A Streamlined PCR-based Fragment Analysis Assay that Resolves Both Single Nucleotide and Poly-T Length Polymorphisms at *APOE* and *TOMM40* Susceptibility Loci in Alzheimer's Disease

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Summary

- Alzheimer's disease (AD) is an irreversible neurodegenerative disorder that progresses slowly over time, affecting 5.4M Americans in 2016, or 1 in 10 Americans age 65 and older.
- Sequence variants in *TOMM40* and *APOE* are two genetic risk predictors of AD. However, these sequences are difficult to genotype using conventional PCR due to their AT- and GC-rich character.
- We unified a proof-of-concept *APOE* assay with our AmplideX® PCR/CE *TOMM40* Kit (RUO) to simultaneously genotype both genetic markers.
- Here we report the first single-assay workflow that achieves reproducible and accurate resolution of *TOMM40* poly-T length polymorphisms and all 6 *APOE* SNP genotypes.

Introduction

Alzheimer's disease (AD) accounts for 60-80% of all dementias in the US[1]. Previous studies have identified an AD susceptibility locus in a region that includes Apolipoprotein E (*APOE*) and Translocase Of Outer Mitochondrial Membrane 40 (*TOMM40*). Of the three common *APOE* alleles, ε4 is the most informative but in only ~25% of Caucasians[2]. Independently, poly-T polymorphisms within the *TOMM40* gene have been reported to influence both age of onset in late-onset AD (LOAD) and measures of cognitive decline elevating informative risk data for LOAD to ~97% of individuals[2,3,4]. The AmplideX® PCR/CE *TOMM40* Kit (RUO) is a commercially available option for this marker. Here we describe the first PCR-based workflow that unifies *APOE* and *TOMM40* genotyping.

Materials and Methods

Cell-line gDNA samples (14 AD, 104 other) were acquired from the Coriell Institute. Whole blood (WB; 97) and matched WB/buccal gDNA samples (77) were isolated from presumed healthy donors (Asuragen, Equitech Enterprises). Sample gDNA was PCR amplified using prototype AmplideX reagents (Asuragen) by different operators on different days. Amplicons tagged with FAM (*TOMM40*) and HEX (*APOE*) were resolved on a 3500xL Genetic Analyzer (Thermo Fisher Scientific) CE instrument using POP-7™ polymer with 2.5kV, 20 sec injection and 20 min run time. Genotype analysis utilized a ROX 400HD size ladder (Thermo Fisher Scientific) in separate color channels from target amplicon patterns (*APOE*) or from the mobility of target amplicon (*TOMM40*) relative to a three-point cell-line DNA calibrator (16T, 29T, 36T poly-T lengths).

Amplidex PCR/CE *TOMM40* Kit - Research Use Only - Not for Use in Diagnostic Procedures
Prototype *APOE* reagents used to gather preliminary research data. The performance characteristics of this assay have not yet been established.
Presented at AAIC 2017

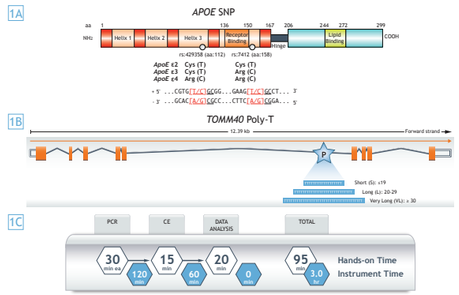

Figure 1. Genotyping *APOE* and *TOMM40* in a streamlined workflow from sample-to answer. A) *APOE* is found on human Chromosome 19 (figure adapted from Liu et. al.[5]). Three common alleles designated ε2, ε3, and ε4 corresponding to 2 SNP variants in Exon 4 were associated with AD in 1993[6]. Unique primers were designed to specifically amplify each allele differentiated based by amplicon size. **B)** The variable poly-T tract of *TOMM40* is located in intron 6 of the gene (blue star). This length polymorphism was binned into the following genotypes: Short (≤19 Ts), Long (20-29 Ts), and Very Long (≥30 T). **C)** For the prototype assay, PCR and thermocycling conditions were independent but similar for the two targets. Dye-labelled amplicons were combined in a single CE injection after PCR. The unified assay has a turnaround time of ~4.5 hrs for 24 samples and a total hands-on time of ~1.5 hrs.

