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Failure of X Inactivation in the Autosomal Segment of an X/A Translocation

CATHERINE G. PALMER,[1] THOMAS W. HUBBARD,[2] G. WILLIAM HENRY,[2] AND DAVID D. WEAVER[1]

SUMMARY

A newborn with an X/A translocation (46,X,der X,t(X;17)(17pter→-17p13::Xp22→Xqter) demonstrated multiple anomalies. X-replication studies in leukocytes of the patient with RBG (R Bands by BrdU using Giemsa stain) showed the abnormal X, t(X;17), to be late replicating except for the translocated segment. Clinical findings and replication studies suggest failure of inactivation of the translocated segment.

INTRODUCTION

In mice, coat-color genes are used to demonstrate that limited spread of X inactivation to autosomal segments translocated to the X occurs [1]. While X autosome (X/A) translocations are valuable tools for studying spread of inactivation in mice, their usefulness to demonstrate failure of spread of inactivation in man is limited by the absence of suitable genetic markers and by the relative imprecision of the autoradiographic technique used for this purpose.

We investigated an X/A translocation t(X;17)(p22;p13) in a child with multiple congenital anomalies using the RBG technique for X-replication studies. With this technique we have shown early replication, that is, failure of inactivation, of all or part of the autosomal segment translocated to the X chromosome.

The clinical and cytogenetic findings of the patient are presented here, and the literature is reviewed regarding X/A translocations with failure of inactivation of translocated autosomal region on an otherwise inactive X/A translocation.

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[1] Department of Medical Genetics, Indiana University School of Medicine, Indianapolis, IN 46223.

[2] Department of Pediatrics, Indiana University School of Medicine.

CASE REPORT

The pertinent features of this patient are presented in figure 1 and list 1. The patient (Dept. of Medical Genetics, Family no. 25680), a Caucasian female, was born after an estimated 35-week gestation to a 16-year-old primigravida mother and an 18-year-old father. The pregnancy was uncomplicated. At delivery, the amniotic fluid was meconium-stained, and Apgar scores were three and five at 5 and 10 min, respectively. Initial laboratory evaluation revealed acidemia, hypoxemia, and anemia. Birth weight, length, and occipitofrontal head circumference were 2300 gm, 46.5 cm, and 31.5 cm, respectively, all appropriate for her gestational age.

Cardiac catheterization at 2 weeks revealed dextroposition of the heart, a small ventricular septal defect with some left to right shunting, a small patent ductus arteriosus, and moderate pulmonary artery hypertension (55/14 mm Hg). Subsequently, she had recurrent emesis and poor weight gain and developed congestive heart failure. Her condition worsened, and she expired at 11 weeks. Permission for an autopsy was denied.

FAMILY HISTORY

The mother has one full sister as well as a half-sister and a half-brother. The full sister was born with a cleft palate and has produced two normal daughters. The half-siblings are normal and have had no children. The maternal grandmother, the common parent of the patient's mother and her mother's sibling and half-siblings, has had two midtrimester miscarriages. These pregnancies were by the maternal grandfather, who was not available for evaluation. No family information is available on the father's side.

CYTOGENETIC STUDIES

Chromosomal studies of the patient's peripheral lymphocytes using G banding by trypsin (GTG) showed a small extra segment on the p arm of one of the X chromosomes, while the rest of her chromosomes appeared normal (fig. 2). Her mother was found to carry a reciprocal translocation involving chromosomes X and 17 (fig. 3). The breakpoints of the translocation were at 17p13 and Xp22. The segment of 17p was translocated close to the tip of the X chromosome, Xp22. However, we could not establish whether a piece of the X was reciprocally positioned on chromosome 17. The maternal grandmother and aunt both had normal chromosomes, and the maternal grandfather was not available for study.

