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Molecular Analysis of Male-viable Deletions and Duplications Allows Ordering of 52 DNA Probes on Proximal Xq

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Summary

While performing a systematic search for chromosomal microdeletions in patients with clinically complex X-linked syndromes, we have observed that large male-viable deletions and duplications are clustered in heterochromatic regions of the X chromosome. Apart from the Xp21 band, where numerous deletions have been found that encompass the Duchenne muscular dystrophy gene, an increasing number of deletions and duplications have been observed that span (part of) the Xq21 segment. To refine the molecular and genetic map of this region, we have employed 52 cloned single-copy DNA sequences from the Xcen-q22 segment to characterize two partly overlapping tandem duplications and two interstitial deletions on the proximal long arm of the human X chromosome. Together with a panel of somatic cell hybrids that had been described earlier, these four rearrangements enabled us to order the 52 probes into nine different groups and to narrow the regional assignment of several genes, including those for tapetochoroidal dystrophy and anhidrotic ectodermal dysplasia.

Introduction

The practical value of X-linked DNA markers for diagnosis and molecular elucidation of X-linked gene defects is limited by two fundamental problems. First, accurate estimation of small genetic distances between a given pair of syntenic genes requires that their segregation pattern be studied in numerous large families. In the absence of recombination, several hundred doubly informative individuals have to be examined to establish that the true genetic distance does not exceed 1 centimorgan (cM). Given the rarity of most Mendelian disorders, this implies that reliable estimation of the risk of recombination between a disease locus and a closely linked diagnostic marker is not possible. Second, there is no universally applicable formula to convert genetic distances into physical distances, and, in general, even very small genetic distances are still too large to be bridged by conventional molecular cloning. For a variety of X-linked defects, a solution to these problems has come from the analysis of clinically complex, X-linked syndromes that could be explained as small X-chromosomal deletions encompassing several genes and random DNA probes (Francke 1984; Lange et al. 1985). Minute deletions have been employed very successfully to identify useful diagnostic markers in the vicinity of the Duchenne muscular dystrophy (DMD) gene and elsewhere on the X chromosome, and deletions have played a crucial role for the isolation, by reverse genetics strategies, of the genes for DMD (Monaco et al. 1985) and chronic granulomatous disease (Royer-Pokora et al. 1986).

In the course of family studies, our group encountered various microdeletions in patients with complex X-linked disorders (Gal et al. 1985, 1986; Wieringa et al. 1985*a*, 1985*b*). These findings encouraged us to perform a systematic search for minute deletions in clinically complex X-linked syndromes by employing X chromosome–specific DNA sequences as probes. Since

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several of the deletions that had been described were detectable by cytogenetic examination (Francke 1984; Francke et al. 1985; Wieringa et al. 1985*a*, 1985*b*), we speculated that, on average, deletions spanning several genes might encompass at least 1 million bp. Assuming that the human X chromosome comprises 200 million bp, we reasoned that 200 random probes should suffice to detect more than 60% of all deletions spanning several X-chromosomal genes, and this convinced us that such a search would be economically and logistically feasible. Therefore, in collaboration with several European and American colleagues, we collected DNA, blood, or cell lines from patients with X-linked syndromes that could be interpreted either as combinations of several X-linked diseases or as known X-linked disorders with additional clinical features. In parallel, numerous X chromosome–specific DNA probes were isolated and obtained from various laboratories, characterized, and, if necessary, regionally assigned by employing a panel of somatic cell hybrids (Wieacker et al. 1984).

