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Research paper

Rare splicing mutation in COL1A1 gene identified by whole exomes sequencing in a patient with osteogenesis imperfecta type I followed by prenatal diagnosis: A case report and review of the literature

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ABSTRACT

Background: Osteogenesis imperfecta (OI) is a rare disease characterized by increased bone fragility and predisposition to fractures, bone deformities and other major signs such as dentinogenesis imperfecta, blue sclera and deafness. Over 90% of OI cases are caused by mutations in the COL1A1 and COL1A2 genes and the inheritance is autosomal dominant.

Methods: We present a case of a couple requesting genetic counseling, because the man was diagnosed with OI on a clinical and radiological basis and the woman was pregnant. Whole exomes sequencing (WES) was performed in order to identify the mutation (s), followed by prenatal diagnosis.

Results: WES identified a rare splicing mutation c.1155 + 1G > C in the COL1A1 gene recognized to be pathogenic and subsequently confirmed by next generation sequencing. The carrier state of the mutation was excluded for the fetus, so the pregnancy was further pursued and a healthy baby was born at term.

Conclusions: WES is a new and effective technique for detecting pathogenic variants in monogenic diseases and it is preferable to use such a technique in diseases with genetic heterogeneity especially when time does not allow another time-consuming diagnostic technique such classical Sanger sequencing. WES offers possibility to expand the global spectrum of OI pathogenic variants enabling the diagnosis of the disease.

1. Introduction

Osteogenesis imperfecta (OI) represents a heterogeneous group of connective tissue diseases that have major clinical signs of bone fragility that cause fractures, bone deformities and small stature. Prevalence is estimated at between 1/10,000 and 1/20,000 (https://www.omim.org (accessed on 14 June 2019; https://www.orpha.net/consor/cgi-bin/index.php (accessed on 10 June 2019).

Abbreviations: OI, Osteogenesis imperfecta; WES, Whole exomes sequencing; OMIM, Online Mendelian Inheritance in Man database; N, Normal; DI, Dentinogenesis imperfecta; AD, Autosomal dominant; AR, autosomal recessive; XR, X linked recessive; *COL1A1*, Collagen, type I, alpha-1; *COL1A2*, Collagen, type I, alpha-2; *IFITM5*, Interferon induced transmembrane protein 5; *SERPINF1*, Serpin peptidase inhibitor, clade F, member 1; *CRTAP*, Cartilage associated protein; P3H1, prolyl 3-hydroxylase 1; *PPIB*, Peptidylprolyl isomerase B/cyclophilin B; *SERPINH1*, Serpin peptidase inhibitor, clade H,member 1/heat shock protein 47; *FKBP10*, FK506 binding protein 65; *SP7*, Transcription factor 7/osterix; *BMP1*, Bone morphogenic protein1/procollagen C proteinase; *TMEM38B*, Transmembrane protein 38B; *WNT1*, Wingless-type MMTV integration site family, member 1; *CREB3L1*, cAMP responsive element binding protein 3 like 1; *SPARC*, SPARC/osteonectin; *TENT5A*, Terminal nucleotidyl transferase 5a; *MBTPS2*- Membrane-bound transcription factor protease, site 2; NGS, Next Generation Sequencing Splicing Clinically Applicable Pathogenicity prediction, S-CAP; HGMD, Human Gene Mutation Database; ACMG, American College of Medical Genetics and Genomics; BDGP, Berkeley Drosophila Genome Project; S-CAP, Splicing Clinically Applicable Pathogenicity prediction

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Table 1
Osteogenesis imperfecta (OI) classification – genetic and clinical characteristics (https://www.omim.org (accessed on 14 June 2019; Lim et al., 2017; Forlino and Marini, 2016; Valadares et al., 2014; Marini et al., 2017).

