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Lack of Association of the rs11655081 ARSG Gene with Blepharospasm

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Abstract

Blepharospasm (BSP) is a sub-phenotype of focal dystonia. A few genetic risk factors are considered to be implicated in the risk of developing BSP. There is recent evidence, based on results from GWAS and meta-analyses, to suggest that arylsulfatase G (ARSG), and more specifically rs11655081, is implicated in focal dystonia. The aim of the present study was to evaluate the effect of rs11655081 ARSG on BSP. A Greek cohort, which consisted of 206 BSP patients and an equal number of healthy controls, was genotyped for rs11655081. Only a marginal trend for the association between rs11655081 and the risk of BSP was found in the over-dominant model of inheritance [odds ratio, OR (95% confidence interval, CI): 0.64 (0.38–1.07), $p = 0.088$]. It is rather unlikely that rs11655081 across ARSG is a major genetic risk contributor for BSP.

Keywords ARSG · Blepharospasm · Focal dystonia · Polymorphism · SNP

Introduction

Dystonia is a neurological movement disorder, which can be caused by a number of etiologies and comes with great variety and heterogeneity as regards phenotypic appearance (Albanese et al. 2018; Balint et al. 2018; Hallett 2015). Despite its heterogenic nature, there is some overlap between the dystonia sub-phenotypes at a clinical and genetic level (Siokas et al. 2017b). However, the different dystonia syndromes are not supposed to share a unique etiology or pathophysiology (Albanese 2017). Consequently, diagnostic criteria for dystonia have yet to be confirmed, despite the fact that a few have been put together over time (Albanese 2017). Therefore, the guide for dystonia's clinical assessment in the recent updated classification of dystonia is based on these two distinct axes: the etiology and the clinical appearance (Albanese et al. 2013a).

Dystonia is far from rare as a movement disorder, and previous epidemiological studies have underestimated its prevalence (Albanese et al. 2018). Focal dystonia with age at onset during adulthood is estimated to be the most common dystonia sub-phenotype (Wang et al. 2016; Williams et al. 2017). Among the focal sub-types, blepharospasm (BSP) is considered the second most frequent, following cervical dystonia (CD) (Defazio et al. 2004). However, there are studies on Japanese and Italian populations, reporting that BSP prevails (Valls-Sole and Defazio 2016).

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The genetic architecture of dystonia has been investigated with candidate gene association studies (CGASs), linkage analyses, genome-wide association studies (GWAS), whole-exome sequencing (WES), and meta-analyses (Lohmann et al. 2014; Mok et al. 2014; Ohlei et al. 2018; Siokas et al. 2017b; Tian et al. 2018). To date, these efforts have yielded a few pathogenic genetic variants which result in monogenic dystonia cases (Lohmann and Klein 2017; Xiromerisiou et al. 2012) and have also revealed genetic variants that are considered to confer susceptibility to dystonia (Siokas et al. 2017b).

The arylsulfatase G (ARSG) protein is encoded by the *ARSG* gene. ARSG is implicated in several pathophysiological processes such as cell signaling, neuronal ceroid lipofuscinosis, and protein degradation, through the hydrolyzation of sulfate esters, among others (Sardiello et al. 2005).

Two GWAS have been conducted thus far, aiming to identify variants that may indicate a predisposition to dystonia (Lohmann et al. 2014; Mok et al. 2014). Lohmann et al. (2014) reported that the intronic rs11655081 across the *ARSG* gene was associated with specific sub-phenotypes of focal dystonia (musician's dystonia (MD) and writer's cramp (WC)) (Lohmann et al. 2014). These results were also replicated in a recent meta-analysis, which reported that rs11655081 is in fact associated with MD (Ohlei et al. 2018). Regarding the same meta-analysis, rs7342975 and rs9972951 in ARSG gene exhibited a trend for association before the correction for multiple testing (Ohlei et al. 2018). Finally, the ARSG missense variant rs61999318 has been reported to be more frequent in patients with WC compared to European Americans in the EVS database (Nibbeling et al. 2015). On the contrary, rs11655081 failed to reach the statistical significance threshold regarding its association with other phenotypes (CD, BSP, other forms of focal dystonia) (Lohmann et al. 2014).