X-replication studies were carried out on both the child's and mother's leukocytes using the RBG technique [2, 3]. BrdU was added during the last 6 hrs of culturing, and after staining with

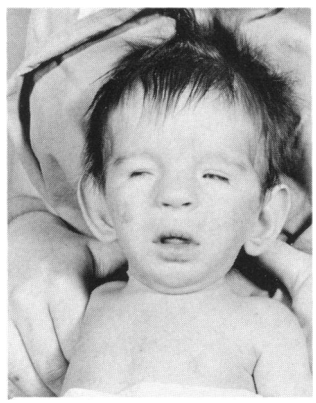

FIG. 1.—Proband at 8 wks. Note prominent, simple ears, short palpebral fissures, short nose, and long philtrum.

LIST 1

MAJOR CLINICAL FEATURES OF PATIENT WITH t(X;17) TRANSLOCATION

Ocular:
Microphthalmia
Microcornea
Corneal clouding
Intraocular malformations, type undetermined
Short palpebral fissures
Deeply set eyes
Craniofacial:
Cupped and simple ears
Short nose
Long philtrum
Triangular-shaped face
Trunk:
Dextroposition of the heart
Ventricular septal defect, small
Patent ductus arteriosus, small
Probable hypoplasia of right pulmonary artery and lung
Eleven ribs bilaterally
Single umbilical artery
Performance:
Hypotonia
Poor feeding and recurrent emesis
Failure to thrive
Early death

Hoechst 33258 (Frankfurt, Germany) and exposure to sunlight, the slides were restained with Giemsa. When BrdU is added during this terminal period of replication, chromosomes or segments which have completed replication stain darkly, while those incorporating BrdU during the terminal 6 hrs of DNA synthesis are decondensed and stain lightly with Giemsa. In the child's cells, the abnormal X was consistently late replicating (116 cells). The mother's normal X chromosome was predominately inactivated. Of 124 cells of the mother in which differential X replication could be observed, the normal X was late replicating in 107 cells. In 17, the abnormal X appeared to replicate later than the normal X.

The severity of the clinical findings in this patient would be puzzling if the X/A translocation was completely inactivated. They differed from those seen in cases of deleted Xp in that there was no evidence of any Turner syndrome stigmata. We compared the terminal replication pattern of the late X of the patient with her mother's abnormal X and with normal females B-pulsed (terminal pulsed with BrdU) for 5 and 6 hrs. The child's abnormal X was unique in that the translocated segment appeared to have completed replication prior to the remainder of the abnormal X chromosome (fig. 4*A* and *B*). The child's abnormal X chromosome was late replicating with the exception of the translocated portion, which replicated earlier than any other part of the X chromosome when observed by the BrdU technique. However, in none of the mother's 17 cells in which the abnormal X appeared late could we delimit later replication of the autosomal segment.

DISCUSSION

The clinical findings might be considered the result of partial trisomy 17. There are limited clinical reports [4, 5] other than those observed in malignancies of partial trisomies involving chromosome 17 (table 1). In these reports, the identification of an

