Mutational spectrum of *GNAL*, *THAP1* and *TOR1A* genes in isolated dystonia: study in a population from Spain and systematic literature review

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ABSTRACT

Objective. We aimed to investigate the prevalence of *TOR1A*, *GNAL* and *THAP1* variants as the cause of dystonia in a cohort of Spanish patients with isolated dystonia and in the literature.

Methods. A population of 2028 subjects (including 1053 patients with different subtypes of isolated dystonia and 975 healthy controls) from southern and central Spain was included. The genes *TOR1A*, *THAP1* and *GNAL* were screened using a combination of high resolution melting analysis and direct DNA resequencing. In addition, an extensive literature search to identify original articles (published before August 10th 2020) reporting mutations in *TOR1A*, *THAP1* or *GNAL* associated to dystonia was performed.

Results. Pathogenic or likely pathogenic variants in *TOR1A*, *THAP1* and *GNAL* were identified in 0.48%, 0.57%, and 0.29% of our patients, respectively. Five patients carried the variation p.Glu303del in *TOR1A*. A very rare variant in *GNAL* (p.Ser238Asn) was found as a putative risk factor for dystonia.

In the literature, variations in *TOR1A*, *THAP1* and *GNAL* accounted for about 6%, 1.8%, and 1.1% of published dystonia patients, respectively.

Conclusions There is a different genetic contribution to dystonia of these three genes in our patients (about 1.3% of patients) and in the literature (about 3.6% of patients), probably due the high proportion of adult-onset cases in our cohort. As regards age at onset, site of dystonia onset, and final distribution, in our population there is a clear differentiation between DYT-*TOR1A* and DYT-*GNAL*, with DYT-*THAP1* likely to be an intermediate phenotype.

INTRODUCTION

Dystonia (DT) is a movement disorder characterized by sustained or intermittent involuntary muscle contractions, frequently causing twisting and repetitive movements, abnormal postures or both.[1] Based on the etiology, it can be acquired, inherited or idiopathic. Regarding the associated features, isolated dystonia (formerly referred to as primary dystonia) refers to a disorder in which dystonia is the only neurologic sign. Therefore, this article is exclusively focused on isolated, inherited dystonia.

Although the molecular mechanisms underlying isolated dystonia are largely unknown, it is known that its pathogenesis is genetically heterogeneous. Indeed, several genes have been related to isolated focal/segmental dystonia. Causative mutations in three genes (*TOR1A*, *THAP1*, and *GNAL*) have been identified in patients from different populations.[2-4] Mutations in *TUBB4*, *CIZ1* and *ANO3* have been reported[5-7]. Pathogenic role of *TUBB4* and *CIZ1* variations in isolated dystonia is still under debate because they are very rare.[8,9] In the case of *ANO3,* many different rare variations have been identified but their pathogenicity is often not clear;[10] however the detection of de novo mutations in this gene provide increasing evidences for a pathogenic role of *ANO3*.[11] On the other hand, mutations in *COL6A3* and *HPCA* have been rarely associated with isolated dystonia.[12,13]

Therefore, three genes have been unequivocally established as related to autosomal dominant isolated dystonia: *TOR1A*, *THAP1* and *GNAL* (Table 1). Mutations in these genes are often present with incomplete penetrance. In addition, mutations in *THAP1* or *GNAL* can also be inherited in a recessive manner.[14]

The *TOR1A* gene is located on chromosome 9q34.1 and encodes the endoplasmic reticulum luminal protein Torsin A, an adenosine triphosphatase that participates in a range of cell activities. An in-frame three-nucleotide deletion in *TOR1A* (c.907-909delGAG; p.Glu303del) is the most common cause of an early-onset autosomal dominant form of dystonia called DYT-*TOR1A*,[2,15] which has been described as the most common and severe genetic form of isolated dystonia.[1] In addition, several studies have shown that some variants in this gene seem to influence the dystonia phenotype or confer susceptibility to developing dystonia.[16,17] Moreover, the phenotypic spectrum associated with *TOR1A* mutations has been expanded due to the description of biallelic *TOR1A* disease, characterized by severe arthrogryposis, developmental delay, strabismus and tremor.[18,19]

Next, the gene *THAP1* is located on chromosome 8p11.21 and encodes a DNA-binding transcription factor named THAP1 (Thanatos-associated domain containing apoptosis-associated protein 1), ubiquitously expressed. Numerous disease-determining variants in *THAP1* have been associated with adolescent or adult-onset dystonia called DYT-*THAP1* in patients from various ethnicities. DYT-*THAP1* presents an autosomal dominant inheritance with incomplete penetrance,[3] but apparently, recessively inherited mutations have also been described.[20,21] In addition, for reasons still unclear, DYT-*THAP1* appears to be slightly more prevalent in women than in men.

Finally, the gene *GNAL*, on chromosome 18p11.21, encodes the stimulatory alpha subunit (Gαolf) of a GTP-binding protein (G protein), important for dopamine D1 receptor function and olfactory signal transduction.[22] Gαolf is expressed in the olfactory epithelium and in the brain, especially motor regions previously associated with dystonia, such as the striatum.[4,23] In the brain, *GNAL* presents the alternative use of upstream promoters

and first exons, giving rise to two different proteins, the normal Gαolf (Isoform 1) and an extra-large variant known as XL-Golf (isoform 2).[24] A collection of variations in *GNAL* has been associated with a type of dystonia called DYT-*GNAL*, an adult-onset cranio-cervical dystonia.[23]

The prevalence of causative mutations in these three forms of inherited dystonia (DYT-*TOR1A*, DYT-*THAP1* and DYT-*GNAL*) varies with genetic background, which can have important implications in clinical investigation. The study of genes underlying these forms of isolated dystonia will lead to a better understanding of its pathophysiology. In this study we therefore aimed to investigate the prevalence of *TOR1A*, *GNAL* and *THAP1* variants as the cause of dystonia in a cohort of Spanish patients with isolated dystonia and in the literature.

SUBJECTS AND METHODS

Subjects

In this study, a total of 2028 subjects were recruited at the various Spanish hospitals, forming the "Multicenter study of genetic factors in primary dystonia consortium", including 1053 unrelated isolated dystonia patients and 975 healthy controls. A family history of dystonia was present in 4.84% of patients.

Isolated DT was diagnosed by senior neurologists using accepted clinical criteria agreed beforehand. Age at onset of dystonia, body distribution, disease course, and associated features were analyzed in each patient. The selection of controls was clinic-based since the subjects were either spouses of the patients or patients from other outpatient clinics at the same hospitals, and they did not have any neurologic disease. Demographic characteristics of subjects included in this study are summarized in Table 2.

The study was approved by the ethics committees of all participating centers and was conducted according to the principles expressed in the Declaration of Helsinki. All subjects, whether with isolated DT or controls, signed an informed consent form before blood withdrawal.

Genetic Analysis

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

Polymerase chain reaction (PCR) primer couples were designed on the basis of the known genomic sequences to cover completely the genes *TOR1A* (NM_000113.2; NP_000104.1), *THAP1* (NM_018105.2; NP_0600575.1), and *GNAL*. Therefore, our analysis included five exons of *TOR1A*, and three exons of *THAP1*. In addition, we took into account both *GNAL* isoforms expressed in the brain, the longest (NM_182978; NP_892023.1) and the major isoform (NM_001142339.2; NP_001135811.1). Because the major isoform differs from the longer transcript at exon 1, we included in our study 13 exons of *GNAL*. All primer pairs generated single amplicons that produced single well-defined bands on agarose gel electrophoresis. Furthermore, Sanger sequencing confirmed the identity of these bands.

