Clinical Research Article

Familial Short Stature—A Novel Phenotype of Growth Plate Collagenopathies

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Abbreviations: ACMG, American College of Medical Genetics and Genomics; COL, collagen type; FSS, familial short stature; GH, growth hormone; GHD, growth hormone deficiency; NGS, next-generation sequencing; SGA, small for gestational age; SH/H ratio, sitting height to total height ratio; SHOX-D, SHOX deficiency.

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Abstract

Context: Collagens are the most abundant proteins in the human body. In a growth plate, collagen types II, IX, X, and XI are present. Defects in collagen genes cause heterogeneous syndromic disorders frequently associated with short stature. Less is known about oligosymptomatic collagenopathies.

Objective: This work aims to evaluate the frequency of collagenopathies in familial short stature (FSS) children and to describe their phenotype, including growth hormone (GH) treatment response.

Methods: Eighty-seven FSS children (pretreatment height ≤ –2 SD both in the patient and his or her shorter parent) treated with GH were included in the study. Next-generation sequencing was performed to search for variants in the COL2A1, COL9A1, COL9A2, COL9A3, COL10A1, COL11A1, and COL11A2 genes. The results were evaluated using American College of Medical Genetics and Genomics guidelines. The GH treatment response of affected children was retrospectively evaluated.

Results: A likely pathogenic variant in the collagen gene was found in 10 of 87 (11.5%) children. Detailed examination described mild asymmetry with shorter limbs and mild bone dysplasia signs in 2 of 10 and 4 of 10 affected children, respectively. Their growth velocity improved from a median of 5.3 cm/year to 8.7 cm/year after 1 year of treatment. Their height improved from a median of –3.1 SD to –2.6 SD and to –2.2 SD after 1 and 3 years of therapy, respectively. The final height reached by 4 of 10 children differed by –0.67 to +1.0 SD and –0.45 to +0.5 SD compared to their pretreatment height and their affected untreated parent’s height, respectively.
Conclusion: Oligosymptomatic collagenopathies are a frequent cause of FSS. The short-term response to GH treatment is promising.

Key Words: familial short stature, growth plate, collagenopathies, next-generation sequencing, growth hormone treatment

Collagens are the most abundant proteins in the human body. They play an important structural role and by interacting with cellular receptors, they also participate in the regulation of cell growth, differentiation, and migration. The collagen family comprises 28 members, each exhibiting a high degree of tissue specificity. Collagen types II, IX, X, and XI are present in the extracellular matrix of a growth plate (1).

Collagen type II (COL2) is the major type of collagen synthesized by chondrocytes. It is a homotrimer composed of 3 identical chains encoded by the COL2A1 gene (1, 2). On the other hand, collagen type XI (COL11) is a heterotrimer composed of 3 different chains: alpha 1 chain (COL11A1 gene), alpha 2 chain (COL11A2 gene), and alpha 3 chain (COL2A1 gene—the final product is more extensively posttranslationally modified compared to COL2 molecule) (1, 3). The COL2 and COL11 molecules together form collagen fibrils, each consisting of a core and an envelope. The core is made of 2 COL2 microfibrils and 2 COL11 microfibrils. The core is surrounded by an envelope made of 10 COL2 microfibrils (4). Collagen type IX (COL9) is also a heterotrimer encoded by the COL9A1, COL9A2, and COL9A3 genes. COL9 does not form fibrils. It is attached to the surface of COL11 and is crucial for tissue integrity and for interaction with other proteins within the extracellular matrix (1, 5). Type X collagen (COL10) consists of 3 identical chains encoded by the COL10A1 gene. COL10 expression is limited to the matrix of hypertrophic chondrocytes. It forms a membrane-like structure around each chondrocyte and is thought to facilitate the process of calcification and endochondral ossification (6).

