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Tremor and Other Hyperkinetic Movements

Reviews

Risk Factor Genes in Patients with Dystonia: A Comprehensive Review

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Abstract

Background: Dystonia is a movement disorder with high heterogeneity regarding phenotypic appearance and etiology that occurs in both sporadic and familial forms. The etiology of the disease remains unknown. However, there is increasing evidence suggesting that a small number of gene alterations may lead to dystonia. Although pathogenic variants to the familial type of dystonia have been extensively reviewed and discussed, relatively little is known about the contribution of single-nucleotide polymorphisms (SNPs) to dystonia. This review focuses on the potential role of SNPs and other variants in dystonia susceptibility.

Methods: We searched the PubMed database for peer-reviewed articles published in English, from its inception through January 2018, that concerned human studies of dystonia and genetic variants. The following search terms were included: "dystonia" in combination with the following terms: 1) "polymorphisms" and 2) "SNPs" as free words.

Results: A total of 43 published studies regarding *TOR1A*, *BDNF*, *DRD5*, *APOE*, *ARSG*, *NALC*, *OR4X2*, *COL4A1*, *TH*, *DDC*, *DBH*, *MAO*, COMT, *DAT*, *GCH1*, *PRKRA*, *MR-1*, *SGCE*, *ATP1A3*, *TAF1*, *THAP1*, *GNAL*, *DRD2*, *HLA-DRB*, *CBS*, *MTHFR*, and *MS* genes, were included in the current review.

Discussion: To date, a few variants, which are possibly involved in several molecular pathways, have been related to dystonia. Large cohort studies are needed to determine robust associations between variants and dystonia with adjustment for other potential cofounders, in order to elucidate the pathogenic mechanisms of dystonia and the net effect of the genes.

Keywords: Dystonia, genetic polymorphism, single nucleotide polymorphism, variant, cervical dystonia, blepharospasm, movement disorders

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Introduction

Dystonia is a movement disorder with high heterogeneity regarding phenotypic appearance and etiology.[1] The prevalence of dystonia is estimated to be 16:100,000.[2,3] In 2013, a new general definition and classification of dystonia were introduced by an international panel of dystonia experts.[4] The two main axes of this classification are considered to be the etiology and the clinical features.[4] However, the pathophysiology and cause of most dystonia cases remain largely unknown.[5]

A polymorphism is a variation in the DNA sequence that occurs in a population with a frequency of 1 % or higher.[6,7] When a variation occurs in a single nucleotide, at a specific position in the genome, it is called an SNP (single-nucleotide polymorphism).[8,9] SNPs can occur within coding sequences of genes, non-coding sequences, introns, or the regions between genes (also known as intergenic regions).[10,11] An SNP across a coding sequence of a gene can be characterized as synonymous (when the protein sequence is not affected) and non-synonymous (when the amino acid sequence of the protein is altered).[10,12] The non-synonymous SNPs are divided into missense (when they result in a different amino acid) and nonsense (when they result in a premature stop codon).[10,12] Recently, it has been recommended that both terms, "mutation" and "polymorphism", be replaced by the term "variant".[13,14] An additional modifier (e.g. pathogenic, benign) to the term "variant" should be used, in order for its pathogenic or benign effect to be declared.[13,14]

The importance of genetic factors was unambiguously demonstrated with the identification of causative pathogenic variants in monogenic cases of familial dystonia under the autosomal dominant, autosomal recessive, or X-linked mode of inheritance.[3] Furthermore, a few candidate gene association studies (CGASs) have suggested that the presence of specific genetic loci may confer susceptibility to dystonia.[15] Genetic variations may affect dystonia's phenotypic appearance, age at onset, and spread to adjacent body regions, and may also affect the penetrance of other pathogenic variants suspected for dystonia.[15]

Previous reviews have mainly discussed the genetics of dystonia in general its monogenic forms and its phenotypic divergence.[3,16–25] However, genetics of sporadic forms of dystonia with no clearly discernible family history, and results from case–control studies are relatively rarely discussed.[15,18,25] Therefore, in the present review article, we discuss the current state of knowledge regarding genetics of dystonia, by emphasizing the CGASs that have linked single nucleotide polymorphisms and variants across genes that predispose to dystonia. Owing to the lack of a widely accepted nomenclature gene classification system for dystonia, we have used gene names for loci identification.[26] The main aim of the current comprehensive review is to shed some light on which polymorphisms predispose for dystonia, and to what extent.