While this work was in progress, several other male-viable deletions were found (Tabor et al. 1983; Old et al. 1985; Bartley et al. 1986; Hodgson et al. 1987; Nussbaum et al. 1987), and it became apparent that large deletions are not evenly distributed on the human X. Almost invariably, deletions that can be detected by cytogenetic analysis map within the two most prominent, dark-staining Giemsa bands of the human X chromosome, Xp21 or Xq21. Because dark Giemsa bands are thought to be relatively devoid of functional genes (Kurnit and Hoehn 1979), we speculated that in these regions male-viable deletions might be larger than average. It followed that the inverse might be true for the remainder of the X, suggesting that detection of deletions outside these regions would require many more probes than originally anticipated. To some extent, this speculation was corroborated by the limited success of our deletion screening, which revealed only a single additional deletion (P. Braakhekke, personal communication). Since significant further enlargement of the probe panel did not seem practicable, we felt that instead we should concentrate on specific segments of the X and on complex syndromes comprising clinical features of regionally assigned gene defects. In addition, we decided that, apart from deletion screening, we would employ the available probes for the molecular analysis of previously described structural rearrangements of the X chromosome. The present paper describes the molecular analysis of male-viable deletions and duplications spanning several gene loci on the proximal long arm of the X chromosome, as well as the fine-mapping of 52 cloned DNA sequences in the Xcen-q22 segment.

Material and Methods

Clinical and Cytogenetic Data

Patient C.N. (Cremers et al. 1987*b*) suffered from muscular hypotony, growth retardation, psychomotor retardation, cryptorchidism, and Pelizaeus-Merzbacher disease (PMD). Cytogenetic and molecular analyses revealed a duplication of the Xq21-Xq22 segment, resulting from unequal crossing-over between the two maternal X chromosomes.

Patient K.M. (Vejerslev et al. 1985) showed psychomotor retardation, cryptorchidism, and various minor congenital malformations. Cytogenetic analysis suggested an inherited tandem duplication of the Xq13.1-q21.2 segment, but an insertion of autosomal material into the proximal long arm of the X chromosome could not be excluded.

Patient N.P. (Tabor et al. 1983) had cleft lip and palate (CLP), agenesis of the corpus callosum, and mental retardation. At the age of 5, retinal changes indicative for choroideremia were observed (Rosenberg et al. 1986; Schwartz et al. 1986). Cytogenetic and molecular examination showed an Xq21.1-q21.33 deletion which spanned the DXYS1 locus and was also present in N.P.'s mother (Rosenberg et al. 1986).

Patient R.v.D. is a mentally retarded boy with congenital heart defect (VSD), facial and skull deformities, frontal lobe atrophy, and various minor anomalies. His hearing is severely impaired (up to 75 dB), but even at the age of 10 ophthalmologic examination yielded no clinical signs of choroideremia. Chromosome analysis revealed an interstitial deletion encompassing a large portion of the Xq21 band and possibly part of band Xq22 (fig. 1). The same deletion was found in his mother who, like R.v.D., has a normal ocular fundus (J. Beverstock, personal communication).

Southern Blot Analysis

Chromosomal DNA from peripheral blood, Epstein-Barr virus–immortalized B cells, or fibroblast cell lines was isolated according to the method of Aldridge et al. (1984), with minor modifications. Restriction endonuclease–cleaved chromosomal DNA was resolved electrophoretically on a 0.7% (w/v) agarose gel. Following depurination in 0.15 N HCl for 10 min, DNA was denatured in 0.4 N NaOH and blotted onto a ny-

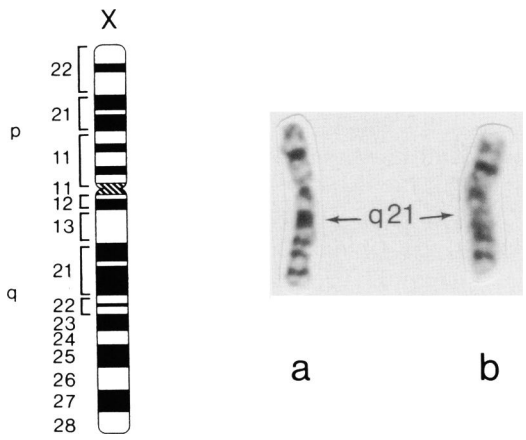

Figure 1 Cytogenetic analysis of the X chromosome of a normal male (*a*) and R.v.D. (*b*). GTG-banding reveals an interstitial deletion within Xq21, possibly extending into Xq22.

