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Phenotypic and genotypic correlation evaluation of 148 pediatric patients with Fanconi anemia in a Chinese rare disease cohort

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ABSTRACT

Background: Fanconi anemia (FA) is a rare autosomal recessive, X-linked or autosomal dominant disease. Few large-scale FA investigations of rare disease cohorts have been conducted in China.
Methods: We enrolled 148 patients diagnosed with FA according to evidence from the clinical phenotype, family history, and a set of laboratory tests. Next, the clinical manifestations and correlation between the genotype and phenotype of FA pediatric cases were investigated.
Results: The most common FA subtype in our cohort was FA-A (51.4 %), followed by FA-D2 and FA-P. Finger (26 %) and skin (25 %) deformities were the most common malformations. Based on family history, blood system diseases (51 %) had the highest incidence rate, followed by digestive system tumours. A set of new or prognosis-related mutation sites was identified. For example, c.2941 T > G was a new most common missense mutation site for *FANCA*. *FANCP* gene mutation sites were mainly concentrated in exons 12/14/15. The mutations of FANCI/FANCD2 were mainly located at the α helix and β corners of the protein complex. FA-A/D1 patients with splicing or deletion mutations showed more severe disease than those with missense mutations. Chromosome 1/3/7/8 abnormalities were closely linked to the progression of FA to leukemia.
Conclusion: Our study investigated the clinical features and genotype/phenotype correlation of 148 Chinese pediatric FA patients, providing new insight into FA.

1. Introduction

As a rare autosomal recessive, X-linked or autosomal dominant disease, Fanconi anemia (FA) is the most common congenital bone marrow failure syndrome in children [1–5]. The main clinical manifestations of FA patients are congenital abnormalities, progressive bone marrow failure and a high risk of acute myeloid leukemia (AML) or other solid tumours [6–11]. To date, twenty-three DNA repair genes, such as *FANCA*, *FANCB*, *FANCC*, *BRCA2*, and *FANCD2*, have been identified in the FA-related signalling pathway [1–5,12]. Genetic mutations in these genes contribute to the occurrence or pathogenesis of Fanconi anemia [1–5,12,13]. Increasing evidence supports the links between the FA signalling pathway and tumorigenesis [6–8,10,13].

Characterizing FA patient cohorts and exploring their genotype/phenotype links are warranted?. An increasing number of investigations targeting FA cohorts at different geographical locations have been reported [14–25]. In 2019, Kesici, S. et al. enrolled 175 FA cases at a single centre in Turkey to analyse the clinical and prognostic characteristics of FA, but the pathogenic mutation features were not involved [16]. Previously, we enrolled five Chinese pediatric patients for whole-exome sequencing and reported multiple heterozygous FA gene mutations [15]. Very recently, Altintas, B. et al. included 203 FA

Abbreviations: FA, Fanconi anemia; AML, acute myeloid leukemia; MDS, myelodysplastic syndromes; MMC, mitomycin C; SCGE, single-cell gel electrophoresis; MLPA, multiplex ligation-dependent probe amplification; WES, whole-exome sequencing; VACTERL-H, verbal, anal, cardiac, tracheo-oesophageal fistula, oesophageal atresia, renal, upper limb, and hydrocephalus; PHENOS, skin pigmentation, small head, small eyes, nervous system, otology, and short stature; RAEB, refractory anemia with excess blasts.

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patients in a predominantly white population from a National Cancer Institute cohort to evaluate the links between genotype-phenotype and outcomes [17]. However, to our best knowledge, investigations are lacking on the comprehensive genotype/phenotype correlation of FA based on a relatively large-scale Chinese rare disease cohort.

In the present study, we explored the clinical characteristics of 148 Chinese FA cases. We analysed the links between genotype and phenotype in 105 cases with FA genotyping results and the genetic mutations in patients who progressed to myelodysplastic syndromes (MDS) or AML.

2. Materials and methods

2.1. Patients

In total, 148 FA patients from 140 families (2003.10–2021.10) were diagnosed according to family history, clinical phenotype, and clinical measures, including the mitomycin C (MMC) induction chromosome breakage test, single-cell gel electrophoresis (SCGE), targeted sequencing, multiplex ligation-dependent probe amplification (MLPA) and whole-exome sequencing (WES). Our cases were enrolled from 23 provinces, cities or autonomous regions of China. We used Tableau software (Pro Edition 2018.2.0) to visualize the distribution characteristics of FA patients in different regions of China. Because no genetic test data were obtained for the patients before 2010, only 105 patients after 2010 (including five previously reported cases) [15] obtained gene sequencing results for genotyping. Our study was reviewed and approved by the Clinical Research Ethics Committee of Blood Diseases Hospital & Institute of Hematology, Chinese Academy of Medical Sciences [IIT2021010-EC-2]. Because our study was retrospective, we could not obtain the application for informed consent.

