Clinical Study: XIST Gene and Pattern of X-Inactivation in Children with Ring-X Turner Syndrome

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Abstract

Objectives: To evaluate the presence of XIST on ring X and to correlate the pattern of ring X replication to the IQ level. Subjects: Fifteen cases with TS who had 45,X/46,X,r(X). They were subjected to I.Q. estimation, FISH using WCP for X, locus specific identifier for XIST, differential replication pattern with BrdU for r(X). Results: By FISH, it was noted that these rings were derived entirely from the X chromosome. XIST was not deleted in all cases, a very small percentage of mosaic cell line was found in three cases who had deleted XIST and also two of them had double r(X). Ten cases had small rings, 5 had large rings. MR was found in 90% of cases with small r(X) while the large r(X) had normal mentality. A significant correlation was found between the presence of small ring X and lower IQ level. The correlations between the IQ level and age at presentation, percentage of r(X) and presence of active r(X) were not reaching a significant level. Conclusion: FISH is useful in identification of hidden cell line. The mentality in TS is affected by the presence of small r(X) and the presence of functioning XIST gene on the r(X).

Key words: Ring X, XIST gene, Turner syndrome, X inactivation, Mental retardation.

Introduction

RING X chromosomes are special cases of T.S. with loss of different portions of the second X. Patients with ring X often have some features of T.S., also in addition patients with a small ring X usually have mental retardation [1,2].

It has been suggested that deletion of a gene present on both X and Y chromosomes outside the pseudoautosomal region and escaping X-inactivation may be responsible for somatic features of T.S. The position of this gene close to the centromere means that it is likely to be preserved in ring X chromosomes. The presence of unusual two functioning copies of such a gene or genes may explain the less classic phenotype of ring X [3].

X inactivation is the method used by mammals to compensate for the sex difference in the number of X chromosomes [4]. The XIST (X Inactivation Specific Transcript) locus, residing in the region considered to be the XIC (X Inactivation Center) is the only locus transcribed exclusively from the inactive X chromosome and is thought to play an essential role in the initiation of mammalian X inactivation [5-7]. Preliminary studies of the genetic content of these ring chromosomes associated with severe

phenotypes reveal heterogeneity with respect to the presence of the XIST locus, some lack the locus [8], while others have sequences homologous toXIST [9].

5-Bromo-2-deoxyuridine (BrdU) is a thymidine analog that can be incorporated into the replicating DNA in place of thymidine. Its photodegradable property has enabled microscopic demonstration of chromosomal regions by differential staining. The Brdu continuous labeling is used to investigate the replication events that occur during the end of S phase. (i.e. late replicating pattern). For late replicating pattern the darkly stained DNA have little incorporated Brdu (e.g. the heterochromatic region of chromosome 1,9,16, Y and the inactive X chromosome in females) [10]. The aim of this work was to evaluate the presence of XIST gene on ring X and to correlate the pattern of ring X replication to the IQ level.

Subjects and Methods

The subject of this study were 15 cases selected among Turner syndrome referred to the Human Genetic Clinic, National Research Center, Egypt. Cytogenetically they all had 45,X/46,X,r(X). The mean age at referral among them was 10.4 years. Mohamed/ Kamel/Kayed/ Meguid/Hussein. Each patient was subjected to thorough clinical examination and assessment of intelligence quotient using Stansford or Binet test. Fluorescence in situ Hybridization (FISH) procedures were carried out according to Lichter et al [11].

FISH was done using whole chromosome paint (WCP) for X chromosome to identify the origin of the ring and to detect mosaicism. Also we used locus specific identifier (LSI) for XIST to detect the copy number of XIST gene. Visualization was done with an epifluorescence microscope (Zeiss), with aligned filter combinations. The number and chromosomal locations of the signals were scored.

Differential replication studies were conducted using 5-Bromo-2-deoxyUridine, flurodeoxyuridine, uridine solution, following Dutrillaux et al [12]. For each case 30 metaphases were analyzed .The inactive X chromosomes remain darkly stained [13].

Results

Cases with ring X were divided into 2 groups: 10 cases with small r(X) and 5 cases with large r(X) Figs (1&2). FISH analysis for cases with ring chromosomes could identify that the origin of the rings was derived entirely from X chromosome Figs (3A&3B). LSI for XIST demonstrated the presence of two copies in all cases, but three cases showed a very small percentage of cell line with deleted XIST and two of them showed double ring X with deletion of XIST in both rings Fig (4 A&4B).

