PRENATAL PREDICTION OF AUTOSOMAL DOMINANT DISEASES BY LINKAGE STUDIES: MYOTONIC DYSTROPHY

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Remarkable progress in adapting specific chromosomal and biochemical tests to amniotic fluid samples has allowed intrauterine diagnosis for an increasing number of inherited conditions. However, the lack of any specific tests for autosomal dominant diseases, especially those affecting the nervous system and muscle, has precluded such prenatal evaluation in genetic counseling.

Genetic linkage offers a potentially useful alternative approach to such diseases. The gene for a particular disorder must be closely linked to a genetic marker which can be analyzed in amniotic fluid or cells obtained in the second trimester of pregnancy.

We have recently applied linkage studies to the prediction of myotonic dystrophy (Dm) *during pregnancy.* Dm *is known to be closely linked to the secretor locus* (Se), *which determines the secretion of ABH blood-group substances into saliva and other body fluids, including the amniotic fluid of the fetus.*

Only certain families will be suitable for this analysis: the affected parent must have the secretor-positive phenotype and be heterozygous (Se/se) *at the secretor locus; the coupling of the* Dm *allele to the* Se *or* se *allele must be determined; and the spouse must be either secretor-negative or heterozygous secretor-positive. Secretor genotypes can be inferred from secretor phenotypes of close relatives, including the fetus at risk.*

Unfortunately, these criteria will be satisfied in only 5-10 percent of couples at risk to transmit myotonic dystrophy. In suitable couples, however, linkage analysis can be quite helpful.

Autosomal dominant disorders of the nervous system and muscle present major difficulties in genetic counseling, because of the delayed age of onset of clinical manifestations, incomplete penetrance, and the almost total lack of diagnostic tests for the asymptomatic carrier of the abnormal gene. Thus, families with Huntington's disease, myotonic dystrophy, olivo-pontine or spino-cerebellar degenerations, and other diseases, come for genetic counseling when a middle-aged parent has become affected but long before we can advise the adult children whether they are at risk to pass the disease on.

We know little of the biochemistry of autosomal dominant diseases, except for certain hemoglobinopathies. Mutations of the alpha or beta chain subunits lead to unstable tetrameric hemoglobin molecules. If one subunit of a multisubunit protein is abnormal, therefore, the entire protein structure is abnormal. We may predict confidently that similar dominant expression of abnormal protein subunits will be found among collagen, actin and myosin, microtubular, and membrane proteins. Unfortunately, techniques for analysis of the detailed

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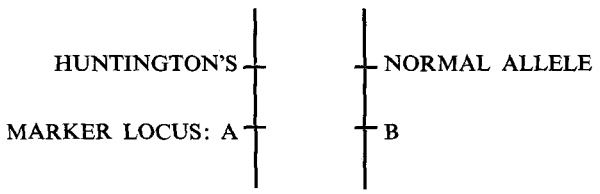
structure and function of poorly soluble complex proteins and of membrane components involved in neurotransmitter reuptake mechanisms, for example, are not yet available. In the absence of basic knowledge of the pathogenesis of these diseases, early diagnosis depends upon recognition of very mild abnormalities in cognitive, motor, coordinating, visual, or electromyographic functions. An indirect alternative approach, though limited in its application, will be discussed here: detection of gene carriers by linkage analysis.

The basis of a linkage approach is the expectation that the gene for a particular disease may be situated on any given chromosome close enough to another gene locus, whose product is detectable, that recombination between the two loci is infrequent. If the genes were on different chromosomes or far apart on the same chromosome, the alleles (forms of the gene) at the loci on the homologous chromosomes would segregate independently. For example, a mother might transmit the allele for Huntington's disease together with allele *A* at the marker locus, while the father might transmit the normal allele (not causing Huntington's) together with allele *B* at the marker locus. If the two loci are close together, then the two homologous chromosomes in the child can be distinguished:



The further apart the two loci, the more likely is recombination to occur, with a reversal of the " coupling " of the Huntington's alleles and the marker alleles. For linkage purposes, it does not matter which marker allele is coupled to the Huntington's allele and which to the normal allele at the Huntington's locus; however, key individuals in the pedigree must be heterozygous at the marker locus, so that the two chromosomes can be distinguished.

