Association of *COL1A1 Sp1* polymorphism with the effect of subcutaneously injected recombinant hGH in GH-deficient adults

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Objective: Collagen type I is a common structural protein in bone and skin. Similar to its association with the mechanical properties of the skeleton and, thus, bone-fracture risk, the collagen type I α (*COL1A1*)-1 specific protein (*Sp*)-1 polymorphism may be related to variations in the collagen type I-containing subcutaneous tissue and its biological properties. In this study, we analyzed a possible influence of the *COL1A1 Sp1* polymorphism on the effect of subcutaneously injected recombinant human growth hormone (hGH) in GH-deficient adults. **Materials & methods:** We determined the *COL1A1 Sp1* polymorphism in 122 adults with GH deficiency of different origin, who were derived from the prospective Pfizer International Growth Database (KIMS) Pharmacogenetics Study. Inclusion criteria were subcutaneous applied treatment with hGH for over 12 months, finished dose titration of hGH by following serum IGF-1 concentrations until desired levels were achieved, and centralized, standardized IGF-1 measurements. The genotypes (GG/GT/TT) were statistically related to clinical data from the KIMS database. **Results:** The dose of injected hGH was significantly related to the *COL1A1 Sp1* genotypes ($p = 0.049$), whereby the GG homozygotes were treated with a significantly higher dose of hGH than TT homozygotes ($p = 0.03$). Accordingly, the IGF-1:GH ratios were significantly lower in GG compared with TT homozygotes ($p = 0.04$). Both groups showed no significant differences in their IGF-1 serum concentrations ($p = 0.98$) and IGF-1 SDS ($p = 0.79$). **Conclusion:** The *COL1A1 Sp1* polymorphism is related to the dose of individually required, subcutaneous injected hGH in GH-deficient adults, probably because of an alteration of the subcutaneous collagen type I structure, content and/or biological/biomechanical properties. GG homozygotism, which is related to a more stable bone structure and decreased fracture risk, may impact skin resistance to subcutaneous injected protein-based drugs, as shown for hGH in this study.

Collagen type I, a common component of bone, skin, blood vessels, periosteum, ligaments and tendons in humans, represents the most abundant extracellular matrix protein in subcutaneous tissue [1,2].

The collagen type I triple-helix molecule consists of two $\alpha 1$ - and one $\alpha 2$ -protein chains, which are encoded by two different genes, collagen type I α (*COL1A1*)-1 and *COL1A2*, located on chromosomes 17 and 7, respectively [2].

The *COL1A1* gene is extensively studied for susceptibility to osteoporosis. A G-to-T SNP affecting a binding site for the ubiquitously expressed zinc-finger transcription factor specific protein (*Sp*)-1 within the first intron of the *COL1A1* gene has been shown to be important for bone quality by influencing collagen gene regulation and transcription [3–5]. A large multicenter study confirmed that the *COL1A1 Sp1* polymorphism is associated with bone mineral density (BMD) of the lumbar spine and femoral neck with a recessive mode of inheritance after evaluating 20,786 European individuals [6]. Furthermore, the *COL1A1*

Sp1 polymorphism has been associated with the modulus of elasticity of bones, determined by acoustic measurement [7].

The extracellular connective tissue matrix of bone and skin both contain collagen type I. An association of BMD and skin thickness is well described [8–10]. Several exogenous predictors of BMD are also stated to predict skin thickness [8], but genetic predictors have not been demonstrated.

More recently, studies were published concerning the identification of regulatory elements in the promoter region of the *COL1A1* gene, which are also involved in transcriptional regulation of collagen production in normal and systemic sclerosis fibroblasts [11,12].

The effect of the *COL1A1* promoter region both on systemic sclerosis, a connective-tissue disorder with excessive production of extracellular matrix components, especially collagen type I, and on BMD leads to the hypothesis that there might also be an effect of the *COL1A1 Sp1* polymorphism on the mechanical and biological properties of collagen type I-containing subcutaneous tissue.

The interindividual variability of response to subcutaneous applied protein-based drugs is not completely understood. Exogenous influencing factors such as subcutaneous blood flow, injection site, room and skin temperature, physical exercise, skinfold and subcutaneous thickness, local massage, subcutaneous edema, injection technique, smoking, orthostatic changes and ketosis have been described as influencing factors for variable insulin absorption [13,14].

