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# Genetic backgrounds and genotype-phenotype relationships in anthropometric parameters of 116 Japanese individuals with Noonan syndrome

Yasuko Shoji[1, 2, #], Ayaha Hata[1, #], Takatoshi Maeyama[1], Tamaki Wada[1], Yuiko Hasegawa[3], Eriko Nishi[3], Shinobu Ida[4], Yuri Etani[1], Tetsuya Niihori[5], Yoko Aoki[5], Nobuhiko Okamoto[3], and Masanobu Kawai[1, 6]

[1]*Department of Gastroenterology and Endocrinology, Osaka Women's and Children's Hospital, Osaka, Japan*
[2]*Department of Epidemiology and Health Policy, University of Toyama, Toyama, Japan*
[3]*Department of Genetics, Osaka Women's and Children's Hospital, Osaka, Japan*
[4]*Department of Clinical Laboratory, Osaka Women's and Children's Hospital, Osaka, Japan*
[5]*Department of Medical Genetics, Tohoku University School of Medicine, Miyagi, Japan*
[6]*Department of Bone and Mineral Research, Research Institute, Osaka Women's and Children's Hospital, Osaka, Japan*

## Highlights

- Pathogenic variants were identified in 86% of clinically diagnosed NS subjects.
- Height-SDS was higher in subjects with *RIT1* variants.
- No genotype-phenotype relationships were detected in the context of body mass index.

**Abstract.** Noonan syndrome (NS) is caused by pathogenic variants in genes encoding components of the RAS/MAPK pathway and presents with a number of symptoms, including characteristic facial features, congenital heart diseases, and short stature. Advances in genetic analyses have contributed to the identification of pathogenic genes in NS as well as genotype-phenotype relationships; however, updated evidence for the detection rate of pathogenic genes with the inclusion of newly identified genes is lacking in Japan. Accordingly, we examined the genetic background of 116 individuals clinically diagnosed with NS and the frequency of short stature. We also investigated genotype-phenotype relationships in the context of body mass index (BMI). Genetic testing revealed the responsible variants in 100 individuals (86%), where *PTPN11* variants were the most prevalent (43%) and followed by *SOS1* (12%) and *RIT1* (9%). The frequency of short stature was the lowest in subjects possessing *RIT1* variants. No genotype-phenotype relationships in BMI were observed among the genotypes. In conclusion, this study provides evidence for the detection rate of pathogenic genes and genotype-phenotype relationships in Japanese patients with NS, which will be of clinical importance for accelerating our understanding of the genetic backgrounds of Japanese patients with NS.

**Key words:** Noonan syndrome, genotype, short stature, body mass index

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[#] These authors contributed equally to this study.
Corresponding author: Masanobu Kawai, M.D., Ph.D., Department of Gastroenterology and Endocrinology, Osaka Women's and Children's Hospital and Department of Bone and Mineral Research, Research Institute, Osaka Women's and Children's Hospital, 840 Murodo-cho, Izumi, Osaka 594-1101, Japan
E-mail: kawaim@wch.opho.jp

## Introduction

Noonan syndrome (NS, OMIM#163950) was first reported by Jacqueline Noonan and is a congenital disorder associated with characteristic facial and physical features, congenital heart diseases, short stature, and other distinct symptoms (1, 2). NS is caused by pathogenic variants in the genes that encode components of the RAS/MAPK signaling pathway (3). Although NS is clinically diagnosed according to the diagnostic criteria proposed by van der Burgt (4), genetic diagnosis has become available and more prevalent since the identification in 2001 of *PTPN11* as one of the genes responsible for NS (5). This was followed by the identification of other genes associated with this disease, including *SOS1* (6, 7), *RAF1* (8, 9), *KRAS* (10), *NRAS* (11), *BRAF* (12), *RIT1* (13), and *LZTR1* (14). These breakthroughs have contributed to updating the genetic background of NS (2, 15). Although there is evidence demonstrating the genetic background of NS in Japan (16–18), recently identified genes have not been well-studied in previous reports. This underscores the need for updated studies investigating the genotypic background of NS in the Japanese population.

