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DIAGNOSTIC DILEMMA: MILD AND MODERATE FORMS OF OSTEOGENESIS IMPERFECTA

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DIAGNOSTIC DILEMMA: MILD AND MODERATE FORMS OF OSTEOGENESIS IMPERFECTA

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ABSTRACT - Osteogenesis imperfect (OI) is a group of genotypically and phenotypically diverse disorders of connective tissue characterized by bone fragility. The classic typing of OI includes: type I (mildest), type II (perinatally lethal), type III (most severe non-lethal) and type IV (moderate). Diagnosis and accurate typing of mild and moderate types of OI are challenging due to the heterogeneity of OI and the lack of diagnostic criteria. We did a retrospective review of 84 patient charts with a diagnosis of mild and moderate forms of OI. We report on their diagnostic experience with the goal of evaluating the role of genotyping in establishing and confirming a diagnosis of mild or moderate OI. Our analysis supports the fact that no set of clinical data is diagnostic in these cases and genetic testing has valuable utility. We further constructed a genotype-phenotype map of our patient population, which can serve as an expandable tool for clinicians and families in the ongoing management of patients with mild and moderate types of OI.

INTRODUCTION

Classification of OI – Osteogenesis Imperfecta (OI) is a heterogenous group of skeletal dysplasias characterized by bone fragility and therefore susceptibility to fractures. The spectrum of severity of OI ranges from perinatally lethal to nearly asymptomatic, with individuals who only have a mild propensity to fractures. The most widely accepted classification of OI, which has remained the basis of the present day nosology, came in 1979 by Sillence *et al*. based on clinical characteristics and radiographic features. Type I OI is the most common and mildest presentation with decreasing frequency of fractures after puberty. Type II is perinatally lethal with multiple congenital fractures,

micromelia and severe lung disease. Types III is the most severe non-lethal form presenting with multiple (congenital) fractures and severe and progressive bone deformities. Type IV is an intermediate group that encompasses presentations that cannot otherwise be categorized into the other types.

In 2004, Glorieux and Rauch expanded the original Sillence classification by adding types V–VII. As more genetic causes of OI were discovered, nomenclature revisions have been made to include up to OI type XIV (Forlino *et al*., 2011). Currently, there are a total of 17 genetic causes of OI described in the literature (Van Dijk and Sillence, 2014). There is ongoing debate about classification and subdivisions into different types since many new types of OI are not distinguishable clinically from the classically defined Sillence types (van Dijk *et al*., 2010).

Genotype-Phenotype Relationship – OI is as diverse genotypically as it is phenotypically. The genetic defect is either directly or indirectly related to the biosynthesis of type 1 collagen, the main structural constituent of bones, tendons and ligaments. The individuals that fall into the four classic Sillence categories comprise over 90% of cases of OI and are heterozygous for dominant mutations of either the *Col1α1* or *Col1α2* genes, which respectively encode the proα1(I) and proα2(I) chains of type I procollagen (Engel and Prockop, 1991; Byers *et al*. 1991). Type I collagen is a heterotrimer composed of two alpha 1 and one alpha 2 chains. The peptide sequence of the chains contains uninterrupted repeats of Gly-X-Y. The glycine residue in every third

position of the chains is necessary for the correct formation of the triple helix (Ben Amor *et al*, 2011).

The mildest phenotype (type I OI) is usually a consequence of a quantitative defect of collagen type I (Byers 2000 and 2002). In these cases only about half the normal amount of type I collagen is produced because a nonsense, frameshift or splice site mutation has introduced premature termination of *Col1α1*, resulting in degradation of non-sense mRNA. Rarely, substitutions for glycine by small amino acids (cysteine, alanine and serine) near the N-terminus of either the alpha 1 or alpha 2 chains result in type I OI as well.