Methods: study identification and selection

In order for any potentially relevant study to be identified, we searched through the Pubmed database (https://www.ncbi.nlm.nih.gov/pubmed) for peer-reviewed articles published in English, from its inception to January 2018, that concerned human studies of dystonia and genetic polymorphisms. The following search terms were included: "dystonia" in combination with 1) "polymorphisms" and 2) "SNPs" as free words. The complete search algorithm is available in the S1 Appendix. The last literature search was performed on February 20, 2018. Additionally, reference lists of retrieved articles were examined in order to identify missing from the initial database search results. The flowchart presenting the selection procedure of the studies is presented in Figure 1. Published studies between 1996 and 2017 were included.

The following data were extracted from each study, when possible: author, year of publication, ethnicity of the studied population, numbers of cases and controls, age at disease onset, mean age and gender distribution, tested variants, family history of the participants, screening or not for the *TOR1A* ΔGAG pathogenic variant, correction for multiple comparisons, assessment of Hardy–Weinberg equilibrium, and the tested dystonia phenotypes.

Results and discussion

Published studies between August 2001 and September 2017 were included. Baseline characteristics from studies regarding *TOR1A*, *BDNF*, *DRD5*, *APOE*, *ARSG*, *NALC*, *OR4X2*, *COL4A1*, *TH*, *DDC*, *DBH*, *MAO*, *COMT*, *DAT*, *GCH1*, *PRKRA*, *MR-1*, *SGCE*, *ATP1A3*, *TAF1*, *THAP1*, *GNAL*, *DRD2*, *HLA-DRB*, *CBS*, *MTHFR*, and *MS* are presented in Supplementary Tables 1–5. GnomAD frequencies (http://gnomad.broadinstitute.org/) and the type of individual variants are available at the S2 Appendix.

TOR1A

The *TOR1A* gene is a five-exon gene that covers an 11-kb region in chromosome 9. The TOR1A protein, called TorsinA, belongs to the family of the AAA+ ATPases. It can be found in the endoplasmic reticulum and the nuclear envelope of most cells,[8] including those of the central nervous system.[18] The function of TorsinA and how *TOR1A* gene pathogenic variants lead to dystonia remains largely unknown.[27] TorsinA acts mainly as a molecular chaperone.[28] The molecular and cellular processes in which TorsinA is involved include the interactions between cytoskeleton and membrane, important functions of the endoplasmic reticulum and the nuclear envelope, and the regulation of cellular lipid metabolism.[18,29–31] It is known that TorsinA needs to bind to TOR1AIP2 (Torsin 1A Interacting Protein 2) or to Heat Shock Protein Family A (Hsp70) Member 8 (HSPA8) in order to be activated,[32] a procedure that is impaired by the GAG deletion, as has been confirmed by crystallography.[33,34]

TOR1A remains the most extensively studied gene in both monogenic and sporadic forms of dystonia.[15] However, results from case–control studies yielded conflicting results, with the association being affected by body distribution, ethnicity, and other phenotypic manifestations. A number of case–control studies have been conducted so far[35–52] and quite a few *TOR1A* variants have also been investigated (rs1801968, rs2296793, rs1182, rs3842225, rs13283584, rs11787741, rs13297609, rs2287367, rs1043186, and rs35153737). Apart from case–control studies, a number of variants have been identified through mutational screening (rs766483672, rs80358233, rs75881350, rs1183, rs563498119, rs573629050, rs1045441, rs144572721).[53] Additionally, three meta-analyses have been conducted so far examining the effects of *TOR1A* gene variants on dystonia.[15,39,40] The most recent evidence stemming from a meta-analysis, reveals a significant association of the rs1182 (allele frequency [AF]=0.1666) and the rs1801968 (AF=0.1236 for the G allele and AF=4.061e-6 for the C allele) *TOR1A* variants with the development of focal dystonia (FD) and writer's cramp (WC) respectively.[15] Moreover, variants within 3′-UTR (untranslated region) encoded by exon 5 represent an additional functional genetic locus of *TOR1A*, though it may be under synergistic action with other *TOR1A* genetic variants.[15] This comes in accordance with a recent case–control study, suggesting an association of the rs35153737 in the 3′-UTR of *TOR1A* with dystonia; a result, though, that has been attributed to functional variants that are in high linkage disequilibrium (LD).[52]

