

## Muscular dystrophy in girls with X;autosome translocations

YVONNE BOYD, VERONICA BUCKLE, SHEILA HOLT, ELIN MUNRO, DAVID HUNTER, AND IAN CRAIG

*From the Genetics Laboratory, Department of Biochemistry, South Parks Road, Oxford OX1 3QU.*

**SUMMARY** Twenty known cases of X;autosome translocations with breakpoints at Xp21 associated with Duchenne or Becker muscular dystrophy in girls are reviewed. The variable severity described for different persons may reflect differences in X inactivation or in the nature of the genomic target disrupted. High resolution cytogenetic studies on 12 cases indicate breakpoints on the X chromosome at Xp21.1 or Xp21.2. Translocation chromosomes from several of these cases have been isolated in human/mouse somatic cell hybrids. Molecular heterogeneity in the breakpoint positions has been established by probing DNA from these hybrids with a range of cloned sequences known to be located within, or closely linked to, the Duchenne region. The minimum separation between the most distal and the most proximal breakpoints is 176 kb suggesting that, if a single gene is involved, it must be large. Alternatively, the translocations may affect different genes, or confer alterations to regulatory sequences which operate at a distance.

Approximately 1 in 3000 boys are affected by Duchenne muscular dystrophy (DMD) and many of these are sporadic cases with no previous family history.<sup>1,2</sup> The first report of DMD in a female was of a girl who also manifested Turner's syndrome (45,XO), the condition presumably resulting from the presence of a defective locus on the single X chromosome.<sup>3</sup> In 1977, two brief reports described girls with progressive muscular dystrophy and de novo translocations affecting the X chromosome short arm.<sup>4-6</sup> Two years later, more extensive descriptions of two further female patients with translocations involving a similar position (Xp21) on the X chromosome were published.<sup>7,8</sup> It was suggested that the translocations had disrupted the X chromosome at the site of the Duchenne locus and that the consequent damage, coupled with the non-random inactivation of the intact X chromosome, was responsible for the occurrence of the disease. Non-random inactivation patterns are a usual feature in females with balanced X;autosome translocations.<sup>9,10</sup> It is presumed that cells with inactive translocation chromosomes are at a selective disadvantage, as the inactivation of the X portion may spread to the adjacent autosomal regions.<sup>11</sup> There are now 14 published cases of

females with translocations and DMD of varying severity,<sup>4-7,12-21</sup> and a further six known through personal communication. All have translocations with breakpoints in the Xp21 band of the X chromosome short arm, but involve different autosomes (table 1). No affected males have been reported in the families of the girls described in table 1, a finding consistent with the interpretation that the de novo translocation is responsible for the manifestation of the disease. There is little doubt that disruption within a region of the Xp21 band will give rise to Duchenne-like muscular dystrophy in girls. It is reasonable to suppose that this region contains the gene, or genes, responsible for the disorder and that mutations affecting this same region will result in Duchenne muscular dystrophy in boys. High resolution cytogenetic studies on a few boys have failed to detect any abnormalities<sup>22</sup> except for one DMD patient who suffered from multiple disorders and where a minor deletion of the Xp21 band was observed.<sup>23</sup> The assignment of disease loci on the basis of manifestation in translocation carrying patients has been recently extended to other sex linked disorders.<sup>24</sup> In the case of DMD, and the less severe Becker muscular dystrophy, the assignment to Xp21 has been subsequently confirmed by family studies using probes which detect restriction fragment length polymorphisms of the X chromosome short arm.<sup>24</sup>

TABLE 1 Reported cases of muscular dystrophy and translocations affecting Xp21.

Translocation	Reference	Clinical features	X chromosome breakpoint	Autosomal breakpoint	X inactivation pattern (Xn/X)
X;1	8		Xp21.2 (inv Xp11.4-Xp21.2)	1p34.1 or 34.3	75/75 L
X;2	*		Xp21	2q14	
X;2	15	Moderate mental retardation	Xp21.2	2q37.3	
X;3	†		Xp21	3q27	
X;3	7	Mental retardation, dysmorphic	Xp21.2 or Xp21.3	3q13.2 or 3q13.32	55/58 L
X;4	19		Xp21.1	4q26	104/104 L
X;5	21	Moderate mental retardation	Xp21.2	5q31.1	35/35 L
X;5	12		Xp21.1	5q35.3, 566, 576 L	146/146 F
X;6	17		Xp21	6q16	
X;6	13		Xp21.2	6q21	55/56 L
X;8	20	Mild	Xp21.1	8q24.3	80% L
X;9	18	+ Turner's syndrome, epilepsy, mental retardation	Xp21 (extreme proximal)	9p21 (extreme proximal)	24/24 L
X;9	16	Moderate mental retardation	Xp21.2	9p22.3	84/86
X;11	4	(Mother with polycythaemia vera)	Xp21.1	11q13.5	
X;11	16	Mild	Xp21.2	11q23.3	(Xn inactivated) L
X;15	‡	(Parents 1st cousins)	Xp21	15q26	93% L 95% F
X;21	6 7	Mild	Xp21.1	21p12	39/42 L 35/37 F
X;22	*		Xp21	22q13	

