Mineral particle size in children with osteogenesis imperfecta type I is not increased independently of specific collagen mutations

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Abstract

Osteogenesis imperfecta (OI) type I represents the mildest form of OI and is usually caused by two classes of autosomal dominant mutations in collagen type I: haploinsufficiency leading to a reduced quantity of normally structural collagen (quantitative mutation), or sequence abnormalities generating structurally aberrant collagen chains (qualitative mutation). An abnormally high bone matrix mineralization has been observed in all OI cases investigated so far, independently of mutation type. This raises the question whether the increased amount of mineral is due to mineral particles growing to larger sizes or to a higher number of more densely packed particles. For this reason, we revisit the problem by investigating the mineral particle size in cancellous bone from two subsets of the previously analyzed biopsies (patient’s age: 2–4.2 and 7.6–11 years) comparing OI quantitative mutations (n = 5), OI qualitative mutations (n = 5) and controls (n = 6). We used a combined small-angle X-ray scattering (SAXS) and wide-angle X-ray diffraction (WAXD) setup with a beam diameter of 10 μm of synchrotron radiation, which allows the determination of mineral particle characteristics in 10 μm thick sections at the same positions where the matrix mineralization density was previously determined. The thickness parameter of mineral particles (T) was obtained from SAXS data and the mineral volume fraction was calculated from the mean calcium content of the bone matrix determined by quantitative back-scattered electron imaging (qBEI). The combination of these two quantities allowed calculating the true particle width (W) of the plate-like mineral crystals. T was larger in the older than in the younger age-group independently of genotype (p < 0.004) and was larger in the controls than in each OI group. The qBEI results showed that the mineral volume fraction increased from 32.45wt.% in controls to 36.44wt.% in both OI groups (corresponding to a 12% increase in relative terms). Combining these data, we find that also W was larger in the older than in the younger age-group (p < 0.002), but stayed equal or smaller in both OI genotypes (controls: 2.3 nm ± 0.04, OI qualitative: 2.2 ± 0.05; OI quantitative 2.3 ± 0.04, mean ± SEM). A linear regression analysis even suggests a slower increase of W in qualitative OI as compared to quantitative OI and controls, where the particle sizes stayed similar at all ages. We thus conclude that the high mineral density in human OI is not due to increased particle size but rather to increased particle packing density. The lack of an observed difference between the two classes of mutations suggests the occurrence of a bone cell defect downstream of the collagen mutation.

Introduction

Osteogenesis imperfecta (OI) is a clinically heterogeneous, heritable connective tissue disorder with high bone fragility [1,2]. More than 90% of the patients carry autosomal dominant mutations, in one of the two genes that encode collagen type I alpha chains, COL1A1 and COL1A2, altering either the quantity or the structure of collagen type I. The resulting phenotypes are extremely broad and have traditionally been classified according to Sillence [3] into four groups based on clinical, radiographic and genetic criteria whereby type I represents the mildest, non-deforming form, type IV and III are progressively deforming and increasingly severe and type II is perinatal lethal. More recently, proteins have been described that interact with collagen biosynthesis and their deficiency results in recessive forms of OI sharing clinical and radiological criteria of “classical” forms but lacking primary defects in type I collagen [2,4]. Consequently the Sillence classification has been expanded including novel forms (OI type V to XI) based on the underlying mutations and/or the distinctive clinical phenotype [1,2,5–7].

The hallmark of OI is bone fragility that is manifest independently of clinical severity. Previous studies agree that the susceptibility to

