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Inherited mtDNA variations are not strong risk factors in human prion disease

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Abstract

Aside from variation in the prion protein gene, genetic risk factors for sporadic Creutzfeldt-Jakob disease (sCJD) remain elusive. Given emerging evidence implicating mitochondrial dysfunction in the pathogenesis of the disorders, we studied the role of inherited mitochondrial DNA (mtDNA) variation in a 2255 sporadic prion disease cases and 3768 controls. Our analysis indicates that inherited mtDNA variation does not have a major role in the risk of developing the disorder, adding to recent nuclear genome wide association studies that failed to define additional genetic risk loci.

1 Introduction

Prion disorders are a group of inherited and acquired neurodegenerative diseases which are caused by the misfolding and aggregation of normal cellular prion protein (PrP^C) into a disease-associated form.¹ A small number of genetic risk and modifying factors for prion disease have been proposed based on work with mouse models or human association study.^{2–4} Aside from variants of the prion protein gene,^{5,6} confirmed risk factors for the most common human prion disease, sporadic Creutzfeldt-Jakob disease (sCJD), remain elusive.⁷

Several studies have shown the disruption of mitochondrial structure and function in human and animal brains infected by prion proteins, raising the possibility that mitochondrial mechanisms might be involved, as seen several sporadic and inherited neurodegenerative disorders. In support of this hypothesis, a reduction of respiratory chain complex IV activity

has been shown to correlate with synaptic loss in murine models of prion disease,⁸ and increased PrP was associated with a decrease in mitochondrial resistance to reactive oxygen species,⁹ predisposing neurons to mitochondrially-mediated apoptosis. However, it is not clear whether these effects are a cause, or a consequence of the prion pathology. One way of separating cause from effect is to determine whether genetic variation in mitochondrial DNA (mtDNA) predisposes to prion diseases, as has been shown for other neurodegenerative disorders where specific mtDNA genetic variants are associated with different risk of developing the disorder.^{10–13,14}

Here we investigated the role of 230 inherited mtDNA variants in a large cohort of cases (n=2255) and ethnically matched controls (n=3768) from 3 independent cohorts. *A priori* power calculations indicated that we had >80% power to detect given an estimated prevalence of 1/20,000, an estimate risk allele frequency of 0.01 and a odds ratio of >2.25 (2-tailed significance with $\alpha=5\%$).¹⁵

2 Experimental Procedure

Sample Cohorts

We studied ~230 mitochondrial variants, present on Illumina genotyping platforms, in 2254 sporadic Creutzfeldt-Jakob disease (CJD) cases (686=UK, 649=German and 919=USA) and 3768 controls (847=UK as part of the GERAD consortium and 2921=German). All samples were genotyped using compatible Illumina array systems (Supplementary Table 1). Probable or definite sporadic CJD patients (sCJD, according to World Health Organisation criteria) were recruited by the NHS National Prion Clinic, London, the National CJD Research and Surveillance Unit, Edinburgh, the National Reference Centre for Transmissible Spongiform Encephalopathy, Georg-August University Goettingen, the Institut für Neuropathologie, Ludwig-Maximilians Universität, Munich, Germany; and the National Prion Disease Pathology Surveillance Centre, Case Western University, Cleveland, Ohio, USA. All patients of known non-white ethnicity were excluded.

Data QC

Stringent quality control (QC) was applied to each individual cohort.¹⁶ Briefly, each cohort was pruned of missing phenotypes (defined as -9 in the pedigree/sample files). Poorly performing SNPs (genotyped=0.05),¹⁶ and subsequently, samples were removed (individual missingness=0.05)¹⁶ using PLINK v2.050.¹⁷ Additionally non-European mtDNA sequences (defined with m.8701A, m.8540T and 10873T) were also removed.^{18–20} Finally, to verify the quality of genotypes cluster plots of normalized intensity for each SNP were generated using R (<http://www.R-project.org>) and inspected. Case datasets were then merged with complimentary control data: UK cases with UK (GERAD) controls and German cases with German controls. USA samples, Caucasian individuals with European ancestry, were combined with UK controls as a third cohort. Differential missingness tests, which statistically compare the frequency of 'missing' genotype data between cases and controls were performed on each case-control dataset.¹⁶ Variants were removed when missingness was significantly different ($P<10^{-4}$).¹⁶ Allelic association was implemented in