2.2. Chromosome breakage assay

We conducted an MMC-induced chromosome breakage assay to assess the DNA damage repair status, as described previously [26,27]. After adding MMC at different concentrations (0 ng/ml, 40 ng/ml and 80 ng/ml), the cells were cultured for 48 h and harvested for prefixation, hypoosmosis and sectionalization. The aberration rate was then observed by ordinary light microscopy.

2.3. SCGE

We also measured DNA damage repair defects using single-cell gel electrophoresis (SCGE), as described previously [26,27]. Briefly, mononuclear cells were isolated from the peripheral blood or bone marrow. After boiling, 100 μl of 7.5 g/L agarose gel was quickly and evenly spread on the microelectrophoresis board and incubated in a refrigerator at 4 °C for 1 min. Next, 25 μl of leukocyte suspension and 75 μl of 7.5 g/L low melting point agarose gel were mixed and evenly spread on the first layer of gel. The freshly prepared lysate was added to the gel and lysed at 4 °C for 1 h. After electrophoresis, the cells were stained with ethidium bromide and observed using a fluorescence microscope. A total of 100 cells were calculated for the proportion of positive cells in the comet tail. More than 100 cell electrophoresis images were collected using CASP (comet assay software project) system analysis, and the comet cell rate was calculated. Two times higher than the normal control was considered positive.

2.4. Targeted sequencing

Targeted sequencing analysis was performed to identify the FA-related gene mutation sites of patients. A targeted capture sequencing assay was designed to detect 417 blood disease genes, including FA-related genes. A biotinylated capture probe (MyGenostics, Baltimore, MD, USA) was used to enrich the targeted genes. Next, the specific mutation sites were confirmed through Sanger sequencing [28].

2.5. MLPA

As reported previously [29], we performed multiplex ligation-dependent probe amplification (MLPA) to detect large fragment deletions in patients with clinical manifestations and positive results in the MMC-induced chromosome breakage assay but not in the targeted sequencing analysis.

2.6. WES

We performed whole-exome sequencing (WES) analysis of five patients and their parents [15]. Briefly, we amplified the extracted DNA using ligation-mediated PCR, which was then hybridized to the SureSelect Biotinylated RNA Library (Agilent). A HiSeq2000 Platform was used to load each captured library.

2.7. Mutation site visualization

To visualize the locations of mutations within the three-dimensional structure of several FA proteins, we obtained the crystal structures (6VAD) for FANCD2 (Q9BXW9) and FANCI (Q9NVI1) from the protein data bank database (https://www.rcsb.org/) and the predicted protein structure (AF-Q8IY92-F1) for FANCP (Q8IY92) from the alphafold protein structure database (https://alphafold.ebi.ac.uk/). PyMOL software was then used to visualize specific sites of mutation.

3. Results

3.1. Clinical characteristics

A total of 148 FA patients from 23 provinces, cities or autonomous regions of China (2003.10–2021.10) were included in our rare disease cohort. The distribution characteristics of FA patients in different regions of China (Fig. 1) showed that our participants were primarily from Shandong Province and Hebei Province near our hospital.

Our cohort comprised 84 men and 64 women. The median age of onset was four years (0 ~ 18 years). Finger and skin deformities were the most common malformations (Fig. 2A), accounting for 26 % and 25 %, respectively. The detailed malformations of different systems are shown in Table S1. Two classical congenital abnormalities described in the VACTERL-H (verbal, anal, cardiac, tracheo-oesophageal fistula, oesophageal atresia, renal, upper limb, and hydrocephalus) association and PHENOS (skin pigmentation, small head, small eyes, nervous system, otology, and short stature) [30,31] were detected in 94.7 % (215/227) of FA patients. In 148 patients, VACTERL-H malformation accounted for 35.6 % (81/227) of all malformations, including 79 % (64/81) of upper limb malformations, 71.6 % (58/81) of finger deformities, and 7.4 % (6/81) of thenar muscular dysplasia, followed by 9.9 % (8/81) of cardiac deformities, 7.4 % (6/81) of renal deformities, and 3.7 % of vertebral deformities. The proportion of patients with VACTERL-H 2/8 features was 3.4 %, and that with 3/8 features was 0.7 %, less than the data previously reported in Europe [32]. There was on anal, tracheo-oesophageal fistula, oesophageal atresia, or hydrocephalus malformation. We also found an ocular macular absence malformation, which was not reported previously. Additionally, finger deformities were detected in 41 % (43/105) of patients with mutated FA genes. Patients with FANCA mutations accounted for 79.1 % (34/43) of finger deformities, and patients without FANCA mutations accounted for 20.9 % (9/43).

