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CYP1A2 rs762551 and ADORA2A rs5760423 Polymorphisms in Patients with Blepharospasm

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Abstract

Blepharospasm (BSP) is a neurological movement disorder. Coffee consumption has been found to have a protective effect against BSP. BSP and apraxia of eyelid opening are particularly common among patients with PD. The CYP1A2 rs762551 and ADORA2A rs5760423 variants have been previously marginally associated with the risk of PD and are also implicated in caffeine metabolism pathways. The aim of the present study was to evaluate the effect of the CYP1A2 rs762551 and ADORA2A rs5760423 variants on BSP. A Southeastern European Caucasian (SEC) cohort of 206 BSP patients and 206 healthy controls was genotyped for rs762551 and rs5760423. CYP1A2 rs762551 was associated with a decreased BSP risk in the dominant (OR (95% CI) 0.62 (0.41–0.92), $p = 0.017$), log-additive (OR (95% CI) 0.68 (0.51–0.92), $p = 0.011$), and co-dominant modes (for the CC genotype OR (95% CI) 0.49 (0.25–0.93), $p = 0.038$). We provide preliminary evidence that CYP1A2 rs762551 is associated with BSP. Further studies and replication of our results are needed.

Keywords CYP1A2 · ADORA2A · Caffeine · Blepharospasm · Focal dystonia · Polymorphism · SNP · Genetics

Introduction

Blepharospasm (BSP) is a neurological movement disorder and it is characterized by bilateral, synchronous, and stereotypical spasms of the orbicularis oculis muscle (Marsden 1976). Until today, specific diagnostic criteria for BSP have not been established, and therefore, the diagnosis of BSP is based on clinical findings (Defazio et al. 2013; Defazio et al. 2017). On this front, in order for the clinical assessment of BSP and dystonia to be facilitated, the recent updated classification of dystonia and expert recommendations (Albanese et al. 2013; Defazio et al. 2019; Defazio et al. 2013) represents a useful guide.

The prevalence of BSP has been reported to be as high as 133 patients per million (Steeves et al. 2012). Results from epidemiological studies suggest that BSP could be even considered as the commonest phenotypical sub-type of dystonia, with a prevalence higher than even that of cervical dystonia (CD) (Valls-Sole and Defazio 2016).

The etiology of BSP and of dystonia as well have not been fully elucidated yet (Albanese et al. 2019; Jellinger 2019; Sharma 2019). Despite the fact that the basal ganglia appear to be involved in BSP pathogenesis (Defazio et al. 2017), the evidence derived from studies in humans and animals suggests that different regions are involved in dystonias and seem to be in favor of the motor network model for this family of disorders (Jinnah et al. 2017; Pirio Richardson and Jinnah 2019).

To date, some studies have revealed a few risk factor genes for dystonia and BSP (Siokas et al. 2018, 2019a, b). However,

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they have failed to produce consistent and reproducible results (Ohlei et al. 2018; Siokas et al. 2017b), suggesting a variability in the architecture of BSP genetics and also highlighting the need for ongoing research regarding targeting new genetic loci through candidate-gene association studies (CGASs). The identification of genetic loci that confer susceptibility to pathologies of nervous system is of particular interest, as they could provide new insight into ongoing research regarding novel biomarkers for nervous system diseases (Nery et al. 2019).

Cytochrome P450 1A2 (CYP1A2) is an inducible enzyme, found in most brain regions across humans (Koonrungsesomboon et al. 2018). CYP1A2 metabolizes numerous drugs and is considered as the main caffeine-metabolizing enzyme, converting over 90% of caffeine to paraxanthine in the liver (Sachse et al. 1999). Rs762551 (also known as -164A>C or -163C>A) is a single-nucleotide polymorphism (SNP) encoding the CYP1A2*1F allele of the *CYP1A2* gene. The carriers of the rs762551 appear to have lower CYP1A2 activity (Sachse et al. 1999).

