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TITLE PAGE

Truncating *SLC12A6* variants cause different clinical phenotypes in humans and dogs

running title: SLC12A6 variants in humans and dogs

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ABSTRACT

Clinical, pathological and genetic findings of a primary hereditary ataxia found in a Malinois dog family are described and compared with its human counterpart. Based on the family history and the phenotype/genotype relationships already described in humans and dogs, a causal variant was expected to be found in *KCNJ10*. Rather surprisingly, whole exome sequencing identified the *SLC12A6* c.178_181delinsCATCTCACTCAT (p.(Met60Hisfs*14)) truncating variant. This loss-of-function variant perfectly segregated within the affected Malinois family in an autosomal recessive way and was not found in 562 additional reference dogs from 18 different breeds, including Malinois. In humans, *SLC12A6* variants cause “agenesis of the corpus callosum with peripheral neuropathy” (ACCPN, alias Andermann syndrome), due to a dysfunction of this K^+-Cl^- cotransporter. However, depending on the variant (including truncating variants), different clinical features are observed within ACCPN. The variant in dogs encodes the shortest isoform described so far and its resultant phenotype is quite different from humans, as no signs of peripheral neuropathy, agenesis of the corpus callosum nor obvious mental retardation have been observed in dogs. On the other hand, progressive spinocerebellar ataxia, which is the most important feature of the canine phenotype, hindlimb paresis and myokymia-like muscle contractions have not been described in humans with ACCPN so far. Since this is the first report of a naturally occurring disease-causing *SLC12A6* variant in a non-human species, the canine model will be highly valuable to better understand the complex molecular pathophysiology of *SLC12A6*-related neurological disorders and to evaluate novel treatment strategies.

Keywords: *SLC12A6, KCC3,* Andermann syndrome, hereditary ataxia, Malinois dogs,

causal variant.

INTRODUCTION

It has long been proven that research on human and canine diseases can benefit from each other because humans and dogs share hundreds of analogous diseases. On the one hand, existing knowledge typically flows towards canine research, because human diseases are far more studied than canine diseases. On the other hand, new candidate genes for complex and/or rare human diseases are easier identified in dogs because they are often monogenic and common in dog breeds, and the availability of cells or tissues from a canine model might aid in the characterization of the underlying pathophysiology especially when appropriate human material is scarce.[1]

This also applies to hereditary ataxias, a very heterogeneous group of neurological spinocerebellar disorders characterized by a lack of coordinated muscle movement. Ataxia can be present as an isolated symptom or as part of a syndrome. In the more than 100 described human hereditary ataxias, similar phenotypes can be caused by variants in different genes and different variants in the same gene can cause different phenotypes.[2] Described genetic variants, often in genes from conserved pathways, are repeat expansions, SNVs and INDELs, and follow a dominant, recessive, X-linked or mitochondrial inheritance. The prevalence of hereditary ataxias in humans varies in different populations, but ranges between 1-9 in 100 000 (ref. 3).

Ataxia-related phenotypes are also described in several dog breeds, with causal variants in *KCNJ10*, *GRM1*, *ITPR1*, *SNX14*, *SPTBN2*, *CAPN1*, *ATP1B2*, *RAB24* and *SEL1L*. Some of the *KCNJ10* variants have been associated with a particular syndrome known as spinocerebellar ataxia, myokymia, seizures or both (SAMS) in Jack Russel Terriers[4] and Malinois[5] dogs. Variants in the first 5 genes are also described to cause a

spinocerebellar ataxia phenotype in humans, a variant in *CAPN1* has been reported to cause spastic paraplegia, but in the last 3 genes no human counterparts have been identified yet (Supplementary Information File 1).

Here we report a new ataxia-related phenotype of slowly progressive spinocerebellar ataxia, paraparesis and myokymic-like muscle contractions in a Malinois dog family caused by a truncating *SLC12A6* variant, and compared it with its human counterpart.

