Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology)
ISSN 1673-1581 (Print); ISSN 1862-1783 (Online)
www.zju.edu.cn/jzus; www.springerlink.com
E-mail: jzus@zju.edu.cn

Association between *SMN2* methylation and disease severity in Chinese children with spinal muscular atrophy*#

Yan-yan CAO§†, Yu-jin QU§, Sheng-xi HE, Yan LI, Jin-li BAI, Yu-wei JIN, Hong WANG, Fang SONG†‡

(*Department of Medical Genetics, Capital Institute of Pediatrics, Beijing 100020, China*)

†E-mail: yanyancao2@163.com; songf_558@263.net

Received Mar. 24, 2015; Revision accepted Nov. 7, 2015; Crosschecked Dec. 16, 2015

Abstract: The homozygous loss of the survival motor neuron 1 (*SMN1*) gene is the primary cause of spinal muscular atrophy (SMA), a neuromuscular degenerative disease. A genetically similar gene, *SMN2*, which is not functionally equivalent in all SMA patients, modifies the clinical SMA phenotypes. We analyzed the methylation levels of 4 CpG islands (CGIs) in *SMN2* in 35 Chinese children with SMA by MassARRAY. We found that three CpG units located in CGI 1 (nucleotides (nt) −871, −735) and CGI 4 (nt +999) are significantly hypomethylated in SMA type III compared with type I or II children after receiving Bonferroni correction. In addition to the differentially methylated CpG unit of nt −871, the methylation level of the nt −290/−288/−285 unit was negatively correlated with the expression of *SMN2* full-length transcripts (*SMN2*-fl). In addition, the methylation level at nt +938 was inversely proportional to the ratio of *SMN2*-fl and lacking exon 7 transcripts (*SMN2*-Δ7, fl/Δ7), and was not associated with the *SMN2* transcript levels. Thus, we can conclude that *SMN2* methylation may regulate the SMA disease phenotype by modulating its transcription.

Key words: CpG island, Methylation, Survival motor neuron 2 (*SMN2*), Spinal muscular atrophy
http://dx.doi.org/10.1631/jzus.B1500072 **CLC number:** R746.4

1 Introduction

Degeneration of motor neurons in the anterior horn of the spinal cord causes progressive muscle weakness and atrophy in the autosomal recessive disorder, proximal spinal muscular atrophy (SMA). The disease forms are categorized by order of increasing age and decreasing clinical severity as follows: childhood onset of SMA type I (MIM #253300), SMA type II (MIM #253550), SMA type III (MIM #253400), and adult onset of SMA type IV (MIM #271150). SMA is caused by deletions or mutations in the survival motor neuron 1 (*SMN1*) gene on chromosome 5q. The copy gene, *SMN2*, varying in a nucleotide in exon 7, modifies the SMA clinical symptoms. For example, patients with deleted or altered *SMN1* often have increased copies of *SMN2*, which contributes to SMA phenotypes. The *SMN2* copy number inversely correlates with the SMA severity (Prior *et al.*, 2009). However, siblings with the identical *SMN1* genetics and *SMN2* copies show marked clinical discrepancies, indicating that epigenetic modification may control the individual variations in the *SMN2* function (Bernal *et al.*, 2011). In fact, SMA type I and III patients have two differentially methylated CpG sites (CpGs), which colocalize within the first transcriptional start site of *SMN2*, correlating with the disease severity (Hauke *et al.*, 2009). Thus, DNA methylation regulates gene

---

‡ Corresponding author

§ The two authors contributed equally to this work

* Project supported by the National Natural Science Foundation of China (Nos. 81050034 and 81500979), the Research Foundation of the Capital Institute of Pediatrics (No. Fangxiang-2014-01), and the Beijing Talents Fund (No. 2014000021469G228)

Electronic supplementary materials: The online version of this article (http://dx.doi.org/10.1631/jzus.B1500072) contains supplementary materials, which are available to authorized users

ORCID: Fang SONG, http://orcid.org/0000-0002-8844-1331 expression by altering the transcriptional activity, and is responsible for non-equivalent *SMN2* functions. However, the role of *SMN2* DNA methylation in the SMA progression, particularly in young patients, still needs further elucidation.

Therefore, in this study, we analyzed the DNA methylation levels of *SMN2* in 35 children with SMA and evaluated the correlation between the methylation modification and childhood onset of SMA progression. This is a second study being conducted after Hauke *et al.* (2009) completed their study on *SMN2* promoter methylation in connection to SMA severity.

