American Journal of Medical Genetics 80:252–259 (1998)

Molecular Analysis of Mosaicism for Two Different De Novo Acrocentric Rearrangements Demonstrates Diversity in Robertsonian Translocation Formation

Sue Ann Berend,[1] Sonia Canún,[2] Christopher McCaskill,[1] Scott L. Page,[1] and Lisa G. Shaffer[1]*

[1]Department of Molecular and Human Genetics, Baylor College of Medicine, One Baylor Plaza, Houston, Texas
[2]Hospital General Dr Manuel Gea Gonzalez, Mexico City, Mexico

Robertsonian translocations (ROBs) involving chromosome 21 occur in about 5% of individuals with Down syndrome. ROBs are the most common chromosomal rearrangements in humans and are formed through whole arm exchanges of any two acrocentric chromosomes. The de novo formation of ROBs occurs at exceptionally high rates. The present case concerns a child with mosaic Down syndrome who has two cell lines that contain two different de novo ROBs: 45,XX,rob(14;21)(q10;q10) and 46,XX,rea(21;21)(q10;q10),+21. To elucidate the mechanisms by which the rearrangements formed, somatic cell hybrids were constructed to allow the parental origins of the chromosomes involved in the ROBs to be distinguished. The analysis of the hybrids showed that the rob(14q21q) must have formed postzygotically because it contained a maternal chromosome 14 and a paternal chromosome 21. Furthermore, hybrid analysis of the rea(21q21q) demonstrated two copies of the same chromosome from the mother and thus, by definition, was an isochromosome [i(21q)]. All free-lying chromosomes 21 isolated in hybrids were of maternal origin. These chromosomes may have originated from either of the patient's cell lines. We present four hypotheses for the formation of the two cell lines of this child. This case is part of an ongoing project to determine the mechanism(s) of de novo ROB formation and the results differ from the other de novo rob(14q21q) studied in our laboratory (n = 7) in that all previously studied translocations were maternally derived, leading to the conclusion that most de novo rob(14q21q) occur in oogenesis. The current case illustrates that other mechanisms may contribute to ROB formation. Am. J. Med. Genet. 80:252–259, 1998. © 1998 Wiley-Liss, Inc.

KEY WORDS: mosaicism; Robertsonian translocation; Down syndrome; isochromosome

INTRODUCTION

Robertsonian translocations (ROBs) are the most common chromosomal rearrangements occurring in humans and other organisms [Hamerton et al., 1975; Jacobs, 1981; Nielsen and Wohlert, 1991; White, 1973] and are formed when the long arms of two acrocentric chromosomes are translocated near the centromere, forming a single chromosome. ROBs occur at a frequency of ~1 in 1,000 in the general population [Hamerton et al., 1975; Jacobs, 1981; Nielsen and Wohlert, 1991] and have a relatively high rate of de novo formation with a mutation rate of approximately 3.92×10^{-4} [Jacobs, 1981]. Even though most of the short arms of the human acrocentric chromosomes are lost after translocation formation, balanced ROB carriers are phenotypically normal. However, carriers are at an increased risk of having an aneuploid child because of abnormal segregation of the rearranged chromosome and the corresponding homologues; unbalanced ROBs are a significant cause of birth defects and fetal wastage in our population.

Molecular genetic analyses of ROBs comprised of nonhomologous acrocentric chromosomes have indicated that ROB formation occurs more frequently during maternal meiosis I [Page and Shaffer, 1997]. Of 15 de novo ROBs analyzed, 14 were found to be exclu-

Contract grant sponsor: National Institutes of Health; Contract grant numbers: RO1 GM56845, T32-GM07526; Contract grant sponsor: National Science Foundation; Contract grant number: DGE 9255674.

*Correspondence to: Lisa G. Shaffer, Ph.D., Department of Molecular and Human Genetics, Baylor College of Medicine, One Baylor Plaza, Room 015E, Houston, TX 77030. E-mail: lshaffer@bcm.tmc.edu

Received 15 April 1998; Accepted 21 July 1998

sively maternal in origin, and only one was found to be paternally derived [Page and Shaffer, 1997]. Although formation of ROBs comprised of homologous acrocentric chromosomes have been reported to occur postzygotically in some cases [Robinson et al., 1994; 1996; Blouin et al., 1994; Cheung et al., 1997], no postzygotic formation of nonhomologous ROBs has been proven to date. This is partly because of the difficulties of distinguishing the parental origins of the ROBs from the corresponding homologues using molecular genetic techniques on total genomic DNA samples. To unequivocally determine the parental origin of the chromosomes comprising the ROB, the ROB must be isolated from the free-lying homologous chromosomes. Somatic cell hybrids are an effective method to isolate the chromosomes and allow the unequivocal determination of the parental origin of the ROB [Page and Shaffer, 1997]. Similarly, cytogenetic studies are not sufficient to distinguish true ROBs between homologous acrocentric chromosomes that exhibit two distinct chromosome arms from isochromosomes with two genetically identical chromosome arms [Shaffer et al., 1991; 1993]. Molecular studies using highly polymorphic microsatellite markers have been used in the analysis of the parental origins of chromosomes involved in homologous acrocentric rearrangements and indicate that the majority of rea(21q21q) are, in fact, isochromosomes and only a small percentage are true ROBs [Grasso et al., 1989; Antonarakis et al., 1990; Brahe et al., 1990; Shaffer et al., 1991; 1993]. The parental origins of i(21q) are equally divided between maternal and paternal origins [Shaffer et al., 1991; 1993; Grasso et al., 1989; Antonarakis et al., 1990; Brahe et al., 1990] indicating an equivalent predisposition to isochromosome formation in either oogenesis or spermatogenesis or perhaps during mitosis in the zygote.

