European Journal of Human Genetics (2008) 16, 153–162

www.nature.com/ejhg

ARTICLE

X inactivation in triploidy and trisomy: the search for autosomal *trans*factors that choose the active X

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Only one X chromosome functions in diploid human cells irrespective of the sex of the individual and the number of X chromosomes. Yet, as we show, more than one X is active in the majority of human triploid cells. Therefore, we suggest that (i) the active X is chosen by repression of its *XIST* locus, (ii) the repressor is encoded by an autosome and is dosage sensitive, and (iii) the extra dose of this key repressor enables the expression of more than one X in triploid cells. Because autosomal trisomies might help locate the putative dosage sensitive *trans*-acting factor, we looked for two active X chromosomes in such cells. Previously, we reported that females trisomic for 18 different human autosomes had only one active X and a normal inactive X chromosome. Now we report the effect of triplication of the four autosomes not studied previously; data about these rare trisomies – full or partial – were used to identify autosomal regions relevant to the choice of active X. We find that triplication of the entire chromosomes 5 and 11 and parts of chromosomes 1 and 19 is associated with normal patterns of X inactivation, excluding these as candidate regions. However, females with inherited triplications of 1p21.3–q25.3, 1p31 and 19p13.2–q13.33 were not ascertained. Thus, if a single key dose-sensitive gene induces *XIST* repression, it could reside in one of these locations. Alternatively, more than one *dosage-sensitive* autosomal locus is required to form the repressor complex.

European Journal of Human Genetics (2008) **16,** 153–162; doi:10.1038/sj.ejhg.5201944; published online 31 October 2007

Keywords: X inactivation; *XIST* repressor; triploidy; trisomy; autosomal *trans*factor; choice of active X

Introduction

Studies of the molecular mechanisms underlying X inactivation – the means of dosage compensation of X-linked genes in mammals – show that the non-coding *XIST* RNA is a key player.[1] Transcription of *XIST* at the critical time in embryonic development is sufficient to initiate a cascade of events that silences all the X chromosomes in the cell except the one that remains active. How one X in diploid cells escapes inactivation and is chosen to be the future active X remains to be determined. However, it is likely that autosomes participate in this process. The two active X chromosomes in many 69, XXY and 69, XXX triploid cells[2–6] suggest a role for the extra set of autosomes in protecting X chromosomes from inactivation in triploid cells and, by extension, a role for autosomes in choosing the future active X in diploid cells. The easiest way for an autosomal gene(s) to do this is by repressing the *XIST* locus on one X chromosome.[7] We previously tried to identify candidate autosomes by examining X inactivation in spontaneously aborted females trisomic for individual autosomes. At that time, we determined the X inactivation status for 18 of the 22 possible autosomal trisomies and

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Received 26 June 2007; revised 22 September 2007; accepted 26 September 2007; published online 31 October 2007

showed that, in each case, the specimen had one active and one inactive X.[8] Assuming a single dosage sensitive gene is sufficient to repress *XIST*, these chromosomes could be excluded. However, it was impossible to study the rarest trisomies, 1, 5, 11 and 19, which are the subjects of the present study. We now have observations on four females, two with trisomy of chromosome 11, one with trisomy of chromosome 5 and another with tetrasomy of chromosome 5p; in all four cases there was a single active X and a normal inactive X. Because of their rarity, we could not examine the remaining two chromosomes directly. Therefore, to identify candidate regions for the critical autosomal *trans*factor we ascertained partial trisomies for these chromosomes among live-borns. In addition, we reconsidered the effect of triploidy on X inactivation using direct examination of *XIST* to look at the characteristics of X inactivation in triploid cells.

Materials and methods

Cells and tissues

Triploid and trisomic cell cultures were derived from spontaneous abortions. Most of the triploid specimens were reported previously by Jacobs *et al*[5] or Migeon *et al*[4] and have been maintained in liquid nitrogen. The others were obtained at the time of miscarriage, according to an IRB-approved protocol. Supplementary Table 1 shows the triploid specimens that are new or restudied by different methods for this paper.

