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Muscle-Targeted Gene Therapy of Charcot Marie-Tooth Disease is Dependent on Muscle Activity

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1. Introduction

Charcot-Marie-Tooth disease (CMT) is the most common inherited neurologic disorder. Reportedly 10-28 cases exist per 100,000 in Western societies. CMT patients suffer from a variable degree of motor dysfunction which adversely affects locomotion and balance. These deficits explain by a slowing of impulse conduction in motor and sensory nerves. This affects the recruitment of muscle fibres for contraction whereby slow motor units are preferentially affected (Gale et al., 1982; Roy et al., 1996). This results in muscle weakness and wasting in the extremities of the body.

The inefficient propagation of excitatory signals in moto neurons towards peripheral muscle in CMT disease is caused by a myelination defect which has a genetic origin. Some 20 genes for axon proteins and myelin have been implicated in CMT, and allow the classification into specific subtypes of the disease. Frequently affected of these genes are peripheral myelin protein 22 (pmp22) and myelin protein zero (MPZ) which are mutated in a majority of cases (Braathen et al., 2011). CMT subtype 1A (CMT1A) is the most predominant disease type being associated with a duplication of the pmp22 gene (Wise et al., 1993; Magyar et al. 1996). The pmp22 gene encodes for a factor that is incorporated into the myelin sheet of neurons and is thought to control myelin thickness. The pathology on the CMT1A disease types is explained by slowed saltatory transduction in moto neurons due to the aberrant nerve insulation (Sereda & Griffiths, 1996).

Currently there is no cure for CMT. There are however a number of occupational interventions that can be used to effectively manage its symptoms (http://www.ninds.nih.gov/disorders/charcot_marie_tooth/detail_charcot_marie_tooth.htm). The most effective treatment consists of a rehabilitation programs with physical therapy as an active ingredient. This involves daily heel cord stretching exercises to prevent Achilles tendon shortening. Concomitantly, resistant training of CMT patients can lead to impressive

increases in maximal voluntary force (Lindeman et al., 1999). Exercise is thus encouraged within each individual patient's capability to condition motor performance in this neurological disease. Physical activity however only retards the irreversible degeneration and may have detrimental consequences if inappropriately administered (Vinci et al., 2003). Gene transfer therapy is a promising route for future cures of muscular disorders. However major limitations appear regarding the treatment of neurological diseases such as CMT. In this case one would normally expect that the therapy must directly target moto neurons to initiate a compensatory mechanism that can correct the missing myelination. This is currently not feasible. In this regard the important positive influence of exercise on motor performance in CMT patients demands consideration as this may offers an indirect means to improve neuromuscular function. This relates to the well established feed forward control of the muscle phenotype by muscle use (Fluck & Hoppeler, 2003). It has been pointed out that the effects of increased muscle recruitment with exercise involve the promotion of the slow-oxidative expression program (Schmutz et al., 2010; Baumann et al., 1987). Recently, has been established that electrically imposed muscle recruitment can re-establish muscle functionality in denervated muscle of tetraplegic patients through the remodelling of the contractile and metabolic makeup (Boncompagni et al., 2007). The positive influence of exercise in CMT patients suggests that the use-dependent pathway importantly affects neuro-muscular function.

We reasoned that altered muscle use combined with gene therapy for focal adhesion kinase (FAK), an enzyme that regulates recruitment dependent slow oxidative gene expression, would be a suitable venue to promote pmp22 expression in skeletal muscle. Towards this end we investigated the use dependence of pmp22 expression in human skeletal muscle and assessed the nature of contractile defects in muscles of a mouse model for CMT at the molecular, cellular and functional level. Subsequently we tested whether overexpression of FAK can enhance pmp22 expression in skeletal muscle (Durieux et al., 2009).

2. Methods

Response to exercise in human muscle - A resting biopsy was collected with a conchotome from the non-exercising leg after an overnight fast and prior to exercise. Thereafter, each participant performed an acute single-legged endurance exercise bout at 60% of their Pmax on an Ergoline bicycle ergometer (Jaeger). There was an incremental warm-up period to reach their 60% Pmax, and after 20 minutes of cycling at 80 rpm, the resistance was increased by 5 W every 10 seconds until volitional exhaustion. A biopsy was collected with the ACECUT needle system (UK Surgical Ltd) from the exercised leg 8 hours after the bout of exercise. Biopsy position was standardized based on an ultra-sound measurement at 50% of femur length. This study was performed at Manchester Metropolitan University (United Kingdom) with permission of the institutional ethics Committee, in compliance with the Helsinki Convention for research on human subjects.

Model of altered muscle activity - Hindlimb suspension of rats was performed for 7 days as previously described (Fluck et al., 2005) at the Université Lyon 1 (France) and started 2 days after transfection. Subsequent reloading was provoked by allowing the animals to return to normal cage activity for 1 or 5 days.

Hindlimb suspension of mice was performed for 3 days as previously described at the University of Berne (Switzerland) (Dapp et al., 2004). This was achieved by attaching the tail via a swivel hook to a movable X-Y system; thereby preventing the mouse from touching the

ground with its hindlimbs while permitting free movement within the entire cage. Soleus muscles were extracted from anesthetized animals (5% isoflurane).

Animal model of CMT - Pmp22-tg mouse (pmp22tg) as CMT model (strain C3Hb6) were bred at the University of Fribourg. The original strain was a gift of U. Suter (University of Zurich). The pmp22-tg mice carry approximately 16 and 30 copies of the pmp22 gene and display a severe congenital hypomyelinating neuropathy due to the accumulation of overexpressed pmp22 in the Golgi compartment, and the concomitant interference with myelin assembly during Schwann cell differentiation (Niemann et al., 2000). This is characterized by an almost complete lack of myelin and marked slowing of nerve conductions (Magyar et al., 1996). The animals entering the study were between 1.5- and 3-month of age.

