### Newborn Screening

The main purpose of newborn screening is to identify affected children prior to the development of clinical symptoms for early treatment interventions. The most suitable technology for newborn screening, in our opinion, may be HRMA, which is a simple and rapid but low-cost test for detecting human disease-associated mutations, especially for samples with mutations of low incidence in the population (Li et al., 2011).

Regarding the implementation of newborn screening, there should be availability of an accepted treatment for patients with a recognized disease as a prerequisite for such screening, following the Wilson-Junger criteria (Orzalesi & Danhaive, 2009). There are a number of genetic conditions for which newborn screening is routinely carried out in a number of countries. An example is that for phenylketonuria (PKU) deficiency, which was the first newborn screening test implemented in the USA in the 1960s. PKU is a condition in which early treatment will make a significant difference to clinical outcome, thus meeting the Wilson-Junger criteria. This is in contrast with SMA which is still an incurable disease.

There are two challenges in recommending newborn screening for SMA, namely cost-effectiveness and psychosocial issues. In principle, any newborn screening program should achieve maximum public health benefits with economic savings to costs ratio. A pilot population-based carrier screening for SMA showed it not to be cost effective (Little et al., 2010), suggesting that this may be true for newborn screening too. The second issue with such newborn screening relates to the psychosocial effects affecting the relationship between parents and child (Kerruish & Robertson, 2005), resulting in emotionally draining experiences. In addition, current diagnostic methods can identify SMA mutations but cannot ascertain the SMA subtype or accurately predict disease onset or severity. In particular, we cannot identify the SMA subtype in pre-symptomatic children with any of the current methods. Early diagnosis of SMA in a presymptomatic child may have a negative impact on bonding with the parents and bring about uncertain psychosocial effects. Raising such a presymptomatic offspring with an incurable disease and uncertain prognosis may be a traumatic experience for many parents.

However, Swoboda and her colleagues pointed out that newborn screening may be important even in the absence of a curative treatment for SMA (Swoboda et al., 2005). Citing the experience in cystic fibrosis, they argued that newborn screening can lead to significant improvements in quality of life for patients as supportive interventions can be implemented early. According to them, the prerequisite for newborn screening is the presence of early supportive care. A recent study showed that presymptomatic treatment of SMA mice ($Smn^{-/-}$ $SMN2^{+/+}$ $SMN\Delta 7^{+/+}$) at the earliest postnatal time was the most effective, lending further support to the usefulness of early neonatal screening in humans (Porensky et al., 2012). In this context, such newborn screening can identify appropriate patient cohorts for enrolment into clinical trials at the earliest possible time before disease onset (Prior, 2010). However, this needs to be balanced against the ethical viewpoint that without a known cure for the disease, the implementation of newborn screening to accelerate clinical trials cannot be justified.

In theory, newborn screening is useful for providing a framework in place to identify children as early as possible when better treatments become available. At this point in time, it may be a rational decision to accept the statement of Wirth et al. (2006b) that "Neonatal screens will be crucial for a successful SMA therapy. Children at risk to develop SMA should be recognized as soon as possible before first symptoms occur. However, as long as we do not have a clear answer whether any drug is sufficiently beneficial to SMA patients, a neonatal screening for SMA should not be offered." Newborn screening may be indicated if an effective treatment is found to cure or slow disease progression.

## Molecular Pathophysiology

### Biogenesis of Small Nuclear Ribonucleoprotein

SMN is a 38 kDA protein that is ubiquitously expressed in both neuronal cells and non-neuronal cells. SMN interacts and forms a complex with binding partner proteins in a variety of cellular activities; including pre-mRNA splicing (Fischer et al., 1997; Pellizzoni et al., 1998), biogenesis of small nuclear ribonucleopoteins (snRNPs) (Burghes & Beattie, 2009), transcription (Strasswimmer et al., 1999), stress responses (Zou et al., 2011), apoptosis (Iwahashi et al., 1997), axonal transport (Pagliardini et al., 2000) and cytoskeleton dynamics (Bowerman et al., 2007).

In 1996, Dreyfuss' group first reported the presence of SMN in both the nucleus and cytoplasm of HeLa cells, an immortalized cell line derived from cervical cancer cells. In the nucleus, SMN is localized within a nuclear structure, so called "Gems," or gemini of coiled bodies (Cajal bodies), interacting with RNA-binding proteins (Liu & Dreyfuss, 1996). In any type of cell, SMN exists as a part of a stable multiprotein complex in cytoplasm and nuclear Gems. SMN, together with Gemin2-Gemin8 and UNRIP, plays an essential role in the assembly of snRNPs and their transport from the cytoplasm into the nucleus. In the cytoplasm, the SMN complex functions as an assemblyosome in the formation of snRNP. The SMN complex facilitates the assembly of a small nuclear RNA (snRNA) with RNA binding proteins (known as Sm proteins) to form snRNP (Gubitz et al., 2004). The SMN complex re-enters the nucleus with the snRNP-cargo through the help of snurportin and importin (Pellizzoni, 2007). In the nucleus, the SMN complex is liberated from its snRNP-cargo and then shuttles back to the cytoplasm to help assemble the new snRNPs (Matera et al., 2007; Burghes & Beattie, 2009; Cauchi, 2010). In addition, snRNPs join the spliceosome to participate in splicing. According to Zhang et al. (2008), SMN-deficient mouse tissues show alteration in the stoichiometry of snRNAs as well as widespread pre-mRNA splicing defects in numerous transcripts of diverse genes. These findings highlight the role of the SMN complex in RNA metabolism and splicing regulation, suggesting that SMA is a general splicing disease that is not restricted to motor neurons (Zhang et al., 2008).

Even so, the main pathological finding of SMA is the loss of motor neurons. It remains to be resolved whether loss of function in spliceosomal assembly, resulting in widespread defects in mRNA splicing, is directly responsible for motor neuron death. Bäumer et al. (2009) assessed the degree of altered splicing in the spinal cord of SMA mice ($Smn^{-/-}$ $SMN2^{+/+}$ $SMN\Delta 7^{+/+}$), using exon-specific microarrays. According to them, the vast majority of splicing changes are a late feature of the disease and are therefore unlikely to contribute to early disease pathogenesis. These findings noted that splicing defects may not be a primary effect of the loss of SMN. However, the authors could not fully rule out the presence of significant early changes in a small number of transcripts crucial to motor neuron survival (Bäumer et al., 2009).

Two hypotheses have been presented to explain the basis of the motor neuron defects in SMA (Burghes & Beattie, 2009). The first suggests that disturbed snRNP synthesis due to decrease of SMN affects the splicing of genes that are important for the circuit formation of motor neurons. Although defects in these genes may not cause the same SMA phenotype, it is thought that they affect motor neuron functioning that contributes toward the clinical symptoms in SMA (Jablonka et al., 2002). Even though the identities of these genes have yet to be fully clarified, it was recently reported by Lotti and colleagues that SMN deficiency perturbs splicing and decreases the expression of a subset of U12 intron-containing genes in animal models, including a protein called Stasimon (Lotti et al., 2012). Restoration of Stasimon expression in the motor circuit corrects defects in neuromuscular junction (NMJ) transmission and muscle growth in Drosophila SMN mutants, and corrects aberrant motor neuron development in SMN-deficient zebrafish (Lotti et al., 2012). These findings link defective splicing of critical neuronal genes induced by SMN deficiency to motor circuit dysfunction, contributing toward further understanding on the role of the snRNP complex in SMA pathogenesis.

The second hypothesis suggests that SMN has some critical role, independent of snRNP synthesis, in the motor neuron function. SMN deficiency has been reported to produce defects in $\beta$-actin mRNA axonal transport, neurofilament dynamics, neurotransmitter release, and synapse maturation (Torres-Benito et al., 2011). Some of the SMN functions in the motor axon outgrowth may be independent of the functions required for snRNP synthesis, because SMN oligomerization or Sm binding does not correlate with the motor axon growth (Carrel et al., 2006).

### Axonal Transport of Motor Neurons and NMJ Maturation

Rossoll and colleagues demonstrated that a complex of Smn (the ortholog of human SMN) with its binding partner hnRNP-R has been found to interact with $\beta$-actin (Rossoll et al., 2002; Rossoll et al., 2003). In Smn-deficient motor neurons from an SMA mouse model ($Smn^{-/-}$ $SMN2^{+/+}$), reduced axon elongation and small-sized growth cones correlated with reduced $\beta$-actin protein and mRNA levels in distal axons and growth cones (Rossoll et al., 2003). The model for pathogenesis based on their findings was that defects in dynamic processes in axons may hamper axonal elongation, synapse formation, and presynaptic function of the motor endplate in SMA.

Zhang and colleagues also showed that SMN is localized in granules that are actively transported into neuronal processes and growth cones (Zhang et al., 2006). According to them, in cultured motor neurons, SMN granules co-localize with ribonucleoprotein Gemin proteins but not with spliceosomal Sm proteins that are required for snRNP assembly. SMN–Gemin complex containing granules are distributed to both axons and dendrites of differentiated motor neurons. In addition, high-speed dual channel imaging of live neurons depicted rapid and bidirectional transport of the SMN–Gemin complex.

Recently, it has been reported that SMN also interacts with RNA-binding protein, HuD (Hubers et al., 2010; Fallini et al., 2011; Akten et al., 2011). HuD is a neuron-specific RNA-binding protein. The complex of SMN and HuD regulates localization of poly(A) mRNA (Fallini et al., 2011). It also binds to the candidate plasticity-related gene, *cpg15* (Akten et al., 2011). The *cpg15* protein is highly expressed in the developing ventral spinal cord and can promote motor axon branching and neuromuscular synapse formation, suggesting a crucial role in the development of motor axons and NMJs. All these findings support the model for pathogenesis mentioned above, and imply that motor neuron degeneration could begin before the formation of NMJs.

**Table 3** Current advances in SMA therapeutic strategies.

| 1. *SMN2*-targeting strategies | | | | | |
|---|---|---|---|---|---|
| Treatment categories | Compounds/Drugs | Clinical trial phase | Subjects | Outcome | References |
| (1) Increase in FL-SMN levels by pharmacological compounds | | | | | |
| HDAC inhibitors | Benzamide M344 | In vitro | SMA fibroblasts | Increase in SMN protein and gem numbers | (Riessland et al., 2006) |
| | Hydroxamic acid LBH589(panobinostat) | In vitro | SMA fibroblasts | Increase in SMN protein and gem numbers | (Garbes et al., 2009) |
| | Sodium butyrate | In vitro | SMA lymphoblastoid cells | Increase in SMN protein | (Chang et al., 2001) |
| | | In vivo | mice ($Smn^{-/-}SMN2^{+/+}$) | Improvement in lifespan and motor functions | (Chang et al., 2001) |
| | Sodium phenylbutyrate (PBA) | In vitro | SMA fibroblasts | Increase in SMN protein and gem numbers | (Andreassi et al., 2004) |
| | | HT-CS Phase 0 | 4 patients (type 2) and 2 patients (type 3) | Increase in full-length *SMN2* transcript of leukocytes and in muscle strength | (Brahe et al., 2005) |
| | | HT-RCT Phase 2 | 107 patients (type 2) | No benefit in motor function | (Mercuri et al., 2007) |
| | Suberoylanilide hydroxamic acid (SAHA) | In vitro | Neurectodermal tissues | Increase in SMN protein | (Hahnen et al., 2006) |
| | | In vivo | mice ($Smn^{-/-}SMN2^{+/+}$ and $Smn^{-/-}SMN2^{+/-}$) | Improvement in lifespan, motor function | (Riessland et al., 2010) |
| | Trichostatin A (TSA) | In vivo | SMA mice ($Smn^{-/-}SMN2^{+/+}$ $SMN\Delta7^{+/+}$) | Increase in weight gain<br>Improvement in lifespan, and motor function<br>Increase in weight gain | (Avila et al., 2007) |
| | Valproic acid (VPA) | | | | |
| | a. VPA only | In vitro | SMA fibroblasts | Increase in SMN protein | (Brichta et al., 2003) |
| | b. VPA only | In vitro | SMA fibroblasts | Increase in SMN protein | (Sumner et al., 2003) |
| | c. VPA only | HT-CS Phase 0 | 7 patients (type 3 and 4) | Improvement in muscle strength and subjective functions | (Weihl et al., 2006) |
| | d. VPA only | HT-OL Phase 2 | 42 patients (type1,2 and 3) | Improvement of motor function in patients with SMA type 2 | (Swoboda et al., 2009) |
| | e. VPA + Carnitine | HT-OL Phase 2 | 61 patients (type 2 and 3) | No benefit in motor function | (Swoboda et al., 2010) |
| | f. VPA + Carnitine | HT-OL Phase 2 | 33 patients (type 3) | No benefit in motor function | (Kissel et al., 2011) |
| Non-HDAC inhibitors | Aclarubicin | In vitro | SMA fibroblasts | Increase in SMN protein and gem numbers | (Andreassi et al., 2001) |
| | Hydroxyurea (HU) | In vitro | SMA lymphoblastoid cells | Increase in SMN protein and gem numbers | (Grzeschik et al., 2005) |
| | | HT-RCT Phase 2 | 28 patients (type 2) and 29 patients (type 3) | No benefit in motor function | (Chen et al., 2010) |

*Continued*





However, the deteriorating mechanisms of motor neurons in SMA are more complicated than expected. Cifuentes-Diaz et al. (2002) reported that SMA mice ($Smn^{F7}/Smn^{\Delta7,NSE\text{-}Cre+}$) display a drastic and progressive loss of motor axons, consistent with the skeletal muscle denervation process in SMA. Interestingly, they also found accumulation of neurofilaments in terminal axons of the remaining NMJs, associated with a defect of axonal sprouting and postsynaptic apparatus formation. These findings suggested that loss of motor neuron cell bodies results from a "dying-back" axonopathy in SMA. Such denervation likely resulted from defects in synapse maintenance rather than defects in the initial formation of nerve-muscle contact (Kong et al., 2009; Ling et al., 2012). Based on these findings, motor neuron degeneration may begin after NMJ formation. Such understanding on the timing of the beginning of motor neuron degeneration may be critical for effective treatment.