MATERIALS AND METHODS

Clinical examination

Four 6-12 month old intact Malinois dogs (3 males and 1 female) from 2 related litters were presented at the Small Animal Department of the Faculty of Veterinary Medicine of Ghent University for an uncoordinated gait since the age of 3-6 months. Two additional affected littermates were seen on video footage and blood was collected from 1 of them. A clinical and neurological examination was performed on all presented dogs. A complete blood count and serum biochemistry (including glucose and Na^+, K^+, Cl^-, Ca^{2+} and Mg^{2+} electrolytes) was obtained from 5 dogs. Cerebrospinal fluid (CSF) analysis was performed in 3 dogs. Urinalysis (including electrolyte clearance for Na^+, K^+, Cl^- and Ca^{2+}) was performed in 2 dogs. Electromyography (EMG) and motor nerve conduction velocity (MNCV) studies of the sciatic nerves were performed under general anesthesia in 2 dogs. EMG recordings were made from facial, truncal and appendicular muscles of the front and hind limbs. Brainstem auditory evoked responses (BAER) was performed under sedation in 1 dog. Magnetic resonance imaging (MRI; 0.2 Tesla magnet) of the brain and complete spinal cord was done in 1 dog. A commercially available electrophysiological unit (Natus Synergy UltraPro, Acertys Healthcare NV, Aartselaar, Belgium) was used for electrodiagnostic recordings. A summary of the

clinical investigations performed in each of the 6 affected Malinois dogs (Figure 1) is shown in Supplementary Information File 2.

Pathological examination

Post-mortem examination was performed in the 4 presented dogs, immediately after euthanasia. Both central and peripheral nervous tissue samples and skeletal muscle samples were collected, fixed in 10% neutral buffered formaldehyde, embedded in paraffin, sectioned at 5 µm and stained with haematoxylin and eosin (H&E). Additional histochemical stainings on selected sections included luxol fast blue (LFB) and toluidine blue (TB) on semi-thin sections. Immunohistochemistry (IHC) was performed for neurofilament (monoclonal mouse anti-human neurofilament protein clone 2F11, Cat No. M076229-2, Dako, Glostrup, Denmark), synaptophysin (monoclonal mouse anti-human synaptophysin clone DAK-SYNAP, Cat No. M731501-2, Dako, Glostrup, Denmark), glial fibrillary acidic protein (polyclonal rabbit anti-glial fibrillary acidic protein, Cat No. Z033401-2, Dako, Glostrup, Denmark) and ubiquitin (rabbit polyclonal ubiquitin IHC antibody, Cat No. IHC-00420, Bethyl laboratories, Montgomery TX, USA).

Genetic analysis

EDTA blood was sampled from 5 affected Malinois and 13 healthy family members (Figure 1). In addition, EDTA blood was sampled from 118 reference Malinois dogs and 444 reference dogs from 17 breeds related to Malinois or known to suffer from ataxia (Supplementary Information File 3). DNA isolation from blood and the structural variant analysis of *KCNJ10* was performed as described in Van Poucke *et al*[5]. Details about the known ataxia-related causal variants in dogs are described in Supplementary Information File 1. Whole exome sequencing was performed as described in Broeckx *et*

130 *al*[6]. The reads were aligned to the reference genome using BWA v0.7.15 (ref. 7).
131 Duplicate reads were marked with Picard tools v2.1.1. Using the GATK v3.8-0, variants
132 were called according to the GATK Best Practices.[8] From the total list of putative
133 variants, only those were retained that (1) passed the "hard" quality filter suggested
134 from the GATK Best Practices, (2) were not found in an internal variant database, nor in
135 the public database of the online Variant Effect Predictor tool, (3) followed an
136 autosomal recessive mode of inheritance and (4) were predicted to be nonsynonymous.[9-
137 [10] Filtering was performed with VCFtools v0.1.14 and custom R-scripts.[11] A genotyping
138 assay for the new variant is described in Supplementary Information File 4.
139 Cerebrum (unaffected), cerebellum (affected) and cervical spinal cord (affected) tissue
140 samples were taken from 2 affected dogs. Tissue sampling, RNA isolation and cDNA
141 synthesis were performed as described by Van Poucke *et al*[12]. RT-qPCR assays are
142 described in Supplementary Information File 5.

143 **RESULTS**

144 **Clinical features**

145 Clinical examination was unremarkable in all presented dogs, except for a mild
146 palmigrade stance. Neurological examination revealed a severe generalized hypermetric
147 ataxia (worse on the hindlimbs) associated with a mild to moderate degree of
148 paraparesis and absent patellar reflexes in all dogs (Supplementary Information File 6a).
149 Generalized (including tongue and eyelids) involuntary vermicular muscle contractions,
150 strongly resembling myokymia but only triggered by sedation, were also seen in 3 dogs
151 (Supplementary Information File 6b).
152 Complete blood count and serum biochemistry were normal. Urinalysis was
153 unremarkable. EMG was silent in all clinically normal muscles. Unfortunately, EMG

could not be performed in the muscles with involuntary vermicular contractions as those were short-lasting and transient. MNCV and BAER did not differ from age-matched Malinois control dogs. MRI of the brain and whole spinal cord and CSF analysis were unremarkable.

