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# Spinal muscular atrophy (5qSMA): best practice of diagnostics, newborn screening and therapy

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**Abstract:** Proximal spinal muscular atrophy (SMA) is an autosomal-recessive inherited neuromuscular disorder caused by the degeneration of alpha motor neurons in the anterior horn of the spinal cord. Patients show hypotonia, muscular atrophy and weakness of voluntary proximal muscles. SMA is one of the most common genetic diseases, with a frequency of about 1 in 7,000 newborns in Germany. The vast majority of patients carry a homozygous deletion of exons 7 and 8 of the survival motor neuron (*SMN*) 1 gene on chromosome 5q13.2; only about 3–4 % of patients are compound heterozygous for this common mutation and an additional subtle mutation in *SMN1*. The severity of the disease is mainly influenced by the copy number of the highly homologous *SMN2*.

Since the discovery of the underlying genetic defect 25 years ago, both the diagnostics of SMA and its treatment have undergone constant and in recent times rapid improvements. SMA has become one of the first neuromuscular disorders with effective therapies based on gene targeted strategies such as splice correction of *SMN2* via antisense oligonucleotides or small molecules or gene replacement therapy with a self-complementary adeno-associated virus 9 expressing the *SMN1*-cDNA. With the availability of treatment options, which are most effective when therapy starts at a pre-symptomatic stage, a newborn screening is indispensable and about to be introduced in Germany. New challenges for diagnostic labs as well as for genetic counsellors are inevitable.

This article aims at summarising the current state of SMA diagnostics, treatment and perspectives for this disorder and offering best practice testing guidelines to diagnostic labs.

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## Introduction

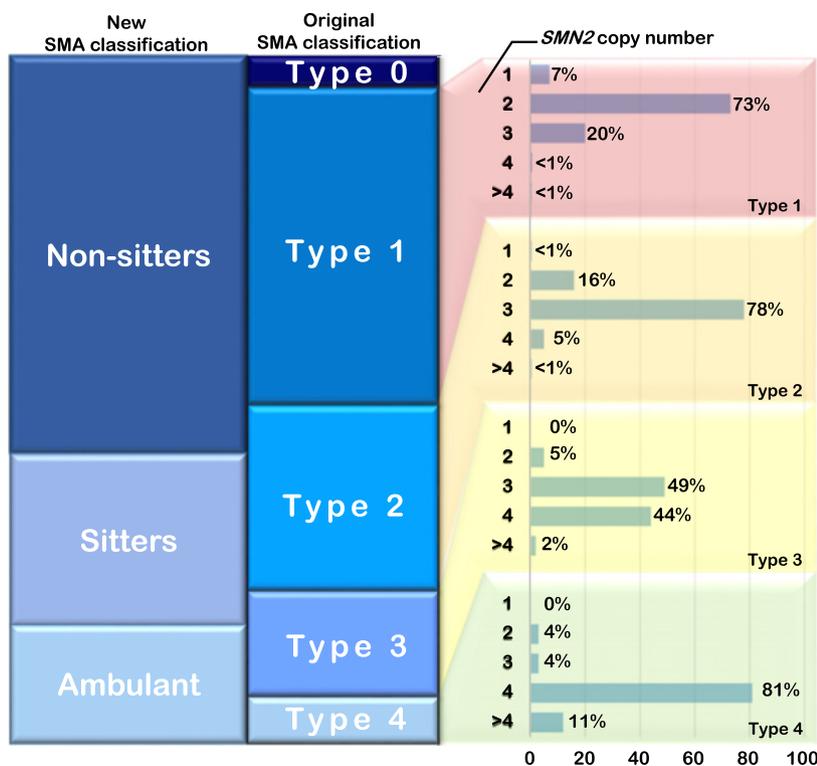
Spinal muscular atrophy (SMA) is an autosomal-recessive neuromuscular disorder caused by mutations in the survival motor neuron (*SMN*) 1 gene, while the disease severity is mainly determined by the number of *SMN2* copies (reviewed in [1]). *SMN1* and its copy gene *SMN2* are localized within a copy number variation (CNV) on chromosome 5q13.2. Each segment of the CNV comprises approximately 500 kb. The CNV is highly polymorphic with respect to orientation, length and structure; individuals may carry zero to four segments per chromosome, resulting in different numbers of *SMN1* and *SMN2* per genome. The majority of healthy individuals carry two *SMN1* and two *SMN2* genes per genome; however, both *SMN1* and *SMN2* genes may vary from zero to four copies per chromosome. Individuals with SMA usually harbour a homozygous deletion of *SMN1*, while the *SMN2* copy number varies between one and six. The SMA carrier frequency is 1:51 worldwide and 1:41 in Europe (Table 1) [2].