On the other hand, more severe phenotypes are typically a result of mutations that disrupt the structure of either chain of type I collagen. Most commonly, mutations that replace the glycine residues of the triple helix are responsible because they delay helix formation and prolong duration of post-transcriptional modifications, which ultimately produce structurally defective collagen. The resulting phenotypes vary in severity, causing the dominantly inherited OI types II-IV (van Dijk *et al.,* 2012). Less commonly these phenotypes can result from mutations in the C-terminus, insertion/deletion or missense mutations that cause structural abnormalities in collagen type I chains as well.

These genotype-phenotype correlations are by no means absolute. Establishing correlations are further complicated by the fact that most OI mutations are unique. To

date, there have been more than 2000 different mutations of type I collagen reported in a public access database (http://www.le.ac.uk/ge/collagen/) (Dalgleish, 1998).

The pathogenic pathways responsible for the remaining cohort of OI patients who do not have a dominantly inherited collagen mutation, have been attributed to null mutations of genes that interact with collagen post-translationally (Marini and Blissett, 2013). Type V OI is an exception overall in that it has distinct clinical features and is a result of one specific dominantly inherited mutation in *IFITM5*. The other non-Sillence types of OI range in severity from moderate to lethal and are inherited in an autosomally recessive manner. So far, genes have been identified that are involved in collagen folding (*CRTAP, LEPRE1* and *PPIB)*, chaperoning (*FKBP10* and *SERPINH1*), modification and cross-linking (*PLOD2*), bone mineralization (*SERPINF1*) and bone cell differentiation (*SP7, TMEM38B* and *WNT1)* (Marini and Blissett, 2013).

Diagnostic Dilemma – There are no specific criteria set for the diagnosis of OI. Clinically, the hallmark feature is the presence of fractures in the absence of trauma. Blue/grey sclera, dentinogenesis imperfecta (DI), hearing loss and other bone deformities vary in presentation. Historically, biochemical collagen screening has been the gold standard of confirming an OI diagnosis. Collagen screening involves taking a skin biopsy, followed by culture of the dermal fibroblasts, and biochemical assay of the excreted collagen showing reduced or abnormal procollagen (Steiner *et al.*, 1996; Cabral *et al*., 2006). This is an invasive, time-consuming procedure, often taking up to two months, and typically providing definitive identification of a collagen defect in up to

90% of cases (Wenstrup *et al*., 1990). With the discovery of causative mutations in *Col1α1* and *Col1α2*, confirmatory molecular testing for mutations in these genes can be performed on a blood or tissue sample providing results in 4-8 weeks. With accurate clinical diagnosis, more than 95% of individuals with OI have a mutation detected with genomic sequencing (Sykes *et al.,* 1990; Körkkö *et al*., 1998).

The heterogeneity of OI can be a confounding factor in its diagnosis. When a severe phenotype is present, any qualified physician can diagnose the disease. Milder phenotypes are more difficult to diagnose. There are other conditions that have significant clinical overlap with OI including Ehlers Danlos Syndrome (EDS) kyphoscoliotic type (type VIA), EDS arthrochalasia type (types VIIA and VIIB) and EDS dermatosparaxis type (type VIIC) to name some (Steiner *et al*., 2013). The differential diagnosis also includes premature aging syndromes in which fractures may be the first manifestation and precede other clinical features (Van Dijk and Sillence, 2014). Furthermore, non-accidental injury as a result of child abuse can present similar to OI and importantly needs to be distinguished, though the two can coexist.

The diagnostic dilemma of OI is two-fold. In the mildest form or type I, it is not always obvious whether OI exists or fracturing is due to another bone disease, (child) abuse or an unknown cause. In the moderate form or type IV, the true severity of the condition and whether the patient has been typed accurately can be subjective and based on presenting clinical features at any given time. For example, if an unrelated 5 year old and 50 year old both have had 5 fractures and do not exhibit other features, the

younger patient's condition is likely to be classified as more severe (type IV) while the older patient is likely to be classified as more mild (type I) even though they may have the same genotype and similar phenotypes. Therefore, genetic testing has valuable utility in establishing and confirming a diagnosis of OI in a quick, non-invasive and accurate fashion. This in turn informs medical management, administration of appropriate molecular therapies and evaluation of responses, likely prognosis and recurrence risks. The goal of the present work has been to report on the diagnostic criteria of patients with mild and moderate OI based on clinical and laboratory features, at the Skeletal Dysplasias Clinic at the Hospital for Special Surgery. We further used this information to catalogue genotype-phenotype correlations in our patient population and compare our findings to the published literature.