### Cytoskeleton Dynamics Regulated by SMN Downstream Signaling

Recent observations have also revealed an association between cytoskeleton dynamics and the pathogenesis of SMA. Axonogenesis of motor neurons, as well as axonogenesis of other neurons, is mediated by changes in cytoskeletal dynamics, i.e., assembly and disassembly of cytoskeleton proteins including actin and tubulin. Thus, much of the research in this area has been focused on the relationship between SMN and cytoskeleton dynamics.

It has been reported that two SMN-binding proteins, profilin IIa and plastin 3, are closely related to actin dynamics (Bowerman et al., 2007, 2005; Oprea et al., 2008). Bowerman's group showed that Smn knockdown in neuronal cells increased profilin IIa isoform, resulting in an increased formation of Rho-associated kinase (ROCK)/profilin IIa complex (Bowerman et al., 2007). In the activated RhoA/ROCK pathway, Rho (a well-characterized member of the family of Rho GTPases) and its effector ROCK mediate enhancing signals to the downstream proteins. The increased ROCK/profilin IIa complex activated the RhoA/ROCK pathway inappropriately, resulting in altered cytoskeletal integrity and a subsequent defect in axonogenesis (Bowerman et al., 2007). Meanwhile, Oprea et al. (2008) found that overexpression of *PLS3*, a gene encoding plastin 3, rescued the axonal growth defect in culture motor neurons. They also reported that overexpression of *PLS3* was found in unaffected siblings that shared the same SMN genotype as children with SMA. In addition, a decrease in plastin 3 levels was observed in the brain and spinal cord of SMA mice ($Smn^{-/-}SMN2^{+/+}$) and this is now considered to be related to the pathophysiology of SMA (Bowerman et al., 2009).

In the context of dysregulation of cytoskeleton in SMA, stathmin, a microtubule-destabilizing protein, should also be considered. Upregulated stathmin has been shown to correlate with a decrease in polymerized tubulin level in distal axons of SMA mice ($Smn^{-/-}SMN^{2/-}$) and in Smn-deficient cells (Wen et al., 2010). It was observed that knockdown of stathmin restored the microtubule network defects of Smn-deficient cells, and promoted axonal growth in motor neurons of SMA mice.

## Potential Treatment Strategies and Clinical Trials for SMA

### Classification of SMA Treatment Strategies

Current SMA therapeutic strategies can broadly be classified into three major groups. We summarize the therapeutic strategies and the compounds that have been used as candidate drugs for SMA in Table 3. Some compounds in each group have already been tested while others are still in the test phase. Table 3 also provides the information on the different test phases of the compounds: in vitro, in vivo, and human trial (HT) phases. HT phases include case series (CS), open label study (OL), and randomized controlled trial (RCT).

The first group which we term "*SMN2*–targeting strategies" involves strategies to increase FL-SMN protein either using pharmacological compounds or through splicing correction of *SMN2* mRNA by antisense oligonucleotides (ASO), and to produce a stable form of Δ7-SMN protein with additional C-terminal peptides by a translational read-through method.

The second group, "*SMN1*-introduction strategies," involves strategies to introduce exogenous *SMN1* gene copies using vector-mediated gene delivery methods (gene therapy), and stem cell transplantation methods (stem cell therapy)

The third group, "Non-*SMN*-targeting strategies," involves strategies to protect motor neurons or improve the pathological conditions of non-neuronal tissues including muscles. This group also includes modulation of SMN-downstream signaling systems including the RhoA/ROCK pathway.

In this review, we put stress on the new treatment candidates. Some clinical trials have already been conducted for SMA based on new strategies for increasing FL-SMN protein or protection of motor neurons.

### *SMN2*-Targeting Strategies

*Increasing FL-SMN protein using pharmacological compounds*

In 2001, Chang and colleagues reported that a histone deacetylase (HDAC) inhibitor, sodium butyrate, increased FL-SMN protein in SMA lymphoid cells and prolonged the

**Table 3** Continued

| Treatment categories | Compounds/Drugs | Clinical trial phase | Subjects | Outcome | References |
|---|---|---|---|---|---|
| Bifunctional oligonucleotide | Oligonucleotide with ESE mimic sequence | In vitro | SMA fibroblasts | Increase in SMN protein | (Skordis et al., 2003) |
| | | In vivo | SMA mice (*Smn*$^{-/-}$*SMN2*$^{+/+}$*U7-ESE-B+*) | Increase in weight gain<br>Improvement in lifespan and motor neuron | (Meyer et al., 2009) |
| Trans-splicing | a. Oligonucleotide with *SMN1* exon 7 sequence | In vitro | SMA fibroblasts | Increase in SMN protein | (Coady et al., 2007) |
| | | In vivo | SMA Mice (*Smn*$^{-/-}$*SMN2*$^{+/+}$*SMNΔ7*$^{+/+}$) | Improvement in lifespan and phenotype | (Coady & Lorson, 2010) |
| | b. Oligonucleotide with *SMN1* exon 7 in sinergy with *IGF1* expressed vector | In vivo | SMA Mice (*Smn*$^{-/-}$*SMN2*$^{+/+}$*SMNΔ7*$^{+/+}$) | Improvement In lifespan<br>Increase in weight gain | (Shababi et al., 2011) |
| ISS masking | Antisense oligonucleotide | | | | |
| | a. Oligomer against ISS-N1 | In vitro | SMA fibroblasts | Increase in SMN protein | (Singh et al., 2006) |
| | | In vivo | SMA Mice (*Smn*$^{-/-}$*SMN2*$^{+/+}$*SMNΔ7*$^{+/+}$) | Increase in weight gain,<br>Improved motor function | (Porensky et al., 2012) |
| | b. ASO-10—27 (or ISIS-SMNRx) | In vivo | SMA mice (*Smn*$^{-/-}$*SMN2*$^{+/+}$*SMNΔ7*$^{+/+}$) | Improvement in lifespan and motor function<br>Increase in weight gain | (Hua et al., 2010, Hua et al., 2011, Passini et al., 2011) |
| | c. ASO-10—27 (or ISIS-SMNRx) | HT-RCT Phase 1 | 28 Patients (type 1,2 and 3) | The compound was well tolerated<br>Improvement in HFMSE, MUNE and CMAP score | http://www.isisph.com/pdfs/AAN_Isis_Investor-Event.pdf |
| (3) Stabilization of Δ7-SMN protein via read-through strategy | | | | | |
| Stop-codon read-through technology | Aminoglycosides | | | | |
| | a. TC007 (PTC-X) | In vitro | SMA fibroblasts | Increase in SMN protein and gem numbers | (Mattis et al., 2006) |
| | b. TC007 (PTC-X) | In vitro | SMA mice (*Smn*$^{-/-}$*SMN2*$^{+/+}$*SMNΔ7*$^{+/+}$) | Improvement in lifespan and phenotype | (Mattis et al., 2009) |
| | c. G418 (Geneticin) | In vitro | SMA fibroblasts | Increase in SMN protein | (Heier & DiDonato, 2009) |
| | | In vivo | SMA mice (*Smn*$^{-/-}$*SMN2*$^{+/+}$*SMNΔ7*$^{+/+}$) | Increase in motor function, no significant benefit in lifespan and body weight | |

*Continued*



**Table 3** Continued

| Treatment categories | Compounds/Drugs | Clinical trial phase | Subjects | Outcome | References |
|---|---|---|---|---|---|
| | Indoprofen | In vitro | SMA fibroblasts | Increase in SMN protein and and gem numbers | (Lunn et al., 2004) |
| | | In vivo | SMA mice embryos (*Smn*$^{-/-}$*SMN2*$^{+/-}$) | Improvement in viability and lifespan | |
| | Prolactine | In vivo | SMA mice (*Smn*$^{-/-}$*SMN2*$^{+/+}$*SMNΔ7*$^{+/+}$) | Increase in SMN protein, weight gain, improvement in lifespan, and motor function | (Farooq et al., 2011) |
| | Salbutamol (Albuterol) | HT-CS Phase 0 | 5 patients (type 2) and 8 patients (type 3) | Increase in muscle mass and strength | (Kinali et al., 2002) |
| | | HT-CS Phase 0 | 12 patients (type 2 and 3) | Increase in full length *SMN2* transcripts of leucocytes | (Tiziano et al., 2010) |
| | | In vitro | SMA fibroblasts | Increase in SMN protein and gem numbers | (Angelozzi et al., 2008) |
| | | HT-OL Phase 0 | 23 patients (type 2) | Increase in muscle mass and strength | (Pane et al., 2008) |
| | Tetracycline-like compound PTK-SMA1 | | | | |
| | | In vitro | SMA fibroblasts | Increase in SMN protein | (Hastings et al., 2009) |
| | | In vivo | SMA mice (*Smn*$^{-/-}$*SMN2*$^{+/+}$, and *Smn*$^{-/+}$ *SMN2*$^{+/+}$) | Increase in SMN protein and improvement in motor function | |
| | Quinazolines | | | | |
| | a. D156844 | In vivo | SMA mice (*Smn*$^{-/-}$*SMN2*$^{+/+}$*SMNΔ7*$^{+/+}$) | Improvement in lifespan and motor function | (Butchbach et al., 2010b) |
| | b. Quinazoline-495 (or RG 3039) | In vivo | SMA Δ7-mice | Improvement in lifespan and motor function | (Van Meerbeke et al., 2011) |
| | | HT-RCT Phase 1 | patients (type 2 and 3) | Ongoing | |
| | Triptolide (PG490) | In vitro | SMA fibroblasts | Increase in SMN protein and gem numbers | (Hsu et al., 2012) |
| | | In vivo | SMA Mice (*Smn*$^{-/-}$*SMN2*$^{+/+}$) | Increase in SMN protein, improvement in weight loss and improvement in motor function | |
| (2) Increase in FL-SMN levels by antisense oligonucleotides | | | | | |
| Splice-site targeting | Antisense oligonucleotide against 3′ splice-site | In vitro | In-vitro splicing assay | Increase in exon 7 inclusion into *SMN2* mRNA | (Lim & Hertel, 2001) |
| Bifunctional peptide nucleic acid | Peptide nucleic acid with splicing factor mimic peptides (ESSENCE) | In vitro | In-vitro splicing assay | Increase in exon 7 inclusion into *SMN2* mRNA | (Cartegni & Krainer, 2003) |

*Continued*

**Table 3** Continued

| 3. Non-*SMN*-targeting strategies | | | | | |
|---|---|---|---|---|---|
| Treatment Categories | Compounds/Drugs | Clinical trial phase | Subjects | Outcome | References |
| 1. Neuroprotection therapy | | | | | |
| Reduction in glutamate mediated excitotoxicity | Gabapentin | HT-RCT Phase 2<br>HT-RCT Phase 2 | 84 patients (type 2 and 3)<br>120 patients (type 2 and 3) | No benefit in motor function<br>Slight improvement in muscle strength | (Miller et al., 2001)<br>(Merlini et al., 2003) |
| | Riluzole | HT-CS phase 1 | 10 patients type 1 | No adverse event, possible benefit in lifespan | (Russman et al., 2003) |
| | | HT-RCT phase 2 | 141 patients (type 2 and 3) | Ongoing | |
| | Beta-lactam antibiotics | In vivo | SMA mice ($Smn^{-/-}SMN2^{+/+}SMN\Delta 7^{+/+}$) | Improvement in lifespan, muscular phenotype, & neuromuscular function<br>Increase in SMN protein in spinal cord | (Nizzardo et al., 2011) |
| Neurotrophic effect | Thyrotropin releasing hormone (TRH) | HT-CS Phase 0 | 3 patients SMA type 1 and | No benefit in SMA patients type 1<br>Increase in muscle strength for patient type 2 | (Takeuchi et al., 1994) |
| | | HT-CS Phase 0<br>HT-RCT Phase 0 | 1 patient<br>9 patients (type 2 and 3) | Increase in muscle strength<br>Improvement in motor function | (Kato et al., 2009)<br>(Tzeng et al., 2000) |
| | rhIGF1-rhIGF1BP-3 (IPLEX™) | In vivo | SMA mice ($Smn^{-/-}SMN2^{+/+}SMN\Delta 7^{+/+}$) | No improvement in lifespan and weight gain.<br>Improvement in motor function, increase in muscle fiber size | (Murdocca et al., 2012) |
| Protection of mitochondria | Olesoxime (TRO-19622) | HT-RCT Phases 2 and 3 | 150 patients (type 2 and 3) | On going | |