Cytogenetic studies

Data about the sex chromosomes in triploids and non-mosaic trisomics were obtained from the laboratories of co-authors in Honolulu and Wessex (PAJ), Baltimore (GS), New York (CT), from reports in the literature as cited in Table 1, from the Coriell Institute, and the American Cytogenetics Forum List. Queries to cytogenetic laboratories of colleagues did not yield further cases.

X inactivation studies

The presence of an inactive X chromosome was detected by *XIST* expression and late replication; *XIST* transcription was analyzed by RTPCR.[15] *XIST* and *TSIX* RNA were detected by FISH; unfixed metaphase cells were labeled by indirect immunofluorescence. *XIST* DNA was detected on the same specimens used for *XIST* RNA in sequential hybridizations.[16] Late replicating X chromosomes were identified using the standard BrdU and acridine orange method. X inactivation studies of all the autosomal trisomies *except* 1, 5, 11, and 19 had been reported by us previously.[8]

Mapping candidate regions

For chromosomal trisomies that could not be examined directly we looked for partial trisomies among live-borns. Our strategy was based on the assumptions that(i) three copies of the region containing the putative *trans*factor would allow a second X to remain active and (ii) any fetus with two active X chromosomes would die before birth, as no diploid live-born with two intact active X chromosomes has ever been observed. Information was gathered mainly from two sources: the literature (PubMed) and unpublished data from The Wessex Regional Genetics Laboratory at Salisbury District Hospital, UK. Our inquiry to cytogenetics data banks and cytogeneticist colleagues was not productive, except for six partial trisomies from the Coriell Institute, included in Figure 2. As this approach was initiated prior to our ascertaining cells from the trisomy 5 specimens, we considered any chromosome anomaly that resulted in a partial trisomy of chromosomes 1, 5 and 19. In each case we determined if the evidence was sufficient to document a viable partial trisomy.

The candidates for our region of interest were based on four criteria: (i) the subject must be female, as only females could have two active X chromosomes; (ii) the subject must be live-born, as the ability to survive gestation eliminates the possibility of having two intact active X chromosomes; (iii) the partial trisomy must involve three separate chromosomes, as is the case in unbalanced translocations; (iv) the translocation must be inherited, as *de novo* translocations may have arisen after the choice of the active X and would not affect the choice process. In addition, because the unbalanced translocations were associated with congenital malformations and were inherited from a phenotypically normal balanced translocation carrier, it is likely that the extra copy of the triplicated

Table 1 Relative frequency of the various human triploids among 820 specimens

Specimen[a]	*Trunca,*[b] *N Y*	*Jacobs, Hawai*	*Jacobs, Wessex*	*Stetten, MD*	*Other reported studies*[c]	*Total (%)*
69, XXX	50	75	70	23	138	356 (43.4%)
69, XXY	81	107	51	30	176	445 (54.3%)
69, XYY	6	5	1	1	6	19 (2.3%)
Total	137	187	122	54	320	820

[a]Karyotype of the triploid specimens.
[b]Investigator and place of study.
[c]Cases reported in the literature include the following papers: Zaragoza *et al*,[9] McFadden *et al*,[10,11] Baumer *et al*,[12] Daniel *et al*[13] and Kajii *et al*.[14]

segment was expressed. Direct duplications in males and females were also mapped but were not used for exclusions, as both copies are on the same chromosome and might function as one copy rather than two and might be subject to position effects. Because a triple dose of the putative *trans*factor in males should not affect the activity status of their single X chromosome, we expected to see trisomic regions in males not seen in females. Therefore, we noted the occurrence of familial partial trisomies in males but did not use them for exclusions.

In the case of data reported at a resolution of less than the 700-band level, we interpreted breakpoints conservatively, so as to exclude the smallest region possible. For example, if the breakpoint was reported as 1p21, we interpreted it as 1p21.3.

Finally, for all the candidate regions on human autosomes we identified the orthologous regions in the mouse genome using the NCBI map viewer: comparative maps of *Homo sapiens* (Build 36.2), at the NCBI website.