Both nonhomologous and homologous ROBs involving chromosome 21 can lead to Down syndrome. When the age of the mother is disregarded, approximately 92.5% of all children with Down syndrome have free trisomy 21, 4.8% have translocation Down syndrome due to an ROB involving chromosome 21, and the remaining cases (~2.7%) are due to mosaicism [Giraud and Mattei, 1975]. Mosaicism usually presents as two chromosomally distinct cell lines, and the differences commonly involve whole chromosomes. Mosaicism involving structural chromosomal rearrangements is uncommon, and mosaicism for cell lines with different chromosomal rearrangements is very rare, reported in only a few cases [Zellweger and Abbo, 1965; Atkins and Bartsocas, 1974; Vianna-Morgante and Nunesmaia, 1978; Leiber and Shah, 1982; Tharapel et al., 1984; Clarke et al., 1989; Leal-Garza et al., 1996]. The cytological findings of each case reported in the literature are presented in Table I (with the exception of Zellweger and Abbo [1965], as this analysis was of nondifferentially stained chromosomes and chromosomal assignments were suggested by morphology).

The present case concerns a child with Down syndrome and two cell lines involving two distinct de novo ROBs: 45,XX,rob(14;21)(q10;q10) and 46,XX,rea(21;21)(q10;q10),+21. To unequivocally determine the parental origins of the chromosomes involved in each of the ROBs and understand the mechanism of their formation, somatic cell hybrids were first constructed. These were then analyzed by polymerase chain reaction (PCR) using highly polymorphic markers for chromosomes 14 and 21 to determine the parental origins of the chromosomes involved in the rearrangements. Herein, we present the results of this analysis and several hypotheses for the mechanisms involved in the origin of these cell lines.

MATERIALS AND METHODS

Case Report and Cytogenetic Analyses

The proposita was referred at age 2 months for chromosome analysis because of apparent Down syndrome. She was the first child born to young parents (mother, 19 years; father, 20 years). Her birth weight was 2,900 g. Physical examination showed brachycephaly, flat facial profile, up-slanted palpebral fissures, bilateral epicanthal folds, telecanthus, a small nose with a low nasal bridge, short neck, clinodactyly of both of the fifth fingers, a wide gap between the first and second toes, and hyperflexibility of the joints. At 2 years, her weight was 10 kg, length was 83 cm, and OFC was 46.5 cm. These measurements were all normal compared with charts for Mexican girls [Ramos-Galván, 1976]. Of the 50 cells analyzed initially, 33 contained a balanced karyotype of 45,XX,rob(14;21)(q10;q10) and 17 contained an unbalanced karyotype of 46,XX,rea(21;21)(q10;q10),+21.

Blood samples were obtained from the proposita and both parents. Lymphocyte cultures were established, and metaphase chromosomes were prepared using standard procedures. A repeat chromosome analysis was performed using the GTG-banding technique. Of the 30 metaphases analyzed, 16 contained a balanced karyotype of 45,XX,rob(14;21)(q10;q10) and 14 were unbalanced with a karyotype of 46,XX,rea(21;21)(q10;q10),+21 (Fig. 1). Chromosome analyses of both parents revealed normal karyotypes indicating de novo formation of both rearranged chromosomes in the index case.

TABLE I. Mosiac Cases Identified That Involve More Than One Acrocentric Rearrangement

Cytological findings	Reference
45,XX,rob(15;21)(q10;q10)/46,XX,rea(21;21)(q10;q10),+21	Atkins and Bartsocas, 1974
45,XY,rob(15;21)(q10;q10)/46,XY,rea(21;21)(q10;q10),+21	Vianna-Morgante and Nunesmaia, 1978
46,XY/45,XY,rob(13;21)(q10;q10)/46,XY,rea(21;21)(q10;q10),+21	Lieber and Shah, 1982
45,XX,rob(21;22)(q10;q10)/46,XX,rea(21;21)(q10;q10),+21	Tharapel et al., 1984
46,XX/45,XX,rob(13;21)(q10;q10)/46,XX,rea(21;21)(q10;q10),+21	Clarke et al., 1989
45,XX,rob(14;21)(q10;q10)/46,XX,rea(21;21)(q10;q10),+21	Leal-Garza et al., 1996
45,XX,rob(14;21)(q10;q10)/46,XX,rea(21;21)(q10;q10),+21	Present case

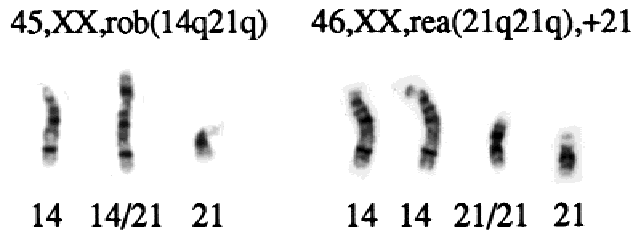

Fig. 1. Partial karyotype of the proposita. Shown are the rearranged chromosomes and the free-lying homologous chromosomes for each cell line.

Somatic Cell Hybrids

Transformed lymphoblast cultures were established on the proposita and both parents. To isolate the rearranged chromosomes from the corresponding free-lying homologous chromosomes, somatic cell hybrids were constructed by fusing the lymphoblast cells from the proposita to HPRT-deficient RJK88 hamster cells (a derivation of the Chinese hamster lung cell line V79), as described by Zoghbi et al. [1989]. This allowed separate, unequivocal analyses of the alleles of the rearranged chromosomes and alleles of the corresponding free-lying homologues [Page and Shaffer, 1997]. Colonies were isolated using cloning cylinders, and cells were tested by fluorescent in situ hybridization (FISH) to insure pure colonies that contained the chromosome of interest. The colonies that were not pure were discarded. Cell lysates were made for each hybrid colony by incubating the cells in lysis buffer (10 mmol/L Tris-HCl (pH 8.3), 50 mmol/L KCl, 2.5 mmol/L MgCl, 0.10 mg/ml gelatin, 0.45% (v/v) TWEEN-20, 0.40% (v/v) Nonidet P-40, and 60 μg/ml proteinase K) at 55°C for 12 hours. The lysates were used for the rapid identification, by PCR, of colonies that contain chromosomes of interest (e.g., the chromosomes involved in the rearrangements).

