Assignment of Emery-Dreifuss Muscular Dystrophy to the Distal Region of Xq28: The Results of a Collaborative Study

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Summary

Emery-Dreifuss muscular dystrophy (EDMD) is an X-linked humeroperoneal dystrophy associated with cardiomyopathy that is distinct from the Duchenne and Becker forms of X-linked muscular dystrophy. Linkage analysis has assigned EDMD to the terminal region of the human X chromosome long arm. We report here further linkage analysis in two multigenerational EDMD families using seven Xq28 marker loci. Cumulative lod scores suggest that EDMD is approximately 2 cM from DXS52 (lod = 15.67) and very close to the factor VIII (F8C) and the red/green color pigment (R/GCP) loci, with respective lod scores of 9.62 and 10.77, without a single recombinant. Several recombinations between EDMD and three proximal Xq28 markers suggest that the EDMD gene is located in distal Xq28. Multipoint linkage analysis indicates that the odds are 2,000:1 that EDMD lies distal to DXS305. These data substantially refine the ability to perform accurate carrier detection, prenatal diagnosis, and the presymptomatic diagnosis of at-risk males for EDMD by linkage analysis. The positioning of the EDMD locus close to the loci for F8C and R/GCP will assist in future efforts to identify and isolate the disease gene.

Introduction

Dreifuss and Hogan (1961) and Emery and Dreifuss (1966) reported an X-linked form of muscular dystrophy with onset in late childhood. The disease is characterized by slowly progressive muscle wasting and weakness with humeroperoneal distribution in the early stages of the disease, early contractures of the elbows, Achilles tendons, and postcervical muscles. Cardiomyopathy is present as a progressive A-V atrioventricular block eventually requiring ventricular pacing (Emery 1987). Cardiac conduction abnormalities were observed in one of six female carriers of Emery-Dreifuss muscular dystrophy (EDMD) described by Hopkins et al. (1981).

EDMD can be distinguished from the Duchenne and Becker forms of muscular dystrophy (DMD and BMD, respectively) by the absence of muscle pseudo-hypertrophy and the distribution of involved muscles. Early flexion contractures of the elbows and atrial conductive defects further differentiate these muscular dystrophies. Mental retardation, commonly observed in DMD, is not found in EDMD. EDMD can be clearly distinguished by genetic linkage from both DMD and BMD, which are allelic and map to Xp21. Thomas et al. (1972) described linkage between color blindness, which maps to Xq28, and a myopathy with contractures which is now thought to be EDMD (Goldblatt et al. 1989). This assignment was subsequently confirmed by demonstration of linkage to DXS15 (Boswinkel et al. 1985) and DXS52 and factor VIII (F8C) (Hodgson et al. 1986a, 1986b; Thomas et al. 1986; Yates et al. 1986; Romeo et al. 1988).

We report here the expanded study of the exception-
ally large, multigenerational pedigree reported by Thomas et al. (1986), as well as the study of another EDMD pedigree not previously studied by linkage analysis. Seven Xq28 loci, including one having a newly identified VNTR polymorphism (DXS455; Consalez et al., submitted), were used to assign EDMD to distal Xq28.

**Subjects and Methods**

**Families**

The two families studied live in north Georgia (fig 1, family 1) and Alabama (fig. 1, family 2). Families 1 and 2 were described by Waters et al. (1975), Wright and Elsas (1980; family 1), and later Hopkins et al. (1981). Linkage studies in a portion of family 1 have been published (Thomas et al. 1986). Affected males in both families presented as typical EDMD with abnormal electrocardiograms and elevated creatine phosphokinase levels. All family members studied in this report were reexamined, including those previously reported by Thomas et al. (1986). Penetrance was assumed complete in adult males (20 or older) and null in carrier females. Presymptomatic males or males less than 20 years old with insufficient clinical signs for diagnosis were excluded, and only obligate carrier females, based on transmission of EDMD, were included in linkage calculations. However, linkage studies were performed on numerous at-risk females and presymptomatic males for genetic counseling purposes and will, in time, contribute significantly to the data reported below.

