Number of C-bands of human isochromosome Xqi and relation to 45,X mosaicism^{1,2}

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SUMMARY With combination of C and G banding techniques, three morphologically different types of isochromosome for the long arm of X (Xqi) have been identified, i.e. those with one C-band and symmetrical banding patterns of both arms, those with two C-bands and symmetrical banding patterns of two arms, and those with two C-bands but with asymmetrical banding patterns of two arms. The last type is a heterogeneous group with various different asymmetrical patterns. We have studied 6 cases of Xqi with C, G, and Q banding: 3 showed one C-band and symmetrical arms and all these were without 45,X mosaicism; the other 3 cases showed two C-bands, 2 of the cases having symmetrical arms and being mosaic for 45X/46,XXqi/47,XXqiXqi (those 2 were a pair of identical twins). One other had asymmetrical arms and was mosaic for 45,X/46,XXqi. Including our 6 cases, there have been a total of 30 reported cases of Xqi with C and G banding studies. Two-thirds of Xqi's were found to have 2 C-bands and one-third to have 1 C-band. Mosaicism was found in 85% of Xqi's with 2 C-bands and in only 44% of Xqi's with 1 C-band. Apparently, Xqi's with 2 C-bands have a greater tendency for anaphase lag and mitotic nondisjunction. Several possible mechanisms for the formation of the different types of Xqi's are discussed.

Isochromosome for the long arm of X, that is Xqi, is the most common structural aberration of the X chromosome associated with Turner syndrome. Of 288 cases with ovarian dysgenesis collected by Hamerton (1971), 74 were found to have structural aberrations of the X chromosome and 50 of these were reported as having Xqi. It is also known that Xqi is frequently associated with 45,X mosaicism. In Hamerton's series, 38 out of 50 cases, i.e. 75%, were mosaic. An earlier collection by Ferguson-Smith (1965) of 35 cases with Xqi also showed mosaicism in 66% of the cases. Apparently a structurally abnormal chromosome has a tendency for either anaphase lag or mitotic nondisjunction. With the various banding techniques now available we have attempted to determine the relation between the nature of the Xqi and its potential mosaic tendency.

Materials, methods, and results

During the past decade we have detected a total of 12 cases of Xqi. Five of these were studied before the banding era. The other 7 were identified with quinacrine fluorescence (Q-banding) and/or trypsin Giemsa (G-banding) studies (Paris Conference, 1972). Of these latter 7 cases we were able to study centric-heterochromatin (C-banding) patterns in 6 patients. Of the 12 cases, Xqi mosaicism was found in 6; 3 of these were from the 6 cases that we were able to study with both G or Q and C-banding techniques.

The clinical manifestations of these 6 patients are shown in Table 1. The patients ranged from age 10 to 24 years. Cases 4 and 5 were a pair of identical twins. All 6 cases presented with short stature. The 4 preadolescent girls (cases 1, 2, 3, and 6) had heights ranging from 4 to 6 years below the mean for their chronological ages. The two adult patients (cases 4 and 5) both had primary amenorrhoea. Short neck without webbing was seen in only 2 patients (cases 3 and 6). Cubitus valgus was seen in 5 patients.

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Table 1 Clinical summary of patients studied with both G and C-banding

Case No.	Age (y)	Short stature	1° Amenorrhoea	Short neck	Cubitus valgus	Pigmented naevi	Short 4th and/or 5th metacarpal	Low hair line	Wide-spaced nipples	BP (mmHg)	Total finger ridge count	% cells positive for X-chromatin in buccal smear
1	14	+ (55" HA 10 2/12)		—	+ (25°)	+	+ (slight)	—	—	N1	228	40%
2	13	+ (50 7/8" HA 8 7/12)		—	+ (25°)	+	—	±	+ (slight)	N1	129	43%
3	13	+ (47 3/4" HA 7)		+ (no webbing)	+	+	+	±	—	N1	124	27%
4*	24	+ (59 1/4" HA 12)	+	—	+ (slight)	+	—	—	—	170/105	177	8%
5*	24	+ (58 1/2" HA 12)	+	—	+ (slight)	+	—	—	—	145/95	172	16% (Few cells with 2 X-cnromatin)
6	10	+ (47" HA 6 1/2)		+ (no webbing)	—	+	+	—	—	N1	150	62%

*Identical twins.

