Linkage analysis using multiple Xq DNA polymorphisms in normal families, families with the fragile X syndrome, and other families with X linked conditions

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SUMMARY Multipoint linkage analysis was undertaken with eight Xq cloned DNA sequences which identify one or more restriction fragment length polymorphisms in 26 families. These families comprise seven phase known normal families with three or more males in the third generation, seven families segregating for haemophilia B, one large family with dyskeratosis congenita, and 11 families with the fragile X syndrome. Phase known meioses informative for three or more loci supported the order centromere—*DXYS1*—*DXS107*—*DXS102*, *DXS51*—*F9*—*FRAXA*—*DXS15*, *DXS52*, *F8*—Xqter in each group of families studied. One of the normal families was segregating for protan colour blindness and showed a phase known recombination which would support the order centromere—*F9*—*DXS52*—*CBP*—Xqter.

With the exception of *DXYS1*, all of these sequences have been localised to Xq27→qter by in situ hybridisation or hybridisation to Xq fragment panels, and on this basis should lie within 20 cM of one another. No recombination was observed between the sequences localised to Xq28, namely *DXS52*, *F8*, and *DXS15* (between *DXS15* and *DXS52* $\hat{Z}=12.25$ at $\theta=0$ with confidence limits of 0 to 5 cM). However, an excess of recombination was apparent in the region of *FRAXA* with maximal lod scores as follows: *F9* versus *FRAXA* ($\hat{Z}=2.05$, $\hat{\theta}=0.19$), *DXS52* versus *FRAXA* ($\hat{Z}=1.85$, $\hat{\theta}=0.26$), and *DXS15* versus *FRAXA* ($\hat{Z}=1.33$, $\hat{\theta}=0.27$). No consistent differences were observed in the frequency of recombination when families with the fragile X syndrome were compared with normal families or families segregating for other X linked conditions. These results are compared with other published work and support the conclusion that although measurable linkage exists between these flanking markers and *FRAXA*, the intervals as measured by the frequency of meiotic recombination will seriously limit their clinical usefulness.

With an estimated frequency of 1 in 2000 males,¹ the fragile X syndrome (Martin-Bell syndrome, McKusick catalogue number 30955) is a common, recognisable cause of mental retardation. The characteristic male phenotype includes an elongated face, large ears, large testes, and mental retardation with marked verbal disability.² The phenotype is, however, variable both within and between families and the most consistent feature has been mental retardation with an IQ usually in the range of 40 to 60.³ X linked inheritance is favoured by pedigree analysis and by the association with the fragile site at

Xq27.3 in up to 50% of cells from affected males.⁴ About one-third of female carriers are phenotypically abnormal with either mental retardation (usually mild) or less frequently an elongated facies. Cytogenetic demonstration of the Xq fragile site is usually possible in these clinically abnormal carriers but is not possible in about 50% of clinically normal female carriers.⁸ Counselling of affected families is further hampered by the knowledge that clinically normal males in older generations of some pedigrees have transmitted the trait or a predisposition to the trait to daughters and hence to affected grandsons.^{9 10}

These difficulties have encouraged attempts to

develop closely linked DNA markers which might be clinically useful for carrier detection and prenatal diagnosis. In 1983, Camerino *et al*¹¹ reported tight linkage between a coagulation factor IX (F9) DNA polymorphism and the locus for the fragile X syndrome (*FRAXA*). This conclusion was largely based on a large French family in which transmission from a phenotypically normal grandfather to six of his 10 grandsons was suspected. Subsequent published linkage studies with the factor IX polymor-

phisms have failed to confirm such tight linkage.¹²⁻¹⁷ These studies and pertinent abstracts from Human Gene Mapping 8 are briefly summarised in the proceedings of that meeting¹⁸ and are considered further in the discussion. Tight linkage has also been reported between *FRAXA* and distal markers from Xq28, such as *G6PD* or *CBP*.^{19,20} and between *FRAXA* and *DXS52*.²¹ However, other investigators¹⁴ have been unable to confirm tight linkage with another marker from Xq28 (*DXS15*)

FIG 1 Families 1 to 7 are phase known normal families. In pedigree 3823, III.2 has deutan colour blindness and in pedigree 3824 III.2 and III.4 have protan colour blindness. Families 8 to 14 are portions of families which are segregating for haemophilia B. Family 15 (pedigree 4543) is segregating for dyskeratosis congenita.