Adenosine receptor subtype A2a (ADORA2A) is a G protein–coupled adenosine receptor which is implicated in mechanisms including synaptic plasticity in glutamatergic synapses, neurogenesis, and neuroinflammation (Horgusluoglu-Moloch et al. 2017). Knockout mice with non-functioning ADORA2A receptor appear to be protected against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) toxicity, similarly to the blockade of the receptor by caffeine, suggesting the implication of ADORA2A to PD (Chen et al. 2001; Chuang et al. 2016; Popat et al. 2011).

Prior coffee consumption has been found to have a protective effect against BSP (Defazio et al. 2007). The CYP1A2 rs762551 and ADORA2A rs5760423 variants possibly affect the caffeine metabolism (Popat et al. 2011; Sachse et al. 1999). Moreover, the CYP1A2 rs762551 and ADORA2A rs5760423 variants have been previously reported to confer susceptibility to the risk of PD (Chuang et al. 2016; Palacios et al. 2010; Rayaprolu et al. 2013). Additionally, BSP and apraxia of eyelid opening are particularly common among patients with PD (Hallett et al. 2008; Shetty et al. 2019). In view of the former considerations, the aim of the current study is to broaden apprehension regarding the role of polymorphism in genes that are possibly implicated to caffeine-related pathways and that have been previously associated with PD, in a SEC cohort with BSP patients.

Methods

Study Population

Two-hundred and six patients with BSP and 206 controls (SEC) were recruited, as previously described (Siokas et al. 2019a). BSP patients were examined at the Neurology and Ophthalmology outpatient clinics of the University Hospital of Larissa, Greece, and were diagnosed by neurologist and ophthalmologist specialists. This research study was approved by the Local Ethics Committee and was performed according to the Declaration of Helsinki. Following verbal and written explanation of the experimental protocol all subjects gave their written informed consent, with the option of withdrawing from the study at any time.

Isolation of DNA and Genotyping

Using the method of salting out, DNA was extracted from peripheral blood samples (Dardiotis et al. 2017; Siokas et al. 2017a, 2020). 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, CA, USA) (Dardiotis et al. 2015; Siokas et al. 2017c). The laboratory personnel who performed the genotyping were unaware of the individuals' phenotype. Two variants (CYP1A2 rs762551 and ADORA2A rs5760423) were genotyped in each participant.

Statistical Analysis

With the CaTS Power Calculator, we performed the power of the analysis (Skol et al. 2006). In the terms of the exact test, we calculated the Hardy–Weinberg equilibrium (HWE) and using the SNPStats software (Sole et al. 2006) we calculated odds ratios (ORs) and the respective 95% confidence intervals (CIs), by assuming the following genetic modes of inheritance: co-dominant, over-dominant, dominant, recessive, and additive. The p value of 0.05 was set as the statistical significance threshold.

Results

Two-hundred and six BSP patients (45.1% male) and an equal number of matched (regarding age and sex) controls were recruited in total (mean age of blood sample collection of the BSP cohort was 67.32 (±12.02) years, and the mean BSP age of onset was 61.15 (±12.03) years).

The percentage of the genotype call rate was equal to 97.57% (402/412) for the CYP1A2 rs762551 and 97.82% (403/412) in both BSP cases and controls, for the ADORA2A rs5760423. Our sample had a power of 80.6% to detect an association of a variant with a genetic relative risk of 1.43, in the multiplicative model, with a minor allele frequency of 35%. No deviation from the HWE was found for CYP1A2 rs762551 and ADORA2A rs5760423, in cases and controls. Total numbers and frequencies regarding alleles and genotypes of the study's cohort for all the examined variants

(CYP1A2 rs762551 and ADORA2A rs5760423) can be found in Table 1.