OI type	OMIM	Gene	Mechanism	Inheritance	Bone deformity	Sclerae	Hearing loss	DI
I	166,200	*COL1A1/ COL1A2*	Impairment of collagen synthesis and structure	AD	Rare to very severe	N, grey to dark blue	Absent to common	Absent to common
II	166,210	*COL1A1/ COL1A2*		AD	Rare to very severe	N, grey to dark blue	Absent to common	Absent to common
III	259,420	*COL1A1/ COL1A2*		AD	Rare to very severe	N, grey to dark blue	Absent to common	Absent to common
IV	166,220	*COL1A1/ COL1A2*		AD	Rare to very severe	N, grey to dark blue	Absent to common	Absent to common
V	610,967	*IFITM5*	Compromised bone mineralization	AD	Variable	N -blue	Infrequent	Absent
VI	613,982	*SERPINF1*		AR	Moderate/severe	N	Absent	Absent
VII	610,682	*CRTAP*	Abnormal collagen post-translational modification	AR	Severe rhizomelia	N, grey	Absent	Absent
VIII	610,915	*P3H1*		AR	Severe rhizomelia	N	Absent	Absent
IX	259,440	*PPIB*		AR	Severe	Grey	Absent	Absent
X	613,848	*SERPINH1*	Compromised collagen processing and crosslinking	AR	Severe	Blue	Absent	Present
XI	610,968	*FKBP10*		AR	Mild to severe	N, grey	Absent	Absent
XII	613,849	*SP7*		AR	Severe	N	Absent	Absent
XIII	614,856	*BMP1*	Altered osteoblast differentiation and function	AR	Mild to severe	N	Absent	Absent
XIV	615,066	*TMEM38B*		AR	Severe	N to blue	Absent	Absent
XV	615,220	*WNT1*		AR/AD	Severe	White	Absent	Absent
XVI	616,229	*CREB3L1*		AR	Severe	–	–	–
XVII	616,507	*SPARC*		AR	Progressive bone fragility	White to slightly grey	Absent	Absent
XVIII	617,952	*TENT5A*		AR	Moderate	Blue	–	–
XIX	259,440	*MBTPS2*		XR	Moderate/severe	Blue	–	–

N – normal; DI - dentinogenesis imperfecta; AD – autosomal dominant; AR – autosomal recessive; XR – X linked recessive; *COL1A1* - Collagen, type I, alpha-1; *COL1A2* - Collagen, type I, alpha-2; *IFITM5* - Interferon induced transmembrane protein 5; *SERPINF1* - Serpin peptidase inhibitor, clade F, member 1; *CRTAP* - Cartilage associated protein; *P3H1*- prolyl 3-hydroxylase 1; *PPIB* - Peptidylprolyl isomerase B/cyclophilin B; *SERPINH1* - Serpin peptidase inhibitor, clade H,member 1/heat shock protein 47; *FKBP10* - FK506 binding protein 65; *SP7* - Transcription factor 7/osterix; *BMP1* - Bone morphogenic protein1/procollagen C proteinase; *TMEM38B* - Transmembrane protein 38B; *WNT1* - Wingless-type MMTV integration site family, member 1; *CREB3L1* - cAMP responsive element binding protein 3 like 1; *SPARC* - SPARC/osteonectin; *TENT5A* - Terminal nucleotidyl transferase 5a; *MBTPS2*- Membrane-bound transcription factor protease, site 2.

The earliest evidence of OI is a mummy, dating from ancient Egypt, but the first scientific description was made 1788 (Peltier, 1981). 1979, Sillence classified this group of diseases into four entities on the basis of clinical, radiological and inheritance patterns, most of which being caused by mutations in the *COL1A1* and *COL1A2* genes. The discovery of other genes involved in the pathogenesis of OI allows a genetic classification of diseases, which uses as the main element the genes and theirs variants that determine the disease. Thus now, in the Online Mendelian Inheritance in Man (OMIM) database, 19 types of OI are recognized (Table 1).