Additionally, the accumulation of genetic data has highlighted the presence of genotype-phenotype relationships in NS. For example, the prevalence of hypertrophic cardiomyopathy was observed to be higher in individuals with pathogenic variants of the *RAF1* gene (8) but lower in those with pathogenic variants of *PTPN11* (19). A genotype-phenotype relationship has also been reported for the prevalence of short stature (20, 21). We previously investigated the genetic characteristics and clinical features of 48 Japanese patients clinically diagnosed with NS and reported a genotype-phenotype relationship with height (16); however, there were several limitations in our previous publication such as the small number of subjects and recently identified genes not being thoroughly investigated. Furthermore, although height is significantly affected by the timing of puberty, this was not incorporated into the study design.

Therefore, in this study we analyzed the genetic backgrounds of 116 Japanese patients clinically diagnosed with NS to provide updated genetic information. Additionally, we expanded our research to investigate the genotype-phenotype analysis of height using anthropometric data obtained during the prepubertal period. We also evaluated genotype-phenotype relationships in the context of body mass index (BMI) that serves as a surrogate marker for nutritional status, as accumulating evidence indicates changes in the nutritional status and bioenergetics of patients with NS (22).

## Methods

### Ethical considerations

This study was approved by the Ethics Committee of Osaka Women's and Children's Hospital (approval No.1138-2). The ethics review board approved the opt-out recruitment method for participation in this study.

### Participants

We retrospectively evaluated 116 patients (M:71, F:45) who were clinically diagnosed with NS based on their medical records between May 1993 and September 2023 at Osaka Women's and Children's Hospital. All individuals underwent genetic testing. Geneticists made clinical diagnoses using the *van der Burgt* clinical scoring system. There was an overlap with the subjects in our previous studies (16, 23–28).

### Study design

We retrospectively assessed the anthropometric parameters of height and weight based on medical records. Supine length and standing height were measured using an infant meter and stadiometer, respectively. Weights were measured using a digital scale. BMI was calculated by dividing weight by height$^2$. Height standard deviation scores (SDS) and BMI-SDS were assessed based on normal growth standards for Japanese children from a national survey conducted in 2000 (29, 30). The NS-specific height SDS was calculated based on growth references for NS (18). Short stature was defined as height-SDS < –2.0 for age and sex.

To exclude the effects of puberty on growth, we included prepubertal participants. The initiation of puberty was defined as the time of Tanner stage II or greater with breast development for girls or a testicular volume of ≥ 4 mL for boys. Additionally, to exclude the effects of delayed puberty, we used data obtained before the age of 14 yr for boys and before the age of 12 yr for girls. Those younger than 1 yr of age were also excluded from the genotype-phenotype analysis based on evidence that growth restriction often occurs during this period in NS. Anthropometric parameters were obtained prior to the initiation of GH treatment to avoid the effects of GH on height. Subjects with GH deficiency were excluded from the genotype-phenotype analysis.

### Genetic analysis

Genomic DNA was purified from whole blood obtained from the subjects. First, Sanger sequencing or targeted next-generation sequencing (NGS) was performed for the genetic analysis. Sanger sequencing revealed exons 1–15 in *PTPN11*, all exons in *SOS1*, exons 7, 14, and 17 in *RAF1*, exons 1–5 in *KRAS*, exons 6 and 11–16 in *BRAF*, exon 1 in *SHOC2*, all exons in *RIT1*, exons 2 and 3 in *MAP2K1*, and exons 2 and 3 in *MAP2K2*. Targeted NGS was performed using MiSeq (Illumina, San Diego, CA, USA) and included *PTPN11*, *SOS1*, *RAF1*, *RIT1*, *KRAS*, *NRAS*, *SHOC2*, *CBL*, *BRAF*, *HRAS*, *MAP2K1*, and *MAP2K2*. When pathogenic variants were not identified, whole-exome sequencing was performed using a HiSeq2500 sequencing system (Illumina, San


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Diego, CA, USA). All variants were confirmed using Sanger sequencing as appropriate. Identified variants (with the exception of deletions) were evaluated according to ACMG/AMP standards and guidelines for the interpretation of sequence variants (31).

### Statistical analysis

All data were expressed as medians (interquartile range: IQR). Statistical analyses were performed using the Kruskal-Wallis test followed by the post-hoc Steel-Dwass test. Statistical significance was set at $P < 0.05$.