Somatic transgenesis for FAK - Gene transfer of pCMV-FAK into soleus muscle of 3-month old male Wistar rats was essentially carried out as previously described (Durieux et al., 2009). In brief, soleus muscles of anaesthetized rats (60 mg of sodium pentobarbital per kg body) were surgically exposed. 70 microgram per 70 microliter of endotoxin-free plasmid in Tris-EDTA buffer (plasmidfactory, Bielefeld, Germany, www.plasmidfactory.de) was injected in the belly portion of the soleus muscle and three trains of 80-pulses of 100 microseconds duration, each at 100 mA, were delivered using needle electrodes with the GET42generator (Electronique et Informatique du Pilat, Jonzieux, France). The wound was closed with sutures and animals transferred to single cages. Right soleus muscles were injected with cytomegalovirus promoter-driven plasmids for the constitutive overexpression of chicken FAK (pCMV-FAK). The plasmid was a gift from Tony Parsons (University of Virginia, Charlottesville, USA). The (left) soleus muscles of the contralateral leg were injected with empty pCMV plasmid.

The experiments were performed at the Universities of Berne (Switzerland) and Lyon (France) with the permission of the local Animal Care Committee of the Canton of Berne (Switzerland) and following the recommendations provided by the European Convention for the protection of Vertebrate Animals used for Experimental and Scientific purposes (Strasbourg, 18.III.1986).

Muscle sampling - Collected muscles were frozen in nitrogen cooled isopentane and stored in sealed cryotubes at -80°C until use.

Transcript profiling - Transcript levels were assessed with microarray in total RNA. In brief, 25 micrometer cross-sections were prepared for 10 mm³ of muscle volume and total RNA extracted with the RNeasy mini-protocol (Qiagen, Basel, Switzerland) for skeletal muscle as described (Fluck, Schmutz et al. 2005). Integrity of the RNA was checked with denaturing agarose gel electrophoresis, and the concentration was quantified against ribosomal standard using the RiboGreen RNA quantification kit (Molecular Probes, Eugene, OR).

Subsequently RNA was labelled during reverse-transcription and hybridized to different platforms dependent on the species:

For human samples this involved the use of a full human genome Affimetrix platform through a commercial provider (DNA vision, Gosselies, Belgium). For statistical analysis raw signals were normalized to total mRNA signal on the array and assessed with statistical analysis of microarrays between pre and 8-hours post samples with T-tests (SAM).

For studies with rat and mouse soleus muscle, 3 micrograms of total RNA were reverse transcribed with SuperScript II (Life Technologies, Basel, Switzerland) using [α -³²P]dATP and gene-specific primers. Radio-labelled target cDNA was hybridised in ExpressHyb solution overnight at 68°C to nylon membranes which spotted cDNA probes (BD

Biosciences, Allschwil, Switzerland). The membranes were washed (four times for 1 h in 2x SSC (0.3 M sodium chloride, 0.03 M sodium citrate), 1% SDS and once for 30 min in 0.1x SSC, 0.5% SDS at 68°C and exposed for 5 days to a phosphorimager (Molecular Dynamics, Sunnyvale, CA) for quantification with AIDA Array Easy software (Raytest Schweiz, Urdorf, Switzerland).

For the rat experiment cDNA microarrays (Atlas Rat 1.2, no. 7854; Clontech Laboratories, Ozyme, France) were used. More details to this cDNA array can be found on Gene Expression Omnibus (GEO, <http://www.ncbi.nlm.nih.gov/geo/>) under GPL153. Data sets have been deposited under sample codes GSM10045–GSM10062, and GSM17357–GSM17361. For statistical analysis, raw signals were normalized to beta actin signal on the array and assessed with T-tests.

For the mouse experiment custom-designed low-density Atlas cDNA expression arrays with 229 double-spotted probes of mouse cDNAs associated with skeletal muscle form and function were employed. More details to this cDNA array can be found on GEO under GPL1097. For statistical analysis raw signals were normalized to total mRNA signal on the array and assessed with SAM.

Microscopy - Fibre type analysis of mouse muscle was carried out on cryosections with specific antibodies using a two-step detection protocol as described (Fluck et al., 2002). In brief, 12 micrometer cryosections were prepared, fixed in cold acetone and wetted in phosphate-buffered saline (PBS). Tissue peroxidase activity was then quenched (10 min, 3% H₂O₂ in methanol), sections were washed in PBS and blocked with 3% BSA in PBS for 0.5 h. Subsequently the sections were incubated for 1 h at room temperature (20 °C) with 1:100 of a monoclonal antibody in 0.3 % BSA/PBS against slow (Chemikon Juro, Lucerne, Switzerland) or fast type myosin heavy chain (Sigma Chemicals, (Buchs, Switzerland), respectively. Following, 3 brief washing in PBS, the section was reacted for 30 min with peroxidase-conjugated goat anti-rabbit IgG (diluted 1:2000 in 0.3 % BSA/PBS; Jackson Laboratories, West Grove, PA, USA) and again washed with PBS. Immunoreactivity was detected with 3-amino-9-ethylcarbazole substrate (Sigma Chemicals, Buchs, Switzerland); the nuclei were counterstained with haematoxylin, and the sections were embedded in Aquamount (BDH Laboratory Supplies Poole, UK). The stain was visualised on film (Ektachrome 64T, Kodak) using a microscope/photograph system (Vanox-S, Olympus).

Micrographs were taken from corresponding fields and the fibre types were classified. Slow and fast type fibres were differentiated on the basis of the presence of immunoreactivity for either type I or type II myosin heavy chain isoforms. Subsequently the percentages of each fiber population were counted.

The overexpression of FAK in muscle fibres was detected out with a Leica TCS SP5 confocal microscope on a DMI6000 stage powered by Argon laser and He-Ne lasers (Leica Microsystem CMS, Milton Keynes, UK). In brief, 12 micrometer cryosections were reacted with a 1 : 100 dilution of rabbit FAK antibody A-17 (Santa Cruz) and MHC2 antibody in 0.3% BSA in phosphate-buffered saline (PBS) and reacted with fluorescent-labelled secondary antibodies (Alexa488-conjugated anti-rabbit IgG, and Alexa555-conjugated anti-mouse IgG, Molecular Probes/Invitrogen). Sections were embedded in fluorescence-compatible mounting medium (DAKO, Denmark). Signal for FAK and the co-detected myosin protein was inspected with fluorescence after excitation at 458 nm, 476 nm and 488 nm with sampling in channels between 510–533 nm (Alexa 488) and 593–614 nm (Alexa 555).