To systematically test for sequence variants, we screened all coding exons, exon/intron boundaries, and the 5'and 3' UTR regions, using a combination of high resolution melting (HRM) analysis and subsequent direct DNA resequencing. HRM reactions were performed on a LightCycler480 (LC480) instrument using the LC480 HRM Master Mix (Roche Applied Science, Indianapolis, IN, USA). HRM curve acquisition and analysis were

performed on all samples using the LC480 software version 1.3. As an internal control, we duplicated 21.85% of the samples. Subsequently, samples showing abnormal melting profiles were analyzed using a new PCR product and sequenced on both strands using the BigDye terminator cycle sequencing kit (Applied Biosystems, Foster City, CA, USA) and subsequently resolved on an ABI3500 genetic analyzer (Applied Biosystems). Sequences were analyzed using VariantReporter and SeqScape softwares (Applied Biosystems).

Bioinformatic analysis

Because DYT-*TOR1A*, DYT-*THAP1* and DYT-*GNAL* are rare conditions, we focused our analyses on rare sequence variants. The population frequencies were obtained from the Trans-Omics for Precision Medicine program (TOPMed, https://www.nhlbi.nih.gov/science/trans-omics-precision-medicine-topmed-program), the Genome Aggregation Database (gnomAD, https://gnomad.broadinstitute.org/), and the 1000 genomes phase 3 (https://www.internationalgenome.org/category/phase-3/).

The potential pathogenicity of a variant was assessed according to the recommendations of the American College of Medical Genetics and Genomics (ACMG) consensus[25] using VarSome.[26] In addition, we also assessed the clinical significance reported in ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), Rare Exome Variant Ensemble Leaner (REVEL)[27], and ClinPred (https://sites.google.com/site/clinpred/).[28] Variants were considered likely to be disease-relevant in the presence of: interpretation of ACMG as pathogenic, or likely pathogenic, and interpretation of ACMG as VOUS with REVEL and ClinPred scores >0.5.

Statistical analysis

All association analyses of the variants and isolated dystonia, cervical dystonia, and blepharospasm risk in our case-control study were carried out with PLINK software.[29] Genotypes in the control group were first assessed for departures from the Hardy-Weinberg equilibrium using the chi-square test and using a cut off p-value of 0.01. Allelic associations between categorical groups were tested using the chi-square test and genotype-specific risks were estimated by calculating odds ratios (OR) and 95% confidence intervals. P values of <0.05 were considered statistically significant.

Literature review

We performed a detailed and systematic search for articles using the PubMed database and using the key words of *GNAL*, *TOR1A*, *THAP1*, dystonia, isolated dystonia, genetics and mutation, in various combinations. Only articles fully available online (published before August 10th 2020) were included and manually reviewed to get the available clinical and molecular information on dystonia and mutations. Single case reports were excluded when no novel mutation was reported.

RESULTS

In our population, a total of 55 variants were detected: 19 variants in *TOR1A*, 12 in *THAP1*, and 25 in *GNAL* (Table e-1). However, only fourteen patients carried pathogenic or likely pathogenic variants. In addition, we reviewed the literature, and 139 studies about these genes and their relationship with isolated dystonia were retrieved. The study selection process is described in Figure 1.

Analysis of *TOR1A*

We detected a total of 19 variants across the *TOR1A* sequence (Table e-1), including 8 coding variants (3 synonymous and 5 non-synonymous) and 9 non-coding variants. All previously described variants were detected in our cohort at frequencies comparable to those in databases. Only one variant (c.369T>G; p.Gly123Gly) was not previously reported.

Four variants were present only in DT patients. Indeed, five DT patients (prevalence = 0.48%) presented the recurrent 3bp deletion (c.904_906delGAG; p.Glu303del), the most common pathogenic variation in *TOR1A*. The other three variants were synonymous (p.Leu136Leu) or non-coding (c.*10T>C, and c.*672_*673insC), but our analysis suggested they were not responsible of the dystonia.

The intronic variant c.445-22G>A (rs10988526) was found in two controls and five patients with DT. However; this variation has been previously reported as benign and, in addition, the analysis of its putative effect on the splicing process was negative. Therefore, it was considered not responsible for the development of dystonia in the carriers.

Finally, because the frequent variants p.D216H (rs1801968) and c.*191G>T (rs1182) have been previously associated with the risk of dystonia, we performed an association analysis (adjusted for sex and age) for these two variations with the risk of isolated dystonia or specific types of dystonia (such as blepharospasm, or cervical dystonia) and no evidence of an association was observed.

Therefore, only the variation p.Glu303del has been considered unequivocally associated with the development of isolated dystonia in our population. Clinical characteristics of patients carrying this variant are shown in Table 3.

A comprehensive literature review of all reported *TOR1A* variations associated with dystonia was performed. In total, 22 variations have been published (Table e-2). However, only 10 are considered as pathogenic or likely pathogenic. Distribution of the variants on the gene and the protein are shown in Figure 2A and Figure 3A, respectively.

Analysis of *THAP1*

We detected a total of 12 variants across the *THAP1* sequence (Table e-1), 5 coding variants (1 nonsense and 4 non-synonymous) and 7 non-coding variants. Five variants were not previously reported, two in 5'-UTR (c.-211-205delGGAGATG and c.-57G>A), one in exon 1 (c.37C>G), and two in exon 3 (c.292G>T and c.605C>T).

All previously reported variants were detected in our cohort at frequencies comparable to those in databases.

Each of the genetic variants c.-6G>A, p.Arg13Gly, p.Arg29Gln and Pro202Leu was found in just one patient with dystonia. The genetic variant p.Glu98Ter (c.292G>T) was found in 3 DT patients (0.29 %) and not previously described.

Taking into account the bioinformatic data (Table e-1), we considered only four variants (p.Arg13Gly, p.Arg29Gln, p.Glu98Ter, and Pro202Leu) as causative of the development of DT in our population, carried by a total of 6 DT patients (prevalence = 0.57%). Clinical characteristics of patients carrying these variants are shown in Table 3.

A comprehensive literature review of all reported *THAP1* variations associated with dystonia was performed, finding that 104 variations have been published (Table e-3). Taking into account these variants and

those from our study, this results in a total of 108 variants, of which 96 have been considered as pathogenic or likely pathogenic. Their distributions on the gene and the protein are shown in Figures 2B and 3B, respectively.

Analysis of *GNAL*

We detected a total of 26 variants across the *GNAL* sequence (Table e-1), including 12 coding variants (1 insertion, 5 synonymous and 6 non-synonymous) and 14 non-coding variants (6 in 5'-UTR, 6 intronic, and 2 in 3'-UTR). Three variants were not previously reported, one in 5'-UTR (c.-308G>A), one in intron 6 (c.722+31C>A), and one in exon 8 (c.828C>A).

Three variations (p.Glu155lys, p.Phe199Leu, and p.Arg367Cys) were predicted as pathogenic in the bioinformatics analysis (Table e-1), all of them carried by only 1 DT patient (prevalence = 0.29%). Clinical characteristics of patients carrying these variants are shown in Table 3.