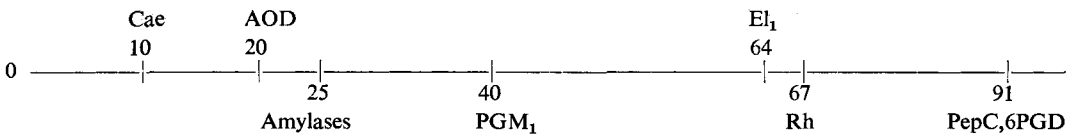
Obviously, the human genome is very large, and the likelihood that close linkage will be found for any particular disease under study is small. There are 22 pairs of autosomes and one pair of sex chromosomes (XX, XY) in normal humans. A middle-sized chromosome like the X chromosome is large enough (250 centimorgans) that two loci would have to be within a segment corresponding to only about 1/10 of its length in order to detect linkage (Sanger and Race 1970). Nevertheless, there is renewed optimism about linkage studies with the rapid growth in the number of potential marker loci (McKusick et al. 1972, Ruddle 1972 and 1973). New antigens and electrophoretic variants of enzymes have been found with common variation (polymorphisms) in human population studies, and work on somatic cell hybridization has localized a number of markers and pairs of markers to specific human chromosomes. The recently-described chromosome banding techniques may provide polymorphic morphological markers in the karyotype, as well. At present, there are very few diseases, let alone neurological diseases, whose gene locus is known to be closely linked to any convenient marker. The known examples are listed in Table 1. There is always the severe limitation that the particular family analyzed for linkage must be "informative" for the linked genetic marker; that is, members of the family must carry two different alleles at the marker locus.

TABLE 1

DISEASES LINKED TO GENETIC MARKERS

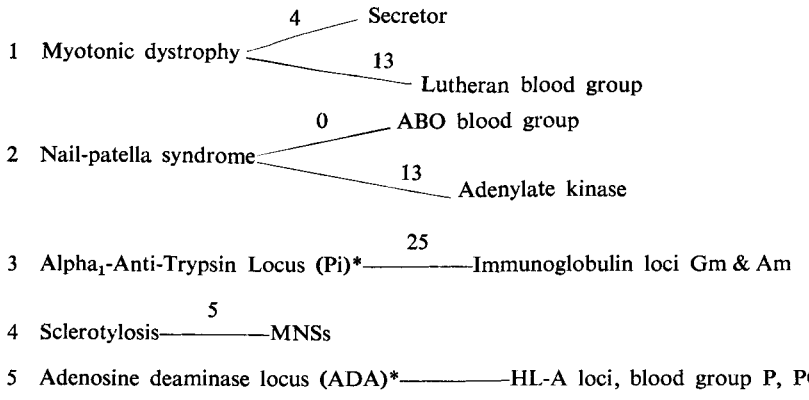
Chromosome Number 1

Elliptocytosis (El), Auricular osteodysplasia (AOD), and zonular pulverulent cataract (Cae)



Tentative Map
(numbers indicate estimated map distances)

Linkages on Unknown Autosomes



X-Chromosome Linkages

Retinoschisis, retinitis pigmentosa, ocular albinism, ichthyosis——Xg blood group
Hemophilia A & B, color blindness deutan & protan————G6PD
Also enzymopathies: alpha-galactosidase deficiency (Fabry's disease)
HGPRT deficiency (Lesch-Nyhan syndrome)
G6PD deficiency (hemolytic anemia)
PGK deficiency (hemolytic anemia + neurological abnormality)

* Presumably deficiency of alpha$_1$-anti-trypsin, resulting in pulmonary emphysema or congenital cirrhosis of the liver, and deficiency of ADA, resulting in combined immune deficiency syndrome, are determined at the same loci as the polymorphic proteins.