The ataxia, paraparesis and palmigrade stance of all dogs progressively worsened, resulting in non-ambulatory paraparesis by 2.5-3 years of age (Supplementary Information File 6c) and therefore euthanasia was performed.

Pathological features

Weight of the 4 presented dogs ranged from 22 to 26 kg at the time of euthanasia (weight of healthy littermates at about the same age ranged between 28 and 35 kg). At necropsy, no gross abnormalities were noted. The histopathologic findings were consistent with a severe bilateral symmetrical axonopathy of the white matter, characterized by prominent axonal swelling with vacuolation, affecting the whole spinal cord, as well as the dorsal and ventral nerve roots, brain stem and cerebellum (in declining order of severity). Cerebrum, peripheral nerves and skeletal muscles showed no or rare abnormalities. The lesions showed variation both in degree and in their location along the spinal cord, as well as within the same animal as in different animals, but the same pathways were consistently affected in all dogs. The most severe lesions were noted in some descending motor pathways (prominent in the ventral corticospinal tract and the vestibulospinal tract and to a lesser extent the lateral corticospinal tract) as well as in some sensory ascending pathways (prominent in the dorsal spinocerebellar tract and some mild lesions in the ventral spinocerebellar tract). The lesions consisted of sharply delineated swollen axons, often appearing optically empty (‘axonal vacuoles’) yet sometimes filled with a light eosinophilic, amorphous to slightly granular material

('axonal spheroids'). Axonal swelling was often extreme with diameters up to 140 µm. Neuronal perikarya in the cerebrum, cerebellum, brain stem, spinal cord, and dorsal root sensory ganglia appeared normal, except for the presence of some slight perikaryal retraction, probably representing an artefact. LFB stains and semi-thin sections stained with TB demonstrated well preserved myelin sheaths, both in the central and peripheral nervous system, surrounding the normal axons, the axonal spheroids and the empty vacuoles. Only around the extremely dilated axons, the myelin sheath appeared thinned or could not be visualized. IHC for neurofilament revealed a dilated aspect of almost all axons in the affected areas and scattered, extremely large axonal spheroids (diameter up to 140 µm), compatible with the large 'eosinophilic material filled spheroids' on H&E. The latter were also highlighted on IHC for synaptophysin, suggesting this to be axoplasm of severely dilated axons. No abnormalities were detected in the spinal cord neuronal cell bodies with synaptophysin or ubiquitin IHC, and no areas of gliosis were noted on glial fibrillary acidic protein IHC. See Supplementary Information File 7 for the histopathological lesions on H&E, and on IHC for neurofilament and synaptophysin.

Genetic analysis

Because of the similarities of the syndrome in the dogs of the present study with SAMS and the fact that SAMS was so far only associated with *KCNJ10* variants in dogs, we first tested the 3 previously described *KCNJ10* variants in the affected Malinois dogs. The c.627C>G[4] and c.986T>C[5] variants were not present. One dog carried 1 allele of the g.22141027insC[13] variant. Although this variant is not causal in heterozygous state, we analyzed this variant in the rest of the affected family and in 57 additional reference Malinois dogs, because it was the first time that this variant was detected in the Malinois breed. Also the healthy father of the affected Malinois dog and a healthy

offspring of that father carried 1 allele (Figure 1), and the allele frequency in the reference Malinois dogs was 4.4%. In addition, we did not find any of the 8 other described ataxia-related canine variants in the affected Malinois dogs (Supplementary Information File 1). Next, we followed the candidate gene approach and performed a structural variant analysis on *KCNJ10* in 1 affected Malinois dog. Since no potential causal variants were found, whole exome sequencing was performed on 4 animals (2 healthy parents and 2 affected siblings; Figure 1). A frameshift inducing INDEL in *SLC12A6* was further investigated as the most likely causal variant after filtering.

SLC12A6 (solute carrier family 12 member 6, alias *KCC3*; Gene ID: 478239) is located on canine chromosome 30. Its canonical transcript is encoded in 25 exons and is translated into a 1151 aa long integral transmembrane protein involved in K^+-Cl^- cotransport, predicted to contain 12 transmembrane domains and large hydrophilic intracellular termini. Alternative transcripts, caused by alternative promoters, alternative transcript initiation sites, alternative exons (e.g. exon 1a and 1b) and alternative splicing (e.g. exon 2), give rise to a complex mix of isoforms in many tissue/cell types.[14-16]

The INDEL involves a 12-bp insertion (CATCTCACTCAT) and a 4-bp deletion (ATGA), most probably generated by a template switch process with an inverted repeat and an inverted spacer (Figure 2). The variant is located in exon 1a and causes a frameshift at codon 60 leading to a premature stopcodon 14 codons downstream in all transcripts containing exon 1a (Figure 2). The *SLC12A6* c.178_181delinsCATCTCACTCAT (p.(Met60Hisfs*14)) variant was deposited in the EVA database (Project: PRJEB30850; Analyses: ERZ802317).

