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Genotype-phenotype correlation among Malaysian patients with osteogenesis imperfecta

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ABSTRACT

Background: Osteogenesis imperfecta (OI) is a rare genetic bone disease characterized by bone fragility and low bone mass. OI was mainly caused by genetic mutations in collagen genes, *COL1A1* and *COL1A2*. Nevertheless, new genes have been identified to be causally linked to OI. The clinical features between each OI groups share great similarities and it is sometimes difficult for clinicians to diagnose the disease accurately. Here, we identify the genetic mutations of OI patients from Malaysia and correlate the genetic mutations with the clinical features.
Method: Targeted sequencing of fourteen genes panel was performed to identify the mutations in 29 OI patients with type I, III, IV and V disease. The mutations were determined using Ion Torrent Suite software version 5 and variant annotation was conducted using ANNOVAR. The identified mutations were confirmed using Sanger sequencing and *in silico* analysis was performed to evaluate the effects of the candidate mutations at protein level.
Results: Majority of patients had mutations in collagen genes, 48% (n = 14) in *COL1A1* and 14% (n = 4) in *COL1A2*. Type I OI was caused by quantitative mutations in *COL1A1* whereas most of type III and IV were due to qualitative mutations in both of the collagen genes. Those with quantitative mutations had milder clinical severity compared to qualitative mutations in terms of dentinogenesis imperfecta (DI), bone deformity and the ability to walk with aid. Furthermore, a few patients (28%, n = 8) had mutations in *IFITM5*, *BMP1*, *P3H1* and *SERPINF1*.
Conclusion: Majority of our OI patients have mutations in collagen genes, similar to other OI populations worldwide. Genotype-phenotype analysis revealed that qualitative mutations had more severe clinical characteristics compared to quantitative mutations. It is crucial to identify the causative mutations and the clinical severity of OI patients may be predicted based on the types of mutations.

1. Introduction

Osteogenesis Imperfecta (OI) is a group of genetic bone disorders primarily characterized by low bone mass, which results in increased bone fractures and disrupted growth [1]. Additional extra-skeletal phenotypes include blue sclera, dentinogenesis imperfecta (DI) and hearing impairment [2]. There is a wide spectrum of clinical features and severity among patients, ranging from mild with minimal or no bone deformities to severe with multiple bone fractures and also perinatal lethality [3]. The variable types of OI occurs in approximately 1 in every 15,000–20,000 births with majority of them being autosomal dominant inheritance [4]. The classic Sillence classification categorized OI into four numerical groups (OI type I to IV) according to its clinical findings and inheritance patterns [5]. Type I is often the mildest form, with normal or near-normal skeletal features. Type II is lethal in the perinatal period, with multiple intrauterine fractures and skeletal deformities of the limbs. Type III is the severe form with frequent fractures and bone deformities that progress with age. Type IV has various phenotypes from mild to moderate skeletal deformities and varies in height and statures [5]. On the other hand, type V OI was mainly identified by the presence of hyperplastic callus and interosseous membrane [6].

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The initial assumption was that this disease was caused by a mutation in either *COL1A1* or *COL1A2* genes which codes for two collagen type I alpha chains, α1 (1) and α1 (2) [7]. However, with the advancement of genetic testing, mutations in non-collagenous genes have been detected such as *LEPRE1*, *PPIB*, *SERPINH1*, *FKBPIO*, *SP7*, *IFITM5*, *BMP1*, *WNT1*, *TMEM38B*, *CRTAP* and *SERPINF1* genes, all of which are linked to the autosomal recessive OI except for *IFITM5* mutation [8]. There are two groups of mutational defects in type I collagen. The first is quantitative mutations due to frameshift, nonsense and splice-site mutations, which introduces a premature termination codon in the coding sequence of *COL1A1* allele. The second group is qualitative mutations resulted from the synthesis of collagen molecules that has structural abnormalities, most frequently caused by glycine substitution [9].

The phenotypic severity of OI patients depends on the affected gene, position of mutation, substituted amino acid and the final protein product [10]. However, OI has variable phenotypes and to date, the exact correlation between genotype and phenotype is not fully understood. Hence, we aimed to determine the genetic mutations involved in our local OI patients. Subsequently, the phenotype of these patients will be linked to their genotype and the data obtained may be useful in the clinical diagnosis, genetic counselling and prenatal diagnosis for OI patients

2. Materials and methods

2.1. Subjects

The study was implemented in compliance with the ethical principles formulated in the declaration of Helsinki and was approved by our local Ethics Committee. Patients granted their informed, written consent to participate. Patients were recruited from the UKM Medical Centre (UKKMC) and Hospital Putrajaya. Written informed consent was given by patients, their parents or legal representatives. The inclusion criteria for OI patients include being Malaysian and with the clinical features of OI.

