Genotype-phenotype relationship in a large cohort of osteogenesis imperfecta patients with *COL1A1* mutations revealed by a new scoring system

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Abstract

Background: Osteogenesis imperfecta (OI), a heritable bone fragility disorder, is mainly caused by mutations in *COL1A1* gene encoding α1 chain of type I collagen. This study aimed to investigate the *COL1A1* mutation spectrum and quantitatively assess the genotype-phenotype relationship in a large cohort of Chinese patients with OI.

Methods: A total of 161 patients who were diagnosed as OI in Department of Endocrinology of Peking Union Medical College Hospital from January 2010 to December 2017 were included in the study. The *COL1A1* mutation spectrum was identified by next generation sequencing and confirmed by Sanger sequencing. A new clinical scoring system was developed to quantitatively assess the clinical severity of OI and the genotype-phenotype relationship was analyzed. The independent sample *t*-test, analysis of variance, Mann-Whitney *U*-test, Chi-squared test, Pearson correlation, and multiple linear regression were applied for statistical analyses.

Results: Among 161 patients with OI, 32.9% missense mutations, 16.8% non-sense mutations, 24.2% splice-site mutations, 24.8% frameshift mutations, and 1.2% whole-gene deletions were identified, of which 38 variations were novel. These mutations led to 53 patients carrying qualitative defects and 67 patients carrying quantitative defects in type I collagen. Compared to patients with quantitative mutations, patients with qualitative mutations had lower alkaline phosphatase level (296 [132, 346] U/L *vs.* 218 [136, 284] U/L, $P=0.009$) and higher clinical score (12.2 ± 5.3 *vs.* 7.4 ± 2.4, $P<0.001$), denoting more severe phenotypes including shorter stature, lower bone mineral density, higher fracture frequency, more bone deformity, vertebral compressive fractures, limited movement, and dentinogenesis imperfecta (DI). Patients would not present with DI if the glycine substitutions happened before the 79th amino acid in triple helix of α1 chains.

Conclusions: This presented distinctive *COL1A1* mutation spectrum in a large cohort of Chinese patients with OI. This new quantitative analysis of genotype-phenotype correlation would be helpful to predict the prognosis of OI and genetic counseling.

Keywords: Osteogenesis imperfecta; *COL1A1*; Clinical scoring system; Genotype; Phenotype

Introduction

Osteogenesis imperfecta (OI) is a rare heritable bone disorder with incidence of 1/15,000 to 20,000 births, which is characterized by decreased bone mineral density (BMD), impaired bone strength, recurrent bone fractures, and resulting in progressive bone deformity (BD).[1] Moreover, OI can also cause a variety of complex extra-skeletal manifestations including dentinogenesis imperfecta (DI), ligamentous laxity, blue sclera (BS), hearing impairment, and so on.[2] OI is traditionally classified as type I, type II, type III, and type IV, which indicate the mild type, perinatal lethal type, most severe type of survival and moderate type, respectively.[2] However, as the high heterogeneity of patients with OI, the traditional classification could not express the degree of clinical severity of OI quantitatively.

As we know, the pathogenesis of OI is closely correlated to the abnormal structure or quantity of type I collagen. As the most abundant bone matrix protein, type I collagen plays crucial roles in maintaining the integrity of bone structure and bone strength. It is an ordered heterotrimer that consisted of 2 α1 chains and 1 α2 chain, which are encoded by *COL1A1* and *COL1A2* genes, respectively.[3] Qualitative or quantitative defects of type I collagen induced by gene mutations would lead to OI.[4] Generally,

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in the random selection of abnormal chains, 75% of mutant heterotrimers contain 1 or more defective α1 chains.[5] Recently, collagen alterations induced by *COL1A1* mutations are divided into 2 categories, including mutations causing a defect to synthesize the products and leading to quantitative defects of type I collagen, or mutations leading to produce structural abnormal collagen molecules and causing the substitution of a glycine within the Gly-X-Y triplet domain of the triple helix.[6]

Although a lot of *COL1A1* mutations had been reported, the genotype-phenotype relationship of patients with OI have not been fully illuminated,[6-11] and results of such studies were elusive in China because of the limited sample size of OI.[12-14] However, OI not only damages the life quality of the patients, but also brings heavy social and economic burden on their families. Therefore, it is critically important to assess clinical severity and reveal the genotype-phenotype relationship of patients OI, which will be helpful to genetic counseling and predicting the prognosis of OI. This study investigated the *COL1A1* mutation spectrum and evaluated clinical severity of OI quantitatively by a clinical scoring system in a large Chinese cohort of OI. In addition, this study analyzed the genotype-phenotype relationship in these patients with OI.

Methods

Ethical approval

The study was conducted in accordance with the *Declaration of Helsinki* and was approved by the Scientific Ethics Committee of Peking Union Medical College Hospital. All participants or their parents provided written informed consents before they participated in this study.

Subjects

A total of 161 patients from 146 unrelated families who were diagnosed as OI in Department of Endocrinology in Peking Union Medical College Hospital from January 2010 to December 2017 were included in the study. There were 107 males and 54 females with median visiting age of 10.7 years (range: 0–64 years).