Myography - Soleus muscles were harvested from anesthetized animals (pentobarbital, 50 microgram/gram body weight) and equilibrated in Tyrod solution (mM/l: 118.0 NaCl, 4.7 KCl, 2.0 CaCl₂, 1.2 MgSO₄, 1.1 KH₂PO₄, 24.0 NaHCO₃, 4.5 glucose, pH 7.3–7.4; 5% CO₂ / 95% O₂ @ 25°C). Muscles were transferred to Tyrod solution in the contraction chamber,

connected to the force transducer (KG7; Muscle tester ORG (SI-Heidelberg, Germany) and equilibrated for 5-10 minutes by gazing with 5% CO₂ / 95% O₂ @ 25°C. Contractions were initiated by point stimulation via an Ion Optix myopacer. Signals were recorded using a Powerlab system (AD instruments, Germany) and using Chart 5 software. Measurement of single twitch, tetanus and fatigue were carried out: First the length of the inserted muscle was optimized for maximal force at a stimulation of 1 Hz, 10 V, 0.4 ms. For single twitch contractions, the muscle was stimulated at 1 Hz, 10 V, 0.4 ms. For the assessment of fatigue, a repeated 3-minute tetanic contraction protocol was used (55/60 Hz, 10 V, 0.4 ms in trains of 4 seconds on and off). Fatigue was defined as the contraction cycle when force dropped below 50% of original force. Before each measurement the Tyrod solution was exchanged. The measured muscles were frozen in liquid nitrogen-cooled isopentane to determine fiber size and distribution. Twitch parameters (latency, twitch-time to peak, t1/2 relaxation, maximal force) were determined from the digital recording. Measures from contralateral muscles were considered as separate biological replica. Statistical analysis was carried out with an unpaired t-test (Statistica 6.1).

2.1 Results
2.1.1 Regulation of myelin-specific transcripts by muscle activity

Muscle fibres of anti-gravitational muscles are active for a considerable duration during free movement and quiet standing (Hennig & Lomo, 1985). We wished to investigate transcript regulation of factors of the myelin sheet by muscle activity. Towards this end we carried out an exploratory microarray analysis with the slow oxidative soleus muscle of rats that was subjected to changes in muscle activity by hindlimb suspension. The model allows reducing load bearing activity to slow oxidative soleus muscle by non-invasive means with the suspension of hindlimbs with the option to increase muscle activity with subsequent reloading (Morey Holten model as referred by Dapp et al., 2004). Transcript profiling of rat soleus muscle showed lowered expression of the myelin-specific transcripts, peripheral myelin protein 22 (pmp22), myelin protein zero (MPZ), myelin basic protein (mbp) as well as myelin proteolipid protein and myelin-associated glycoprotein with reduced muscle activity (Fig. 1). With reloading the abundance of all of these transcripts were sizably increased. We assessed the response of myelin-specific factors in human knee extensor muscle to exhaustive exercise. It has been pointed out before that this exercise stress induces a compensatory expression response during the first 24 hours of recovery from exercise (Pilegaard et al., 2000; Fluck, 2006). The analysis in the mixed *vastus lateralis* muscle consolidated the observation on the regulation of myelin-specific transcripts by muscle use. Pmp22, mpz and mbp transcript levels were increased by 1.2 to 1.8-fold (table 1).

gene	abbr	mean	SE	p-value
myelin basic protein	MBP	1.7	0.3	2%
myelin expression factor 2	MYEF2	1.4	0.2	3%
peripheral myelin protein 22	PMP22	1.8	0.4	5%
myelin associated glycoprotein	MAG	1.1	0.1	5%
myelin protein zero	MPZ	1.2	0.1	21%
myelin oligodendrocyte glycoprotein	MOG	1.2	0.1	25%

Table 1. Expressional alterations in myelin-specific transcripts in human *vastus lateralis* muscle after endurance exercise.

