New method for detection of C34-T mutation in the AMPD1 gene causing myoadenylate deaminase deficiency

Myoadenylate deaminase (MAD) is the muscle isofrom of AMP deaminase (EC 3.5.4.6). MAD deficiency is the most common enzyme defect in human skeletal muscle and seems to cause a metabolic myopathy which is found in 2-5% of all muscle biopsies. Therefore it might be one of the most common causes of exercise-induced myalgias and early fatigue.

MAD is encoded by the AMPD1 gene. Recently, the nonsense mutation C34-T, which is a single base pair mutation in exon 2, in this gene was reported to cause all cases of MAD deficiency studied so far. This mutation affects the last nucleotide of exon 2 and destroys the MAEI restriction site at the exon 2-intron 2 boundary. This mutation can therefore be detected by MAEI restriction analysis of the AMPD1 gene after PCR of exon 2 and the adjacent intron sequences.2

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Studies on small numbers of subjects revealed a frequency of 0-119 of the mutant allele in white populations. About 1-4% of the population therefore is assumed to be heterogeneous for that mutation. Several asymptomatic homozygotes were identified in these studies. This finding raises questions about the pathogenic significance of MAD deficiency. Determination of allele frequency in a large number of patients with this clinical condition is required to provide specific concerning the clinical importance of MAD deficiency. The C34-T mutation with the above characteristics appears to be of random origin and might not be detected in the Asian gene pool.

The MaeII restriction analysis faces two major disadvantages: first of all, this enzyme is expensive. Secondly, it is only sufficiently active under specific assay conditions. The reaction sample after PCR cannot be amplified to allow the digestion with MaeII. Instead, the PCR product must be precipitated and resuspended in the MaeII assay buffer.

Therefore, a new method for detection of the C34-T mutation is presented that is both less expensive and less time consuming. DNA is extracted from blood by standard procedures. Exon 2 and part of intron 2 of the AMPD1 gene are amplified by PCR using Bethesda primer. The PCR product starts in intron 1 and ends at the second nucleotide of exon 2. This corresponds to the wild type AMPD1 gene and the expression of the second nucleotide of the mutant allele which is C instead of A (5'-CATACGCTG-AAAGACA-3'). After amplification of the mutant allele, the PCR product contains the sequence CATAGT at the exon 2-intron 2 boundary which presents a NsiI restriction site. This NsiI site is not present after amplification of the wild type allele. The primer P2 starts at the 83rd nucleotide of intron 2 (5'-AACACGCTGAAAGATAC-3'). The PCR product has been found to be homozygous for the wild type allele, the primer P2 is therefore of no use. Only the C34-T mutant allele is cut by NsiI into two fragments of 20 and 99 base pairs. NsiI digestion of heterozygous DNA results in three fragments: two fragments of the mutant allele and one of the unrestricted band of the wild type allele.

The PCR is carried out in a DNA thermal cycler (Perkin Elmer Cetus Instruments) for 30 cycles at 94°C denaturing temperature, 44°C annealing temperature, and 72°C extension temperature, each for one minute. The 100 μl PCR reaction volume contains 10 mM Tris-HCl, 50 mM KCl, 1.5 mM MgCl2, 0.001% Tween 20, and 2.5 U AmpliTaq DNA polymerase (Perkin Elmer Cetus) and 10 ng of genomic DNA.

After PCR, 1/10 volume of 100 mM Tris-HCl pH 7.5, 100 mM MgCl2 and 10 mM diethiothreitol (L-buffer from USB, Cleveland, Ohio) are added to the reaction volume. The PCR product is mixed with 10 U NsiI (USB, Cleveland, Ohio) and incubated at 37°C for one hour. After incubation of the DNA mixture to separate by electrophoresis in a 2.5% agarose gel followed by ethidium bromide staining. The 20 base pair fragment is hardly detectable. Only the bands of 99 and 119 base pairs are seen clearly (figure).
DNA of 11 patients with MAD deficiency and 100 randomly selected subjects were analysed for the C34-T mutation using both methods and always demonstrated identical results. The mismatch in primer 1 did not prevent sufficient PCR amplification in any experiment.

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Ethidium bromide staining of an agarose gel after NsPI digestion of PCR product. The PCR amplified exon 2 and part of intron 2 of the AMPD1 gene using P1 and P2 primers. 40 μl of digested PCR product was loaded into a 2.5% gel. (1, 4) homozygous patients with MAD deficiency; (2) heterozygous mother of patient (1); (3) PCR size marker with band of 50, 100, 200, 300, 400, 500, 700 and 1000 bp; (5) wild type DNA.
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