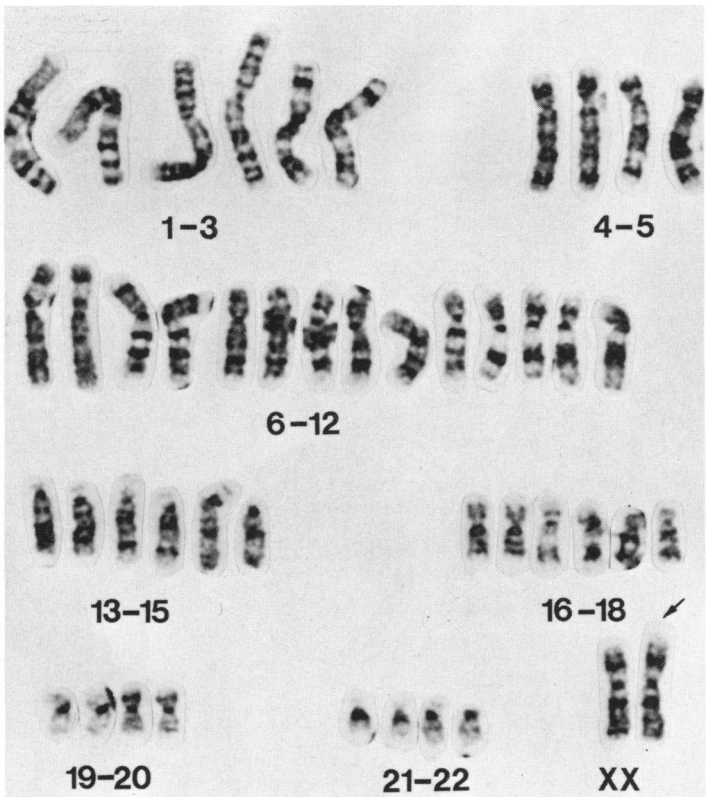

FIG. 2.—Karotype of patient showing the derived t(X;17)(p22;p13)

extra small chromosome as a deleted chromosome 17 is tenuous, and the clinical findings are disparate. Our patient has not only a small trisomic segment, but as the translocation involves the X chromosome, some degree of X inactivation of the translocated segment may have occurred. Thus, the clinical findings might not be completely typical of partial trisomy 17.

Inactivation patterns seen in X/A translocations are hypothesized to be the result of selection in a cell population in which random X inactivation has occurred [6]. In most cases of X/A translocations, the apparent non-random X inactivation probably results from random inactivation followed by selection for the most viable cell type [7].

Individuals with balanced translocations involving an X and an autosome may be normal and fertile and show the normal X to be inactive, as the mother of this patient demonstrates.

If an individual is unbalanced for an X/A translocation, the patient may be normal if the X/A translocation is inactive and the extra autosomal genes are not expressed.

If selection is incomplete, the two populations of randomly inactivated X's may be present with resulting expression of the autosomal segment. The alternative this patient demonstrates is inactivation failure of all or part of the translocated segment in an otherwise inactive X. The inactivation failure of the autosomal segment of the

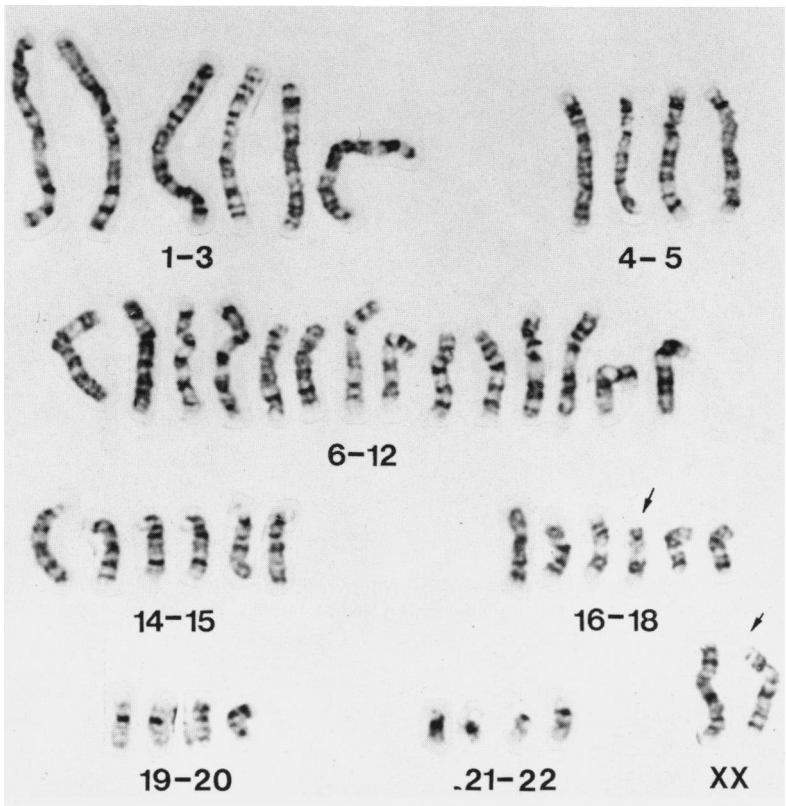

FIG. 3.—Karotype of proband's mother with balanced reciprocal translocation t(X;17)(p22;p13)

translocated piece of chromosome 17 may be responsible for this patient's clinical features.