Fluorescence In Situ Hybridization

The colonies were additionally analyzed using FISH to identify those that contained either the rearranged chromosomes or those that contained the corresponding chromosomes 14 and 21. FISH was initially performed on metaphase chromosomes from the hybrids using D13Z1/D21Z1 (Oncor, Gaithersburg, MD), which is specific for centromeres of chromosomes 13 and 21, and D14Z1/D22Z1 (Oncor), which is specific for centromeres of chromosomes 14 and 22. The D13Z1/D21Z1 probe and the D14Z1/D22Z1 probe were obtained labeled with digoxigenin and biotin, respectively (Oncor). FISH analysis with the centromeric probes was performed as described by Shaffer et al. [1994]. The cells were counterstained with 4′,6′-diamidino-2′-phenylindole (DAPI) and were viewed with a Zeiss Axiophot fluorescent microscope equipped with a triple-band pass filter. Rearrangements were identified as dicentric by the appearance of two distinct regions of hybridization of the alpha-satellite probe on metaphase chromosomes or interphase nuclei.

The hybrids found to contain an isolated chromosome 21 using the D13Z1/D21Z1 centromere probe were also analyzed using the LSI™ 13 SpectrumGreen™/LSI™ 21 SpectrumOrange™ Dual Color DNA Probe Mixture in Hybridization Buffer (Vysis, Downers Grove, IL), specific for regions on the long arms of chromosomes 13 and 21. The slides were prepared as described by the manufacturer. The rea(21q21q) was distinguished from a free-lying chromosome 21 as having signals on both chromosome arms.

Molecular Analyses

DNA was extracted from lymphoblast cultures from the proposita and each of the parents using standard methodology. PCR was performed as previously described [Shaffer et al., 1993], and all markers were obtained from Research Genetics (Huntsville, Alabama). To determine the parental origin of the chromosomes involved in the rearrangement, two informative polymorphic microsatellite markers for each chromosome were amplified by PCR for the hybrids containing the rearranged chromosomes, hybrids containing the free-lying homologues, and genomic DNA from the parents. Alleles corresponding to the ROB and the free-lying homologues in the child were compared with the alleles from the parents. Primers for D21S210, D21S267, D14S45, D14S51, and D14S52 were found to be informative in this family and, thus, were used to analyze the parental origins of chromosomes 14 and 21 involved in the rearrangements and corresponding homologues. Additionally, six proximal markers (D21S120, D21S258, D21S215, D21S369, D21S192, and D21S16) and five distal markers (D21S1245, APP, D21S1235, D21S198, and D21S212) for chromosome 21 were studied. The proximal chromosome 21 markers were used to determine if the (21q21q) was an isochromosome or a true ROB. Distal markers were used to assess for the presence of three alleles, which would be helpful in establishing the mechanism of formation.

RESULTS

The initial PCR and FISH analyses of the hybrid colonies demonstrated that 26 of 86 colonies contained, either singularly or in combination, the rob(14q21q), the rea(21q21q), or the free-lying homologous chromosomes 14 or 21. The rob(14q21q) was isolated in three colonies (Fig. 2A), and the rea(21q21q) was isolated in a single colony (Fig. 2B). The free-lying homologous chromosomes 14 and 21 were isolated from the rearranged chromosomes in four and six colonies, respectively. Additionally, FISH analysis of hybrids containing the rob(14q21q) confirmed its presence and documented that both chromosome 14 and 21 centromeres were present (Fig. 2A), indicating a dicentric rearrangement. FISH analysis using the 21q specific probe confirmed that one hybrid contained the rea(21q21q) (Fig. 2B).

Results of the polymorphic molecular marker analyses are presented in Table II and Figure 3. Hybrids isolating a free-lying chromosome 14 were equally divided between maternal (n = 2) and paternal origin (n = 2). Hybrids, which contained an isolated chromosome 21 separated from the rearranged chromosomes, showed all chromosomes 21 to be of maternal origin (n = 6). The chromosome 14 of the rob(14q21q) was of

