SUPPLEMENT

Identification of secondary effects of hyperexcitability by proteomic profiling of myotonic mouse muscle; by Lisa Staunton, Harald Jockusch, Christiane Wiegand, Timo Albrecht and Kay Ohlendieck

Molecular and physiological characterization of the myotonic mouse mutant MTO*5J

1. Origin of the allele *mto-5J* of the muscular chloride channel gene *Clc1*. The mutation *mto-5J* has arisen spontaneously on a 129 background at the Jackson Laboratory and was shown to be allelic to *mto ("myotonia")*. A stock carrying a *Fcgr2b*^{tm1Ttk} (Fc gamma2b receptor = CD32) transgene was obtained in 2001 from the Jackson laboratory. The mutations *adr "arrested development of righting response")* and *mto* had previously been shown to be allelic.[1] They are both null mutations.[2,3]

2. Molecular identification of the *mto-5J* mutation in the *Clc1* gene. Using primers based on sequence information on the exons and introns of the mouse *Clc1* gene[4], a single base (A) insertion in codon 93 of the *Clc1* gene has been identified. This insertion, due to frameshift, changes the amino acid sequence from position 76 to position 92 and leads to an opal stop codon (TGA) at position 93, thus terminating the polypeptide at about one tenth of its length (**Fig. S1**).

3. **Overt symptoms of myotonia**. Like in ADR and MTO mice, cramps could be induced in the hindlegs of MTO*5J mice by light squeezing. In contrast to severely

affected mutants, however, MTO*5J mice had a normal appearance, only slightly reduced body weights and near normal life spans.

4. Mechanophysiology of mutant muscles. Whereas all tested muscles from adult ADR and MTO mice showed after-contractions (**Table S1**)[5], isolated *extensor digitorum* (EDL) muscle of young adult MTO*5J mice relaxed like wild type muscle (**Fig. S2**); only in very old individuals, moderate after-contractions were observed (**Fig. S3**). Whereas myotonic indices M (for definition see Füchtbauer and co-workers[6]) in adult ADR and MTO mice vary from 0.7 to 1.0, those in MTO*5J mice range from zero to 0.25, depending on their age (**Table S1**). The EDL of MTO*5J mice behaved as a typical fast muscle, as judged from time-to-peak values (**Fig. S3; Table S1**). Most of these data are from the unpublished thesis by Albrecht[7].

5. Muscle histochemistry. Like in ADR and MTO muscle, the sarcolemmal chloride channel ClC-1 was not detectable by antibody staining (not shown). This result was to be expected on the basis of a stop codon near the N terminus of the coding region of the $Clc1^{mto\text{-}5J}$ allele. In contrast to ADR and MTO muscles which are fully transformed to an oxidative phenotype[5], fast muscles from MTO*5J mice (e.g. *M. gastrocnemius* and EDL) retained a pattern of glycolytic and oxidative fibres similar to that in the wild type (**Fig. S4**). Unexpectedly, numerous central nuclei were seen in MTO*5J muscles (**Fig. S4**) [7]. Central nuclei usually result from muscle fibre regeneration, e.g. in muscular dystrophies, like in the dystrophin-deficient MDX mouse; they were not observed in the severe mouse myotonias ADR and MTO.

6. What causes the mild phenotype of MTO*5J mice? In general, the myotonic

phenotype is quite independent of the genetic background, as shown e. g. by crossing mice carrying the *Clc1*^{adr} mutation on the A2G background with MDX mice on a BL10 background.[8] A role of the *Fcgr2b*^{tm1Ttk} transgene for the myotonia symptoms has been excluded: Removal of the transgene from the stock carrying the *Clc1*$^{mto\text{-}5J}$ mutation did not aggravate myotonia of homozygous MTO*5J animals. Crossing the transgene into the strain carrying *Clc1*^{mto} did not cause milder symptoms in MTO mice.

7. Conclusion. The reason for the mild phenotype of MTO*5J despite the *Clc1*$^{mto\text{-}5J}$ null mutation is not known. However, the MTO*5J mutant is a valuable research tool: whereas the severe symptoms of ADR and MTO mice result in dramatic and easily characterized differences to the wild type, the milder symptoms of MTO*5J mice are closer to those of human patients.