## Classification of SMA

Historically, SMA had been divided into three subgroups (types I to III) based on age of onset and achieved milestones [3]; the spectrum was then expanded by types 0 (congenital) [2] and IV (adult) [4]. With the development of treatment and the resulting dynamic clinical picture of the treated patients, an adapted re-classification system was required. Following treatment, overlaps between subgroups are observed and in the light of ongoing clinical studies, an adjusted classification has been proposed. According to the current consensus, SMA patients are now classified as ‘Non-sitters’, ‘Sitters’ and ‘Walkers’ [5, 6] (Figure 1). In this system each subgroup comprises patients that either never achieve the ability to sit or walk or lose/achieve the respective ability during their lifetime.

**Table 1:** 5q-SMA carrier frequency of various ethnicities (based on [7] and references therein).

Country-ethnicity	Carriers (one <i>SMN1</i> copy)	Total number of tested individuals	Ratio of carriers in the population
Europeans	90	3,704	1:41
USA-Caucasians	558	26,839	1:48
USA-Asian	110	6,908	1:63
USA-Black	64	6183	1:97
USA-Jewish	115	7536	1:66
USA-Hispanic	110	8968	1:82
USA-Mixed (newborn screening)	38	1530	1:40
Australia/New Zealand-Caucasians	3	147	1:49
Asian	2,407	116,162	1:48
Sub-Saharan Africa	6	868	1:145
Israel-Jewish	294	14,741	1:50
<b>Total</b>	<b>3,795</b>	<b>193,586</b>	<b>1:51</b>

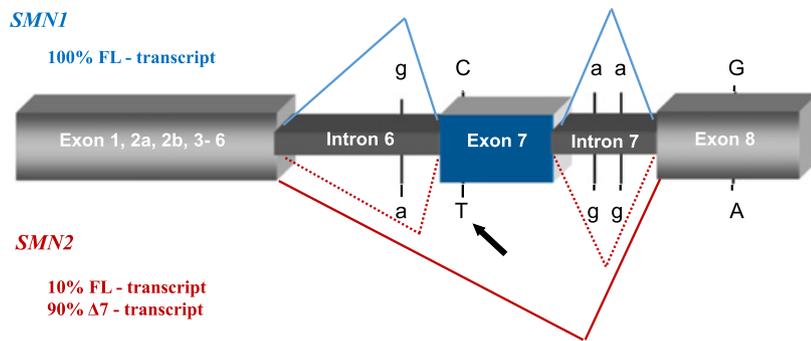
**Figure 1:** Clinical forms of SMA according to the new and original classifications and their correlations with *SMN2* copy numbers. See also [1].

## Gene structure of *SMN1*

Both *SMN* genes consist of nine exons that are historically designated as 1, 2a, 2b and 3 to 8 (Figure 2).

There are only five single nucleotide variants (SNVs) distinguishing *SMN1* and *SMN2*. Two of these are in the coding sequence of the genes: in exon 7, SNV c.840C is

*SMN1*-specific and c.840T is *SMN2*-specific (p.Phe240=) (rs4916) (ENST00000380707; NM\_000344.4); the SNV in exon 8 is located in the 3' non-translated region. Additionally, there is one SNV in intron 6 and there are two in intron 7 [8]. All other SNVs described are not unique to one of the two *SMN* genes and do not allow a direct assignment. However, these five unique SNVs are valuable tools for the



**Figure 2:** Schematic diagram of *SMN1* and *SMN2* gene structure. The five nucleotide differences between the two *SMN* genes are shown. The arrow points to the main difference in exon 7, which disrupts an exonic splicing enhancer in *SMN2*, causing aberrant splicing. FL-transcript: Full-length *SMN* transcript;  $\Delta 7$ -transcript: *SMN* transcript lacking exon 7.

distinction of *SMN1* and *SMN2* in sequencing analysis (Figure 2).

