The genetics of spinocerebellar ataxia and dystonia

Nibbeling, Esther

Accumulation of rare variants in arylsulfatase G in task-specific dystonia

E. Nibbeling, MS[1], S. Winkler, BS [2], M.A. Tijssen, MD[3], A. Weissbach, MD[2,4], J.L. Groen, MD[5], E. Altenmüller, MD[6], D.S. Verbeek, PhD[1], K. Lohmann, PhD[2]

1. *Department of Genetics, University Medical Center Groningen, Groningen, The Netherlands*
2. *Institute of Neurogenetics, University of Lübeck, Lübeck, Germany*
3. *Department of Neurology, University Medical Center Groningen, Groningen, The Netherlands*
4. *Department of Neurology, University of Lübeck, Lübeck, Germany*
5. *Department of Neurology and Genome Analysis, Academic Medical Center, Amsterdam, The Netherlands*
6. *Institute of Music Physiology and Musicians' Medicine, Hanover University of Music, Drama and Media, Hanover, Germany*

J Neurol. 2015; 262(5):1340-3; doi: 10.1007/s00415-015-7718-3

6

Abstract

Musician's dystonia (MuD) and writer´s cramp (WC) are examples of task-specific dystonia. Recently, the arylsulfatase G (*ARSG*) locus was suggested to be associated with MuD and WC by a genome wide association study. To test for the presence of causal variants, the coding region of *ARSG* was sequenced in 158 MuD patients which were collected at the University of Music, Drama, and Media (Hanover, Germany), and 72 WC patients which were recruited at the Academic Medical Centers in Amsterdam and Groningen, the Netherlands. We identified 11 single nucleotide variations (SNVs) within exons of *ARSG* in both MuD and WC cases. All of these are known in the EVS database and are *in silico* predicted to be benign. Only rs61999318 in the WC group was shown to be significantly enriched compared to European frequency of the EVS database. In conclusion, we did not detect any conclusive mutation but showed an association for rs61999318 ($p=0.0013$) and an overall enrichment for rare protein-changing variants in WC patients compared to controls ($p<0.001$). Our data provide further support for a role of *ARSG* variants in task-specific dystonia, especially WC.

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 a 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 but distinct pathomechanism for MuD and WC.

The ARSG protein belongs to the sulfatase enzyme family that is involved in cell signaling, protein degradation, and hormone biosynthesis.[7] *ARSG*-deficient mice were shown to accumulate heparan sulfate in visceral organs and the central nervous system and developed neuronal cell death and behavioral deficits,[8] whereas a homozygous *arsg* mutation in dogs lead to neuronal ceroid lipofuscinosis, which includes dystonic symptoms.[9]

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), which were all used in the original GWAS study, and 72 WC patients recruited at the Academic Medical Centers in Amsterdam and Groningen. 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) of the NHLBI Exome Sequencing Project (ESP). The frequency of variants were compared between MuD/WC patients and samples in EVS using a 2-tailed Fisher´s exact test and P-values < 0.05 were considered statistically significant.

Results

The entire coding region of the *ARSG* gene was successfully screened and led to the identification of 11 single nucleotide variations (SNVs) within exons 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 significantly higher frequencies of rs62000424 (p.T444M) and rs61999318 (p.I493T) among WC patients. However, only the association of rs61999318 with WC remained significant after Bonferroni correction for multiple testing. Further, we detected the variant rs370852507 (p.E481K) in a single MuD patient. This variant has not been reported in EVS (Table 1) and is predicted to be benign *in silico* by multiple 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 reported variants in EVS and found 33 variants, reported with a minor allele frequency (MAF) <1% and an effect on the protein sequence. This group comprised 28 missense, 2 nonsense, 2 frameshift, and 1 splice site variant (Supplementary Table 2) yielding a total of 77 variant and 283,031 wild type alleles in EVS. This accounts for a frequency of rare, protein-changing variants of 0.027% in *ARSG*. In our patients such variants were detected in 1.4% (4/284) of the alleles in WC patients and 0.16% (1/632) of alleles among MuD patients. Notably, the difference in WC patients is highly significant indicating an enrichment of rare protein-changing variants.