PLINK v2.050 17. Given the discovery nature of the experiment, statistical significance was defined as $P < 0.05$.

mtDNA Haplogroup Assignment

MtDNA haplogroups were determined by comparing post QC array variant in each subject to the mtDNA reference sequence (rCRS) to identify haplogroup specific motifs allowing 100% of subjects to be assigned to a European haplogroup.¹²

Power Calculations

Associative power calculations were conducted using Genetic Power Calculator (GPC).¹⁵

3 Results

Case-control association analysis revealed a number of SNP associations (Table 1), although none were observed in more than one cohort. Analysis of the UK cohort identified a single SNP associated with prion disease: m.13879T>C ($P=1.4 \times 10^{-2}$, OR=4.48) which is a rare non-synonymous variant in *MTND5* (S515P) (MAF=0.01, in 7729 mtDNA sequences) and is predominantly present on mtDNA haplogroup J. Analysis of the German cohort revealed significant associations to the phylogenetically linked m.499G>A ($P=2.8 \times 10^{-3}$, OR=0.35, present in the regulatory *DLOOP*) and m.15693T>C ($P=2.74 \times 10^{-3}$, OR=0.33, a non-synonymous variant in *MTCYTB* - M316T), both members of the mtDNA sub-haplogroup U4. In addition, there were associations with m.1811A>G ($P=4.8 \times 10^{-2}$, OR=0.73), present in the 16s rRNA; m.15693T>C ($P=2.74 \times 10^{-3}$, OR=0.33), a non-synonymous variant in *MTCYTB* (M316T) and m.15924A>G ($P=2.09 \times 10^{-3}$) in tRNA^{thr}. No SNPs were associated with prion disease in the US cohort and we found no association with canonical mtDNA population haplogroups in any cohort (Table 2).

4 Discussion

Given that neither single SNPs nor any phylogenetically related SNPs were consistently associated with prion disease in more than one cohort, and that the significant P-values would not withstand a Bonferroni correction, it is highly unlikely that mtDNA variation contributes to the risk of developing prion disease. Although we cannot exclude the possibility that specific mtDNA SNPs might be associated with prion disease in a particular population – perhaps because of a specific nuclear genetic background, or an environmental trigger – this would be without precedent, and is therefore unlikely. However without high-resolution genotyping in very large cohorts of patients and carefully matched controls, ideally at the whole mtDNA genome level, it is unlikely that this could be resolved further. Studies of this kind would be particularly challenging for prion disorders because they are rare in the population. Our findings do not rule out the possibility of mitochondrial involvement in the pathogenesis of prion diseases, but indicate that a strong mtDNA-mediated heritable component is unlikely.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Association between inherited mtDNA variants and prion disease in three independent cohorts, where rCRS is position in mtDNA, A1 is the associated allele, MAF is the corresponding frequency in n=7729 full mtDNA population sequences (showing the frequency and % of dominant haplogroup carrying each variant), haplogroup according to phylotree.org, F_A is frequency in cases, F_U is frequency in controls, P probability and OR is odds ratio implemented through PLINK. Shown are the most significant variants (bold) and the comparative association in the other cohorts. The last columns show the result of a fixed effect meta-analysis of all three data sets implemented in PLINK.

