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Genetic Linkage Confirmed between the Locus for Myotonic Dystrophy and the ABH-Secretion and Lutheran Blood Group Loci

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INTRODUCTION

Myotonic dystrophy (dystrophia myotonica) was one of the earliest autosomal diseases of man to be studied for its linkage relationships. In 1954 Mohr [1] examined the blood groups of 27 sibships with myotonic dystrophy, using the clinical data of Thomasen [2] who had made an extensive study of the disease in Denmark. Mohr found evidence suggesting linkage between the locus for myotonic dystrophy and those for the Lewis and Lutheran blood groups; the results, calculated by Penrose's sib-pair method, were significant at the 2% and 3% levels, respectively, though this method may exaggerate the significance[3]. Mohr also showed a highly significant linkage between the Lewis and Lutheran blood groups themselves [1, 4].

The situation was, however, complicated by the discovery that the Lewis red cell antigens were not determined by one locus alone, but were dependent on the locus controlling secretion of the ABH blood group antigens; the supposed Lewis-Lutheran linkage was shown to be a secretor-Lutheran linkage [5]. The possible linkages with myotonic dystrophy remained unconfirmed, and it was the aim of the present work to resolve the question.

A study to determine whether the secretor status of the fetus could be accurately defined by amniocentesis in early pregnancy was undertaken concurrently with the linkage study in the hope of providing a practical application of the linkage in antenatal diagnosis. This appears to be possible and the results have already been reported [6, 7].

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MATERIALS AND METHODS

Clinical Methods

A total of 48 kindreds with myotonic dystrophy was studied; of these 391 persons, 167 were affected. The families were ascertained from two principal sources: those seen at the Johns Hopkins Hospital, principally residents of Maryland and neighboring states, and a group of families living in Indiana, studied originally by one of us (P. R. Dyken) and visited again for the present study. Further families were seen by courtesy of a number of hospitals and physicians. In some instances, the families had already been fully investigated, while in others, many new affected individuals were discovered.

The accurate scoring of individuals as affected or unaffected, of fundamental importance to any linkage study, proved to be a considerable difficulty, for while there was in general little problem in recognizing the affected members, the exclusion of minimal signs of the disease was often difficult, especially in younger members. The following criteria were adopted to minimize error:

1. All individuals, affected and unaffected, were examined personally by at least one of us (P. S. Harper), the only exceptions being three affected patients, documented by medical records, one of whom died before she could be examined.

2. Myotonia of grip and on percussion of the thenar muscles was sought in every case, together with weakness of grip and of facial, jaw, and neck muscles, and other suggestive features. Because the great majority of people were seen in their homes, a full neurological examination was not generally attempted, though in many instances records of such were available.

3. Cataract was looked for by ophthalmoscopy and, in the great majority of individuals, unaffected as well as affected, a slit-lamp examination of the lenses under mydriasis was performed. A portable instrument giving high resolution (Kowa Instrument Company) was used on home visits, and whenever patients could be examined at a hospital or ophthalmologic center, standard fixed instruments were used. In some individuals with otherwise equivocal or absent signs of the condition, the use of the slit lamp enabled a positive diagnosis to be made by recognition of the multicolored crystalline lens opacities characteristic of this disease [8]; in addition, normal individuals with senile cataract or various ocular lesions, which might have been misinterpreted using ophthalmoscopy alone, could be excluded from the affected category in which they might erroneously have been placed.

In spite of the use of these combined criteria, a small number of individuals (14 of 391) who showed equivocal muscular signs or lens opacities of uncertain significance could not be scored conclusively either as affected or unaffected. These were disregarded in the linkage analysis.

Linkage and Statistical Methods

Members of families with myotonic dystrophy were typed for each of the following marker traits: ABO, Rh, MNS, Kidd (Jk), Duffy (Fy), Lutheran (Lu), P, Kell (K), Lewis (Le), secretor (Se), haptoglobin (Hp), transferrin (Tf), and Gc. The linkage computer program of Renwick and Schulze [9] was utilized in the analysis of the genotype data and in the computation of lods for each family. The multipoint mapping program MAPIN [10, 11] was used to provide the probability for each of the various orders of three linked loci, estimates of the map distances for each of the three orders, and the overall probability that all three loci were on the same chromosome.

In the linkage analysis, unaffected individuals were included only if they had reached 35 years of age at the time of physical examination. Although the use of this strict age limit may discard a considerable amount of linkage information, such information as is retained is likely to be more accurate.