lon membrane (BioTrace,® Gelman Sciences, Inc.), using the same solution. Probe insert DNAs were isolated from low-gel-temperature agarose gels and labeled by primed synthesis with Klenow DNA polymerase I and α-^{32}P-dCTP, as described by Feinberg and Vogelstein (1983, 1984). Prehybridization of the DNA blots was done at 65 C for 6–18 h in 6 × SSC, 0.1% (w/v) BSA, 0.1% (w/v) Ficoll, 0.1% (w/v) polyvinylpyrrolidone, 10% (w/v) dextran sulfate, 0.2% (w/v) SDS, and 250 μg sonicated, single-stranded herring sperm DNA/ml. Hybridization was performed for 16–20 h at 65 C in the same solution at a probe concentration of 1–3 ng/ml (2–5 × 10^8 cpm ^{32}P/μg). Washing was done at 65 C with stringencies increasing stepwise from 2 × SSC/0.5% (w/v) SDS to 0.1 × SSC/0.5% (w/v) SDS. Filters were rinsed in 2 × SSC and exposed to Kodak X-Omat film for 8 h–3 days at −80 C, using two intensifying screens.

Isolation of Single-Copy Sequences from Repetitive Probes

For some probes containing low or moderately repetitive X-chromosomal inserts, various insert segments were tested to obtain unambiguously interpretable hybridization patterns for signal density scanning. To this end, 2 μg of plasmid DNA was digested with endonuclease *Sau*3A and fragments were separated on a 1.5% (w/v) low-gel-temperature agarose gel. Distinct nonvector fragments were isolated, labeled with α-^{32}P-dCTP as described above, and hybridized to *Eco*RI-digested human control DNA. Unique sequences of probes pXG8b (0.6 kb), pXG12 (1.3 kb), p722 (1.2 kb), p776 (0.4 kb), and pTAK2 (1.4 kb) subsequently were used in the analysis of the rearrangements throughout this study. Occasionally, as for probes pX65H7, pX104f, pF1, and pF8, which failed to yield single-copy *Sau*3A subfragments, labeled insert DNAs were prehybridized to sheared human DNA (Cot = 3,600 mg × min/ml), essentially as described by Litt and White (1985), to reduce background hybridization.

Dosage Determination

Signal intensities were quantitated using an LKB 2202 Ultroscan Laser Densitometer (scan speed 20 mm/min, focus 50 μm). Hybridization signals obtained with probe p8 (DXS1), probe p708 (DXS82), or Y-specific signals (i.e., the 15-kbp *Taq*I band detected with pDP34 [DXYS1]) were used as internal references, and signal ratios were normalized through comparison with ratios of male control DNA. Values ranging from 0.7 to 1.3 and from 1.7 to 2.3 were considered to represent one or two copies, respectively, of the relevant DNA sequence.

Results

All 52 X-specific probes employed in this study, as well as their map positions, are listed in figure 2. For several of these, regional assignments have been reported elsewhere (Chance et al. 1983; Goodfellow et al. 1985; Willard and Riordan 1985; Riddell et al. 1986; Cremers et al. 1987*b*, 1987*c*). Others were regionally mapped by making use of a cytogenetically reanalyzed subset of the hybrid cell lines originally described by Wieacker et al. (1984). Recent reexamination of the breakpoint in hybrid 749, formerly given as Xq12 (Gerald and Brown 1974), has indicated that it may be at the interface of bands q12 and q13 or even at q13.1 (J. Zonana, personal communication). Therefore, the positioning

Figure 2. Signal dosage determination of hybridization intensities for probes located in the proximal Xq region. Horizontal lines indicate the cytogenetic assignment of the various breakpoints employed in this study. Lines with arrowheads at the right indicate the breakpoints in the hybrid cell lines, whereas lines without arrowheads indicate duplication and deletion breakpoints. The order of probes within each segment is arbitrary. The regional assignments of probes given in the third column refer to (a) the present study, (b) Cremers et al. (1987*b*), (c) Goodfellow et al. (1985), (d) Chance et al. (1983), (e) Willard et al. (1985), (f) Riddell et al. (1986), and (g) Willard and Riordan (1985). Probes cpX8, cpX12, cpX58, cpX205, cpX210, and cpX251 were isolated by one of us (M.H.H.); pXG8b was a gift from P. Szabo, New York; pF1 and pF8 were provided by T. A. Kruse, Aarhus. Quantitation of signal intensities was as described in Material and Methods.