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fractures in OI arises, on the one hand, from low bone mass, due to low bone volume [1,2,8] and, on the other hand, from alterations of bone material properties [9]. Histomorphometric evaluations of bone biopsy samples from children with OI type I, III and IV revealed that bone acquisition during growth is profoundly disturbed due to abnormal bone modeling, decreased production of secondary trabeculae during endochondral ossification as well as decreased thickening of existing trabeculae by bone remodeling [8]. At the bone material level, early high-resolution transmission electron microscopy observations of OI bone fragments revealed overmineralized regions, with generally small unorganized apatite crystals [10]. Boyde and coworkers first studied iliac crest biopsy cores from children with OI by back-scattered electron imaging and reported that the bone matrix in OI children is more highly mineralized than in aged-matched controls and hypothesized that this change in tissue characteristics increases bone stiffness and may account for the “brittleness” observed in OI [9,11]. The abnormally high matrix mineralization in OI was further confirmed in mice models using Fourier transform infrared spectroscopy (FTIR) [12–15] and/or quantitative backscattered electron imaging (qBEI) to assess bone mineralization density distribution (BMDD) [13,16,17]. Moreover, the qBEI analyses conducted so far in bone biopsies from children with OI showed that the abnormal mineralization density did not depend on clinical severity and that treatment with bisphosphonate did not further increase the mineral content of the bone matrix, presumably because of the inherent mineral saturation of the bone matrix [18–20].

To get further insights into the abnormal bone mineralization in OI we have previously compared BMDD in bone biopsy samples from pediatric patients with the same clinical phenotype but different genotypes. Indeed, OI type I can result either from stop or frameshift mutations in COL1A1, leading to haploinsufficiency and consequently to formation of a reduced quantity of structurally normal collagen (quantitative mutation), or from mutations affecting a glycine residue in the triple helical domain in one of the two alpha chains. The latter mutations lead to the generation of structurally aberrant collagen chains that may be incorporated into the bone matrix (qualitative mutation) reviewed by [2,6,21]. Unexpectedly, we found in both mutation groups, therefore also in subjects where the collagen structure was intact, the same histomorphometric alterations, i.e., reduced bone size and mass, increased bone turnover and the same increased bone matrix mineralization [18]. These observations suggested that the abnormally high mineralization in OI bone might not be primarily caused by local defects in the collagen chain structure, but by a modification of the structure and composition of the mineralized collagen fibril, the basic building block of bone material [22]. The mineral particles in the collagen matrix are typically elongated plate-like particles of width W and length L. The dimension of W is in the range of several nanometers, the dimension of L of several tens of nanometers [23]. Conflicting models have been proposed as the origin for the increased mineral content in the OI bone matrix. In the first one, mineral particles are able to grow to larger sizes because of larger available space in defective collagen fibrils [9]. This model would suggest that quantitative and qualitative mutations should behave differently since there is no structural collagen defect in the quantitative mutation. Following results in the oim mouse model, originally obtained by small-angle X-ray scattering (SAXS) [24] and later confirmed by transmission electron microscopy [17], it was concluded that in this mouse model mineral particles are not larger but rather more densely packed than normal. Similar data do not exist to date for human OI bone.

The goal of the present study was to determine size and shape of hydroxyapatite mineral particles in transiliac biopsy samples from children with OI, either with quantitative or qualitative mutations, as well as, in age-matched healthy controls. The population in this study was evaluated previously by bone histomorphometry and qBEI [8,18,25,26] and we now applied a synchrotron X-ray scattering technique to evaluate mineral particle size in bone within the same sample positions where mineral content was previously measured [27].

Subjects and methods

Subjects

The study population comprised in total of 16 children, 11 males and 5 females, aged 2.1 to 11.0 years from the Shriners Hospital for Children in Montreal, Canada and are part of a larger cohort published previously [8,18,25,26]. For the present study we compared two age-groups: 2–4.2 years and 7.6–11 years.

The diagnosis of OI type I was based on clinical presentations, as described by Silence [3]. Patients were eligible for the present study if molecular diagnostic studies had revealed a mutation affecting collagen type I, and if an iliac bone biopsy sample had been obtained before the start of any osteotropic medication. Five patients had quantitative mutations, and 5 qualitative mutations. The age-matched control samples were obtained during surgery for various orthopedic conditions. All subjects were ambulatory, had normal renal function as assessed by measurement of serum creatinine and had no evidence of any metabolic bone disease. Orthopedic conditions included lower limb deformities, scoliosis, clubfoot and other problems that require corrective surgery (exostoses, cubitus valgus, equinovarus of the foot). Clinical characteristics of all subjects are summarized in Table 1.

