

Short communication

Susceptibility to chronic inflammatory demyelinating polyradiculoneuropathy is associated to polymorphic GA repeat in the SH2D2A gene

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Received 5 December 2007; received in revised form 3 April 2008; accepted 8 April 2008

Abstract

The SH2D2A gene encodes a T-cell-specific adapter protein involved in the negative control of T-cell activation. The genotype GA13-16 homozygote of the SH2D2A gene promoter has been associated with the susceptibility to develop multiple sclerosis. Chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) is an immune-mediated neuropathy sharing several pathogenetic mechanisms with multiple sclerosis. We genotyped the SH2D2A promoter region in 105 controls and 48 patients with CIDP. We found a significant association between CIDP and the genotype GA13-16 homozygote (OR 3.167; p 0.013). We hypothesize that this genotype is associated with the susceptibility to develop CIDP and may be implicated in the persistence of the disease.

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Keywords: Apoptosis; Chronic inflammatory demyelinating polyradiculoneuropathy; Persistence of inflammatory reaction; SH2D2A gene polymorphism

1. Introduction

Chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) is a rare (1.2 to 7.7/100,000) autoimmune disorder of the peripheral nerve system in which both T-cells and antibodies are involved (Hughes et al., 2006a). In investigating the immunogenetics of CIDP two studies suggested trends toward increased frequency of the HLA-DR molecules DR3 and DR2 whereas other studies did not show any significant association (Stewart et al., 1978; Feeney et al., 1990; Vaughan et al., 1990; Van Doorn et al., 1991). Another examination showed a significant increase in the frequency of protease inhibitor type M3 allele in CIDP and also in

Guillain–Barré (GBS) syndrome and multiple sclerosis (MS) patients (McCombe et al., 1985). Although the immunodominant antigen in CIDP remains unknown, antibodies to ganglioside GM1 have been reported in up to 23% of patients (Van Schaik et al., 1994). The CD1 antigen-presenting molecules are a conserved family of MCH-like transmembrane glycoproteins specialized in capturing and presenting a variety of microbial and self-glycolipids to antigen-specific T-cells (Porcelli and Modlin, 1999). An association between polymorphisms of CD1 genes and the GBS has been reported (Caporale et al., 2006). Therefore we investigated the polymorphisms of CD1A and CD1E genes in CIDP but we did not find any association (De Angelis et al., 2007). Recent studies in mice and humans have identified the SH2D2A gene as an important regulator of autoimmune diseases (Marti et al., 2005). The SH2D2A gene is located on the chromosome 1 in a region conferring the susceptibility to develop chronic allergic encephalomyelitis (Dai et al., 2000; Sundvall et al., 1995). A variable number of GA tandem repeats were present in the promoter region of the SH2D2A gene and short SH2D2A alleles were associated

Abbreviations: CIDP, chronic inflammatory demyelinating polyradiculoneuropathy; GBS, Guillain–Barré syndrome; HWE, Hardy–Weinberg equilibrium; MS, multiple sclerosis; TSA, specific adapter protein.

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Table 1
Hardy–Weinberg equilibrium

		Genotype			Chi-squared test
		GA13-16/ GA13-16	GA13-16/ GA17-33	GA17-33/ GA17-33	
SH2D2A	Observed number	10	52	36	$\chi^2=2.99$ $p=0.08$
	Expected proportion	0.134444	0.464444	0.401111	

with the susceptibility to develop MS and juvenile rheumatoid arthritis (Dai et al., 2001; Smerdel et al., 2004).

The above observations and the fact that CIDP shares several pathogenetic mechanisms with MS prompted us to investigate the possible role of the SH2D2A gene polymorphisms in the susceptibility to develop CIDP (Toyka and Gold, 2003).

2. Subjects and methods

Forty-eight CIDP patients (30 males; mean age: 52 years; range 8 to 81) were enrolled in the study. According to the diagnostic criteria and categories proposed by the joint task force of the European Federation of Neurological Societies and the Peripheral Nerve Society, 40 patients had a definite and 8 a probable CIDP (Hughes et al., 2006b). Eleven patients had CIDP with concomitant diabetes and none had paraproteins. One-hundred and five unrelated randomly selected healthy subjects, from the same geographic area, constituted the control group for genetic studies.