FIG 2 Families 16 to 26 are segregating for the fragile X syndrome.

which is known to be tightly linked to DXS52.²² These findings have led to conflicting opinions about the frequency of recombination in the distal portion of Xq in families with the fragile X syndrome, which may be decreased,²⁰ decreased only when transmitted by a normal male,¹⁷ or increased¹⁴ in comparison with normal families.

TABLE 1 Cytogenetic findings in *fra(X)* positive subjects in 11 families with the fragile X syndrome.

Pedigree	Subject	Sex	<i>fra(X)</i> (%)	No of <i>fra(X)</i> / No counted
665	III.3	M	10	10/100
	III.5	F	9	9/100
	III.6	F	12	12/100
	III.7	M	1	1/100
2051	IV.2	F	32	24/75
	IV.7	F	44	22/50
	V.2	F	33	25/75
	V.3	M	8	4/50
	V.6	F	13	10/75
	V.7	M	12	7/60
3475	II.13	F	2	1/50
	III.2	F	6	3/50
	III.8	F	4	2/50
	III.9	F	6	3/50
	IV.1	F	24	12/50
	IV.3	M	4	2/50
	IV.5	M	10	5/50
	IV.6	M	20	5/25
3653	II.2	F	0.5	1/200
	III.1	M	24	24/100
	III.2	M	3	3/100
3940	III.3	F	3	2/75
	IV.1	M	48	12/25
	IV.2	M	48	12/25
4521	I.2	F	2	2/100
	III.6	M	10	5/50
	III.7	M	33	10/30
	III.9	F	18	9/50
	III.10	F	12	6/50
4523	IV.4	M	20	10/50
	II.4	F	6	3/50
	II.5	F	20	10/50
	III.1	M	28	14/50
	III.2	M	52	26/50
	III.3	M	24	12/50
4524	III.6	M	44	22/50
	II.3	F	4	2/50
	III.1	M	4	2/50
	III.2	M	24	12/50
4658	II.2	F	2	2/100
	II.4	F	1	1/100
	III.2	F	21	21/100
	IV.2	M	8	4/50
	IV.3	M	16	8/50
4669	II.2	F	1	1/105
	III.2	F	1	1/75
	IV.1	M	3	2/75
	IV.2	M	28	21/75
4769	III.4	F	10	9/88
	III.6	M	54	27/50
	IV.1	M	15	9/61
	IV.3	M	13	8/60
	IV.4	M	38	19/50

The present study addressed these difficulties by comparing linkage relationships of multiple Xq DNA markers in families with and without the fragile X syndrome.

Patients and methods

The seven phase known normal families were all from the west of Scotland and are illustrated in fig 1. Each family was screened for colour blindness using Ishihara plates. Family 2 (3823) contained a male with deutan colour blindness and family 3 (3824) contained two males with protan colour blindness. The families with haemophilia B were all from the west of Scotland and the application of the factor IX DNA polymorphisms to these families has already been described.^{23 24} Families 8 to 14 which were used for linkage analysis are also shown in fig 1. Family 15 with dyskeratosis congenita is from Liverpool and has been described elsewhere.²⁵ Nine of the families with the fragile X syndrome are from the west of Scotland and two (665 and 3653) are from Liverpool (fig 2). Cytogenetic details of each *fra(X)* positive family member are shown in table 1 and in this study clinically normal males in the family were also examined cytogenetically. Blood samples for cytogenetic analysis were cultured for 72 hours in either TC199 without folic acid (10% newborn calf serum) or in Iscove's medium, and the Xq27 fragile site was confirmed by destaining and trypsin banding. A minimum of 25 cells was scored for each male and a minimum of 50 cells was scored for each female. No evidence for transmission by normal males was apparent in these families and all retarded males were fragile X positive. One male (family 26, III.1) was considered normal by his family but showed 15% *fra(X)* and was abnormal on formal psychological assessment. All retarded or dull females were *fra(X)* positive but 50% (nine of 18) of obligate carriers were cytogenetically normal (table 1, fig 2).