The study was approved by the Shriners Hospital Institutional Review Board and informed consent was obtained from subjects and/or legal guardians.

Methods

Mutation analyses

Genomic DNA from peripheral blood leukocytes from all OI-I patients was analyzed as reported previously [18,28].

Quantitative backscattered electron imaging (qBEI)

Full details of the technique and its precision have been published elsewhere [29]. Blocks containing polymethylmethacrylate-embedded undecalcified iliac bone samples were prepared by grinding and polishing in order to obtain plane parallel surfaces. The sample surface was then carbon coated. Quantitative BEI was performed in a digital scanning electron microscope (DSM 962, Zeiss, Oberkochen, Germany) using a pixel resolution of 2 μm. CaMean, the weighted mean calcium concentration of the bone area was deduced from the bone mineralization density distribution curve [16].

Small angle X-ray scattering (SAXS) and wide angle X-ray diffraction (WAXD)

SAXS requires different sample thickness than qBEI and therefore the qBEI analysis was performed in a first step on a block and later thin slices of about 10 μm were cut head on from this block. The top slice with the carbon coating was discarded and the most adjacent slice to the top was used for the SAXS analysis. The individual sections were then mounted into lead foils with circular windows of about 2 mm and the bone structure was pre-characterized using light microscopy. Scanning small angle X-ray scattering (SAXS) and wide-angle X-ray diffraction (WAXD) experiments with a micrometer beam were performed at the microspot beamline at the synchrotron radiation source BESSY II (Helmholtz-Zentrum Berlin für Materialien und Energie GmbH). For each bone sample, two-dimensional scattering patterns (measurement range in q-space: 0.3 nm−1 to 25 nm−1) were obtained from at least 35 positions (on average: 42; see Table 1) in the cancellous bone compartment, and were used to calculate parameters describing the mineral particle size. Fig. 1a shows a qBEI image of a sectioned trabecular bone feature on the sample block adjacent to the 10 μm section studied in the synchrotron. The positions where the single SAXS measurements were performed are indicated exemplarily for one sample. The combination of SAXS and qBEI measurements allowed obtaining information on the width W of the plate-like mineral particles (see
Patients’ characteristics with all synchrotron (SAXS, WAXD) and qBEI results.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Type I collagen mutation</th>
<th>Age (years)</th>
<th>Sex</th>
<th>SAXS (n/sample)</th>
<th>SAXS T mean [nm] (SD)</th>
<th>qBEI Ca Mean [%] (FWHM)</th>
<th>qBEI ΦqBEI [%]</th>
<th>SAXS qBEI W mean (nm)</th>
<th>WAXD (n/sample)</th>
<th>WAXD L mean (nm)</th>
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<td>44</td>
<td>3.0 (0.29)</td>
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<td>F</td>
<td>42</td>
<td>2.9 (0.22)</td>
<td>20.86 (3.12)</td>
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<td>M</td>
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<td>22.30 (2.77)</td>
<td>36.00</td>
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<td>M</td>
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<td>2.8 (0.18)</td>
<td>22.46 (2.95)</td>
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<td>2.2</td>
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<td>18.6</td>
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<td>F</td>
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<td>3.0 (0.18)</td>
<td>22.56 (2.95)</td>
<td>36.61</td>
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<td>OI-I qual Gly 190-STOP in COL1A2</td>
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<td>M</td>
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<tr>
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<td>F</td>
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<td>9.4</td>
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<td>41</td>
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<td>20.99 (3.29)</td>
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<td>11.0</td>
<td>M</td>
<td>36</td>
<td>2.9 (0.28)</td>
<td>21.26 (2.77)</td>
<td>33.61</td>
<td>2.2</td>
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</table>

Clinical characteristics and results of the collagen mutation analysis for each OI patient (with quantitative mutation, OI-I quant; with qualitative mutation, OI qual); and controls; quantitative backscattered electron microscopy (qBEI), small angle X-ray scattering (SAXS) and wide-angle X-ray diffraction (WAXD) of the corresponding bone biopsy.

