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Letter to the Editors-in-Chief

Whole-exome sequencing identified novel mutations in *FGA* and *FGG* genes in the patients with decreased fibrinogen

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1. Introduction

Fibrinogen plays a major role in clot formation through conversion into insoluble fibrin assisted by thrombin (activated factor II). Congenital fibrinogen deficiency is a group of rare coagulation disorders characterized by lower level of fibrinogen. The compromised fibrinogen may be introduced by qualitative or quantitative genetic changes, referred as dysfibrinogenemia and hypodysfibriogenemia or hypofibrinogenemia and afibriogenemia, respectively [1]. Patients with congenital dysfibrinogenemia are presented as bleeding including umbilical cord hemorrhage, epistaxis, gum bleeding, menorrhagia, gastrointestinal bleeding. Individuals with hypofibrinogenemia are mostly asymptomatic and carry heterozygous causative mutations. In symptomatic patients, bleeding phenotype is usually related to trauma, surgery, or concomitant coagulopathies, and thrombotic phenotypes are associated with fibrinogen beta chain variants [2]. More than 300 DNA mutations in fibrinogen gamma (*FGG*), fibrinogen alpha (*FGA*), and fibrinogen beta (*FGB*) genes have been found to be associated with dysfibrinogenemia, afibrinogenemia and related diseases [3]. Most of the studies employed traditional Sanger sequencing to identify those genetic changes in the exons of *FGA*, *FGB*, and *FGG*. Recent years, whole-exome sequencing (WES) technology is widely used in the dissection of genetic causes of diseases. As the cost of performing WES is significantly decreased, WES might be a technology with high cost-effective performance used in the future for identifying the mutations of congenital fibrinogen disorders.

2. Patients and methods

Of the three female patients studied in present work, two were pregnant (Patient 1 and 2) and visited our Obstetrics Department for coagulation evaluation before delivery. The third patient (Patient 3) was an outpatient from the Infertility Center of Beijing Obstetrics and Gynecology Hospital. The patients' demographic information and clinical presentations were summarized in Supplementary Table 1. The same basic coagulation panel tests were ordered for all three patients by their physicians.

The coagulation tests including prothrombin time (PT), D-dimer, thrombin time (TT), activated partial thromboplastin time (APTT) and fibrinogen (Fib), were performed on the Sysmex CS 5100 automatic coagulation analyzers. Fibrinogen activity was determined by both of the Clauss and PT-derived methods (Siemens, catalog No. B4233-27, Marburg, Germany). The fibrinogen antigen level was determined with the immunoturbidimetric assay kit manufactured by Medical System (Fibrinogen Kit, Ningbo, China).

This study was approved by the Ethics Committee of Beijing Obstetrics and Gynecology Hospital. Written informed consent was obtained from each patient, and then 5 mL of peripheral blood was collected from each of the patient for genomic DNA extraction and sequencing analysis. The genomic DNA was extracted from the whole blood using a DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA, USA). The exome of each patient was captured using a SureSelect Human All Exon V6 kit (Agilent Technologies). The enriched sequence was subjected to whole-exome sequencing using the HiSeq X10 Sequencer (Illumina). The raw reads were aligned to the hg19 reference genome with Burrows–Wheeler Aligner. Variants were called and filtered using the Genome Analysis Toolkit (GATK) software package. Functional annotation of variants were performed using the ANNOVAR software. Sanger sequencing was used to validate the mutations in the *FGA* and *FGG* genes in the patients. *In silico* analysis was performed by using the online prediction tools including PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/), SIFT (http://sift.jcvi.org/www/SIFT_enst_submit.html), PROVEAN (http://provean.jcvi.org/index.php), MutationTaster (http://www.mutationtaster.org/), SNPs&GO (http://snps.biofold.org/snps-and-go/snps-and-go.html), and PhD-SNP (http://snps.biofold.org/phd-snp/phd-snp.html).

