Multi-site Evaluation of the AmplideX® PCR/CE *TOMM40* Kit (RUO) for Rapid and Accurate Genotyping of Poly-T Length Polymorphisms at rs10524523 of the *TOMM40* Gene

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Summary

- Alzheimer's disease (AD) is the most common cause of dementia, accounting for 60-80% of all dementias and impacting 5.4M Americans.
- An improved risk test may provide insights for AD clinical research, having the potential to improve patient management in the future.
- A poly-T repeat in the *TOMM40* gene is associated with age-of-onset risk of AD in some studies. However, this region is very AT-rich and resists efficient, high-fidelity PCR amplification and detection.
- The AmplideX® PCR/CE *TOMM40* Kit (RUO) achieved rapid and reliable genotyping of the rs10524523 poly-T polymorphism with single-nucleotide resolution across multiple operators, days, instruments and laboratories, and in different sample types.

Introduction

There is increasing interest in identifying Alzheimer's disease (AD) populations that can benefit from early intervention. Poly-T polymorphisms at rs10524523 ('523) of the *TOMM40* gene have been reported to influence age of onset in late-onset AD (LOAD)[1] and the rate of cognitive decline[2]. Studies that have examined this locus, however, have been limited by assays with cumbersome workflows and/or suboptimal resolution. Here we describe a two-site evaluation of a fast, simple and accurate PCR assay that reproducibly reports *TOMM40* poly-T length.

Materials and Methods

The assay was evaluated at both Asuragen (Site 1) and the University of Pennsylvania (Site 2). Both sites genotyped a common set of cell-line samples. Site 1 assessed additional cell-line materials and 14 matched buccal and peripheral blood samples. Site 2 assessed 93 brain tissue samples, covering 2 cohorts. The AD cohort had 64 neuropathologically confirmed AD cases, of which 11 had matching peripheral blood samples, while the control cohort had 29 without AD neuropathology. Sample gDNA was PCR amplified using the AmplideX® PCR/CE *TOMM40* Kit (RUO) and amplicons were resolved by capillary electrophoresis (CE) on the 3500xL instrument (Thermo Fisher Scientific). Genotypes were determined from the mobility of target peaks relative to a calibration curve.

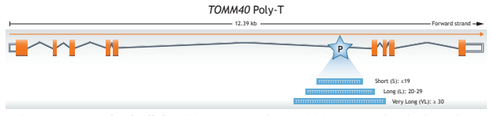

Figure 1. *TOMM40* gene and risk alleles. *TOMM40* (Translocase Of Outer Mitochondrial Membrane 40) is a protein-coding gene that is a part of the translocase of the outer mitochondrial membrane pore subunit. A variable poly-T tract, rs10524523 ('523), located in intron 6 of the *TOMM40* gene (blue star), was found to have a strong relationship with the age of onset, further refining the *APOE* association with AD[1]. This poly-T polymorphism was binned into the following *TOMM40* genotypes: Short (S, ≤19 Ts), Long (L, 20-29Ts), and Very Long (VL, ≥30 Ts), with L showing the highest risk[1].

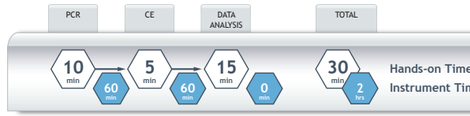

Figure 2. Time-motion for the AmplideX PCR/CE *TOMM40* (RUO) assay workflow. An overview of the *TOMM40* assay workflow, highlighting the rapid turnaround time of ~2.5 hours from gDNA to answer. Total hands on time is ~30 min for 24 samples.

Results

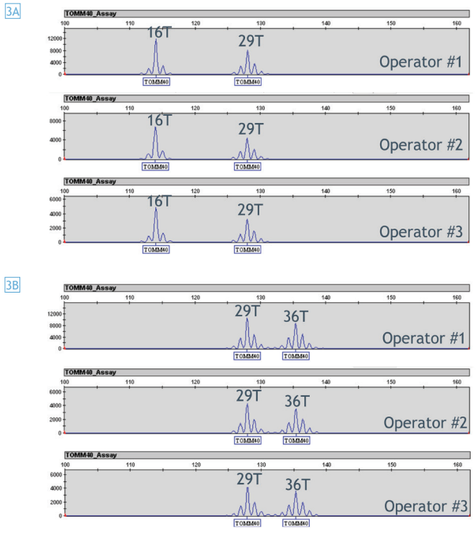

Figure 3. The *TOMM40* PCR/CE assay produces reproducible poly-T genotypes across multiple operators and days. DNA samples with 16-36 Ts were assessed by three operators across two sites, three different days and three 3500xL instruments. All results were in agreement within a single nucleotide repeat. Representative electropherograms are shown for **A)** an S/L sample of 16/29 Ts and **B)** an L/VL sample of 29/36 Ts.

