

Abstract

Field inversion gel electrophoresis (FIGE) for the analysis of the CCTG repeat in the ZNF9 gene causing myotonic dystrophy type 2

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Myotonic dystrophy type 2 (DM2) is an autosomal dominant disorder resulting in myotonia and primarily proximal weakness. It is caused by an expansion of a CCTG repeat in intron 1 of the ZNF9 gene on chromosome 3. Those individuals with DM2 have CCTG repeats ranging in size between 75 and 11,000 and, therefore, this region can be as large as 44,000 bp in size. In addition, there is considerable somatic heterogeneity of expanded alleles resulting often in a smear of DNA on Southern blot rather than a distinct band. These features can complicate the molecular diagnosis of DM2. Recently Day et. al. (2003), reported that 20% of known carriers were not detected by Southern analysis and introduced a third method of detecting repeat expansions which consisted of PCR amplification of the DM2 repeat followed by Southern analysis of the PCR products probed with an internal probe. In this report I show that by using field Inversion Gel Electrophoresis instead of conventional electrophoresis, repeat expansions can reliably be detected by Southern blot. When used in conjunction with PCR amplification across the repeat, at least 92% of all expansions can be detected.

In our lab, we have tested a total of 54 probands for a CCTG expansion. Out of these, 28 were heterozygous for a normal sized repeat (52%). Out of the remaining 26 individuals, 24 had an expansion (44%) and 2 appeared homozygous normal (4%). In both cases the patients were homozygous for a 140 bp allele which is the most common allele in our population (25%) and in the control population reported by Liquori et. al. (2001) (~21%). If these two homozygotes are individuals who have expansions and were missed by Southern blot analysis, then a maximum of 2/26 (8%) have been misdiagnosed. Clearly we are not missing 20% of positive cases using field inversion gel electrophoresis suggesting this is likely a superior technique to conventional electrophoresis in detecting large and somatically unstable repeats.