# Wormian Bones in Osteogenesis Imperfecta: Correlation to Clinical Findings and Genotype

Oliver Semler, Moira S Cheung, Francis H Glorieux, and Frank Rauch*
Genetics Unit, Shriners Hospital for Children and McGill University, Montreal, Quebec, Canada

The presence of a larger than usual number of Wormian bones (accessory skull bones completely surrounded by a suture line) is a well-known radiographic sign of osteogenesis imperfecta (OI), but the phenotypic and genotypic correlates are not well characterized. In the present study we retrospectively analyzed skull radiographs of 195 OI patients (median age 11.8 years, range 0.4–48 years; 100 female). A significant number of Wormian bones (SNWB, defined as the presence of 10 or more Wormian bones) were found in at least one patient in all of the OI types studied (I, III to VII). SNWB were observed in 35% of patients with OI type I, in 96% of patients with OI type III and 78% of patients with OI type IV. SNWB were present in 28% of patients with haploinsufficiency (nonsense and frameshift) mutations in *COL1A1*, in 96% of patients with helical glycine substitutions in the alpha 1 chain of collagen type I and in 72% of patients with helical glycine substitutions in the alpha 2 chain of collagen type I. Stepwise multivariate logistic regression analysis showed that height z-score, an indicator of disease severity, was inversely related with the prevalence of SNWB. SNWB were visible in 19 of the 26 patients who had skull radiographs in the first year of life, including a 2-week-old newborn. Thus, it appears that SNWB occur more frequently in more severely affected OI patients and seem to develop mostly in utero. © 2010 Wiley-Liss, Inc.

**Key words:** collagen type I; cranial abnormalities; osteogenesis imperfecta; phenotype–genotype correlation; Wormian bones

Patients with mild to moderate bone deformities and variable short stature are classified as OI type IV.

Two broad categories of type I collagen mutations result in OI types I–IV. The first are haploinsufficiency mutations that are caused by the failure to synthesize the products of one *COL1A1* allele and consistently result in a clinical picture of OI type I. This can result from frame shifts due to small insertions or deletions, point mutations that create termination codons and some splice site mutations [Byers, 2000]. The second class of mutations are those that result in the synthesis of collagen molecules with structural abnormalities. This is most frequently caused by the substitution of glycine by another amino acid in the helical domain of either the $\alpha 1(I)$ or the $\alpha 2(I)$ chain. Helical glycine mutations can lead to the whole spectrum of clinical severity of OI, from mild OI type I to lethal OI type II, depending on the type of alpha chain affected, the type of amino acid substituted for glycine and the position of the mutation within the alpha chain [Marini et al., 2007].

Apart from these "classical" types of OI, three conditions called OI types V, VI, and VII have been identified over the past decade. OI types V–VII resemble OI types I, III, or IV on clinical grounds, but also have some distinguishing features, and are not caused by *COL1A1* or *COL1A2* mutations. The genetic defects underlying

## INTRODUCTION

Osteogenesis imperfecta (OI) is a hereditary disease characterized by bone fragility and short stature [Rauch and Glorieux, 2004]. The clinical spectrum represents a continuum ranging from perinatal lethality to nearly asymptomatic individuals with occasional fractures and normal stature. The majority of individuals with a clinical diagnosis of OI have an identifiable mutation in *COL1A1* or *COL1A2*, the genes that encode the two collagen type I alpha chains, $\alpha 1(I)$ and $\alpha 2(I)$ [Rauch and Glorieux, 2004]. OI patients with collagen type I mutations can be classified into four clinically defined types. OI type I comprises patients with absence of bone deformities and normal or near normal stature. Type II is lethal in the perinatal period. OI type III is the most severe form in children surviving the neonatal period and leads to extreme short stature.

OI types V and VI are unknown at present, but OI type VII is caused by mutations in the *CRTAP* gene [Morello et al., 2006].