In fact, linkage analysis of neurological diseases inherited as autosomal dominant traits requires a collaborative effort to generate enough informative families of large size and long-term follow-up to determine the disease status of individuals typed for various markers. Some efforts to identify two- and three-generation families with Huntington's disease have been reported (Lindstrom et al. 1973).

Because of delayed onset and rapid deterioration, young members of the family cannot be typed with regard to the disease and older members of the family who were affected have often died before the linkage study is performed. Diseases in which the affected members of the family have long survival are better suited to two- and three-generation analysis; since these diseases are less severe, the urgency to make a diagnosis may be less great. In other diseases, some clinical signs may be recognized much earlier. Thus, the detection of typical cataracts in adolescents at risk for myotonic dystrophy allowed Mohr and others to carry out extensive family studies for this disease. Even so, linkage analysis may be useful in extending the time of carrier detection to newborn or even fetal periods.

Almost twenty years ago, Mohr concluded that the gene for myotonic dystrophy (*Dm*) is closely linked to the secretor (*Se*) locus. The linkage has been confirmed recently by Renwick et al. (1971) and by Harper et al. (1972). The average estimate of the recombination frequency between the two loci (*Dm* and *Se*) is 0.08. Secretor status can be tested conveniently at any age by analysis of body fluids, particularly saliva samples, for ABH blood-group substances. The secretor status of the fetus can be determined by direct test of the amniotic fluid (Harper et al. 1971).

We have had an opportunity to apply this approach to a family with myotonic dystrophy (Schrott et al. 1973), an experience which illustrates the potential usefulness and some of the difficulties of such linkage analysis.

A 21-year-old married woman, presented to our Medical Genetics Clinic in the 13th week of pregnancy, inquired whether there was any chance that her children might develop myotonic dys-

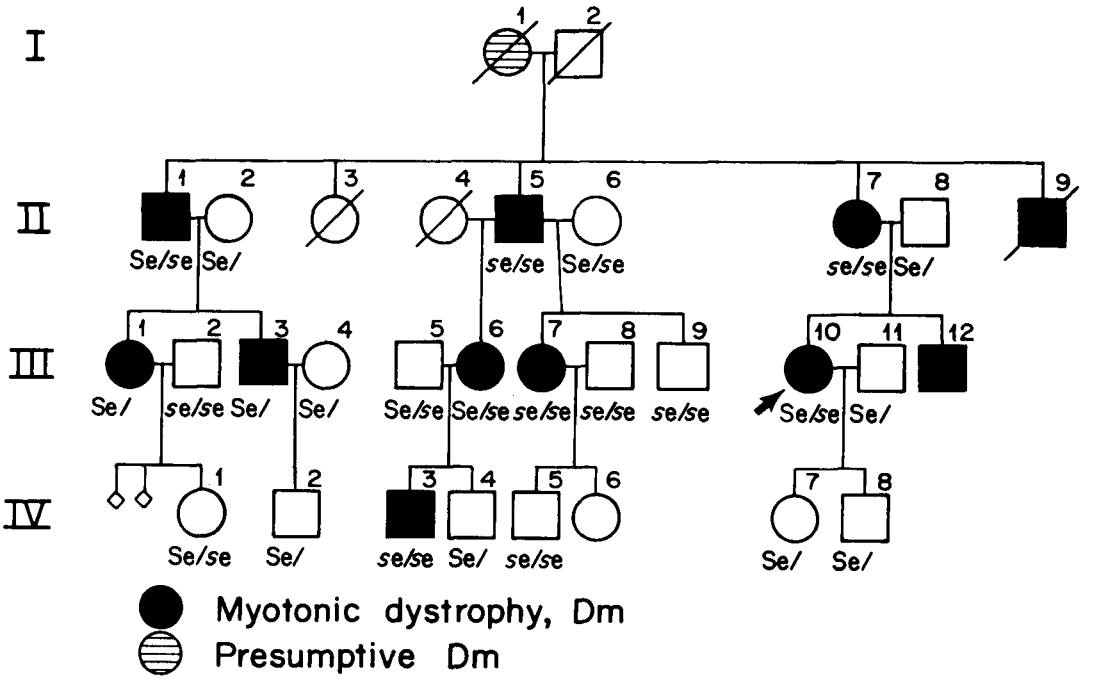


Figure. Pedigree of a family with myotonic dystrophy, showing genotypes at the secretor locus inferred from determination of secretor phenotypes.

