INTRODUCTION

Idiopathic short stature (ISS) is diagnosed by the exclusion of identifiable diseases, including growth hormone deficiency, chromosomal abnormalities, and other hormonal or nutritional disorders. However, novel genetic causes of ISS, which were not considered in the past, have been recently proposed owing to new diagnostic technologies (Kang, 2017; Wit et
al., 2016). These genetic defects include paracrine factors affecting the growth plate such as C-type natriuretic peptide (CNP) and its receptor (natriuretic peptide receptor-B, NPR-B) system.

The CNP is the third member of the natriuretic peptide family and was first purified in 1990 (Sudoh, Minamino, Kangawa, & Matsuo, 1990). It is highly expressed in brain, chondrocytes, and endothelial cells (Potter, Yoder, Flora, Antos, & Dickey, 2009). The NPR-B is the principal receptor of CNP and it is expressed in brain, kidney, liver, lung, uterus, and vascular smooth muscle tissue (Potter et al., 2009). Mice with a disruption in the CNP (Nppc) or NPR-B genes (Npr2) displayed dwarfism (Chusho et al., 2001; Tamura et al., 2004), and an overexpression of Nppc or NPR2 showed overgrowth (Miura et al., 2012; Yasoda et al., 2004), which all suggest their critical roles in skeletal growth. In humans, the homologous loss-of-function mutation in NPR2 is well-known as acromesomelic dysplasia, type Maroteaux (AMDM, OMIM# 602875) (Bartels et al., 2004).

The potential role of NPR2 in ISS patients was identified during family studies of AMDM patients. Bartels et al. (2004) found that 30 adult carriers of AMDM were on average 5.7 cm shorter than the general population. Olney et al. (2006) observed a single family of 1 AMDM patient and found that 16 adult mutation carriers (mean height z-score of −1.8) were significantly shorter than 23 noncarriers (mean height z-score of −0.4). The average reduction in adult height of carriers was 9 cm for women and 10 cm for men compared to the general population (Olney et al., 2006). Several studies have reported the prevalence of heterozygous NPR2 mutations in 2%–6% of patient with nonsyndromic short stature (Amano et al., 2014; Vasques et al., 2013; Wang et al., 2015).

Based on this evidence, we conducted an NPR2 and NPPC mutation study along with functional analysis in Korean patients with ISS to identify the prevalence of heterozygous mutations in the CNP and NPR-B system and to confirm their associations with short stature.

2 | METHODS

2.1 | Subjects

One hundred and sixteen ISS subjects (60 males and 56 females) who visited the Hallym Medical Center and Korea Medical Center due to their short stature were enrolled. ISS was diagnosed after satisfying all of the following criteria: height z-score < −1.88; peak growth hormone (GH) after stimulation ≥7.0 ng/ml (Hawkes, Grimberg, Dzata, & De Leon, 2016; Wit et al., 2008); full term (gestational age between 37 and 41 weeks); normal birth weight (between 2.5 and 4.2 kg); and no documented systemic, endocrine, nutritional, or chromosomal abnormalities. Medical records were reviewed to check anthropometric measurements (at baseline and during GH treatment), bone age (BA), and laboratory test results, including a GH stimulation test. Using data from 94 subjects treated with GH, growth velocity (cm/year) was calculated before and after 1 year of GH treatment. If GH stimulation test results were not available, growth velocity before GH treatment was checked and only 20 prepubertal subjects who showed normal growth velocity (>4 cm/year) were included. Height was measured twice to the first decimal place with a Harpenden stadiometer (Holtain Ltd.) and weight to the first decimal place with a digital scale (150A, Cas Co. Ltd.). The z-scores for height, weight, and body mass index (BMI) were calculated using the 2017 Korean growth standard (Kim et al., 2018).

