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Molecular determination of X inactivation pattern correlates with phenotype in women with a structurally abnormal X chromosome

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Purpose: To correlate the X inactivation pattern, as determined by one or more molecular assays, with phenotype in individuals with structurally abnormal X chromosomes. **Methods:** We utilized methylation analysis of androgen receptor (AR) and Fragile X (FMR1) genes and expression studies of an XIST polymorphism to assess X inactivation patterns of 28 females with structurally abnormal X chromosomes. Individuals were placed in one of three categories: (1) completely nonrandom inactivation of one X chromosome, (2) preferential or skewed inactivation of one X chromosome, or (3) random inactivation of either X chromosome. **Results:** In 19 of 21 cases with complete (>97%) skewing of X inactivation, the phenotype was either normal, consistent with a single gene disorder, or consistent with classical Turner syndrome; two cases with completely nonrandom X inactivation had unexplained mental retardation phenotypes. In contrast, six of seven cases that did not exhibit completely nonrandom X inactivation were phenotypically abnormal. Carriers of two balanced translocations, two duplicated Xs, one deleted X, and one 45,X/46,X,r(X) presented with mental retardation and/or multiple congenital anomalies. **Conclusion:** In patients with random or skewed X inactivation, the abnormal phenotype was hypothesized to be due to functional nullisomy or disomy of X-linked genes. Based on these results, we propose that X inactivation studies should be performed on all women with structurally abnormal X chromosomes. This should aid in the understanding of abnormal phenotypes in liveborn individuals with abnormal X chromosomes and may help to predict phenotypes for prenatally detected cases in the future. ***Genetics in Medicine*, 2000:2(2):136–141.**

Key Words: *X inactivation, abnormal X chromosome, aberrant phenotype, phenotype correlation*

In chromosomally normal mammalian females, one of the two X chromosomes becomes randomly inactivated early in embryogenesis to allow for dosage compensation of X-linked genes between XX females and XY males.[1] The selection of which X is inactivated is random in the normal situation, and once made, the inactivation pattern is maintained and inherited in the clonal descendants of the precursor cell.[2] Random inactivation generally results in approximately equal numbers of cells with inactivation of the maternal or paternal X chromosome.

Nonrandom or skewed X inactivation is the result of one X chromosome becoming inactivated in most or all of the female's cells. There is a very small chance that extremely skewed X inactivation may arise by chance, due to stochastic variation, but completely nonrandom inactivation is more commonly observed as the result of a secondary selection against cells carrying a genetic alteration.[3,4] Recently, skewing of X inactivation has also been shown to arise from a primary effect of the X inactivation process.[5] Secondary cell selection has been documented in women who carry X-linked disease alleles[6–9] and in women who carry structurally abnormal X chromosomes.[10–13] In women with abnormal X chromosomes, this selection against one cell line ensures maintenance of the most genetically "balanced" situation, and these women are usually phenotypically normal or present with features of Turner syndrome.[12,14]

Historically, late replication banding has been used to assess the X inactivation pattern in women with structurally abnormal X chromosomes.[15] This technically challenging and subjectively interpreted testing modality is not routine for most clinical cytogenetic laboratories. While late replication banding has the advantage of allowing for the identification of the chromosome inactivated, this method is not useful for cases with a low mitotic index or for cases with subtle aberrations in which the X chromosomes are not easily distinguishable cytologically. Recently, several quantitative molecular methods have been described to directly assess X inactivation patterns.[16–18] Molecular methods sample material (DNA or RNA) isolated from a large population of cells, yielding a cost-effective test that is likely to be more reflective of an individual's true X inactivation pattern than assays based on the analysis of

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<100 cells. We have studied 28 females with structurally abnormal X chromosomes using three molecular techniques (methylation analysis at the AR and/or FMR1 loci and expression of an XIST polymorphism) to evaluate the X inactivation pattern. This data were used to correlate the phenotype of the cases with the X inactivation pattern.

