Inhibition of C1q Reduces Nerve Damage as Measured by Neurofilament Light in the HD R6/2 Mouse Model

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INTRODUCTION

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder caused by expansion of CAG repeats in the *Huntingtin (HTT)* gene. There is evidence of complement involvement in HD, however, efficacy of complement-based therapy has not been examined. Using the R6/2 mouse model of HD, we first evaluated levels of early classical complement components and activation during disease progression. We also measured plasma and cerebral spinal fluid (CSF) levels of the neurodegenerative biomarker neurofilament light chain (NFL) and found correlation between NFL and complement changes during disease. Finally, we treated R6/2 mice with anti-C1q (ANX-M1, administered systemically) and showed reduction of CSF NfL that accompanied inhibition of the classical pathway in the plasma and brain.

OBJECTIVE

To investigate the role of classical complement pathway in driving neurodegeneration in the R6/2 mouse model of HD.

METHODS

In vivo work with R6/2 animals (hemizygous, 120 CAG repeats, Jackson Laboratory) and their littermate controls was performed at PsychoGenics. Longitudinal changes in NFL levels were assessed in CSF and plasma, and complement components were measured in plasma and brain lysates obtained from R6/2 animals at 6, 10 and 15 weeks of age (n=15 per group). Nontransgenic littermate mice at 14 weeks of age were used as wild type healthy controls. The treatment study with ANX-M1 (Annexon Biosciences) enrolled three experimental groups (*Table 1*). Animals were dosed twice per week, via intraperitoneal (IP) injection (16mL/kg, 100mg/kg). Levels of NFL were measured in CSF and plasma using Quanterix Single Molecule Array (SIMOA) Technology. Complement signature in plasma and brain protein lysate was defined using ELISA based assays.

Group	N Number	Genotype / Study Nomenclature	Treatment	Route	Frequency of Administration	Duration of Treatment
1	18	Hemizygous – R6/2	mIgG1 Isotype control at 100 mg/mL	IP (16mL/kg)	Twice per week	~4.5W – 13W
2	18	Hemizygous – R6/2	M1 at 100 mg/mL	IP (16mL/kg)	Twice per week	~4.5W – 13W
3	10	WT Littermates	NT			13W

Table 1*:* Treatment groups

RESULTS

Figure 1. Increased level of NFL, biomarker of neuronal damage, in CSF and plasma of R6/2 HD mice

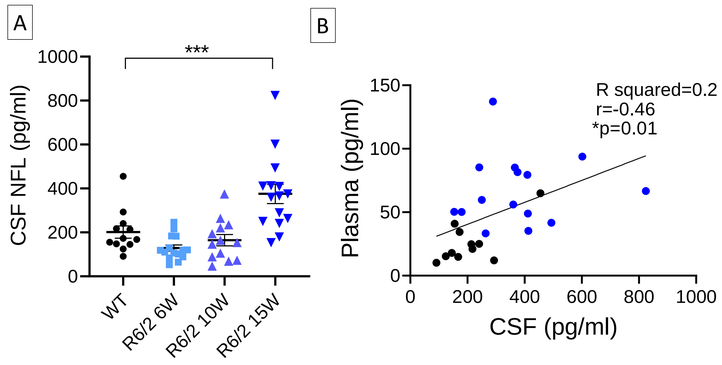

Figure 1: A) Longitudinal levels of NFL measured in CSF of R6/2 mice and their littermate WT control showing significant increase with disease. B) Correlation analysis showing significant positive correlation between CSF and plasma NFL levels in R6/2 mice at 15 weeks of age (Pearson r=0.46; *p=0.01).

Figure 2. Changes in complement expression and activation in plasma and brain of R6/2 HD mice with disease progression