From a functional aspect, loci containing the aforementioned variants appear to have consequences; variants across exon 4 and 3′-UTR encoded by exon 5, in particular, appear to overall influence the function of the *TOR1A* gene.[15] More specifically, rs1801968 was confirmed to be associated with reduced penetrance of the ΔGAG pathogenic variant in humans.[54,55] Regarding the 3′-UTR of exon 5, there is only some indication that specific variants across this region may have some functional consequences under synergistic action.[15,52] Interestingly, based on the results regarding frequencies, computational analyses and function experiments, rs563498119 in the 3′-UTR of *TOR1A* was reported to change the expression of the *TOR1A* gene.[53]

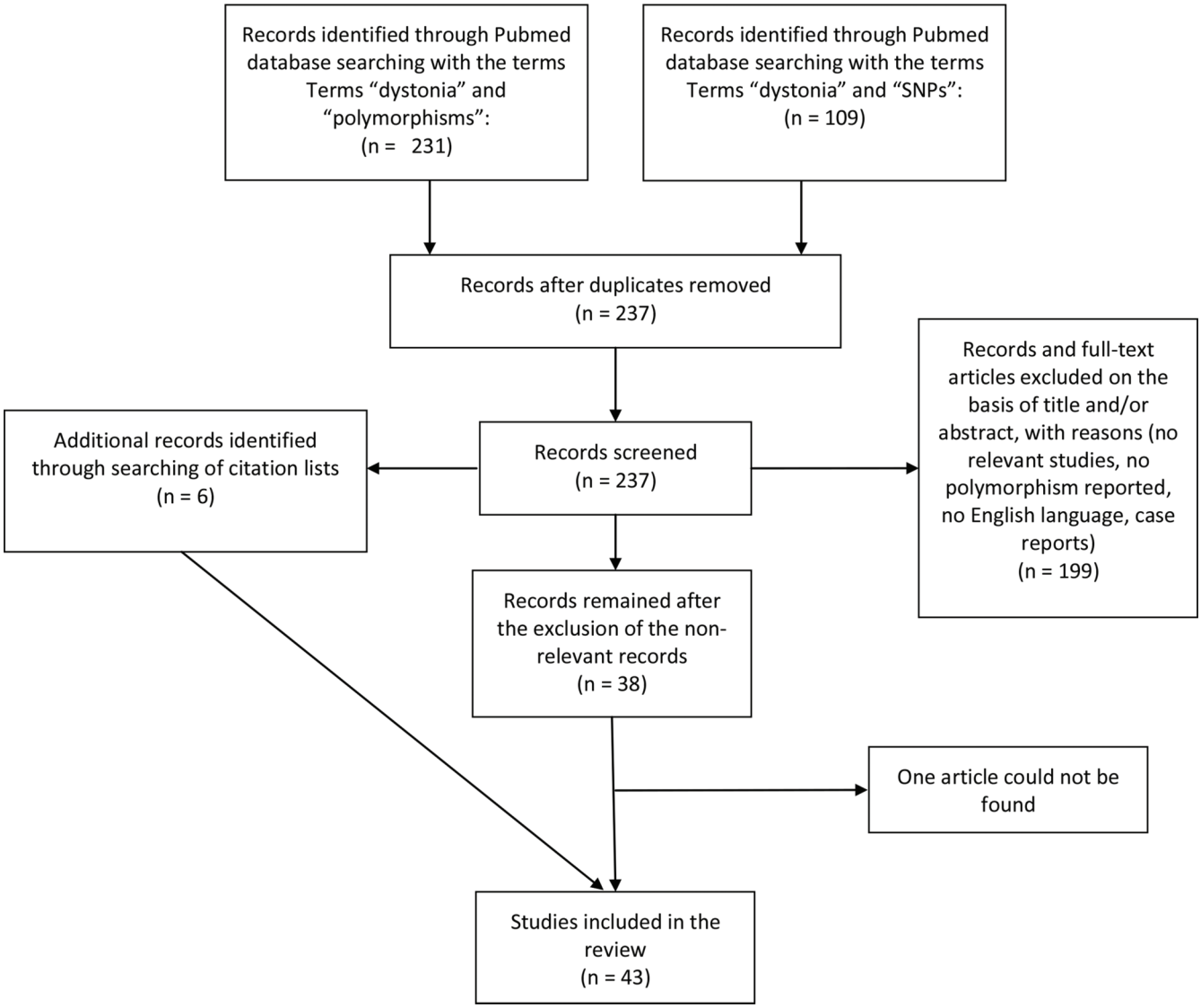

Figure 1. Flow chart presenting the selection of the studies included in the current review.