The DNA polymorphisms used in these families are outlined in table 2 and are described in more detail elsewhere²⁶ with the exception that the

TABLE 2 Probes used.

HGM name	Other name	Cytogenetic localisation
DXYS1	pDP34	Xq13→q21.1
DXS107	cpX234	Xq27→q28
DXS102	p38.1	Xq27→q28
DXS51	S2A	Xq27
F9	F9(VIII)	Xq27
F8	F8A	Xq28→qter
DXS15	DX13	Xq28→qter
DXS52	St14-1	Xq28→qter

DXS51/TaqI polymorphism is 1.3 kb/0.7 kb +0.6 kb¹⁴ not 1.6 kb/1.3 kb. The techniques for DNA extraction, digestion, and fragment identification have been described previously.²³⁻²⁵

Linkage analysis was undertaken using LIPED 3,²⁷ and in these calculations an allele frequency for fragile X of 0.01 was used and scoring included fragile X positive males, fragile X negative males, and fragile X positive female offspring, all of whom had more than 4% fra(X), but not fragile X negative female offspring. The inclusion of fra(X) negative male offspring in these calculations was considered justified, as in the families with normal male transmission these subjects rarely have mentally retarded sibs.⁹ Confidence intervals were calculated corresponding to values of the recombination fraction at a lod score one unit less than the maximum value as recommended at HGM8.²⁸

Results

Table 3 shows the polymorphic fragment frequencies for the probes used in this study in unrelated Scottish persons. *DXS52* identifies at least 10

TABLE 3 Polymorphic fragment frequencies in unrelated subjects from the west of Scotland.

Probe	Fragment (kb)	Frequency	No of chromosomes
<i>DXYS1</i>	11.8	0.34	45
	10.6	0.66	
<i>DXS107</i>	3.2	0.35	52
	1.1	0.65	
<i>DXS102</i>	10	0.57	47
	1.7	0.43	
<i>DXS51</i>	1.3	0.48	50
	0.7+0.6	0.52	
<i>F9/TaqI</i>	1.8	0.69	46
	1.3	0.31	
<i>F9/XmnI</i>	11.5	0.68	60
	6.5	0.32	
<i>F9/DdeI</i>	1.75	0.23	30
	1.7	0.77	
<i>F8</i>	1.2	0.22	40
	0.9	0.78	
<i>DXS15</i>	5.8	0.4	62
	2.8	0.6	

TABLE 4 Number of phase known recombinant chromosomes providing support for the proposed position of each locus relative to the others on the basis of one recombination per chromosome.

	<i>DXS52</i>	<i>DXS15</i>	<i>FRAXA</i>	<i>F9</i>	<i>DXS51</i>	<i>DXS102</i>	<i>DXS107</i>	<i>DXYS1</i>
<i>DXS52</i>	—	0	2	4	4	3	4	5
<i>DXS15</i>		—	3	6	3	3	10	2
<i>FRAXA</i>			—	1	2	4	5	
<i>F9</i>				—	4	4	7	5
<i>DXS51</i>					—		5	3
<i>DXS102</i>						—	2	
<i>DXS107</i>							—	
<i>DXYS1</i>								—

TABLE 5 Summary of lod scores for *FRAXA* versus other markers.

Combination 0-4	+	Recombination fraction				
		0	0.05	0.1	0.2	0.3
<i>FRAXA</i> v <i>DXYS1</i>	—00	—5.08	—3.19	—1.53	—0.73	
—0.27	2/2	5/12	7/14			
<i>FRAXA</i> v <i>DXS107</i>	—00	—3.72	—2.18	—0.92	—0.43	
—0.23	5/6	1/11	6/17			
<i>FRAXA</i> v <i>DXS51</i>	—00	—3.08	—1.57	—0.4	—0.02	
0.01	5/7	1/11	6/18			
<i>FRAXA</i> v <i>DXS102</i>	—00	—2.9	—0.82	0.57	0.75	
0.42	4/7	2/19	6/26			
<i>FRAXA</i> v <i>F9</i>	—00	0.24	1.52	2.04	1.67	
0.88	1/11	6/27	7/38			
<i>FRAXA</i> v <i>DXS15</i>	—00	—3.39	—0.79	1.01	1.32	
0.89	3/14	5/20	8/34			
<i>FRAXA</i> v <i>DXS52</i>	—00	—2.77	—0.04	1.69	1.75	
1.07	3/8	3/19	6/27			

TABLE 6 Pairwise marker lod scores in families without the fragile X syndrome.