*Continued*





**Table 3** Continued

| 2. *SMN1*-introduction strategies | | | | | |
|---|---|---|---|---|---|
| Treatment categories | Vector (administration route)/ Stem cells | Clinical trial phase | Subjects | Outcome | References |
| (1) Gene therapy | | | | | |
| Vector mediated gene delivery | EIAV- SMN (muscle injection and retrograde axonal transport) | In vivo | SMA mice ($Smn^{-/-}SMN2^{+/+}SMN\Delta 7^{+/+}$) | Improvement in lifespan, increase in weight gain, reduction in motor neuron death | (Azzouz et al., 2004) |
| | AAV8-SMN, scAAV8-SMN (cerebral lateral ventricle injection) | In vivo | SMA mice ($Smn^{-/-}SMN2^{+/+}SMN\Delta 7^{+/+}$) | Improvement in lifespan and motor function.<br>Increase in weight gain.<br>Histological improvement in NMJ formation | (Passini et al., 2010) |
| | scAAV9-SMN(intravenous injection) | In vivo | SMA mice ($Smn^{-/-}SMN2^{+/+}SMN\Delta 7^{+/+}$) | Improvement in lifespan, increase in weight gain, rescue in motor function. | (Foust et al., 2010) |
| | scAAV9-SMNopti (intravenous injection) | In vitro<br>In vivo | SMA astrocyte cells<br>SMA mice ($Smn^{-/-}SMN2^{+/+}SMN\Delta 7^{+/+}$) ($Smn^{-/-}SMN2^{+/+}SMNA2G^{+/-}$) | Increase in SMN protein<br>Improvement in lifespan, increase in weight gain, rescue of motor neuron function | (Dominguez et al., 2011) |
| (2) Stem cell therapy | | | | | |
| Cell transplant technology | Neuronal stem cells | | | | |
| | a. Spinal cord-derived neuronal stem cells | In vivo | SMA mice ($Smn^{-/-}SMN2^{+/+}SMN\Delta 7^{+/+}$) | Improvement in lifespan, locomotor activity and exploratory behavior.<br>Increase in weight gain | (Corti et al., 2008) |
| | b. Embryonic stem cell-derived neural stem cells | In vivo | SMA mice ($Smn^{-/-}SMN2^{+/+}SMN\Delta 7^{+/+}$) | Improvement in muscle innervation, lifespan, and behavior endpoint.<br>Increase in weight gain. | (Corti et al., 2010) |

*Continued*

lifespan of the SMA mice ($Smn^{-/-}$ $SMN2^{+/+}$) (Chang et al., 2001). In the same year, a cancer drug, aclarubicin, was also reported to increase FL-SMN protein in SMA fibroblasts (Andreassi et al., 2001). These reports led to a number of studies investigating potential therapeutic candidates for SMA.

Several HDAC inhibitors were found to activate *SMN2* transcription and correct splicing of *SMN2* exon 7, leading to a significant increase in FL-SMN. These compounds include sodium butyrate (Chang et al., 2001), valproic acid (VPA) (Brichta et al., 2003; Sumner et al., 2003), sodium phenylbutyrate (PBA) (Andreassi et al., 2004; Brahe et al., 2005; Mercuri et al., 2007), suberoylanilidehydroxamic acid (SAHA) (Hahnen et al., 2006; Riessland et al., 2010), benzamide M344 (Riessland et al., 2006), trichostatin A (Avila et al., 2007), and hydoroxamic acid LBH589 (panobinostat) (Garbes et al., 2009). Among them, VPA, a drug that is widely used for epilepsy patients, was first shown to increase SMN protein level in SMA fibroblasts via upregulation and correction of exon 7 splicing of *SMN2* (Brichta et al., 2003; Sumner et al., 2003).

Weihl et al. (2006) reported that VPA was also able to increase muscle strength and subjective function of seven adult patients with SMA type 3–4. A Phase 2 open label study conducted by Swoboda et al. (2009) also showed that VPA improved the motor function in SMA type 2 patients. According to them, significant improvement was limited to the patients who were under 5 years of age.

However, a double-blind, randomized placebo controlled trial demonstrated no benefits from 6 to 12 months treatment with VPA and carnitine in a cohort of non-ambulatory subjects with SMA type 2–3 (sitters, 2–8 years of age) (Swoboda et al., 2010), with similar results from another prospective single-armed trial on a cohort of ambulatory subjects with SMA type 3 (standers and walkers, 3–17 years of age) (Kissel et al., 2011). In both studies, carnitine was given to the patients for two reasons: SMA patients may have a limited carnitine synthetic capacity due to reduced skeletal muscle mass, and VPA itself may inhibit carnitine transport and deplete carnitine levels. Thus, the combination therapy of VPA and carnitine was chosen in order to avoid concerns about a confounding effect of carnitine depletion (Swoboda et al., 2010).

Inconsistent data relating to VPA effects may be explained by the coexistence of responders and non-responders to the drug, suggesting the necessity of pre-selecting potential responders (Pruss et al., 2010). A recent study has reported that an increase in fatty acid translocase CD36 expression may account for VPA non-responsiveness (Garbes et al., 2013). Pretreatment analysis of genetic background including CD36 expression may be useful for identification of potential responders.

The change in *SMN* transcript levels or SMN protein levels in the blood cells or cultured fibroblasts treated with VPA could be a measurable and informative biomarker for the biochemical/pharmacological effect of VPA treatment. However, it should be noted that the increase in *SMN* transcript levels or SMN protein levels would not necessarily guarantee the amelioration of SMA disease progression. Any valid biomarkers for SMA disease progression have yet to be identified or validated. Hence, currently there are no useful biomarkers to predict the outcome of the clinical trials.

Non-HDAC inhibitor drugs may also activate *SMN2* transcription and correct splicing of *SMN2* exon 7, leading to a significant increase in FL-SMN protein. These drugs include aclarubicin (Andreassi et al., 2001), hydroxyurea (HU) (Grzeschik et al., 2005; Chen et al., 2010), salbutamol (Kinali et al., 2002; Angelozzi et al., 2008; Pane et al., 2008; Tiziano et al., 2010), indoprofen (Lunn et al., 2004), PTK-SMA1 (Hastings et al., 2009), quinazolines (Singh et al., 2008), and triptolide (Hsu et al., 2012).

Hydroxyurea has been reported to increase FL-*SMN2* transcripts and SMN protein levels, but without changing total *SMN* mRNA, suggesting that it promotes the inclusion of exon 7 during *SMN2* transcription in SMA fibroblast cell lines (Grzeschik et al., 2005). However, a clinical trial of HU in 2007 showed no significant clinical improvements in motor function (Chen et al., 2010).

Among the $\beta$-adrenergic agonists, only salbutamol has been identified as a candidate drug for SMA. Early studies had shown that salbutamol enhanced muscle strength in patients with SMA type 2–3 (Kinali et al., 2002; Pane et al., 2008), and it was recently proven that it increases FL-*SMN2* mRNA in fibroblasts and leukocytes from SMA patients (Angelozzi et al., 2008; Tiziano et al., 2010).

Indoprofen has been used as a non-steroidal anti-inflammatory drug and cyclooxygenase inhibitor. This therapeutic candidate was selected from a high-throughput screen using a splicing reporter mini-gene and was found to selectively increase *SMN2* exon 7 inclusion and therefore increase the amount of FL-SMN protein produced from transfected cells (Lunn et al., 2004). Recently, NINDS (National Institute of Neurological Disorders and Stroke, USA) announced the start of clinical trials of indoprofen derivatives for SMA (http://www.ninds.nih.gov/news).

PTK-SMA1, a tetracycline-like compound, stimulates *SMN2* exon 7 inclusion and increases SMN production in vitro and in vivo (Hastings et al., 2009). There is a structural similarity between tetracyclines and aclarubicin which also activates FL-*SMN2* transcription. It is notable, that the former is far less toxic compared to the latter. According to Paratek Pharmaceuticals, clinical trials of PTK-SMA1 could begin in 2013 (http://www.ricercasma.it/).







**Table 3** Continued

| Treatment Categories | Compounds/Drugs | Clinical trial phase | Subjects | Outcome | References |
|---|---|---|---|---|---|
| 3. Non-*SMN*-targeting strategies | | | | | |
| 2. Improvement of pathological conditions of non-neuronal tissues | | | | | |
| Amendment of affected muscles | Inhibition of myostatin | | | | |
| | a. follistatin | In vivo | SMA mice ($Smn^{-/-}$ $SMN2^{+/+}$ $SMNA7^{+/+}$) | No improvement in lifespan | (Sumner et al., 2009) |
| | b. ActRIIB-Fc | In vivo | SMA mice ($Smn^{-/-}$ $SMN2^{+/+}$ $SMNA7^{+/+}$) | Slight improvement in motor function, but no improvement in lifespan | |
| | Expression of IGF-1 | In vivo | SMA mice ($Smn^{-/-}$ $SMN2^{+/+}$ $SMNA7^{+/+}$ $mIGF\text{-}1^{+/-}$) ($Smn^{-/-}$ $SMN2^{+/+}$ $SMNA7^{+/+}$ $mIGF\text{-}1^{-/-}$) | Increase in muscle fiber size and body weight gain. Improvement in lifespan | (Bosch-Marcé et al., 2011) |
| Inhibition of ROCK pathway | Y-27632(ROCK inhibitor) | In vivo | SMA mice ($Smn^{2B/-}$ mice, $Smn^{2B/+}$ mice and $Smn^{-/-}$ $SMN2^{+/+}$) | Improvement in lifespan, muscle size and motor end plate maturation. Increase in weight gain | (Bowerman et al., 2010) |
| | Fasudil | In vivo | SMA mice ($Smn^{2B/-}$ mice and $Smn^{2B/+}$ mice) | Improvement in lifespan, muscle size and motor end plate maturation. Increase n weight gain | (Bowerman et al., 2012) |

*NB*. Olexosime clinical trial was recently completed and showed no significant improvement in patients with ALS; HT: human-trial phases (CS: case series; RCT: randomized controlled trial; OL: open label trial).

Quinazolines have also been reported to be potent *SMN2* promoter activators (Jarecki et al., 2005; Thurmond et al., 2008). These compounds work by binding to the scavenger decapping enzyme, DcpS, and potently inhibit its decapping activity. DcpS is a nuclear shuttling protein that binds and hydrolyzes the m7GpppN mRNA cap structure and is a modulator of RNA metabolism. The potency of DcpS inhibition correlates with potency for *SMN2* promoter activation (Singh et al., 2008; Butchbach et al., 2010b; Van Meerbeke & Sumner, 2011). A clinical trial (phase 1) of a C5-substituted quinazoline called quinazoline495 (or RG3039) is currently ongoing (Van Meerbeke et al., 2011)

Another promising candidate drug for SMA is Triptolide (PG490) that was reported to increase FL-*SMN2* transcript and SMN protein levels in fibroblast cells derived from SMA patients (Hsu et al., 2012). In addition, injection of the drug improved survival in SMA mice ($Smn^{-/-}SMN2^{+/+}SMN\Delta 7^{+/+}$). Triptolide is a diterpene triepoxide antibiotic isolated from extracts of the herb *Tripterygium wilfordii* Hook F (TWHF). TWHF has been used as an herbal drug for rheumatoid arthritis in traditional Chinese medicine because of its immunosuppressive and anti-inflammatory properties.

Recently, prolactin (PRL) was shown to increase SMN expression PRL is a 199–amino acid 23-kDa polypeptide hormone that binds to the PRL receptor and activates the JAK2/STAT5 pathway, resulting in SMN upregulation in both SMA mouse models ($Smn^{-/-}SMN2^{+/+}SMN\Delta 7^{+/+}$) and human motor neuron-derived cell lines (Farooq et al., 2011). PRL may be a good candidate for SMA therapy; however, clinical experience with PRL is still limited, and there are some potential side effects associated with high levels of PRL. Hyperprolactinaemia can lead to precocious puberty, infertility, and osteoporosis (Advis et al., 1981; Aguilar et al., 1988; Farooq et al., 2011). Thus, careful monitoring for hyperprolactinaemia-related symptoms is essential if long-term PRL treatment is given to the patients.

Charbonnier and colleagues reported that NMDA receptor activation leads to an increase in SMN expression through AKT/cAMP response element-binding protein (CREB) pathway (Biondi et al., 2010; Branchu et al., 2013). According to them, because of the reciprocal crosstalk in the level of extracellular signal-regulated kinase (ERK) and AKT kinases, pharmacological inhibition of the MEK/ERK/Elk-1 pathway using experimental compound UO126 or selumetinib may efficiently activate the AKT/CREB pathway, resulting in an increase in SMN expression (Branchu et al., 2013).

Once SMN is expressed, the problem of the SMN degradation comes next. Makhortova and colleagues carried out an image based screen to identify regulators of SMN levels, and found that glycogen synthase kinase (GSK)-3 is a key regulator of SMN degradation. Its activity is also controlled by certain neurotransmitter ligands. Here, certain sets of kinase inhibitors may be able to promote motor neuron survival (Makhortova et al., 2011). It is conceivable that some drugs increasing SMN stability might provide an adjunctive effect in addition to *SMN2*-targeting strategies.

*Splicing correction of FL-SMN2 mRNA by oligonucleotides*

Correction of *SMN2* splicing (incorporation of *SMN2* exon 7) may increase FL-SMN protein production. In 2001, Lim and Hertel reported that an antisense oligonucleotide (ASO) targeting the 3′ splice site of exon 8 was able to incorporate exon 7 into *SMN2* mRNA. Since then, at least five methods, including that of Lim and Hertel have been developed to modulate *SMN2* mRNA. The first strategy of Lim and Hertel (2001) involves blocking splice sites in the exon-intron boundaries (Table 3).

The second method promotes exon-specific splicing enhancement using bifunctional peptide nucleic acid as chimeric effectors (ESSENCE). The "nucleic acid" part of the synthetic compound binds to exon 7 sequences and the "peptide" part with serine-arginine repeats exercises the ESE-dependent function of positive splicing proteins, thus facilitating inclusion of the index exon (Cartegni & Krainer, 2003).

The third method uses bifunctional oligonucleotides, such that one half of the oligonucleotide binds to exon 7 sequences and the second half contains ESE motifs which facilitates the index exon to be included (Skordis et al., 2003; Meyer et al., 2009).