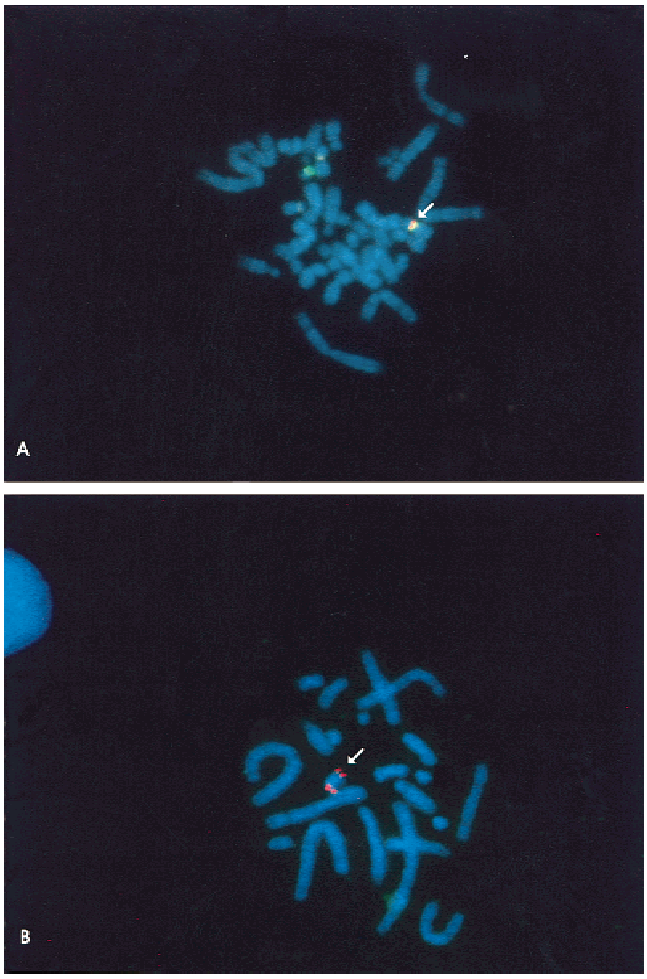

Fig. 2. Two-color FISH analyses of the somatic cell hybrids isolating the rearranged chromosomes from the free-lying homologues. **A:** A hybrid (clone 11) isolating the rob(14q21q). The arrow indicates the rearranged chromosome. The two signals indicate that the rob(14q21q) is a dicentric chromosome. A chromosome 22 is also included in this hybrid. **B:** FISH analysis of the somatic cell hybrid (clone 14) isolating the i(21q) from the free-lying homologues. This figure shows hybridization with a probe that is specific for 21q22.3 (Vysis). The 21q probe hybridized to both arms of the rearranged chromosome indicating that the hybrid contains the i(21q).

maternal origin and the chromosome 21 of the rob(14q21q) was of paternal origin, indicating that the formation of the rob(14q21q) in this proposita occurred postzygotically. The parental origins of the free-lying 14 and 21 of the rob(14q21q) cell line were unequivocally determined because of the simultaneous occurrence of the ROB with either of the chromosomes 14 or 21 in different hybrids (Table II). The free-lying 14 was of paternal origin and the free-lying 21 was of maternal origin. Finally, the chromosomes 21 involved in the rea(21q21q) were both of maternal origin and had identical alleles at all polymorphic loci tested (n = 13) consistent with an isochromosome. Additional comparisons of proximal and distal markers on chromosome 21 in genomic DNA of the mother, father, and proposita showed only two distinct chromosomes 21 in the index case (three alleles were not observed for any of the loci).

TABLE II. Molecular Analysis of Parental Origin of Rearranged Chromosomes and Free-Lying Homologous Chromosomes Isolated in Hybrid Clones*

		Parental origin	
Clone	Isolated chr.	Chr. 14	Chr. 21
1	14	mat	
2	14	pat	
3	14	mat	
4	14	pat	
5	21		mat
6	21		mat
7	21		mat
8	21		mat
9	21		mat
10	21		mat
11	rob(14q21q)	mat	pat
12	rob(14q21q)	mat	pat
13	rob(14q21q)	mat	pat
14	rob(14q21q), 21	mat	mat, pat
15	rob(14q21q), 14	mat, pat	pat
16	i(21q)		mat

*mat, chromosomes of maternal origin; pat, chromosomes of paternal origin.

DISCUSSION

Acrocentric rearrangements are involved in 5% of all Down syndrome cases and 95% of these involve Rob-

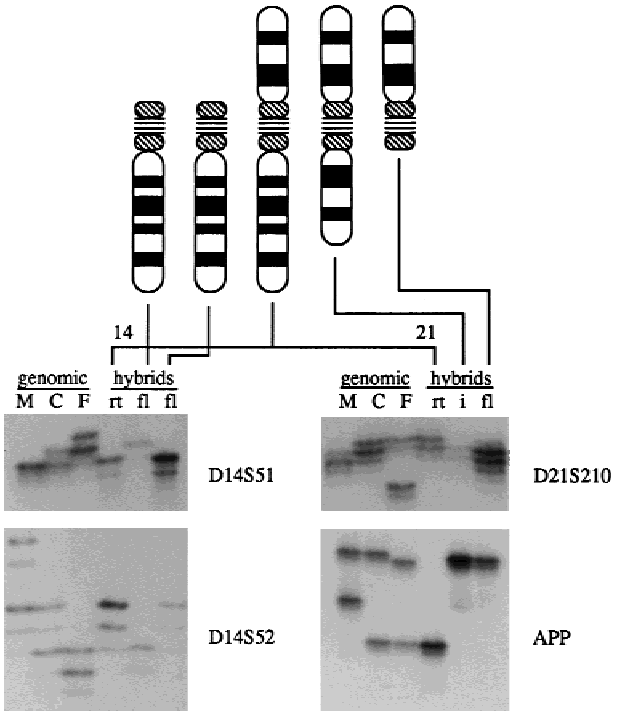

Fig. 3. Comparisons of polymorphic markers of somatic cell hybrids and genomic DNA of the proposita and both parents. PCR analysis of two polymorphic markers for each chromosome involved in the rearrangements was used to determine parental origins. PCR products of the genomic DNA of the mother (M), proposita (C), and father (F) are in lanes next to somatic cell hybrids isolating the Robertsonian translocation 14q21q (rt), free-lying homologues (fl), and the isochromosome 21q (i). The rob(14q21q) was comprised of a maternal chromosome 14 and a paternal chromosome 21, indicating a postzygotic error, and the i(21q) was of maternal origin. Both maternal and paternal free-lying chromosomes 14 were observed and only maternal free-lying chromosomes 21 were observed.