## Results

### Genetic characteristics in clinically diagnosed NS

Pathogenic variants were identified in 100 of the 116 patients (86%), where *PTPN11* was the most prevalent (43%), and this was followed by *SOS1* (12%), *RIT1* (9%), *RAF1* (7%), and *KRAS* (5%) (**Table 1**). Additional less prevalent causative genes are summarized in **Table 1**. Notably, we observed one patient harboring a deletion variant of *LZTR1* (NM_006767.4: c.605-606del). Whole-exome sequencing failed to detect any additional variants associated with NS. Information regarding the identified variants is listed in **Table 2** and illustrated in **Fig. 1**. A number of these variants have been reported in our previous publications as presented in **Table 2** (16, 23–28). Four variants were categorized as variants of unknown significance according to the ACMG/AMP standards and guidelines for the interpretation of sequence variants (31); however, based on the clinical characteristics, we collectively judged that these variants were responsible for the NS phenotypes in each individual.

### Genotype-phenotype analyses of anthropometric parameters in genetically diagnosed NS

We assessed the frequency of short stature for each NS genotype as presented in **Table 3**. Height and BMI data were available for all 96 participants. The medians (IQR) of height-SDS and BMI-SDS were –2.52 (–3.12 to –1.78) and –0.24 (–0.78 to 0.28), respectively. The prevalence of short stature was 70% (**Table 3**). When comparing genotypes for which at least three data were available, individuals with pathogenic variants in *RIT1* exhibited the lowest frequency of short stature, whereas all subjects with pathogenic variants in *KRAS* or *SHOC2* exhibited short stature. We also compared height-SDS among genotypes and observed a significant difference, where the highest value was observed in subjects with pathogenic variants of *RIT1* (**Table 4**). We then examined the relationship between BMI-SDS and genotypes for which at least three data were available, and we observed no significant differences. We next examined the relationship between BMI-SDS and genotypes for which at least three data points were available, and we observed no significant differences (**Table 4**).

**Table 1.** Genetic characteristics in clinically diagnosed NS

| Gene | N = 116 | Detection rate |
|---|---|---|
| *PTPN11* | 50 | 43.1% |
| *SOS1* | 14 | 12.1% |
| *RIT1* | 10 | 8.6% |
| *RAF1* | 8 | 6.9% |
| *KRAS* | 6 | 5.2% |
| *SHOC2* | 4 | 3.4% |
| *BRAF* | 2 | 1.7% |
| *PPP1CB* | 2 | 1.7% |
| *LZTR1* | 1 | 0.9% |
| *MAP2K1* | 1 | 0.9% |
| *NRAS* | 1 | 0.9% |
| *CBL* | 1 | 0.9% |
| Total | 100 | 86% |

### Genotype-phenotype analyses of *PTPN11* variants with anthropometric parameters

Variants identified in *PTPN11* are presented in **Fig. 1**. Eighty-two percent of the variants were identified in the protein tyrosine phosphatase (PTP) domain, and the amino acid substitution variant NP_002825.3: p.(N308D/S) was the most prevalent (29%). The PTP domain plays a crucial role in the function of SHP2 that is a product of *PTPN11* (32). Therefore, we hypothesized that variants in this region may lead to distinct phenotypes. Accordingly, we compared height-SDS in subjects harboring variants in the PTP domain to those harboring variants outside this domain and observed no significant differences (–2.8 ± 0.9 [N = 34]) in the PTP domain *vs* (–2.8 ± 1.5 [N = 11]) outside the PTP domain ($p = 0.92$). We then compared height-SDS between subjects with variants in the PTP domain in the presence or absence of p.N308D/S variants and observed no significant differences (–3.0 ± 1.3 [N = 13]) in subjects with this variant *vs* (–2.6 ± 0.7 [N = 21]) in those without ($p = 0.19$).

## Discussion

We investigated the genetic background of patients clinically diagnosed with NS and determined that the percentage of subjects harboring pathogenic variants was 86%. The detection rates of pathogenic variants vary among studies and range from 50% to more than 90% in patients with NS or RASopathies (3, 33–36). A recent review by Tartaglia *et al.* reported a detection rate of approximately 80% (15). Although the higher detection rate in the present study may be partially attributed to the inclusion of recently identified genes, it may also be a consequence of the involvement of geneticists familiar


