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SHORT COMMUNICATION

No common founder for *C9orf72* expansion mutation in Sweden

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Hexanucleotide expansion mutations in the chromosome 9 open reading frame 72 (*C9orf72*) gene is the most common genetic cause for frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS). SNP haplotype analyses have suggested that all *C9orf72* expansion mutations originate from a common founder. However, not all *C9orf72* expansion mutation carriers have the same haplotype. To investigate if the *C9orf72* expansion mutation carriers in Sweden share a common founder, we have genotyped SNPs flanking the *C9orf72* expansion mutation in cases with FTD, FTD–ALS or ALS to perform haplotype analysis. We have genotyped 57 SNPs in 232 cases of which 45 carried the *C9orf72* expansion mutation. Two risk haplotypes consisting of 31 SNPs, spanning 131 kbp, were found to be significantly associated with the mutation. In summary, haplotype analysis on Swedish *C9orf72* expansion mutation carriers indicates that the *C9orf72* expansion mutation arose on at least two risk haplotypes.
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A hexanucleotide, G_4C_2, expansion mutation in the chromosome 9 open reading frame 72 (*C9orf72*) gene is the most common genetic cause for frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS).[1–5]

The mutation frequencies of *C9orf72* expansion mutations in FTD and ALS vary, with the highest frequency in European countries, especially in Finland (29% in FTD cases and 28% in ALS cases), Sweden (21% in FTD cases and 35% in ALS cases) and Spain (25% in FTD cases).[2,4–6] A common founder haplotype, of 232 kbp, has been reported in two-thirds of the Finnish *C9orf72* expansion mutation carriers (MC).[4,7] European MC shared a 110 kbp haplotype which corresponds to a part of the Finnish founder haplotype.[6,8]

To investigate if the high mutation frequency of *C9orf72* expansion mutations in Sweden is due to a common founder we have performed genetic screening for the *C9orf72* expansion mutation, using repeat-primed PCR complemented by a short tandem repeat-assay and genotyped 68 tagged SNPs (tSNPs) spanning 281 kbp on chromosome 9p21.2 in 256 patients diagnosed with FTD, FTD–ALS or ALS. The tSNPs covering three linkage disequilibrium (LD) blocks were identified in Haploview 4.2 (http://www.broadinstitute.org/mpg/haploview) using SNP data from 527 neurologically healthy controls from Sweden (provided by Professor Tomas Olsson[9]). Immunohistochemical staining for the detection of poly-dipeptides translated from the *C9orf72* expansion mutation (NBP2-25018, Novus Biologicals, Littleton, CO, USA 1/5000) were performed on cases with available post mortem tissue from cerebellum. See Supplementary Information for details regarding the methods used.

After quality control, 57 SNPs and 232 cases remained (the success rate for SNPs in the 232 cases was 98.9%). Among the cases, 45 index *C9orf72* MC were detected. The demography of the cases is described in Table 1. SNPs in LD block 2 were highly associated in the *C9orf72* MC ($n=45$) when compared to controls ($n=527$) (Table 2). LD block 2 includes 31 SNPs, from rs10511816 to rs2477518, and spans 131 kbp. Haploview 4.2 was used to estimate the frequencies of the most likely haplotypes based on the genotypes in *C9orf72* MC and controls in LD block 2. Association analysis of the haplotype frequencies between MC and controls demonstrated two haplotypes A ($P=5.2\times10^{-20}$) and B ($P=1.2\times10^{-7}$) segregating with the *C9orf72* expansion mutation (Table 3). These haplotypes differed at two SNPs (rs7868845 and rs2477518) and were found at a haplotype frequency of 39 and 16% respectively among *C9orf72* expansion MC, compared with 8 and 4% of the controls. Haplotypes A and B were also confirmed by segregation analysis in additional family members. Moreover, haplotype A was found in four heterozygote *C9orf72* MC who were homozygous for the A haplotype. In a UK population a similar founder haplotype was observed by Mok *et al.*[8] However, Mok *et al.* allowed for both alleles C and T at both rs7868845 and rs2477518 which therefore does not distinguish between our haplotypes A and B (see Table 2). Furthermore, in cases without the expansion mutation the haplotype frequencies of A and B were similar to controls (data not shown). Three of the *C9orf72* MC were observed

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Table 1 Demography of the patient cohort and those with or without the *C9orf72* expansion mutation

