

REVIEW ARTICLE

Disease mechanisms in spinal muscular atrophy with respiratory distress type 1 (SMARD1): what about motoneurons?

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Abstract

Spinal muscular atrophy with respiratory distress type 1 (SMARD1), described as a fatal motoneuron disorder in children is characterized by α -motoneuron loss. Most of the SMARD1 patients suffer from diaphragmatic palsy leading to permanent ventilation at very early stages of disease. As muscular atrophy is the predominant clinical sign in SMARD1 patients, the question arises whether motoneuron degeneration occurs cell-autonomously. *IGHMBP2*, the disease causing gene in SMARD1, encodes for a helicase with still unknown function in motoneurons. Studies from a SMARD1 mouse model (*Nmd^{2J}* mouse) revealed that degeneration of motor axons precedes muscle fiber atrophy in the gastrocnemius muscle aside from a pure myopathy of the diaphragm without affected motor nerve innervation. *Nmd^{2J}* mice suffer from a reduced IGF1 level which can be compensated by external application of a polyethylene glycol (PEG)-coupled variant of IGF1 (PEG-IGF1). The beneficial effects in striated muscles corresponded to a marked activation of the IGF1 receptor (IGF1R), resulting in enhanced phosphorylation of Akt (protein kinase B) and the ribosomal protein S6 kinase. Unfortunately, no protective effects of PEG-IGF1 were observed at the level of motoneuron survival. Fast motor axon loss innervating the gastrocnemius muscle of *Nmd^{2J}* mice does not correspond to morphological and functional alterations at the neuromuscular endplate as it is described in mouse models for proximal spinal muscular atrophy (SMA). However, the high capacity for axonal sprouting in *Nmd^{2J}* mice indicates the functionality of the remaining motoneurons. These observations argue for additional non-cell-autonomous disease mechanisms which do not primarily affect the neuromuscular endplate and in general the functionality of *Ighmbp2* deficient motoneurons.

Keywords: SMARD1, IGHMBP2, helicase, motoneuron.

Abbreviations: Nmd=neuromuscular disorder; *Ighmbp2*=Immunoglobulin μ -binding Protein 2; IGF1=Insulin like growth factor 1; PEG=polyethylene glycol; iPSC=induced pluripotent stem cell

1. Introduction

Spinal muscular atrophy with respiratory distress type 1 (SMARD1), also referred to as DSMA1 (distal spinal muscular atrophy type 1), is a fatal motoneuron disorder which occurs in infancy and early childhood (1, 2) characterized by a very heterogenic clinical phenotype. Like the proximal form of spinal muscular atrophy (SMA), SMARD1 is characterized by a dysfunction and progressive degeneration of α -motoneurons in the ventral horn of the spinal cord, resulting in a neurogenic atrophy of striatal and skeletal muscle fibers in trunk, extremities and diaphragm (3). Despite similar pathological signs, progression of the disease is very distinct from proximal spinal muscular atrophy (SMA) (4). The most prominent and defining symptom of SMARD1 is a serious and life-threatening respiratory distress due to a severe paralysis of the diaphragm (5), resulting in permanent ventilation (3, 6, 7). As opposed to this, patients suffering from SMA develop respiratory distress at later stages of the disease due to paralysis of the intercostal muscles (5). The age of onset and progress of the disease distinguish two types of the disorder, infantile and juvenile SMARD1 (8). In patients with the rapidly progressing infantile disease, respiratory distress arises in the first months of life, whereas in cases of the very rare and more slow progressing juvenile disease, patients stay mobile for the first years (7, 9-12). Initially, muscle weakness in SMARD1 patients predominantly affects distal muscle groups, usually starting in the lower limbs. Congenital foot deformities followed by contractures of the fingers and fatty pads on the proximal phalanges are typical for this disorder (6, 7). Infants affected by SMARD1 can still move their arms against gravity but not their hands and fingers, while patients with infantile SMA present with an opposite phenotype (5).