HGM8 nomenclature	Probe designation	Regional assignment		Signal dosage duplications C.N.	K.M.	deletions N.P.	R.v.D.
DXS62	MGU22	p11-q12	(c)	+	+	+	+
	cpX8	p11.1-q12	(a)	+	+	+	+
	cpX210	p11.1-q12	(a)	+	+	+	+
DXS136	pX12g	cen-q12	(c)	+	+	+	+
DXS1	p8	q11-q12	(a,c)	+	+	+	+
DXS153	cX37.1	p11.1-q12	(a,c)	+	++	+	+
	cpX12	p11.1-q12	(a)	+	++	+	+
DXS132	cpX23	p11.1-q12	(a,c)	+	++	+	+
DXS133	cpX30	p11.1-q12	(a,c)	+	++	+	+
	cpX58	p11.1-q12	(a)	+	++	+	+
DXS106	cpX203	p11.1-q12	(b,c)	+	++	+	+
	cpX205	p11.1-q12	(a)	+	++	+	+
PGK1P1	pHPGK-7e	q11-q12	(d,e)	+	++	+	+
	cpX251	p11.1-q12	(a)	+	++	+	+
DXS162	cpX289	p11.1-q12	(a)	+	++	+	+
DXS171	pX63c	q12-q13	(a,c)	+	++	+	+
DXS131	cpX21	q12-q21.3	(b,c)	+	++	+	+
PGK1	pHPGK-7e	q12-q13	(d,e)	+	++	+	+
DXS159	cpX73	q12-q21.3	(b,c)	+	++	+	+
DXS135	cpX93	q12-q21.3	(a,c)	+	++	+	+
DXS56	pL2.98	q12-q21.3	(c)	+	++	+	+
DXS128	cX65.3	q12-q21.3	(b,c)	+	++	+	+
DXS227	p1bB4	q12-q22	(a)	+	++	+	+
DXS169	pX104f	q13-q21.1	(f)	++	++	-	-
DXS72	pX65H7	q13-q22	(c)	++	++	-	-
DXYS1	pDP34	q13-q21	(c)	++	+	-	-
DXYS5	p47b	q12-q22	(c)	++	+	-	-
DXYS12	pSt25.2	q13-q22	(c)	++	+	-	-
DXYS13	pTAK2	q12-q22	(b)	++	+	-	-
DXS165	p1bD5	q12-q21.3	(c)	++	+	-	-
DXS95	pXG7c	q21	(a,c)	++	+	-	-
	pXG8b	q12-q22	(a)	++	+	-	-
DXS110	p722	p11.1-q21.3	(c)	++	+	-	-
DXS121	p784	q12-q21.3	(b,c)	++	+	-	-
DXS214	pPA20	q12-q22	(a)	++	+	-	-
DXS73	pX20R42	q13-q22	(c)	++	+	-	-
DXS3	p19.2	q21.3-q22	(c)	++	+	-	-
DXS112	p753	q21.3-q22	(b,c)	++	+	-	-
DXS96	pXG3b	q12-q22	(b)	++	+	+	-
DXS118	p776	q21.3-q22	(b,c)	++	+	+	-
	pF1	q12-q22	(a)	++	+	+	-
	pF8	q12-q22	(a)	++	+	+	-
DXS94	pXG12	q12-q22	(b)	++	+	+	+
DXS17	pS9	q21.3-q22	(c)	++	+	+	+
DXS147	cX4.4	q21.3-q22	(c)	++	+	+	+
PLP	pLP1	q13-q22	(g)	++	+	+	+
DXS101	cX52.5	q21.3-q22	(c)	++	+	+	+
DXS211	pD3	q13-q22	(b)	++	+	+	+
DXS24	pX-5	q13-q22	(c)	++	+	+	+
DXS173	pX76a	q22	(b,f)	++	+	+	+
DXS111	p733	q22-q24	(c)	++	+	+	+
DXS82	p708	q22-q24	(c)	+	+	+	+
DXS11	p22.33	q24-q26	(c)	+	+	+	+

of this breakpoint in figure 2 may be somewhat arbitrary, and the same applies to the hybrid 676 × 175K37 breakpoint, whose exact location within band Xq22 is not known.