Table 1. Single nucleotide variants found in dystonia patients and their population frequency in European American controls (EVS database). * This p-value remains significant after correcting for multiple testing (11 tests; $p_{corrected} = 0.014$)

				Musician´s dystonia		Writer´s cramp		EVS		p-value	p-value WC
Location	SNP ID	cDNA change	Amino Acid change	Variant alleles	Wildtype alleles	Variant alleles	Wildtype alleles	Variant alleles	Wildtype alleles	MuD vs. EVS	vs. EVS
17:66364691	rs1558876	c.707C>G	p.T236S	123	193	64	76	3769	4831	0.0938	0.7983
17:66364749	rs1558877	c.765T>C	p.P255=	147	169	78	64	4478	4122	0.0584	0.5535
17:66364770	rs146307895	c.786G>A	p.A262=	2	314	0	142	89	8511	0.7730	0.4069
17:66364804	rs1558878	c.820T>C	p.W274R	140	176	67	75	4276	4324	0.0589	0.5550
17:66366659	rs144503106	c.976C>G	p.R326G	8	308	4	138	186	8414	0.5588	0.3774
17:66391232	rs7342975	c.1110A>G	p.P370=	8	308	4	138	121	8479	0.1407	0.1456
17:66391276	rs9972951	c.1154G>A	p.R385H	17	299	8	134	292	8308	0.0817	0.0967
17:66391314	rs11657051	c.1192C>T	p.R398W	11	305	3	139	353	8247	0.7712	0.2887
17:66416357	rs62000424	c.1331C>T	p.T444M	46	270	12	130	1302	7298	0.8728	**0.0242**
17:66416467	rs370852507	c.1441G>A	p.E481K	1	315	0	142	0	8600	**0.0354**	1
17:66416504	rs61999318	c.1478T>C	p.I493T	0	316	4	138	26	8574	1	**0.0013***

Table 2. *In silico* prediction of the pathogenicity of the variants found in dystonia patients.

SNP ID	cDNA change	Amino Acid change	SIFT	Allign GVGD	Mutationtaster	Polyphen 2	MutPred	SNPs & GO
rs1558876	c.707C>G	p.T236S	benign	benign	damaging	benign	benign	benign
rs1558877	c.765T>C	p.P255=	NA	NA	benign	NA	NA	NA
rs146307895	c.786G>A	p.A262=	NA	NA	benign	NA	NA	NA
rs1558878	c.820T>C	p.W274R	benign	benign	benign	benign	benign	benign
rs144503106	c.976C>G	p.R326G	benign	benign	damaging	benign	benign	benign
rs7342975	c.1110A>G	p.P370=	NA	NA	benign	NA	NA	NA
rs9972951	c.1154G>A	p.R385H	benign	benign	benign	benign	benign	benign
rs11657051	c.1192C>T	p.R398W	benign	benign	benign	benign	unknown	benign
rs62000424	c.1331C>T	p.T444M	benign	benign	benign	benign	benign	benign
rs370852507	c.1441G>A	p.E481K	benign	benign	benign	benign	benign	benign
rs61999318	c.1478T>C	p.I493T	benign	benign	benign	damaging	unknown	benign

Discussion

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<1%). We provide evidence for an association of one of these rare variants (rs61999318) with WC ($p_{corrected}$=0.014). Further, we demonstrated an enrichment of rare, protein-changing variants among patients compared to samples from the EVS; this enrichment was significant for WC patients (p<0.001).

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,[10] one would expect not more than one dystonia patient among the 4,300 European American samples from the EVS. Second, the large amount of data was generated by high throughput, next-generation 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 >27x, 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.

The lack of causal variants (mutations) in our patients might also be explained by the fact that we only sequenced the coding (exon-intron boundaries) part of *ARSG*, and not the intronic regions were regulatory domains might be present.

In conclusion, we did not identify protein changing, causal variants in *ARSG* in WC and MuD, however accumulation of rare SNVs in the coding region of *ARSG* may increase the disease risk to develop task-induced focal dystonia, especially WC. These findings suggest that task-induced focal dystonia is not a monogenic disorder, but seems to have a genetically complex etiology which fits well with the two-hit hypothesis in dystonia. Given the relatively small sample size in our study, the known population differences in frequencies of some *ARSG* variants,[6] and the possibility of causal intronic variants, further analysis of the role of ARSG in focal task specific dystonias is warranted.