In this highly homologous region, a gene conversion between *SMN1* and *SMN2* can occur via fusion of *SMN1* exon 8 with *SMN2* exon 7 (hybrid gene), thus converting *SMN1* into *SMN2* or vice versa [9, 10]. Therefore, a deletion of *SMN1* exon 7 only also confirms 5q-SMA. Note, the term “deletion” here refers to deletion and gene conversion.

According to its high variability, the chromosomal region 5q13.2 shows a high *de novo* mutation rate. Approximately 2% of affected patients show a *de novo* mutation that may arise by unequal crossing over or gene conversion [11].

## Genetic pathomechanism

None of the five SNVs distinguishing *SMN1* and *SMN2* is predicted to lead to an altered amino acid sequence. So what is the pathomechanism behind the disease?

Interestingly, SNV c.840C>T in exon 7 is located at cDNA position +6 in an exonic splicing enhancer (ESE) sequence and the nucleotide change creates a novel exonic splicing silencer in *SMN2* [12–14]. While *SMN1* mainly produces full-length transcripts, the single nucleotide variant in *SMN2* leads to aberrant splicing and the generation of approximately 90% of transcripts lacking exon 7 [12]. *SMN2 $\Delta$ 7* transcripts are translated into a truncated SMN $\Delta$ 7 protein that is degraded rapidly [15]. The remaining 10% of *SMN2* transcripts encode an SMN protein that is identical to the one generated from *SMN1* (Figure 2). There are further *cis*-regulatory splice domains that influence the splicing process of *SMN2* like an intronic splicing silencer (ISS) in intron 7, ISS-N1 [16]. Current therapeutic

approaches address these regulatory elements (→ Therapeutic approaches).

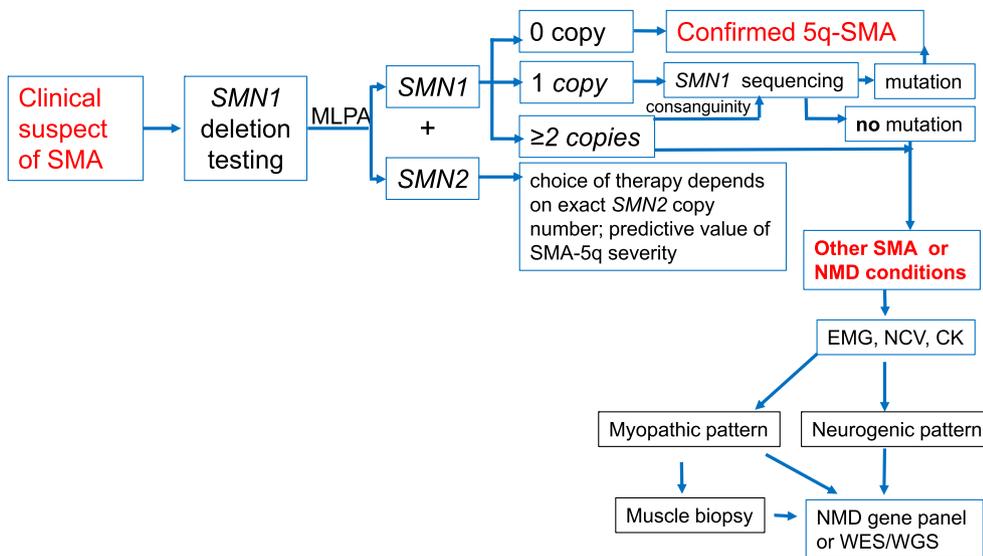
## Protein function

The SMN protein is highly conserved and ubiquitously abundant. Its canonical function is in small nuclear ribonucleoprotein (snRNP) biogenesis and splicing – a crucial process for most genes in all cells. A full-body *SMN* knockout causes very early embryonic lethality in all species. Beside the main canonical function, SMN is implicated in a plethora of additional cellular functions such as translation, microRNA biogenesis, apoptosis, ubiquitin homeostasis, mitochondrial dysfunction, cytoskeleton dynamics and endocytosis, neuromuscular junction development, maturation and maintenance [1, 17].

Low full-length SMN protein levels mainly affect the U12 minor spliceosome and aberrantly spliced transcripts accumulate. Mis-spliced transcripts also affect calcium homeostasis and voltage-gated calcium clustering, processes that are impaired in SMA patients [18–20].