8. Statement on animal welfare. The results described did not involve experiments on live animals. The breeding of mutants were done in accordance with the German law on the protection of laboratory animals and approved by the local authorities.

References

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Figure and Table legends:

Table S1: Mechanophysiological properties of MTO*5J EDL in comparison to ADR and wild type muscles.

Fig. S1: Molecular basis of the *Clc1*$^{mto\text{-}5J}$ mutation. Codons 55 to 105 (out of a total of 995) are shown including the primers used for the mutation analysis are shown (Forward, mut, magenta = mutant *Clc1*$^{mto\text{-}5J}$; WT, green = wild type; blue= backward, including an intron sequence). The insertion and the resulting opal stop codon TGA are in red.

Fig. S2: Mechanophysiology of adult MTO*5J EDL in comparison to ADR and wild type (two different background strains). 50 Hz stimulation resulting in incomplete

tetani. In the ADR mouse (severe myotonia) the contractions in the plateau are completely fused and there are strong and long lasting after-contractions (myotonia indices 0.71 and 0.82). These symptoms are not seen in MTO*5J muscle, but there is a loss of force during the plateau phase, not observed in ADR and WT muscles.

Fig. S3: Mechanophysiology of EDLs from a very old (285 d) MTO*5J mouse. Stimulation at100 Hz resulting in a complete tetanus with a moderate after-contraction.

Fig. S4: Fibre types and position of nuclei in wild type (WT, left) and MTO*5J (right). Shown is the border between the *M. gastrocnemius* (G) and the *M. soleus* (S). Staining was for succinate dehydrogenase (SDH) activity, nuclei were stained with Hoechst DNA dye (light blue fluorescence upon UV illumination). In the WT G large diameter glycolytic fibres (low SDH) are prominent; in the S (lower right) fibres are smaller and predominantly oxidative (high SDH). Nuclei of WT muscle fibres are always peripheral. In MTO*5J muscle there is still a pattern of glycolytic and oxidative fibres whereas in ADR muscle all fibres are oxidative.[5]

Table S1: Mechanophysiological properties of MTO*5J versus ADR versus WT muscle

Property	Frequency	WT	ADR	MTO*5J
Force twitch	3Hz	80.0 ± 20 mN	20.0 ± 10 mN	49.0 ± 15 mN
Incomplete tetanus	50Hz	180 ± 35 mN	85 ± 30 mN	140 ± 60mN
Complete tetanus	100Hz	250 ± 35 mN	125 ± 35 mN	205 ± 40 mN
Time to peak	3Hz	22.5 ± 3 ms	36.5 ± 20 ms	21.0 ± 2,5 ms
Half relaxation time	50Hz	24.5 ± 7 ms	--	19.0 ±6.5 ms
	100Hz	28.0 ± 5 ms	--	28.2 ±6.5 ms

