CX3CR1 gene common polymorphisms influence ALS outcome: a population-based study

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Background

The involvement of neuroinflammation in the pathogenesis of ALS has been a subject of increasing interest in recent years. Microglia activation and the crosstalk between immune cells appear to play a significant role in neuronal death in both in vivo (McCombe et al., 2011) and in vitro studies and correlate with disease progression and symptoms. The *CX3CR1* gene (chemokine (C-X3-C motif) receptor 1, also known as *Fractalkine receptor* (OMIM: 601470), in the brain is only expressed by microglia (Hickman et al., 2013) where it is a key mediator of the neuron-microglia interactions that are upregulated in neurodegenerative diseases of the central nervous system that course with neuroinflammation, microglia and/or t-cell recruitment (Cardona et al, 2006; Ransohoff et al., 2010).

Aim

Recently, it was reported that the p.Val249Ile and p.Thr280Met polymorphisms of the *CX3CR1* gene may modify ALS phenotype (Lopez-Lopez et al., 2014), in small number of cases. The aim of this study is to assess whether the two common polymorphisms of the CX3CR1 gene (rs3732379 and rs3732378) alter the risk of developing ALS and/or modify phenotype in a large population-based series of Italian ALS patients.

Methods

The study includes 755 ALS patients diagnosed in Piemonte between 2007 and 2012 and 369 age- and gender-matched controls from the same geographical area, identified through the patients' general practitioners. Both familial and apparently sporadic ALS patients have been included in the present study.

Cases and controls were genotyped using Illumina Infinium II HumanHap550 SNP chips (Illumina, San Diego, CA, USA). Genotype data for rs3732379 (build hg19, chr3:39307256C>T) and rs3732378 (chr3:39307162G>A) were extracted from the larger dataset.

The quality of genotyping has been assessed using Polar and Cartesian cluster plots for SNPs rs3732378 and rs3732379; the quality control metric of genotyping accuracy for this SNP was 0.835, indicating a high level of precision in assigning genotypes to samples (Figure 3).

Factor	ALS cases (n=755)	Healthy controls (n=369)	P
Mean age at onset (years, SD)	63.7 (11.1)	63.5 (10.9)	0.76
Gender, female (%)	357 (47.3%)	177 (48.0%)	0.93
Site of onset bulbar (%)	226 (29.9%)	-	

Table 1. Demographic and clinical characteristics of cases and controls.

	Haplotype	# of cases	Median survival, years (IQR)	p
V241I				
Dominant model	**VV**	387	2.6 (1.7-4.4)	**0.02***
	VI + II	368	3.1 (1.7-5.3)	
Recessive Model	**VV+VI**	692	2.7 (1.9-5.3)	0.46
	II	63	2.8 (1.8-4.7)	
Additive Model	**VV**	387	2.6 (1.7-4.4)	0.07
	VI	305	3.2 (1.9-5.3)	
	II	63	2.7 (1.9-5.3)	
T280M				
Dominant Model	**TT**	558	2.8 (1.8-4.6)	0.08
	TM+MM	197	2.9 (1.8-5.6)	
Recessive Model	**TT+TM**	733	2.8 (1.8-4.7)	0.10
	MM	22	3.7 (1.9-6.8)	
Additive Model	**TT**	558	2.7 (1.8-4.6)	**0.04***
	TM	175	2.8 (1.8-5.6)	
	MM	22	3.7 (1.9-2.8)	

Table 2. Single marker analysis for survival (years) according to different types of models. V241I and T280M polymorphisms

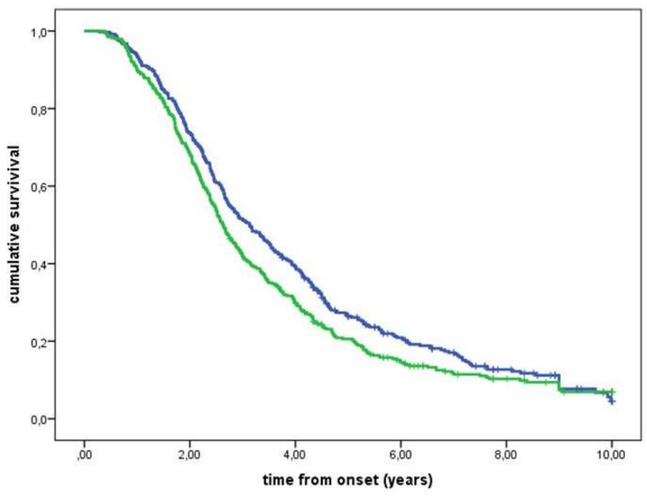

Figure 1. Kaplan-Meier survival curves for CX3CR1 V249I genotypes according to a dominant model in patients with spinal onset (p=0.02). The blue line is for genotypes VI+II (262 cases), the green line is for genotype VV (268 cases).

