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PRENATAL DIAGNOSIS OF FRIEDREICH ATAXIA: IMPROVED ACCURACY BY USING NEW GENETIC FLANKING MARKERS

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SUMMARY

Friedreich ataxia is a neurodegerative disorder with autosomal recessive inheritance. Since the gene causing mutation has not yet been identified, prenatal, predictive, and carrier diagnoses are based on indirect haplotype analysis with closely linked markers. Until recently, only distal markers were available and their physical distance to the Friedreich ataxia (FRDA) gene remained elusive. The identification of close flanking markers that mark out the boundaries of the FRDA locus and reduce the critical genomic region which contains the gene allows for the first time misdiagnosis due to undetectable recombination to be avoided and diagnosis accuracy to be greatly improved. In this sense, we have verified a prenatal diagnosis in which the fetus was diagnosed as an unaffected carrier last year with a confidence of 95 per cent. By using the new flanking markers, the diagnosis improved and confidence reached almost 100 per cent.

KEY WORDS: Friedreich ataxia; prenatal diagnosis; microsatellite markers.

INTRODUCTION

Friedreich ataxia is an autosomal recessive inherited neurodegenerative disorder of unknown aetiology. Requirements for prenatal diagnosis are not frequent, since the age of onset is around puberty and most parents are beyond childbearing age when the disease appears in the family. The lack of gene identification precludes both direct analysis of the disease causing mutation and the typing of intragenic markers. Prenatal or predictive diagnosis is based on indirect haplotype analysis of closely FRDA-linked markers, and can only be performed when there is an affected sib in the family.

The Friedreich ataxia (FRDA) gene was mapped to chromosome 9q in 1988 (Chamberlain *et al.*, 1988). The first genomic candidate region around D9S5–D9S15 (Fig. 1) was discarded in 1993 through the description of six recombinant events which, for the first time, orientated the FRDA-linked markers in the chromosome and positioned the FRDA locus proximal to the linkage group (Belal *et al.*, 1992; Chamberlain *et al.*, 1993). Genomic walking towards the centromere allowed Rodius *et al.* (1994) to isolate the new linked microsatellite markers FR1, FR2, FR8, FR7, and FR5, and analysis of recombinations and haplotype divergence in patients homozygous by descent permitted the FRDA locus to be located in a region of 300 kb flanked by markers FR2 and FR8 (Fig. 1) (Rodius *et al.*, 1994; Duclos *et al.*, 1994; Monrós *et al.*, 1994). The delimitation of the FRDA gene in such a small genomic region flanked by highly informative markers now allows us to perform prenatal or predictive diagnosis for Friedreich ataxia with a confidence very close to 100 per cent. Nevertheless, special care has to be taken regarding the clinical diagnosis and several aspects have to be stressed: (1) late onset (De

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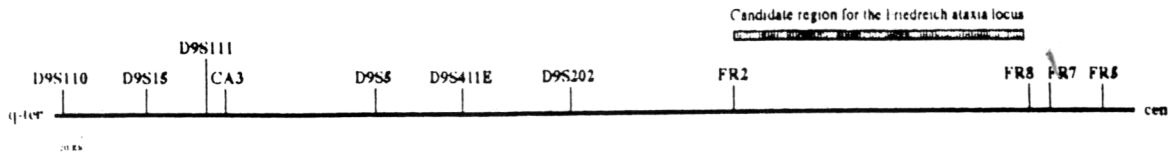

Fig. 1. Physical map of the FRDA-linked markers in chromosome 9q. The dashed bar on the top of the figure indicates the genomic region containing the FRDA locus

Michele *et al.*, 1994) and retained lower deep tendon reflexes (Palau *et al.*, 1995) are compatible with Friedreich ataxia linked to chromosome 9q markers, but prenatal diagnosis is not advisable when Harding's criteria are not fulfilled and linkage to chromosome 9q cannot be demonstrated; (2) the presence of cardiomyopathy (Smeyers *et al.*, 1994) and the exclusion of vitamin E deficiency (Ben Hamida *et al.*, 1993) must be documented to ensure linkage.

In this report we demonstrate how the accuracy of prenatal diagnosis of Friedreich ataxia is improved by using the new FR markers, as compared with the results obtained with previous markers from the D9S15–D9S5 linkage group.

MATERIALS AND METHODS

Case report

The proband was a 20-year-old girl, the oldest of four sibs. The parents were second cousins and of Portuguese ancestry. Friedreich ataxia was diagnosed according to Harding (1981). The clinical picture was onset at 8 years of age with progressive ataxic gait, decreased vibratory sense, normal position sense, and abolished lower deep tendon reflexes. Neurophysiological studies revealed the presence of a sensory axonal neuropathy. Secondary signs included pes cavus and scoliosis. ECG findings showed an abnormal repolarization pattern, suggesting the existence of cardiomyopathy. Normal glucose serum levels indicated no diabetes mellitus and vitamin E deficiency was discarded (serum levels = 12·1 μg/ml; normal range 5–20 μg/ml). Her mother was at 15 weeks' gestation when prenatal diagnosis for Friedreich ataxia was requested.