**DNA Studies**

DNA was isolated from peripheral blood by a modification of the method described by Miller et al. (1988). Immortalized lymphoblastoid cells were established for 59 individuals as described elsewhere (Neitzel 1986). Restriction digestion was carried out according to the manufacturer’s recommendations, and Southern blotting who performed as described elsewhere (Southern 1975) using nylon membrane. Probe DNA was oligo-labeled according to the method of Feinberg and Vogelstein (1983), and hybridization was carried out at 65°C (Sambrook et al. 1989). Xq28 marker loci were genotyped with the following probes: U6.2 (DXS304), 346.8 and 346.72 (DXS455), 1A1 (DXS305), Dx13 (DXS15), Sr14.1 (DXS52), F8 (F8C), and Hs7 (red/green color pigment [R/GCP] locus). Specific restriction endonucleases are shown in table 1. Probe 346.8, detecting locus DXS455, is a 1.4-kb BstYI fragment cloned into the BamHI site of the pBluescript SK-polyn linker (Stratagene Cloning Systems). A repeat-free 1.1 HindIII fragment was used to detect a multiallelic BclI polymorphism. The three alleles observed in the two families reported here are 8.3, 8.0, and 7.7 kb in size; an example is shown in figure 2. Probe 346.72, a 750-bp Sau3AI fragment cloned into the BamHI site of pBluescript 1, detects both the BclI polymorphism (above) and a BsrYI polymorphism with bands of 4.3, 4.0, and 3.7 kb. A more complete characterization of this multiallelic locus will be described elsewhere (Consalez et al., submitted).

**Linkage Analysis**

Linkage data were entered with the program LINKSYS version 4.11 (Attwood and Bryant 1988) and were double-checked by hand and by using the “knowledge base” routine to minimize the occurrence of logical and typing errors. Linkage analysis was performed using the LINKAGE software package (version 4.7) as indicated elsewhere (Lathrop et al. 1984, 1985; Lathrop and Lalouel 1984, 1988). The routine MLINK was used to calculate lod scores correspond-

**Table 1**

**Probes Used and Relative Information Contents**

<table>
<thead>
<tr>
<th>Probe</th>
<th>Locus</th>
<th>RLFP</th>
<th>No. of Alleles</th>
<th>PIC</th>
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<tr>
<td>U6.2</td>
<td>DXS304</td>
<td>BclI</td>
<td>2</td>
<td>.26</td>
</tr>
<tr>
<td>346.8</td>
<td>DXS455</td>
<td>BclI</td>
<td>5</td>
<td>.70</td>
</tr>
<tr>
<td>1A1</td>
<td>DXS305</td>
<td>TaqI</td>
<td>2</td>
<td>.35</td>
</tr>
<tr>
<td>Dx13</td>
<td>DXS15</td>
<td>BglII</td>
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<td>.36</td>
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<tr>
<td>Sr14</td>
<td>DXS52</td>
<td>TaqI</td>
<td>7</td>
<td>.74</td>
</tr>
<tr>
<td>F8</td>
<td>F8C</td>
<td>BclI</td>
<td>2</td>
<td>.33</td>
</tr>
<tr>
<td>Hs7</td>
<td>R/GCP</td>
<td>SstI</td>
<td>3</td>
<td>.50</td>
</tr>
</tbody>
</table>

Note.—Other polymorphisms used included F8-BglII, F8-MspI, 346-BglII, and 346-BsrYI.
ing to recombination fractions (θ's) between 0 and .2, at increments of .01. Given the size of our data set (up to 162 individuals), approximate 90%–95% confidence intervals were calculated on four markers as described elsewhere (Conneally et al. 1985). For three- and four-point linkage analysis (Lathrop et al. 1984), the ILINK and LINKMAP programs of the LINKAGE package were used. With LINKMAP, genetic distances were derived by using Haldane’s mapping function. The VMS version of LINKAGE was used in a mainframe computer to increase efficiency and speed with some constants modified in the course of compilation of the Pascal source file.

Results

The families studied are shown in figure 1. Previously reported individuals in family 1 were re-examined, particularly those males who appeared unaffected during prior examinations and those individuals who were classified as recombinants. One male (VII-120 in fig. 1) had been previously classified as normal at age 17 and subsequently reported by Thomas et al. (1986) as a recombinant between EDMD and DXS52/DXS15. Rereevaluation at age 21 showed clear signs of early involvement of the disease, such as contractures of the elbow and lower spine, first-degree atrioventricular heart block (PR interval of 0.24 ms), and elevated creatine phosphokinase levels (282 U/l; normal range 30–170 U/l). This individual has been therefore reclassified as affected and a nonrecombinant at the above loci. It should be noted that the other male previously reported as unaffected and a recombinant between the above loci (VII-125 in fig. 1) was rereevaluated at age 29 and remains normal with no neuromuscular or cardiac abnormalities noted. However, the reported recombination event between DXS52-DXS15 and EDMD in this individual is now known to be due to sample error. Likewise, several other individuals in family 1 who had previously been reported as recombinants with R/GCP locus (Thomas et al. 1987) have also been retyped and shown to have been misclassified because of sample error. As a result, R/GCP and EDMD are now linked with a peak lod score of 10.77 at no recombination. All data conflicting with previous reports (Thomas et al. 1986, 1987) were double-checked independently by both groups participating in this study. Given the above corrections of previous mistypings, the distances established at HGM10 by Keats et al. (1989) between EDMD and R/GCP, as well as between EDMD and DXS52/DXS15, are no longer valid.