rCRS	A1	MAF	Population Based Haplogroup		Phylotree.org	UK			Germany			USA						
			n=7729	Dominant Hap.		Haplogroup(s)	F_A	F_U	P	OR	F_A	F_U	P	OR	F_A	F_U	P	OR
499	A	0.03	204	U (94.6%)	T2/U49	-	-	-	-	0.02	0.05	2.9E-3	0.35	-	-	-	-	-
1719	A	0.05	399	X1 (35.8/33.3%)	I/X/H/K	0.05	0.06	6.2E-1	0.89	0.04	0.06	6.6E-2	0.62	0.03	0.06	2.9E-1	0.47	
1811	G	0.14	1073	K (60.48%)	U/K/H1/J1	0.13	0.14	3.5E-1	0.86	0.11	0.15	4.8E-2	0.73	0.15	0.14	9.1E-1	1.04	
3010	A	0.20	1539	H (69.01%)	H1/J1/U3	0.25	0.29	7.6E-2	0.80	0.26	0.22	1.5E-1	1.18	0.22	0.29	1.9E-1	0.68	
10463	C	0.09	696	T (96.84%)	H6/H13/J1/T	0.11	0.08	6.2E-2	1.41	0.11	0.12	5.4E-1	0.90	0.12	0.08	2.3E-1	1.56	
13879	C	0.01	81	J (74.07%)	U2X3/J1	0.02	0.01	1.4E-2	4.48	-	-	-	-	-	-	-	-	-
14793	G	0.04	320	U (96.88%)	V2/J2/U5	-	-	-	-	-	-	-	-	0.09	0.05	8.5E-2	2.06	
15693	C	0.02	187	U (100.0%)	U4	0.02	0.03	6.3E-1	0.84	0.02	0.05	2.7E-3	0.33	0.03	0.03	9.8E-1	0.98	
15924	G	0.04	291	I (43.99%)	N1/X2/H1/J1/U5/K1	0.05	0.06	2.7E-1	0.76	0.02	0.05	2.1E-2	0.49	0.11	0.06	9.1E-2	1.95	

Table 2

Association between mtDNA haplogroup and prion disease in three independent cohorts, where F_A is frequency in cases, F_U is frequency in controls, P is permuted probability (x1000 permutations) by max(T) implemented within PLINK

Haplogroup	UK			Germany			USA		
	F_A	F_U	P	F_A	F_U	P	F_A	F_U	P
HV	0.021	0.013	8.7E-01	0.021	0.019	7.1E-01	0.019	0.013	4.5E-01
H	0.458	0.448	7.0E-01	0.448	0.426	3.9E-01	0.421	0.448	2.9E-01
V	0.030	0.027	7.5E-01	0.039	0.030	3.2E-01	0.033	0.027	4.9E-01
R	0.000	0.002	5.2E-01	0.000	0.002	1.0E+00	0.001	0.002	6.1E-01
J	0.108	0.125	3.5E-01	0.109	0.097	4.5E-01	0.112	0.125	4.1E-01
T	0.099	0.083	3.4E-01	0.107	0.114	6.9E-01	0.106	0.083	1.2E-01
U	0.145	0.138	2.5E-01	0.146	0.174	1.4E-01	0.159	0.138	2.3E-01
K	0.081	0.096	3.4E-01	0.071	0.068	8.4E-01	0.072	0.096	6.9E-02
N	0.005	0.002	4.0E-01	0.004	0.003	6.8E-01	0.009	0.002	1.1E-01
I	0.025	0.033	4.3E-01	0.013	0.023	2.3E-01	0.033	0.033	1.0E+00
W	0.012	0.015	8.2E-01	0.028	0.019	2.2E-01	0.019	0.015	7.1E-01
X	0.014	0.015	1.0E+00	0.015	0.023	3.1E-01	0.015	0.015	1.0E+00
Super Haplogroup	F_A	F_U	P	F_A	F_U	P	F_A	F_U	P
HV	0.510	0.488	4.5E-01	0.507	0.475	1.9E-01	0.473	0.488	5.7E-01
JT	0.207	0.211	8.9E-01	0.216	0.214	9.0E-01	0.219	0.211	6.8E-01
UK	0.227	0.235	7.5E-01	0.216	0.242	2.4E-01	0.231	0.235	9.1E-01
OTH	0.057	0.067	5.0E-01	0.060	0.069	5.5E-01	0.076	0.067	4.6E-01