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ARTICLE

Mutation-Based Growth Charts for SEDC and other *COL2A1* Related Dysplasias

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From data collected via a large international collaborative study, we have constructed a growth chart for patients with molecularly confirmed congenital spondylo-epiphyseal dysplasia (SEDC) and other *COL2A1* related dysplasias. The growth chart is based on longitudinal height measurements of 79 patients with glycine substitutions in the triple-helical domain of COL2A1. In addition, measurements of 27 patients with other molecular defects, such as arginine to cysteine substitutions, splice mutations, and mutations in the C-terminal propeptide have been plotted on the chart. Height of the patients progressively deviate from that of normal children: compared to normal WHO charts, the mean length/height is −2.6 SD at birth, −4.2 SD at 5 years, and −5.8 SD in adulthood. The mean adult height (male and female combined) of patients with glycine substitutions in the triple-helical region is 138.2 cm but there is a large variation. Patients with glycine to cysteine substitutions tend to cluster within the upper part of the chart, while patients with glycine to serine or valine substitutions are situated between +1 SD and −1 SD. Patients with carboxy-terminal glycine substitutions tend to be shorter than patients with amino-terminal substitutions, while patients with splice mutations are relatively tall. However, there are exceptions and specific mutations can have a strong or a relatively mild negative effect on growth. The observation of significant difference in adult height between affected members of the same family indicates that height remains a multifactorial trait even in the presence of a mutation with a strong dominant effect.
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KEY WORDS: growth; *COL2A1*; spondylo-epiphyseal dysplasia congenita

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INTRODUCTION

The type II collagenopathies are a heterogeneous group of chondrodysplasias with a broad phenotypic spectrum and variable outcome ranging from perinatal death (achondrogenesis type 2, hypochondrogenesis) to normal life expectancy with degenerative joint disease as the only major problem (Stickler syndrome, familial Legg–Calve–Perthes) [Warman et al., 2011]. This variability is also reflected in the growth pattern of the affected individuals. In severe cases, there is profound antenatal micromelia; whereas in mild cases a normal adult height is attained. The aim of this study was to analyze growth in patients with heterozygous mutations in the *COL2A1* gene and to develop growth charts based on molecularly proven cases.

The *COL2A1* gene encodes the α1 chain of procollagen type II which is the major fibrillar protein of hyaline cartilage. Three α1 chains are folded together in a triple-helical configuration to form the procollagen homotrimer. The triple-helical domain of the pro-α1(II) chain is characterized by repeating Gly-X-Y triplets. Mutations in *COL2A1* have been identified in a wide spectrum of chondrodysplasias including achondrogenesis type 2 (ACG2), hypochondrogenesis, platyspondylic dysplasia-Torrance type, Kniest, SEDC, SEMD-Strudwick type, spondyloperipheral dysplasia (SPPD), Czech-dysplasia metatarsal type, Stickler syndrome, and familial Legg–Calve–Perthes.

Loss-of-function mutations leading to either a truncated protein or nonsense-mediated mRNA decay result in Stickler syndrome [Ahmad et al., 1991; Hoornaert et al., 2010]. Individuals with Stickler syndrome usually are of normal stature but suffer from early-onset arthrosis and variable orofacial and ocular abnormalities. Missense mutations on the other hand usually result in a chondrodysplasia phenotype with disproportionate short stature. The most common and well-known example is spondylo-epiphyseal dysplasia congenita (SEDC). Most patients with SEDC carry either glycine substitutions affecting the Gly-X-Y triplets or splice site mutations causing in-frame deletions. Glycine substitutions in the triple-helical domain are not unique to SEDC patients, as they also have been identified in SEMD Strudwick type [Tiller et al., 1995; Walter et al., 2007], Kniest dysplasia [Wilkin et al., 1994], and even in individuals with isolated avascular necrosis of the femur (Legg–Calvé–Perthes disease) [Liu et al., 2005; Miyamoto et al., 2007; Su et al., 2008]. Splice site mutations and in-frame deletions or duplications are frequently found in Kniest dysplasia patients [Spranger et al., 1997; Wilkin et al., 1999].

Arginine to cysteine substitutions constitute a different and distinctive class of missense mutations in the *COL2A1* gene. They are less frequent than glycine substitutions and may result in specific phenotypes. For example, the recurrent p.Arg275Cys seems to be specific for Czech dysplasia metatarsal type [Hoornaert et al., 2007]. Arginine to cysteine substitutions can result in SEDC as is exemplified by the p.Arg989Cys mutation but can also cause mild phenotypes with just arthropathy as the major clinical problem. (for review see [Hoornaert et al., 2007]).

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Mutations in the C terminal region (C-propeptide) are associated with Torrance type platyspondylic skeletal dysplasia and with SPPD. Platyspondylic skeletal dysplasia Torrance type is characterized by platyspondyly, short bones with metaphyseal abnormalities, brachydactyly, and typical histological features. The disorder usually results in stillbirth or neonatal death. SPPD is characterized by a short stature, brachydactyly, platyspondyly, and epiphyseal abnormalities. Missense or truncating mutations in the C-propeptide can lead to either one of these disorders by a dominant negative mechanism [Nishimura et al., 2004; Zankl et al., 2005].