From the 18 sampled Malinois family members, all 5 affected dogs were homozygous for the variant allele, 10 healthy dogs carried 1 variant allele and 3 healthy dogs did not

carry the variant allele, following perfectly an autosomal recessive segregation (Figure 1). The variant was not found in 118 additional reference Malinois dogs, neither in 444 reference dogs from 17 breeds related to Malinois or known to suffer from ataxia (Supplementary Information File 3).

Because the variant does not affect transcripts containing exon 1b, RT-(q)PCR was performed, focusing on the first exons, to identify which *SLC12A6* transcript variants (TVs) are transcribed in affected tissues (cerebellum and the cervical spinal cord) compared to unaffected tissue (cerebrum) from 2 affected Malinois dogs. Sequencing RT-PCR products identified TVs starting with both exon 1a or 1b, and both with or without exon 2 (data not shown). To quantify these end-point detection results, we performed RT-qPCR with specific assays for the 4 observed TVs. Both TVs containing exon 1a (with or without exon 2) were highly transcribed, while both TVs containing exon 1b were very weakly transcribed (at least 50 fold less) in all 3 investigated tissues from both animals (Supplementary Information File 5).

DISCUSSION

SLC12A6 encodes 1 of the 4 distinct K^+-Cl^- cotransporters that belong to the cation-Cl^- cotransporter family. Their structure, function and regulation are highly conserved across evolution, and despite their high homology, they exhibit unique patterns of distribution and fulfill distinct biophysical and physiological roles.[16-17] The functional properties of SLC12A6 are even more complex because it can exist in many isoforms that can be organized as homo- or hetero-oligomers with other cation-Cl^- cotransporters.[18] The SLC12A6 cotransporter is broadly expressed throughout the brain, spinal cord and peripheral nervous system, amongst other various tissue locations.[16,19] It is inactive under isotonic conditions, but gets activated (by dephosphorylation of its C-

terminus) upon cell swelling where it regulates cell volume by the efflux of K^+ and Cl^- ions together with water molecules across the plasma membrane. It is therefore believed to have a key role in cell volume homeostasis and neuronal activity control.[16-18]

Naturally occurring disease-causing variants in *SLC12A6* are so far only described in humans, where they cause "agenesis of the corpus callosum with peripheral neuropathy" (ACCPN, alias Andermann syndrome; phenotype MIM number 218000).[20] It is a rare (prevalence rate of less than 1 in 1 000 000 individuals worldwide)[16], multisystemic disorder, characterized by sensorimotor polyneuropathy, variable degree of agenesis of the corpus callosum, mental retardation and dysmorphic features.[21-22] Psychotic episodes with visual and auditory hallucinations also have been reported.[23] The histopathologic lesions of ACCPN are a combination of axonal degeneration (axonal spheroids) with variable myelin swelling or loss (dependent on the location). They are most pronounced in the peripheral nervous system with progression to axonal loss and endoneurial and perineurial fibrosis. There is no obvious damage to neurons, no evidence of active myelin degradation or inflammation. Muscle biopsies show signs of denervation such as mildly atrophic and angulated fibers.[22,24-25]

Most causal variants associated with ACCPN are randomly distributed truncating variants, due to premature stop codons caused by INDEL, nonsense or splice site variants (recessive; homozygous or compound heterozygous).[16-18,20,26] Contrary to what would be expected, *SLC12A6* mRNAs harboring premature termination codons are not degraded by nonsense-mediated mRNA decay, but are translated as truncated proteins.[26] They are associated to loss-of-function because of an aberrant structure or a defective transit to the plasma membrane.[27] Interestingly, Uyanik *et al*[28] described a recessive missense variant (p.Arg207Cys, modifying a region crucial for oligomerization)

associated with a milder form of ACCPN, and Kahle *et al*[29] a dominant missense variant (p.Thr991Ala, abolishing a phosphorylation site crucial for deactivation) associated with a distinct form of ACCPN due to a gain-of-function. Despite research on human patients with inherited disease-causing variants or experiments in mouse, Xenopus, Caenorhabditis and Drosophila model systems, the underlying pathological mechanisms that account for the neurological manifestations of ACCPN are still not clearly understood.[17-18,30]

