17. Cheng FB, Wan XH, Feng JC, Wang L, Yang YM, Cui LY. Clinical and genetic evaluation of DYT1 and DYT6 primary dystonia in China. Eur J Neurol 2011;18:497-503.
18. LeDoux MS, Xiao J, Rudzińska M, et al. Genotype-phenotype correlations in THAP1 dystonia: molecular foundations and description of new cases. Parkinsonism Relat Disord 2012;18:414-425.
19. Campagne S1, Saurel O, Gervais V, Milon A. Structural determinants of specific DNA-recognition by the THAP zinc finger. Nucleic Acids Res 2010;38:3466-3476.
20. Kaiser FJ, Osmanoric A, Rakovic A, et al. The dystonia gene DYT1 is repressed by the transcription factor THAP1 (DYT6). Ann Neurol 2010;68:554-559.
21. Klein C, Liu L, Doheny D, et al. Epsilon-sarcoglycan mutations found in combination with other dystonia gene mutations. Ann Neurol 2002;52:675-679.
22. Esapa CT, Waite A, Locke M, et al. SGCE missense mutations that cause myoclonus-dystonia syndrome impair epsilon-sarcoglycan trafficking to the plasma membrane: modulation by ubiquitination and torsinA. Hum Mol Genet 2007;16:327-342.
23. Yokoi F, Yang G, Li J, DeAndrade MP, Zhou T, Li Y. Earlier onset of motor deficits in mice with double mutations in Dyt1 and Sgce. J Biochem 2010;148:459-466.

Supporting Data

Additional supporting information may be found in the online version of this article at the publisher's web-site.

BDNF Val66Met Polymorphism in Primary Adult-Onset Dystonia: A Case-Control Study and Meta-analysis

Pilar Gómez-Garre, PhD,[1,2†] Ismael Huertas-Fernández, MSc,[1†] María Teresa Cáceres-Redondo, MD,[1] Araceli Alonso-Canovas, MD,[3] Inmaculada Bernal-Bernal, MSc,[1] Alberto Blanco-Ollero, MD,[4] Marta Bonilla-Toribio, MSc,[1] Juan Andrés Burguera, MD, PhD,[5] Manuel Carballo, MD, PhD,[1] Fátima Carrillo, MD,[1] María José Catalán-Alonso, MD, PhD,[6] Francisco Escamilla-Sevilla, MD, PhD,[7] Raúl Espinosa-Rosso, MD,[8] María Carmen Fernández-Moreno, MD,[9] Juan García-Caldentey, MD,[10] José Manuel García-Moreno, MD,[11] Pedro José García-Ruiz, MD, PhD,[10] Sandra Giacometti-Silveira, MD,[12] Javier Gutiérrez-García, MD,[13] Silvia Jesús, MD, PhD,[14] Eva López-Valdés, MD,[6] Juan Carlos Martínez-Castrillo, MD, PhD,[3] Irene Martínez-Torres, MD,[5] María Pilar Medialdea-Natera, MD, PhD,[15] Carolina Méndez-Lucena, MD,[11] Adolfo Mínguez-Castellanos, MD, PhD,[7] Miguel Moya, MD,[8] Juan José Ochoa-Sepulveda, MD,[16] Tomás Ojea, MD,[12] Nuria Rodríguez, MD,[17] Miriam Sillero-Sánchez, MD,[17] Laura Vargas-González, RN[1] and Pablo Mir, MD, PhD[1,2*]