OI type I-IV are concordant with Sillence classification, being determined by mutations in *COL1A1* and *COL1A2* genes. The transmission pattern is an autosomal dominant one for OI type I-V, autosomal recessive for OI type VI-XVIII and X-linked recessive for OI type XIX. The pathophysiological mechanisms in which the products of the genes involved interfere are: impairment of collagen synthesis and structure (*COL1A1, COL1A2)*, compromised bone mineralization (*IFITM5, SERPINF1*), abnormal collagen post-translational modification (*CRTAP, P3H1, PPIB*), compromised collagen processing and crosslinking (*SERPINH1, FKBP10, SP7, PLOD2*), altered osteoblast differentiation and function (*BMP1, TMEM38B, WNT1, CREB3L1, SPARC, TENT5A, MBTPS2*) (Table 1) (Lim et al., 2017; Forlino and Marini, 2016). The penetrance for pathogenic variants in COL1A1 and COL1A2 genes is 100%. Phenotypic expressivity is variable even within the same family (Steiner et al., 2013).

The primary feature is increased bone fragility with a high prevalence of fractures (> 90% of total cases) and osteoporosis. Other features presented in some types of OI are: blue sclerae, young adult onset hearing loss, dentinogenesis imperfecta, joint hypermobility, short stature and progressive skeletal deformity (Van Dijk and Sillence, 2014).

In the mild form of OI hereditary transmission of mutation was identified in 40% of cases while the progressive deforming disease with perinatal mortality is produced only by the *de novo* variants (Steiner et al., 2013).

We aimed to identify the mutation in a man diagnosed with OI according to clinical and radiological criteria, considering that his partner was pregnant and the couple requested genetic counseling.

2. Methods

The couple asked genetic counseling because the woman was pregnant (first trimester) and the partner was diagnosed with OI. The patient (37 year old) was diagnosed at birth with OI because he had a clavicle fracture and blue sclera. Since birth, he had many fractures (over 60), mostly affecting his legs, consequently resulting a shortened stature (1.36 m coresponding at 5th Percentile). In childhood, due to numerous fractures, he had a steady decrease in his condition compared to other kids. At the time of genetic consultation the patient had short stature and blue sclera without dentinogenesis imperfecta and deafness (Supplementary figure 1).

His family history is negative for OI (Fig. 1). The theoretical risk of the fetus to have the same disease is 50%, but in order to establish the fetus heterozygous status it was necessary to make a molecular prenatal diagnosis. The patient was not previously molecularly tested. The written informed consent for molecular genetics studies was obtained

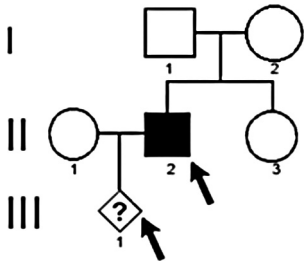

Fig. 1. Pedigree (arrows indicate the persons tested).

from the patient. A Clinical Whole Exome Sequencing test was performed on blood of patient focusing on 3583 OMIM disease genes and Exome of 20,370 genes with target region capture followed by Next Generation Sequencing (NGS). Sequencing covered also the intron–exon junction. After identification of a pathogenic variant in *COL1A1* gene we applied a prenatal diagnosis. Biological sample from fetus was obtained by amniocentesis and amniocytes were cultured. After that, DNA was extracted and a targeted sequencing was performed on both DNA strands of the relevant *COL1A1* region (the reference sequence is: COL1A1: NM_000088.3.).

3. Results

Direct DNA sequencing analysis of *COL1A1* gene revealed a splicing mutation (c.1155 + 1G > C or IVS17 + 1G > C) in heterozygous state. The mutation was located at the beginning of the 17th intron of *COL1A1* gene and was characterized by replacement of guanine with cytosine at the level of the 1155th nucleotide of the gene sequence. This mutation implies the border between 17th exon and 17th intron of *COL1A1* gene. The mutation causes abnormal mRNA processing, causing a splicing-type modification in the corresponding protein resulting in the transcriptional deletion of exon 17 (Fig. 2).

The c.1155 + 1G > C mutation has been reported for its pathogenicity and is present in the LOVD database with 7 entries, all as substitutions within intron 17, classified as pathogenic splice-site variants. The frequency of this mutation in the normal population is very low (< 1% in 1000 human genome project, dbSNP, ESP6500, BGI internal databases).