Many individuals with OI have an abnormally large number of Wormian bones, which are accessory skull bones completely surrounded by a suture line. These bones are named after Ole Worm, a 17th century anatomist. Although a few Wormian bones can often be found on skull radiographs of individuals without any skeletal disorder, 99% of healthy subjects have less than 10 Wormian bones [Cremin et al., 1982]. Consequently, OI patients are generally diagnosed with having a "significant number of Wormian bones" (SNWB) when 10 or more Wormian bones are visible on a skull radiograph [Cremin et al., 1982].

SNWB are not specific to OI but can also occur in other skeletal diseases like hypophosphatasia, cleidocranial dysplasia, hypothyroidism, and in many other rare syndromes [Sanchez-Lara et al., 2007]. SNWB can also arise after craniosynostoses and can be induced by "purposeful skull deformation," a practice in some ancient cultures, whereby the cranium of young infants was shaped through outside forces [O'Loughlin, 2004]. It has been proposed that abnormal mechanical stresses across cranial sutures are the common denominator of the various conditions that are associated with SNWB [Sanchez-Lara et al., 2007].

Although they do not give rise to clinical problems, the presence of SNWB can be an important clinical finding to confirm the diagnosis of OI and to differentiate OI from non-accidental injury [Paterson and McAllion, 2006]. It is therefore important to characterize Wormian bones associated with OI in more detail. Until now, few studies have examined the presence of Wormian bones in patients with OI. Cremin et al. [1982] compared skull X-rays from 81 OI patients to those of healthy controls and concluded that none of the controls but all of the OI patients had more than 10 Wormian bones. However, this conclusion is difficult to interpret, because the report did not provide any clinical information on the OI patients. In a group of 54 adult OI patients, Kovero et al. found that most patients with OI types III and IV, but less than half of patients with OI type I had SNWB. SNWB were more frequent in patients with platybasia (an abnormality of the shape of the skull base), but no other clinical correlations were undertaken.

In the present study we therefore determined the prevalence of SNWB in a larger group of OI patients and analyzed which phenotypic and genotypic factors were associated with SNWB.

## PATIENTS AND METHODS
## Patients

The study population comprised patients with a clinical diagnosis of OI who were examined at the Shriners Hospital for Children in Montreal between October 1999 and June 2009 and for whom at least one lateral or antero-posterior skull radiograph was available. The majority of OI patients were referred in order to assess whether bisphosphonate treatment was indicated. Skull radiographs were typically performed at the initial diagnostic work-up unless a skull X-ray had already been obtained by the referring physician. In addition to the X-rays taken at the initial work-up, lateral skull radiographs were obtained systematically in patients with a clinical diagnosis of OI who were examined after April 2007 in order to screen for the presence of cranial base abnormalities.

One hundred ninety five patients (100 female, 95 male; age: median 11.8 years, range 0.4–48 years) fulfilled the inclusion criteria for the present analysis. When more than one skull X-ray was available, the most recent radiograph was used for the cross-sectional analyses presented here. In 110 of these patients two or more skull radiographs were available. The first and the most recent skull X-ray of these patients were used for the longitudinal analysis of SNWB. The diagnosis of OI type (based on clinical assessment), bisphosphonate treatment history, height, weight and results of mutation analyses were obtained from the medical chart of each patient.

Out of the 195 patients included in this study, 166 patients were affected by one of the OI types that are commonly caused by mutations in collagen type I (OI type I, III, or IV). DNA sequence analysis of the *COL1A1* and *COL1A2* genes were offered to all patients as part of the standard clinical work-up of OI. At the time of the database lock-in for the present study, the results of DNA sequence analysis were available for 138 of these patients, whereas the results were still outstanding for 22 patients. In nine patients, no mutation was found by full sequence analyses of all exons and exon–intron boundaries of the *COL1A1* and *COL1A2* genes. The 129 patients who were positive for a mutation in *COL1A1* or *COL1A2* were included in the analysis on the relationship between genotype and SNWB.

Five of the patients had a clinical diagnosis of OI type VII [Ward et al., 2002]. In all of these patients, DNA sequence analysis of the *CRTAP* gene was performed and a mutation was discovered in each case, as described elsewhere [Morello et al., 2006]. *CRTAP* sequence analysis was not performed in patients who had a clinical diagnosis other than OI type VII.

