

CASE REPORT

Clinical whole-exome sequencing analysis reveals a novel missense COL11A1 mutation resulting in an 18-week Iranian male aborted fetus with Fibrochondrogenesis 1: A case report

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Abstract

Fibrochondrogenesis 1, an autosomal recessive syndrome, is a rare disease that causes short-limbed skeletal dysplasia. Mutations in the gene encoding the $\alpha 1$ chain of type XI collagen (COL11A1) are seen to be the main cause of this disease. We present an 18-week Iranian male aborted fetus with Fibrochondrogenesis 1 from consanguineous parents. Whole-exome sequencing revealed a novel missense variant from G to A in exon 45 of 68 in the COL11A1 gene (NM_080629.2: c.3440G > A, [p.G1147E, g.103404625]). The mutation was confirmed by Sanger sequencing and further, MutationTaster predicted this variant to be disease-causing. Bioinformatic analysis suggests that this variant is highly conserved in both nucleotide and protein levels, suggesting that it has an important function in the proper role of COL11A1 protein. *In silico* analysis suggests that this mutation alters the COL11A1 protein structure through a Glycine to Glutamic acid substitution.

KEYWORDS

aborted fetus, collagen type XI $\alpha 1$ (COL11A1), Fibrochondrogenesis, whole-exome sequencing (WES)

Hanifeh Mirtavoos-Mahyari and Sanaz Ajami contributed equally to the work.

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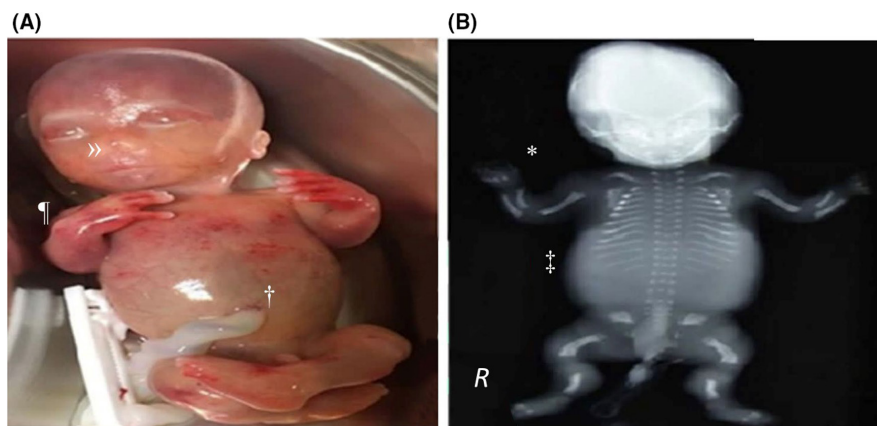


FIGURE 1 Clinical Photograph (A) and Radiograph (B) of Fibrochondrogenesis Case from 18-week Iranian male aborted fetus. (») the 18-week Iranian male aborted fetus; flat midface, a small nose, and anteverted nares, (¶) significant shortening of all limb segments but relatively normal hands and feet, and (†) small bell-shaped thorax with a protuberant abdomen. (*) Ribs are typically short and wide and have metaphyseal cupping at both ends, and (‡) the long bones are short and have broad metaphyseal ends, giving them a dumb-bell shape.

1 | BACKGROUND

Fibrochondrogenesis 1 (FBCG1; MIM 228520) is known as an autosomal congenital syndrome, which is related to short-limbed skeletal dysplasia.¹ The disease is clinically distinguished by a distinctive face, protuberant eyes, a flat small nose, and a small mouth accompanied by a long upper lip. Radiographically, the vertebral bodies are flat, the long bones are short and have broad, large metaphyseal ends, giving them a dumbbell shape. Moreover, the ribs are typically short, wide, and own metaphyseal cupping at both ends.²

According to different studies, the disease has occurred around the world at different levels of disease, gender, and race.³ Thereby, a case of FBCG1 was diagnosed by Hunt and Vujanic in a fetus of 17 weeks, with severe micrognathia, and bifid tongue.⁴ Lazzaroni-Fossati and colleagues described this disorder in an infant from a consanguineous marriage.¹

Studies have illustrated that mutations in *COL11A1* are the main cause of FBCG1 incidence.² Currently, several methods are being used for FBCG screening and diagnosis, electron microscope analysis, immunofluorescence assays, and genetic testing.² Among these methods, genetic testing is available for the most common mutations.⁵ Nonetheless, rare mutations cannot be discovered using conventional methods. Clinical whole-exome sequencing (WES), as a newborn screening strategy, is an effective, successful diagnostic tool to track rare genetic alterations in a methodical and organized way for patients who have failed to receive a diagnosis despite extensive testing and follow-ups.⁶

We present an analysis of the outcomes of WES that was performed in a diagnostic laboratory on an 18-week Iranian male aborted fetus with FBCG1. Besides, a detailed interpretation of the identification of a novel variant in a single case with FBCG1 is described. We believe

that this report can provide insight into the phenotypic variability of this disease.

