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Genetic Homogeneity at the Friedreich Ataxia Locus on Chromosome 9

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Summary

Classical Friedreich ataxia, a progressive, neurodegenerative disorder involving both the central and peripheral nervous systems, has been subclassified according to the observed clinical heterogeneity. The variations in the age at onset and in the spectrum and severity of symptoms have previously been interpreted as evidence of genetic heterogeneity. We have studied the linkage between the disorder and closely linked DNA markers in families of distinct ethnic origins, including the "typical" French–Canadians and the Acadian population of Louisiana. The disease in these two populations, both of continental French origin, has a very similar initial clinical picture. However, a marked difference in the rate of progression of the obligatory symptoms after 10 years of apparent disease is observed. A total of 553 individuals from 80 families with 202 affected members have been typed with the chromosome 9 marker MCT112, which we have previously shown to be closely linked to the disease locus. Evidence for linkage was observed in all families with the generation of a combined total lod score of 25.09 at a recombination fraction of $\theta = .00$, providing strong evidence for genetic homogeneity at this locus for the classical form of this disease.

Introduction

Clinical classification of the hereditary ataxias has been difficult historically because of a failure to define precisely a set of symptoms that are diagnostically precise and universally recognized. Classification systems have often been regarded either as being too simplistic, resulting in the lumping of distinct clinical entities, or, in contrast, of being too complex, splitting a disease according to variants. Such criticisms will eventually stand or fall on whether mutations in a single gene are shown to be responsible for the pathology of a specific disorder.

Friedreich ataxia, an autosomal recessive ataxia with progressive degeneration of the central and peripheral nervous system is one such disorder in which precise definition of the clinical criteria has been difficult, primarily owing to the comparative rarity of the disorder. Although it is the commonest of the hereditary ataxias, as recently as 10 years ago even the disease's mode of inheritance remained in dispute in the literature. In 1976, a major advance to the absolute definition of the disease was made by Geoffroy et al. (1976); strict diagnostic criteria were delineated for the "classical" form of the disease, providing a sound basis for future research. These criteria included autosomal recessive inheritance, onset usually before puberty and never after the age of 20 years, and primary presentation of ataxia, followed by dysarthria, absence of deep tendon reflexes, posterior column signs, and muscle weakness.

Clinical variability in certain ethnic patient groups led to the description of several variants, defined primarily by differing rates of progression: "Rimouski type," with rapidly progressing ataxia and early onset

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(Bouchard et al. 1979), and the "recessive Roussy-Levy syndrome," commonly seen in the descendants of Acadian families from New Brunswick, with a much slower course of progression and survival into the fifth decade of life (Barbeau et al. 1984). In a clinical review of British patients confirming the essential diagnostic criteria, Harding (1981) argued in support of heterogeneity, but with one mutation accounting for the majority of cases. The identification of biochemical abnormalities, candidates for the pathology, in the Canadian patients split opinion further, with failure to confirm these observations in geographically separate patient populations being interpreted as direct evidence for the existence of distinct clinical entities.

Until the assignment of the Friedreich ataxia mutation to the centromeric region of chromosome 9 (Chamberlain et al. 1988), it had been impossible to attribute these variations to either the involvement of two distinct genetic loci or the presence of different mutations within the same gene locus, with variable expression dependent on the influence of different gene pools.

We have now investigated heterogeneity in apparently unrelated families from the United Kingdom and Germany and from a consanguineous population from Cantabria, Spain. Particular emphasis was placed on the analysis of the two populations with founder effects, "typical" French–Canadian patients and the variant, Acadian, population of Louisiana. Both have their ancestral origins in France, but, following an initial period of parallel development of the disease, the latter then exhibits a more slowly progressing peripheral involvement (muscle weakness and loss of vibratory perception) and a lower incidence or an absence of cardiomyopathy, leading to a longer life span than commonly found among Friedreich ataxia patients.

Subjects and Methods

Subjects

A total of 553 individuals from 80 families with two or more affected members, 202 patients in total, were typed for genetic linkage to the locus defined by the anonymous marker MCT112. Conformation to the strict diagnostic criteria was observed in all families to minimize the possibility of heterogeneity due to misdiagnosis.

