ORIGINAL ARTICLE

Diagnosis and Genetic Counseling for Friedreich's Ataxia: A time for consideration of TP-PCR in an Indian Setup

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Abstract

Background and Introduction: Expansion of GAA triplet repeats in the first intron of the frataxin gene causes Friedreich's ataxia. Genetic testing in such condition is important to initiate the appropriate genetic counseling for the family members. The conventional genetic tests used in the diagnosis of Friedreich's ataxia are southern blot, short and long PCR. Recently, triplet repeat primed polymerase chain reaction (TP-PCR) methodology was described in the diagnosis of Friedreich's ataxia, especially for detection of long repeats. Accurate genetic diagnosis of Friedreich's ataxia helps in differentiating it from other ataxias and helps provide appropriate genetic counseling for such families. Extended family screening and genetic counseling can prevent birth of children with Friedreich's ataxia in these families.

Materials and Methods: TP-PCR was carried out in 37 samples obtained from Neurology clinic, Sanjay Gandhi Post Graduate Institute of Medical Sciences. The amplified products were subjected to genotyping on a ABI 310 genetic analyser. For heterozygosity, the samples were processed for short and long range PCR.

Results: A total of 37 samples of suspected cases of Friedreich ataxia were analysed. Of these, 81% samples were confirmed as Friedreich ataxia and 19% of samples were found to be negative for Friedreich's ataxia by TP-PCR. Extended family screening was done in 2 of the families. Among the 7 individuals screened, 4 were identified as carriers and genetic counseling was provided to them.

Conclusions: This is first report from India which describes the molecular diagnosis of Friedreich's ataxia by TP-PCR, its utility in extended family screening and genetic counseling. It qualifies as a highly reliable, sensitive and robust technique that can easily be set up in any laboratory.

Keywords: Triplet repeat primed-PCR, Friedreich's ataxia, trinucleotide repeat disorder, genetic counseling, family screening

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Introduction

Friedreich's ataxia (FRDA) was named after Nicholaus Friedreich of Germany[1]. Among the various types of ataxias, FRDA is the most common hereditary ataxia with an estimated prevalence of 1:50,000-1:29,000[2]. Progressive, unremitting ataxia that is mostly accompanied with clumsiness of gait is the principle characteristic of FRDA[3]. The onset of the disease is usually around puberty, but there is a clinical variability of about 2-3 years of age. In a case of late onset, FRDA may occur at the later age of 25 years[2].

The genetic defect in this disease has been assigned to chromosome 9q13-q21, *FXN*[4] with the expansion of a GAA triplet repeat in the first intronic region being responsible for the disease phenotype. Approximately 95% of individuals with FRDA have expanded GAA triplet-repeat mutations in intron 1 of *FXN* on both alleles while 5% of individuals have an expanded GAA repeat mutation in the disease-causing range in one *FXN* allele and an intragenic inactivating *FXN* mutation (i.e. a point mutation or exon deletion outside of the GAA repeat region) in the other allele[5]. These individuals are designated as "compound heterozygotes".

Clinical diagnosis of FRDA is difficult as the clinical picture overlaps with Charcot-Marie-Tooth, ataxia with a Vitamin E deficiency and others[6]. Therefore, genetic testing plays a critical role in accurate diagnosis, which in turn will lead to better management of patient and genetic counseling for family[7].

The gold standard of genetic testing for FRDA is Southern blot. Short PCR is used for the detection of alleles in the normal range and long PCR is used for expanded alleles[8]. A new methodology called triplet repeat primed PCR (TP-PCR) has been described for screening

of expanded alleles in the FRDA gene. TP-PCR was developed by Warner et al. for screening the CAG repeat expansion in myotonic dystrophy and it was implemented in FRDA diagnosis by Schmitt et al[9,10]. It provides a characteristic peak pattern which confirms the existence of expanded triplet repeats in the FRDA gene[8].