RESULTS

The total lods for the linkage between myotonic dystrophy and secretor, and myotonic dystrophy and Lutheran loci are given in table 1 together with those for the already established Lutheran-secretor linkage [12]. Total lods for the other marker loci and myotonic dystrophy are given in table 2. There is no striking evidence of linkage with any markers other than Lutheran and secretor, and indeed close linkage can be excluded in some instances on the basis of highly negative scores.

Odds (prior and posterior) for various hypotheses regarding the synteny of the myotonic dystrophy, Lutheran, and secretor loci are given in table 3. Part A lists the three possible orders of these loci, given that they are all on the same chromosome (3-synteny); part B gives the possibilities if only two of the three loci are on the same chromosome (2-synteny), and part C considers the final alternative that all three loci are not on the same chromosome (asynteny). Column 1 of this table gives the prior odds on synteny, calculated from the relative autosomal lengths [13]; column 2 represents the "change in odds" for each hypothesis as calculated from the total lods in table 1 by the multipoint mapping program. The posterior odds, shown in column 3, are the product of columns 1 and 2. After simplifying the hypotheses into two basic alternatives—either all three loci are on the same chromosome or they are not—the final posterior probability is obtained. The lods obtained in our study provide a final probability of .89 that the three loci, myotonic dystrophy, secretor, and Lutheran, are syntenic.

The most probable map distances for each interval within each of the orders possible under 3-synteny are given in table 4.

Segregation analysis of data on marker loci displaying dominance was performed according to the method described by Morton [14]. There was no distortion of segregation ratios at the Lutheran and secretor loci, nor was there evidence of association between myotonic dystrophy and any of the phenotypes at these loci.*

DISCUSSION

The results of this study strongly suggest, as did those of Mohr [1], that linkage exists between the locus for myotonic dystrophy and those controlling secretor status and the Lutheran blood group system. The probability of 3-synteny from our data is .89. There is no evidence to suggest other factors operating that could mimic linkage, such as direct association of phenotypes or distortion of segregation ratios. However, the problem of misscoring individuals as affected or unaffected, though minimized by strict clinical criteria and the use of an age limit, is impossible to overcome entirely. Such errors will, if present, tend more

* For full results of the segregation analysis and of tests for association, together with complete pedigree and typing data and the calculated lods for individual families, order document NAPS 01686 from ASIS-National Auxiliary Publications Service, c/o CCM Information Corporation, 866 Third Avenue, New York, New York 10022, remitting $2.00 for each microfiche or $5.00 for each photocopy.

TABLE 1

LODS FOR LINKAGE BETWEEN MYOTONIC DYSTROPHY AND THE SECRETOR AND LUTHERAN LOCI

Recombination fraction (θ)	0	0.05	0.10	0.15	0.20	0.25	0.30	0.35	0.40	0.45	0.482	0.495
Map distance (Morgans)	0	0.05	0.10	0.15	0.20	0.25	0.31	0.37	0.44	0.55	0.69	0.95
Myotonic dystrophy–secretor ...	$-\infty$	1.681	2.230	2.242	2.035	1.720	1.354	0.974	0.606	0.276	0.092	0.012
Myotonic dystrophy–Lutheran ..	$-\infty$	0.777	0.875	0.848	0.749	0.605	0.439	0.275	0.133	0.036	0.012	0.001
Secretor-Lutheran	$-\infty$	12.479	17.724	18.256	16.673	14.138	10.794	7.183	3.749	1.103	0.368	0.046

NOTE.—The lods for Se:Lu reflect published and unpublished data from many laboratories, particularly those of Drs. Mohr, Race, Greenwalt, Cleghorn, Lamm, Harris, Lawler, and Renwick.

TABLE 2

SUMMED LODS FOR LOCI OTHER THAN SECRETOR AND LUTHERAN

LOCUS	INFORMATIVE FAMILIES	RECOMBINATION FRACTION θ					
		0	.05	.10	.20	.30	.40
ABO	34	$-\infty$	−11.536	−6.707	−2.604	−.921	−0.216
Rh	31	$-\infty$	−10.868	−5.929	−1.976	−.549	−0.089
P	36	$-\infty$	−5.339	−2.951	−0.979	−.275	−0.056
MNS	34	$-\infty$	−16.900	−10.182	−4.473	−1.982	−0.664
K	20	$-\infty$	−2.558	−1.438	−0.480	−0.096	+0.034
Fy	27	$-\infty$	−8.042	−4.281	−1.157	−.063	+.128
Tf	2	−.004	+.014	+.024	+0.029	+0.020	+0.007
Hp	28	$-\infty$	−6.582	−3.192	−0.673	+0.072	+0.157
Gc	27	$-\infty$	−5.307	−2.892	−0.861	−.123	+.072
Jk	29	$-\infty$	−2.637	−1.556	−0.630	−.244	−.070

often to mask linkage than to favor it, and it is perhaps surprising that in Mohr's original study, which included individuals of all ages and used rather variable clinical criteria, the data actually suggested linkage.