Acknowledgements

We thank M. Boerrigter for practical assistance, R.J. Sinke for critically reviewing the manuscript, and J. Senior for editing of the manuscript. This work was supported by funding from the German Research Foundation (DFG) to KL (LO 1555/4-1) and from the Prinses Beatrix Fund (WAR08-06) to MAT. The authors declare that they have no conflict of interest.

References

1. Albanese, A., Bhatia, K., Bressman, S.B., Delong, M.R., Fahn, S., Fung, V.S.C., Hallett, M., Jankovic, J., Jinnah, H.A., Klein, C., et al. (2013). Phenomenology and classification of dystonia: a consensus update. Mov. Disord. *28*, 863–873.
2. Paudel, R., Hardy, J., Revesz, T., Holton, J.L., and Houlden, H. (2012). Review: genetics and neuropathology of primary pure dystonia. Neuropathol. Appl. Neurobiol. *38*, 520–534.
3. Berman, B.D., Hallett, M., Herscovitch, P., and Simonyan, K. (2013). Striatal dopaminergic dysfunction at rest and during task performance in writer's cramp. Brain *136*, 3645–3658.
4. Rietveld, A.B.M., and Leijnse, J.N. A L. (2013). Focal hand dystonia in musicians: a synopsis. Clin. Rheumatol. *32*, 481–486.
5. Schmidt, A, Jabusch, H.-C., Altenmüller, E., Hagenah, J., Brüggemann, N., Lohmann, K., Enders, L., Kramer, P.L., Saunders-Pullman, R., Bressman, S.B., et al. (2009). Etiology of musician's dystonia: familial or environmental? Neurology *72*, 1248–1254.
6. Lohmann, K., Schmidt, A., Schillert, A., Winkler, S., Albanese, A., Baas, F., Bentivoglio, A.R., Borngräber, F., Brüggemann, N., Defazio, G., et al. (2013). Genome-wide association study in musician's dystonia: A risk variant at the arylsulfatase G locus? Mov. Disord. *00*, 1–7.
7. Frese, M.-A., Schulz, S., and Dierks, T. (2008). Arylsulfatase G, a novel lysosomal sulfatase. J. Biol. Chem. *283*, 11388–11395.
8. Kowalewski, B., Lamanna, W.C., Lawrence, R., Damme, M., Stroobants, S., Padva, M., Kalus, I., Frese, M.-A., Lubke, T., Lullmann-Rauch, R., et al. (2012). Arylsulfatase G inactivation causes loss of heparan sulfate 3-O-sulfatase activity and mucopolysaccharidosis in mice. Proc. Natl. Acad. Sci. *109*, 10310–10315.
9. Abitbol, M., Thibaud, J.-L., Olby, N.J., Hitte, C., Puech, J.-P., Maurer, M., Pilot-Storck, F., Hédan, B., Dréano, S., Brahimi, S., et al. (2010). A canine Arylsulfatase G (ARSG) mutation leading to a sulfatase deficiency is associated with neuronal ceroid lipofuscinosis. Proc. Natl. Acad. Sci. U. S. A. *107*, 14775–14780.
10. Steeves, T.D., Day, L., Dykeman, J., Jette, N., and Pringsheim, T. (2012). The prevalence of primary dystonia: a systematic review and meta-analysis. Mov. Disord. *27*, 1789–1796.

Supplementary Table 1. Primer sequences for the screening of coding exons of *ARSG*. Exons 1 and 2 are non-coding and thus not analyzed.

Exon	Forward Primer	Reverse Primer	Product Length
3	AGCTGCGGTGAGGAAACAC	AGCATGGGTCCATCAATGAC	615bp
4	CCATTTCCTGACTTGTTCC	GCTGAGAGCATCCCTAACTC	458bp
5	GCTAATGGAGAGCTGAGTGTG	CTGTTCATGGAGTCTTACACAG	215bp
6	CTCCACTTCCTCCCAAACTG	TGCACAGAATGTACCGTGTATC	501bp
7	GCATTAAAGCATTTAGTTCTGC	AAGGGACATGGTGTGTGTG	283bp
8	AGAGGGAGGATCCCTGAG	CTCTGCTCTTCACAGGTAATG	347bp
9	GGATATAGGGCCAGAGTGG	AGGCAGAATCCCACACC	232bp
10	GTTGGGGTTATTGGAAACTCAG	CAAAGGCAAGTGGTGAATGAC	366bp
11	CTCTGGGCTGGTTCAGG	TGTCTGCACAGAGGTGAGAG	287bp
12	TCGACCTCCCAAGGTATTG	GGTGAGAGTCCATGGGG	247bp
13	GTAGAATCACTCGCAGCTCTC	TCCAAGACTAAACTAAAGCGTG	466bp