The fourth is a *trans*-splicing method incorporating an exogenous RNA sequence of SMN1 exon 7 into the FL-SMN transcript (Coady et al., 2007; Coady & Lorson 2010). The *trans*-splicing RNA containing *SMN1* exon 7 sequence binds to endogenous *SMN* pre-mRNA at the intron 6 region by complementary base-pairing. The mRNA product includes *SMN1* exon 7 sequence followed by a poly-adenylation signal. More recently, Shababi and colleagues reported the synergistic effect of *trans*-splicing RNA and a neurotrophic factor, insulin-like growth factor (IGF)-1. Intracerebroventricular injection of the *trans*-splicing/IGF-1 vector significantly increased SMN protein levels in brain and spinal cord of SMA mice ($Smn^{-/-}SMN2^{+/+}SMN\Delta 7^{+/+}$), extended lifespan, and increased the body weight (Shababi et al., 2011). However, it should be noted that a vector with IGF-1 alone has similar efficacy as one containing the splicing modulator that promotes exon 7 inclusion and IGF-1. We will discuss the effect of IGF-1 again in the section on "protection of motor neurons."

The fifth is an ISS-masking method to facilitate the inclusion of exon 7 into *SMN2* mRNA since a target ISS has been found in *SMN2* intron 7 (Singh et al., 2006; Hua et al., 2008; Porensky et al., 2012). Singh et al. (2006) showed that an antisense oligonucleotide (ASO) against ISS-N1 in intron 7, Anti-N1, facilitated the inclusion of exon 7 into *SMN2* mRNA leading to increased SMN production in SMA cell lines. Krainer's group also reported that an ASO against the ISS, named ASO-10–27, effectively corrected SMN2 splicing (Hua et al., 2010) and demonstrated that it restored SMN expression in motor neurons of SMA mice ($Smn^{-/-}SMN2^{+/+}SMN\Delta 7^{+/+}$) after intracerebroventricular injection (Passini et al., 2011). They also clarified that systemic administration (subcutaneous injection) of ASO-10–27 to neonates extended the median lifespan of SMA mice, although distribution of the ASO was limited and the *SMN2*-splicing changes were moderate in the CNS (Hua et al., 2011).

ISIS Pharmaceuticals recently announced the results of a phase 1 clinical trial of an ASO-10–27 delivering system, ISIS-SMNRx, using intrathecal administration. In the trial, a single dose (1, 3, 6, and 9 mg) was given intrathecally as a lumbar puncture (LP) bolus injection in male and female SMA patients 2–14 years old who are medically stable. According to their report with a total of 28 patients enrolled in the trial (http://www.isisph.com/pdfs/AAN_Isis_InvestorEvent.pdf), (1) ISIS-SMNRx was well tolerated, (2) the LP injection procedure was shown to be feasible in SMA children, (3) Improvement in Hammersmith Functional Motor Scale Examination (HFMSE) scores and electrophysiology measurements (motor unit number estimation (MUNE) with stable compound muscle action potential (CMAP)) were observed at the highest dose level. They are now planning controlled phase 2/3 registration-enabling studies in infants and children with SMA.

*Producing a stable form of Δ7-SMN*

It has been reported that the Δ7-SMN is unable to oligomerize or self-associate as well as FL-SMN (Lorson et al., 1998). The exon 7 domain is also necessary for localization of SMN into the cytoplasm (Zhang et al., 2003). However, interestingly, Δ7-SMN itself was reported to be capable of extending survival of SMA mice ($Smn^{-/-}SMN2^{+/+}SMN\Delta 7^{+/+}$) (Le et al., 2005). It was postulated that Δ7-SMN may produce such phenotypic improvement either through partial functionality or by "seeding" oligomerization with functional FL-SMN. In this context, stabilization of Δ7-SMN may present a viable therapeutic strategy for SMA (Heier & DiDonato, 2009).

Aminoglycosides are an FDA-approved class of drug that acts within cells by binding to ribosomes to affect the translation of proteins from mRNA transcripts, i.e., by misreading stop codons (Wolstencroft et al., 2005). Aminoglycosides can lessen the severity of the SMA mouse model ($Smn^{-/-}SMN2^{+/+}SMN\Delta 7^{+/+}$) via a Δ7-SMN translational read-through mechanism which enhances the stability of the Δ7-SMN protein with additional C-terminal peptides (Heier & DiDonato, 2009). Recently, two aminoglycosides, G418 and TC007, have been reported as candidate dugs for SMA (Mattis et al., 2006; Heier & DiDonato, 2009). G418 improved motor function of SMA mice, but did not extend their lifespan. On the contrary, TC007 demonstrated improved phenotypic measures and prolonged the lifespan of SMA mice ($Smn^{-/-}SMN2^{+/+}SMN\Delta 7^{+/+}$) (Mattis et al., 2009).

## *SMN1*-Introduction Strategies

Introduction of exogenous SMN1 by gene or stem cell therapies may prevent or alleviate the symptoms associated with motor neuron defects in SMA. With regards to gene therapy, researchers devoted their ingenuity and resources to developments in vector-gene construction, delivery systems and maximization of treatment effect. In 2004, Azzouz and colleagues first reported successful rescue of SMA mice ($Smn^{-/-}SMN2^{+/+}SMN\Delta 7^{+/+}$) using a vector-mediated gene delivery approach (Azzouz et al., 2004). They injected SMN-expressing lentivector [Equine Infectious Anemia Virus (EIAV) vector] in various muscles of SMA mice on postnatal day 2. The vector reached the motor neurons by retrograde axonal transport and restored SMN levels. This gene therapy resulted in body weight gain and extension of the lifespan of SMA mice.

In 2010, Passsini and colleagues published a report on gene therapy using a self-complementary adeno-associated virus (scAAV) 8 vector expressing SMN (Passini et al., 2010). The scAAV vector is a recombinant virus defined as having a double-stranded DNA genome resulting in earlier onset of gene expression compared with regular single-stranded AAV. In their study, scAAV 8-SMN was injected on postnatal day 0 into the CNS (cerebral lateral ventricle and upper lumber spinal cord) of SMA mice ($Smn^{-/-}SMN2^{+/+}SMN\Delta 7^{+/+}$). This resulted in increase in body weight gain and muscle strength as well as in lifespan extension in the treated SMA mice. Interestingly, they also demonstrated that the CNC-directed gene therapy partially resolved the abnormal architecture of the NMJ. This rescue may have been achieved by improved axonal transport and/or efficient spliceosomes modifying gene expression related to NMJ function.

In the same year, Foust and colleagues reported successful rescue of SMA mice ($Smn^{-/-}SMN2^{+/+}SMN\Delta 7^{+/+}$) using an intravenous injection approach with scAAV-9 vector (Foust et al., 2010). They injected scAAV-9 carrying SMN1 into the facial vein of mice pups on postnatal days 1, 5, and 10. According to them, scAAV9-mediated vascular gene delivery at postnatal day 1 successfully introduced SMN into SMA pups and rescued motor function, neuromuscular physiology and lifespan. Treatment on postnatal day 5 resulted in partial correction, whereas postnatal day 10 treatment had little effect. These experimental data with SMA mice suggested the

presence of a critical period when a sufficient amount of SMN protein is required during motor neuron development. In addition, the maturation of the blood brain barrier may hamper the transport of the SMN-expressing vectors to the target neurons, suggesting a finite period for efficient gene therapy.

In 2011, Dominguez and colleagues reported that they used postnatal day 1 systemic injection of self-complementary adeno-associated virus (scAAV9) vectors carrying a codon-optimized *SMN1* sequence and a chimeric intron placed downstream of the strong phosphoglycerate kinase (PGK) promoter (SMNopti) to overexpress the human SMN protein in a mouse model of severe SMA ($Smn^{-/-}SMN2^{+/+}SMN\Delta 7^{+/+}$) (Dominguez et al., 2011). Codon optimization is a gene optimization technology that can alter both naturally occurring and recombinant gene sequences to achieve the highest possible levels of productivity in any given expression system. This treatment increased life expectancy from 27 to over 340 days (median survival of 199 days) in mice that normally survive about 13 days. The systemic scAAV9 therapy mediated complete correction of motor function, prevented motor neuron death and rescued the weight loss. This study also showed sex differences in the responsiveness to the treatment. Male SMA mice displayed a lower body weight gain than age-matched control mice, whereas the body weight of females was not statistically different from the controls.

As for stem cell therapy, it has been shown that "spinal cord-derived stem cells" and "embryonic stem cell-derived neural stem cells" can differentiate into motor neurons in vivo (Corti et al., 2008; Corti et al., 2010). In addition, following intrathecal transplantation into SMA mice ($Smn^{-/-}SMN2^{+/+}SMN\Delta 7^{+/+}$), the administrated neural stem cells survived and migrated extensively to appropriate areas. Here, the transplanted embryonic stem cells were found to work by secreting soluble neuroprotection factors, such as glial-cell derived neurotrophic factor (GDNF), brain derived neurotrophic factor (BDNF), and tumor growth factor (TGF)-$\alpha$. These growth factors were confirmed to play a role in the improved functional recoveries of SMA mice following transplantation, showing an increase in myofiber number and size, axon length and body weight gain, suggesting that neural stem cell transplantation resulted in successful amelioration of behavioral end points and life span extension in SMA mice (Corti et al., 2010).

Gene and stem cell therapies may be very promising treatments for SMA patients, especially for pre-symptomatic patients. However, the efficacy of such therapies should further be tested in non-human primates before such approaches are applied to the patients, especially in considering if blood brain barrier function may hinder the delivery of therapeutic agents to the neurons (Tsai, 2012). Foust and colleagues investigated whether scAAV9 can traverse the blood–brain barrier in a nonhuman primate, *cynomolgus macaque* (Foust et al., 2010). They intravenously injected scAAV9 carrying the green fluorescent protein (GFP) gene on postnatal day 1, and demonstrated that scAAV9 crossed the blood brain barrier and reached motor neurons in the nonhuman primate model, suggesting that gene therapy targeting motor neurons can also be done in human.

## Non-*SMN*-Targeting Strategies

*Protection of motor neurons*

Neuroprotection therapy with riluzole and gabapentin, which had originally been used for the patients with amyotrophic lateral sclerosis (ALS), was also applied to SMA patients (Russman et al., 2003; Merlini et al., 2003). Glutamate excitotoxicity may be an important factor in the pathogenesis of ALS since the cell bodies of motor neurons receive afferent innervation from glutamate neurons. Hence, pharmacologic agents that rescue glutamate excitotoxicity may be effective in slowing disease progression in ALS (van den Bosch, 2006). Similarly, glutamate excitotoxicity may also be an important factor in the pathogenesis of SMA. Riluzole inhibits the presynaptic release of glutamate, while gabapentin reduces the pool of releasable glutamate in the pre-synaptic neurons. Russman et al. (2003) reported the outcome of a clinical trial of riluzole in 10 patients with SMA type 1 (phase 1 trial with randomization of 2:1, i.e., 2 riluzole to 1 placebo). None of the subjects in this study experienced adverse effects. Even though the study sample size was small, some benefits of riluzole were suggested in the treated patients: three of the seven patients taking riluzole lived to more than 5, 4, and 2 years of age respectively with only BiPAP respiratory assistance at night. On the contrary, the placebo-controlled trials of gabapentin showed no significant benefit in motor function of the patients with SMA type 2/3 (Miller et al., 2001; Merlini et al., 2003).

Another group of compounds, $\beta$-lactam antibiotics, can also provide neuroprotection against glutamate-mediated excitotoxicty by increasing the expression level of the glutamate transporter EAAT2/GLT-1. However, to date these observations have only been demonstrated using model mice. A treated ALS mouse model showed a delay in loss of neurons and muscle strength, and increase in survival rate (Rothstein et al., 2005). Nizzardo and colleagues demonstrated that a $\beta$-lactam antibiotic, ceftriaxone, also ameliorated the neuromuscular phentotype in SMA mice ($Smn^{-/-}SMN2^{+/+}SMN\Delta 7^{+/+}$) (Nizzardo et al., 2011). Treatment with ceftriaxone increased general weight, muscle size, motor neuron numbers and NMJs, which are likely the reasons for the increased life span and muscle strength of the SMA mice. According to them, the neuroprotective effect of the $\beta$-lactam antibiotic in the SMA mice seems to be mediated not only through the process of increasing EAAT2/GLT-1, but also by other mechanisms that increase transcription factor Nrf2 and SMN.

Thyrotropin-releasing hormone (TRH, L-pyroglutamyl-L-histidyl-L-prolinamide) has trophic effects on spinal motor neurons, and it has also been tried for ALS and SMA patients. TRH was administered intravenously (Takeuchi et al., 1994; Tzeng et al., 2000) or orally (Kato et al., 2009) to SMA patients in small clinical trials but only transient improvement was observed in some patients.

Olesoxime (TRO19622), a small molecule with a cholesterol-like structure, has protective properties for motor neurons. It targets proteins associated with the mitochondrial permeability pore (Bordet, 2007). Olesoxime has been granted orphan drug status for the treatment of ALS and SMA, and clinical trials for ALS and SMA have been started in the US and Europe (http://clinicaltrials.gov/ct2/show/NCT01285583; http://clinicaltrials.gov/ct2/show/NCT01302600). In December 2011, Trophos SA announced the results from the phase 3 study of olesoxime in 512 patients with ALS; olesoxime was well tolerated but did not demonstrate a significant increase in survival of patients receiving riluzole (Rilutek®). Olesoxime trials for SMA are still ongoing and the results are to be expected in 2013 (http://www.trophos.com/news/pr20111213.htm).