MATERIALS AND METHODS

Patient samples and cell lines

The X inactivation patterns of 28 females with structurally abnormal X chromosomes were assayed (Table 1). These included 15 women with balanced X/autosome translocations (cases #1–15); three with a deleted X (cases #16–18); three with a duplicated X (cases #19–21); three with an isochromosome X (cases #22–24); and four with small ring X chromosomes (cases #25–28) (Table 1). For some cases, cell lines were purchased from Coriell Human Genetic Cell Repository (Table 1; http://locus.umdnj.edu/nigms/sitemap.html).

Methylation analyses of androgen receptor and FMR1

X inactivation patterns of women with an abnormal X chromosome were assayed via methylation of androgen receptor (AR) and the Fragile X mental retardation gene (FMR1). For AR analysis, methylation of the CpG island adjacent to the polymorphic CAG repeat in exon 1 of the gene was evaluated essentially according to the method described by Allen *et al.*[16] The FMR1 analysis was performed according to the method of Carrel and Willard.[17] For both methods, DNAs from the patient and appropriate controls were digested with the methylation-sensitive enzyme *Hpa*II along with *Rsa*I, which allows for more thorough *Hpa*II digestion. To ensure complete digestion of the sample, additional *Hpa*II was added the following morning following the overnight digestion, and the samples were incubated for an additional 2 hours. Digested and undigested DNA samples were PCR amplified using a ^{32}P end-labeled forward primer. Samples were loaded onto a 6% acrylamide gel and electrophoresed. X inactivation ratios were determined using visual comparison of the cut and uncut samples.

X inactivation ratios were also assessed by comparing the ratios of an expressed polymorphism in the XIST gene,[18] as described in Carrel *et al.*, 1999.[19] Patient cDNA was amplified for 29 cycles, followed by a single cycle primer extension using a ^{32}P-labeled forward primer. The PCR products were digested with *Hin*fI and were electrophoresed in a 6% polyacrylamide gel. Relative expression of each XIST allele was determined visually.

For the three methods, X inactivation patterns were divided into three readily-distinguishable categories, based on visual inspection: (1) completely nonrandom inactivation (with no detectable band for one allele, *e.g.*, pt. # 13, Fig. 1), (2) skewed inactivation (with easily detectable differences between two bands, *e.g.*, pt. #19, Fig. 1), and (3) random X inactivation (with no visible difference between the band intensities, *e.g.*, pt. #18, Fig. 1). The X inactivation pattern was considered "completely nonrandom" if only one band of a heterozygous individual was visualized on an overexposed autoradiograph using one or more of the methods described. A monoallelic amplification pattern was indicative of complete inactivation of either the maternal or paternal chromosome. Because an X inactivation ratio of 97:3 revealed two visible alleles, as determined using titration analyses (L.C., unpublished data), the sensitivity was demonstrated to be >97%.

RESULTS

Completely nonrandom X inactivation was demonstrated for eight phenotypically normal carriers of aberrant X chromosomes (Table 1) including six carriers of balanced translocations involving the X chromosome (cases #1, 2, 3, 11, 12, 13) (case #13, Fig. 1), and two carriers of deleted X chromosomes (cases #16, 17) (Table 1). Completely nonrandom inactivation was also observed in five individuals with a breakpoint-associated single gene disorder and a balanced X/autosome translocation (case #4 - X-linked hypohidrotic ectodermal dysplasia, case #5 - Duchenne muscular dystrophy (DMD), case #8 - Klippel Feil syndrome, case #9 - sporadic retinoblastoma, case #10 - DMD). Case #7, with secondary amenorrhea and a balanced X/autosome translocation, exhibited the predicted completely nonrandom X inactivation pattern, as did six individuals with Turner syndrome, three with an isochromosome X (cases #22, 23, 24) and three with small ring(X) chromosomes (cases #25, 26, 27). Two cases with completely nonrandom X inactivation had mental retardation of unexplained origin (cases #7 and 25). Late replication data available from lymphocyte analysis of nine cases with completely nonrandom X inactivation revealed a 100% correlation (Table 1).

Seven cases did not show completely nonrandom X inactivation, such that a proportion of cells were either monosomic or disomic for X-linked genes (Table 1). Six of these individuals were reported to be phenotypically abnormal; #19, who had a small duplication of Xq, had a percentage of cells with an active abnormal X, but exhibited a normal phenotype (Fig. 1).

