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Mutation of the start codon in the FRDA1 gene: linkage analysis of three pedigrees with the ATG to ATT transversion points to a unique common ancestor

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Abstract Friedreich ataxia (FRDA) is a progressive neurodegenerative disorder caused by loss-of-function mutations in the gene encoding frataxin. Most patients with FRDA have trinucleotide repeat expansions in both alleles of the FRDA1 gene. In patients heterozygous for the expansion the second allele may be inactivated by a point mutation. We identified the ATG→ATT (M1I) mutation of the start codon in three independent families. Individuals with symptoms of FRDA in these families are compound heterozygous for the repeat expansion and the ATG mutation. To look for a common founder of the M1I mutation, a detailed linkage analysis employing six polymorphic chromosome 9 markers was performed. We found complete haplotype identity for two of the three chromosomes with the point mutation. The third case shows partial conformity and may be the result of a single recombination event.

Introduction

Friedreich ataxia (FRDA) is an autosomal recessive neurodegenerative disorder characterized by progressive gait and limb ataxia, loss of tendon reflexes, and dysarthria. FRDA is the most frequent inherited ataxia (1:50,000) with early onset, usually before the age of 25 years. In 1988, the FRDA locus was mapped to chromosome 9q by linkage analysis (Chamberlain et al. 1988). The FRDA1 gene has recently been described by Campuzano et al. (1996). A (GAA) trinucleotide expansion in intron 1 of the X25 gene is the major mutation in patients with FRDA. The majority of patients are homozygous for this repeat expansion. Linkage analyses indicate that these FRDA chromosomes could have originated from a single or a few initial mutations (Monros et al. 1996; Cossee et al. 1997b; Zühlke et al., in preparation). In normal alleles, the repeat varies in size between 7 and 36 units (Montermini et al. 1997), whereas in mutated alleles the repeat ranges in length from 110 to about 1200 units (Dürr et al. 1996; Filla et al. 1996). In patients heterozygous for the expansion the second allele may be inactivated, e.g., by a point mutation, insertion, or deletion. To date, the last two types of mutation have not been described for the FRDA1 gene. Point mutations that cause single amino acid changes in the C-terminal region of the 210-amino-acid protein, named frataxin, have been described. Five patients from three Italian families were compound heterozygous for the (GAA) expansion and the I154F mutation (Campuzano et al. 1996; Filla et al. 1996). A second missense mutation (G130V) was reported (Bidichandani et al. 1997) as well as a splice-site mutation and the stop mutation L106X (Campuzano et al. 1996). Furthermore, mutations of the ATG initiation codon in addition to the repeat expansion of the second allele will cause the disease. Two cases of M1I mutations in the FRDA1 gene are known (Cossee et al. 1997a; Laccone and Schloesser 1997). A third family with this mutation is reported here. As shown for the I154F missense mutation, alteration of the start codon could be demonstrated in unrelated families. Therefore, we performed linkage studies with six highly polymorphic markers on chromosome 9 to determine whether the ATG→ATT transversion is linked to a common haplotype or whether it could have arisen from different chromosomes. Furthermore, we compared the M1I haplotype with linkage analysis data from FRDA chromosomes with (GAA) repeat expansions.

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Materials and methods

Patients

Patients G, L, and S with symptoms of FRDA from three unrelated families were investigated. For patients G and S, samples of both parents were available; for patient L, only the mother could be included in the analysis.

DNA analysis

Genomic DNA was isolated from proteinase K, SDS digests of blood samples. To test for repeat expansions, polymerase chain reaction (PCR) amplification from 50 ng DNA was performed using primers GAA-F and GAA-R (Campuzano et al. 1996) in a volume of 25 µl containing 5 pmol of each primer, 200 µM dNTPs and 1 U *Taq* DNA polymerase (Qiagen) in the recommended buffer. The PCR conditions were 94°C for 3 min, followed by 35 cycles of 94°C for 1 min, 65°C for 1 min, and 72°C for 4–6 min in a RoboCycler (Stratagene). The products were separated by agarose (1.5%) gel electrophoresis and visualized by ethidium bromide staining.

Single-strand chain polymorphism (SSCP) analysis and sequencing

To search for point mutations, SSCP analyses were performed using exon-specific PCR primer pairs. For exon 1, oligonucleotides 5′ AGT CTC CCT TGG GTC AGG GGT CCT GG 3′ and 5′ CCG CGG CTG TTC CCG G 3′ were used, yielding a PCR product of 414 bp. Five µl of PCR product was added to 3 µl of denaturing buffer (98% formamide, 20 mM EDTA, 0.05% xylene cyanol, 0.05% bromophenol blue). Samples were heated for 5 min to 95°C followed by cooling on ice. After a pre-run, the mixture was loaded on a 6% polyacrylamide gel (29:1 acrylamide:bisacrylamide) containing 1×TBE and 10% urea. Electrophoresis was in 1×TBE at 30 W and 4°C for 4 h. The SSCP banding pattern was detected by silver staining.

