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Accumulation of rare variants in the arylsulfatase G (ARSG) gene in task-specific dystonia

Esther Nibbeling¹ · Susen Schaake² · Marina A. Tijssen³ · Anne Weissbach²,4 · Justus L. Groen⁵ · Eckart Altenmüller⁶ · Dineke S. Verbeek¹ · Katja Lohmann²

Abstract Musician’s dystonia and writer’s cramp are examples of task-specific dystonia. Recently, the arylsulfatase G (ARSG) locus was suggested to be associated with musician’s dystonia and writer’s cramp by a genome-wide association study. To test for the presence of causal variants, the entire coding region and exon–intron boundaries of ARSG were sequenced in DNA samples from 158 musician’s dystonia patients which were collected at the University of Music, Drama, and Media (Hanover, Germany), and 72 patients with writer’s cramp which were recruited at the Academic Medical Centers in Amsterdam and Groningen, the Netherlands. The frequency of variants within ARSG was compared to publically available data at the exome variant server (EVS) from the NHLBI GO Exome Sequencing Project. We identified 11 single nucleotide variants (SNVs) in the patients including eight non-synonymous substitutions. All variants have previously been reported at EVS including two SNVs with a reported minor allele frequency <1%. One rare missense variant, rs61999318 (p.I493T), was significantly enriched in the group of writer’s cramp patients compared to European Americans in EVS database (p = 0.0013). In patients with writer’s cramp, there was an overall enrichment for rare, protein-changing variants compared to controls (p < 0.01). In conclusion, we did not detect any conclusive mutation in ARSG. However, we showed an association with rs61999318 in patients with writer’s cramp that contributed to an overall enrichment for rare, protein-changing variants in these patients. Thus, our data provide further support for a role of ARSG variants in task-specific dystonia, especially writer’s cramp.

Keywords Genetic risk factors · Association · ARSG · Musician’s dystonia · Writer’s cramp

Introduction

Dystonia comprises a group of hyperkinetic movement disorders characterized by involuntary muscle contractions, resulting in twisting movements and abnormal postures, or both [1]. Focal dystonia affects only a single body part and includes different subtypes of dystonia like blepharospasm, cervical/cranial dystonia, writer’s cramp (WC), or musician’s dystonia (MuD) [2]. The latter two are task-specific forms of focal dystonia that only occur when a certain task is performed, i.e., while writing or playing an instrument [3, 4]. As both WC and MuD affect mainly the hand and/or arm, it is thought that these subtypes might exhibit a shared etiology.
Very little is yet known about the underlying disease mechanisms of focal task-specific dystonias, but a genetic background is very likely as about 20 % of all MuD patients show a positive family history with one or more relatives affected with either MuD or WC [5]. Recently, an intronic variant (rs11655081) in the arylsulfatase G (ARSG) gene was shown to be associated with MuD with genome-wide significance as revealed by a genome-wide association study and respective replication. Importantly, the association was also shown for WC in validation samples [6]. In contrast, the ARSG variant was not associated with other, non-task-specific forms of dystonia such as cervical dystonia or blepharospasm [6]. These data further strengthen the possibility for a shared disease mechanism for MuD and WC that is distinct from the one for other forms of focal dystonia.

To investigate whether mutations or rare variants in ARSG may underlie task-induced focal dystonia, we sequenced the entire coding region of ARSG in German MuD and Dutch WC patients.

Subjects and methods

After obtaining informed consent, we included 158 MuD patients collected at the University of Music, Drama, and Media (Hanover, Germany) and 72 WC patients recruited at the Academic Medical Centers in Amsterdam and Groningen. Patients with MuD have been included in the GWAS [6]. The study was approved by the regional ethical review boards of the Academic Medical Center, Amsterdam, the University Medical Center Groningen (The Netherlands), and the University of Lübeck (Germany).

We screened the eleven coding exons and exon–intron boundaries of ARSG (HGNC: 24102, NM_014960.4) by Sanger sequencing using an ABI3700 capillary sequencer (Applied Biosystems). The primers used for sequencing are listed in the Supplementary Table 1. The sequences were analyzed using the Mutation Surveyor program (Softgenetics). Detected variants were tested for novelty using data from the exome variant server (EVS; http://www.evs.gs.washington.edu/EVS/) of the NHLBI Exome Sequencing Project (ESP). The frequency of variants was compared between MuD/WC patients and samples in EVS using a two-tailed Fisher’s exact test and p values <0.005 (according to Bonferroni correction of 11 independent tests) were considered statistically significant.

Detected rare variants were in silico tested for their pathogenicity using different prediction programs and information about conservation of respective amino acids. We used SIFT (http://www.sift.jcvi.org/www/SIFT_seqSubmit2.html), Polyphen 2 (http://www.genetics.bwh.harvard.edu/pph2/), MutationTaster (http://www.mutationtaster.org/), Align GVD (http://www.avgd.iarc.fr/avgd_input.php), SNPs&GO (http://www.snps-and-go.biocomp.unibo.it/snps-and-go/) and check the following scores: GERP (from the EVS server), PhastCons and CADD-PHR (http://www.cadd.gs.washington.edu/score).