The CYP1A2 rs762551 was associated with a decreased risk of BSP in the dominant (OR (95% CI) 0.62 (0.41–0.92), $p = 0.017$), log-additive (OR (95% CI) 0.68 (0.51–0.92), $p = 0.011$), and co-dominant modes (for the CC genotype OR (95% CI) 0.49 (0.25–0.93), $p = 0.038$). No statistically significant association regarding the ADORA2A rs5760423 and BSP was revealed ($p > 0.05$).

ORs, CIs, and p values for the analyses of CYP1A2 rs762551 and ADORA2A rs5760423 regarding BSP are summarized in Table 2.

Discussion

In the current study, two polymorphisms were genotyped across two genes (CYP1A2 rs762551 and ADORA2A rs5760423), which were previously associated with PD and are also implicated in caffeine metabolism pathways, aiming to find a possible association with BSP risk susceptibility in a cohort of SEC patients with BSP compared with healthy controls. We detected that CYP1A2 rs762551 was associated with a decreased risk of BSP. Our results provide preliminary indication for a potential contribution of CYP1A2 genetic variability in the risk of BSP.

Research regarding genetic risk factors for BSP has not produced steady and easily replicated results. Whole exome sequencing (WES) has detected some possible damaging variants across a few genes in patients with BSP (Tian et al. 2018). Recently, two non-synonymous single-nucleotide variants (SNVs), the rs201870669 (c.538 C>T, R180W) and the rs117450750 (c.661 C>T, R221C), have emerged as damaging, through sequencing of the receptor expression-enhancing protein 4 (REEP4) gene (Hammer et al. 2019).

Table 2 Single locus analysis for association between CYP1A2 rs762551, ADORA2A rs5760423, and BSP, in co-dominant, dominant, recessive, over-dominant, and log-additive modes

Variant/mode	Genotype	OR (95%CI)	p value
CYP1A2 rs762551			
Co-dominant	A/A	1.00	*0.038*
	C/A	0.65 (0.43–1.00)	
	C/C	*0.49 (0.25–0.93)*	
Dominant	A/A	1.00	*0.017*
	C/A-C/C	*0.62 (0.41–0.92)*	
Recessive	A/A-C/A	1.00	0.11
	C/C	0.61 (0.33–1.12)	
Over-dominant	A/A-C/C	1.00	0.19
	C/A	0.77 (0.52–1.14)	
Log-additive	–	*0.68 (0.51–0.92)*	*0.011*
ADORA2A rs5760423			
Co-dominant	G/G	1.00	0.5
	T/G	0.87 (0.57–1.34)	
	T/T	0.69 (0.37–1.29)	
Dominant	G/G	1.00	0.38
	T/G-T/T	0.83 (0.55–1.26)	
Recessive	G/G-T/G	1.00	0.32
	T/T	0.75 (0.42–1.33)	
Over-dominant	G/G-T/T	1.00	0.88
	T/G	0.97 (0.66–1.44)	
Log-additive	–	0.84 (0.62–1.13)	0.25

BSP, blepharospasm; *CI*, confidence interval; *OR*, odds ratio; *CYP1A2*, cytochrome P450 1A2; *ADORA2A*, adenosine receptor subtype A2a; *NA*; non-available, Statistical significant values are presented in italics

Table 1 Allelic and genotype frequencies for CYP1A2 rs762551 and ADORA2A rs5760423 in healthy controls, in BSP cases, and whole sample

Variant	Genotypes/alleles	Healthy controls $n = 206$ n (%)	BSP $n = 206$ n (%)	Whole sample $n = 412$ n (%)
CYP1A2 rs762551				
Genotype	A/A	74 (0.36)	96 (0.48)	170 (0.42)
	A/C	99 (0.49)	84 (0.42)	183 (0.46)
	C/C	30 (0.15)	19 (0.01)	49 (0.12)
Allele	A	247 (0.61)	276 (0.69)	523 (0.65)
	C	159 (0.39)	122 (0.31)	281 (0.35)
ADORA2A rs5760423				
Genotype	G/G	64 (0.32)	72 (0.36)	136 (0.34)
	G/T	107 (0.53)	105 (0.52)	212 (0.53)
	T/T	31 (0.15)	24 (0.12)	55 (0.14)
Allele	G	235 (0.58)	249 (0.62)	484 (0.60)
	T	169 (0.42)	153 (0.38)	322 (0.40)