**Calculation of the parameters** $T$, $Φ_{q\text{BEI}}$, $W$ and $L$

SAXS is based on the measurement of the electron density distribution, which in the case of bone reflects at the nanometer scale the different phases, of the hydroxyapatite particles embedded in collagen [30–32]. SAXS results refer to an average value describing the mineral particle size within the volume irradiated by the X-ray beam [22]. To obtain high spatial resolutions via small X-ray beam diameters of a few microns or lower, synchrotron sources have to be used [33,34].

In the present experiments, the beam (diameter = 10 μm, wavelength = 0.82656 Å) was defined by a toroidal mirror to focus the beam horizontally and vertically [27] and with a MoBC-Multilayer monochromator. To record the scattering patterns, a two-dimensional CCD-based MAR Mosaic detector was used. The calibrations of the sample-to-detector distance and beam center were carried out by using the software Fit2d (Andy Hammersley, ESRF, v.12.077) and the parameter calculation was performed by using AutoFit (Chenghao Li, MPI, v.12.3). The size of the mineral particles can be obtained from the SAXS intensity $I(q, Φ)$. The parameter $T$ is defined as

$$T = \frac{4Φ(1-Φ)}{σ}$$

with $Φ$ being the mineral volume fraction and $σ$ the total surface of the mineral particles per total tissue volume. Using the assumption that mineral particles have the shape of platelets and that the volume fraction of mineral particles is in the range of $Φ ≈ 0.5$, the $T$ parameter represents directly the average mineral particle thickness [24,30,35–37]. If the volume fraction deviates notably from $Φ ≈ 0.5$ and/or is changing within the sample, SAXS and qBEI data can be combined to determine the particle thickness more precisely as described elsewhere [34,38]. The parameter $W$ (Eq. (2)), where $Φ_{q\text{BEI}}$ is the volume fraction of the
mineral particles determined by qBEI (Eq. (3)), describes the true width of the plate-like particles [34].

\[ W = \frac{T}{2(1-\Phi_{qBEI})} \]  

(2)

\[ \Phi_{qBEI} = \frac{HA_{\text{weight}}}{HA_{\text{weight}} + (1-HA_{\text{weight}})(\rho_{\text{HIA}}/\rho_{\text{org}})}. \]  

(3)

When combining and interpreting both data types from SAXS and qBEI to use \( W \) instead of \( T \), one has to be cautious [34] because both methods are working with different signal sampling volumes. The signal sampling depth in qBEI is around 2 \( \mu \)m, whereas SAXS uses the entire thickness of the sample sections (in our case, around 15 \( \mu \)m).

WAXD was carried out at the same synchrotron beamline as described above, but using a double crystal monochromator with a Si 111 crystal. The particle parameter \( L \) (particle length (c-axis)) is an average value and can be easily calculated by the following equation

\[ L = \frac{K\lambda}{\beta_m \cos \theta} \]  

(4)

where \( K \) is a constant which was chosen 0.9 for plate shaped crystallites, \( \lambda \) is the wavelength, \( \beta_m \) is the line width of the pure diffraction profile resulting from small crystallite size and \( \theta \) is the diffraction angle [39]. The lengths for mineral crystals determined by X-ray diffraction is presumably shorter than the real crystal length since microstrain effects also influence the peak broadening [39,40]. The determined values of the WAXD 002 peak widths have been converted to a length through the Scherrer equation to allow a comparison between controls and cases, for a small subset of our specimens. Theoretically microstrain and crystallite size could differ significantly in the OI versus control samples in such a way as to make the total broadening indistinguishable. But we doubt this for two reasons: (i) crystallite size broadening affects the peaks much more than microstrain and (ii) microstrains seem to be rather consistent in bone [39,40]. For this reason, we report the broadening of the 002 peak despite the fact that higher orders could not be measured to distinguish between microstrain and crystallite size.