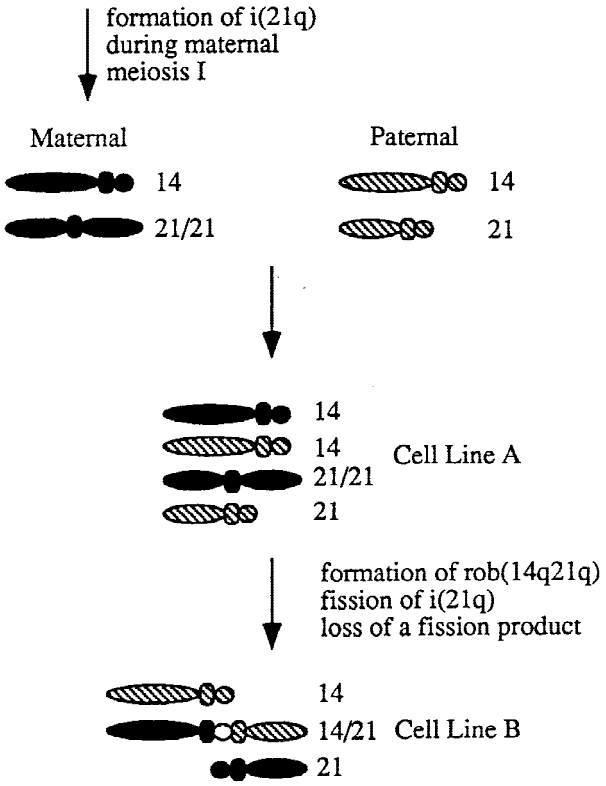

Fig. 4. Diagrammatic representation of the first hypothesis for the formation of the two cell lines. The first hypothesis proposes the formation of the two cell lines from an unstable trisomic conceptus. Isochromosome formation would occur during maternal meiosis. After fertilization with a normal sperm, the conceptus would be trisomic for chromosome 21 with two copies represented in the isochromosome. The rob(14q21q) would form postzygotically from the free-lying chromosomes. For the final rob(14q21q) cell line, a fission of the i(21q) would occur and one chromosome 21 would be lost.

ertsonian translocations [de Grouchy and Turleau, 1984]. The two most common acrocentric rearrangements in Down syndrome are rob(14q21q) and rea(21q21q), and these occur at approximately equal frequencies. About half of rob(14q21q) are inherited; however, over 95% of rea(21q21q) arise de novo [Shaffer et al., 1993]. Although i(21q) are indistinguishable from rob(21q21q) by using cytogenetic techniques, molecular techniques have revealed that the majority of rea(21q21q) are isochromosomes. The isochromosomes are equally divided between those that are of paternal origin and those of maternal origin [Shaffer et al., 1991; 1993; Grasso et al., 1989; Antonarakis et al., 1990; Brahe et al., 1990].

Mosaic Down syndrome involving cell lines that contain two different de novo rearrangements is quite rare and has only been reported in a small number of cases (Table I) [Zellweger and Abbo, 1965; Atkins and Bartsocas, 1974; Vianna-Morgante and Nunesmaia, 1978; Leiber and Shah, 1982; Tharapel et al., 1984; Clarke et al., 1989; Leal-Garza et al., 1996]. Many mechanisms were suggested to account for the various cell lines in these studies, but molecular analyses were not performed in any of the cases to support one particular mechanism.

Most mechanisms resulting in complex karyotypes involve multiple steps. The present case involves a child with Down syndrome and mosaicism for the following two cell lines: 45,XX,rob(14;21)(q10;q10) and 46,XX,i(21)(q10),+21. The parental origins of the rearrangements were determined by analyzing somatic cell hybrids that isolated the rearranged chromosome from the free-lying homologous chromosomes. The rob(14q21q) was comprised of a paternal chromosome 21 and a maternal chromosome 14, indicating postzygotic formation of the ROB. Because the ROB involved a chromosome from each of the parents, the rearrangement must have occurred after fertilization. Additionally, the parental origin of the free-lying chromosomes 14 and 21 of the rob(14q21q) cell line were unequivocally determined to be a chromosome 14 of paternal origin and a chromosome 21 of maternal origin, excluding uniparental disomy in this cell line. The rea(21q21q) was determined to be an isochromosome of maternal origin.

The first hypothesis put forward to explain this mosaicism is that the formation of the two cell lines resulted from an unstable trisomic conceptus (Fig. 4). In this situation, isochromosome formation would occur during maternal meiosis. After fertilization with a normal sperm, the conceptus would be trisomic for chromosome 21 with two copies represented in the isochromosome. The rob(14q21q) would form postzygotically from the free-lying chromosomes. For the final rob(14q21q) cell line, a fission of the i(21q) would occur and one 21 would be lost. This scenario requires a number of sequential events: formation of an isochromosome 21, a fusion of the rob(14q21q), a fission of the i(21q), and a subsequent loss of the extra 21q. Additionally, the fission of the isochromosome would be required to occur in such a way that would result in satellited stalks on the retained free-lying chromosome

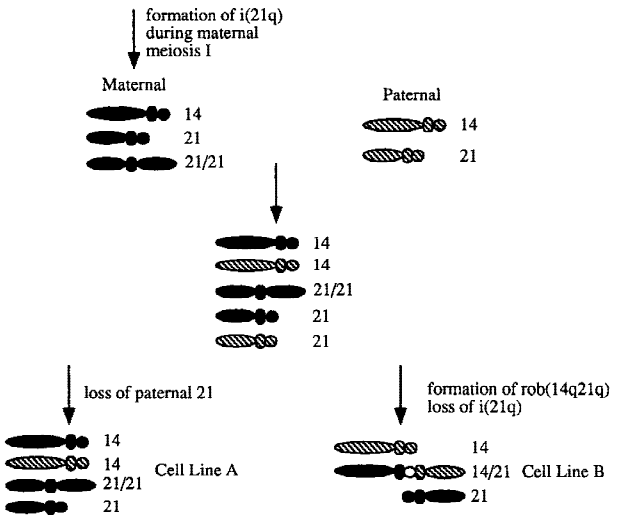

Fig. 5. Diagrammatic representation of the second hypothesis for the formation of the two cell lines. The second hypothesis involves a tetrasomic conceptus due to isochromosome formation and nondisjunction occurring at maternal meiosis I. The aneuploid ovum is fertilized by a normal sperm forming a tetrasomic conceptus. The tetrasomic cell line is unstable. The rob(14q21q) forms and this cell line loses the isochromosome. The cell line containing the isochromosome is formed by the loss of a chromosome 21.