IGF-1 is reported to modulate multiple fundamental cellular processes, such as cellular growth, proliferation, and survival (Vardatsikos et al., 2009). Most recently, Tsai and colleagues reported that CNS-directed IGF-1 delivery could reduce motor neuron death in SMA mice ($Smn^{-/-}SMN2^{+/-}$) (Tsai et al., 2012). Murdocca and colleagues also reported the effects of IPLEX™ [recombinant human insulin-like growth factor 1 (rhIGF-1) combined with recombinant human IGF-1 binding protein 3 (rhIGFBP-3)] on a severe SMA mouse model ($Smn^{-/-}SMN2^{+/+}SMN\Delta 7^{+/+}$) (Murdocca et al., 2012). According to them, perinatal administration of IPLEX™ results in reduced degeneration of motor neurons, increased muscle fiber size and in amelioration of motor functions in SMA mice, suggesting this compound as a plausible therapeutic candidate to hinder the progression of the neurodegenerative process in SMA. However, it should be noted that CNS-directed IGF-1 delivery could not improve motor function in SMA mice (Tsai et al., 2012) and that IPLEX™ did not improve lifespan and body weight gain of the treated mice (Murdocca et al., 2012). IGF-1 may provide at least some beneficial effects on the survival of motor neurons. However, it is necessary to further study the systemic effect of IGF-1 administration before clinical application, because IGF-1 has multiple functions in various organs.

*Protection of non-neuronal tissues*

Nutrition may be critical for the care of SMA patients, especially SMA type 1 patients (Oskoui et al., 2007). The improved survival of SMA type 1 patients observed in recent years can be attributed to noninvasive pulmonary support and aggressive nutrition with gastrostomy feedings. Butchbach and colleagues observed that maternal diet can significantly modify survival and the motor neuron disease phenotype in SMA mice ($Smn^{-/-}SMN2^{+/+}SMN\Delta 7^{+/+}$). According to them, SMA mice from dams that were fed a higher fat diet survived longer than those from dams on a lower fat diet (Butchbach et al., 2010a). The effect of nutritional support on survival of SMA patients and model animals indicates that improvement of pathological conditions of non-neuronal tissues including muscles should be considered in SMA therapy.

Treatments that directly target muscles and improve muscle mass have been reported: inhibition of myostatin by overexpression of follistatin (Sumner et al., 2009) and expression of IGF-1 (Bosch-Marcé et al., 2011). Although inhibition of myostatin did not ameliorate motor function or survival of severe SMA mice ($Smn^{-/-}SMN2^{+/+}SMN\Delta 7^{+/+}$) (Sumner et al., 2009), overexpression of IGF-1 resulted in enlarged myofibers, but not in improvement of motor function (Bosch-Marcé et al., 2011). Murine IGF-1 administration had been proven to give different positive effects when it was expressed locally in muscle of SMA mice ($Smn^{-/-}SMN2^{+/+}SMN\Delta 7^{+/+}mIGF\text{-}1^{+/-}$) (Bosch-Marcé et al., 2011) or in motor neurons (Murdocca et al., 2012).

Recently, there has been emerging evidence that the RhoA/ROCK pathway may play an important role in the pathogenesis of SMA (Bowerman et al., 2009; Nölle et al., 2011) since SMN depletion leads to an increased activation of ROCK, a major regulator of actin dynamics. Bowerman et al. (2010) reported that ROCK inhibitors, Y-27632 and Fasudil, dramatically improved the survival of the $Smn^{2B/-}$ mice, an intermediate SMA mouse model. They emphasized that lifespan extension in SMA mice with ROCK inhibitors was accompanied by an improvement in the maturation of NMJs and an increase in muscle fiber size (Bowerman et al., 2010; Bowerman et al., 2012). However, Bowerman and colleagues showed that administration of Y-27632 had no beneficial effect on the $Smn^{-/-}$ mouse model with the most severe SMA phenotype. Here, they suggested that there may be a need for differential therapies for the different types of SMA severities and that the "one size fits all" approach may not be tenable (Bowerman et al., 2010).

## Challenges in Clinical Trials

Despite the large number of candidate compounds evaluated, there has yet to be any effective drug treatment reported for all types of SMA (Wadman et al., 2012a; Wadman et al., 2012b). A number of clinical trials for SMA have already been conducted in the past decade, some of which are still ongoing (Miller et al., 2001; Mercuri et al., 2007; Pane et al., 2008;

Swoboda et al., 2009; Tiziano et al., 2010; Kissel et al., 2011). However, all clinical trials reported so far failed to show significant effectiveness of the therapeutic approaches, which may indicate the difficulties of designing clinical trials for this disorder. An adequate design should take into account the rarity of the patients, clinical disease heterogeneity (subtypes, onset age, sex, stage of disease progress, timing of enrollment, and intervention relative to disease progression), treatment plans (selection of the drug with possible ameliorating effects on the clinical symptoms, sufficient dose, and duration to see some measurable effects) and outcome measures [laboratory biomarkers including *SMN* transcript and SMN protein amounts, muscle mass and strength, motor function testing, respiratory function testing, MUNE, questionnaires for quality of life (QOL)] (Swoboda et al., 2007; Kissel et al., 2011). Clinical endpoints, i.e., the target outcome of the clinical trials: such as extension of the survival period in the patients with SMA type 1 (which will be discussed again below), improvement of motor function in the patients with SMA type 2, and extension of the walking period in the patients with SMA type 3, need to be specified. However, great subtlety may be required for the accurate evaluation of these outcomes. Even if a therapeutic approach could ameliorate the symptoms in some patients, these outcomes may not be detected if the trials are not adequately designed.

To address the challenges due to the rarity of SMA, Mercuri's group (2012) called for clinical trials to be carried out as large multicenter international trials. Such large-scale collaborations would increase the numbers of patients enrolled and would enable randomized placebo studies to be carried out. This approach could also overcome the problems due to clinical heterogeneity as a stratification method could be used to provide a fair evaluation of the treatments (Mercuri et al., 2012).

The selection of appropriate outcome measures to test the efficacy of a therapy remains one of the most difficult problems to be resolved. As for laboratory biomarkers, only *SMN* transcript or SMN protein levels have been established. However, determination of *SMN* transcript or SMN protein levels may not be enough, because these cannot be used to evaluate treatments targeting biochemical reactions downstream of SMN-related signaling (Crawford et al., 2012). Recently, metabolomics studies have suggested that some proteins and metabolites can be used as laboratory biomarkers to reflect responsiveness to treatment (Finkel et al., 2012). Further studies are still required for future clinical usage.

The Hammersmith Functional Motor Scale (HFMS) (Main et al., 2003), Modified HFMS (MHFMS) (Krosschell et al., 2006), and gross motor function measure (GMFM) (Nelson et al., 2006) have been established as standard measures of functional ability in children with SMA types 2 and 3 for use in longitudinal multicenter clinical trials. The Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP INTEND) may also be used for the evaluation of children with SMA type 1 (Glanzman et al., 2010). However, it is difficult to evaluate the actual change in motor scales in SMA patients with any motor function measurements. Thus, it is necessary for investigators in multicenter networks to share the test skills and scoring criteria in order to improve inter-rater reliability and objectivity. For that purpose, training of test skills and collaboration in the scoring criteria should be implemented across centers with different expertise (Mercuri et al., 2012).

In an SMA mouse model, extension of lifespan has been considered to reflect the effectiveness of therapeutic approaches. However, lifespan cannot be simply applied to evaluate the therapeutic approaches in human SMA patients because not only the administered therapy, but the type of supportive care including respiratory management can also change the lifespan of patients. In addition, the use of an artificial respirator in SMA type 1 management is still controversial. Such differences in clinical care may hamper simple comparison using lifespan outcomes in international clinical trials. The occurrence of death and the requirement for an artificial respirator may be considered as equivalent events when evaluating the efficacy of clinical trials in patients with SMA type 1 because improvement of motor scale cannot be expected from these patients (Oskoui et al., 2007; Mercuri et al., 2012). Currently, using lifespan as the only available outcome measure, is not ideal anymore. If it is possible to measure improvements in respiratory function or restoration of motor function, alternative outcome measures for SMA type 1 may become achievable. Highly effective therapies which will improve motor scale of patients with SMA type 1 can then be sought.

## Conclusions

SMA is an incurable motor neuron disease with autosomal recessive inheritance. Molecular biology studies of SMA have been greatly advanced in two directions, namely diagnostic applications and pathophysiological studies, since the discovery of the SMN genes in 1995. Molecular diagnostics has enabled us not only to diagnose SMA in patients, but has also provided the ability to carry out carrier and newborn screening of SMA for populations. Pathophysiological studies have provided an improved understanding of the underlying pathogenesis of SMA, including alternative splicing of *SMN2*, aberrant splicing due to the defect of snRNPs, impairment of motor circuit formation and/or NMJ development, and dysregulation of cytoskeleton dynamics. To date, there has been no successful therapy for SMA, but an in-depth understanding of the pathophysiology underlying the disease can offer useful insights for development of effective treatment approaches. Some therapeutic strategies have already been devised based on current pathophysiological knowledge of the disease, namely *SMN2*-targeting, *SMN1*-introduction and non-*SMN* targeting strategies. With multiple approaches in therapeutic strategies for SMA being pursued, some of which are already in clinical trials, it is expected that some candidate compounds may emerge as potential therapeutic agents in the near future. These exciting developments offer promising outcomes for SMA patients in overcoming this debilitating disease.

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原　著

# 日本における脊髄性筋萎縮症の臨床実態調査

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## Clinical Epidemiological Investigation of Spinal Muscular Atrophy in Japan

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No clinical epidemiological investigations of spinal muscular atrophy (SMA) have been carried out in Japan. We performed a population-based study of SMA to survey the number of patients visiting the departments of pediatrics and internal medicine and to clarify clinical features. Simultaneously, we studied the clinical features and laboratory findings of 110 individuals from whom informed consent had been obtained. The number of patients visiting the hospital was estimated to be 0.5-1 per 100,000 people. The male to female ratio was 1 to 1.14. As to the maximum motor functional level, severity varied among SMA subtypes. Eighty-seven percent of patients with type I demonstrated poor head control. More than half of type II patients could sit unsupported at the same point. All patients with type III were able to walk. Patients with all types of SMA showed the same pattern of muscle weakness, with proximal and upper limb dominance. However, there were some patients showing atypical symptoms. Although most SMA patients have homozygous deletion of *SMN1*, the range of clinical severities is broad. We will continue with additional study to elucidate the disease mechanisms in both typical SMA patients and atypical individuals.

**Key Words:** spinal muscular atrophy (SMA), clinical investigation, maximum motor functional level, muscle weakness, epidemiology



## 緒　言

脊髄性筋萎縮症（spinal muscular atrophy：SMA）は，脊髄前角細胞，脳神経核の変性・脱落により，進行性の神経原性筋萎縮を示す常染色体劣性遺伝性疾患である．1891年にWerdnigにより最初の臨床例が報告されて以来，現在に至るまで病態解明のために様々な基礎研究が重ねられている．根本的な治療法はいまだに確立されてはいないが，各国で臨床研究が進められている．

SMAの臨床症状は多様であり，近年まで定義が統一されていなかった[1)2)]．1992年，International SMA Consortiumにより，診断基準と分類が確立された[3)]．I型（Werdnig-Hoffmann病）は，生下時から6ヵ月までの発症で坐位保持は不可能，人工呼吸管理をしなければ2歳までにほとんどが亡くなる重症型である．II型は，1歳6ヵ月頃までに発症し，起立または歩行が不可能であるが，2歳以降も生存可能な中間型とされている．III型は，小児期から成人期に発症し，歩行が可能な軽症型である．臨床的重症度は，それぞれの病型のなかでも多様性が認められ[2)]，III型は発症年齢により，IIIa型，IIIb型に分類されることもある[4)]．神経内科では，診断基準を満たすSMAのみならず，下位運動ニューロンが障害される病態を広くSMAとして認識していることが多く，診断が曖昧であることもあった．成人発症で，進行は緩徐であり，呼吸障害や嚥下障害をほとんど認めない例は脊髄性進行性筋萎縮症と診断していたが，国際的な分類に合わせて2009年より脊髄性筋萎縮症IV型とされた．わが国では，2009年に，厚生労働科学研究費補助金（難治性疾患克服研究事業）神経変性疾患に関する調査研究班において，SMAの認定基準が作成された[5)]．同年，特定疾患治療研究事業の対象疾患に指定されたことにより，SMAにおいて医療社会福祉的な環境は改善されつつある．





SMAは，ほぼ全身の臓器に存在する蛋白質であるsurvival motor neuron（SMN）蛋白の欠損あるいは機能障害によって生じる．SMN蛋白は*survival motor neuron*（*SMN*）遺伝子（*SMN1*）によりコードされており，主に*SMN1*の欠失によりSMAが発症する．*SMN*遺伝子（*SMN1*）は5番染色体長腕5q13にあり，向反性に重複したコピー遺伝子（*SMN2*）も存在する[6)]．また，*SMN*遺伝子の近傍には，*neuronal apoptosis inhibitory protein*（*NAIP*）遺伝子も存在し，重症度に関与するといわれている．SMAの遺伝子診断は，*SMN1*と*SMN2*のexon 7とexon 8の領域における塩基配列の5塩基の相違を利用して行われる[7)]．