Phenotype assessment

We obtained a detailed medical history of the patients and performed a physical examination on them, including BD, BS, DI, and ligamentous laxity. Patients' height and weight were measured by Harpenden stadiometer (Seritex Inc., East Rutherford, NJ, USA) and RGZ-120 weighing scale (Xiheng, Wuxi, China). The age- and gender-specific *Z*-scores of height and weight were calculated on the basis of reference data from the Chinese National Centers for Disease Control and Prevention.[15] BMD at lumbar spine 2–4 and proximal hip were measured by Dual energy X-ray absorptiometry (DXA, Lunar Prodigy Advance; GE Healthcare, Chicago, IL, USA), and the BMD *Z*-scores were calculated based on BMD data from age- and gender-matched normal Chinese children.[16,17] Information of bone fractures were evaluated including time of the initiation, frequency and site. Features of bone were assessed by X-ray films. Then patients were diagnosed as OI type I, III, or IV according to the Sillence classification based on clinical and radiological characteristics. As type II OI was neonatal lethal, no patient with this type OI was included.

To quantitatively evaluate the phenotypes, we used clinical scores to assess the severity of OI.[18,19] Clinical score = number of fractures (1–4) + fracture frequency (1–4) + vertebral compressive fracture (VCF) (0–1) + scoliosis (0–1) + bowing (0–8) + height *Z*-score (1–3) + BMD *Z*-score (1–4) + limited movement (0–1). Number of fractures scoring: 1, number of fractures < 10; 2, $10 \leq$ number of fractures < 20; 3, $20 \leq$ number of fractures < 30; 4, number of fractures ≥ 30. Frequency of fracture scoring: 1, frequency of fractures (per year) < 1; 2, $1 \leq$ frequency of fractures (per year) < 2; 3, $2 \leq$ frequency of fractures (per year) < 3; 4, frequency of fractures (per year) ≥ 3. VCF scoring: 0, non VCF; 1, with VCF. Scoliosis scoring: 0, non scoliosis; 1, with scoliosis. In the scoring system, bowing of the tibia, femur, humerus or forearm accounts for 1 point each. Height Z-score scoring: 1, height Z-score ≥ -2.0; 2, $-3.0 \leq$ height Z-score < -2.0; 3, height Z-score < -3.0. BMD Z-score scoring: 1, BMD Z-score ≥ -2.0; 2, $-3.0 \leq$ BMD Z-score < -2.0; 3, $-4.0 \leq$ BMD Z-score < -3.0; 4, BMD Z-score < -4.0. Limited movement scoring: 0, autonomic activity; 1, limited movement. A higher score meant severer clinical phenotype of OI.

Serum levels of alanine aminotransferase (ALT), creatinine (Cr), calcium (Ca), phosphate (P), alkaline phosphatase (ALP), and a bone formation marker were measured by automated analyzers. An automated electrochemiluminescence system (E170; Roche Diagnostics, Basel, Switzerland) was used to detect serum levels of beta cross-linked carboxy-terminal telopeptide of type I collagen (β-CTX, a bone resorption marker), 25-hydroxyvitamin D (25OHD), and intact parathyroid hormone (PTH). All these serum biochemical parameters were measured in the central clinical laboratory of Peking Union Medical College Hospital.

Detection of COL1A1 mutation

The patients' genomic DNA was isolated from peripheral leukocytes under the standard procedures (QIAamp DNA Mini Kit (50); Qiagen, Duesseldorf, Germany). Genetic detection was performed through paired-end sequencing using a panel for next generation sequencing (NGS), which covered more than 700 genes associated with genetic bone disorders, including 19 candidate genes of OI (*COL1A1*, *COL1A2*, *IFITM5*, *SERPINF1*, *CRTAP*, *P3H1*, *PPIB*, *SERPINH1*, *FKBP10*, *BMP1*, *PLOD2*, *SP7*, *TMEM38B*, *WNT1*, *CREB3H1*, *SPARC*, *PLS3*, *P4HB*, *SEC24D*). The experimental procedures of the present research followed a protocol as described previously. The genomic DNA from patients were fragmented and ligated with end-repaired adaptors oligonucleotides. Then DNA fragments with adaptor molecules were purified and enriched by polymerase chain reaction (PCR). Each barcoded library product was pooled and subjected to enrichment of targeted genomic sequences, and sequenced on one lane of the IlluminaHiSeq 2000 (Illumina Inc., San Diego, CA, USA)

with paired-end 100 bases read length. Illumina pipeline (version 1.3.4) was used to perform bioinformatic analysis processes of reads mapping, variants detection (including polymorphisms, single-nucleotide variants and small indels), and annotation.[20]

All the sequencing results were analyzed by Nucleotide BLAST software (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to compare with the standard sequences in the database. We only further analyzed those mutations expected to produce damaged protein, including non-sense mutations, missense mutations, frameshift mutations, splice-site mutations, and whole-gene deletion. Prediction of missense mutations was performed by Polyphen2 (http://genetics.bwh.harvard.edu/pph2/) and SIFT software (http://sift.jcvi.org/). Frameshift mutations were predicted using Genome Analysis Toolkit (https://software.broadinstitute.org/gatk/). Potential splice-site mutations were predicted with Berkeley Drosophila Genome Project (http://www.fruitfly.org/). The mutations identified by NGS were further confirmed by PCR using primers (Supplementary Table 1, http://links.lww.com/CM9/A2) and Sanger sequencing and co-segregation analysis in each OI family.

