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COMMENTARY

PLS3 expression and SMA phenotype: a commentary on correlation of *PLS3* expression with disease severity in children with spinal muscular atrophy

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MODIFIERS OF SMA PHENOTYPE

Spinal muscular atrophy (SMA; OMIM 253300) is an autosomal recessive neuromuscular disorder characterized by the loss of motor neurons.[1] It is clinically heterogeneous and can be classified into three subtypes depending on the age of onset and achievement of motor milestones: SMA type 1 (a severe type with onset before the age of 6 months, patients are unable to sit without support), SMA type 2 (an intermediate type with onset before the age of 18 months, patients are unable to stand or walk without support) and SMA type 3 (a mild type with onset after the age of 18 months, patients are able to stand and walk independently until the disease progresses).[1]

Causative mutations of SMA are mainly homozygous deletions of the *SMN1* gene located on chromosome 5q13. The gene product, the SMN protein, has critical roles in a variety of cellular activities.[1] The *SMN2* gene, an almost identical homolog of *SMN1*, is retained in all SMA patients and produces low levels of SMN protein, but does not fully compensate for mutated *SMN1*. *SMN2* is now considered to be a modifier of the SMA phenotype, as high copy numbers of *SMN2* ameliorate the clinical severity in SMA patients.[1] However, some asymptomatic individuals inherit the same *SMN* genotype (homozygous *SMN1* deletion and identical *SMN2* copy numbers) as their affected siblings. The presence of such SMA-discordant families suggests the influence of modifier genes other than *SMN2*.[2]

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PLS3 EXPRESSION IN FEMALE SMA PATIENTS

Oprea *et al.*[3] identified six SMA-discordant families with eight fully asymptomatic females who shared the same *SMN* genotype as their affected siblings. The authors found that *PLS3*, a gene encoding the actin-bundling protein plastin 3 (PLS3, T-plastin or T-fimbrin; MIM300131, Xq23), was highly expressed in lymphoblastoid cell lines from unaffected female siblings. They also obtained experimental evidence that the overexpression of *PLS3* rescues the axonal growth defect associated with low-SMN levels in motor neurons of SMA-mouse embryos and zebrafish. They concluded that PLS3 (or *PLS3*) may be a gender-specific SMA modifier.

To examine whether *PLS3* is a gender-specific modifier, Stratigopoulos *et al.*[4] analyzed 88 SMA patients (41 males and 47 females), and found that in postpubertal female patients, the amount of the *PLS3* transcript was highest in type 3 patients, followed by type 2 patients and lowest in type 1 patients. In these SMA patients, *PLS3* expression was related to *SMN2* copy number, gross motor function measure and clinical subtype. Interestingly, *PLS3* expression in either pre- or postpubertal male patients or prepubertal female patients did not correlate with clinical subtype or *SMN2* copy number. The authors concluded that *PLS3* is an age- and/or puberty-specific and sex-specific modifier of the SMA phenotype.

In previous issue of the journal, Cao *et al.*[5] also suggested that *PLS3* is a gender-specific modifier for SMA phenotype, based on their analysis of 65 SMA patients (36 males and 29 females) and 59 healthy controls (31 males and 28 females). According to this study, among the older female patients (>3 years of age), *PLS3* expression was significantly higher in type 3 than type 2, which is consistent with the results of Stratigopoulos *et al.*,[4] although the age cut-off levels differed between the two studies. Cao *et al.*[5] demonstrated two important findings in this article. The first was that *PLS3* expression is age-dependent and decreases in type 1–2 SMA patients and healthy controls above 3 years of age, suggesting that the patient's age should always be considered when evaluating *PLS3* expression. The second was that *PLS3* expression of type 3 patients who can walk is higher than in patients unable to do so, suggesting that it could be used as a biomarker of disease progression. However, as stated in the report, this will be necessary to verify in a larger sample.

PERSPECTIVE ON SMA TREATMENT

The relationships between SMN protein levels (or *SMN2* copy number) and PLS3 protein levels (or *PLS3* expression) in motor neurons are still poorly understood and studies have shown conflicting results. For example, PLS3 was suggested to be expressed independently of SMN (or *SMN2* copy number) in the report by Oprea *et al.*,[3] which described unaffected siblings of the patients, and that by Ackermann *et al.*[6] using SMA mice. By contrast, the study of Hao *et al.*[7] on zebrafish showed that Pls3 levels were dependent on Smn levels.

Hao *et al.*[7] demonstrated that human *PLS3* overexpression was able to rescue neuromuscular junction defects in SMA mutants

of transgenic zebrafish, suggesting that decreased PLS3 contributes to SMA motor phenotypes. Indeed, when Smn protein levels were severely decreased in SMA mutants, they found that Pls3 translation was compromised, leading to SV2 presynaptic defects at the neuromuscular junction. In addition, as human SMN expression increased the Pls3 level in SMA mutants, this led them to conclude that Pls3 levels are dependent on Smn levels.

Ackermann *et al.*[6] generated a conditional PLS3-overexpressing mouse bred into an SMA background to show that PLS3 overexpression restored defects in motor neurons and neuromuscular junctions. In this study, they also observed that Pls3 was not decreased in the brain and spinal cord of SMA mice compared with controls. This finding suggests that Pls3 levels are not regulated by Smn levels. Thus, it was postulated that Pls3 has an Smn-independent ameliorative action on the phenotype of SMA mice.

As PLS3 expression restores the motor neuron and rescues neuromuscular junction defects, it could be a potential therapeutic target for SMA treatment.[3,6] This has already been hypothesized by Bowerman *et al.*[8] who have suggested possible treatment strategies including the upregulation of PLS3 to target actin cytoskeletal dynamics.

CONCLUSIONS

Cao *et al.*[5] confirmed that *PLS3* expression modifies the phenotype of female SMA patients and suggested that its expression in peripheral blood cells will be a useful biomarker of disease progression in female patients with SMA. Investigation of the modifiers including PLS3 expression, *SMN2* copy numbers and additional unknown factors will help our understanding of the mechanism underlying SMA pathology (Figure 1), and lead to the establishment of new treatments for SMA.

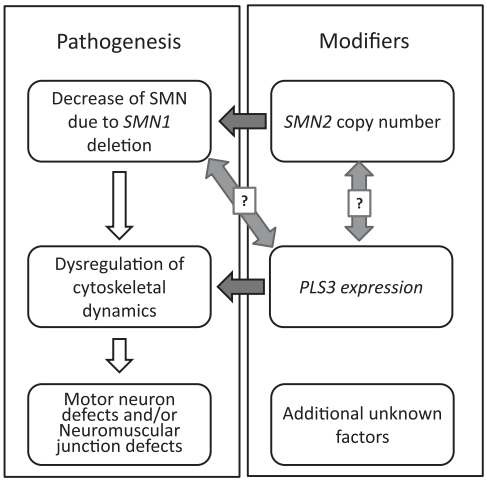

Figure 1 Modifiers of phenotype in female SMA patients. *SMN2* copy number and *PLS3* expression are recognized as phenotype modifiers in female SMA patients. Both the SMN and PLS3 proteins are involved in the regulation of cytoskeleton dynamics. However, the relationship between SMN protein levels (or *SMN2* copy number) and PLS3 protein levels (or *PLS3* expression) remain to be elucidated. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

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