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How genetic modifiers influence the phenotype of spinal muscular atrophy and suggest future therapeutic approaches

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Both complex disorders and monogenetic diseases are often modulated in their phenotype by further genetic, epigenetic or extrinsic factors. This gives rise to extensive phenotypic variability and potentially protection from disease manifestations, known as incomplete penetrance. Approaches including whole transcriptome, exome, genome, methylome or proteome analyses of highly discordant phenotypes in a few individuals harboring mutations at the same locus can help to identify these modifiers. This review describes the complexity of modifying factors of one of the most frequent autosomal recessively inherited disorders in humans, spinal muscular atrophy (SMA). We will outline how this knowledge contributes to understanding of the regulatory networks and molecular pathology of SMA and how this knowledge will influence future approaches to therapies.

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Introduction

Identification of modifiers is challenging and, therefore, relatively few genetic modifiers have been detected so far (reviewed in [1–6]; only some diseases have been selected due to a restricted number of references). Modifiers can act in multiple ways on the expression or stability of RNA or proteins: The predominant disease-determining gene (in monogenic or oligogenic disorders) can be modulated by *cis*- and *trans*-acting factors, by epigenetic factors, by proteins belonging to the same pathway or network, by proteins involved in stand-alone pathways but converging on a common final pathway or ending in the same biological function or, finally, by extrinsic non-genetic or environmental factors. In concert with the disease determinant, modifiers give rise to a large phenotypic variability, sometimes conferring full protection to an individual carrying a disease-causing mutation, a phenomenon defined as incomplete penetrance. One of the most impressive 'monogenic' diseases shown to be modulated by a large variety of factors is spinal muscular atrophy (SMA), selected herein to exemplify the role of modifiers and the use of this knowledge gained in developing target-driven therapies.

SMA is a common genetic neuromuscular disorder most often leading to childhood lethality. However, this devastating disease has some remarkable and almost unique features. The identification of the modifying factors influencing the SMA phenotype enlarged our understanding of the pathology, molecular and biochemical mechanisms underlying SMA and, most importantly, allowed the development of therapies.

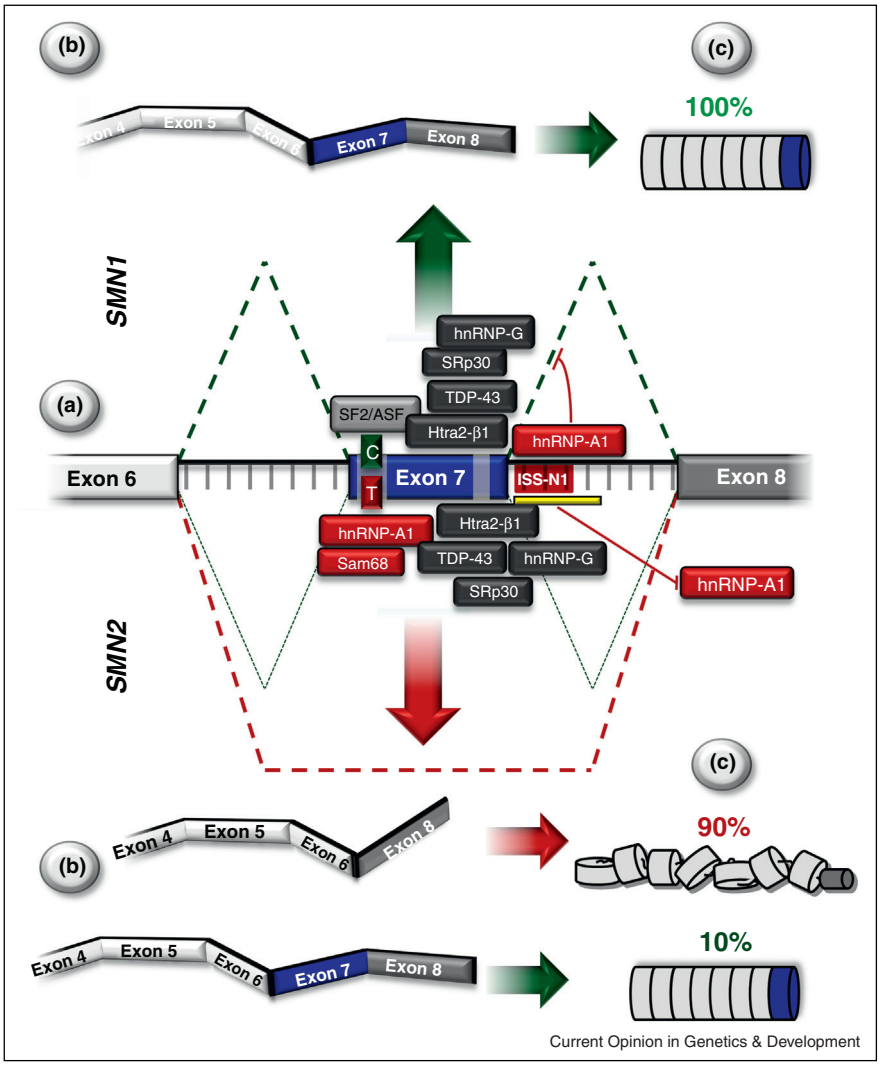
What makes SMA so exceptional and different from other genetic conditions and what do we have to consider for future therapies in order to be successful?