2 Materials and methods

2.1 Subjects

Thirty-five Chinese patients with SMA, determined by homozygous exon 7 deletion in *SMN1*, were enrolled with the Department of Medical Genetics in the Capital Institute of Pediatrics (Beijing, China). Informed consent was obtained from the minors' parents and the Ethical Committee of the Capital Institute of Pediatrics approved the study protocol.

2.2 DNA extraction and *SMN* copy number

Genomic DNA was extracted from peripheral blood samples by proteinase K digestion and phenol/chloroform extraction. The *SMN1* and *SMN2* copy numbers were determined by multiplex ligation-dependent probe amplification (MLPA) (MRC-Holland, Amsterdam, the Netherlands), according to the manufacturer's instructions.

2.3 Bisulfite conversion and *SMN2* DNA methylation analyses

For epigenetic analyses, unmethylated cytosines were bisulfite converted, using EpiTect Bisulfite Kit (Qiagen, Hilden, Germany), following the manufacturer's protocol. Four polymerase chain reaction (PCR) fragments were cloned into T1 vectors, using a pEASY™-T1 Cloning Kit (TransGen Biotech, Beijing, China). Subsequently, ten positive clones were sequenced by ABI 3730 XL (Life Technologies, Carlsbad, USA) to evaluate the methylation status of four CpG islands (CGIs) in *SMN2*.

Quantitative DNA methylation analysis was performed using a MassARRAY platform (Sequenom, San Diego, CA, USA). Four CGIs were amplified by nested PCR using AmpliTag Gold DNA polymerase (Life Technologies, Carlsbad, USA) and previously described primers with some modifications (Hauke *et al.*, 2009). Each reverse primer for the inner PCR contained a T7 promoter sequence for in vitro transcription. The PCR conditions were as follows: 95 °C for 5 min, followed by 40 amplification cycles (95 °C for 15 s, 52−60 °C for 45 s, 72 °C for 60 s) and then a final extension for 5 min at 72 °C. Following dephosphorylation and in vitro transcription, the samples were purified through a Clean Resin (Sequenom, San Diego, CA, USA), according to the manufacturer's instructions. EpiTyper software (Sequenom, San Diego, CA, USA) analyzed and generated methylation data on the CpG-positive fragments. The primers used for bisulfite sequencing and MassARRAY are shown in Tables S1 and S2.

2.4 RNA extraction and *SMN2* expression level

Total RNA was extracted from the peripheral blood using an RNAsimple Total RNA Kit (Tiangen, Beijing, China) and reverse-transcribed by an Moloney murine leukemia virus (M-MLV) reverse transcriptase (Invitrogen, Carlsbad, CA, USA). Then, real-time PCR determined the transcript levels using the following primer pairs: (1) full-length *SMN2* (*SMN2*-fl), 5'-TAC ATG AGT GGC TAT CAT ACT GGC TA-3' and 5'-AAT GTG AGC ACC TTC CTT CTT TT-3'; (2) truncated *SMN2* lacking exon 7 (*SMN2*-Δ7), 5'-CAT GGT ACA TGA GTG GCT ATC ATA C-3' and 5'-AGT GGT GTC ATT TAG TGC TGC TCT A-3'. TaqMan MGB probes, 5'-FAM-TAT GGG TTT TAG ACA AAA-MGB-NFQ-3' and 5'-FAM-CTA TTA TAT GGA AAT GCT GG-MGB-NFQ-3', were used to specifically detect *SMN2*-fl transcripts and *SMN2*-Δ7 transcripts, respectively. Overall, the *SMN2* transcript levels were calculated as the sum of the *SMN2*-fl and *SMN2*-Δ7 transcripts. Endogenous reference primers and previously described TaqMan probes (Tiziano *et al.*, 2010) were used for the internal control, glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*). The construction of a standard curve and thermal cycling conditions were adapted from Qu *et al.* (2012). The quantities of *SMN2*-fl transcript, *SMN2*-Δ7 transcript, and *GAPDH* were verified against the appropriate standard curve. All samples were analyzed in triplicate.