Written informed consents were obtained from all subjects and their parents before enrollment. If the variant was detected, blood samples were also obtained from consenting family members. The institutional Review Boards (IRB) of Hallym Medical Center (IRB# KANGDONG 2017-07-003) and Korea Medical Center (IRB# 2015GR0134) approved this study.

2.2 | Gene sequencing for NPPC and NPR2

Genomic DNA was isolated from peripheral blood leukocytes by following the manufacturer’s instructions (Qiagen, QIAGEN GmbH). Primers were designed to amplify all exons and exon–intron boundaries according to the published NPPC and NPR2 genomic DNA sequences (GenBank accession number: NC_000009.12 for NPPC; NC_000002.12 for NPR2) (Tables S1 and S2). PCR products were bidirectionally sequenced and nested PCR was performed for the NPPC (Table S2). Variants were filtered with references from the dbSNP and Korean reference genome databases (http://coda.nih.go.kr/coda/KRGDB). The locations of mutations in the NPR-B protein were assumed by a graphic model illustrated previously (Irfanullah et al., 2018).

2.3 | In silico analysis and variant classification

To predict the possible impact of sequence variants on the protein function or structure, Mutation Taster (http://mutationtaster.org) (Schwarz, Rodelsperger, Schuelke, & Seelow, 2010), PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2) (Ramensky, Bork, & Sunyaev, 2002), Mutation Assessor (http://mutationassessor.org) (Reva, Antipin, & Sander, 2011), and SNPs & GO (http://snps-and-go.biocomp.unibo.it/snps-and-go) (Capriotti et al., 2013) programs were used.
We also performed the American College of Medical Genetics and Genomics (ACMG)/Association for Molecular Pathology (AMP) criteria (Richards et al., 2015) to classify the variants. We interpreted the sequence variant according to the ACMG/AMP guidelines using the InterVar web site (http://wintervar.wglab.org) and performed manual adjustments.

2.4 | In vitro functional study

In vitro functional analysis was performed at Keio University (Tokyo, Japan). The hemagglutinin (HA)-tagged wild-type (WT) human NPR-B construct (HA-WT-NPR-B) has been described previously (Hachiya et al., 2007). Mutation expression constructs for novel variants (R495C, Y598N) were generated by site-directed mutagenesis (QuickChange XL site-directed mutagenesis kit; Agilent Technologies). A known pathogenic mutation (R110C) was used as positive control (Amano et al., 2014). Sequences of the constructs were confirmed by direct sequencing (mutagenic primer sequences are available on request). COS-7 cells were grown in DMEM supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. Cells were transiently transfected with Lipofectamine 2000 (Life Technologies) according to the manufacturer’s protocol.

COS-7 cells, seeded in 12-well plates, were transiently transfected with WT, R110C, mutants, or empty vector (1,000 ng of plasmid per well). To evaluate a dominant-negative effect of the novel NPR-B mutants, COS-7 cells were cotransfected with constant amounts of HA-WT-NPR-B cDNA (500 ng/well) plus empty vector or plus each mutant NPR-B cDNA at a WT to mutant ratio of 1:1. Forty-eight hours after transfection, the cells were incubated with or without 100 nM CNP-22 (Bachem, Ltd) in Optimem (Invitrogen) for 20 min at 37°C. Then 0.1 M HCl was added to stop the reaction, and cells were centrifuged at 600 × g for 10 min. cGMP in the supernatant (pmol/ml) was measured via a competitive enzyme immunoassay according to the manufacturer’s protocol (cGMP Complete; Enzo Life Science). All experiments were performed three times independently.

2.5 | Statistical analyses

Clinical data were expressed as mean±standard deviation. Sexual differences of clinical parameters were analyzed using the student’s t test. Comparisons of growth velocity before and after 1 year of GH treatment were analyzed using a paired t test. cGMP concentrations in transfected cells were expressed as mean and standard error of mean. Differences in cGMP concentrations were analyzed using Welch’s t test. A value of p < .05 was considered statistically significant.