欧米では，その発症頻度は約10,000出生に1人とされ，保因者頻度は約50人に1人といわれている．わが国においては，1978年に福山，大澤らが81家系101例について臨床遺伝学的研究を行ったが[8)]，それ以降，本格的な臨床調査は実施されたことがなかった．我々は，SMAの特定疾患治療研究事業の対象疾患への認定を目標として，2003年に臨床調査を施行した．今回は，その結果に基づき，患者数，病型別の臨床症状，などについて分析したので報告する．

## 対象および方法

### 1．推定患者数調査

東京女子医科大学倫理委員会の承認のもと，2003年に郵送によるアンケート方式で疫学調査を施行した．対象は，全国の国公立（当時）病院・療養所，大学病院，療育施設および無作為に抽出した全国の主要病院の小児科，内科または神経内科などで，総施設数は2,620であった．病床規模別にみた施設数は，500床以上は720，200～499床は1,455，199床以下は283，無床は162であった．一次調査として，まず，各施設における患者の有無を尋ねた．一次調査で返信のなかった医療機関へは，一次追加調査として同内容のアンケートを送付した．その結果を，橋本らの「難病の全国疫学調査に基づく患者数の区間推定」[9)]の方法に基づいて解析し，その時点でのわが国における通院中の患者数を推定した．

### 2．臨床症状調査

一次調査，または一次追加調査で「患者あり」と返信のあった施設（科）に対して，二次調査としてアンケート方式の質問票を郵送した．質問票には，臨床病型，診断方法，最高到達運動機能（生涯で獲得し得た最高の運動能力），筋力低下の状態，筋線維束性収縮の有無，中枢神経障害の有無，遺伝子検査所見などの質問項目を設けた．遺伝子検査に関しては，*SMN*遺伝子および*NAIP*遺伝子の欠失について調査した．最高到達運動機能は，大川らの「Werdnig-Hoffmann病における運動機能レベル」[10)]を用いて，0（定頸不可能），1（定頸可能），2（坐位保持可能），3（坐位保持可能かつ，その場まわり可能），4（坐位での移動可能），5（立位保持可能），6（介助ありで歩行可能），7（介助なしで歩行可能），8（介助なしで階段昇降可能）と評価した．さらに，国際SMA協会による診断基準[3)]では，筋力低下の特徴を左右対称・近位筋優位・下肢優位・体幹筋罹患としていることから，筋力低下の評価についてはこれらを質問項目に入れた．

## 結　果

### 1．推定患者数調査

質問票の発送総数は2,620，返信数は888であり，無効（廃院，統合などにより返却）の数を除いて計算すると，回答率は34％となった．各施設から報告された患者総数は455例で，診療科別には，小児科365例，内科81例，整形外科5例，不明が4例であった．橋本らの「難病の全国疫学調査に基づく患者数の区間推定」の方法により，患者実数をもとにして，その時点での患者総数を求めた．対象の医療機関を病床数で分類して検討すると，2003年当時，通院中の国内の推定患者数は741～1,391人となった．総務省によると，2003年のわが国の総人口は127,619,000人であることから，SMAの患者数は100,000人当たり0.5～1人と概算できる．

### 2．臨床症状調査

二次調査で報告された患者総数455例のうち，臨床調査の項目別アンケートの返信があった110例について検討した．病型別で分類すると，I型は39例，II型は46例，III型は21例，不明は4例であった．男女比は全体では1：1.14で，各臨床病型別ではI型が1：1.43，II型が1：1.14，III型が1：0.75であった．SMAの発症に男女差はないとされているが，今回の調査でも有意な差異は認めなかった．

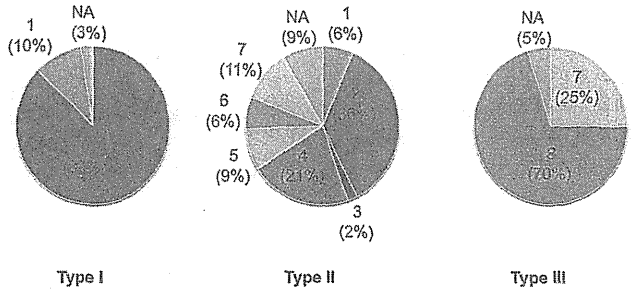


Figure Analysis of maximum motor functional level
The maximum motor functional level represents the patient's peak motor functional abilities. Motor functional level 0: designated as no head control, 1: head control feasible, 2: being able to sit, 3: being able to sit and turn on buttocks, 4: being able to shuffle on buttocks in sitting position, 5: standing with support, 6: walking with support, 7: walking unaided, 8: climbing up stairs without support. NA: (information) not available.

診断方法としては，遺伝子診断が64例（58%），筋生検による病理診断が33例（30%），電気生理学的診断が4例（4%），臨床診断のみが2例（2%），記載なしが6例であった．遺伝子診断は，72例（65%）において施行され，病型別では，I型は31例(74%)，II型は29例(63%)，III型は11例(52%)であり，診療科別では小児科においての実施が多かった．*SMN* 遺伝子はI型の27例(遺伝子検査を施行したI型例の90%)，II型の24例（同II型例の86%)，III型の6例(同III型例の75%)に欠失を認めた．*SMN* 遺伝子欠失例のうち，exon7, 8欠失はI型で80%，II型で71%，また，exon7のみ欠失はI型で10%，II型で14%であった．*NAIP* 遺伝子はI型の11例(37%)，II型の2例(7%)に欠失を認め，III型での欠失例はなかった．*NAIP* 遺伝子欠失例は全例 *SMN* 遺伝子欠失を伴っていた．

最高到達運動機能についての結果をFigureに示す．I型では，定頸不可能例は34例(87%)，定頸可能例は4例(10%)であった．II型では，坐位保持可能まで到達した例が16例（36%）と最も多く，介助なしで歩行まで可能であった例は5例(11%)であった．III型では，介助なしで階段昇降まで可能であった例が15例（70%）と多数であり，歩行は全例で可能であった．

筋力低下の状態については，左右の対称性・遠近の優位性・上下肢の優位性・体幹筋罹患の有無を調査した．筋力低下が左右対称であるのは，I型で34例(87%)，II型で37例(80%)，III型で18例(86%)に認めた．筋力低下が左右非対称であるのは，I型で4例(10%)，II型で9例(20%)，III型で3例(14%)に認めた．筋力低下が左右非対称で，かつ，*SMN* 遺伝子欠失のある例は，I型で3例(8%)，II型で5例(11%)，III型で2例(10%)存在した．近位筋優位の筋力低下はI型では23例（59%），II型では26例（57%），III型では15例（71%）に認め，遠位筋優位の筋力低下はI型で2例（5%），II型で5例(11%)，III型で4例(19%)存在した．I型，II型では遠近の優位性が不明瞭な例はともに30%程度認めた．遠位筋優位の筋力低下を認め，かつ，*SMN* 遺伝子欠失のある例は，I型で2例(5%)存在した．II,III型で遠位筋優位の筋力低下を認めた例では，筋生検による病理診断で確定診断されていた．筋力低下が下肢優位か否かについても検討したところ，I型は17例（44%），II型は23例（50%），III型は13例（62%）と大多数が下肢優位であり，上肢優位の症例もI型で1例（3%），II型で5例（11%），III型で1例（5%）存在した．上肢優位の筋力低下を認め，かつ，*SMN* 遺伝子欠失のある例は，I型で1例(3%)認めた．体幹筋罹患は，I型で37例(95%)，II型で42例(91%)，III型で12例(57%)に認めた．また，顔面筋の罹患は，I型で17例(44%)，II型で2例（4%）にみられた．顔面筋罹患のある例は，I型では全例，II型では1例が人工呼吸管理を受けていた．舌の筋線維束性収縮は，I型で28例(72%)，II型で25例（54%），III型で2例（10%）に認められた．中枢神経系障害は，I型では8例（21%）に認められ，低酸素性脳症1例，顔面神経麻痺1例，球麻痺1例，詳細不明が5例であった．II型では中枢神経系障害を1例(2%)に認めたが詳細は不明であり，III型では認められなかった．





## 考　察

SMAの発症頻度は，欧米では約10,000出生に1人とされ，保因者頻度は約50人に1人といわれている[1]．2003年当時の総人口を基にして検討すると，わが国の推定患者数は100,000人当たり0.5〜1人となった．今回の調査方法では，発症頻度や保因者頻度を算出するのは困難であったため，この結果は概算値にとどまる．諸外国から，発症率あるいは患者数が報告されているが[12)〜18)]，調査方法や対象は統一されておらず，数値にも若干の違いがある．民族による発症率の差はないとされてきたが，近年では，その差異を示唆する報告例も散見される．今後，新しい認定基準を踏まえた上で，再度，わが国における患者数調査を施行することは意義のあることと考える．

SMAの診断方法としては，今回の調査では遺伝子診断が58%，筋生検による病理診断が30%，電気生理学的診断が3.6%，臨床診断のみが1.8%という結果となり，遺伝子診断が多かった．これは，2003年当時の結果であり，2008年に *SMN* 遺伝子検査が保険収載されたことを考慮すると，現在では遺伝子診断の比率はさらに増加しているものと考える．遺伝子検査は，確定診断をする上では必須であるが，臨床遺伝専門医による遺伝カウンセリングを行うことが望ましい．

診断基準を満たすSMAは，*SMN* 遺伝子の欠失を認めることが多い．わが国では，I型の98%，II型の95%，IIIa型の52%，IIIb型の42%，IV型の15%に *SMN* 遺伝子欠失を認めている[19)]．本調査では，I型の90%，II型の86%，III型の75%に *SMN* 遺伝子の欠失を認めた．前述の *SMN* 遺伝子欠失率より，I, II型の欠失率が低いのは，各施設におけるSMAの診断そのものが若干曖昧であった可能性も示唆される．*NAIP* 遺伝子はI型の37%，II型の7%に欠失を認め，III型での欠失例はなかった．*NAIP* 遺伝子欠失例は全例 *SMN* 遺伝子欠失を伴っていた．一般に，*SMN* 遺伝子とその近傍遺伝子（*NAIP* 遺伝子など）の欠失範囲が広いほど，重症であることも明らかになっている[20)]．また，本調査において，*SMN* 遺伝子exon7のみの欠失を認めた例は，I型では10%，II型では14%存在した．*SMN* 遺伝子（*SMN1*）exon7のみの欠失を認める例の中には，*SMN1* からコピー遺伝子である *SMN2* への遺伝子変換を示す例も含まれる可能性もある．*SMN1* から *SMN2* への遺伝子変換を示す例では，臨床症状が軽症になる傾向もあることが示唆されている[21)]ことから，今回は未施行であるが，今後，このような *SMN* 遺伝子exon7のみの欠失を認めた例において更なる臨床像の分析を進めていくことは有意義である．

SMAの運動機能の評価法として，2003年の調査時は，大川らの「Werdnig-Hoffmann病における運動機能レベル」[10)]を用いた．このレベル0からレベル8までの分類により，病型別の大まかな臨床像をみることは可能である．今回の調査でも，各病型の最高到達運動機能を分析し，I型は坐位保持不可能，II型は起立または歩行が不可能，III型は歩行が可能，という診断基準にほぼ合致する結果を得た．現在では，評価者（医師や理学療法士など）による判定の相違を少なくするために，「Hammersmith運動機能評価スケール（Modified Hammersmith Functional Motor Scale)」[22)]を用いてSMAの運動機能を評価することが試みられている．

SMAはその臨床病型の範囲が幅広く，前述のI型からIV型のほかに，胎児期発症の最重症例を0型とすることもある．*SMN* 遺伝子欠失があっても非典型的な症状を示す例や，*SMN* 遺伝子欠失（あるいは同定困難な *SMN* 遺伝子変異）がなくてもほぼ典型的な症状を示す例も存在する[20)22)]．今回の調査において，筋力低下という臨床症状に限って検討しただけでも，*SMN* 遺伝子欠失があり，かつ，上肢優位，あるいは，遠位筋優位の筋力低下を示す非典型例の存在も明らかとなった．これらの非典型例の存在からも，SMAの病態の複雑さが示唆される．

遺伝子検査では診断できず，臨床診断により確定する例は成人発症例に多い[23)]．特定疾患治療研究事業の対象疾患としての認定を受けるためにも，臨床診断は非常に重要といえる．呼吸や嚥下機能障害，側弯症などの合併症へ早期に対応するためにも，早期診断は重要である．現在，SMAの治療法開発に向けて治験の開始準備も進められている．その一環として，希望者が罹患者リストに登録するシステムも構築されつつある．SMAおよびその周縁疾患の臨床像を分析することは非常に重要であり，今後も継続していく予定である．

## 結　論

2003年当時のわが国におけるSMAの推計通院

患者数は 0.5〜1 人/100,000 人であり，諸外国からの既報告例と概ね同様であった．発症者数，保因者数などについては，引き続き検討を要する．今回の調査では，SMA の確定診断には遺伝子検査を用いた例が半数を占めることが明らかとなった．最高到達運動機能を検討すると，I 型では定頸不可能例が，II 型では坐位保持可能例が最も多く，III 型では歩行は全例で可能であった．臨床症状については多様性が認められた．罹患年齢が幅広い SMA の臨床像の分析は，複数の診療科による協力が不可欠である．診断基準を満たす例のみではなく，SMA の周縁疾患の範疇にある例も含めて，今後も臨床研究を進めていく必要がある．わが国でも統一基準をもって多施設共同研究が可能となるような基盤ができれば，医療的ケアの充実，治療法開発に向けての研究が今後も進展していくと思われる．

本研究は，平成 15 年度文部省科学研究費基盤研究(課題番号 B12470173) の助成によって開始され，平成 20 年度本学女性医学研究者支援室の助成を受けて進められた．現在は，平成 20 年度厚生労働科学研究費補助金(難治性疾患克服研究事業)「神経変性疾患に関する調査研究班」(研究代表者　中野今治，分担研究者　斎藤加代子)，および，平成 22-24 年度厚生労働科学研究費補助金(難治性疾患克服研究事業)「脊髄性筋萎縮症の臨床実態の分析，遺伝子解析，治療法開発の研究」(研究代表者斎藤加代子) において継続して行われている．

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開示すべき利益相反状態はない．

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総　説

# 脊髄性筋萎縮症の遺伝カウンセリング

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## Genetic Counseling of Spinal Muscular Atrophy

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In recent years, significant progress has been made in molecular genetics, and researchers have identified many gene mutations responsible for diseases. The range of useful information in genetic counseling has increased not only for definitive diagnosis but in terms of medical treatment and prevention. Additionally, gene information is now applicable to prenatal diagnosis. Thus, it has become more important to provide appropriate genetic counseling. We herein describe genetic counseling for spinal muscle atrophy.