MATERIALS & METHODS

We obtained approval for this research from the Institutional Review Board of the Hospital for Special Surgery and conducted a retrospective chart review of 84 patients who were diagnosed with mild and moderate forms of OI at the Kathryn O. and Alan C. Greenberg Center for Skeletal Dysplasias at Hospital for Special Surgery. the Skeletal Dysplasias Clinic. We excluded patients diagnosed with more severe forms of OI (types II and III) or other metabolic bone or connective tissue disorders where fractures are a main clinical finding.

In addition to the demographics, we collected all pertinent clinical information and evaluations leading to the diagnosis of mild or moderate OI in an index case. We

collected data on fracture history, items identified in the physical exam and medical history including evaluation of auditory, ophthalmologic, cardiopulmonary, and skeletal systems and any treatment regimens and surgical procedures. In addition we reviewed radiographic findings (including X-rays, DXA scans, CAT scans, MRI, and ultrasounds), family history, and laboratory studies (biochemical and genetic testing, and other serum and urinalysis). We hypothesized that definitive determination of a diagnosis of mild or moderate OI is by direct genomic sequencing.

We de-identified our cohort for the analytic portion of this work and examined what correlation, if any, existed between clinical findings, laboratory test results, family history, and imaging results for individuals diagnosed with mild and moderate OI. In this work, we report on our findings on the nine most commonly expressed features of OI: fracture frequency, presence of blue/grey sclera, dentinogeneis imperfecta, hearing loss, short stature, scoliosis, bone density loss, bone deformity and ligament laxity. Our 84 patients were grouped as (likely) having type I, type IV, or some other non-classic Sillence type OI. For each of the nine features, the proportions of patients in each group who exhibited the feature were calculated. To shed light on what role genotyping played in the clinical diagnosis, we subdivided each group into those with genotype information and those without. We then compared the percentage of genotyped patients versus un-genotyped patients, exhibiting each of the nine cardinal features.

33 of the 84 patients had genotype information available and we used 30 of these individuals who had a mutation in either *Col1α1* or *Col1α2* to further examine their

genotype-phenotype correlations. We catalogued the mutations of these patients, using the OI Variant Database when possible and mapped the mutations on the corresponding genes.

RESULTS

Figure 1A illustrates the number of patients in each category. Generally, there were more type I patients than type IV patients and within each group, most patients had not been genotyped. Figure 2 shows the fracture frequencies observed in each group and Figure 1B summarizes the proportion of patients in each group exhibiting the other eight cardinal features of OI.