The regulation of *TOR1A* expression, by mutating the conserved region of the binding site of the human microRNA (hsa-miR-494), where rs563498119 is located, hints towards hsa-miR-494 being a possible therapeutic target.[53]

BDNF and *APOE*

Among the major mechanisms in dystonia, the reduced inhibition of the motor system and the increased plasticity are included.[56] In greater detail, increased plasticity in the hand representation area of the motor cortex has been observed in focal hand dystonia, blepharospasm (BSP), and cervical dystonia (CD) using high-resolution transcranial stimulation.[57] Consequently, abnormal plasticity within certain motor cortical circuits may represent a lineament of adult-onset dystonia forms.[57,58]

Synaptic plasticity is influenced by the brain-derived neurotrophic factor (BDNF). A common SNP across the *BDNF* gene within the prodomain region is the rs6265 (G/A) (AF=0.1896) and it results in the substitution of Val in amino acid position 66 with Met (Val→Met), which may influence synaptic plasticity[59–61] and is possibly involved in dystonia development. Healthy carriers of the val66met appear to have differences in brain structure and abnormal motor cortex plasticity as well.[62,63] Rs6265 has been found to be associated with quite a few diseases such as Parkinson's disease, Alzheimer disease (AD), schizophrenia, bipolar disease, depressive disorder, and panic disorders, although strong evidence has yet to be presented.[64–69]

The studies that have been conducted so far regarding the role of the rs6265 on dystonia have yielded conflicting results. More precisely, rs6265 has been reported to be associated with CD and BSP in

multiethnic and Chinese cohorts respectively.[70,71] Additionally, higher frequency of bilateral postural arm tremor in CD patients with the *BDNF* Met66Met variant than inVal66Met and Val66Val carriers has also been observed.[72] However, these results have not been replicated in Serbian, Chinese, Italian, or Caucasian dystonia cohorts.[58,73–75] To date, two meta-analyses have evaluated the effects of rs6265 variant on dystonia.[75,76] The most recent reports a statistically significant overall effect of the AA genotype on the development of idiopathic dystonia.[76] However, the lack of reproducibility of the positive results could be attributed, among others, to the culture of null hypothesis significance testing,[77] the possible influence of epigenetic mechanisms in the gene function (such as DNA methylation),[78] and to the fact that additional variants across the *BDNF* may regulate the level of serum BDNF and its function.[79] BDNF could be considered a potential therapeutic target in dystonia, as in neurological and psychiatric disorders.[80–82]

Apolipoprotein E is the product of the *APOE* gene, which connects to lipids in order to form lipoproteins. There are at least three alleles (e2, e3, and e4) of the *APOE* gene, with the commonest one being the e3. The main function of lipoproteins is to package cholesterol and other fats, and transport them throughout tissues including the central nervous system.[83] The e4 allele is associated with an increased risk of AD compared with the e3 allele, whereas the e2 allele is associated with decreased risk.[84] Like BDNF, APOE may also influence neural plasticity and remodeling.[71,85] In a Japanese cohort, E4 carriers were shown to develop dystonia on average approximately 10 years earlier than e4 non-carriers.[58] TorsinA is also involved in cellular lipid metabolism.[29] Therefore, variants that influence lipid biology may contribute to dystonia. Matsumoto et al.[85] suggested that the e4 allele may severely affect neuronal reorganization and this impairment of neuronal repair may contribute to an earlier age of dystonia onset. Consequently, it is possible that variants within *TOR1A*, *BDNF*, *APOE* or even other genes under synergistic action, influence the phenotypic manifestation of dystonia.