A written informed consent was obtained from each subject and the study was approved by the local Ethic Committee.

3. Genetic analysis

Peripheral venous blood was sampled and genomic DNA was extracted by standard procedure (Coligan et al., 1992). Amplification by polymerase chain reaction (PCR) was performed and PCR products containing the SH2D2A GA repeats were obtained from genomic DNA using the following primers: primer 1 5' TCCCCATCCCCTGCTC 3'; primer 2 5' CCTGACCTT-CATCCCTCC 3'. One of the primers was fluorescence-labelled. Sequencing products were loaded on an ABI Prism 3100 (Applied Biosystem, Foster City, CA) and analyzed with GeneScan software (Applied Biosystem).

4. Statistical analysis

Associations may be spurious if the distribution of genotypes in the healthy control groups in genetic case–control studies

Table 3
SH2D2A genotypes as risk factor

Genotype	OR	1 tailed Fisher's p	Bonferroni significance
GA13-16/GA13-16	3.167	0.013	*
GA13-16/GA17-33	0.505	0.039	NS
GA17-33/GA17-33	1.058	0.507	NS

NS: not statistically significant.

*: statistically significant — overall alpha level of 0.05.

deviates from HWE. HWE depends on a series of assumptions about the tested population, including, for example, no new mutation, no selection, and random mating. Departures from HWE, if not due to chance or violation of these assumptions, therefore may point to genotyping error or other biases (Mitchell et al., 2003; Trikalinos et al., 2006). Departures from HWE were tested by using an asymptotic χ^2 test among the disease-free controls.

The association between SH2D2A genotypes and presence/absence of CIDP was evaluated using odds ratio and Fisher's exact test. The frequency of each genotype was compared to the overall frequency of the other genotypes in patients and controls. GA13-16 homozygote genotype was hypothesized as a possible risk factor for the development of CIDP and compared to all other genotypes; to perform this comparison, a one-sided test was used since, in case of a statistically significant difference, the results are more informative than those obtainable from a two-sided test. Since GA13-16 homozygote genotype was considered as a possible risk factor, GA17-33 homozygote genotype and the heterozygote genotype were considered as possible non-risk factors. The Bonferroni adjustment was used to control the type I error in multiple tests. The thresholds for 1 tailed statistical significance in each Fisher's test was adjusted to 0.01667 to obtain in 3 tests an overall alpha level of 0.05 in the case of the SH2D2A gene that had 3 different genotypes. The choice to use Bonferroni adjustment was based on the consideration that, despite the shortcomings evidenced in this procedure, the evaluation of association of a disease with genotypes is one of the few situations in which Bonferroni adjustment is acceptable (Perneger, 1998).

5. Results

The Hardy–Weinberg equilibrium (HWE) was not violated by the control group for either of the three genotypes of the SH2D2A gene promoter (Table 1). The SH2D2A promoter region has a characteristic bimodal frequency distribution. The frequency of long alleles (GA17-33) was increased in both controls and CIDP patients compared with the frequency of

Table 2
Genotype and allele frequency for SH2D2A in patients and controls

Group	Genotype			% of allele frequency		Number of subjects (%)	
	Number of subjects (%)					Positive for allele	
	GA13-16/GA13-16	GA13-16/GA17-33	GA17-33/GA17-33	GA13-16	GA17-33	GA13-16	GA17-33
CIDP	12 (25%)	18 (38%)	18 (38%)	44%	56%	30 (63%)	36 (75%)
Controls	10 (10%)	57 (54%)	38 (36%)	37%	63%	67 (64%)	95 (90%)

Table 4
Genotype and allele frequency for SH2D2A in definite CIDP patients (excluding patients with diabetes and probable CIDP) and controls