There is little information about growth in patients with *COL2A1* mutations. In one of the first studies on SEDC, adult height varied between 94 and 132 cm [Spranger and Langer, 1970]. Horton et al. [1982] published growth charts for SEDC based on measurements in 62 patients. The mean adult height in this patient cohort was 115 cm, with a standard deviation of 15 cm [Horton et al., 1982]. In both studies, the diagnosis of SEDC was not molecularly proven since the causative gene was not known at that time. Nishimura et al. [2005] studied 17 patients with SEDC caused by a missense mutation in the *COL2A1* gene. Adult height varied between 93 and 151 cm [Nishimura et al., 2005]. From this report and subsequent publications, it became clear that growth in patients with SEDC can vary substantially and that adults can be much taller than what had been estimated from the earlier growth studies that may have been biased towards more severe cases [Horton et al., 1982; Sellick et al., 2006].

The aim of this study was to develop growth charts for patients with bona fide mutations in the *COL2A1* gene. Patients with loss-of-function mutations were excluded since these individuals have Stickler syndrome with normal stature.

MATERIALS AND METHODS

Study Design, Inclusion and Exclusion Criteria

The study was approved by the Institutional Review Board of the University Medical Centre Utrecht. The patients were recruited through two laboratories that offered DNA analysis of the *COL2A1* gene. The study population included patients with a heterozygous mutation in the *COL2A1* gene. Patients with loss-of-function mutations (leading

to Stickler syndrome) were excluded as well as patients with a perinatally lethal phenotype (hypochondrogenesis, ACG2, or Torrance dysplasia). Patients with developmental disability, severe birth asphyxia, or an abnormal karyotype were not included. For patients who received growth hormone therapy, only growth data before the start of growth hormone therapy were incorporated in the study. Patients born before 30 weeks of pregnancy were excluded. If born prematurely (>30 weeks but <36 weeks of pregnancy) height measurements of the first 2 years were excluded from analysis. After written consent was obtained from the patient or his/her responsible family members, the referring physician was asked to fill in a standard checklist. In this checklist information about growth, weight, and head circumference was gathered and a growth curve was requested.

The COL2A1 mutation-specific growth chart was established based on the biometric data obtained from a total of 79 patients with a glycine substitution in the triple-helical domain of *COL2A1* (c.1–c.3754, reference sequence NM_001844.4). Height measurements (n = 381) from 33 male and 46 female patients originating from14 different countries were used for constructing the growth chart. Height measurements (n = 96) from 27 patients with other mutations in the *COL2A1* gene (splice mutations, mutations in the C-terminal propeptide, arginine to cysteine substitutions, and a duplication in the triple-helical domain) were superimposed on this baseline growth chart. Data from patients aged over 20 years were plotted at the age of 20 years, as most individuals have reached adult height by that age.

Statistical Analysis

We constructed a height-for-age reference chart with longitudinal height measurements of only the patients with glycine substitutions in the triple helix (n = 79) by using GAMLSS in R Version 2.9.0 [Rigby and Stasinopoulos, 2004]. The distribution of height was determined by three parameters, the Box-Cox power transformation (L), the median (M), and the coefficient of variation (S) [Cole and Green, 1992]. The values of L, M, and S changed smoothly with age leading to values that could be used to construct the chart. The choice of the smoothing parameters (effective degrees of freedom) for the L, M, and S curves was made by creating worm plots (detrended Q–Q plots where "Q" stands for quantile) [van Buuren and Fredriks, 2001]. The curves were fitted as cubic splines. A weighing factor was applied such that boys and girls contributed equally to the construction of the chart.

After construction of the growth chart, measurements from patients with different glycine substitution groups were plotted on the chart. The patients with the splice mutations, arginine to glycine substitutions and mutations in the C-terminal propeptide were also plotted separately.

Next, all height measurements were converted in Z-scores or standard deviation scores (SDS) according to the new references. We then tested whether there was a significant relation between codon number and height SDS by a mixed-effects model (multi-level model) for the total group as well as for different glycine substitution groups separately. To explore whether there were specific locations in the *COL2A1* gene that affect height more or less than others, we plotted the last height SDS of the patients against the specific position in the gene on the X-axis.

RESULTS

Study Population

Data were obtained from 79 patients with a glycine substitution in *COL2A1* (33 males, 41.8%; 46 females, 58.2%). Most patients (n = 69, 87.3%) are living in Europe (the Netherlands, Belgium, Germany, France, Austria, United Kingdom, Scotland, Denmark, and Spain). A relatively large part comes from the Netherlands (n = 21, 26.6% of the total group with glycine substitutions). Other patients originate from outside Europe (n = 9, 11.4%): Australia (n = 5), Israel (n = 1), and Lebanon (n = 3). One patient has South American parents but lives in Europe.

Our patient cohort also included 27 patients with mutations other than glycine substitutions: 11 patients had a mutation in the C-terminal propeptide, nine patients had a splice site mutation, six patients had an arginine to cysteine substitution, and one patient had a duplication in the triple-helical domain (Supplementary eTable I).