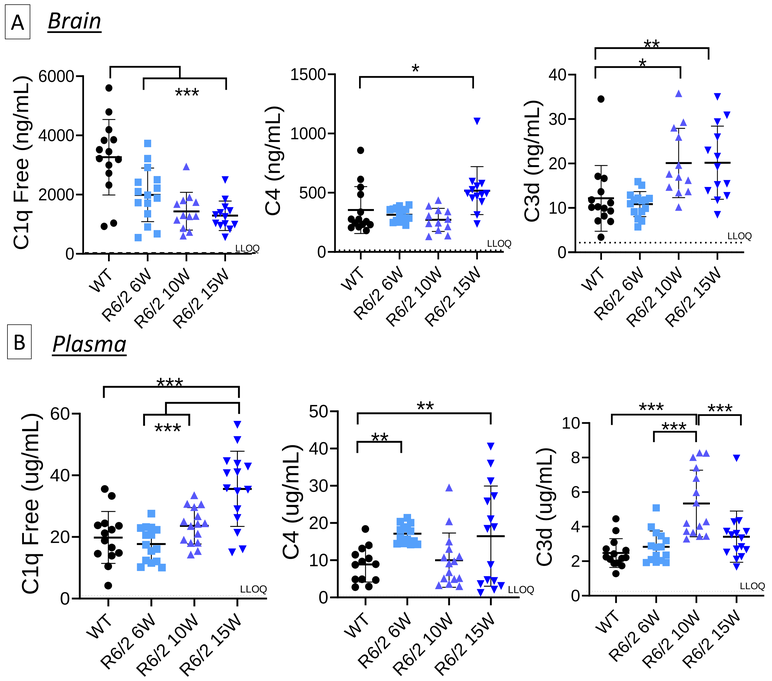

Figure 2: Longitudinal levels of complement proteins in brains (A) and plasma (B), showing changes in C1q, C4, and C3d levels with disease progression

RESULTS

Figure 3. Anti C1q treatment with ANX-M1 significantly inhibited classical complement pathway in brain and plasma

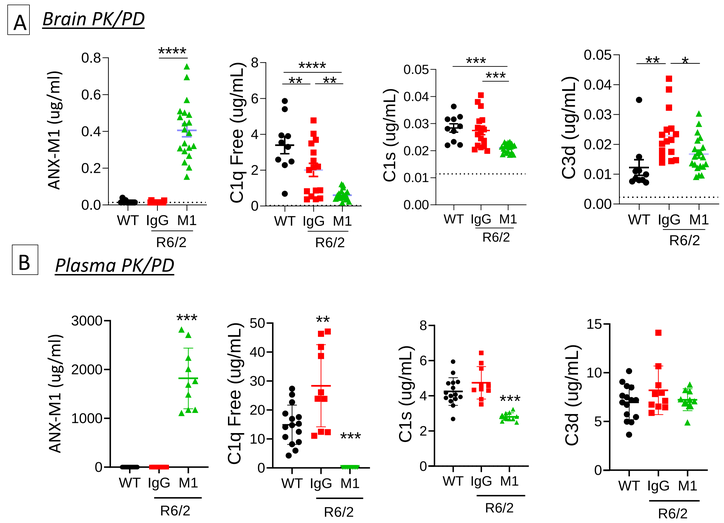

Figure 3: A-B) PK/PD analysis showing detectable drug levels in R6/2 mouse brains and plasma, corresponding to significant decrease in complement C1q, C1s and C3d. *Note that levels of C1q in the brain lysate reflect extracellular C1q (accessible to drug in vivo during the study) and intracellular C1q (increased with disease, but not accessible to drug)*

Figure 4. Correlation of plasma C1q and CSF NfL levels and reduction of CSF NfL with systemic anti-C1q treatment

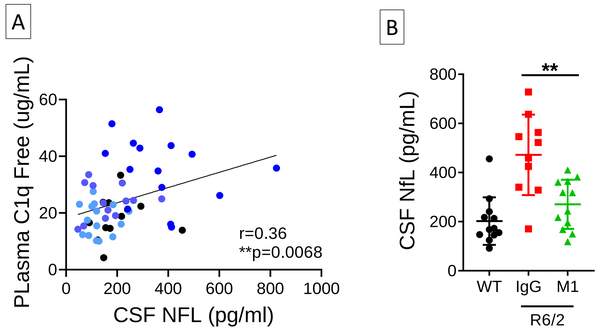

Figure 4: A) Correlation analysis showing significant positive correlation between plasma C1q and CSF NFL levels during disease (Pearson r=0.36; **p=0.006). B) Reduced level of NFL in R6/2 mice upon treatment suggests neuroprotection.

CONCLUSIONS

Classical complement component C1q represents a potential pharmacological target in HD. A Phase 2 study of ANX005 anti-C1q therapy in HD patients is ongoing (clinical trials.gov NCT04514367).