Comparison of Two Real-Time PCR Assays for Apolipoprotein E Genotyping

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Apolipoprotein E (*APOE*) polymorphisms are used as biological markers to assess the risk of cardiovascular disease, dyslipidemia, and Alzheimer's disease. Consequently, *APOE* genotyping is one of the most frequently conducted tests in clinical molecular laboratories. Although *APOE* genotyping may appear to be uncomplicated and a relatively easy test to perform, genotyping errors can still occur due to polymorphisms near codons 112 and 158 in the human *APOE* gene. Therefore, validation and verification of *APOE* genotyping assays before clinical use are essential. So far, we have been using the TaqMan SNP Genotyping Assay (Life Technologies, USA). However, recently, the Real-Q ApoE genotyping kit (BioSewoom, Korea) was approved by the Korean Ministry of Food and Drug Safety, which led us to compare the results obtained from this genotyping kit to those of the TaqMan SNP Genotyping Assay. The Real-Q ApoE genotyping kit yielded correct genotyping results for all six *APOE* genotypes and provided concordant results with the TaqMan SNP Genotyping Assay in a series of blinded comparison samples. Thus, we validated its use in clinical tests.

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Apolipoprotein E (*APOE*) polymorphisms are used as biological markers to assess the risk of cardiovascular disease, dyslipidemia, and Alzheimer's disease [1,2]. The human *APOE* gene, located on chromosome 19q13.2, is polymorphic at codons 112 and 158, yielding three common codominant alleles (ϵ 2, ϵ 3, and ϵ 4) and six different genotypes [2-5]. Although sequencing analysis is currently the standard method for *APOE* genotyping, it is laborious, expensive, and time-consuming. Hence, several molecular methods, including allele-specific polymerase chain reaction [6,7], PCR-restriction fragment length polymorphism [8], and real-time PCR assays using

fluorescence resonance energy transfer or TaqMan probes, have been developed [9-13].

We have used the TaqMan SNP Genotyping Assay (Life Technologies, Grand Island, NY, USA). However, recently, the Real-Q ApoE genotyping kit (BioSewoom, Seoul, Korea) was approved by the Korean Ministry of Food and Drug Safety, leading us to evaluate this genotyping kit and compare its results to those of the TaqMan SNP Genotyping Assay.

Twenty-one specimens with known *APOE* genotypes (set 1), as determined by the TaqMan SNP Genotyping Assay, and another 53 specimens with unknown *APOE*

genotypes (set 2) were used to compare the two assays. Genomic DNA (gDNA) was extracted from EDTA-anticoagulated whole blood using the MagNA Pure LC 2.0 System (Roche Applied Science, Penzberg, Germany), according to the manufacturer's instructions. For the TaqMan SNP Genotyping Assay, 5 μ L of TaqMan reagent (2 $\times$ TaqMan GTXpress Master Mix), 0.5 μ L of 20 $\times$ TaqMan SNP Genotyping Assay reagent, and 4 μ L of sterile water were mixed with 0.5 μ L of gDNA (100 ng/ μ L). After a pre-heating step at 95°C for 20 seconds, 40 reaction cycles were performed using the Applied Biosystems 7500 Real-Time PCR System platform (Applied Biosystems, Foster City, CA, USA) with a denaturation step at 95°C for 3 seconds and annealing and extension at 60°C for 30 seconds each. After the procedure, the genotypes were determined using the Sequence Detection System software (Applied Biosystems).

For the Real-Q ApoE genotyping assay, 21 μ L of master mix (including 17.5 μ L of reaction mixture, 3 μ L of a primer mixture for codon 112 and 158, and 0.5 μ L of sterile water) was mixed with 4 μ L of gDNA. A pre-heating step at 95°C for 10 minutes was followed by 40 reaction cycles, which were performed on the Applied Biosystems 7500 Real-Time PCR System platform, was carried out along with a denaturation step at 95°C for 20 seconds and annealing and extension at 60°C and 72°C, respectively, for 30 seconds each. When the reaction cycles were completed, the results were analysed by the standard curve method (Applied Biosystems).