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trophy. Her family history revealed that three maternal uncles, four maternal cousins, her mother, and her brother suffered from myotonic dystrophy, with early cataracts, myotonia, atrophy of skeletal muscle, and premature coronary heart disease. She was not aware that she herself was affected, but physical examination revealed atrophy of the temporalis and sternocleidomastoid muscles, receding hairline, expressionless facies, grip myotonia, and percussion myotonia of the tongue and thenar eminence. Slit-lamp examination disclosed peripheral lens opacities. The relevant pedigree is shown in the Figure, with myotonic dystrophy and inferred secretor genotypes indicated. Amniocentesis was performed in the 17th week of gestation to determine the secretor status of the fetus.

Before analyzing the results in this case, let us consider the general approach to linkage analysis for myotonic dystrophy.

As always, amniocentesis for intrauterine diagnosis is indicated only if the family is considering abortion for a fetus likely to be affected. Otherwise, linkage analysis can be performed sometime after birth much more conveniently with a sample of saliva. Also it is essential to determine as reliably as possible whether the parent at risk for myotonic dystrophy is affected or not. Then we can proceed to sequential testing of secretor phenotypes (see Table 2).

TABLE 2

CLINICAL APPROACH TO PRENATAL PREDICTION OF MYOTONIC DYSTROPHY BY LINKAGE TO SECRETOR LOCUS

	Testing order	Prenatal prediction possible
1	Determine secretor status of affected mate	→ Affected mate secretor-positive
2	Determine secretor genotype of affected mate and coupling of secretor allele to *Dm* allele by testing parents, sibs, and children	→ Affected mate *Se/se*, coupling ascertained
3	Test secretor status of normal mate ↓ Normal mate secretor positive	→ Normal mate *se/se*
4	Test parents, sibs, and children of normal mate	→ Normal mate *Se/se*

NOTE. Prenatal approach is impossible if the affected mate is homozygous for either allele at the secretor locus. Since it is not possible to prove homozygosity for the dominant *Se* allele by testing secretor status, pregnancies involving a secretor-positive unaffected mate may be studied in the hope that the individual is, in fact, a heterozygote.

First, the affected mate must be secretor-positive. Then the genotype of the affected mate and the coupling of secretor allele to *Dm* allele must be determined by testing the first-degree relatives; the affected mate must be heterozygous at the secretor locus and the coupling must be known. Then we can proceed to test the normal mate; intrauterine prediction is the simplest when the spouse is secretor-negative, but is still feasible if the spouse is a heterozygote. Tests of first-degree relatives of the normal mate may allow inference of the genotype. In fact, determinations of the secretor phenotype of the fetus may reveal heterozygosity in the parents, if the fetus proves to be secretor-negative.

These requirements for linkage analysis will eliminate most families from prenatal prediction of myotonic dystrophy. Since the frequencies of the alleles are approximately $Se = 0.5$

and *se* = 0.5, 62.5% of all matings cannot possibly be informative. These situations include those matings in which the affected spouse is homozygous for the non-secretor (25%) or for the secretor allele (25%) and in which the nonaffected spouse is homozygous secretor-positive when the affected spouse is heterozygous (12.5%). The remaining 37.5% of matings are potentially informative, *if* sufficient data about genotype and coupling can be obtained, which will be impossible in many cases.

