Genetic architecture of dystonia
Groen, Justus

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2.2

RELN RARE VARIANTS IN MYOCLONUS-DYSTONIA

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Submitted
ABSTRACT

Myoclonus-Dystonia (M-D) syndrome is a neurological movement disorder with myoclonic jerks combined with dystonia of the upper part of the body. By combining homozygosity mapping and exome sequencing we found co-segregation of a rare missense variant in the RELN gene with the MD phenotype in a 3-generation family (Thr1904Met). By screening a cohort of 24 additional M-D patients we identified 2 families (Thr1904Met, Ile1217Met) and two sporadic RELN mutation carriers (Pro1703Arg, Leu411Ile). Reelin, the protein product of RELN, is a large secreted glycoprotein that plays essential roles in the cytoarchitecture of laminated brain structures and modulation of synaptic transmission and plasticity, hallmarks of physiological findings in dystonia. We propose that RELN mutation contribute to the genetic heterogeneity of M-D.
2.2 RELN rare variants in Myoclonus-Dystonia

**MAIN TEXT**

Myoclonus-Dystonia (M-D, MIM #159900) syndrome is a rare hyperkinetic movement disorder with myoclonic jerks combined with dystonia in predominantly the upper part of the body. Symptoms often improve after alcohol ingestion and psychiatric comorbidity is prevalent. M-D patients are classified into definite, probable and possible cases based on their phenotypic presentation and family history. In as much as 21-85% of definite M-D cases mutations in the SGCE gene (epsilon-sarcoglycan, DYT11, MIM159900) can be identified. Genetic heterogeneity of M-D is illustrated with genetic linkage to chromosome 18p11 (DYT15, OMIM 607488), but thus far no additional genes have been identified. Here, by combining homozygosity mapping and exome sequencing, we identified a predicted deleterious single nucleotide variant (SNV) in the RELN gene (MIM 600514) co-segregating in a 3-generation M-D pedigree. Screening of RELN in a cohort of 24 M-D patients resulted in identification of four additional missense SNVs: two segregating with disease in respective families; two rare missense variants were present in sporadic M-D patients.

The study was approved by the local ethics committee and all participants gave informed consent. DYT11-negative M-D patients were recruited from the Academic Medical Center Movement Disorder outpatient clinic in the period June 2008 – December 2011 and clinically evaluated by a movement disorders specialist (MAJT). All patients exhibited the classical M-D phenotype with 17 having a positive family history (definite cases) and 9 being sporadic (probable cases).

We performed linkage analysis in a large M-D family (Family MD1, Figure 1) and combined these results with the exome sequence data obtained from the index case of this family (Patient IV-1).

Exome sequencing was performed on a SOLiD v4 platform (Applied Biosystems) with a mean coverage of 70.3x and 95.1% of target bases covered over 5x. Exome sequencing was performed using enriched libraries (NimbleGen SeqCap EZ Exome v2 Sequence Capture beads) sequenced on a SOLiD v4 platform (Applied Biosystems), which created paired end reads of 50 bases forward and 35 bases reverse. Colour space reads were mapped to the reference human genome (hg18) with SOLiD Bioscope software version 1.3 (Life Technologies). PCR duplicates were removed prior to variant calling using MarkDuplicates from the Picard software (picard.sourceforge.net/index.shtml). Reads were realigned around known ‘indels’ with the Genome Analysis Toolkit (Broad Institute). Variants were called using the GATK’s Unified Genotyper and GATK’s VariantAnnotator was used to annotate variant calls in preparation of filtering. ANNOVAR was used to find non-synonymous variants in coding regions that are not present in dbSNP130. A total of 12.91Gb exome data was generated, with 52.93% of the total bases mapped to the reference genome (mean coverage 70.3x and 95.1% of target bases covered over 5x). From 430,605 high quality variants generated, 14,648 variants were located in protein coding regions of the genome. Over 95% of
Figure 1. Pedigrees of MD families with RELN SNVs. Index family MD1 and family MD2 with co-segregation of RELN c.5711C>T/p.thr1904Met and family MD3 [c.3651C>G/p.Ile1217Met]. Unaffected and affected family members are indicated by clear symbols and blackened symbols, respectively. Diagonal bars through the symbols denote deceased individuals. Question marks in the symbols denote lacking information about the phenotype. + denote examined, genotyped for the RELN variant and carrying the mutation; # denote examined, genotyped for the variant in RELN, no mutation; 0 denotes examined, no genotyping performed.* denotes not tested.