The absence from the Osteogenesis Imperfecta Variant Database (https:// oi.gene.le.ac.uk/home.php) was the criterion to identify the novel mutations. Mutations in *COL1A1* that led to stop codons or frameshifts were classified as quantitative mutations. Mutations that induced amino acid substitution in α1 chains in type I collagen were regarded as qualitative mutations.

Statistical analysis

Data with normal distribution were expressed as mean ± standard deviation and analyzed by independent sample *t*-test or analysis of variance, while those with abnormal distribution were presented as median (Q1, Q3) and analyzed by Mann-Whitney *U*-test. Covariance analysis was used to compare serum levels of biochemical parameters in different groups after adjusted by age and gender. The Chi-squared test was used to analyze categorical variables. Relationships between the positions of helical glycine substitutions in α1 chains of type I collagen and phenotypic features of patients with OI were analyzed using Pearson correlation. Multiple linear regression analysis was applied to examine the associations between bone turnover biomarker level and clinical scores. The SPSS software version 23.0 (SPSS Inc., Chicago, IL, USA) was used to perform all statistical analyses. A two-tailed $P<0.05$ was considered statistical significance.

Results

Phenotypes of patients with OI carrying COL1A1 mutations

According to Sillence classification, this study included 90 (55.9%) patients with type I, 36 (22.4%) patients with type III, and 35 (21.7%) patients with type IV OI. There was no significant difference in gender among these OI types ($P=0.980$). Patients with type III OI were the earliest onset with median age of 0.5 years ($U=26.35$, $P<0.001$), but their median visit age (12.3 years) was the oldest ($U=9.43$, $P=0.009$). The 69.4% patients with type I OI, 34.3% with type III and 51.4% with type IV presented positive family history ($\chi^2=13.18$, $P=0.001$). The clinical scores were the lowest in patients with type I OI (6.6 ± 2.0), and the highest in patients with type III OI (16.0 ± 3.9, $F=178.79$, $P<0.001$). Compared with patients with type I OI, type III had shorter height and lower weight, severer skeletal lesions, including lower BMD *Z*-score at lumbar spine and proximal hip, higher fracture number and frequency, more proportion of BD, VCFs, limited movement, wormian bones and DI, and patients with type IV OI were in moderate severity of phenotypes. The phenotype difference between OI types was consistent with the clinical score. BS (94.4–97.7%) and ligamentous laxity (61.3–80.0%) were the common manifestations among different OI types, while hearing impairment was infrequent (2.9–5.7%). There was no significant difference in serum levels of Ca, P, β-CTX, 25OHD, PTH, ALT, and Cr among different OI types, but serum ALP level was lowest in patients with type III OI ($U=8.21$, $P=0.016$) [Table 1]. In addition, as shown in Supplementary Figure 1, http://links.lww.com/CM9/A2, there was an independent negative correlation between ALP level and clinical score, which indicated that lower ALP level maybe predicted severer clinical manifestation.

COL1A1 mutation spectrum of Chinese patients with OI

In 161 patients with OI, 53 (32.9%) missense mutations, 27 (16.8%) non-sense mutations, 39 (24.2%) splice-site mutations, 40 (24.8%) frameshift mutations, and 2 (1.2%) whole-gene deletions in *COL1A1* were found, 38 of which were novel variants, including 10 missense mutations, 3 non-sense mutations, 18 frameshift mutations, and 7 splice mutations [Table 2]. Sanger sequencing results for a part of these mutations are shown in Figure 1. The details of *COL1A1* mutation spectrum are shown in Figure 2. In patients with type I OI, frameshift mutation was the most common mutation and in patients with type III/IV OI, missense mutation was the most common mutation. There were 14 mutations found in more than 2 unrelated families. Among the 53 missense mutations, 42 (79.2%) led to of a glycine substitution in the Gly-X-Y triplet domain of the triple helix. Among the 42 glycine mutations, substitutions by serine were the most common ($n=24$, 57.1%), followed by arginine ($n=8$, 19.0%), alanine ($n=3$, 7.1%), asparaginic acid ($n=2$, 4.8%), valine ($n=2$, 4.8%), cysteine ($n=1$, 2.4%), glutamic acid ($n=1$, 2.4%), and lysine ($n=1$, 2.4%) substitutions.

Genotype-phenotype correlation

The OI phenotypes were found to be correlated with genotypes of *COL1A1*. As shown in Table 3, the median onset age of patients with qualitative mutations was 1.0 year, which was earlier than patients with quantitative mutations (2.0 years, $P=0.005$). Compared with quantitative mutations in *COL1A1* gene, patients with qualitative mutations had significantly higher clinical score (7.4 ± 2.4 for quantitative and 12.2 ± 5.3 for qualitative, $t=-6.61$, $P<0.001$), which denoted severer phenotype.