2. SMARD1: genetic and cellular background

SMARD1 is caused by a mutation in the *Ighmbp2* gene that encodes the Immunoglobulin μ -binding Protein 2 (IGHMBP2), a DNA/RNA helicase belonging to the helicase superfamily 1 (SF1). *Ighmbp2*, mapped to chromosome 11 (11q13.2-q13.4) is ubiquitously expressed (3). The majority of mutations (homozygous or compound heterozygous) in *Ighmbp2* are located within or near the helicase domain and seem to affect the enzymatic activities of the protein (12). No direct correlation between type of mutation and phenotype in SMARD1 patients with infantile forms has been observed (6, 10). However, patients with juvenile SMARD1 showed residual elevated steady-state levels of IGHMBP2 protein indicating a correlation of abundance of the SF1 helicase and the mode of clinical progression (13). Neuropathological aspects observed in SMARD1 patients are Wallerian degeneration of motor nerves and failing regeneration of nerve fibers (4). The diameter of myofibrils in skeletal muscles of patients is reduced whereas the diaphragm displays a variation in the diameter of muscle fibers (5). Neurophysiological studies describe an acute or chronic distal denervation and a reduction in sensory or motor conduction velocities. Decreased sizes of myelinated fibers are observed on a sural nerve biopsy (14). Furthermore, axonal atrophy with hypo- and hypermyelination could be detected in SMARD1 patients. These alterations in axonal and myelin structures are reminiscent of other hereditary neuropathies such as Charcot-Marie-Tooth disease (4). Recently, Cottenie et al. has identified two compound heterozygous mutations in IGHMBP2 that associate to Charcot-Marie-Tooth type 2 (CMT2) (15). Patients with CMT2 exhibit slow progressive weakness, sensory loss and an

axonal neuropathy, but no significant respiratory compromise (15) that differs from SMARD1 patients. Studies from fibroblast and lymphoblastoid cell lines of CMT2 patients show an IGHMBP2 protein level higher than that from SMARD1 patients (15). These observations suggest a clear phenotype/genotype correlation due to IGHMBP2 protein levels with regard to peripheral neuropathies.

3. The helicase family SF1

Helicases are ubiquitous and highly conserved enzymes which use ATP hydrolysis to remodel nucleic acids or nucleic acid-protein complexes with numerous functions in virtually all aspects of DNA and RNA metabolism. Helicases are categorized into six superfamilies (SFs) based on their sequence, structure and functionality. Ring-forming helicases comprise SFs 3 to 6 and are mainly found in bacteria and viruses, whereas SF1 and SF2 are composed of non-ring forming helicases and contain all eukaryotic RNA and DNA helicases (16, 17). All helicases of SFs 1 and 2 have a highly conserved helicase core formed by two RecA-like domains arranged in tandem. The helicase domains contain several characteristic sequence motifs, whereof seven are present in all SF1 and SF2 helicases and additional motifs define the classification into subfamilies (17, 18). As previously described, IGHMBP2 belongs to the superfamily 1 of helicases, which can be divided further into three subgroups, namely UvrD/Rep, Pif1-like and Upf1-like helicases (16). Based on its sequence, IGHMBP2 is placed in the group of Upf1-like helicases, which work on both DNA and RNA (13, 17). Members of the SF1 helicases in general have been indicated to participate in various processes of RNA metabolism including transcription, splicing, translation and RNA decay (17). UPF1, a key factor involved in nonsense-

mediated decay (NMD) (19), is another member of the SF1 superfamily. This group also includes Senataxin (SETX), which is involved in resolving RNA-DNA hybrids during transcription (20). IGHMBP2 shows a very high structural similarity to Upf1 and a 42% identity of the helicase domain with SETX (21). Human Upf1 was shown to reduce FUS- and TDP43-associated toxicity in a neuronal model of amyotrophic lateral sclerosis (ALS) through a NMD-mediated mechanism (22) whereas mutations in the *SETX* gene are associated with AOA2 (ataxia with oculomotor apraxia type 2) and ALS4 (amyotrophic lateral sclerosis 4) (20).