Results and discussion

Evidence that triploid cells mainly have two active X chromosomes

The phenotype of human triploids is influenced by the parental origin of the extra haploid set of chromosomes. When it is paternal (diandric), the fetus tends to be more poorly developed and the placenta is abnormal. Zargosa *et al*[9] noted that the majority of human triploids are diandric because dispermy is relatively common; the maternally derived ones result from less frequent errors in oocytes during meiosis II. Because the relative frequency of XYY, XXY and XXX dispermic triploids should be 1:2:1,[17] one expects many triploids of the 69 XYY type. However, Table 1 shows that XYY triploids are extremely rare among recognizable triploid specimens, only 19 of 820 triploids (2.3%) having an XYY karyotype. It seems that XYY triploids are created at their expected frequency,[18] but do not survive beyond implantation. Table 2 summarizes the results of our X inactivation analysis of XXX and XXY triploid specimens. See Supplementary Table 1 for details. Also included in Table 2 are the results of previous studies based on the presence of a late-replicating X,[5] and/or heterodimers for G6PD[4] and a recent study based on *XIST* expression.[6] We re-analyzed some specimens and studied new ones using *XIST* and *TSIX* RNA as markers for an inactive X.[16] In humans, unlike mice, *TSIX* is a marker for the inactive X chromosome as it is co-expressed with *XIST* from the inactive X and not from the active one[16] (See Figure 1). In all cases, the FISH assay confirmed the findings based on chromosome replication. Of the 47 cases of XXY and XXX triploids studied, two active X chromosomes were seen in every cell in 24 cases and in some cells in 17 cases; they were not seen in only 6 cases. Therefore, 87% of these triploids had two active X's in at least some of their cells (Table 2 and Figure 1a–d).

The mixed population of cells (some with two active X's and others with only one) seen in 17 of the 47 cases might reflect some instability of the process. Gartler *et al*[6] suggest that the two active X chromosomes result either from reactivation of the inactive X or reduplication of the active one. However, such reactivation or reduplication events are inconsistent with most previous observations: spontaneous reactivation of the whole chromosome has not been seen in any human somatic cell, and duplication has been excluded in cases where the two X's can be distinguished by polymorphic variants.[3,4] Moreover, previous studies show that the 'two active X phenotypes' in triploid skin fibroblasts is highly stable[4] and this is confirmed in the present study (see JWD clones in Supplementary Table 1). In fact, the mixed population of cells is consistent with expectations – if two copies of an autosomal *trans*-acting factor are needed for one active X chromosome, then three copies may not always be sufficient for two active X chromosomes. Thus, stochastic variation in *trans*factor abundance may result in a mixture of cells – some with one active X and others with two of them. In this case, the cells differing in the number of active X's may be subject to selection as they proliferate.[6] Jacobs *et al*[5] suggested that the advantage might be tissue specific, and hence reflect the predominant tissue present in the specimen.

In any case, one must conclude that (i) irrespective of their parental origin or sex, most triploid specimens have two active X chromosomes (X^a) in at least some of their cells and often in all of them, and (ii) the one X^a/two X^a mixed cultures originate at the time when the active X is chosen. Therefore, studies of triploids tell us that the factor(s) enabling the activity of more than one X

Table 2 X Inactivation studies in triploid cells

Specimen		*No. of active X's/triploid cell*			
Karyotype	*No.*	*One*	*Two*	*Mixed*[a]	*References*
69, XXY	29	5	15	9	Jacobs *et al*;[5] this paper
69, XXX	12	1	6	5	Jacobs *et al*;[5] this paper
69, XXX	6	0	3	3	Gartler *et al*[6]
Total	47	6	24	17	

[a]'Mixed' refers to specimens having cells with one active X and two active X chromosomes.