All previously reported variants were detected in our cohort at frequencies comparable to those in databases, with the exception of rs774061970. This rare variation causes a likely pathogenic missense change p.Ser238Asn. The association study showed that it was in HWE in the control group. Minor allele frequency was higher in DT patients (OR=3.38 (95% CI 1.25-9.13); p=0.011)

A comprehensive literature review of all reported *GNAL* variations associated with dystonia was performed. Thirty-six variations were described (Table e-4). Taking into account these variants and those from our study makes a total of 38 variants, of which 33 have been considered as pathogenic or likely pathogenic. Their distribution on the gene and the protein are shown in Figure 2C and Figure 3C, respectively.

DISCUSSION

To date, an increasing number of articles have reported variants in *TOR1A*, *THAP1* and *GNAL* associated with isolated dystonia. This work represents a systematic synopsis of the implication of variants in *TOR1A*, *THAP1* and *GNAL* genes in isolated dystonia. Overall, we present the results of the analysis of these three genes in 2028 subjects (including 1053 patients with various subtypes of isolated dystonia) from our southern population and a comprehensive analysis of the current state of knowledge about their contribution to the etiopathogenesis of isolated dystonia.

The results of our cohort are compatible with those of published studies, with a higher percentage of women with isolated dystonia than men[30], and the *TOR1A* gene being more frequently mutated.

Five unrelated patients in our cohort (0.48% of patients) presented the pathogenic three-base pair deletion in *TOR1A*, with mean age at onset of 22.6 years (range 10-43), with symptoms starting in limbs and with progression to generalized or multifocal dystonia. Interestingly, one patient started with cervical dystonia. In *TOR1A* cases, the onset of the symptoms usually spares bulbar and craniocervical muscles. However, in some late-onset cases, facial and cervical involvement has been reported at disease onset, including patients from the same families.[31]

Variations in *TOR1A* account for about 6% of published dystonia patients with a total of 23 variants in *TOR1A* (mainly in exon 5), but only half (11) seem to have a pathogenic role in dystonia. This does not mean that the other variants have no effect on dystonia; they may be modifier factors or undergo synergistic action with other variants. However, this should be interpreted with caution for several reasons. *TOR1A* is the most

frequently studied gene, but a number of studies have investigated only exon 5 (where the tri-nucleotide deletion is located). This could be the reason why most variants in *TOR1A* have been found in this exon. Moreover, several studies are case reports. Other studies refer to the p.D216H variation (that seems to be not pathogenic but a modifier factor) or variants within 3'-UTR (untranslated region). In addition, it is important to note that several studies did not use a control cohort. Hence, results yielded controversial results, probably due to the aforementioned factors and others such as ethnicity or body distribution (Figure e-1).

In our cohort no evidence of an association was observed for rs1182 or rs1801968 variants (previously associated with focal dystonia and writer's cramp, respectively)[17] with the development of isolated dystonia nor, more specifically, focal dystonia, cervical dystonia or blepharospasm. However, this result should be interpreted with caution owing to our small sample sizes. Indeed, for this reason we could not perform an analysis on an association with writer's cramp.

Regarding the *THAP1* gene, pathogenic variants were found in 6 dystonia patients in our cohort. The age at disease onset was 38.2 years (range 15-69), with 50% of early-onset cases as the most frequent presentation. The dystonia patterns involved craniocervical regions or limbs in most patients at onset. However, in two cases, a larynx or orofacial distribution was noticed at presentation. These peculiar features have also been reported in other cohorts, expanding the clinical picture of DYT-*THAP1*.[32]

In *THAP1* there is no frequent variation such as the tri-nucleotide deletion in *TOR1A*. Conversely, a high variety of rare variants (108) have been observed in patients with dystonia, mostly with a pathogenic role in dystonia, throughout most of the coding region of the THAP1-domain. Therefore, about 1.8% of published patients in which this gene has been analyzed presented a variant in this gene. In contrast, the frequency of pathogenic or likely pathogenic variants in *THAP1* in our cohort (0.57% of patients) has been very similar to that found in *TOR1A*. This could be because we found three patients in our cohort with a nonsense variation not previously described, p.Glu98Ter. Due to the origin of subjects with this variant, the existence of a founder effect may be possible. However, a limitation of our study was the impossibility of assessing the haplotype around this variation in order to test the existence of this effect.

Variants in *GNAL* have been observed in about 1.1% of published individuals with dystonia. In total, 37 variants have been found in *GNAL* in individuals with dystonia, mainly pathogenic, and are spread over the entire gene. However, it is important to note that this gene has been much less studied (in terms of the origin of populations and number of patients with dystonia studied) than *TOR1A* or *THAP1*. Despite this, not unexpectedly, pathogenic variants in *GNAL* were infrequent in patients with dystonia from our cohort. Therefore, three dystonia patients in our cohort carried pathogenic variants in the *GNAL* gene, with a mean age at onset of 43 years (range 29-59) and with craniocervical dystonia, according to the previously described features.[33] In addition, we observed a likely pathogenic missense variant p.Ser238Asn (c.713G>A, rs774061970). This is a very rare variant which was present in 18 dystonia patients and 5 healthy controls in our population, supporting the idea that it could be a risk factor for dystonia in Spain.

We identified several pathogenic or likely pathogenic variants in *TOR1A*, *THAP1* and *GNAL*. Therefore, genetic contribution to dystonia of these three genes was about 1.3% in our cohort of patients with isolated dystonia, while in the literature this was about 3.6% of patients, with *TOR1A* having the largest impact.

However, it is important to note that this contribution can be disturbed because several factors: first, the majority of studies did not analyze promoter regions or large deletions; in addition, the potential role of *TOR1A* could be underestimated because screening from many studies was limited to exon 5 (Figure e-1); moreover, in our study the involvement of TOR1A may be undercalculated because in those familial cases we included only the proband member; finally, our population is mainly from adult centers (with a mean age of onset of 51.2 years), which could affect the diagnostic yield as it is higher in cases with an early onset.

Another important limitation of our study was that the sensitivity of high resolution melting (the screening technique used in our cohort) is not 100%; therefore, the prevalence of *TOR1A*, *THAP1* and *GNAL* in our population might be slightly underestimated.

Finally, it is important to note that in our study the reported family history is lower than in other cohorts. This fact could be partially explained by the low sensitivity in detecting family cases by clinical interview in focal dystonia.[34]

In summary, this study is the largest and most complete study of *TOR1A*, *THAP1* and *GNAL* genes in the Spanish population and a review of the recent genetic findings in these genes. Our data suggest that the incidence of pathogenic or likely pathogenic variants in *TOR1A*, *THAP1* and *GNAL* in our population is low. There is genetic heterogeneity that includes not only the *TOR1A* tri-nucleotide deletion but also a *THAP1* variant with a putative founder effect as well as other different variations in *THAP1* and *GNAL*. However, there is a relationship between phenotype and genotype. Therefore, in terms of age at onset, site of dystonia onset, and final distribution, there is a clear differentiation between DYT-*TOR1A* and DYT-*GNAL*, with DYT-*THAP1* resembling an intermediate phenotype.

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DATA AVAILABILITY STATEMENT

All data that supports the findings of this study are available in this article.