## Main pathological hallmarks in SMA and implications for therapy

Alpha-motor neurons in the spinal cord and especially the pre-synapse of the neuromuscular junctions (NMJs) are the mainly affected cell types in SMA. Impaired endocytosis, F-actin dynamics and calcium homeostasis are the main underlying cellular mechanisms [20–24]. The lack of development and maturation of NMJs in severe and intermediate SMA types and of maintenance in the mild types are



**Figure 3:** Diagnostic algorithm for diagnostics in spinal muscular atrophy. EMG: electromyography; NCV: nerve conduction velocity; CK: creatine kinase; NMD: neuromuscular disorder; WES: whole exome sequencing; WGS: whole genome sequencing.

hallmarks of SMA. This causes muscle weakness and atrophy of proximal voluntary muscles with an earlier involvement of lower than upper limbs. Respiratory insufficiency in type I SMA is the major cause of death. Additional organs such as heart, lung, intestine, spleen, pancreas, liver and bone are involved especially in the severe type, making SMA type I a multiorgan disorder. This spectrum of pathology implies two important considerations for therapy: therapy in neonates carrying homozygous *SMN1* deletions has to start as early as possible to provide sufficient SMN during NMJ development and maturation and in individuals with type I a systemic application is likely required.

## Molecular genetic diagnostics

In case 5q-SMA is suspected in a patient, molecular genetic diagnostics should be initiated immediately. A flow chart of genetic testing in individuals suspected with SMA is shown in Figure 3. The laboratory carrying out the diagnostics should be aware that due to the therapeutic relevance, the duration of this diagnostics should ideally not exceed five working days.

MLPA is the golden standard for the determination of the *SMN1* and *SMN2* copy number and carried out by nearly all diagnostic labs as shown in the annual European Molecular Quality Network (EMQN) schemes. We suggest using the MLPA kit MRC-P021-B1, which provides a maximum of information. It is a straightforward, robust and

cost-efficient method to determine the number of *SMN1* and *SMN2* copies within a few days. MLPA kit MRC-P021-B1 contains specific probes for sequences present in *SMN1* exon 7 and exon 8 or *SMN2* exon 7 and exon 8 as well as probes detecting sequences that are present in the coding exons of both *SMN1* and *SMN2*. *SMN1* is distinguished from *SMN2* by the exon 7 and 8 specific probes that have their ligation sites at the single nucleotide differences in the respective exons (see Figure 2). Exact knowledge of the *SMN2* copy number for each patient is relevant for SMA treatment and the monitoring of its success. Next generation sequencing (NGS) based approaches on the other hand are currently not suitable for the determination of *SMN* copy numbers and diagnostics of 5q-SMA.

A PCR based approach for the detection of a homozygous deletion of exons 7 and 8 of *SMN1* may lead to a quick diagnosis in certain situations (e. g. prenatal diagnosis) but it does not replace the exact quantification of *SMN1* and *SMN2* copies. Furthermore, it has to be kept in mind that in this test the presence of *SMN1* exons 7 and 8 does not exclude 5q-SMA.

## Interpretation of patients' results

For the interpretation of the MLPA results and the exact quantification of *SMN1* and *SMN2* copy numbers, exact knowledge of the genotype of the control DNA is necessary. Any wrong assessment of control DNAs will lead to

a wrong interpretation of results and thereby harm patients and/or relatives. To achieve best results, control DNAs should originate from the same tissue as the probes to test and be isolated by the same methods. Regular participation in quality control schemes (EMQN, BVDH), as required by RilibÄK, probe exchanges with cooperating laboratories and/or the use of MRC Holland's standardized control DNAs should minimize interpretation errors due to unsuitable control DNAs.

(1) If a homozygous deletion of *SMN1* exons 7 and 8 is detected, the diagnosis of 5q-SMA is confirmed. Approximately 96% of patients carry a homozygous deletion of *SMN1*.

A homozygous deletion of *SMN1* exon 7 only also confirms 5q-SMA. This usually indicates the presence of a so-called hybrid gene, i. e. a fused *SMN* gene carrying exons 1–7 of *SMN2* origin and exon 8 of *SMN1* origin [9, 10].

In very rare cases SNVs within MLPA probes have been reported that lead to disturbed annealing and misinterpretation of correct *SMN1* and/or *SMN2* copies. Single exon deletions that are not associated with a hybrid gene should therefore be checked by sequencing.