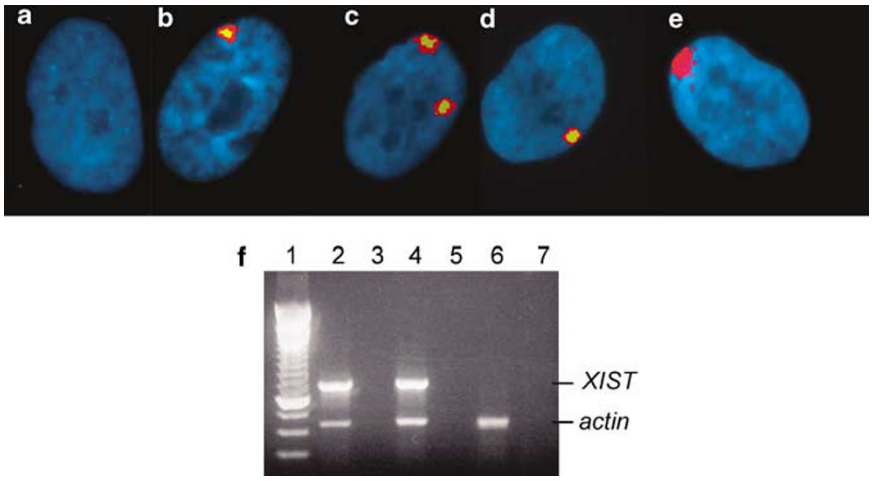

Figure 1 Analysis of X inactivation in triploid (**a**–**d**) and trisomic (**e** and **f**) cells using *XIST* and *TSIX* as markers for the inactive X. Red indicates the signals for XIST RNA and green for TSIX RNA. Yellow shows where the XIST and TSIX signals overlap, as both are expressed from the inactive X chromosome in humans, unlike the situation in the mouse.[16] RNA FISH analysis of 69, XXY triploids (**a** and **b**) showing no *TSIX* or *XIST* signals in specimen 288 indicating two active Xs (**a**) and specimen 322 with one *TSIX/XIST* overlapping signal indicating a single active X chromosome (**b**). The same analysis of 69, XXX triploid specimen JWD (**c** and **d**) showing cells with one active X (**c**) and two active X chromosomes (**d**) respectively. *XIST* expression in cells from a 47, XX female with a trisomy for chromosome 11 showing *XIST* RNA FISH signal (**e**) and RT-PCR analysis (**f**).lane 1,marker; lanes 2 and 3, control female; lanes 6 and 7,control male and lanes 3 & 4,47, XX + 11 . Even lanes show results in presence of the reverse transcriptase.

chromosome is not present in sufficient amounts to ensure two active X chromosomes in *every* cell. On the other hand, because there are two active X's in most triploid specimens, the additional set of chromosomes must in some way enable this to happen, presumably because of extra doses of an autosomal gene(s).

Hypothesis: the human active X is chosen by *XIST* repression

One way that the extra set of autosomes in triploids could enable the activity of more than one X is if they provide an extra dose of *trans*-acting factor, and if the *trans*factor is an *XIST* repressor. Because an *XIST*-expressing chromosome cannot be the active X, the *XIST* locus on that chromosome needs to be repressed. The concept of a blocking factor that protects the active X from inactivation was suggested by Rastan[19] and Lyon[20] long before the *Xist* locus was discovered. We suggest that this blocking factor is an *XIST* repressor, and that in diploid cells there is only enough *XIST* repressor for a single active X, but in triploid cells there is more. Therefore, based on the observations of triploids our working hypothesis is that (i) the single active X is chosen by *XIST* repression; (ii) there is an autosomal gene or element that is the *XIST* repressor or that initiates the events that silence *XIST* on the future active X; (iii) This *XIST* repressor is a dosage sensitive gene product: two doses of it can repress only one *XIST* gene, but three doses can repress more than one *XIST* gene. Although the *trans*factor might bind directly to *cis* elements within the *XIST* locus, it could work indirectly to repress *XIST* by binding to other elements within the X inactivation center.

The search for *trans*-acting factors, using autosomal trisomies

To test our hypothesis, we embarked upon a search with the long-term goal of identifying factors that when expressed in triple dose could enable the activity of two X chromosomes. If triploids can, and usually do, have two active X's and if the *XIST* repressor was encoded by a single autosomal locus, then trisomies, with three copies of the relevant gene, might also have two active X's. We realized that not all cells trisomic for the putative *trans*-acting factor might have two active X chromosomes. Nevertheless, while the search for such a *trans*factor using trisomies cannot prove that it is absent, it can show that it is present.