[1]Unidad de Trastornos del Movimiento, Servicio de Neurología y Neurofisiología Clínica, Instituto de Biomedicina de Sevilla, Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla, Seville, Spain [2]Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), Spain [3]Unidad de Trastornos del Movimiento, Servicio de Neurología, Hospital Ramón y Cajal, IRYCIS, Madrid, Spain [4]Servicio de Neurología, Hospital Juan Ramón Jiménez, Huelva, Spain [5]Unidad de Trastornos del Movimiento, Servicio de Neurología, Hospital La Fe, Valencia, Spain [6]Unidad de Trastornos del Movimiento, Servicio de Neurología, Hospital Clínico San Carlos, Madrid, Spain [7]Unidad de Trastornos del Movimiento, Servicio de Neurología, Hospital Universitario Virgen de las Nieves, Granada, Spain [8]Servicio de Neurología, Hospital Universitario Puerta del Mar, Cádiz, Spain [9]Servicio de Neurología, Hospital Virgen de Valme, Seville, Spain [10]Unidad de Trastornos del Movimiento, Servicio de Neurología, Fundación Jiménez Díaz, Madrid, Spain [11]Servicio de Neurología, Hospital Universitario Virgen Macarena, Seville, Spain [12]Servicio de Neurología, Hospital Carlos Haya, Málaga, Spain [13]Servicio de Neurología, Hospital Universitario San Cecilio, Granada, Spain [14]Servicio de Neurología, Complejo Hospitalario de Jaén, Jaén, Spain [15]Unidad de Trastornos del Movimiento, Servicio de Neurología, Hospital Universitario Virgen de la Victoria, Málaga, Spain [16]Servicio de Neurología, Hospital Universitario Reina Sofía, Córdoba, Spain [17]Servicio de Neurología, Hospital Universitario Puerto Real, Cádiz, Spain

ABSTRACT

Background: A polymorphism in brain-derived neurotrophic factor (BDNF) (Val66Met) has been reported as a risk factor in primary dystonia. However, overall the results have been inconclusive. Our aim was to clarify the association of Val66Met with primary dystonia, and with the most prevalent clinical subtypes, cervical dystonia and blepharospasm.

Methods: We conducted a Spanish multicenter case-control study (including 680 primary dystonia patients and 788 healthy controls) and performed a meta-analysis integrating our study and six previously published studies (including a total of 1,936 primary dystonia patients and 2,519 healthy controls).

Results: We found no allelic or genotypic association with primary dystonia, cervical dystonia, or blepharospasm risks, for the allele A (Met) from a BDNF Val66Met polymorphism in our case-control study. This was confirmed by results from our meta-analysis in white and mixed ethnic populations in any genetic model.

Conclusion: We did not find any evidence supporting the association of the BDNF Val66Met polymorphism with primary dystonia. © 2014 International Parkinson and Movement Disorder Society

Key Words: BDNF; Primary dystonia; meta-analysis; Val66Met; association study

Dystonia (DT) is a hyperkinetic movement disorder. Recently, the term *isolated dystonia* has been introduced to define those syndromes in which DT is the only motor feature, encompassing many cases previously referred to as "primary".[1] Primary DTs are those in which DT is not associated with other neurological features. The most frequent form is the adult-onset focal DT, which includes, as the most prevalent clinical subtypes, cervical dystonia (CD) and blepharospasm (BSP). The major mechanisms in primary DT

include reduced inhibition of the motor system and increased plasticity.[2]

The main function of the brain-derived neurotrophic factor (BDNF) in the adult brain is to facilitate synaptic plasticity.[3] The Val66Met polymorphism in BDNF has been reported as being associated with abnormalities in motor cortex plasticity,[4-6] being a risk factor for DT.

Several studies have investigated the association between Val66Met polymorphism and primary DT.[7-12] However, overall the results of these studies have been inconclusive.

To clarify any suspicion about its role as a risk factor in primary DT, we first aimed to assess the association of this polymorphism in a large Spanish cohort and, then, to perform a meta-analysis that combined our data with those of previously published studies.