The present study aims to explore the value of using TP-PCR in accurate diagnosis of FRDA patients and its utility for genetic counseling in the present setup with the Department of Genetics, Sanjay Gandhi Post Graduate Institute of Medical Sciences, in Lucknow, India. Genetic counseling is the process through which knowledge about the genetic aspects of illnesses is shared by trained professionals with those who are at an increased risk of either having a heritable disorder or of passing it on to their unborn offspring. The aim of this report is to illustrate the potential usefulness of genetic testing and simultaneous genetic counseling in preventing the disease by using data on the families of probands who have been referred to us.

Materials and methods

Sample: 2 mL of blood was collected in EDTA vial from 37 individuals (30 patients and 7 family members along with written informed consent. DNA extraction was accomplished by QIAamp DNA Mini Kit (Qiagen, Valencia, CA). The quality and quantity of DNA was assured by agarose gel electrophoresis and spectrophotometer respectively.

TP-PCR: TP-PCR assay was executed with the primers described elsewhere[11]. The reaction was carried out in a volume of 25 µL with P1, P3 and P4 primers in the concentration of 20 pmol, 20 pmol and 2 pmol respectively, along with 50 ng of Genomic DNA, 200 µM of dNTPs and 1U of Taq Polymerase. The temperature profile adopted in cycling was 1 min at 94°C, followed by 30 cycles of 94°C for 30 sec, 60°C for 30 sec, 72°C for 1 min and one cycle of 72°C for 10 minutes. Amplification was confirmed by observing shearing of the amplified product on 1.5% agarose gel after the run. For fragment analysis 2 µl of amplified product was added to 10 µl of Hidiformamide and 0.5 µl of LIZ 500. This mixture was denatured for 5 minutes at 95°C followed by rapid cooling in ice and analyzed in ABI™ 310 genetic analyzer.

Short and Long PCR: The primers described elsewhere[8] were used in the following concentration for Short PCR GAAF- 10 pmol, GAAR- 10 pmol along with 50ng of DNA, 200 µM of dNTPs, 1U of Taq Polymerase and final volume was made up to 25 µl with water. The cycling conditions were 94°C for 1 min, followed by 30 cycles of 94°C for 20 sec, 60°C for 40 sec, 72°C for 2 min and one cycle of 72°C for 5 min. The amplified product was analyzed on 1.5% agarose gel.

In Long PCR: GAAF-10 pmol, GAAR- 10 pmol along with 50 ng of DNA, 400 µM of dNTPs, 2.5 U of Long amp Taq Polymerase and final volume was made up to 25 µl with milliQ water. The cycling conditions were 2mins at 94°C, followed by 25 cycles of 30 sec at 95°C, 30 sec at 60°C, 7 min at 65°C and one cycle of 15 min at 65°C. The final products were analyzed in 1% agarose gel.

Results

Representative results from our study using TP-PCR are shown in Figure 1. Of all the samples tested, 81% were confirmed as FRDA by TP-PCR. The remaining 19% samples were confirmed as negative for GAA repeat expansion. However 3.3% of the cases were found as heterozygous as suggested by short and long range PCR (Figure 2). These patients are likely to harbour inactivating mutations in the other allele.

The screening for another 7 individuals of the extended family showed that 4 of them were positive for GAA expansion in the TP-PCR. Further analysis of this sample with short and long range PCR confirmed them as heterozygous for the GAA expansion. The pedigree of the family is shown in Figure 3. For these family members, genetic counseling was offered and they decided to undergo premarital screening.

Discussion

FRDA is the most common autosomal recessive ataxia. The identification of GAA repeat expansion is relevant in confirmatory diagnosis as well as in extended family screening (carrier status identification) as these individuals are at high risk of having their children affected by FRDA and can benefit from follow-up recommendation by premarital screening.

Molecular diagnostic tests are imperative for the clinical and prenatal diagnosis. The molecular diagnos-