Previous studies examining the effect of the ARSG gene in dystonia have presented conflicting results (Lohmann et al. 2014; Nibbeling et al. 2015; Ohlei et al. 2018). Despite dystonia's heterogenic nature, there is some overlap between the sub-phenotypes of dystonia, concerning genetics. It is therefore important for genetic polymorphisms identified by GWAS or meta-analyses to be validated (Katsarou et al. 2018; Theuns et al. 2014) in different ethnic groups and different dystonia sub-phenotypes. Therefore, we aimed to enlarge our comprehension regarding the role of rs11655081 ARSG on BSP, in a Greek cohort. Should an association between rs11655081 and the development of dystonia become evident in our ethnically homogenous cohort, it could shed some light on genetic similarities between the populations that have been studied thus far and dystonia subtypes as well.

Methods

Study Population

Two-hundred six patients with BSP and an equal number of healthy controls (Caucasians) were drafted during this study, as previously described (Siokas et al. 2018). Both BSP patients and healthy controls had a negative family history of dystonia. BSP patients had been examined at the neurology and ophthalmology outpatient clinics of the University Hospital of Larissa, Greece. The diagnosis of BSP was made following the patients' examination both by a neurologist and ophthalmologist specialists. The local ethics committee approved study's protocol. All participants provided informed consent.

Isolation of DNA and Genotyping

With the salting out method, we extracted genomic DNA from peripheral blood samples (Dardiotis et al. 2017; Siokas et al. 2017a). The genotyping was performed with a TaqMan allele-specific discrimination assays method on an ABI PRISM 7900 Sequence Detection System and analyzed with SDS software (Applied Biosystems, Foster City, California, USA) (Dardiotis et al. 2015; Siokas et al. 2017c). The laboratory personnel were unaware of the participants' phenotype. In a percentage of 98.79% of our cohort, the genotyping was successful.

Statistical Analysis

The power of the analysis was calculated with the use of the CaTS power calculator (http://csg.sph.umich.edu//abecasis/cats/gas_power_calculator/index.html) (Skol et al. 2006). We had 80.3% power to identify an association of a variant with a genetic relative risk of 1.72, assuming the multiplicative model, a minor allele frequency of 9% (in BSP cases), type I error level of 0.05. The Hardy–Weinberg equilibrium (HWE) (Dardiotis et al. 2018a) was calculated in terms of exact test.

With SNPStats software (http://bioinfo.iconcologia.net/SNPstats/) (Sole et al. 2006) and by presuming five main genetic modes of inheritance (codominant, over-dominant, dominant, recessive, and additive), odds ratios (ORs), and the respective 95% confidence intervals (CIs) were estimated. Values smaller than 0.05 were considered as statistically significant.

Results

Two-hundred six BSP patients (45.1% male) and an equal number of matched healthy controls were drafted in total. The mean age of blood sample collection of BSP cohort was

67.32 (± 12.02) years, whereas the mean BSP age of onset was 61.15 (± 12.03) years. No deviation of HWE was present (p = 0.42). Allelic and genotypic total numbers and frequencies of study's cohort and of subgroups are presented in Supplementary Table 1.

No statistically significant results regarding the genetic variability of the rs11655081 ant the BSP was perceived in any examined mode of inheritance ($p > 0.05$). Only a marginal trend for association between the rs11655081 and the BSP risk was discerned in the over-dominant mode (odds ratio, OR (95% confidence interval, CI), 0.64 (0.38–1.07), $p = 0.088$). ORs, CIs, and p values are summarized in Table 1.

Discussion

The genetic architecture of Greek BSP patients may share either many or few similarities with the genetic risk factors identified through GWAS of other types of focal dystonia. In the present study, we drafted a relatively large number of BSP patients, aiming to investigate the role of rs11655081 ARSG on BSP. However, only a marginally statistically significant implication of rs11655081 in the risk of BSP was revealed.