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**Table 2.** Identified variants in genes responsible for NS

| Gene | Accession number | Variant | | Variant classification | N | Reference* |
|---|---|---|---|---|---|---|
| *PTPN11* | NM_002834.5 | c.172A>G | p.Asn58Asp | Pathogenic | 2 | |
| | | c.184T>G | p.Tyr62Asp | Pathogenic | 1 | 16 |
| | | c.188A>G | p.Tyr63Cys | Pathogenic | 2 | 16 |
| | | c.215C>G | p.Ala72Gly | Pathogenic | 1 | |
| | | c.218C>T | p.Thr73Ile | Pathogenic | 1 | 16 |
| | | c.228G>T | p.Glu76Asp | Pathogenic | 1 | |
| | | c.317A>C | p.Asp106Ala | Pathogenic | 1 | 16 |
| | | c.417G>C | p.Glu139Asp | Pathogenic | 2 | |
| | | c.663A>G | p.Ile221Met | Pathogenic | 1 | |
| | | c.774G>C | p.Glu258Asp | Likely pathogenic | 2 | |
| | | c.782T>G | p.Leu261Arg | Likely pathogenic | 1 | |
| | | c.836A>G | p.Tyr279Cys | Pathogenic | 2 | 16 |
| | | c.844A>G | p.Ile282Val | Pathogenic | 3 | 16 |
| | | c.846C>G | p.Ile282Met | Pathogenic | 1 | |
| | | c.854T>C | p.Phe285Ser | Pathogenic | 4 | 16 |
| | | c.922A>G | p.Asn308Asp | Pathogenic | 13 | 16 |
| | | c.923A>G | p.Asn308Ser | Pathogenic | 2 | |
| | | c.1403C>T | p.Thr468Met | Pathogenic | 1 | |
| | | c.1471C>G | p.Pro491Ala | Pathogenic | 1 | 16 |
| | | c.1493G>T | p.Arg498Leu | Pathogenic | 1 | |
| | | c.1504T>G | p.Ser502Ala | Pathogenic | 1 | |
| | | c.1505C>T | p.Ser502Leu | Pathogenic | 1 | |
| | | c.1510A>G | p.Met504Val | Pathogenic | 4 | 16 |
| | | c.1528C>G | p.Gln510Glu | Pathogenic | 1 | |
| *SOS1* | NM_005633.4 | c.508A>G | p.Lys170Glu | Pathogenic | 3 | 16 |
| | | c.797C>A | p.Thr266Lys | Pathogenic | 1 | 16 |
| | | c.806T>G | p.Met269Arg | Pathogenic | 1 | 16 |
| | | c.1297G>A | p.Glu433Lys | Pathogenic | 1 | 16 |
| | | c.1300G>C | p.Gly434Arg | Pathogenic | 1 | |
| | | c.1322G>A | p.Cys441Tyr | Pathogenic | 1 | 16 |
| | | c.1654A>G | p.Arg552Gly | Pathogenic | 3 | 16 |
| | | c.1655G>C | p.Arg552Thr | Pathogenic | 1 | |
| | | c.1655G>A | p.Arg552Lys | Pathogenic | 1 | 16 |
| | | c.2536G>A | p.Glu846Lys | Pathogenic | 1 | 23 |
| *RIT1* | NM_006912.6 | c.104G>C | p.Ser35Thr | Pathogenic | 2 | 27 |
| | | c.162T>G | p.Ile54Met | VUS | 1 | |
| | | c.170C>G | p.Ala57Gly | Pathogenic | 3 | 16, 24 |
| | | c.247A>C | p.Thr83Pro | Pathogenic | 1 | |
| | | c.270G>T | p.Met90Ile | Pathogenic | 2 | |
| | | c.284G>C | p.Gly95Ala | Pathogenic | 1 | |
| *RAF1* | NM_001354689.3 | c.770C>T | p.Ser257Leu | Pathogenic | 3 | 16, 28 |
| | NM_001354689.3 | c.776C>T | p.Ser259Phe | Pathogenic | 1 | 16, 28 |
| | NM_001354689.3 | c.786T>G | p.Asn262Lys | Pathogenic | 1 | 16 |
| | NM_001354690.3 | c.1371C>G | p.Asp457Glu | VUS | 1 | |
| | NM_001354689.3 | c.1655A>G | p.Tyr552Cys | VUS | 1 | |
| | NM_001354690.3 | c.1837C>G | p.Leu613val | Pathogenic | 1 | 28 |
| *KRAS* | NM_004985.5 | c.40G>A | p.Val14Ile | Pathogenic | 1 | |
| | | c.458A>T | p.Asp153Val | Likely pathogenic | 5 | 16 |
| *SHOC2* | NM_007373.4 | c.4A>G | p.Ser2Gly | Pathogenic | 4 | 25 |
| *BRAF* | NM_004333.6 | c.1408A>C | p.Thr470Pro | Pathogenic | 1 | 16 |
| | | c.1455G>C | p.Leu485Phe | Pathogenic | 1 | 16 |
| *PPP1CB* | NM_002709.3 | c.146C>G | p.Pro49Arg | Pathogenic | 2 | 26 |
| *CBL* | NM_005188.4 | c.1111T>A | p.Tyr371Asn | Pathogenic | 1 | |
| *LZTR1* | NM_006767.4 | c.605-606del | p.Met202fs | Pathogenic | 1 | 26 |
| *MAP2K1* | NM_138957.3 | c.604T>C | p.Ser202Pro | VUS | 1 | |
| *NRAS* | NM_002524.5 | c.149C>T | p.Thr50Ile | Pathogenic | 1 | |