Two cases with apparently balanced X/autosome translocations, case #14 - t(X; 17)(q11; q11) and case #15 - t(X; 9)(q28; q21), exhibited a skewed and random X inactivation pattern, respectively. The patient with the X; 17 translocation had multiple severe congential anomalies that did not conform with any known syndrome, and the patient expired on day 17 (see Disteche *et al.* for additional clinical information). FMR1 methylation studies revealed that this patient had both alleles present in the *Hpa*II-digested lane, however, one allele was reduced in intensity. This skewed X inactivation pattern correlated with previously reported late replication studies[20] that demonstrated approximately 20–40% of cells analyzed had an inactivated derivative chromosome, with no apparent spreading into the autosomal material. This result is consistent with a significant percentage of this patient's cells being functionally disomic for all of Xp and a portion of Xq.

Likewise, the patient with an apparently balanced X; 9 translocation (case #15) was functionally disomic for Xq28-qter in a proportion of her cells. FMR1 and AR methylation studies re-

Table 1
X inactivation ratios of females with abnormal X chromosomes

	Karyotype as designated by source	Phenotype[a]	Inactivation ratio[b]	Test	Source[c] Specimen type (passage number)	Late replication per source[d]
1	46,X,t(X;19)(q22;q13.3)	NP	completely nonrandom	FMR1	NIGMS GM0089 fibroblast (p.6)	100% normal X
2	46,X,t(X;14)(q22;q24.3)	NP	completely nonrandom	AR	this report lymphocyte	
3	46,X,t(X;20) (cen;cen)	NP	completely nonrandom	XIST, AR	NIGMS GM7792 fibroblast (p.3)	100% normal X
4	46,X,t(X;9)(q12;p24)	SG	completely nonrandom	XIST	NIGMS GM0705 fibroblast (p.17)	
5	46,X,t(X;11)(p21;q13)	SG	completely nonrandom	XIST, AR, FMR1	NIGMS GM1695 fibroblast (p.9)	100% normal X
6	46,X,t(X;Y)(q11;q11)	SA	completely nonrandom	XIST, AR	NIGMS GM2103 fibroblast (p.12)	
7	46,X,t(X;20)(cen;cen)	AB	completely nonrandom	XIST	NIGMS GM7792 fibroblast (p.3)	100% normal X
8	46,X,t(X;16)(q26;q24)	SG	completely nonrandom	XIST	NIGMS GM3884 fibroblast (p.13)	100% normal X
9	46,X,t(X;13)(p22;q21)	SG	completely nonrandom	XIST, AR	NIGMS GM2971 fibroblast (p.15)	100% normal X
10	46,X,t(X;5)(p21.2;q35.3)	SG	completely nonrandom	XIST	NIGMS GM5835 fibroblast (p.7)	100% normal X
11	46,X,t(X;21)(q11;p11)	NP	completely nonrandom	XIST, AR, FMR1	NIGMS GM1411 fibroblast (p.6)	
12	46,X,t(X;21)(q22;q11)	NP	completely nonrandom	AR	NIGMS GM8135 fibroblast (p.8)	
13	46,X,t(X;3)(p21.2;q22.2)	NP	completely nonrandom	AR, FMR1	this report lymphocyte	100% abnormal X
14	46,X,t(X;17)(q11;q11)	AB	skewed	FMR1	reference 20 lymphocyte	20–40% abnormal X
15	46,X,t(X;9)(q28;q12)	AB	random	AR, FMR1	reference 21 lymphocyte	84% normal X
16	46,X,del(X)(q26.3q27.3)	NP	completely nonrandom	AR, FMR1	reference 22 lymphocyte	Xs not discernible
17	46,X,del(X)(p22.1p22.33)	SS	completely nonrandom	AR	this report lymphocyte	Xs not discernible
18	46,X,del(X)(q26.3q27.3)	AB	random	AR, FMR1	reference 22 lymphocyte	Xs not discernible
19	46,X,dup(X)(q26.3q27.2)	NP	skewed	AR	this report lymphocyte	Xs not discernible
20	46,X,dup(X)(q21.2q22.1)	AB	skewed	AR	this report lymphocyte	
21	46,X,dup(X)(q13.1q24)	AB	skewed	AR	this report lymphocyte	
22	46,X,dic(X)(qter-p11.4::p11.4-qter)	TS	completely nonrandom	XIST, AR	NIGMS GM8944 fibroblast (p.7)	100% abnormal X
23	46,X,i(X)(qter-p22::q22-pter)	TS	completely nonrandom	AR	NIGMS GM6960 fibroblast (p.5)	
24	46,X,i(Xq)(qter-p11.21::p11.21-qter)	TS	completely nonrandom	XIST	this report lymphocyte	
25	45,X/46,X,r(X)(p11.2q12)	AB	completely nonrandom	AR	this report lymphocyte	
26	45,X/46,X,r(X)(p11.2q12)	TS	completely nonrandom	AR	this report lymphocyte	
27	45,X/46,X,r(X)(p11.2q13)	TS	completely nonrandom	AR	this report lymphocyte	
28	45,X/46,X,r(X)(p11.2q12)	AB	absent	AR	this report lymphocyte	