HA, height age; N1, normal; BP, blood pressure.

Table 2 Chromosome constitution in 3 cases of Xqi with 2 C-bands

Case no.	Chromosome constitution		
	45,X	46,XXqi	47,XXqiXqi
4*	19	31	2
5*	18	31	1
6	17	33	0

*Identical twins.

Increased numbers of pigmented naevi were seen in all 6. Short 4th and/or 5th metacarpals were seen in 3. Hypertension was found in the identical twins (cases 4 and 5). Case 4 died unexpectedly in late 1976, and necropsy in another hospital disclosed a ruptured dissecting aneurysm of the aorta. Case 5 was referred to a hypertension specialist, but has thus far been resistant to medical advice.

Dermatoglyphic analysis showed an increased total finger ridge count in 3 patients (cases 1, 4, and 5).

In general, the clinical features of these 6 patients were not different from those of the 45,X Turner syndrome.

Buccal smears were positive for a large X-chromatin body in 8 to 62% of the cells (Table 1). Case 5 showed a few buccal mucosa cells containing two large X-chromatin bodies (Fig. 1).

Chromosome analysis was carried out from short-term lymphocyte cultures. The isochromosome for the long arm of X was identified by both trypsin Giemsa (Seabright, 1971) and quinacrine dihydrochloride (Breg, 1972) banding techniques. The G-banding of the normal X's and the abnormal Xqi's and C-banding of only the Xqi's are shown in Fig. 2. The G-banding patterns of the Xqi's are symmetrical in 5 patients, that is cases 1, 2, 3, 4, and 5 showing two identical long arms of X. The Xqi of case 6 shows an asymmetrical banding pattern, with an additional narrow band between the centromere and the major band in one arm.

C-banding was performed according to the method

Fig. 2. G-banding patterns of the normal X's and Xqi's, and C-banding patterns of Xqi's of all 6 cases.

Fig. 1. A buccal mucosa nucleus of case 5 showing two large X-chromatin bodies.

Fig. 4 A C-banded metaphase of case 3 with 47,XXqixXi constitution with both Xqi chromosomes showing two distinct C-bands.

described by Salamanca and Armendaris (1974) using 0.07 N Ba(OH)₂. Three cases (cases 1, 2, and 3) showed one C-band; the other 3 (cases 4, 5, and 6) showed two C-bands. The 3 cases with one C-band were found to have only cells with 46,XXqi chromosomal constitution. In each of these 3 cases, at least 50 cells were examined. No cell with 45,X was detected. On the other hand, mosaicism was evident in all 3 cases with Xqi's containing two C-bands. The chromosome counts of the 3 cases of Xqi with two C-bands are tabulated in Table 2. Triple mosaicism containing 45,X/46,XXqi/47,XXqixXi was found in both cases 4 and 5. 45,X/46,XXqi mosaicism was shown in case 6. Interestingly, the identical twins, cases 4 and 5, showed very similar proportions of the three different cell lines in their peripheral lymphocyte cultures. A C-banded metaphase of case 4 of 47,XXqixXi is shown in Fig. 3; both Xqi chromosomes clearly show two C-bands.

Discussion

Including our 6 cases, a total of 30 cases of Xqi have been studied with C-banding technique in addition to G or Q banding (Table 3). Nine were found to contain one C-band and 21 showed two C-bands. The possibility that certain X isochromosomes are dicentric was first postulated by de la Chapelle *et al.* in

Fig. 4. Schematic drawing of three morphologically different Xqi's.

Table 3 C-banding studies of iso-X-chromosomes (Xqi) 30 cases

Author	One C-band (9 cases)			Two C-bands (21 cases)		
	XXqi	X/XXqi	X/XXqi/XXqiXqi	XXqi	X/XXqi	X/XXqi/XXqiXqi
De La Chapelle and Stenstrand (1974)	1	1			2	
Priest <i>et al.</i> (1975)	1	1			1	
Cohen <i>et al.</i> (1975)					1	
Yanagisawa and Yokoyama (1975)						1
Baranorskaya <i>et al.</i> (1976)				1	2	
Palmer and Reichmann (1976)		2		1	5	
Howell <i>et al.</i> (1976)				1	2	1
This presentation	3				1	2
Total	5	4	0	3	14	4