Genetic factors influencing absorption capacity, bioavailability and/or biological effects of and resistance to subcutaneous applied protein-based drugs by modifying mechanical and/or biological properties of the subcutaneous connective tissue have not yet been described. The aim of this study was to evaluate a possible effect of the *COLIA1 Sp1* polymorphism on the response to subcutaneous injected recombinant human growth hormone (hGH) in GH-deficient adults. The individually required GH dose was determined by following the IGF-1 serum concentrations until the desired levels, based on age- and gender-related reference concentrations, were achieved.

Materials & methods

Study population

The prospective German Pfizer International Growth Database (KIMS) Pharmacogenetics Study is a substudy of the international observational safety surveillance study KIMS, which observes nearly 10,000 adult patients with GH deficiency (GHD) of different origin, most of them receiving recombinant hGH therapy. Approval for the German KIMS Pharmacogenetics Study protocol was obtained from the local ethics committee. Written informed consent was obtained from each patient.

Inclusion criteria for this study were:

- Enrolment into the German KIMS Pharmacogenetics Study by written informed consent, availability of genomic DNA and clinical dataset;
- Diagnosis of GHD and indication for treatment with recombinant hGH according to established criteria [15,16]. According to the current guidelines, all patients had multiple pituitary-hormone deficiency, with deficiency of at least one additional pituitary axis. Patients were on stable regular replacement therapy for other hormonal deficiencies;
- hGH substitution for at least 12 months before inclusion. Recombinant hGH (in all

cases, Genotropin®) was injected subcutaneously at night. Titration of the hGH dose according to age- and gender-adjusted IGF-1 reference serum concentrations had to be finished. Documentation of the final hGH dose was mandatory. IGF-1 serum concentration measured in one central laboratory obtained after finishing the titration period had to be available.

A total of 122 GHD adult patients (66 men, 56 women; mean age: 50.0 years $\pm$ 13.0 standard deviation [SD]; majority Caucasian) fulfilled these criteria and were included in the study.

The *COLIA1 Sp1* genotype was determined and correlated with clinical data, collected independently by German medical centers participating in the KIMS Study: etiology of GHD, gender, age, weight, height, BMI, hGH dose (mg/day), duration of hGH treatment, IGF-1 serum concentration, standard deviation score of age- and gender-adjusted IGF-1 reference serum concentrations (IGF-1 SDS), IGF-1:GH ratio.

IGF-measurement

IGF-1 serum concentrations of all patients were measured with the same Nichols Advantage® Chemiluminescence Assay (Nichols Institute Diagnostics, Bad Nauheim, Germany) in one laboratory (Pfizer, Stockholm, Sweden).

Extraction of genomic DNA

Genomic DNA was extracted from samples of peripheral venous blood according to standard procedures [17].

Genotyping

The G-to-T polymorphism in a *Sp1* binding site of the *COLIA1* gene is listed in the NCBI SNP database (dbSNP rs1800012; [101]) according to the GenBank accession number J03559 [102]. The G-to-T substitution is located at position ex2–348 at the first base of a binding site for the transcription factor *Sp1* in the first intron of the *COLIA1* gene (gene locus 17q21).

The *COLIA1 Sp1* polymorphism was genotyped by MscI restriction digestion as previously described [4,7], except that PCR, using the pair of mismatched primers described by Grant *et al.* [4], was performed using REDtaq™ Ready Mix™ PCR Reaction Mix (Sigma-Aldrich Corp., MO, USA) with a slight modification of the conditions (96°C for 5 min, 35 cycles of 94, 60 and 72°C for 40 s, respectively, followed by 72°C for 5 min). PCR products were digested with 3 units

of MscI restriction enzymes (New England Biolabs Inc., MA, USA) at 37°C for 30 min and separated on a 3% NuSieve® 3:1 agarose gel.

The resulting genotypes were characterized GG/GT/TT, where GG represents the homozygous wild-type alleles with absence of the G-to-T substitution and absence of the MscI restriction site.

Statistical analysis

The distribution of genotypes was analyzed by testing for Hardy–Weinberg disequilibrium using the χ^2 test.

In case of nonsymmetric data, the Mann–Whitney U test and Kruskal–Wallis analysis were used. Symmetric data were analyzed by unpaired student's t-test and one-way analysis of variance. Spearman's correlation coefficient was used to calculate correlations.