Combination 0-4	+	Recombination fraction				
		0	0.05	0.1	0.2	0.3
<i>DXYS1</i> v <i>DXS107</i>	1.23	1.12	1.0	0.75	0.5	
0.26	0/3	0/2	0/5			
<i>DXYS1</i> v <i>DXS51</i>	—00	—5.59	—3.59	—1.76	—0.82	
—0.28	5/8	2/4	7/12			
<i>DXYS1</i> v <i>DXS102</i>	—00	—0.72	—0.44	—0.19	—0.07	
—0.02	1/3	1/3				
<i>DXYS1</i> v <i>F9</i>	—00	—2.62	—1.1	0.06	0.43	
0.36	5/17	1/3	6/20			
<i>DXYS1</i> v <i>DXS15</i>	—00	—0.61	—0.14	0.17	0.21	
0.15	2/4	3/4	5/8			
<i>DXYS1</i> v <i>DXS52</i>	—00	—9.88	—6.66	—3.56	—1.85	
—0.75	11/14	11/14				
<i>DXS107</i> v <i>DXS51</i>	—00	—3.88	—1.45	—0.04	0.33	
0.27	3/12	2/5	5/17			
<i>DXS107</i> v <i>DXS102</i>	—00	—1.76	—1.19	—0.64	—0.35	
—0.16	2/3	2/3				
<i>DXS107</i> v <i>F9</i>	—00	—1.28	—0.08	0.74	0.84	
0.57	5/16	0/2	5/18			
<i>DXS107</i> v <i>DXS15</i>	—00	—3.16	—1.44	—0.15	0.21	
0.17	7/18	7/18				
<i>DXS107</i> v <i>DXS52</i>	—00	—5.17	—3.07	—1.35	—0.67	
—0.29	5/11	1/3	6/14			
<i>DXS51</i> v <i>DXS102</i>	—00	—0.32	—0.08	0.09	0.12	
0.07	1/3	0/1	1/4			
<i>DXS51</i> v <i>F9</i>	—00	1.73	2.35	2.39	1.87	
1.06	1/16	2/9	3/25			
<i>DXS51</i> v <i>DXS15</i>	—00	—1.46	0.11	1.05	1.08	
0.66	4/16	1/4	5/20			
<i>DXS51</i> v <i>DXS52</i>	—00	—0.32	0.91	1.51	1.31	
0.72	3/15	0/2	3/17			
<i>DXS102</i> v <i>F9</i>	—00	—0.16	0.09	0.23	0.21	
0.1	2/5	0/3	2/8			
<i>DXS102</i> v <i>DXS15</i>	—00	—0.44	—0.19	0.01	0.08	
0.06	1/3	1/3				
<i>DXS102</i> v <i>DXS52</i>	—00	—3.9	—2.51	—1.24	—0.62	
—0.23	4/8	4/8				
<i>F9</i> v <i>F8</i>	—00	—1.49	—0.93	—0.42	—0.18	
—0.05	2/4	2/4				
<i>F9</i> v <i>DXS15</i>	—00	0.09	1.15	1.72	1.53	
0.94	3/15	2/4	5/19			
<i>F9</i> v <i>DXS52</i>	—00	0.12	1.2	1.79	1.68	
0.89	3/18	3/4	6/22			
<i>F8</i> v <i>DXS52</i>	1.82	1.67	1.52	1.18	0.82	
0.41	0/5	0/5				
<i>DXS15</i> v <i>DXS52</i>	4.84	4.47	4.05	3.17	2.2	
1.11	0/12	0/5	0/17			

