Disappearing Trisomy 8 Mosaicism*

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ABSTRACT

A case is presented of a patient with disappearing trisomy 8 mosaicism initially thought to have stigmata of the fragile X syndrome. This case is interesting for two reasons. First, it demonstrates the occurrence of “disappearing mosaicism,” a phenomenon first described by LaMarche et al., in 1967. Our patient, initially studied in 1991 by two laboratories and found to be mosaic for chromosome 8 trisomy, was apparently normal by both GTG-banding and fluorescent in situ hybridization (FISH) when studied in 1996. Second, this case further underscores the fact that except under special circumstances, it is important that GTG-banding analysis be performed so that the entire human genome be examined in addition to scoring for the fragile X mutation on Xq27.3. In a recent review of the existing database at Rhode Island Hospital on chromosomal abnormalities found in patients referred because of a question of the fragile X syndrome during the period from January 1, 1990 to June 30, 1995, it was found that the frequency of other chromosomal abnormalities in patients referred because of a question of fragile X syndrome equaled or exceeded that of patients found to be positive for fragile X. Our figures, consistent with those reported in the literature, underscore the value of routine karyotyping in this population of patients.

Introduction

Much emphasis in the recent literature has focused on the occasional findings of fragile X syndrome in the pediatric population with delayed development, while other clinical findings in this same patient population have been somewhat neglected. In a previous study, the existing database at Rhode Island Hospital was reviewed to establish the true frequency of chromosomal abnormalities found in patients referred for fragile X syndrome at our center. In this report, a representative case of a patient from this population is presented in detail. This patient is also interesting from another perspective, in that he provides an example of an under-recognized phenomenon, disappearing mosaicism, first described in 1967.

The patient, referred to us initially to rule out fragile X, was found to have a low percentage mosaicism for trisomy 8. Features of the trisomy 8 syndrome include long facies, prominent forehead, large ears, transverse palmar crease, and mental retardation, which overlap...
those of the fragile X phenotype. Other features of the trisomy 8 syndrome, such as cryptorchidism, skeletal malformations and micrognathia, are not generally present in the fragile X syndrome. Sometimes the only significant findings in trisomy 8 mosaicism are mental retardation or psychological problems.

Case Report

The patient (ME, figure 1) a two-and-one-half-year-old male, was referred to the Rhode Island Hospital Child Development Center in June of 1991 because of developmental delay. He was born prematurely to a 28 year old gravida 1, para 0 female by cesarian section followed by a 33-week pregnancy, which was complicated by cigarette smoking, hypertension and pre-eclampsia. Birthweight was 1900 grams. After a relatively benign course in the intensive care nursery, he was discharged.

When seen at two-and-one-half-years, the patient's mother reported that he had been in good health generally, with the exception of frequent episodes of otitis media. Developmental milestones were delayed, as the patient crawled at 1 year, did not stand or walk independently until 2 years, and had only recently begun to use single words. He was only beginning to spoon feed himself at the time of the evaluation. The patient's physical examination at two-and-one-half-years was remarkable for a large head size in proportion to his body, an unusual hair whorl pattern, ears of normal size, but with mildly underdeveloped antefhelices, mildly low overall muscle tone, and ligamentous laxity. The patient had an unusual cognitive profile with overall functioning at approximately the 19 months level, but with visual motor skills at the 30 month level. He had poor eye contact and some self-stimulatory, stereotypic behaviors, and showed limited interest in social contact with others. He showed a lack of communicative intent, and expressive and receptive language skills were tested to be at the 12 to 15 month level and 18 to 20 month level, respectively.

The global developmental delay, combination of an unusual cognitive profile, and many of the behaviors demonstrated during his evaluation, were consistent with the diagnosis of pervasive developmental disorder (PDD). Given his relative macrocephaly, his low muscle tone and ligamentous laxity, as well as his unusual behavioral characteristics, developmental delay and diagnosis of PDD, chromosome studies were requested in order to rule out the fragile X syndrome. Chromosomal analysis revealed mosaic trisomy 8 at this age.