Molecular analysis of the Xq-proximal rearrangements enabled us to define a total of six new intervals spanning this chromosome area. Results from hybridization with representative probes from within each of these intervals are shown in figures 3*a* and 3*b*. Figure 2 summarizes the quantitation data obtained from densitometric scanning analysis. Out of the 52 sequences tested, 19 were duplicated in K.M.'s DNA. Therefore, in accordance with the cytogenetic findings of Vejerslev et al. (1985), these probes were tentatively assigned to the Xq13.1-q21.2 segment. In C.N., 28 probe sequences could be assigned to the duplicated Xq21.1-q22.3 segment. Two of these, pX65H7 (DXS72) and pX104f (DXS169), were also duplicated in K.M., which indicated that there is a small but detectable overlap between these duplications. As is shown in figure 3*b*, an extremely complex array of bands of single and double intensities was obtained with probe pHPGK-7e (PGK1). Of these, the 1.0- and 5.0-kbp signals, which originate from chromosome 19 and chromosome 6, respectively (Gartler et al. 1985), were used as reference signals for quantitation of X-signal dosage. The X-specific signal at 7.6 kbp is partly obscured by a signal of identical size originating from chromosome 6 (Michelson et al. 1985). Furthermore, the X-specific signals at 0.6, 2.7, and 5.5 kbp represent two loci at proximal Xq, namely, the PGK1 gene at q12-q13 and a PGK pseudogene (PGK1P1), tentatively assigned to q11–q12 (Chance et al. 1983; Willard et al. 1985). As can be inferred from the autoradiograph, clearly all X-specific PGK signals were found to be of double intensity in K.M.'s DNA but not in C.N.'s DNA. We have confirmed this result by a similar analysis using restriction enzyme *Eco*RI to distinguish between the authentic and the pseudo-PGK signals from Xq (not shown).

In a previous study (Cremers et al. 1987*b*), we had concluded that the two X/Y homologous sequences, DXYS1 and DXYS5, one of which had been assigned previously to the interface of bands Xq13 and q21 (Page et al. 1984), map proximal to the duplicated DNA segment of patient C.N. Still, neither of the two was found to be located on the duplicated segment of patient K.M. Critical reexamination of our previous results indicated that, contrary to that previous report, all four X-Y homologous loci tested (i.e., DXYS1, DXYS5, DXYS12, and DXYS13) map inside the C.N. duplication and thus more distally than had been indicated previously.

This is corroborated by the failure of all four probes to yield X chromosome–specific hybridization signals in the DNA of the two deletion patients, N.P. and R.v.D. (figs. 2, 3*a*). In N.P., cytogenetic analysis had revealed a large deletion spanning the q21.1-q21.33 segment (Tabor et al. 1983; Rosenberg et al. 1986), and, in R.v.D., there was cytogenetic evidence for a deletion encompassing part of Xq21 and q22 (see Material and Methods; J. Beverstock, personal communication). Our hybridization experiments suggest that the R.v.D. deletion is slightly larger than the N.P. deletion. The former spans 19 and the latter 15 DNA loci, all of which are located on the segment that is duplicated in patient C.N. Both deletions overlap the K.M. duplication, as evidenced by the fact that they encompass two markers, pX65H7 (DXS72) and pX104f (DXS169), which are duplicated in the DNA of patient K.M. In contrast, none of the four markers that are deleted in R.v.D. but not in N.P.—i.e., pXG3b (DXS96), p776 (DXS118), pF1, and pF8—are duplicated in this patient. This indicates that all of these four markers are situated at the distal end of the R.v.D. deletion. As yet we have not identified probes that distinguish between the proximal endpoints of the two deletions and the C.N. duplication in the vicinity of the Xq13.3-q21.1 boundary.