Growth Data

All patients and growth data are shown in Table I and in Supplementary eTable I. The growth chart of the patients with glycine substitutions in the triple helical domain of type II procollagen is shown in Figure 1. When comparing these data with the WHO growth standards from birth to 5 years [WHO Multicentre Growth Reference Study Group, 2006] and to the general Dutch population in 2009 at 18 years [Talma et al., 2010], it becomes clear that height of the patients progressively falls off the curves for normal children. The median length or height of patients with glycine substitutions is 44.6 cm at birth, 85.9 cm at 4 years, 90.2 cm at 5 years, and 138.2 at 18 years which is respectively −2.6 SD, −4.0 SD, −4.2 SD, and −5.8 SD on the WHO or Dutch growth chart (girls and boys combined).

Glycine to Serine Mutation Group

Patients with a glycine to serine substitution mainly have a growth between the −1 SD and +1 SD of the chart (Fig. 2A). However, there are some outliers. Patients 128b (male) and 128c (female) are patients from the same Dutch family with a relatively mild form of SEDC due to a p.Gly945Ser mutation. Final heights in two other females in this family (128d and 128e, numbers not shown in the picture) were 158.5 and 161.5 cm, respectively, which is around +1 SD on the growth chart. Patient 12 is a boy from the United Kingdom who had a p.Gly1164Ser

TABLE I. Individuals Included in This Study and Proportion of Height Measurements

Mutation group	Number of patients	Number of measurements	Percentage of measurements
Gly → Ser	30	143	30.0
Gly → Asp	15	54	11.3
Gly → Val	14	69	14.5
Gly → Arg	12	76	15.9
Gly → Cys	4	32	6.7
Gly → Glu	3	6	1.3
Gly → Ala	1	1	.2
Arg → Cys	6	23	4.8
splice	9	29	6.1
C-terminal propeptide	11	38	8.0
Duplication in triple helix	1	6	1.3
Total	106	477	100

See text for more explanations concerning the mutation groups.

mutation. This boy was diagnosed with SEMD type Strudwick.

Glycine to Valine Mutation Group

In the group with glycine to valine substitutions, there were two outliers at adult age (Fig. 2B). From Patient 8 (male) only one measurement was available. He had an adult height of 182 cm, mild myopia, hearing loss, and a family history of retinal detachment and myopia. He is heterozygous for the p.Gly315Val mutation. He appeared not to have definite radiological signs of SEDC. Patient 82 is a British female patient with SEMD Strudwick type due to the p.Gly1053Val mutation.

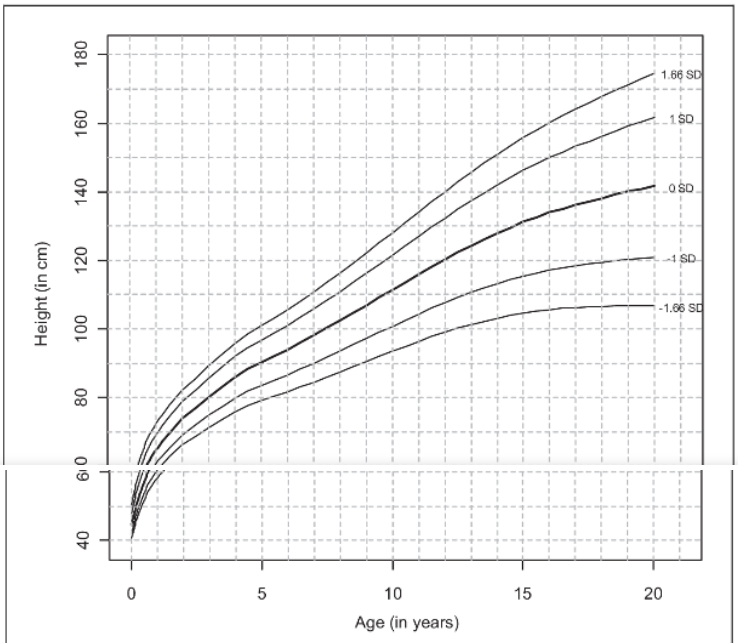

Figure 1. Growth chart for length/height (cm) of males and females with SED associated with a glycine substitution in the triple helix.

Glycine to Arginine Mutation Group

In this group, the range in height seemed to be larger (Fig. 2C). Patient 103 is a female SEDC patient with a p.Gly690Arg mutation. She had approximately the same height (148.5 cm) as her affected sister (148.0 cm) when she was 15 years. Patient 120 was a male with a p.Gly684Arg mutation. His adult height was 158 cm, however his affected father had a final height of 136 cm (specific number not shown). Patient 10 was a female from the United Kingdom who carried the p.Gly1122Arg mutation. This patient was diagnosed by the European Skeletal Dysplasia Network as having typical SEMD type Strudwick.

Glycine to Asparagine Mutation Group

Growth in patients belonging to the glycine to asparagine group tended to proximate the upper parts of the growth chart (Fig. 2D). The tallest male (adult height 172.6 cm) was Patient 9b who had the p.Gly369Asp mutation (specific number not shown). The affected mother of this patient had an adult height of 148 cm. Another patient with a relative tall stature was Patient 39 who had the p.Gly429Asp mutation (specific number not shown). His last measurement at the age of 15 years and 9 months revealed a height of 157 cm (+1.4 SD). However, other patients in this group were much shorter (Patients 37, 32, and 76 with the p.Gly1152Asp, p.Gly725Asp, and p.Gly444Asp mutations, respectively).