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FIGURE LEGENDS

Figure 1. Flow chart showing the literature search and the selection of studies included in the present review. In total, 2684 articles were identified, and 1867 duplicate articles were removed. Then 678 additional articles were excluded on the basis of: no relevant studies, studies about genes other than *GNAL*, *TOR1A* or *THAP1*, no genetic variation reported, no English or Spanish language, no-peer-reviewed, or incorrect type of article (e.g., review, editorial, etc.). Full texts of four articles (published on 1976 and 1975) were not found. 139 studies providing information on *TOR1A*, *THAP1* and *GNAL* in patients with dystonia were retrieved. Of those, 75 studies provided original information on *TOR1A*, *THAP1* and *GNAL* variations in patients with dystonia. 132 studies (of those 139) were included for the study of the prevalence of variations related to dystonia in the three genes worldwide (including reported clinical histories, case reports and smaller case series, larger case series and case-control cohorts).

Figure 2. Schematic representations of exon-intron structure of *TOR1A*, *THAP1* and *GNAL* genes with all variations reported to date associated with dystonia indicated. Variations considered pathogenic or likely pathogenic are highlighted in red above. Variations found in our population are underlined. References for the first describers are given for each variation (in parentheses).

Figure 3. Schematic diagrams of TOR1A, THAP1 and GNAL proteins with all variations reported to date associated with dystonia indicated. Variations considered pathogenic or likely pathogenic are highlighted in red above. Variations found in our population are underlined. References for the first describers are given for each variation (in parentheses). Variants showed in this study are underlined. Functional domains are shown. SS: Signal sequence; HD: hydrophobic domain; W-A: Walker A motif; W-B: Walker B motif; APDB: ATP binding protein domain; SR1: Sensor 1; SR2: Sensor 2; TD: THAP1 domain; PRR: low-complexity Proline-Rich Region; CCD: Coiled-Coil Domain; NLS: Nuclear Localization Signal.

Mutational spectrum of *GNAL*, *THAP1* and *TOR1A* genes in isolated dystonia: study in a population from Spain and systematic literature review

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ABSTRACT

Objective. We aimed to investigate the prevalence of *TOR1A*, *GNAL* and *THAP1* variants as the cause of dystonia in a cohort of Spanish patients with isolated dystonia and in the literature.

Methods. A population of 2028 subjects (including 1053 patients with different subtypes of isolated dystonia and 975 healthy controls) from southern and central Spain was included. The genes *TOR1A*, *THAP1* and *GNAL* were screened using a combination of high resolution melting analysis and direct DNA resequencing. In addition, an extensive literature search to identify original articles (published before August 10th 2020) reporting mutations in *TOR1A*, *THAP1* or *GNAL* associated to dystonia was performed.

Results. Pathogenic or likely pathogenic variants in *TOR1A*, *THAP1* and *GNAL* were identified in 0.48%, 0.57%, and 0.29% of our patients, respectively. Five patients carried the variation p.Glu303del in *TOR1A*. A very rare variant in *GNAL* (p.Ser238Asn) was found as a putative risk factor for dystonia.

In the literature, variations in *TOR1A*, *THAP1* and *GNAL* accounted for about 6%, 1.8%, and 1.1% of published dystonia patients, respectively.

Conclusions There is a different genetic contribution to dystonia of these three genes in our patients (about 1.3% of patients) and in the literature (about 3.6% of patients), probably due the high proportion of adult-onset cases in our cohort. As regards age at onset, site of dystonia onset, and final distribution, in our population there is a clear differentiation between DYT-*TOR1A* and DYT-*GNAL*, with DYT-*THAP1* likely to be an intermediate phenotype.

INTRODUCTION

Dystonia (DT) is a movement disorder characterized by sustained or intermittent involuntary muscle contractions, frequently causing twisting and repetitive movements, abnormal postures or both.[1] Based on the etiology, it can be acquired, inherited or idiopathic. Regarding the associated features, isolated dystonia (formerly referred to as primary dystonia) refers to a disorder in which dystonia is the only neurologic sign. Therefore, this article is exclusively focused on isolated, inherited dystonia.

Although the molecular mechanisms underlying isolated dystonia are largely unknown, it is known that its pathogenesis is genetically heterogeneous. Indeed, several genes have been related to isolated focal/segmental dystonia. Causative mutations in three genes (*TOR1A*, *THAP1*, and *GNAL*) have been identified in patients from different populations.[2-4] Mutations in *TUBB4*, *CIZ1* and *ANO3* have been reported[5-7]. Pathogenic role of *TUBB4* and *CIZ1* variations in isolated dystonia is still under debate because they are very rare.[8,9] In the case of *ANO3,* many different rare variations have been identified but their pathogenicity is often not clear;[10] however the detection of de novo mutations in this gene provide increasing evidences for a pathogenic role of *ANO3*.[11] On the other hand, mutations in *COL6A3* and *HPCA* have been rarely associated with isolated dystonia.[12,13]

Therefore, three genes have been unequivocally established as related to autosomal dominant isolated dystonia: *TOR1A*, *THAP1* and *GNAL* (Table 1). Mutations in these genes are often present with incomplete penetrance. In addition, mutations in *THAP1* or *GNAL* can also be inherited in a recessive manner.[14]

The *TOR1A* gene is located on chromosome 9q34.1 and encodes the endoplasmic reticulum luminal protein Torsin A, an adenosine triphosphatase that participates in a range of cell activities. An in-frame three-nucleotide deletion in *TOR1A* (c.907-909delGAG; p.Glu303del) is the most common cause of an early-onset autosomal dominant form of dystonia called DYT-*TOR1A*,[2,15] which has been described as the most common and severe genetic form of isolated dystonia.[1] In addition, several studies have shown that some variants in this gene seem to influence the dystonia phenotype or confer susceptibility to developing dystonia.[16,17] Moreover, the phenotypic spectrum associated with *TOR1A* mutations has been expanded due to the description of biallelic *TOR1A* disease, characterized by severe arthrogryposis, developmental delay, strabismus and tremor.[18,19]

Next, the gene *THAP1* is located on chromosome 8p11.21 and encodes a DNA-binding transcription factor named THAP1 (Thanatos-associated domain containing apoptosis-associated protein 1), ubiquitously expressed. Numerous disease-determining variants in *THAP1* have been associated with adolescent or adult-onset dystonia called DYT-*THAP1* in patients from various ethnicities. DYT-*THAP1* presents an autosomal dominant inheritance with incomplete penetrance,[3] but apparently, recessively inherited mutations have also been described.[20,21] In addition, for reasons still unclear, DYT-*THAP1* appears to be slightly more prevalent in women than in men.

Finally, the gene *GNAL*, on chromosome 18p11.21, encodes the stimulatory alpha subunit (Gαolf) of a GTP-binding protein (G protein), important for dopamine D1 receptor function and olfactory signal transduction.[22] Gαolf is expressed in the olfactory epithelium and in the brain, especially motor regions previously associated with dystonia, such as the striatum.[4,23] In the brain, *GNAL* presents the alternative use of upstream promoters

and first exons, giving rise to two different proteins, the normal Gαolf (Isoform 1) and an extra-large variant known as XL-Golf (isoform 2).[24] A collection of variations in *GNAL* has been associated with a type of dystonia called DYT-*GNAL*, an adult-onset cranio-cervical dystonia.[23]

The prevalence of causative mutations in these three forms of inherited dystonia (DYT-*TOR1A*, DYT-*THAP1* and DYT-*GNAL*) varies with genetic background, which can have important implications in clinical investigation. The study of genes underlying these forms of isolated dystonia will lead to a better understanding of its pathophysiology. In this study we therefore aimed to investigate the prevalence of *TOR1A*, *GNAL* and *THAP1* variants as the cause of dystonia in a cohort of Spanish patients with isolated dystonia and in the literature.

