MEDICAL PRACTICE

Clinical Topics

Application of a closely linked polymorphism of restriction fragment length to counselling and prenatal testing in families with myotonic dystrophy

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Abstract

The close genetic linkage between the loci for apolipoprotein CII (ApoC2) and myotonic dystrophy makes ApoC2 the closest fully validated marker for prediction of myotonic dystrophy. Application to genetic counselling and presymptomatic and prenatal prediction is reported in seven families with myotonic dystrophy, including one case in which the disorder was excluded prenatally. Only one of the families did not have members with ApoC2 genotypes that allowed prediction, but careful clinical study of older family members was found to be an important factor. ApoC2 typing of families with myotonic dystrophy should be of practical help both in prediction for asymptomatic relatives and for prenatal diagnosis in pregnancies of an affected parent.

Introduction

Myotonic dystrophy (DM) is an autosomal dominant disorder with an incidence of at least 5/100 000. Though the disease is classified as a form of human muscular dystrophy, it also affects other body systems. In the adult form of myotonic dystrophy the main disease manifestation is myotonia of grip associated with a variable progression of muscle weakness, principally affecting the muscles of the face, neck, and distal limbs. The commonest abnormalities in other systems are cataracts, cardiac conduction abnormalities, mild mental retardation, hypersomnia, anaesthetic apnoea, and, in some men, testicular tubular atrophy.

The extreme variability of myotonic dystrophy makes it difficult to give an accurate prognosis and is in itself distressing to patients in whom the disease is diagnosed in early adult life. Affected women face an additional problem, as not only do they have a 50% chance of transmitting the myotonic dystrophy gene, but there is also a considerable risk of an affected child having congenital myotonic dystrophy.[1] These children suffer severe muscle problems, usually associated with mental retardation from the perinatal period. As most young adults with the myotonic dystrophy gene have few, if any, symptoms, it is often only the birth of a child with congenital myotonic dystrophy that leads to the disorder being diagnosed in the mother.

The two sets of circumstances in which families with myotonic dystrophy usually seek genetic counselling are, firstly, when children born to affected parents reach adulthood and wish to know their likelihood of carrying the myotonic dystrophy gene, and, secondly, when mildly affected women who have had one child with congenital myotonic dystrophy wish to know about the risks for future pregnancies and the possibility of prenatal diagnosis. Until recently the assessment of subjects at risk relied solely on clinical examination, including electromyography and slit lamp examination.

Though this approach has been shown by several studies to be capable of detecting most presymptomatic gene carriers,[2-4] it is

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limited because a significant proportion of patients with myotonic dystrophy will show clinical signs of the disease only in later adult life. For a woman who has had a child with congenital myotonic dystrophy the risk of her having similarly affected children is close to 50%, as family studies have shown a high degree of concordance in sibships with this form of the disease.[5] There is therefore a need for a laboratory test that will reliably identify the myotonic dystrophy gene for both prenatal and presymptomatic diagnosis.

The linkage of the myotonic dystrophy locus to the secretor locus[6] has been applied in prenatal prediction.[7,8] Its application is limited, as less than 20% of matings are maximally informative, and predictions can be only 85-90% accurate at best. The application of recombinant DNA technology to the fine mapping of the myotonic dystrophy gene has led to the identification of more polymorphic markers closer to it.[9] The apolipoprotein CII (ApoC2) gene probe is the closest of these markers,[10] the recombination frequency from combined studies being 0·04 with a maximum linkage (lod) score of 23·9.[11]

We report here our experience of the clinical application of the ApoC2-DM linkage in counselling families with myotonic dystrophy, including the results of a prenatal chorionic villus biopsy. None of the families described was included in our initial linkage study of this marker, and they therefore represent family structures and clinical circumstances typical of those arising in the context of genetic counselling.

Patients and methods

The propositi were counselled either in our own genetics clinic or in other centres, which then requested ApoC2 typing by our department. A 96% accuracy was given for predictions. All key members of the family underwent full clinical assessment, and samples of blood were taken for DNA analysis from appropriate members.

Several polymorphisms of restriction fragment length can now be detected with gene probes for ApoC2.[12-14] The cDNA probe detects polymorphism at Taq1, BamH1, and Ban1 restriction sites (fig 1), while the ApoC2 genomic subclone (designated pSCl1) detects a Bgl1 restriction site polymorphism. Table I shows the allele and heterozygote frequencies. The combined heterozygote frequency assumes independent segregation of alleles, as numbers available at present do not allow an accurate estimation of linkage disequilibrium for all the polymorphisms, though it is known to exist between the Taq1 and Scl1 Bgl1 polymorphisms.