Mental subnormality was found in 90% in cases with small r(X); while cases with large ring X had normal mentality (Table 1). A significant correlation was found between the presence of small ring X and lower IQ level. The correlations between the IQ level and each of age at presentation, percentage of r(X) and presence of active r (X) were not reaching a significant level.

5-Bromo-2- Deoxy Uridine Technique Results (Replication Pattern):

Table (1) showed that cases No.2,5,9,10,11,12 and 13 had small r(X) and consistently demonstrated an early replication pattern (light in color) which means that they are in the active status Fig (5A&5B) ,and they all had mental subnormality except in case No.2 who had learning disability (IQ=79). Although, cases 1,3 and 6 had small size r(X) of size in between chromosome 20 to 21, and were late replicating (dark, i.e. inactive), they had M.R. Cases 4, 7, 8, 14 and 15 had large ring X with late replication pattern (dark) and had normal intelligence.

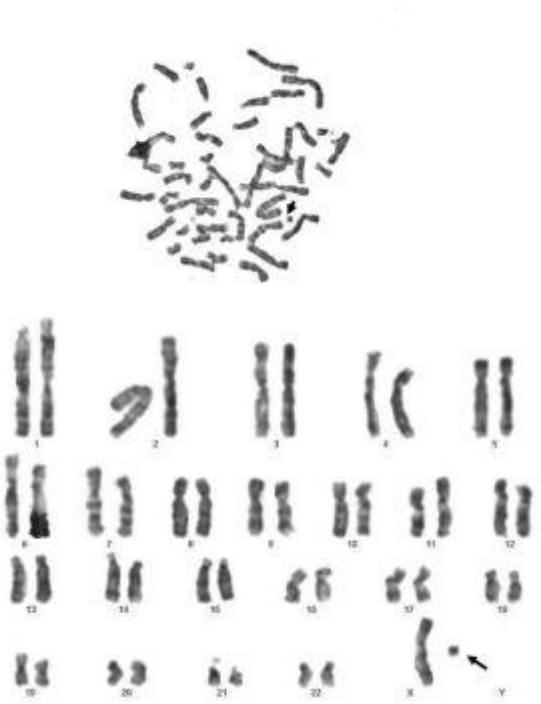


Fig. 1: G-banded Karyotype showing very small ring (X).

Fig 2: G-banded Karyotype showing large ring (X).