Usually, when an autosome and an X chromosome are involved in a translocation, the entire X/A translocation is inactivated. The literature reveals, however, a number of cases studied either by autoradiography or by the RBA (R bands by BrdU and acridine orange) or RBG techniques, showing clinical expression of an autosomal segment in an X/A translocation and early replication of the autosomal piece (table 2).

We review these to demonstrate that not all cases of failure of inactivation may be due to a common cause. For example, the patient described by Neuhauser and Back [8] demonstrated early replication of a segment of a C-group chromosome translocated to Xp of an otherwise late replicating abnormal X. This patient, with 45 chromosomes, was reported to have clinical features which we currently might identify as partial monosomy 9. Inactivation of the autosomal segment in the translocation would have rendered the cells monosomic for the entire C (9?) chromosome. Thus, the cells with the active segment of the t(X;A) were selected.

Gaal and Laszlo [9] described a proband with gonadal dysgenesis and mental retardation with an X/6 translocation of the X chromosome. Cells with three different kinds of inactivation were demonstrated. In 16.6% of the cells, the entire t(X;6) was

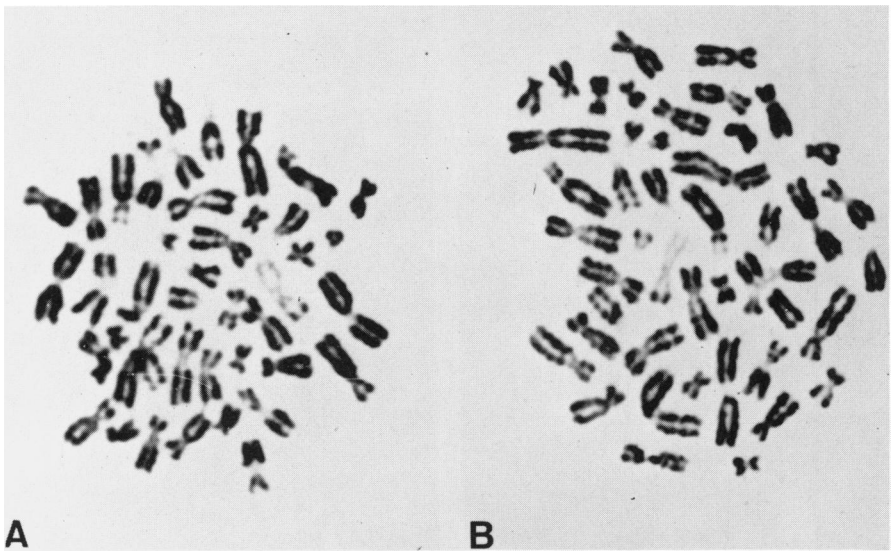

FIG 4. —*A* and *B*, Two cells showing abnormal X chromosome of proband lightly staining with exception of translocated segment, which appears to have completed its replication prior to rest of X after B-pulse.

inactivated. The normal X was inactivated in 13.49% of the cells, and only the X portion of the translocation chromosome in 70%. This case is unique in reportedly involving a translocation of Xp on to 6p. If true, this would support the concept of a second inactivating center on the p arm.

In our patient, the failure of inactivation of the translocated autosomal segment is suggested clinically by the dysmorphic features of the child and cytologically by early replication of this chromosomal segment in an otherwise late replicating X/A translocation.

Accumulated evidence supports the presence of an inactivation initiation center or centers. It most likely is located proximally in Xq, and from it, X inactivation theoretically spreads to the remainder of the X chromosome [10, 11]. This spreading effect is well established in mouse X/A translocations, and failure of inactivation may have an analogous relation to the size of the translocated segment or the distance from such an inactivating center, resulting in inactivation failure over the entire autosomal segment. This might be considered in several instances cited above where failure of inactivation occurred in large segments of autosome positioned distally in either Xp or Xq arms [8, 12–15]. The explanation is probably more complex than this since both cases reported by Hajemeijer et al. [16] and the case reported here represent small segments of autosome which have been translocated distally on the Xp arm.

