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Gender Effects on the Clinical Phenotype in Japanese Patients with Spinal Muscular Atrophy

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Background: Spinal muscular atrophy (SMA) is a neuromuscular disease caused by a mutation in *SMN1*. SMA is classified into three subtypes (types 1, 2, 3) based on achieved motor milestones. Although *NAIP* and *SMN2* are widely accepted as SMA-modifying factors, gender-related modifying factors or gender effects on the clinical phenotype are still controversial. Methods: A total of 122 Japanese patients with SMA, of which *SMN1* was homozygously deleted, were analyzed from the perspective of the achieved motor milestone, *NAIP* status and *SMN2* copy number. Results: A predominance of male patients was observed in SMA type 3 (the walker group) without *NAIP*-deletion or with high *SMN2* copy number (3 or 4 copies). Conclusion: We suggest the presence of gender-related modifiers on disease severity in SMA patients. The modifiers may contribute only in the presence of *NAIP* and a high copy number of *SMN2*.

INTRODUCTION

Spinal muscular atrophy (SMA) is a common neuromuscular disorder with autosomal recessive inheritance. SMA is clinically classified into three subtypes: type 1 (severe form, onset of symptoms at age of <6 months, unable to sit without aid); type 2 (intermediate form, onset of symptoms at age of 6-18 months, able to sit alone but unable to stand or walk without aid); and type 3 (mild form, onset of symptoms at age of >18 months, able to stand and walk without aid) (11). The responsible gene of the disease is the survival motor neuron 1 gene (*SMN1*), which was identified in the SMA locus in chromosome 5q (7). *SMN1* is homozygously deleted in 95% of SMA patients and deleteriously mutated in the remaining patients (7).

The neuronal apoptosis inhibitory protein gene (*NAIP*) was also identified as an SMA-related gene within the SMA locus (14). *NAIP* deletion is found most frequently in SMA type 1 (~50% of the patients with SMA type 1), and seldom in other subtypes (14). On the other hand, the survival motor neuron 2 gene (*SMN2*) in the SMA locus, a highly homologous gene of *SMN1*, encodes the same SMN protein as *SMN1* does, and multiple copies of *SMN2* may compensate to some degree for the lack of *SMN1* (17). Thus, *NAIP* and *SMN2* are now widely accepted as SMA-modifying genetic factors.

Gender effect on the clinical phenotype of SMA has been controversial until now. Some researchers reported that the symptoms in female siblings were milder than those of the male siblings in the affected SMA families (4,13). In addition, predominance of female patients with SMA type 3 was reported (3). However, other researchers observed a predominance of male patients in milder forms of SMA (5,6,18). In this study, to clarify the gender effects on the disease severity or clinical phenotype of SMA, we analyzed the SMA patients referred to our laboratory.

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PATIENTS AND METHODS

Patients

A total of 122 Japanese patients (60 females and 62 males) were enrolled in this study. They were referred to Kobe University from 1996 to 2015, and fulfilled the diagnostic criteria defined by the International SMA Consortium (11). Informed consent was obtained from these patients and/or their parents. This study, including the genetic analysis, was approved by the Ethics Committee of Kobe University Graduate School of Medicine, Japan.

SMN and *NAIP* deletion test

To detect *SMN* and *NAIP* deletion, PCR restriction fragment length polymorphism analysis was performed according to the method of van der Steege *et al.* (16). *NAIP* exon 5 was detected using the PCR method of Roy *et al.* (14).

Copy number analysis of the *SMN* genes

The copy number of the *SMN* genes was determined by real-time PCR (15) or the multiplex-dependent probe amplification (MLPA) assay (1).

Statistics

Correlation of *SMN2* copy number with clinical subtype was determined using chi-squared tests and logistic regression analysis. A *P*-value of less than 0.05 was considered to indicate a significant difference. To avoid α-error inflation in the separate analyses of *NAIP*-deleted and non-*NAIP*-deleted patients, and of low- and high-*SMN2*-copy-number patients, a *P*-value of less than 0.025 was considered to indicate a significant difference. The software used for statistical analysis was Statistical Program for Social Science (SPSS) version 16 (IBM Corporation, Palo Alto, CA, USA).

RESULTS

To clarify the effect of gender on the disease severity of SMA, we analyzed the relations between gender and clinical subtypes in 122 Japanese SMA patients. A significant predominance of male patients (or a rarity of female patients) was found in SMA type 3 ($p<0.01$) (Table I). Then, the patients were divided into two groups based on the "obtaining the ability to walk without aid in the life", the non-walkers (SMA types 1 and 2) and the walkers (SMA type 3). A 2×2 contingency table ([male and female] × [non-walker and walker]) demonstrated a significantly higher frequency of male patients (or a significantly lower frequency of female patients) in the walkers ($p<0.01$) (Table I).

Then, we examined the gender effect in the patients with or without *NAIP* deletion, and we found a significant difference between males and females only in the patients without *NAIP* deletion. Only in the patients without *NAIP* deletion, the 2×2 contingency table demonstrated a significantly higher frequency of male patients (or a significantly lower frequency of female patients) in the walkers ($p<0.05$) (Table II).