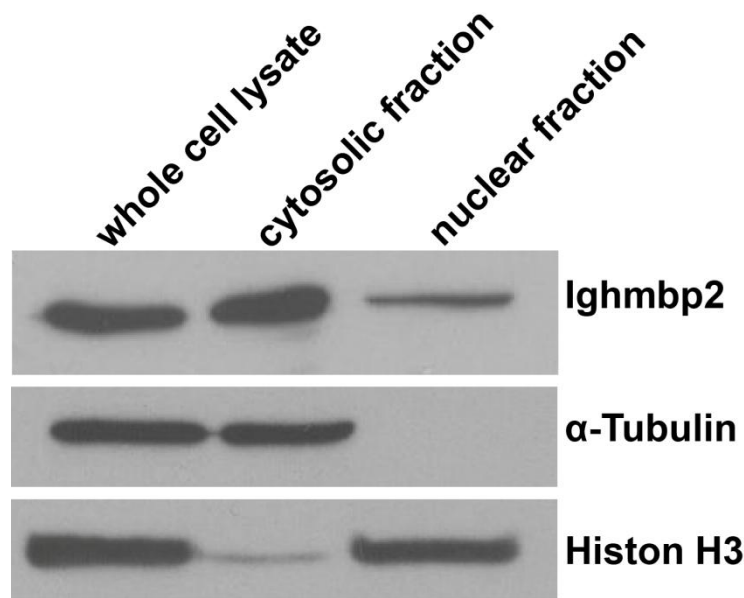
3.1 The helicase IGHMBP2

IGHMBP2 is a multidomain protein consisting of 993 amino acids (23) and is composed of a DExxQ-type helicase/ATPase domain, a R3H domain and a zinc finger domain (13, 24). The helicase core of IGHMBP2 contains four domains: two RecA-like domains including the seven conserved sequence motifs typical for SF1 and SF2 helicases and two subdomains (24). The R3H domain was shown to bind single-stranded nucleic acids (25) and supposedly has a regulatory role in promoting nucleic acid binding and stimulating ATPase activity (24). IGHMBP2 is a 5'-3' helicase which uses ATP hydrolysis to unwind RNA and DNA duplexes with 5' overhangs (13). It is predominantly located in the cytoplasm of cultured motoneurons (Figure 1) including the perinuclear cytoplasm as well as axons and growth cones. Guenther et al. found that IGHMBP2 is a ribosome-associated helicase and co-localizes among others with translation factor eIF4G2 and ribosomal proteins (13). Furthermore, an association of IGHMBP2 with tRNA as well as factors important for tRNA transcription and ribosome maturation has been shown (26). These findings suggest a role for IGHMBP2 in regulatory mechanisms of mRNA

translation rather than a function on DNA or pre-mRNA in the nucleus. Although many genes which cause motoneuron diseases are ubiquitously expressed - such as genes encoding RNA helicases discussed above - motoneurons seem to be especially

susceptible to mutations. In the case of SMARD1, this elevated susceptibility might be explained by a particular requirement of IGHMBP2 for translation of certain mRNAs in motoneurons as well as in non-neuronal cells.

A Lysate from HEK293 cells



B Primary cultured motoneuron

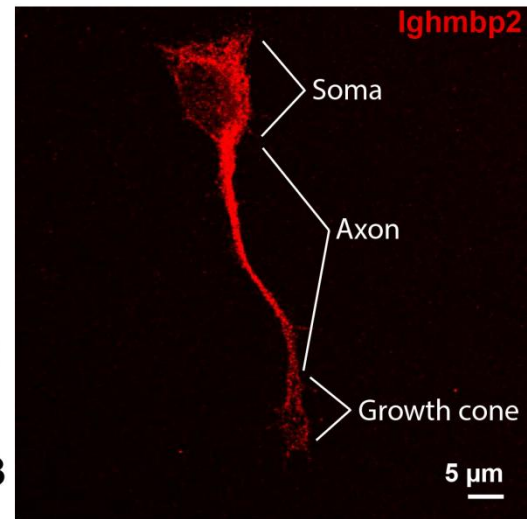


Figure 1: Intracellular distribution of Ighmbp2

(A) Representative Western Blot for cytosolic and nuclear fractions from HEK293 cells. Ighmbp2 is pre-dominant detectable in the cytosolic fraction. Histone H3 and α -tubulin were used as markers for nuclear and cytosolic fractions, respectively. (B) Intracellular distribution of Ighmbp2 in primary cultured motoneurons revealed a predominant localization in the cytoplasm of the cell body and the axonal compartment by partially omitting the growth cone.