## Collagen Type I Mutation Analysis

Total genomic DNA was isolated from peripheral blood using the QIAamp DNA Blood Midi Kit (Qiagen, Inc., Mississauga, Canada). All exons of the *COL1A1* and *COL1A2* genes, including the exon–intron boundaries, were amplified by polymerase chain reaction using primers described before [Korkko et al., 1998]. The sequencing reaction was performed using a BigDye Terminator cycle sequencing kit (Applied Biosystems, Foster City, CA) and the nucleotide sequence was determined using an Applied Biosystems 3100 DNA sequencer.

Sequence traces were aligned with the GenBank reference sequences of the *COL1A1* genomic DNA (AF017178) and cDNA (NM_000088.3), and the *COL1A2* genomic DNA (AF004877.1) and cDNA (NM_000089.3). The helical domains of each α(I) chain correspond to the residues encoded by codons 179–1192 of the *COL1A1* transcript and codons 91–1104 of *COL1A2*, when expressed following the convention, which starts with the translation initiator methionine as amino acid +1, and the A of the ATG codon as nucleotide +1 (http://www.hgvs.org/mutnomen/recs.html).

Mutations in *COL1A1* causing frameshifts or point mutations creating termination codons were predicted to lead to haploinsufficiency. Even though some splice site mutations may also result in haploinsufficiency, the effect of such mutations on mRNA processing is often complex and can not be predicted on the basis of DNA analysis alone [Byers, 2002]. Therefore patients with splice site mutations were not included in the haploinsufficiency group.

## Radiological Methods

Skull radiographs had been obtained using a film-focus distance of 100 cm. These radiographs were assessed independently by two pediatricians specialized in the care of children with OI (O.S. and F.R.). As proposed by Cremin et al. [1982], a radiograph was classified as positive for SNWB when 10 or more Wormian bones were present. The presence of SNWB was assessed for 305 radiographs (as 110 of the 195 patients had two evaluations). For 277 of the 305 (91%) assessments, a lateral skull X-ray was available. In the other 28 (9%) cases the evaluation was based on an antero-posterior skull radiograph. The independent assessments by the two observers was discrepant in 16 cases (5%). The corresponding radiographs were assessed together by the two observers who then identified the individual Wormian bones and thus reached consensus as to the classification of the case.

Lateral skull radiographs were also used to measure the anterior cranial base angle as described by Kovero et al. [2006]. The angle is measured between the nasion-sella turcica line and the sella turcica-basion (tip of clivus, denoting the anterior lip of the foramen magnum) line.

Lumbar spine (L1–L4) areal bone density (LS-aBMD) had been determined by dual-energy X-ray absorptiometry (Hologic QDR 2000W or 4500A; Hologic, Inc., Waltham, MA) in the posterior-–anterior direction. Results were transformed to age-specific z-scores combining reference data from Salle et al. [1992] and data provided by the densitometer manufacturer.

## Statistical Analyses

Height and weight measurements were converted to age- and sex-specific z-scores on the basis of reference data published by the Centers for Disease Control and Prevention [Ogden et al., 2002].

Logistic regression analysis was used to evaluate the relationship between patient characteristics and the presence of SNWB. Results were expressed as odds ratios (OR) with 95% confidence intervals (95% CI). The effect of potential predictor variables was initially assessed in univariate models and then in stepwise multivariate models. To assess the effect of prior bisphosphonate therapy, bisphosphonate treatment status at the time of the radiograph was included as a dichotomous variable (no/yes). OI type I was selected as the reference category for the analyses of OI types, because this

group of patients constitutes one end of the spectrum of disease severity.

All tests were two-tailed. P values below 0.05 were considered significant. Calculations were performed using PASW Statistics software version 17.0 for Windows (SPSS, Inc., Chicago, IL).

## RESULTS

### Analysis by Clinical OI Type

Among the 195 OI patients included in this analysis, 29 were diagnosed with OI types V–VII (Table I). These OI types were considered too rare for detailed statistical analysis, but in each group at least one patient was positive for SNWB. Notably, SNWB were observed in two of the five patients with OI type VII, who all had a mutation in the CRTAP gene.