Genetic analysis

DNA samples were obtained from peripheral leukocytes from both parents and the four siblings, by standard phenol extraction and ethanol precipitation protocols. Fetal DNA was obtained by chorionic villus sampling. Southern blot analysis was performed for the probe 26P/BstXI (D9S5) labelled with digoxigenin-11-dUTP (Genius System, Boehringer Manheim) and detected by chemiluminescence using Lumi-Phos (Boehringer Manheim). The microsatellite markers GS4 (D9S110), MCT112/MS (D9S15), GS2 (D9S111), CA3, FD1 (D9S411E), FR1 (D9S202), FR2, FR8, FR7, and FR5 were typed by using the polymerase chain reaction and electrophoresed on non-denaturing polyacrylamide gels. Alleles were visualized after gel silver-staining.

RESULTS AND DISCUSSION

When prenatal diagnosis was requested in November 1993, the markers available for genetic analysis were D9S110, D9S15, D9S111, CA3, D9S5, D9S411E, and D9S202 (Fig. 1). It was known that the disease locus was located proximal to D9S5 (Belal *et al.*, 1992; Chamberlain *et al.*, 1993) and that a hot spot for recombination probably existed between D9S111 and D9S5 (Pugachev *et al.*, 1993 and personal observations).

Despite most of the above-mentioned markers displaying high heterozygosity, haplotype construction showed that the father was uninformative for all markers but CA3 (Fig. 2). Phase information given by this microsatellite marker allowed us to indirectly diagnose the fetus as a healthy carrier of the FRDA maternal mutation, with a confidence of 95 per cent. This figure was obtained by using the upper confidence limit of the estimated recombination fraction with the disease.

Recently, the Friedreich ataxia locus has been narrowed to within the 300 kb interval flanked by markers FR2 and FR8 (Fig. 1) (Rodius *et al.*, 1994; Duclos *et al.*, 1994; Monrós *et al.*, 1994). Analysis of the family with these new markers, together with FR5, has allowed us to confirm the prenatal diagnosis (Fig. 2). Full informativity for all markers in both parents removes the possibility

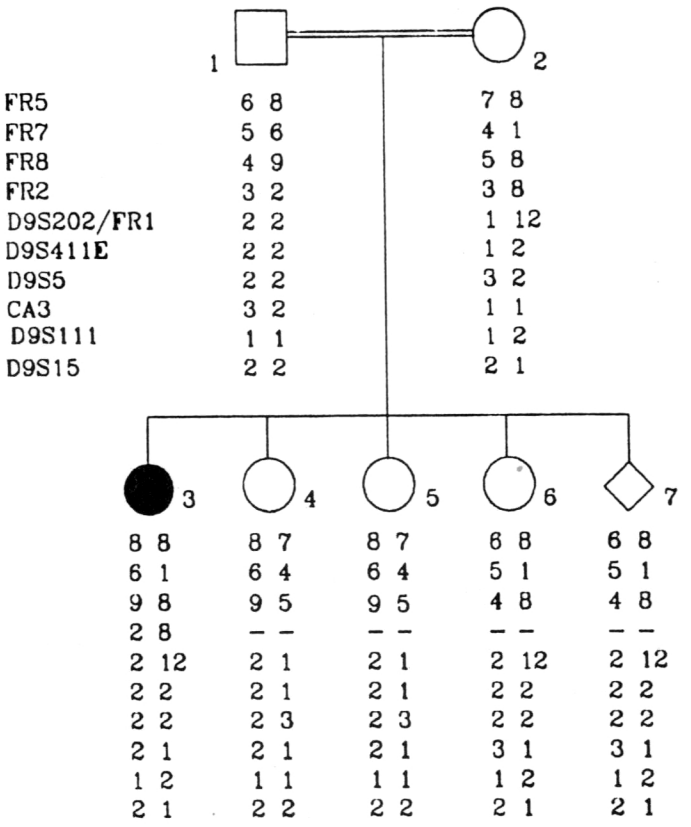

Fig. 2--Pedigree and FRDA-linked haplotypes for each member of the family

of recombination between the disease locus and marker loci. As the chance of an undetectable double crossover between markers FR2 and FR8 is negligible, the accuracy of the diagnosis approaches 100 per cent.

It is interesting to point out the fact that the proband was heterozygous for all markers but D9S5, D9S411E, and FR5, despite her parents being second cousins. In recessive diseases, affected children of consanguineous marriages are *a priori* expected to be homozygous by descent at the mutation loci and neighbour markers. Nevertheless, the chance of not being homozygous by descent (i.e. being affected due to random mating of disease alleles), according to Lander and Botstein (1987), is 0·22, assuming a disease allele frequency of 1/220 and a coefficient of inbreeding $F = 1/64$ for third-degree consanguinity. This probability is high enough to support the finding that two different mutations are segregating in the family.

This is, to date, the second reported prenatal diagnosis of Friedreich ataxia. The first one was published by Wallis *et al.* in 1989, when only two scarcely informative linked RFLPs at D9S15 were available (MCT112/MspI and MCT112/AccI) and a precise estimation of the confidence limits for the distance between marker loci and the disease locus was not possible.

In conclusion, we have shown how the existence of highly informative markers that closely flank the FRDA locus clearly improves the accuracy of prenatal and predictive diagnosis of Friedreich ataxia, with a risk of error of almost 0 per cent.

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