BSP, blepharospasm; *CYP1A2*, *cytochrome P450 1A2*; *ADORA2A*, *adenosine receptor subtype A2a*

Additionally, in another recent study, Dong et al., performed genetic testing in patients with BSP on 151 genes related to movement disorders and concluded that SYNE1 and CIZ1 mutations may be implicated to the etiology of BSP (Dong et al. 2019). Moreover, results from case-control studies have also shown an association between specific polymorphisms (in TOR1A, BDNF, DRD5, and D1 receptor genes), but overall, the results remain conflicting (Groen et al. 2012; Siokas et al. 2019a). This highlights the obvious need for further studies regarding the genetics of BSP, targeting in new candidate genes.

The CYP1A2 gene is located on chromosome 15 (region 17q24.2, 74,748,845-74,756,607) and consists of 7 exons. The CYP1A2 enzyme encoded by the CYP1A2 gene is the main enzyme that metabolizes caffeine and converts over 90% of caffeine to paraxanthine in the liver (Gunes and Dahl 2008; Sardiello et al. 2005). Studies examining the involvement of environmental factors to BSP development have tied factors (e.g., head trauma, coffee consumption, and smoking status) to having either protective, neutral, or deleterious effects on the disease (Defazio et al. 2017). Coffee consumption was inversely associated with BSP, and this inverse association tended to be also increased following coffee consumption, as it was even stronger when those who were drinking two cups of coffee per day were compared with those who never drank coffee (Defazio et al. 2007). However, as we did not perform an interaction analysis between CYP1A2 rs762551 and caffeine consumption, it is difficult to connect CYP1A2, caffeine, and BSP risk.

In the present study, ADORA2A, a gene that is also probably involved in caffeine metabolism pathways, was also investigated. However, no significant differences in allele and genotype frequencies regarding ADORA2A rs5760423 between the patients with BSP and controls were found. However, as in the case of CYP1A2 rs762551, we did not perform an interaction analysis between ADORA2A rs5760423 and caffeine consumption, and so any assumptions regarding ADORA2A rs5760423, caffeine intake, caffeine metabolism, and BSP risk cannot be extracted with certainty.

At this point, the limitations of the current study must be acknowledged. Gene-gene interaction (G×G) and gene-environment (G×E) analyses, and also adjusted logistic regression analyses for other potential co-triggers, especially caffeine intake, would have given more power to our conclusions (Dardiotis et al. 2018; Ruszkiewicz et al. 2019). Since CYP1A2 is highly inducible by environmental and life style factors such as smoking or dietary habits (Docea et al. 2017), the use of the real-life risk simulation approach, whose aim is to calculate cumulative risk assessment (Aloizou et al. 2020; Tsatsakis et al. 2017, 2018, 2019), would have increased the likelihood of disclosing the net effects of CYP1A2 rs762551 to BSP. Moreover, the association between CYP1A2 rs762551 should have been further validated in another cohort with a different ethnic background. Finally, our study would have more robustness if additional genetic loci, containing additional polymorphisms, implicated in caffeine metabolism pathways or affect habitual caffeine intake, would have been genotyped (Yoshihara et al. 2019).

In conclusion, we hereby provide preliminary evidence that the CYP1A2 rs762551 is associated with BSP. Further studies and replication of our results are needed. Given the complexity of the BSP pathogenesis, more studies regarding the role of CYP1A2 rs762551 in BSP are of great necessity, so that better insight into the etiology and the genetic architecture of BSP can be provided.

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Compliance with Ethical Standards

This research study was approved by the Local Ethics Committee and was performed according to the Declaration of Helsinki.

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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