Table 1 shows the marker loci examined and the RFLPs used. This study reports a revision of previously published data on family 1, as well as the addition of 78 new individuals in that family and of 26 members of family 2. DNAs were prepared and, in most instances, lymphoblastoid cells established (59 individuals). The revision of the data by Thomas et al. (1986) and the addition of new individuals from families 1 and 2 resulted in peak lod scores of 7.47, 13.71, 6.12, and 10.77 (with no recombinants) for DXS15, DXS52, F8C, and R/GCP, respectively, in our families (table 2).

Table 3 shows the combination of our DXS52, DXS15, and F8 data with that previously published by Yates et al. (1986) and Hodgson et al. (1986a). (Family 3 described by Hodgson et al. [1986a] was excluded from these calculations because of the unusual clinical picture. This family was described to include calf hypertrophy, absent cardiac involvement, unusual muscle involvement, and very markedly elevated creatine phosphokinase values, none of which are observed in typical EDMD patients. Furthermore, autosomal inheritance could not be ruled out because of the small family size.) Table 4 shows the peak lod scores and approximate 95% confidence intervals resulting from these combinations (Conneally et al. [1985] suggest that a 95% or higher confidence interval can be obtained from the study of large data sets: the combined data reported here include 162 individuals and six families). Also shown is the approximate 90% confidence interval relative to R/GCP-EDMD, obtained from calculations for family 1 of this study. Of the loci presented in table 4, only R/GCP and F8C do not show any recombinations with EDMD. However, neither in this study nor in any of the previous reports was it possible to document a recombination event between DXS52-DXS15 and F8-R/GCP in an Emery-Dreifuss family. Therefore, the order of these loci versus the EDMD gene is still unclear.

Three recently identified Xq28 loci (DXS304, DXS455, and DXS305) were also tested (table 2), although their order could not be established based on our family data alone. However, previous studies (Vincent et al. 1989b), ongoing CEPH family studies (N. B. Freimer, S. T. Warren, and T. C. Gilliam, unpublished data), and the analysis of several breakpoints in fragile X families (Consalez et al., submitted) suggest the order FRAXA-DXS304-DXS455-DXS305-DXS52.
Figure 1  Pedigrees of two families segregating EDMD studied in this report. Affected males and carrier females are indicated by closed and partially closed symbols, respectively.
as the most likely order. These loci have been shown to map close to the fragile X syndrome (Dahl et al. 1989; Patterson et al. 1989; Vincent et al. 1989; Rousseau et al., in press), proximally to DXS52, DXS15, F8C, G6PD, and R/GCP. Locus DXS304 shows two recombination events with EDMD in 15 informative meioses, yielding a $Z_{max}$ of 1.42 at a $\theta$ of .12. With DXS455, a peak lod score of 0.40 was obtained at a $\theta$ of .20. Only one recombination event in 16 informative meioses was documented in family 1 between EDMD and locus DXS305, giving a peak lod score of 2.32 at a $\theta$ of .06.

For multipoint linkage, 0's between DXS305 and DXS52 and between DXS305 and F8C were established as .03 and .08, respectively (Patterson et al. 1989). A $\theta$ of .06 between DXS52 and F8C was assumed, according to HGM10 (Keats et al. 1989). The order FRAXA-DXS305-DXS52-F8C was preferred to the order FRAXA-DXS305-F8C-DXS52 (Patterson et al. 1989; Keats, personal communication). LINKMAP was used to establish the most likely location of EDMD with respect to DXS304-DXS305-DXS52-F8C (Lathrop et al. 1984) in families 1 and 2 of this study, families 1 and 2 of Yates et al. (1986), and families 1 and 2 of Hodgson et al. (1986a). This analysis (fig. 3) shows that EDMD is about four times more likely to map distal to DXS52 than between DXS52 and DXS305 (future study with proximal markers of family 1 described by Yates et al. [1986] may clarify this point). Locations for EDMD proximal to DXS305 or to DXS304 are, respectively, 2,454 and 229,000 times less likely than the most probable location. An antimode in correspondence to the F8C locus is not observed because of the absence of recombinants in the six families analyzed. DXS15 was not tested for multipoint linkage, since it showed no recombinants.