A summary of outcomes for the potentially informative matings is given in Table 3. The

TABLE 3

INFORMATIVE MATINGS FOR LINKAGE ANALYSIS OF MYOTONIC DYSTROPHY
[*After Schrott et al. 1973*]

	Matings			Frequency	Offer to terminate pregnancy only if secretor status of fetus is...	Proportion (%) affected with myotonic dystrophy		Ratio of abortion to live births
	Affected		Normal			Among abortions[a]	Among live births[a]	
1	Se^b/se	×	*se/se*	0.0625	Positive	100 (92)	0 (8)	1 : 1
2	Se/se^b	×	*se/se*	0.0625	Negative	100 (92)	0 (8)	1 : 1
3	Se^b/se	×	*Se/se*	0.125	Positive	67 (64)	0 (8)	3 : 1
4	Se/se^b	×	*Se/se*	0.125	Negative	100 (92)	33 (36)	1 : 3

[a] First value assumes no recombination: values in parentheses represent the best estimate that any specified gamete is a recombinant, 0.078, based upon the skewed distribution of the posterior probabilities for θ, the recombination fraction (Renwick & Bolling 1971, p. 405, Fig. 2).

[b] Secretor-positive allele in coupling with myotonic dystrophy allele in matings 1 and 3; secretor-negative allele (*se*) in matings 2 and 4.

first two types of matings involve a secretor-negative unaffected mate and coupling of *Dm* to *Se* or to *se* in the affected mate. In these cases, prediction of inheritance of the *Dm* allele for myotonic dystrophy is clear-cut, except for recombination. We have used a value of 8% for recombination per generation; this value is intermediate between the estimates of 0.04 and 0.11 for most likely map interval (above) and agrees with the analysis by Renwick and Bolling (1971) of the skewed distribution they obtained for the posterior probabilities of θ, the recombination fraction. It should be noted that two recombinational events could occur in those pedigrees in which the affected mate received the *Dm* allele from a parent who was heterozygous at secretor. When the normal spouse is secretor-positive, difficulties arise. First, the procedure is worthless if he is homozygous. When he is heterozygous, as required, either a large proportion of fetuses will have to be aborted to prevent birth of an affected child or there will remain a substantial probability that the liveborn children may still develop myotonic dystrophy, depending upon the *Dm/Se* coupling in the affected mate (mating types 3 and 4 in Table 3).

In the case of our family, the probable mating type was type 4. The affected was definitely heterozygous at secretor, with coupling of *se* to *Dm*. Since she received the *Dm* allele from her secretor-negative mother, there was no recombination in the parental generation. We were

unable to rule out the possibility that her husband was a homozygote, but proceeded with the analysis since there was a probability of approximately 72% that he is heterozygous (Schrott et al. 1973). Had the fetus been secretor-negative, there would have been proof that he is heterozygous and the fetus, except for recombination, would have been expected to develop myotonic dystrophy. In that event, the parents would have requested termination of the pregnancy. However, the fetus was secretor-positive. Allowing for recombination and also for the possibility that the father was homozygous, we have reduced the risk for myotonic dystrophy in this child only from 50% to 40%. (For detailed analysis, see Schrott et al. 1973.)

Further examination of other family members and their spouses uncovered an even more informative mating, III-1 × III-2, corresponding to mating type 2 in Table 3. Here the mother III-1 has approximately 92% likelihood of being heterozygous at the secretor locus and having the myotonic dystrophy gene in coupling with the nonsecretor allele. The prior probability of 92% results from the possibility of a crossover in her father (II-1) when transmitting the myotonic dystrophy allele. Her spouse is secretor-negative. The secretor-positive child IV-1 thus has approximately 85% chance $(0,92)^2$ of not carrying the myotonic dystrophy allele and 15% risk of developing the disease. Any secretor-negative child of this mating would have an 85% risk for the disease.

In summary, families in which the criteria of Table 2 can be met will benefit from linkage analysis, particularly those families in which the unaffected spouse is secretor-negative. For the large majority of families with myotonic dystrophy, however, this approach will not be feasible. For other autosomal dominant diseases, prenatal prediction awaits the finding of closely-linked testable markers. It should be noted that recombination between linked loci reintroduces uncertainty into the genetic counseling situation: one of the major advantages of prenatal diagnosis based upon karyotype or enzyme analyses has been the certainty with which a fetus can be said to be affected or unaffected with the condition for which tests were performed. Nevertheless, substantial improvement in the estimate of risk in suitable families can be achieved with the linkage approach, even after allowing for recombination, as is demonstrated in our family with myotonic dystrophy.

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