J Med Genet 1993; 30: 314–315

SHORT REPORTS

A cytogenetic survey in Menkes disease: implications for the detection of chromosomal rearrangements in X linked disorders

N Tommerup, Z Tümer, T Tønnesen, N Horn

The detection of specific chromosome rearrangements has facilitated the mapping and isolation of genes associated with several important mendelian disorders.[1] Classical examples are X;autosomal translocations in girls affected with X linked recessive disorders,[2] microdeletions in subjects suffering from different, but closely linked, specific disorders (contiguous deletion syndromes),[3] and deletions in patients with non-specific symptoms like mental retardation/congenital anomalies, who also suffer from an easily recognisable mendelian disorder, such as retinoblastoma.[4] In classical mendelian disorders without conspicuous combinations of clinical features, data on types and frequencies of specific chromosome mutations are scanty. Therefore, we performed a systematic cytogenetic analysis of 181 unrelated boys with Menkes disease, a serious X linked recessive disorder of copper metabolism which has been mapped to Xq13.3.[5] Conventional Q band metaphase analysis was applied in the majority of cases, either on PHA stimulated peripheral blood lymphocytes or on cultured skin fibroblasts. Both fibroblasts and lymphocytes were cultured in medium TC 199, and elongated metaphase chromosomes were obtained routinely with an approximate resolution of 550 to 600 bands per haploid genome. High resolution RBA banding[6,7] (800 to 1000 band resolution) was applied in 36 of the cases (table).

Only one patient had an abnormal karyotype, involving a unique rearrangement of the X chromosome with an insertion of the long arm segment Xq13.3-q21.2 into the short arm at band Xp11.4, karyotype 46,XY,ins(X)(p11.4q13.3q21.2)mat. The rearrangement was not associated with a cytogenetically detectable deletion of chromosomal material. The same rearranged X chromosome was present de novo in his phenotypically normal mother, in whom it was preferentially inactivated and of paternal origin. Details are reported elsewhere.[5]

Implications for chromosomal rearrangements in X linked disorders

The two most frequent types of chromosome mutations reported in mendelian disorders are deletions and reciprocal translocations.[2] All visible deletions of the X chromosome detected in males have been located within the regions Xp22.3, Xp21, Xq21, and Xq25,[2,8] probably because large deletions outside these areas are lethal in males. Thus, one might expect that in the majority of X linked disorders, visible deletions would not occur in affected males.

Most de novo chromosomal rearrangements have been found to be of paternal origin,[9,10] including all the X;autosomal translocations examined so far.[11] As a consequence, de novo rearrangements of the X chromosome must occur predominantly in females, where cellular selection favouring the least genetic imbalance

Origin and number of male patients with Menkes disease studied by metaphase and prometaphase techniques.

	Number of cases analysed by				
	Metaphase technique		Prometaphase technique		
Country of origin	Lymphocytes	Fibroblasts	Lymphocytes	Fibroblasts	Total No
Denmark	1	5			6
Sweden		1	1		2
Norway		3			3
Finland			1		1
Germany	5	20	4	1	30
UK	2	17	3	1	23
France		14	4	3	23
Holland	1	12			13
Belgium		4			4
Italy		6			6
Greece		1			1
Spain		1			1
Switzerland		4			4
Turkey		1			1
Hungary		1			1
Israel				1	1
USA	4	34	16	1	55
Canada		7			7
Argentina		1			1
Total	13	132	29	7	181

The John F Kennedy Institute, Gl Landevej 7, DK-2600 Glostrup, Denmark.
N Tommerup
Z Tümer
T Tønnesen
N Horn

Correspondence to
Dr Tommerup.

Received 10 August 1992.
Revised version accepted
5 October 1992.

may result in non-random X inactivation patterns.[12] In female carriers of balanced X;autosomal translocations, it is usually the normal X which is inactivated.[12] If a disease locus has been truncated or otherwise functionally impaired by the translocation, this non-random inactivation of the normal X chromosome will lead to phenotypic expression of that particular disease in the female carrier. If the disease is sufficiently serious, this may prohibit transmission of the translocation to the next generation and hence transmission to males. Furthermore, most breakpoints within a 'critical region' Xq13-q25 appear to be associated with ovarian dysfunction and impaired fertility in female carriers, a second factor which may prevent transmission of some X;autosomal translocations to males.[13]

In many X linked disorders, therefore, both visible deletions and X;autosomal translocations must be rare in affected males. This was corroborated by the present survey. The preponderance of X;autosomal translocations in affected females is further illustrated by the presence of one X;autosomal translocation[14] among the five females with Menkes syndrome known to us.[14–16] Since reciprocal translocations and deletions are among the most common types of chromosomal rearrangement which occur in man,[17] cytogenetic mutations in general will be very rare in males affected with many X linked disorders.

This study also indicates that the rare cases of chromosome mutation which occur in such affected males will be balanced intrachromosomal rearrangements, balanced (at least visibly) because a cytogenetically visible deletion would have been male lethal, and intrachromosomal because it is believed that there are selective factor(s) against cells in females carrying inactive translocated chromosomes. These are the spreading of inactivation onto the autosomal segments and/or the inability to inactivate the translocated part of the X chromosome which does not contain the X inactivation centre.[18] Such females therefore have an active translocated chromosome which, as mentioned previously, is unlikely to be passed on to the next generation. Neither of these factors will be at work in intrachromosomal rearrangements such as inversions and insertions. Hence either random inactivation or, as shown in the mother of the cytogenetically abnormal patient detected in the present survey, non-random inactivation of the rearranged X chromosome may occur.[5] Female carriers of such rearrangements will thus tend to be unaffected and will be able to transmit the rearrangement to male offspring.