2.5 Statistical analysis

A Kolmogorov-Smirnov test was used to assess the normal distribution for all variables. The methylation levels of *SMN2* CGIs and units in different groups were compared using an analysis of variance (ANOVA) or Brown-Forsythe test when appropriate, and followed by a least significant difference (LSD) or Tamhane's T2 post-hoc comparison test. The correlation between the CpG methylation and *SMN2* transcript levels was determined by Spearman correlation analysis. Statistical analyses were performed using SPSS 19.0 for Windows (SPSS Inc., Chicago, IL, USA). $P<0.05$ was considered to be statistically significant. However, after Bonferroni correction, the statistically significant *P*-value should be adjusted based on the number of comparing units.

3 Results

3.1 Subjects' characteristics

Thirty-five *SMN1*-deleted patients were all carrying three copies of *SMN2*, including 12 type I, 15 type II, and 8 type III samples. The ages of these patients ranged from 3 months to 5.5 years (mean, (1.4±1.3) years) and the male to female ratio was 19:16 (Table 1). Among them, 3 of type I, 13 of type II, and 8 of type III patients were evaluated for the related association between *SMN2* methylation and transcript levels. The ages ranged from 3 months to 5.5 years (mean, (1.8±1.4) years) and the male to female ratio was 12:12.

3.2 *SMN2* CGIs methylation pattern

Putative CGIs within the genomic region, 3000 nucleotides (nt) upstream and downstream of the translational *SMN2* start site, were predicted through a CGI finder algorithm (http://www.EBI.ac.uk/emboss). For the four CGIs, 1–4 extended from nt −896 to −645, nt −469 to −247, nt −151 to +295, and nt +844 to +1146, respectively.

To identify the *SMN2* CGIs methylation in Chinese SMA children, the bisulfite-treated DNA derived from the peripheral blood samples of three *SMN1*-deleted subjects with SMA types I, II, and III (*SMN2* copy number=3) were examined by cloning and sequencing. The results demonstrated that the hypermethylations of CGIs 1 and 4 (mean, (98.67±1.15)% and (89.33±3.06)%, respectively) were independent of the SMA type. CGI 2 had intermediate methylation ((47.33±2.89)%), but CGI 3 was hypomethylated ((0.67±0.29)%) (Fig. 1).

3.3 *SMN2* CGIs methylation correlation to SMA type, age, and gender

We subsequently employed a MassARRAY to examine the degree of methylation in *SMN2* CGIs from the 35 SMA children. The few closely located CpG dinucleotides were measured as one unit. Methylation was measured at 9, 8, 26, and 12 CpG units in CGIs 1, 2, 3, and 4, respectively. Consistent with the results of cloning and sequencing, the MassARRAY revealed the same methylation pattern: CGIs 1 and 4 were hypermethylated and CGI 3 was hypomethylated, while CGI 2 was at the intermediate level of the methylation (Fig. 2). There were no differences in methylation status from CGI 1 to CGI 3 by the SMA type. However, the status of CGI 4 was still different among the SMA types after consideration of the patients' ages. The patients with SMA type II had significantly higher CGI 4 methylation than the SMA type III patients ($P=0.017$; Table 1). Gender did not influence the methylation status in any SMA patients (data not shown, $P>0.05$).

Table 1 Characteristics of SMA children and *SMN2* methylation according to their clinical types

Type	Age (year)	Gender male/female	Methylation (%)			
			CGI 1	CGI 2	CGI 3	CGI 4
SMA I (*n*=12)	0.6±0.5	8/4	76.7±8.6	59.9±5.0	13.5±4.6	70.0±3.8
SMA II (*n*=15)	1.3±1.1	7/8	79.6±3.9	58.6±3.5	13.1±3.1	73.4±6.3
SMA III (*n*=8)	2.9±1.3	4/4	77.8±3.1	56.8±2.8	12.8±1.7	68.0±1.7
P-value	**0.000**	0.558	0.453	0.236	0.916	**0.032**
Adjusted *P*-value			0.474	0.098	0.824	**0.014**

Results are shown as mean±standard deviation (SD) unless otherwise stated. Statistically significant results are shown in bold. The *P*-value was obtained by performing an ANOVA test or chi-square test when appropriate. The adjusted *P*-value was obtained by an analysis of covariance and age was considered as the co-variable