References are given to the original reports, unless these have been followed by a more detailed account. In addition several cases reported at meetings in Britain are included: \*Dr M Zatz, Brazil, †Professor P Pearson and Professor M Ferguson-Smith, ‡Ribiero *et al*, manuscript submitted. Any clinical features in addition to classical DMD are given in column 3. The cytogenetic analysis presented in columns 4 and 5 is the most detailed analysis available either in the original reports or in later references.<sup>23,36</sup> At the Vth International Congress on Neuromuscular Diseases (July 1986) two further patients were described, both X;4 translocations (Dr U Francke, USA, Professor R G Worton, Canada). L, F=X inactivation analysis on fibroblasts or lymphocytes respectively.

There has been much debate about the precise genetic relationship between Duchenne and Becker muscular dystrophy in boys and it remains to be established whether or not they are allelic. The separate clinical classification arises from the later stage of onset and milder progression of the disorder in patients suffering from Becker muscular dystrophy. While in many cases the clinical picture is incomplete, several of the translocation carrying girls have symptoms more consistent with a diagnosis of Becker muscular dystrophy than of DMD<sup>25</sup> (table 1). Four reasons can be suggested to explain the appearance of both types of dystrophy in the girls. The first possibility is that mutations affecting a muscular dystrophy gene may exhibit varying penetrance depending upon the contribution of other loci in the genetic background. The second is that Becker and Duchenne dystrophy are genetically distinct, either representing different classes of mutation of the same gene, or mutations of different, closely linked loci. A third explanation is that the severity of the symptoms reflects variability in the pattern of X chromosome inactivation. In several cases, a small percentage of cells have been observed in which the normal X is early replicating and, therefore, presumably active (table 1). The presence of some gene product(s) from the normal X chromosome at the right time in development may give rise to a milder form of dystrophy which mimics the clinical picture presented by Becker muscular dystrophy. The existence of manifesting

carriers lends support to the idea that partial expression of Duchenne muscular dystrophy is dependent upon differential X inactivation.<sup>26</sup> A final explanation for the bimodal severity of the disease in these girls is that the various autosomal sequences may affect, to different extents, the expression of the X chromosome region immediately adjacent to the translocation breakpoints.

Several points of clinical and scientific interest can be investigated through studies on this cluster of translocations in Xp21. The reasonable supposition that the translocation breakpoints identify the Duchenne locus provides a direct strategy for the characterisation and cloning of the gene or genes involved.<sup>27</sup> This can be achieved through the identification of the X chromosome DNA sequences around the translocation breakpoint, as in the case of the X;21 translocation where the autosomal breakpoint was in the middle of the tandem array of ribosomal RNA genes.<sup>5,6</sup> Characterisation of the ribosomal gene complex provided probes which detected an abnormal restriction fragment comprising DNA spanning the translocation breakpoint, referred to as the 'junction fragment'.<sup>28</sup> This has permitted the cloning of X chromosome sequences on both sides of the breakpoint.<sup>29</sup> This strategy will undoubtedly be extended to other translocations in the near future.