Linkage analysis using multiple Xq DNA polymorphisms

polymorphic fragments and overall 80% of Scottish females were heterozygous. The DNA results on each family member are available from the authors in the form of an appendix and are summarised here. The three point cross technique in phase known meioses supported the order centromere—*DXYS1*—*DXS107*—*DXS102*, *DXS51*—*F9*—*FRAXA*—*DXS15*, *DXS52*, *F8*—Xqter in each group of families studied. The numbers of phase known meioses supporting this order are indicated in table 4. Family 3824 was informative for *DXYS1*, *F9*, protan colour blindness (*CBP*), and *DXS52*. A phase known recombination has occurred in III.4, family 3 (fig 1) between *CBP* and *DXS52* which would support the order *F9*—*DXS52*—*CBP*—Xqter. Unfortunately this family was not informative for *F8* or *DXS15*.

TABLE 7 Pairwise marker lod scores in families with the fragile X syndrome.

Combination 0-4	+	Recombination fraction				
		0	0-05 +/-	0-1	0-2	0-3
<i>DXYS1</i> v <i>DXS107</i>	No information					
<i>DXYS1</i> v <i>DXS51</i>	-0-4	-0-29	-0-21	-0-1	-0-04	
	1/2	1/2				
<i>DXYS1</i> v <i>DXS102</i>	-0-0	-4-12	-2-7	-1-35	-0-66	
	-0-25	2/2	3/6	5/8		
<i>DXYS1</i> v <i>F9</i>	-0-0	-4-28	-2-63	-1-14	-0-45	
	-0-1	7/13	7/13			
<i>DXYS1</i> v <i>DXS15</i>	-0-0	-2-3	-1-43	-0-64	-0-25	
	-0-06	2/6	2/6			
<i>DXYS1</i> v <i>DXS52</i>	-0-0	-3-73	-2-11	0-73	-0-15	
	0-06	0/2	6/13	6/15		
<i>DXS107</i> v <i>DXS51</i>	-0-0	-4-51	-2-64	-1-04	-0-39	
	-0-16	3/4	2/9	5/13		
<i>DXS107</i> v <i>DXS102</i>	-0-0	0-48	0-8	0-83	0-62	
	0-21	0/2	2/14	2/16		
<i>DXS107</i> v <i>F9</i>	-0-0	-2-51	-1-24	-0-24	0-05	
	0-03	4/6	0/9	4/15		
<i>DXS107</i> v <i>DXS15</i>	-0-0	-7-06	-4-54	-2-23	-1-09	
	-0-43	6/6	4/11	10/17		
<i>DXS107</i> v <i>DXS52</i>	-0-0	-5-9	-3-9	-2-03	-1-04	
	-0-43	4/4	4/9	8/13		
<i>DXS51</i> v <i>DXS102</i>	-0-0	0-34	1-37	1-21	0-87	
	0-39	0/2	2/11	2/13		
<i>DXS51</i> v <i>F9</i>	-0-0	0-02	0-62	0-88	0-68	
	0-26	3/4	0/11	3/15		
<i>DXS51</i> v <i>DXS15</i>	-0-0	-6-48	-3-99	-1-74	-0-68	
	-0-16	5/6	3/9	8/15		
<i>DXS51</i> v <i>DXS52</i>	-0-0	-5-6	-3-61	-1-65	-0-82	
	-0-28	2/3	3/7	5/10		
<i>DXS102</i> v <i>F9</i>	-0-0	-0-12	0-48	0-77	0-34	
	0-27	2/3	1/12	3/15		
<i>DXS102</i> v <i>DXS15</i>	-0-0	-3-11	-1-31	0-04	0-38	
	0-25	4/5	5/16	9/21		
<i>DXS102</i> v <i>DXS52</i>	-0-0	-6-68	-3-92	-1-56	-0-6	
	-0-23	5/6	5/14	10/20		
<i>F9</i> v <i>F8</i>	0	0/3	0-33	0-3	0-17	0-06
	0	0/3	1/5	1/8		
<i>F9</i> v <i>DXS15</i>	-0-0	-5-32	-2-94	-0-91	-0-11	
	0-14	3/11	5/14	8/25		
<i>F9</i> v <i>DXS52</i>	-0-0	-1-79	-0-37	0-62	0-69	
	0-39	2/6	6/24	8/30		
<i>F8</i> v <i>DXS52</i>	0-53	0-51	0-47	0-36	0-21	
	0-06	0/3	0/3			
<i>DXS15</i> v <i>DXS52</i>	7-41	6-76	6-07	4-63	3-05	
	1-4	0/13	0/26			

TABLE 8 Pairwise marker lod scores for all families (tables 6 and 7 combined).