The families were typed initially with the most common polymorphism (Taq1 restriction site) and subsequently with the other enzymes as necessary. If the propositus had requested presymptomatic counselling the results of ApoC2 typing were combined with the age dependent risk of being an asymptomatic gene carrier (table II).

Results

Seven families were counselled from the results of investigations on the ApoC2-DM linkage. In five (families 1-5) the propositus wished to know if prenatal diagnosis of myotonic dystrophy would be possible in future pregnancies, while in the other two families (6 and 7) typing was performed to establish whether three asymptomatic subjects were carriers of the myotonic dystrophy gene. Figure 2 shows the pedigrees.

Family 1—The propositus (II-2) requested prenatal prediction for future pregnancies after the birth of her first child, who had congenital myotonic dystrophy. Using the BamH1 polymorphism, prenatal prediction was possible. The propositus was heterozygous and her spouse was homozygous, and the linkage phase was determined from the grandparents: the myotonic dystrophy gene was segregating with allele 1. Thus by allowing for a 4% risk of crossover at meiosis in the propositus the predicted risk of the subsequent fetus carrying the myotonic dystrophy gene could be modified from 50% to either 4% or 96%, depending on the ApoC2 BamH1 typing of that fetus.

Family 2—Myotonic dystrophy in the propositus (II-3) was diagnosed after his sister had had two children with congenital myotonic dystrophy. He wanted to know about the possibility of prenatal diagnosis. His parents were both asymptomatic and knew of no relatives with myotonic dystrophy. The results of their clinical and electromyographic examinations were normal, but on slit lamp examination I-1 had the polychromatic lens crystals typical of the early lens changes in myotonic dystrophy. The propositus and his wife (II-4) were both heterozygous for all polymorphisms so accurate prediction would be possible only if the fetus were homozygous. A heterozygous fetus would have an unaltered 50% chance of being normal.

TABLE I—*Polymorphisms of the gene for apolipoprotein CII (ApoC2) and genomic subclone (Scl1)*

	Restriction enzyme	Allele	Size (kilobases)	Allele frequency	Heterozygote frequency (%)	Combined heterozygote frequency (assuming no linkage disequilibrium) (%)
ApoC2	Taq1[18]	1	3·8	0·50	50	
		2	3·5	0·50		
	BamH1	1	4·9	0·9	18	59
		2	6·0	0·1		
	Ban1	1	2·5	0·68	43	77
		2	1·6	0·32		
Scl1	Bgl1[19]	1	12·0	0·47	49	88
		2	9·0	0·53		

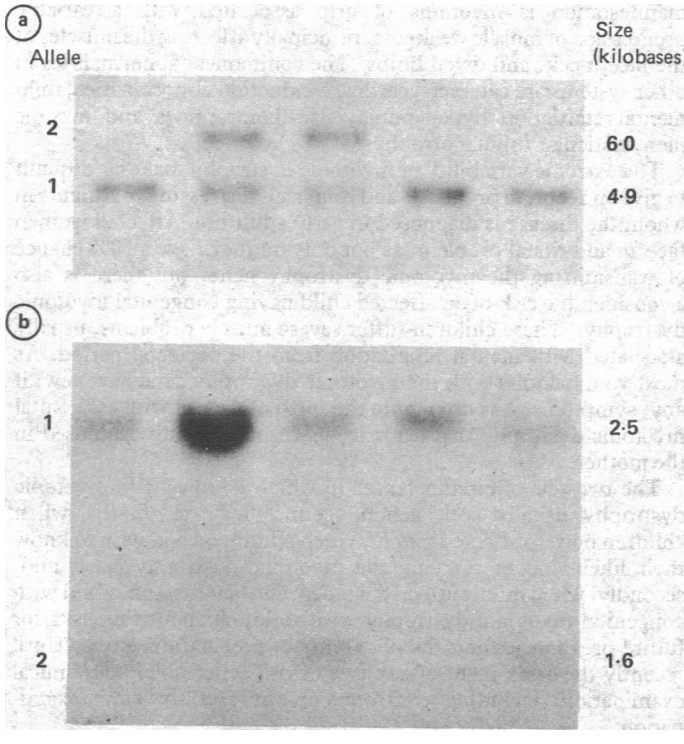

FIG 1—ApoC2 restriction fragment length polymorphisms: (*a*) cut with BamH1, and (*b*) cut with Ban1.