[a]NP, normal phenotype; SG, single gene disorder; SA, secondary amenohea; TS, Turner syndrome; AB, abnormal phenotype with dysmorphia and mental retardation; SS, short stature.
[b]Based on visual assessment of band intensities; see text for definitions.
[c]NIGMS, Coriell Human Genetic Cell Repository.
[d]For cases with late replication study results reported by the source, the ratios are given, along with the data on the identity of the late replicating chromosome. Some cases had subtle abnormalities that did not allow for the Xs to be discriminated.

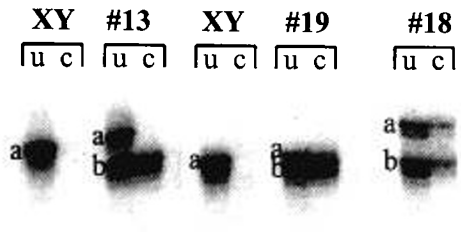

Fig. 1 Examples of results obtained using the androgen receptor methylation analysis. For each paired lane set, PCR products from undigested DNA (U) and *Hpa*II-cut reaction (C) are shown. Two male control samples (XY) reveal complete digestion in the *Hpa*II-cut lanes. Case #13 [46, X,t(X; 3)(p21.2; q22.2)] had completely nonrandom X inactivation with no amplification product of allele "a" in the *Hpa*II-cut lane. A skewed X inactivation pattern is demonstrated by Case #19 [46, X,dup(X)(q26.3q27.2)], and Case #18 [46, X,del(X)(q26.3q27.3)] revealed a random X inactivation pattern.

Fig. 2 Androgen receptor methylation analysis of two 46, X,r(X) samples. No amplification product is seen in the *Hpa*II-cut lane (C) for Case #28 consistent with both alleles being active. Completely nonrandom X inactivation of the small ring X is demonstrated by the results of Case #27.

vealed a random pattern of X inactivation in this patient's lymphocytes.[21] Case #15 exhibited dysmorphic features and was mentally impaired.[21] While the molecular data suggested random X inactivation, late replication studies of a limited amount of cells (N = 25) revealed a skewed pattern of X inactivation with 84% of cells exhibiting inactivation of the normal X.[21]

Case #18, with a Xq27.3 - q28.3 deletion, exhibited an abnormal phenotype with mental and growth retardation, seizures, and dysmorphic features.[22] Both FMR1 and AR methylation analyses revealed a random X inactivation pattern (case #18, Fig. 1). Late replication studies were not useful in this case because the Xs could not be distinguished in the banded preparations.[22]

A skewed X inactivation pattern was seen for all three of the X duplication cases (#19, 20, 21), suggesting that, at least in a percentage of cells, these patients were disomic for X sequences. One case was phenotypically normal (#19). One case (#20) had a duplication of Xq21.2 - q22.1 and presented with growth retardation and developmental delay. Case #21, with a duplication Xq13.1 - q24, also had an abnormal phenotype.