**Key Words:** spinal muscular atrophy, genetic counseling, prenatal diagnosis, self-determination



## はじめに

分子遺伝学の目覚ましい進歩により，多くの遺伝性疾患の原因遺伝子が特定されてきた．遺伝学的検査による確定診断により，罹患者の自然歴や予後が推定できるようになった．原因遺伝子を特定する意味だけでなく，治療や予防に有用な情報の範囲が増加したため，適切な遺伝カウンセリングの提供がますます重要になり，十分な情報提供の元に自己の治療に役立つ選択が可能になっていくと考えられる．

本稿では，脊髄性筋萎縮症（spinal muscular atrophy：SMA）の遺伝カウンセリングについて述べる．

### 1．遺伝カウンセリングとは

遺伝カウンセリングとは，National Society of Genetic Counselors によって「疾患の医学的心理的，家族的影響を理解し，それに適応できるように援助するプロセスである．このプロセスは以下の3つの事項を統合したものである．」と定義されている[1]．

①疾患の発生もしくは再発の可能性を評価するための家族歴と解釈

②遺伝，検査，マネージメント，予防，資源および研究についての教育

③リスクもしくは状況に対するインフォームドチョイス（説明を受けた上での選択同意）と，適応を促進するカウンセリング

つまり，遺伝カウンセリングは，ある遺伝性疾患に関して，どのくらいの発症リスクがあるか，または再発のリスクがあるかなどについての情報を求める患者（クライエント）が心の負担や重荷を感じることなく，様々な情報を得て，自己決定できるようにすることが重要と考えられている．

その過程では，クライエントにはチームで関わることが望ましく，臨床遺伝専門医や遺伝カウンセラー，看護師，心理士などの臨床遺伝専門職は心理的，社会的な支援を通して，クライエントへの援助を行うことを求められている．

例えば，再発のリスクの受け止め方は人によって異なる．受け止める背景には，その人の教育や文化的背景，数学的確率の理解度，不安になりやすさなどの性格傾向によって影響を受ける．ある染色体異常の児をもつ母親は，再発率は一般人のリスク（3～5%）より数倍くらい高くなると伝えられた．ある母親は「そんなにリスクが高くては，とても次の子は望めません．」と答えたが，別の母親は「一般人ともそんなに違わないので安心しました．」と話した．





| Genetic counseling | Process |
|---|---|
| appointment | major complaint, point to be checked for accompanying person, gathering information about their disease |
| ↓ | |
| pre-counseling meeting | preparations for the institution which can possibly carry out the genetic testing |
| ↓ | |
| date of first visit | genetic information on proband, plus clinical features, pedigree construction, family tree construction, providing information to the patient about medical genetics |
| ↓ | |
| staff conference | case conference |
| ↓ | |
| after the 2nd visit | supplementary information on the proband, provide information to the patient about medical genetics |
| ↓ | |
| follow-up care | |

**Fig. 1**　Process of Genetic counseling

このようにリスクの捉え方，解釈の仕方は人それぞれで，それゆえに遺伝カウンセリングの展開は家族ごとに違ってくる．そこで，様々な職種がチームで関わることによって，多様なクライエントの価値観，解釈にきめ細やかな対応が可能になると考えられる．

通常，遺伝カウンセリングは Fig. 1 のような流れで行われる．

まず，遺伝カウンセリングではクライエントがどんな相談で来院したかを把握するのが重要である．ときには漠然とした不安を持って来院することもあるが，そのようなときにはクライエントの不安に共感しつつ，問題点を明確にする必要がある．

本人とその家族の遺伝学的情報を聴取し，家系図をまとめる．家系図では，医学的な情報の収集のみならず，家族の情緒的な交流の度合いなども推し量れることもあり，その後のカウンセリングには有用な情報をもたらすことも多い．

次に家系や疾患の情報を収集した上で重要なのはやはり診断の確定である．診断が正しくなければ，その後の方向性も変わってくるので留意する．

その後，遺伝的なリスク，対応などの情報提供を行うが，クライエントの理解度を確認しながら行うのが望ましい．また医学的な情報のみではなく，心理社会的な支援の状況などを加えながら伝えることが大切である．

SMA の相談では，遺伝子による診断確定の希望で来院されることも多いが，「子どもが SMA と診断されているが，次の子どもも同じ症状をもつかどうか心配なので相談したい．」あるいは「兄弟姉妹が SMA と診断されている．自分が保因者かどうか，が知りたい．」といって来院される．遺伝カウンセリングで得られた情報を元に遺伝子検査を受ける，受けないなどの選択がスムーズにできるよう支援していくことになる．

### 2．SMA の遺伝カウンセリング

#### 1）情報提供

SMA は脊髄前角細胞の消失による筋萎縮と進行性の筋力低下を特徴とする常染色体劣性遺伝性疾患の1つである．SMA の責任遺伝子は染色体長腕 5q13 に存在し，運動神経生存（survival motor neuron：*SMN*）遺伝子の欠失により症状がおこる．国際 SMA 協会により発症年齢，臨床経過に基づいて，I 型（重症 Werdnig-Hoffman 病），II 型（中間 Dubowitz 病），III 型（軽症 Kugelberg-Welander 病），IV 型（成人発症）に分類される[2]．

I 型は生後6ヵ月までに発症し，呼吸管理をしなければ2歳までに死亡することが多い．II 型は1歳6ヵ月頃までに発症し，坐位保持までは可能であるが，自立歩行は困難である．III 型は1歳6ヵ月以降に転びやすい，歩けないなどの症状で気づくが発症年齢は個人差がある．自立歩行を獲得するが，次第に筋力低下，歩行困難が目立つようになる．IV 型は成人型で，孤発例が多く，20歳以降，老年にかけて発症する．

小児期発症の SMA の原因遺伝子は *SMN1* 遺伝子で，5番染色体長腕 5q13 に存在している．両親から受け継いだ *SMN1* 遺伝子が欠失していることにより発症する場合が多い．I 型，II 型では 95% 以上，III 型では 40～50% に *SMN1* 遺伝子の exon7, 8 の両方もしくは exon7 のみの欠失が認められる．常染色体劣性遺伝性形式を取るので，患者の両親から生まれる同胞は 25%（1/4）の確率で遺伝子変異を持つ．また症状がない患者の同胞は 2/3 の確率で保因者の可能性がある．また，次子の出生前診断につい

(1) Prenatal testing and diagnosis options during the first half of pregnancy include cytogenetics, biochemical genetics, molecular genetics, and histopathological methods employing amniotic fluid, villus cells, and other embryonic samples, as well as the physical procedure of ultrasound examination.
(2) In performing prenatal genetic inspection and diagnosis, care must be taken regarding ethical, including social, concerns. Care must especially be taken in terms of the following points.
  (a) Before prenatal testing, we provide relevant explanations about the possibility of an infant having a disease, the diagnostic limits of laboratory procedures, and risks and adverse effects on the mother and the fetus. We also offer sufficient genetic counseling.
  (b) Implementation of testing necessitates sufficient fundamental training and must be carried out by obstetricians and gynecologists who have mastered safe and appropriate inspection techniques, or have been given adequate instruction.
(3) Invasive prenatal testing and diagnosis employing chorionic villus sampling, amniocentesis, and so on, have given couples hope about pregnancy for the following reasons, and can be performed when understanding of the meaning of a test is sufficient.
  (a) When either the husband or the wife is a gene carrier of a chromosome abnormality
  (b) When there is a past history of having given birth to a child suffering from a chromosome abnormality.
  (c) In pregnant women of advanced age
  (d) In pregnant women who are heterozygote carriers of an X-linked hereditary disease associated with a serious illness in the newborn period or during childhood
  (e) When both the husband and the wife are heterozygotes for an autosomal-recessive-inheritance disease associated with a serious illness in the newborn period or during childhood
  (f) When either the husband or the wife is a heterozygote with a critical autosomal-dominant-inheritance disease associated with a serious illness in the newborn period or during childhood
  (g) In addition, when a fetus is suspected to possibly have serious of life-threatening disease.

**Fig. 2** Prenatal diagnosis (Genetic testing guidelines derived from consensus of ten related societies)

ては遺伝子変異が判明している場合には可能になるが，倫理的な問題を含むため，遺伝カウンセリングで十分に話し合う必要がある．

2）遺伝子による確定診断

SMA の遺伝子による確定診断は臨床診断に基づいて行われる．採血よりリンパ球を取り出し，DNA を抽出して *SMN* 遺伝子が欠失しているかどうかを調べる．

確定診断を行い，結果開示の遺伝カウンセリングでは，診断を告げられた両親は衝撃を受け，説明がほとんど頭に残らなかったと後に語ることも多い．両親の受け止めを見ながら，わかりやすい言葉で，段階を踏んで説明していく必要がある．また，SMA の児は知的障害がなく，理解力を備えているので，年齢に即した本人への説明も考慮されなければならない．

3）出生前診断について

本邦では，出生前診断は遺伝関連10学会（2003年5月）に作成されたガイドライン[3]に基づき，実施範囲が決定される．ガイドラインでは以下のように定義されている（Fig. 2）．

SMA ではガイドラインの（e）に相当し，重篤と考えられるⅠ型，Ⅱ型が本学倫理委員会でも審議を通過している．しかし，そこには倫理的な問題が含まれていることを忘れてはならない．ガイドラインで乳児期，小児期に発症する重篤な疾患，と定義されているが，実際にはっきりとした線引きは存在しない．重篤という観点については家族，特に両親の想いは様々である．Ⅲ型やⅣ型は出生前診断の対象にはなっていないが，ある地方在住の SMA Ⅲ型の家族は，出生前診断について話を聞きたいと来院した．この家族にとっては，家業を継ぐ後継者としては障害がない子でないと困る，と出生前診断を切望した．遺伝カウンセリングの中で，Ⅲ型は出生前診断のガイドラインに合致せず，本学倫理委員会を通過していないこと，また成人に達し社会で活躍している方が多いことなどの説明を受けた．両親は数回の遺伝カウンセリングで出生前診断は実施できないことを納得した．この両親のように，医療に関する自己決定は容易ではない．生命倫理の課題が含まれていることが多いため，意思決定においての困難さが増す．そのため，問題解決のための遺伝カウンセリングが重要な意味をもつと考えられる．

さらに，出生前診断を実施し，児が遺伝子変異を有していない場合にはその後の妊娠を継続できるが，児が病気であると判明した場合には，子どもをあきらめるのか，あるいは病気と知って子どもを迎えるのか，重い選択を迫られることになる．また，人工妊娠中絶術を受けた母親は通常の分娩とは異なり，大きなストレスを抱える．中絶後の女性の心理的な葛藤は中絶のすぐ後で大きく，たいていの女性は時間を経て弱まるが，何人かの女性は苦悩の状態





が続くとしている[9]．中絶した悲しみ（grief）からの回復には十分な時間がかけられることが大切である．このように出生前診断の遺伝カウンセリングに際しては，夫婦あるいは家族が納得のいく決定を支え，その後の心理的葛藤にも援助をすることが重要になるだろう．

3．遺伝カウンセリングの実際

**症例**：SMA Ⅰ型の児を亡くした夫婦

**家族歴**：神経筋疾患の既往がある家系員はなし．

**家族**：夫30代，妻30代．血縁関係はなし．

**第一子の経過**：在胎週数41週3日，遷延分娩，帝王切開にて出生した．体重3,200g．生下時より四肢の動きが少ないと感じていた．6ヵ月時，筋力低下，運動発達遅滞などを指摘され，地元の大学病院を受診した．精査入院し，SMA が疑われ，東京女子医科大学附属遺伝子医療センターに遺伝子検査の依頼があった．検査の結果，*SMN* 遺伝子の欠失が認められた．診断後，徐々に呼吸困難症状が出現し，呼吸不全により1歳4ヵ月時死亡した．次子の希望があることから主治医より紹介され，当センターに来院した．

**遺伝カウンセリング初回（臨床遺伝専門医と筆者らは毎回同席）**：児が亡くなって約4ヵ月後に夫婦で来院した．臨床遺伝専門医により，疾患のこと，遺伝と遺伝子，次子の出生前診断について説明を受けた．児の経過を話すときにも母はすぐに涙を見せ，まだ情緒的に不安定である様子が見受けられた．夫婦でよく話し合い，次回，出生前診断についての考えを伺うことになった．

スタッフカンファレンスでは，児が亡くなって日が浅く，両親が児の亡くなったことの受け入れにはほど遠い状態であることが懸念された．ここですぐに出生前診断を行って，もし次の子が罹患だった場合，心理的な負担が大きいと思われた．時間をかけた方がよいだろうということになり，心理の継続面接も勧めてみる．