4. The *Nmd^{2J}* mouse as a model for SMARD1

The neuromuscular degeneration (*Nmd^{2J}*) mouse as a model system for juvenile form of SMARD1 disease (27) carries a point mutation in *Ighmbp2*, leading to a ubiquitously reduced Ighmbp2 level (28). The mutation was initially discovered and described at the Jackson Laboratories (29). It is a point mutation in the fourth intron of the *Ighmbp2* gene on chromosome 19. An A to G transition induces a new splice donor site which leads to alternatively

spliced *Ighmbp2* mRNA. A premature stop codon results in a truncated protein (28). As a consequence, the amount of functional Ighmbp2 protein is reduced to 20-30 % in all tissues (27) and in primary cultured motoneurons (Figure 2). The pathological features of the *Nmd^{2J}* mouse are comparable to humans. Motoneuron cell loss in *Nmd^{2J}* mice (~30%) is initially detectable ten days after birth (P10) when these mice appear clinically completely unaffected (27, 30). Loss of motoneurons remains within a constant level of about 30% until three weeks after birth (P21), a

time point at which *Nmd^{2J}* mice have developed hindlimb paralysis and a substantial number of degenerating axons in the sciatic nerve becomes detectable (27). At later disease stages motoneuron loss is mitigated (27). Thus, *Nmd^{2J}* mice resemble patients with a milder course of the disorder, representing the juvenile form of SMARD1 (27). Mice develop muscle weakness and atrophy, and also show myopathic changes in the diaphragm and a cardiomyopathy at later disease stages (27, 28, 31). Former studies revealed that degeneration of muscle fibers in the gastrocnemius muscle appears relatively early in the second to third week after birth (27). Muscle fiber degeneration in the diaphragm starts around six to eight weeks after birth and does not correlate with motor

axon loss in the phrenic nerve (27, 30). The survival rate is highly variable ranging from six to ten weeks up to eleven months presumably depending on unknown modifying polymorphisms on chromosome 13 (28, 32). *Nmd^{2J}* mice are considerably smaller than control mice. The group of Maddatu et al. could show that motoneuron degeneration can be ameliorated by transgenic expression of wildtype *Ighmbp2* cDNA in neurons (31). However, these transgenic *Nmd^{2J}* mice still developed a severe cardiomyopathy suggesting an important role of *Ighmbp2* expression in motoneurons as well as in myocytes (31). Ubiquitous up-regulation of the intracellular *Ighmbp2* level via adenoviral gene transfer leads to a more effective phenotypic rescue (32).

Cell lysate from primary cultured motoneurons

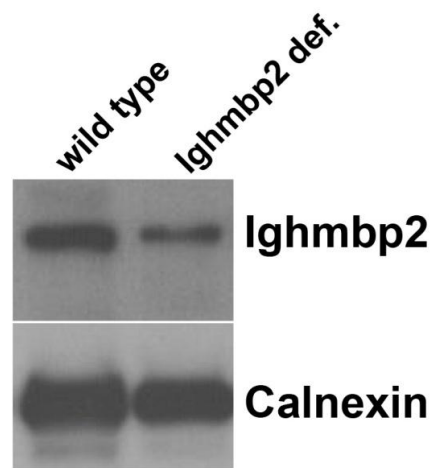


Figure 2: Ighmbp2 protein level in Ighmbp2 deficient motoneurons

Representative Western Blot from primary cultured wild type and *Nmd^{2J}* (*Ighmbp2* def.) motoneuron lysates shows *Ighmbp2* protein reduction in *Nmd^{2J}* motoneurons.

4.1 Cellular dysfunctions in striated muscles of *Nmd^{2J}* mice

Nmd^{2J} mice typically exhibit muscle fiber degeneration characterized by reduced caliber of gastrocnemius and diaphragm muscle fibers that is strictly associated to

affected differentiation of type I and type II fibers (2, 3, 9, 30, 33). *Nmd^{2J}* mice exhibit increased spontaneous activity (SpA) and reduced compound muscle action potentials (CMAPs) amplitudes in the gastrocnemius muscle which are characteristic signs of muscle fiber denervation and degeneration.