Generally, a patient with a small ring X chromosome presents a special case. Because several small marker X chromosomes that hybridize with an XIST DNA probe have been shown to be active,[23] the androgen receptor assay may be used to see if the alleles from both the normal and the small marker X chromosome are expressed. (XIST and FMR1 assays may not be helpful because they may not be present on the tiny marker chromosomes.) We used this analysis to study 4 patients with the karyotype 46,X,r(X): two cases (#25 and #28) with an abnormal phenotype and two cases (#26 and #27) with a typical Turner syndrome phenotype. Cases #25, 26, and 27 revealed a pattern consistent with completely nonrandom X inactivation (only one allele detectable; Fig. 2), and each of these cases was positive for XIST DNA using FISH (data not shown). No alleles were detected in the digested sample from case #28 (although DNA was readily amplified from an unrelated locus), consistent with both the normal X and the r(X) being active. This case was shown to be deleted for XIST DNA (data not shown). Thus, the molecular assay concurred with the XIST DNA FISH results. These results reveal that case #25 appropriately inactivated the ring X; therefore, the abnormal phenotype in this patient is not likely to be due to overexpression of X-linked genes

DISCUSSION

X inactivation, in the normal situation, is random with respect to which X chromosome in the cell is inactivated. Extreme nonrandom X inactivation may reflect a bias in the initial choice, such as the imprinted inactivation of the paternal X in extra-embryonic tissues of the mouse or may be the result of selective pressures.[4] In patients with structurally abnormal X chromosomes, initially X inactivation is random; however, cell selection, to ensure the most genetically balanced situation, occurs in the majority of cases.[12,24] We have studied the X inactivation patterns of 28 women with structurally abnormal X chromosomes. For the majority of cases with a normal phenotype, a Turner syndrome phenotype, or a single-gene disorder phenotype, completely nonrandom X inactivation was demonstrated, consistent with the expected cell selection phenomenon. However, 6 of 7 women with a skewed or random pattern had nonspecific mental retardation and/or congenital abnormalities. These data confirm a correlation between phenotype and the X inactivation pattern as was reviewed by Schmidt and Du Sart.[12]

Early evaluations of X inactivation patterns used late-replication banding[15] or protein isozyme studies[25] to determine ratios of inactivation in women. Late replication, an interpretive test lacking sensitivity, and protein polymorphism analysis, a test with limited utility due to low heterozygosity, were not useful for the study of all of the females with structurally abnormal X chromosomes. More recently, several molecular methods have been described to directly assess X inactivation patterns.[16,17,19] These methodologies have the advantages of

high heterozygosity and the ability to evaluate material from a large numbers of cells leading to increased sensitivity. The major disadvantages of molecular testing are the inability to identify which X is inactive (normal or abnormal) and the lack of detection of spreading of X inactivation into autosomal material. Thus, when inactivation is completely nonrandom, it is unclear which X is inactive in every cell. It is presumed that the skewing is toward the most "genetically balanced" situation. In carriers of a structurally abnormal X chromosome that exhibit skewed or random X inactivation, as evaluated by molecular techniques, functional disomy or monosomy of X chromosomal sequences may be correlated with an anomalous phenotype. For cases of X/autosome translocations, additional information from complementary late replication studies would be useful to further indicate possible abnormal dosage of autosomal genes.

For our study and previous X inactivation studies, the correlation of phenotype and X inactivation pattern is limited by the study of one (at most two) tissue types. In addition, not all clinically relevant cell lineages (for example, brain) may be studied. However, for the majority of cases for which multiple tissues (e.g., blood and skin) have been assayed, the X inactivation patterns are consistent.[12] For eight of our cases, late replication studies on lymphocytes correlated exactly with the molecular findings on fibroblast tissue (Table 1, cases #1, 3, 5, 7–10, 22). Significant differences in X inactivation patterns in various tissues from an individual have been reported rarely.[20,26] Our results suggest that, although the finding of a completely nonrandom pattern in a single tissue of an individual with a structurally abnormal X chromosome cannot ensure a good outcome, a documented random inactivation pattern in a single tissue from a female with a structurally abnormal X may indicate a significant disomy or monosomy of X-linked sequences. For clinical utility of molecular testing, it would be advantageous to sample multiple tissues prior to risk evaluation.