Set 1 included all six possible APOE genotypes: $\epsilon 2/\epsilon 2$ (n=2), $\epsilon 2/\epsilon 3$ (n=7), $\epsilon 2/\epsilon 4$ (n=2), $\epsilon 3/\epsilon 3$ (3), $\epsilon 3/\epsilon 4$ (n=5)

and $\epsilon 4/\epsilon 4$ (n=2), which were all genotyped correctly using the Real-Q ApoE genotyping assay. Among set 2, the $\epsilon 3/\epsilon 3$ genotype (n=37) was the most common, followed by the $\epsilon 3/\epsilon 4$ genotype (n=12). The results of the two assays were completely concordant in the genotypes of all 53 specimens (Table 1).

APOE genotyping is one of the most frequently conducted tests in clinical molecular laboratories, along with the methylenetetrahydrofolate genotyping test. Recent reports on national proficiency testing by the Korean Institute of Genetic Testing Evaluation showed that 41 institutions are performing APOE genotyping. Among these institutions, Seeplex ApoE ACE Genotyping (Seegene, Seoul, Korea) was most commonly used (in 11 Korean institutions, 26.8%), followed by the Bio-Core ApoE genotyping PCR KIT (BioCore, Seoul, Korea), which was used by 7 institutions (17.1%) [14]. Although APOE genotyping may appear to be uncomplicated and a relatively easy test to perform, genotyping errors can still occur due to polymorphisms near codons 112 and 158 in the human APOE gene [15]. Therefore, validation and verification of APOE genotyping assays before clinical use are essential.

The Real-Q ApoE genotyping kit yielded correct genotyping results for all APOE genotypes and provided concordant results with the TaqMan SNP Genotyping Assay in a series of blinded comparison samples. Thus, we validated the use of this kit for clinical tests.

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Table 1. APOE genotyping results of 53 unknown samples

APOE genotype	No. of samples (%)
$\epsilon 2/\epsilon 2$	0
$\epsilon 2/\epsilon 3$	4 (7.5)
$\epsilon 2/\epsilon 4$	0
$\epsilon 3/\epsilon 3$	37 (69.8)
$\epsilon 3/\epsilon 4$	12 (22.6)
$\epsilon 4/\epsilon 4$	0

Abbreviation: APOE, apolipoprotein E.

- protein with expanding role in cell biology. *Science* 1988; 240:622-30.
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아포지단백 E 유전자형 검사를 위한 두 가지 실시간 중합효소연쇄반응법 비교

정유정 • 최리화 • 정재영 • 이명근 • 기창석 • 김종원

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아포지단백 E 유전자(apolipoprotein E, APOE) 유전자형 검사는 분자유전검사실에서 가장 흔히 시행하고 있는 유전자형 검사인데, APOE 유전자 다형성이 심혈관계질환, 이상지질혈증 및 알츠하이머 치매의 위험도를 평가하는 생물학적 지표로 활용되고 있기 때문이다. APOE 유전자형 검사는 비교적 시행이 간편한 검사이지만, APOE 유전자 다형성을 결정하는 112번째 및 158번째 아미노산 주변의 또 다른 다형성으로 인해 유전자형 판별 오류가 일어날 가능성이 있다. 따라서 실제 APOE 유전자형 검사를 위한 시약을 결정할 때 검증과정이 반드시 필요하다. 본 연구에서는 APOE 유전자형 결과를 알고 있는 21개의 검체와 결과를 알지 못하는 53개의 검체를 이용하여, 기존에 사용하고 있던 TaqMan SNP Genotyping Assay (Life Technologies, USA)와 최근 식품의약품안전처로부터 의료기기 승인을 받은 Real-Q ApoE genotyping kit (BioSewoom, Korea)를 비교 검증하고자 하였다. 그 결과 Real-Q ApoE genotyping kit는 결과를 알고 있던 21개의 검체 및 결과를 알지 못했던 53개의 검체 모두에서 기존 검사법과 일치하는 결과를 보여 임상적으로 사용하기에 적합한 검사법으로 판단하였다.

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