Poly-glycine-arginine pathology and *CASP8* intronic expansion variants in Alzheimer's disease

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Alzheimer's disease (AD) is characterized by progressive cognitive decline and affects >10% of people older than 65 years of age. However, the underlying mechanisms of most forms of AD are unclear. Here we show poly-glycine-arginine positive (polyGR+) aggregates are frequently found in postmortem sporadic AD brains but not in age-similar controls in three cohorts of AD and control cases. PolyGR+ aggregates are strongly associated with disease neuropathological hallmarks, including Aβ plaques, phosphorylated Tau tangles, and neuritic plaques. Levels of polyGR+ aggregates are elevated in AD cases who experienced high blood pressure or brain injuries. Transcriptomic analysis shows neurogenesis and oligodendrocytes related pathways are dysregulated in polyGR+ AD brains. To identify putative repeat expansion mutations that express polyGR-containing proteins, we developed and used a repeat pulldown assay to isolate an interrupted (GGGAGA)n intronic expansion located within a SINE-VNTR-Alu (SVA) element in *CASP8* (*CASP8*-GGGAGA^{EXP}). Immunostaining using anti-polyGR and locus-specific C-terminal antibodies demonstrates that the *CASP8*-GGGAGA^{EXP} expresses hybrid poly(GR)n(GE)n(RE)n proteins that accumulate in *CASP8*-GGGAGA^{EXP}(+) AD brains. In cells, expression of *CASP8*-GGGAGA^{EXP} minigenes leads to increased p-Tau levels. Consistent with other types of repeat associated non-AUG (RAN) proteins, poly(GR)n(GE)n(RE)n levels are increased by stress which in turns increase pTau levels. Association studies show that specific interrupted *CASP8* sequence variants increase AD risk (*CASP8*-GGGAGA-AD-R1; OR 2.2, 95% CI [1.5185-3.1896], p = 3.1 x 10^{-5}). Cells transfected with a high-risk *CASP8*-GGGAGA-AD-R1 variant show increased toxicity and increased levels of poly(GR)n(GE)n(RE)n aggregates. Taken together, these data identify polyGR+ aggregates and *CASP8*-GGGAGA^{EXP} alleles are important factors in AD.

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