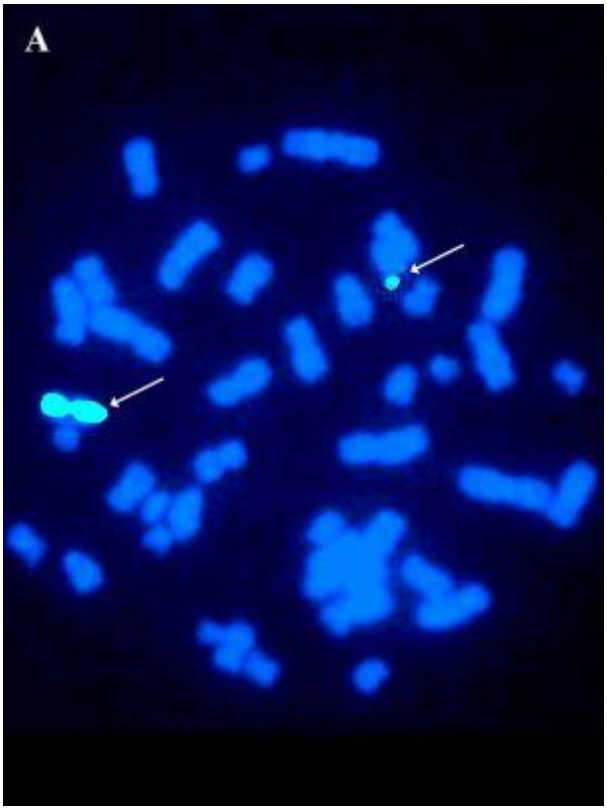


Fig. 3A

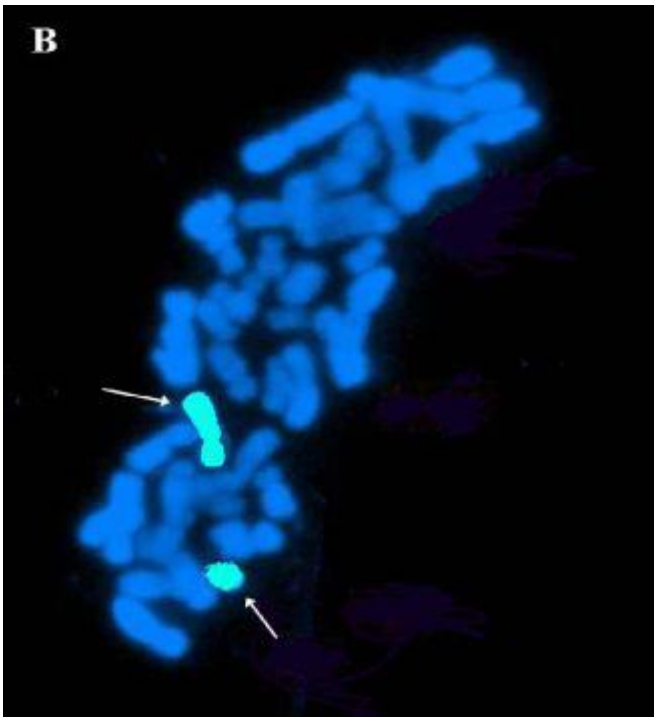
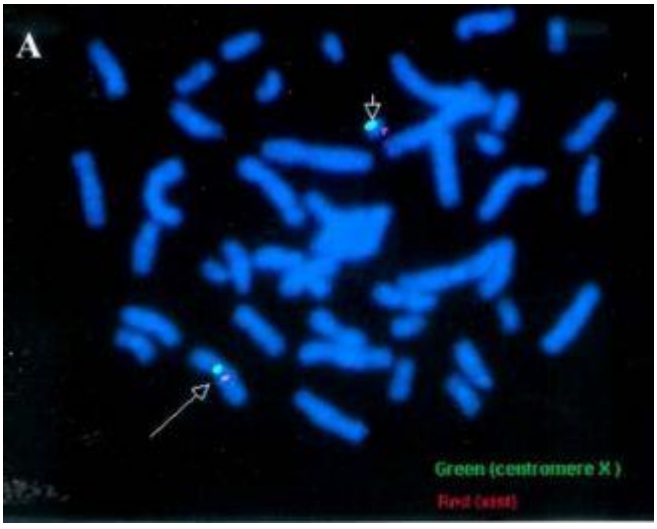


Fig. 3B

Fig 3: Fluorescence is situ hybridization (FISH) to a metaphase using whole chromosome paint for X chromosome, showing (A) normal (X) and small ring (X) & (B) normal (X) and large ring (X).

Fig 4: BrdU banding pattern showing (A) an early replication of a very small ring (X) [light in color] & (B) a late replication of a large ring (X) [dark in color].



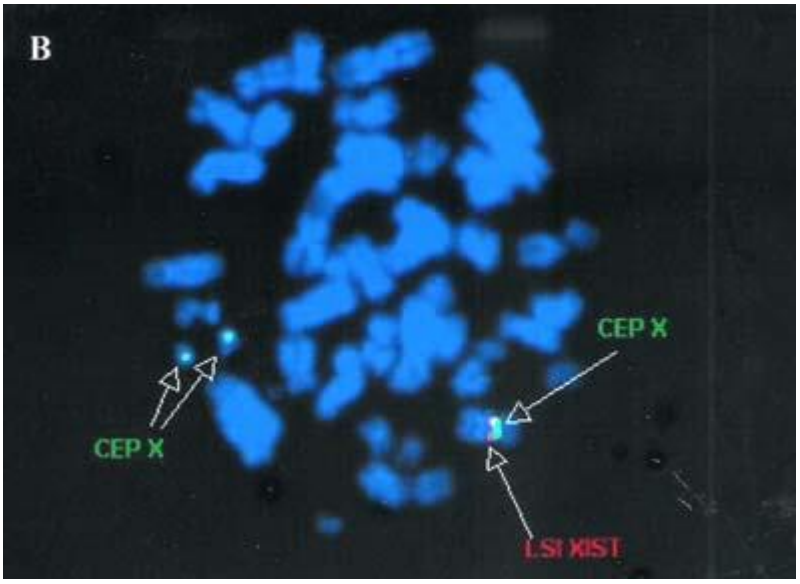


Fig. 5: Fluorescence in situ hybridization (FISH) using alpha satellite probe DXZ1 and LSI for XIST, showing (A) one signal for XIST on both the normal X and ring X. (B) one signal for XIST on the normal X and deleted XIST on both rings. Mohamed/ Kamel/Kayed/ Meguid/Hussein.

Table I: Age at presentation, IQ value, size, % of ring X and replication pattern in cases with ring X.