Table 1: Demographic and clinical characteristics of patients with OI with *COL1A1* mutations in this study

Characteristics	Type I OI (n=90)	Type III OI (n=36)	Type IV OI (n=35)	Statistical values	P
Male	61 (67.8)	24 (66.7)	22 (62.9)	0.04[†]	0.980
Age of onset (years)	2.0 (1.0, 5.0)	0.5 (0.0, 1.0)	1.3 (1.0, 2.9)	26.35	<0.001
Age of visit (years)	11.2 (5.0, 16.0)	12.3 (3.3, 19.8)	5.8 (4.0, 10.7)	9.43	0.009
Positive family history[*]	59 (69.4)	12 (34.3)	18 (51.4)	13.18[†]	0.001
Blue sclera	85 (97.7)	34 (94.4)	34 (97.1)	0.363[†]	0.639
DI	5 (5.9)	16 (45.7)	5 (14.3)	28.78[†]	<0.001
Hearing loss	3 (3.6)	2 (5.7)	1 (2.9)	0.018[†]	0.805
Ligament relaxation	49 (61.3)	28 (80.0)	19 (61.3)	4.18[†]	0.126
Height Z-score	−0.65±2.0[§]	−4.37±3.39[‖,¶]	−1.10±2.04[§]	31.91[‡]	<0.001
Weight Z-score	0.05±1.70[§]	−1.40±1.52[‖,¶]	−0.09±1.46[§]	10.44[‡]	0.041
Lumbar spine BMD Z-score	−1.64±1.68[§,¶]	−3.25±2.02[‖]	−3.18±2.06[‖]	14.00[‡]	<0.001
Proximal hip BMD Z-score	−2.31±1.78[§,¶]	−4.49±2.22[‖]	−4.41±2.76[‖]	18.89[‡]	<0.001
Number of fractures	4.0 (3.0, 5.0)	10.0 (3.0, 30.0)	5.0 (3.0, 6.0)	34.56	<0.001
Frequency of fractures (per year)	0.7 (0.3, 1.3)	1.5 (0.9, 2.4)	1.0 (0.8, 1.8)	8.76	<0.001
Deformity of bone	6 (7.0)	36 (100.0)	14 (40.0)	81.97[†]	<0.001
Wormian bones	35 (50.7)	25 (71.4)	22 (75.9)	7.19[†]	0.025
VCF	31 (50.0)	22 (78.6)	15 (60.0)	6.62[†]	0.038
Scoliosis	7 (10.1)	16 (53.3)	5 (19.2)	23.85[†]	<0.001
Limited movement	0 (0.0)	25 (69.4)	6 (19.4)	53.50[†]	<0.001
Clinical score	6.6±2.0[§,¶]	16.0±3.9[‖,¶]	9.3±2.0[§,‖]	178.79[‡]	<0.001
Ca (mmol/L)	2.44±0.09	2.46±0.11	2.48±0.11	0.81[‡]	0.447
P (mmol/L)	1.57±0.28	1.53±0.32	1.68±0.21	1.39[‡]	0.253
ALP (U/L)	279 (162, 333)	201 (96, 285)	286 (250, 368)	8.21	0.016
β-CTX (ng/mL)	0.700 (0.563, 0.963)	0.400 (0.300, 0.783)	0.760 (0.500, 1.000)	2.70	0.259
25OHD (ng/mL)	21.3±10.8	19.9±9.9	23.3±9.4	0.32[‡]	0.730
PTH (pg/mL)	24.7 (17.7, 39.0)	24.1 (12.6, 33.7)	16.6 (10.9, 28.8)	4.08	0.130
ALT (U/L)	15 (12, 20)	13 (11, 22)	15 (12, 21)	0.46	0.795
Cr (μmol/L)	42±15	38±16	34±11	1.03[‡]	0.361

The data are shown as mean±standard deviation, median (Q1, Q3), or n (%). [*]There were 6 patients who were adopted and could not provide family history. [†]χ^2-value. [‡]F-value, otherwise U-value. [§]$P<0.05$, *vs.* OI type III. [‖]$P<0.05$, *vs.* OI type I; and.[¶]$P<0.05$, *vs.* OI type IV (all $P<0.017$ in Bonferroni *post-hoc* test). OI: Osteogenesis imperfecta; DI: Dentinogenesis imperfecta; BMD: Bone mineral density; VCF: Vertebral compressive fractures; Ca: Serum calcium; P: Serum phosphate; ALP: Alkaline phosphatase; β-CTX: β-isomerized carboxy-telopeptide of type I collagen; 25OHD: 25 Hydroxy-vitamin D; PTH: Parathyroid hormone; ALT: Alanine aminotransferase; Cr: Creatinine.

Patients with qualitative mutations had lower BMD at proximal hip (Z-score, −3.92±2.17) than patients with quantitative mutations (Z-score, −3.02±2.64, t=2.02, P=0.046). And patients with qualitative mutations had higher median fracture number (5 *vs.* 4, U=1275.5, P=0.008) and frequency (1.0 *vs.* 0.8, U=1369.5, P=0.042) and more proportion of BD (52.8% *vs.* 19.7%, χ^2=14.29, P=0.042), wormian bones (75.0% *vs.* 47.1%, χ^2=8.09, P=0.004), VCF (75.0% *vs.* 41.9%, χ^2=9.85, P=0.002), and limited movement (38.0% *vs.* 6.2%, χ^2=17.91, P<0.001), compared with patients with quantitative mutations in *COL1A1*. Patients with qualitative mutations had lower ALP level than patients with quantitative mutations in type I collagen. The height of patients with qualitative mutations in *COL1A1* was significantly shorter than patients with quantitative defects (−0.80±2.12 for quantitative and −2.92±3.41 for qualitative, t=4.16, P<0.001). All patients with quantitative changes of type I collagen had blue sclera, which was 90.6% in qualitative collagen defect group. Patients with qualitative mutations were more prone to suffer from DI (28.8%) than quantitative mutations (4.5%, χ^2=14.29, P<0.001). Moreover, the patients would not present DI when the glycine substitutions happened before 79th amino acid in triple helix of collagen type I α1 chain [Figure 3]. However, there was no association between phenotypes and positions or types of glycine substitution (Supplementary Table 2, http://links.lww.com/CM9/A2 and Supplementary Figure 2, http://links.lww.com/CM9/A2).