Statistics

The \( T \) and \( W \) parameter values obtained from the single SAXS measurements as well as the \( \Phi_{qBEI} \) measurements were averaged for each bone sample. The sample means were used then for the statistical evaluations. Statistical comparisons for \( T \), \( W \) and \( \Phi_{qBEI} \) were based on two-way ANOVA for genotype (OI-quant/OI-qual/controls) and age (2–4.2 years/7.6–11 years) (SigmaPlot 11.0). p-Values for genotype, age and interaction term, and subsequent genotype post-hoc comparison of Holm–Sidak-test are shown. Linear regression analyses of \( W \) against age with statistical comparison of the slopes were performed using Graph Pad Prism 5.0. The platelet length \( L \) was determined in only 6 samples (see Table 1) and therefore, the data are described numerically.

Results

The size and density distribution of mineral within bone material of 10 patients with OI type I were investigated by a combination of qBEI in the scanning electron microscope and SAXS/WAXD using synchrotron X-ray radiation. All these data are summarized in Table 1. Among the 10 patients, 5 had frameshift mutations or stop codons within the COL1A1 gene and were classified as having a quantitative mutation (OI-I quant); 5 patients had mutations affecting a glycine residue in the collagen type I alpha 1 or alpha 2 chains and were classified as having a qualitative mutation (OI-I qual). The table also summarizes the comparative data for 6 normal controls.

First, the thickness of the mineral particles was characterized by the SAXS parameters \( T \) (defined by Eq. (1), Fig. 2). Assorting the samples in two age groups (2–4.2 years and 7.6–11 years, since there were no specimens between 4.2 and 7.6 years of patient age), a two-way ANOVA analysis for \( T \) revealed that: i) the interaction between factors age and genotype was not significant (\( p = 0.371 \)), ii) The factor age had a significant influence on \( T \) (\( p = 0.004 \)). \( T \) was larger in the older age-group than in the younger one. iii) The factor genotype had a significant influence on \( T \) (\( p = 0.008 \)). Post-hoc tests revealed that \( T \) was larger for controls compared to each of the OI types (\( p = 0.016 \) for quantitative OI and \( p = 0.017 \) for quantitative OI), but no significant difference could be found between the OI types (\( p = 0.53 \)). These data indicate that \( T \) is larger in the 7.6–11 year group compared to 2–4.6 year group and is larger in controls than in OI but there are no detectable differences between OI quantitative and OI qualitative.

The advantage of \( T \) is that it can directly be extracted from the SAXS data and is, therefore, independent from any further assumption. The drawback with this definition is that \( T \) is a substitute for mineral particle thickness only if the local mineral content in the bone material \( \Phi \) stays roughly constant. Since in the present case we are interested to understand the origin of the change in \( \Phi \) between the control and the OI bone, we needed to be more careful and extracted the parameter \( W \) via Eq. (2), whereby \( \Phi \) was obtained from the same specimens via qBEI (Eq. (3)). The outcomes for \( W \) were that: i) the interaction term between factors age and genotype was not significant (\( p = 0.316 \)), ii) the factor age had a significant influence on \( W \) (\( p = 0.002 \)), and iii) the factor genotype had no significant influence on \( W \) (\( p = 0.51 \)). This data show that the mean crystal width \( W \) is significantly larger in
the older age-group versus the younger one for controls and OI and there is no obvious difference between controls, OI quantitative and OI qualitative. The smaller values of \( T \) found for both OI types, are obviously due to the influence of the mineral content on the parameter \( T \).

To avoid the somewhat artificial separation into two age groups, we also did a linear regression analysis with a statistical comparison of the slopes of \( W \) versus age, where we found 0.040 ± 0.0075 for controls, 0.048 ± 0.0091 for OI-quantitative and −0.00022 ± 0.017 for OI-qualitative. This difference of slope was significant to the level \( p = 0.05 \), which indicates that the mineral particle thickness did not increase with age for the qualitative OI, in contrast to controls and quantitative OI.