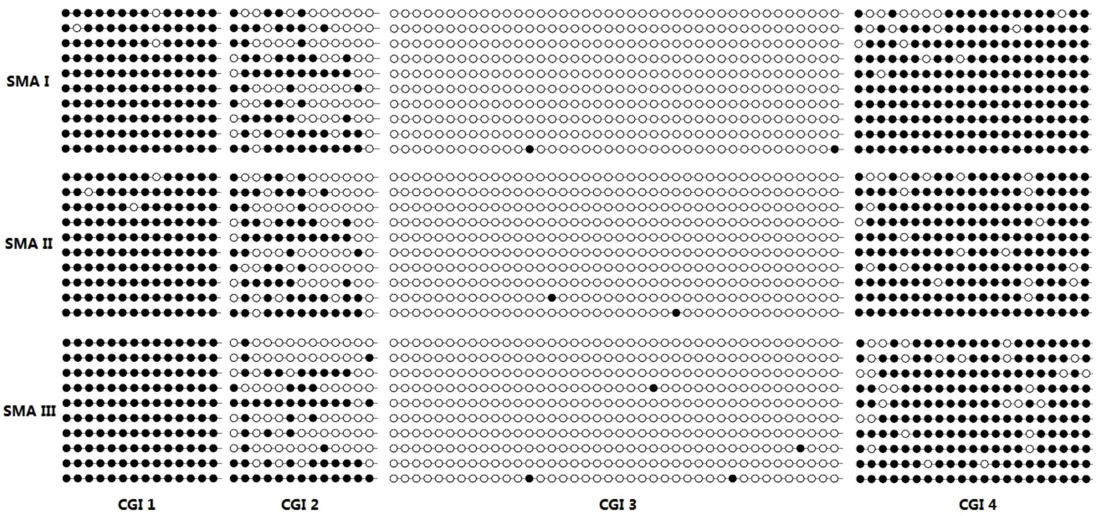

Fig. 1 Methylation analysis of four *SMN2* CGIs by bisulfite treatment, cloning, and sequencing

Three DNA samples were isolated from the peripheral blood of the SMA I, II, and III patients. Ten independent clones for each CGI of each patient were analyzed for methylation levels. Full circles represent methylated CpGs, while empty circles correspond to unmethylation

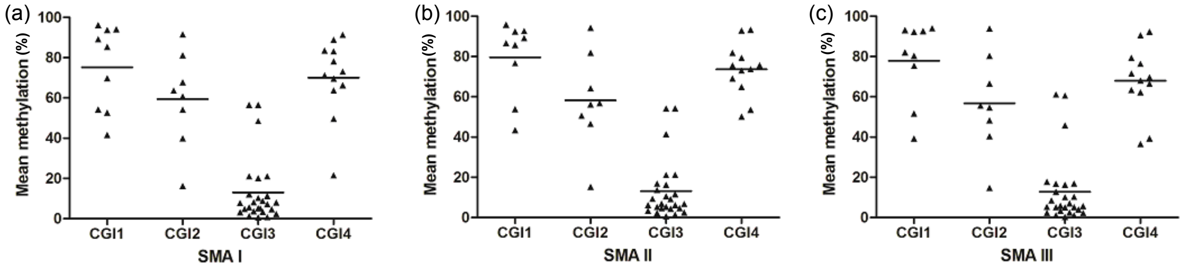

Fig. 2 Methylation analysis of four *SMN2* CGIs by MassARRAY

Black triangles represent mean methylation levels of each CpG unit within each *SMN2* CGI in the SMA patients. The mean methylation levels of each *SMN2* CGI are indicated by a horizontal line: (a) CGI 1: 76.7%, CGI 2: 59.9%, CGI 3: 13.5%, CGI 4: 70.0%; (b) CGI 1: 79.6%, CGI 2: 58.6%, CGI 3: 13.1%, CGI 4: 73.4%; (c) CGI 1: 77.8%, CGI 2: 56.8%, CGI 3: 12.8%, CGI 4: 68.0%

In this current study, thirteen CpG units of *SMN2* CGIs, including 19 CpGs (1–4 CpGs per unit) were detected that significantly varied in their methylation levels based on SMA classification (Table 2). Among these, eight CpG units located in CGI 1 (nt −871, −735), CGI 2 (nt −290/−288/−285), and CGI 4 (nt +890/+894/+898/+900, +938, +988, +999, +1064) had lower methylation levels in SMA type III compared with type I or II patients (Fig. 3). After Bonferroni correction, there were three CpG units (nt −871, −735, and +999) that still remained differently methylated ($P<0.001$).

