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Massive parallel sequencing and osteogenesis imperfecta: An essential tool for forensic investigation over child abuse

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ABSTRACT

Osteogenesis imperfecta (OI) is a rare disease of collagen synthesis causing bone fragility. Also called "glass bone disease" since it manifests as spontaneous fractures, it is classified into nine types, both with dominant and recessive transmission. In 95% of cases OI is caused by mutations in COL1A1 and COL1A2 genes encoding the alpha1 and alpha2 chains of type 1 collagen, mainly null variants caused by frame-shift/nonsense mutations or splicing defects. In infants the differential diagnosis include not-accidental trauma, so child abuse. Families suspected of abuse often provide an unverified history of frequent fractures; conversely, the family history of individuals with OI often does not reveal any other affected individuals because of a de novo pathogenic variant in the proband or the presence of a mild phenotype in relatives. Therefore, legal medicine unit with DNA lab is crucial in these cases since it could early collect living or autopsy samples when a child abuse is suspected and then test DNA. We set up a MPS (massively parallel sequencing) panel including the coding regions of COL1A1 and COL1A2 and other 11 genes known to cause OI. We presented a case of suspected abuses in 2-month-old baby. MPS libraries were sequenced by Ion Torrent PGM platform; pathogenic variants and VUS (variants of uncertain significance) were confirmed by Sanger sequencing and familial segregation study was performed to better characterize the clinical significance of the mutation. This study remarks that MPS could help not only for identification, ancestry/phenotyping or molecular autopsy applications but also for forensic investigation over child abuse. The usefulness of this assay for diagnostic projects on victims of abuse together with post-mortem cases is discussed.

1. Introduction

OI is a rare collagen disease with a very wide range of phenotypes. OI types II–V are easier to diagnose clinically as they usually concern multiple, often prenatal fractures with deformation of bone and other specific features like blue sclerae, dentinogenesis imperfecta and hearing loss. OI type I could be difficult to clinically diagnose because individuals are frequently nearly asymptomatic with a mild predisposition to fractures, normal stature and normal lifespan as is observed in individuals with idiopathic juvenile osteoporosis. In these last cases, DNA helps clinics in diagnosing OI type I, and sometimes IV, that are important for differential diagnostic considerations in case of suspicion of non-accidental injury, so child abuse [1]. When there is a suspected abuse, forensics are involved in a team together with clinicians and psychologists. Therefore, legal medicine unit with DNA lab is crucial since it could early collect living or autopsy samples when a child abuse is suspected and then test DNA. In this study we set up a MPS panel useful to search for sequence variants in the genes involved in OI, and describe the early results of one forensic case of suspected child abuse.

2. Material studied, methods, techniques

A custom Ion targeted AmpliSeq™ On-Demand Panel was designed by the apposite web tool; it included the coding regions for 76 genes useful for some inherited rare diseases studied in our university hospital. Targeted analysis was performed by in-silico panel, filtering the 13 genes involved in OI: BMP1, COL1A1, COL1A2, CREB3L1, FKBP10, IFITM5, P3H1, PPIB, SERPINF1, SERPINH1, SP7, TMEM38B, WNT1. For the OI genes, the design resulted in 31,232 bases covering the coding regions (22 bases missed); the 2-primer pools PCR resulted in 274 amplicons ranged between 94 and 262 bp. Libraries were prepared using Ion Ampliseq Library kit 2.0 and samples were barcoded with Ion Xpress barcode Adapters; each sample libraries were pooled and submitted to emulsion PCR by Ion PGM Hi-Q View OT2 kit. The template-positive Ion PGM Hi-Q View ISPs were enriched on Ion One touch ES Instrument and sequenced on Ion Torrent PGM instrument by using Ion

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PGM Hi-Q View sequencing kit, 318 chip types and 200 base read mode. The data analysis was performed using a custom bioinformatic pipeline. Briefly, alignment of reads against human reference genome was performed on the Torrent Suite version 5.0.4, followed by variant calling of the variants to reference genome hg19. Variants annotation was performed by wANNOVAR and candidate variants were filtered by their function (intronic and synonymous variants excluded) and minor allele frequency (MAF) < 0,1% in public databases. Prioritization and interpretation were accomplished following the American College of Medical Genetics and Genomics (ACMG) guidelines [2] with the help of Varsome and InterVar tools as well as Alamut Visual 2.11 software. Rare variants confirmation and segregation were finally confirmed by Sanger sequencing: PCR products were checked by Agilent Bioanalyzer capillary electrophoresis, purified by EXO-CIP enzyme mix, bidirectionally sequenced by Big Dye Terminator 1.1 cycle sequencing kit. Sequencing product were revealed by 3130 Genetic Analyzer and analyzed by SeqScape 3.0 aligning the sequences to the reference genome hg19.