Glycine to Cysteine Mutation Group

Patients with glycine to cysteine substitutions also plotted in the upper

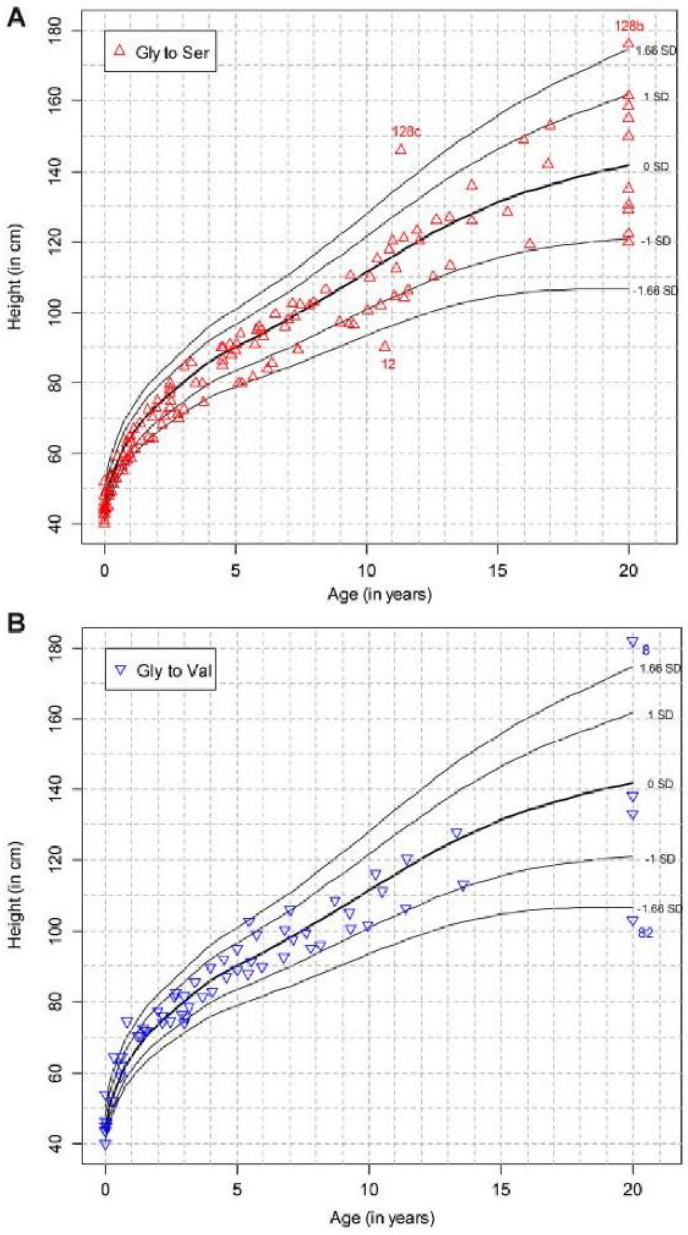

Figure 2. **A**: The constructed growth chart for length/height (cm) for patients with a glycine substitution in the triple helix, the measurements of patients with glycine to serine substitutions in the triple helix are plotted in red. **B**: The constructed growth chart for length/height (cm) for patients with a glycine substitution in the triple helix, the measurements of patients with glycine to valine substitutions in the triple helix are plotted in blue.

