Journal of the Neurological Sciences 372 (2017) 324–328

Contents lists available at ScienceDirect

Journal of the Neurological Sciences

journal homepage: www.elsevier.com/locate/jns

Association of glutathione S-transferase omega polymorphism and spinocerebellar ataxia type 2

Luis E. Almaguer-Mederos [a,*], Dennis Almaguer-Gotay [a], Raúl Aguilera-Rodríguez [a], Yanetza González-Zaldívar [a], Dany Cuello-Almarales [a], José Laffita-Mesa [a], Yaimé Vázquez-Mojena [a], Pedro Zayas-Feria [a], Roberto Rodríguez-Labrada [a], Luis Velázquez-Pérez [a], Patrick MacLeod [b]

[a] Center for the Investigation and Rehabilitation of Hereditary Ataxias (CIRAH), Holguín, Cuba
[b] Division of Medical Genetics, Department of Pathology, Laboratory Medicine and Medical Genetics, Victoria General Hospital, Canada

ARTICLE INFO

Article history:
Received 27 June 2016
Received in revised form 28 November 2016
Accepted 29 November 2016
Available online 5 December 2016

Keywords:
Age at onset
Maximal Saccade Velocity
Modifier genes
Omega class glutathione-S-transferases
Spinocerebellar ataxia type 2

ABSTRACT

Background: Spinocerebellar ataxia type 2 is a neurodegenerative disorder caused by a CAG repeat expansion in *ATXN2* gene. There is high clinical variability among affected patients suggesting the occurring of modifier genes influencing the clinical phenotype.
Objective: The objective is to assess the association of *GSTO1* rs4925 and *GSTO2* rs2297235 SNPs on the clinical phenotype in SCA2 patients.
Methods: A case-control study was performed in a sample of 120 SCA2 Cuban patients and 100 healthy subjects. Age at onset, 60° Maximal Saccade Velocity and SARA score were used as clinical markers. *GSTO1* rs4925 and *GSTO2* rs2297235 SNPs were determined by PCR/RFLP.
Results: Distribution of the *GSTO1* alleles and genotypes was nearly equal between the control group and SCA2 patients. *GSTO1* genotypes were not associated to clinical markers in SCA2 patients. Distribution of the *GSTO2* "G" allele and "AG" genotype differed significantly between SCA2 patients and controls. Symptomatic SCA2 individuals had a 2.29-fold higher chance of carrying at least one "G" allele at *GSTO2* rs2297235 than controls (OR = 2.29, 95% CI: 1.29–4.04). *GSTO2* genotypes were significantly associated to age at onset ($p = 0.037$) but not to 60° Maximal Saccade Velocity or SARA score in SCA2 patients.
Conclusion: The *GSTO1* rs4925 polymorphism is not associated to SCA2. Meanwhile, the *GSTO2* rs2297235 "AG" genotype is associated to SCA2 but failed to show any association with clinical markers, with the exception of a potential association with the age at disease onset.

1. Introduction

Spinocerebellar ataxia type 2 (SCA2) is a neurodegenerative condition due to a CAG repeat expansion mutation in the first exon of the *ATXN2* gene which encodes the protein ataxin-2. The CAG repeat sequence is translated into an abnormally long polyglutamine tract, which is believed to acquire a neurotoxic gain of function in the mutant protein [1]. It is well known that the CAG repeat number at expanded *ATXN2* alleles is the major genetic determinant of clinical severity; however, there is huge variability in the clinical phenotype, even for individuals sharing the same CAG repeat number [2,3]. This observation led to the hypothesis of additional genetic factors probably acting together with environmental and stochastic factors to produce the corresponding clinical phenotypes. Actually, heritability of residual variance in age at onset after correction for *ATXN2* CAG repeats length is about 55% [4].

By means of the candidate gene approach, modifying effects of the CAG repeat polymorphism on *CACNA1A* [4], *ATXN3* [5], *ATXN7* [6], and *RAI1* genes [7,8] as well as the 10398A/G polymorphism on the *MTND3* gene [9] have been found on the age at onset in SCA2 affected individuals. The identification of additional modifier genes is of utmost significance since they may provide further clues concerning pathophysiological mechanisms and pathways underlying SCA2.