3. Results

The coagulation testing results were summarized in Table 1 and they were essentially normal except for D-dimers, fibrinogen and thrombin time. The patient's D-dimer was elevated due to a physiological change of normal pregnancy in the third trimester. The fibrinogen levels (Clauss) for all three patients were profoundly decreased, along with elongated thrombin time as expected. No obvious clot was identified with any of the citrated plasma samples of these patients and the preanalytical errors were excluded. Interestingly, compared with the

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Table 1
Coagulation testing results.

Test	P1	P2	P3	Reference interval
PT	12.5	12.9	13.5	10.0–14.0 s
INR	1.09	1.13	1.14	0.800–1.500
TT	24.6	29	23.4	14.0–21.0 s
APTT	23.6	26.4	31.7	22.80–35.00 s
D-dimer	0.94	0.78	0.19	0.00–0.55 mg/L
FIB (Clauss)	0.85	0.96	0.7	1.80–4.00 g/L
FIB (PT-derived)	3.9	4.51	1.52	1.8–4.00 g/L
FIB (antigen)	3.45	2.08	ND	2.00–4.00 g/L

FIB, fibrinogen; PT, prothrombin time; INR, international normalized ratio; TT, thrombin time; APTT, activated partial thromboplastin time; ND, not determined.

fibrinogen (Clauss) results, the fibrinogen (PT-derived) which partially represents fibrinogen antigen levels [4], was slightly reduced for patient 3 (P3) but within the normal reference range for patients 1 (P1) and 2 (P2). To further confirm above results, the fibrinogen antigen levels were directly measured for P1 and P2 with an immunoturbidimetric assay that was described in the Patients and Methods section. As shown in Table 1, the fibrinogen antigen levels were within normal limits in P1 and P2. Unfortunately, due to the consent disagreement, the same fibrinogen antigen immunoassay was not performed with the sample of P3.

The common conditions that lead to decreased fibrinogen level include fibrinolysis, cirrhosis, hepatitis, disseminated intravascular coagulation and congenital fibrinogen deficiency. With normal liver functions (data not shown) for all three subjects, genetic variations were queried with the WES technique. We focused on the genetic variations in the three fibrinogen genes (*FGA*, *FGB*, and *FGG*). After filtering out the variants located in the non-coding regions (except splicing variants) and the variants with allele frequencies more than 1% in databases including Exome Aggregation Consortium (http://exac.broadinstitute.org/), 1000 Genomes (1000G, http://browser.1000genomes.org/index.html), Exome Variant Server (ESP6500, http://evs.gs.washington.edu/EVS/) and Genome Aggregation database (gnomAD, http://gnomad.broadinstitute.org/), we found that P1 carried a novel *FGA* mutation (FGA:NM_000508:exon2:c.A112T:p.R38W), P2 harbored a known *FGG* mutation (FGG:NM_000509:exon8:c.G902A:p.R301H), and P3 carried a novel *FGG* mutation (FGG:NM_000509:exon9:c.G1202T:p.R401L). These three mutations were heterozygous and have been confirmed by Sanger sequencing (Fig. 1A–C).

In silico analysis predicted that the three mutations are most likely disease-causing mutations as suggested by the six online prediction tools (Polyphen-2, SIFT, PROVEAN, Mutation Taster, SNPs&GO and PhD-SNP) employed in present study (Supplementary Table 2). The two novel mutations (*FGA* c.112A > T and *FGG* c.1202G > T) are rare mutations that were absent from both the ExAC and 1000 Genomes databases (Supplementary Table 2). These three mutations were also 100% conserved from zebrafish to human (Fig. 1D and E), suggesting the critical and functional roles of these three sites.

4. Discussion

Here we report three mutations identified by WES in the *FGA* or *FGG* genes in three patients with congenital fibrinogen deficiencies. Two of the three mutations are novel. All of the three mutations are conserved and are strongly predicted to be pathogenic mutations, which are consistent with their reduced fibrinogen activity (Clauss) results in Table 1.