Combination 0-4	+	Recombination fraction				
		0	0-05 +/-	0-1	0-2	0-3
<i>DXYS1</i> v <i>DXS107</i>	1-23	1-12	1-0	0-75	0-5	
	0-26	0/3	0/2	0/5		
<i>DXYS1</i> v <i>DXS51</i>	-0-0	-5-88	-3-8	-1-86	-0-86	
	-0-29	5/8	3/6	8/14		
<i>DXYS1</i> v <i>DXS102</i>	-0-0	-4-84	-3-14	-1-54	-0-73	
	-0-27	2/2	4/9	6/11		
<i>DXYS1</i> v <i>F9</i>	-0-0	-6-9	-3-73	-1-08	-0-02	
	0-26	5/17	8/16	13/33		
<i>DXYS1</i> v <i>DXS15</i>	-0-0	-2-91	-1-57	-0-47	-0-04	
	0-09	2/4	5/10	7/14		
<i>DXYS1</i> v <i>DXS52</i>	-0-0	-13-61	-8-77	-4-29	-2-0	
	-0-69	11/16	6/13	17/29		
<i>DXS107</i> v <i>DXS51</i>	-0-0	-7-89	-4-09	-1-08	-0-06	
	0-11	6/16	4/14	10/30		
<i>DXS107</i> v <i>DXS102</i>	-0-0	-1-28	-0-39	0-19	0-27	
	0-05	2/5	2/14	4/19		
<i>DXS107</i> v <i>F9</i>	-0-0	-3-79	-1-32	0-5	0-89	
	0-6	9/22	0/11	9/33		
<i>DXS107</i> v <i>DXS15</i>	-0-0	-10-22	-5-98	-2-38	-0-88	
	-0-26	13/24	4/11	17/35		
<i>DXS107</i> v <i>DXS52</i>	-0-0	-11-07	-6-97	-3-38	-1-71	
	-0-72	9/15	5/12	14/27		
<i>DXS51</i> v <i>DXS102</i>	-0-0	0-02	1-29	1-3	0-99	
	0-46	1/5	2/12	3/17		
<i>DXS51</i> v <i>F9</i>	-0-0	1-75	2-97	3-27	2-55	
	1-32	4/20	2/20	6/40		
<i>DXS51</i> v <i>DXS15</i>	-0-0	-7-94	-3-88	-0-69	0-4	
	0-5	9/22	4/13	13/35		
<i>DXS51</i> v <i>DXS52</i>	-0-0	-5-92	-2-71	-0-14	0-49	
	0-44	5/18	3/9	8/27		
<i>DXS102</i> v <i>F9</i>	-0-0	-0-28	0-57	1-0	0-55	
	0-37	4/8	1/15	5/23		
<i>DXS102</i> v <i>DXS15</i>	-0-0	-3-55	-1-5	0-05	0-46	
	0-31	5/8	5/16	10/24		
<i>DXS102</i> v <i>DXS52</i>	-0-0	-10-58	-6-43	-2-8	-1-22	
	-0-46	9/14	5/14	14/28		
<i>F9</i> v <i>F8</i>	-0-0	-1-19	-0-67	-0-25	-0-12	
	-0-05	2/7	1/5	3/12		
<i>F9</i> v <i>DXS15</i>	-0-0	-5-23	-1-79	0-81	1-42	
	1-08	6/26	7/18	13/44		
<i>F9</i> v <i>DXS52</i>	-0-0	-1-67	0-83	2-41	2-37	
	1-28	5/24	9/28	14/52		
<i>F8</i> v <i>DXS52</i>	2-35	2-18	1-99	1-54	1-03	
	0-47	0/3	0/5	0/8		
<i>DXS15</i> v <i>DXS52</i>	12-25	11-23	10-12	7-8	5-25	
	2-51	0/25	0/18	0/43		

Linkage analysis of each marker versus *FRAXA* is presented in table 5. Pairwise linkage analysis of markers is presented for families without the fragile X syndrome in table 6, for families with the fragile X syndrome in table 7, and both sets of data are combined in table 8. Comparison of recombination rates in pairwise crosses revealed no consistent differences between the fragile X and normal families.

