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Research article

Variation in complement protein C1q is not a major contributor to cognitive impairment in Parkinson's disease

Sophia Carbutt[a], Jennifer Duff[a], Alison Yarnall[b], David J. Burn[b], Gavin Hudson[a,*]

[a] Mitochondrial Research Group, University of Newcastle Upon Tyne, United Kingdom
[b] Institute for Ageing and Health, University of Newcastle Upon Tyne, United Kingdom

HIGHLIGHTS

- Cognitive decline is a strong hallmark of PD.
- Genetic variation in *C1Q* – does not account for the cognitive decline seen in PD.
- Genetic variation in *C1Q* – is unlikely to contribute to PD aetiology.

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ABSTRACT

Traditional dogma regarding the brain as an immune exempt organ has changed in recent years. New research has highlighted the role of the classical complement cascade in both synaptic elimination and function, driven largely by the role of the pathway initiating protein C1q. Given the links between C1q and cognitive function we assessed the genetic variability of the C1q encoding genes: *C1QA*, *C1QB* and *C1QC* between PD patients and matched controls. Despite a strong link between C1Q/cognitive decline and PD/cognitive decline we were unable to find a link between common C1Q variation and PD. We conclude that common *C1Q-A/B/C* genetic variation is unlikely to contribute to cognitive decline or the missing heritability in PD.

1. Introduction

Cognitive impairment is rapidly becoming a significant health risk in an already aging population [3]. Mild cognitive impairment (MCI), the transitional state between normality and dementia, has risen in recent years with 10–15% of individuals progressing to Alzhemiers-type dementia. In addition, MCI has become a prognostic marker in Parkinson's disease (PD), where dementia is a frequent complication [21].

Cognitive decline, mediated through synapse elimination, is a hallmark of normal ageing yet remains poorly understood. Synapse elimination, primarily a developmental process, is an increasing feature in a number of neurodegenerative diseases, where it is thought that re-inactivation of the developmental synapse elimination mechanism leads to disease [16]. Complement proteins are expressed in neurons and glia and are localised to developing synapses during synaptic remodelling [17]. C1q, the first subcomponent of the C1 complex of the classical pathway of complement activation is critical to synapse elimination and connectivity [17]; moreover C1q has been shown to mediate CNS synapse formation and contribute to neuroprotection in the CNS [2]. This is supported by recent evidence that specific components of the complement cascade, including C1q, are up-regulated and localised to synapses prior to neuronal loss in Alzheimer's disease (AD) and other brain diseases [2] . At the genetic level, C1q is encoded by three genes: *C1QA*, *C1QB* and *C1QB*, and studies have linked common variation in *C1q*- primarily to systemic lupus erythematosus [13]. However, associated phenotypes have expanded to include; rheumatoid arthritis [18], schizophrenia [22], amyloidotic polyneuropathy [5], cancer metastasis[1].

Despite extensive study, the genetic causes of PD remain largely elusive. Given the link between cognitive impairment and C1q activation, reported synaptic pathology in PD [11], and the role of *C1Q* genetic variation in age-related neurological disorders, we investigated the role of common *C1Q* genetic variation on PD cognitive decline and aetiology. Given that PD progression may not be limited to the post-synaptic neuron, we hypothesise that variation in

* Corresponding author. Tel.: +44 1912418650.
E-mail address: Gavin.Hudson@ncl.ac.uk (G. Hudson).

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Table 1
Analysis of rs172378 (*C1QA*) and rs15940 (*C1QC*) genotypes on mean baseline variables (where; SD is standard deviation, P is ANOVA probability, A is affected, U is unaffected and Comb. is combined, MMSE is mini-mental state exam score, UPDRS is the unified Parkinson's disease rating scale, MoCA is the Montreal cognitive assessment score, PoA is power of attention, PRM is pattern recognition memory testing and SRM is spatial recognition memory testing).