21. All free-lying chromosomes 14 and 21 retained satellited stalks in the proband.

A second scenario involves a tetrasomic conceptus caused by isochromosome formation and nondisjunction occurring at maternal meiosis I (Fig. 5). The aneuploid ovum would be fertilized by a normal sperm forming a tetrasomic conceptus that would be chromosomally unstable. The rob(14q21q) would form, and this cell line would lose the isochromosome 21. The cell line containing the isochromosome would be formed by the loss of one chromosome 21. The formation of these two cell lines would be triggered by the instability of the tetrasomic cell line. Our molecular data are not totally consistent with this mechanism. If nondisjunction occurred during maternal meiosis I, we would expect to identify three alleles at some point along chromosome 21. Analysis of six proximal and five distal chromosome 21 microsatellite markers revealed only two alleles. Consequently, although the majority of nondisjunction occurs during maternal meiosis I [Lamb et al., 1997], our results would not support a meiosis I nondisjunction.

A third explanation would be that the patient is a true chimera, formed when two fertilized ova fused to form a single zygote (Fig. 6). Because the rob(14q21q) is of both maternal and paternal origin, this rearrangement must have occurred postfertilization. However, the isochromosome is of maternal origin and could originate during either maternal meiosis or after fertilization. The two zygotes would have subsequently formed a single embryo. Although true chimeras have been documented [Benirschke, 1974; Mayr, 1981; Altshuler, 1982], this situation may be unlikely based on our results because one would not expect to observe the same chromosomes 14 and the same chromosomes 21 involved in these two cell lines as these chromosomes should independently assort from the parents. None of the markers analyzed showed three or four alleles. The finding of the same parental alleles in both cell lines does not give much support to this hypothesis.

The fourth mechanism proposed for the formation of the two rearranged chromosomes is that their formation is coincidental and independent (Fig. 7). De novo Robertsonian translocation formation is a relatively common phenomenon and the coincident independent formation of two different rearrangements involving the acrocentric chromosomes could occur. Starting from a normal 46,XX conceptus, the rob(14q21q) would form postzygotically and result in the balanced translocation cell line. Likewise, the isochromosome of the maternal chromosome 21 also could occur postzygotically through centromere misdivision or a U-type ex-