Fig. S1

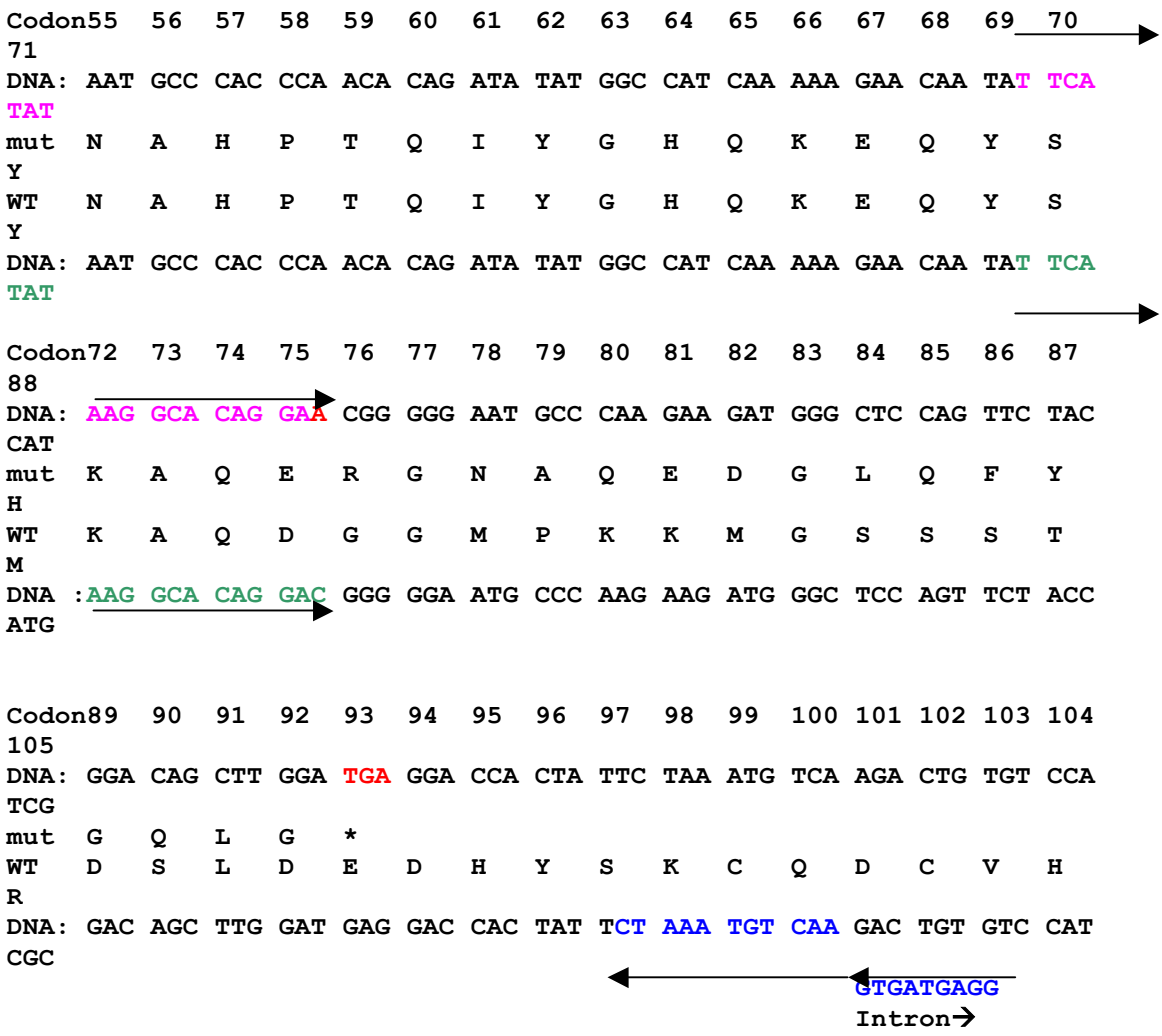

Fig. S2