A repeat physical examination was done in October of 1995 when the patient was six-and-three-quarter-years old. His behavior during this evaluation was significant for occasional mouthing of objects, fair eye contact, perseverative behaviors, communication and socialization difficulties, all consistent with the diagnosis of autism. Non-verbal cognitive testing results showed skills at the 4 years, 9 months level. His mother reported ongoing language, social interaction, and behavioral concerns. Physical examination revealed that his face was mildly narrow, but overall there were no significant dysmorphic features. A repeat peripheral blood specimen was obtained in August of 1996 for the purpose of assessing the status of his trisomy 8 mosaicism. A buccal smear was used to substitute for a more invasive means of obtaining another tissue, as study of other tissues is the recommended approach for cases of mosaicism.

Materials and Methods

Conventional Cytogenetic Techniques

The peripheral blood specimens were collected in heparinized tubes. Lymphocytes were cultured for 72 hours with phytohemagglutinin using standard techniques. Fragile X site expression in the 1991 specimen was induced by one or more standard treatment protocols upon request from the referring physician. Scoring of other sites besides the fragile X site at Xq27.3 was performed to provide an internal control. Briefly, fluorodeoxyuridine (FUDR, 10 µl of 10^{-7} M final concentration),* methotrexate (MTX, 0.1 ml of 10^{-5} M final concentration)* and Trimethoprim (TMP, 0.1 ml of 13 mg/mL final concentration)* were added to each 5 ml of culture 72 hours after the initiation of the culture. Twenty-one hours after the addition of the induction agents, 32 µl of stock Colcemid (10 µg/mL)† were added. Three hours later (or 24 hours after the

* Sigma Chemical Co., St. Louis, MO.
† Gibco Life Technologies, Inc., Grand Island, NY.
addition of additives), cells were harvested according to a modification of the methods of Moorhead et al. The GTG-banding analyses were simultaneously performed to rule out chromosome rearrangements.

When fragile X cytogenetic testing was performed, at least 50 percent of the cells scored for fragile Xq27.3 were derived from cultures in which fragile site expression was induced by anti-metabolites (e.g., 5-fluorodeoxyuridine and methotrexate) in accordance with various guidelines, such as those from the Pacific Northwest Regional Genetics Group. The presence of the fragile site in putative cases was confirmed by GTG-banding. When a fragile X study was performed, it was considered positive when at least 4 percent of metaphases in the cytogenetic analysis contained a fragile X chromosome. Borderline cases were usually repeated and/or confirmed by molecular testing. In a cytogenetic study, at least 100 cells were scored. Scoring of other breaks and gaps was used as a means of internal control. Where indicated, DNA testing was performed by reference laboratories. In general, when DNA testing was ordered, a routine three-day stimulated culture of peripheral blood was also performed to rule out the presence of constitutional chromosomal abnormalities present elsewhere in the genome.

The designation of chromosomal abnormalities were in accordance with An International System for Human Cytogenetic Nomenclature.

FLUORESCENT IN SITU HYBRIDIZATION (FISH)

For FISH, modifications of the procedures of Pinkel et al., Mark et al., Mark et al., Miranda, Mark and Medeiros, Afify, Bland and Mark as well as manufacturer's instructions were followed. A biotin-labeled, chromosome-specific α-satellite probe was used. Alpha-satellite DNA probes hybridize to highly repeated alphoid sequences at the peri-centromeric regions of specific human chromosomes and are particularly suited for the purpose of chromosome enumeration.

Briefly, slides containing metaphase chromosome spreads were pre-treated using RNases and 2XSSC, dehydrated in a cold-graded ethanol series, denatured in formamide at 70° to 71°C and hybridized overnight at 37°C in a humid chamber. On the following day, the coverslips were removed, and the slides were post-washed under stringent conditions using 65% formamide and 2XSSC at 43°C.

For detection, slides were treated with 50 μl of fluorescein-labeled avidin at 37°C for 20 minutes. The interphase nuclei were counterstained with 20 μl of a 1:1 dilution of propidium iodide/antifade for a final concentration of 0.3 μg/ml per slide. The slides were covered with glass coverslips and examined under a Zeiss epifluorescence microscope using an FITC exciter filter set. Suitable fields were photographed using Ektachrome ASA 400 color film.