3 | RESULTS

3.1 | Clinical characteristics of subjects

Mean height z-score of total 116 subjects was −2.65 and males were higher than females (p = .026), but weight, BMI, parental heights, and other clinical parameters were not different between males and females. Sixty-two percent (72/116) of ISS subjects had delayed bone ages more than 1 year compared to their chronological ages. Thirty-five percent (41/116) of the subjects had at least one short parent with height z-score < −1.88. Significant height improvement was obtained after 1 year of GH treatment in 94 ISS subjects (p < .001) (Table 1).

3.2 | Sequencing results

Four different heterozygous variants in NPR2 were identified in four ISS subjects. Three variants were located in the intracellular region of the kinase homology domain (c.2359C>T, R787W and c.1792T>A, Y598N) and the guanylyl cyclase domain (c.2762G>A, R921Q). One variant (c.1483C>T, R495C) was located in the transmembrane domain. In vitro functional studies involving one benign variant (R787W, unchanged guanylyl cyclase activity) (Wang et al., 2015) and one pathogenic mutant (R921Q, decreased guanylyl cyclase activity) (Wang et al., 2016) have been previously reported. Two novel variants (R495C and Y598N), not found in dbSNP nor Korean reference genome databases, were identified in this study. R495C and Y598N were suggested to be highly pathogenic by in silico prediction models. No novel sequence variant in the NPPC was found in all ISS subjects (Table 2).

3.3 | In vitro functional analysis results

COS-7 cells were transfected with HA-WT-NPR-B, HA-R110C-NPR-B, HA-R495C-NPR-B, HA-Y598N-NPR-B, or an empty vector. Treatment with CNP at a dose of 100 nM increased intracellular cGMP levels by 59-fold in HA-WT-transfected cells (70.5 ± 12.9 pmol/ml) compared to empty vector-transfected cells (1.2 ± 0.1 pmol/ml). cGMP productions after stimulation with CNP decreased significantly in HA-R110C-, HA-R495C-, or HA-Y598N-transfected cells (2.0 ± 0.1, 3.6 ± 0.8, or 2.6 ± 0.3 pmol/ml respectively) compared to those of HA-WT-transfected cells (Figure 1a, all p < .05).

To confirm a dominant-negative effect of the novel NPR-B mutants, HA-R495C or HA-Y598N were
coexpressed with the WT. Intracellular cGMP levels increased by 37-fold in HA-WT+empty vector-transfected cells (69.3 ± 4.6 pmol/ml) compared to empty vector-transfected cells (1.9 ± 0.2 pmol/ml). HA-WT+R495C- or HA-WT+Y598N-transfected cells showed decreased cGMP production (15.7 ± 2.1 or 24.4 ± 2.0 pmol/ml respectively) compared to HA-WT+empty vector-transfected cells (Figure 1b, all p < .05).

### 3.4 Clinical phenotypes of three subjects with NPR2 heterozygous mutants and their family analyses

#### 3.4.1 Case 1 with R921Q

At age 10.3 years, he was diagnosed with ISS, presenting a height z-score of −2.57 and 0.8 years of mild delayed BA.