			Clinical diagnosis			*Neuropathological diagnosis*			
Patients from the two recruitment site (n = *232*)	*Gender Female (%)*	*Onset (range)*	*FTD*	*FTD–ALS*	*ALS*	*FTLD*	*FTLD–MND*	*MND*	*Other*
Memory clinic (n = *182*)[a]									
Wild type (*n* = 144)	84 (58%)	61 years (28–82 years)	136	8	–	19	4	–	8[b]
MC (*n* = 38)	22 (58%)	57 years (27–70 years)	29	7	2	15	2	1	1[c]
Division of pathology (n = *50*)[d]									
Wild type (*n* = 43)	14 (33%)	61 years (30–83 years)	–	–	11	3	28	1[e]	
MC (*n* = 7)	3 (43%)	61 years (54–72 years)	–	–	–	–	3	4	–

Abbreviations: FTD, frontotemporal dementia; ALS, amyotrophic lateral sclerosis; FTLD, frontotemporal lobar degeneration; MC, mutation carrier; MND, motor neuron disease.
[a]Patients recruited from Memory clinic at Karolinska University hospital. 50 patients also had neuropathological diagnoses.
[b]Patients with clinical diagnosis FTD but at neurological examination showed: CBD 5 patients (1 patient with Pick bodies), argyrofilic grain disease 1 patient, PSP 1 patient and probable AD 1 patient.
[c]Patient with clinical diagnosis FTD but at neuropathological examination had argyrofilic grain disease.
[d]Patients recruited from Division of Pathology at Karolinska University Hospital. The neuropathological diagnoses were available but limited information about the clinical diagnosis and age at onset (age at onset not available for 16 patients).
[e]Patient with neurodegenerative disease (negative for FUS and pTDP43, and positive for p62 and ubiquitin).

Table 2 The result from the SNP association analysis between the C9orf72 MCs (*n* = 45) and controls (*n* = 527), based on the 57 SNPs

			Allele frequencies							
LD block[a]	*SNP ID*	*Allele*	*MCs*	*Controls*	P-*value*	*Finnish haplotype*[b]	*European haplotype*[c]	*UK haplotype*[d]	*Haplotype A* (n = *30*)[e]	*Haplotype B* (n = *12*)[e]
	rs7849799	A	0.944	0.893	0.121					
	rs12552712	T	0.690	0.616	0.174					
1	rs4483251	G	0.144	0.110	0.322					
	rs10121765	C	0.619	0.547	0.204	C				
	rs17768620	C	0.369	0.213	0.001					
	rs10967916	G	0.545	0.386	0.003					
	rs10812580	G	0.978	0.944	0.171					
	rs1110264	A	0.230	0.230	0.865	A				
	rs1110155	T	0.889	0.762	0.006	T				
	rs1110154	T	0.889	0.803	0.046					
	rs1888382	G	0.202	0.200	0.962					
	rs2150336	G	0.320	0.330	0.907	G				
	rs2225389	G	0.200	0.083	2.00×10^{-4}	G				
	rs17696142	G	0.262	0.237	0.602					
	rs1161680	C	0.330	0.370	0.456	C				
	rs2589054	G	0.500	0.348	0.004	G				
	rs1058326	G	0.589	0.549	0.470	G				
	rs944404	T	0.821	0.701	0.019	T				
	rs10812599	C	0.929	0.851	0.052					
	rs10511817	G	0.966	0.896	0.034	G				
	rs7021930	G	0.690	0.661	0.586					
	rs725804	A	0.557	0.448	0.049	A				
2	rs10511816	A	0.722	0.426	5.87×10^{-8}	A	A		A	A
	rs10967952	T	0.867	0.793	0.093				T	T
	rs1444533	T	0.944	0.821	0.003	A		A	A	A
	rs1822723	C	0.856	0.707	0.003	C		C	C	C
	rs10967958	C	0.878	0.827	0.220				C	C
	rs4879515	T	0.700	0.463	1.56×10^{-5}	T	T	T	T	T
	rs10967959	C	0.933	0.799	0.002				C	C
	rs12350089	T	0.956	0.876	0.025				T	T
	rs895023	A	1.000	0.968	0.084	T		T	T	T
	rs2440622	T	1.000	0.967	0.079	T		T	T	T
	rs1977661	C	0.978	0.892	0.010	C		C	C	C

Table 2 (Continued)