2 | CASE PRESENTATION

The present study comprised a single case of an Iranian aborted male fetus from a 26-year-old mother. The parents were first-degree cousins. The family history for genetic or metabolic history was normal. The case was presented after 18 weeks and 2 days of gestation with absent fetal movements and multiple abnormal ultra-sonographic signs.

The disease was first clinically diagnosed by significantly small bell-shaped thorax with a protuberant abdomen, significant shortening of all limb segments despite moderately normal hands and feet, and flat midface with a small nose and anteverted nares (Figure 1A). The disease was then diagnosed with FBCG1 by radiography. Radiographically, the ribs were typically short and wide and had metaphyseal cupping at both ends. The long bones were short and had broad metaphyseal ends, which gave them a dumbbell shape (Figure 1B).

The genomic DNA was extracted from fetal tissue using the Omega EZNA® Tissue DNA Kit (Omega Bio-Tek) according to the manufacturer's protocols. NanoDrop spectrophotometer was used to evaluate DNA concentration (85 ng/ml). The WES was performed by MacroGen Europe (Amsterdam, The Netherlands). Target Enrichment System (Agilent, Human All Exon Kits SureSelect V7; Agilent Technologies, Inc.), followed by a paired-end high-throughput sequencing on reads of 151 bp using Illumina NovaSeq 6000 (Illumine Inc.). Overall, 75,309 single nucleotide polymorphisms (SNPs) and 10,068 indel variations were detected after removing low quality and out of the boundary of the capture kit in the exome analysis out of 633,000 variants (Table 1).

TABLE 1 Summary of whole-exome sequencing 100X; Quality controls and Variant types

≥10X	92%
≥30X	69%
≥50X	50%
Mean coverage	66.5X
Total number of variants obtained	633,000
Total Indel variants	10,068
Exonic non-synonymous variants	75,309
Frameshift	574
Nonsense	247
Splice site	562

Obtained data from WES were first mapped to the Homo Sapiens genome reference (UCSC hg19), with mapping efficiencies of 99.9% for each paired-end read using the Burrow-Wheeler Alignment Tool (BWA 0.7.15).⁷

Variant calling of indels and SNPs was carried out by using the GenAP pipeline (Cimorgh Medical IT Solutions, Tehran, Iran) employing the Genome Analysis Toolkit (version v4.1.9.0) HaplotypeCaller pipeline, and the Picard tool.⁸ For functional annotation and genetic filtering, the GenAP automated variant annotation, classification, and prioritization were used.⁹ Furthermore, all the recognized recurrent mutations were confirmed using the Integrative Genomics Viewer (IGV) (version 2.8.13).¹⁰ Following the previous step, to remove synonymous and non-exonic variants, common SNPs with minor allele frequency less than 0.02, which were reported in the single nucleotide polymorphism database (dbSNP), the GenAP in house, the 1 K human genome, and the genomAD, and exAC databases were filtered out.¹¹ Ultimately, the filtered variants were sorted based on the Combined Annotation Dependent Depletion-PHRED score (Cut-off = 15) and zygosity. Two homozygous variants were found in *COL11A1*. Only the mutation in the *COL11A1*, which causes FBCG1, was compatible with the clinical findings of the proband. The genomic information regarding this mutation is as follows: transcript: NM_080629.2, nucleotide change: c.3440G > A, amino acid change: p.G1147E, and chromosome position: g.103404625.

For further genetic evaluation, the rare variants with high confidence then were contemplated as disease-causing candidates. To evaluate the pathogenicity of the novel variants, several computational algorithms including Polyphen-2, MutationTaster, and SIFT were recruited.¹² Variants occurring in the well-known phenotype-causing genes along with the candidate genes were selected and evaluated with priority based on known physiological, biological, and/or functional connections to the phenotype. The interpretation and elucidation of variants were

conducted based on the American College of Medical Genetics and Genomics (ACMG) guidelines.¹³ Among the prioritized variants, the mutations which were predicted to be damaging and disease-causing were accounted as the most promising candidates. The results indicate that there is a missense variant from G to A in exon 45 of 68 in the *COL11A1* gene NM_080629.2: c.3440G > A, [p.G1147E, g.103404625], which is found to be disease-causing. This variant is highly conserved in both nucleotide and protein levels suggesting that it has an important function. This variant is not reported in gnomAD, ExAC, or 1000genome, and therefore can be considered as a rare variant.