Initially, we studied female trisomic specimens obtained from miscarriages and could show that for all of them the trisomy did not alter the inactivation process.[8] Recently, we ascertained two specimens of trisomy 11, and in each case *XIST* was expressed from their normal inactive X chromosome (Figure 1d and e).

Being aware that having two active X chromosomes might be lethal prior to the time when a pregnancy is recognized, we examined the sex ratio for the various autosomal trisomies. Conceivably, the trisomy we sought might be more lethal to females than to males as, irrespective of the amount of *trans*-acting factor, only females could have more than one active X. Table 3 shows the small excess of males among these specimens in general – a good deal of it attributable to the excess of males with trisomy 21 and 22. Chromosomes 1 and 5 were also more prevalent in males, but the number of specimens is very small.

Table 3 The sex ratio and X inactivation status of fetal specimens with complete trisomy for one of the human autosomes

	Sex ratio			*X inactivation studies*		
Autosome	*Male*	*Female*	*M:F*	*No. of specimens*	*Method*	*Inactive X present*
1	(2)	(1)	(2.0)	NA		
2	29	29	1.0	3	LR	+
3	13	16	0.81	2	LR	+
4	27	26	1.04	2	LR	+
5	6	3	2.0	1	LR	+
6	16	9	1.78	1	LR	+
7	28	15	1.87	2	LR	+
8	24	23	1.04	2	LR, G6PD	+
9	23	30	0.77	9	LR	+
10	15	17	0.88	1	LR	+
11	4	10	0.40	2	XIST/TSIX	+
12	12	8	1.50	2	LR	+
13	134	113	1.19	4	LR	+
14	31	29	1.07	2	LR	+
15	69	63	1.10	3	LR, G6PD	+
16	211	232	0.91	4	LR	+
17	8	9	0.89	2	LR	+
18	197	235	0.85	2	LR, G6PD	+
19	3	3	1.0	NA		
20	16	28	0.57	2	LR	+
21	613	475	1.29	2	LR	+
22	143	119	1.20	3	LR	+
Total	1626	1486	1.09			

Specimens were obtained from prenatal diagnosis and miscarriages in the laboratories of co-investigators and collegues. Numbers in parentheses for trisomy 1 comprise all the cases in literature, and were not included in the total. A subset of these specimens was examined for X inactivation status by late replication (LR), G6PD heterodimer (G6PD) and RNA FISH for *XIST* and *TSIX*. NA, trisomies that were not available for study.

From the literature we learned of three instances of trisomy 1 in recognizable pregnancies (two males and one female),[21–23] and another male with just eight cells.[24] The sole female conceptus, an empty sac with no sign of fetal development, was miscarried at 39 days post conception[22] relatively close to the time of X inactivation in human fetal tissues (~20 days). We also studied a specimen trisomic for the entire long arm of chromosome 1 that expressed *XIST*. However, as there were two normal 46, XX cells in the culture that could not be definitively attributed to maternal contamination, we could not exclude the possibility that the trisomy occurred after X inactivation.

There are no reports of complete trisomy of chromosome 5 in the literature, but recently we studied two relevant female specimens. The first has a complete trisomy 5 and a gestational age of 8 weeks. In all cells with three chromosome 5, only one of the X chromosomes was active, whereas the other was late replicating. The second fetal specimen had four copies of the short arm of chromosome 5, two on the normal chromosome 5s and the other two as components of an isochromosome of the entire short arm; in each of the 30 cells suitable for study, one X was active and the other late replicating.

Mapping of trisomic regions in partial trisomies

For the chromosomes that could not be studied directly (1 and 19), we looked for partial trisomies among live births. This permitted us to identify triplicated regions in individuals who had a single active X. We are aware that such females might have had mosaic X inactivation patterns, surviving only because selection favored cells with a single active X. However, if they were not mosaic initially, then their very existence could exclude these regions as candidates. Partial trisomies were ascertained from the laboratory of one of us (PAJ), from the Corielle Institute and from the literature. We established strict criteria for exclusion (see Materials and Methods). Triplication needed to involve three separate chromosomes and the translocation or insertion had to be familial to assure that it was present at the time that the active X was chosen. Although the locations of tandem duplications were noted, they were not used to eliminate a candidate region. See Figure 2.