Here, we describe the first non-human naturally occurring truncating *SLC12A6* variant, segregating in a Malinois dog family descending from a common ancestor, due to an INDEL in exon 1a, causing a frameshift at codon 60 and resulting in a premature stopcodon after 13 aberrant codons. It only affects TVs containing exon 1a and encodes the shortest truncated SLC12A6 protein reported so far, comprising only a part of the intracellular N-terminus (Figure 2). As such, it can be considered as a loss-of-function variant. As in ACCPN patients, affected Malinois dog family members are homozygous for the truncating *SLC12A6* variant, and heterozygotes are asymptomatic carriers. Because the *SLC12A6* variant was only found in the affected Malinois family, it is likely a private variant because of a founder effect. In contrary, the g.22141027insC variant[13] has an estimated frequency of 4.4% in the Belgian Malinois population and should be taken into account in breeding schemes.

In agreement with what has been seen in humans[26], RT-qPCR results show that *SLC12A6* mRNAs harboring a premature termination codon (i.e. TV1 and TV2) are not degraded by nonsense-mediated mRNA decay in dogs either, and will probably be translated into truncated proteins as well. The fact that the levels of transcripts containing exon 1b (i.e. TV3 and TV4) are low (at least 50 times lower than transcripts

containing exon 1a) and unchanged in affected tissues compared to unaffected tissue, makes it very unlikely that they can take over the role of the predominant transcripts containing exon 1a.

Electrophysiological findings associated with ACCPN in humans are abnormal resting activity on EMG, increased duration of motor unit potentials, increased polyphasia, decreased MNCV and absent sensory action potentials.[21-22] Electrophysiology was entirely normal in the 2 investigated dogs, and furthermore no signs of sensorimotor neuropathy were found on histopathology. As EMG confirmation of myokymia could not be obtained in the muscles with involuntary vermicular contractions, we decided to refer to those as “myokymic-like muscle contractions”.

Homozygous *SLC12A6* global knockout in mice has been reported to reproduce the typical ACCPN sensorimotor neuropathy, as well as neurogenic hypertension, age-related deafness, renal dysfunction and a reduced threshold to develop epileptic seizures.[31] However, only minor changes of the corpus callosum have been reported in mice, and a complete agenesis has not yet been described in that species.[17] No signs of corpus callosum abnormalities nor signs of sensorineural deafness were seen in the investigated dogs. None of them developed epileptic seizures and their normal blood and urine analysis suggest normal renal function. Phenotypically, the current described syndrome seems quite similar to the SAMS syndrome previously reported in Malinois dogs[5] and Jack Russell Terriers[4,32], both caused by a *KCNJ10* variant. Still, some differences are undeniably present. The age of onset is younger in SAMS (6-8 weeks) and the progression to non-ambulatory status is also more rapid in SAMS (before 6 months of age) compared to the dogs investigated here (respectively 3-6 months and 2.5-3 years of age). Paraparesis and palmigrade stance were seen in this study but were

not described in SAMS. Epileptic seizures have also been described in dogs with SAMS but were not seen here. Myokymia were clearly seen in dogs with SAMS when awake (confirmed by electrophysiological examination) and even progressed to neuromyotonia episodes in some cases, while the myokymic-like muscle contractions were only seen here when the dogs had been sedated and disappeared shortly after the induction of anesthesia. SAMS dogs also repetitively present some degree of subclinical sensorineural deafness, which was not seen here when a BAER test was performed.[5,32] Those differences might be explained amongst others, by the fact that *KCNJ10* encodes a voltage-gated K^+ channel[4,5], while *SLC12A6* encodes an electroneutral K^+-Cl^- cotransporter in the brain and spinal cord.

The marked phenotypic differences between the human and canine phenotype of *SLC12A6* variants are striking, but quite pronounced phenotypic variations have already been reported between human patients, as well as with mice.[17] Interestingly, as in humans where patients with ACCPN have extremely low body weights and heights[25], affected dogs weighted about 25% less than age-matched littermates at the time of euthanasia.