Following the WES analysis, Sanger sequencing was performed to confirm the candidate variants found in WES as well as segregation analysis of the candidate variants within the family. The results illustrated that there is a G to A alteration at position g.103404625 c.3440. The obtained data shows that the proband carried the A/A genotype (homozygous), while the unaffected parents were found to be heterozygous for this mutation.

Finally, in silico analyses were performed to predict the effect of this SNP on the protein structure of *COL11A1*. The amino acid sequence of *COL11A1* in humans with NP-542196 was obtained from the NCBI server (<https://www.ncbi.nlm.nih.gov>). The 3D mature peptide of *COL11A1* structure was built by using the iterative threading assembly refinement (I-TASSER) server, as its structure was not available on the protein data bank.¹⁴ Each model produced by the I-TASSER is given a confidence score (c-score). Higher values of the c-score indicate higher quality of the 3-D structure and a higher confidence level of the predicted structure. Five models were predicted by the I-TASSER. The best-predicted model was model 1 with the highest c-score (1.28). The analysis of the predicted 3D model of proteins was validated using the VADAR web server.¹⁵ The Ramachandran results analysis of proteins by VADAR are as follows: Residue in phi-psi core: 70%, Residue in phi-psi allowed: 24%, Residue in phi-psi generous: 2%, and Residue in phi-psi outside: 2%. The results indicate that the selected model is applicable for mutagenesis studies. Bioinformatics predictions have reported a G1147E (G624E in predicted structure) mutation in the protein structure of *COL11A1*. UCSF Chimera software was then used to generate the structure and identify the energy minimization of models. PyMOL software was recruited for visualization of structures (Figure 2A), superimposing the mutant model with the native one (Figure 2B), and evaluating the surface electrostatic potential of the native and mutated protein. The assessment of the native *COL11A1* and the mutant *COL11A1* displayed that the G624E mutation caused electrostatic alteration from a partial positive charge in native to the negative charge in mutant *COL11A1* (Figure 2C,D).

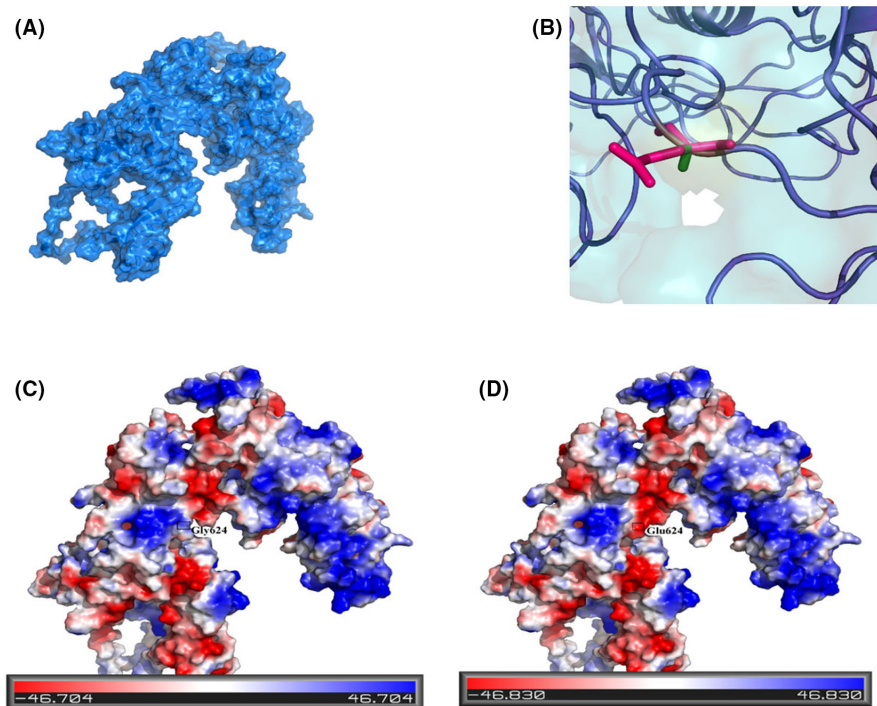


FIGURE 2 3D protein structure and superimposition of native and mutant structure: (A) the predicted 3D protein structure, and (B) superimposition of the native and mutant protein. Green stick represented Gly (G) in the native structure and pink represented Glu (E) in the mutant structure. (C) the surface electrostatic potential of native form and (D) the mutated form of COL11A1 were calculated by PYMOL software. Blue areas symbolize a positive charge, white a neutral charge, and the red-colored area is negatively charged.