SUBJECTS AND METHODS

Subjects

In this study, a total of 2028 subjects were recruited at the various Spanish hospitals, forming the "Multicenter study of genetic factors in primary dystonia consortium", including 1053 unrelated isolated dystonia patients and 975 healthy controls. A family history of dystonia was present in 4.84% of patients.

Isolated DT was diagnosed by senior neurologists using accepted clinical criteria agreed beforehand. Age at onset of dystonia, body distribution, disease course, and associated features were analyzed in each patient. The selection of controls was clinic-based since the subjects were either spouses of the patients or patients from other outpatient clinics at the same hospitals, and they did not have any neurologic disease. Demographic characteristics of subjects included in this study are summarized in Table 2.

The study was approved by the ethics committees of all participating centers and was conducted according to the principles expressed in the Declaration of Helsinki. All subjects, whether with isolated DT or controls, signed an informed consent form before blood withdrawal.

Genetic Analysis

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

Polymerase chain reaction (PCR) primer couples were designed on the basis of the known genomic sequences to cover completely the genes *TOR1A* (NM_000113.2; NP_000104.1), *THAP1* (NM_018105.2; NP_0600575.1), and *GNAL*. Therefore, our analysis included five exons of *TOR1A*, and three exons of *THAP1*. In addition, we took into account both *GNAL* isoforms expressed in the brain, the longest (NM_182978; NP_892023.1) and the major isoform (NM_001142339.2; NP_001135811.1). Because the major isoform differs from the longer transcript at exon 1, we included in our study 13 exons of *GNAL*. All primer pairs generated single amplicons that produced single well-defined bands on agarose gel electrophoresis. Furthermore, Sanger sequencing confirmed the identity of these bands.

To systematically test for sequence variants, we screened all coding exons, exon/intron boundaries, and the 5'and 3' UTR regions, using a combination of high resolution melting (HRM) analysis and subsequent direct DNA resequencing. HRM reactions were performed on a LightCycler480 (LC480) instrument using the LC480 HRM Master Mix (Roche Applied Science, Indianapolis, IN, USA). HRM curve acquisition and analysis were

performed on all samples using the LC480 software version 1.3. As an internal control, we duplicated 21.85% of the samples. Subsequently, samples showing abnormal melting profiles were analyzed using a new PCR product and sequenced on both strands using the BigDye terminator cycle sequencing kit (Applied Biosystems, Foster City, CA, USA) and subsequently resolved on an ABI3500 genetic analyzer (Applied Biosystems). Sequences were analyzed using VariantReporter and SeqScape softwares (Applied Biosystems).

Bioinformatic analysis

Because DYT-*TOR1A*, DYT-*THAP1* and DYT-*GNAL* are rare conditions, we focused our analyses on rare sequence variants. The population frequencies were obtained from the Trans-Omics for Precision Medicine program (TOPMed, https://www.nhlbi.nih.gov/science/trans-omics-precision-medicine-topmed-program), the Genome Aggregation Database (gnomAD, https://gnomad.broadinstitute.org/), and the 1000 genomes phase 3 (https://www.internationalgenome.org/category/phase-3/).

The potential pathogenicity of a variant was assessed according to the recommendations of the American College of Medical Genetics and Genomics (ACMG) consensus[25] using VarSome.[26] In addition, we also assessed the clinical significance reported in ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), Rare Exome Variant Ensemble Leaner (REVEL)[27], and ClinPred (https://sites.google.com/site/clinpred/).[28] Variants were considered likely to be disease-relevant in the presence of: interpretation of ACMG as pathogenic, or likely pathogenic, and interpretation of ACMG as VOUS with REVEL and ClinPred scores >0.5.

Statistical analysis

All association analyses of the variants and isolated dystonia, cervical dystonia, and blepharospasm risk in our case-control study were carried out with PLINK software.[29] Genotypes in the control group were first assessed for departures from the Hardy-Weinberg equilibrium using the chi-square test and using a cut off p-value of 0.01. Allelic associations between categorical groups were tested using the chi-square test and genotype-specific risks were estimated by calculating odds ratios (OR) and 95% confidence intervals. P values of <0.05 were considered statistically significant.

Literature review

We performed a detailed and systematic search for articles using the PubMed database and using the key words of *GNAL*, *TOR1A*, *THAP1*, dystonia, isolated dystonia, genetics and mutation, in various combinations. Only articles fully available online (published before August 10th 2020) were included and manually reviewed to get the available clinical and molecular information on dystonia and mutations. Single case reports were excluded when no novel mutation was reported.

RESULTS

In our population, a total of 55 variants were detected: 19 variants in *TOR1A*, 12 in *THAP1*, and 25 in *GNAL* (Table e-1). However, only fourteen patients carried pathogenic or likely pathogenic variants. In addition, we reviewed the literature, and 139 studies about these genes and their relationship with isolated dystonia were retrieved. The study selection process is described in Figure 1.

Analysis of *TOR1A*

We detected a total of 19 variants across the *TOR1A* sequence (Table e-1), including 8 coding variants (3 synonymous and 5 non-synonymous) and 9 non-coding variants. All previously described variants were detected in our cohort at frequencies comparable to those in databases. Only one variant (c.369T>G; p.Gly123Gly) was not previously reported.

Four variants were present only in DT patients. Indeed, five DT patients (prevalence = 0.48%) presented the recurrent 3bp deletion (c.904_906delGAG; p.Glu303del), the most common pathogenic variation in *TOR1A*. The other three variants were synonymous (p.Leu136Leu) or non-coding (c.*10T>C, and c.*672_*673insC), but our analysis suggested they were not responsible of the dystonia.

The intronic variant c.445-22G>A (rs10988526) was found in two controls and five patients with DT. However; this variation has been previously reported as benign and, in addition, the analysis of its putative effect on the splicing process was negative. Therefore, it was considered not responsible for the development of dystonia in the carriers.

Finally, because the frequent variants p.D216H (rs1801968) and c.*191G>T (rs1182) have been previously associated with the risk of dystonia, we performed an association analysis (adjusted for sex and age) for these two variations with the risk of isolated dystonia or specific types of dystonia (such as blepharospasm, or cervical dystonia) and no evidence of an association was observed.

Therefore, only the variation p.Glu303del has been considered unequivocally associated with the development of isolated dystonia in our population. Clinical characteristics of patients carrying this variant are shown in Table 3.

A comprehensive literature review of all reported *TOR1A* variations associated with dystonia was performed. In total, 22 variations have been published (Table e-2). However, only 10 are considered as pathogenic or likely pathogenic. Distribution of the variants on the gene and the protein are shown in Figure 2A and Figure 3A, respectively.

Analysis of *THAP1*

We detected a total of 12 variants across the *THAP1* sequence (Table e-1), 5 coding variants (1 nonsense and 4 non-synonymous) and 7 non-coding variants. Five variants were not previously reported, two in 5'-UTR (c.-211-205delGGAGATG and c.-57G>A), one in exon 1 (c.37C>G), and two in exon 3 (c.292G>T and c.605C>T).

All previously reported variants were detected in our cohort at frequencies comparable to those in databases.