THAP 1

Around a hundred missense, nonsense, and frameshift pathogenic variants, throughout most part of the coding region of the "thanatos associated protein domain containing, apoptosis associated protein 1" (*THAP1*) gene, have been associated with dystonia[3,86,87] in a genetically diverse population.[18] The *THAP1* gene encodes the transcription factor THAP1, a zinc finger protein with an amino-terminal THAP domain, a proline-rich region, and a carboxy-terminal nuclear domain as well.[88] THAP1 is thought to regulate the transcription of several key genes, *TOR1A* included.[18,89]

Case–control studies regarding *THAP1* variants are limited[35,40] because of the variety and the rarity of *THAP1* variants. Therefore, most findings derive from mutational screening and the comparison between dystonia cases and healthy individuals.[86,87,90–96] However, there is an indication that the frequency of the C allele of the c.71+126T>C pathogenic variant was elevated in British dystonia patients.[90] -237_236GA>TT was also over-represented in dystonia when compared with controls in a European cohort[94] but these results could not be replicated.[90,91,97] Furthermore, the IVS2-87 A>G (rs11989331, AF=0.003428) was over-represented in dystonia in an Indian study.[95] The MAF of rs200209986 was also found to be significantly higher in dystonic patients (MAF=0.359%) than in the controls (MAF=0.0318%, $p<0.05$) in the Vemula et al.[96] study and the 1000 Genomes project (MAF=0.0916%, $p<0.05$), but not when compared with the EVS database (MAF=0.199%, $p=0.13$).

The large amount of *THAP1* pathogenic variants linked to dystonia may suggest an interplay between environmental and genetic factors.[98] Further, the type of work or the exposure to environmental factors, such as pesticides, may possibly predispose to dystonia development in pathogenic variant carriers.[21,86,87]

GNAL, TAF1, GCH1, MR-1 (PNKD), SGCE, ATP1A3, PRKRA, HLA-DRB, CBS, MTHFR, and MS

GNAL (guanine nucleotide-binding protein subunit alpha L) has been identified as responsible for adult-onset dystonia, which is primarily cervical or cranial.[99] A few *GNAL* variants (rs9303742, rs9675415, rs1895689, rs8095592, rs72865259, rs1647556, rs200508915, rs138151459, rs2071140, rs2071141, rs199571902) have been examined for association with generalized, multifocal, segmental, and focal dystonia.[100] Despite the fact that no strong evidence for association with dystonia was found, novel variants are constantly reported in single dystonia patients with various phenotypes,[100] leading to approximately 30 different *GNAL* variants in dystonia patients.[3] *GNAL* encodes guanine nucleotide-binding protein G subunit alpha [Gα(olf)]. Gα(olf)is involved in both the direct and indirect pathway to the activation of adenylate cyclase, by coupling dopamine type 1 receptors and the adenosine A2A receptors in medium spiny neurons, respectively.[99] In fact, the involvement in the indirect pathway of the activation leads to the activation of adenylyl cyclase type 5 (AC5). AC5 is encoded by the adenylyl cyclase 5 (*ADCY5*) gene, which was recently reported to be a co-founder of dystonia.[101] It is possible that epistasis phenomenon with *ADCY5* influences the causative effect of *GNAL* variants.

Newman at al.[40] in 2012, apart from *TOR1A* and *THAP1*, which are described in the above sections, genotyped several variants of other genes as well (*TAF1*, *GCH1*, *MR-1* (*PNKD*), *SGCE*, *ATP1A3*, and *PRKRA*).[40] The results were negative regarding quite a few variants across *TAF1*, *MR-1* (*PNKD*), *SGCE*, *ATP1A3*, and *PRKRA*, yet weak associations were observed for the rs12147422 (AF=0.217), rs3759664 (AF=0.2353), and rs10483639 (AF=0.2539) of *GCH1* (GTP cyclohydrolase 1) variants when the entire, non-homogeneous phenotypic, dystonia group was compared with controls. The lack of reproducibility of these associations could be explained by the low prevalence of dystonia, suggesting the need of collaborative studies.[102] Nevertheless, *GCH1* belongs to the confirmed causative genes of dopa-responsive dystonia. Additionally, the penetrance of GCH1 pathogenic variants appears to be considerably higher in females than in males.[103] The *GCH1* gene encodes the rate-limiting enzyme in the biosynthesis of dopamine via the biopterin pathway. GTP cyclohydrolase1 is involved in tetrahydrobiopterin neo-synthesis from GTP, as it catalyzes the first step of this reaction.[18] Variants of *GHC1* influence enzyme activity,

leading to a deficiency in dopamine and serotonin.[104] Therefore, a possible role of *GCH1* in non-monogenic forms of dystonia should not be dismissed, as scientific reasoning could not be substituted by statistical analysis.[105]