Methods

Case-Control Study

Participants

Subjects were recruited at the various Spanish hospitals forming the "Multicenter study of genetic factors in primary dystonia consortium" and signed an informed consent form. Our case-control study included 680 patients with adult-onset primary DT and 788 unrelated healthy control subjects. Of these patients, 483 had focal DT (252 BSP, 176 CD, 26 writer's cramp, and 29 with other affected sites), 111 segmental DT, 17 multifocal DT, 16 generalized DT, and 52 dystonic tremor. Primary DT was diagnosed by senior neurologists, using accepted clinical criteria agreed on beforehand. The selection of control subjects was clinic-based, and absence of any neurological disease was an essential inclusion requirement. The study was approved by the ethics committees of all participating centers.

Genetic Analysis

Genomic DNA was isolated from the peripheral blood of each subject by automated methods (Maxwell 16 System, Promega Corporation, Madison, WI, USA; MagNA Pure LC, Roche Diagnostics, Indianapolis, IN, USA).

The genotyping was carried out using a TaqMan SNP Genotyping Assay (Applied Biosciences Hispania, Alcobendas, Madrid, Spain) performed in a LightCycler480 instrument (Roche) with a high genotyping quality. Of all genotyping, 3.4% was duplicated to analyze the concordance.

Statistical Analysis

All association analyses of the BDNF Val66Met polymorphism and primary DT, CD, and BSP risk in our case-control study were carried out with PLINK software.[13] Hardy-Weinberg equilibrium in the control group and allelic associations between cases and controls were tested by χ^2-test ($P < 0.05$ was considered significant).

Meta-analysis

Despite the relatively low number of primary DT patients included in the published studies, we conducted a meta-analysis using our data and previously published data on the implication of the BDNF Val66Met polymorphism in primary DT or in CD and BSP. We searched for articles using the PubMed database and included studies published before 15 January 2014. Studies were selected if they reported genotype distribution in case and control groups and, if applicable, genotype distribution in the subgroup of cases with CD and BSP.

Data Extraction

The following data were collected from each study: authors, year of publication, sample collection region, ethnicity, sample size of cases and controls, sex, age range, diagnosis, genotype, and allele frequency (Supplemental Data Table S1).

Data Analysis

All associations were indicated as odds ratio (OR) with the corresponding 95% confidence interval (CI). The meta-analysis was conducted using the R-package "meta" (cran.r-project.org/web/packages/meta/index.html). Heterogeneity between the studies was assessed using the Q-statistic, and its derived metric I^2. When no heterogeneity was observed ($P_Q > 0.10$ and $I^2 < 50\%$), the pooled OR was estimated using a fixed effects model.[14] Otherwise, a random effects model was applied.[15] We pooled the allelic ORs using the Mantel-Haenszel method, and the significance of the overall effect was assessed by Z test ($P < 0.05$

*Correspondence to: Dr. Pablo Mir, Unidad de Trastornos del Movimiento, Servicio de Neurología y Neurofisiología Clínica, Hospital Universitario Virgen del Rocío, Av. Manuel Siurot s/n. 41013, Seville, Spain. E-mail: pmir@us.es

Funding agencies: This study was supported by grants from the Ministerio de Economía y Competitividad de España (SAF2007-60700), the Instituto de Salud Carlos III (CP08/00174, PI10/01674, PI13/01461), the Consejería de Economía, Innovación, Ciencia y Empresa de la Junta de Andalucía (CVI-02526, CTS-7685), the Consejería de Salud y Bienestar Social de la Junta de Andalucía (PI-0377/2007, PI-0741/2010, PI-0437-2012, PI-0471/2013), the Sociedad Andaluza de Neurología, the Fundación Alicia Koplowitz, the Fundación Mutua Madrileña and the Jaques and Gloria Gossweiler Foundation. Dr. Pilar Gómez-Garre was supported by the "Miguel Servet" program at the Instituto de Salud Carlos III.

Relevant conflicts of interest/financial disclosures: Nothing to report. Author roles may be found in the online version of this article.

†These authors contributed equally to the manuscript.