3.4 *SMN2* CGIs methylation frequencies and transcript levels

To determine whether the methylation status of *SMN2* affects its transcript level, we evaluated

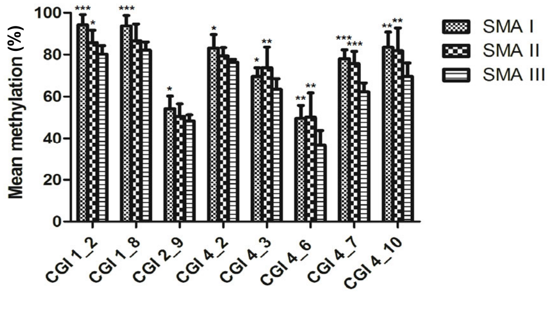

Fig. 3 Comparison of *SMN2* methylation levels by SMA types

Eight CpG units had different methylation frequencies in type I or II compared with type III SMA patients (* $P<0.05$, ** $P<0.01$, and *** $P<0.001$ significantly differ). The bar graph depicts the mean methylation levels (±SD) of each CpG unit within the respective CGIs and each CpG unit includes one to four CpGs. The CpG unit corresponding dinucleotide position is as follows: CGI 1_2: nt −871, CGI 1_8: nt −735, CGI 2_9: nt −290/−288/−285, CGI 4_2: nt +890, CGI 4_3: nt +938, CGI 4_6: nt +988, CGI 4_7: nt +999, and CGI 4_10: nt +1064

Table 2 Differentially methylated units in *SMN2* among SMA types

Methylation unit (dinucleotide position, nt)	Methylation level (%)			*P*-value
	SMA I (*n*=12)	SMA II (*n*=15)	SMA III (*n*=8)	
CGI 1_2 (−871)	94.1±5.3	85.7±5.6	80.3±4.4	0.000
CGI 1_8 (−735)	93.7±5.1	86.6±7.7	82.0±4.4	0.001
CGI 2_9 (−290/−288/−285)	54.2±5.7	50.5±5.6	48.3±3.4	0.046
CGI 3A_7 (−81)	3.2±3.3	6.8±3.4	2.3±1.7	0.003
CGI 3B_5 (+160)	1.3±1.1	2.2±1.2	1.1±0.9	0.046
CGI 3B_10 (+213)	8.0±1.6	9.4±2.5	7.0±1.6	0.032
CGI 3B_11 (+228/+237)	8.1±3.5	11.6±2.6	8.5±2.3	0.008
CGI 4_2 (+855)	21.5±8.6	53.5±18.2	39.3±8.6	0.000*
CGI 4_2 (+890/+894/+898/+900)	83.1±6.5	79.4±4.0	76.4±1.4	0.010*
CGI 4_3 (+938)	69.6±4.1	73.7±9.9	63.4±5.1	0.006*
CGI 4_6 (+988)	49.6±6.2	50.1±11.6	36.5±7.3	0.004
CGI 4_7 (+999)	78.1±4.2	75.7±5.9	62.2±4.3	0.000
CGI 4_10 (+1064)	83.5±7.3	81.9±10.8	69.6±6.4	0.003

Results are shown as mean±SD. *P*-values were obtained using an ANOVA test or a Brown-Forsythe test (*)

SMN2-fl and *SMN2*-Δ7 transcripts in 24 *SMN1*-deleted types I, II, and III SMA patients carrying three *SMN2* copies (Table 3).

Correlation analysis revealed that there was no association between *SMN2* CGIs methylation levels and total *SMN2* transcript levels (*SMN2*-fl+*SMN2*-Δ7) (Crawford *et al.*, 2012). However, 3/8 differentially methylated CpG units correlated with different *SMN2* transcripts after applying a Bonferroni correction (*P*<0.017). The CGI 1_2 (nt −871) methylation frequency was inversely proportional to the *SMN2*-fl, *SMN2*-Δ7, and total *SMN2* transcript levels (*r*=−0.550, *P*=0.007; *r*=−0.512, *P*=0.012; *r*=−5.990, *P*=0.003). Meanwhile, CGI 2_9 (nt −290/−288/−285) and CGI 4_3 (nt +938) inversely correlated with *SMN2*-fl (*r*=−0.500, *P*=0.015) and the ratio reflecting *SMN2* exon 7 inclusion (fl/Δ7, *r*=−0.515, *P*=0.012), respectively.