Results

Figure 2. All six possible *APOE* and *TOMM40* genotypes were resolved using PCR methods. A) Five of six *APOE* genotypes were identified within Coriell cell-line and WB samples. ε2-ε2 was positively identified in PC3 cell line (ATCC). GS is a positive gene specific control. **B)** The *TOMM40* poly-T repeat number was determined within a single nucleotide for all sample genotypes assayed between 15 and 48Ts. **C)** Genotype distribution for 174 WB samples recapitulated the prevalence of *APOE* and *TOMM40* variants that were previously reported. Although 21.5% of samples had *TOMM40* genotypes that fell near allele length boundaries, all calls were repeatable.

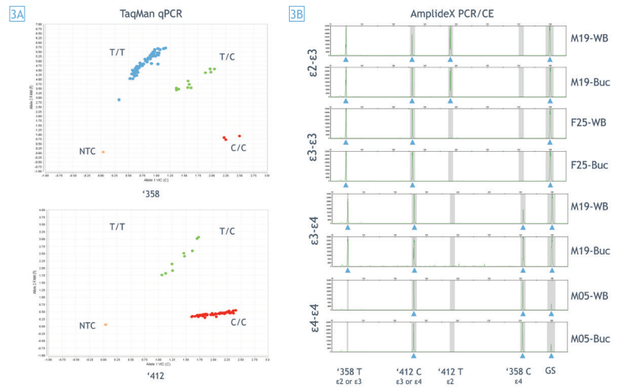

Figure 3. *APOE* genotypes resolved by the prototype assay agreed with reference calls for all 292 cell-line, blood and buccal cell samples. A subset of samples and controls were duplicated across different operators and days. All results were repeatable. Input gDNA concentration was measured by A_{260} and ranged from 5.5ng to 176.8ng. **A)** The *APOE* TaqMan® SNP Genotyping qPCR Assay (Thermo Fisher Scientific) was used as a reference method to determine agreement. Matched WB (43) and each AD Coriell cell-line genotype (4/6) and NTC (1) are shown. **B)** Representative WB and buccal PCR electropherograms demonstrate 4 of the 6 possible *APOE* genotypes.

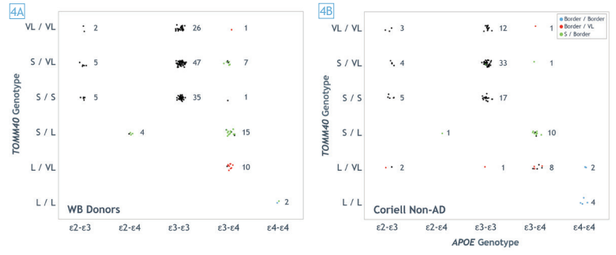

Figure 4. Assayed *APOE* and TOMM40 genotypes reflect known allele-variant associations. A) Distribution of *APOE* and *TOMM40* genotypes matched for all independent samples from WB (160) and **B)** Coriell cell-line non-AD (104). Colors represent samples containing one or both alleles with a poly-T length of 30±2 (L/VL boundary) for all but one WB sample with a 20 T allele (S/L boundary).

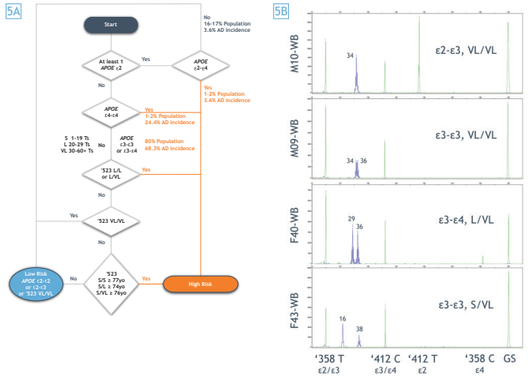

Figure 5. *APOE* and *TOMM40* PCR amplicons could be resolved from a single-assay PCR/CE workflow to assess AD risk. A) Risk algorithm and prevalence were previously published[5,7]. *TOMM40* poly-T length was found to have a strong relationship with age of onset, further refining the *APOE* association with AD with L showing the highest risk[8]. **B)** Co-injection on CE simplified both the workflow and data analysis, and stratified the largest subset of *APOE* genotypes (ε3-ε3 and ε4-ε4) into high and low risk groups.

Conclusions

- A single PCR/CE technology resolved SNP and length polymorphisms in *APOE* and *TOMM40*, respectively, and produced concordant genotypes from 292 samples across three gDNA sources, multiple operators and different days.
- This streamlined workflow analyzes purified DNA with a turn-around time of 4.5 hrs.
- This multimodal approach has the potential to advance clinical research using a standardized assay that can harmonize results across laboratories.

References

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