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Study of leukemia inhibitory factor polymorphism within an Australian multiple sclerosis population ☆

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ABSTRACT

Objective: To examine a polymorphism within the 3′ untranslated region of the leukemia inhibitory factor gene for an association with multiple sclerosis within an Australian case–control population.
Methods: A test group of 121 unrelated multiple sclerosis patients, of Caucasian origin, and 121 controls, matched for ethnicity, sex and age (+/−5 years) were included in the study. The LIF 3′ UTR StuI polymorphism was genotyped by restriction fragment length polymorphism analysis. Statistical analysis of genotype and allele frequencies included Hardy–Weinberg law and conventional contingency table analysis incorporating the standard chi-squared test for independence.
Results: Allelic and genotype frequencies did not demonstrate a significant association between the case and control groups for the tested LIF 3′ UTR StuI polymorphism.
Conclusion: The results indicate that the LIF 3′ UTR StuI polymorphism is not associated with multiple sclerosis, however we cannot exclude the hypothesis that other polymorphic alleles of LIF could be implicated in MS susceptibility.

1. Introduction

Multiple sclerosis (MS) is a central nervous system (CNS) disease and is associated with the formation of demyelinated plaques. These plaques are the result of an ongoing infiltration of leukocytes in the CNS parenchyma causing damage to the axonal insulator, myelin. Demyelination leads to alteration of the action potential conduction along axons and results in broad and various symptoms [1]. The pathology of MS is classified into three clinical types: relapsing–remitting MS (RR-MS), secondary progressive MS (SP-MS) and primary progressive MS (PP-MS). Interestingly, RR-MS coincides with periods of remission, in which regeneration of the myelin is known to involve anti-inflammatory agents, neurotrophins and resident precursors of oligodendrocytes [1].

Particular attention has been focused on the investigation of inflammatory molecules within the immune system. In the presence of myelin proteins, infiltrated leukocytes become reactive and secrete a plethora of pro-inflammatory agents degrading the myelin sheath [2]. All genome-wide screens in MS have revealed a global implication of the major histocompatibility complex (MHC) at the 6p21 chromosomal region [3–7]. Leukemia inhibitory factor (LIF) belongs to the neuropoietic family and is present in MS plaques [8]. The gene coding for LIF is located at 22q12.2, a region associated with MS in a Spanish population [9]. While the systemic action of LIF is wide, LIF triggers an intracellular cascade that is very similar and even indistinguishable from other neurokines, including CNTF in the nervous system, as well as IL-6, in non-neuronal hematopoietic cells [10]. Macrophages and T cells secrete LIF and have been shown to confer oligodendrocyte protection in the CNS [8]. Experiments undertaken in the experimental allergic encephalomyelitis animal model of MS have shown that the level of expression of LIF mRNA is up-regulated in plaques [11]. Additionally, antibodies directly targeted to LIF and inhibiting LIF action showed increase severity in EAE mice [12]. The positive effect on oligodendrocytes appears to be mediated through LIF induced activation of suppressors of cytokine signalling (SOCS) [13]. SOCS have critical roles in regulating numerous cytokines that act through the janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway, creating a negative feedback loop that limits the effect of cytokine signalling on the cell [14]. The up-regulation of SOCS has been shown to ameliorate arthritis in mice, and possibly be a therapeutic strategy for treating the inflammatory disease [15,16]. SOCS regulates an important cytokine interferon-γ (INFγ) which is widely implicated in MS lesions [17,18]. In addition, LIF has opposite effects to the proinflammatory agent INFγ and has been demonstrated to block the cytotoxic effects of INFγ on oligodendrocytes [19].

Interestingly, a polymorphism located within the 3′ untranslated region (UTR) of LIF has been shown to affect the level of stability of LIF

☆ The research undertaken in this article complies with the Australian ethics standards and was approved by the Griffith University Ethics Committee.

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mRNA [20]. This C→T transition reduces stability of LIF mRNA and may potentially reduce the expression of LIF [20]. In this present report, we investigated the genotypes of an Australian MS population ($n=121$) age and sex matched with non neurological disease affected healthy controls ($n=121$) for the C→T transition polymorphism of the LIF gene.

2. Methods

2.1. Subject groups

The study used unrelated participants of Caucasian (Northern European) origin. The study protocol was approved by Griffith University's Ethics Committee for Experimentation on Humans. The test groups consisted of 121 MS patients and 121 controls, matched for ethnicity, sex and age (+/−5 years). The MS population was obtained from patients from the Multiple Sclerosis Clinic at the Royal Brisbane and Women's Hospital, from the South East Queensland region. This case group consisted of 75% women and 25% men and were categorised into three groups according to the clinical course: RR-MS, SP-MS and PP-MS with frequencies of 55%, 25%, and 20%, respectively. MS diagnosis of all patients was per the 2005 revised McDonald criteria [21]. The control group was also obtained from the South East Queensland region through the Genomics Research Centre, Southport, with each control matched to an MS patient for the variables age (+/−5 years), sex, and ethnicity. All individuals gave informed consent before participating in the research. Genomic DNA was extracted from peripheral blood leucocytes using a standard salting-out protocol as previously described [22].