Table 3 Correlation between differentially methylated units and *SMN2* transcription

Transcription	nt −871		nt −290/−288/−285		nt +938	
	r	*P*-value	*r*	*P*-value	*r*	*P*-value
SMN2-fl	−0.550	0.007	−0.500	0.015	−0.383	0.071
SMN2-Δ7	−0.512	0.012	−0.141	0.522	−0.024	0.913
SMN2-fl+ *SMN2*-Δ7	−5.990	0.003	−0.355	0.097	−0.109	0.621
fl/Δ7	0.077	0.726	−0.206	0.346	−0.515	0.012

Spearman correlation analysis was used to evaluate the correlation between differentially methylated units and *SMN2* transcription

4 Discussion

Despite identifying the causative gene for SMA, the contribution of a significantly homologous gene, *SMN2* complicates the molecular interactions leading to SMA phenotypes. Both the *SMN2* copy number and epigenetic modification underly the SMA clinical symptoms. Methylation levels of seven CpGs upstream and downstream to the *SMN2* translational start site significantly varied between the types I and III SMA patients. And CpGs at position nt −296 and −290 were more hypermethylated in SMA type I than type III patients. Moreover, their methylation levels negatively correlated with the activity of the first transcriptional start site of *SMN2* (Hauke *et al.*, 2009). These findings suggest that *SMN2* methylation may modify the predicted SMA phenotypes. Because DNA methylation is affected by an individual's genetic makeup, age, gender, and lifestyle (Murrell *et al.*, 2005; Schumacher and Petronis, 2006; Barrès *et al.*, 2012), we analyzed the *SMN2* methylation status upstream and downstream of the translational start site in 35 Chinese SMA children.

The aim of this study was to clarify whether DNA methylation affects SMA clinicopathology by altering the expression levels of *SMN2*. In accordance to a previous report (Hauke *et al.*, 2009), four CGIs predicted the upstream and downstream of the *SMN2* translational start site, which had different methylation patterns, wherein the CGIs 1 and 4 were hypermethylated, CGI 2 was moderately methylated and CGI 3 was hypomethylated. These results suggest evolutionary conservation of the methylation pattern of CGIs proximal to the *SMN2* translational start site, which is independent of the tissue specificity and race, and has negligible inter-individual variability. It is obvious that the age distributions in SMA types are significantly different due to the criteria for SMA clinical classification. In our study, with or without consideration of patients' ages, the statistical results for the average methylation levels from CGIs 1–4 were the same. Additionally, we also found that gender did not affect the methylation levels of any CGIs. Taken together, our data suggested that neither age nor gender might affect CGI methylation levels. Moreover, our study showed that although the average methylation levels of CGI 4 in children with type III SMA were significantly lower than those in children with type II SMA (P=0.017), this difference was no longer significant after applying the Bonferroni correction. Therefore, it should be further confirmed in a larger sample study.

In this study, 13 differentially methylated units were detected in the four CGIs in the *SMN2* gene, and 8/13 CpG units associated with the disease severity. For example, the methylation levels in type I SMA were significantly higher than those in type III SMA (P<0.05). In comparison to that reported by Hauke *et al.* (2009), seven differentially methylated sites (nt −871, −695, −296, −290, +855, +988, and +1103), our data were consistent for nt −871, −290, and +988 results; however, the methylation levels at nt −695, −296, +855, and +1103 had insignificant differences. Moreover, our data demonstrated significantly higher methylation at nt −735, +890/+894/+898/+900, +938, +999, and +1064 in children with type I SMA than that in children with type III SMA. These results suggest that race may affect the methylation at specific loci despite conservation of methylation of each CGI in the *SMN2* gene.

To determine the influence of *SMN2* methylation on its transcription, we evaluated the correlation between *SMN2* methylation and transcription in 24 children with types I–III SMA. The methylation level of individual CGI did not correlate with total *SMN2* transcription levels. However, the correlation analysis of levels of differentially methylated units and *SMN2* transcriptional levels indicated that at nt −871 and nt −290/−288/−285, the methylation levels negatively correlate with *SMN2*-fl transcript levels. Jan Hauke *et al.* (2009) indicated that the differentially methylated sites at nt −296 and nt −290 correlated with the SMA severity. However, in our study, we did not find differences in the methylation level at nt −296 among the different SMA types. Moreover, based on the other two adjacent sites along with nt −290 in the same differentially methylated unit (nt −290/−288/−285), it is suggested that the variations in methylation of this unit may not be very significantly caused by nt −290 differential methylation (0.001<P<0.05). Thus, the methylation levels at nt −871 and −290 most likely affect *SMN2* transcription. Interestingly, methylation of nt +938, an intragenic methylation site located in *SMN2* CGI 4, inversely correlated with the ratio of fl/Δ7, but not *SMN2* transcript levels. Intragenic methylation is associated with alternative gene splicing (Jones, 2012; Maunakea *et al.*, 2013), and this data suggest that hypermethylation at nt +938 might decrease the ratio of fl/Δ7 by promoting the alternative splicing of exon 7. However, we need to perform further experiments to confirm the underlying mechanism.