The following values were distributed non-symmetrically: gender, etiology, duration of GH treatment, dose of injected hGH and IGF-1:GH ratio. Symmetrical distribution was revealed by the following parameters: age, weight, height, BMI, IGF-1 serum concentration and IGF-1 SDS.

All tests were performed two-tailed. A p-value of less than 0.05 was considered to be significant. Statistical analysis was performed using SPSS for Windows (release 11.5; SPSS Inc., IL, USA).

Results

Clinical characteristics of the study population

Etiologies of GHD in total and according to the *COLIA1 Sp1* genotypes are shown in Table 1. Clinical characteristics, such as age, gender and anthropometric data, in total and according to the *COLIA1 Sp1* genotype are given in Table 2. There were no significant differences in any of the clinical characteristics by genotype (Table 2). Constant results were observed combining the TT homozygotes and GT heterozygotes into a 'T-allele-containing' group (Table 3).

hGH-treatment-related characteristics of the study population

hGH-treatment-related characteristics in total and according to the *COLIA1 Sp1* genotype are given in Table 4.

There was no significant difference between the three genotype groups in IGF-1 serum concentrations ($p = 0.99$), IGF-1 SDS values ($p = 0.73$) and the duration of hGH treatment ($p = 0.21$) (Table 4).

The testing of all three *COLIA1 Sp1* genotype groups (GG/TT/GT) by Kruskal–Wallis analysis revealed a significant difference in the dose of injected recombinant hGH ($p = 0.049$) (Table 4).

In detail, GG homozygotes had been treated with a significantly higher dose of injected hGH than TT homozygotes ($p = 0.03$) (Figure 1), but showed no significant difference in IGF-1 serum concentrations ($p = 0.98$) or IGF-1 SDS ($p = 0.79$) (Table 4). Doses of injected hGH of both groups compared with the doses of hGH of the GT heterozygotes differed, but not significantly (GG vs GT, $p = 0.11$; TT vs GT, $p = 0.48$). Nevertheless, GT heterozygotes required a dose of recombinant hGH between the doses of hGH of GG and TT homozygotes (Figure 1).

Accordingly, the IGF-1:GH ratios of the GG homozygotes were significantly lower than the IGF-1:GH ratios of the TT homozygotes ($p = 0.04$), expressing a higher requirement of hGH in the GG homozygous group in order to achieve comparable IGF-1 serum concentrations as compared with the TT homozygous group (Figure 2 & Table 4). Differences of the IGF-1:GH ratios of both groups compared with IGF-1:GH ratios of the GT heterozygotes were not significant (GG vs GT, $p = 0.11$; TT vs GT, $p = 0.27$), but, again, the IGF-1:GH ratios of the GT heterozygotes were placed between the IGF-1/GH ratios of the GG and TT homozygotes (Figure 2).

Similar results were obtained by comparing the GG homozygotes with the 'T-allele-containing' group after combining TT and GT heterozygotes (Table 3).

Correlation: clinical & hGH-treatment-related characteristics of the study population

IGF-1 serum concentrations, IGF-1 SDS and IGF-1:GH ratios were not highly correlated with gender, age, height, weight or BMI (Spearman's correlation coefficient <0.3).

The dose of injected hGH was not correlated with gender, height, weight or BMI, but was negatively correlated with age (Spearman's correlation coefficient: -0.34 ; $p = 0.00$). Older patients required lower doses of injected hGH, likely owing to lower IGF-1 reference serum concentrations according to physiologically lower IGF-1 serum concentrations with increasing age.

The duration of hGH treatment was not significantly correlated with the dose of injected hGH ($p = 0.62$), IGF-1 serum concentrations ($p = 0.11$), IGF-1 SDS ($p = 0.93$) and IGF-1:GH ratios ($p = 0.52$).

Table 1. Etiologies of growth-hormone deficiency in 122 adults according to the *COLIA1 Sp1* genotype.