| TABLE 1 Clinical characteristics of subjects |
|------------------------------------------------|
| **Total (n = 116)** | **Male (n = 60)** | **Female (n = 56)** |
| Age | 8.0 ± 3.2 | 8.3 ± 3.3 | 7.7 ± 3.1 |
| Height z-score | −2.65 ± 0.40 | −2.57 ± 0.33 | −2.73 ± 0.45 |
| Weight z-score | −1.99 ± 0.73 | −2.02 ± 0.74 | −1.96 ± 0.72 |
| BMI z-score | −0.59 ± 0.95 | −0.71 ± 0.95 | −0.45 ± 0.95 |
| BA-CA (year) | −1.5 ± 1.1 | −1.7 ± 1.2 | −1.3 ± 1.0 |
| Gestational age (year) | 39.4 ± 1.0 | 39.3 ± 1.1 | 39.4 ± 1.0 |
| Birth weight (kg) | 3.1 ± 0.3 | 3.1 ± 0.3 | 3.0 ± 0.3 |
| Father’s height z-score | −0.96 ± 1.02 | −0.86 ± 1.03 | −1.07 ± 1.00 |
| Mother’s height z-score | −1.04 ± 1.05 | −1.11 ± 1.10 | −0.97 ± 1.00 |
| MPH z-score | −0.99 ± 0.77 | −0.95 ± 0.77 | −1.04 ± 0.79 |
| Peak GH (ng/ml) | 16.3 ± 10.2 | 15.4 ± 8.0 | 17.4 ± 12.2 |
| IGF-1 SDS | −0.79 ± 0.73 | −0.68 ± 0.86 | −0.89 ± 0.57 |
| IGFBP-3 SDS | 1.99 ± 1.81 | 1.87 ± 1.90 | 1.88 ± 1.72 |
| Pre-GH treatment GV (cm/year) | 5.0 ± 1.1 | 4.8 ± 1.2 | 5.2 ± 1.0 |
| Post-GH treatment GV (cm/year)* | 8.9 ± 1.6 | 8.7 ± 1.8 | 9.1 ± 1.4 |

Note: Data are expressed as mean±standard deviation.

Abbreviations: BA, bone age; BMI, body mass index; CA, chronological age; GH, growth hormone; GV, growth velocity; IGF-1, insulin-like growth factor-1; IGFBP-3, insulin-like growth factor-binding protein-3; MPH, mid-parental height; SDS, standard deviation score.

*Growth velocity during the first year of GH treatment.

| TABLE 2 Four identified NPR2 variants |
|---------------------------------------|
| Variant | Protein | Exon # | Location in receptor protein | SNP # | Polyphen-2 score | Mutation taster | Mutation assessor | SNPs and GO | ACMG/AMP: InterVar-adjusted |
|---------|---------|--------|--------------------------------|------|-----------------|----------------|-----------------|-------------|-----------------------------|
| c.2359C>T | R787W | Exon 15 | Kinase homology domain | rs114147262 | 0.998 | Heterozygous in TGP or ExAC | Medium (score, 3.14) | Disease US |
| c.2762G>A | R921Q | Exon 19 | Guanylyl cyclase domain | rs770276670 | 0.997 | Protein features (might be) affected | Medium (score, 2.30) | Disease P |
| c.1483C>T | R495C | Exon 8 | Transmembrane domain | novel | 0.999 | Protein features (might be) affected | Medium (score, 2.57) | Disease P |
| c.1792T>A | Y598N | Exon 11 | Kinase homology domain | novel | 1.000 | Protein features (might be) affected | High (score, 4.31) | Disease P |

Abbreviations: ACMG/AMP, American College of Medical Genetics/Association for Molecular Pathology; ExAC, exome aggregation consortium; P, pathogenic; SNP, single-nucleotide polymorphism; TGP, 1000 genome project; US, uncertain significance.
GH stimulation test result was not available but basal IGF-1 and IGFBP-3 levels were within reference ranges, and his growth velocity was 4.5 cm/year. Hand x rays showed short middle phalanx of the fifth fingers but no other evidence of skeletal dysplasia was found. The sitting height: total height ratio was 0.53 which was measured at his age of 13.0 years. Height z-scores of his father, mother, and his older sister (18 years old) were −2.11, −1.69, and −3.28 respectively. Sitting height: total height ratios of his father, mother, and his older sister were 0.55, 0.54, and 0.56 respectively. On molecular study of NPR2, his father and sister showed the same variant, c.2762G>A, and his mother was wild type. Growth velocity improved by 8.0 cm/year during the first year of GH treatment with a GH dose of 0.97 IU/kg/wk. GH injection was discontinued after 5 years and 9 months of treatment at his age of 16.0 years. His height z-score was −0.11 and BA was 16.0 years. After 6 months of GH discontinuation, height z-score was −0.02 at his age of 16.5 years (Table 3, Figure 2).