More comprehensive analyses were carried out in the group of 166 patients who were diagnosed with OI types I, III, or IV (Table I and Fig. 1). Overall, 96 (58%) of these patients were positive for SNWB. The prevalence of SNWB was 35% in OI type I, 96% in OI type III, and 78% in OI type IV.

To elucidate clinical characteristics that were associated with SNWB, logistic regression analyses were performed (Table II). Univariate analyses showed that anterior cranial base angle and a history of bisphosphonate treatment were positively associated with SNWB, whereas z-scores of height, weight and LS-aBMD as well as a diagnosis of OI type I were associated with a lower prevalence of SNWB. Age and gender were not significantly related to the occurrence of SNWB. To find out which of these factors were independently associated with SNWB, they were entered into a stepwise multivariate logistic regression model. The final model included only age (in years) (OR 0.92; CI 0.89–0.97; $P < 0.001$), height z-score (OR 0.47; CI 0.35–0.62; $P < 0.001$) and male gender (OR 0.44; CI 0.22–0.88; $P = 0.02$) as significant determinants of SNWB, whereas OI type, weight z-score, LS-aBMD z-score, anterior cranial base angle and a history of bisphosphonate treatment were not independently associated with SNWB in this model.

### Analysis by Genotype

The association between SNWB and the type of collagen type I mutation underlying OI was investigated in the 129 patients with OI

### TABLE I. Clinical Characteristics According to Clinical OI Type

|  | OI type I | OI type III | OI type IV | OI type V | OI type VI | OI type VII |
|---|---|---|---|---|---|---|
| N (m/f) | 89 (40/49) | 27 (16/11) | 50 (25/25) | 15 (8/7) | 9 (6/3) | 5 (0/5) |
| Age (years); median (range) | 12.0 (0.4; 47.5) | 8.5 (0.7; 22.0) | 11.8 (0.7; 28.7) | 18.1 (3.4; 43.2) | 10.9 (3.0; 28.0) | 7.3 (0.9; 15.6) |
| Height (z-score); mean (SD) | −1.2 (1.2) | −6.9 (1.9) | −3.3 (1.9) | −2.7 (2.3) | −3.9 (3.5) | −2.5 (3.4) |
| Weight (z-score); mean (SD) | −0.5 (1.2) | −2.4 (2.0) | −1.4 (1.4) | −1.0 (1.5) | −1.4 (1.9) | 0.5 (2.6) |
| LS-aBMD (z-score); mean (SD) | −2.3 (1.0) | −3.5 (1.1) | −2.6 (1.4) | −2.2 (1.9) | −2.9 (2.1) | −2.1 (3.8) |
| Bisph tx; n (%) | 52 (58) | 25 (93) | 46 (92) | 12 (80) | 6 (67) | 4 (80) |
| aCBA (degree); mean (SD) | 129 (8) | 147 (10) | 137 (10) | 127 (15) | 129 (1) | 130 (5) |
| SNWB positive, n (%) | 31 (35) | 26 (96) | 39 (78) | 11 (73) | 1 (11) | 2 (40) |