Etiology	All, n (%)	<i>COLIA1 Sp1</i> genotype, n (%)			p-value*
		<i>GG</i>	<i>GT</i>	<i>TT</i>	
Adenoma	72 (59.0)	40 (51.9)	25 (71.4)	7 (70.0)	
Craniopharyngeoma	17 (13.9)	12 (15.6)	4 (11.4)	1 (10.0)	
Idiopathic/congenital	10 (8.2)	8 (10.4)	2 (5.7)	–	
Sheehan syndrome (postpartum necrosis)	5 (4.1)	3 (3.9)	1 (2.9)	1 (10.0)	
Trauma	4 (3.3)	2 (2.6)	2 (5.7)	–	
Other etiologies [†]	14 (11.5)	12 (15.6)	1 (2.9)	1 (10.0)	
Total	122 (100)	77 (63.1)	35 (28.7)	10 (8.2)	0.11

Numbers in parentheses: percentages according to the *COLIA1 Sp1* genotype.

*p-value for difference by *COLIA1 Sp1* genotype (Kruskal–Wallis test).

[†]Other etiologies: *GG*: Clivus chordoma, empty sella, encephalitis, epidermoid cyst, hematoma, intra-/supra-sellar abscess, lymphocytic hypophysitis (*n* = 2), Rathke cyst (*n* = 2), unknown (*n* = 2); *GT*: Rathke cyst; *TT*: Irradiation.

COLIA1: Collagen type I $\alpha 1$; *Sp1*: Specific protein 1.

Genotyping of *COLIA1 Sp1* polymorphism

Genotype frequencies were just in Hardy–Weinberg disequilibrium ($p = 0.05$) (Table 2). The allele frequency of the G allele was 77.5% and of the T allele 22.5%. Genotype distribution and allele frequencies shown in this study are similar to previously published data [6,7].

Discussion

In general, the interindividual variability of response to subcutaneous applied protein-based drugs remains poorly understood, although several influencing factors have been evaluated and discussed. One of the suggested factors is skin thickness. In continuous ambulatory peritoneal dialysis patients, the possibility of dose reduction of daily subcutaneously applied recombinant erythropoietin was correlated with skin thickness [18].

The individual thickness of subcutaneous tissue has been reported to be correlated with BMI and gender [19], as well as with additional factors, such as years past menopause, hours of exercise per week, caffeine ingestion, years of thyroid hormone replacement and lifetime prednisone dose [8]. Looking at bone and fracture data [6], and bearing in mind that collagen type I is the major component of bone matrix and skin [1,2], it seems probable that skin thickness, skin architecture, and biochemical and mechanical properties of the skin and subcutaneous connective tissue should also be determined, at least partially, by common genetic factors. To our knowledge, no data have yet been published addressing this issue.

Defining genetic markers associated with biological effects of subcutaneously injected protein-based drugs may improve the understanding of

Table 2. Clinical characteristics according to the *COLIA1 Sp1* genotype.

Clinical characteristics	All	<i>COLIA1 Sp1</i> genotype			p-value
		<i>GG</i>	<i>GT</i>	<i>TT</i>	
Gender, n (%)					0.12*
Men	66 (54.1)	37 (48.1)	21 (60.0)	8 (80.0)	
Women	56 (45.9)	40 (51.9)	14 (40.0)	2 (20.0)	
Age (years)	50.0 $\pm$ 13.0	49.2 $\pm$ 13.0	52.0 $\pm$ 13.6	49.7 $\pm$ 11.0	0.57 [†]
Weight (kg)	84.7 $\pm$ 16.9	82.9 $\pm$ 16.0	86.1 $\pm$ 18.8	93.7 $\pm$ 14.7	0.14 [†]
Height (cm)	169.9 $\pm$ 10.2	168.6 $\pm$ 10.2	170.9 $\pm$ 9.9	176.3 $\pm$ 9.4	0.07 [†]
BMI (kg/m ²)	29.3 $\pm$ 5.4	29.1 $\pm$ 5.4	29.4 $\pm$ 5.8	30.1 $\pm$ 4.7	0.87 [†]
Total, n (%)	122 (100)	77 (63.1)	35 (28.7)	10 (8.2)	0.05[§]

Values (except gender) are mean $\pm$ standard deviation (SD); numbers in parentheses are percentage according to the *COLIA1 Sp1* genotype.

*p-value for difference by *COLIA1 Sp1* genotype (Kruskal–Wallis test).

[†]p-value for difference by *COLIA1 Sp1* genotype (one-way analysis of variance).

[§]p-value for distribution in Hardy–Weinberg equilibrium.