Discussion

This study with the large sample size investigated the genotype-phenotype relationship in patients with OI carrying *COL1A1* mutations. The results not only outlined an abundant and significant *COL1A1* mutation spectrum in Chinese patients with OI, but also quantitatively indicated an important genotype-phenotype correlation using a clinical scoring system.

This scoring system included 8 major criteria,[18,19] including number of fractures, fracture frequency, VCF, scoliosis, bowing, height Z-score, BMD Z-score, and limited movement. The higher the score, the more severe the condition was. The clinical score of patients with type I was the lowest while the patients with type III OI was the highest, which confirmed that the clinical scoring system

Table 2: Novel variants of *COL1A1* detected by next generation sequencing in this study

Family number	Sillence classification	Clinical score	Nucleotide change	Predicted amino acid change	Mutation effect	Exon or intron
1	III	12	c.802_804+9delAGAGTGAGTCAC	–	Splice	Intron 11
2	III	15	c.1454G>C	p.Gly485Ala	Missense	Exon 21
3	III	20	c.653G>T	p.Gly218Val	Missense	Exon 9
4	I	8	c.2285delT	p.Val762Alafs*4	Frameshift	Exon 33
5	III	12	c.3155G>C	p.Gly1052Ala	Missense	Exon 43
6	III	13	c.2334delT	p.Gly779Valfs*329	Frameshift	Exon 33
7	I	4	c.3814+1G>T	–	Splice	Intron 48
8	I	9	c.1077delG	p.Arg361Glufs*180	Frameshift	Exon 17
9	I	8	c.813_814delTG	p.Ser271Argfs*15	Frameshift	Exon 12
10	IV	7	c.1516–5A>G	–	Splice	Intron 22
11	IV	8	c.1524delT	p.Gly509Valfs*32	Frameshift	Exon 23
12	IV	7	c.3063_3064delAG	p.Gly1022Phefs*13	Frameshift	Exon 42
13	III	11	c.4328C>T	p.Ala1443Val	Missense	Exon 51
14	I	4	c.3633dupT	p.Gly1212Trpfs*8	Frameshift	Exon 48
15	IV	7	c.1003G>A	p.Gly335Ser	Missense	Exon 16
16	IV	11	c.3627delT	p.His1210Thrfs*29	Frameshift	Exon 48
17	IV	11	c.4005+1G>A	–	Splice	Intron 49
18	I	7	c.2482G>A	p.Glu828Lys	Missense	Exon 36
19	I	6	c.284_285insC	p.Asp97Argfs*72	Frameshift	Exon 2
20	IV	12	c.751–2A>C	–	Splice	Intron 10
21	I	7	c.652G>A	p.Gly218Ser	Missense	Exon 9
22	I	7	c.604C>T	p.Gln202*	Nonsense	Exon 8
23	IV	8	c.1408G>T	p.Gly470*	Nonsense	Exon 21
24	I	5	c.2829+4delA	–	Splice	Intron 39
25	I	5	c.1985delG	p.Gly662Valfs*104	Frameshift	Exon 30
26	I	6	c.3258delC	p.Ala1087Profs*21	Frameshift	Exon 44
27	I	7	c.1451delC	p.Pro484Leufs*57	Frameshift	Exon 21
28	I	7	c.1001delC	p.Pro334Leufs*207	Frameshift	Exon 15
29	I	9	c.3898delG	p.Val1300Cysfs*31	Frameshift	Exon 49
30	I	6	c.1922delG	p.G641Dfs*125	Frameshift	Exon 28
31	I	10	c.3421C>T	p.R1141*	Nonsense	Exon 46
32	I	6	c.3209_3218delGTCCTGCTGG	p.Gly1070Valfs*35	Frameshift	Exon 44
33	I	6	c.1373delG	p.Gly458Aspfs*83	Frameshift	Exon 21
34	I	9	c.770G>A	p.Gly257Glu	Missense	Exon 11
35	III	11	c.441_442insC	p.Gly148Argfs*21	Frameshift	Exon 5
36	I	7	c.1200+1G>T	–	Splice	Intron 18
37	III	13	c.2434G>A	p.Gly812Ser	Missense	Exon 35
38	I	11	c.1591G>A	p.Glu531Lys	Missense	Exon 23

–: Not applicable.

could quantitatively reflect clinical severity of OI. Patients with type III OI were early onset and lag in diagnosis, which led to not in time of the treatment and aggravated the condition of OI.[21] In aspect of extra-skeletal manifestation, BS was a common feature of patients with OI.[14,22] Consistent with the previous studies,[21,23] patients with types III and IV OI had higher proportion of DI than type I patients, which may be interpreted as dentin and bone were similar in the composition of the extracellular matrix.[24] The progressive hearing loss was reported in 50% to 60% of adult patients with OI, but it was found in 5.0% to 26.7% of pediatric patients with OI,[14,25] which was similar in the patients of this study. This phenomenon may be related to the short course of OI in children.