Variant classification was based on ACMG/AMP standards and guidelines for the interpretation of sequence variants, with the exception of deletion variants (31). VUS: variant of unknown significance. * Variants reported by our group are referenced.


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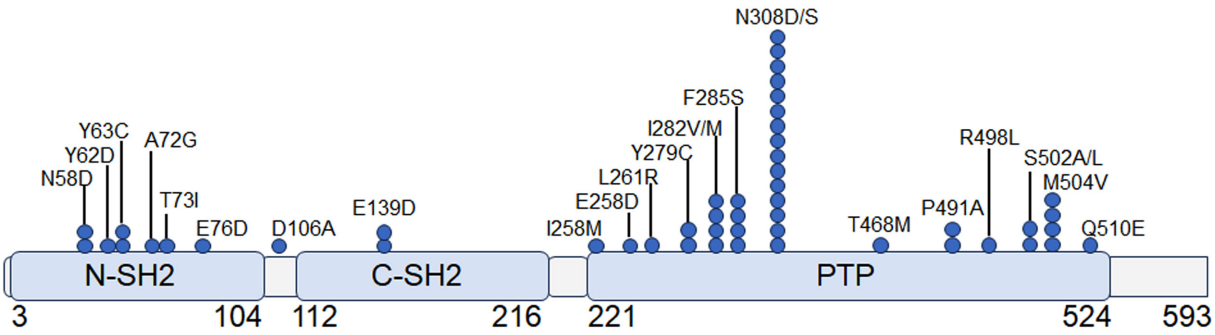


**Fig. 1.** Location and number of variants identified in the *PTPN11* gene. The number at the bottom represents the amino acid number in the SHP2 protein that is a product of *PTPN11*. The circle represents the number of individuals possessing the identified variants. SH2, Src homology 2 domain; PTP, protein tyrosine phosphatase domain.

**Table 3.** Association of genotype with the frequency of short stature

| Gene | N of subjects | N of short stature | Frequency |
|---|---|---|---|
| *PTPN11* | 45 | 37 | 82% |
| *SOS1* | 11 | 5 | 45% |
| *RIT1* | 8 | 2 | 25% |
| *RAF1* | 5 | 4 | 80% |
| *KRAS* | 4 | 4 | 100% |
| *SHOC2* | 4 | 4 | 100% |
| *BRAF* | 2 | 1 | 50% |
| *CBL* | 1 | 0 | 0% |
| *LZTR1* | 1 | 1 | 100% |
| *MAP2K1* | 1 | 0 | 0% |
| *NRAS* | 1 | 0 | 0% |
| *PPP1CB* | 1 | 1 | 100% |
| Not indentified | 12 | 8 | 67% |
| Total* | 96 | 67 | 70% |

*Anthropometric data were available for 96 of 116 individuals.