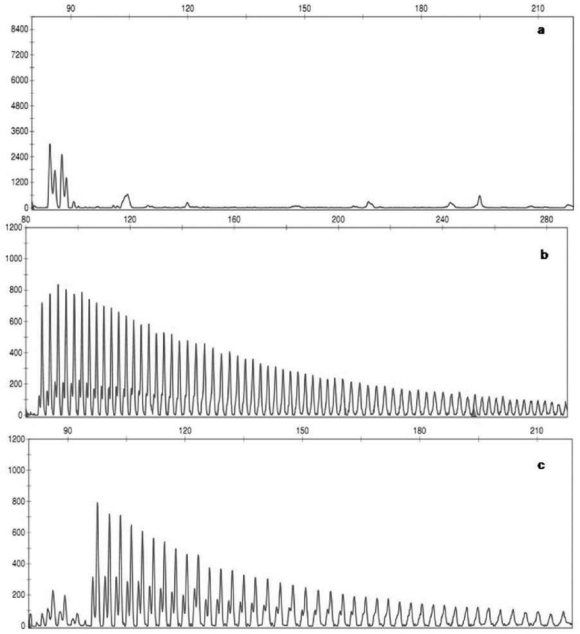

Figure 1: Electropherogram obtained in TP-PCR. The vertical axis represents the signal intensity and the horizontal axis as fragment size in base pairs. a) Normal individual, b) Homozygous individual, c) Heterozygous individual.

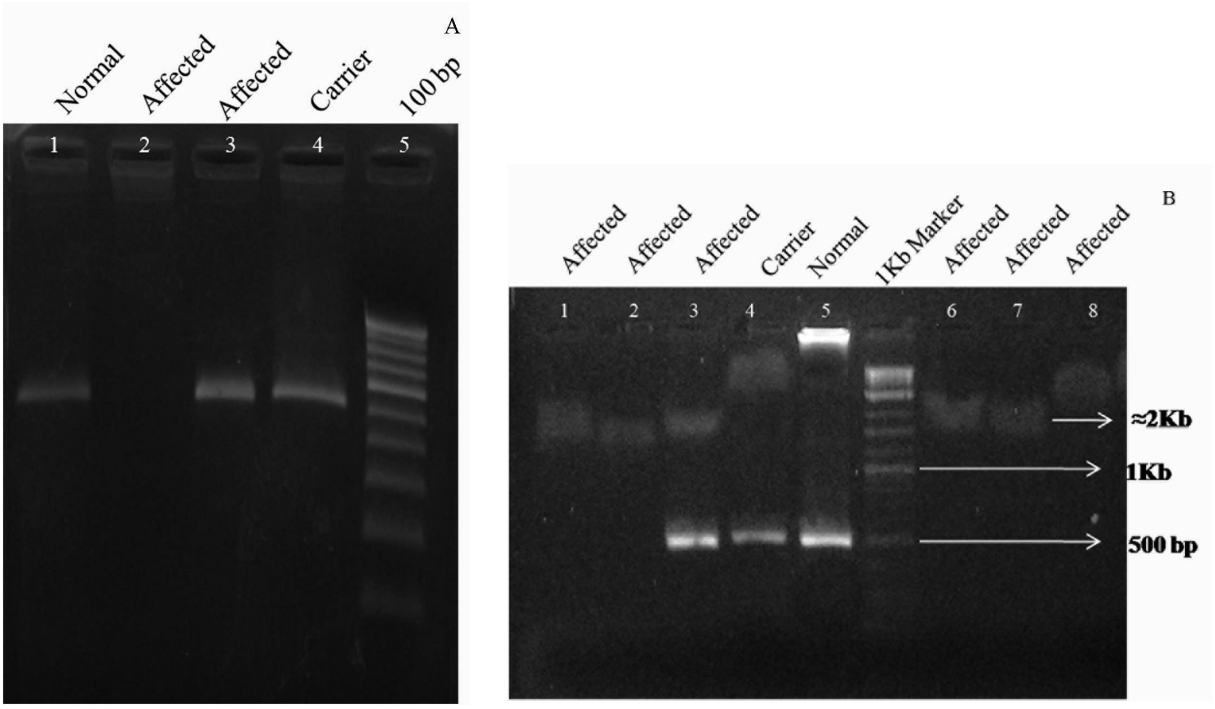

Figure 2: A) Short PCR: In lane 1, 3 & 4 bands were observed within normal range (above 500). Lane 1 was from a normal individual, lane 3 was an affected heterozygous individual and lane 4 was from a carrier individual. In lane 2 absence of band confirms homozygous expansion. B) Long PCR: Lane 1, 2, 6, 7, 8 were samples from an affected individual (homozygous expansion), whose band size are above 1.5Kb. Lane 3: heterozygous affected individual with two bands, one in normal range (500 bp) and another band nearly 2Kb. Lane 4: Carrier individual, two bands observed one in normal range, another above 8kb. Lane 5: Normal individual observed with band of about 500bp.