(2) In case a patient shows two or more *SMN1* copies by MLPA, the diagnosis of 5q-SMA is almost excluded and differential diagnoses should be considered. In consanguineous families, homozygosity for a pathogenic SNV might be considered [25, 26] and Sanger sequencing of *SMN1* is indicated. Compound heterozygosity for two pathogenic sequence variants is extremely unlikely due to the rareness of point mutations in *SMN1*.

For analysis of differential diagnoses, strategies like targeted panel diagnostics or whole exome sequencing should be considered in a close collaboration between clinicians and human geneticists. Finding a diagnosis is relevant for the patients and caregivers, for family planning and reproductive risk calculations as well as for clinicians to provide optimal therapeutic options. Therefore, all undiagnosed patients with SMA-like phenotypes should be analysed for differential diagnoses.

(3) If a patient suspected of having 5q-SMA shows only one *SMN1* copy, a pathogenic SNV might be present on the second allele. In this case, sequencing of the coding sequence and regulatory regions of the *SMN1* gene is necessary to detect the second mutation (→ Sequencing of *SMN1*). There might as well be rare unusual deletions not affecting exons 7 and 8; therefore all irregularities concerning *SMN1* exons 1–6 in MLPA should be analysed further (segregation analyses, RT-PCR, long-range sequencing techniques).

However, with a heterozygosity frequency of approximately 1:41 in Europe a heterozygous deletion of *SMN1*

might be found by chance and it might not be related to the phenotype of the patient. In this case, at least one of the parents is an SMA carrier of a heterozygous *SMN1* deletion. Genetic counselling to explain the consequences of this result should be offered to the family.

(4) A homozygous deletion of *SMN2* has no impact on the individual, and instead excludes the diagnosis of SMA (→ Protein function). Approximately 5% of the general population carry a homozygous deletion of *SMN2*.

The combined MLPA probes for *SMN1* and *SMN2* also identify the frequent variant *SMN2*-ΔE7/8, which has no clinical relevance.

## Sequencing of *SMN1* for subtle mutations

If a patient shows heterozygosity for the common *SMN1* deletion, sequencing of the coding region and regulatory elements of *SMN1* is indicated. Due to the high degree of homology between *SMN1* and *SMN2*, both genes will be sequenced simultaneously using standard Sanger sequencing from genomic DNA, making an unequivocal assignment impossible. Various, more laborious techniques have been developed such as long-range PCR of genomic *SMN1* followed by nested PCR and Sanger sequencing of each exon [27], sequencing of *SMN1* cDNA PCR products [28] or long-read sequencing such as Nanopore whole genome sequencing. In addition, segregation analyses and exact knowledge about *SMN1* and *SMN2* copy numbers are helpful. So far, more than 100 different pathogenic sequence variants, some of them recurrent, have been described in *SMN1*. Compound heterozygosity has been found in 3.4% of 5q-SMA patients [28].

## Relevance of exact determination of *SMN2* copies

The exact quantification of the *SMN2* copy number in patients with a homozygous *SMN1* deletion is relevant for inclusion in therapy programmes, choice of therapy and the monitoring of their outcome. Therefore, the exact *SMN2* copy number quantification is crucial for patients and their clinical management.

While the correlation between the *SMN2* copy number and disease severity is very strong, it is not an absolute correlation (Figure 1). Some SNVs within *SMN2* may facilitate and others suppress correct splicing of exon 7 [1]. The most abundant SNV correcting splicing is c.859G>C, (p.Gly287Arg) (NM\_000344.4) and occurs in about 1% of the *SMN2* copies. In addition, other independent modifiers

influence disease severity (→ Independent SMN genetic modifiers).

Although not suitable for an absolute correlation, the *SMN2* copies still have a strong predictive value [29]. Moreover, it is highly relevant for judging therapies in pre-symptomatic neonates who carry homozygous *SMN1* deletions. While all patients showing clinical symptoms are eligible for one of the currently approved therapies (Spinraza, Zolgensma) or therapies under companionate use (Evrysdi), in case of neonates with four or more *SMN2* copies a different treatment is applied (→ Therapy of SMA).