Previous studies had indicated that, probably as a result of unequal crossing-over between the two maternal X chromosomes, C.N. is heterozygous for several polymorphic DNA markers from the proximal Xq (Cremers et al. 1987*b*). In K.M., the search for heterozygosity was unsuccessful with four probes—cpX203 (DXS106), cpx289 (DXS162), cpX93 (DX135), and pHPGK-7e (PGK1)—all located on the duplicated segment and known to detect two-allelic RFLPs. Given the frequencies of the rare alleles—frequencies that are .35, .33, .06, and .40, respectively—the probability of two different X chromosomes carrying identical alleles at all four loci tested is about 14%. Therefore, our findings suggest that the K.M. duplication did not arise by unequal crossing-over but by a different mechanism.

Discussion

Owing to their dual function as genetic and physical signposts, DNA markers play an essential role in the construction and alignment of genetic and physical chromosome maps. While the goal of covering all human chromosomes with a network of closely linked DNA markers has almost been reached (Donis-Keller et al. 1987; Nakamura et al. 1987), the accuracy of the physical map is still hampered by the limits of

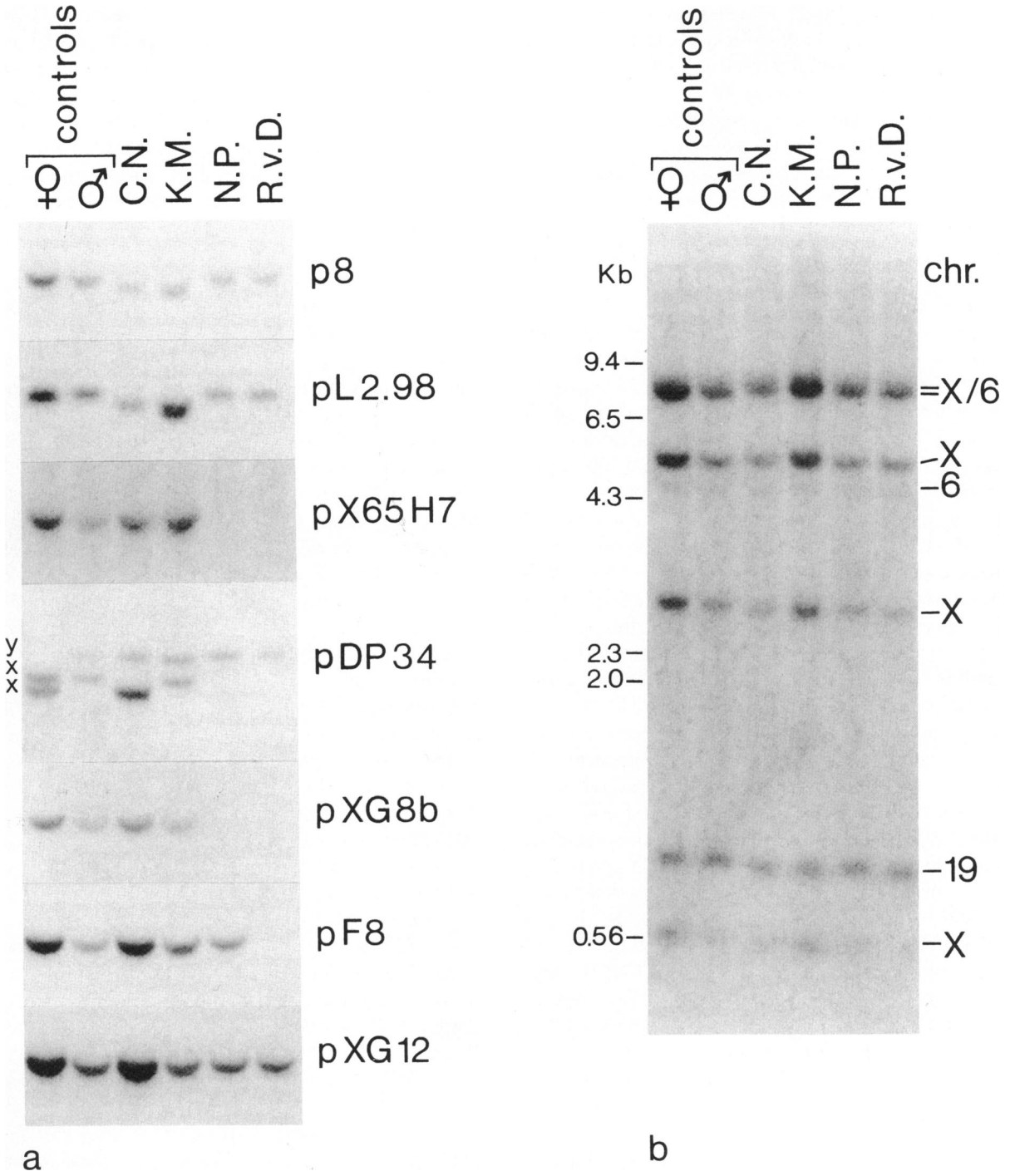

Figure 3 Southern blot analysis of genomic DNAs from patients with rearrangements at proximal Xq, using probes from within several intervals in the Xp11-Xq24 area. *a*, For all probes hybridization analysis was on *Eco*RI-digested (except for pDP34, which was digested with *Taq*I) genomic DNA of two controls (male and female) and four patients. Relevant sections from the various autoradiographs are arranged—according to the position of the probe—from proximal to distal (compare with fig. 2). *b*, Southern analysis using probe pHPGK-7e (PGK1). DNAs of patients and controls were digested with restriction enzyme *Hin*dIII. The chromosomal localisation of various X- and autosome-specific signals is indicated to the right. Size markers are given to the left.

cytogenetic resolution. Here we report on our attempt to correlate the size of two deletions and two duplications on the proximal long arm of the X chromosome with the presence or absence of specific single-copy DNA sequences. This has enabled us to define the endpoints of the duplicated or deleted segments and to refine the assignment of numerous DNA probes that had been mapped previously by employing a panel of somatic cell hybrids (Wieacker et al. 1984).