Thirteen of the samples tested were cultured fibroblasts (Table 1). Cultured cells are not the optimal specimen for molecular testing of X inactivation given the chance of clonality. However, fibroblast cultures, unlike lymphoblast cultures, are outgrowths from a large number of cells and are therefore likely oligoclonal. The majority of cultures analyzed were of a relatively low passage number (<10) and therefore most likely were not clonal. For clinical testing, fresh blood and/or other tissue should be assayed.

One case (#19) with a small duplication of Xq revealed a skewed X inactivation pattern and a normal phenotype. This individual thus had a population of cells with disomy of Xq26.3-q27.2. While the majority of cytogenetically evident duplications of chromosomal material result in an abnormal phenotype, there have been several reports of autosomal duplications in normal individuals.[27–29] It has been hypothesized that increased dosage of small amounts of specific genetic material may be tolerated by cells and not interfere with normal development. Two other cases (#20, 21) with small duplications and skewed X inactivation patterns did exhibit an abnormal phenotype. Thus, correlations of X inactivation pattern and phenotype in patients with small duplications should be interpreted with care.

Patients with small ring X chromosomes are often divided into two categories based upon the presence or absence of the XIST gene DNA.[30–32] While this association holds true for the majority of cases, a molecular analysis of gene expression and/or activity offers the advantage of assessing specific genes on the ring X. This testing may be particularly useful in cases that are positive for XIST DNA, but do not express XIST appropriately.[22] Our results demonstrated concordance between the XIST DNA FISH and the molecular assays. In addition, one patient with a ring X and an abnormal phenotype (case #25) had a pattern consistent with normal XIST expression. Therefore this patient's abnormal phenotype is most likely unrelated to the abnormal karyotype.

For 9 of our cases, two or three methods were used to assess X inactivation pattern (Table 1). The assays gave consistent results between the various testing methods, suggesting that a single test method will yield reliable X inactivation results. It is important to note that the methylation assays are performed on extracted DNA; whereas, cDNA from isolated RNA is needed for the XIST assay. Therefore, clinical laboratories will need to weigh the utility of each extraction and testing methodology.

While molecular determination of the X inactivation ratio is not technically difficult and is of clinical importance, several factors should be considered when performing these assays and interpreting the clinical significance. Complete restriction enzyme digestion for the methylation assays must be documented, because incomplete digestion will give a result compatible with random X inactivation. To ensure complete digestion for our studies, we included *Rsa*I in the *Hpa*II digestions, we doubled the amount of *Hpa*II routinely used for digestion, and we included at least two male controls with each assay.

The informativeness of the gene polymorphisms is an additional consideration. The androgen receptor is polymorphic in approximately 70% of women.[16] The informativeness of the repeat in FMR1 and the expressed XIST polymorphism are somewhat less, 65% and 50%, respectively.[18,33] Thus, it is advantageous to have the capability of performing several assays to increase the likelihood of finding informative polymorphisms for the majority of cases.

The 28 cases presented here represent a biased population of women with structurally abnormal X chromosomes. The majority of our cases were obtained from the NIGMS Mutant Cell Repository or from clinical cytogenetic laboratories that referred interesting cases for evaluation. Thus, it may be presumed that most women with structurally abnormal Xs will present with nonrandom X inactivation. However, in patients with a structurally abnormal X and an unexplained aberrant phenotype, X inactivation studies are warranted.

There is currently no literature on prenatal X inactivation studies and correlation with phenotype. Again, it is presumed that the majority of female fetuses with structurally abnormal X chromosomes will undergo cell selection, and thus, exhibit a

completely nonrandom X inactivation pattern. Prospective X inactivation studies will need to be performed on prenatal samples to assess the feasibility and utility of testing all female fetuses with structurally abnormal X chromosomes.

Our studies indicate that the X inactivation status of women with structurally abnormal X chromosomes and an abnormal phenotype should be assayed as part of a routine clinical work-up. While the clinical significance of slight variations in X inactivation patterns has not been evaluated, our data reveal that the phenotype may be correlated with easily detectable differences in the X inactivation ratios. Clearly, the intensities of bands demonstrating nonrandom X inactivation, random X inactivation, and skewed X inactivation are readily visually distinguishable, and the clinical correlations may be made using these distinctions. Further studies are needed to evaluate the relevance of more subtle variations in the X inactivation patterns.

