Assessing the Influence of TOMM40-523' Enhancer Length on APOE ε3 Expression in Alzheimer's Disease

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The APOE3 allele, the most common APOE variant, is believed to influence Alzheimer's Disease (AD) progression. It has been proposed that the TOMM40 gene – which neighbors APOE3 -- might serve a regulatory function on APOE3 expression, and, therefore, may affect the progression of AD in APOE3-homozygous patients. TOMM40 contains a poly-T repeat known as TOMM40-523', which varies in nucleotide length between individuals ("Short (S)" (<19nt), "Long (L)" (19nt-29nt), "Very Long (VL)" (>29nt)). Prior analysis suggests that TOMM40-523'(VL) may have a protective effect compared to TOMM40-523'(S) against AD progression in APOE3-homozygous patients. It is hypothesized that the TOMM40-523' (VL) repeat may be in gene linkage with variants (located in the TOMM40 enhancer and APOE3 promoter regions) that give it this observed protective effect. In the current study, we aim to investigate the suspected regulatory effect of these variants on APOE3 activation. PGL4.10[luc2] vectors were ligated with APOE promoter/TOMM40 enhancer regions containing these variants. AD-associated cell types (neurons, microglia, astrocytes) were transfected with these vectors and analyzed using luciferase reporter assays. In microglia/astrocytes, a TOMM40-523' (VL) enhancer activated the APOE3 promoter (VL haplotype) significantly more than the TOMM40-523' (S) enhancer did (microglia: $p = 0.003$; astrocytes: $p = 0.001$). In general, a TOMM40-523'(S)/APOE3 (S) pairing displayed less fluorescence than a TOMM40-523'(VL)/APOE3 (VL) pairing in microglia/astrocytes. Enhancer activity of TOMM40-523'(VL) was confirmed in microglia/astrocytes, supporting the initial hypothesis. Future analysis will expand the APOE3 promoter/TOMM40-523' enhancer regions to include other potential regulatory variants.