This linkage is the first to be established for a serious human autosomal disease, and it gives an opportunity for practical application in genetic counseling and antenatal diagnosis, as was predicted in general terms by Edwards [15] and Renwick [16]. In genetic counseling of the young and apparently unaffected individuals at risk, linkage information will be of undoubted value, particularly when

TABLE 3

PROBABILITY OF SYNTENY AMONG MYOTONIC DYSTROPHY, SECRETOR, AND LUTHERAN LOCI

Order of Loci	Prior Odds (1)	Change in Odds (2)	Posterior Odds (3)		Posterior Probability (4)
A. 3-synteny:					
Secretor–myotonic dystrophy–Lutheran	1	0.999×10^{19}	0.999×10^{19}	4.06×10^{19}	.89
Myotonic dystrophy–Lutheran–secretor	1	1.376×10^{19}	1.376×10^{19}		
Lutheran–secretor–myotonic dystrophy	1	1.685×10^{19}	1.685×10^{19}		
B. 2-synteny:					
Lutheran–secretor (myotonic dystrophy asyntenic)	46	11.05×10^{16}	0.51×10^{19}	0.51×10^{19}	.11
Myotonic dystrophy–secretor (Lutheran asyntenic)	46	29.6	1,361		
Myotonic dystrophy–Lutheran (secretor asyntenic)	46	2.3	106		
C. All three loci asyntenic	759	1	759		

TABLE 4

BAYESIAN ESTIMATES OF MAP DISTANCES FOR THE Dm-Lu-Se TRIPLET

Postulated Order of Loci	Relative Odds	Loci	Joint Modal Estimate of Map Distances (Morgans)	95% Probability Limits (Morgans)
Se-Dm-Lu	1	Se-Dm	.08	.02–.14
		Dm-Lu	.05	.01–.12
		Se-Lu	.13	.09–.20
Dm-Lu-Se	1.4	Dm-Lu	.05	.00–.21
		Lu-Se	.13	.07–.19
		Dm-Se	.18	.11–.37
Lu-Se-Dm	1.7	Lu-Se	.13	.07–.19
		Se-Dm	.11	.01–.26
		Lu-Dm	.24	.13–.42

used in conjunction with data on penetrance at different ages. In antenatal diagnosis it now seems possible [6, 7] to establish the secretor status of the fetus in early pregnancy, though this application of linkage data is limited by the necessity of the correct genotypes being present and by our present inability to distinguish between the heterozygous and homozygous secretor phenotypes.

The present linkage represents a new example of a three-point linkage in man, but the gene order is by no means certain at present; the orders Lu-Se-Dm and Dm-Lu-Se are, respectively, only 1.7 and 1.4 times more likely than the order Se-Dm-Lu. As yet a chromosome assignment cannot be made; the only marker chromosome so far recognized in the present study was a D/D translocation in one family (family 8) previously studied [17], and which appears to have its origin outside the affected line of ancestry.

The present data are insufficient to give an accurate estimate of the difference in recombination fraction between the sexes. This phenomenon, first recognized in man for the Lutheran-secretor linkage by Cook [18] and for the ABO-nail-patella syndrome linkage by Renwick and Schulze [19], must be taken into account when linkage is used in genetic counseling; pooling of comparable data will be essential if this is to be done for myotonic dystrophy.

SUMMARY

A genetic linkage study of 48 kindreds with myotonic dystrophy has shown strong evidence of linkage between this locus and those for ABH blood group secretion and the Lutheran blood group, confirming the original suggestion of Mohr that these loci might be linked. The probability of all three loci being on the same chromosome is .89 on the present data alone; the most probable order of the loci is myotonic dystrophy-secretor-Lutheran, and the most probable map intervals, assuming this order, are .11 Morgans between myotonic dystrophy and secretor loci and .24 Morgans between myotonic dystrophy and Lutheran loci. This information, when combined with the determination of secretor status of the

fetus from amniotic fluid in early pregnancy, may allow a prenatal prediction of myotonic dystrophy in families with appropriate genotypes.

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