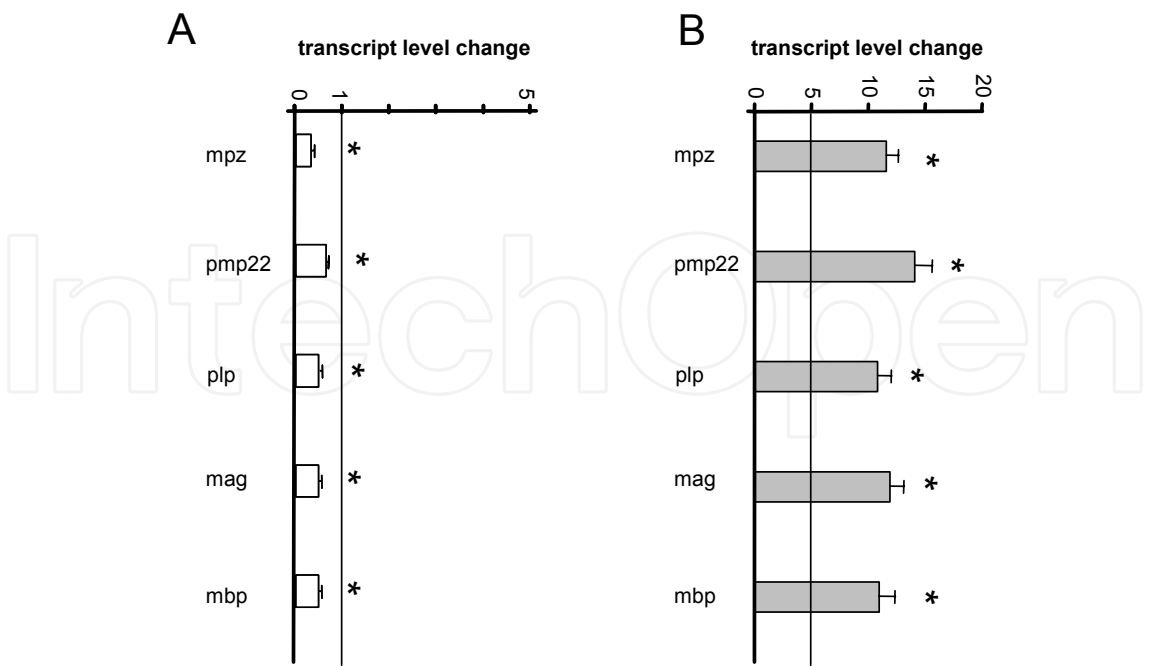


Fig. 1. Expression of myelin-specific transcripts depends on muscle activity. Bar graphs indicating mean \pm standard error (SE) of level changes of five transcripts (per beta actin) in rat m. soleus with 7 days of hindlimb suspension vs. cage controls (A) and one day of reloading vs. hindlimb suspension (B). Asterisks denote significant effect based on a T-test. $n=6$. The line of identity is given. Abbreviations: mpz, myelin protein zero; pmp22, peripheral myelin protein 22; plp, myelin proteolipid protein; mag, myelin-associated glycoprotein; mbp, myelin basic protein.

2.2 Contractile defects in a mouse model of pmp22-dependent CMT

Pmp22-tg mice show reduced pmp22 protein expression, lack myelination, and demonstrate neurogenic muscle atrophy (Magyar et al., 1996). We compared the muscles of pmp22-tg mice vs. strain matched controls to identify contractile defects with pmp22-dependent moto neuron aberration. Soleus muscle of pmp22-tg mice demonstrated a shift towards an atrophic, slow phenotype. This was indicated by the reduced muscle weight (Fig. 2D) and elevated percentage of slow type muscle fibres (Fig. 2A-C). This became manifest in prolonged time-to-peak and half time of muscle relaxation (Fig. 2D). The aberrations resulted in a reduction in RNA messengers for the slow oxidative expression program concomitantly with the elevated abundance of gene transcripts for fast type myogenesis (Fig. 3). The only exceptions were GAPDH and CA3 being involved in pH regulation of slow type muscle fibres.

2.3 Aberrant muscle plasticity in CMT mice with altered muscle use

Towards understanding the functional implication of motor unit recruitment for control of muscle gene expression we subjected pmp22-tg mice to 3 days of hindlimb suspension. Microarray experiments identified important alterations in transcript expression in *soleus* muscles with 3 days of reduced muscle activity (Fig. 4). The differences between suspended and cage control muscle distinguished between wildtype and pmp22-tg mice. This concerned gene ontologies defining the contractile and metabolic muscle phenotype of