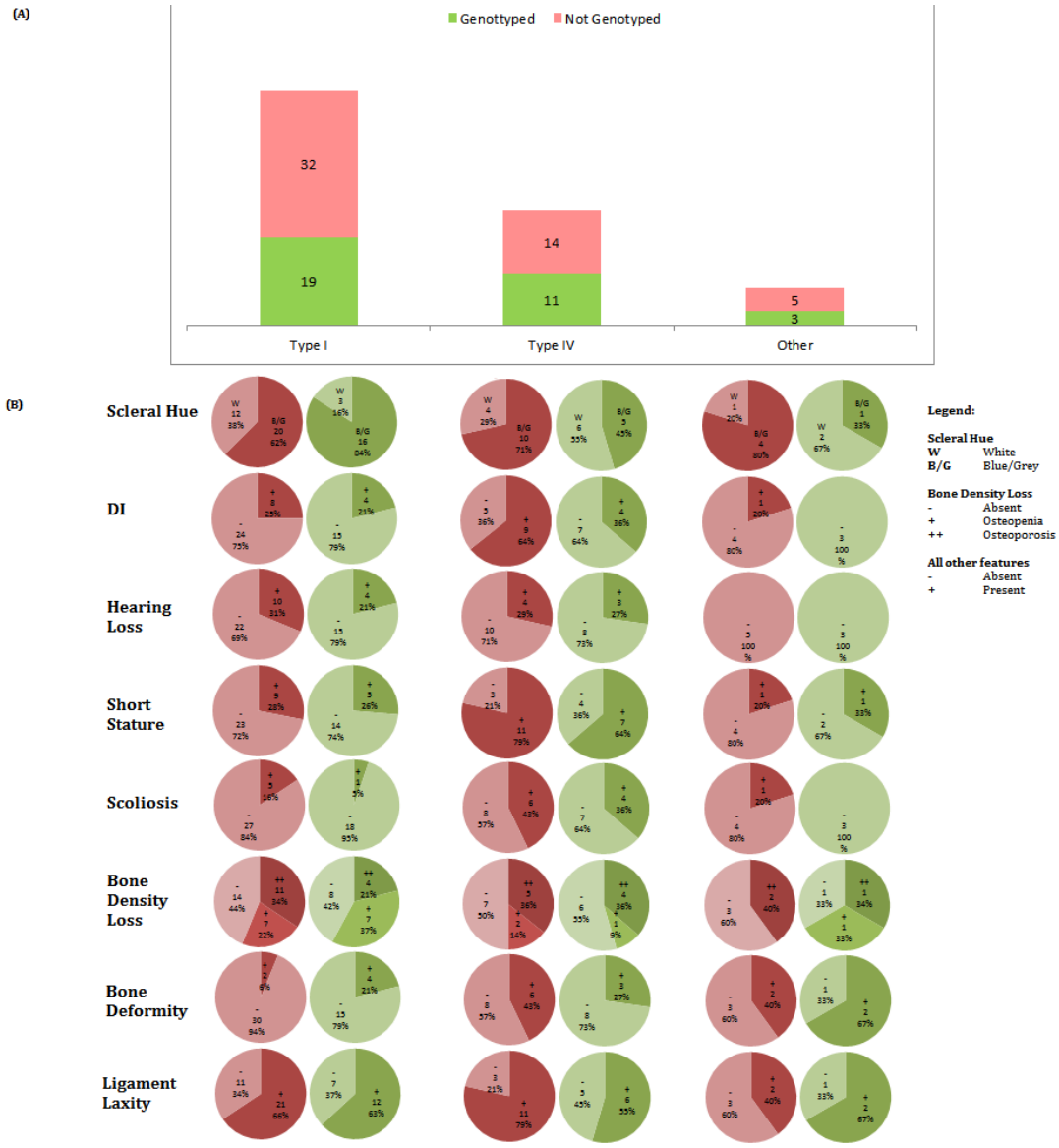

Figure 1 –
(A) Breakdown of study subjects according to typing and genotyping status
(B) Summary of proportions of patients exhibiting phenotypic features of OI.

Figure 2 – Fracture frequencies observed in the study cohort.