The histopathologic findings show a strong correlation with the human ACCPN, as both display a severe axonopathy with striking unnoted neuronal damage, inflammation or gliosis. The dogs developed lesions in both central (mainly spinal cord and brain stem) and peripheral nervous system (mainly nerve roots), broadly consistent with the localization pattern in humans and mouse.[25] An important difference in dogs is the severe, bilateral symmetrical vacuolation of the spinal cord white matter, a feature not well described in humans. A large amount of these vacuoles are severely dilated axons, as IHC for neurofilament and synaptophysin stained positive if axoplasm was still

present. The slightly different distribution pattern and minor interspecies differences can be explained by 2 complementary hypotheses. Firstly, most human case reports do not describe a complete necropsy and histopathology is (only) performed on biopsies of a peripheral sensory nerve (the sural nerve).[24,28,33-34] Auer *et al*[25] did perform a complete necropsy on 8 human patients and described some mild lesions in the spinal cord with scattered vacuoles. Secondly, dogs in this case report are all euthanized for humane reasons at an age of 1 to 3 years, in a disease state where they would not have spontaneously died. This is in contrast to the human patients with ACCPN who died a natural death, mostly due to respiratory failure, at a more advanced stage of disease around 20-30 years old.[25] As this is a chronic, progressive disease, an evolution in type of lesions and distribution pattern can be expected.

We conclude that the loss-of-function *SLC12A6* c.178_181delinsCATCTCACTCAT (p.(Met60Hisfs*14)) truncating variant causes an ataxia-related phenotype of slowly progressive spinocerebellar ataxia, paraparesis and myokymic-like muscle contractions in Malinois dogs. Although the *SLC12A6* variant in dogs resembles genetically the most frequently observed variants in humans, the clinical phenotype in dogs is quite different from ACCPN in humans. Since this is the first report of a naturally occurring disease-causing *SLC12A6* variant in a non-human species, the canine model will be highly valuable to better understand the complex molecular pathophysiology of *SLC12A6*-related neurological disorders and to evaluate novel treatment strategies.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

Supplementary information is available on European Journal of Human Genetics' website.

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TITLES AND LEGENDS TO FIGURES

Figure 1. Pedigree of the affected Malinois family (drawn with the kinship2 package in RStudio[35]). Squares represent males, circles females, grey icons non-sampled dogs, black icons sampled dogs, non-shaded icons healthy dogs, shaded icons affected dogs and strikethrough icons deceased dogs. Numbers correspond to the investigated dogs described in Supplementary Information File 2. Dog X was euthanized because of the same symptoms but no video footage nor blood sample was available to confirm the diagnosis. Whole exome sequencing was performed on dogs marked with a degree sign. The *SLC12A6* c.178_181delinsCATCTCACTCAT genotype is shown as Wt/Wt, Wt/Mt or Mt/Mt. Dogs with an asterisk carry 1 allele of the g.22141027insC variant.[13]

Figure 2. Description, origin, location and influence on protein structure of the *SLC12A6* c.178_181delinsCATCTCACTCAT (p.(Met60Hisfs*14)) variant. The upper part shows a schematic representation of the genomic structure of the first exons of *SLC12A6*. White boxes represent exonic untranslated regions, black boxes exonic

coding regions and the white vertical bar the position of the *SLC12A6* c.178_181delinsCATCTCACTCAT variant. The middle part shows the chromatograms of the wild type (Wt) and the mutated variant (Mt), and the proposed origin of the INDEL by a template-switch process (1-3-4-2) with inverted repeat (arrows) and inverted spacer (dotted line) as described by Löytynoja and Goldman[36]. The lower part shows the predicted structure of the canonical SLC12A6 protein (Wt) and the truncated variant translated from the INDEL-containing transcript (Mt), drawn with Protter[37].

SUPPLEMENTARY INFORMATION

Supplementary Information File 1 (*.pdf)

Table showing genotypes of the described canine ataxia-related variants in Malinois dogs.

Supplementary Information File 2 (*.pdf)

Table showing a summary of the clinical investigations performed in each of the 6 affected Malinois dogs.

Supplementary Information File 3 (*.pdf)

Table showing genotypes of the *SLC12A6* c.178_181delinsCATCTCACTCAT variant in reference dog breed populations.

Supplementary Information File 4 (*.pdf)

Description sheet of the genotyping assay for the *SLC12A6* c.178_181delinsCATCTCACTCAT variant.

Supplementary Information File 5 (*.pdf)

Description sheet of the *SLC12A6* RT-qPCR assays and results.

Supplementary Information File 6a (*.mpg4)

Video showing severe generalized hypermetric ataxia (worse on the hindlimbs) associated with a mild to moderate degree of paraparesis and a mild palmigrade stance at 12-month of age (dog 3).