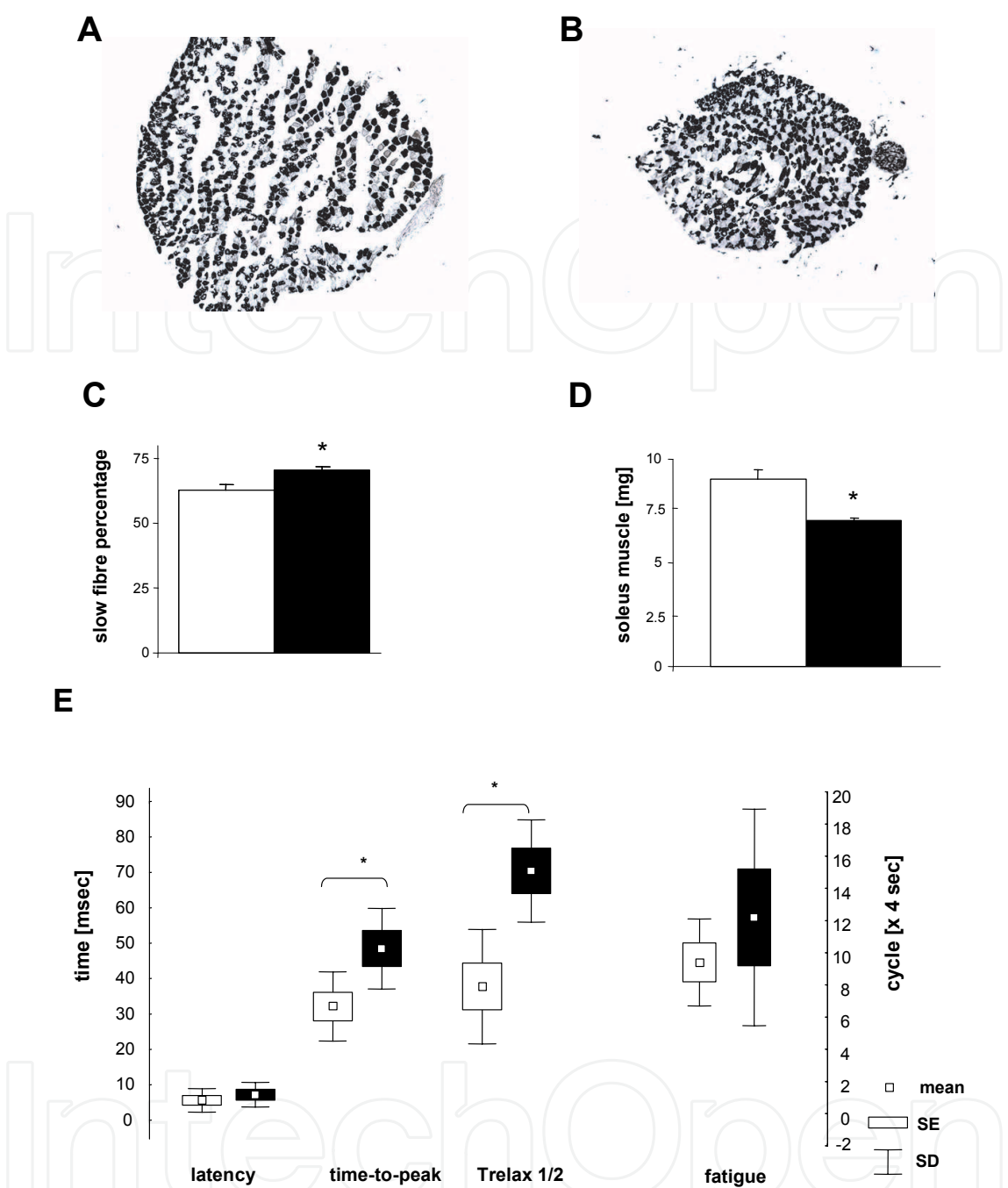


Fig. 2. Contractile aberrations in anti-gravitational muscle of pmp22-transgenic mice. A, B) Micrographs visualizing cross sections of soleus muscle from a wildtype (A) and pmp22-tg mouse (B) after staining for slow type myosin heavy chain. C, D) Bar graphs indicating mean \pm SE of percentage of slow type muscle fibres (C) in soleus muscle and weight (D) of wildtype (white bar, n=5) and pmp22-transgenic mice (black bar, n=6). E) Mean \pm SE and standard deviation (SD) of contractile parameters in soleus muscles of wildtype (n=6) and pmp22-transgenic mice (n=5). Asterisks denote significant effect based on a T-test.

skeletal muscle. Thereby transcript levels of factors of myogenesis, fast contraction, glycolysis and oxidative metabolism altered in opposite ways between wildtype and pmp22-tg muscle.

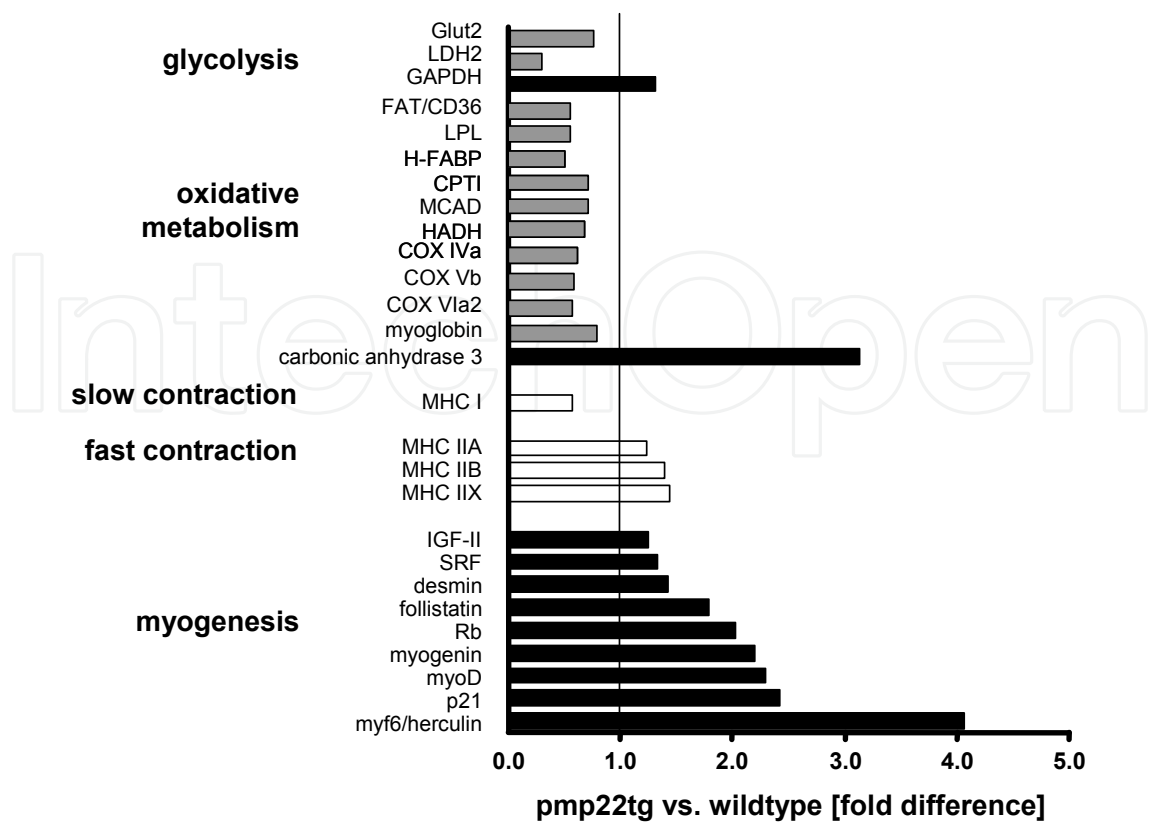


Fig. 3. Aberrant transcript expression in pmp22-transgenic mice. Bar graphs representing the mean fold difference in transcript levels between pmp22tg and wildtype mice for gene ontologies which set the phenotype of soleus muscle. Data were generated with custom microarrays for 229 selected factors, normalized to total mRNA and assessed with unpaired test using SAM (n=4). Black and grey bars denote significantly up-regulated and significantly down-regulated transcripts, respectively. Transcripts reflected by white bars were not significantly altered. The line of identity is given. Abbreviations: Glut2, facilitative glucose transporter member 2; LDH2, lactate dehydrogenase 2; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; COX IVa, cytochrome c oxidase subunit 4 isoform 1; COX Vb, cytochrome c oxidase subunit 5B; COX VIa2, cytochrome c oxidase subunit 6a2; CPTI, carnitine O-palmitoyltransferase 1; FAT/CD36, cluster of Differentiation 36/Fatty acid transport protein; HADH, 3-hydroxyacyl-CoA dehydrogenase; H-FABP, fatty acid binding protein of the heart; LPL, lipoprotein lipase; MCAD, medium-chain specific acyl-CoA dehydrogenase; MHC I, myosin heavy chain type I; MHC IIA, myosin heavy chain type IIA; MHC IIB, myosin heavy chain type IIB; MHC IIX, myosin heavy chain type IIX; IGF-I, Insulin-like growth factor I; SRF, serum response factor; Rb, retinoblastoma-associated protein; myoD, Myoblast determination protein 1; p21, cyclin-dependent kinase inhibitor 1; myf6/herculin; myogenic factor 6.