Different regions in the inactive X could differ in ability to induce or transmit inactivation to the translocated segments. The possibility that some loci or regions of the X chromosome escape inactivation has been the subject of much speculation. Thus, the work of Therman et al. [11], based on studies of isodicentric X's, suggests that the proximal region of Xp(p11) remains active. The failure to demonstrate inactivation of

TABLE 1

CLINICAL FINDINGS IN PATIENTS REPORTED TO HAVE PARTIAL TRISOMY 17p

Clinical findings	17pter→17q11*	17pter→17q21†	17pter→17p13‡
Head and face:			
Wide sutures and fontanelles	+	...	−
Low set and/or malformed ears	+	+	+
Microcephaly	+	...	−
Eyes:			
Microphthalmia/small palpebral fissures	...	...	+
Cloudy cornea	...	...	+
Short nose	+	...	+
Long philtrum	...	...	+
Micrognathia	+	+	−
Performance:			
Hypotonia	...	...	+
Hypertonia	+	+	−
Failure to thrive	+	...	+
Short survival	...	...	+
Other:			
Joint contractures	+	+	−
Congenital heart defects (VSD/ASD)	+	...	+
Dextroposition of the heart	...	...	+
Single umbilical artery	...	...	+

* [4].
† [5].
‡ Source: this study.

the Xg^a locus in man also suggests that a region of the X chromosome which is not subject to inactivation does exist [6]. Bernstein et al. [17] suggest that the Xg^a locus may be located in the terminal portion of Xp(Xp22). Translocations of autosomal segments to this region of the X chromosome might thus escape inactivation. Failure of inactivation does appear to occur more frequently in X short-arm translocations. Of the eight exceptional cases, five of the X breakpoints are found in the short arm of the X and four of these are located distally on this arm. The centromere may possibly prevent spread of inactivation to the p arm. However, since other cases of inactivation of autosomal regions translocated to the p arm have occurred [18], such interference by the centromere is probably not likely or usual.

TABLE 2

UNBALANCED X/A TRANSLOCATIONS WITH FAILURE OF INACTIVATION OF THE AUTOSOMAL SEGMENT

Translocation	Replication studies	X Breakpoint	Source
t(Xq;?)	H_3TDN	Xq	[12]
t(Xp;C)	H_3TDN	Xp	[8]
t(Xq8q)	H_3TDN	Distal Xq	[13]
t(X;6) (p21;p24)	H_3TDN,RBA	Xp21	[9]
t(X;17) (p11;q24)	RBA-RBG	Xp11.2→p11.3	[16]
t(X;13) (q27;q12)	H_3TDN	Xq27	[14]
t(X;17) (p22;p13)	RBG	Xp22	This study

Recently, Willard and Latt [2] using the RBA technique, found that the late and early replicating X chromosomes differed in sequence and timing of specific band replication, and suggested that X replication-control may be mediated at several sites on the X chromosome. Thus, in addition to the center for induction of X replication, control by several regions of the active X could determine the occurrence of autosomal inactivation. Regions of the late X which are somewhat earlier in replication might be the location of those X-linked genes which fail to be inactivated. These regions might also differ in the capacity to permit late replication of an autosomal segment positioned in close proximity. Hajemeijer et al.'s [16] translocation has a breakpoint at Xp11, and both ours and Zabel et al.'s [15] are at Xp22, while Crandall et al.'s [14] is at Xq27. All are earlier replicating regions of the late X. Two of these regions, Xp distal (Xp22) and Xp11, have been hypothesized to be areas where there may be common failure of X inactivation [11, 17].

Alternative causes of inactivation failure in a number of X/A translocations in the literature as well as in our patient have been examined. While it may be premature to ascribe commonality of cause to this phenomenon, it is apparent that study of additional X/A translocations by the RBA-RBG method will give us better information than earlier autoradiographic methods. This method, plus closer attention to details of X chromosome banding, will undoubtedly reveal further cases of inactivation failure of all or part of autosomal segments in X/A translocations.

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