## Independent SMN genetic modifiers

About 0.5 % of individuals with homozygous *SMN1* deletions and three to four *SMN2* copies remain asymptomatic, because they are protected by other SMA protective modifiers such as plastin 3 (PLS3) or neurocalcin delta (NCALD) and others not yet known ([24, 30] and Wirth et al., unpublished data). Upregulation of PLS3, an F-actin binding and bundling protein, has been shown to protect against SMA not only in human, but in various genetically engineered or AAV9-PLS3-treated SMA animal models. Similarly, downregulation of NCALD, a neuronal calcium sensor protein, is protective against SMA in human and other species. Reduction of NCALD levels in genetically engineered SMA animal models or treatment with *Ncald* antisense oligonucleotides ameliorated the SMA phenotype (reviewed in [1]). However, for both modifiers the exact genetic mechanism of up- or downregulation is not understood and therefore cannot be included into direct diagnostic testing to identify these individuals.

## Genetic analyses in relatives

Prior to genetic analysis of relatives, genetic counselling should be offered to the families.

### Parents

The method of choice depends on the mutation detected in the index patient; in the vast majority of cases it will be MLPA.

In case biallelic SMA causing mutations are detected in a patient, it is most likely that both parents are carriers for the disease. However, molecular testing to confirm carrier status should always be performed to provide the basis

for genetic counselling in the family. In case both parents are carriers for 5q-SMA, there is a 25 % recurrence risk for children with a similar form of SMA in future pregnancies.

In most instances, testing will be straightforward and confirm carrier status. However, in about 4–5 % cases two *SMN1* copies can be identified but still being false negative due to the following scenarios:

- (1) Carriers may harbour a deletion of *SMN1* on one chromosome 5 and have two *SMN1* copies on their second chromosome 5. In Germany in 2.4 % of carriers this genotype (*SMN1*[2+0]) was found [29]. Quantitative methods cannot distinguish between a genotype *SMN1*[2+0], i. e. a hidden deletion, and *SMN1*[1+1], i. e. one *SMN1* gene copy on each chromosome 5. This situation may only be solved by segregation analyses in the family; one of the grandparents or siblings of the respective parent may show three *SMN1* copies. For genetic counselling of the family, the knowledge about a hidden deletion is essential.
- (2) In approximately 1.7 % of parents pathogenic sequence variants in *SMN1* can be found that will not be detected by MLPA but only by other techniques (→ Sequencing of *SMN1* for subtle mutations). However, the index patient should carry the same mutation and thus already hint towards this situation.
- (3) One of the parents is indeed a non-carrier and their affected child has a *de novo* mutation in the *SMN1* gene. The *de novo* mutation rate in SMA patients is approximately 2 %, meaning that 1 % of parents are non-carriers. *De novo* mutations may occur both in the paternal and in the maternal germline [11].

## Siblings and other relatives

Molecular genetic testing of siblings for 5q-SMA requires knowledge of the genotypes in the patient and parents. Without this information, interpretation will always be limited if 2 *SMN1* copies are detected and carrier status cannot be fully excluded.

If both parents carry only one *SMN1* copy, a sibling with two *SMN1* copies is most likely a non-carrier and a sibling with only one copy a carrier. If one of the parents of a patient with a homozygous *SMN1* deletion carries two *SMN1* gene copies, a sibling with two *SMN1* copies might well be a carrier and molecular testing of the spouse is indicated to estimate the probability for children with 5q-SMA. If the patient harbours a pathogenic sequence variant in *SMN1*, sequencing of the gene is required in siblings with two *SMN1* copies.

To avoid unnecessary testing, further relatives should be tested in a cascade screening if possible, and again, information on the genotype of the patient and the parents facilitates the interpretation of results.

## Spouses and general population

For reproductive issues, a 5q-SMA carrier might want to know the carrier status of the spouse. In case there is no SMA family history, a person with a European background has an *a priori* risk of approx. 1 in 40 of being a 5q-SMA carrier. With a heterozygous partner, this couple would have an *a priori* risk of 1/160 ( $1 \times 1/40 \times 1/4$ ) of getting a child with 5q-SMA. This risk may be reduced considerably by exclusion of heterozygosity for the common deletion of *SMN1* in the spouse.

In individuals with two *SMN1* copies and without a family history of 5q-SMA, there remains the risk of being a carrier of an undetected deletion with a genotype *SMN1*[2+0] or a subtle *SMN1* mutation which cannot be resolved any further. The probability for an individual with two *SMN1* copies of still being a 5q-SMA carrier is therefore 1/1,000 ( $1/40 \times 4/100$ ), thus resulting in a risk for an SMA offspring of 1 in 4,000 in a partnership with a heterozygous individual. The more *SMN1* gene copies there are, the less likely is a hidden deletion, decreasing the risk figure.