3 | DISCUSSION AND CONCLUSION

FBCG1 as an autosomal congenital is related to short-limbed skeletal dysplasia. With a prevalence rate of 1.6 per 10,000, this disease is known to be infrequent and rare.¹⁶ According to previous studies, the disease has two main types that are caused by mutations in the COL11A1 gene. The purpose of this study was to determine the FBCG1-causing mutation in the COL11A1 gene by performing WES on extracted genomic DNA from an Iranian aborted male fetus from consanguineous parents.

WES provided comprehensive and complete information on the sequence to screen 34 candidate genes for FBCG1. After processing raw FASTQ data, variant calling, strict variant filtering, and confirming the mutation with results obtained from Sanger sequencing, the pathogenic mutations were identified.

In this case, there was a missense variant from G to A in exon 45 of 68 in the COL11A1 gene (NM_080629.2: c.3440G > A, [p.G1147E, g.103404625]). MutationTaster predicted this variant to be disease-causing. Since this variant is highly conserved in both nucleotide and protein levels, it has an important function. As this variant is not reported in gnomAD, ExAC, or 1000genome, it can be considered a rare and novel variant. *In silico* analyses have revealed that this SNP imposes a G624E mutation at the protein structure level in the predicted structure. Substitution of Glutamate, a negatively charged residue, with Glycine, a partial positive charge residue, influences the electrostatic potential, which can ultimately affect the function of the protein.

The results of bioinformatics analysis, segregation analyses, and clinical phenotype coherence have supported that the compound missense variant of COL11A1 was the pathogenic mutation of the patient, which was first reported.

Although WES provides data about the protein-coding regions of genes and provides a rapid and cost-effective approach to finding novel mutations in FBCG1, it should be noticed that WES does not cover the entire exome, and not all the exons are captured. Additionally, WES cannot include the sequencing of intron regions. This is a matter of concern as non-coding regions participate in the central dogma of molecular biology by regulating gene expression. Hence, recruiting whole genome sequencing could be also valuable to determine the role of non-coding regions in the incidence of FBCG1. The readers should also consider that our findings are obtained based on only one case. Despite the fact that FBCG1 is an extremely rare condition, the authors suggest further experiments on this mutation and its effect on the downstream pathways. Moreover, employing the above-mentioned methods can also unravel new participants in the development of FBGS1 as potentially, other mutations can also lead to this condition.

Overall, disease-causing mutations have yet to be identified for many patients with FBCG1. Many genotype–phenotype correlations remain unknown. The authors suggest that specific mutations, including the NM_080629.2: c.3440G > A that was investigated in this study could be tested for in cases where none of the more common mutations are detected. The identification of novel mutations will improve the diagnosis and clinical treatment of FBCG1 patients.

AUTHOR CONTRIBUTIONS

Hanifeh Mirtavoos-Mahyari: Conceptualization; investigation; methodology; validation; writing – original draft. **Sanaz Ajami:** Conceptualization; validation; writing – review and editing. **Amirhosein Mehrtash:** Data curation; writing – review and editing. **Seyedeh Mahya Marashiyani:** Data curation; writing – review and editing. **Farbod Bahreini:** Formal analysis; writing – original draft. **Kambiz Sheikhy:** Formal analysis; methodology; writing – review and editing. **Sogol Ghanbari:** Investigation; writing – original draft. **Amin Ardeshirdavani:** Supervision; validation; writing – review and editing.

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CONFLICT OF INTEREST

Amin Ardeshirdavani discloses that he is an equity shareholder of Cimorgh Medical IT Solution. Other authors declare no potential conflict of interests.

DATA AVAILABILITY STATEMENT

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

ETHICAL APPROVAL

The study design was approved by the ethics committee of Shahid Beheshti University of Medical Sciences. The Ethics committee approval number is IR.SBMU.NRITLD.REC.1399.197.

CONSENT

Written informed consent was obtained from the parents of the aborted fetus to publish this report in accordance with the journal's patient consent policy.

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