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References

1. Lyon MF. Gene action in the X-chromosome of the mouse (Mus musculus L). *Nature* 1961;190:372–373.
2. Willard HF. The sex chromosomes and X-chromosome inactivation. In: Scriver CR, Beaudet AL, Sly WS, Valle D, editors. The metabolic and molecular bases of inherited disease. McGraw-Hill, New York, pp. 717–737, 1995.
3. Willard HF, Brown CJ, Carrel L, Hendrich B, Miller AP. Epigenetic and chromosomal control of gene expression: molecular and genetic analysis of X chromosome inactivation. *Cold Spring Harb Symp Quant Biol* 1993;58:315–322.
4. Belmont JW. Genetic control of X inactivation and processes leading to X-inactivation skewing. *Am J Hum Genet* 1996;58:1101–1108.
5. Plenge RM, Hendrich BD, Schwartz C, Arena JF, Naumova A, Sapienza C, Winter RM, Willard HF. A promoter mutation in the XIST gene in two unrelated families with skewed X inactivation. *Nature Genet* 1997;17:353–356.
6. Nyhan WLA, Bakay B, Connor JD, Marks JF, Keele DK. Hemizygous expression of glucose-6-phosphate dehydrogenase in erythrocytes of heterozygotes for the Lesch-Nyhan syndrome. *Proc Natl Acad Sci USA* 1970;65:214–218.
7. Puck JM, Nussbaum RL, Conley ME. Carrier detection in X-linked severe combined immunodeficiency based on patterns of X chromosome inactivation. *J Clin Invest* 1987;79:1395–400.
8. Redonnet-Vernhet I, Ploos van Amstel JK, Jansen RPM, Wevers RA, Salvayre R, Levade T. Uneven X inactivation in a female monozygotic twin pair with Fabry disease and discordant expression of a novel mutation in the α-galactosidase A gene. *J Med Genet* 1996;33:682–688.
9. Devriendt K, Matthijs G, Legius E, Schollen E, Blockmans D, van Geet C, Degreef H, Cassiman J-J, Fryns J-P. Skewed X-chromosome inactivation in female carriers of Dyskeratosis Congenita. *Am J Hum Genet* 1997;60:581–587.
10. Mattei MG, Mattei JF, Ayme S, Giraud F. X-autosome translocations: cytogenetic characteristics and their consequences. *Hum Genet* 1982;61:295–309.
11. Camargo M, Cervenka J. DNA replication and inactivation patterns in structural abnormality of sex chromosomes. I. X-A translocations, rings, fragments, isochromosomes, and pseudo-isodicentrics. *Hum Genet* 1984;67:37–47.
12. Schmidt M, Du Sart D. Functional disomies of the X chromosome influence the cell selection and hence the X inactivation pattern in females with balanced X-autosome translocations: a review of 122 cases. *Am J Med Genet* 1992;42:161–169.
13. Hatchwell E, Robinson D, Crolla JA, Cockwell AE. X inactivation analysis in a female with hypomelanosis of Ito associated with a balanced X;17 translocation: evidence for funtional disomy of Xp. *J Med Genet* 1996;33:216–220.
14. Shapiro LJ, Mohandas T. DNA methylation and the control of gene expression on the human X chromosome. *Cold Spring Harb Symp Quant Biol* 1983;47:631–637.
15. Zuffardi O. Cytogenetics of human X/autosome translocations. In: A Sandburg, editor. Cytogenetics of the Mammalian X Chromosome, Part B: Basic Mechanisms of X chromosome Behaviour, A. New York: Alan R Liss, Inc., 193–210, 1983.
16. Allen RC, Zoghbi HY, Moseley AB, Rosenblatt HM, Belmont JW. Methylation of HpaII and HhaI sites near the polymorphic CAG repeat in the human androgen-receptor gene correlates with X chromosome inactivation. *Am J Hum Genet* 1992; 51:1229–1239.
