsonian translocations is attributed to the fact that translocation formation requires the occurrence of an unusual U-type recombination.

Previous to the proposal of the recombination model, the breakage/reunion model was the accepted model for Robertsonian translocation formation. The breakage/reunion model suggested that random breakage occurring either spontaneously or as a result of a mutagen would typically rejoin in the original order by repair processes. Sometimes, however, a new rearrangement would arise resulting from breakage within the short arms, loss of p-arm material and subsequent fusion of the remaining segments. From analyses of the association of nonhomologous acrocentric chromosomes during prophase I of meiosis in human oocytes and spermatocytes, Stahl (1983) argued in favor of the breakage/reunion for the origin of Robertsonian translocation formation. Analyses of nonhomologous acrocentric chromosomes during prophase I of meiosis in human oocytes and spermatocytes determined that acrocentric chromosomes consistently pair side-by-side. These data are inconsistent with the recombination model that postulates frequent end-to-end pairing of chromosome 14 with either chromosome 13 or 21 due to homologous sequences located on the p-arms of those chromosomes resulting in the high frequency of observed rob(13q21q) and rob(14q21q) translocations. A study of 56 nonhomologous Robertsonian translocations provided additional evidence in support of the breakage/reunion model (Page *et al.* 1996). Localization of the breakpoints in these 56 translocations determined that while in nearly all of the rob(13q14q) and rob(14q21q) translocations the breakpoints mapped to the same region, the breakpoint locations in the remaining "less common" nonhomologous Robertsonian translocations were highly variable (Page *et al.*, 1996). While this observed variability is consistent with the breakage/reunion model of Robertsonian translocation formation, Page *et al.* (1996) suggested that these data provided direct evidence that rob(13q14q) and rob(14q21q) form through a specific mechanism (recombination model) which is distinct from the mechanism(s) that contributes to the formation of the remaining types of Robertsonian translocations.

The breakage/reunion model provides a plausible model which explains all observed types of pre- and postzygotically derived Robertsonian translocations: monocentric and dicentric with/without NOR and β-satellite sequences (Berend *et al*., 1998; Catalan *et al*., 2000). It does not require (in translocations other than those involving chromosome 14 and either chromosomes 13 or 21) the occurrence of an unusual U-type recombination and is more consistent with the general absence of β-satellite signal within the translocation region of human Robertsonian chromosomes (Cheung *et al.,* 1990; Earle *et al.,* 1992; Gravholt *et al.,* 1992; Wolff and Schwartz, 1992). Additionally, Robertsonian translocation is a common mode of chromosomal reorganization in many species in which the rearrangements do not involve progenitor chromosomes characterized by the presence of NOR regions. Fragility at the 14/21tr region, as the result of fragility inherited from the progenitor chromosomes, would be consistent with the breakage/reunion model and with the expectations of a causal relationship between common fragile sites and chromosomal rearrangements. This novel finding, therefore, warrants further investigation of the breakage/reunion model and additional chromosomal breakage studies involving other D- and G-group Robertsonian translocation carriers.

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