Group	Genotype			% of allele frequency		Number of subjects (%)	
	Number of subjects (%)					Positive for allele	
	GA13-16/GA13-16	GA13-16/GA17-33	GA17-33/GA17-33	GA13-16	GA17-33	GA13-16	GA17-33
CIDP	9 (30%)	10 (33%)	11 (37%)	47%	53%	19 (63%)	21 (70%)
Controls	10 (10%)	57 (54%)	38 (36%)	37%	63%	67 (64%)	95 (90%)

short alleles (GA13-16) (Table 2). The genotype GA13-16 homozygote was more frequent in all CIDP patients (25%) compared to controls (10%) and conferred a three fold risk to develop CIDP (OR 3.167; p 0.013) (Tables 2 and 3). Moreover the genotype GA13-16 homozygote was more frequent in definite CIDP patients without diabetes compared to controls, conferring a four fold risk to develop definite CIDP (Tables 4 and 5). The frequencies of the genotypes GA13-16/GA17-33 heterozygote and GA17-33 homozygote were not significantly different in controls and CIDP patients (Tables 2–5).

6. Discussion

In humans two polymorphic variants of the SH2D2A gene promoter, differing in the number of GA repeats and located at position-340 from the initiator ATG codon, have been recognized (Dai et al., 2000). Short alleles have 13-16GA repeats whereas long alleles contain up to 33GA repeats. The genotype GA13-16 homozygote has been associated with the susceptibility to develop multiple sclerosis (Dai et al., 2001). Although further studies, including larger numbers of patients in different populations, are necessary to confirm our results, we found a strong association between the genotype GA13-16 homozygote and CIDP.

SH2D2A gene encodes a T-cell specific adapter protein (TSAd) and the GA13-16 homozygote genotype is associated with lower expression of TSAd in T-cells (Dai et al., 2001; Smerdel et al., 2004). TSAd is restricted to T and NK cell lineages and it is involved in the inhibition of early T-cell activation (Nejad et al., 2004; Sundvold et al., 2000). TSAd-deficient T-cells secrete reduced quantities of some cytokines, IL2 being the most affected (Rajagopal et al., 1999). In vivo IL2, the major controller of the autoimmunity through the production of suppressive CD4+CD25+ T regulatory cells, induces T-cell death (Kneitz et al., 1995; Malek, 2003). Patients with MS have reduced IL2 and IL10 mRNA expression and IL2 resulted undetectable in cerebral spinal fluid and sera of CIDP patients (Musette et al., 1996; Sivieri et al., 1997).

Table 5
SH2D2A genotypes as risk factor in definite CIDP patients (excluding patients with diabetes and probable CIDP)

Genotype	OR	1 tailed Fisher's p	Bonferroni significativity
GA13-16/GA13-16	4.071	0.008	*
GA13-16/GA17-33	0.421	0.034	NS
GA17-33/GA17-33	1.021	0.562	NS

NS: not statistically significant.

*: statistically significant — overall alpha level of 0.05.

Moreover, TSAd-deficient T-cells showed a resistance to superantigen-induced T-cell death in vivo and partial resistance to Fas-mediated death in vitro (Marti et al., 2005). Patients with CIDP, similarly to MS patients, show a significantly lower Fas-induced T-cell death resulting in a defective control of autoreactive T-cells (Dianzani et al., 2003; Comi et al., 2006). Activated T-cells are crucial in CIDP as well in MS pathogenesis, because they produce cytokines modulating the activation of macrophages, found at the site of demyelination, and help antibody production by B-cells (Rezania et al., 2004). In chronic autoimmune diseases, such as CIDP, whether the immune response stops or persists may depend on the immune response shutting off system, which involves Fas-dependent cell death (Rezania et al., 2004).

All the above observations suggest that increased frequency of the genotype GA13-16 homozygote in CIDP patients may result in a defective control and elimination of autoreactive T-cells, conferring a susceptibility to develop the disease and favouring a chronic course.

Acknowledgements

We are grateful to all the study participants. This study has been supported by a grant from the Italian Ministry of Health (Ricerca Ordinaria 2004).

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