In the genotype analysis, missense mutation in *COL1A1* was dominant in this cohort, but the proportion was lower than reports in Canada (47.3%),[6] Sweden (60.9%),[21] Vietnam (67.6%),[26] and India (85.7%).[27]We further found that missense mutation in *COL1A1* could explain 21.1% mild OI, 34.3% moderate, and 61.1% severe OI. Similar to previous studies, glycine substituted by serine was the most common.[14,22,28] Moreover, this study found 38 novel variations, which contributed to extended the gene mutant spectrum of OI and reveal its pathogenesis. In addition, c.769G>A (p.Gly257Arg), c.1678G>A (p.Gly560Ser), c.2299G>A (p.Gly767Ser), c.2461G>A (p.Gly821Ser), c.3505G>A (p.Gly1169Ser), c.1081C>T (p.Arg361*), c.1792C>T (p.Arg598*), c.2644C>T (p.Arg882*), c.3076C>T (p.Arg1026*), c.1155+1G>A

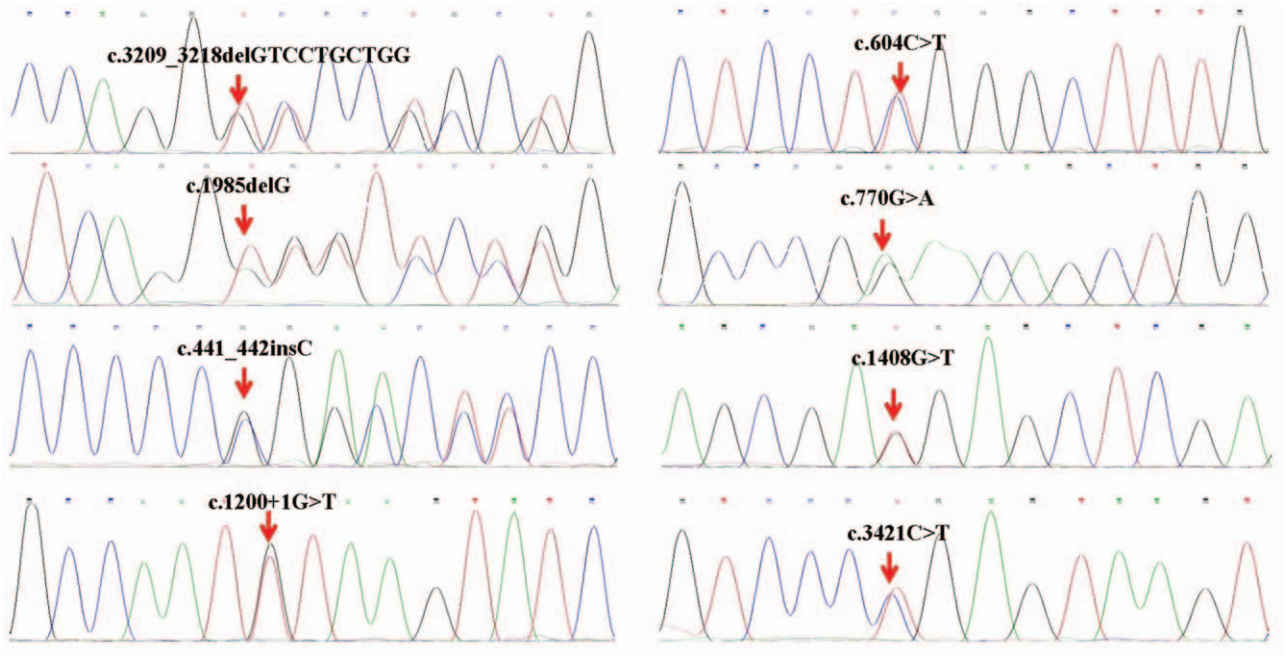

Figure 1: Sanger sequencing for a part of *COL1A1* mutations.

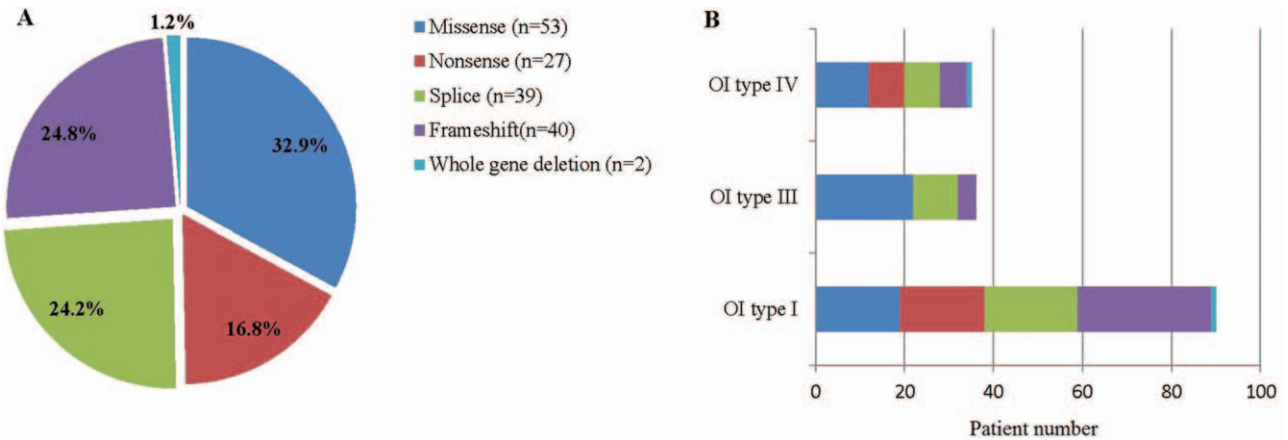

Figure 2: Mutation spectrum of *COL1A1* mutations in patients with OI. (A) Mutation types of *COL1A1* in patients with OI. (B) *COL1A1* mutation spectrum in patients with different Sillence classification. OI: Osteogenesis imperfecta.