In the setting of genetic counselling, sequencing of *SMN1* may be discussed for spouses with two *SMN1* copies. However, it has to be pointed out that exclusion of pathogenic sequence variants in *SMN1* only marginally reduces the risk of being a carrier.

In the near future, there might be a greater demand for testing members of the general population with respect to pre-conception counselling. This should be discussed carefully within the setting of genetic counselling with particular respect to the limitations of testing as explained above.

## Therapy of spinal muscular atrophy

Fortunate enough, there are several approved or nearly approved highly efficient therapeutic options for SMA. One therapeutic strategy is based on correcting endogenous *SMN2* splicing and thus increasing the functional SMN protein level; the other option is gene replacement therapy (reviewed in [1]).

For the splice-correction strategy two compounds are available:

**Nusinersen (Spinraza™)** (Biogen/Ionis Pharmaceuticals) is an antisense oligonucleotide that blocks a splice silencer ISS-N1 within intron 7 of *SMN2* pre-mRNA, facilitating exon 7 inclusion into the *SMN2* mRNA. Hence more full-length *SMN1* transcripts and SMN proteins are produced [31]. Nusinersen has been approved by the US Food and Drug Administration (FDA) and the European Medicine Agency (EMA) and is reimbursed by the health insurance system in Germany for all paediatric and adult SMA patients since 2017. It is injected intrathecally three times within 2 months (loading doses), and then every 4 months, for a lifetime. Various studies including all three types of SMA patients reported safety, tolerability and clinical efficacy (reviewed in [1]). All patients seem to benefit to a certain extent from this therapy [32]. However, pre-symptomatically treated neonates with three *SMN2* copies achieve the most spectacular improvement with motor milestones similar to controls while those with two *SMN2* copies fall slightly behind (e. g. 77 % achieve independent walking). In general, neonates treated before 6 weeks of age develop better than those treated after 6 weeks of age [33, 34]. These data clearly show that all steps from identification of an infant with homozygous *SMN1* deletion by newborn screening until start of the therapy should take no longer than 3 to 6 weeks. However, it has to be kept in mind that the patients' long-term outcome still has to be awaited.

**Risdiplam (Evrysdi)** (Roche) is a small molecule identified by high-throughput screening that facilitates *SMN2* exon 7 retention within the mRNA and thus increases SMN protein levels. It is not as specific as Nusinersen, but seems to act on only very few additional transcripts [35]. Risdiplam is administered orally every day and has a good biodistribution able to cross the blood–brain barrier. Indeed, the various clinical studies reported good tolerability and clinical efficacy [1]. Moreover, in one clinical study (NCT 03032172) patients previously treated with Nusinersen or Zolgensma were recruited to evaluate the potential of follow-up therapy with Risdiplam. In August 2020 Risdiplam has been FDA-approved for all SMA patients older than 2 months and is under companionate use in Europe.

**Onasemnogene abeparvovec-xioi (Zolgensma™; Avexis/Novartis)** is a self-complementary adeno-associated virus 9 expressing the *SMN1*-cDNA under a cytomegalovirus enhancer/chicken  $\beta$ -actin hybrid promoter. The gene replacement therapy is based on a single intravenous injection of  $1.1 \times 10^{14}$  viral genomes /kg body weight. To achieve this viral load, children have to weigh less than 13.5 kg. Only SMA patients or pre-symptomatic infants who have no or very low levels of antibodies with

AAV9 titres of  $\leq 1:50$  are qualified for Zolgensma therapy [36]. With increasing age, the risk of developing antibodies against AAV9 is dramatically increased; therefore an early injection is advisable. As for Nusinersen, an early pre-symptomatic administration dramatically improves motor milestones of treated individuals (SPR1NT, NCT 03505099; MDA-media release). Zolgensma has been approved by the FDA for infants with biallelic *SMN1* mutations under 2 years of age. Recently, EMA gave a marketing authorisation of Zolgensma for children with biallelic *SMN1* mutations with two or three *SMN2* copies and a weight of less than 21 kg.