17. Carrel L, Willard HF. An assay for X inactivation based on differential methylation at the Fragile X locus, FMR1. *Am J Med Genet* 1996;64:27–30.
18. Rupert JL, Brown CJ, Willard HF. Direct detection of non-random X chromosome inactivation by use of transcribed polymorphism in the XIST gene. *Eur J Hum Genet* 1995;3:333–343.
19. Carrel L, Willard HF. Heterogeneous gene expression from the inactive X chromosome: an X-linked gene that escapes X inactivation in some human cell lines but is inactivated in others. *Proc Natl Acad Sci USA* 1999;96:7364–7369.
20. Disteche CM, Swisshelm K, Forbes S, Pagon RA. X-inactivation patterns in lymphocytes and skin fibroblasts of three cases of X-autosome translocations with abnormal phenotypes. *Hum Genet* 1984;66:71–76.
21. Wolff DJ, Schwartz S, Montgomery T, Zackowski JL. Random X inactivation in a female with a balanced t(X;9) and an abnormal phenotype. *Am J Med Genet* 1998; 77:401–404.
22. Wolff DJ, Gustashaw KM, Zurcher V, Ko L, White W, Weiss L, Van Dyke DL, Schwartz S, Willard HF. Deletions in Xq26.3-q27.3 including FMR1 result in a severe phenotype in a male and variable phenotypes in females depending upon the X inactivation pattern. *Hum Genet* 1997;100:256–262.
23. Migeon BR, Luo S, Jani M, Jeppesen P. The severe phenotype of females with tiny ring X chromosomes is associated with inability of these chromosomes to undergo X inactivation. *Am J Hum Genet* 1994;55:497–504.
24. Disteche CM, Eicher EM, Latt SA. Late replication in an X-autosome translocation in the mouse: correlation with genetic inactivation and evidence for selective effects during embryogenesis. *Proc Natl Acad Sci USA* 1979;76:5234–5238.
25. Nance WE. Genetic tests with a sex-linked marker: Glucose-6-phosphate dehydrogenase. *Cold Spring Harb Symp Quant Biol* 1964;29:415–424.
26. Hellkuhl B, de la Chapelle A, Grzeschik K-H. Different patterns of X chromosome inactivity in lymphocytes and fibroblasts of a human balanced X:autosome translocation. *Hum Genet* 1982;60:126–129.
27. Li SY, Gibson LH, Gomez K, Pober BR, Yang-Feng TL. Familial dup(5)(q15q21) associated with normal and abnormal phenotypes. *Am J Med Genet* 1998;75:75–77.
28. Robin NH, Harari-Shacham A, Schwartz S, Wolff DJ. Duplication 14(q24.3q31) in a father and daughter: Delineation of a possible imprinted region. *Am J Med Genet* 1997;71:361–365.
29. Wolff DJ, Raffel LJ, Ferre MM, Schwartz S. Prenatal ascertainment of an inherited dup(18p) associated with an apparently normal phenotype. *Am J Med Genet* 1991; 41:319–321.
30. Van Dyke DL, Wiktor A, Palmer CG, Miller DA, Witt M, Babu VR, Worsham MJ, Roberson JR, Weiss L. (1992) Ullrich-Turner syndrome with a small ring X chromosome and presence of mental retardation. *Am J Med Genet* 43:996–1005.
31. Wolff DJ, Brown CJ, Schwartz S, Duncan AM, Surti U, Willard HF. Small marker X chromosomes lack the X inactivation center: Implications for karyotype/phenotype correlations. *Am J Hum Genet* 1994;55:87–95
32. Jani MM, Torchia BS, Pai GS, Migeon BR. Molecular characterization of tiny ring X chromosomes from females with functional X chromosome disomy and lack of cis X inactivation. *Genomics* 1995;27:182–188.
33. Fu YH, Kuhl DP, Pizzuti A, Pieretti M, Sutcliffe JS, Richards S, Verkerk AJ, Holden JJ, Fenwick RG Jr, Warren ST. Variation of the CGG repeat at the fragile X site results in genetic instability: resolution of the Sherman paradox. *Cell* 1991;67:1047–1058.