In conclusion, in many X linked disorders specific chromosomal mutations will be less frequent than in autosomal disorders. The male Menkes patient with the abnormal X chromosome studied by us did not deviate in any significant way from other typical Menkes patients, either clinically or biochemically.[5] He was a male, so an X;autosomal translocation was not suspected. He did not suffer from any additional mendelian disorders suggesting a contiguous deletion syndrome, and there were no reports of infertility or miscarriages in the family which might have called attention to this inherited chromosome rearrangement. Thus, without a systematic survey, this chromosome rearrangement which has facilitated the mapping of the Menkes disease locus would not have been detected.

The message is clear if rare, disease specific rearrangements are to be detected: chromosomes of all unrelated affected patients should be analysed. The present survey also suggests that high resolution cytogenetic techniques may not be needed for screening males affected with X linked disorders mapping outside the regions Xp22.3, Xp21, Xq21, and Xq25. High resolution chromosome analysis is a costly and tedious technique, which is mainly aimed at the detection of microdeletions. These are unlikely to be found (outside the aforementioned bands). and the present study suggests that intrachromosomal rearrangements visible by routine banding techniques may be more common, thus facilitating cytogenetic screening of affected males.

This study was supported by the Danish Centre for Human Genome Research. Anni Hallberg is thanked for expert technical help and Carll Goodpasture for reading the manuscript.

1 Collins FS. Positional cloning: let's not call it reverse anymore. *Nature Genet* 1992;**1**:3–6.
2 Frézal J, Schinzel A. Report of the committee on clinical disorders, chromosome aberrations and uniparental disomy. *Cytogenet Cell Genet* 1991;**58**:986–1052.
3 Schmickel RD. Contiguous gene syndromes: a component of recognizable syndromes. *J Pediatr* 1986;**109**:231–41.
4 Turleau C, de Grouchy J. Constitutional karyotypes in retinoblastoma. *Ophthal Paediatr Genet* 1987;**8**:11–17.
5 Tümer Z, Tommerup N, Tønnesen T, Kreuder J, Craig IW, Horn N. Mapping of the Menkes locus to Xq13.3 distal to the X-inactivation center by an intrachromosomal insertion of the segment Xq13.3-q21.2. *Hum Genet* 1992;**88**:668–72.
6 Couturier J, Dutrillaux B, Lejeune J. Etude des fluorescences spécifiques des bandes R et des bandes Q des chromosomes humains. *C R Acad Sci Ser D* 1973;**276**:339–42.
7 Søndergaard F, Kristensen M, Tommerup N. High resolution chromosomes from first trimester trophoblast cultures. *Prenat Diagn* 1985;**5**:291–4.
8 Cremers FPM, van de Pol TJR, Wierenga B, *et al*. Molecular analysis of male-viable deletions and duplications allows ordering of 52 DNA probes on proximal Xq. *Am J Hum Genet* 1988;**43**:452–61.
9 Chamberlin J, Magenis RE. Parental origin of de novo chromosome rearrangements. *Hum Genet* 1980;**53**:343–7.
10 Chandley AC. On the parental origin of de novo mutation in man. *J Med Genet* 1991;**28**:217–23.
11 Robinson DO, Boyd Y, Cockburn D, Collinson MN, Craig I, Jacobs PA. The parental origin of de novo X-autosome translocations in females with Duchenne muscular dystrophy revealed by M27β methylation analysis. *Genet Res Camb* 1990;**56**:135–40.
12 Mattei MG, Mattei JF, Ayme S, Giraud F. X-autosome translocations: cytogenetic characteristics and their consequences. *Hum Genet* 1982;**61**:295–309.
13 Therman E, Laxova R, Susman B. The critical region on the human Xq. *Hum Genet* 1990;**85**:455–61.
14 Kapur S, Higgins JV, Delp K, Rogers B. Menkes syndrome in a girl with X-autosome translocation. *Am J Med Genet* 1987;**26**:503–10.
15 Gerdes AM, Tønnesen T, Horn N, *et al*. Clinical expression of Menkes syndrome in females. *Clin Genet* 1990;**38**:452–9.
16 Iwakawa Y, Niwa T, Tomita M. Menkes' kinky hair syndrome: report of an autopsy case and his female sibling with similar clinical manifestations. *Brain Dev (Tokyo)* 1979;**11**:260–6.
17 Hook EB, Hamerton JL. The frequency of chromosome abnormalities detected in consecutive newborn studies – differences between studies – results by sex and by severity of phenotypic involvement. In: Hook EB, Porter IH, eds. *Population cytogenetics*. New York: Academic Press, 1977:81–97.
18 Schmidt M, Du Sart D. Functional disomies of the X chromosome influence the cell selection and hence the X inactivation pattern in females with balanced X-autosome translocations: a review of 122 cases. *Am J Med Genet* 1992;**42**:161–9.