Finally there is no strong evidence for the association between *HLA-DRB* variants or variants in the homocysteine pathway (cystathionine β-synthase [*CBS*], methionine tetrahydrofolate reductase [*MTHFR*], methionine synthase [*MS*] genes) with dystonia.[50]

Dopamine pathway genes *(DAT1, DRD1, DRD2, DRD3, DRD4, DRD5, COMT, DAT, TH, MAO-A* and *-B, DDC,* and *DBH)*

Dystonic movements are considered the result of impaired function and abnormalities of dopaminergic neurotransmission and signaling in the basal ganglia.[106] The involvement of the dopaminergic system in the pathophysiology of dystonia has also been enhanced via the mutated genes of the dopamine pathway in monogenic forms of dystonia (*GCH1*).[107] Allele 2 of the *DRD5* has been associated with increased risk of CD and BSP in British cohorts.[108,109] Allele 6 and allele 4 of the *DRD5* have been associated with CD in British and Italian cohorts respectively,[109,110] thus strongly supporting the involvement of the *DRD5* gene in dystonia.[111] However, DRD5 has not been associated with dystonia in Italian, US, and German studies.[49,50] Dopamine receptor genes regulate neurotransmission in response to dopamine.[112] Dopamine receptors are divided into two families, based on either the activation (D1-like receptors: DRD1 and DRD5) or the inhibition (D2-like receptors: DRD2, DRD3, and DRD4) of adenylate cyclase.[113] Although the negative results in genes of dopamine signaling pathway (dopamine receptors) are few,[49,114,115] Groen et al.[115] suggested that changes in dopamine levels may be secondary during the dystonia course and that rare single nucleotide variants of dopamine genes are possibly associated with dystonia.[116]

ARSG, NALC, OR4X2, COL4A1

To date, only two genome-wide association studies (GWASs) have been performed in order to identify variants that may predispose to dystonia.[117,118] According to their results, there is a preliminary indication that arylsulfatase G (*ARSG*) and sodium leak channel (*NALCN*) variants play that role.[117,118]

In a GWAS executed by Lohmann et al.,[117] it was suggested that the intronic rs11655081 (AF=0.181) of the *ARSG* gene was associated with musician's dystonia and writer's cramp. The missense rs61999318 (AF=0.002619) was significantly higher in the group of writer's cramp patients than in European Americans in the EVS database ($p=0.0013$).[119] Functional analysis suggested that rs61999318 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.[119] ARSG is the protein encoded by *ARSG*; it hydrolyzes sulfate esters and is therefore implicated in cell signaling, synthesis of hormones, and protein degradation.[120] Moreover, it may be involved in neuronal ceroid lipofuscinosis,[121] which can present itself as dystonia.[117] In view of the former considerations, *ARSG* could be targeted as a gene for further study mainly in task-specific dystonias.

According to the GWAS from Mok et al.,[118] the cluster of variants near exon 1 of *NALCN* was found nearest to the significance threshold in a British population with CD. The most statistically significant variants were *NALCN* (rs61973742, rs1338051, rs9518385, rs9518384, rs1338041 rs3916908), *COL4A1* (rs619152), *RGL1* (rs12132318), *OR4X2 3* (rs67863238), intergenic (rs1249277, rs1249281, rs9416795), *KIAA1715* (rs10930717), *OR4B1* (rs35875350).[118] However, a replication of this GWAS case–control study did not report any association of *NALCN*, *OR4X2*, *COL4A1*, and intergenic variants,[122] and the results for *NALC* (rs1338041, rs61973742) were also not reproduced in a Chinese population.[123] NALCN is a voltage-independent and cation-non-selective channel. Its main function is the leaky sodium transport across neuronal membranes and the regulation of neuronal excitability.[124] In general, variants in genes, whose protein also acts like an ion channel, are crucial components and may be additional factors for dystonia development.[117] *ANO3* is among the confirmed genes that cause a monogenic form of late-onset craniocervical dystonia, with a possible effect on the calcium-activated chloride channel.[3,125]