(Splicing variant), and c.1821+1G>A (Splicing variant) could be hotpot mutations for human OI, which had been reported for 6 to 37 times previously (https://oi.gene.le.ac.uk/home.php).

As for the analysis of the relationship between genotype and phenotype, this study confirmed that the clinical phenotype of patients with collagen qualitative defects was severer than patients with collagen quantitative defects, which was similar to previous reports.[6,22] BMDs of patients with collagen qualitative defects were lower than the patients with collagen quantitative defects, especially at proximal hip. In consistence with the results of this study, previous studies had revealed that *COL1A1* quantitative mutations led to milder bone fragility than *COL1A1* qualitative mutations.[6,11,22] Lower than Caucasian,[29] this study observed that 18.0% and 29.8% of patients with quantitative and qualitative mutations had scoliosis, respectively. Similarly, this study also found that the height of patients with collagen quantitative defects was taller than the patients with triple helical mutations.[6] In addition, this study also found wormian bone was more common in patients with qualitative mutations than patients with quantitative mutations. Two studies revealed patients with serine substitutions presented more severe bone phenotypes,[11,30] but this study did not observe this

Table 3: Comparison of phenotypes between quantitative and qualitative mutations in *COL1A1*

Items	Quantitative mutations (n=67)	Qualitative mutations (n=53)	Statistical values	P
Male	42 (62.7)	38 (71.7)	1.08*	0.298
Age of onset (years)	2.0 (1.0, 5.0)	1.0 (0.0, 3.5)	1225.5	0.005
Age of visit (years)	10.5 (4.0, 16.8)	10.5 (5.4, 16.7)	1714.5	0.747
OI types (I/III/IV)	49/4/14	19/22/12	24.56*	<0.001
Blue sclera	67 (100.0)	48 (90.6)	8.45*	0.004
DI	3 (4.5)	15 (28.8)	13.29*	<0.001
Hearing loss	4 (6.3)	1 (1.9)	1.41*	0.234
Ligament relaxation	37 (61.7)	31 (63.3)	0.03*	0.864
Height Z-score	−0.80±2.12	−2.92±3.41	4.16†	<0.001
Weight Z-score	−0.06±1.52	−0.54±2.13	1.44†	0.152
Lumbar spine BMD Z-score	−2.24±2.24	−2.58±1.91	0.87†	0.387
Proximal hip BMD Z-score	−3.02±2.64	−3.92±2.17	2.02†	0.046
Number of fractures	4 (3, 5)	5 (3, 10)	1275.5	0.008
Frequency of fractures (per year)	0.8 (0.3, 1.5)	1.0 (0.6, 2.0)	1369.5	0.042
Deformity of bone	13 (19.7)	28 (52.8)	14.29*	<0.001
Wormian bones	24 (47.1)	36 (75.0)	8.09*	0.004
VCF	18 (41.9)	33 (75.0)	9.85*	0.002
Scoliosis	9 (18.0)	14 (29.8)	1.86*	0.173
Limited movement	4 (6.2)	19 (38.0)	17.91*	<0.001
Clinical score	7.4±2.4	12.2±5.3	−6.61†	<0.001
Ca (mmol/L)	2.42±0.09	2.47±0.11	3.74†	0.056
P (mmol/L)	1.56±0.30	1.57±0.27	0.216†	0.643
ALP (U/L)	296 (132, 346)	218 (136, 284)	1251.5	0.009
β-CTX (ng/mL)	0.700 (0.400, 0.900)	0.652 (0.400, 1.012)	1222.5	0.909
25OHD (ng/mL)	22.0±9.3	18.9±9.0	3.60†	0.061
PTH (pg/mL)	25.0 (14.6, 42.1)	17.6 (13.4, 31.9)	868.0	0.073
ALT (U/L)	14 (12, 21)	15 (11, 22)	1616.5	0.686
Cr (μmol/L)	38±15	39±14	0.683†	0.410

The data are shown as mean±standard deviation, median (Q1, Q3), or n (%). *χ^2 value. †t-value, otherwise U-value. OI: Osteogenesis imperfecta; DI: Dentinogenesis imperfecta; BMD: Bone mineral density; VCF: Vertebral compressive fractures; Ca: Serum calcium; P: Serum phosphate; ALP: Alkaline phosphatase; β-CTX: β-isomerized carboxy-telopeptide of type I collagen; 25OHD: 25 hydroxy-vitamin D; PTH: Parathyroid hormone; ALT: Alanine aminotransferase; Cr: Creatinine.

phenomenon. In this study, the position and type of glycine substitutions within the α1 (I) triple helical domain were not found to have association with bone lesions of OI. Rauch *et al*[28] observed that OI patients with mutations close to carboxyl-terminal end of type I collagen may not present BS, but this study did not found this phenomenon. This study did not find relationship between hearing loss and genotypes in *COL1A1*, which was similar to previous studies.[25,31] In Europe studies, patients with glycine substitutions before the 127th amino acids of the α1 (I) triple helical domain would have no DI,[21,28] but this study found the point was the 79th amino acids.