In addition, we searched on the EVS server for rare (minor allele frequency <3 %), protein-changing variants within ARSG and counted the alleles with such variants (Supplementary Table 2). The frequency of these alleles was then compared with the frequency of rare, protein-changing variants in our patient sample.

Results

The entire coding region of the ARSG gene was successfully screened and led to the identification of 11 exonic single nucleotide variants (SNVs) in both MuD and WC cases (Table 1). This included eight missense and three synonymous SNVs. We did neither detect any deletion or insertion nor any variant at exon–intron boundaries defined as the first and last four nucleotides of an intron. All SNVs were reported in the EVS database including two very rare (minor allele frequency <0.005), missense variants (rs61999318, rs370852507); of which rs370852507 was only reported in one African American sample in EVS. Comparison of the frequency of the 11 SNVs with the reported frequencies in European Americans from the EVS revealed higher frequencies of rs62000424 (p.T444M) and rs61999318 (p.I493T) among WC patients (p < 0.05). However, only the association of rs61999318 with WC remained significant after Bonferroni correction for multiple testing. None of the SNVs is clearly predicted to be disease causing by multiple in silico prediction programs (Table 2).

Since more than 80 variants are reported in the coding region of ARSG, we also tested whether there is an enrichment of rare, protein-changing variants in our patients compared to EVS controls. For this, we filtered the variants in EVS and found 34 variants, reported with a minor allele frequency (MAF) <3 % and an effect on the protein sequence. This group comprised 29 missense, 2 nonsense, 2 frameshift, and 1 splice site variant (Supplementary Table 2) yielding a total of 263 variant alleles among 8600 chromosomes of European Americans in EVS. This accounts for a frequency of rare, protein-changing variants of 3.1 % in ARSG. In our patients such variants were detected in 7.7 % (11/144) of the alleles in WC patients and 2.8 % (9/316) of alleles among MuD patients. Notably, the difference in WC patients is highly significant (p = 0.005) indicating an enrichment of rare protein-changing variants.
In this study, we addressed the role of rare variants within the coding region of ARSG in two groups of patients with focal task-specific dystonia. For this, we sequenced the 11 coding exons of ARSG in 230 MuD and WC patients. We did not detect any clear mutation among our patients but found 11 known variants including two rare variants (MAF $\leq 1\%$). We provide evidence for an association of one of these rare variants (rs61999318) with WC ($p_{\text{corrected}} = 0.014$). Interestingly, this variant results in an amino acid substitution at position 493 (p.I493T) which is highly conserved. Thus, the substitution is predicted to be disease causing by two prediction programs suggesting that it might be functionally important. In this context, it is important to note that all prediction programs have certain limitations [7]. In addition, we demonstrated an enrichment of rare, protein-changing variants among patients compared to samples from the EVS; this enrichment was highly significant for WC patients ($p < 0.01$).

Using data from EVS as controls has two limitations. First, these samples are not neurologically examined and may include patients with dystonia. However, given the fact that dystonia is a rare disorder with an estimated prevalence of 16.3 per 100,000 [8], one would expect not more than one dystonia patient among the 4300 European American samples from the EVS. Second, the large amount of data was generated by high-throughput, next-generation

<table>
<thead>
<tr>
<th>Location</th>
<th>SNP ID</th>
<th>cDNA change</th>
<th>AA change</th>
<th>Musician’s dystonia Variant alleles</th>
<th>Wild-type alleles</th>
<th>Writer’s cramp Variant alleles</th>
<th>Wild-type alleles</th>
<th>EVS (European) Variant alleles</th>
<th>Wild-type alleles</th>
<th>$p$ value MuD vs. EVS</th>
<th>$p$ value WC vs. EVS</th>
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<td>78</td>
<td>64</td>
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<td>8511</td>
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</tr>
</tbody>
</table>

Bold values indicate $p < 0.05$

* This $p$ value remains significant after testing for multiple testing (11 tests): $p_{\text{corrected}} = 0.0013 * 11 = 0.014$

**Table 1** Single nucleotide variants found in dystonia patients and their population frequency in European American controls (EVS database)

**Table 2** In silico prediction of the pathogenicity of the variants found in dystonia patients
sequencing and not all variants in EVS have been validated (possibility of false-positive variants) and some variants may have been missed (false-negative variants). Notably, the average read depth for the coding ARSG exons was $>27\times$, indicating a high coverage and a small likelihood to miss variants. Therefore, the strong enrichment of variants in our Sanger-sequenced patients is likely to be true.

In conclusion, we did not identify any clear-cut causal variant in ARSG in WC and MuD, however, accumulation of rare SNVs in the coding region of ARSG further supports a role of ARSG variants in task-induced focal dystonia, especially WC. Given the relatively small sample size in our study, and the uncertainty about pathogenicity of rs61999318, further analysis of the role of ARSG in focal task-specific dystonias is warranted.

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Conflicts of interest None of the authors has any conflict of interest.

Ethical standard The study has been approved by the appropriate ethics committees and has therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. All patients gave their informed consent prior to their inclusion in the study.

References