Blood DNA isolation. Blood DNA isolation was performed using PAXgene blood DNA kit (Cat. no. 761133, PreAnalytiX, Qiagen, Switzerland) following the manufacturer's protocol. Briefly, frozen whole blood samples collected in PAXgene blood DNA tubes (Cat. no. 761115, PreAnalytiX, Qiagen, Switzerland) were thawed in 37°C water bath for 15 min. Samples were poured into individual 50ml processing tubes with 25 ml of BG1. Tubes were capped, inverted 5 times, centrifuged at 2500 x g for 5min in a swing-out rotor, and discarded supernatant carefully. 5ml of BG2 was then added, followed by vigorous vortexing for 5 sec, tubes were centrifuged at 2500 x g for 3 min in a swing-out rotor, and discarded supernatant. 5 ml of BG3 with 50 µl reconstituted PreAnalytiX Protease (included in the kit) was added, tubes were vortexed for 20 sec, placed in 65°C water bath for 10 min, and vortexed again for 5 sec at high speed. 5 ml of 100% Isopropanol was added, tubes were inverted for at least 20 times, centrifuged at 2500 x g for 3 min in a swing-out rotor, and discarded supernatant. Tubes were kept inverted for 1 min on an absorbent paper. 5 ml 70% Ethanol was added, tubes were vortexed at high speed for 1 sec, centrifuged at 2500 x g for 3 min in a swing-out rotor, and discarded supernatant. Tubes were kept inverted for at least 5min on an absorbent paper. 1 ml BG4 was added into each tube, incubated for 1 hr at 65°C water bath, and further incubated overnight at RT to fully dissolve the DNA. DNA quantification and quality assessment were done using Nanodrop (Thermo Fisher Scientific, MA, USA). Genomic DNA samples were stored in -80°C freezer until use for downstream experimentations/analyses.

APOE genotyping. APOE genotyping was conducted using Real-Time PCR described by Calero et al. with modifications [1]. Primers used were as follows: ApoE.112C Forward – 5' CGGACATGGAGGACGTGT 3'; ApoE.112R Forward – 5' CGGACATGGAGGACGTGC 3'; ApoE.158C Reverse – 5' CTGGTACACTGCCAGGCA 3'; and ApoE.158R Reverse – 5'

CTGGTACACTGCCAGGCG 3'. Primer combinations for each specific allele target were as follows: ε2 – ApoE.112C and ApoE.158C; ε3 – ApoE.112C and ApoE.158R; and ε4 – ApoE.112R and ApoE.158R. Power SYBR Green PCR Master Mix (Cat. no. 4368577, Applied Biosystems, CA, USA) executed on Bio-Rad CFX96 Real-Time PCR detection system was used to determine the expression of each allele per sample. PCR reaction per allele were as follows: Power SYBR Green PCR Master Mix (1x final concentration); Primers (0.3 uM final concentration each); and 50 ng genomic DNA. Negative control (no DNA template) was also prepared. All reactions were run in triplicate. Real-Time PCR setting: 95°C, 10min initial activation; followed by 40 cycles of 95°C, 15 sec (denaturation), 62°C, 1 min (annealing/extension), and plate read. Melt-Curve Analysis was set at 65°C-95°C, 0.5°C increment, 5 second/step. The results were analyzed using the expected Ct range ($15 \leq Ct \leq 25$ cycles) used by Calero et al [1]. Assay was considered failed if Ct value was below the lower and above the upper limits.

REFERENCE

[1] Calero O, Hortigüela R, Bullido MJ, Calero M. Apolipoprotein E genotyping method by Real Time PCR, a fast and cost-effective alternative to the TaqMan® and FRET assays. J Neurosci Methods 2009. https://doi.org/10.1016/j.jneumeth.2009.06.033.