Received: 31 January 2014; **Revised:** 27 March 2014; **Accepted:** 8 May 2014

Published online 12 June 2014 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/mds.25938

TABLE 1. Demographic characteristics and distribution of the genetic frequencies for the population from Spain

		Demographic characteristics				Genotypes, *n* [%]				Allele A vs allele G	
		Sex (M/F)	Age (y)[a]	AO (y)[a]	*n*	AA	AG	GG	P_{geno}	OR [95 CI %]	$P_{allelic}$
Healthy controls		324/464	56 ± 15	-	788	42 [5.3]	264 [33.5]	482 [61.2]	-	-	-
Primary dystonia	Total	246/434	61 ± 15	48 ± 17	680	39 [5.7]	226 [33.2]	415 [61.0]	0.94	1.02 [0.85, 1.21]	0.86
	Cervical dystonia	65/111	55 ± 14	47 ± 15	176	9 [5.1]	59 [33.5]	108 [61.4]	0.99	0.99 [0.75, 1.31]	0.93
	Blepharospasm	70/182	68 ± 10	57 ± 11	252	12 [4.8]	94 [37.3]	146 [57.9]	0.53	1.08 [0.85, 1.37]	0.53
	Other subtypes	111/141	57 ± 16	42 ± 19	252	18 [7.1]	73 [29.0]	161 [63.9]	NC	NC	NC

AO, age at onset; *n*, number of samples; OR, odds ratio; CI, confidence interval; NC, not calculated; y, years; P_{geno}, genotypic *P* value.
[a]Data are presented as the mean ± standard deviation.

was considered significant). Studies were further grouped in the meta-analysis by ethnicity to look for possible population stratification effects. Finally, we carried out further meta-analyses for dominant and recessive inheritance models.

Results

Case-Control Study

The BDNF Val66Met polymorphism was genotyped in 680 patients with primary DT and 788 healthy controls. The main demographic and genotypic features of cases and controls are summarized in Table 1.

The distributions of the allele frequencies in controls were in harmony with the Hardy-Weinberg equilibrium. Primary DT and control subjects showed a similar distribution of genotypes and alleles. When CD and BSP subgroups versus controls group were analyzed, similar results were obtained for both genotype distribution and allele frequencies.

Meta-analysis

In addition to our study, six other articles were included in the meta-analyses of primary DT. Two of these six articles reported only series of CD patients, but patients entered the meta-analysis of primary DT as well. Only three of the six studies contained a cohort of patients with BSP. Genotypic and allelic frequencies distributions for the polymorphism in each population are shown in Supplemental Data Tables S2 and S3.

Our meta-analysis consisted of 1,936 primary DT patients (1,091 CD, 271 BSP) and 2,519 healthy controls from seven different studies, comprising five Caucasian populations (four from previous studies and our case-control association study)[7-9,12] and two studies with samples of Asian origin.[10,11]

No heterogeneity was detected for allele contribution OR in the primary DT and CD meta-analyses under any genetic model. However, a certain degree of heterogeneity was noted when pooling across ethnicities in the BSP meta-analysis.

Results of meta-analysis for primary DT, CD, and BSP under allelic, dominant, and recessive genetic models are summarized in Table 2.

The pooling of the allelic ORs across the seven studies on primary DT showed no effect of the Met allele in the global population meta-analysis. We only found a significant contribution for the recessive model (OR = 1.32; CI, 1.06-1.65), and with an effect common for both populations ($P_{ethnicity} > 0.05$).

All analyses for CD were negative, and for BSP we observed that the significant contribution for the recessive model seen in Chen et al. was not replicated in the white populations.

The forest plots of the meta-analysis for the allelic model in all primary DT, CD, and BSP samples are shown in Supplemental Data Figures S1, S2, and S3, respectively.