aCBA, anterior cranial base angle; Bisph tx, bisphosphonate treatment; LS-aBMD, lumbar spine areal bone mineral density; SNWB, significant number of Wormian bones.
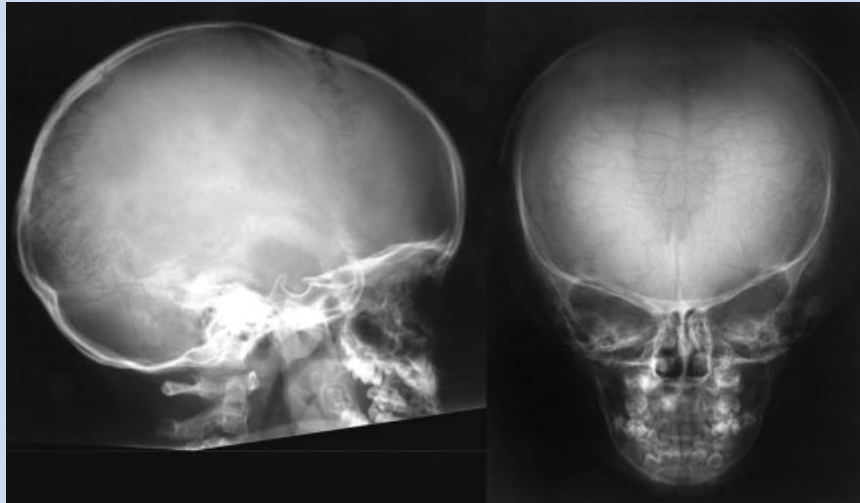
**FIG. 1. Examples of radiographs examined in this study. Left panel: Lateral skull X-ray of a 4-year-old girl with OI type IV. Right panel: Antero-posterior skull X-ray of a 3-year-old boy with OI type III. A significant number of Wormian bones are present in both images.**

type I, III, or IV who had a known mutation in either the *COL1A1* or the *COL1A2* gene (Table III).

SNWB were present in 28% of patients with haploinsufficiency mutations, in 96% of patients with α1(I) helical glycine substitutions, in 72% of patients with α2(I) helical glycine substitutions, in 48% of patients with splice-site mutations, in two of the six patients with C-propeptide mutations, in two of three patients with in-frame deletions, but not in the one patient with an N-propeptide mutation. Further statistical assessment was performed only in the groups of patients with haploinsufficiency mutations and helical glycine substitutions, because propeptide mutations and deletions were too rare for statistical analysis and the effect of splice site mutations on gene transcription can not be judged on the basis of DNA analyses alone.

Univariate logistic regression analysis in the group of patients with haploinsufficiency mutations or helical glycine substitutions showed that the presence of a glycine substitution, anterior cranial base angle and a history of prior bisphosphonate treatment were positively associated with SNWB, whereas higher z-scores of height, weight and LS-aBMD were associated with a lower prevalence of SNWB (Table IV). Gender and age were not related to SNWB in univariate analysis. When all of these variables were entered into a multivariate stepwise logistic regression model, only the presence of glycine substitutions (OR 3.6, CI 1.1–11.6; $P = 0.03$), age (in years) (OR 0.87; CI 0.80–0.94; $P < 0.001$), and height z-score (OR 0.45; CI 0.28–0.74; $P = 0.001$) emerged as significant independent predictive factors of SNWB.

## Longitudinal Analysis

The preceding analyses were performed on the last available radiograph of each patient. However, in 110 patients more than one skull X-ray was available. This allowed for the evaluation of changes in SNWB status. The first skull radiograph had been obtained at a median age of 3.9 years (range: 1 day to 23 years) and the median time interval between the first and the last skull X-ray was 7.4 years

**TABLE II. Univariate Logistic Regression Analysis of Factors Associated With the Presence of Wormian Bones in Patients With Clinical Type I, Type III, or Type IV OI**

|  | n | OR (95% CI) | *P*-Value |
|---|---|---|---|
| Gender (male vs. female) | 166 | 0.98 (0.64, 1.49) | 0.91 |
| OI type (I vs. III and IV) | 166 | 0.53 (0.35, 0.83) | 0.005 |
| Age (years) | 166 | 1.00 (0.98, 1.03) | 0.75 |
| Height (z-score) | 166 | 0.75 (0.67, 0.84) | <0.001 |
| Weight (z-score) | 166 | 0.68 (0.57, 0.82) | <0.001 |
| LS-aBMD (z-score) | 159 | 0.82 (0.73, 0.93) | 0.001 |
| Bisph tx (yes vs. no) | 166 | 1.79 (1.22, 2.61) | 0.003 |
| aCBA (degree) | 157 | 1.003 (1.000, 1.005) | 0.03 |

aCBA, anterior cranial base angle; Bisph tx, bisphosphonate treatment; CI, confidence interval; LS-aBMD, lumbar spine areal bone mineral density; OR, odds ratio.