Remarkable features of SMA

First, patients with SMA show a homozygous functional loss of the *survival motor neuron 1* gene (*SMN1*), but all patients carry one or more *SMN2* copies that modulate the severity of the disease [7,8••,9] and can be targeted by small molecules and drugs (reviewed in [10]). Second, about 94% of all SMA patients carry the same type of mutation, which allows simple molecular genetic testing [11]. Third, SMA carriers with frequencies varying between 1:8 and 1:105 among various populations can be easily and reliably identified by use of quantitative PCR or MLPA [7,12,13]. In the European population the frequency of SMA carriers is 1:35 which makes SMA the second most frequent autosomal recessively inherited condition. Fourth, a duplication of about 500 kb on chromosome 5q13.2 including *SMN* occurred late in evolution, in primates, while the development of two different *SMN* genes is human-specific [14]. The location of the *SMN* copies within a CNV makes this region prone to *de novo* deletions, duplications and gene conversions which are found in 2% of SMA patients [15]. The transgenic insertion of the human-specific *SMN2* onto a null Smn background of other species allowed the generation of humanized SMA mouse and pig models, which have been extremely useful in deciphering the pathology of SMA and for development of SMA therapies [16,17••]. Fifth, the main functional difference between *SMN1* and *SMN2* is a single, translationally silent nucleotide exchange in exon 7. It affects an exonic splicing enhancer and thereby impairs correct splicing of *SMN2* so that only very low amounts of full-length (FL) transcripts are produced while the majority of transcripts lack exon 7 [8••,18••]. Sixth, importantly, both FL-*SMN1* and FL-*SMN2* transcripts

encode an identical SMN protein — a finding that is essential for therapeutic strategies aiming to increase *SMN* expression levels [8••]. Seventh, discordant families with large phenotypic discrepancies pointed towards factors/pathways able to circumvent the detrimental impact of reduced SMN levels on motor neurons. Modifiers such as Plastin 3 (PLS3) have shown to fully protect against SMA in humans carrying a homozygous deletion of *SMN1* [19••]. This knowledge can be further used for the development of SMN-independent therapeutic strategies [20]. Eighth, exogenous factors such as lack of nutrition or hypoxia reduce FL-*SMN2* [21••,22•]. Additional support of SMA patients or SMA mice with oxygen and appropriate nutritional support can partially counteract this negative impact [23,24]. Ninth, despite ubiquitous SMN expression, reduced SMN levels mainly affect motor neurons in the ventral horns of the spinal cord. However, also other neuronal circuits including sensory and interneurons seem

Figure 1



Cis and *trans* regulation of *SMN1* and *SMN2* splicing and its impact on translation. Schematic overview of splicing processes of the *SMN* exon 7 and its consequences on RNA level and protein level. **(a)** Magnification of the exons 6–8 of the *SMN1* and *SMN2* pre-mRNA. The C to T transition at the beginning of exon 7 is highlighted. Both exonic splicing enhancers within exon 7 are shown in gray. Splice factors promoting exon 7 inclusion are depicted in dark gray, while factors favouring exon 7 skipping are marked in red. SF2/ASF, which is displaced by hnRNP-A1 and Sam68 in the *SMN2* splice process, is marked in light gray. Dashed lines indicate the joining of exons during the splicing processes, which is adumbrated by the arrows. Shown in red is the intronic splicing silencer ISS-N1, which is bound by hnRNP-A1. A therapeutic antisense oligo preventing hnRNP-A1 binding is highlighted in yellow. **(b)** Mature mRNA products of both *SMN1* and *SMN2*. While splicing of *SMN1* pre-mRNA produces 100% of full-length transcripts comprising exons 1 to 8 (FL-*SMN1*), *SMN2* produces only about 10% FL-*SMN2*. The remaining 90% of *SMN2* transcripts lack exon 7 (*SMN2Δ7*). Arrows indicate the translation process. **(c)** Final protein products of both *SMN* genes. In contrast to *SMN1*, *SMN2* predominantly produces an unstable truncated protein, which is rapidly degraded.