Inherited deletion of subband Xp21.13 in a male with Duchenne muscular dystrophy

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SUMMARY The chromosomes of a male patient who suffers from Duchenne muscular dystrophy (DMD) with a molecular deletion were examined with an improved high resolution R type replication banding technique. High resolution cytogenetic analysis of the proband revealed a deletion of the Xp21.13 subband. His healthy mother was heterozygous for the deletion, which is subject to random X inactivation in lymphocytes. The X chromosomes of the proband's grandmother were normal, suggesting that the deletion of the Xp21.13 subband in the mother was a new mutation. The finding of a very small, cytologically visible Xp21.1 deletion in a male DMD patient with a molecular deletion emphasises the importance of resolving the fine structure in the Xp21 region.

Duchenne muscular dystrophy (DMD) is the most common X linked fatal disease, affecting approximately 1 in 3500 male livebirths.¹ Most DMD patients are sporadic cases with no previous family history of muscular dystrophy; however, theoretically, two-thirds of the patients' mothers are DMD carriers. The DMD locus was first localised to band Xp21 through the study of de novo balanced X;autosome translocations in female DMD patients. To date 13 cases of de novo balanced X;autosome translocations with breakpoints in the Xp21 band have been described in female patients with X linked muscular dystrophy.^{2,3} It was suggested that the translocation breakpoint within Xp21 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.

Since 1985, several partial deletions of the Xp21 band have been described in male DMD patients with complex phenotypes of various combinations of distinct syndromes, including ornithine transcarbamylase (OTC) deficiency, retinitis pigmentosa (RP), chronic granulomatous disease (CGD), glycerol kinase (GK) deficiency, adrenal hypoplasia (AH), and mental retardation.⁴⁻¹² In contrast to these cases, no X chromosome anomaly was observed in a few male patients with the complex

phenotype of DMD, GK deficiency, AH, and mental retardation.^{7,8,13} Moreover, cytologically detectable minor Xp21 deletions were described in male DMD patients with mental retardation not known to have any additional pathology.^{14,15} Such interstitial Xp21 deletions have proved useful for the isolation and cloning of specific gene sequences of the OTC and DMD loci.^{16,17} In addition to cytologically detectable deletions of 5000 to 10 000 kb in DMD patients with complex phenotypes, submicroscopic deletions of the XJ1.1-pERT87 region were found in 7 to 10% of DMD/BMD males.¹⁸⁻²¹

Although molecular deletions at the DMD locus are more frequent than large, cytologically detectable deletions, one might assume that the size of cytologically visible deletions and submicroscopic DMD deletions varies continuously. Therefore, we perform a systematic cytogenetic examination of DMD patients with molecular deletions by an improved high resolution R type replication banding technique to look for very small cytologically visible deletions. In this paper we report a case of inherited deletion of an Xp21 subband in a male DMD patient.

Case report

Our proband is considered by one of us (AWJS) to be a classical DMD patient²² of normal intelligence and without any additional pathology. He is an only child with no known family history of muscular dystrophy. Several measurements of serum creatine kinase, when he was eight years old, varied between

39 000 and 60 000 nmol/l·s (normal value <2500 nmol/l·s). The patient's mother was identified as a carrier on the basis of a very raised creatine kinase activity of 8850 nmol/l·s (normal value <1250 nmol/l·s).²³ The healthy and asymptomatic grandmother of our patient could not be identified as a carrier because of normal creatine kinase activities.

Methods

CYTOGENETIC ANALYSIS

Peripheral blood lymphocytes from the proband, his healthy mother, and grandmother were cultured in RPMI 1640 medium supplemented with 20% fetal calf serum and 200 µg/ml gentamycin using a

FIG 1 High resolution R type replication banding of the active, early replicating X chromosome (EX) from four early prometaphase cells, showing the presence of a very small deletion within the Xp21.1 subband in our DMD patient (b) in comparison with the structurally normal EX of a 45,X patient with Turner's syndrome, but without muscular dystrophy (a). The same Xp21.1 deletion chromosome (d) or the structurally normal X chromosome (c) of the patient's mother is the active, early replicating X, reflecting random inactivation of the deleted X chromosome. The R positive subband Xp21.2 is marked by an arrow, and the Xp21.12 faint R subbands are indicated by bars.

standard protocol. The synchrony of cell division required for high resolution cytogenetic analysis was induced by addition of methotrexate (MTX, final concentration 0.05 µg/ml) at 48 hours. The methotrexate block was released at 65.5 hours by centrifugation. The cells were resuspended in fresh medium supplemented with 5-BrdU (final concentration 20 µg/ml) and Hoechst 33258 (final concentration 5 µg/ml). At 72 hours the cells were harvested without the use of colcemid, treated with 0.075 mol/l KCl for 25 minutes at 37°C, and fixed in methanol:glacial acetic acid (3:1). The fixative was changed at least four or five times before slide preparation by air drying. The slides were differentially stained using a modification of the FPG technique.²⁴ The preparations were stained in Hoechst 33258 (0.5 µg/ml deionised water), exposed

to ultraviolet light for 30 to 45 minutes, and stained in 3% Giemsa.

