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Short communication

DRD1 rare variants associated with tardive-like dystonia: A pilot pathway sequencing study in dystonia

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ABSTRACT

The dystonias are a clinical heterogeneous group with a complex genetic background. To gain more insight in genetic risk factors in dystonia we used a pathway sequence approach in patients with an extreme dystonia phenotype ($n = 26$). We assessed all coding and non-coding variants in candidate genes in D1-like subclass of dopamine receptor genes (DRD1, DRD5) and the synaptic vesicle pathway linked to torsinA (TOR1A, STON2, SNAPIN, KLC1 and THAP1), spanning 96 Kb.

Two rare missense variants in DRD1 were found: c.68G>A(p.Arg23His) in the screening group and c.776C>A(p.Ser259Tyr) in an additional screen of 15 selected dystonia patients. Genetic burden analysis of DRD1 rare variants in patients (4.8%) versus European American controls from ESP (0.72%) reveals an OR 5.35 (95% CI 1.29–23.1). No rare missense SNVs in the synaptic vesicle pathway were found. Sequencing of TOR1A showed variant enrichment in haplotype 2, possibly accountable for contradictive results in previous association studies. Two new rare SNVs were detected in THAP1, including a nonsense mutation (p.Gln167Ter) and a splice site variant (c.72-1G>A). Screening for rare SNV of candidate pathways in a phenotype extreme population appears to be a promising alternative method to identify genetic risk factors in complex disorders like primary torsion dystonia. These findings indicate a role for rare genetic variation in dopamine processing genes in dystonia pathophysiology.

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1. Introduction

The dystonias are a group of movement disorders characterized by involuntary twisting, repetitive movements or abnormal postures. Sporadic, adult-onset primary torsion dystonias (prevalence 1/2500 [1]) are genetically complex disorders [2]. More rare are the familial, mostly severe and early onset forms of primary torsion dystonia. So far, three genes for these autosomal dominant familial primary torsion dystonia syndromes (FPTD) have been well established: the *TOR1A* gene (904_906delGAG/907_909delG AG) [3], mutations in *THAP1* [4] and in *GNAL* [5]. Mutations in these genes are responsible for a small portion of patients; in our clinic only 25% of early onset dystonia patients and less than 2% of late onset patients carry mutations in one of these genes [2]. FPTDs show a reduced penetrance (in *TOR1A* families 30%, *THAP1* 60%, *GNAL* not known yet). This reduced penetrance, combined with a lack of biomarkers and variability in the phenotypic expression make genetic family studies in dystonia a challenge, even with utilization of exome sequencing.

From the known dystonia-genes, no clear pathophysiological pathway can be deduced as yet. TorsinA is implicated in several cellular pathways, notably the synaptic vesicle trafficking pathway. TorsinA shows a vesicle-like distribution pattern, it partially co-localizes with synaptic vesicle markers and with the SNARE-associated protein SNAPIN [6]. Also it interacts with proteins in synaptic vesicle processing such as STON2 and the kinesin light chain 1 (KLC1) [7]. Another interesting torsinA-related pathway is dopaminergic signaling: dysfunctional striatal dopamine signaling system can induce dystonic symptoms [8] and a role for torsinA in dopamine transport and abnormal neurotransmission has been suggested [6]. Furthermore, alterations in basal ganglia dopamine signaling have been observed in dystonia, a feature also found in *TOR1A* mouse models [9].

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We test the hypothesis that genetic variation in candidate pathways increases risk for dystonia and holds part of the unknown portion of genetic risk factors for dystonia. We screened, besides *THAP1* and *TOR1A*, genes in synaptic vesicle recycling (*STON2*, *SNAPIN*, *KLC1*) and D1-like dopamine receptors (*DRD1*, *DRD5*). We selected patients with severe, early onset primary torsion dystonia ($n = 26$), as patient samples from the extremes of phenotypes are likely enriched for genetic risk alleles [10].

2. Patients and methods

2.1. Patients

Patients were seen at the movement disorders clinic in the Academic Medical Centre in Amsterdam (AMC). Written informed consent was obtained for all participants. The study was approved by the Medical Ethical Committee of the AMC. All patients were examined by a movement disorder specialist (MAJT) and diagnosed with primary dystonia based on the accepted criteria [11]. Secondary causes were excluded by patient history, additional neuroimaging and laboratory tests. Selection criteria were a primary dystonia with an early age at onset (under 30 years of age) and segmental or generalized spread of dystonia (Cohort 1). To expand the initial genetic screening of the *DRD1* gene, a second group of patients was selected (Cohort 2), based on the presence of segmental dystonia of the neck, spreading to the upper extremities or larynx and predominant an early age at presentation of dystonia (E-Suppl. Table 1).

2.2. Genotyping

454 genotyping – We sequenced 98 Kb including the intronic and coding regions of *TOR1A*/DYT1 (NM_000113.2), *THAP1*/DYT6 (NM_018105.2), *STON2* (NM_033104.3), *SNAPIN* (NM_012437.5), *KLC1* (NM_005552.4), *DRD1* (NM_000794.3) and *DRD5* (NM_000798.4) (E-Suppl. Text). *Sanger sequencing* – Sanger sequencing of the coding regions of *DRD1* was performed in 15 additional segmental dystonia patients. *TaqMan SNP genotyping* – A selection of variants (*DRD1* c.68G>A and c.776C>A (rs74414188)) were genotyped in 1024 healthy Dutch Blood Bank samples using TaqMan endpoint genotyping (Applied Biosystems).