Probes in the Xcen–Xq22 region could be ordered into nine segments defined by eight discernible chromosome breakpoints (see fig. 2). This is two less than expected because the proximal borders of the deletions and of one duplication appear to coincide: all three are located between the DXS227 and DXS169 loci. Apart from coincidence, which seems highly implausible, or enhanced proneness of this region to chromosome breakage, for which there is no evidence, it is possible that DNA sequences from the chromosome segment that harbors these breakpoints may be underrepresented in our panel of probes. Alternatively, it is conceivable that there are functional constraints that prohibit a small region near the border of band Xq13 and Xq21 from being deleted or duplicated (although, for duplications, these constraints cannot be absolute because there is a small but detectable overlap between the two duplications: both encompass DXS72 and DXS169).

Though there is no evidence for obvious clustering or scarcity of probes within the Xcen-q22 segment, this possibility cannot be ruled out, because several of the breakpoints given may not be precisely defined owing to the limited resolution of conventional cytogenetic analysis. Indeed, the recent observation (J. Zonana, personal communication) of a more distal localization of the breakpoint in hybrid 749 is supported by the fact that nine probes that map proximal to this breakpoint are still comprised in the duplicated Xq13.1-Xq21.2 segment of patient K.M. (Vejerslev et al. 1985; see fig. 2). Whether these nine probes are derived from nine different loci is, however, questionable, as all probes except cX37.1 were subcloned from pooled cosmids (Hofker et al. 1987). Nonetheless, based on the molecular analysis, our data suggest a more proximal location of the duplicated DNA segment in patient K.M. (Xq12.2-q21.1; see fig. 2). It is interesting that the 749 breakpoint may be located within the locus for AED since this hybrid had been originally established from fibroblasts of a female with an X-autosome translocation, t(X;9) (q12;p24) and AED (Grzeschik and Siniscalco 1976; K. H. Grzeschik, personal communication). This would imply that the anhidrotic ectodermal dysplasia locus is flanked by the phosphoglycerate kinase (PGK1) gene and the PGK pseudogene (PGK1P1), which map distal and proximal to this breakpoint, respectively (Chance et al. 1983; see fig. 2).