The carrier mutation state was excluded for the fetus, so the pregnancy has been pursued further and a healthy female baby was born at term (height 3400 g, weight 49 cm, Apgar score 9).

To predict the consequences of mutation we used BDGP (Berkeley Drosophila Genome Project) algorithm that showed a significant impairment of exon 17 binding with intron 17 (boundaries) resulting in the transcriptional escape of exon 17. Prediction analysis of pathogenicity using Human Splicing Finder indicates alteration of the WT donor site, most probably affecting splicing. By using of S-CAP (Splicing Clinically Applicable Pathogenicity prediction) 5′ core dominant we obtained a score of 0.834 corresponding to the heterozygous pathogenic variant status.

4. Discussion

The prenatal diagnosis for OI should be considered in three main situations: 1) risk of the birth of a child with OI in a family with autosomal dominant form of OI; 2) risk of OI type AD when the affected parent is the only one in the family; 3) risk of germinal mosaicism in a family with negative history of disease but more than two children with

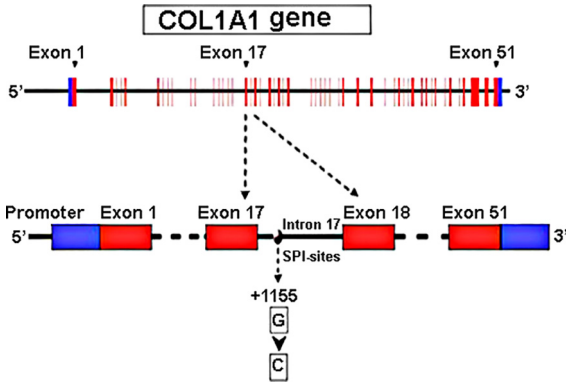

Fig. 2. The diagram shows the mutation site in the COL1A1 gene, in intron 17 at the splice site.

OI (Pepin et al., 1997; Gug et al., 2020a; Gug et al., 2018; Popovici et al., 2011; Gug et al., 2019). In our case we are in the second situation: the patient's familial history was negative for OI, so our patient presents a de novo mutation with dominant autosomal inheritance, and therefore there is a 50% risk of having a child with this mutation for every future pregnancy.

Because OI is a disease with important genetic heterogeneity and we needed an urgent prenatal diagnosis, the patient did not undergo ordinary Sanger sequencing, which is time consuming and expensive. In this situation, the effective approach for screening the mutation of osteogenesis imperfecta is whole exome sequencing (Keller et al., 2018; Mackenroth et al., 2016; Wang et al., 2019). Whole exome sequencing takes into account both information about the target genes associated with the main signs, enabling checking of the genes indicated by the American College of Medical Genetics (Gug, 2016; Gug et al., 2018; Gug et al., 2020b). Cases with OI have been reported in Romania but this is the first case confirmed at molecular level trough NGS and followed by prenatal diagnosis. This is the main particularity of the presented case. In addition, there is an explanation for the “de novo” appearance of the mutation because the patient's mother worked in a toxic environment before conception. Our patient can benefit from specific treatment with Pamidronat Torrex, which is free in Romania for patients with identified mutation (Stoicanescu and Belengeanu, 2009; Marginean et al., 2017).

The *COL1A1* gene is located 17q21.33 and contains 52 exons. Gene expression of the *COL1A1* gene is not characterized by alternative splicing, a phenomenon that occurs in over 50% of human genes. There are two different transcripts (5.8 kb and 4.8 kb differentiated by the 3′ noncoding region), but the translation of mRNA produces a single protein of 140 kDa (http://atlasgeneticsoncology.org/Genes/GC_COL1A1.html (accessed on 27 July 2019). This fact is explained by the alternative polyadenylation that determines posttranscriptional changes, that modify translation rate of mRNA, change lifetime of mRNA and in this way modulate quantity of protein synthetized (Popovici et al., 2017; Miskulin et al., 1986). The transcription of exons 6–49 allows the production alpha helical domain. All these exons have a common characteristic: the number of base pairs is a multiple of 9 (http://atlasgeneticsoncology.org/Genes/GC_COL1A1.html (accessed on 27 July 2019).