Each of the genetic variants c.-6G>A, p.Arg13Gly, p.Arg29Gln and Pro202Leu was found in just one patient with dystonia. The genetic variant p.Glu98Ter (c.292G>T) was found in 3 DT patients (0.29 %) and not previously described.

Taking into account the bioinformatic data (Table e-1), we considered only four variants (p.Arg13Gly, p.Arg29Gln, p.Glu98Ter, and Pro202Leu) as causative of the development of DT in our population, carried by a total of 6 DT patients (prevalence = 0.57%). Clinical characteristics of patients carrying these variants are shown in Table 3.

A comprehensive literature review of all reported *THAP1* variations associated with dystonia was performed, finding that 104 variations have been published (Table e-3). Taking into account these variants and

those from our study, this results in a total of 108 variants, of which 96 have been considered as pathogenic or likely pathogenic. Their distributions on the gene and the protein are shown in Figures 2B and 3B, respectively.

Analysis of *GNAL*

We detected a total of 26 variants across the *GNAL* sequence (Table e-1), including 12 coding variants (1 insertion, 5 synonymous and 6 non-synonymous) and 14 non-coding variants (6 in 5'-UTR, 6 intronic, and 2 in 3'-UTR). Three variants were not previously reported, one in 5'-UTR (c.-308G>A), one in intron 6 (c.722+31C>A), and one in exon 8 (c.828C>A).

Three variations (p.Glu155lys, p.Phe199Leu, and p.Arg367Cys) were predicted as pathogenic in the bioinformatics analysis (Table e-1), all of them carried by only 1 DT patient (prevalence = 0.29%). Clinical characteristics of patients carrying these variants are shown in Table 3.

All previously reported variants were detected in our cohort at frequencies comparable to those in databases, with the exception of rs774061970. This rare variation causes a likely pathogenic missense change p.Ser238Asn. The association study showed that it was in HWE in the control group. Minor allele frequency was higher in DT patients (OR=3.38 (95% CI 1.25-9.13); p=0.011)

A comprehensive literature review of all reported *GNAL* variations associated with dystonia was performed. Thirty-six variations were described (Table e-4). Taking into account these variants and those from our study makes a total of 38 variants, of which 33 have been considered as pathogenic or likely pathogenic. Their distribution on the gene and the protein are shown in Figure 2C and Figure 3C, respectively.

DISCUSSION

To date, an increasing number of articles have reported variants in *TOR1A*, *THAP1* and *GNAL* associated with isolated dystonia. This work represents a systematic synopsis of the implication of variants in *TOR1A*, *THAP1* and *GNAL* genes in isolated dystonia. Overall, we present the results of the analysis of these three genes in 2028 subjects (including 1053 patients with various subtypes of isolated dystonia) from our southern population and a comprehensive analysis of the current state of knowledge about their contribution to the etiopathogenesis of isolated dystonia.

The results of our cohort are compatible with those of published studies, with a higher percentage of women with isolated dystonia than men[30], and the *TOR1A* gene being more frequently mutated.

Five unrelated patients in our cohort (0.48% of patients) presented the pathogenic three-base pair deletion in *TOR1A*, with mean age at onset of 22.6 years (range 10-43), with symptoms starting in limbs and with progression to generalized or multifocal dystonia. Interestingly, one patient started with cervical dystonia. In *TOR1A* cases, the onset of the symptoms usually spares bulbar and craniocervical muscles. However, in some late-onset cases, facial and cervical involvement has been reported at disease onset, including patients from the same families.[31]

Variations in *TOR1A* account for about 6% of published dystonia patients with a total of 23 variants in *TOR1A* (mainly in exon 5), but only half (11) seem to have a pathogenic role in dystonia. This does not mean that the other variants have no effect on dystonia; they may be modifier factors or undergo synergistic action with other variants. However, this should be interpreted with caution for several reasons. *TOR1A* is the most

frequently studied gene, but a number of studies have investigated only exon 5 (where the tri-nucleotide deletion is located). This could be the reason why most variants in *TOR1A* have been found in this exon. Moreover, several studies are case reports. Other studies refer to the p.D216H variation (that seems to be not pathogenic but a modifier factor) or variants within 3'-UTR (untranslated region). In addition, it is important to note that several studies did not use a control cohort. Hence, results yielded controversial results, probably due to the aforementioned factors and others such as ethnicity or body distribution (Figure e-1).

In our cohort no evidence of an association was observed for rs1182 or rs1801968 variants (previously associated with focal dystonia and writer's cramp, respectively)[17] with the development of isolated dystonia nor, more specifically, focal dystonia, cervical dystonia or blepharospasm. However, this result should be interpreted with caution owing to our small sample sizes. Indeed, for this reason we could not perform an analysis on an association with writer's cramp.

Regarding the *THAP1* gene, pathogenic variants were found in 6 dystonia patients in our cohort. The age at disease onset was 38.2 years (range 15-69), with 50% of early-onset cases as the most frequent presentation. The dystonia patterns involved craniocervical regions or limbs in most patients at onset. However, in two cases, a larynx or orofacial distribution was noticed at presentation. These peculiar features have also been reported in other cohorts, expanding the clinical picture of DYT-*THAP1*.[32]

In *THAP1* there is no frequent variation such as the tri-nucleotide deletion in *TOR1A*. Conversely, a high variety of rare variants (108) have been observed in patients with dystonia, mostly with a pathogenic role in dystonia, throughout most of the coding region of the THAP1-domain. Therefore, about 1.8% of published patients in which this gene has been analyzed presented a variant in this gene. In contrast, the frequency of pathogenic or likely pathogenic variants in *THAP1* in our cohort (0.57% of patients) has been very similar to that found in *TOR1A*. This could be because we found three patients in our cohort with a nonsense variation not previously described, p.Glu98Ter. Due to the origin of subjects with this variant, the existence of a founder effect may be possible. However, a limitation of our study was the impossibility of assessing the haplotype around this variation in order to test the existence of this effect.

Variants in *GNAL* have been observed in about 1.1% of published individuals with dystonia. In total, 37 variants have been found in *GNAL* in individuals with dystonia, mainly pathogenic, and are spread over the entire gene. However, it is important to note that this gene has been much less studied (in terms of the origin of populations and number of patients with dystonia studied) than *TOR1A* or *THAP1*. Despite this, not unexpectedly, pathogenic variants in *GNAL* were infrequent in patients with dystonia from our cohort. Therefore, three dystonia patients in our cohort carried pathogenic variants in the *GNAL* gene, with a mean age at onset of 43 years (range 29-59) and with craniocervical dystonia, according to the previously described features.[33] In addition, we observed a likely pathogenic missense variant p.Ser238Asn (c.713G>A, rs774061970). This is a very rare variant which was present in 18 dystonia patients and 5 healthy controls in our population, supporting the idea that it could be a risk factor for dystonia in Spain.

We identified several pathogenic or likely pathogenic variants in *TOR1A*, *THAP1* and *GNAL*. Therefore, genetic contribution to dystonia of these three genes was about 1.3% in our cohort of patients with isolated dystonia, while in the literature this was about 3.6% of patients, with *TOR1A* having the largest impact.

However, it is important to note that this contribution can be disturbed because several factors: first, the majority of studies did not analyze promoter regions or large deletions; in addition, the potential role of *TOR1A* could be underestimated because screening from many studies was limited to exon 5 (Figure e-1); moreover, in our study the involvement of TOR1A may be undercalculated because in those familial cases we included only the proband member; finally, our population is mainly from adult centers (with a mean age of onset of 51.2 years), which could affect the diagnostic yield as it is higher in cases with an early onset.