### TABLE III. Clinical Characteristics According to the Type of COL1A1 or COL1A2 Mutation

| | Haplo-insufficiency | Helical α1(I) mutation | Helical α2(I) mutation | Splice site mutation | C-Propeptide mutation | Deletion | N-Propeptide mutation |
|---|---|---|---|---|---|---|---|
| N (m/f) | 31 (11/20) | 24 (13/11) | 39 (26/13) | 25 (10/15) | 6 (1/5) | 3 (2/1) | 1 (0/1) |
| Age (years); median (range) | 11.7 (0.4; 42) | 12.0 (2.3; 22) | 13.3 (2.3; 29) | 8.7 (0.4; 24) | 8.0 (1.2; 13) | 7.1 (6.0; 16) | 2.6 |
| Height (z-score); mean (SD) | −0.9 (1.1) | −4.5 (2.8) | −3.6 (2.9) | −1.7 (1.5) | −2.7 (1.5) | −3.1 (2.5) | 0.3 |
| Weight (z-score); mean (SD) | −0.3 (1.2) | −1.5 (2.1) | −1.4 (1.6) | −0.8 (1.2) | −1.7 (1.1) | −2.6 (1.3) | 0.6 |
| LS-aBMD (z-score); mean (SD) | −2.1 (0.8) | −2.8 (1.3) | −2.8 (1.2) | −2.3 (1.0) | −1.1 (0.8) | −3.2 (0.5) | −4.8 |
| Bisph tx; n (%) | 19 (59) | 22 (92) | 37 (95) | 16 (64) | 6 (100) | 1 (33) | 0 (0) |
| aCBA (degree); mean (SD) | 130 (8) | 142 (11) | 136 (13) | 129 (7) | 144 (7) | 140 (8) | |
| SNWB positive, n (%) | 9 (29) | 23 (96) | 28 (72) | 12 (48) | 2 (33) | 2 (67) | 0 (0) |

aCBA, anterior cranial base angle; Bisph tx, bisphosphonate treatment; LS-aBMD, lumbar spine areal bone mineral density; SNWB, significant number of Wormian bones.

### TABLE IV. Univariate Logistic Regression Analysis of Factors Associated With the Presence of Wormian Bones in Patients With Haploinsufficiency Mutations or Triple Helical Glycine Substitutions

| | n | OR (95% CI) | P-Value |
|---|---|---|---|
| Gender (male vs. female) | 94 | 1.32 (0.72, 2.39) | 0.37 |
| Type of mutation (helical glycine vs. haploinsufficiency) | 94 | 4.25 (2.27, 7.97) | <0.001 |
| Age (years) | 94 | 1.04 (1.00, 1.07) | 0.03 |
| Height (z-score) | 94 | 0.63 (0.51, 0.78) | <0.001 |
| Weight (z-score) | 94 | 0.59 (0.45, 0.78) | <0.001 |
| LS-aBMD (z-score) | 89 | 0.76 (0.64, 0.89) | <0.001 |
| Bisph tx (yes vs. no) | 94 | 2.12 (1.32, 3.41) | 0.002 |
| aCBA (degree) | 90 | 1.005 (1.002, 1.008) | 0.004 |

aCBA, anterior cranial base angle; Bisph tx, bisphosphonate treatment; CI, confidence interval; LS-aBMD, lumbar spine areal bone mineral density; OR, odds ratio; SNWB, significant number of Wormian bones.

(range: 6 months to 22 years). In 105 of the 110 patients (95%) no change in SNWB status occurred. All patients who were positive for SNWB in the first radiograph also had SNWB in the most recent X-ray. However, in five patients who did not have SNWB at the time of the first skull radiograph (obtained at an age ranging from 1 day to 1.4 years), SNWB were present in the most recent X-ray (Fig. 2).

No minimum age was found for the presence of SNWB. In 26 patients the first skull radiograph had been obtained in the first year of life. SNWB were visible in 19 of these radiographs. The youngest patient in whom SNWB were detected was 2 weeks old at the time when the skull X-ray was taken.