Collagen type I consists of a triple helix including two alpha1 chains (encoded by the *COL1A1* gene) and one alpha 2 chain (encoded by the *COL1A2* gene). Type I collagen is predominantly in the connective tissue of bones, corneas, dermis and tendons. Obviously, mutations in the *COL1A1* gene are more deleterious than those in the *COL1A2* gene (Popovici et al., 2017). The defect in OI 1 is a quantitative reduction of type 1 collagen or an impaired structure (qualitative defect). In the first situation the phenotype is milder, while in the second situation the phenotype is more severe (Tournis and Dede, 2018; Forlino and Marini, 2000). The types of mutations in the *COL1A1* included in the Human Gene Mutation Database (HGMD®) are: missense/nonsense mutations (476 variants); splicing mutation (234 variants); regulatory (4 variants); small deletion (20 pb or less) – (192 variants); small insertions (20 pb or less) – (61 variants); small indels (20 pb or less) – (10 variants); gross deletions – (24 variants); gross insertions – (2 variants); and complex – (2 variants). In general, splicing mutations represent 8.72% of the total mutations inventoried in HGMD (accessed 9.06.2019; HGMD professional 2019.1) (Stenson et al., 2017).

In LOVD database the total number of variants was 2007. Most variants were reported in exon 37 (80 variants), exon 44 (69 variants) and exon 11 (68 variant). Of the total variants most are substitutions (1541), and 302 of them are intronic as in the case of our proband. A total of 16 variants were described in intron 17 (https://www.le.ac.uk/ge/collagen/ (accessed on 6 June 2019). The splicing mutations is the second most frequent and could generate exon skipping, intronic inclusion or activation of cryptic sites in introns and exons (Marini et al., 2007).

Table 2
Characteristics of splicing mutations (Anna and Monika, 2018).

Type	Location	Effects
I	Canonical acceptor site	Single exon skipping
II	Deep intronic	Cryptic exon inclusion
III	Exonic sequences	Introduce a new 5′ or 3′/activate a cryptic splice site
IV	Canonical donor site	Inclusion of the intron fragment/removal of the exon fragment
V	Exonic sequences	Entire exon skiping

The splicing process involves the interaction between *cis* and *trans* elements. *Trans* elements are: spliceosome proteins and splicing repressors or activators. The *cis* elements are: donor (5′) and acceptor (3′) splice sites, branch point, polypirimidine sequences, splicing silencers and enhancers. In 98.7% of cases at both ends of the intron the normal nucleotide pairs GT respectively AG (5′ and 3′ end of the intron) are present. Splicing mutations can be classified into 5 categories (I-V) depending on the location of the mutation and its effects (Table 2) (Anna and Monika, 2018).

In our case, WES focused on 3583 OMIM diseases and exome of 20,370 genes. Point mutations, micro-insertions, deletions, duplications (< 20 bp) in the analyzed genes can be detected simultaneously. The global coverage is over 95%.

According to the American College of Medical Genetics and Genomics (ACMG) there are five mutation types: “pathogenic”, “likely pathogenic”, “uncertain significance”, “likely benign” and “benign” (Richards and Aziz, 2015). Splice donor mutations are more common compared to splice acceptor mutations (1.5:1). The classic mutations are located in positions + 1 and + 2 at level 5′, respectively −1 and −2 at level 3′ (Krawczak, 2007). In the case of our patient the prediction algorithm of the link sites concluded that the c.1155 + 1G > C variant of COL1A2 occurs in the strictly conserved + 1 nucleotide sequence of the end 5′ in the donor / acceptor binding sites (Exon - intron) exactly at the edge points. This mutation significantly affected the binding of Exon 17 with intron 17 and caused the transcriptional deletion of exon 17.

As shown in Table 3, the mutation identified in our patient was described in other seven cases: five mutations in OI type 1, two in OI III and one in OI IV. Only in two cases were the mutations identified through WES.