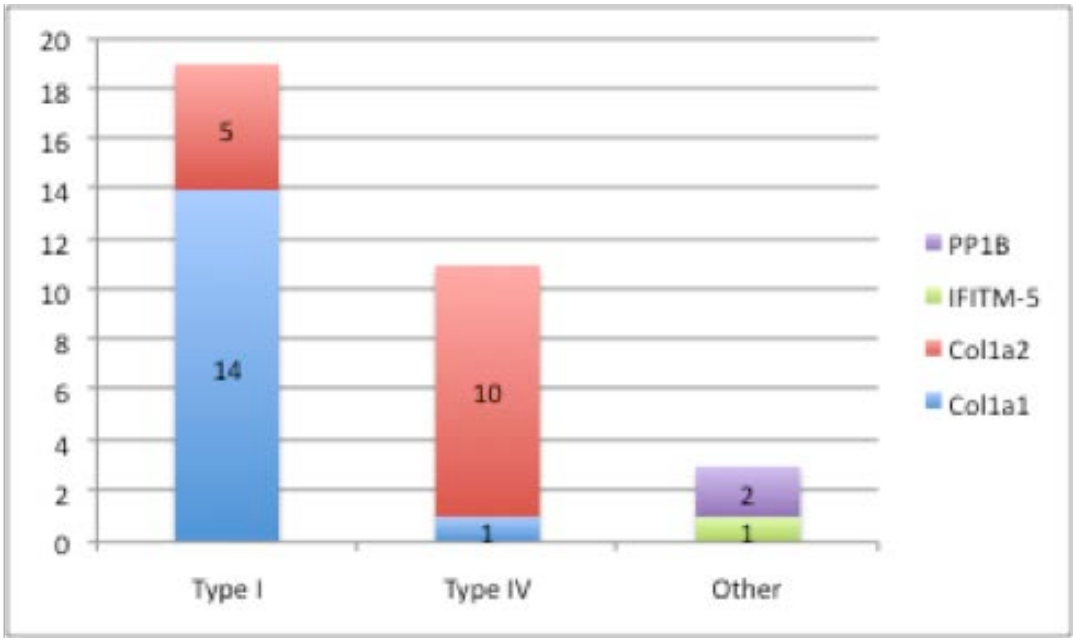

Figure 3 – Genes implicated in genotyped patients.

Type I – Patients in this group had a wide range in the number of fractures they had experienced from none (due to young age) to over 100 (due to multiple traumatic incidents such as motor vehicle accidents), however the majority had had 20 fractures or fewer. The presence of blue/grey sclera was the most prominent feature in this group, particularly if the patient was genotyped. 84% of the genotyped patients had blue/gray sclera while only 63% of the non-genotyped patients had blue/gray sclera, a difference of 21%. However the difference was not statistically significant (Chi-Square (1) = 1.76, p = 0.18). More than half of type I patients also had some degree of bone density loss (osteoporosis or osteopenia) and ligament laxity. Roughly a quarter of this group had DI, hearing loss and short stature. Bone deformity and scoliosis were only present in a small percentage of this group. While there were only six out of 51 patients with bone deformity, four or 21% of patients who were genotyped had bone deformity, while only two patients or 6% of those from the non-genotyped group had bone deformity. However, given the small sample of those with bone deformity, the difference was not statistically significant (Fisher's Exact test = 0.18). Scoliosis was present in only six cases, but represented 16% of those in the non-genotyped group and only 5% of those in the genotyped group. The difference was not statistically significant, given the small sample of those with scoliosis (Fisher's Exact probability = 0.39).

Type IV – The sample size in this group was 25 patients. he fracture frequency in this group was quite variable from a few to over 100 with no major pattern of distribution. The other phenotypic features observed in most patients were short stature and

ligament laxity. Roughly half of the patients had blue/grey sclera, DI, and bone density loss. Hearing loss, scoliosis and bone deformity were seen in roughly a third of the patients in this group. Besides hearing loss which was observed at the same approximate frequency in the genotyped, and not genotyped groups, features other than fractures were observed less frequently in the genotyped group. In the Type IV group 63% (15) had blue/gray sclera. This was 71% of those in the non-genotyped group and only 45% of those in the genotyped group. The difference was not statistically significant (Fisher's Exact probability = 0.24). Likewise, 52% (13) had dentinogenesis imperfecta. This represented 64% of those in the non-genotyped group and only 36% of those in the genotyped group, a descriptive difference that was not statistically significant (Fisher's Exact probability = 0.24). In the Type IV group, 36% (9) had bone deformity. 43% of those in the non-genotyped group and only 27% of those in the genotyped group, a difference that was not statistically significant (Fisher's Exact probability = .68). In the type IV group, 68% (17) had ligament laxity. This was 79% of the non-genotyped group and only 55% of those in the genotyped group, a difference that was not statistically significant (Fisher's Exact probability = 0.39)