DNA templates were sequenced using the dideoxy chain-termination method in combination with dye terminator chemistry or in the presence of ^{35}S. Fluorescent sequencing reactions were electrophoresed on ABI Sequencers.

Haplotype analysis

In order to test whether FRDA chromosomes with ATG→ATT transitions display different haplotypes, genotyping with six polymorphic markers flanking the FRDA1 gene was performed. The marker locus references were as follows: FR5 D9S889 (Rodius et al. 1994), FR7 D9S887 (Rodius et al. 1994), FR8 D9S888 (Rodius et al. 1994), FR6 (Montermini et al. 1995), FR1 D9S202 (Pandolfo et al. 1993), FD1 D9S411 (Pianese et al. 1994). Genotypes were determined by PCR amplification. The PCR conditions for each of the loci were as described. The PCR products were resolved on 6% polyacrylamide gels and visualized by silver staining.

Results

Analyses of the (GAA) repeat lengths in intron 1 of the FRDA1 gene of patient L with the clinical diagnosis FRDA revealed heterozygosity for the repeat expansion (Fig. 1A). To search for point mutations, SSCP analyses were performed. Using this procedure a deviating SSCP pattern was found in exon 1 for this heterozygous patient (Fig. 1B) representing a mutation in one allele of the FRDA1 gene. Sequencing of the PCR product revealed a mutation of the start codon. Two heterozygous patients with an M1I mutation have previously been reported elsewhere (Cossee et al. 1997a; Laccone and Schloesser 1997). As in these patients (S and G) the initiation codon is changed from ATG to ATT in patient L (Fig. 1C). The ATG→ATT transversion destroys the recognition sequence CATG of the restriction enzyme *Nla*III (Cossee et al. 1997a). Cleaving the PCR products of exon 1 of these patients with *Nla*III confirmed the heterozygous presence of the mutation (Fig. 1D). This effi-

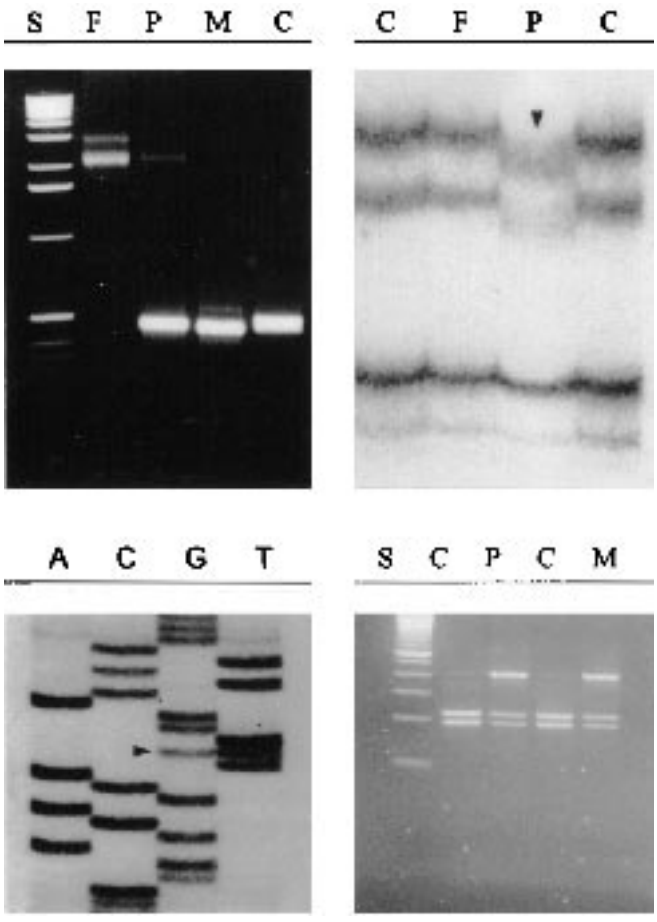

Fig. 1A–D DNA analyses of the FRDA1 gene. (*C* control DNA, two unrelated samples in **B** and **D**, *F* FRDA patient with expansions on both alleles, *M* patient L's mother, P compound heterozygous FRDA patient L, *S* molecular weight marker) **A** Agarose gel electrophoresis of polymerase chain reaction (PCR) products generated using primers that flank the (GAA) triplet repeat in intron 1 of the FRDA1 gene. Homozygous (*F*, *M*), heterozygous (*P*, *C*), normal (*M*, *C*), and expanded (*F*, *P*) alleles for this locus are shown. **B** Detection of a mutation in exon 1 of the FRDA1 gene by single-strand conformation polymorphism analysis. The *arrowhead* indicates the shifted PCR fragment in patient L. **C** Identification of the ATG→ATT mutation of the FRDA1 start codon in patient L by sequence analysis. The *arrowhead* indicates the mutated nucleotide. **D** Agarose gel electrophoresis of PCR products of FRDA exon 1 digested with *Nla*III. For the normal allele, the 414-bp PCR product with the wild-type ATG is cleaved into two fragments, whereas in the mutated allele with the sequence ATT the restriction site is destroyed. *F* and *C* are homozygous for the ATG; patient L and her mother are heterozygous