TABLE II—*Probability that a person at initial 50% risk of myotonic dystrophy carries the gene despite normal results of clinical examination. Based on data of Harper*[7]

Age (years):	0	10	20	30	40	50	60	70
Percentage probability:	48·0	42·5	32·9	22·5	11·9	6·5	2·9	0·0

Family 3—Myotonic dystrophy in the propositus (II-2) was diagnosed after the death of her first child soon after birth with myotonic dystrophy. Her family were assessed: both her parents yielded normal results on clinical and slit lamp examination, but on electromyography her father showed changes typical of myotonic dystrophy. One of her brothers (II-4) also had signs of mild myotonic dystrophy. II-1, aged 35 years, yielded normal results of clinical, electromyographic, and slit lamp examinations. I-1, I-2, II-2, and II-3 were initially typed for the TaqI polymorphism. As I-1 and I-2 were heterozygous we were unable to establish the linkage phase for the propositus. We therefore typed her brothers, who were both homozygous pregnant, and he requested prenatal diagnosis. Though both his parents were asymptomatic, clinical assessment showed his father to be affected, and typing with BanI was informative. Chorionic villus biopsy was performed at 11 weeks' gestation, and typing indicated that the fetus had a 4% risk of myotonic dystrophy. The couple elected to continue the pregnancy. (This pregnancy has been reported briefly previously.[15])

Family 6—The propositus (II-2) in this family had mild myotonic dystrophy that started when he was an adult and that was diagnosed after completion of his family. Assessment of family members showed that his brother (II-3) was also affected. Both their parents were asymptomatic and