Discussion

In a pioneer study, no association between the BDNF Val66Met polymorphism and cranial/CD was observed.[8] However, a subsequent study in samples from the United States suggested a role for this polymorphism in the pathogenesis of CD in some subjects.[7] These inconsistent results could in theory be explained by different inter-ethnicity genetic backgrounds. However, several other reports also failed to replicate this association in different populations,[9-12] including our case-control study. Moreover, the observed minor allele (A, Met) frequencies were very similar in all analyzed white populations, also supported by data in the International HapMap project (Release 28). Therefore, the positive result for the study with patients from the United States was probably attributable to a very small number of cases (n = 34), and the CI around the estimated OR was large.

Similarly, we did not observe an effect of Val66Met polymorphism on BSP risk, in contrast to a recently study performed in a Chinese population,[11] but in agreement with other studies in white populations.[8,12] Although this discrepancy might again be attributed to population differences, the positive finding was obtained once more from a small number of patients (n = 37) and with large CI around the estimated OR, whereas our negative results come from 252 patients with BSP. However, the observed minor allele frequencies of the polymorphism is higher in the reported Chinese populations than in the white populations.

TABLE 2. Pooled measures for the associations of BDNF Val66Met polymorphism with primary DT, CD and BSP

Population	Genetic model	Primary dystonia				Cervical dystonia				Blepharospasm			
		Pooled OR (95% CI)	*Z* score	*P*-value	$P_{ethnicity}$	Pooled OR (95% CI)	*Z* score	*P*-value	$P_{ethnicity}$	Pooled OR (95% CI)	*Z* score	*P*-value	$P_{ethnicity}$
Overall	Allelic	1.10 [0.99; 1.21]	1.85	0.06	0.32	1.09 [0.97; 1.23]	1.38	0.17	0.86	1.19 [0.83; 1.70]	0.96	0.34	0.01
	Dominant	1.06 [0.94; 1.21]	0.96	0.34	0.73	1.07 [0.91; 1.24]	0.82	0.41	0.82	1.18 [0.92; 1.50]	1.32	0.19	0.10
	Recessive	1.32 [1.06; 1.65]	2.44	0.01	0.43	1.07 [0.82; 1.40]	0.51	0.61	0.41	1.29 [0.53; 3.13]	0.56	0.57	0.01
Caucasian	Allelic	1.06 [0.95; 1.20]	1.05	0.29		1.08 [0.94; 1.25]	1.05	0.29		1.04 [0.84; 1.29]	0.39	0.70	
	Dominant	1.05 [0.92; 1.21]	0.72	0.47		1.08 [0.91; 1.28]	0.84	0.40		1.10 [0.86; 1.42]	0.75	0.45	
	Recessive	1.21 [0.88; 1.65]	1.19	0.23		1.21 [0.82; 1.79]	0.96	0.34		0.83 [0.45; 1.54]	0.58	0.56	
Asian	Allelic	1.19 [0.99; 1.44]	1.82	0.07		1.11 [0.89; 1.37]	0.91	0.36		2.07 [1.25; 3.44]	2.82	0.0048	
	Dominant	1.12 [0.82; 1.50]	0.72	0.47		1.03 [0.73; 1.46]	0.16	0.87		2.58 [0.96; 6.92]	1.88	0.06	
	Recessive	1.45 [1.05; 2.00]	2.26	0.024		0.97 [0.67; 1.40]	0.17	0.86		2.94 [1.39; 6.25]	2.81	0.0049	

BDNF, brain-derived neurotrophic factor; CD, cervical dystonia; BSP, blepharospasm; DT, dystonia; OR, odds ratio; CI, confidence interval; $P_{ethnicity}$, *P* value of the inter-ethnicity comparison.

Therefore, more well-designed association studies including a larger sample size from a Chinese population with BSP are required to further validate the findings in that population.