### 3.4.2 Case 2 with R495C

At age 9.5 years, he was diagnosed with ISS, presenting a height z-score of −2.49 and 2.0 years of delayed BA. Basal IGF-1 and stimulated GH levels were within reference ranges, but the basal IGFBP-3 level was elevated. No evidence of skeletal dysplasia was found in hand x rays. Height z-scores of his father and mother were −0.81 and 0.59 respectively. Molecular study of NPR2 was not performed in his family members because they refused genetic testing. He was born as a twin at full term and his birth weight was 3.0 kg. Growth velocity improved by 8.0 cm/year during the first year of GH treatment with a GH dose of 0.87 IU/kg/wk. On his last visit, he was 11.7 years old and his height z-score was −1.29. He was still prepubertal and showed 1.8 years of delayed BA.

### 3.4.3 Case 3 with Y598N

At age 12.1 years, he was diagnosed with ISS, presenting a height z-score of −2.77 and 2.6 years of delayed BA. Stimulated GH response was at the lower limit of normal ranges but basal IGF-1 and IGFBP-3 levels were within reference ranges. He was prepubertal at the time of diagnosis and his growth velocity was 4.7 cm/year. No evidence of skeletal dysplasia was found in hand x rays. Height z-scores of his father and mother were −0.81 and −1.91 respectively. On molecular study of NPR2, his mother showed the same variant, c.1792T>A. Growth velocity improved by 7.2 cm/year during the first year of GH treatment with a GH dose of 0.84 IU/kg/wk, but treatment response was blunted afterward. On his last visit, he was 13.8 years old and his height z-score was −2.28. The testicular volumes measured by the Prader orchidometer showed 3–4 ml and his BA was 13 years.
In this study, we identified three pathogenic mutants (R921Q, R495C, and Y598N) and one benign variant (R787W) of the \( \text{NPR2} \) in 116 Korean ISS subjects. Two pathogenic mutants (R495C and Y598N) were novel. Functional studies revealed a dominant-negative effect of two novel mutants which suppressed CNP-dependent cGMP production in vitro.

Although the height \( z \)-score criteria of ISS varied between \(-2.25\) to \(-1.80\), recent studies have reported that the prevalence of a heterozygous loss-of-function mutation of the \( \text{NPR2} \) in ISS patients were 2% to 6%, with or without abnormal body proportions (Amano et al., 2014; Hisado-Oliva et al., 2015; Olney et al., 2006; Vasques et al., 2013), which were similar to our observation (2.6%, 3 pathogenic mutants of 116 ISS subjects). However, the rate of probably damaging \( \text{NPR2} \) mutations, as estimated by large databases (1000 Genome Project, NHLBI Exome Sequencing Project), is 0.4% to 0.9% in the general population (Vasques et al., 2013; Wang et al., 2015). ISS includes a diverse group of people with short stature, such as familial short stature or constitutional delay of growth. In this study, 35% of 116 subjects, who had at least one short parent and two \( \text{NPR2} \) mutation cases (R921Q and Y598N), had family histories of short stature. Olney et al. (2006) studied 39 family members of an AMDM patient and found 41% of them were carriers. Wang et al., (2015) reported a prevalence of \( \text{NPR2} \) haploinsufficiency in 13.6% of familial cases. Therefore, \( \text{NPR2} \) screening would considerably help clinicians to find the cause of ISS, especially patients with familial short stature.