Mild cardiac hypertrophy at P42 is indicated by a significantly increased ratio of heart weight to body weight. *Nmd^{2J}* mice show an up-regulation of IGF1R in gastrocnemius muscle and diaphragm that is not observed in spinal cord tissue two weeks after birth (33). *Nmd^{2J}* mice have been shown to be deficient in IGF1, a growth factor involved in muscle and neuron survival as well as differentiation including axon growth during development. Deficiency can be compensated by external application of PEG-IGF1 (33). This external application leads to a full compensation of muscle fiber typing in diaphragm and a partial rescue in gastrocnemius muscle (33). The proper fiber typing corresponds to the activation and down-regulation of IGF1R and enhanced phosphorylation of Akt as well as the ribosomal protein S6 kinase (33). These observations support the notion that

myopathic changes in the *Nmd^{2J}* mouse results from IGF1 deficiency and can be compensated by external application of PEG-IGF1 (33).

Unfortunately, the compensatory effect of PEG-IGF1 could not be observed in spinal cord tissue (33). Cell body and axon loss of motoneurons is still present in treated *Nmd^{2J}* mice. This raises the question of about dysregulation of growth factors primarily affecting motoneuron development. Neuroprotective effects by growth factor release have been already shown in transplantation studies of wild type iPSC-derived neuronal stem cells (NSCs) in *Nmd^{2J}* mice (34). However, the capability of *Nmd^{2J}* mice to induce additional axonal sprouts (Figure 3) due to muscle fiber denervation exceeds that of SMA mice and indicates non-autonomous cellular pathomechanisms in *Nmd^{2J}* mice leading to motoneuron degeneration.