2.2. Clinical characteristics

All 29 patients from 28 families which comprise of 16 males and 13 females were recruited. They underwent clinical and physical examination to evaluate their clinical features and all medical histories were recorded. The clinicians classified the patients into four groups (OI-I, OI-III and OI-IV) according to Sillence classification. OI-V was categorized according to description by Glorieux et al. [6]. The key features observed were blue sclera, bone deformities, degree of disability, dentinogenesis imperfecta (DI) and hearing ability. DI was characterized by the formation of translucent and brittle teeth. For patient's degree of disability, Bleck's 5-point scale was used to assess mobility and walking ability [11].

2.3. Sample preparation

Total genomic DNA was extracted from EDTA-preserved peripheral blood using Macherey-Nagel Nucleospin Blood QuickPure kit (Macherey-Nagel, Germany. The Gender validation test was performed for each DNA sample as part of quality assessment. Briefly, PCR was performed using *SRY* and *ATL1* primers and assessed by agarose gel electrophoresis.

2.4. DNA sequencing

Targeted sequencing was performed using Ion PGM Semiconductor ™ (Thermo Fisher Scientific, USA) sequencer with a total of 14 genes with 214 exons selected for the gene panel. The selected genes are associated with osteogenesis imperfecta: *COL1A1*, *COL1A2*, *CRTAP*, *LEPRE1*, *PPIB*, *SERPINH1*, *SERPINF1*, *FKBP10*, *P3H1*, *BMP1*, *TMEM38B*, *IFITM5*, *PLOD2* and *WNT1*. Two primer pools to amplify 320 amplicons were designed using Ion AmpliSeq Designer version 4.48 using the 'standard DNA' (225-bp amplicon target sizes) and 'Gene + UTR' options (https://www.ampliseq.com). The number of amplicons and base covered for each gene are listed in Supplementary Table 1. A total of 10 ng DNA per sample was used for target enrichment by multiplex PCR and barcoded libraries from 16 samples were pooled in a single tube (Thermo Fisher Scientific, USA). Library pools were subjected to Ion Chef ™ instrument (Thermo Fisher Scientific, USA) for template preparation and enriched Ion Sphere Particles (ISPs) were loaded on Ion 316 v2 BC Chip for sequencing. Potentially pathogenic variants were validated using Sanger sequencing. The candidate mutations were also compared against 667 chromosomes with similar age, gender and ethnicity.

2.5. Sequencing data analysis

Data from sequencing run were processed using Ion Torrent Suite software (version 5.0.4; Thermo Fisher Scientific, USA) for base calls, read alignments and variant calling using the reference genomic sequence (hg19). Called variants were annotated using ANNOVAR [12] and the variants were filtered out if the minor allele frequency was equal or higher than 1% in 1000 Genomes Project database and National Heart, Lung, and Brain Institute-Exome Sequencing Project (NHLBI-ESP) with 6500 exomes. Pathogenic variants were predicted using SIFT score (< 0.05) [13], PolyPhen2 score (> 0.4) [14], and MutationTaster [15]. The Integrative Genomics Viewer (IGV) software was used for visualization of protein deleterious effects [16,17]. Novel mutations were identified using Osteogenesis Imperfecta and Ehlers Danlos Syndrome database (https://oi.gene.le.ac.uk/home.php).

2.6. Data analysisfor genotype-phenotype correlation

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) version 21. Age differences between the patients and normal was calculated using Independent T Test. Qhi-square was used to determine the gender differences between the patients and normal group. Fisher test was carried out to determine the correlation between gender, OI groups and types of colagen with the patients phenotype. P less than 0.05 was considered as significant.

3. Results

3.1. Clinical characteristics

Most of our patients (62.1%) were OI type III, followed by 17.2% of type I and IV respectively. One patient was classified as type V. Table 1 shows the relationship between the clinical characteristics of OI patients and types of OI. None of the patients had OI type II. No group differences were found in the prevalence of blue sclera, however bone deformities were significantly prominent in patients with OI type III as

Table 1
Phenotypic characteristics among OI patients.

Characteristics, n (%)/ Types of OI, n (%)	OI type I 5 (17.2)	OI type III 18 (62.2)	OI type IV 5 (17.2)	OI type V 1 (3.4)	*p* value
COL1A1/COL1A2	5/0	6/3	3/1	–	0.392
Gender (Male/Female)	3/2	9/9	3/2	0/1	1.000
Blue sclera	5 (17.2)	18 (62.2)	5 (17.2)	1 (3.4)	–
DI	3 (14.3)	15 (71.4)	3 (14.3)	–	0.145
Hearing loss	1 (50)	–	1 (50)	–	0.623
Bone deformity	1 (4.5)	18 (81.8)	2 (9.1)	1 (4.5)	< 0.001
Walk with aid	–	12 (80)	2 (13.3)	1 (6.7)	0.028

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