Sample #	Site 1	Site 2
Sample1	16/16	16/16
Sample2	16/35	16/35
Sample3	16/29	16/29
Sample4	35/36	35/36
Sample5	34/36	34/36
Sample6	29/36	29/36
Sample7	16/36	16/36
Sample8	16/30	16/30
Sample9	16/35	16/35
Sample10	16/16	16/16
Sample11	16/29	16/29
Sample12	16/34	16/34
Sample13	16/22	16/22
Sample14	16/29	16/29
Sample15	16/16	16/16
Sample16	16/36	16/36

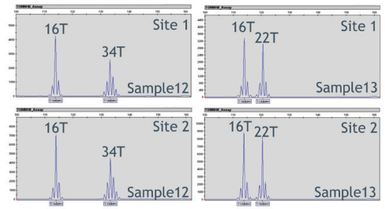

Figure 4. *TOMM40* genotypes generated at two different laboratories are identical across 16 cell-line/whole blood gDNA samples. Evaluation of the AmplideX PCR/CE *TOMM40* Kit at both Asuragen (Site 1) and the University of Pennsylvania (Site 2) resulted in 100% agreement for 16 samples. An example of the output from both sites is shown for two blood samples.

Sample #	*APOE* genotype	Gender	Age at Onset	Age at Death	*TOMM40* Brain	*TOMM40* Blood	*TOMM40* genotype
Sample1	E3/E3	M	76	85	16/16	16/16	S/S
Sample2	E3/E3	F	83	90	36/37	36/37	VL/VL
Sample3	E3/E3	F	67	78	36/37	36/37	VL/VL
Sample4	E3/E4	M	85	87	16/34	16/34	S/VL
Sample5	E3/E4	M	75	79	16/29	16/29	S/L
Sample6	E3/E4	M	75	86	16/29	16/29	S/L
Sample7	E3/E4	F	66	85	29/34	29/34	L/VL
Sample8	E4/E4	F	N/A	80	29/29	29/29	L/L
Sample9	E4/E4	M	66	72	22/29	22/29	L/L
Sample10	E4/E4	M	74	77	29/29	29/29	L/L
Sample11	E4/E4	F	79	85	29/30	29/30	L/VL

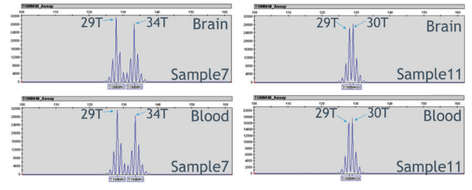

Figure 5. *TOMM40* genotypes agree in matched brain and whole blood samples. Site 2 demonstrated full agreement in the number of poly-T repeats for 11 paired samples, with a median time difference between paired collections of 1520 days. Sample7 and Sample11 electropherograms are shown as an example of the genotype profiles. A separate sample set also revealed genotype concordance for matched buccal and whole blood samples at Site 1 (not shown).

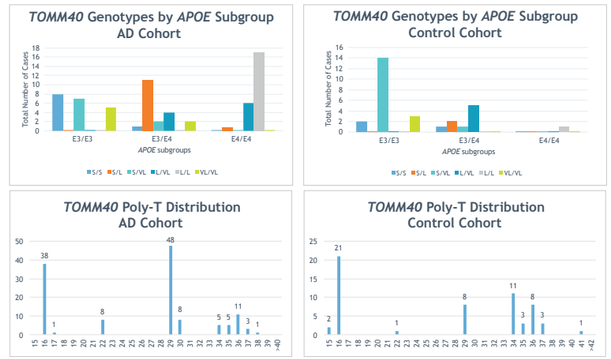

Figure 6. Distribution of *TOMM40* genotypes across 93 patient samples. Distribution of *TOMM40* genotypes in a cohort of neuropathologically confirmed AD cases (AD cohort, n=64) and a control cohort without AD neuropathology (n=29) from the Center for Neurodegenerative Disease Research (CNDR) brain bank at the University of Pennsylvania. All were genotyped on the 3500xL instrument as described.

Conclusions

- The AmplideX® PCR/CE *TOMM40* Kit (RUO) enabled single-base poly-T resolution, reproducible genotyping, and unambiguous data interpretation from cell-line, blood, buccal, and brain DNA.
- Matched specimens from blood and brain cells, and blood and buccal cells, demonstrated identical *TOMM40* '523 genotypes.
- The combination of reproducible assay results and a simple, rapid, and scalable workflow may help standardize findings across many different sites in large-scale research studies.
- This assay has potential to improve risk assessments for LOAD, particularly when combined with *APOE* genotyping and/or other prognostic information.

References

1. Crenshaw DG, et al. Using genetics to enable studies on the prevention of Alzheimer's disease. Clin Pharmacol Ther. 2013;93(2):177-85.
2. Yu L, et al. *TOMM40* '523 variant and cognitive decline in older persons with *APOE* ε3/3 genotype. Neurology. 2017 Feb 14;88(7):661-668.

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