Another important limitation of our study was that the sensitivity of high resolution melting (the screening technique used in our cohort) is not 100%; therefore, the prevalence of *TOR1A*, *THAP1* and *GNAL* in our population might be slightly underestimated.

Finally, it is important to note that in our study the reported family history is lower than in other cohorts. This fact could be partially explained by the low sensitivity in detecting family cases by clinical interview in focal dystonia.[34]

In summary, this study is the largest and most complete study of *TOR1A*, *THAP1* and *GNAL* genes in the Spanish population and a review of the recent genetic findings in these genes. Our data suggest that the incidence of pathogenic or likely pathogenic variants in *TOR1A*, *THAP1* and *GNAL* in our population is low. There is genetic heterogeneity that includes not only the *TOR1A* tri-nucleotide deletion but also a *THAP1* variant with a putative founder effect as well as other different variations in *THAP1* and *GNAL*. However, there is a relationship between phenotype and genotype. Therefore, in terms of age at onset, site of dystonia onset, and final distribution, there is a clear differentiation between DYT-*TOR1A* and DYT-*GNAL*, with DYT-*THAP1* resembling an intermediate phenotype.

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DATA AVAILABILITY STATEMENT

All data that supports the findings of this study are available in this article.

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FIGURE LEGENDS

Figure 1. Flow chart showing the literature search and the selection of studies included in the present review. In total, 2684 articles were identified, and 1867 duplicate articles were removed. Then 678 additional articles were excluded on the basis of: no relevant studies, studies about genes other than *GNAL*, *TOR1A* or *THAP1*, no genetic variation reported, no English or Spanish language, no-peer-reviewed, or incorrect type of article (e.g., review, editorial, etc.). Full texts of four articles (published on 1976 and 1975) were not found. 139 studies providing information on *TOR1A*, *THAP1* and *GNAL* in patients with dystonia were retrieved. Of those, 75 studies provided original information on *TOR1A*, *THAP1* and *GNAL* variations in patients with dystonia. 132 studies (of those 139) were included for the study of the prevalence of variations related to dystonia in the three genes worldwide (including reported clinical histories, case reports and smaller case series, larger case series and case-control cohorts).

Figure 2. Schematic representations of exon-intron structure of *TOR1A*, *THAP1* and *GNAL* genes with all variations reported to date associated with dystonia indicated. Variations considered pathogenic or likely pathogenic are highlighted in red above. Variations found in our population are underlined. References for the first describers are given for each variation (in parentheses).

Figure 3. Schematic diagrams of TOR1A, THAP1 and GNAL proteins with all variations reported to date associated with dystonia indicated. Variations considered pathogenic or likely pathogenic are highlighted in red above. Variations found in our population are underlined. References for the first describers are given for each variation (in parentheses). Variants showed in this study are underlined. Functional domains are shown. SS: Signal sequence; HD: hydrophobic domain; W-A: Walker A motif; W-B: Walker B motif; APDB: ATP binding protein domain; SR1: Sensor 1; SR2: Sensor 2; TD: THAP1 domain; PRR: low-complexity Proline-Rich Region; CCD: Coiled-Coil Domain; NLS: Nuclear Localization Signal.

Table 1. Most frequent isolated forms of dystonia with an established genetic cause

Locus symbol	OMIM	Inheritance	Mutations	Acronym	Phenotype	Mean age of onset of dystonia	Penetrance
DYT-1	128100	AD	Only a few. Mainly p. Glu303del	DYT-*TOR1A*	Early-onset generalized dystonia	In the teens	~ 30% -40%
DYT-6	602629	AD	More than 100	DYT-*THAP1*	Adolescent-onset cranial or generalized dystonia	Around twenty	~ 60%
DYT-25	615073	AD	About 40	DYT-*GNAL*	Adult-onset focal/segmental dystonia	In the thirties	High but not full

Table 2. Demographic characteristics of the 2029 subjects from Spanish population enrolled in this study

Clinical diagnosis	Total number of Subjects	Sex: N (P)		Age mean ± SD	Age at onset mean ± SD	Dystonia type: n (p)	
		Male	Female				
Healthy control	975	368 (37.74%)	607 (62.26%)	55 ± 15	-	-	
Isolated Dystonia	1053	331 (31.43%)	722 (68.57%)	61.2 ± 15.5	51.3 ± 14.8	Focal: 727 (69.04%)	Cervical: 247 (33.98%[a])
							Writer's cramp: 15 (2.06%[a])
							Blepharospasm: 391 (53.78%[a])
							Oromandibular: 7 (0.96%[a])
							Hand: 39 (5.37%[a])
							Other: 28 (3.85%[a])
						Segmental: 143 (13.58%)	
						Generalized: 30 (2.85%)	
						Multifocal: 29 (2.75%)	
						Dystonic tremor: 124 (11.78%)	

N: number of subjects; P: Percentage over total number of subjects; N: Number of subjects; SD: Standard deviation; Ages are given in years; n: Number of subjects with each type of dystonia; p: Percentage over total of dystonias; a: Percentage over the total of focal dystonias

Table 3. Clinical features of patients carrying putative pathogenic variants

Gene	Variant	Sample	Sex	Age at onset (y)	Distribution of dystonia	Site of dystonia onset	Family history
TOR1A	p.E303del	Case 1	Female	43	Multifocal	Neck	Yes[1]
		Case 3	Male	10	Generalized	Lower right limb	Yes[2]
		Case 4	Female	11	Generalized	Lower right limb	No
		Case 5	Male	27	Focal (Hand)	Hand	Yes[3]
		Case 7	Female	22	Segmental	Upper left limb	No
THAP1	p.Arg13Gly	Case 8	Female	21	Focal (CV)	Neck	No
	p.Arg29Gln	Case 9	Female	40	Focal (CV)	Neck	No
	p.Glu98Ter	Case 10	Male	22	Generalized	Lower left limb	No
		Case 11	Female	69	Focal	Vocal chords	No
		Case 12	Female	15	Segmental (CV/BF)	Mouth	No
	p.Pro202Leu	Case 13	Female	62	Focal (CV)	Neck	Yes[4]
GNAL*	p.Glu155Lys	Case 14	Male	29	Focal (CV)	Neck	No
	p.Phe199Leu	Case 15	Female	45	Focal (CV)	Neck	no
	p.Arg367Cys	Case 16	Male	59	Focal (BF)	Eyes	No

CV: cervical; BF: Blepharospam. *: Isoform 2; 1: Family history of focal, segmental and generalized dystonia in several relatives. The family member with the youngest age at onset was at 9 years old; 2: Family history of focal, segmental and generalized dystonia in several relatives. The proband was the family member with the youngest age at onset; 3: Family history with a daughter with generalized dystonia; 4: Family history of familial dystonic tremor.