Table 3. Clinical and hGH-treatment-related characteristics according to the COL1A1 Sp1 genotype group (GG homozygotes versus GT heterozygotes and TT homozygotes combined).

Characteristics	COL1A1 Sp1 genotype		p-value
	GG homozygotes	GT and TT combined	
n (%)	77 (63.1)	45 (36.9)	
Gender: men (%) / women (%)	37 (48.1) / 40 (51.9)	29 (64.4) / 16 (35.6)	0.08*
Age (years) [‡]	49.2 ± 13.0	52.0 ± 13.0	0.35 [§]
Weight (kg) [‡]	82.9 ± 16.0	87.7 ± 18.1	0.12 [§]
Height (cm) [‡]	168.6 ± 10.2	172.1 ± 10.0	0.07 [§]
BMI (kg/m ²) [‡]	29.1 ± 5.4	29.5 ± 5.6	0.71 [§]
Duration of hGH treatment (months) [¶]	60.0 ± 3.6	71.0 ± 4.9	0.19*
Dose of injected hGH (mg/day) [¶]	0.4 ± 0.03	0.2 ± 0.03	0.03*
IGF-1 serum concentration (ng/ml) [‡]	180.4 ± 85.1	182.3 ± 80.1	0.90 [§]
IGF-1 SDS [‡]	0.4 ± 1.4	0.6 ± 1.1	0.44 [§]
IGF-1:GH ratio [¶]	460.0 ± 60.1	650.0 ± 60.3	0.03*

*p-value for difference by COL1A1 Sp1 genotype (Mann–Whitney U test).

[‡]Values are mean ± standard deviation (SD).

[§]p-value for difference by COL1A1 Sp1 genotype (unpaired student's t-test).

[¶]Values are median ± standard error mean.

COL1A1: Collagen type I $\alpha 1$; hGH: Human growth hormone; Sp1: Specific protein 1.

individual variability of response and even drug resistance, for example, as observed in some diabetic patients requiring exorbitant high doses of insulin in so-called insulin resistance [20].

Predicting individual responsiveness to hGH treatment in adult patients with GHD is also not yet possible. Requirement of higher doses may sometimes be misdiagnosed as 'non-compliance'.

As subcutaneous tissue consists not only of connective collagen type I fibers but also of fat cells, differences in subcutaneous tissue thickness and properties could therefore be correlated to differences in fat and collagen content and/or composition.

BMI is an established marker reflecting body fat mass. Our data revealed no association between hGH dose and body weight or BMI.

Table 4. hGH-treatment-related characteristics according to the COL1A1 Sp1 genotype.

hGH-treatment-related characteristics	All	COL1A1 Sp1 genotype			p-value	GG versus TT
		GG	GT	TT		
Duration hGH treatment (months)*	64.0 ± 2.9	60.0 ± 3.6	76.0 ± 5.4	58.5 ± 11.2	0.21 [§]	0.780 [‡]
Dose of injected hGH (mg/day)*	0.4 ± 0.02	0.4 ± 0.03	0.3 ± 0.04	0.2 ± 0.03	0.049 [§]	0.03 [‡]
IGF-1 serum concentration (ng/ml) [¶]	181.1 ± 83.0	180.4 ± 85.1	182.7 ± 88.3	180.9 ± 44.2	0.99 [#]	0.98**
IGF-1 SDS [¶]	0.5 ± 1.3	0.4 ± 1.4	0.6 ± 1.2	0.5 ± 0.7	0.73 [#]	0.79**
IGF-1:GH ratio*	503.8 ± 44.1	460.0 ± 60.1	568 ± 72.1	842.5 ± 101.3	0.05 [§]	0.04 [‡]

*Values are median ± standard error mean.

[‡]p-value for difference by COL1A1 Sp1 genotype (Mann–Whitney U test).

[§]p-value for difference by COL1A1 Sp1 genotype (Kruskal–Wallis test).

[¶]Values are mean ± standard deviation.

[#]p-value for difference by COL1A1 Sp1 genotype (one-way analysis of variance).

**p-value for difference by COL1A1 Sp1 genotype (unpaired student's t-test).

COL1A1: Collagen type I $\alpha 1$; hGH: Human growth hormone; Sp1: Specific protein 1.

Figure 1. hGH dose according to the *COLIA1 Sp1* genotype.

