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A PCR-SSP method for detecting the Cys282Tyr mutation in the HFE gene associated with hereditary haemochromatosis

David Smillie

Abstract

Hereditary haemochromatosis is a common genetic disorder that causes hyper-absorption of dietary iron, leading to increased deposition and various organic diseases. Early diagnosis is important if effective treatment is to be applied and the iron overload corrected before the onset of clinical symptoms. Recently, a candidate gene has been identified in which a single point mutation shows a very close association with hereditary haemochromatosis. A polymerase chain reaction method using sequence specific primers (PCR-SSP) is described that, in conjunction with a simple DNA extraction method, would provide a specific diagnostic test or rapid screening procedure for this putative haemochromatosis associated mutation.

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Keywords: haemochromatosis; PCR-SSP; HFE gene; rapid screen

Haemochromatosis is transmitted as an autosomal recessive trait with a homozygosity frequency of 0.005-0.008 and an estimated carrier frequency of 10% among individuals of North European origin. Although a link between haemochromatosis and the human leucocyte antigen (HLA) allele, HLA-A3, was described over 20 years ago,¹ a much closer association has now been found with a mutation in a newly recognised gene, assigned the name HFE (the accepted representation for the haemochromatosis locus approved by the Genome Database Nomenclature Committee).² This gene belongs to the major histocompatibility complex (MHC) class I related gene family and is situated 4.5 Mb telomeric to HLA-A. The mutation, whereby

G is replaced by A at nucleotide position 845 in the cDNA sequence (845G→A), results in the substitution of tyrosine for cysteine at amino acid position 282 in the transcribed protein (Cys282Tyr); this critically affects an intramolecular disulphide bond.³ It is possible that the products of MHC class I related genes are involved in iron metabolism and that this function is compromised by a mutation that affects the structure of the gene product.

Over 83% of haemochromatosis patients are homozygous for the Cys282Tyr mutation in the HFE gene,³⁻⁶ compared with ~0.2% in normal controls. The PCR-SSP method described here allows rapid and specific typing for this haemochromatosis associated mutation.

Methods

DNA was extracted from 2 ml anticoagulated blood by a simple salting out method⁷ and DNA concentration adjusted to 50 ng/μl.

Polymerase chain reaction (PCR) amplification was carried out in a reaction volume of 10 μl, consisting of 20 mM ammonium sulphate, 75 mM Tris-HCl (pH 9.0), 0.01% Tween, 1.5 mM MgCl₂, 200 μM dNTPs, 1.0 μM specific primers (Oswel, Southampton,

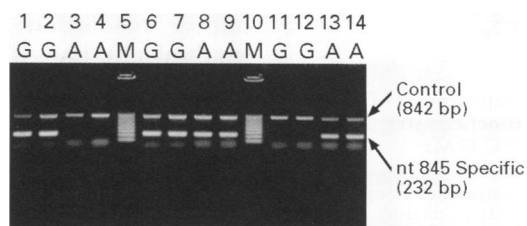


Figure 1 Gel electrophoresis of PCR products from three individuals (I-III). Lanes 1-4 (individual I), nucleotide 845G/G, Cys282Tyr -/-; Lanes 6-9 (individual II), nucleotide 845G/A, Cys282Tyr +/-; Lanes 11-14 (individual III), nucleotide 845A/A, Cys282Tyr +/+; Lanes 5 and 10, molecular markers (100 base pair ladder).

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UK), 0.25 μ M control primers, 0.5 U DNA polymerase (Advanced Biotechnologies, Epsom, UK), and 50 ng genomic DNA. One duplicate reaction set was specific for the normal sequence (845G) and a second reaction set was specific for the mutated sequence (845A); both reaction sets used a common forward primer (5'-AAGGTGACACATCATGTGACC-3'), with a reverse primer specific for either 845G (5'-CTGGGTGCTCCACCTGGC-3'), or 845A (5'-CTGGGTGCTCCACCTGGT-3'), resulting in a PCR product of 232 base pairs. Control primers, which amplified an 842 base pair fragment of the human growth hormone gene⁸ were included in all reactions. Amplification conditions involved an initial denaturation step for one minute at 96°C, followed by five cycles consisting of denaturation for 25 seconds at 96°C, annealing for 45 seconds at 70°C, and extension for 30 seconds at 72°C, then a further 21 cycles where the annealing temperature was lowered to 65°C, plus four cycles where annealing was for one minute at 55°C, and extension was for two minutes at 72°C. A final extension step for five minutes at 72°C completed the amplification.

Results and discussion

The duplicate reaction sets typing for either 845G or 845A were analysed by electrophoresis on a 1.5% agarose gel; PCR products were

visualised by staining with ethidium bromide and photographed under ultraviolet illumination. Results from three representative individuals are shown in fig 1. The results demonstrate that the PCR-SSP method is highly specific for the haemochromatosis associated mutation 845G→A in the HFE gene and that the method could be used as an aid to diagnosis, or for screening purposes.

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High resolution single strand conformation polymorphism analysis using formamide and ethidium bromide staining

T Xie, S L Ho, O C K Ma

Abstract

Single strand conformation polymorphism (SSCP) analysis using ethidium bromide can be improved by adding formamide as the denaturant. This gives higher resolution than previous SSCP methods; it had 100% sensitivity in the discrimination of 14 PCR samples from two different genes, even for a long fragment close to the upper limit of 250 base pairs. This modified procedure is a rapid, simple, safe, and yet highly sensitive method for detecting structural differences in DNA fragments.

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Keywords: single strand conformation polymorphism; formamide; ethidium bromide

Polymerase chain reaction (PCR) amplification of DNA fragments followed by single strand conformation polymorphism (SSCP) analysis is a sensitive method for detecting genetic polymorphisms or mutations. Detection by autoradiography¹ or silver staining² is time consuming, costly, and inconvenient. Non-isotopic SSCP (cold SSCP) using ethidium bromide staining^{3,4} facilitates the rapid identification of structural changes in PCR products. Currently, the denaturant used most frequently in cold SSCP is either sodium hydroxide⁴ or methylmercury hydroxide.³ The former has been reported in some cases to yield inconsistent results³; the latter is an extremely toxic and volatile compound⁵ and great care has to be taken to avoid skin contact with methylmercury and inhalation of its aerosol.

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