Despite the association of differentially methylated CpG units with SMA severity, the exact pathogenesis needs to be elucidated, and will be our focus in the future. For example, the methylation of other genes, such as *CHML*, *ARHGAP22*, *CYTSB*, *CDK2AP1*, and *SLC23A2*, also contributes to the SMA phenotype modification (Zheleznyakova *et al.*, 2013). SMA clinicopathology may also be affected by other epigenetic modifications such as acetylation. In fact, histone deacetylase (HDAC) inhibitors can increase the in vitro transcript levels of full-length *SMN2* proteins, which in turn affect SMA severity in animal models (Chang *et al.*, 2001; Andreassi *et al.*, 2004; Hahnen *et al.*, 2006). In-depth studies of these modifying factors will improve our understanding of SMA pathogenesis, and allow for the development of effective therapeutic regimens.

In conclusion, our studies indicate that *SMN2* methylation, particularly at nt −871 and −290, regulates SMA clinical phenotypes by possibly regulating *SMN2* transcription. Moreover, nt +938 potentially affects *SMN2* transcription by regulating the alternative splicing, which will be studied extensively in the near future.

Acknowledgements

We would like to thank the patients and their parents for their collaborations in this investigation. We would also like to thank Editage for providing editorial assistance.

Compliance with ethics guidelines

Yan-yan CAO, Yu-jin QU, Sheng-xi HE, Yan LI, Jin-li BAI, Yu-wei JIN, Hong WANG, and Fang SONG declare that they have no conflict of interest.

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008 (5). Informed consent was obtained from all patients for being included in the study.

References

Andreassi, C., Angelozzi, C., Tiziano, F.D., *et al.*, 2004. Phenylbutyrate increases SMN expression in vitro: relevance for treatment of spinal muscular atrophy. *Eur. J. Hum. Genet.*, **12**(1):59-65. http://dx.doi.org/10.1038/sj.ejhg.5201102

Barrès, R., Yan, J., Egan, B., *et al.*, 2012. Acute exercise remodels promoter methylation in human skeletal muscle. *Cell Metab.*, **15**(3):405-411. http://dx.doi.org/10.1016/j.cmet.2012.01.001

Bernal, S., Also-Rallo, E., Martínez-Hernández, R., *et al.*, 2011. Plastin 3 expression in discordant spinal muscular atrophy (SMA) siblings. *Neuromuscul. Disord.*, **21**(6): 413-419. http://dx.doi.org/10.1016/j.nmd.2011.03.009

Chang, J.G., Hsieh-Li, H.M., Jong, Y.J., *et al.*, 2001. Treatment of spinal muscular atrophy by sodium butyrate. *PNAS*, **98**(17):9808-9813. http://dx.doi.org/10.1073/pnas.171105098

Crawford, T.O., Paushkin, S.V., Kobayashi, D.T., *et al.*, 2012. Pilot Study of Biomarkers for Spinal Muscular Atrophy Trial Group. Evaluation of SMN protein transcript and copy number in the biomarkers for spinal muscular atrophy (BforSMA) clinical study. *PLoS ONE*, **7**(4):e33572. http://dx.doi.org/10.1371/journal.pone.0033572

Hahnen, E., Eyüpoglu, I.Y., Brichta, L., *et al.*, 2006. In vitro and ex vivo evaluation of second-generation histone deacetylase inhibitors for the treatment of spinal muscular atrophy. *J. Neurochem.*, **98**(1):193-202. http://dx.doi.org/10.1111/j.1471-4159.2006.03868.x

Hauke, J., Riessland, M., Lunke, S., *et al.*, 2009. Survival motor neuron gene 2 silencing by DNA methylation correlates with spinal muscular atrophy disease severity and can be bypassed by histone deacetylase inhibition. *Hum. Mol. Genet.*, **18**(2):304-317. http://dx.doi.org/10.1093/hmg/ddn357