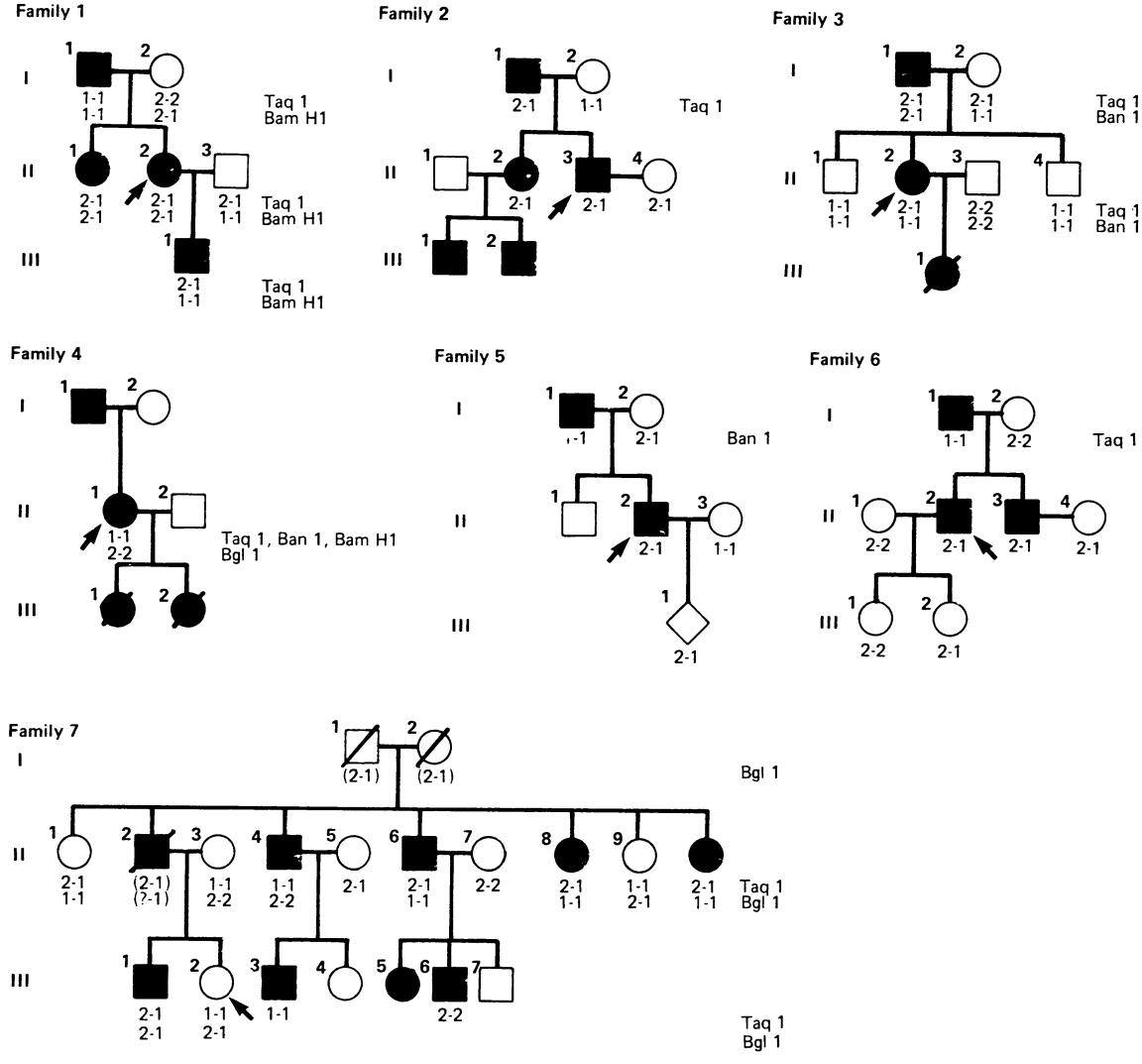

FIG 2—Pedigrees of seven families showing DNA typing. Arrows indicate propositi. Brackets in family 7 indicate inferred genotypes.

1-1. This could have arisen either because of a crossover between the marker and the myotonic dystrophy gene in one of the brothers or because II-1 was an asymptomatic gene carrier. The family was typed with the other enzymes, and the results obtained with ApoC2 BanI, though not informative for prenatal diagnosis for the propositus, showed that the myotonic dystrophy gene was segregating with allele 1 of the BanI polymorphism. Assuming that the likelihood of crossover between the TaqI site and the BanI site was negligible, we were able to use an ApoC2 haplotype to establish that the propositus received the myotonic dystrophy gene with allele 1 of the TaqI polymorphism. If DNA from her first child (III-1) had been available the linkage phase in II-2 could have been established from it.

Family 4—The propositus (II-1) requested prenatal prediction for future pregnancies after the death of her first two children with congenital myotonic dystrophy. As she was homozygous for all four enzyme polymorphisms no prenatal prediction was possible.

Family 5—Myotonic dystrophy in the propositus (II-2), aged 31 years, was diagnosed after cataract surgery. Shortly afterwards his wife became knew of no relatives with myotonic dystrophy, but on examination I-1 had temporal hollowing and electromyographic changes typical of the disease. The children of the propositus were both normal on clinical and slit lamp examination. The ApoC2 TaqI polymorphism typing showed that the myotonic dystrophy gene was segregating with allele 1 in this family, and by combining the risk figure from DNA typing with the age dependent risk the overall risk for III-1 and III-2 being affected became 2·7% and 94·7%, respectively. The reduced risk for III-1 precluded the need for prenatal diagnosis, but chorion villus biopsy could be offered to III-2 in the event of a pregnancy. In this family both II-3 and his wife (II-4) were heterozygous for all the polymorphisms so accurate prenatal prediction would be limited to the 50% of instances in which the fetus is homozygous.

Family 7—The propositus (III-2) was referred to see if ApoC2 typing would reduce her predicted risk of carrying the myotonic dystrophy gene. She was 30 years old, and results of clinical, slit lamp, and electromyographic examinations were normal. Her father had myotonic dystrophy, and her brother was also affected. Her age dependent risk before DNA typing

was therefore 22%. As her father and grandparents were dead, however, it was necessary to type her father's siblings and their offspring to establish the linkage phase. From studying the ApoC2 TaqI polymorphism, together with the ScII BglI polymorphism, and computing the results from various family members with the program Linkage,[16] her predicted age dependent risk of carrying the myotonic dystrophy gene was reduced from 22% to 1-2%.

Discussion

We have reported on our initial experience in applying the ApoC2-DM linkage when counselling families with myotonic dystrophy using the various polymorphisms identified by the DNA probes for the ApoC2 locus. In five of seven families at least one of the polymorphisms was fully informative for prediction. Its use clarified the myotonic dystrophy gene carrier state of three apparently asymptomatic members of two families, allowed prenatal diagnosis in one family, and could allow a similar test in two others. In two families, however, its use was limited, as prenatal prediction would be possible in only the 50% of fetuses that were homozygous. In only one of the seven families was the application of the ApoC2-DM linkage unable to alter the predicted genetic risks.