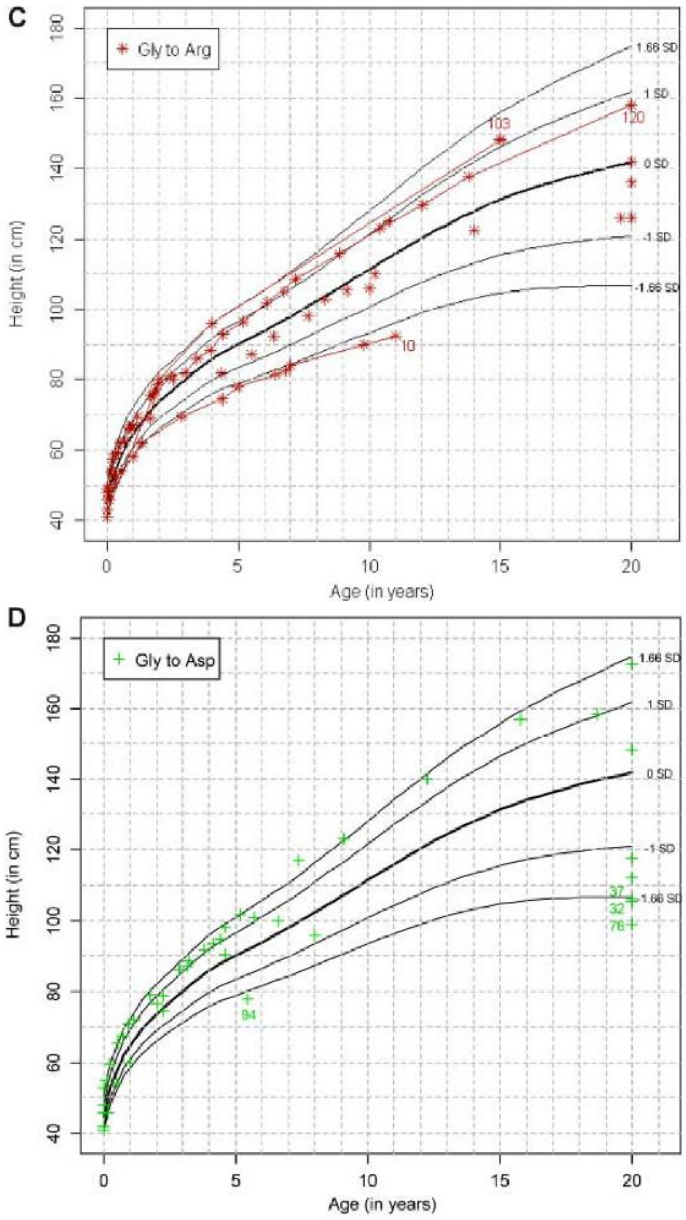

Figure 2. **C**: The constructed growth chart for length/height (cm) for patients with a glycine substitution in the triple helix, the measurements of patients with glycine to arginine substitutions in the triple helix are plotted in orange. Measurements of some patients are connected to show that it is the same patient. **D**: The constructed growth chart for length/height (cm) for patients with a glycine substitution in the triple helix, the measurements of patients with glycine to asparagine substitutions in the triple helix are plotted in green.

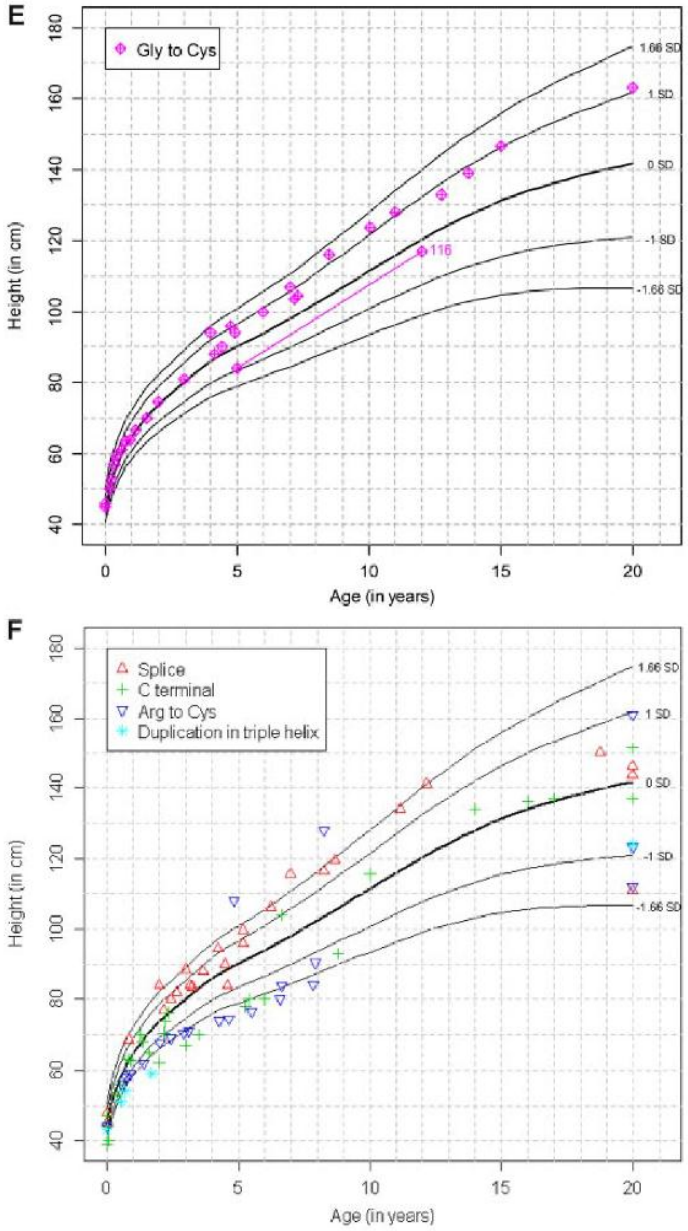

Figure 2. **E**: The constructed growth chart for length/height (cm) for patients with a glycine substitution in the triple helix, the measurements of patients with glycine to cysteine substitutions in the triple helix are plotted in pink. Measurements of one patient are connected. **F**: The constructed growth chart for length/height (cm), plotted with mutations of patients with splice mutations, mutations in the C-terminal propeptide, arginine to cystine substitutions and a patient with a duplication in the triple helix.

part of the curve (shown in Fig. 2E). The only patient below the mean was Patient 116 who carried the p.Gly918Cys mutation which is the most carboxy-terminal mutation within this group.

Splice Mutations, Mutations in the C-Terminal Propeptide, Arginine to Cysteine Substitutions

Patients with the other *COL2A1* mutations are depicted in Figure 2F. The splice mutations (in red) were located in the upper part of the chart (or above the mean). Patient 119 (specific number not shown in the picture) was an exception, his final height was 111 cm. We did not detect specific differences in height between patients with frameshift or missense mutations in the C-terminal propeptide. In both groups, height was variable. The patients with a p.Arg989Cys mutation cluster at the lower end and patients with a p.Arg719Cys mutations at the upper end of the curve. The only patient with a duplication (Patient 136) in the triple helix had a growth that followed the lower regions of the chart (in light blue.)