The most extensively examined polymorphism regarding BSP risk appears to be rs6265, of the brain-derived neurotrophic factor (BDNF), with conflicting results thus far (Groen et al. 2012; Siokas et al. 2018). Apart from BDNF, the DRD5, D1 receptor gene and TOR1A have been reported to either confer susceptibly to BSP or to modify BSP's phenotypic course (Defazio et al. 2009; Misbahuddin et al. 2002). Moreover, a few variants in at least 9 genes have been detected through WES in BSP patients (Tian et al. 2018).

Table 1 Single locus analysis for association between rs11655081 (ARSG) and BSP, in codominant, dominant, recessive, over-dominant, and log-additive modes

Mode	Genotype	OR (95%CI)	p value
Codominant	T/T	1.00	0.23
	C/T	0.64 (0.38–1.07)	
	C/C	0.95 (0.19–4.76)	
Dominant	T/T	1.00	0.1
	C/T-C/C	0.66 (0.40–1.09)	
Recessive	T/T-C/T	1.00	0.98
	C/C	1.03 (0.20–5.14)	
Over-dominant	T/T-C/C	1.00	0.088
	C/T	0.64 (0.38–1.07)	
Log-additive	–	0.72 (0.46–1.12)	0.14

ARSG, arylsulfatase G; *BSP*, blepharospasm; *CI*, confidence interval; *OR*, odds ratio

The arylsulfatase G (ARSG) protein is encoded by the *ARSG* gene. The human ARSG gene is located within chromosome 17, in region 17q24.2, (68,259,182-68,422,731) and consists of 12exons (https://www.ensembl.org/index.html). The ARSG protein is implicated in several pathophysiological processes such as cell signaling, neuronal ceroid lipofuscinosis, and protein degradation, through the hydrolyzation of sulfate esters, among others (Sardiello et al. 2005).

Rs11655081 frequencies vary across populations, indicating that it is rather unlikely for it to be the causal variant across the ARSG gene (Lohmann et al. 2014). The latter could possibly explain, to a certain degree, the lack of association in our study. Apart from rs11655081, the rs61999318 ASRG variant has been reported to be more frequent in patients with WC compared to European Americans (Nibbeling et al. 2015). The rs61999318 variant may represent a functional variant, as the underlying amino acid substitution of isoleucine at position 493 with threonine (p.I493T) appears to be disease-causing (Nibbeling et al. 2015). Finally, ARSG, apart from dystonia, has also been associated with amyotrophic lateral sclerosis (ALS). More precisely, the rs1558878 missense variant has been detected in a GWAS study on ALS patients (Cronin et al. 2008).

Our study carries some limitations which should be addressed. Firstly, the adjustment for other potential cofounders (Dardiotis et al. 2018b; Tsatsakis et al. 2011; Tsatsakis et al. 2009), such other genetic variants (e.g., TOR1A) (Siokas et al. 2017b), or of additional risk exogenous factors (e.g., exposure to bright light) (Hallett et al. 2008) in the regression models would have provided more robustness to our results. Additionally, a tagging SNP selection approach could have possibly revealed the effect of the entire ARSG gene and not only of variants in high proximity to rs11655081. Moreover, our results would have more robustness if they had been validated in another cohort or accompanied from a functional analysis. Finally, a prospective analysis, including other outcomes (e.g., spread to adjusted body regions (Defazio et al. 2009)), BSP scales (Albanese et al. 2013b) or botulinum toxin treatment response (Lee et al. 2013; Vlata et al. 2012), in association with the rs11655081 genetic status, would have also been an advantage.

In conclusion, we did not identify any connection between rs11655081 ARSG and the BSP risk. Given the uncertainty over the pathogenicity of BSP and other types of focal dystonia, further analysis of the role of ARSG in dystonia is warranted, so that it will be eventually made clear whether ARSG is a genetic contributor to dystonia's architecture or not.

Compliance with Ethical Standards

The local ethics committee approved study's protocol. All participants provided informed consent

Informed Consent Informed consent was obtained from all individual participants included in the study.

Conflict of Interest The authors declare that they have no conflict of interest.

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