Genotype-Phenotype Relationship – Figure 3 illustrates the number and distribution of patients who were genotyped. There were more individuals with type I than type IV who had been genotyped. The majority of patients with type I OI had a mutation in *Col1α1* while the majority of type IV patients had a mutation in *Col1α2*. The three patients with mutations in other genes are not currently typed within the classical Sillence types. We constructed a genotype-phenotype map of the individuals who had a

mutation in either collagen type I gene (Figure 4). The mutations in our study subjects were all private and only common amongst family members. As Figure 4 illustrates, we observed nonsense, missense, frameshift and splice site mutations of *Col1α1* at roughly the same frequency. For *Col1α2,* missense mutations were predominant and there were also frameshift mutations and one whole exon deletion. There appeared to be no correlation between the type, identity or location of mutations with the resulting phenotype.

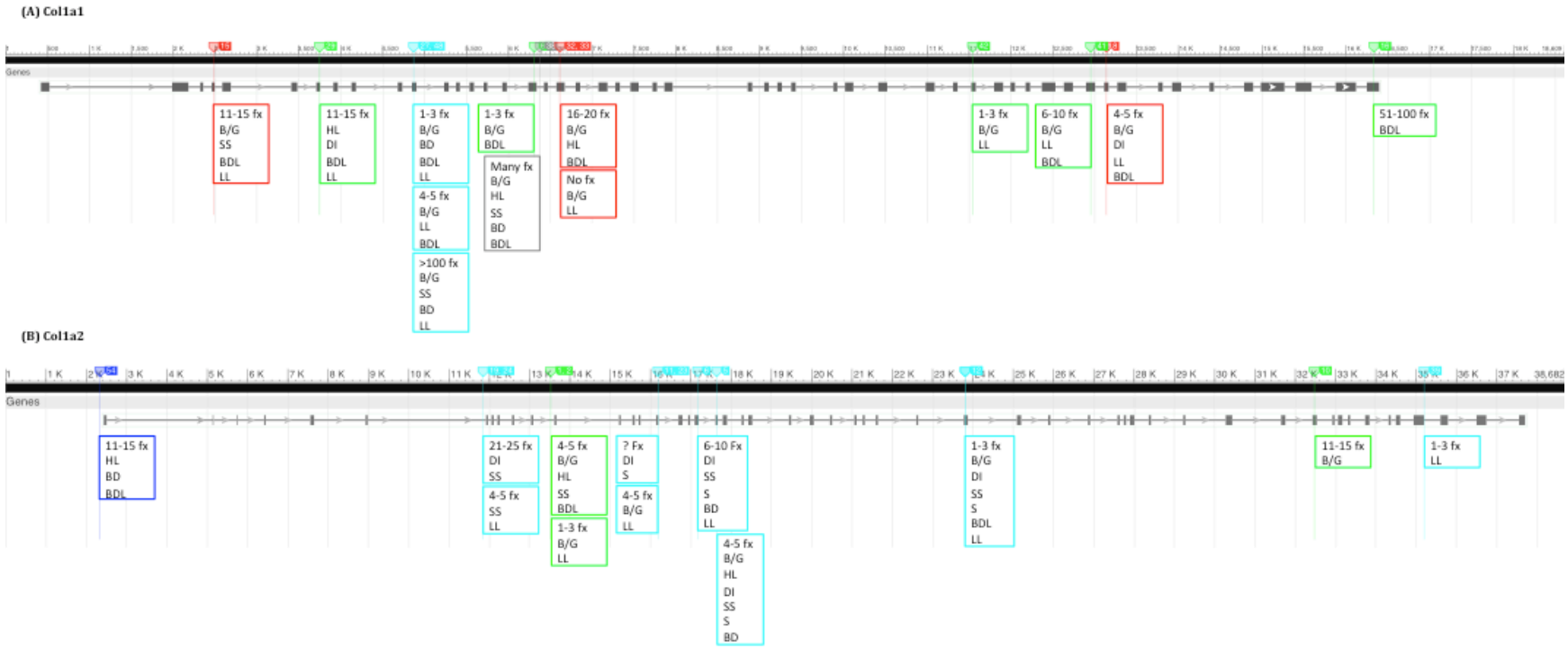

Figure 4 – Genotype-Phenotype Map. (A) Col1a1 (B) Col1a2

Abbreviations:

Fx	Fractures
B/G	Blue/Grey Sclera
HL	Hearing loss
DI	Dentinogenesis Imperfecta
SS	Short Stature
S	Scoliosis
BD	Bone Deformity
BDL	Bone Density Loss
LL	Ligament Laxity

Mutation Types:

- Non-sense
- Mis-sense
- Frameshift
- Splicesite
- Whole exon deletion

DISCUSSION

Diagnostic Odyssey – One of the aims of this retrospective study was to examine how genetic testing is utilized in the clinic to address the diagnostic dilemma of mild and moderate forms of OI. Overall, we did not identify a set criteria that would be clinically diagnostic of OI type I or IV. Fracture frequency, the hallmark feature of OI, was highly variable in both patient groups ranging from few to over a hundred. Additionally, individuals who were typed outside the classical Sillence types (based on genotyping or a known mutation in the family), overlapped in terms of fracture frequency with type I and IV groups. Secondary features associated with OI including scleral hue, DI and hearing loss were also observed at variable rates and did not contribute to a definitive diagnosis or typing. As expected the skeletal phenotype was more involved in individuals with type IV with short stature and ligament laxity being predominant. Our data supports the claim that diagnosing and typing of mild and moderate types of OI strictly based on clinical features can be subjective. We observed that genotyping appears to be utilized most often in the most ambiguous cases. For example, a larger proportion of type I patients had been genotyped had bone deformity (a feature usually absent in these mild cases), compared to those not genotyped. In other words patients who had few fractures and were therefore likely to be type I but had bone deformities atypical of type I, presented as ambiguous cases that were resolved by genotyping. Likewise, in the type IV population, individuals who had not been genotyped were more likely than the genotyped individuals to exhibit all features except hearing loss. These are likely to have appeared as ambiguous cases in that their fracture frequency implied type

IV, however, they appeared mildly or not at all affected with other features commonly seen in type. The exception to this observation is that type I patients with scoliosis were not genotyped at a higher rate, even though scoliosis is generally not expected to be present in mild cases. This could indicate that presence or absence of scoliosis does not provide strong clinical evidence in the diagnosis of OI. Of note, while there were the mentioned descriptive differences between genotyped and non-genotyped groups in the features, the differences were not statistically significant due in part to the small sample size and the small number of patients with the particular feature.

Genotype-Phenotype Correlations – Our analysis of the genotype-phenotype data revealed that mutations in either *Col1α1* or *Col1α2* can lead to both type I and type IV OI with no identifiable correlation with to mutation type, identity or location along the genes. Still, the majority of type I cases were due to *Col1α1* mutations and almost all type IV cases were a result of *Col1α2* mutations. In other words, mutations in *Col1α1* appear to result in milder phenotypes than *Col1α2*. This would not be expected from a strictly stoichiometric standpoint given that each collagen polymer is composed of two alpha 1 and one alpha 2 chains. The explanation for the increased severity of a *Col1α2* mutations compared to *Col1α1*, could instead be in the functional consequences of the mutations. This is consistent with the idea that type I OI is a result of quantitative defects caused by mutations in *Col1α1* that result in collagen haploinsufficiency and more severe OI types are a result of qualitative defects due to mutations in *Col1α2* that cause structural defects of collagen. These

hypotheses may be answered with further functional studies that examine the protein products of these genes.