Chromosome 1 A large part of the short arm of chromosome 1 can be eliminated by two case reports. Halal *et al*[46] described a female infant with trisomy 1p31→1pter, resulting from a translocation between chromosomes 1 and 2. It is likely that one of the parents (who refused testing) carries a balanced translocation, as there were seven previous miscarriages. The second case is a 30-year-old female with a trisomy resulting from a direct insertion of 1p21→p31 into the long arm of the chromosome 13 inherited from her mother.[49] Based on phenotypic similarities, two other relatives (one male and one female) were also affected. In addition, because of the conservative

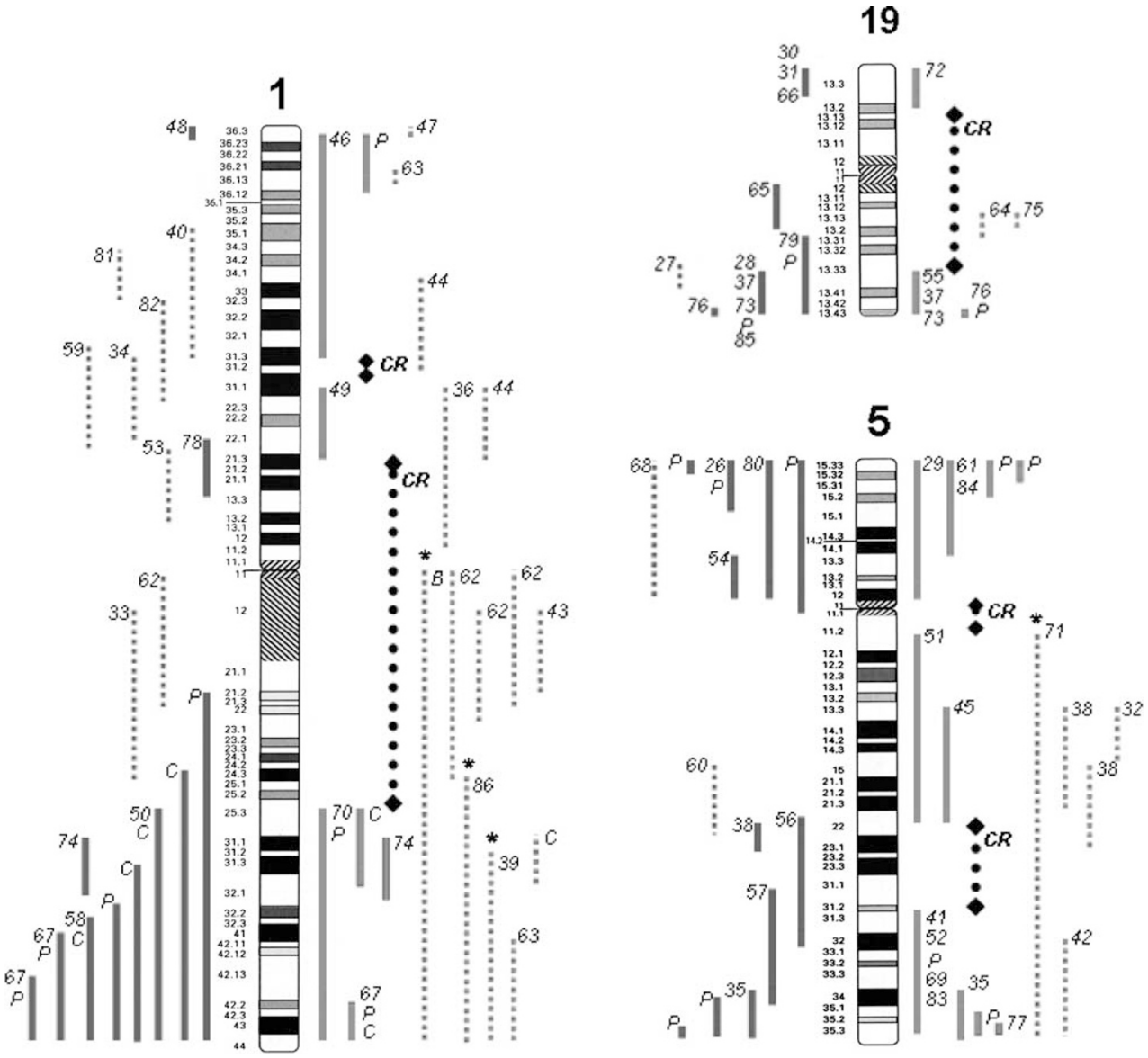

Figure 2 Map locations of partial trisomies for chromosomes 1, 5 and 19, for which no trisomic specimens were available. A full trisomy 5 was ascertained only after these studies were initiated (see text). Ideograms of metaphase chromosomes (700 bands, ISCN, 2005)[25] showing the location of trisomic regions. Males are shown on the left and females on the right. The numbers ([26–86]) refer to literature citations; the letters P and B refer to cases studied by PAJ and BRM respectively and C refers to specimens from the Coriell collection. *Samples shown to have an inactive X, ▬ trisomic regions due to inherited unbalanced translocations. ┈┈ trisomic regions due to *de novo* or possible *de novo* duplications, ◆••••◆ candidate Regions (CR) for autosomal *trans*factor.

interpretation of breakpoints, a small region from 1p31.1–p31.3 cannot be excluded. Therefore, the candidate region on the short arm of chromosome 1 is reduced to 1p10→p21.3 and the small interval in 1p31 (Figure 2).

The distal long arm of chromosome 1 can be eliminated because of two females with a duplication of 1q25→qter resulting from a familial balanced translocation (Rosenthal *et al*[70] and PAJ). A girl with a trisomy for 1q24-qter caused by a non-familial X/1 translocation had an inactive X chromosome with spreading of inactivation into the translocated 1q segment ameliorating her phenotype;[86] this suggests that 1q24.3→qter could be excluded (Figure 2, asterisk). The previously noted female fetus with full trisomy 1q and *XIST* transcripts was not used to eliminate candidate regions because of uncertainty as to when the trisomy occurred. Using strict criteria, the major candidate region for the entire chromosome is 1p21.3→q25.3, but, based on the spreading of X inactivation into the