Jones, P.A., 2012. Functions of DNA methylation: islands, start sites, gene bodies and beyond. *Nat. Rev. Genet.*, **13**(7):484-492. http://dx.doi.org/10.1038/nrg3230

Maunakea, A.K., Chepelev, I., Cui, K., *et al.*, 2013. Intragenic DNA methylation modulates alternative splicing by recruiting MeCP2 to promote exon recognition. *Cell Res.*, **23**(11):1256-1269. http://dx.doi.org/10.1038/cr.2013.110

Murrell, A., Rakyan, V.K., Beck, S., 2005. From genome to epigenome. *Hum. Mol. Genet.*, **14**(Suppl. 1):R3-R10. http://dx.doi.org/10.1093/hmg/ddi110

Prior, T.W., Krainer, A.R., Hua, Y., *et al.*, 2009. A positive modifier of spinal muscular atrophy in the *SMN2* gene. *Am. J. Hum. Genet.*, **85**(3):408-413. http://dx.doi.org/10.1016/j.ajhg.2009.08.002

Qu, Y.J., Du, J., Li, E.Z., *et al.*, 2012. Subtle mutations in the *SMN1* gene in Chinese patients with SMA: p.Arg288Met mutation causing *SMN1* transcript exclusion of exon7. *BMC Med. Genet.*, **13**:86. http://dx.doi.org/10.1186/1471-2350-13-86

Schumacher, A., Petronis, A., 2006. Epigenetics of complex diseases: from general theory to laboratory experiments. *Curr. Top. Microbiol. Immunol.*, **310**:81-115.

Tiziano, F.D., Pinto, A.M., Fiori, S., *et al.*, 2010. SMN transcript levels in leukocytes of SMA patients determined by absolute real-time PCR. *Eur. J. Hum. Genet.*, **18**(1):52-58. http://dx.doi.org/10.1038/ejhg.2009.116

Zheleznyakova, G.Y., Voisin, S., Kiselev, A.V., *et al.*, 2013. Genome-wide analysis shows association of epigenetic changes in regulators of Rab and Rho GTPases with spinal muscular atrophy severity. *Eur. J. Hum. Genet.*, **21**(9):988-993. http://dx.doi.org/10.1038/ejhg.2012.293

List of electronic supplementary materials

Table S1 Primers for *SMN2* CGIs cloning sequencing
Table S2 Primers for *SMN2* CGIs nested PCR

中文概要

题　目：*SMN2*基因甲基化与中国儿童型脊肌萎缩症疾病严重程度的相关分析

目　的：分析我国脊肌萎缩症（SMA）患儿*SMN2*基因甲基化水平与其转录水平，并初步探讨该基因的甲基化修饰是否影响我国儿童型SMA疾病的严重程度。

创新点：首次在中国儿童型SMA人群中分析*SMN2*基因甲基化状态，提示该基因的甲基化模式在不同人种间具有一定保守性，而甲基化单元的甲基化状态可能具有种族差异。本研究也初步提示*SMN2*基因甲基化水平除了可能影响该基因的转录，还可能影响基因的可变剪接。

方　法：应用MassARRAY的方法检测35例SMA患儿（*SMN1*基因纯合缺失，*SMN2*基因3拷贝）外周血细胞中*SMN2*基因甲基化状态；应用实时聚合酶链反应（real-time PCR）的方法检测*SMN2*基因不同转录本的表达水平；分析*SMN2*基因甲基化与该基因的转录以及SMA疾病严重程度的关系。

结　论：位于甲基化岛1的两个甲基化单元（nt −871和nt −735）和位于甲基化岛4的nt +999甲基化单元的甲基化水平在III型患儿中显著低于II型和I型患儿；nt −871和nt −290/−288/−285甲基化单元的甲基化水平与*SMN2*基因全长转录本（*SMN2*-fl）的转录水平呈负相关。此外，nt +938甲基化单元的甲基化水平与*SMN2*基因全长转录本与跳跃外显子7转录本的比值（fl/Δ7）呈负相关，但与*SMN2*的转录水平无关。因此，我们初步得出*SMN2*基因甲基化可能通过调控其转录而影响我国儿童型SMA的疾病表型。

关键词：CpG岛；甲基化；运动神经元存活基因2；脊肌萎缩症