Emery-Dreifuss muscular dystrophy: linkage to markers in distal Xq28


Abstract

Emery-Dreifuss muscular dystrophy (EMD) is characterised by (1) early contractures of the Achilles tendons, elbows, and postcervical muscles, (2) slowly progressive muscle wasting and weakness with a predominantly humero-peroneal distribution in the early stages, and (3) cardiomyopathy with conduction defects and risk of sudden death. Inheritance is usually X linked recessive but can be autosomal dominant. Family linkage studies have mapped X linked EMD to the distal long arm of the X chromosome. Precise genetic localisation has been hampered by the rarity of the condition. Published linkage data are only available for 13 EMD families showing unequivocal X linked inheritance and only a minority of these have been comprehensively typed for Xq27-pter and three previously published families typed for additional markers. No recombination was observed with the red/green cone pigment locus, RGCp (lod score, Z = 2.46), the factor VIII coagulant gene locus, F8C (Z = 6.39), or with DXS115 (Z = 4.94). Two recombinants were observed which mapped EMD distal to DXS15 (DX13) and DXS52 (St14) respectively. Multipoint linkage analysis gave odds exceeding 200:1 for EMD being distal to these markers. A multipoint analysis incorporating published data gave the map cen-DXS304-9cM-DXS15-3cM-DXS52-2cM-(RGCp,EMD)-3cM-F8C-2cM-DXS115 with odds of 120:1 in favour of a location for EMD between DXS52 and F8C as compared to the next best position distal to F8C.


Emery-Dreifuss muscular dystrophy (EMD) is characterised by (1) early contractures of the Achilles tendons, elbows, and postcervical muscles, (2) slowly progressive muscle wasting and weakness with a predominantly humero-peroneal distribution in the early stages, and (3) cardiomyopathy with conduction defects. Cardiac involvement carries a substantial risk of sudden death preventable by the insertion of a pacemaker. The clinical features have been described in detail by Emery1 in a recent review. Inheritance is usually X linked recessive (McKusick catalogue no 310300) but can be autosomal dominant (McKusick catalogue no 181350). Family linkage studies have mapped X linked EMD to the distal long arm of the X chromosome. Precise genetic localisation has been hampered by the rarity of the condition. Published linkage data are only available for 13 EMD families showing unequivocal X linked inheritance and only a minority of these have been comprehensively typed for Xq27-pter and three previously published families typed for additional markers.

Patients

Three new families with X linked Emery-Dreifuss muscular dystrophy were studied. The family pedigrees are shown in the figure.
Pedigrees of EMD families 1, 2, and 3. Affected males are shaded and obligate carrier females are indicated by a central dot.

**Family 1**

Family 1 was evaluated by FD. The diagnosis of EMD was based on the findings in the three affected males II-4, II-5, and III-20 of (1) childhood onset of slowly progressive muscle wasting and weakness with a scapulohumero-peroneal distribution, (2) early contractures of the Achilles tendons, elbows, and neck muscles, and (3) cardiac conduction defects (necessitating cardiac pacemaker insertion in II-4 and II-5). Serum creatine kinase levels were normal or mildly raised. A muscle biopsy in II-5 showed dystrophic changes. The pedigree shows unequivocal X linked inheritance. Full details of this family will be published elsewhere.

**FAMILY 2**

Family 2 from England was evaluated by JRWY. The index case, III-3, had difficulty climbing stairs from 3 years of age and was unable to straighten his arms from early childhood. He had lengthening of his Achilles tendons at 9 years of age. First degree heart block with right bundle branch block was diagnosed at 27 years of age. Heart rate varied from 35 to 87 beats per minute with pauses of up to 1-9 seconds. A cardiac pacemaker was inserted. Examination at the age of 31 years showed a waddling gait with lumbar lordosis. There were contractures of the elbows, ankles, and postcervical muscles. There was wasting and weakness most marked in the humeral, pelvic, and peroneal muscles. Tendon reflexes were absent. Spinal flexion and extension were weak. There was no calf hypertrophy. Intellect was normal. Serum creatine kinase was four times the upper limit of normal. EMG was myopathic. Muscle biopsies showed myopathic changes. III-4 suffered a similar but milder pattern of disease. Cardiological assessment at 21 years of age showed first degree heart block and partial right bundle branch block. A cardiac pacemaker was inserted. Examination at the age of 28 years showed a waddling gait with marked lumbar lordosis. There were contractures of the elbows and postcervical muscles. There was weakness of the humeral muscles. In the lower limbs there was marked peroneal weakness and lesser involvement of proximal muscles. Tendon reflexes were absent. Spinal flexion and extension were weak. There was no calf hypertrophy. Intellect was normal. Serum creatine kinase was five times the upper limit of normal. Muscle biopsy showed myopathic changes. II-2 walked on his toes from early childhood with an abnormal gait said to resemble that of the index case. In adult life he complained of episodes of chest pain and at 28 years of age he unexpectedly collapsed dead. If he was indeed affected by EMD then the pedigree shows X linked inheritance.

**Methods**

Blood samples were obtained from available family members, DNA extracted by routine methods, and DNA polymorphisms analysed by Southern analysis or the polymerase chain reaction using standard protocols. The families were typed for the DNA markers DXS304 (probe U6/2/TaqI restriction digest), DXS52 (StuI-1/TaqI), DXS15 (DX13/BglII), RGC (HS7/SacI), F8C (p114.12/Bell), and DXS115 (767/BstXI) as detailed elsewhere. Additional linkage data were obtained by completing the typing of the above markers in two families we have reported previously (EMD Consortium families 5 and 6) and in a
third family reported by Paquis et al.\(^a\) (EMD Consortium family 97). All six families studied met the diagnostic criteria drawn up by the EMD Consortium for families being used in linkage studies in X linked EMD.\(^{12}\) The clinical features in four of the families (EMD Consortium families 5, 6, 9, and 17) were reviewed at the European Workshop on EMD (1991) and accepted as meeting these criteria.\(^{12}\)

Linkage data were analysed using the computer programmes LIPED\(^a\) and LINKMAP\(^b\) for two point and multipoint analyses respectively. Confidence intervals were obtained by taking values of the recombination fraction corresponding to a lod score one unit less than the maximum.\(^{16}\) Published multiple pairwise data were analysed with the computer programme MAP\(^c\) using the Rao mapping function\(^c\) with an interference parameter value of 0.35.