DNA ANALYSIS

Data of the DNA analysis of our male DMD patient have recently been published in detail (pat 1426).²⁵

FIG 2 Cut out of EX from five early prometaphase (a) and late prophase (b) cells, showing the more precise location of the Xp21.1 deletion to subband Xp21.13. The position of the proximal faint R subband of Xp21.12 (bar) in relation to the positive R subband Xp21.2 (arrow) indicates the size and location of the Xp21.13 deletion. The deleted portion of the Xp21.1 subband is marked on the structurally normal EX on the left side in (a) and (b) in comparison with four deleted EX from our DMD patient and his mother. All X chromosomes in figs 1 and 2 are of the same magnification.

FIG 3 Schematic representation of the improved high resolution R type replication banding pattern of the X chromosome in late prophase with the location of the deletion of subband Xp21.13 observed in our DMD patient, and the DNA probes which fail to hybridise to his DNA, respectively.

Results

Using an improved high resolution replication banding technique we are able to obtain more elongated prometaphase and prophase chromosomes.²⁶ After FPG differential staining the chromosomes display a highly contrasted R type replication pattern. The genetically active, early replicating X chromosome (EX) shows a large number of easily distinguished R type replication bands. The late replicating X (LX) has an overall pale blue colouration with only a few dark, early replicating R bands. The high resolution R type replication pattern of the early replicating X (EX) at prometaphase stages is in good agreement with the reversal of high resolution G banding.²⁷ Additional fine structures, especially of the Xp21 region, can be visualised in more elongated EX chromosomes at late prophase and early prometaphase stages.²⁸

The high resolution examination of prometaphase EX chromosomes of our DMD patient showed a very small deletion of subband Xp21·1 (fig 1b) in 85 out of 101 cells analysed. The deletion could not be identified with certainty in 16 cells. However, the large number of EX chromosomes with unequivocally detectable Xp21·1 deletion excludes an artefact. The same interstitial Xp21·1 deletion was observed in prometaphase EX chromosomes of the proband's mother (fig 1d). The high resolution RBG banding technique simultaneously allows us to estimate the inactivation pattern of the Xp21·1 deletion chromosome. In 48 prometaphase cells the deleted chromosome was the early replicating X (fig 1d), while in 57 prometaphase cells the normal

X chromosome was the early replicating one (fig 1c), and therefore random inactivation of the deleted X chromosome may be assumed. More elongated late prophase and early prometaphase EX chromosomes, in which the R positive subbands Xp21·12 and Xp21·32 are clearly visible, were analysed in detail. In this way, the interstitial Xp21·1 deletion has been more precisely localised to subband Xp21·13 (figs 2 and 3).

The X chromosomes of the proband's grandmother were normal. The Xp21·1 deletion, therefore, was a new mutation in the proband's mother.

On the basis of the physical map around the DMD gene, constructed through pulsed field gel electrophoresis,^{25 29} it can be calculated that the size of this DMD deletion is approximately 2000 kb. It seems probable that this deletion is smaller than all other interstitial Xp21 deletions published so far. Cytologically detectable interstitial Xp21 deletions have been observed in male patients who suffer from complex phenotypes incorporating several distinct syndromes: OTC deficiency, RP, CGD, DMD, GK deficiency, AH, and mental retardation.^{4-12 14 15} A comparison of clinical, cytogenetic, and molecular data is given in the table. There are inconsistencies between apparently similar cytogenetic and molecular data on one hand and different clinical phenotypes of male patients on the other hand. In male patients suffering from GK deficiency and AH alone a deletion of the subband Xp21·2 was found,^{8 11} whereas in a few male patients with DMD, GK deficiency, and AH no X chromosome anomaly was found.^{7 8 13} Moreover, the findings of various combinations of syndromes in

TABLE Comparison of clinical, molecular, and cytogenetic data of male patients with minor Xp21 deletions.