The p.R301H (c.902G > A) mutation in *FGG* exon 8 of P2 has been reported as causative mutation of dysfibrinogenemia. *FGG* Arg301 is one of the mutation hotspots of congenital dysfibrinogenemia (CD). For instance, the Arg301His mutation was reported to account for up to 18.9% of all dysfibrinogenemia patients [5]. With these mutations, functionally impaired fibrinogen fails to form normal coagulating clot, resulting in coagulating mechanism related disorders. Dysfibrinogenemia clinical manifestations vary from asymptomatic to abnormal bleeding or arteriovenous thrombosis. Even asymptomatic patients could suffer from bleeding or thrombosis events with injury, surgery or delivery procedures. Pregnant women with CD presented with relative higher risk of miscarriage, postpartum hemorrhage than the normal [5]. Two main mechanisms has been proposed to explain the development of thrombosis with dysfibrinogenemia: (i) increased thrombin level due to impaired fibrinogen-thrombin binding ability (ii) decreased t-PA mediated fibrinolysis as a consequence of molecular changed fibrin [6].

The p.R38W (c.112A > T) mutation in *FGA* of P1 is novel and has been predicted as pathogenic mutation in present work. The R38 site and its flanking regions have been found to be mutated for several times in the patients with congenital dysfibrinogenemia, such as R38G, R36D, R35H, R35S, R35C. The mutations on the R38 and its neighboring sequences were postulated to be associated with defective fibrinopeptide cleavage or impaired thrombin binding [7]. With reduced level of functional fibrinogen (Clauss) and normal antigen level , the diagnosis of dysfibrinogenemia can be made for P1. It is the first time to report the p.R38W (c.112A > T) mutation in *FGA* as a pathogenic mutation of CD.

The p.R401L (c.1202G > T) in *FGG* of P3 is also a novel mutation. As on the same site another mutation of p.R401W has been reported to cause hypofibrinogenemia [8], the defect by R401L might be similar to that of R401W, which introduced a conformational change and abnormal aggregation within the endoplasmic reticulum [8]. Since we were not able to perform the fibrinogen antigen assay for P3, the definitive diagnosis of her fibrinogen deficiency could not be reached at this point.

As fibrinogen antigen assay is not routinely available in most clinical laboratories, whether PT-derived fibrinogen correlated with the fibrinogen level directly measured by immunoassay became an interesting question. In theory, as the recommended screening test by the National Committee for Clinical Laboratory Standards, the Clauss method employs excessive thrombin to determine the concentration of functional fibrinogen. In contrast, the PT-derived method is an indirect estimation of fibrinogen antigen concentration by measuring plasma turbidity introduced by its converted product fibrin. Although it was controversial that if the PT-derived method could be applied as a routine screening method for different patient groups, more recent studies supported that the PT-derived method correlated well with the fibrinogen antigen concentration determined with immunoassays. In current work, all three patients presented with decreased fibrinogen by the Clauss method. Both P1 and P2 had normal fibrinogen levels by the PT-derived method, which were consistent with those determined by immunoassay. According to the laboratory characteristics of P1 and P2, dysfibrinogenemia should be diagnosed [9]. Genetically, P2 harbored a previously reported mutation (p.R301H, c.902G > A) leading to dysfibrinogenemia; P1 was identified with a novel mutation (p.R38W, c.112A > T) that is predicted to introduce dysfibrinogenemia. P3 displayed proportional reduced fibrinogen with both Clauss and PT-derived methods, suggesting quantitatively compromised fibrinogen production. Therefore, even without direct fibrinogen immunoassay for P3, the coagulation study results favored the diagnosis of hypofibrinogenemia [9].