these variants (n=13,987) were present in dbSNP130, while 661 were novel coding variants. Linkage analysis was performed using SNP data from Affymetrix Gene-Chip Human Mapping 250K Nsp Array using 5 affected (III-3, IV-1, IV-3, IV-5, V-1) and 3 unaffected siblings (III-8, VI-2, IV-4) from Family MD1. (Figure 1) The program PedCheck was applied to detect Mendelian errors and data for SNPs with such errors were removed from the data set, non-Mendelian errors were identified by using the program MERLIN and unlikely genotypes for related samples were deleted. Linkage analysis was performed with the program ALLEGRO making use of a reduced marker panel of approximately 20000 SNPs. The reduced marker panel was obtained by choosing SNPs with a minimal inter-marker distance of 100 kb, having a minor allele frequency of 0.15 or higher. We assumed a dominant inheritance with full penetrance and a disease allele frequency of 0.0001. AD mode of inheritance with full penetrance was assumed and a disease allele frequency of 0.0001. For the linked regions, again calculations with ALLEGRO were made with all SNPs from 250K-Chip to determine more accurately the limiting SNPs of the linkage regions. This analysis revealed 9 regions co-segregating with the phenotype in this family with a maximum parametric LOD score of 1.5. The DYT15 locus on chromosome 18p11 was not linked in this pedigree. The linkage peaks form a region of interest spanning approximately
RELN rare variants in Myoclonus-Dystonia

79.6 MB and comprised 374 coding SNV from the exome sequencing data. To reduce the number of candidate genes we applied further filtering strategies by selecting only heterozygous variants (161 SNV); from this group missense, nonsense or (possible) splice site variants (98 SNV); and subsequently variants with minor allele frequency below 0.05 (frequencies from '1000 Genome' dataset). This resulted in two rare missense SNVs: ADAM22 c.G2666A/p.R889Q (Chr 7, Refseq NM_021722) and RELN c.C5711T/p.Trp1904Met (rs114190729, Chr 7, Refseq NM_005045.3) segregating with disease in this family. The variant in RELN, and not in ADAM22 was also present in an additional affected family member (III-4) included later in the study. RELN Thr1904Met had a MAF of 0.002 (3/1410) in healthy controls from the Dutch Blood Bank (Custom TaqMan assay, Applied Biosystems, Life Technologies) and was predicted to be damaging (SIFT and PolyPhen).

Subsequently we screened all 65 exons of RELN (cDNA length of 10.383bp) in a cohort of 24 SGCE-negative patients with a probable and definite M-D phenotype (Table 1) using standard PCR amplification and Sanger sequencing. (ABI big dye v3.1 chemistry, ABI 3730 capillary system, Applied Biosystems).
Here, we identified 4 additional rare, missense variants in the RELN gene: c.5711C>T/p.Thr1904Met (rs114190729, identical to the variant found in Family MD1), c.5108C>G/p.Pro1703Arg (rs2229860), c.3651C>G/p.Ile1217Met (rs56342240) and c.1231C>A/p.Leu411Ile (rs144978163). Patients carrying Thr1904Met and Ile1217Met were familial cases (MD family 2 and 3, respectively. See Figure 1). Co-segregation of these variants with the phenotype was confirmed using Sanger sequencing: in MD family 3 (Ile1217Met), six family members were tested. All 3 affected members carried the variant; the 3 unaffected siblings did not. In MD family 2 (Thr1904Met), 10 family members were available for testing (3 affected, 7 unaffected). The three affected members all carried the RELN Thr1904Met SNV. However, 3 of 7 tested unaffected individuals (IV-6, IV-7 and V-4) also carried this SNV, suggesting reduced penetrance of the M-D phenotype associated with the RELN SNV in this pedigree. At the time of examination the unaffected mutation carriers were 59, 71 and 45 years old, respectively (see Figure 1). A reduced penetrance is present in all familial dystonias, like DYT6 (mutations in THAP1 have a 60% penetrance) and DYT1 (the delGAG mutation in TOR1A has a 30% penetrance). In addition, we identified two sporadic M-D patients carrying rare missense variants: MD-ID1 with young onset M-D carried Pro1703Arg and Leu411Ile was identified in MD-ID2. More difficult to interpret, is a non-coding variant at c.806-170T>A found in two patients (MD-fam4 and MD-ID3, MD-ID4).