| TABLE 3 | Clinical characteristics of four ISS subjects with identified \( \text{NPR2} \) heterozygous variants |
|-----------------|-----------------|-----------------|
| **Variant**     | **Case 1**      | **Case 2**      | **Case 3**      |
|                 | c.2762G>A       | c.1483C>T       | c.1792T>A       |
| **Sex**         | M               | M               | M               |
| **Before GH treatment** |                  |                  |                  |
| Age (years)     | 10.3            | 9.5             | 12.1            |
| Height \( z \)-score | -2.57           | -2.49           | -2.77           |
| BMI \( z \)-score | -1.19           | -0.86           | 0.43            |
| BA-CA (years)   | -0.8            | -2.0            | -2.6            |
| Peak GH (ng/ml) | NA              | 23.6            | 7.3             |
| IGF-1 SDS       | -1.05           | 1.59            | -1.23           |
| IGFBP-3 SDS     | -1.62           | 4.53            | -0.88           |
| Pre-GH treatment GV (cm/year) | 4.5             | 5.4             | 4.7             |
| **After 1st year of GH treatment** |                  |                  |                  |
| Height \( z \)-score | -1.85           | -1.81           | -2.49           |
| BMI \( z \)-score | -0.86           | -1.13           | 0.32            |
| GH dose (mg/kg/wk) | 0.32            | 0.29            | 0.28            |
| IGF-1 SDS       | 1.04            | 2.50            | -0.72           |
| IGFBP-3 SDS     | 0.20            | 4.90            | 1.18            |
| GV during the 1st year of GH treatment (cm/year) | 8.0             | 8.0             | 7.2             |
| **At last visit during GH treatment** |                  |                  |                  |
| Age (years)     | 16.0            | 11.8            | 13.8            |
| Height \( z \)-score | -0.11           | -1.29           | -2.28           |
| BMI \( z \)-score | 0.34            | -1.22           | 0.17            |
| BA-CA (years)   | 0.0             | -1.8            | 0.8             |
| GH dose (mg/kg/wk) | 0.22            | 0.32            | 0.31            |
| IGF-1 SDS       | 1.97            | 1.40            | -1.39           |
| IGFBP-3 SDS     | -1.57           | 4.62            | 0.03            |

Abbreviations: BA, bone age; BMI, body mass index; CA, chronological age; F, female; GH, growth hormone; GV, growth velocity; IGF-1, insulin-like growth factor-1; IGFBP-3, insulin-like growth factor-binding protein-3; M, male; NA, not available; SDS, standard deviation score.

\( ^{a} \)Corresponds to age at diagnosis of idiopathic short stature.

4 | DISCUSSION

In this study, we identified three pathogenic mutants (R921Q, R495C, and Y598N) and one benign variant (R787W) of the \( \text{NPR2} \) in 116 Korean ISS subjects. Two pathogenic mutants (R495C and Y598N) were novel. Functional studies revealed a dominant-negative effect of two novel mutants which suppressed CNP-dependent cGMP production in vitro.

Although the height \( z \)-score criteria of ISS varied between \(-2.25\) to \(-1.80\), recent studies have reported that the prevalence of a heterozygous loss-of-function mutation of the \( \text{NPR2} \) in ISS patients were 2% to 6%, with or without abnormal body proportions (Amano et al., 2014; Hisado-Oliva et al., 2015; Olney et al., 2006; Vasques et al., 2013), which were similar to our observation (2.6%, 3 pathogenic mutants of 116 ISS subjects). However, the rate of probably damaging \( \text{NPR2} \) mutations, as estimated by large databases (1000 Genome Project, NHLBI Exome Sequencing Project), is 0.4% to 0.9% in the general population (Vasques et al., 2013; Wang et al., 2015). ISS includes a diverse group of people with short stature, such as familial short stature or constitutional delay of growth. In this study, 35% of 116 subjects, who had at least one short parent and two \( \text{NPR2} \) mutation cases (R921Q and Y598N), had family histories of short stature. Olney et al. (2006) studied 39 family members of an AMDM patient and found 41% of them were carriers. Wang et al., (2015) reported a prevalence of \( \text{NPR2} \) haploinsufficiency in 13.6% of familial cases. Therefore, \( \text{NPR2} \) screening would considerably help clinicians to find the cause of ISS, especially patients with familial short stature.