The mechanism of genotype-phenotype correlation in OI could relate to that *COL1A1* quantitative mutations resulted in the amount of type I procollagen synthesis reduced by half of the amount and causing mild bone fragility. However, qualitative mutations in *COL1A1* gene led to the structural abnormalities in collagen molecules, which could result in more severe bone lesions. Another finding in this study was that patients with qualitative mutations had lower serum ALP level than patients with quantitative mutations, and ALP level was negatively correlated to clinical severity of OI. ALP, as a bone formation marker, had important roles in promoting bone mineralization.[32,33] Therefore, this study conjectured that patients with OI with *COL1A1* qualitative mutations may have been more severely damaged of osteoblasts function, which aggravated bone lesions.

Although this research expanded the spectrum of mutations in *COL1A1*, and revealed meaningful correlation between genotype and phenotype of OI, there were some limitations. Firstly, it was a cross-sectional study, which could not reveal the relationship of genotype and efficacy of bisphosphonates in patients with OI. Secondly, this study did not perform functional experiment to verify the protein variation of splice-site mutations. Thirdly, this study did not intensively evaluate the influence of gene mutations in *COL1A1* on bone microstructure and bone biomechanical properties.

In conclusion, this study presented the distinctive *COL1A1* mutation spectrum in the large Chinese patients with OI. In particular, this study identified 38 novel mutations, which expanded the known pathogenic spectrum of *COL1A1* gene. Furthermore, this new quantitative analysis of genotype-phenotype correlation would be helpful to predict the prognosis of OI and genetic counseling.

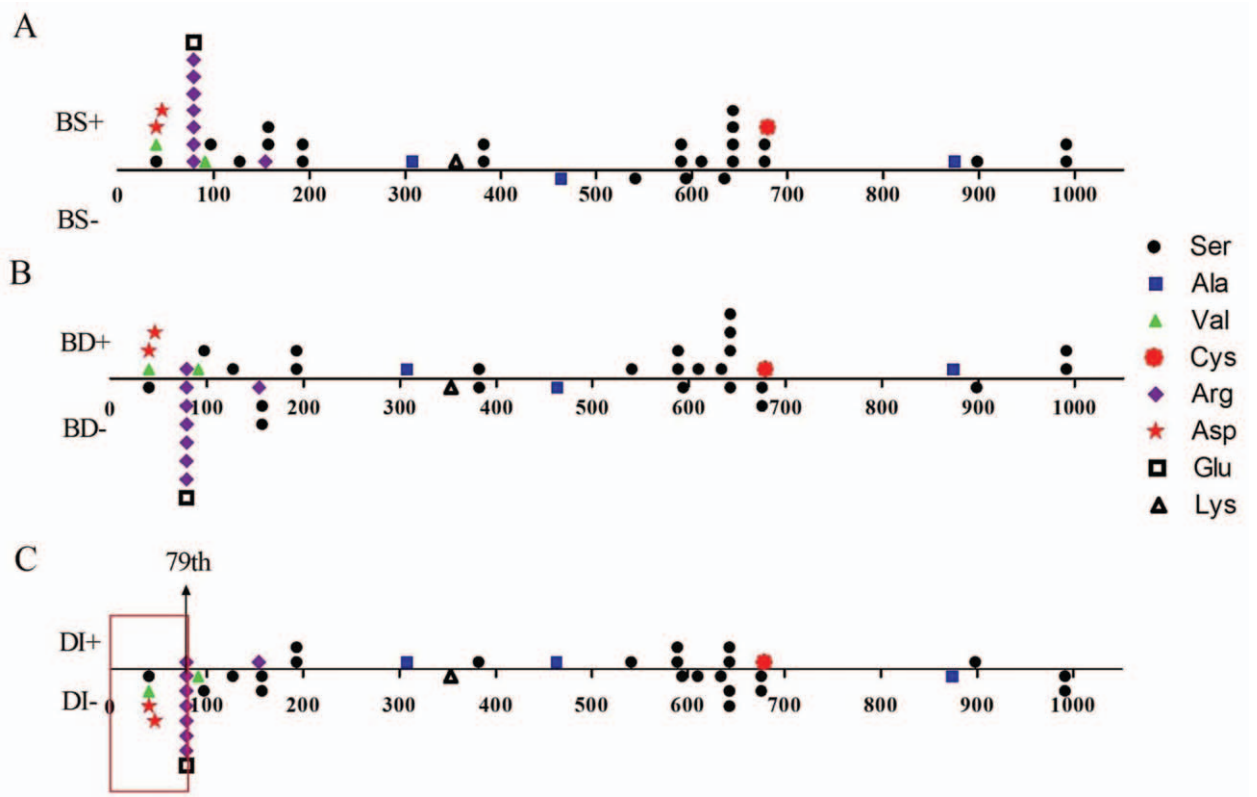

Figure 3: Relationships between helical glycine substitutions positions in collagen type I α1 chains and phenotypes. (A) Relationship between the positions of glycine substitutions and BS. (B) Relationship between the positions of glycine substitutions and BD. (C) Relationship between the positions of glycine substitutions and DI. The red frame indicated that patients would not present DI if the glycine substitutions happened before the 79th amino acid in triple helix of collagen type I α1 chains. BS: Blue sclera; BD: Bone deformity; DI: Dentinogenesis imperfecta.

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Conflicts of interest

None.

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