2.4 Muscle-targeted overexpression of the mechano-sensor FAK elevates myelin factor expression

We assessed whether overexpression of the governor of slow-oxidative gene expression, focal adhesion kinase (FAK), can control myelin factor expression and pmp22-dependent gene transcripts. Using gene electro transfer we introduced a constitutively active expression plasmid for FAK, pCMV-FAK, in soleus muscle of the right leg of Wistar rats.

Transfection of the contralateral muscle with empty pCMV plasmid served as intra-animal control. Immunofluorescence experiments showed that overexpression of FAK was confined to muscle fibres but was not observed in contralateral controls (Fig. 5). Microarray analysis demonstrated that FAK overexpression increased transcript levels of pmp22 along with the one of MBP in soleus muscle of active rats. Concurrently the expression of a battery of slow oxidative genes was increased (Durieux et al., 2009).

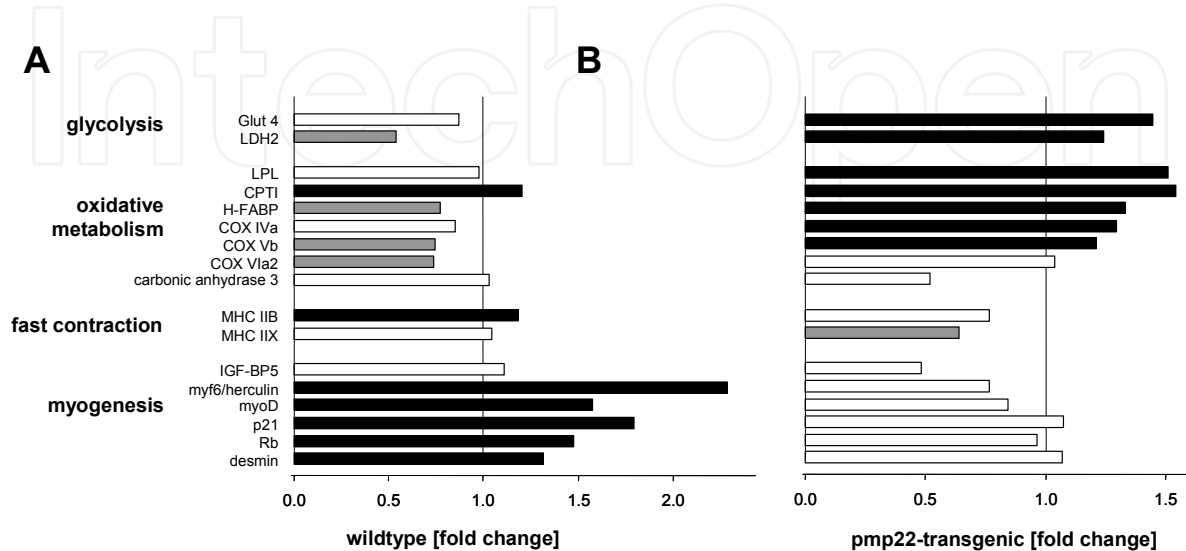


Fig. 4. Anomalous expression response of pmp22-transgenic mice to reduced muscle activity. Bar graph representing the mean fold difference in transcript levels in soleus muscle between wildtype mice (A, n=8) and pmp22tg (A, n=4) with 3 days of reduced load bearing (suspension) vs. cage controls. Data were generated with custom microarrays for 229 selected factors, normalized to total mRNA and assessed with a paired test between values from suspended and cage control muscle using SAM. Black and grey bars denote significantly up-regulated and significantly down-regulated transcripts, respectively. Transcripts reflected by white bars were not significantly altered. The line of identity is given.

Abbreviations: Glut 4, facilitative glucose transporter member 4; IGF-BP5, Insulin-like growth factor binding protein 5. For others consult legend to figure 3.

2.5 FAK dependent elevation of pmp22 expression is use dependent

FAK signals mechanical stimuli in muscle to gene expression and protein synthesis (Durieux et al. 2009; Klossner et al., 2009). We subjected the FAK-transfected soleus muscles to 7 days of hindlimb suspension and subsequent one day of reloading to assess the interaction of muscle activity with gene therapy. Pmp22 transcript expression was little affected by reduced load bearing. With subsequent reloading however pmp22 levels were reduced. This effect was transient and was gone 5 days after reloading

3. Discussion

Skeletal muscle is the largest tissue of vertebrate species and most muscle groups rely on muscle activity to maintain their phenotype (Booth & Thomason, 1991). This is most pronounced in slow oxidative muscle types which are typically involved in control of gait and posture (Roy et al., 1996). This resembles the preferential affection of oxidative muscles