Discussion

High resolution light microscopy and scanning electron microscopy have localised the fragile site associated with the fragile X syndrome to Xq27-3.^{29 30} Although the precise relationship of

this site to the clinical syndrome is unknown, there is general agreement that the locus for *FRAXA* is either localised to or closely linked to this site.⁸ Physical localisation of the DNA probes used in this study, with the exception of *DXS1*, has indicated that all lie in Xq27→qter.³¹⁻³³ This area represents about 5% of the metaphase X chromosome or about 18 000 kb.³³ If recombination were proportional to physical length, then loci in Xq27 should be less than 20 cM from more distal loci.³⁴ However, recombination is greater than expected in this area and a growing body of evidence, as reviewed by Szabo *et al*,²⁰ tends to exclude measurable linkage between the locus for haemophilia B (in Xq27) and members of the *G6PD* cluster in Xq28. Thus, for the subtelomeric loci *G6PD*, haemophilia A, adrenoleucodystrophy, and protan and deutan colour blindness, only five recombinants have been observed in 411 meiotic events, whereas for *G6PD* and haemophilia B 16 recombinants in 37 meioses have been observed.²⁰

Against this background close linkage between both *F9* and *G6PD* with an intervening locus would be unexpected. Yet Szabo *et al*²⁰ have only observed two recombinants in 29 meioses between *FRAXA* and *G6PD* ($\hat{Z}=5.57$ at $\hat{\theta}=0.07$). Similarly, Oberle *et al*²¹ supported this distance with a maximal lod score of 10.65 at 10% recombination for *FRAXA* versus *DXS52* (90% confidence limits 4 to 18.5%). Furthermore, this latter group, on the basis of 16 families with the fragile X syndrome, support a recombination distance of 12% between *FRAXA* and *F9* (90% confidence limits 4.4 to 22.5%).²¹ These data include the exceptional family of Camerino *et al*¹¹ in which, if transmission by the normal grandfather is assumed, there were no recombinants in 15 meiotic events.

The present study excludes close linkage (up to 5% recombination) for both *DXS52* and *DXS15* and suggests a likely meiotic distance to *FRAXA* of 26% and 27%, respectively. Table 9 compares reported studies on *FRAXA* versus *F9*. The combined data support a meiotic distance between *FRAXA* and *F9* of 26% ($\hat{Z}=8.67$ at $\hat{\theta}=0.26$, confidence intervals 0.14 to 0.28). In view of the suggestion by Brown *et al*¹⁷ that tight linkage was confined to families with male transmission, this table also indicates the numbers of families with known or suspected male transmission. Exclusion of these families as far as possible results in $\hat{Z}=4.97$ at $\hat{\theta}=0.22$.

In situ hybridisation data are available to support the order proposed by the present study with respect to *DXS51*, *F9*, *FRAXA*, *DXS15*, and *DXS52*.^{14 31 33} This order is also supported in part by Drayna and White,²² whose studies on large normal families from Utah favour the order

TABLE 9 Comparison of linkage data in fragile X families for *FRAXA* v *F9*.

Recombination fraction						Families*	Reference
0	0-05	0-1	0-2	0-3	0-4		
—00	—0.7	—0.27	—0.01	—0.01	—0.05	5 —	15
—00	—0.69	0.15	0.57	0.45	0.16	5 1	12
—00	—0.72	—0.44	—0.19	—0.08	—0.02	1 —	13
—00	—4.6	—2.4	—0.28	0.04	0.14	5†	14
—00	5.4	6.15	5.56	4.0	2.05	16> 1‡	21
—00	—3.79	—2.39	—1.1	—0.48	—0.06	4 —	16
—00	0.2	1.1	1.81	1.56	0.91	8 2	17
—00	0.24	1.52	2.04	1.67	0.88	11 —	Present study
Total	—4.66	3.42	8.4	7.15	4.01		

*The first column is total families and the second is families with known or suspected male transmission.