### Table 2.

<table>
<thead>
<tr>
<th>Patient/Family ID</th>
<th>rs</th>
<th>SNV (NM_005045)</th>
<th>MAF Blood bank Controls</th>
<th>MAF ESP American Europeans</th>
<th>SIFT/ PolyPhen-2 (HumDiv)</th>
<th>Grantham distance</th>
</tr>
</thead>
<tbody>
<tr>
<td>MD-fam1, MD-fam2</td>
<td>rs114190729</td>
<td>c.5711C&gt;T/p.Thr1904Met</td>
<td>0.0010 (A=3/G=1407)</td>
<td>0.0004 (A=4/G=8596)</td>
<td>D/P.D.</td>
<td>81</td>
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<tr>
<td>MD-fam3</td>
<td>rs56342240</td>
<td>c.3651C&gt;G/p.Ile1217Met</td>
<td>0.0005 (C=4/G=795)</td>
<td>0.0053 (C=46/G=8554)</td>
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<td>103</td>
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<td>c.5108C&gt;G/p.Pro1703Arg</td>
<td>0.0005 (C=4/G=795)</td>
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<td>D/P.D.</td>
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<td>MD-ID2</td>
<td>rs144978163</td>
<td>c.1231C&gt;A/p.Leu411Ile</td>
<td>0.0001 (T=1/G=8599)</td>
<td>0.0009 (C=8/T=8592)</td>
<td>D/P.D.</td>
<td>5</td>
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<td>MD-fam4</td>
<td>rs141617635</td>
<td>c.4275A&gt;G/p.Gly1425=?</td>
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<td>-</td>
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<td>MD-ID3, MD-ID4</td>
<td>-</td>
<td>c.806-170T&gt;A/p.=?</td>
<td>-</td>
<td>-</td>
<td>unknown</td>
<td>NA</td>
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### Rare SNV (MAF<0.01)

<table>
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<tr>
<th>Patient/Family ID</th>
<th>rs</th>
<th>SNV (NM_005045)</th>
<th>MAF Blood bank Controls</th>
<th>MAF ESP American Europeans</th>
<th>SIFT/ PolyPhen-2 (HumDiv)</th>
<th>Grantham distance</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 patients</td>
<td>rs362691</td>
<td>c.2989C&gt;G/p.Leu997Val</td>
<td>-</td>
<td>0.1038 (C=893/G=7707)</td>
<td>benign</td>
<td>32</td>
</tr>
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</table>

Common SNV

<table>
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<tr>
<th>Patient/Family ID</th>
<th>rs</th>
<th>SNV (NM_005045)</th>
<th>MAF Blood bank Controls</th>
<th>MAF ESP American Europeans</th>
<th>SIFT/ PolyPhen-2 (HumDiv)</th>
<th>Grantham distance</th>
</tr>
</thead>
<tbody>
<tr>
<td>MD-ID1</td>
<td>rs141617635</td>
<td>c.4275A&gt;G/p.Gly1425=?</td>
<td>-</td>
<td>-</td>
<td>unknown</td>
<td>NA</td>
</tr>
</tbody>
</table>
ID3, MD-ID4; Table 2) which is predicted to activate a cryptic splice site. This variant was not found in 1080 Dutch Blood Bank controls or in the 1000 Genomes database (www.1000genomes.org). Moreover, one patient with familial M-D (MD-fam4) carried the synonymous variant c.4275A>G/p.Gly1425Gly (rs141617635) with possible effect on splicing. However, this variant did not show co-segregation with phenotype in the three generation family of this patient. The common missense SNV RELN Leu-997Val (rs362691, MAF 0.104 in 1000 Genomes) was found in 10/25 M-D patients.