The defects in individual collagen molecules cause various types of growth plate disorders that are frequently associated with short stature (1, 6). Mutations in the COL2A1 gene are known to cause 16 different syndromic bone dysplasias with significant clinical heterogeneity (eg, Kniest dysplasia, Stickler syndrome, achondrogenesis type 2). Bone dysplasia signs (eg, disproportionate short stature, scoliosis, brachydactyly, metaphyseal abnormalities), facial stigmatization (eg, cleft palate, midface hypoplasia), ocular complications (eg, myopia, retinal detachment), sensorineural hearing loss, and joint deformities are common (2, 7). Heterozygous mutations in the COL11A1 gene are known to cause Stickler syndrome, Marshal syndrome, and phenotypes overlapping both disorders (8). In addition to the syndromic forms, COL11A2 mutations cause nonsyndromic cleft palate and nonsyndromic hearing loss (9). Heterozygous mutations in COL9 genes cause multiple epiphyseal dysplasia (10), which can be associated with proximal muscle weakness (11). Heterozygous mutations in the COL10A1 gene cause Schmid-type metaphyseal chondrodysplasia characterized by short stature, widened growth plates, and bowing of the long bones (12). The characteristic phenotypes of specific collagenopathies are summarized in Supplementary Materials Table 1 (13).

As described earlier, growth plate collagenopathies are known to cause very heterogenic syndromic disorders. On the other hand, we have recently described that mutations in genes encoding various collagen molecules may also cause nonsyndromic short stature (14). Little is known about the frequency and subtle phenotypic signs of various collagenopathies in children with apparent nonsyndromic short stature, and data about their response to growth hormone (GH) treatment are lacking. The aim of our study was to evaluate the frequency of collagenopathies using next-generation sequencing (NGS) methods among children with familial short stature (FSS). In addition, we aimed to describe their phenotype including the response to GH treatment.

Materials and Methods

Patients

Inclusion criteria

The database of children treated with GH in our center currently includes 747 individuals. After exclusion of patients with known SHOX deficiency (SHOX-D), Turner syndrome, and Prader-Willi syndrome (genetic diagnoses established in routine practice prior to the study after clinical suspicion of a specific genetic disorder) and those with secondary causes of their short stature (eg, chronic renal insufficiency, secondary GH deficiency due to intracranial tumor and/or irradiation), 522 children remained for further evaluation. Within this group, 117 children had FSS defined as life-minimum height less than or equal to ~2 SD both in the patient and his or her shorter parent. In 87 children with FSS, their legal guardians agreed with genetic testing and were enrolled in the study. All study participants or their legal guardians signed written informed consent prior to genetic testing. The study was approved.
Clinical evaluation prior to the study
The heights of all the children were obtained by anthropometric measurement, which also included body proportionality (sitting height to total height ratio; SH/H ratio). The birth parameters were obtained from medical records. All parental heights were measured to the nearest 1 mm, and the heights of more distant relatives were reported by the parents. All the data were standardized according to recent normative values (15-17). Growth hormone deficiency (GHD) and small for gestational age (SGA) were evaluated according to current guidelines (18, 19) as previously described (14).

The median age of children with FSS at inclusion in the study was 12 years (range, 5-19 years), and their life-minimum height was –3.0 SD (–2.1 to –6.3 SD), and their shorter parent height was –2.6 SD (–2.0 to –4.2 SD). The children had been treated with GH for 5 years (median; range, 1-14 years) with an initial dose of 33 µg/kg/day (median; range, 21-45 µg/kg/day). Within this group, 59 of 87 (68%) children were classified as GHD. Their birth weights ranged from –0.6 to –3.0 SD (median –2.1 SD), and their birth lengths ranged from –1.2 to –4.7 SD (median –2.6 SD). Twenty-three children (26%) were classified as having combined GHD and SGA.

Genetic Examination
Genomic DNA was extracted from peripheral blood in all patients included in the study. DNA from 28 patients with severe FSS (life-minimum height < –2.5 SD both in the patient and his or her shorter parent) was analyzed using whole-exome sequencing, and DNA of the remaining 59 patients was analyzed using a custom-targeted NGS panel of 398 genes known or potentially associated with growth (Supplementary material [13]). The genetic analysis was described in detail in our previous articles (14,20).

Evaluation of the Genetic Results
All variants in the COL2A1, COL9A1, COL9A2, COL9A3, COL10A1, COL11A1, and COL11A2 genes obtained from the NGS examination were confirmed using Sanger sequencing as described previously (21) and subsequently evaluated using the American College of Medical Genetics and Genomics (ACMG) standards and guidelines (22). For evaluation of population frequencies of evaluated variants, the Exome Aggregation Consortium database was used. The Exome Aggregation Consortium European frequency of less than 0.01% was considered sufficiently low to support the pathogenicity of the variant. For the evaluation of segregation of genetic variants with short stature within the families, DNA and height information about distant relatives was obtained from the probands’ parents. Finally, all variants were classified based on ACMG classification as pathogenic, likely pathogenic, benign, likely benign, or as variants of uncertain significance.