SCA2 pathophysiology has been linked to oxidative stress, particularly to altered activity of key antioxidant enzymes including glutathione S-transferases [10–12]. Consequently, functional polymorphisms in genes coding for antioxidant enzymes have potential modifying effects on the clinical phenotype of SCA2 individuals. In particular, it has been indicated that polymorphisms in the *GSTO1* and *GSTO2* genes region are associated with age at onset in Alzheimer's (AD) and Parkinson's diseases (PD), suggesting this region as a probable candidate to modify age at onset in additional neurodegenerative conditions [13]. Additionally, *GSTO1–GSTO2* haplotypes can cause an age at onset

* Corresponding author.
E-mail addresses: leam@cristal.hlg.sld.cu, lalmaguermederos@gmail.com (L.E. Almaguer-Mederos).

http://dx.doi.org/10.1016/j.jns.2016.11.075
0022-510X/

delay of as long as 13 years in both AD and PD [14], and *GSTO* locus variants are associated with increased AD risk in older age [15].

Human glutathione S-transferases (GSTs, EC 2.5.1.18) are a family of related isoenzymes that protect against genotoxic xenobiotic chemicals and endogenous compounds, including drugs, carcinogens, and reactive oxygen species that results from aerobic metabolism [16]. The role of *GSTO1* and *GSTO2* loci in SCA2 has not been investigated before. Therefore, a non-synonymous polymorphism of the *GSTO1* gene (rs4925, which results in an Ala140Asp amino acid change) and a transition polymorphism in the *GSTO2* gene (rs2297235, −183A to G) were examined looking for associations with the clinical phenotype in SCA2 patients.

2. Patients and methods

2.1. Study design

A case-control study was performed in 120 SCA2 (male/female: 69/51) Cuban patients and 100 healthy subjects (male/female: 46/54). Healthy controls were selected from the same geographical and ethnic backgrounds as the SCA2 patients. Informed consent was obtained from both patients and controls.

2.2. Clinical assessment

Diagnosis of SCA2 was based on clinical and molecular criteria. Clinical diagnosis was based on the presence of gait ataxia, dysarthria, dysmetria, dysdiadochokinesis, dysphagia and slow saccadics. Age at onset was defined as the onset of motor impairment, and an approximate estimation of it was performed through a retrospective review of patient's clinical records and interviews with patients and close relatives. Maximal Saccade Velocity (MSV, in 60 °/s) was determined as previously reported [17]. The Scale for the Assessment and Rating of Ataxia (SARA score) [18] was applied to assess disease severity.

2.3. Molecular studies

The *ATXN2* CAG repeat number was determined by PCR amplification [19]. Aliquots of the PCR products were then precisely sized by fragment analysis on an ALF Express II apparatus (Amersham Biosciences, Sweden) in comparison to internal and external size markers using Allele Links 1.0 software (Amersham Biosciences, Sweden). Cases with 32 or more repeats were designated SCA2 gene carriers in accordance with published association with disease expression [2]. *GSTO1* rs4925 and *GSTO2* rs2297235 SNPs were genotyped by PCR/RFLP according to standard protocols [20].

2.4. Bioinformatics analysis

Function of *GSTO1* rs4925 SNP was predicted by using F-SNP (http://compbio.cs.queensu.ca/F-SNP/) [21] and iPTREE-STAB (http://210.60.98.19/IPTREEr/iptree.htm) [22].

2.5. Statistical analysis

The calculation for deviation from Hardy-Weinberg equilibrium among SCA2 patients and control individuals and the calculation of linkage disequilibrium between *GSTO1* and *GSTO2* loci were computed by using the Genetic Data Analysis (GDA) software (version 1.0) [23].

Frequencies of the alleles and genotypes among the case and control individuals were compared by the Fisher's exact test with the values predicted under the assumption of Hardy-Weinberg equilibrium in the sample and under three genetic models (dominant, additive and recessive). Odd ratios were calculated as a measure of the association of the genotype with the phenotype of SCA2 using the Mantel-Haenszel test. For each odd ratio 95% confidence intervals were calculated. Descriptive statistics were applied to quantitative clinical and molecular variables under study. Pearson's correlation coefficient was used to assess for significant associations between quantitative clinical and molecular variables.