Clinically, a homogeneous phenotype for RELN mutation carriers is present (Textbox 1, Table 3). Summarizing the clinical findings, RELN missense variant carriers present with myoclonus and mild dystonia of the neck and upper limbs. In addition, a high rate of anxiety and depressive disorders were present in the RELN mutation carriers. Stress was an aggravating factor in all RELN M-D patients and alcohol had a registered positive effect on motor symptoms in 6/13 carriers. Magnetic resonance imaging of the brain of index patient of MD family 1 does not show cerebellar or cortical abnormalities.

Textbox 1. Clinical description of M-D patients with RELN rare missense variants.

The index patient of family MD1 (c.5711C>T/p.thr1904met) is a 55 year old woman with myoclonic jerks of her hands since age 30 and jerky dystonia of the neck since age 40 years. Her clinical history reveals periods of depression and anxiety. She does not use alcohol and is not on any medication. Her father (III-3) is 78 years old and suffers from low frequency myoclonic jerks in trunk and neck (EMG: 2-3Hz jerks, posture dependence) with mild dystonic posture since his fifties. Alcohol use results in instant relieve of the symptoms. Patient’s history shows cardiac arrhythmias, treated with ablation therapy, and panic attacks. Her son (V-1) developed myoclonic jerks of his arms at very young age. On examination at age 13 he has myoclonus of his trunk, arms and legs and dystonia of the neck and trunk, as well as writer’s cramp. Two sisters of the index patient show cervical dystonia starting at age 35 and 20 and mild myoclonic jerks of the arms. One of the sisters (IV-5) has experienced panic attacks and suffers of compulsive behavioural problems.

The index patient of Family MD2 (c.5711C>T/p.thr1904met) is a 71 year old man with jerky movements of both hands since age 12 years. A decade ago he developed additional shaking of the head and abnormal posture of the neck, worsening with stress. Alcohol has a positive effect on complaints and sensory trick is noticed to relieve his neck dystonia. His sister (IV-3) has a jerky cervical dystonia and tremulous hands. Also a brother was affected with cervical dystonia, treated with botulinum toxin injections. He passed away before examination. His father (III-5) was reported to have some tremulousness of the hands and some slight lateroflexion of the neck, however he passed away before examination. Three sisters and two brothers of his father were also affected. The youngest sister has one affected daughter, with complaints jerks that mainly occurs in the upper limbs and trunk. Also the mother of his father had similar complaints.
The 44 year old female index of Family MD3 (c.3651C>G/p.Ile1217Met) has jerky movements of her arms in posture and rest as long as she can remember. Additionally, she has an intermittent myoclonus of her left shoulder since 10 years. Alcohol diminishes the symptoms; however the jerks do not disappear. Patient has two sons who have similar complaints since birth. In her fathers family schizophrenia is present in two uncles (no DNA available). She previously was on levodopa without any effect on the jerks or tremor. With examination she has myoclonic jerks of face musculature and distal upper limbs, left more than right sided. The jerks of the arms are stimulus sensitive. There is a dystonic endorotation of the left arm and cervical dystonia. Both sons have a disabling jerky dystonia of the upper limbs. There is a history of increased startling in all affected family members.

Sporadic M-D patient MD ID1 (c.5108C>G/p.Pro1703Arg) is a 28 year old man complaints started at age 15 with myoclonic jerks of both hands, worsening with action and stress. Over a course of months additional complaints of jerky dystonia of the neck developed. Alcohol had a positive effect on jerks. No medication was used. Family history is negative for movement disorders. Patient MD ID2 (c.1231C>A/p.Leu411Ile) is a 44 year old woman with a jerky dystonia of the neck since age 28. She does not drink any alcohol, so alcohol response is unknown. She has a history of migraine with aura. EMG of neck musculature shows tonic activity in sternocleidomastoid, splenic and semispinal muscles, especially on the left side. Also spontaneous series of short jerks (30-50 msec) are observed in mentioned muscles. Family history is negative for movement disorders.

