Mutation analysis and prenatal diagnosis of a Chinese family with Fanconianemia

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Abstract

Fanconi anemia (FA) is a rare genetic blood disorder. Because there are at least 15 genetic subtypes that are associated with its development, and large deletions, duplications, or sequence variations are frequently found in some of these genes, customary genetic testing for FA is complicated and time-consuming. Therefore, we used the diagnostic result of a Chinese boy with Fanconi anemia by the next generation sequencing (NGS) as the reference, we confirmed the mutations in him, his father and pregnant mother using Sanger sequencing technique and performed the prenatal diagnosis to the fetus. A heterozygous nucleotide deletion c.989_995del7 (p.H330LfsX2) and nucleotide substitution c.3971C>T (p.P1324L) in \textit{FANCA} gene were detected in the patient. Further analysis confirmed that the p.H330LfsX2 was derived from his mother and the p.P1324L from his father. As for the fetus, both of the same parental mutations were detected in amniotic fluid and cultured amniotic fluid. Our result shows that NGS combined with Sanger sequencing greatly improve the speed of prenatal diagnosis of FA.

Key words

Prenatal diagnosis, Next generation sequencing, \textit{FANCA}, Fanconi anemia

1 Introduction

Fanconi anemia (FA) is an inherited disorder characterized by diverse defects, including progressive pancytopenia, hyperpigmentation, skeletal malformations, small stature, hematologic malignancies and solid tumor \cite{1}. Currently, FA shows a genetic heterogeneity, using cell fusion and complementation analysis, with 15 defined genes corresponding for FA complementation groups: \textit{FANCA}, \textit{FANCB}, \textit{FANCC}, \textit{FANCD1}, \textit{FANCD2}, \textit{FANCE}, \textit{FANCE}, \textit{FANCF}, \textit{FANCG}, \textit{FANCI}, \textit{BRIP1}, \textit{FANCL}, \textit{FANCM}, \textit{PALB2}, \textit{RAD51C}, \textit{SLX4} \cite{2}. Fourteen of above genes are autosomal recessively inherited while \textit{FANCB} is inherited in an X-linked manner.

Because 15 subtypes genes are linked to FA, and there is a variety of mutant forms in each gene \cite{3}, such as large deletions or duplications, missense and frameshift mutations, it is a challenge to make molecular diagnosis. The Sanger sequencing approach we currently used for diagnosis of FA is time-consuming and costly, especially for pregnant women with a classification unknown FA proband. The rapid and accurate prenatal diagnosis will provide a chance to parents to have a
healthy neonate. We described here a successful prenatal diagnosis to a Chinese pregnant woman with a FA proband by combining the next-generation sequencing and regular DNA sequencing approaches.

2 Materials and methods

2.1 Patients
A male patient, 4 years old, has been diagnosed of FA for one year. The patient was born to a healthy non-consanguineous family with 40 gestational weeks. The patient’s height was 112.2cm (p50), and weight was 20kg with unremarkable physical urological examination. His initial results of blood cell count were as follows: white blood cell 2.73×10⁹/L, hemoglobin 58g/L, platelets 20×10⁹/L. Bone marrow examination showed bone marrow failure. No family members had similar symptoms to the patient. At the time, the patient's mother was 14 weeks pregnant.

2.2 Genomic DNA preparation
Informed consent for the study was obtained from the patient’s parents. Peripheral venous blood from the proband and his parents was collected in tubes containing EDTA. Amniotic puncture was performed at 17th week of pregnancy according to the requirement of prenatal diagnosis. Genomic DNA from peripheral blood leukocytes, amniotic fluid cell and cultured amniotic fluid was extracted by TIANGEN RelaxGene Blood DNA System.

2.3 DNA sequencing analysis
Patient’s next-generation sequencing was performed by Shanghai Genesky Biotechnology Co., Ltd. We used software [4-6] to check the mutations in all FA genes and a low-frequency frameshifts and a truncating mutation in FANCA gene were considered as pathogenic compound mutations.

2.4 PCR amplification and Sanger sequencing
According to the results of next-generation sequencing, potential mutations were verified by Sanger sequencing. Reaction mixtures included 25μlDreamTaq™ Green PCR Master MIX, 2μl (5μM) of each primer (see Table 1), 2μl DNA and 19μl nuclease-free water. The amplification was carried out as follows: denaturation at 95°C for 5 min, followed by 33 cycles of denaturation at 95°C for 40 s, annealing at 58°C for 40 s, extension at 72°C for 50 s, and a final extension at 72°C for 10 min.