Results and Discussion

The fragile X syndrome is considered to be one of the major inherited causes of mental retardation in males. Clinical features usually associated with the fragile X syndrome include developmental delay, learning disabilities, mental retardation, autism, avoidance behavior as well as hyperactivity and attention deficit, speech and language delay, unusual hand mannerisms, long and narrow faces with moderately increased head circumference (>50th percentile), prominence of the jaw and forehead with particularly large and mildly dysmorphic ears, hyperextensible joints, high arched palate, pes planus, pectus excavatum, mitral valve prolapse and macroorchidism. Some clinical features of the syndrome overlap those associated with low level mosaicism for trisomy 8, such as the long face, prominent forehead, large ears, transverse palmar crease and mental retardation. It is probably for this reason that this patient with the mosaic trisomy 8 syndrome was referred for fragile X analysis.
Our patient was initially referred in 1991 for the purpose of ruling out fragile X syndrome. Chromosomal analysis revealed a modal chromosome number of 46 per cell with a male sex constitution. A single cell with trisomy 8 (5 percent) was found in the first 20 cells analyzed. An additional 100 cells were therefore analyzed. Results of this and subsequent analysis yielded no additional cells with fragile X or trisomy 8, while autosomal fragile sites were induced at 8 percent. Fluorescent in situ hybridization by a reference laboratory using probe pJM128 was performed because FISH permits the examination of a large number of cells. It revealed the presence of 22 out of 1000 nuclei (2.2 percent) with three positive signals. Similar results were obtained in our laboratory using a commercial chromosome 8 specific α-satellite probe (D8Z1).‡

After an exhaustive search of slides prepared from a second specimen, metaphases showing hybridization of D8Z1 to three chromosomes (figure 2) were found among numerous metaphases and interphase nuclei showing only two signals. The findings of three positive signals for the chromosome 8 probe were consistent with the findings of three of the chromosome 8 in a GTG-banded cell originally detected by the reference laboratory in the metaphase study (data not shown). These results, from two independent laboratories, were interpreted as low level mosaicism for trisomy 8. It was further noted that this level of mosaicism could have been easily missed in a standard 20 cell GTG-banded analysis.

Because of the observation that trisomy 8 mosaicism declines with age, a finding consistent with the hypothesis of “disappearing mosaicism”, another sample of blood was taken at the age of approximately 7 years and 8 months, in August of 1996.1,19 Results of chromosomal analysis of a routine three-day culture of 120 peripheral blood leukocytes derived from such a sample revealed the number of chromosomes to be 46 per cell with a male sex constitution and normal appearing banding patterns in all the cells examined. No abnormal cells were detected in this metaphase study.

Furthermore, fluorescent in situ hybridization using a chromosome 8-specific α-satellite probe (D8Z2)‡ revealed 19 interphase nuclei out of a total of 3160 nuclei (0.6 percent) examined to be trisomic. This is compared with a frequency of 0.2 percent positivity for 3 signals in normal controls.

When a blood smear slide was analyzed using FISH and the same probe, only one interphase out of 512 cells (0.2 percent) was found to be trisomic.

A buccal smear specimen was also obtained in an attempt to score cells from another tissue, as the optimal approach in cases of mosaicism is the analysis of more than one tissue. Out of 224 buccal mucosal cells, only 1 interphase nucleus, representing 0.4 percent of the total cell population, was trisomic. The quality of buccal smear preparations, however, is in general not comparable to blood and skin. The possibility of a skin biopsy to obtain cells for a fibroblast culture, although previously explored, was ultimately ruled out because the patient’s mother was not enthusiastic about this option.

Taken together, the results of the study conducted five years after the initial study are interpreted as apparently normal.

LaMarche et al1 first described the phenomenon of disappearing mosaicism in a female with mosaic trisomy 18 syndrome at birth. At 10 months of age, chromosome analysis revealed only normal metaphases. A growth advantage of the normal cell line over the abnormal population of cells with time was suggested by these authors for this particular mosaicism. Since the original findings by these authors were first described, we have not encountered other follow-up reports on this hypothesis. However, it has been known for some time that trisomy 8 mosaicism declines with age leading one to question if there is a steady decline in trisomic cells so that eventually these cells disappear completely.19 This hypothesis is indeed intriguing. However, to

‡ Oncor, Gaithersburg, MD.
the best of our knowledge, very little pertaining to this topic has appeared in the cytogenetic literature.

Acknowledgments

Thanks are extended to Dr. John F. Stone for stimulating discussions, personnel from our laboratory as well as the reference laboratory (Genetrix, Phoenix, AR) for technical help, and Drs. Paul H. LaMarche and Erlinda Polvorosa for helpful comments. The continued support of Dr. Roger Mark and the staff of the Laboratory of Cytogenetics, FISH and Genotoxicology at Rhode Island Hospital is acknowledged.

References