Relation Codon Number (X-Axis) and Height in SDS (Y-Axis) of Last Measurement of Each Patient, With a Glycine Substitution in the Triple Helix

Statistical analysis in the group of patients with glycine substitutions showed a significant decrease in SDS when the mutation was more carboxy-terminal ($P < 0.001$). However, when this was analyzed in the different mutation groups, there was only a significant decrease in the glycine to cysteine ($P < 0.001$) and the glycine to asparagine group, the latter containing only few patients ($P = 0.02$). In the glycine to serine and glycine to arginine group there was a decrease in SDS, however not significant. There was no increase or decrease in SDS in patients with glycine to valine substitutions when comparing amino-terminal with carboxy-terminal located mutations.

DISCUSSION

This study provides a reference growth chart for individuals with a nonlethal type II collagen disorder associated with a glycine substitution in the triple helical domain of the pro-α1(II) chain. The growth chart is based on a series of 79 patients from different countries and combines both sexes. The median length at birth is 44.6 cm and median height at the age of 18 years is 138.2 cm. Two general limitations apply to our study: first, there may be a selection bias in that we included many patients from the Dutch population that is relatively tall when compared to other populations in Europe and worldwide. Analyses with a mixed-effects model showed a significant difference of +0.59 SD in mean height in children from the Netherlands compared to other ethnicities ($P = 0.007$). Therefore, a correction of +0.59 SD to height may be applied in patients from non-Dutch countries where average normal height is lower. Secondly, we had to combine the measurements of males and females to obtain sufficient power to construct the chart. Mixed-effects analysis revealed that males are on average +0.60 SD taller than females ($P = 0.008$). Once again, a correction can be applied if one wants to differentiate between boys and girls. We have not taken into account the effect of the presence or absence of a scoliosis or surgical operations (like valgus trans-trochanteric osteotomy) and the possibility that we have relatively more measurements from mildly affected patients.

A glycine substitution in the triple-helical domain is the most common type of pathogenic mutation in the COL2A1 gene. For this reason, we chose to calculate the growth curves of patients carrying this type of mutation as the "standard" curves against which other mutation types were then compared. The phenotype of patients with glycine substitutions ranged from classic SEDC to milder forms of SED with premature osteoarthritis (Fig. 3, mildly affected female with the p.Gly945Ser mutation, Fig. 4, severely affected female with the p.Gly1155Val mutation). In Family 8, with the p.Gly315Val mutation, the two affected individuals had a normal stature with no spondylo-epiphyseal changes on radiographs, so that even the diagnosis of Stickler syndrome could be considered. Glycine substitutions have been described in about 5% of Stickler patients [Hoornaert et al., 2010]. No other patients with Stickler syndrome compatible phenotype are present in our cohort.

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The effect of glycine substitutions on growth may depend on several factors including the localization of the glycine residue within the triple-helical domain and the nature of the newly incorporated amino acid. Our study shows that the substituting amino acid has a rather small effect on the growth pattern of the affected individuals. However, patients with a glycine to cysteine substitution tend to be taller than patients with another glycine substitution. In a recent study on osteogenesis imperfecta caused by mutations in either the *COL1A1* or *COL1A2* genes, glycine to serine substitutions in COL1A1 were reported to result in more severe short stature, compared with glycine to arginine substitutions [Rauch et al., 2010]. This was not the case in our study population. The heights of patients with a glycine to serine (and valine) substitution tended to cluster between the +1 SD and −1 SD and patients with glycine to arginine substitutions were found both in the upper and lower part of the chart. In the osteogenesis imperfecta study,

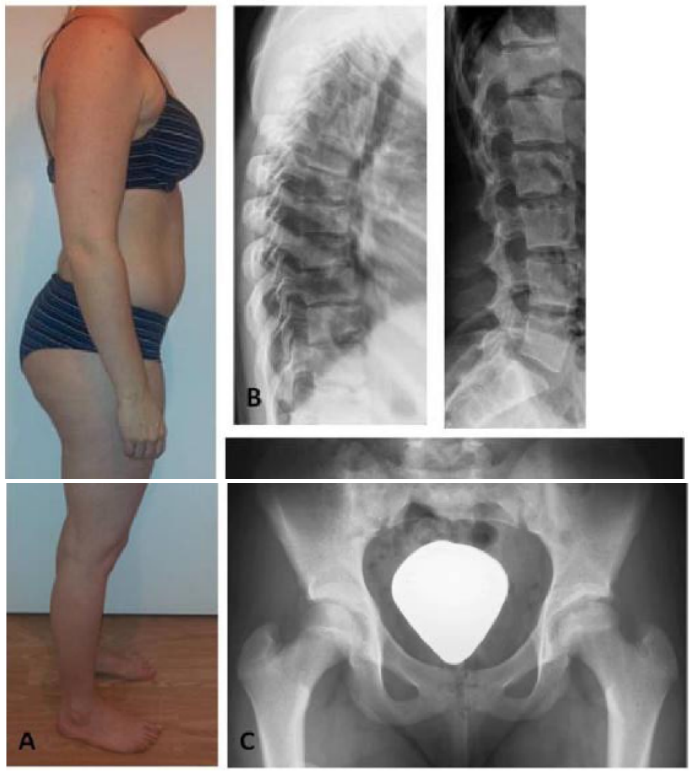

Figure 3. **A**: Clinical picture of Patient 128e at age 23 years. Note proportionate stature, adult height 161.5 cm. **B**: X-ray of the thoracic and lumbar spine of Patient 128e at age 19 years, showing platyspondyly and irregular endplates of multiple vertebrae. **C**: X-ray of the pelvis of Patient 128e at the age of almost 13 years, showing a flat and irregular femoral head on the left side and a somewhat small femoral head on the right side.

glycine to asparagine substitutions in COL1A2 resulted in a severe short stature [Rauch et al., 2010], whereas in our study many patients with glycine to asparagine mutations were rather tall (Fig. 2D).