The detailed characterisation of the region identified by the translocation breakpoints may also reveal features that contribute to the high mutation

rate for DMD. This may simply be a reflection of a large target region, or, possibly, a predisposition to frequent DNA rearrangements and consequent disruption of genes in the region. The same features might be expected to increase the frequency of translocations in this region in comparison with other regions of the X chromosome. Nevertheless, in spite of a possible bias in ascertainment due to the severity of the disorder, there appears to be no evidence for an increased incidence of translocation events in Xp21.<sup>30 31</sup>

Finally, these translocation breakpoints and others in the same region of the X chromosome which are not associated with muscular dystrophy can be used to identify those sequences likely to be nearest to the locus and therefore presumed to be most valuable for genetic studies on families. Breakpoint clusters are particularly useful for fine mapping of small regions of the genome. Cytogenetic analysis of translocation breakpoints by direct microscopic examination has an approximate resolution of the order of 1000 to 4000 kb, depending upon the chromosomal regions involved. If there are DNA sequences available which map within a translocation cluster, an order for both the sequences and the translocation breakpoints can be obtained by established physical mapping techniques. At the same time, information about the size of the target region is obtained. The results from cytogenetic and molecular studies on several of the X chromosome breakpoints associated with DMD are discussed in detail below.

### Cytogenetic and molecular observations

The assumption that the translocation events directly disrupt the Duchenne locus follows from the observation that the autosomal sites involved are different in each case, with the possible exception of two on the short arm of chromosome 9 (table 1). Nevertheless, there are some noteworthy features about the positions of the autosomal translocation breakpoints; eight are coincident with positions of known fragile sites,<sup>32 33</sup> three are situated in the same cytogenetic bands as proto-oncogenes,<sup>34</sup> and one, the X;21, enters a region coding for a tandem array of ribosomal genes. Two of the autosomal sites (9p21/2;11q23) are involved in the reciprocal translocation associated with acute monocytic and non-lymphocytic leukaemias.<sup>35</sup> The characterisation of the autosomal breakpoints is important as it may provide a means of cloning further junction fragments whose subsequent analysis will be extremely valuable for the delineation of the Duchenne region.

A detailed cytogenetic examination of lymphoblastoid cell lines established from nine patients

has recently provided evidence for an apparent heterogeneity in breakpoint position<sup>36</sup>; fine assignments subsequently reported for other cases support this<sup>19-21 23</sup> (table 1). There are five cases which appear to have X chromosome breakpoints in the sub-band Xp21·1, six cases have an apparent breakpoint in the sub-band Xp21·2, and there are seven cases where a precise localisation for the breakpoint within Xp21 has not been determined. Of these seven, one has a complex rearrangement involving two breakpoints on the X chromosome and the breakpoint of another may be in either Xp21·2 or Xp21·3. The amount of DNA between the Xp21·1 and Xp21·2 breakpoints on a normal X chromosome can be estimated to be between 1000 and 3000 kb. Assuming that there is no translocation dependent alteration to the banding pattern and that the translocations are simple, this implies that either the gene responsible is very large (or that there are several), or that it can be affected by events more than a thousand kilobases away.

These findings have stimulated our interest in the molecular mapping of the translocation breakpoints with a variety of probes which had been assigned to Xp21 and made available by other laboratories. These include the junction fragment of the X;21 translocation and subclones from the pERT87 region which detect cryptic deletions in a minority of boys affected with DMD<sup>29 37-39</sup> (table 2). The two approaches commonly used in physical mapping, in situ hybridisation and analysis of somatic cell hybrids retaining translocation chromosomes, have both been applied to a detailed study of the translocation breakpoints in Xp21.

A combination of replication banding and in situ hybridisation techniques using I<sup>125</sup> labelled probes can be applied to provide a molecular characterisation of translocation breakpoints.<sup>40 41</sup> Analysis of the grain distribution over both reciprocal translocation chromosomes and their normal counterparts is sufficient to determine whether a previously assigned sequence lies above or below a particular breakpoint (fig 1). A summary of the results obtained from in situ hybridisation experiments per-

TABLE 2 Probes mapping to the X chromosome short arm.<sup>24</sup>

HGM8 symbol	Probe	Regional assignment
<i>DXS85</i>	782	Xp22·2→Xp22·3
<i>DXS43</i>	pD2	Xp22·1→Xp22·2
<i>DXS67</i>	B24	Xp21·3
<i>DXS164</i>	pERT87	Xp21
—	pXJ1·1	Xp21·1
<i>DXS84</i>	754	Xp21·1→Xp21·2
<i>OTC</i>	pOTC	Xp21
<i>DXS7</i>	L1-28	Xp11·3