1966. Eight years later, de la Chapelle and Stenstrand (1974) reinvestigated several of their Xqi patients with both G and C banding techniques. They found that the Xqi of 2 patients contained two regions of centromeric heterochromatin while 2 other cases of Xqi showed only one C-band. The 2 cases with two C-bands were mosaic and 1 of the 2 cases with one C-band was also mosaic. By 1975, it became clear that there were two types of human Xqi chromosomes, namely those with one C-band and those with two C-bands. Five cases of Xqi reported in 1975 included 3 with two C-bands that were mosaic, and 2 with one C-band, 1 of whom was mosaic (Cohen *et al.*, 1975; Priest *et al.*, 1975; Yanagisawa and Yokoyama, 1975). In 1976, 15 more cases of Xqi were studied with C-banding. The vast majority, that is, 13 cases were found to have two C-bands of which 10 were mosaic (Baranorskaya *et al.*, 1976; Palmer and Reichmann, 1976; Howell *et al.*, 1976). Therefore, in a total of 30 cases studied 21 showed two C-bands, of which 18 were mosaic, while 9 had one C-band, 4 of which were mosaic. In other words, thus far C-banding studies have shown that two-thirds of Xqi have two C-bands and one-third have one C-band. Mosaicism was found in 85% of Xqi with two C-bands and in only 44% of Xqi with one C-band.

The prevalence of mosaicism in Xqi's with two C-bands is most likely the consequence of the dicentric nature of these chromosomes, though without C-banding only one centromere is visible. It is known that chromosomes with two centromeres are unstable and the Xqi's with two C-bands, being theoretically dicentric, behave more like a dicentric chromosome than a monocentric chromosome. It is interesting to point out here that triple mosaicism of 45,X/46,XXqi/47, XXqiXqi has only been associated with Xqi's with two C-bands. This suggests that Xqi's with two C-bands have not only a greater tendency for anaphase lag resulting in 45,X/46,XXqi mosaicism but also for mitotic non-disjunction leading to 45,X/46,XXqi/47,XXqiXqi mosaicism. De la Chapelle and Stenstrand (1974) in fact noticed abnormal anaphase configurations such as anaphase bridges and

lagging in two cases of Xqi with two C-bands. Both these cases were mosaic.

As to the possible mechanisms of isochromosome formation, Darlington in 1940 postulated that an isochromosome may be the result of a misdivision of the centromere, and Nusbacher and Hirschhorn in 1968 suggested that an isochromosome can also be the result of a small pericentric inversion followed by a crossover event within the inversion which leads to a chromosome with essentially identical arms. With the banding studies, it is evident that several mechanisms can account for the formation of Xqi's. Thus far, at least three morphologically different Xqi's have been described (Fig. 4), that is those with one C-band and symmetrical banding patterns of both arms, those with two C-bands and symmetrical banding patterns of the two arms, and those with two C-bands but with asymmetrical banding patterns of the two arms. The last type is actually a heterogeneous group with various different asymmetrical patterns.

Priest *et al.* (1975) suggested two different mechanisms for the formation of the two different types of Xqi's with two C-bands. She suggested that the type with two C-bands and symmetrical arms is probably a result of a single break in the short arms of normal X's at p11 followed by chromatid duplication and joining of chromatids from one side of the break. The other type with two C-bands and asymmetrical arms could be the result of an inversion involving the centromere similar to the model postulated by Nusbacher and Hirschhorn (1968). It is apparent that different forms of Xqi's can be formed when the inversion involves different regions of the X-chromosome. Priest *et al.* also believe that the best interpretation of the Xqi with one C-band and symmetrical arms is to assume transverse breakage of the centromeric region to produce an isochromosome with identical arms, though a small pericentric inversion could explain this as well. In addition to these mechanisms, Xqi could also be caused by a translocation of two X-chromosomes involving regions adjacent to the centromere.

In conclusion, by employing various banding techniques, one can begin not only to study the mechanisms

of formation of Xqi chromosomes, but also the relation of their structure to the tendency for mosaicism.

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Addendum

Very recently, Fujita *et al.* (1977) reported 10 cases of Xqi. Of the 6 containing one C-band, 5 were 46,XXqi without mosaicism. Of the 4 containing two C-bands, 3 were 45,X/46,XXqi mosaic. Therefore, Fujita's observation has further strengthened our speculation.