Table 1 FRDA haplotypes. The alleles for six chromosome 9 loci for FRDA patients G, L, and S are given. Haplotypes for the chromosomes with the point mutation and those with the (GAA) repeat expansion are shown

Patient	D9S889	D9S887	D9S888	FRDA	FR 6	D9S202	D9S411
G	8	5	7	ATG→ATT	3	2	3
L	8	5	7	ATG→ATT	3	2	3
S	5	6	5	ATG→ATT	3	2	3
G	9	4	7	GAA	2	3	2
L	9	8	8	GAA	2	3	2
S	9	2	7	GAA	2	3	1

cient and fast method was applied for investigating the start codons in the parents of the patients (this work). Patients G and L inherited the point mutation from their mothers, patient S from his father. Expansion of the (GAA) repeat could be proved for father G and for mother S. No DNA sample was available for father L.

Haplotype analyses were performed with six polymorphic markers spanning a region of approximately 1000 kb around the FRDA1 gene. The order of loci was taken from Rodius et al. (1994) and Montermini et al. (1995). The six FRDA haplotypes, three with the M1I mutation and three with the repeat expansion, are given in Table 1. Patients G and L show identical allele combinations for their M1I chromosomes. The equivalent from patient S corresponds to theirs for FR6, D9S202 and D9S411 but is different for D9S889, D9S887 and D9S888. The three (GAA) expansion chromosomes are different for the linkage group D9S889–D9S887–D9S888 but uniform for the FR6 and D9S202 alleles. FR6 is a marker very close to the FRDA1 gene (approximately 250 kb, Montermini et al. 1995). While the (GAA) expansion mutation is linked to FR6 allele 2, the transversions occurred on FR6 allele 3.

Discussion

Sequencing of the FRDA1 gene in a third patient heterozygous for the repeat expansion revealed a mutation of the start codon, which is changed from ATG to ATT. All three unrelated patients are compound heterozygotes as determined by investigation of their parents. In combination with the repeat expansion the mutation of the translation initiation codon causes the FRDA symptoms. If the second methionine can be used for translation initiation, the loss of the first ATG codon may result in a shortened protein. The second initiation codon is located 75 amino acids downstream. The use of this ATG would mean that one-third of frataxin would be missing. At the amino acid level the deletion would comprise the entire exon 1 and parts of exon 2 and would lead to the loss of the mitochondrial localization target signal and mislocation of the protein. On the other hand, the functionally important C-terminus would be present. We do not know whether the shortened protein is expressed. However, the function of the aberrant frataxin should be reduced, producing the affected phenotype in these cases.

The three unrelated families live in Germany. Recently, the allele frequencies for the markers used have been estimated (Zühlke et al. in preparation). The strongest linkage disequilibrium could be identified for the four-allele marker FR6, for which 76% of FRDA chromosomes displayed allele 2, while this allele is present in only 16% of control chromosomes. Inverse frequencies were found for allele 3 at this locus: allele 3 was found in 60% of control chromosomes and in 19% of FRDA chromosomes. The linkage data of these three families agree with this finding. The (GAA) repeat expansion in all three cases is linked to FR6 allele 2, whereas the point mutations originated from allele 3, the most frequent "normal" FR6 allele.

The haplotypes associated with the point mutation are completely identical for patients G and L and correspond in one part to that of patient S also (Table 1). This finding may be explained by a common ancestor. The grandparents with the point mutation of patient L were born in the Dutch-German borderland, the grandparents of patient G originate from Saxony and Poland, and the grandparents of patient S from Pomerania. Saxony and Pomerania became part of East Germany before the second world war and are close to Poland, geographically. Although no relationship between these families is known, a common root is highly probable. The differences in the alleles D9S889, D9S887, and D9S888 between patient S on one side and patients G and L on the other may be the result of a unique recombination event. Therefore, a common ancestor and a relationship of these three families may be postulated.

The conformity of haplotypes with the M1I mutation points to an ancestor in the close past. For ancient mutations, e.g., the (GAA) repeat expansion, multiple haplotypes could be shown (Zühlke et al., in preparation). Nevertheless, a common founder has also been postulated for these FRDA chromosomes (Monros et al. 1996; Cossee et al. 1997b; Zühlke et al., in preparation), and the haplotype diversity is proposed to be the result of numerous recombinations and of microsatellite instability.

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