Table 1. Primers for Amplifying Sequences of FANCA

<table>
<thead>
<tr>
<th>Exon</th>
<th>Sense primer</th>
<th>Anti-sense primer</th>
</tr>
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<tbody>
<tr>
<td>FANCA-11</td>
<td>CACAGTTTGTGCTGGGATTG</td>
<td>GGACCCAGTCTCTGGTCAA</td>
</tr>
<tr>
<td>FANCA-40</td>
<td>ATGTCCAGAGTTGCTGAGC</td>
<td>GGTGATGCGAAGGGATACTG</td>
</tr>
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</table>

3 Results

3.1 Identification of FANCA gene mutations in patient
Two potential heterogeneous mutations were found in the FANCA gene from the patient by Sanger sequencing those were consistent with the found by the next-generation sequencing: one mutation has been reported (HGMD) and the other was a small fragment deletion [7]. The reported mutation was c.3971C>T (p.P1324L) in exon 40 (see Figure 1), and the frameshift mutation was c.989_995del7 (p.H330LfsX2), in exon 11 (see Figure 2). The patient’s father carried a
heterozygous c.3971C>T (p. P1324L) mutation and the mother had a heterozygous c.989_995del7 (p.H330LfsX2) mutation.

3.2 Prenatal diagnosis
DNA sequencing confirmed the fetus had two heterozygous mutations c.3971C>T (p.P1324L) (see Figure 1) and c.989_995del7 (p.H330LfsX2) (see Figure 2) in the FANCA gene. The p.P1324L was inherited from father and p.H330LfsX2 from mother.

![Figure 1. Sequencing results of the FANCA gene show p. P1324L](image1)

![Figure 2. Sequencing results of the FANCA gene show p. H330LfsX2](image2)

4 Discussion
FA is usually diagnosed according to clinical presentations and chromosome breakage test. The chromosome breakage testing is used widely in detection of amniotic cells, chorionic epithelium cells and fetal blood cells, so it can be used for prenatal diagnosis [8]. Though chromosome breakage test is highly specific to the diagnosis of FA, it sometimes gives in false-negative results, similar to those observed in cases of somatic mosaicism patients [9]. Therefore, molecular testing is a preferred method for FA diagnosis [2].

The fifteen genetic subtypes of FA have been distinguished as FA-A, -B, -C, -D1, -D2, -E, -F, -G, -I, -J, -L, -M, -N, -O, and -P. The majority of patients belong to the subtypes A (60%-70%), C (10-15%), or G (10%), and the prevalence of the remaining 12 subtypes is between 1% to 5% [10,11]. There are variable mutational sites covering the entire FA gene, so it is
a challenge to genetic diagnosis, especially for a pregnant mother when the proband’s molecular diagnosis is unclear. The molecular diagnosis of FA usually uses western blot analysis, immunoprecipitation, RT-PCR or whole FA gene exons sequencing combined with MLPA \[10, 12\]. These methods can effectively detect protein expression, point mutations and large fragment deletion/duplications, but it often requires several methods used in combination. Therefore, the diagnosis of FA becomes complicated and time-consuming. The large amounts of sequence-data output pose a bioinformatics challenge for the clinical laboratory if the patient is etiology unknown or unclassified\[13\].

In this study, when the proband’s mother came to our hospital she was already 14 weeks pregnant, so prenatal diagnosis was needed urgently. Disease like FA with severe genetics heterogeneity, it is difficult to identify all the diverse mutations in a short period of time by traditional DNA analysis. So we considered NGS as a screening method and used the result of the patient tested by this method as reference, we first checked the FA subtype gene mutations in a large number of target genes and then verified potential mutations in the patient and his parents by Sanger sequencing. Compared to the current applications of NGS in FA \[7, 14, 15\], we combined the NGS and Sanger sequencing to speed up the processing of molecular diagnosis. The total turn round time of the diagnosis of FA was reduced from regular 4 weeks to 2 weeks that provide enough time to the pregnant mother to make decision to her fetus. The parents made decision of abortion according to the positive result, unfortunately we could not obtain the aborted fetus due to most traditional Chinese would not let their loss was disturbed. Otherwise, we should verified the mutations in fetus to clarify our prenatal diagnosis.

In conclusion, using the next-generation sequencing combined with Sanger sequencing can reduce the time of sequencing and can quickly find abnormal mutational sites. It greatly improves the detection speed and provides the reliability of prenatal diagnosis.

Conflict of interest
All authors declare no conflict of interest.

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References