Our results from the meta-analysis indicated, based on the recessive model, the Val66Met polymorphism as a light risk allele in the overall population. However, when stratified by ethnicity, only the Chinese population showed a risk role for the polymorphism in primary DT. This might be partially explained because the genotypic prevalence of this polymorphism in the cohorts was higher in Chinese populations than in white populations. Hence, the analysis of the BSP subgroup showed an ethnic-specific risk association but with a significant heterogeneity among the studies. We conjecture that this could be attributable to many factors. First, the number of recruited studies and sample size in each study is relatively limited, mostly for the Chinese population. This fact did reach an extreme significance in the BSP analysis, where the total number of Chinese subjects included was very small and from only one study, but with a high contribution to positive findings from overall analyses. Furthermore, the existence of interstudy heterogeneity could be attributable to differences such as sample selection or different genotyping methods, which could not be matched well among studies.

In conclusion, our results showed that the BDNF Val66Met polymorphism is not a risk-conferring factor for the development of primary DT in white and Chinese populations. ■

Acknowledgments: We thank the patients and healthy individuals who participated in this study. We also thank Raquel Gómez, from the Genomic Service of the Instituto de Biomedicina de Sevilla (IBiS), for her technical support. This research has been conducted using samples from the Andalusian Public Health System Biobank (ISCIII-Red de Biobancos RD09/0076/00085).

References

1. Albanese A, Bhatia K, Bressman SB, et al. Phenomenology and classification of dystonia: a consensus update. Mov Disord 2013; 28:863-873.
2. Kojovic M, Parees I, Kassavetis P, et al. Secondary and primary dystonia: pathophysiological differences. Brain 2013;136:2038-2049.
3. Lu B, Nagappan G, Guan X, Nathan PJ, Wren P. BDNF-based synaptic repair as a disease-modifying strategy for neurodegenerative diseases. Nat Rev Neurosci 2013;14:401-416.
4. Kleim JA, Chan S, Pringle E, et al. BDNF val66met polymorphism is associated with modified experience-dependent plasticity in human motor cortex. Nat Neurosci 2006;9:735-737.
5. Cheeran B, Talelli P, Mori F, et al. A common polymorphism in the brain-derived neurotrophic factor gene (BDNF) modulates human cortical plasticity and the response to rTMS. J Physiol 2008;586:5717-5725.
6. Lee M, Kim SE, Kim WS, et al. Interaction of motor training and intermittent theta burst stimulation in modulating motor cortical plasticity: influence of BDNF Val66Met polymorphism. PLoS One 2013;8:e57690.
7. Cramer SC, Sampat A, Haske-Palomino M, Nguyen S, Procaccio V, Hermanowicz N. Increased prevalence of val(66)met BDNF genotype among subjects with cervical dystonia. Neurosci Lett 2010;468:42-45.
8. Martino D, Muglia M, Abbruzzese G, et al. Brain-derived neurotrophic factor and risk for primary adult-onset cranial-cervical dystonia. Eur J Neurol 2009;16:949-952.
9. Groen JL, Ritz K, Velseboer DC, et al. Association of BDNF Met66Met polymorphism with arm tremor in cervical dystonia. Mov Disord 2012;27:796-797.
10. Ma L, Chen Y, Wang L, et al. Brain-derived neurotrophic factor Val66Met polymorphism is not associated with primary dystonia in a Chinese population. Neurosci Lett 2013;533:100-103.
11. Chen Y, Song W, Yang J, et al. Association of the Val66Met polymorphism of the BDNF gene with primary cranial-cervical dystonia patients from South-west China. Parkinsonism Relat Disord 2013;19:1043-1045.
12. Svetel MV, Djuric G, Novakovic I, et al. A common polymorphism in the brain-derived neurotrophic factor gene in patients with adult-onset primary focal and segmental dystonia. Acta Neurol Belg 2013;113:243-245.
13. Purcell S, Neale B, Todd-Brown K, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 2007;81:559-575.
14. Mantel N, Haenszel W. Statistical aspects of the analysis of data from retrospective studies of disease. J Natl Cancer Inst 1959;22:719-748.
15. Laird NM, Mosteller F. Some statistical methods for combining experimental results. Int J Technol Assess Health Care 1990;6:5-30.

Supporting Data

Additional supporting information may be found in the online version of this article at the publisher's web-site.