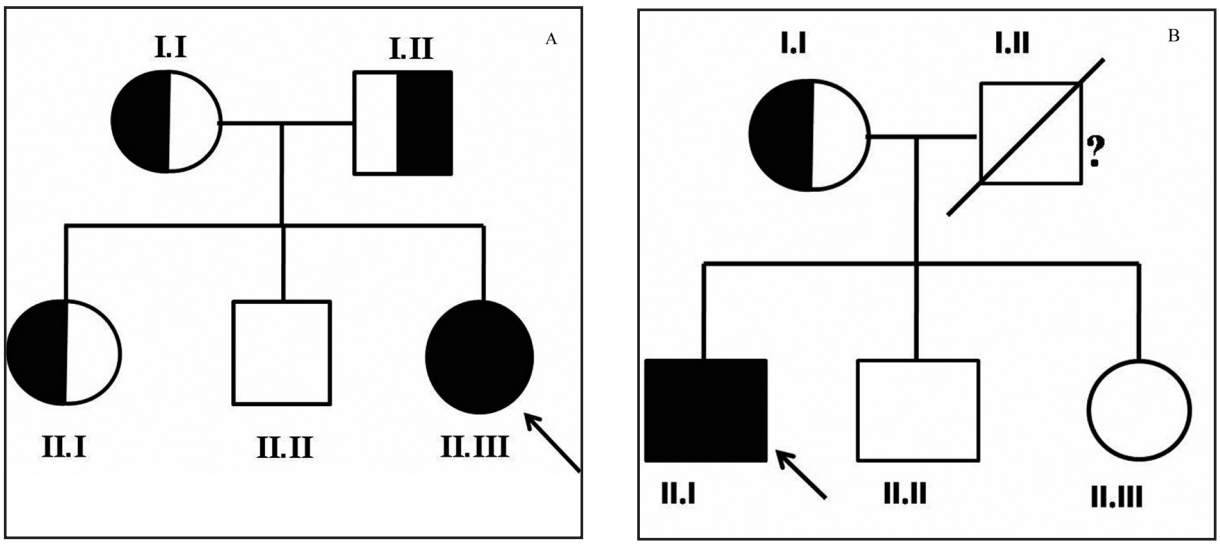

Figure 3: A) Pedigree of Family 1: Mother (I.I) and (I.II) father were healthy carriers with an allele positive for GAA expansion. Among the siblings, elder daughter (II.I) was a healthy carrier, son (II.II) had a GAA expansion in normal range and younger daughter (II.III) was positive for GAA expansion in both the alleles and presented with clinical symptoms for FRDA. B) Pedigree of family 2: Mother (I.I) a healthy carrier, father (I.II) was deceased and genotyping data was unavailable. Among the siblings, the elder son (II.I) was affected and compound heterozygous for expansion.

tic tests are also helpful in carrier detection. Routine molecular diagnostics involves a traditional PCR, amplifying the repeat region followed by agarose gel electrophoresis whereas the gold standard is southern blotting to determine the size of the repeats for FRDA. However, the larger expanded alleles get frequently missed by these traditional PCR approaches and southern blotting is a very cumbersome and expensive method for routine diagnostics. TP-PCR method has many advantages and suits well especially for rapid handling and testing of few samples as required in routine laboratory practice since the method is PCR-based, rapid, and not labor intensive. The analysis can be done using a small amount of DNA. It is a closed tube system that does not require post-PCR handling, and has a high sample throughput. Our study also has revealed that TP-PCR is much more reliable for the detection of expanded repeats in FRDA.

In conclusion, TP-PCR qualifies as a highly reliable, sensitive and robust technique which overcomes the shortcomings of a traditional PCR by detecting the very large expanded alleles of even >5 kb accurately. It can be easily set up in any laboratory as is evident from our study and will help in better genetic counseling of these families.

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Conflicts of Interest

We authors do not have conflicts of interest.

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