Figure 1

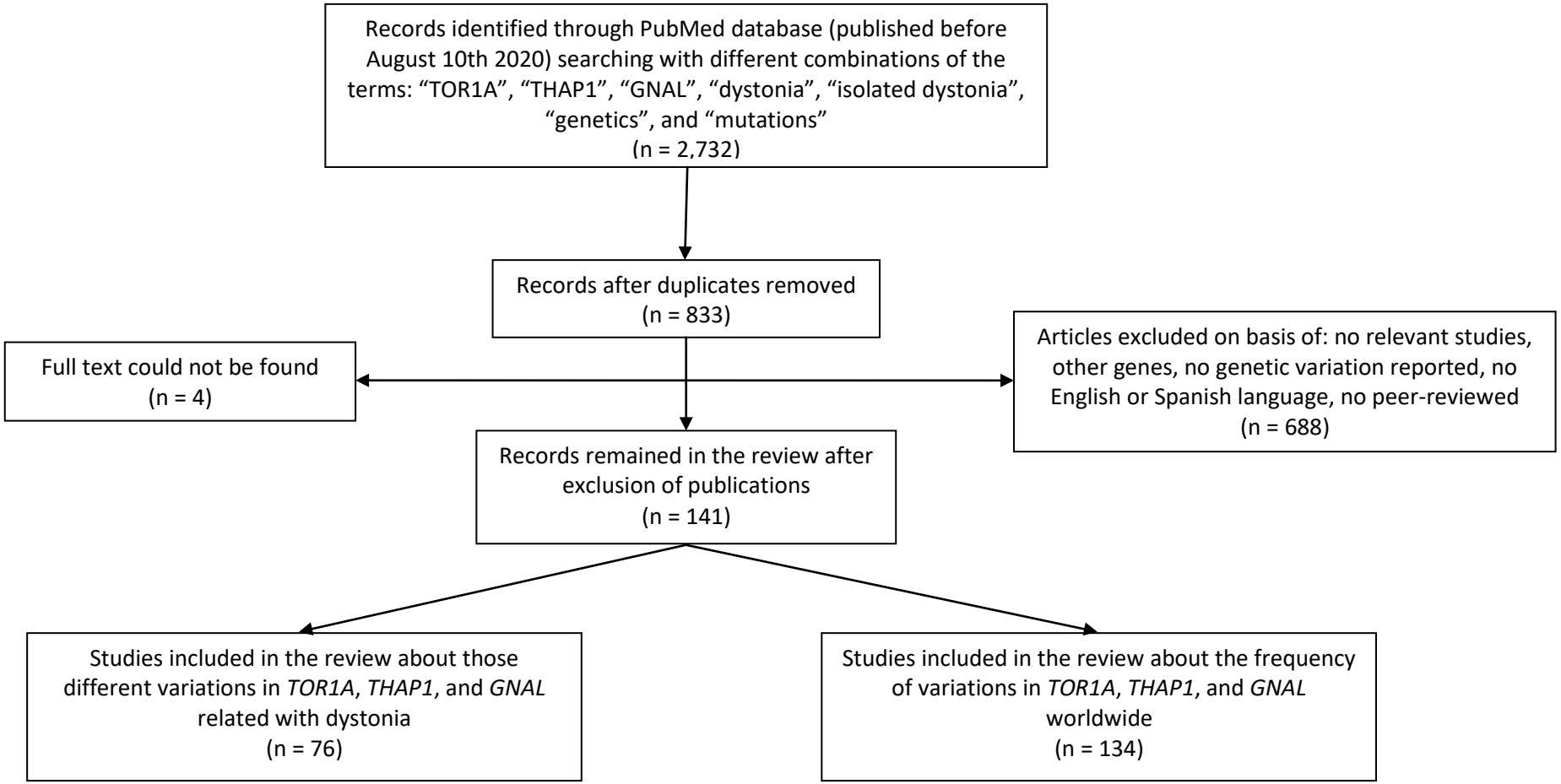

Figure 2

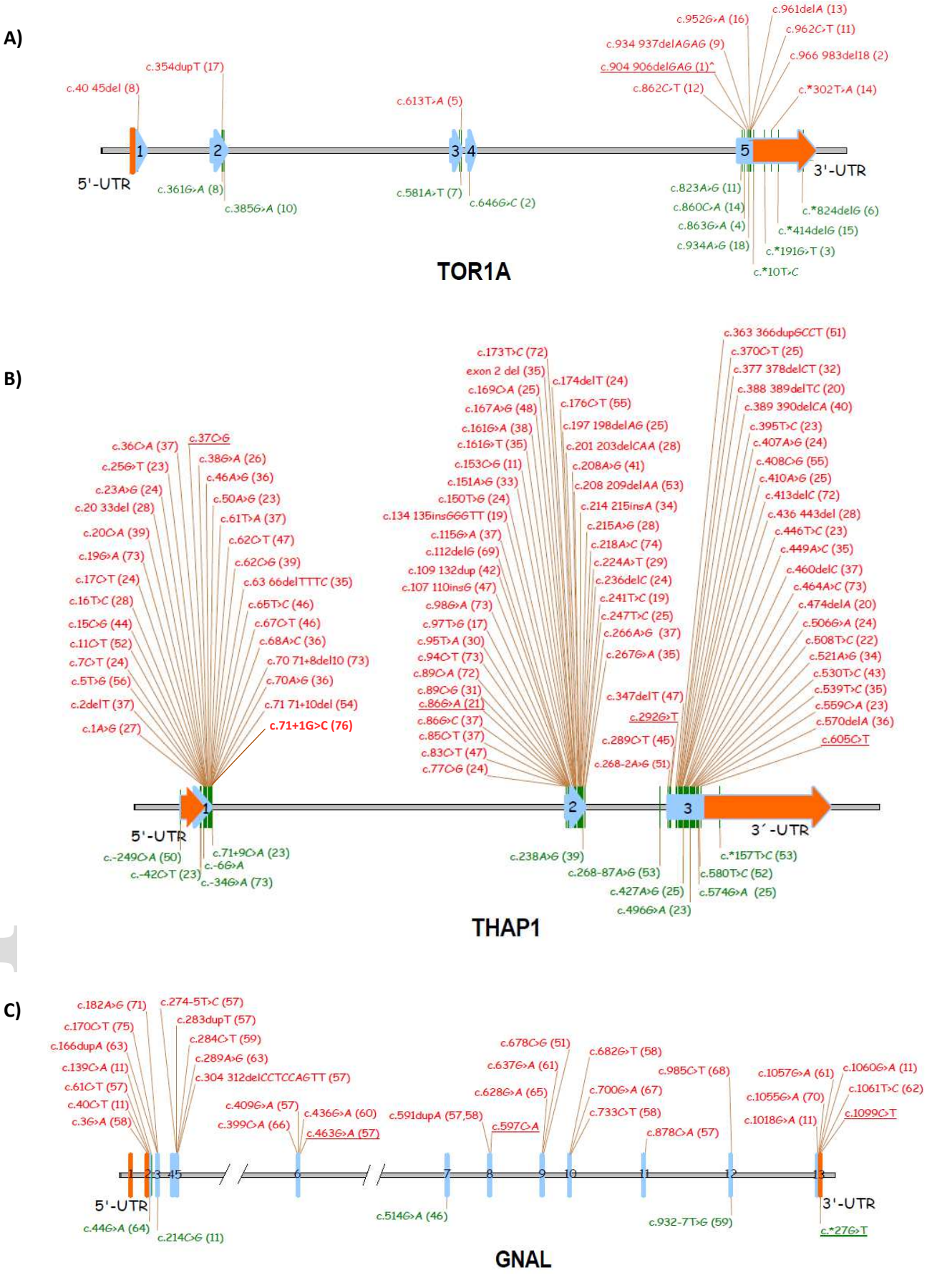

Figure 3