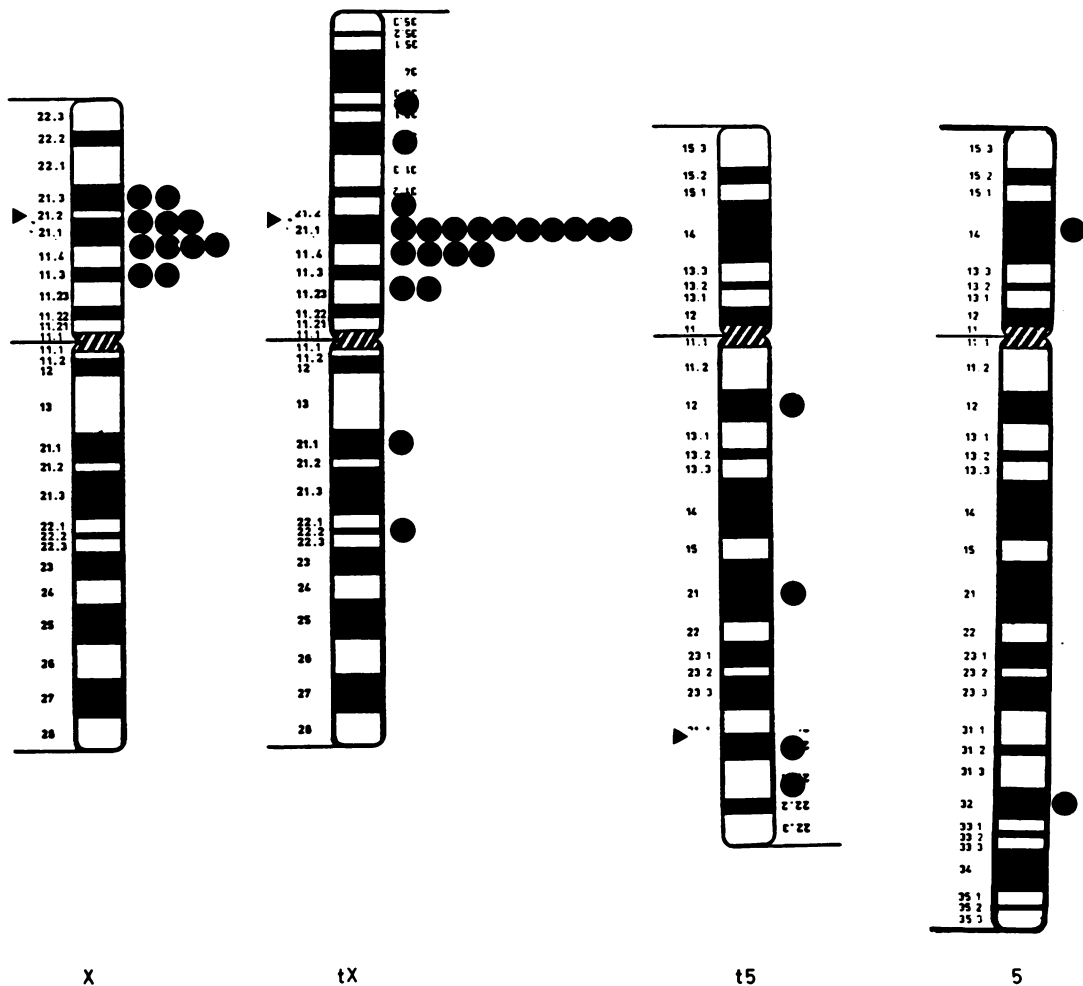


FIG 1 Grain distribution after hybridisation of pOTC to replication banded metaphase chromosomes of a lymphoblastoid cell line established from a female DMD patient with  $t(X;5)(p21.2;q31.1)$ .<sup>21</sup>

formed in our laboratory are summarised in table 3. It can be seen that probes 754 (*DXS84*) and OTC recognise sequences below, and that the probes B24 (*DXS67*) and D2 (*DXS43*) recognise sequences above, the five breakpoints examined. The gene for OTC has also been shown to lie below the X;9 translocation breakpoint by in situ hybridisation.<sup>42</sup>

A second approach for the characterisation of chromosomal breakpoints is to separate individual translocation chromosomes from each other and the intact inactive X chromosome in somatic cell hybrids.<sup>43</sup> Various strategies which take advantage of selectable systems for the X chromosome have been used to construct such a panel in our

laboratory.<sup>44</sup> Information on the pattern of human X chromosome retention by hybrid cell clones has been obtained by enzyme and karyotype analysis. In addition, total DNA prepared from the hybrids has been examined with a range of probes already known to map either above or below the Xp21 region. Once characterised in this way, hybrids can be analysed for the presence or absence of sequences thought to be located within the Xp21 band. The results obtained from mapping studies on a range of hybrids clearly show that the X chromosome breakpoints are not immediately adjacent to one another; all of the pERT subclones and the junction fragment mapped within the most proximal

TABLE 3 Summary of *in situ* hybridisation results.