			Mean (SD)			
Variable	rs172378	Group	AA	AG	GG	P
MMSE	*AA v AG v GG*	*A*	28.8 (1.1)	28.4 (1.5)	28.8 (1.2)	1.4×10^{-1}
		U	28.9 [1.3]	29.2 [1.0]	28.9 [1.2]	5.6×10^{-1}
		Comb.	28.9 [1.2]	28.7 [1.4]	28.9 [1.2]	4.8×10^{-1}
UPDRS	*AA v AG v GG*	*A*	28.7 [13.4]	25.7 [10.8]	27.5 [13.0]	3.7×10^{-1}
		U	–	–	–	–
		Comb.	–	–	–	–
MoCA	*AA v AG v GG*	*A*	25.5 [3.4]	24.8 [3.7]	25.2 [3.9]	6.9×10^{-1}
		U	26.9 [2.5]	27.0 [2.5]	26.8 [2.3]	9.8×10^{-1}
		Comb.	26.1 [3.1]	25.7 [3.5]	25.9 [3.5]	5.8×10^{-1}
PoA	*AA v AG v GG*	*A*	1375.0 (255.6)	1390.9 (176.3)	1346.2 (214.3)	6.7×10^{-1}
		U	1313.2 (161.2)	1242.2 (101.5)	1264.6 (108.4)	5.8×10^{-2}
		Comb.	1347.9 (220.4)	1341.4 (170.2)	1316.8 (185.7)	7.1×10^{-1}
PRM	*AA v AG v GG*	*A*	2303.3 (785.8)	2729.1 (2735.1)	2400.8 (816.7)	4.8×10^{-1}
		U	2288.4 (447.8)	2323.6 (486.3)	2316.2 (725.3)	9.5×10^{-1}
		Comb.	2296.5 (649.9)	2587.6 (2227.9)	2370.3 (775.5)	4.1×10^{-1}
SRM	*AA v AG v GG*	*A*	2265.0 (840.7)	2802.3 (2221.0)	2514.0 (1072.7)	2.2×10^{-1}
		U	2352.4 (595.4)	2282.4 (528.5)	2123.2 (410.4)	4.1×10^{-1}
		Comb.	2305.1 (736.1)	2620.9 (1831.0)	2372.9 (904.1)	2.4×10^{-1}
			Mean (SD)			
Variable	rs15940		CC	CT	TT	P
MMSE	*CC v CT v TT*	*A*	28.6 [1.3]	28.5 [1.5]	28.8 [1.2]	7.8×10^{-1}
		U	28.9 [1.3]	29.3 [1.0]	29.0 [1.1]	3.8×10^{-1}
		Comb.	28.7 [1.3]	28.8 [1.4]	28.8 [1.2]	9.1×10^{-1}
UPDRS	*CC v CT v TT*	*A*	27.2 [12.9]	24.5 [9.5]	35.5 [12.1]	1.8×10^{-2}
		U	–	–	–	–
		Comb.	–	–	–	–
MoCA	*CC v CT v TT*	*A*	25.0 [3.7]	25.3 [3.4]	25.3 [4.4]	9.1×10^{-1}
		U	26.7 [2.7]	27.8 [1.9]	25.5 [1.8]	4.9×10^{-2}
		Comb.	25.7 [3.4]	26.3 [3.1]	25.3 [3.7]	3.1×10^{-1}
PoA	*CC v CT v TT*	*A*	1380.9 (233.9)	1377.7 (162.3)	1363.0 (218.0)	9.6×10^{-1}
		U	1287.1 (154.2)	1262.5 (106.9)	1269.8 [80.6]	7.2×10^{-1}
		Comb.	1345.9 (212.2)	1331.3 (152.8)	1331.9 (186.3)	8.5×10^{-1}
PRM	*CC v CT v TT*	*A*	2320.4 (737.2)	2920.6 (3429.5)	2600.1 (871.4)	2.6×10^{-1}
		U	2395.4 (545.9)	2196.9 (401.5)	1947.8 (213.1)	5.2×10^{-2}
		Comb.	2350.0 (667.3)	2617.4 (2638.6)	2408.2 (792.0)	4.9×10^{-1}
SRM	*CC v CT v TT*	*A*	2368.1 (817.7)	2868.3 (2758.2)	2906.2 (1289.3)	2.1×10^{-1}
		U	2315.9 (546.6)	2299.8 (564.0)	1989.5 (445.8)	4.4×10^{-1}
		Comb.	2347.5 (721.3)	2630.1 (2141.9)	2636.6 (1173.8)	2.9×10^{-1}