translocation chromosome, it is likely that more of the long arm could be excluded. The orthologous regions in the mouse genome are on mouse chromosomes 3 and 1.

Chromosome 19 Based on viable trisomies, ~one-third of chromosome 19 can be excluded. The distal segment of the short arm of chromosome 19 is excluded by a female infant with a partial trisomy of 19p13.2→pter derived from a paternal balanced 3/19 translocation.[72] Therefore, the candidate region for the short arm of chromosome 19 is 19p10→p13.2 (Figure 2).

The distal long arm of chromosome 19 is represented by several females with familial trisomies 19q13.33→qter.[55,37,73] Therefore, the candidate region is 19q10→q13.33 for the q arm, and 19p13.2–q13.33 for the entire chromosome. Two trisomies in males overlap this candidate region (Figure 2). The orthologous regions have been fragmented among several mouse chromosomes, predominantly chromosomes 7 and 8 with some sequences on 10 and 17.

Chromosome 5 Our search for partial trisomies of chromosome 5 among live-borns was initiated before we ascertained the complete trisomy 5 and tetrasomy 5p specimens. We found that the short arm of chromosome 5 could be excluded because of a female with a trisomy of the entire short arm of chromosome 5 inherited from a maternal balanced translocation.[29] This finding was confirmed by our studies of the fetal specimen with 5p tetrasomy. Also, most of the long arm of chromosome 5 is not a candidate region based on evidence from live-borns.[45,52,69,83,77,35] Although several males have been reported,[56,57,38] there are no females with trisomy spanning any part of 5q22–q31.1. The trisomy of the entire long arm of chromosome 5 reported as due to unproven gonadal mosaicism[71] is shown as a dotted line in Figure 2. The only regions of chromosome 5 not represented by partial trisomies (5q22–q31.1 and 5q10–q11.2) can now be excluded by our X inactivation studies of the female specimen with complete trisomy 5.