**Table 2**

<table>
<thead>
<tr>
<th>Locus</th>
<th>.001</th>
<th>.05</th>
<th>.10</th>
<th>.15</th>
<th>.20</th>
<th>.30</th>
<th>.40</th>
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<tbody>
<tr>
<td>DXS52</td>
<td>13.71</td>
<td>12.62</td>
<td>11.48</td>
<td>10.28</td>
<td>9.02</td>
<td>6.29</td>
<td>3.21</td>
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<td>R/GCP</td>
<td>10.77</td>
<td>9.74</td>
<td>8.67</td>
<td>7.54</td>
<td>6.35</td>
<td>3.80</td>
<td>1.11</td>
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<td>7.47</td>
<td>6.89</td>
<td>6.27</td>
<td>6.53</td>
<td>4.94</td>
<td>3.44</td>
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<tr>
<td>F8C</td>
<td>6.12</td>
<td>5.60</td>
<td>5.05</td>
<td>4.49</td>
<td>3.91</td>
<td>2.65</td>
<td>1.26</td>
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<tr>
<td>DXS305</td>
<td>$-\infty$</td>
<td>2.31</td>
<td>2.25</td>
<td>2.06</td>
<td>1.82</td>
<td>1.26</td>
<td>.65</td>
</tr>
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<td>DXS304</td>
<td>$-\infty$</td>
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<td>1.39</td>
<td>1.24</td>
<td>.75</td>
<td>.13</td>
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<tr>
<td>DXS455</td>
<td>$-\infty$</td>
<td>.71</td>
<td>.07</td>
<td>.33</td>
<td>.40</td>
<td>.29</td>
<td>.08</td>
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**Table 3**

<table>
<thead>
<tr>
<th>Locus</th>
<th>.00</th>
<th>.05</th>
<th>.10</th>
<th>.15</th>
<th>.20</th>
<th>.30</th>
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<tbody>
<tr>
<td>DXS15</td>
<td>$-\infty$</td>
<td>11.03</td>
<td>10.22</td>
<td>9.23</td>
<td>8.13</td>
<td>5.64</td>
<td>2.80</td>
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<tr>
<td>DXS52</td>
<td>$-\infty$</td>
<td>15.22</td>
<td>14.06</td>
<td>12.69</td>
<td>11.21</td>
<td>7.88</td>
<td>4.08</td>
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<tr>
<td>F8C</td>
<td>9.62</td>
<td>8.82</td>
<td>7.99</td>
<td>7.13</td>
<td>6.23</td>
<td>4.30</td>
<td>2.14</td>
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</table>
with any of the other markers tested. Although recombinants, as a single DXS15 and DXS52 haplotype, with EDMD have been previously reported (Hodgson et al. 1986a, family 2), placement of EDMD between DXS52 and DXS15 is unlikely since the physical distance between the DXS52 and DXS15 estimated to be 195 kb (Feil et al. 1990).

Other loci, whose location is not yet well established (DXS455, R/GCP), were used for multipoint linkage with ILINK by adopting two-point data from our families as estimates of genetic recombination between fixed loci and testing EDMD for each interval. Table 5 confirms that the odds against a placement of EDMD proximal to DXS305, DXS455, or DXS304 are quite high. Figure 4, showing individuals from two sibships of family 1 of this study, positions the recombination between DXS455 and R/GCP in one sibship (none of the other five loci was informative in this mating) and between DXS305 and DXS52 in the second one (DXS15 was not informative in this case).

**Discussion**

Seven Xq28 marker loci have been tested in two families segregating EDMD. Data relative to family 1 represent a revision and expansion (78 new individuals) of a previous study by Thomas et al. (1986). Three of the marker loci utilized, DXS304, DXS455, and DXS305 have not previously been used in EDMD linkage studies.

The studies reported above exclude tight linkage between proximal Xq28 and EDMD by finding several recombinations between more proximal Xq28 loci (DXS304-DXS-DXS305) and the disease locus. The suggestion of a distal placement of EDMD is supported by multipoint linkage analysis that excludes a placement of the disease gene proximally to DXS305.