Ref	Identification	Complex phenotype							DNA sequence present for						Cytogenetic deletion
		OTC	RP	CGD	DMD	GK	AH	MR	OTC	754	p84	p87	C7	B24	
4		+	?	?	?	+	+		+	-			+		Xp11·2→Xp21 from his mother
5	BB	-	+	+	+	-	-	+	+	-	-	-	+	+	Xp21·1±parts of Xp21·2 and/or Xp21·3
6	NF	-	-	+	+	-	-	+	-	-		-			Xp21·1→Xp21·3
7	1 fam	-	-	-	+	+	+	+	+	-			+	+	Xp21·2
	2 fam	-	-	-	+	+	+	+	+	-			+	+	No X chromosome anomaly
8	1 fam	-	-	-	+	+	+	+	+	-					Xp21·2→Xp21·3
	3 fam	-	-	-	+	+	+	+	+	+	+				No X chromosome anomaly
	4 fam	-	-	-	+	+	+	+	+	+	+				No X chromosome anomaly
	2 fam	-	-	-	-	+	+	+	+	+	+				Xp21·2
13	pat 1	-	-	-	+	+	+	+	+	-			+		No X chromosome anomaly
12	pat 1	-	-	-	+	+	+	+	+	-			-		Xp21·1→Xp21·11
14	SS/JD	-	-	-	+	-	-	±	+	-	-	-	+	+	Xp21·1→Xp21·3
15		-	-	-	+	-	-	+	+	-		-			Xp21·2
This report	AW	-	-	-	+	-	-	-		+	-	-	+	+	Xp21·13
9	III.3	-	-	-	+	+	+	+							Xp21·2→Xp21·3
10		-	-	-	+	+	+	+							Xp21
11	1 fam	-	-	-	-	+	+	+							Xp21·2 and part of Xp21·1 and/or Xp21·3
	2 fam	-	-	-	+	+	+	+							Xp21·2 and part of Xp21·1

such male patients suggest different breakpoint localisation and size of interstitial Xp21 deletions, but this has not yet been confirmed by standard high resolution G banding.

Discussion

In a male patient, who suffers from DMD alone, and in his healthy carrier mother a very small deletion of the high resolution subband Xp21.1 has been identified using an improved high resolution replication banding technique. The interstitial deletion Xp21.1 has been localised more precisely to subband Xp21.13. The presence of a deletion in our DMD patient has been confirmed through molecular analysis of his DNA which showed a deletion of the pERT84-pERT87 region.²⁵ Hence, the proximal part and the 5' starting point of the DMD gene or gene complex would map to subband Xp21.13. The localisation of our DMD deletion in Xp21.13 is in good agreement with the recent finding of cytogenetic heterogeneity of X chromosome breakpoints from female DMD patients carrying balanced X;autosome translocations. Some of these X chromosome translocation breakpoints have been mapped to subband Xp21.2 and others to subband Xp21.1.^{30,31} Therefore, the identification of an inherited deletion of subband Xp21.13 in our DMD patient shows that improved high resolution R type replication banding patterns of prophase and prometaphase chromosomes contribute to better regional gene mapping within the Xp21 region.

It was shown that the Xp21.13 deletion chromosome of the patient's mother is subject to random X inactivation, which is similar to the case reported by Francke.³² These findings are in contrast to the observation of preferential inactivation of a deleted Xp21.1→Xp22.11 chromosome in the phenotypically normal mother of a male patient with a complex phenotype of DMD, GK deficiency, and AH.¹²

The observation of a very small interstitial Xp21.1 deletion in our male DMD patient may explain whether minute deletions are confined to complex syndromes or whether they may be found in patients with typical monogenic disorders as well. Clarke *et al.*¹² performed cytogenetic analysis in nine cases of DMD with molecular deletions, but no X chromosome anomaly was found by standard high resolution G banding. However, as was shown in our case, large molecular DMD deletions may be cytologically observable applying an improved high resolution banding technique. Molecular analysis of the DNA of our DMD patient provided evidence that the sequences for probes pERT84, HIP25, XJ1.1, and pERT87 are deleted.²⁵ However, it can

not be ascertained whether the whole DMD gene or only the proximal portion of it is deleted. Based on data from pulsed field gel electrophoresis,^{25,29} the size of the deletion of subband Xp21.13 could be approximately 2000 kb. It is now obvious that the DMD gene or gene complex in man is very large at about 2000 kb. Hence, complete or large intragenic deletions of the DMD region might be visible cytologically.

The identification of an inherited deletion of subband Xp21.13 in our DMD patient and in his heterozygous mother emphasises the importance of resolving the fine structure of the Xp21 region by an improved high resolution banding technique. Furthermore, improved high resolution replication banding could possibly narrow the existing gap between the resolution levels of cytogenetic and molecular techniques.

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