### Newborn screening (NBS)

Since the introduction of the therapies, it has become evident that an early pre-symptomatic treatment of patients is crucial for the therapeutic outcome [33, 37]. In January 2018, a pilot project for a newborn screening for patients with a homozygous *SMN1* deletion started, initiated and recently published by the cystinosis foundation [38]. After two years, a total of 279,163 infants were screened: 200,901 between 01/2018 and 05/2019 in the pilot project, and 78,262 in a following project between 06/2019 and 01/2020. 43 positive cases with a homozygous *SMN1* deletion were identified (K. Vill et al., under publication), leading to an incidence of approximately 1:7,000.

We expect that the NBS for 5q-SMA will be implemented in Germany by the end of 2020 or at the latest early 2021. Neonates detected with homozygous *SMN1* deletion by NBS must be confirmed by a second probe and method; the exact number of *SMN2* copies has to be determined and parents must be offered a genetic counselling according to German law (GenDG). In the light of different treatment strategies for patients with different *SMN2* copy numbers, we strongly recommend testing the *SMN2* copy number by two independent expert laboratories. Paediatricians will have to be aware of the fact that compound heterozygosity for the deletion and a subtle *SMN1* mutation in 5q-SMA patients escapes detection in NBS due to methodological reasons. NBS will therefore leave some SMA patients undetected.

### Prenatal diagnosis (PD) and preimplantation genetic testing for monogenic disease (PGT-M)

Although the current therapeutic approaches are highly promising, SMA cannot be regarded as a fully treatable

condition yet; too little is known about the long-term effects of the different therapies. Therapies are challenging for the patients, their families and clinicians, and the families' compliance is not always satisfying. Therefore, the possibilities of prenatal diagnosis and preimplantation genetic diagnostics should be discussed with couples at risk and appropriate counselling should be offered. Prenatal diagnostics for SMA has been implemented for years and is offered after detailed genetic counselling of the families by several human genetic laboratories. Preimplantation genetic testing for monogenic disease (PGT-M) is only possible at specialised centres that are approved by the government authorities ('Zentren für Präimplantationsdiagnostik'), and is subject to a case-by-case application procedure and approval by an ethics committee. Application and technical procedures are complex and should be thoroughly discussed and prepared by the genetic counsellor. PGT-M always involves *in vitro* fertilization procedures.

PGT-M of SMA is technically challenging because of the high homology of *SMN1* and *SMN2*. Various multiplex PCR protocols with linked polymorphic markers (short tandem repeats) in combination with the direct detection of nucleotide differences between *SMN1* and *SMN2* were developed. PGT-PCR protocols are susceptible to contamination and allelic drop-out. Informative polymorphic markers closely flanking the *SMN1* and *SMN2* duplicated region must be identified for each individual family. Recently karyomapping was introduced for PGT-M. This method uses multiple displacement amplification, a method of whole genome amplification in an isothermal reaction, followed by genotyping of a large number of SNVs. Genome-wide karyomapping is extremely accurate and facilitates PGT-M for SMA and it allows the simultaneous detection of chromosomal aneuploidies in one test.

### Conclusion

SMA is one of the most common autosomal recessive inherited disorders worldwide. It can be easily genetically tested since 96 % of patients show homozygous deletion of *SMN1* exon 7 and/or exon 8. The *SMN2* copy gene is the most relevant modifier of disease severity, while few other independent protective modifiers can influence the phenotype. However, the latter are found in very rare cases and should not compromise rational decision for the remaining 99.5 % of all 5q-SMA patients, who will strongly benefit from an early therapy. Therapies acting directly on the endogenous *SMN2* gene or gene replacement therapies are approved or will be soon approved. All three therapies

can lead to dramatic motor improvement, especially when applied pre-symptomatically. Pre-symptomatically treated neonates with at least three *SMN2* copies seem to develop normal, while the majority of pre-symptomatically treated neonates with two *SMN2* copies do in fact show most of the motoric milestones albeit not to the level of a control individual and delayed. Therefore, combinatorial therapies seem to be required for these patients. To achieve this ultimate goal of pre-symptomatic therapy newborn screening needs to be implemented as fast as possible. Identification of the exact *SMN1* and *SMN2* copy number in newborns is crucial to judge further motor milestones in the treated individual. Moreover, close follow-up of treated patients is recommended since new, previously unrecognized phenotypes may arise with ageing, especially in individuals with only two *SMN2* copies.

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