**Table 4.** Anthropometric findings for each genotype

| Gene | *PTPN11* | *SOS1* | *RIT1* | *RAF1* | *KRAS* | *SHOC2* | p value |
|---|---|---|---|---|---|---|---|
| N | 45 | 11 | 8 | 5 | 4 | 4 | |
| Age | 5.4 (3.6 to 7.6) | 7.8 (3.0 to 9.6) | 5.6 (3.0 to 7.4) | 9.7 (3.5 to 11.3) | 5.4 (3.8 to 7.7) | 1.7 (1.3 to 2.6) | ns |
| M/F | 29/16 | 7/4 | 4/4 | 3/2 | 1/3 | 2/2 | |
| Height-SDS | –2.70 (–3.07 to –2.22) | –1.70 (–1.25 to –2.50) | –0.75 (–0.43 to –1.65) | –3.5 (–4.10 to –3.20) | –2.50 (–2.76 to –2.40) | –3.65 (–4.35 to –3.18) | < 0.01 * |
| NS-height-SDS | –0.14 (–0.68 to 0.29) | 0.57 (–0.59 to 1.1) | 1.69 (0.63 to 2.13) | –0.70 (–1.05 to –0.36) | –0.18 (–0.28 to –0.05) | –1.26 (–1.40 to –1.16) | < 0.01 ** |
| BMI-SDS | –0.38 (–0.70 to 0.01) | –0.24 (–1.19 to 0.30) | –0.21 (–0.96 to 0.61) | 0.69 (0.69 to 1.06) | 0.24 (–0.18 to 0.60) | –0.04 (–0.69 to 0.31) | ns |

NS-height-SDS: the NS-specific height SDS was calculated based on the growth references for NS (18). Post-hoc Steel-Dwass test revealed significant differences in * *RIT1* compared to *PTPN11* ($p < 0.01$). **: *RIT1* compared to *PTPN11* ($p < 0.05$).

with dysmorphology. This indicates the importance of a thorough and comprehensive evaluation of clinical symptoms prior to genetic testing for the accurate diagnosis of NS.

The percentage of genes identified in the present study was consistent with that reported previously (2). The frequency of *PTPN11* was 35.4% in our previous study (16) and was 43% in the present study. Therefore, an increase in the number of subjects that were examined appears to have provided more accurate information regarding the genetic background of NS in Japanese patients, as the global frequency of *PTPN11* variants in NS was reported to be approximately 50% (15). In the present study, the detection rate of *RIT1* variants was


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9%, and this was higher than that reported in Europe and Brazil (5–6%) (33–35). In Eastern Asia, the detection rates of *RIT1* are 7.5% in Japan (27) and 6.8% in China (36). These findings suggest that pathogenic variants of *RIT1* are more prevalent in eastern Asia; however, accumulation of real-world evidence is required to clarify the rates of *RIT1* variants in NS.

Short stature is a characteristic feature of NS. Previous studies have reported that the frequency of short stature is high in NS and ranges between 59 and 70% (16, 17, 19, 20). As delayed puberty that is associated with higher adult height is a characteristic of NS, the timing of puberty must be considered when evaluating height in children with NS. To avoid this limitation, we collected anthropometric data during the prepubertal period before the age of 14 yr in boys and 12 yr in girls, and we observed that the rate of short stature was 70%, thereby providing more accurate information regarding the frequency of short stature during childhood. The mechanisms by which short stature develops in NS remain unclear; however, a number of mechanisms have been proposed. Feeding difficulties during infancy that are partially caused by gastroesophageal reflux may have contributed to growth retardation (37). Moreover, activation of the RAS/MAPK pathway has been demonstrated to suppress the induction of insulin-like growth factor 1 (IGF-1) by GH (38). Therefore, reductions in IGF-1 may play a role in the development of short stature in NS. Previous studies reported reduced IGF-1 levels in individuals with pathogenic variants of *PTPN11* (38–40). Activation of the RAS/MAPK pathway has also been associated with suppression of chondrocyte proliferation (41, 42), thus suggesting additional mechanisms by which activation of the RAS/MAPK pathway is associated with the development of short stature.

Relationships have been detected between short stature and genotypes. Zenker *et al*. observed shorter statures in patients with pathogenic variants of *PTPN11* compared to that in patients without these variants (20). A similar finding was reported in another study (18). As pathogenic variants have been most frequently detected in *PTPN11*, the relationship between short stature and variant locations in *PTPN11* was examined in the present study; however, no relationships with anthropometric parameters were observed. Malaquians *et al*. observed that shorter stature was more common in individuals with pathogenic variants of *RAF1* and *SHOC2*, whereas taller stature was more frequently observed in those possessing pathogenic variants of *SOS1* and *BRAF* (21). All subjects with pathogenic variants of *RAF1* or *SHOC2* in the present study consistently exhibited short stature; however, the number of subjects that were examined was limited. There is also evidence that individuals with pathogenic variants of *RIT1* exhibit taller stature than those with *PTPN11* or *RAF1* variants (27). Kouz *et al*. observed that 20 of 28 subjects possessing *RIT1* pathogenic variants exhibited a height-SDS > –2.0 (43). Similar findings have been reported by Bertola *et al*. (44). The present study also demonstrated that the frequency of short stature was low (25%) in the subjects with *RIT1* variants.