C1Q-A/B/C may modulate PD cognitive function and may provide an insight into the missing heritability.

2. Materials and methods

2.1. Subjects

We compared 158 community based cases with PD fulfilling UK-PD Society brain bank criteria for the diagnosis of PD [7] (mean age 66.0 years, SD = 8.04, 61% male) from the North East of England to an ethnically age and gender matched control group ($N = 100$, mean age 69.89 years, SD = 7.88, 56% male), with no clinical evidence of PD. All were of Caucasian origin.

At baseline, all subjects underwent cognitive assessment; global cognitive function was assessed using the mini-mental state examination (MMSE) [15] and the Montreal Cognitive Assessment (MoCA) [10]. Attention was measured using Cognitive Drug Research (CDR, Goring-on-Thames, UK) computer testing [19]. Memory was assessed using Pattern Recognition Memory (PRM) and Spatial Recognition Memory (SRM) from the computerized Cambridge Neuropsychological Test Automated Battery (CANTAB) battery [14,20].

MCI was determined using published criteria (using 1.5 SD as a cut-off) [20]. Highers scores represent worse performance and directly reflect the ability to maintain concentration to a particular task, conversely greater scores in SRM and PRM represent better performance [6,19].

2.2. Molecular studies

The entire coding region of C1QA, C1QB and C1QC was sequenced and compared between UK community PD cases ($n = 158$) and matched controls ($n = 100$). Sequencing was performed using BDT v3 sequencing chemistry and an ABI3130xl Genetic Analyser (Applied Biosystems).

2.3. Statistical power

Power estimates[S8] indicate >0.85 power to detect a significant allelic association (MAF = 0.05) with alpha an <0.05, given a prevalence of 1% and relative risk of a dominant allele >2.2.

Additionally, QTL power estimates [12] indicate 75%> power to detect genotype: phenotype differences in MMSE, UPDRS, MoCA, PoA (power of attention), PRM (pattern recognition memory testing) and SRM (spatial recognition memory testing), given a QTL variance of 0.10 and an allele frequency of 1%.

2.4. Statistical analysis

Case-control allele and genotype comparisons and comparisons between common C1Q- variants and cognitive function were made using SPSS (v21).

3. Results

3.1. Case-control genotype comparison

Sequence analysis of *C1Q-* genes identified several previously identified single nucleotide polymorphisms, six in *C1QA*; rs149230484, rs17887074, rs41507347, rs180679721, rs20169349, rs172378 (previously associated with, lupus, breast cancer survival and polyneuropathy) [1,4,5] and rs180679721 and two in *C1QC*; rs15940 and rs140444929. The majority of variants were rare (MAF < 0.01), with only three with a MAF > 0.01 (rs172378, rs172378 and rs15940). Variant prediction (mutationtaster.org) predicts benign function outcomes, classifying all nine variants as polymorphic variants (probability = 0.998–0.999). Case-control analysis revealed no direct relationship to the onset of PD, when comparing genotypes or alleles (Supplementary Table 1). Additionally, we found no significant association between *C1Q-A/B/C* genotypes and age of onset when compared using Cox-proportional hazards model. Additionally, multi-marker analysis of non-synonymous variants (rs149230484), invoked through the Cumulative Minor Allele Test (CMAT) [23], failed to find a significant association between rare-variant burden and PD. Analysis of *C1QB* sequence revealed no genetic variation in cases or controls.