COLIA1: Collagen type I $\alpha 1$; hGH: Human growth hormone; Sp1: Specific protein 1.

However, our study was able to demonstrate a significant association between the *COLIA1 Sp1* polymorphism and the individually required, subcutaneously injected hGH dose in GHD adults by using different statistical approaches. T-allele carriers were treated with significantly lower hGH doses compared with the GG homozygotes, but came to an equivalent clinical outcome, reflected by IGF-1 response to hGH substitution.

Referring to the association of the *COLIA1 Sp1* polymorphism with osteoporosis and fracture risk, gel-shift assays showed that the T allele has increased affinity for Sp1 protein binding compared with the G allele [5]. This was accompanied by an increased abundance of T-derived transcripts in unspliced DNA extracted from bone samples in individuals who were heterozygous for the *COLIA1 Sp1* polymorphism [5]. Analysis of collagen protein production by metabolic labeling showed a higher production of the *COLIA1* chain relative to the *COLIA2* chain in osteoblasts derived from GT heterozygotes compared with GG homozygotes. This was accompanied by relative overexpression of *COLIA1* mRNA relative to *COLIA2* mRNA in osteoblasts [5]. In conclusion, the abnormal ratio of *COLIA1* relative to *COLIA2* was identified to be responsible for impaired mechanical bone strength [5].

The molecular mechanisms by which the *COLIA1 Sp1* polymorphism modulates the response to subcutaneously injected hGH remains unsolved. Similar to the findings related to impaired bone strength [5], alterations of the subcutaneous collagen type I structure, content and/or biological/biomechanical properties may be strongly assumed. This will probably be determined by modification of the *COLIA1:COLIA2* ratio, leading to different absorption capacities and thus different bioavailability/biological effects of subcutaneously applied protein-based drugs.

In summary, the existence of one or two T alleles might alter the *COLIA1:COLIA2* ratio in subcutaneous tissue and thus determine diffusion and/or absorption capacity, resulting in a decreased requirement of hGH to achieve equivalent therapeutic IGF-1 serum concentrations.

The biomechanical properties of the skin are significantly influenced by age [8,21,22]. Approximately 80% of skin aging is attributed to UV irradiation by inducing functional vitamin A deficiency of the skin and intensifying the potency of free radicals [22]. During the cutaneous aging process, epidermal, dermal and subcutaneous tissue components and skin collagen content decrease [22,23]. Subcutaneous tissue reduction comprises the reduction of subcutaneous fat as well as the decrease of collagen synthesis [22,24]. Therefore, the absorption capacity of subcutaneously injected protein-based drugs might also change during the aging process. In our study, the hGH dose was, in fact, significantly lower in patients with older age. Nevertheless, as the hGH dose was titrated referring to the age- and gender-adjusted IGF-1 serum concentrations of the patients, this finding seemed to be rather caused by lower IGF-1 reference serum concentrations according to physiologically lower IGF-1 serum concentrations with increasing age than by age-related skin alterations.

Injected recombinant hGH is also known to influence subcutaneous tissue by itself. Human skin is a GH target organ, expressing GH [25] and IGF-1 receptors [26]. Monoclonal antibodies against IGF-1 inhibited GH effects on skin thickness in nude mice, which were human-skin transplanted [27].

Under GH excess, skin thickness in acromegalic patients is increased [28] and could be significantly reduced by successful treatment of acromegaly [29]. Opposite findings have been histomorphologically demonstrated in GHD patients [30]. In GHD, decreased skin thickness with decreased collagen content occurs [31,32].

Figure 2. Ratio of IGF-1 serum concentrations divided through the hGH dose according to the *COL1A1 Sp1* genotype.

COL1A1: Collagen type I $\alpha 1$; GH: Growth hormone; hGH: Human growth hormone; Sp1: Specific protein 1.

After 6 months of hGH-replacement therapy in GHD patients, skin thickness, measured mechanically and by ultrasound, increased, indicating a stimulation of collagen type I synthesis by hGH therapy, leading to a deposition of collagen type I in the skin [33]. Skin thickness and/or skin collagen content in GHD patients may therefore depend on their individual duration of hGH-replacement therapy.