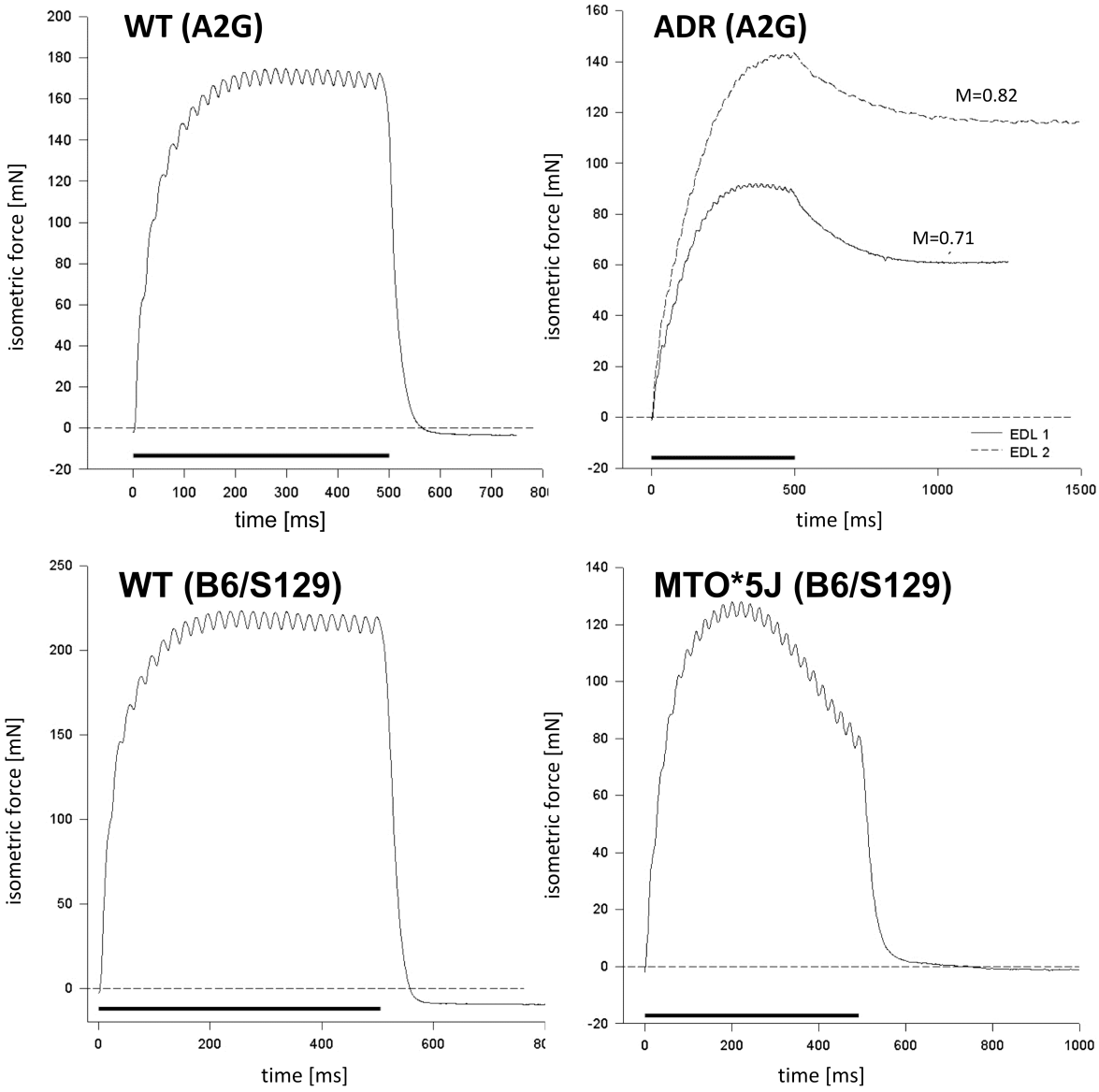

Fig. S3

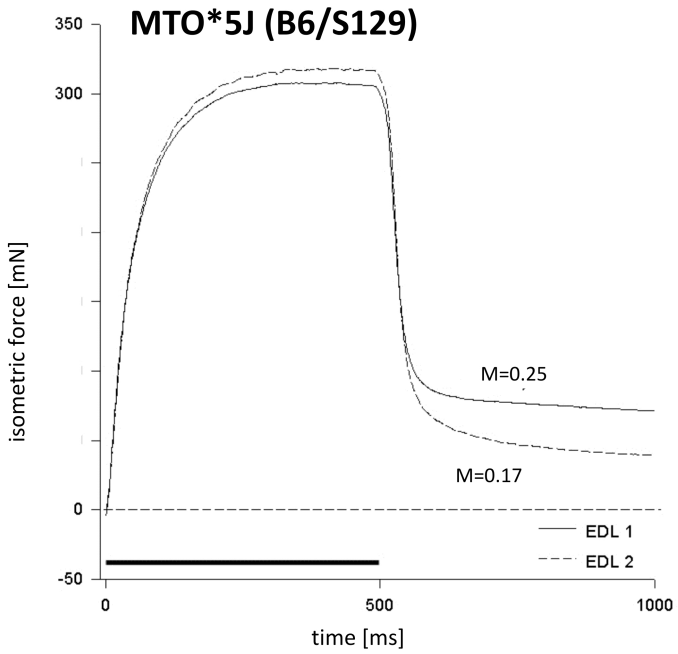

Fig. S4