Supplementary Table 2: Frequency of rare (minor allele frequency <1%), protein-changing variants reported in European Americans on the EVS server (http://evs.gs.washington.edu/EVS/).

Location	dbSNP ID	cDNA change	Amino Acid change	Functional Effect	Variant alleles	Wildtype alleles	MAF
17:66303829	rs138635679	c.195T>A	D65E	missense	2	8598	0,000232558
17:66339779	rs141748845	c.253T>C	S85P	missense	1	8599	0,000116279
17:66339810	rs369315697	c.284G>A	R95Q	missense	1	8599	0,000116279
17:66339837	rs139424653	c.311G>A	R104H	missense	1	8599	0,000116279
17:66339860	rs141406045	c.334G>A	G112R	missense	2	8598	0,000232558
17:66347790	rs150020444	c.529C>T	P177S	missense	4	8596	0,000465116
17:66352808	rs368689864	c.567G>T	R189S	missense	1	8599	0,000116279
17:66352810	rs147696203	c.569A>G	N190S	missense	1	8599	0,000116279
17:66352836	rs186474907	c.595G>A	V199M	missense	3	8597	0,000348837
17:66352938	rs144846341	c.697C>T	R233C	missense	1	8599	0,000116279
17:66352941	-	c.700_701insC	S235Kfs*54	frameshift	1	8253	0,000121153
17:66364750	rs144631983	c.766G>A	V256M	missense	3	8597	0,000348837
17:66364769	rs138604336	c.785C>T	A262V	missense	1	8599	0,000116279
17:66364805	rs370504807	c.821G>A	W274*	stop-gained	2	8598	0,000232558
17:66364811	rs149548617	c.827T>C	M276T	missense	4	8596	0,000465116
17:66364886	rs372699563	c.901+1G>A	NA	splice-5	1	8599	0,000116279
17:66366621	rs374175059	c.938C>T	A313V	missense	1	8599	0,000116279
17:66366660	rs372096059	c.977G>T	R326L	missense	1	8599	0,000116279
17:66381226	rs147264809	c.1004C>T	T335M	missense	1	8599	0,000116279
17:66381279	rs376148240	c.1057G>C	V353L	missense	1	8599	0,000116279
17:66391215	rs185381597	c.1093G>A	V365M	missense	1	8599	0,000116279
17:66391272	rs150072981	c.1150C>T	R384W	missense	3	8597	0,000348837

Supplementary Table 2: Frequency of rare (minor allele frequency <1%), protein-changing variants reported in European Americans on the EVS server (http://evs.gs.washington.edu/EVS/). (continued)

Location	dbSNP ID	cDNA change	Amino Acid change	Functional Effect	Variant alleles	Wildtype alleles	MAF
17:66391275	rs370480016	c.1153C>T	R385C	missense	1	8599	0,000116279
17:66391288	rs375017370	c.1166T>C	V389A	missense	1	8599	0,000116279
17:66397562	rs140303640	c.1274T>G	L425R	missense	1	8599	0,000116279
17:66416339	rs368990704	c.1313G>C	R438T	missense	1	8599	0,000116279
17:66416349	-	c.1324del1	S443Afs*12	frameshift	1	8253	0,000121153
17:66416491	rs373638226	c.1465G>A	V489I	missense	2	8598	0,000232558
17:66416504	rs61999318	c.1478T>C	I493T	missense	26	8574	0,003023256
17:66416512	rs143160105	c.1486G>A	D496N	missense	3	8597	0,000348837
17:66416527	rs202165247	c.1501G>A	A501T	missense	2	8598	0,000232558
17:66416586	rs142933223	c.1560C>A	C520*	stop-gained	1	8599	0,000116279
17:66416588	rs150683466	c.1562G>A	R521H	missense	1	8599	0,000116279
Total					77	283031	