The underlying mechanisms that determine the genotype-phenotype association in the context of NS remain to be elucidated; however, the distinct activation capacity of each pathogenic variant in the RAS/MAPK pathway may be responsible, as each pathogenic gene in NS has been implicated in the activation of the RAS/MAPK pathway through distinct mechanisms (15). For example, SHP2 (Src Homology Region 2 Domain-containing Phosphatase-2), a product of *PTPN11*, functions as a phosphatase upstream of RAS activation, whereas KRAS, NRAS, and RIT1 belong to the RAS family that operates downstream of SHP2 in the RAS/MAPK pathway. Thus, pathogenic variants in *PTPN11* may exert differential effects on the RAS/MAPK pathway compared to those exerted by pathogenic variants in genes belonging to the RAS family. Additional mechanisms other than the RAS/MAPK pathway may be involved in the signaling pathways influenced by pathogenic variants. For example, in addition to its role in the activation of the RAS/MAPK pathway (45), RIT1 has been implicated in other signaling pathways, including the p38-dependent AKT pathway (46) and ELK transactivation (24). As demonstrated in the current study, individuals with *RIT1* variants exhibited distinct phenotypes from those with *PTPN11* variants. Thus, differences in signaling pathways beyond the RAS/MAPK pathways may contribute to phenotypic variations among genotypes in NS. However, it is important to note that this speculation lacks concrete evidence, and further studies are clearly required.

Based on evidence that activation of the RAS/MAPK pathway in the hypothalamus triggers the leptin-POMC pathway that results in suppressed appetite and increased metabolism (22), we also investigated BMI-SDS as a surrogate marker for nutritional status and bioenergetics in NS. Consistent with previous findings demonstrating reduced BMI-SDS in NS (21, 47, 48), the results obtained herein revealed a lower mean BMI-SDS in subjects clinically diagnosed with NS, and this was not significantly different from that reported by da Silva *et al*. (mean BMI: –0.4 ± 1.48 [N = 62]) ($p = 0.60$) (47). Despite consistent findings of a lower BMI in NS, the genotype-phenotype relationship in BMI varies among studies. Previous studies have reported that BMI is reduced in NS, with the lowest values observed in patients harboring pathogenic variants in *KRAS* or *SHOC2* (21, 47). In contrast, Cessans *et al*. reported no relationship between the genotype and BMI (48). Although we did not detect any differences in BMI-SDS among genotypes, the number of subjects with *KRAS* or *SHOC2* variants was very limited. Therefore, further studies are required to establish the genotype-phenotype relationship in BMI for NS.

The present study possessed several limitations that must be addressed. This study was performed at a single center, and therefore, the detection rate of


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pathogenic variants may have been biased. Furthermore, this single-center study only included a small number of subjects, thus leading to an inability to perform statistical analyses to detect genotype-phenotype relationships for recently identified genes. Additionally, this study is an extension of our previous study (16). Therefore, genotype-phenotype analysis (aside from anthropometric parameters) was not conducted to prevent potential breaches of ethical standards related to duplication. Anthropometric data regarding the height of parents were not available in most cases, and this may have created bias, as the anthropometry of parents is strongly associated with the height of their child. Moreover, adult height was not evaluated in this study due to insufficient data regarding adult height. Furthermore, data from nutritional interviews were not included in the study design, and therefore, we were unable to perform detailed analyses on the influence of nutritional status on BMI. Additional studies involving analyses of body composition, indirect calorimetry, and nutritional interviews are required to obtain a more detailed understanding of nutritional status and its impact on body composition in NS.

In conclusion, we investigated the genetic backgrounds of subjects clinically diagnosed with NS and provided evidence for the detection rate of pathogenic variants of genes involved in the RAF/MAPK signaling pathway. Data detailing genetic signatures were largely a recapitulation of previous findings from overseas. However, due to the paucity of Japanese data and the inclusion of recently identified genes in the analysis, the present study is of clinical importance, as it provides more accurate and updated knowledge of the genetic backgrounds of Japanese NS patients. Notably, the detection rate of the pathogenic genes was high in this study. As specialized geneticists were involved in the clinical diagnosis of NS, the results obtained herein indicate the importance of a thorough and comprehensive evaluation of clinical symptoms prior to genetic testing.

**Conflict of interests:** The authors have no conflicts of interest to disclose.

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