2.2. Genotyping

The C→T polymorphism was genotyped with a restriction fragment length polymorphism (RFLP) used to detect each variant. The 3′ UTR of LIF was investigated using the StuI restriction enzyme to detect, at the nucleotide position 3951, a C to T transition. The polymorphic region was amplified by standard, unlabeled oligonucleotides followed by restriction enzyme digestion corresponding to the RFLP. Oligonucleotide primers used for the StuI RFLP were: forward primer 5′-AGGGGCAGGTTGC-TAAGTCAG-3′; reverse 5′-CCCCATTCTCTCAGATCCGA-3′. For detection of the LIF marker, 20–30 ng genomic DNA was amplified with 1× polymerase chain reaction (PCR) buffer, 2.5 mM MgCl2, 0.2 mM each dNTP, 0.2 μM each primer and Taq polymerase in a 25-μL final volume on a Corbett (Sydney, Australia) PC-960 thermocycler. Cycles consisted of a 4-min denaturation at 95 °C followed by 40 cycles of 95 °C for 30 s and 59 °C for 30 s and 72 °C for 30 s with a final extension at 72 °C for 5 min. PCR products were digested with StuI (1 U at 37 °C) and electrophoresed on 2% ethidium bromide stained agarose gels. Genotypes were denoted as TT (488 bp), TC (488, 124, 364 bp) or CC (364,124 bp).

2.3. Statistical analysis

Genotype and allele frequencies for the LIF variants were calculated from observed genotype counts. As a statistical control for systematic genotyping error and population stratification, the expected genotype proportions according to the Hardy–Weinberg law were calculated and compared to observed genotypes. Genotype and allele frequencies were initially assessed for association with MS using conventional contingency table analyses incorporating the standard chi-squared test for independence. This analysis produces a χ^2 statistic with one or two degrees of freedom and corresponding P-values for allele and genotype distributions, respectively.

3. Results

We could clearly determine the type of LIF StuI polymorphism in all patients and controls. The proportion of the three LIF StuI genotypes, denoted as TT (488 bp), TC (488, 124, 364 bp) or CC (364,124 bp), are tabulated in Table 1. The expected genotype proportions were according to Hardy–Weinberg equilibrium for patient and control cohorts (MS $P=0.52$, Control $P=0.92$). Analysis was conducted by the 2×3 chi-square test for patient and control groups genotype frequency with results indicating no statistically significant association ($\chi^2=0.58$, $P=0.75$). Similar analysis by 2×2 chi-square test was conducted for patient and control groups allele frequency with results showing no statistically significant association ($\chi^2=0.32$, $P=0.57$). In addition, no significant association was shown with any of the patient subtypes compared to control allele or genotype frequencies ($P>0.05$).

Table 1
Distribution of LIF StuI variant (genotype and allele) frequencies in MS case and control groups.

Group	Genotypes[a]			N (genotypes)	Alleles[b]	
	C/C	C/T	T/T		C	T
MS[c]	50 (41%)	53 (44%)	18 (15%)	121	153 (63%)	89 (37%)
RR-MS	29 (44%)	25 (38%)	12 (18%)	66	83 (63%)	49 (37%)
SP-MS	12 (40%)	17 (57%)	1 (3%)	30	41 (68%)	19 (32%)
PP-MS	9 (36%)	11 (44%)	5 (20%)	25	29 (58%)	21 (42%)
Control[d]	52 (43%)	55 (45%)	14 (12%)	121	159 (66%)	83 (34%)

[a] Genotype distribution comparison for total MS ($\chi^2=0.58$, $P=0.75$).
[b] Allele distribution comparison for total MS ($\chi^2=0.32$, $P=0.57$)
[c] Hardy–Weinberg equilibrium $P=0.52$.
[d] Hardy–Weinberg equilibrium $P=0.92$.

4. Discussion

The aim of this study was to investigate a C/T polymorphism in the LIF gene for its involvement in MS susceptibility within an Australian cohort. The investigated was distinguishable by StuI restriction enzyme digest and genotyped in 121 MS Australian individuals and 121 matched controls. A StuI restriction site is located within the 3′ UTR LIF. Our results showed no significant association between cases and controls for the tested LIF 3′ UTR StuI polymorphism.

During CNS inflammation, LIF is secreted by astrocytes [23] acts on oligodendrocytes to prevent their loss and increase their survival [24,25]. Furthermore, protective effects of LIF include roles in reducing cytotoxic activity of INFγ, a pro-inflammatory molecule secreted by infiltrating T cells. Additionally, the Th1 to Th2 shift observed in MS remissions correlates with differences in LIF secretion levels between these types of cells [8]. Th2 cells show higher LIF concentrations compared to Th1 clones demonstrating a LIF Th2 mediated anti-inflammatory effect. However, LIF neurotrophin has been shown to be reduced RR-MS compared to controls [26]. Interestingly, Simvastatin, a cholesterol lowering drug with associated anti-inflammatory effects that may possibly be used in the future to treat MS [27], has previously been shown to increase LIF level secretions from both CD4+ and CD8+ T cells [8]. Despite results implicating a role for LIF in MS, our study could not demonstrate any association between the tested LIF functional variant and MS. Similarly, results of a recent Belgium study also showed no association for the C→T transition of LIF in MS [28]. We cannot however exclude the hypothesis that other polymorphic alleles of LIF could be implicated in MS susceptibility.

LIF acts on the LIF receptor (LIFR), a gene localised to 5p13, a region strongly associated with MS especially in Finnish populations [29]. Of note, recent studies from UK, Scandinavia, Belgium and USA revealed that interleukin 7 receptor alpha (IL7RA) also localised to 5p13 might be implicated in MS [21,30,31]. Association studies for LIFR have not yet been carried out and may be useful for determining whether LIFR plays a role in MS susceptibility.

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