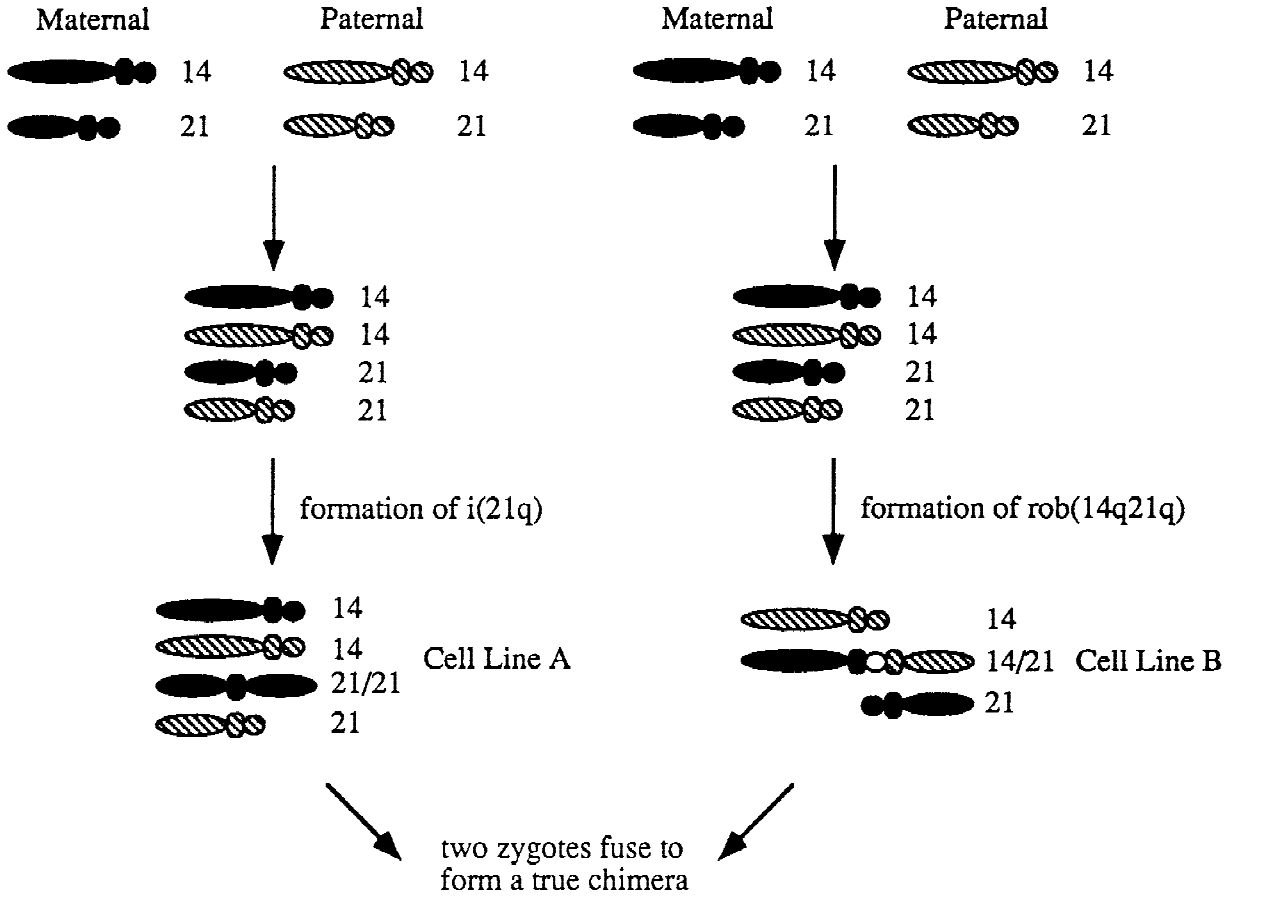

Fig. 6. Diagrammatic representation of the third hypothesis for the formation of the two cell lines. The third explanation for the formation of the cell lines is that the patient is a true chimera formed when two fertilized ova fused to form a single zygote. Because the rob(14q21q) is of both maternal and paternal origin, this rearrangement must have occurred after fertilization. However, the isochromosome is of maternal origin and could originate during either maternal meiosis or postfertilization. The two zygotes later fused and developed into a single embryo.

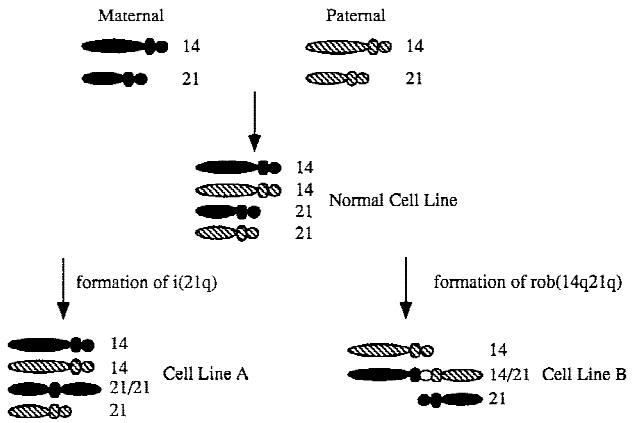

Fig. 7. Diagrammatic representation of the fourth hypothesis for the formation of the two cell lines. The fourth mechanism proposes the independent formation of the two rearranged chromosomes. Starting from a normal 46,XX conceptus, the rob(14q21q) forms postzygotically and results in a balanced cell line. Likewise, the isochromosome of the maternal chromosome 21 also occurs postzygotically through centromere misdivision or a U-type exchange. The normal cell line is lost or is not present in the peripheral blood sample.

change [Van Dyke et al., 1987; Therman and Susman, 1993; Shaffer et al., 1991; 1993]. In this situation, we would expect to observe at least a minimal number of cells consistent with the primary normal cell line (46,XX), as one may expect the normal cell line to be more stable than either cell line containing the rearranged chromosomes. However, no karyotypically normal cells were observed. There are two possible explanations: 1) this mechanism may have occurred very early in zygote development and the normal cells were lost and 2) the only tissue available for analysis was peripheral blood. The normal cell line may be present in other tissues of this patient, and thus, the normal cell line was undetected during cytogenetic analysis of the peripheral blood.

Whereas all four explanations are possible, we favor this last hypothesis. Although the normal cell line was not observed in peripheral blood, this last hypothesis only requires two steps from a karyotypically normal conceptus: the formation of the rob(14q21q) and the formation of the isochromosome. Mechanisms to account for mosaic structural rearrangements necessarily contain multiple steps. The surprising finding of two different rearrangements that did not share a common chromosome reinforces the notion that the formation of acrocentric rearrangements is relatively common. It is interesting that all previously reported cases of two acrocentric rearrangements in a Down syndrome individual involved a balanced, nonhomologous ROB and an unbalanced homologous rea(21q21q). Two of the cases also showed a karyotypically normal cell line. The previously reported cases, plus the current case, led us to erroneously speculate that the i(21q) cell line was derived from the ROB cell line, perhaps through misdivision of the centromere. The surprising finding of no common chromosomes in the two rearrangements leads us to believe that the rearrangements formed independently. Although no molecular studies were performed in the other reported cases, the finding of a normal cell line in two of the cases additionally supports the hypothesis of independent origins. It may be possible that the same mechanism was responsible for the mosaicism that occurred in all reported cases. Finally, although all rob(14q21q) studied previously appear to arise through a common directed mechanism [Page et al., 1996; Page and Shaffer, 1997], the current study demonstrates the diversity that may exist in ROB formation.

ACKNOWLEDGMENTS

We thank the family for participating in this study, and we thank Kelli Daily and Catherine Kashork (Kleberg Cytogenetics Laboratory, Baylor College of Medicine, Houston, Texas) for their help with Figure 2. This work was supported in part by a grant from the National Institutes of Health (NIH) (RO1 GM56845) (L.G.S.), an NIH training grant (T32-GM07526) (S.A.B.), and a National Science Foundation predoctoral scholarship (DGE 9255674) (S.L.P.).

REFERENCES

Altshuler G (1982): Developmental aspects of twins, twinning, and chimerism. Perspect Pediatr Pathol 7:121–136.

Antonarakis SE, Adelsberger PA, Petersen MB, Binkert F, Schinzel AA (1990): Analysis of DNA polymorphisms suggests that most *de novo* dup(21q) chromosomes in patients with Down syndrome are isochromosomes and not translocations. Am J Hum Genet 47:968–972.

Atkins L, Bartsocas CS (1974): Down syndrome associated with two Robertsonian translocations, 45,XX,-15,-21,+t(15q;21q) and 46,XX -21,+t(21q;21q). J Med Genet 11:306–309.

Benirschke K (1974): Chimerism and mosaicism–two different entities. Obstet Gynecol Annu 3:33–45.