Statistical analysis in the entire group of patients with glycine substitutions showed overall a significant decrease in SDS when the mutation was located closer to the carboxy-terminal end (Fig. 5). However, when analyzing the data for each individual substitution group, this decrease was only significant for glycine to cysteine and glycine to asparagine substitutions. In a recent study on 17 patients with SEDC, the patients with carboxy-terminal substitutions (p.Gly624Asp, p.Gly672Ser, p.Gly822Ser, and p.Gly1188Ala) were shorter (adult height between 93 and 109.5 cm) than the patients with the p.Gly393Ser and p.Gly504Ser mutation (adult height between 136.5 and 151 cm) [Nishimura et al., 2005]. The study on OI also showed an inverse relationship between height and the location of the mutation in the COL1A2 chain [Rauch et al., 2010]. In our study this correlation was not absolute and there were many exceptions. For example, patient 128b with a p.Gly945Ser mutation had an adult height of 176 cm, whereas patient 76 with a p.Gly444Asp was less than 100 cm tall. In addition, a patient with a p.Gly492Asp mutation, which is located almost 50 amino acids further downstream in the carboxy-terminal direction, has been reported with Stickler syndrome and a normal stature [Hoornaert et al., 2010].

The effect of mutations on growth of the affected individual seems to be much more complex than expected from its relative position within the triple-helical domain. Other factors such as possible interactions with other matrix proteins surrounding the collagen fibril and the flanking amino acids may influence and determine the phenotypic outcome of the mutation.

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In addition, the intrafamilial variability in height (illustrated by family 120) underscores the multifactorial nature of growth. That the severity of the phenotype can be very location-specific is also demonstrated by the fact that mutations (often glycine substitutions) that cause a lethal ACG/hypochondrogenesis phenotype are scattered throughout the *COL2A1* gene, in the close neighbourhood of mutations causing SEDC or other milder phenotypes [Korkko et al., 2000; Mortier et al., 2000, LOVD database Gent].

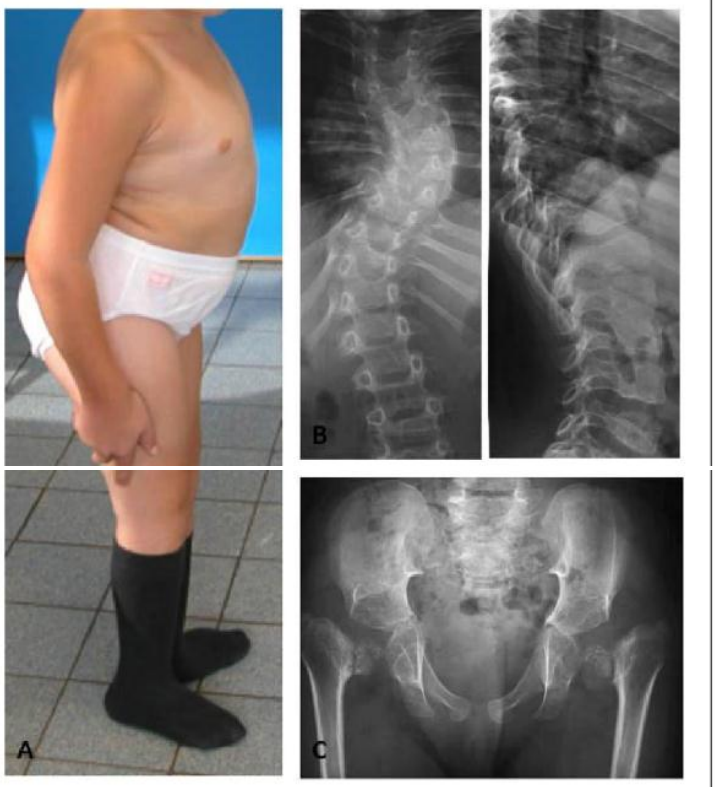

Figure 4. **A**: Clinical picture of Patient 123 at the age of 13.5 years. Note disproportionate short stature with short trunk, height 113 cm. **B**: X-ray of the thoracolumbar spine of Patient 123 at age 8 years, showing scoliosis and severe platyspondyly, more pronounced in the posterior part of the vertebrae. **C**: X-ray of the pelvis of Patient 123 at age 8 years, showing coxa vara, bilateral severe underdevelopment of the femoral neck, dysplastic acetabula, bilateral fragmentation of the proximal femoral epiphysis, and caudally displaced femoral heads.