	Age	IQ	Ring X %	Ring size	Replication pattern by BrdU
1	16	65	20	Small	Inactive (dark)
2	6:02	79	40	Very small	Active (light)
3	7	49	30	Small	Inactive (dark)
4	4:02	96	30	Large	Inactive (dark)
5	3:07	68	10	Small	Active (light)
6	10:11	70	9	Small	Inactive (dark)
7	12	90	20	Large	Inactive (dark)
8	12:08	95	10	Large	Inactive (dark)
9	11	65	27	Small	Active (light)
10	10	60	50	Very small	Active (light)
11	6:02	68	50	Very small	Active (light)
12	15:05	60	60	Very small	Active (light)
13	15:05	65	55	Small	Active (light)
14	12	90	30	Large	Inactive (dark)
15	13	95	15	Large	Inactive (dark)

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IQ- Intelligence Quotient
BrdU- 5Bromo -2-Deoxy Uridine

Table 2: Relation between the size of the ring X-chromosome and the level of IQ

Ring size	Number of Cases	Mean (IQ)	Std. deviation	Median	Minimum	Maximum
Very small	7	66.43	± 6.4513	65.0	60.0	79.0
Small	3	61.33	± 10.9697	65.0	49.0	70.0
Large	5	93.67	± 3.2146	95.0	90.0	96.0

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IQ – Intelligence Quotient

Discussion

Turner syndrome (T.S.) occurs approximately in 1/2500 to 1/5000 female live births. Around 6-15% of them possess a cell line with ring chromosomes, the majority of which are X chromosome derived [14,15].

In the present study FISH technique using whole chromosome painting probe, demonstrated that the origin of the ring in all cases was X chromosomal. This finding is similar to that reported by Cole et al [16] , Cervantes et al [17] in which all rings in their study on T.S. were derived entirely from X chromosome after application of FISH.

Turner et al [14] , used X-alpha-satellite probe to confirm the X chromosomal origin of the rings. Scanning a large number of cells with the appropriate probe in cases of mosaicism is a distinct advantage of FISH over routine cytogenetics [18,19].

The age at presentation of our patients ranged between 3 and 16 years with a mean of 10.4 years. In some studies, about one third of cases who had 45,X was ascertained at neonatal period [3,20]. In a German study, Simm et al [21] found that almost 50% Turner syndrome was diagnosed between the age of 6 and 11 (median 8.9 years) and that short stature was the most important diagnostic symptom. Leppig et al reported that MR is the presenting complaint in cases of r (X) [22]. It is assumed that the physical features of T.S. are due to monosomy of genes present on both the X and Y chromosomes and that normally escape X-inactivation. The reported absence of some physical stigmata from patients with a cell line containing an r(X) suggested that at least some genes controlling such features may be located pericentromerically, so that the r(X) prevents functional monosomy of the genes included in the ring, and so the diagnosis is often not suspected until later childhood [23].

The genetic content and/or level of mosaicism for r(X) were among the factors possibly contributing to the variability in phenotype seen among 45,X /46,X,r(X) cases In the present study the level of mosaicism varied widely in these individuals ranged between 9-60% of studied cells. In other studies the percentage of cells containing the r(X) ranged from 6-86% [1,24].

Statistical correlation in our study showed that as the percentage of the ring (X) increased, the IQ level decreased but it did not reach a significant level. Dennis et al [1] reported that a significant greater proportion of cases with at least 50% mosaicism for the r(X) had mental retardation / developmental delay.

Cole et al [16], reported that among the studied cases with active r(X) and mental retardation/ developmental delay there was no correlation between presence of mental retardation/ developmental delay and the level of mosaicism. The activation status (active or inactive) of the r(X) chromosome could be expected to contribute to phenotypic variability in addition to their level of mosaicism and genetic content [25].

In our cases a significant correlation was found between the presence of small ring X and lower IQ level. In the present work, 9 out of the 15 cases had a small ring X with mental retardation,. Vandyke et al [24], proposed a hypothesis to explain the etiology of the unusual phenotype observed in cases with a small r(X) and mental retardation/ developmental delay, speculating that the rings may lack an X inactivation center, thus leading to a lack of dosage compensation for the genes found on the rings (the 'loss of X-inactivation-center hypothesis).

Matsuo et al [26], suggested that the size and the frequency of the active r(X) chromosomes also influence phenotypic severity. Leppig et a1.[22] also found that r (X) in cases with M.R. were consistently smaller than those in individuals with normal intelligence, perhaps indicating inability for small rings to undergo structural changes associated with complete X inactivation or lethality in cases with a large non-inactivated r (X).

Cole et al [16] and Dennis et al [20], reported that the X-inactivation status of the ring was determined by replication studies using BrdU at the end of the S-period. These results agreed with our study.

According to BrdU results we had four types of r(X), large inactive with normal mentality, small active with normal mentality, small active with MR and small inactive with MR.