**Results**

Two point lod scores between EMD and marker loci are given in the table. No recombinants were observed with RGCP (HST7), F8C, or DXS115 (767). EMD Consortium family 5 showed a recombinant between EMD and DXS52 (St14-1) as we have previously reported\(^3\) with two affected brothers having different restriction fragment patterns at this locus. They had the same marker patterns for DXS15 (DX13) and F8C but their obligate carrier mother was dead and could have been homozygous at these loci. In the present study these results were confirmed on repeat blood samples and additional typing showed that they were also recombinant for the more proximal marker DXS304 (U6.2) but had the same marker patterns for RGCP and DXS115. Consortium family 9 showed a recombinant between EMD and DXS15 as previously reported\(^d\) with two affected brothers having different restriction fragment patterns at this locus. More proximal markers were also recombinant, DXS52 was uninformative, and F8C was non-recombinant. In the present study these results were confirmed on repeat blood samples and additional typing showed that RGCP was non-recombinant.

For the multipoint analysis we used the marker order DXS304 (proximal), DXS15, DXS52, RGCP, F8C, and DXS115 (distal) with recombination fractions of 0.09, 0.03, 0.02, 0.03, 0.02, respectively, calculated by multiple pairwise analysis of published linkage data.\(^{16}\) This order is consistent with recent physical mapping data\(^{20}\) except that the order of DXS15 and DXS52 is not well established. The pairwise analysis provided weak support for DXS15 being the more proximal marker.

In the LINKMAP analysis the maximum location score was 38.5 with EMD coincident with F8C. This position was favoured by odds of 200:1 compared to a location between DXS52 and DXS15 and by odds exceeding 1000:1 compared to all locations proximal to these markers. If the order of DXS15 and DXS52 were reversed, the maximum location score was 38.6 with EMD coincident with F8C and this position was supported by odds exceeding 1000:1 compared to all locations proximal to DXS15. In the absence of recombinants it was not possible to determine order with respect to RGCP, F8C, or DXS115.

A second LINKMAP analysis incorporated published data for which haplotype information is available, taken from Thomas et al. 1972,\(^2\) family A of Romeo et al. 1988,\(^2\) and Cole et al. 1992\(^2\) (EMD Consortium families 1, 2, 3, and 87). The maximum location score was 66·1 with EMD coincident with RGCP. This position was supported by odds of 120:1 compared to the next highest location score when EMD was coincident with DXS115. The odds against locations proximal to DXS52/DXS15 exceeded 10 000:1.

**Discussion**

The results presented here map the Emery-Dreifuss muscular dystrophy locus (EMD) close to the factor VIII coagulant gene locus (F8C) in Xq28 and are consistent with previously reported data. Until recently there was uncertainty about the order of markers in the vicinity of F8C but from the physical mapping data of Poustka et al.\(^{20}\) it now seems that the most likely order is cen-DXS304-DXS52, DXS15-RGCP-F8C-DXS115-qter. Provided this order is correct, the recombinants reported previously\(^9\) and now confirmed and further characterised in this report map EMD distal to DXS15 (DX13) and DXS52 (St14). This is consistent with the recombinant with DXS52 and DXS15 identified by Hodgson et al.\(^a\) and confirmed in a subsequent report.\(^21\) Taken together these data provide strong evidence for a distal location for the Emery-Dreifuss muscular dystrophy locus within a region estimated at 3 Mb from DX13 to the telenet.

A recombinant recently reported by Cole et al.\(^a\) maps EMD proximal to F8C. This finding has been fully validated by clinical review of key family members, repeat blood sampling, and independent confirmation of the marker.

<table>
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<th>Locus</th>
<th>Lod score (at recombination fraction)</th>
<th>Zmax</th>
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<th>Confidence interval (θ)</th>
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</table>

Two point lod scores between Emery-Dreifuss muscular dystrophy and the DNA markers DXS304 (U6.2), DXS15 (DX13), DXS52 (St14), RGCP, F8C, and DXS115 (767).
typing by a second laboratory. Conflicting data from another family apparently mapping EMD distal to F8C\(^2\) has recently been withdrawn due to the clinical status of the relevant family members.\(^3\)

All the current data are therefore consistent in placing EMD between DXS15/DXS52 and F8C. This location is supported by odds of 120:1 in the LINKMAP analysis presented here incorporating published data for which haplotype information is available. This corresponds to a physical distance of approximately 2 Mb.\(^30\) Further family studies will be needed to confirm F8C as a distal flanking marker. Better localisation will also be dependent on having new markers from this interval so that flanking recombinants can be mapped precisely.

We are most grateful to the families who have cooperated with this study and to the many colleagues who have provided clinical information and helped with the collection of samples, particularly Dr Barbara Badurska, Dr Clare Davison, Dr Graham Cook, Professor Alan Emery, Mrs Marie Ferguson-Smith, Dr Anna Fidzianska, Dr David Gardner-Medwin, Professor Jean Pouget, and Dr Barbara Ryniewicz. For technical help we thank Doreen Jamieson, Susan Newby, and Dr Rob McMahon. The support of the Muscular Dystrophy Group of Great Britain is gratefully acknowledged.