Conclusions based on observations of X inactivation in trisomies

The results show that for the 20 trisomies (one to nine female specimens of each kind) that were studied directly, there was only a single active X, so X inactivation had occurred in each of the 51 fetal specimens analyzed. Therefore, three copies of these autosomes neither induced the activity of more than one X nor interfered with the ability to inactivate an X. Unlike the triploids, not one of the 51 specimens had cells with two active Xs. Although we observed no mosaicism among any of the 51 trisomic specimens – many of them early first trimester embryos – it is conceivable, but unlikely, that the extra dose of *trans*factor invariably leads to a mosaic fetus, and the cells with a single active X have a major advantage so that we ascertain only those cells. The time when specimens with two active X chromosomes die is not known, but if all trisomic specimens with two active X chromosomes were lost prior to implantation, then one might expect significantly more male specimens with the relevant trisomy among recognized abortions. Although the numbers are relatively small, there is no obvious selective loss of females for any trisomy (Table 3).

In lieu of the fetal specimens unavailable for study, we ascertained live-born females with partial trisomies of chromosomes 1, 5 and 19 who came to attention because of congenital abnormalities. Not all of the partial trisomies had been studied specifically for their X inactivation pattern, but as no live-born with two intact active X chromosomes has ever been identified, it seemed reasonable to expect that they had an inactive X. We found that some chromosomal regions were not represented in the females with partial trisomies; some of these regions were seen in males. The regions not observed may merely reflect the relatively small number of samples. This seems to be the case for the missing segment of chromosome 5, as we found that complete trisomy for chromosome 5 was associated with an inactive X chromosome. With regard to chromosomes 1 and 19, the alternative explanation for regions that are not represented in females is that they are the ones in which autosomal *trans*factors lie, and their triplication is incompatible with intrauterine survival.

Using ENU mutagenesis, Percec *et al*[87] looked for autosomal dominant mutations affecting X-inactivation choice in mice and reported some evidence supporting the existence of candidate regions on mouse chromosomes 5, 10 and 15. However, till now no autosomal genes have been definitively identified. We have identified regions of the mouse genome that correspond to our human candidate regions, so as to facilitate experimental attempts to find the relevant genes or elements in mouse models. Searching for physical interactions between X and an autosome in embryos prior to the onset of X inactivation might identify relevant autosomes, as this has been able to identify interacting chromosomes involved in the choice of olfactory alleles.[88]

We have presented evidence that the two active X phenotype is characteristic of the majority of triploid embryos. We have also proposed that three copies of a single dosage-sensitive *trans*factor are sufficient for the activity of two active X's, at least in some cells. As a preliminary test of the hypothesis, we have identified potential candidate regions of human autosomes and their corresponding location in the mouse. We are aware that our studies cannot eliminate the possibility of more than one dosage-sensitive locus; in this case, trisomy for a single autosome might not be sufficient to repress more than one *XIST* locus. In any case, further studies of these candidate regions may identify a key dosage-dependent autosomal

locus involved in choosing the active X chromosome. We hope that publication of these results (i) encourages the search for autosome/X interactions within the X inactivation center of mouse embryos during the appropriate window of development, and (ii) elicits clinical and cytogenetic reports of regions of the human chromosome that have not yet been ascertained. If triplication of all of the genome fails to identify a candidate for the putative *trans*factor, then other possibilities need to be considered. Future studies will either support or refute our hypothesis.

Acknowledgements

We gratefully acknowledge Cathy Lee, Ashis Chowdhury and Conor Steuer for their contributions to the FISH analysis, RTPCR study and literature search, respectively, and Daniel Saul and Sarah Dowey for DNA replication studies. This work was supported in part by Grant HD 05465 from the National Institute of Child Health and Development to BRM and from The Wellcome Trust to PAJ.

Web Resources

The URLs for data presented herein are as follows:
NCBI, URL: http://www.ncbi.nlm.nih.gov/Homology, accessed November 15, 2006.

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