Probe	Translocation					
	X;1 <sup>8</sup>	X;3 <sup>7</sup>	X;5 <sup>21</sup>	X;5 <sup>12</sup>	X;6 <sup>13</sup>	X;21 <sup>6</sup>
D2	tA	tA	tA	tA	tA	tA
B24	-	tA	tA	-	-	-
754	tX	-	tX	-	-	tX
OTC	-	tX	tX	tX	tX	tX

tA, probe recognised sequences on translocated autosome (that is, maps above breakpoint); tX, probe recognised sequences on the translocated X. See fig 1 for example of a typical analysis.

and the most distal breakpoints (fig 2). Three breakpoints were proximal to pXJ1.1 and the pERT87 region and two were distal to both the junction fragment and to the pERT87 region (fig 3, manuscript submitted). The X;21 breakpoint marks the distal edge of the junction fragment, but is proximal to the pERT87 region.<sup>39</sup> Preliminary analysis of a further two translocations places these distal to both pXJ1.1 and to the pERT87 region. The results from the molecular work completed so far indicate that the most proximal and most distal breakpoints examined must be separated by at least 176 kb. With the exception of the one complex translocation,<sup>8</sup> the relative positions of the translocation breakpoints are consistent with those suggested by cytogenetic analysis which indicates a target area of well over a thousand kilobases. These findings can be put into context by the observation that the largest characterised gene on the X chromosome, HEMA, which codes for factor VIIIc, extends over 186 kb.<sup>45</sup> The mutation rate for haemophilia A is, however, about 10 times lower than that for Duchenne muscular dystrophy.<sup>46</sup> It is tempting to speculate that this difference in mutation rate results from the existence of a proportionately larger target region for the lesions which give rise to muscular dystrophy.

### Towards a map of Xp21

As shown in fig 3, the positions of six translocation breakpoints associated with Duchenne muscular dystrophy in girls have been defined in relation to the four DNA segments *DXS84*, pXJ1.1, *DXS164*, and *DXS67*. This map will be refined and extended in the near future through studies of further translocation breakpoints and additional sequences mapping to the region. The inclusion of translocation breakpoints in the region which are not associated with DMD will assist in the identification of those regions in which disruption gives rise to muscular dystrophy.

The observations reported above and the variety of cryptic deletions reported in affected boys<sup>39</sup> indicate that two models may be proposed for the nature of the Duchenne locus and the mechanisms by which the translocation (or deletion) events cause a disruption. The first possibility is that the locus (that is, coding sequences and necessary adjacent controlling elements) itself is enormous, of the order of at least 1000 kb, and that disruptions or deletions in any part of that region can give rise to Duchenne or Becker muscular dystrophy. This model would also cover the existence of several neighbouring loci all coding for products necessary to prevent the appearance of muscular dystrophy. Characterisation of translocation breakpoints in Xp21 not associated with muscular dystrophy will help to clarify this point. If they lie outside the breakpoint cluster region of the Duchenne translocations the idea of a large single transcript would be supported; if within, then strong evidence for the existence of multiple genes would be obtained. An interesting feature of three of the five such translocations known to us (table 4) is that the positions of the autosomal breakpoints are similar to those of translocations associated with muscular dystrophy (table 1). Preliminary molecular charac-

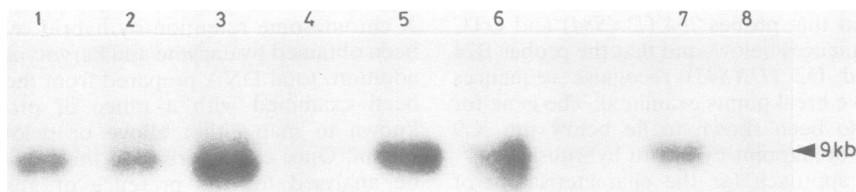


FIG 2 Hybridisation of pERT87-8 to *Hind*III digests of human and hybrid DNA. Tracks 1 to 3, human controls; track 4, mouse control; track 5, hybrid retaining 21;X translocation chromosome<sup>8</sup>; track 6, hybrid retaining 5;X translocation chromosome<sup>12</sup>; track 7, hybrid retaining X;6 translocation chromosome<sup>13</sup>; track 8, hybrid retaining X;1 translocation chromosome.<sup>8</sup>