Recently, Arveiler et al. (1987*b*) have presented a refined genetic map of the Xq11–q22 region which includes several of the probes dealt with in the present study. Our results are in keeping with their data, except for two loci. According to Arveiler et al., the DXS72 locus maps distal to the sequences within band Xq21 that show homology to the Y chromosome. In contrast, our study assigns this marker proximal to these X–Y homologous sequences, and this assignment is corroborated by linkage studies which point to a location proximal to the DXYS1 marker (B. van Oost, personal communication). Second, there is an inconsistency pertaining to the regional assignment of the DXS159 locus (probe cpX73). Using the same somatic cell hybrid line (749 or ANLY1), Arveiler et al. place this marker proximal to the breakpoint while we have mapped it to the distal side. This discrepancy can only be resolved by assuming that Arveiler et al. have employed a different probe. In fact, this probe sequence detects a *Pst*I polymorphism (Arveiler et al. 1987*a*), whereas DXS159 is not polymorphic for this enzyme (M.H.H., unpublished data).

Because of the strikingly different phenotypes associated with the two deletions studied, N.P. and R.v.D., it is tempting to speculate that they encompass several different genes. In part, this is corroborated by the cytogenetic finding that the R.v.D. deletion extends into the q22 band while the N.P. deletion is confined to q21 (Tabor et al. 1983; Rosenberg et al. 1986). Our hybridization studies confirm these findings by defining four sequences—pXG3b (DXS96), p776 (DXS118), pF1, and pF8—that are only absent in the larger of the two deletions. These findings may account for the presence of deafness in patient R.v.D. and its apparent absence in patient N.P. Deafness due to stapes fixation is a well-known X-linked disorder (McKusick 30440; Cremers et al. 1985), and the gene responsible for this disorder has recently been assigned to the proximal long arm of the X chromosome by demonstration of linkage to the DXYS1 marker (H. G. Brunner, personal communication). Deafness with stapes fixation has also been described in another patient with a deletion encompassing part of the Xq21 band (Ayazi 1981; Nussbaum et al. 1987).

Presence of CLP, agenesis of the corpus callosum, and choroideremia in patient N.P.—and the absence of these features in R.v.D., the patient with the larger

deletion—points to the possibility that the N.P. deletion may extend farther proximal than the R.v.D. deletion, a possibility for which cytogenetic analysis provides some support. This would further substantiate our speculation that there is a gap in our probe panel and may assign the genes responsible for these differences to the segment between these two breakpoints. In particular, this may apply to a gene involved in the etiology of CLP, since linkage studies have recently mapped a cleft palate gene to the same chromosome region (Moore et al. 1987). On the other hand, deletions of the X-linked CLP gene may not always give rise to CLP. Indeed, CLP has not been found in any of the other large Xq21 deletions that have been described (Hodgson et al. 1987; Nussbaum et al. 1987). In contrast, tapetochoroidal dystrophy (TCD) has been observed repeatedly and is an almost constant feature of these deletions. Comparison of the relevant chromosomal breakpoints has provided clear evidence that TCD cannot be located near the interface of Xq13 and Xq21 but should map farther distal, probably within the Xq21.1-q21.2 segment (F.P.M.C., unpublished data). Therefore, it is likely that the TCD gene is deleted in R.v.D., too, and that the absence of clinical signs in this patient may be a consequence of his young age.

These examples illustrate the mutual usefulness of (1) DNA probes for the genetic and molecular characterization of chromosomal rearrangements and (2) duplications and deletions for the fine-mapping of probes. The precise regional assignment and ordering of 52 cloned DNA sequences from the proximal long arm of the X chromosome into nine different groups should provide the framework for the construction of a contiguous molecular map of this chromosome segment by field-inversion gel electrophoresis (FIGE; Carle et al. 1986) or related techniques. Moreover, the alignment achieved between the physical and the genetic map should facilitate the precise regional assignment of other genes and gene defects in this region. Known physical distances and gene orders will permit a more directed search for highly informative diagnostic markers, which appear to be less abundant on the X than on other human chromosomes (Hofker et al. 1986); and this information should be a great asset for the isolation of genes by chromosome walking and hopping strategies. For TCD, we have already been able to confirm this prediction by defining a DNA sequence in the immediate vicinity of the choroideremia gene, as evidenced by the fact that this sequence is deleted in two of eight TCD patients tested (Cremers et al. 1987*a*). Future research in our laboratory will concentrate (1) on the physical mapping of the TCD gene region by employing FIGE and related techniques and, eventually, (2) on the isolation of the TCD gene itself.

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