Supplementary Information File 6b (*.mpg4)

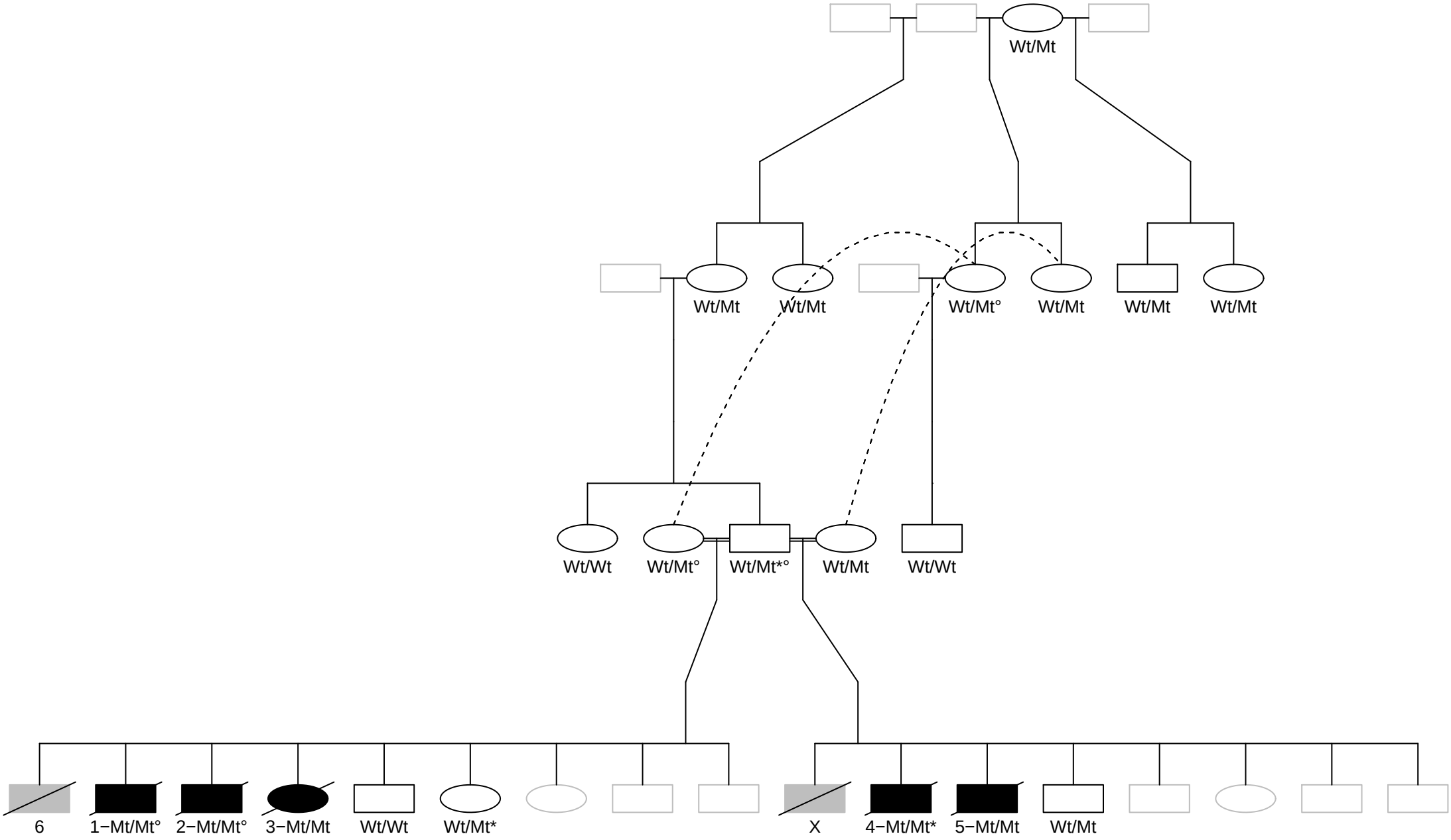
Video showing short-lasting, transient generalized involuntary vermicular muscle contractions (myokymia-like muscle contractions) triggered by sedation (dog 3).