The RELN M-D patients seem to have a higher age at onset and a milder course of disease compared to SGCE M-D patients. In SGCE M-D over 80% of patients have disease onset under 18 years of age. In our study, mean age at onset was 22 years, with the oldest being 53 years of age at the onset of motor symptoms. Only in the RELN family MD3 (Ile1217Met) two patients had a young age at onset. Psychiatric abnormalities are common among M-D patients, both in RELN (Table 2) and SGCE M-D patients. Studies on common genetic variation in RELN have shown association with a variety of psychiatric abnormalities, like schizophrenia, autism, bipolar disorder, major depression and pain sensitivity. Also some functional evidence for a relation of reelin dysfunction with psychiatric disorders is published.

Reelin is a large secreted glycoprotein that plays essential roles in the cytoarchitecture of laminated brain structures. The protein is secreted by Cajal-Retzius cells, cortical and hippocampal GABAergic interneurons and cerebellar granule cells. In neuronal development, reelin controls the migration and positioning of cortical neurons and the foliation of the cerebellum via phosphorylation of tau. It interacts with the receptors apolipoprotein E receptor 2 (ApoER2) and very low density lipoprotein receptor (VLDLR) on target neurons; binding of reelin to these receptors induces tyrosine phosphorylation of Dab1. This results in (among others) activation of a kinase cascade including phosphatidylinositol-3-kinase (PI3K) and protein kinase B (PKB/AKT), ultimately leading to the inhibition of glycogen synthase kinase (GSK3b) which normally phosphorylates the microtubule stabilizing protein tau. This cascade promotes neuronal migration. The DYT1-associtiated torsinA also
Table 3. Summary of clinical presentation of all RELN rare SNVs carriers. Three families and two sporadic MDS patients with RELN mutations and MDS. M=myoclonus, D=dystonia, AaO=age at onset, NP complaints=neuropsychiatric complaints).

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Myoclonus (M)</th>
<th>Dystonia (D)</th>
<th>AaO</th>
<th>Site at onset</th>
<th>Medication</th>
<th>Alcohol responsive</th>
<th>Aggravating factors</th>
<th>Other NP complaints</th>
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<tr>
<td><strong>Family 1</strong> (Thr1904Met)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>IV-1</td>
<td>56</td>
<td>f</td>
<td>hands, neck</td>
<td>neck</td>
<td>43</td>
<td>hands (M)</td>
<td>BonT-A</td>
<td>unknown</td>
<td>stress, action</td>
<td>anxiety, depression</td>
</tr>
<tr>
<td>V-1</td>
<td>19</td>
<td>m</td>
<td>hands, arms, trunk, neck</td>
<td>hands, trunk, neck</td>
<td>2</td>
<td>hands (M)</td>
<td>Artane</td>
<td>unknown</td>
<td>stress, action</td>
<td>anxiety</td>
</tr>
<tr>
<td>III-3</td>
<td>79</td>
<td>m</td>
<td>hands, trunk, neck</td>
<td>neck, trunk</td>
<td>53</td>
<td>trunk (M)</td>
<td>none</td>
<td>unknown</td>
<td>good</td>
<td>stress</td>
</tr>
<tr>
<td>IV-3</td>
<td>51</td>
<td>f</td>
<td>none</td>
<td>neck</td>
<td>35</td>
<td>neck (D)</td>
<td>none</td>
<td>not clear</td>
<td>unknown</td>
<td>panic attacks</td>
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<td>37</td>
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<td>20</td>
<td>neck (D)</td>
<td>none</td>
<td>good</td>
<td>stress, action</td>
<td>anxiety</td>
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<td><strong>Family 2</strong> (Thr1904Met)</td>
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<tr>
<td>IV-2</td>
<td>71</td>
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<td>neck</td>
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<td>none</td>
<td>good</td>
<td>stress, action</td>
<td>depression, anxiety</td>
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<td>68</td>
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<td>stress</td>
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<tr>
<td>II-1</td>
<td>44</td>
<td>f</td>
<td>hands, face</td>
<td>neck, arms, voice</td>
<td>3</td>
<td>hands (M)</td>
<td>BoNT-A</td>
<td>some</td>
<td>stress, action</td>
<td>anxiety, enhanced startle reflex</td>
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<tr>
<td>III-1</td>
<td>15</td>
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<td>III-2</td>
<td>14</td>
<td>m</td>
<td>hands, legs</td>
<td>hands</td>
<td>4</td>
<td>legs (M)</td>
<td>none</td>
<td>unknown</td>
<td>action, reflex (?)</td>
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<tr>
<td><strong>Non-familial</strong></td>
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<td>MD-ID1 (Pro1703Arg)</td>
<td>28</td>
<td>m</td>
<td>hands</td>
<td>neck</td>
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<td>stress, action</td>
<td>none</td>
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<td>f</td>
<td>hands, neck, trunk, neck</td>
<td>neck</td>
<td>28</td>
<td>neck (D)</td>
<td>none</td>
<td>unknown</td>
<td>unknown</td>
<td>migraine, enhanced startle reflex</td>
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relates to microtubule dynamics and intracellular trafficking: torsinA binds to kinesin light chain 1 (KLC1, a subunit of the kinesin 1 motor complex)\(^1\) and is an important component of the cytoskeletal network, responsible for maintaining the shape of the nuclear envelope and endoplasmatic reticulum.\(^1\) Recently, mutations in beta-tubulin 4a (TUBB4), coding for a major constituent of microtubules, have been found to cause DYT4 dystonia\(^1\), pointing to the importance of microtubule dynamics in dystonia pathophysiology. The reeler mouse, an autosomal recessive reln mutant mouse, is the first described movement disorder mouse model\(^1\), showing an ataxic and ‘reeling’ gait. Brain tissue samples of this mouse show structural alterations owing to a defect in positioning of neurons in the cerebral cortex and the cerebellum.\(^1\) In human, patient with homozygous RELN mutations present with a developmental brain disorder showing lissencephaly with severe cerebellar hypoplasia\(^1\), showing the importance of reelin in cerebellar development. Cerebellar dysfunction has increasingly gain attention as important in myoclonus-dystonia aetiology\(^1\)–\(^2\) also in relation to improved symptoms after alcohol ingestion seen in SGCE and RELN mutation carriers.