Evaluation of the Phenotype of Parents With Causative Collagen Gene Variants
All the parents with causative collagen gene variants were asked to undergo detailed anthropometric measurements focused on body proportionality (SH/H ratio) and on clinical signs of bone dysplasia. Their SH/H ratio data were standardized compared to the same normative data as their children (17).

Results
In 16 of 87 (18.4%) GH-treated FSS children, we found a genetic variant in collagen genes with potential clinical significance. Finally, pathogenic or likely pathogenic variants were identified in 10/87 (11.5%) patients. Five patients...
had variants in the \( \text{COL2A1} \) gene, 4 had variants in the \( \text{COL11A1} \) gene, and a single patient had a variant in the \( \text{COL11A2} \) gene. Genetic variants in 2 children were classified as (likely) benign - in the \( \text{COL2A1} \) and \( \text{COL9A2} \) genes. The variants in the remaining 4 children were classified as variants of uncertain significance (\( \text{COL9A2} \) gene in 2, \( \text{COL10A1} \) and \( \text{COL11A1} \) genes each in a single patient). The results are summarized in Table 1. For detailed information about variant evaluation including their population frequencies in specific patients and about noncausative collagen gene variants, see the Supplementary materials [13].

In the 10 children with proven collagenopathies, the median age at the last check-up was 12.5 years (range, 6-17 years), and their height before GH treatment was –3.1 SD (–2.4 to –4.3 SD). The birth length (median =–2.8 SD; range, –0.7 to –4.1 SD) was more severely affected than birth weight (median =–2.1 SD; range, –1.0 to –2.7 SD). Eight children (80%) were born SGA. The remaining 2 of 10 had birth lengths or weights between –2.0 and –1.0 SD and were indicated for GH treatment as mild GHD (maximum level of stimulated GH was 8.0 and 9.7 µ/L). The brain magnetic resonance imaging and the examination of other pituitary hormones of both GHD patients were normal. Detailed anthropometric examination described proportional short stature (SH/H ratio within the range 0.99 to 0.99 SD) in 8 of 10 affected children; the remaining 2 of 10 children had mildly shorter limbs (SH/H ratio 1.2 and 1.9 SD, respectively). Moreover, 4 of 10 affected children had mild signs of bone dysplasia discovered through detailed examinations (scoliosis, more pronounced lumbar lordosis, genua valga, limited elbow extension), 2 of 10 affected children had myopia, and none had hearing problems. For more detailed information about the phenotype in specific patients, see Table 1. Subgroups of children with \( \text{COL2} \) and \( \text{COL11} \) causative variants did not differ significantly in any of evaluated clinical parameters (for a detailed evaluation, see Supplementary Table 2 [13]).

The affected parents of patients with proven collagenopathy with the corresponding causative collagen gene variant had a median height of –2.8 SD (range, –2.2 to –3.5 SD). Nine of them (90%) agreed to undergo detailed anthropologic examination. The parents had more pronounced body disproportionality than their affected children. Only 2 of 9 had clearly proportionate short stature (SH/H ratio –0.2 SD and –0.6 SD, respectively), and 5 of 9 affected parents had mildly shorter limbs (SH/H ratio 1.0-2.0 SD). The remaining 2 of 9 parents had disproportionate short stature -1 with shorter limbs (SHH ratio 2.9 SD) and 1 with a shorter trunk (SHH ratio –3.1 SD). One affected parent had bilateral Perthes disease and saber-like tibia, another had brachydactyly, and none had severe vision or hearing problems.