Fig. 3 shows the \( W \)-parameter plotted versus the average calcium concentration \( \text{Ca}_{\text{mean}} \) obtained by qBEI. Square symbols show data for the older age-group and circles for the younger one. It can be observed that the green symbols (controls) separate nearly completely from the red and yellow ones, representing both OI types (same color code as previously): the OI groups are shifted towards higher mineralization density (without any statistical difference in \( W \) between any of the genotypes, as stated above). Two-way ANOVA outcomes for corresponding \( \Phi_{\text{BEI}} \) values were: i) the interaction term between factor age and genotype was not significant (\( p = 0.551 \)), ii) factor age had no significant influence on \( \Phi_{\text{BEI}} \) (\( p = 0.446 \)), and iii) factor genotype had a significant influence on \( \Phi_{\text{BEI}} \) (\( p = 0.001 \)). Post-hoc tests indicated, that OI quantitative (\( \Phi_{\text{BEI}} = 36.76 ± 1.56 \)) and OI qualitative (\( \Phi_{\text{BEI}} = 36.11 ± 1.42 \)) were significantly higher (+12.3%) compared to controls (\( \Phi_{\text{BEI}} = 32.45 ± 0.88 \), \( p = 0.002 \) and \( p = 0.005 \), respectively), but there was no significant difference between both OI groups (0.704).

This data indicate that the mean mineral volume fraction (\( \Phi_{\text{BM}} \)) corresponding to the mineral volume to tissue volume is significantly higher in both OI groups than in controls. This increase in mineral content is not paralleled by an increase in particle width.

WAXD measurements were performed in four OI samples and two controls and it appears clearly that particle length \( L \) as determined by the width of the (002) reflection peak as described above, was not different between controls and OI samples (see Table 1). These results strongly suggest that the crystals are not much different. Due to the small number of data points, we did not include a statistical analysis of \( L \).

**Discussion**

It has been discussed for many years that bone fragility in OI is not only due to low bone mass but also to altered bone material properties [10]. In particular, the higher stiffness of the more mineralized bone matrix might contribute to the observed brittleness in patients with OI [9,11] Our results show that the high mineralization density in OI bone tissue is not due to an increased particle size, in line with the observation in the oim mouse model [17]. This implies that the larger mineral content must be achieved by an increased number of mineral particles per unit volume.

The combination of synchrotron SAXS with qBEI was used here to characterize the mineral particle size in children with OI having different mutation types, either a reduced quantity of structurally normal collagen (quantitative mutations), or structurally aberrant collagen chains (qualitative mutation) and in age-matched controls. SAXS was used to determine the \( T \)-parameter of mineral particles and qBEI to measure the volume fraction of mineral (\( \Phi_{\text{BEI}} \)) in the same tissue. Both values combined (see Methods formula 2) allow obtaining the real width (\( W \)) of the mineral particles. The SAXS \( T \)-parameter alone has been widely used in previous studies [37,41]. In particular, \( T \) has been determined in the human L4 vertebral body at ages 15 weeks post-conception to 97 years and was found to increase rapidly until the age of 4 years and more slowly afterward [41]. In the present study, we classified our biopsies in two age-groups below 5 years and above 7 years. The main reason for this choice was that translacine bone biopsies of children of both genotypes as well as age-matched controls were not available for each age-point to properly discriminate age-related from genotype-related variation of \( T \). In perfect agreement with this former study, we found that \( T \) to be significantly smaller in younger children with or without OI (age group below 5 years) than in the cohort above 7 years. Moreover, the dimension of \( T \) in the iliac bone measured in the present study: 2.8 to 3 nm in younger controls and up to 3.4 nm in the older group appears very similar to the values of \( T \) measured in the L4 vertebral at similar age [41].