In our study, we evaluated the effect of the *COL1A1 Sp1* genotype on the GH dose and needed to exclude any possible effect of the injected hGH on the subcutaneous tissue by itself. All included patients had received hGH treatment over at least 12 months before inclusion in this study (mean duration of hGH treatment: 64 ± 2.9 months). We found no relationship between the duration of hGH treatment and hGH dose, IGF-1 serum concentrations, IGF-1 SDS values and IGF-1:GH dose ratios. Therefore, we conclude that an effect of the duration of the hGH treatment on hGH dose or skin thickness can be neglected, which affects the interpretation of our data.

In conclusion, we found an association of the *COL1A1 Sp1* polymorphism, affecting a binding site for the transcription factor *Sp1* in a regulatory

region of the *COL1A1* gene, with the individually required, subcutaneously injected hGH dose in GHD patients. This is the first description of a genetic factor influencing responsiveness to subcutaneously applied protein-based drugs. The molecular mechanisms remain unsolved, but probably seem to be a consequence of an altered *COL1A1*:*COL1A2* ratio, resulting in an influence on biological/biomechanical tissue properties.

Other polymorphisms might also interact with the *COL1A1 Sp1* polymorphism, which may affect skin properties. Different polymorphisms have been identified in the promoter region of the *COL1A1* gene that are in linkage disequilibrium or strong interaction with the *COL1A1 Sp1* polymorphism, and may affect bone mass [34,35] and gene transcription [36]. Further studies are ongoing, addressing this issue. Furthermore, it will be helpful to confirm our results in long-term studies of larger populations, also receiving other subcutaneously applied protein-based drugs, in order to evaluate the clinical and economic consequences.

Future perspective

As there is an association of the *COL1A1 Sp1* polymorphism with the effect of subcutaneously injected recombinant hGH in GHD adults, the *COL1A1 Sp1* polymorphism may also have an impact on the effect of other subcutaneously injected protein-based hormones and drugs. This may lead to a better understanding of the interindividual variation of responsiveness to subcutaneously applied protein-based hormones and drugs in common. In future, molecular diagnostic approaches in certain patient subgroups and identification of 'poor responders' may have clinical and economic consequences.

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

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Executive summary

- The collagen type I α (*COL1A1*)-1 specific protein (*Sp*)-1 polymorphism influences systemic sclerosis, a connective-tissue disorder with excessive production of extracellular matrix components, especially type I collagen, and bone mineral density.
- The *COL1A1 Sp1* polymorphism might also have an effect on the mechanical and biological properties of collagen type I-containing subcutaneous tissue and might therefore influence the response to subcutaneously injected recombinant human growth hormone (hGH) in GH-deficient adults.
- The *COL1A1 Sp1* polymorphism was determined in a total of 122 GH-deficient adult patients (66 men, 56 women; mean age: 50.0 years $\pm$ 13.0 standard deviation; majority Caucasian) included into the Pfizer International Growth Database (KIMS) Pharmacogenetics study.
- *COL1A1 Sp1* genotype was correlated with etiology of GH deficiency, gender, age, weight, height, BMI, hGH dose (mg/day), duration of hGH treatment, IGF-1 serum concentration, IGF-1 SDS and IGF-1:GH ratio.
- The *COL1A1 Sp1* polymorphism was genotyped by MscI restriction digestion and the resulting genotypes were characterized as GG/GT/TT, where GG represents the homozygous wild-type alleles with absence of the G-to-T substitution and absence of the MscI restriction site.
- In GH-deficient adults, the dose of injected hGH was significantly related to *COL1A1 Sp1* genotype ($p = 0.049$).
- GG homozygotes were treated with a significantly higher hGH dose than TT homozygotes ($p = 0.03$).
- IGF-1:hGH ratios were significantly lower in GG homozygotes compared with TT homozygotes ($p = 0.04$).
- *COL1A1 Sp1* genotypes differed, but not significantly, in their IGF-1 serum concentrations ($p = 0.98$) and IGF-1 SDS ($p = 0.79$).
- According to its relationship with a more stable bone structure and decreased bone fracture risk, the *COL1A1 Sp1* polymorphism seems to determine hGH dose in GH-deficient adults.
- The *COL1A1 Sp1* polymorphism may impact skin resistance to subcutaneously injected protein-based drugs as shown for hGH in this study.
- This is the first description of a genetic factor influencing responsiveness to subcutaneously applied protein-based drugs.

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