			Allele frequencies							
LD block[a]	*SNP ID*	*Allele*	*MCs*	*Controls*	P-*value*	*Finnish haplotype*[b]	*European haplotype*[c]	*UK haplotype*[d]	*Haplotype A* (n = *30*)[e]	*Haplotype B* (n = *12*)[e]
	rs2166128	C	0.967	0.899	0.037				C	C
	rs10812605	C	0.667	0.334	2.77×10^{-10}		C		C	C
	rs11792285	C	0.789	0.659	0.012				C	C
	rs13290599	G	0.967	0.911	0.068				G	G
	rs3849942	T	0.625	0.200	1.34×10^{-19}	T	T	T	T	T
	rs10967976	G	0.711	0.435	4.70×10^{-7}				G	G
	rs10122902	G	0.911	0.787	0.005	G		G	G	G
	rs10757665	T	0.867	0.741	0.008	T		T	T	T
	rs774359	C	0.619	0.213	6.60×10^{-17}	C	C	C	C	C
	rs2282241	C	0.791	0.540	6.64×10^{-6}	C		C	C	C
	rs2282240	C	0.889	0.732	0.001				C	C
	C9orf72[f]									
	rs1948522	C	0.911	0.781	0.004	C		C	C	C
	rs1982915	G	0.722	0.462	2.12×10^{-6}	G		G	G	G
	rs12002175	G	1.000	0.975	0.132				G	G
	rs7868845	T	0.466	0.306	0.002			T/C	T	C
	rs10757670	T	0.811	0.638	0.001				T	T
	rs2453556	G	0.674	0.393	3.57×10^{-7}	G		G	G	G
	rs702231	A	0.844	0.746	0.038	A		A	A	A
	rs696826	G	0.956	0.842	0.004	G		G	G	G
	rs2477518	T	0.767	0.711	0.258	T		T/C	T	C
3	rs17779794	T	0.929	0.881	0.191					
	rs17779800	A	0.267	0.243	0.614					
	rs536635	A	0.631	0.616	0.783					
	rs10968018	A	0.895	0.893	0.941					

Abbreviations: MC, Mutation carrier.
[a]The three LD blocks identified using the SNP data from the controls ($n=527$).
[b]The Finnish founder haplotype defined by Laaksovirta et al.[7]
[c]The European founder haplotype defined by Smith et al.[6]
[d]The UK haplotype defined by Mok et al.[8]
[e]The two major haplotypes found to be significantly associated with the C9orf72 expansion mutation. n = number of MCs.
[f]Location of the C9orf72 expansion mutation relative to the genotyped SNPs.

Table 3 Haplotype frequencies in MCs and controls

	Haplotype frequencies[a]		
Haplotype	*MC* n *(%)*	*Controls* n *(%)*	P-*value*
Haplotype A	34 (39)	54 (8)	5.2×10^{-20}
Haplotype B	14 (16)	34 (4)	1.2×10^{-7}

P-values were calculated using HaploView 4.2.
[a]Input file contained 45 MCs and 527 controls, whereas haplotypes were calculated for 44 MCs and 475 controls.

to be heterozygous for three shorter variants of haplotypes A and B (data not shown).

Of the 57 SNPs, 29 were part of the previously reported Finnish risk-haplotype which spans 230 kbp (between rs10121765 and rs2477518), that is, which is longer than LD block 2 identified in this study in Swedish control samples (Table 2). Performing haplotype association analysis based on those 29 SNPs showed that 11 out of 45 MC in Sweden had the Finnish founder haplotype. Furthermore, there were four carriers in our cohort that were of known Finnish origin and three of them carried the founder haplotype.

The finding of two haplotypes segregating with the *C9orf72* expansion mutation argues for multiples origin for the *C9orf72* expansion mutation in Sweden. The most common alleles at rs7868845 and rs2477518 are C (69%) and T (71%), respectively, but none of the associated haplotypes carries this combination which is in line with the hypothesis that the mutation has arisen more than once. The most frequent haplotype A in *C9orf72* MC has the minor allele T at rs7868845 and the common allele T at rs2477518 whereas haplotype B carries the opposite allele combination (C–C). However, it cannot be excluded that there was one original haplotype that gave rise to several haplotypes due to recombination and/or SNP mutation events taking place over time.

In summary, there are two significantly associated haplotypes present in the *C9orf72* expansion MC indicating that there is no common founder in patients from Sweden.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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