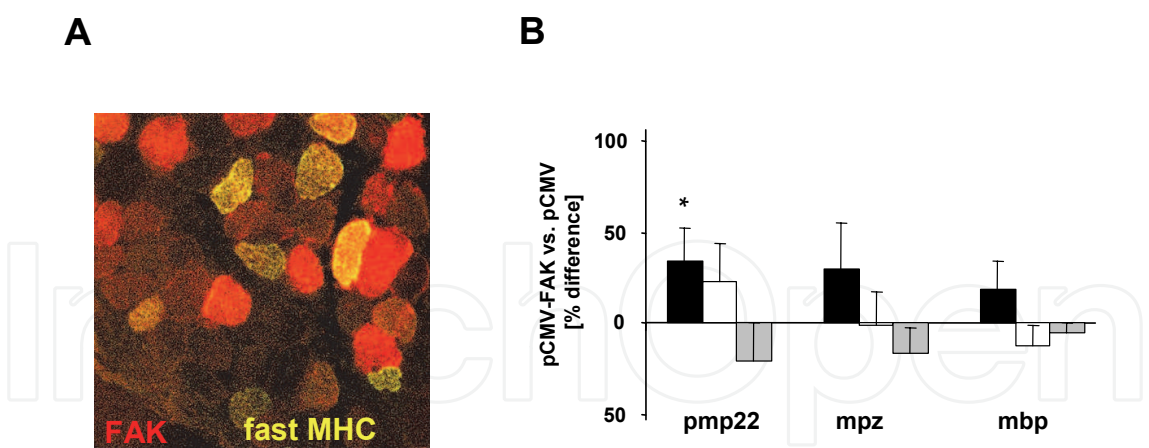


Fig. 5. FAK driven expression of myelination factors is dependent on muscle activity. A) Representative micrograph from a cryosections of a pCMV-FAK transfected soleus muscle of a rat after double staining for FAK (red) and fast type myosin heavy chain (MHC, yellow) as recorded with a confocal microscope. B) Bar graph representing the mean difference + SE of transcripts for three myelination factors pmp22, mpz and mbp between pCMV-FAK vs. empty (pCMV) transfected soleus muscle. Black, white and grey bars reflect data from cage controls, 7 days suspended and one day reloaded mice. Asterisks denote significant effect based on a T-test.

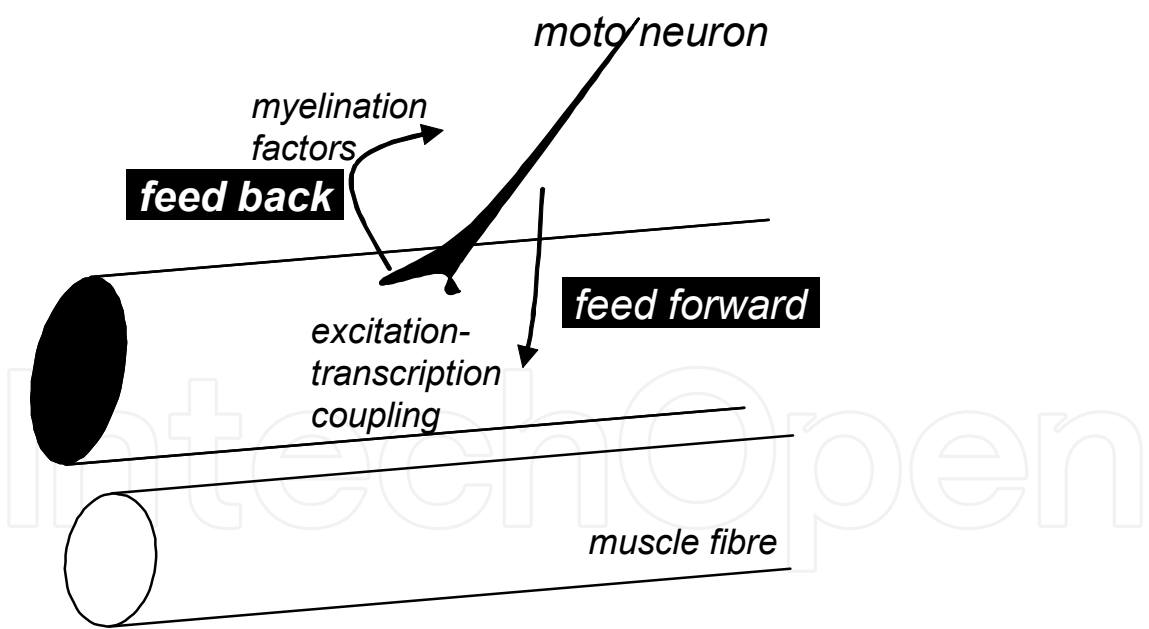


Fig. 6. Implication of excitation-transcription coupling in muscle control. Sketch summarizes a concept whereby moto neuron excitation (feed forward) with muscle use triggers the expression of myelination factors which exert feedback control on the muscle-nerve interaction.

in the trembler model of CMT disease (Gale et al., 1982). This use-dependent control of gene expression and protein synthesis (Fluck & Hoppeler, 2003; Wilkinson et al., 2008) is not taken into consideration in current gene therapy of neurological muscle disorders.

Here we prove the concept that muscle fibre targeted somatic transgenesis can affect nerve-related gene expression. The results point out that muscle activity importantly modifies the pre-translational effects of the somatic overexpression of the governor of the slow oxidative muscle program, FAK, on the expression of myelin-specific transcripts. This was shown by a similar regulation of pmp22, MPZ and MBP transcripts by muscle disuse with reduced load-bearing (down-regulation) in rats, increased load-bearing muscle activity in rats and with endurance exercise (up-regulation) in man (table 1, Fig. 1). The direction of the transcript levels points out that that myelin-specific transcript expression is related to fibre recruitment. Such a mutual control of the muscle-nerve interaction is known from the action of neurotrophic factors during normal development of the slow muscle fiber phenotype (Carrasco & English, 2003). These findings support the novel concept that in addition to feed forward control of muscle gene expression by muscle use (Calvo et al., 2001) a feedback mechanism exists whereby muscle activity in return regulates moto neurons (Fig. 6).

The functional consequences of use dependent expressional alterations in myelin sheath-specific factors in skeletal muscle remain to be explored. Pmp22 is a major component of myelin expressed in the compact portion of essentially all myelinated fibres in the peripheral nervous system and is produced predominantly by Schwann cells. Pmp22 is expressed in cranial nerves but not in the mature central nervous system. Studies in injured nerve suggested a role during Schwann cell growth and differentiation and maturation of the neuromuscular junction (Spreyer et al., 1991; Patel et al., 1992; Magyar et al., 1996). Interestingly the identified transcript level changes in our study are in line with the degeneration of NMJ with disuse (Deschenes et al., 2001) and neurological adaptation which improves fibre recruitment early with exercise (Roy et al., 1996). This supports a hypothesis whereby elevated myelin-specific transcript expression may allow the preservation and improvement of neuromuscular control of muscle fibres.