Replication studies demonstrated a late replication pattern for the large r(X) in cases No. 4, 7, 8, 14 and 15. These results agree with the findings of Van Dyke et al [24] and Cantu et al [27]. These cases have normal intelligence and large-sized ring X, and their ring X is preferentially inactivated.

Van Dyke et al [24] and Migeon et al [28], reported that usually females with an X monosomy or structural abnormal X chromosomes {i(X) chromosomes, X deletions, or large ring X chromosomes} have the relatively benign condition of Turner syndrome presumably because these abnormal X chromosomes are usually inactive in all cells, and the normal X chromosome is always the functional one and this opinion agreed with our data. Although case No. 2 had very small active ring X, (by BrdU replication pattern) but she had normal mentality. An explanation for such cases was made by Matsuo et al and Turner et al [14] as they reported that when the r(X) chromosome is tiny it will be just preserving the peri centromeric region. Other investigators found that failure of X-inactivation was not necessarily associated with a severe phenotype. The degree of impairment in IQ depends on the size of the active ring, and hence was proportionate to the number of genes whose functional disomy affected brain development and functioning

Turner et al [14] found 7 r(X) chromosomes lacking the XIST locus, 6 of them with an unexpectedly mild phenotype and they proposed explanations for this phenomenon. The rings contained limited amounts of X chromosome material, and subsequently disomic effect in a severe phenotype was absent, or the r(X) chromosomes weregenerated in the postzygotic period or the mosaic cell line may be absent from the brain tissue which leads to normal mentality. In our r(X) cases LSI for XIST demonstrated the presence of two copies in all cases, but three cases showed a very small percentage of cell line with deleted XIST and two cases of them showed double ring X with deletion of XIST in both rings.

Although cases No. 1, 3 and 6, had small ring X and mental retardation, but the ring X was inactive as proved by the BrdU replication studies. This means that the XIST gene was functioning and caused inactivation of the ring X. There are many explanations for the preserved XIST inactivation function and presence of mental retardation, since mental retardation is a highly heterogenous phenotype, some genetic or environmental factor(s) irrelevant to the X chromosome abnormalities may be responsible for mental retardation, and since multiple genes for mental retardation have been postulated on the X chromosome such a gene(s) may be mutated on the preferentially expressed single normal X chromosome.

Similar results were reported by Migeon et al [9] who carried out genetic analysis of the r(X) chromosomes from two girls with mental retardation and severe phenol Mohamed/ Kamel/Kayed/ Meguid/Hussein.

type due to X-disomy. The ring (X) chromosome included intact XIST locus which was expressed. Additional studies of uncultured fibroblasts showed a second ring in a small percentage of the cells. The association of severe phenotype with an inactive X chromosome most likely reflects the presence of a second ring X chromosome which was active at least in some tissues during embryogenesis but is no longer prominent in the other tissues.

Lespinasse et al [32] suggested that different tissues other than lymphocytes should be subjected to a karyotype analysis when the observed genotype does not correlate with the clinical phenotype. This is in agreement with our study. We screened a large number of cells to search for an additional cell line, we could detect a very small percentage of cells with deleted XIST and two cases of them showed double ring X with deletion of XIST in both of them.

We had 6 cases [5,10,11,12,13,14] with small ring X and showed no evidence of inactivation, they were all early replicated and therefore active. These cases had mental retardation. Also XIST gene was not deleted in all of them. Leppig et al [22], reported that of 9 cases with r(X) and M.R., 8 cases hadXIST on their r(X), and the majority of cases with M.R. had an early replicating r(X). They concluded that the unusual phenotypic features and M.R. associated with the presence of a r(X) cannot be explained solely on the basis of presence or absence of XIST.

Tomkins et al [33], reported that the clinical findings were consistent with the phenotype described in a limited number of patients with small r(X) lacking the XIST locus, in their patient, FISH demonstrated that the XIST locus was present on the r(X). However, expression studies showed that there was no XIST transcript in peripheral blood cells, suggesting that the r(X) had not been inactivated. The active nature of the ring X would presumably result in over expression of genes that may account for the developmental delay observed in the patient.

On the contrary, other investigators found that failure of X-inactivation was not necessarily associated with a severe phenotype. The degree of impairment in IQ depends on the size of the active ring, and hence was proportionate to the number of genes whose functional disomy affected brain development and functioning [20,22,29].

Conclusion

FISH is useful in identification of a very small percentage of mosaic (hidden) cell line with XIST deletion. The mental status in females with r(X) chromosomes is affected by the presence of small ring X chromosome and presence or absence of functioning XIST on the r(X).

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