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Enhanced capillary electrophoretic screening of Alzheimer based on direct apolipoprotein E genotyping and one-step multiplex PCR

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ABSTRACT

Human apolipoprotein E (ApoE) is associated with high cholesterol levels, coronary artery disease, and especially Alzheimer's disease. In this study, we developed an ApoE genotyping and one-step multiplex polymerase chain reaction (PCR) based-capillary electrophoresis (CE) method for the enhanced diagnosis of Alzheimer's. The primer mixture of ApoE genes enabled the performance of direct one-step multiplex PCR from whole blood without DNA purification. The combination of direct ApoE genotyping and one-step multiplex PCR minimized the risk of DNA loss or contamination due to the process of DNA purification. All amplified PCR products with different DNA lengths (112-, 253-, 308-, 444-, and 514-bp DNA) of the ApoE genes were analyzed within 2 min by an extended voltage programming (VP)-based CE under the optimal conditions. The extended VP-based CE method was at least 120–180 times faster than conventional slab gel electrophoresis methods In particular, all amplified DNA fragments were detected in less than 10 PCR cycles using a laser-induced fluorescence detector. The detection limits of the ApoE genes were 6.4–62.0 pM, which were approximately 100–100,000 times more sensitive than previous Alzheimer's diagnosis methods In addition, the combined one-step multiplex PCR and extended VP-based CE method was also successfully applied to the analysis of ApoE genotypes in Alzheimer's patients and normal samples and confirmed the distribution probability of allele frequencies. This combination of direct one-step multiplex PCR and an extended VP-based CE method should increase the diagnostic reliability of Alzheimer's with high sensitivity and short analysis time even with direct use of whole blood.

1. Introduction

Apolipoprotein E (ApoE) plays an important role in lipoprotein metabolism by serving as a ligand for ApoE receptors [1]. Human ApoE exists in three main isoforms (E2, E3, and E4) related to two polymorphic sites at codons 112 and 158 of the gene located on chromosome 19 [2,3]. These isoforms arise from three alleles (ε2, ε3, and ε4) combined into six different genotypes [4,5]. The ApoE4 isoform has been associated with Alzheimer's disease (AD) as a major risk factor [6–8]. Individuals with heterozygosity of ApoE4 alleles were three to four times more likely to develop AD than those without the ApoE4 allele, and homozygosity of the ApoE4 allele increased the risk more than 10-fold [9]. Thus, determination of the ApoE genotypes will enhance the assessment of patients for associated risk of coronary heart disease or late-onset familial Alzheimer's [10].

By employing the polymerase chain reaction (PCR) to amplify the appropriate parts of the ApoE gene, various methods have been developed to analyze the ApoE genotypes [11–16]. In the last decades, in particular, several PCR-based methods that include PCR [17], PCR in combination with hybridization [18], immuno-PCR that combines monoclonal antibody-coated magnetic beads with PCR [19], PCR in combination with conventional culture and serotyping [20], and real-time PCR [21–26] for detection have been reported. However, all of these methods require pre-enrichment steps of 6–28 h, followed by tedious DNA isolation and purification procedures [11–26].

To reduce the time and complexity of detection, direct PCR methods have been described. The direct PCR methods aim to use raw samples as the template for PCR amplification, thus eliminating the need for DNA purification [27]. In addition, the multiplex PCR method is a useful method that employs several primer pairs in the same amplification

Abbreviation: AD, Alzheimer's disease; ApoE, apolipoprotein E; CE, capillary electrophoresis; EtBr, ethidium bromide; LIF, laser-induced fluorescence; PTFE, polytetrafluoroethylene; PVP, poly(vinylpyrrolidone); SGE, slab gel electrophoresis; TBE, tris-borate-EDTA; VP, voltage programming

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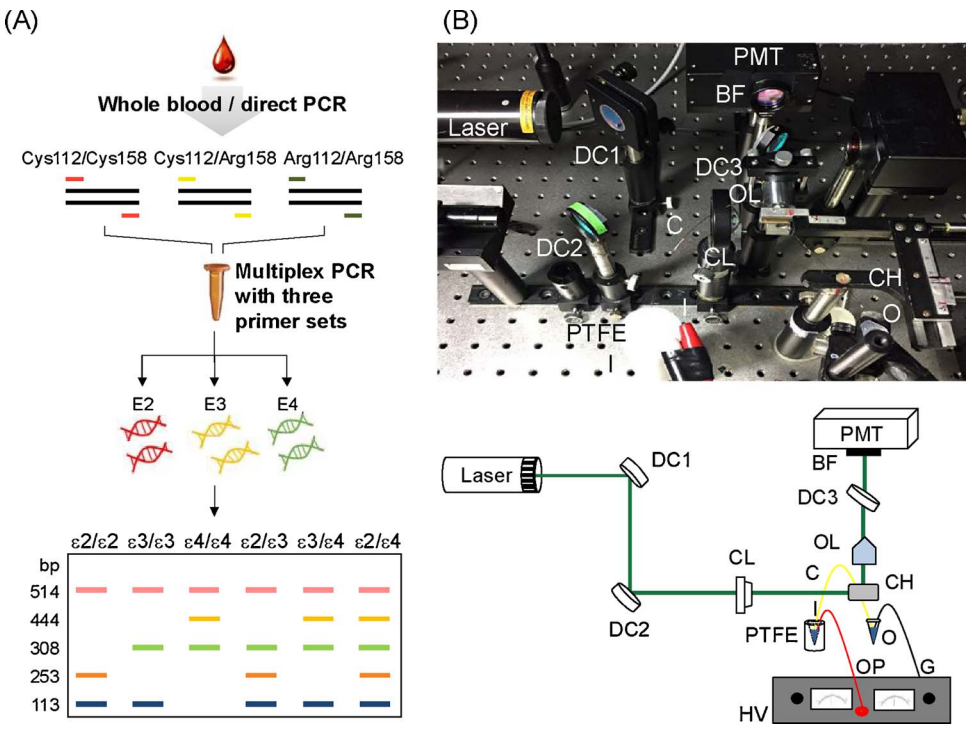