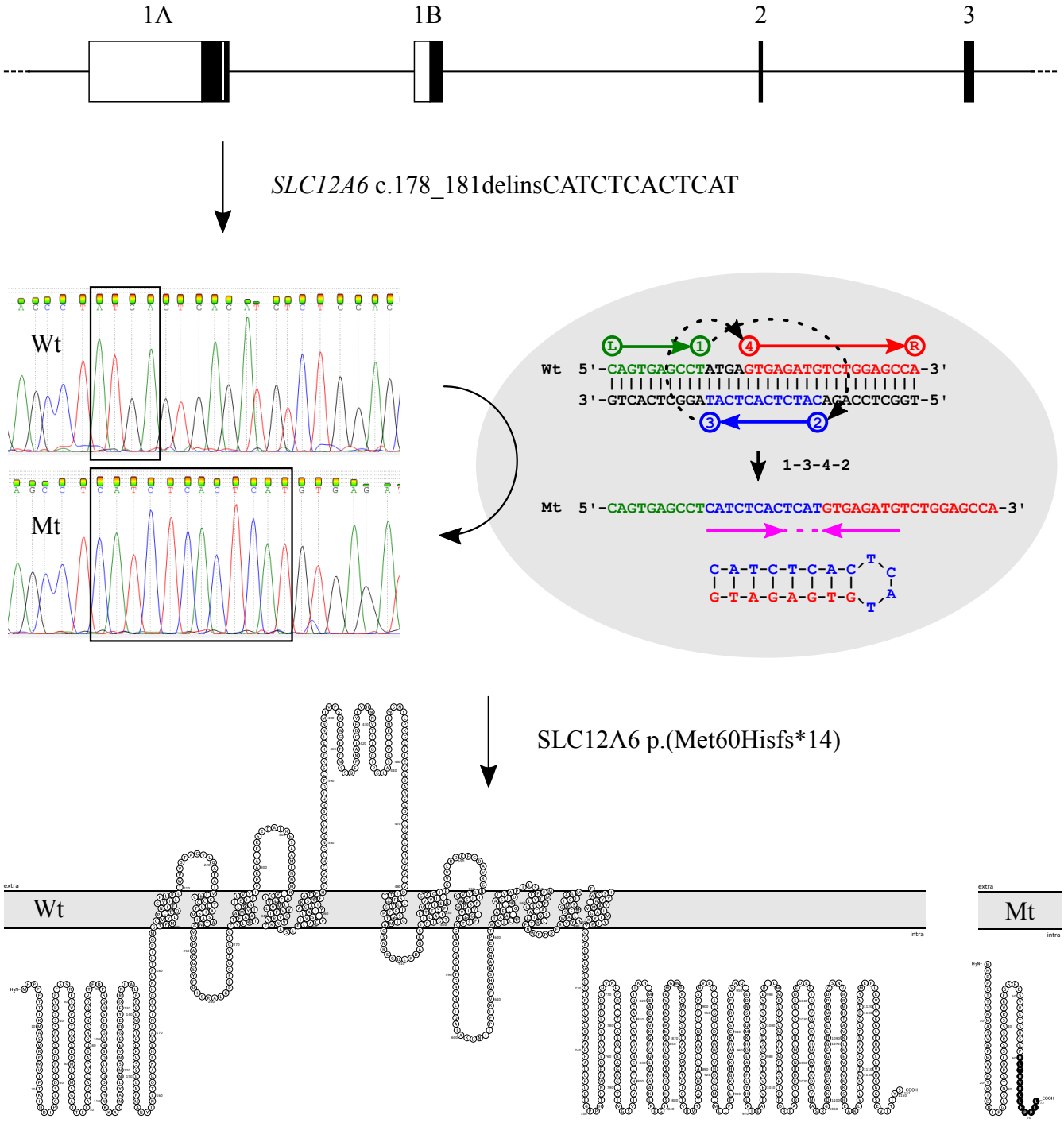
Supplementary Information File 6c (*.mpg4)

Video showing the progression of the ataxia and paraparesis resulting in non-ambulatory paraparesis by 3 years of age (dog 3). The palmigrade stance is also notably worse.

Supplementary Information File 7 (*.pdf)

Figures showing histopathological lesions on haematoxylin and eosin (H&E), and on immunohistochemistry (IHC) for neurofilament and synaptophysin.


Wt/Mt
Wt/Mt
Wt/Mt
Wt/Mt°
Wt/Mt
Wt/Mt
Wt/Mt
Wt/Wt
Wt/Mt°
Wt/Mt*°
Wt/Mt
Wt/Wt
6
1−Mt/Mt°
2−Mt/Mt°
3−Mt/Mt
Wt/Wt
Wt/Mt*
X
4−Mt/Mt*
5−Mt/Mt
Wt/Mt


1A
1B
2
3
SLC12A6 c.178_181delinsCATCTCACTCAT
Wt
Mt
Wt 5'-CAGTGAGCCTATGAGTGAGATGTCTGGAGCCA-3'
3'-GTCACTCGGATACTCACTCTACAGACCTCGGT-5'
1-3-4-2
Mt 5'-CAGTGAGCCTCATCTCACTCATGTGAGATGTCTGGAGCCA-3'
C-A-T-C-T-C-A-C-T-C
G-T-A-G-A-G-T-G-A
T
SLC12A6 p.(Met60Hisfs*14)
extra
intra
Wt
Mt