Blouin J-L, Binkert F, Antonarakis SE (1994): Biparental inheritance of chromosome 21 polymorphic markers indicates that some Robertsonian translocations t(21;21) occur postzygotically. Am J Med Genet 49:363–368.

Brahe C, Tassone F, Moscetti A, Millington-Ward A, Bova R, Serra A (1990): Molecular study of parental origin of extra chromosome 21 in regular and de novo translocation trisomies. Am J Med Genet 7:125–128.

Cheung SW, Shaffer LG, Richards CS, Page SL, Riconda DL (1997): Prenatal diagnosis of a fetus with a homologous Robertsonian translocation of chromosomes 15. Am J Med Genet 72:47–50.

Clarke MJ, Thomson DAG, Griffiths MJ, Bissenden JG, Aukett A, Watt JL (1989): An unusual case of mosaic Down syndrome involving two different Robertsonian translocations. J Med Genet 26:198–201.

de Grouchy J, Turleau C (1984): "Clinical Atlas of Human Chromosomes." New York: John Wiley and Sons, p 344.

Giraud F, Mattei JF (1975): Aspects epidémiologiques de la trisomie 21. J Génét Hum 23:1.

Grasso M, Uzielli, MLG, Pierluigi M, Tavellini F, Perroni L, Bricarelli FD (1989): Isochromosome not translocation in trisomy 21q21q. Hum Genet 84:63–65.

Hamerton JL, Canning N, Ray M, Smith J (1975): A cytogenetic survey of 14,069 newborn infants: Incidence of chromosomal abnormalities. Clin Genet 8:223–243.

Jacobs PA (1981): Mutation rates of structural chromosome rearrangements in man. Am J Hum Genet 33:44–54.

Lamb NE, Freeman SB, Savage-Austin A, Pettay D, Taft L, Hersey J, Gu Y, Shen J, Saker D, May KM, Avramopoulos D, Petersen MB, Hallberg A, Mikkelsen M, Hassold TJ, Sherman SL (1997): Susceptible chiasmate configuration of chromosome 21 predispose to non-disjunction in both maternal meiosis I and meiosis II. Nat Genet 14:400–405.

Leal-Garza CH, Cortez-Gutierrez EI, Ortiz-Jalomo R, Garcia-Cavazos R (1996): A case of mosaic Down syndrome with two Robertsonian translocations. Rev Invest Clin 48:385–388.

Leiber E, Shah P (1982): Two Robertsonian translocations in a boy with mental retardation. J Med Genet 19:229–232.

Mayr WR (1981): Human chimerism. Rev Fr Transfus Immunohematol 24:19–26.

Nielsen J, Wohlert M (1991): Chromosome abnormalities found among 34910 newborn children: Results from a 13-year incidence study in Aarhus, Denmark. Hum Genet 87:81–83.

Page SL, Shaffer LG (1997): Nonhomologous Robertsonian translocations form predominantly during female meiosis. Nat Genet 15:231–232.

Page SL, Shin J-C, Han J-Y, Choo KHA, Shaffer LG (1996): Breakpoint diversity illustrates distinct mechanisms for Robertsonian translocation formation. Hum Mol Genet 5:1279–1288.

Ramos-Galván R (1976): Patrones de referencia para peso y talla en niños mexicanos. Bol Med Hosp Infant Mex [Suppl]:1–107.

Robinson WP, Bernasconi F, Basaran S, Yuksel-Apak M, Neri G, Serville F, Balicek P, Haluza R, Farah LMS, Luleci G, Schinzel AA (1994): A somatic origin of homologous Robertsonian translocations and isochromosomes. Am J Hum Genet 54:290–302.

Robinson WP, Bernasconi F, Dutly F, Lefort G, Romain DR, Binkert F, Schinzel AA (1996): Molecular studies of translocations and trisomy involving chromosome 13. Am J Med Genet 61:158–163.

Shaffer LG, Jackson-Cook CK, Meyer JM, Brown JA, Spence JE (1991): A molecular genetic approach to the identification of isochromosomes of chromosome 21. Hum Genet 86:375–382.

Shaffer LG, McCaskill C, Haller V, Brown JA, Jackson-Cook CK (1993): Further characterization of 19 cases of rea(21q21q) and delineation as isochromosomes or Robertsonian translocations in Down syndrome. Am J Med Genet 47:1218–1222.

Shaffer LG, McCaskill C, Han J-Y, Choo KHA, Cutillo DM, Donnenfeld AE, Weiss L, Van Dyke DL (1994): Molecular characterization of *de novo* secondary trisomy 13. Am J Hum Genet 55:968–974.

Tharapel AT, Redheendran R, Mankinen CB, Kukolich MK (1984): Mosaic Down syndrome with *de novo* 45,XX,-21,-22,+t(21q;22q)/46,XX,-21,+t(21q;21q) rearrangement. J Med Genet 21:391–395.

Therman E, Susman M (1993): "Human Chromosomes: Structure, Behavior, and Effects." 3rd Edition. New York: Springer-Verlag.

Van Dyke DL, Babu VR, Weiss L (1987): Parental age, and how extra isochromosomes (secondary trisomy) arise. Clin Genet 32:75–80.

Vianna-Morgante AM, Nunesmaia HG (1978): Dissociation as probable origin of mosaic 45,XY,t(14;21)/46,XY,i(21q). J Med Genet 15:305–310.

White MJD (1973): "Animal Cytology and Evolution." 3rd edition. London: Cambridge University Press, pp 198–230.

Zellweger H, Abbo G (1965): Familial mosaicism attributable to a new gene. Lancet 1:455–457.

Zoghbi HY, Sandkuyl LA, Ott J, Daiger SP, Pollack M, O'Brien WE, Beaudet AL (1989): Assignment of autosomal dominant spinocerebellar ataxia (SCA1) centromeric to the HLA. Am J Hum Genet 44:255–263.