Fig. 1. (A) Scheme of direct one-step multiplex PCR from whole blood. (B) Photograph and schematic diagram of the VP-based CE system with LIF detection. Abbreviations: L1, 543-nm laser; DC 1, dichroic mirror 1; DC 2, dichroic mirror 2; DC 3, dichroic mirror 3; CL, convex lens; C, capillary; I, inlet reservoir; PTFE, polytetrafluoroethylene holder; O, outlet reservoir; H, capillary holder; OL, objective lens; BF, bandpass filter; PMT, photomultiplier tube; HV, high-voltage power supply; OP, output wire; G, ground wire; AB, amplification board.

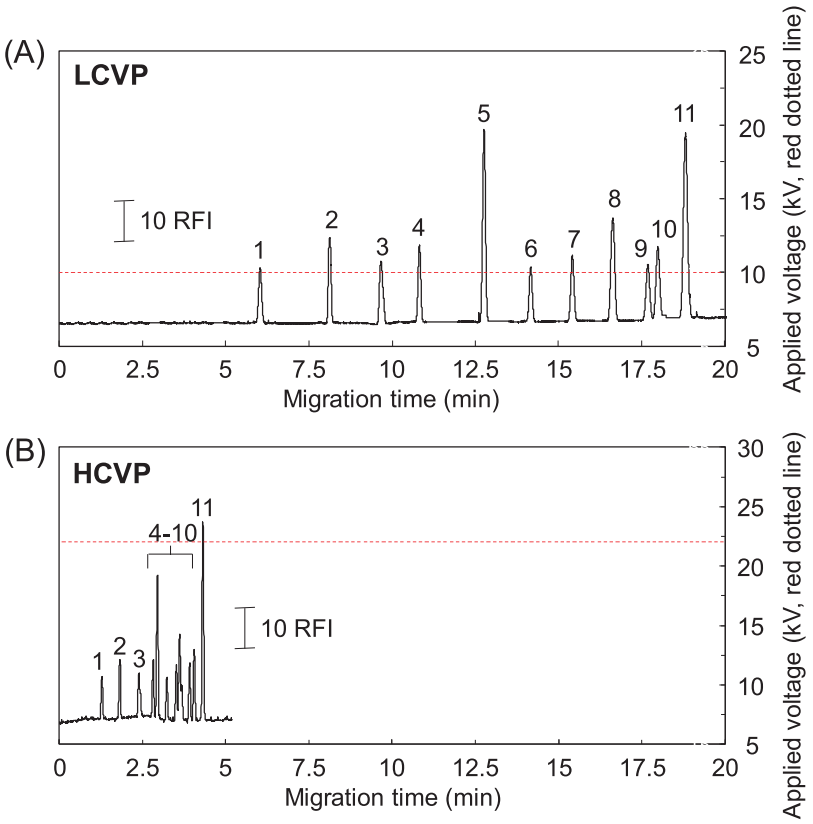

Fig. 2. Representative CE electropherograms of the 100-bp DNA ladder produced by (A) low constant voltage (LCV) and (B) high constant voltage (HCV) conditions. CE conditions: running buffer, 1 × TBE buffer (pH 8.5) with 0.5 ppm EtBr; sieving gel matrix, 1.75% PVP (M_r = 1,000,000); sample injection voltage, 1.7 kV for 15 s; capillary total length, 35 cm (75 μm inner diameter); capillary effective length, 21 cm; applied separation voltage, 10 kV for LCV and 22 kV for HCV. Indications: 1 = 100-bp, 2 = 200-bp, 3 = 300-bp, 4 = 400-bp, 5 = 500-bp, 6 = 600-bp, 7 = 800-bp, 8 = 1000-bp, 9 = 1500-bp, 10 = 2000-bp, and 11 = 3000-bp fragments.

reaction and can detect multiple target DNA sequences by simultaneous amplifications in one tube [28,29] (Fig. 1A). Several methods using multiplex PCR have been studied for the detection of six instances of the ApoE genotype (ε2/ε2, ε3/ε3, ε4/ε4, ε2/ε3, ε2/ε4, and ε3/ε4) [30–32]. The amplified PCR products have been analyzed by slab gel electrophoresis (SGE). However, conventional SGE methods are still quite time consuming, labor intensive, hard to automate, and require significant amounts of sample. In addition, data acquisition has to take place after the separation step in an off-line fashion; moreover, small DNA fragments usually are difficult to detect, giving rise to faint and ambiguous bands.

Capillary electrophoresis (CE) has become an alternative method for separating DNA fragments [33–37]. In conventional CE, as the electric field is increased, the mobility of the analyte and the resolution are

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