3. Results

Among the analyzed samples, three family studies and two isolated subjects were included, both clinical and forensic cases, here we describe the results of one forensic case. A 2-month-old baby presented to the emergency room with multiple head, thorax and arm and leg bones fractures. The parents referred only minimal accidental hits the days before and the court ordered investigations. An extensive medical work-up was done, and the hospital's multidisciplinary child abuse team, including the forensic expert, was consulted. The history, clinical findings and radiographic findings were not definitively consistent with child abuse. DNA test was then requested and a heterozygote missense mutation was observed by MPS, then confirmed by Sanger, in COL1A1 (collagen type I alpha 1 chain) gene, c.2735 G > A (p.Glu912Gly). The variant, never described in literature, was interpreted as VUS (variant of uncertain significance) for the following features:

-the variant is located in a critical and well-established functional domain with few benign variation (UniProt protein CO1A1_HUMAN region of interest 'Triple-helical region' has 350 classified pathogenic, 87%, variants;

-the variant is absent from controls in the main databases (Exome Sequencing Project, 1000 Genomes Project, Exome Aggregation Consortium Variant and GnomAD exomes or genomes despite the good coverage);

-multiple lines of computational evidence support a deleterious effect on the gene or gene product according to conservation, evolutionary, splicing impact (pathogenic computational verdict because 7 pathogenic predictions from DANN, FATHMM, LRT, MutationTaster, PROVEAN, FATHMM-MKL and SIFT versus 1 benign prediction from MutationAssessor; CADD phred = 25.2).

In order to reveal additional information to benign or pathogenic effect of the mutation we conducted a segregation analysis in the family. We observed the mutation in the child's father, that referred to suffered of bone fragility in the youth, and in the grandmother. However, she reported that she did not remember about fractures in her childhood/youth. Finally, functional study has been planned to definitively confirm the effect of the variant on the collagen.

Since the uncertain clinical significance of the mutation for osteogenesis imperfecta and no other child abuse evidence, the court exonerated the parents from imputation.

4. Discussion

The test showed a high analytical sensitivity, with a high sequencing uniformity of coverage along the 13 genes. Clinical sensitivity can be dependent on variable factors such as age or family history but genomic DNA sequencing of the known genes should identify causative variants in more than 95% of individuals with clinically confirmed OI in most populations [3]. On the contrary, when a child abuse is suspected, the frequency of expected positive test (a pathogenetic variant is observed) should decrease to about 5% [4]. This data suggest to better evaluate clinical evidences and perform the DNA test only if blue sclera, osteopenia, and/or a positive family history is present. However, when the clinic signs are uncertain, genetic test and MPS could help, especially now that MPS is cheaper and available in many forensic laboratories.

The major limit we met was the further classification of the uncertain variants. In order to classify unknown non-synonymous sequence variation as pathogenetic, co-segregation and functional analysis are required. Protein studies and mRNA/cDNA analysis isolated from cultured fibroblasts can explain the function of variants, for example those suspected to alter splicing [3]. Checking for a new status of the VUS is planned once a year and a re-classification is reported, if necessary [5].

In this view, our MPS approach become a useful tool for diagnostic projects on victims of abuse in hospitals and has the advantage to be early applied in autopsy cases when the deceased child is suspected to be victim of abuse.

5. Conclusion

Child abuse is a major public health concern that can explain a proportion of fractures in children. The osteogenesis imperfecta is the most common inherited syndrome that predisposes to bone fractures. A detailed clinical evaluation is sufficient in most cases and the genetics helps the uncertain diagnosis.

We described a robust MPS method to reveal sequence variants in the main genes involved in this disease. This approach could help to confirm that a bone pathology is present in the infant, so absolving parents from the crime. However, we could also get a not resolutive result when a variant of uncertain significance is observed, as in the case we described. In this case the definitive role must be reached in segregation and in complex functional studies.

Declaration of Competing Interest

None.

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