†Excluding Camerino *et al*,¹¹ pedigree A.

‡Including Camerino *et al*,¹¹ pedigree A.

DXS51—F9—DXS15—F8, DXS52. This would indicate that, currently, the physically closest probes to *FRAXA* are *F9* and *DXS15*. Drayna and White²² found 17 recombinants in 57 meioses informative for *F9* and *DXS52* and 13 recombinants in 33 meioses informative for *F9* and *DXS15*. These figures are similar to those found in the present study, 14 recombinants of 52 meioses and 13 of 42 respectively (table 8), and indicate that linkage might exist between these loci at the limit of measurement. Drayna and White²² have so far observed only one recombinant between *F9* and *DXS51* in 55 meioses and Drayna *et al*³⁵ found two recombinants in 28 meioses. Linkage of these two loci was also seen in the present study but six recombinants in 40 meioses favoured a greater distance apart ($\hat{Z}=3.66$, $\hat{\theta}=0.19$).

The present study found a similar high frequency of recombination in distal Xq in both normal families and in families with the fragile X syndrome. This would not support either the hypothesis that recombination was suppressed in this area in fragile X families²⁰ or that it was abnormally increased in these families.¹⁴ Close linkage was apparent for the *F8/G6PD* cluster with no recombination observed in the present study between *DXS15* and *DXS52* in 43 meiotic events ($\hat{Z}=12.25$, $\hat{\theta}=0$, confidence interval 0 to 0.05). Thus, these data would favour a subtelomeric cluster with tight linkage and then a region with a particularly high rate of recombination which declines towards the centromere. This conclusion is supported by other investigators.^{14 22 36} The similarity of this pattern to autosomal chiasma distributions³⁷ suggests that this may be a general feature of meiotic recombination.

The loci for deutan and protan colour blindness (*CBD* and *CBP*) are believed to lie on either side of the *G6PD* locus on the basis of a single recombinant in a Sardinian family, but the most distal locus is unknown.^{36 38} The observed phase known recom-

bination between *DXS52* and *CBP* in the present study suggests that *CBP* lies distal to *DXS52*. Janco *et al.*³⁹ found no recombinants between *DXS52* and *F8* in 118 meiotic events, and no recombinants were observed between *F8* and *G6PD* in 58 meioses.²⁰ Thus, it seems likely that *CBP* lies on the telomeric side of *G6PD* (and *F8*). The situation may be more complicated if there is a paracentric inversion in the *G6PD* region in Sardinian populations as proposed by Purrello *et al.*³⁸ These authors sought to explain the observed linkage disequilibrium between *G6PD* and *CBP*, but not *CBD*, in Sardinians⁴⁰ and proposed that such a paracentric inversion would suppress crossing over in this area of the chromosome and enhance the known positive adaptiveness of *G6PD* deficiency. This hypothesis might also explain the anomalous order of loci suggested by Purrello *et al.*⁴¹ on the basis of one multiply informative Sardinian family which favoured the order centromere—*HGPRT*—*G6PD*—*FRAXA*.

Population differences might exist which would explain the discrepancies in linkage estimates from Southern France and Sardinia with other areas. The suggestion of Brown *et al.*¹⁷ that *FRAXA* is tightly linked to *F9* only in families with normal transmitting males might also account for some of the differences, or there might be heterogeneity of molecular pathology which alters linkage estimates. However, whichever reason is correct, the conclusion from this study is that in these families at least the frequencies of recombination between the studied flanking markers are too high to be of clinical value for carrier detection or prenatal diagnosis.

We wish to acknowledge the help of the late Dr S Walker in obtaining samples from families 665 and 3653. We are also indebted to the following scientists for the gifts of DNA probes used in this study: Professor G G Brownlee, Dr K Davies, Dr M H Hofker, Dr R Lawn, Dr J-L Mandel, Dr D Page, and Professor P Pearson. We are also grateful to the Wellcome Trust, the Scottish Hospital Endowments Research Trust, and Action Research for financial support.

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Details of the results of DNA analyses on these families are available from the authors on request.