Modulation of synaptic transmission and plasticity are the hallmarks of electrophysiological findings in dystonia.\(^2\) The high expression of reelin in GABAergic interneurons suggests a role for reelin in the inhibitory neurotransmission in the adult brain.\(^1\) Also, functional studies show that reelin modulates synaptic transmission and plasticity\(^2\) (reviewed by Herz and Chen\(^8\)). Moreover, in the heterozygote reeler mice an altered plasticity is exhibited.\(^2\)

In contrast to the M-D patients in the presented autosomal dominant families, reports of heterozygous carriers in autosomal recessive families with variable mutations in RELN did not show movement disorders.\(^1\)–\(^2\) The absence of motor deficits in these heterozygous mutation carriers could on clinical level relate to difficulties in the diagnosis of the mild M-D phenotype, especially in the adult onset cases. Alternatively it could be due to the complex signaling mechanisms of reelin: intact reelin forms a covalent dimer via intermolecular disulfide bonds. It seems that a special higher order structure maintained by both covalent and non-covalent intermolecular interactions is required for the full activity.\(^2\) In reeler mice, heterozygosity (heterozygote reeler mouse, HRM) presents with both anatomical changes, such as decreased frontal dendritic spine density\(^2\)), alteration of plasticity\(^2\) and behavioral abnormalities, such as decreased prepulse inhibition and fear conditioning defects.\(^2\)

Although there is evidence that rare SNVs in RELN contribute to the M-D, this study has limitations. With an exome coverage of about 95% we might have missed the true disease-causing mutation. Because analysis was focused on rare, protein-changing variants under the assumption of an autosomal dominant mode of inheritance. It is possible, that other (non-coding) variants are linked to the M-D phenotype in these patients. However, the frequency of missense variants in our cohort and the co-segregation with the disorder in the pedigrees favors a role for Reelin in M-D.
We show that rare missense variants in RELN are associated with M-D syndrome. RELN mutations segregate in an autosomal dominant fashion with disease in three M-D families and are present in two additional sporadic M-D patients. To further understand the importance of RELN in M-D pathophysiology, additional DYT11-negative patient cohorts need to be screened for RELN variants. Also, functional studies need to investigate the effect of identified variants on Reelin protein function and whether there is a link to the other M-D associated protein epsilon-sarcoglycan.

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