There were several findings that were not consistent with other studies of genotype-phenotype correlations in OI, though we must keep in mind that the other studies acknowledge possible exceptions to their generalizations. First, since peptide translation occurs in the direction of C to N-terminus, it was expected that the earlier the mutation occurred in the sequence, the more severe the outcome. We did not observe any distinct pattern supporting this. In fact a notable exception was a type IV patient who had experienced over 50 fractures who had a frameshift mutation in the last exon (52) of *Col1α1* (Figure 4). We hypothesize that either the mutation causes a structural defect in the alpha 1 chain or the patient has modifier genes that in combination with this mutation result in a more severe phenotype. Second, we observed that individuals in the same family with the same genotype had different phenotypes. This again could be due to modifier gene effects and in turn makes predicting likely prognoses based on genotype less reliable. Third, contrary to a general consensus that glycine substitutions result in structural defects of either chain and therefore more severe phenotypes, we found that glycine substitutions in the alpha 1 chain usually led to type I OI. Further functional analysis is needed to elucidate the mechanisms underlying these observations.

Utility of Genetic Testing – In this study we illustrated that within our patient cohort, it was not practical to identify a set of clinical criteria for the definitive

diagnosis or accurate typing of mild and moderate forms of OI. Traditionally, biochemical analysis has served as the (confirmatory) diagnostic test for OI, identifying culprits in about 90% of individuals with a clinical diagnosis (Wenstrup *et al*., 1990). In contrast, genetic testing is both minimally invasive and can identify the causative variants in more than 95% of affected individuals (Sykes *et al.,* 1990; Körkkö *et al*., 1998). In addition, in the prenatal setting, biochemical testing is limited to chorionic villus samples and can only identify qualitative defects, with a delay of 2-4 weeks for diagnosis in a time-sensitive context (Engel and Prockop, 1991).

When the primary purpose of investigation is to distinguish cases of child abuse and OI (though they are not mutually exclusive), genetic testing is extremely valuable. It is estimated that about 5% of suspected cases of child abuse are found to have OI (Marlowe *et al*., 2002). With the high detection rate of genomic, the residual risk that a child has OI after a negative genetic testing is less than 0.5%.

Given these considerations, we conclude that genetic analysis should be used as a first tier testing modality in establishing and confirming a diagnosis of OI (in mild and moderate types). Biochemical testing still has value in follow-up studies that can shed light on to the pathogenicity of unclassified variants and functional analysis of splice-site mutations. Though in our study we could not establish any definitive genotype-phenotype correlations, as we collectively expand the variant database, we may start to see reoccurrences of previously assumed “private”

mutations. In this way, having a genotype-phenotype map can help clinicians, patients and families gain insight into the likely course of the condition and therapeutic approaches.

Genotype information is particularly helpful in appropriate selection of available therapies. Bisphosphonates and teriparatide are the two classes of medications currently prescribed in the treatment of OI. The mechanism of action of both drugs is to increase bone mass. Promoting an increase in bone mass is a wise approach in OI types that are due to a quantitative defect of collagen. However in patients whose gene alteration results in structurally defective collagen, promoting an increase in defective collagen synthesis is counter-productive. Furthermore, those with recessive types of OI that are phenotypically indistinguishable from classic types, would not respond in the same way to these treatments because the therapeutic target of these drugs do not align with the pathogenic pathways of recessive OI types. Additionally, bisphosphnates appear to be less effective in adults (Chevrel *et al*., 2006). Thus establishing an early and accurate diagnosis of OI plays an important role in optimizing the clinical management of OI patients.

From a genetic counseling perspective, genotype information provides the most accurate assessment of recurrence risks and is perhaps most useful in the setting of prenatal and pre-implantation genetic diagnosis. This retrospective study reported on the diagnostic experience of patients with mild and moderate forms of OI at the Skeletal Dysplasias Clinic at the Hospital for Special Surgery and will serve as an

expandable tool to inform both clinicians and patient families about the practical intricacies of the diagnosis and care of these patients.

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