European population.—In addition to the core pedigree used to assign Friedreich ataxia to chromosome 9 (Chamberlain et al. 1988), linkage was tested for a further 36 U.K. and 7 German families each with two or more affected individuals. No evidence of consanguinity was apparent in these families.

French-Canadian population.—Eight families from Montreal and the surrounding region of Quebec province were sampled. Genealogical studies have demonstrated that many of the Quebec families derive from a common ancestral couple—immigrants from Perche, northwestern France, who arrived in Quebec in 1634. These families belong to group 1a: "typical" Friedreich ataxia—complete picture (Geoffroy et al. 1976).

Acadian population.—This group is also descended from persons originally from France, although in this instance primarily from the Vienne and Anjou regions of Northern France; many of the early settlers have been traced to the village of Aulnay in Loudunais. In the early 17th century, these settlers colonized the region known as Acadia, including Nova Scotia and New Brunswick. After the colony passed to English rule, there was extensive deportation of the Acadians and the families dispersed, finally resettling in Louisiana, where they blended with the inhabitants to create the Cajun culture (Arsenault 1978). The Cajun families appear to be derived from a common ancestral Acadian couple.

Spanish population.—Four families were collected from Cantabria in northern Spain, where the incidence of Friedreich ataxia is more than double that found elsewhere in Europe (4.7/100,000) as a result of consanguinity. Several consanguinous loops, leading to pseudodominant transmission, have been identified in one family living in a remote village with only 25 inhabitants.

DNA Analysis

Blood samples from family members were taken in EDTA, and genomic DNA was prepared using standard techniques. Five micrograms of DNA were digested with the restriction enzyme *Msp*I, which detects an RFLP with the probe MCT112 (Carlson et al. 1987). The fragments were separated on 0.8% agarose gels and transferred to nylon membrane (Hybond® N, Amersham) by the method of Southern (1975).

The probe MCT112 (D9S15) was linearized and labeled with (^{32}P-dCTP) by using random oligonucleotide-primed synthesis (Feinberg and Vogelstein 1984). Competition with human placental DNA prior to addition to the hybridization mix was necessary owing to the presence of repeat sequences in the probe. Hybridization and autoradiography were performed according to a method described by Gilliam et al. (1984); filters were washed to a stringency of 0.1 × SSC/0.1% SDS.

Table 1

Pairwise Lod Scores between the Friedreich Ataxia Locus and MCT112

Population	z	θ	Recombination Fraction .01	.05	.10	.20	.30	.40
European	17.05	0	16.33	14.41	11.98	7.40	3.51	.84
Acadian	5.06	0	4.98	4.51	3.96	2.80	1.64	.61
French-Canadians	2.98	0	2.91	2.61	2.25	1.50	.79	.23
Total	25.09	0	24.22	21.53	18.19	11.70	5.94	1.68

Results

Pairwise lod scores between the marker MCT112 and the Friedreich ataxia locus, calculated using the LINKAGE computer program package of Lathrop et al. (1984), are shown in table 1. No recombination was observed between MCT112 and the Friedreich ataxia locus in any patient population. Combining the data for the individual groups generated a maximal lod score ($\hat{z}$) of 25.09 at a recombination fraction of $\theta = .00$.

Discussion

The demonstration, in all patient populations, of close linkage to the locus defined by the probe MCT112 argues strongly for a mutation or a set of mutations at a single locus for the classical form of the disease. Constitutive combined lod scores for the Acadian and French-Canadian patient groups each independently provide conclusive evidence for linkage.

Linkage disequilibrium was not observed in either the general European population or the French-Canadian patients. However, in both the Acadian and Spanish patients, allelic association was observed with the mutation cosegregating preferentially with the rare allele. The observation of linkage disequilibrium in these isolated populations argues for a strong founder effect and will facilitate accurate genetic counseling and presymptomatic and prenatal diagnosis for these families.

The various clinical manifestations such as presence or absence of cardiomyopathy, diabetes, differing ages at onset, and slower disease progression may represent a spectrum of different mutations within the Friedreich ataxia locus. Alternatively, the concurrent but familial cosegregation of other genes causing diabetes and cardiomyopathy may explain the presence or absence of these clinical conditions.

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