Table I. Gender and motor function

(A) Gender and clinical subtype

	Type 1	Type 2	Type 3
M (n=62)	28	15	19
F (n=60)	33	22	5

χ 2 = 9.87, df = 2, P < 0.01

(B) Gender and walking ability

	Non-walker	Walker
M (n=62)	43	19
F (n=60)	55	5

χ 2 = 9.61, df = 1, P < 0.01

Table II. Gender, *NAIP* and walking ability

(A) Patients with *NAIP* deletion

	Non-walker	Walker
M (n=17)	15	2
F (n=25)	25	0

(B) Patients without *NAIP* deletion

	Non-walker	Walker
M (n=45)	28	17
F (n=35)	30	5

χ^2=5.45, df=1, P<0.05

We also examined the gender effect in the patients with low *SMN2* copy number (1 or 2) and high *SMN2* copy number (3 or 4). Only in the patients with high *SMN2* copy number, the 2×2 contingency table demonstrated a significantly higher frequency of male patients (or a significantly lower frequency of female patients) in the walkers ($p<0.01$) (Table III).

Table III. Gender, *SMN2* and walking ability

(A) Patients with 1–2 *SMN2* copies

	Non-walker	Walker
M (n=25)	24	1
F (n=29)	29	0

(B) Patients with 3–4 *SMN2* copies

	Non-walker	Walker
M (n=37)	19	18
F (n=31)	26	5

χ^2=5.45, df=1, P<0.05

To clarify which of the studied parameters, gender (male or female), *NAIP* (presence or absence), and *SMN2* (copy number) affects the ability to walk without aid in the life, we performed a multiple logistic regression analysis with "the ability to walk without aid in the life" as a dependent variable. Here, we used the forward stepwise selection method to evaluate the relative contribution of gender, *NAIP*, and *SMN2* to the outcome. The best model we obtained in this stepwise selection process included gender and *SMN2*, but not *NAIP* (Table IV). According to our calculation, the adjusted odds ratios of <male to female> and <high copy number of *SMN2* to low copy number of *SMN2*> for "the ability to walk without aid" were 3.902 and 37.382, respectively.

Table IV. Logistic regression analysis

Variables	B	Standard Error	Wald	df	P Value	Exp (B)	Confidence Intervals
Gender	1.362	0.633	4.623	1	<0.05	3.902*	1.128-13.501
SMN2	3.621	1.022	12.547	1	<0.001	37.382*	5.040-277.243

"B" designates coefficient values. "Exp(B)" designates odds ratios. * designates odds ratio of male to female.

DISCUSSION

Our study revealed that a relatively larger number of male patients were observed in SMA type 3, though there were no significant gender differences with regard to the number of patients in SMA type 1 or 2.

This finding suggested two possible gender-related modifiers: (1) One is the presence of a male-related modifier which lead to increasing the number of male patients with SMA type 3, and (2) the other is the presence of a female-related modifier which lead to decreasing the number of female patients with SMA type 3. The male-related modifier might delay the onset of SMA, while the female-related modifier might prevent the development of SMA. Oprea et al. reported that asymptomatic *SMN1*-deleted females exhibited significantly higher expression of plastin 3 (PLS3, T-plastin or T-fimbrin; MIM 300131, Xq23) in their lymphoblasts than did their SMA-affected counterparts (13), supporting the hypothesis of female-related modifiers. PLS3 is an actin-binding protein that is expressed in normal cells of solid tissues and transformed fibroblasts (8). PLS3 has a calcium-binding domain (9), and calcium binding may be essential for plastin 3 function in SMN-deficient motoneurons (10).

As for the relationships between gender and *NAIP*, or between gender and *SMN2*, we found a significantly higher frequency of male patients in the walker group without *NAIP*-deletion or with a high *SMN2* copy number (3 or 4 copies). It means that gender-related modifiers may have some effects on the clinical phenotype of SMA in the presence of *NAIP* and a high copy number of *SMN2*. According to the report of Oprea et al., non-symptomatic individuals with *SMN1* deletion carried a high *SMN2* copy number (3 or 4 copies) (13).

Then, a question arises why the gender-related modifiers have effects only in the presence of *NAIP* and a high copy number of *SMN2*. The absence of *NAIP* may mark the extent of the deletion involving *SMN1* in SMA chromosome (2). On the other hand, the presence of *NAIP* implies no deletion of *SMN1*; but it suggests that an *SMN1*-to-*SMN2* gene conversion event has occurred at least on one chromosome, which may decrease the *SMN1* copy numbers and increase the *SMN2* copy numbers (12). Thus, the question is paraphrased into "why the gender-related modifiers have effects only in the patients with a high copy number of *SMN2*." Our answer to this question is that in SMA patients, negative effect of a low *SMN2* copy number on the clinical phenotype may be much larger than positive effect of the gender-related modifiers on the clinical phenotype. To say in other words, positive effect of the gender-related modifiers may be masked by negative effect of a low *SMN2* copy number.

In this study, we suggested the presence of gender-related modifiers on disease severity in SMA patients, and the modifiers work only in the presence of *NAIP* and a high copy number of *SMN2*. Indeed, we could not fully neglect the possibility that our study may be subject to selection bias or sampling bias, i.e. the patients studied may not be representative of the Japanese SMA population. If more Japanese patients with SMA could have been recruited for our study, we could draw more solid conclusions about gender-related modifiers.

DECLARATION OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this paper.

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