Two important clinical points arise from this study. Firstly, before linkage can be applied the clinical state of the family members must be determined. In three of the seven families the affected person in the first generation was only identified after electromyographic examination (two cases) and slit lamp examination (one). This has important implications for the timing of prenatal diagnosis. For example, in family 5 the propositus was first diagnosed only shortly before a pregnancy was confirmed, making it difficult to clarify in time which of his parents was affected.

Secondly, it is important to store DNA samples from family members who die, as it may be only subsequently that their children (as in family 7) or their parents (as in family 5) request counselling.

In conclusion, the application of any linked marker in genetic counselling is limited by family structure and heterozygote incidence. We are now searching for other polymorphic markers flanking the myotonic dystrophy gene so that we can further increase the proportion of families suitable for counselling and, in addition, greatly reduce the chance of erroneous prediction. The ideal circumstances will only arise when a specific and sensitive test for myotonic dystrophy is available, which will probably be developed only after the gene itself has been isolated. Meanwhile, many families can be helped by the application of the ApoC2-DM linkage.

A further DNA probe closely linked to myotonic dystrophy has been reported by Roses *et al* (*Nucleic Acids Res* 1986;**14**:5569), which has so far shown no recombination and which would be useful in prediction.

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MATERIA NON MEDICA

Full Marx for duck soup

I have always admired ducks. Not only do they combine the skills of swimming, diving, walking, and flying, but I also like their version of quackspeak. My interest in ducks is, however, like their gait, broadly based, so when invited to dinner in a restaurant where up to 1000 ducks are stripped, cooked, and eaten each day the opportunity was not to be missed.

The Beijing Roasted Duck Restaurant on Wangfujing, Beijing, is a four storey building totally devoted to the duck. The front entrance gates are topped with metal ducks, and the rear entrance is piled high with sacks of down and feathers. This restaurant meets the two great challenges in cooking duck better than I ever have: the cooks achieve a crisp exterior without drying the flesh and they remove the fattiness from the meat, making each mouthful a joy.

The magic lies in the cooking. First, the entrails are removed from the stern. The skin is cut round the neck and in several other places. The duck's feet are tied together and the duck hung upside down. The carcase is filled with boiling water and transferred, still upside down, to the oven to hang over a glowing fire. Fat from under the outer skin drips out through the cuts, further fuelling the fire. As the duck cooks the water inside the duck simmers, cooking the duck from the inside and freeing much internal fat, which then floats to the top and will be decanted with the water. This is the secret of the true Beijing Duck.

Our meal for six people consisted of three ducks, sauces, pancakes, orange juice, sticky red wine, and Red Lion beer. We started with tea and thin slices of duck gizzard. This was a pleasantly sharp aperitif. The cook came to show us naked duck number one and then went off to cook it. Duck breast in aspic was followed by duck paté. Naked duck number two was shown to us, approved, and taken off to the kitchen while duck number one returned as a mound of roasted duck to be dipped into communal sauces en route to the mouth. Naked duck number three appeared and then disappeared to the oven, and duck number two reappeared as a pile of boiled duck meat that had to be wrapped in pancakes (tricky using chopsticks) and eaten. Duck number three appeared sautéed and was dipped in sauce and eaten.

Each time a duck was carved in the kitchen the leg muscles were carefully separated and the psoas muscles removed intact. These were laid across the rest of the meat as a delicacy for the most honoured guest. The number of psoas muscles allowed you to cross check the number of ducks the party had consumed. After being carved, each carcase was brought to the table and displayed with the head cracked open. The legs and webs were also eaten as delicacies, though not by us, but the bill was given to the host.

We finished with a cup of duck soup and went to watch the kite fliers on Tien An'amen Square.—J GRAHAM WATSON, consultant paediatrician, Newcastle upon Tyne.

Correction

Report from the PHLS Communicable Disease Surveillance Centre

We regret that in the most recent report from the PHLS (15 November, p 1293) figs 2 and 3 were accidentally transposed. The histogram on page 1294 shows numbers of cases of AIDS and the graph on page 1295 shows notifications of cases of salmonellosis.