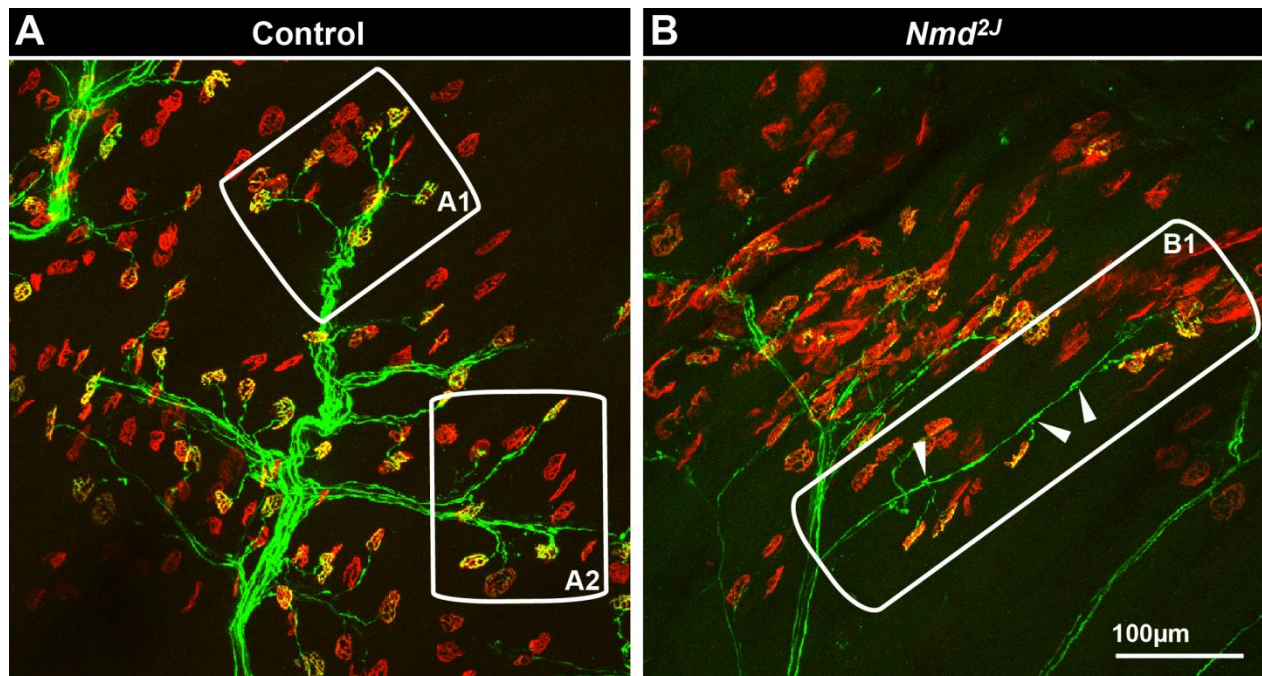


Figure 3: Additional axonal sprouting in *Nmd^{2J}* mice

(A) YFP labeled motor axons in the gastrocnemius muscle from control mice terminate in a neuromuscular endplate (A1 and A2). (B) Motor axons from *Nmd^{2J}* mice display a marked increase in additional sprouts along the axon ending up in a motor endplate (white arrow heads in B1). Modified from ©2013 Krieger *et al.* (30).

4.2 Does motoneuron degeneration occur cell-autonomously in *Nmd^{2J}* mice?

Testing for a neuromuscular transmission defect in the foot muscle by repetitive tibial nerve stimulation revealed a slightly abnormal decremented response as a sign of synaptic dysfunction in only 9% of *Nmd^{2J}* mice (33). This indicates that a transmission defect at the neuromuscular endplate is rather the exception than the rule in *Nmd^{2J}* mice. The detected failures upstream of neurotransmitter release at higher stimuli (30) are most likely caused by defective axonal conduction of action potentials, which in addition indicates a motor axon defect independent from a defective neurotransmission at the endplate. The hypothesis of less pronounced transmission defects in *Nmd^{2J}* mice is supported by confocal and electron-microscopy approaches which clearly demonstrated that morphological abnormalities of the motor endplate are missing (30). At the early symptomatic stages P16 and P21, half of the endplates show an intact innervation and normal synaptic morphology, half of the endplates are denervated, and only a minority of the endplates exhibits a fragmented presynaptic structure with degenerating axons and invading Schwann cells (30). Neurofilament inclusions are not detectable in any of these neuromuscular endplates in contrast to SMA mouse models (35, 36). Compared to the *Nmd^{2J}* mice, the ultrastructural as well as immunohistochemical analysis of motor endplates from SMA mice reveals less vesicles within the presynaptic compartment and the majority of endplates exhibits a fragmented structure at the axon terminals (37, 38). This coincides with reduced neurotransmission found at all disease stages at neuromuscular junctions in SMA mice (36-39). The insights in the *Nmd^{2J}* mouse led to the conclusion that motoneuron loss under *Ighmbp2* deficiency does not primarily correspond to

neurotransmission failures. The unaffected function of the neuromuscular endplates in *Nmd^{2J}* mice that corresponds to proper re-establishment of neuromuscular endplates during the procedure of “additional axonal sprouting” at very early disease stages (30) is completely missing in intermediate forms of SMA mouse models (40). Moreover, transplantation of neuronal stem cells to increase the number of spinal motoneurons extends only moderately survival time of *Nmd^{2J}* mice (41, 42). Summarizing these observations in *Nmd^{2J}* mice argue for a non-cell-autonomous disease mechanism and against a “dying back” pathomechanism which primarily starts out from the endplates.

5. Conclusion

SMARD1 is described as a fatal motoneuron disorder. However, despite a markedly and tremendous motoneuron loss at presymptomatic stages (P10) in *Nmd^{2J}* mice, no signs of defective neurotransmission are detectable. As well as due to the compensatory capability via the induction of additional axonal sprouts in gastrocnemius muscle, the question about cell-autonomous disease mechanisms in motoneurons arises. The answer can exclusively be achieved by appropriate morphological and functional approaches in enriched primary cultured motoneurons or iPSCs from *Nmd^{2J}* mice. Therefore, up until this moment, it can be concluded that the neurodegenerative process in *Nmd^{2J}* mice does not primarily affect the neuromuscular endplate and the neurotransmission machinery, in contrast to other SMA models. These observations argue for additional non-cell-autonomous disease mechanisms that affect motoneuron survival in *Nmd^{2J}* mice at very early stages of the disease.

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