Concluding remarks

Genetic factors confer susceptibility to dystonia development. More precisely, based on our review, exon 4 and the 3′-UTR of exon 5 represent loci that appear to have a strong influence on the function of the *TOR1A* gene, and their pathogenic variants may be associated with sporadic forms of dystonia, specifically with focal distribution. Moreover, rs6265 of *BDNF* appears to be strongly associated with dystonia as well. As the function of the *BDNF* gene may be influenced by other variants, additional loci across it may be worth examining. Further analysis of the *ARSG* gene, notably the rs61999318 in focal task-specific dystonia cohorts and the *DRD5* gene in focal dystonia, is warranted. Additional studies of *GCH1* may be required. Owing to their rarity, *THAP1* gene variants are insecure targets for future case–control studies. The continuing identification of pathogenic variants that cause monogenic forms of dystonia will lead us to new possible targets for case–control studies.[1,3]

Next-generation sequencing has led to the identification of new dystonia genes on a monthly basis.[3,125,126] Therefore, a large amount of common and rare genetic variants that may predispose to dystonia have been identified. Also, a few identified variants may affect penetrance, age at onset, spread to adjacent body, or the phenotype of dystonia.[15] However, it is not prudent to assume that all these genes truly lead to dystonia, and therefore results need to be interpreted with caution. Therefore, applying the CGASs approach to next-generation data could possibly shed some light on the mechanisms of the complex traits.[127]

The understanding of the genetic basis of monogenic and sporadic forms of dystonia will permit the identification and deeper knowledge of dystonia's pathogenesis. This will provide physicians with more personalized tools to manage dystonia in the future, even from the time of diagnosis, and they may also be used for assessing the biological progression of the disease and guide the treatment decisions.[128] Implications, even at the DNA and/or RNA level, are already

considered as new possible targeted therapeutic approaches.[53,80] The stronger grasp on dystonia's genetic susceptibility will also improve genetic testing and counseling.

The lack of validation reproducibility of the positive results could be attributed to several factors; firstly, the culture of null hypothesis significance testing.[77] Moreover, low power CGASs because of relatively small sample sizes is a common phenomenon, as the effective population should ideally be very large (~10,000 individuals) in order for a modest genetic effect to be identified.[129] The interplay between environmental (e.g. pesticides)[86,98] and genetic factors, as well as among genetic factors,[1] may variably determine the penetrance of pathogenic variants and the phenotype.[18,20,86,98,130,131] Furthermore, the phenotypic divergence of dystonia and the possible classification bias should be considered, as the majority of the studies were performed before the new dystonia classification.[4,15] Finally, epigenetic mechanisms may represent an additional explanation for the lack of result validation.[78]

Therefore, it is of great necessity that more collaborative studies[132,133] with adjustment for other potential cofounders (e.g. gene–environment interactions with adjustment for pesticide exposure,[86] air pollution,[134] diseases of the anterior segment of the eye, preceded injury, trauma, surgical intervention or sore throat,[130] time spent handwriting per day and the writing time before dystonia onset,[135] genome methylation status) and a supportive functional analysis be conducted in the future. In this way, the pathogenic mechanisms of dystonia and the net effect of the genes could be elucidated and, consequently, the inherent limitations of association studies will be avoided.[136]

Certain limitations of the present review need to be acknowledged. Firstly, supportive data regarding functional analysis of variants would give more robustness to our conclusions. Moreover, we included relevant studies regardless of the sample power and without any prior quality assessment. Therefore, a possible confounding by population stratification or technical factors cannot totally be excluded. Finally, based on our search strategy procedure, it is possible that some eligible studies might not have been identified. However, this is an inherent limitation of such studies, and the inclusion of a large number of studies does not affect the major conclusions of our results.

We should bear in mind that positive results from genetic association studies require biological and functional evidence that the risk variant is actually involved in the pathophysiology and the pathogenesis of the relevant disease. Pathway-based analysis could facilitate more robust analysis even of GWAS and provide additional biological insights on the mechanisms of disease complex traits.[137,138] Therefore, the scientific reasoning could not be replaced by any single statistical value, index, or test.[105,139] As a consequence, by the correct interpretation of statistical values, the misinterpretation of results could be avoided.[140]

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