GH treatment of affected children was initiated at age 6 years (median; range, 3-13 years) with a dose of 34 µg/kg/day (range, 32-37 µg/kg/day). Their GV improved from 5.3 cm/year (range, 3.8-7.4 cm/year) in the year prior to GH treatment to 8.7 cm/year (range, 6.0-10.3 cm/year) in the first year of therapy (\( P < 0.001 \)). Importantly, patients 3 and 7 had apparently normal GV prior to GH treatment (6.8 cm/year and 7.4 cm/year, respectively); their subsequent GV increase was not therefore high (+1.9 cm/year in patient 3; patient 7 even mildly reduced her GV by 0.1 cm/year after GH was started). The height of children with proven collagenopathies improved from a median of –3.1 SD (range, –2.4 to –4.3 SD) at the time of GH treatment initiation to –2.6 SD (–2.0 to –3.6 SD) after 1 year of therapy (\( P < 0.001 \)) and to –2.2 SD (–0.8 to –2.9 SD) after 3 years of therapy (completed in 8 of 10 children; \( P = 0.001 \)). The height development in the first 3 years of GH treatment is summarized in Figure 1. The final height was reached in 4 of 10 children. Three of them (patients 1, 2, and 10) had a final height SDS that was better than their height SDS at GH treatment initiation (+0.37 SD, +1.0 SD, and +0.85 SD, respectively). However, the final height SDSSs in patients 1 and 10 were almost identical in comparison with the height SDSs of their untreated parents with the same causative collagen gene variant. Patient 2 had a final height that was 0.3 SD better than his affected father. On the other hand, the final height SDS of patient 8 was 0.67 lower than his pretreatment height and 0.42 lower than his affected father. Importantly, the final height in this patient was influenced by scoliosis that developed during GH treatment during puberty.

**Discussion**

This is the first study evaluating oligosymptomatic collagenopathies in children with short stature. Using NGS methods, we have described that oligosymptomatic collagenopathies are relatively frequent among children with FSS treated with GH (10 of 87 children; 11.5%). Our study also provided initial information about the mild phenotypic features and the efficacy of GH treatment.

In mutations of several genes affecting the growth plate, a wide phenotypical spectrum has been described. A typical example is the \( \text{SHOX} \) gene; homozygous variants cause severe Langer mesomelic dysplasia, and the phenotype associated with heterozygous variants ranges from Léri-Weil syndrome (bone dysplasia with mesomelic limb shortening and typical Madelung deformity) to apparently symmetric short stature [23]. Similar phenotypic variability has also been described in \( \text{NPR2} \), \( \text{FGFR3} \), and \( \text{ACAN} \) gene mutations [24-27]. Until now, various collagenopathies have been associated only with syndromic short stature (see
Table 1. Phenotype and growth hormone treatment response in children with proven collagenopathies