2.3. Data analysis

454 data – The identified variants were dichotomized in two groups *rare coding variants* (below 1% in population frequency) and *common variants*. Both groups require a different approach in analysis: as variants from the first group could have a higher impact as disease alleles, the latter could induce dystonia risk on a group level. For rare variants in *DRD1* the total number of variants in the gene were compared to variant data from Exome Variant Server, NHLBI Exome Sequencing Project (ESP; date: 16/12/2012). For the identified common variants allele frequencies were compared with minor allele frequencies (MAF) in Americans of Europeans ancestry from dbSNP132. Chi^2 and unadjusted *p*-values (Pearson) were calculated. Impact of amino acid change on protein function was estimated using prediction programs PolyPhen and SIFT. Alamut 2.0 splicing modules were used to predict splice site variants.

Haplotype construction in TOR1A–H2 haplotype was constructed according to previous studies [12] using the alleles of rs3842225 (del-allele), rs1182 (T-allele) and rs2296793 (T-allele).

3. Results

3.1. Patients

Patients of Cohort 1 ($n = 26$) had a segmental or generalized PTD with a mean age at onset of 18.3 (range 6–30) years and a family history of dystonia was present in 61.5%. Cohort 2 consists of a selection of 15 segmental dystonia patients with a mean age at onset of 25.8 (range 4–45) and a positive family history of 66.7% (E-Suppl.Tables 1 and 2).

3.2. Genetic variation in PTD patients

3.2.1. 454 sequence characteristics

Total number of uniquely mapped reads in the target region (96 Kb) was 1.55 Mb in 26 samples. Median read length counted 335 base pairs. Minimal depth of variant call was set to 5 reads, with an overall mean read depth of 38,4 reads for all called variants.

3.2.2. New rare coding SNVs (frequency <1%)

D1-like dopamine receptors – Patient ID3 carries missense variant in *DRD1* c.68G>A (p.Arg23His), not present in 1024 Dutch controls and 4300 ESP controls (Table 1). This 30 year old female has a severe segmental dystonia, resembling tardive dystonia. Cervical dystonia with prominent rotational component and a severe retrocollis was first noted at age 11 and dystonia gradually spread to the right arm and laryngeal-oromandibular region. Clinical history reveals anxiety and moderate depression. The patient did not receive any medication prior to disease onset. The unaffected father of the index patient also carried this *DRD1* variant as a part of a rare haplotype. Subsequently, we Sanger sequenced the *DRD1* gene in 15 additional dystonia patients (Cohort 2) selected on phenotypic resemblance with Patient ID3. Here, we detected another rare missense SNV in *DRD1* c.776C>A(p.Ser259Tyr) (rs74414188; MAF of 0,0029 in 1016 Dutch controls and MAF of 0,0024 in ESP). This 16 year old man (Cohort2-ID1, E-Supplemental Table 2) had dystonia onset in the neck at age 4, which spread to trunk and right arm. Parents of patient did not want to cooperate in the study. Both variants were predicted to be deleterious (Table 1). In control populations the variability in the *DRD1* gene is extremely rare: 31 missense variants in 4300 ESP control subjects (0.72%), against a frequency of two in 41 PTD patients (4.8%, OR 5.35 (95% CI 1.29–23.1). (E-Suppl. Table 4) *Synaptic vesicle trafficking pathway* – No rare SNVs were found in coding regions of *TOR1A*, *STON2*, *SNAPIN* and *KLC1*.

Enrichment of TOR1A Haplotype 2 – Screening of the 11 Kb region spanning *TOR1A* in Cohort 1 showed 28 different SNVs. Minor allele frequencies of these SNVs range from 0.5% to 37.5% in general population (dbSNP132, CEU dataset). Previous studies identified three common haplotypes in the *TOR1A* region (H1, H2 and H3), tagged by rs3842225, rs1182 and rs2296793. In these studies, *TOR1A* – H1 was present in 75% of patients and controls, H2 in ~20% and H3 in ~2.5% [12]. After clustering the SNVs per haplotype, a higher number of SNVs was found in patients carrying haplotype 2 (del-T-T), compared to the remaining haplotype groups (H1 and haplotypes with lower frequencies): H2 was carried by 25%

Table 1
Rare missense SNVs in DRD1 and THAP1.

Genotype					Phenotype			
Gene	Variant (c)	Variant (p)	MAF	Prediction	Age onset	spread	other	notes
DRD1	68G>A	Arg23His	None[a]	GD = 29; PP/S = deleterious	11	F,V, N, UE	anxiety and depression	unaffected father also carries this variant
DRD1	776C>A	Ser259Tyr	0.002	GD = 144; PP/S = deleterious	3	V, N, T, UE	anxiety	Cohort 2; no family follow up
THAP1	499C>T	Gln167X	none	x	13	N, T, UE		AD
THAP1	72-1G>A	splice	none	x	23	N, T, UE		non-familial

Rare missense SNVs in DRD1 and THAP1 with predicted possible functional effects in dystonia patients. MAF = minor allele frequency: calculated from ESP data (cohort of 4300 European Americans from the ESP cohort. (http://evs.gs.washington.edu/EVS/).

[a] *1024 Dutch Caucasian controls* (*custom TaqMan assay*). *Refseq THAP1* (*NM_018105.2*), *DRD1* (*NM_000794.3*). *GD* = Grantham distance, PP/S=PolyPhen/SIFT prediction, F = face, V = voice, N = neck, T = trunk, UE = upper extremities, LE = lower extremities.

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