## DISCUSSION

In this study we found that SNWB occurred in all OI types and that 58% of patients with OI types I, III, and IV were positive for SNWB. The occurrence of SNWB was strongly correlated to clinical indicators of disease severity. Correspondingly, we found a clear genotype–phenotype correlation: Patients with haploinsufficiency mutations (that lead to a milder phenotype) were less likely to have SNWB than patients with helical glycine substitutions.

These prevalence data confirm and extend the results obtained by Kovero et al. [2006], who noted the presence of SNWB in 63% of

their adult OI patients. The frequency of SNWB among individual OI types I, III and IV was also similar between the present study and the report by Kovero et al. Our observations might also be reconciled with the statement by Cremin et al. [1982] that "all" OI patients have SNWB, if one assumes that these authors only investigated very severely affected OI patients. The finding that SNWB also occurred in patients with CRTAP mutations confirms a recent report on the phenotype of patients with mutations in that gene [Van Dijk et al., 2009].

Our univariate logistic regression analyses showed a strong correlation between the presence of SNWB and all clinical indicators of disease severity (OI type, height, weight, bone density, history of bisphosphonate treatment). In the multiple regression analysis, height z-score displaced the other clinical indicators of severity. This is probably due to the fact that even though the other measures provide largely similar information about disease severity, height z-score is the most precise indicator of overall disease severity.

To our knowledge, this is the first study to correlate the presence of SNWB with the results of sequence analyses in COL1A1 and COL1A2. These analyses showed that glycine substitutions in α1(I) were almost always associated with SNWB, whereas only about three quarters of patients with glycine substitutions in α2(I) and
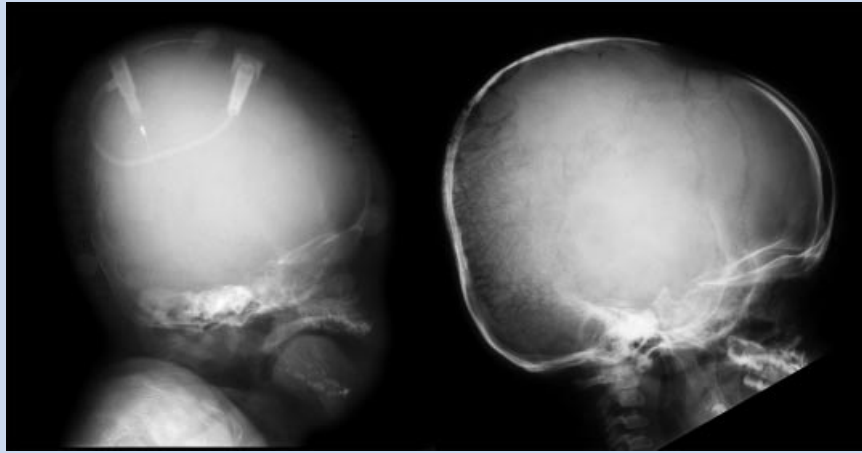
FIG. 2. Longitudinal follow up in a boy with OI type III. Left panel: At the age of 3 months the skull appears undermineralized and no Wormian bones are visible. An infusion system is in place for intravenous bisphosphonate application. Right panel: At the age of 3 years a large number of Wormian bones are present in the occipital area.

one quarter of patients with haploinsufficiency mutations had SNWB. These results demonstrate the importance of the genetic determinants of SNWB. Nevertheless, the fact that the group of patients with haploinsufficiency mutations were discordant with regard to SNWB is intriguing. Haploinsufficiency mutations are expected to have a rather uniform consequence on collagen type I protein production, namely a decrease in expression levels to half the normal amount [Byers, 2000]. The fact that patients with haploinsufficiency mutations varied with regard to SNWB status therefore suggests that the development of SNWB is influenced by factors other than the type of collagen type I mutation. This is also suggested by the finding that height z-score remained significantly associated with SNWB even after the type of collagen type I mutation was entered into the multivariate regression model.