It should be noted that the statistical sample in the present study was not large enough to obtain significant statements about the age dependence within genotypic groups. However, the particle thickness \( W \) (as well as its proxy \( T \)) shows a trend of increasing with age for all groups, except perhaps for the qualitative OI (see Fig. 3). To clarify this point, we further performed a linear regression analysis of \( W \) and the comparison of the slopes showed indeed a significant difference in OI qualitative, suggesting a lack of increase with age. However, it should be noted that two patients with the same mutation of qualitative OI (patients 7 and 16, see Table 1) have quite different \( W \) values. Remarkably, the largest values for \( W \) were observed in the older healthy controls and not in OI cases. Although this trend was not statistically significant, it reinforces the concept that the increased mineral content in OI is not paralleled by an increase in mineral particle width. Please note that the statistical power of the test at a significance level of 0.05 is only 0.05 for genotype, while it is 0.957 for age. Clearly, a much larger number of cases would be needed to exclude a difference between genotypes (in particular between qualitative and quantitative OI). However, it would be difficult to sufficiently increase the number of biopsies from patients with this rare disease and, even more important, the difference between mean values is so small (less than 0.05 nm!) that it is not expected to have any influence on the mechanical behavior of the mineralized tissue.

Moreover, even though crystal length \( L \) was not measured in all samples, the data for \( L \) obtained for several patients show the length of the crystals smaller, not larger in OI than in controls.

The overall emerging picture is that OI type I independent of the underlying mutation has similar particle width and length than controls, though the mineral volume fraction \( \Phi \) is relatively increased by about 12% (Fig. 4). These findings may shed new light on bone matrix mineralization in general and in particular in OI:

- i) The similarity of increased bone matrix mineralization density and mineral characteristics in OI-I quantitative and qualitative mutations does not support the common assumption, that size...
and shape of the mineral crystals are determined by the structure of the collagenous scaffold.

ii) The findings of the present work support our previous hypothesis that the density of mineral nucleation centers might be increased in OI bone (caused by a bone cell defect downstream of the collagen mutation), allowing the simultaneous growth of a larger number of mineral particles and, consequently, to an accelerated increase and a higher overall degree of mineral content of the bone matrix [18]. We speculate that the final degree of bone matrix mineralization might predominantly be determined by the seeding density of the mineral particles. Indeed, numerous SAXS measurements performed previously in bone tissue, dentine and mineralized cartilage gave evidence that the hydroxyapatite mineral particle size reaches a similar plateau level with time [34,41,42].

iii) Finally, the assumption that the bone mineral content is increasing until the available space (first occupied by water) is fully replaced by mineral is challenged by the similarity of the mineral content in the two OI-I collagen mutations [9]. As already pointed out for the oim mouse model [17,24], our current findings in human OI bone revealed that the differences in the available space for mineral associated with the differences in the collagen structure of the two OI-I mutations does not necessarily result in differences in the final degree of mineralization, i.e. the available space for mineral replacement is likely not the limiting factor for the mineral uptake.

Based on these observations, it is likely that the increased mineral content, which seems to be a hallmark for all types of OI, is due to impaired osteoblastic function rather than to the altered structure of the collagen matrix. Increased cellularity, increased osteocyte lacunar density and abnormal areas of woven bone have been observed in different types of OI [11,43]. Bone histomorphometry revealed osteoblasts from affected patients produce only half the amount of collagen matrix [8]. Also, impaired osteoblast differentiation, abnormal metabolism and altered secretion of non-collagenous proteins are a common feature in OI [44,45], reviewed by [2]. Moreover, very recently, striking abnormalities in the differentiation pathway of bone marrow progenitor cells towards osteoblasts were demonstrated in an OI mouse model [46]. Taken together, these results suggest that at least dominantly transmitted types of OI share common defects from aberrant bone cell development to hypermineralization of the bone matrix. The results of the present study show that the latter is due to abnormal mineralization kinetics leading to increased density of mineral particles without increase in particle size.

In summary, we have further characterized bone material properties in bone biopsy samples from children with OI-type I by providing information on crystal size and density. The SAXS results revealed that bone from OI patients (both qualitative and quantitative) have a larger mineral content but not larger particle width W that we interpret as larger number of mineral particles in the same matrix volume. In line with our previous qBEI findings that the increase of mean degree of mineralization was similar in both patient groups we found similar mineralization patterns between both classes of mutations. We suggest that such an increased density of mineral particles led to the generally observed hypermineralization in OI bone that in combination with the decreased bone mass might contribute to the observed compromised bone strength in OI.

Disclosures

The authors have no conflict of interest.

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