**2回目**：初回から2ヵ月後に夫婦で来院した．出生前診断を希望したいと話した．しかし，「診断は受けたいが，もし結果が病気だった場合に，命の選別をすることになるのではないか．迷いもある．でも前の子どものときと同じようなつらい思いはしたくない．」と話した．夫婦は，事前の検査はしておきたいとのことで，多型解析のための同意書を作成し，採血を行った．

**3回目**：夫婦で来院し，多型解析の結果を聞いた．夫婦の気持ちを出生前診断の前に整理することを提案し，心理面接も個別に行った．

**4回目**：初回から2年後，妊娠の連絡を受けた．遺伝カウンセリング終了後，妻のみ心理面接を行う．子どもが病気だった場合にどうするか，という点について話を聞いた．出生前診断を受けるということで，夫婦の意見は一致したということであった．もし，病気だった場合に，母は覚悟をしているようではあったが，戸惑いもある様子だった．

**5回目**：第二子も病気であることが判明した．結果を医師から伝えられると，涙する．夫婦とも言葉少なく，とてもショックを受けている様子だった．

**6回目**：人工妊娠中絶術1ヵ月後，母のみ来院した．次の子どもについては怖くて考えられない，夫婦でも話し合いはまだしていないことなどを語った．その後，電話でも話をしたが，少しずつ外出もできるようになり，落ち着いてきているとのことだった．次の子どもについても前向きに考えられるようになったと報告があった．

**症例の考察**：遺伝学的検査による確定診断が行われることも多くなったが，その際には検査によってどのようなことが判明するか，家族にはどのような影響があるか，などについても説明を行い，結果が出た際に，自分がどのように考えるか，家族にはどう伝えればよいかなど，シミュレーションできることは重要である．また，遺伝学的検査によって，遺伝子変異が検出されなかった場合，臨床症状がある場合には診断の否定にはならず，検査が100% 検出可能ではないことを検査実施前に十分理解を促すことも必要であろう．

このクライエントのように遺伝学的検査によって診断が確定すれば，次の子どもの出生前診断が可能になるが，倫理的な問題も含まれるため，実施の際には遺伝関連10学会によるガイドラインに即しながら，遺伝カウンセリングを行っていく必要がある．

当センターでは1996年から2010年までに SMA Ⅰ型，Ⅱ型の102例66家系，双胎2組を含み，診断対象児は104名について出生前診断を実施した経験がある．診断の結果は104名中，28例（26.9%）が罹患，76例（73.1%）が非罹患であり，常染色体劣性遺伝性疾患の次子再発率から予測される確率に相応している結果となった．

出生前診断を希望する夫婦は，児には異常がない75% の確率を得たくて来院することが多い．しかし，25% の確率，つまり児に病気があると診断され

る場合が必ずあることに焦点を当てなければならない．児に病気があるという，夫婦にとって悪い知らせ（bad news）を聞くときには不安が生じやすく，内容を聞くのが困難になる[9]．心の準備となる出生前診断を受ける前の遺伝カウンセリングでは，夫婦それぞれの病気に対する考えや児に対する思いを聞き，また次子が病気だったときの受け止めについて話し合うことが重要になる．つまり，両親が不安や悲しみなどを表出した際に，医療側の対応が可能になるように，出生前診断を実施する以前から，夫婦の考えや想いを把握しておくことが大切になる．

さらに中絶を前提とするのではなく，児の状態を把握し，出産時の体制を整えるために受けたいという夫婦もいた．また，SMA の子どもを看取ったが，十分なケアができなかったという思いが強く残った夫婦は，遺伝カウンセリングの中で SMA の児が生まれてくることを望んでいると話した．周囲の反対などもあったが，夫婦の決定としては，出生前診断を受けるのをやめ，調べずに出産を迎えた．このように夫婦の選択は様々であり，その多様な思いに対応していくことが医療側にも求められる．

## おわりに

近年，遺伝性疾患の原因遺伝子の同定によって病態の解明も加速している．遺伝子が判明することにより，病態を把握し，臨床的なマネージメントに役立つ情報が得られるが，遺伝子が判明し病気が確定することで新たな悩みを生み出す側面も持っていることを忘れてはならない．治療法の確定していない疾患の出生前診断では，胎児が疾患を持つかどうか判明するが，その後の夫婦の決定には，命の選別につながる重い選択が含まれている．そのような場合には遺伝カウンセリングのようなコミュニケーションのプロセスが重要になる．正確な情報提供とともに，患者の心情を推し量る姿勢や，配慮をした言葉かけや対応などにも重点を置いたケアの実践はどの医療にも通じる姿勢であろう．

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開示すべき利益相反はありません．

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III. トピックス

# 神経筋疾患における小児医療から成人医療への移行：遺伝子診断および遺伝カウンセリングを通した介入

KeyWords
◎神経筋疾患
◎遺伝子医療
◎思春期の成長と課題
◎ライフステージと医療

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**Headline**

1. 神経筋疾患にて通院中の10歳以上の患者・家族に思春期の変化について，アンケート調査を実施した．
2. 遺伝医療の場では，確定診断に伴う開示，告知，その後の身体的・心理的フォローの役割を担う．
3. 思春期はアイデンティティの確立のために葛藤が生じやすく，神経筋疾患をもつ子どもは運動機能障害の進展により，不安を増強させやすいため，介入が必要．
4. 成人医療の移行として遺伝カウンセリングの介入は意義が大きい．

## はじめに

神経筋疾患を抱える小児は，成長とともに運動機能を獲得するが，思春期を境にして，運動機能障害の進展が顕著になり，自由な行動に制限が生じることが多い．小児科から内科への移行期である思春期は，神経筋疾患を抱える小児にとって，喪失する運動機能について悩み，周りの子達と比較して劣等感を抱き，情緒不安定になり，将来への不安が増す時期であり，家族への反抗，不登校などが生じうる．一方，親は，子どもが障害をもつことへの拒否的・否定的な感情と，その反動としての過保護や溺愛となることもある．この時期を乗り越えて，独立心が芽生え，素晴らしい成人に達する人たちも少なくない．進行性に筋力低下を示し，思春期において歩行機能などの運動機能を喪失し，成人期には呼吸器の装着を考えていく場合もある神経筋疾患を有する人々の医療に携わる者は，この時期の課題を把握し，個別医療としての医療的・心理的な対応が求められる．

遺伝診療の現場では，十分な遺伝カウンセリングのもとに遺伝子診断による確定診断を行い，その結果を患者と家族に開示し，その後の身体的・心理的フォローを主治医と協力して実施している．患者が小児の場合には知的な発達に応じて，わかりやすい言葉で説明を行うか，発達経過の時期をみて疾患の説明や開示を行う．

わが国の神経筋疾患の医療には，小児期から成人期への移行のプログラムが存在するわけではない．海外においても，移行プログラムやカウンセリングの必要性が報告されてきている[1,2,3]．本稿では，遺伝カウンセリングの立場から，神経筋疾患をもつ小児の思春期の問題を考察し，遺伝子診断および遺伝カウンセリングを通して，遺伝医療が介入することによる小児科から内科，在宅医療など成人医療への円滑な移行について考察する．

## 神経筋疾患をもつ小児の運動機能の進展課程

神経筋疾患として，Duchenne型筋ジストロフィー（Duchenne muscular dystrophy;DMD）と脊髄性筋萎縮症（spinal muscular atrophy;

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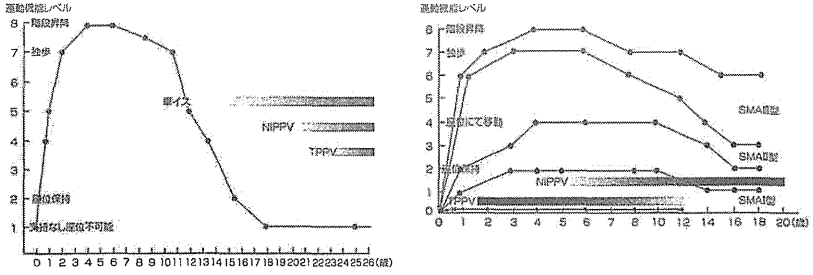


図1　神経筋疾患の運動機能の経過
A：Duchenne型筋ジストロフィー，B：脊髄性筋萎縮症

SMA）をあげる．図1に示すように，DMDでは，10歳前後から歩行不可能となり車椅子使用の生活となる．17〜18歳頃から非侵襲的陽圧人工換気（noninvasive positive pressure ventilation;NPPV）を，20歳を過ぎると気管切開陽圧人工換気（tracheostomy positive pressure ventilation;TPPV）を受ける可能性が出てくる（図1A）．SMAでは，III型において，小学校中学年以降に車椅子使用の生活となる場合が多い．II型においては，幼児期から夜間のみNPPVを使う患児もいるが，思春期頃からNPPV導入の場合も多い（図1B）．このように，神経筋疾患においては，思春期の身長が伸びる時期に体幹・四肢が伸長し，筋力低下の急速な進行，脊柱変形，歩行機能の喪失，呼吸機能の低下を認める．

## 思春期における精神的な成長と問題点

東京女子医科大学附属遺伝子医療センターに神経筋疾患にて通院の10歳以上の患者がいる110例の家庭を対象に，質問紙を郵送してアンケート調査を行った．有効回答数は72通（回答率65.5%）であった．そのなかで「精神的な成長」について41例（57%）が「あり」と，30例（42%）が「なし」と回答した．「あり」と回答した内容は，「思いやり」22例（38%），「友人との関係」17例（29%）であった．具体的には，「周囲の様子を見ながら考えて行動ができるようになった」「人の気持ちがわかるようになった」「客観的な視点から物事をとらえることができる」「感情のコントロールができるようになり穏やかになった」などである．

一方，思春期は，アイデンティティの確立のプロセスとしての葛藤や悩みが生じ，劣等感や無力感を感じる時期である．神経筋疾患をもつ子どもたちにとっても，思春期前後に，「自分の病気は何なのか？」「歩けなくなり，動けなくなったらどうなるのか？」「自分は死んでしまうのか？」という不安や疑問を抱く．親に不安を表明する，親や兄弟に八つ当たりする，親にも言えず一人で悩む，インターネットで検索し，より不安を増強させる，身体・精神症状を引き起こす場合などがある．また，運動機能障害の進展に伴い，思春期になるとむしろ親からのケア・介助を受けることが必須となる．そのため，親との心理的な距離を保ちにくくなる場合も多い．

## 神経筋疾患をもつ人のライフステージと医療―小児科からの移行における遺伝医療の介入

神経筋疾患において，幼児期から学童期は，発達と共に運動機能獲得の時期である．そして，思春期以降，身体的な急激な成長の時期に運動機能喪失に転じていく．このような自然経過のなかで，運動機能障害に対して，さらに呼吸・嚥下機能障害に対して理学療法が，脊柱変形に対しては脊柱固定術などの手術療法が必要である（図2）．神経筋疾患においては，運動発達遅滞により両親が気づき小児科を受診して，乳幼児期において遺伝子検査によって確定診断がなされることが一般的となった．本人が疾患を自覚しない頃から，理学療法が開始されている．しかし，学童期になると運動機能の喪失について，理学療法の必要性について，場合によっては脊柱固定術の必要性について，本人への説明と理解が必要となる．したがって，小学校入学以降から患児の疑問に答える形で，また両親から患児への診断開示に関する相談に対応する形で，遺伝カウンセリングとしての介入は，患児本人の疾患の理解と受容において大きな意義がある．遺伝カウンセリングにおいては，本人が疾患を理解するために，疾患の発症メカニズムのわかりやすい説明と，遺伝子や遺伝の知識の解説を行う．また，青年期となった患者がパートナーとともに，妊娠，出産，子育てを考えて，遺伝医療施設に遺伝カウンセリングを受ける目的で受診するケースも増えている．患者本人と配偶者に情報を提供すること，生活と医療におけるサポート体制を整えることなども，遺伝カウンセリングにおける重要な役割である．遺伝カウンセリングとは，「疾患の遺伝学的関与について，その医学的影響，心理学的影響および家族への影響を人々が理解し，それに適応していくことを助けるプロセス」である（日本医学会「医療における遺伝学的検査・診断に関するガイドライン，2011年2月」より）[4]．すなわち，遺伝医療施設には，神経筋疾患の当事者における疾患の理解→受容→自立/自律の過程の支援という役割がある．



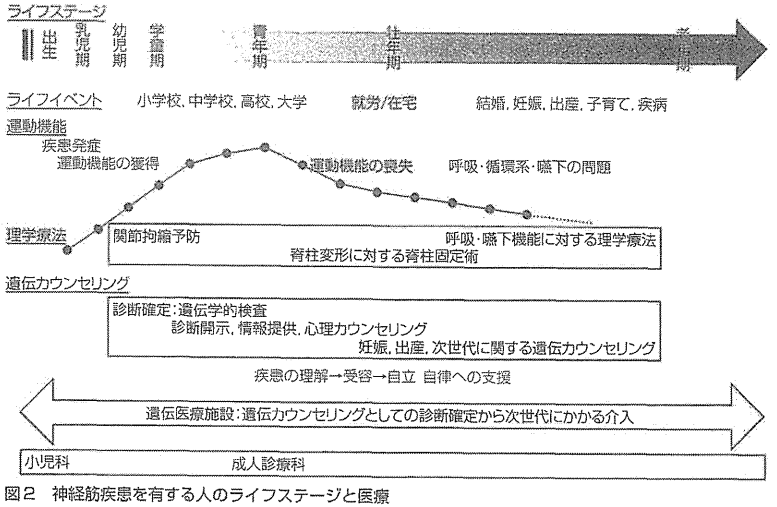


図2　神経筋疾患を有する人のライフステージと医療