When CNP binds to NPR-B, dimerization of NPR-B activates guanylyl cyclase in the cytosolic domain, and then, cGMP activates the type II cGMP-dependent protein kinase.
This antagonizes the fibroblast growth factor receptor 3 signaling by inhibiting the MAPK pathway. Therefore, CNP–NPR-B signaling increases the proliferation and differentiation of chondrocytes (Olney, 2006). Some previous studies investigated possible mechanisms by which NPR2 mutants exert their loss-of-function effects according to their location in the NPR-B (Amano et al., 2014; Vasques et al., 2013). Those mechanisms include glycosylation impairments, endoplasmic reticulum retention of receptor, protein misfolding, or trafficking error from the plasma membrane/endoplasmic reticulum to Golgi apparatus. However, no definite genotype–phenotype correlation was found. In this study, all three mutations occurred at different locations in NPR-B; R921Q, guanylyl cyclase domain; R495C, transmembrane domain; and Y598N, kinase homology domain. Regardless of its location in the receptor protein, all mutant constructs showed decreased cGMP production after stimulation with CNP. And there were no differences in cGMP levels between R495C- and Y598N-construct transfected cells (data not shown).

Human recombinant GH treatment has been approved by the Food and Drug Administration in ISS patients. From a clinical point of view, response to GH treatment is an important factor in deciding whether to keep or terminate GH therapy. In this study, case 1 showed an excellent response to GH treatment, case 2 showed partial catch-up growth, while, case 3 showed a disappointing response. In previous studies of ISS patients with NPR2 mutations, height improvement by GH treatment was

**FIGURE 2** Pedigrees of families with NPR2 variants and their sequencing results: (a) R921Q, (b), R495C, and (c) Y598N. Height z-scores of each family member are described in symbols. Subjects with confirmed NPR2 variants by molecular study are indicated by solid symbols, while those unconfirmed by molecular study or that do not have normal result are indicated by open symbols. For comparisons, reference sequences of the wild type (WT) are also presented.
quite variable (Hisado-Oliva et al., 2015; Vasques et al., 2013, 2017; Wang et al., 2015). Parental height, IGF-1 or IGFBP-3 levels before GH treatment, or BA delay, which are factors known to be correlated with GH response, could not be applied in our cases. Therefore, it is still uncertain whether GH treatment could effectively improve height in short patients with NPR2 haploinsufficiency, but it should be attempted.

We also screened for the NPPC mutation but no novel variant was found. In humans, the NPPC is not well known to date. However, Hisado-Oliva et al. (2018) recently identified and reported, for the first time, two heterozygous NPPC mutations in two families with proportionate short stature and small hands. The prevalence of NPPC mutations in their cohort was about 0.6%, but the prevalence is expected to be much lower in an unselected ISS cohort, assuming there are less than a fifth of NPR2 mutations (Hisado-Oliva et al., 2018). A new therapy involving a CNP analog is now under clinical trials in some short patients with skeletal dysplasia (Vasques et al., 2014). That ISS patients who have CNP−NPR-B signaling problem could get help from a new therapy is promising (Hisado-Oliva et al., 2018).

In conclusion, heterozygous NPR2 mutations were found in 2.6% of ISS Korean subjects. This prevalence and the dominant-negative effect of mutant NPR-B on growth signals imply that it is definitely one of the genetic causes of ISS. Since numerous factors are involved in growth retardation, clinicians could get help from novel insights into the genetic causes of ISS, especially when their nonsyndromic short stature patients do not respond well to conventional GH treatment.

CONFLICT OF INTEREST
This study was supported and funded by the National Research Foundation of Korea (NRF-2015-054988) and the Foundation for Growth Science.

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**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section.