3.2. C1Q and baseline cognitive function

Priori comparisons of cognitive assessment between PD cases and controls revealed significant differences in mean MMSE ($P = 2.0 \times 10^{-2}$), MoCA ($P = 2.5 \times 10^{-5}$), PoA ($P = 1.0 \times 10^{-4}$) PRM ($P = 6.0 \times 10^{-3}$) and SRM ($P = 1.0 \times 10^{-3}$) scores. Comparison of MCI status (presence/absence) was significantly different between PD cases and controls ($P = 1.410^{-3}$).

Variants with MAF > 0.01 (rs15940 and rs172378) were selected for further analysis and compared to mean baseline cognitive assessment variables (Table 1). We found no significant association between rs15940(*C1QA*) genotypes and any baseline variable (Table 1). Additionally, we found no significant association between rs172378(*C1QC*) genotypes and MMSE, PoA and SRM. However, we did find a significant difference in the mean UPDRS scores of PD patients when compared to rs15940 (*C1QC*) genotype ($P = 1.8 \times 10^{-2}$, Table 1) and the mean MoCA score of controls when compared to rs15940 (*C1QC*) genotype ($P = .9 \times 10^{-2}$, Table 1), although correcting for multiple testing removed the statistical significance of these observations.

3.3. C1Q and MCI

Binary logistic regression comparing cognitively normal (CN) to mildly cognitive impaired (MCI), with age and gender as covariates, failed to find a significant association to either rs15940 (*C1QA*) or rs172378 (*C1QC*) when analysed in just PD cases (rs15940 $P = 0.640$, rs172378 $P = 0.985$), just controls (rs15940 $P = 0.384$, rs172378 $P = 0.059$) or combined (rs15940 $P = 0.637$, rs172378 $P = 0.757$).

4. Discussion

Our results indicate that *C1Q-A/B/C* genotypes do not have a strong effect on the progression of PD but, more importantly, do not affect cognitive decline when compared to several baseline measurements of cognitive function. Previous work has shown that complement activation occurs in the substantia nigra of PD patients [9] and variation in *C1Q-A/B/C* genetic variation has been shown to affect neuronal function [5]. However, despite a strong association between C1Q/cognitive decline and PD/cognitive decline we were unable to find a link between *C1Q-A/B/C* variation and PD.

4.1. How do we rationalise our results?

Our sequence analysis indicates that *C1Q-A/B/C* is highly conserved. We were able to identify several, previously reported, variants, however only three had a MAF > 0.01 (rs172378, rs172378 and rs15940). Taking the significantly higher population frequency of PD, and the benign functional prediction of the *C1Q-A/B/CC* variants, we conclude that they are unlikely to be dominant causes of PD. Multi-locus analysis, useful in assessing the effect of very rare alleles, failed to identify a significant burden in *C1Q-A*, however this is likely a result of the high conservation, which result in a low number of measurable variants and is consistent with other studies using small sample sizes [8].

Another possible reason for our lack of association may be PD disease progression. If the effect of *C1Q-A/B/C* genotype on PD is weak in healthy individuals, then it may be masked in a PD cohort whose attention capacity is already markedly reduced due to disease itself. Accepting these limitations we conclude that common genetic variation in *C1Q-A/B/C* is unlikely to contribute to the pathology or cognitive decline seen in PD. However, we would recommend that a, larger, longitudinal study following the effects of cognitive decline and *C1Q-A/B/C* may be warranted, given that these patients exhibit MCI. Moreover, a larger study could identify rare, pathogenic variants which affect the aetiology of PD which are beyond the power of this study.

Disclosure statement

The authors have no actual or potential conflicts of interest. This work has both ethical and institutional review board approval.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.neulet.2015.03.048.

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