Simple and stepwise multiple linear regression analysis were performed looking for significant associations between clinical and molecular variables. Because the association of age at onset (AO) to the *ATXN2* CAG repeat number is best represented by a logarithmic relationship [2], regression analysis was performed using the logarithmic transformed AO as dependent variable, and the *ATXN2* CAG repeat number at expanded alleles and the genotypes for *GSTO1* rs4925 and *GSTO2* rs2297235 SNPs or its genotype combinations as independent variables. The contribution of each of the *GSTO1* and *GSTO2* polymorphisms to unexplained variance of AO was calculated as the percentage of variance in AO out of unexplained variance in AO after accounting for the effect of *ATXN2* expanded alleles alone. Student *t*-test was used for comparing AO between *GSTO2* genotypes under a dominant genetic model.

In a separate analysis, 60° MSV was considered as dependent variable and the *ATXN2* CAG repeat number at expanded alleles and the genotypes for *GSTO1* rs4925 and *GSTO2* rs2297235 SNPs or its genotype combinations as independent variables. In an additional analysis, SARA score was considered as dependent variable, and the disease duration, *ATXN2* CAG repeat number at expanded alleles and the genotypes for *GSTO1* rs4925 and *GSTO2* rs2297235 SNPs or its genotype combinations as independent variables.

All tests were 2-tailed, and statistical significance was defined as $p < 0.05$. Bonferroni correction for multiple testing was applied when necessary. SPSS software (version 20.0) was used for all statistical analysis [24].

3. Results

3.1. Association of GSTO1 and GSTO2 polymorphisms with SCA2

Allelic and genotype frequencies were obtained for the *GSTO1* rs4925 and *GSTO2* rs2297235 SNPs in the full sample. The most frequently observed *GSTO1* allele was the "C" variant accounting for a 69.0% of the total, and the "A" variant was the most common *GSTO2* allele (79.5%). The most frequent genotypes for the *GSTO1* rs4925 and *GSTO2* rs2297235 SNPs were "CC" (51.8%) and "AA" (62.7%), respectively. In both study groups the *GSTO1* and *GSTO2* genotypes were in Hardy-Weinberg equilibrium ($p > 0.05$). *GSTO1* and *GSTO2* loci were found to be in strong linkage disequilibrium (LD; $r^2 = 0.69$).

Genotype and allele frequency distributions for the studied polymorphisms in SCA2 patients and controls are summarized in Table 1. No significant association was found between SCA2 and *GSTO1* rs4925 alleles or genotypes distributions. However, significant associations were observed between SCA2 and *GSTO2* rs2297235 SNP alleles and genotypes distributions; symptomatic SCA2 individuals had a 2.29-fold higher chance of carrying at least one "G" allele at *GSTO2* rs2297235 than controls (OR = 2.29, 95% CI: 1.29–4.04). Nevertheless, this association did not remain significant after correction for multiple testing ($p = 0.52$). Assuming the wild-type "AA" genotype as the reference, a significant higher frequency of the "AG" genotype (43.3% vs. 22.0%; $p = 0.0014$) was observed in SCA2 patients than in controls; this association remained significant even after correction for multiple testing ($p = 0.017$). In addition, a significant association was noted between SCA2 and *GSTO2* genotypes under the dominant model; however, this association did not remain significant after correction for multiple testing ($p = 0.06$).

The joint effect of *GSTO1* rs4925 and *GSTO2* rs2297235 SNPs was explored by evaluating the association between haplotypes and SCA2. The *GSTO1–GSTO2* CC-AA haplotypes were observed in 42.5% of cases and 47.0% of controls; meanwhile, the CA-AA or the AA-AA haplotypes were observed in 8.30% of cases and 24.0% of controls. The haplotypes CC-AG or CC-GG reached no statistically significant results ($p > 0.05$). Conversely, symptomatic SCA2 individuals had a 62% chance reduction