The anterior cranial base angle was assessed as a marker of skull deformity and because Kovero et al. [2006] had found a relationship of this parameter with SNWB. We indeed found a correlation between the anterior cranial base angle and SNWB. However, the multivariate logistic regression analysis showed that the anterior cranial base angle was not an independent predictor of SNWB once height z-score was entered into the model. This suggests that the correlation between the anterior cranial base angle and SNWB is caused by a common determinant (e.g., disease severity and thus the underlying bone weakness) rather than by a cause and effect relationship between cranial base deformation and the development of SNWB.

The effect of gender on the prevalence of SNWB differed in the analysis by clinical OI type and the analysis by genotype. Males had a lower prevalence of SNWB in the analysis by OI type, but not when genotype was taken into account. This may be due to the fact that the genotype distribution were different between males and females. Thus it seems unlikely that there is a real sex-difference in the occurrence of SNWB once the type of disease-causing mutation is taken into account.

Age was significantly associated with SNWB in both the analysis by OI type and the analysis by genotype, and the odds ratios were below 1 in both analyses. This indicates that the percentage of patients who were positive for SNWB decreased with age. In contrast to this, our longitudinal analyses showed that once SNWB are detectable in a patient they persist. It is therefore likely that in the cross-sectional study the older patients differed from the younger patients in aspects that were not captured in our analysis but that have an influence on the occurrence of SNWB.

Even though the present analysis does not provide mechanistic data, some observations may contribute to elucidate the pathogenesis of SNWB in OI. In cultural cranial deformation, Wormian bones arise as a consequence of external forces that are applied to the skull during the first year of life [O'Loughlin, 2004]. As patients with OI also often have skull deformation, one might hypothesize that, in analogy to cultural cranial deformation, Wormian bones in OI develop secondary to postnatal skull deformation. However, our observations that the majority of patients had SNWB already in the first year of life and that babies as young as 2 weeks were positive for SNWB rather suggest that Wormian bones in OI mainly develop in utero. It is still possible that, similar to cultural cranial deformation, abnormal mechanical stresses across sutures play a role in the pathogenesis of SNWB in OI. We are not aware of data on the mechanical forces that the uterine wall exerts on the fetal skull, but it is certainly conceivable that the softness of the skull bones in OI might lead to increased tension across sutures.

In a few cases we found that patients who were negative for SNWB in the first 18 months of life, had SNWB on radiographs later in life. It is therefore possible that Wormian bones may develop postnatally in some cases. However, it can not be ruled out that SNWB in these infants were present already at the time of the first radiograph but escaped detection because of skull undermineralization, and became visible later as skull mineralization increased.

This study has limitations. This was a hospital-based retrospective chart review rather than a prospective population-based study. Skull radiographs were obtained in an institution that receives many referrals of OI patients who are deemed candidates

for bisphosphonate treatment. Consequently, the study cohort probably contains a higher proportion of more severely affected OI patients than if a population-based approach had been taken. Nevertheless, one might expect that this bias towards more severe phenotypes mostly affected the relative distribution of OI types and of mutations types and less the findings within a given OI type or mutation type.

Another limitation of this study is that skull radiographs were classified as being positive or negative for SNWB (defined as the presence of 10 or more Wormian bones), rather than determining the exact number of Wormian bones. This dichotomous approach followed the methodology of previous studies on this topic, but is less informative than recording the exact number of Wormian bones. For example, even though we found that 65% of patients with OI type I did not have SNWB, this does not mean that Wormian bones were completely absent in all of these patients. A few Wormian bones are frequently found in skull radiographs of healthy subjects but the exact frequency distribution in the healthy population is unknown [Cremin et al., 1982]. In future studies it might therefore be advisable to include a control group of healthy subjects and to determine the precise number of Wormian bones that is visible on each radiograph.

In conclusion, the current study provides evidence that SNWB in OI are more frequent in more severely affected patients, but that they occur also in about a quarter of patients who have haploin-sufficiency mutations in *COL1A1*. It appears that Wormian bones in OI develop mostly in utero. Therefore, in cases of diagnostic uncertainty, SNWB can be a clinically useful sign of OI even in young babies.

## ACKNOWLEDGMENTS

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