High-throughput sequencing is now widely used in clinical diagnosis and genetic studies. However, by searching the literature we found only one published study using WES to identify the genetic causes of congenital dysfibrinogenemia [10]. Nowadays the financial cost for WES is significantly reduced, and is even comparable to that of traditional Sanger sequencing. As the patients with congenital fibrinogen deficiencies could harbor unknown mutations in the *FGA*, *FGB*, and *FGG* genes, WES may provide a powerful tool identifying

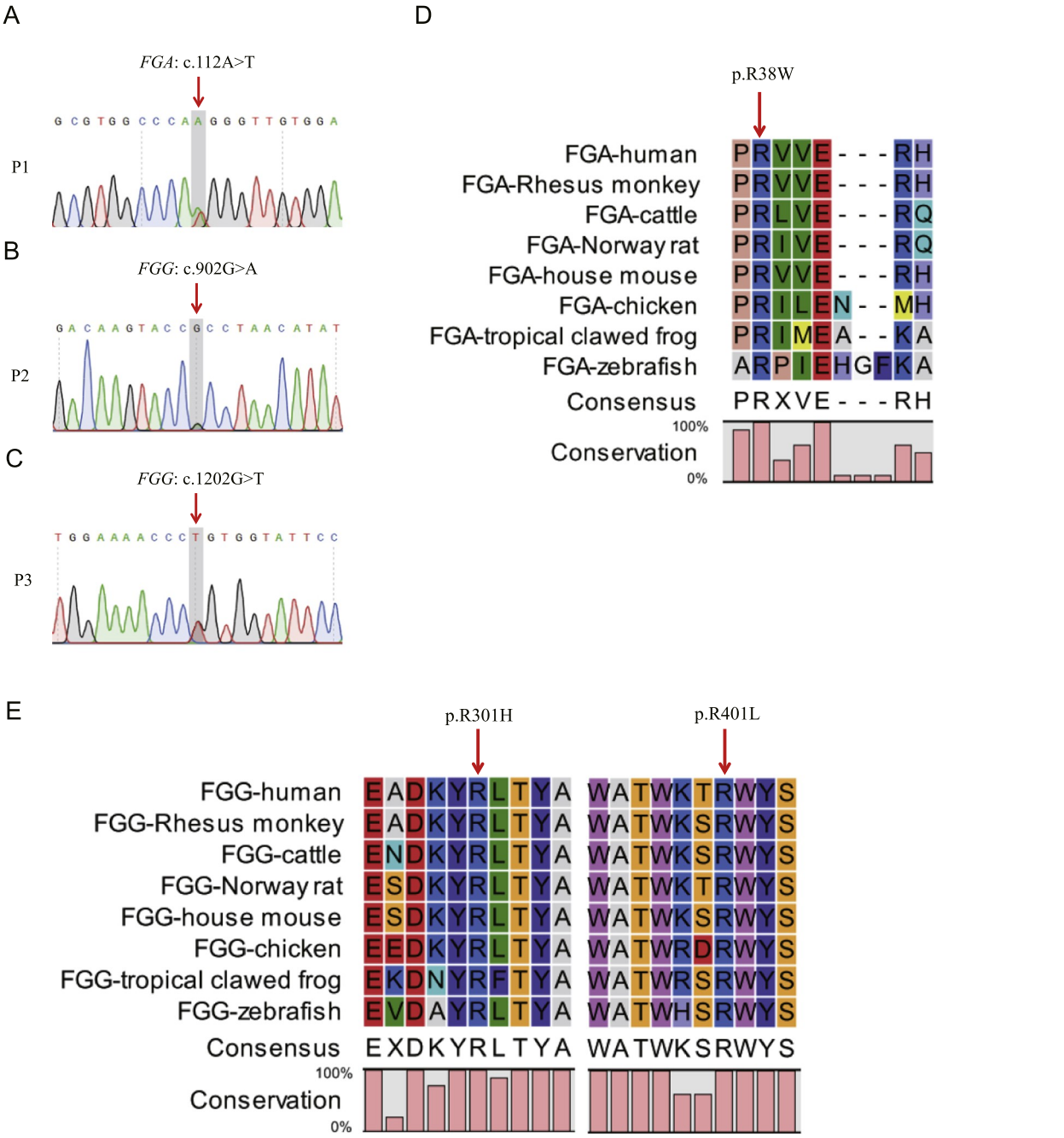

Fig. 1. Sanger sequencing validation of the results from whole-exome sequencing, and Alignment of the mutational sites. (A) Sanger sequencing confirmed the *FGA* mutation in patient 1 (P1). P1 carried a heterozygous *FGA* mutation (c.112A > T). The red arrow points to the mutation site. (B) Sanger sequencing confirmed the *FGG* mutation in patient 2 (P2). P2 carried a heterozygous *FGG* mutation (c.902G > A). The red arrow points to the mutation site. (C) Sanger sequencing confirmed the *FGG* mutation in patient 3 (P3). P3 carried a heterozygous *FGG* mutation (c.1202G > T). The red arrow points to the mutation site. (D) Alignment of FGA proteins in different species. The R38 site of human FGA was highly conserved in the aligned sequences. (E) Alignment of FGG proteins in different species. The R301 and R401 sites of human FGA were highly conserved in the aligned sequences. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

novel mutations in combination with the traditional Sanger sequencing.

In conclusion, our study by WES identified two novel mutations in *FGA* or *FGG* genes, which may be associated with congenital dysfibrinogenemia and hypofibrinogenemia respectively. Their reduced fibrinogen function (Clauss) levels could be introduced by the polymerization deficiency of FGA or FGG erroneous aggregation in the endoplasmic reticulum. The above results suggested that WES is also a powerful tool to dissect the molecular causes of congenital fibrinogen deficiency.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.thromres.2019.03.002.