The pathogenicity of nucleotide substitutions was confirmed in 112 cases and in 906 cases pathogenicity prediction programs were used. In our case, the prediction algorithm using BDGP (Berkeley Drosophila Genome Project) showed significant impairment of exon 17 binding with intron 17 (boundaries) resulting in the transcriptional escape of exon 17. Prediction analysis of pathogenicity using Human Splicing Finder indicates alteration of the WT donor site, most probably affecting splicing.

The use of the S-CAP (Splicing Clinically Applicable Pathogenicity prediction) 5′ core dominant showed a score of 0.834 corresponding to the heterozygous pathogenic variant status. The program uses the classification of mutations into 6 regions: 5′ GT (5′ core) and 3′ AG (3′ core) dinucleotides, intronic variants upstream of a 3′ splice site (3′ intronic), variants lying in the canonical U1 snRNA– binding site, excluding the core 5′ SS (5′ extended) intronic variants downstream of a 5′ SS, and synonymous variants within the protein-coding gene (exonic). For each region the S-CAP score has a threshold that differentiates between benign and pathogenic mutations (Jagadeesh et al., 2019).

Jagadeesh et al divides the variants into the regions mentioned above and, using the score on each region, classifies them into pathogenic and benign. It can thus be observed that the most common pathogenic variants are in the 3′ core region and the 5′ core region, and most benign variants in the 3′ intronic, exonic, 5′ intronic region (Table 4) (Jagadeesh et al., 2019).

5. Conclusions

Osteogenesis imperfecta is a rare condition characterized by genetic heterogeneity (allelic, nonallelic, clinical). We described a rare case of a de novo mutation with dominant autosomal inheritance: a splicing mutation (c.1155 + 1G > C or IVS17 + 1G > C) in heterozygous state. WES is the technique to be applied as the first intention to screen for mutations of genes involved in OI pathogenesis. In silico analysis of the pathogenic variant using dedicated programs is absolutely necessary to classify the variant in one of the 5 categories of mutations, an aspect extremely important in the prenatal genetic counselling.

The detailed molecular and clinical features will be useful for exploring phenotype-genotype correlations. WES offers possibility to expand the global spectrum of OI pathogenic variants that allows a better diagnosis of disease.

6. Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform Requirements for manuscripts submitted to Biomedical journals.

7. Consent

Written informed consent was obtained from the patient and his wife for the presentation of clinical data and any accompanying pictures.

8. Financial support

None declared

Table 3
Characteristics of the mutation present in the proband (No 8) compared to other cases described in the literature (https://www.le.ac.uk/ge/collagen/ (accessed on 6 June 2019).

Number	Type	OI	Methods	Remarks
1	Splice site	OI type I	PCR, SEQ	ni
2	Splice site	OI type I	unknown	Skips exon 17 (minor), activates exon 17 cryptic splice site
3	Splice site	OI type III	PCR, SEQ	ni
4	Splice site	OI type IV	unknown	Skips exon 17
5	Splice site	OI type I	PCR, SEQ	ni
6	Splice site	OI type I	HRM, SEQ	ni
7	Splice site	OI type III	WES	ni
8	Splice site	OI type I	WES	Skips exon 17

ni-no information; no 8 – our proband.

Table 4
Comparison between pathogenic and benign variants in different regions of the gene (Jagadeesh et al., 2019).

Region Variant	3′ intronic	3′ core	exonic	5′ core	5′ extended	5′ intronic
Pathogenic	1419	5835	317	7044	2279	165
Benign	2,357,091	42,880	1,899,048	58,942	210,618	2,191,871

9. The author contribution

GC, LC performed the study design and wrote the first draft of the manuscript. **GC** performed genetic counseling, indicated the genetic test and interpreted the genetic outcome in relation to the clinical characteristics. **EVG, DS** and **DA** made a decisive contribution to the literature search and contributed to the details of the discussions**. IM** and **MG** collected data and contributed to the iconography. **LC** and **IM** are the corresponding authors. All authors verified the final manuscript version.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gene.2020.144565.

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