Hoornaert et al. [2010] observed that glycine substitutions amino-terminal to residue 303, always result in Stickler syndrome with a normal stature. The only patient in our study with a substitution amino-terminal to residue 303 (the Dutch Patient 137 with the p.Gly210Glu mutation) had a height in the lower range of healthy Dutch children (−1.9 SD on the Dutch growth chart). However, when evaluating the radiographs, she appeared to have irregular vertebral endplates, a scoliosis of 16 degrees, severe spinal stenosis as well as distinct epiphyseal abnormalities in hips and upper arms, more compatible with SEDC than with Stickler syndrome [Snead and Yates, 1999]. This leads to the conclusion that, although the height is generally unaffected if mutations occur before codon 303, the patients can have radiographic changes of SEDC.

Patients with splice site mutations grow better than patients with a glycine substitution (Fig. 2F). In our study, nine patients with splice site mutations were included. In seven patients the diagnosis of Kniest syndrome was made; the two remaining patients had SEDC. There could be several reasons for the relative mild effect of splice site mutations on height. The splicing machinery could partially compensate for deleterious effects on the protein by producing alternative splice forms. Another possibility is the "loop-out" hypothesis. Weis et al. [1998] performed trypsin digestion experiments in a patient with Kniest syndrome and a splice site mutation. They found evidence for selective cleavage of the normal pro-α1(II) chain at the location which was predicted to be spliced out in the abnormal chain. They hypothesized that the normal pro-α1(II) chain forms a loop out of the triple helix at the site of the mutation [Weis et al., 1998]. One could imagine that the abnormal pro-α1(II) chain with the deletion could then more easily be incorporated into the triple helix, potentially even better than in case of some amino acid substitutions.

Mutations in the C-terminal propeptide lead to SPPD or platyspondylic skeletal dysplasia Torrance type. As Torrance dysplasia is mostly lethal, only patients with SPPD were included in our study. The C-terminal propeptide is important in the association of the three procollagen chains to allow formation of the triple helix. We investigated if frameshift mutations have a different effect on growth in comparison to amino acid substitutions. Due to their extreme carboxy-terminal location, the frameshift mutations probably escape nonsense-mediated mRNA decay and result in a truncated pro-α1(II) chain that nevertheless participates in helix formation, unlike the more precociously truncated chains associated with Stickler syndrome [Zankl et al., 2005]. However, we could not detect differences in growth when we compared both groups. Patients with a frameshift mutation were plotted either at the +1 SD of the curve (Patient 55) or below the −1.66 SD (Patient 49). In patients with missense mutations resulting in the incorporation of an extra cysteine in the C-propeptide, height clustered around the mean.

Previous studies had suggested that patients with a p.Arg719Cys or a p.Arg275Cys mutation had normal or only mildly reduced stature. Patients with a p.Arg989Cys mutation, however, have a severe SEDC phenotype with disproportionate short stature, possibly due to reduced thermostability of the triple helix [Steplewski et al., 2004;

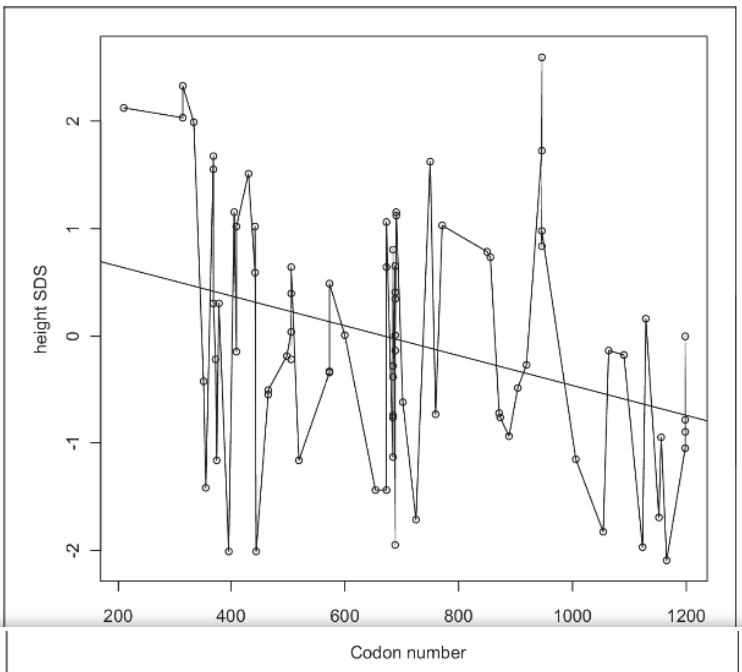

Figure 5. Relation codon number (X-axis) and height in SDS (Y-axis), only patients with glycine substitutions in the triple helix, last measurement of each patient.

Hoornaert et al., 2006]. Our study confirms these observations. Patients with a p.Arg719Cys mutation were plotted above the upper line and around +1 SD, whereas the four patients with the p.Arg989Cys were located in the lower parts of the growth chart.

In conclusion, while our study provides new growth charts for individuals with a type II collagen disorder, it also illustrates the difficulties in predicting the final height in a young child after identification of a specific mutation in the *COL2A1* gene. Our study underscores the polygenic and multifactorial knowing one mutation in the human genome does not allow us to explain the full phenotypic outcome, certainly not in case of complex traits like body height.

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