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Conflicts of interests

The authors declare no conflict of interest.

References

[1] P. de Moerloose, A. Casini, M. Neerman-Arbez, Congenital fibrinogen disorders: an update, Semin. Thromb. Hemost. 39 (2013) 585–595.

[2] M. Neerman-Arbez, A. Casini, Clinical consequences and molecular bases of low fibrinogen levels, Int. J. Mol. Sci. 19 (2018) 192.

[3] Casini A, Blondon M, Tintillier V, Goodyer M, Sezgin ME, Gunes AM, et al. Mutational epidemiology of congenital fibrinogen disorders. Thromb. Haemost. 2018;118:1867–74.

[4] W. Miesbach, J. Schenk, S. Alesci, E. Lindhoff-Last, Comparison of the fibrinogen Clauss assay and the fibrinogen PT derived method in patients with dysfibrinogenemia, Thromb. Res. 126 (2010) e428–e433.

[5] Alessandro C, Marc B, Aurélien L, Jérémie K, Véronique T, Emmanuel DM, et al. Natural history of patients with congenital dysfibrinogenemia. Blood. 2015;125:553–61.

[6] F. Haverkate, M. Samama, Familial dysfibrinogenemia and thrombophilia. Report on a study of the SSC subcommittee on fibrinogen, Thromb. Haemost. 73 (1995) 151–161.

[7] Sugo, T., Endo, H., Matsuda, M., Ohmori, T., Madoiwa, S., Mimuro, J., et al. A classification of the fibrin network structures formed from the hereditary dysfibrinogens. Journal of Thrombosis & Haemostasis. 2010;4:1738–46.

[8] S.O. Brennan, G. Maghzal, B.L. Shneider, R. Gordon, M.S. Magid, P.M. George, Novel fibrinogen gamma375 Arg– > Trp mutation (fibrinogen Aguadilla) causes hepatic endoplasmic reticulum storage and hypofibrinogenemia, Hepatology 36 (2002) 652–658.

[9] A. Casini, A. Undas, R. Palla, J. Thachil, P. de Moerloose, Diagnosis and classification of congenital fibrinogen disorders: communication from the SSC of the ISTH, J. Thromb. Haemost. 16 (2018) 1887–1890.

[10] J. Zhou, P. Zhu, X. Zhang, A Chinese family with congenital dysfibrinogenemia carries a heterozygous missense mutation in FGA: concerning the genetic abnormality and clinical treatment, Pak J Med Sci 33 (2017) 968–972.

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