Linkage and recombination between fragile X-linked mental retardation and the factor IX gene

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Summary. Linkage analysis on a family with fragile X-linked mental retardation was performed using a Taq I restriction fragment length polymorphism detected by a cloned human coagulation factor IX cDNA. Two affected brothers in this sibship were found to have different factor IX RFLP alleles, indicating a recombinational event occurred between the two genes. Our data therefore indicate that the gene responsible for fragile X-linked mental retardation is not as tightly linked to the factor IX gene as the previously published data may suggest.

Introduction

A major cause of genetically determined mental retardation is fragile X-linked mental retardation [fra(X)-linked MR]. This condition results in moderate to profound mental retardation in hemizygous males and is associated with a fragile site near the vicinity of band Xq27 (Jacobs et al. 1980; Turner and Jacobs 1983). Since carrier detection and prenatal diagnosis of this condition are associated with technical and penetrance problems (Glover 1983; Sherman et al. 1984), close linkage of a marker gene to fra(X)-linked MR would be of considerable benefit.

The human coagulation factor IX gene recently has been cloned and mapped to the region from Xq27 to Xq28 by several laboratories, including our own, and a common Taq I restriction fragment length polymorphism (RFLP) has been identified (Karachi and Davie 1982; Choo et al. 1982; Jaye et al. 1983; Camerino et al. 1984; Jagadeeswaran et al. 1984a). Camerino et al. (1983) recently reported linkage between the fra(X)-linked MR gene and the factor IX Taq I RFLP. Out of 17 informative meioses in two families, recombination between these genes was not observed, resulting in an estimate of the genetic distance as less than 12 centimorgans (cM). However, in the absence of recombination, it was not clear how tightly linked these genes are.

We have carried out linkage analyses with a factor IX cDNA probe in families with fra(X)-linked MR and have identified a recombinant individual. Thus, our data indicate that the fra(X)-linked MR and factor IX genes are not as close as the data of Camerino et al. (1983) might suggest.

Materials and methods

Cytogenetic analyses. Heparinized blood was cultured by conventional whole blood microculture techniques for 96h. To induce expression of the fra(X) site, cells were grown in either RPMI 1640 medium with 5% fetal bovine serum (FBS) but without folate (Invitrogen), or in folate containing RPMI 1640 medium with 10% FBS to which 0.1 uM 5-fluorodeoxyuridine (FUDR) was added at the beginning of the culture period (Glover 1981).

Epstein-Barr virus (EBV) transformed lymphoblastoid cell lines were established from some family members by the method of Jacobs et al. (1982). For fra(X) expression, lymphoblastoid cells were cultured in RPMI 1640 medium with 10% FBS. FUDR (0.1 uM) was added 24h prior to chromosome harvest. For all cultures, air dried slides were stained with Giemsa or Q-banded and at least 50 cells scored for fragile X expression.

DNA isolation and Southern blot analyses. High molecular weight DNA was isolated from either lymphocytes or lymphoblastoid cells and subjected to Southern blot analyses (Southern 1975), using as probe a 1.0kb Hpa II fragment, containing the factor IX cDNA insert (Jagadeeswaran et al. 1984).

Lod score calculations. The relative probability of linkage and lod scores were calculated at various recombination intervals from 0 to 50% (Morton 1955; Conneally and Rivas 1980).

Results and discussion

Figure 1 shows the pedigree of the family described in this report. This family, of English/Irish descent, was ascertained through individuals III-2 and III-3, who were diagnosed as having fra(X)-linked MR on the basis of profound psychomotor retardation and expression of the fragile site at Xq27. The fragile site was expressed, with FUDR induction, in both lymphocytes and lymphoblastoid cells from both individuals (III-2, 22–25% fra(X) expression; III-3, 14–23% fra(X) expression). A phenotypically normal brother, III-4, did not express the fra(X) in 10 lymphocyte metaphases examined. Two female siblings were mentally normal. However, one sister (III-5) was determined to be a carrier of fra(X)-linked MR on the basis of expression of the fra(X) site (10–27% fra(X) expression in lymphocytes and lymphoblastoid cells).

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The elder sister, III-1, was chromosomally normal with 200 mitoses scored negative for fra(X) expression. The mother of this sibship, II-2, was designated an obligate heterozygote, with 5-8% expression of the fra(X) in both lymphocytes and lymphoblastoid cells. She is mentally normal as is the father, II-1, who did not express an observable fra(X) site out of 100 lymphocyte metaphases examined. The maternal grandparents, I-1 and I-2, are both phenotypically normal with no evidence of mental deficiency and neither grandparent expressed the fra(X) chromosome (100 lymphocyte metaphase scored for each). There was no additional evidence of mental retardation or fra(X)-linked MR in the extended pedigree.

Fig. 2. Hybridization of Taq I-digested DNA from individuals shown in Fig. 1 with human factor IX cDNA clone. Lane numbers correspond to pedigree numbers in Fig. 1 and the polymorphic 1.8kb and 1.3kb bands are labeled A and a, respectively

| Table 1. Linkage data between fragile X-linked mental retardation and the factor IX gene |
|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| Q    | Lod scores from current study | Data from Camerino et al. (1983) | Total lod scores |
| 0.00 | -                             | 3.12                          | -              |
| 0.01 | -1.49                        | 5.04                          | 3.64           |
| 0.05 | -0.72                        | 4.74                          | 4.02           |
| 0.10 | -0.44                        | 4.34                          | 3.96           |
| 0.15 | -0.20                        | 3.92                          | 3.63           |
| 0.20 | -0.19                        | 3.47                          | 3.28           |
| 0.25 | -0.13                        | 3.00                          | 2.87           |
| 0.30 | -0.08                        | 2.48                          | 2.41           |
| 0.35 | -0.04                        | 1.94                          | 1.90           |
| 0.40 | -0.02                        | 1.35                          | 1.33           |

Data are expressed as lod scores (log of the odds) for different values of the recombination fraction (Q). The total number of informative kindreds for the total lod scores is 3 and the 95% confidence intervals of Q is 0.01 to 0.23. Data for the current study are calculated at phase unknown and excluding III-1.

1.8kb allele (A), since this phase corresponds to a single recombination in generation III, as opposed to three recombinational events out of four meioses if the phase were trans. If the fra(X)-linked MR gene in II-2 were inherited from the grandmother (I-2), as opposed to a new mutation in II-2, the
lack of expression of the fragile site in I-2 is consistent with her normal intelligence, since there is an inverse correlation between fra(X) expression and intelligence in carrier females (Turner and Jacobs 1983).

Lod scores for this family as well as cumulative values including the two previously reported families (Camerino et al. 1983) are shown in Table 1. Since the phase of the genes in I-2 is uncertain, lod score calculations were performed assuming phase unknown. Additional stringency in this calculation was introduced by excluding the apparently normal daughter (I-1), since she could be a non-penetrant carrier (Sherman et al. 1984). The peak lod score of 4.02 corresponding to θ of 0.05 suggests that these two genes may be approximately 5 cM apart (95% confidence interval of 1 to 21 cM) and the odds favoring measurable linkage being 3350:1. Although more data is required to verify this distance, the recombination reported here clearly places the gene responsible for fra(X)-linked MR farther from the factor IX gene than the previous data might suggest.

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References


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Note added in proof

Since the submission of this manuscript other investigators have observed recombination between fra(X)-linked MR and the factor IX gene (Choo et al. 1984).