| Patient No. | Sex | Age at last checkup, y | Height of parent with causative collagen gene mutation, SD | Birth weight, SD | Birth length, SD | Sitting height to height ratio, SD | Stimulated GH, μg/L | GH treatment initiation, y | GH treatment initiation dose, y | Additional phenotypic features | Pre-treatment height, SD | Height after 1 y on GH therapy, SD | Height after 3 y on GH therapy, SD | Pre-treatment GH peak, μg/L | Pre-treatment IGF-1, SD | Pre-treatment IGF-1, SD | Final height, SD | Pre-treatment growth velocity, cm/y | Mutation status | Transcript variant | Protein variant | ACMG classification |
|-------------|-----|------------------------|---------------------------------------------------------|-----------------|-----------------|----------------------------------|---------------------|------------------------|------------------------|--------------------------------|---------------------|-----------------------|------------------------|----------------------|-----------------|---------------------|--------------------------|------------------------|----------------------|-----------------|-----------------|
| 1           | F   | 13                     | -2.8/-1.8                                              | -2.7            | -4.1            | 1.9                 | -1.0               | NA                     | -2.95                 | -1.95                 | -0.84               | -2.58               | 4.9                   | 10.3               | 1.9                  | -3.41                  | -2.92                 | -2.23               | -2.41               | 4.1               | 8.7               | M1/M2               | c.410G>A               | p.Arg137His | LP |
| 2           | M   | 16                     | -2.9                                                  | -2.4            | -3.0            | 0.3                 | -1.6               | 8.4                    | -3.41                 | -2.92                 | -2.23               | -2.41               | 4.1                   | 8.7               | M/n                 | -3.14                  | -2.65                 | NA                  | NA                  | 6.8               | 8.7               | M/n               | c.3106C>G               | p.Arg1036Gly | LP |
| 3           | M   | 6                      | -3.3                                                  | -1.4            | -3.5            | 0.3                 | -1.3               | NA                    | -3.11                 | -2.58                 | -2.05               | NA                  | 5.5                   | 7.8               | M/n                 | -3.11                  | -2.58                 | NA                  | NA                  | 5.5               | 7.8               | M/n               | c.3106C>G               | p.Arg1036Gly | LP |
| 4           | F   | 10                     | -2.2                                                  | -2.3            | -4.0            | 0.1                 | 0.4                | NA                    | -3.61                 | -3.50                 | -2.85               | NA                  | 4.9                   | 6.0               | M/n                 | -3.61                  | -3.50                 | -2.85               | NA                  | 4.9               | 6.0               | M/n               | c.2129C>T               | p.Pro710Leu | LP |
| 5           | M   | 9                      | -3.2                                                  | -1.9            | -2.1            | 0.6                 | 2.2                | 17.1                  | Genua valga, myopia  | -2.95                 | -1.95                 | -0.84               | -2.58               | 4.9                   | 10.3               | M1/M2               | c.410G>A               | p.Arg137His | LP |
| 6           | F   | 12                     | -3.5                                                  | -1.6            | -3.1            | 0.9                 | 1.7                | 11.0                  | Mild scoliosis        | -4.29                 | -3.59                 | -2.70               | NA                  | 6.2                   | 9.1               | M/n                 | c.2921C>A              | p.Pro974Gln | LP |
| 7           | F   | 14                     | -2.3                                                  | -1.5            | -1.9            | 1.2                 | 1.3                | 9.7                    | Mild scoliosis        | -2.82                 | -2.62                 | -1.65               | NA                  | 7.4                   | 7.3               | M/n                 | c.475A>G               | p.Ile519Val | LP |
| 8           | M   | 17                     | -2.8                                                  | -2.4            | -2.6            | 0.6                 | 0.2                | 19.6                  | Scoliosis developed during puberty, myopia | -2.83                 | -2.35                 | -2.85               | -3.32               | 5.1                   | 9.0               | M/n                 | c.1543C>G              | p.Gln515Glu | LP |
| 9           | F   | 8                      | -2.4                                                  | -2.4            | -2.6            | 0.3                 | 0.8                | NA                     | -2.43                 | -1.96                 | NA                  | 6.4                 | 9.1               | M/n               | c.2881G>A              | p.Gly961Ser | LP |
| 10          | M   | 16                     | -2.4                                                  | -1.0            | -0.7            | -0.6               | 1.2                | 8.0                    | -3.35                 | -2.68                 | -2.07               | -2.50               | 3.8                   | 7.8               | M/n                 | -3.35                  | -2.68                 | -2.07               | -2.50               | 3.8               | 7.8               | M/n               | c.3706C>T              | p.Arg1236Cys | LP |

Abbreviations: ACMG, American College of Medical Genetics and Genomics; F, female; GH, growth hormone; GV, growth velocity; IGF-1, insulin-like growth factor-1; LP, likely pathogenic; M, male; M1/M2, compound heterozygous variant; M/n, heterozygous variant; NA, not available, P, pathogenic; PP, prepubertal.
Recently, Zhang et al published a study in which mutations in the \( \text{COL2A1} \) gene caused short stature in 9 of 82 (11%) Chinese patients with clear signs of bone dysplasia (28). Our study has extended the known phenotypical spectrum of collagenopathies by proving that short stature with no apparent signs of bone dysplasia is frequently caused by mutations in the genes encoding collagen molecules.

No apparent features of bone dysplasia were observed during routine clinical evaluation of affected children from our study. However, a detailed examination performed by an experienced clinical anthropologist revealed subtle signs of bone dysplasia in some affected children (see Table 1). These signs corresponded with features known to be associated with collagen gene mutations. Asymmetric short stature, joint problems, bone deformities, and myopia have all been described in collagenopathies (see Supplementary Table [13]). Interestingly, in patient 8, scoliosis did not develop until puberty, and parents of the affected children with the same causative collagen gene variants had more pronounced body disproportionality. Various signs of bone dysplasia might become aggravated with age.

Recognizing the genetic etiology of short stature and clarifying the genetic disorder-specific reaction to GH treatment pose two of the important challenges in pediatric endocrinology today. Children with SHOX-D and Turner syndrome undoubtedly benefit from GH treatment (29). Recently, we demonstrated a good effect of GH treatment in children with heterozygous NPR2 gene mutations (20). On the other hand, the effect of GH therapy on patients with achondroplasia is at least controversial (height SD mildly improves from \(-5\) SD to \(-4\) SD after 5 years of treatment, and a negative effect on body disproportion has not been excluded) (27). Our study is the first to evaluate GH treatment in children with growth plate collagenopathies.