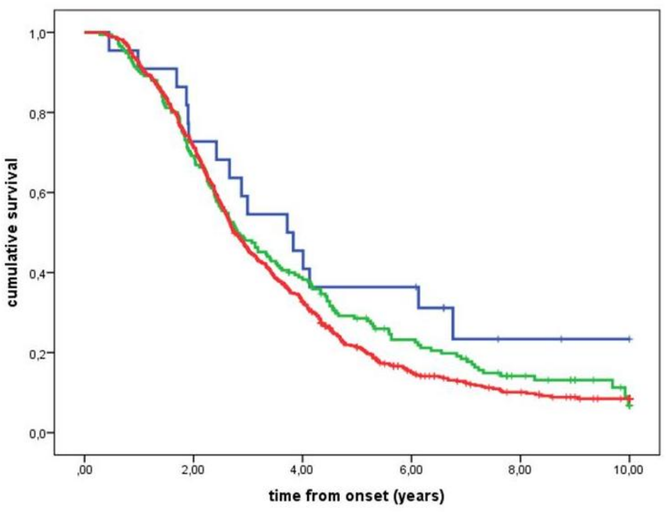

Figure 2. Kaplan-Meier survival curves for CX3CR1 T280M genotypes according to an additive genetic model (p=0.04, test for trend). The blue line is for genotype MM (22 cases), the green line for genotype TM (175 cases); and the red line is for genotype TT (558 cases).

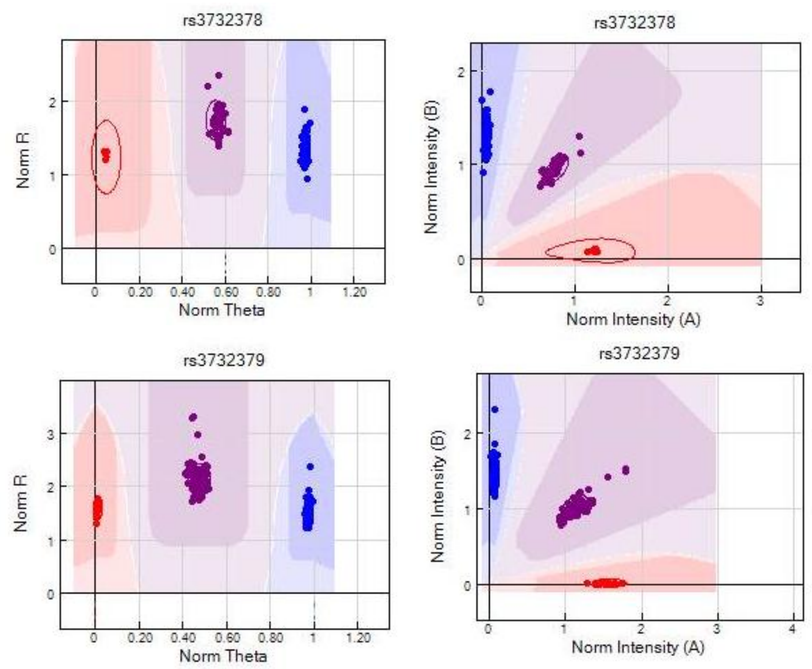

Figure 3. Polar and Cartesian cluster maps for rs3732378 and rs3732379

Results

Neither V249I nor T280M variants were associated with increased risk of ALS under any of the tested statistical models. Age at onset and site of onset of ALS were not influenced by either the V249I or T280M variants under any of the considered statistical models.

The V249I genotype was related to a 6 months shorter survival in patients with a $249^{V/V}$ genotype (387 cases, median survival time 2.6 years, IQR 1.7-4.4) compared to those with a $249^{I/I}$ and $249^{V/I}$ genotype (368 cases, median survival time 3.1 years, IQR 1.7-5.3, p=0.02) under the dominant model (Figure 1, Table 2). Considering the T280M genotype, additive model showed a significant difference between the 3 genotypes (p=0.04, test for trend; Figure 2,Table 2). Since the presence of the minor allele for the two tested SNPs (249I and 280M) was associated with improved survival, we assessed the interaction between the V249I and the T280M genotypes; to do this we subdivided the cases according to their genotypes into two groups. The first group consisted of patients that carried 3 or 4 minor alleles (249I/I and 280T/T or 249I/I + 280T/M and 249V/I + 280M/M) and the second group consisted of patients with 2 or less minor alleles (249V/V + 280T/T or 249V/I and 280T/M or 249V/V + 280M/M or 249I/I + 280T/T). The group of patients with 3 or 4 minor alleles had a significant better survival (p=0.01). In Cox multivariable analysis, the interaction between the two functional variants of the *CX3CR1* gene remained independently significant.

Discussion

In this population-based cohort of Italian patients we found that the 249^{I} variant of the *CX3CR1* gene increased survival by 6 months. This effect was limited to spinal onset patients. The 280^{M} variant had a significant influence on survival under an additive genetic model. We also found an interaction between the two variants of the *CX3CR1* gene with a significantly better survival in patients carrying 3 or 4 minor alleles. These findings were confirmed by Cox multivariable analysis. The tested variants did not differ in frequency between cases and matched controls, indicating that the *CX3CR1* gene is not a risk factor for ALS. Our findings are consistent with a smaller study on 232 ALS patients of Spanish ancestry (Lopez-Lopez et al, 2014).

Considering the effects of these variants on microglia activation, we have provided further clues about the substantial role of neuroinflammation in ALS degenerative process, potentially opening new avenues for the treatment of this terrible disease.

References

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