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**Table 4**

<table>
<thead>
<tr>
<th>Locus</th>
<th>( Z_{\text{max}} )</th>
<th>( \theta_{\text{max}} )</th>
<th>Approximate 95% Confidence Interval</th>
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<tr>
<td>DXS52</td>
<td>15.67</td>
<td>.02</td>
<td>.001 to .075</td>
</tr>
<tr>
<td>DXS15</td>
<td>11.28</td>
<td>.02</td>
<td>.001 to .097</td>
</tr>
<tr>
<td>F8C</td>
<td>9.62</td>
<td>.00</td>
<td>.00 to .062</td>
</tr>
<tr>
<td>R/GCP</td>
<td>10.77</td>
<td>.00</td>
<td>.00 to .082</td>
</tr>
</tbody>
</table>

**Table 5**

<table>
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<tr>
<th>Tested Order (EDMD Gene Position)</th>
<th>Odds against Order</th>
</tr>
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<tbody>
<tr>
<td>DXS304-DXS305-(EDMD-R/GCP)........</td>
<td>1:1</td>
</tr>
<tr>
<td>DXS304-EDMD-DXS305-R/GCP..........</td>
<td>3,891:1</td>
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<tr>
<td>EDMD-DXS304-DXS305-R/GCP..........</td>
<td>18,435:1</td>
</tr>
<tr>
<td>DXS455-DXS305-(EDMD-R/GCP)........</td>
<td>1:1</td>
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<tr>
<td>DXS455-EDMD-DXS305-R/GCP..........</td>
<td>4,182:1</td>
</tr>
<tr>
<td>EDMD-DXS455-DXS305-R/GCP..........</td>
<td>72,984:1</td>
</tr>
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</table>

Notes.—\( \theta \)'s between fixed loci were established based on two-point data obtained from families 1 and 2 of this study. EDMD was tested for each interval.

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**Figure 3** Likelihood of map location of EDMD with respect to three linked loci DXS304, DXS305, and F8C. *Horizontal axis,* Genetic distance from locus DXS304. *Left vertical axis,* LOD scores. *Right vertical axis,* Location scores. Dashed line indicates lower limit for location with odds of less than 1,000:1 relative to overall maximum.

**Figure 4** Diagram of two meiotic recombinations between proximal and distal Xq28 marker loci indicating distal placement of EDMD locus.
EDMD Linkage in Distal Xq28

Of the probes and enzymes tested here, those that appear most reliable for clinical applications of two-point linkage analysis are St14/TaqI (locus DXS52), F8/BclI (locus F8C), and DX13/BglII (locus DXS15). Hs7/Sts1 (locus R/GCP), while often informative, results in a complex restriction pattern requiring a cautious interpretation. It should also be noted that a number of phenotypically similar but genetically unrelated defects need to be ruled out clinically, before diagnosis based on linkage is attempted (for a review of these EDMD phenocopies, see Emery 1989).

Assignment of EDMD to distal Xq28 also suggests that EDMD is less likely to be allelic with X-linked myotubular myopathy (MTM1). The absence of recombination between MTM1 and the proximal locus DXS304, as well as the recombination with F8C (Lehesjoki et al. 1990), supports the notion of separate loci in Xq28 for MTM1 and EDMD, as do markedly different clinical pictures. Allelism of EDMD with another Xq28 muscular dystrophy, myopathy with excessive autophagae (Saviranta et al. 1988), cannot be ruled out because of the minimal linkage data available for that disorder.

Physical mapping data by Arveiler et al. (1989) indicate that DXS15 and DXS52 are in close proximity to each other, at a distance greater than 1,500 kb from the F8C locus, whereas the same authors show that F8C and R/GCP genes are within a 1,100-kb region. Further linkage with additional marker loci should allow the fine mapping of EDMD relative to these physical domains.

In summary, the data reported here refine the genetic mapping of EDMD, positioning this locus in distal Xq28. The extensive characterization of two multigenerational EDMD pedigrees, with established lymphoblastoid cell lines for 59 members, provides a significant resource for efforts to isolate the gene responsible for EDMD. Furthermore, the revision and expansion of previously published data, will improve the ability to perform accurate carrier detection, prenatal diagnosis, and the presymptomatic diagnosis of males at risk for EDMD by linkage analysis.

Acknowledgments

This work was supported by grants from the Muscular Dystrophy Association to S.T.W. and L.J.E. (Atlanta) and P.S.H. and N.S.S.T. (Cardiff) and from the National Institutes of Health (HD20521) to S.T.W. The assistance of the Clinical Research Center of Emory University School of Medicine (supported by NIH grant MO1-RR-00039) is gratefully acknowledged. We are indebted to Stephanie Sherman, Jerry Boss, and Angus Clarke for helpful discussion. We thank Meraida A. Polak for her assistance with our patients and their families, and D. Brendan Moriarty for helpful assistance in the adaptation and compilation of the LINKAGE software. We also thank J. Gitschier, K. Davies, P. Drayna, J. L. Mandel, and J. Nathans for probes.

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Consalez GG, Stayton CL, Freimer NB, Goonewardena P, Brown WT, Gilliam TC, Warren ST. Isolation and characterization of a highly polymorphic locus in human Xq28 distal to the fragile X syndrome (submitted)