Previous examinations of soleus and EDL muscle in the trembler mouse model of CMT revealed that the deep/oxidative and soleus muscles are particularly affected by CMT but fibre type differences were not described (Gale et al., 1982). Our measures in the pmp22-tg model of CMT emphasize that in addition to altered muscle contractility, slow fibre size and transcript expression in the anti-gravitational muscle, m. soleus, is anomalous. The reduction in RNA messengers for the slow oxidative expression program (Fig. 3) is possibly explained by volumetric alterations in fibre size as suggested by visually larger CSA ratio between fast and slow type fibres reflecting atrophy of slow type fibres. Smaller type I fibres in animal models for pmp22-dependent motor and sensory neuropathy type has been noted before (Schuierer et al., 2005). This would increase the contribution of transcripts in fast type muscle fibres. The concomitantly elevated abundance of transcripts for fast type myogenesis between pmp22-tg and wildtype mice likely reflects altered regulation in fast muscle fibres (Deschenes et al., 2001).

A main finding of our investigation was that differences between pmp22 and wildtype mice were preserved in an 'opposite' transcript response of soleus muscle to reduced load-bearing. Altered transcript expression is a potentially important indicator of pathological changes with reduced muscle activity (Bey et al. 2003; Chen et al., 2007). In this regard it is important that the response of soleus muscle from wildtype mice to 3 days of unloading reproduced the changes reported in a different mouse strain after 7 days of reduced load-bearing (Dapp, Schmutz et al. 2004). This overlap concerned contractile (MHCIIB) and metabolic factors (COXVb, COXVIa2, LDH2). Exceptions concerned those involved in myogenesis which were not altered after 3 days in this study. Strikingly, the transcript

response of soleus muscle of pmp22-tg mice to suspension “mirrored” the differences seen between the pmp22-tg mice and wildtype mice at baseline. These observations imply the existence of use-dependent mechanism that “inverts” expression responses in model of CMT.

This observation relates to the effects of current occupational therapy by exercise. We have shown before that FAK overexpression controls contractile performance of soleus muscle in a activity-dependent manner via the regulation of transcript expression (Durieux et al., 2009). Intriguingly with FAK overexpression and the resumption of loading bearing muscle activity, the transcript level of myelination factors were reduced compared to controls (Fig. 5). Our previous findings in the suspension model show that reduced myogenic factor RNA expression after 24 hours of the reloading stimulus is related to a concomitant increase in the encoded protein (Fluck et al., 2008). This relates to the observation that FAK acts as a molecular switch for transition between anabolic and catabolic reactions in skeletal muscle and controls the protein synthetic pathway via p70S6K (Durieux et al., 2009; Klossner et al., 2009). This suggests that transcript level changes in the early phase of reloading may reflect inverse alterations in protein synthesis. These relationships imply that the ‘muscle activity’ is a confounding variable which would be valuable to be considered in somatic gene therapy of skeletal muscle. The fact that activity dependent gene regulation is reflected by the mechano-regulation of FAK activity by pTyr-397 phosphorylation (Durieux et al., 2009) indicates possible venues to stimulate this directly in situations where muscle activity is not an option.

The extent to which differences between slow and fast motor neurons are involved in the coupling between muscle excitation and gene expression (excitation-transcription coupling) is currently not well understood (Schiaffino & Serrano, 2002; Wakeling & Syme, 2002). Based on a preferential atrophy of slow type muscle fibres (Roy et al., 1996; Dapp et al., 2004), hindlimb suspension is expected to affect slow motor units more than fast type units. Our findings on the distinction of basal and disuse-induced transcript expression with slowed signal propagation down the motoneuron (Magyar et al., 1996) highlight the importance of correct excitation for control of gene expression (Calvo et al., 2001).

4. Conclusion

Our observations indicate that muscle dysfunction with Charcot-Marie-Tooth (CMT) is not due to a single, central mechanism. A novel, contraction-dependent feedback mechanism is identified that controls myelin-specific transcripts via a muscle fibre-related pathway. Somatic transfection of muscle fibres with the mechanosensor FAK prove the concept that pmp22 expression which is lowered in CMT can be stimulated when combined with skeletal muscle use. This indicates that muscle activity is a confounding variable that warrants exploration in future gene therapeutic strategies to treat and manage neuromuscular disease.

5. Acknowledgment

The study was financially supported by grants from the Région Rhône-Alpes, the Association Française contre les myopathies, and the Swiss National Science Foundation. The experiments were performed at the University of Berne (Switzerland), the University of Lyon (France), and Manchester Metropolitan University. The assistance of Dominique

Desplanches, Anne-Cécile Durieux, Damien Freyssenet and Prof. Hans Hoppeler during the suspension experiments is greatly acknowledged.

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Targets in Gene Therapy

Edited by Prof. Yongping You

ISBN 978-953-307-540-2

Hard cover, 436 pages

Publisher InTech

Published online 23, August, 2011

Published in print edition August, 2011

This book aims at providing an up-to-date report to cover key aspects of existing problems in the emerging field of targets in gene therapy. With the contributions in various disciplines of gene therapy, the book brings together major approaches: Target Strategy in Gene Therapy, Gene Therapy of Cancer and Gene Therapy of Other Diseases. This source enables clinicians and researchers to select and effectively utilize new translational approaches in gene therapy and analyze the developments in target strategy in gene therapy.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Stephan Klossner, Marie-Noëlle Giraud, Sara Sancho Oliver, David Vaughan and Martin Flück (2011). Muscle-Targeted Gene Therapy of Charcot Marie-Tooth Disease is Dependent on Muscle Activity, Targets in Gene Therapy, Prof. Yongping You (Ed.), ISBN: 978-953-307-540-2, InTech, Available from: <http://www.intechopen.com/books/targets-in-gene-therapy/muscle-targeted-gene-therapy-of-charcot-marie-tooth-disease-is-dependent-on-muscle-activity>

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