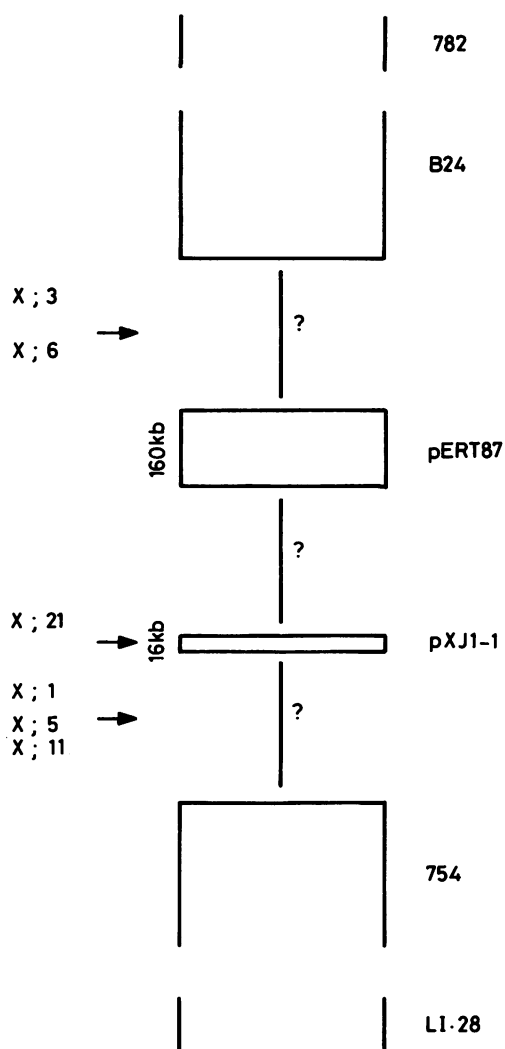


FIG 3 Towards a map of Xp21. For explanation see text.

TABLE 4 X;autosome translocations reported in the Xp21 region not associated with muscular dystrophy.

Translocation	Reference	X inactivation pattern
(X;1)(p21;p34)	47	13/15
(X;6)(p21;p24)	48	21/30
(X;6)(p21;q26)	8	59/70
(X;8)(p11-4;q24-1)	*	-
(X;22)(p21-2;q13-3)	49	-

\*This patient was brought to our attention by Dr R H Lindenbaum, Medical Genetics Unit, Oxford; the probe 754 (*DXS84*) lies proximal to this breakpoint<sup>24</sup> and therefore it is included in this category.

alteration to these sites coincident with the translocation events.<sup>51</sup> New DNAase I hypersensitive sites were observed, in one case, in the immunoglobulin gene region into which the *c-myc* gene had been introduced by translocation. This emphasises the possible role of autosomal sequences in the modification of adjacent X chromosomal regions in the translocations associated with DMD.

Identification and characterisation of the coding sequences and their transcripts in the Xp21 region will be necessary to differentiate between the two models. However, the immediate questions concerning the size and nature of the target region implicated in the aetiology of Duchenne and Becker muscular dystrophies can be resolved by further studies on translocation breakpoints in girls.

We thank Sally Craig for analysis of the OTC/X;5 hybridisation illustrated in fig 1; John Pearson for the detailed cytogenetic analysis of the X;22 translocation (table 4); Kay Davies, Lou Kunkel, Peter Pearson, Ron Worton, and their colleagues for providing probes; the many physicians who provided blood samples and information; and Professor John Edwards for encouragement and advice. This work was supported by the Medical Research Council and a grant from the Muscular Dystrophy Group of Great Britain to IWC.

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terisation in our laboratory of two of these indicates that both lie distal to *DXS84* and proximal to *DXS67*.

The second model that may be proposed is that the translocation (or deletion) events indirectly affect the expression of genes lying in the Xp21 band. Limited uncoiling of the chromatin adjacent to translocation breakpoints has already been suggested as an explanation for band modifications observed in de novo balanced translocations.<sup>50</sup> However, studies on the distribution of DNAase I hypersensitive sites around the *c-myc* in Burkitt lymphoma lines have indicated that there is no

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Correspondence and requests for reprints to Dr I W Craig, Genetics Laboratory, Biochemistry Department, University of Oxford, South Parks Road, Oxford OX1 3QU.