There is no general definition of successful GH therapy; however, children from our study cohort have an initial treatment response that corresponds to previously published data in children with SHOX-D (a genetic diagnosis automatically indicating GH treatment). In the GeNeSis observational program, 521 children with SHOX-D improved their height SDS by an average of 0.53 SD after 1 year of therapy (30) (compared to 0.5 SD in children with collagenopathies in our study). Moreover, in the original study that led to the legal approval of GH treatment in SHOX-D children, Blum et al described a GV increase from 4.8 cm/year to 8.7 cm/year in the first year of treatment and a height increase of 1.2 SD after 2 years of therapy (20, 31), which is also similar to our observations (GV increase from 5.3 to 8.7 cm/year, height improvement by 0.9 SD in children with collagenopathies).

On the other hand, the data from the very limited number of children who had reached their final height may indicate that GH treatment in some children with growth plate collagenopathies may not meet expectations. We can speculate on the reasons. First, early puberty with more rapid progression of the bone age could be more frequent in SGA children (32, 33). A typical example from our study cohort is patient 1, who started puberty at age 8 years; her bone age rapidly progressed during puberty, and she reached her final height before her eleventh birthday. Delaying puberty similarly to that described in children with ACAN gene mutations (34) could have led to better final height in this patient. Second, the GH dosage of approximately 33 \( \mu \)g/kg/day used in SGA children in Europe may not be sufficient. We can speculate that higher dosages used in patients with Turner syndrome, SHOX-D, or Noonan syndrome could be more effective in children with collagenopathies.

Table 1. Height SDS development in children with proven collagenopathies.

<table>
<thead>
<tr>
<th>Patient</th>
<th>GH treatment (years)</th>
<th>Height SDS</th>
<th>Bone age (yrs)</th>
<th>Body disproportionality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>0</td>
<td>-3.3</td>
<td>4.2</td>
<td>0.5</td>
</tr>
<tr>
<td>Patient 2</td>
<td>1</td>
<td>-3.0</td>
<td>4.8</td>
<td>0.7</td>
</tr>
<tr>
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<td>2</td>
<td>-2.7</td>
<td>5.5</td>
<td>0.8</td>
</tr>
<tr>
<td>Patient 4</td>
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<td>-2.5</td>
<td>6.3</td>
<td>0.9</td>
</tr>
<tr>
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<td>7.0</td>
<td>1.0</td>
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<td>7.5</td>
<td>1.2</td>
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</tr>
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<td>Patient 8</td>
<td>7</td>
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<td>8.5</td>
<td>1.6</td>
</tr>
<tr>
<td>Patient 9</td>
<td>8</td>
<td>0.0</td>
<td>9.0</td>
<td>1.8</td>
</tr>
</tbody>
</table>

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Figure 1. Height SD score development in children with proven collagenopathies. Height SD score (SDS) development is demonstrated in individual patients with proven collagenopathies over 3 years of growth hormone (GH) treatment. Box-and-whisker diagram shows median, interquartile ranges, and ranges of height SDS development of a studied group of children. The dots and lines represent height SDS development in individual children.
We must acknowledge that our study had several limitations. First, no functional studies were performed in our study. However, according to current guidelines, other methods can be used to prove the pathogenicity of genetic variants (22). In our cohort of patients with FSS, the most important was the segregation of the variants with short stature within the families. In most cases, we have performed genetic testing in multiple family members from different generations. The supportive methods to evaluate the genetic variants were their frequency in population databases or various in silico studies (for information about specific families, see the Supplementary materials [13]). Second, the number of children with proven collagenopathy (especially those with final height available) in our study is relatively small, and other factors, such as age of GH treatment initiation, puberty onset, dose of GH, or type of damaged collagen molecule, might have influenced the growth outcome. Thus, larger studies are needed to clearly evaluate the efficacy of GH treatment in children with collagenopathies.

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Additional Information

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Disclosures: The authors have nothing to disclose.

Data Availability: The data sets generated during and/or analyzed during the present study are not publicly available but are available from the corresponding author on reasonable request.

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