Amongst the 148 FA cases, PHENOS symptoms accounted for 59 % (134/227), amongst which skin pigmentation was the most common malformation (42.5 %, 57/134), followed by small eye (18.7 %, 25/134), microcephaly (17.9 %, 24/134), short stature (17.2 %, 23/134) and ear deformity (3.7 %, 5/134). The FA cases with 3/6 PHENOS symptoms accounted for 8.8 %; 4/6 PHENOS symptoms accounted for 2

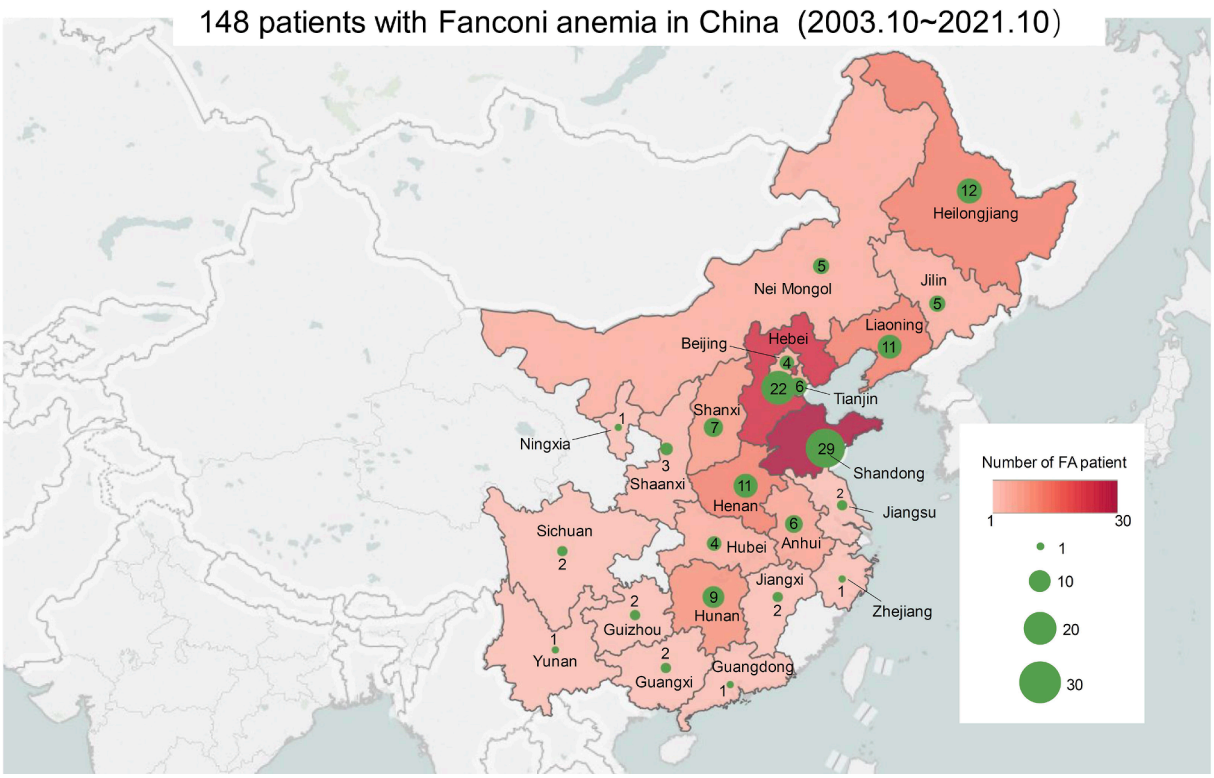

Fig. 1. Distribution characteristics of FA patients in different regions of China.

%. No patients with nervous system malformations were detected. Amongst 105 patients eligible for FA genotyping, 12.3 % had 3/6 PHENOS symptoms and 3.8 % had 4/6 PHENOS symptoms, less than previously reported data [14], and all patients were of the FA-A subtype.

In addition to the anomalies in VACTERL-H and PHENOS symptoms, we observed congenital hypospadias in four cases, penile microsomia in one case, cryptorchidism in one case, toe deformity in three cases, nasal bridge collapse in three cases, and scapula deformity in one case.

3.2. Family history

Based on family history, blood system diseases (51 %) had the highest incidence rate, followed by digestive system tumours (Fig. 2B). Amongst children with a family history, we observed the digestive system tumours, gynaecological tumours, and blood system diseases at the same time. Additionally, we provided information on the coexistence of liver cancer and thyroid cancer, liver cancer and blood system diseases, and the coexistence of breast cancer and lung cancer in Table S2. We identified one case of FANCJ mutation (FA-J), one case of FANCN mutation (FA-N), and two cases of FANCS mutation (FA-S) without a family history or deformity.

3.3. Overall genetic mutation

We identified 19 cases (12.8 %) of homozygous mutations—13 cases of FANCA homozygous mutation, 3 cases of FANCB hemizygous mutation, 1 case of FANCD1 mutation, and 2 cases of FANCE mutation. Among 105 patients with genotyping data, 13 subtypes of FA were found—FA-A, B, C, D1, E, G, I, D2, J, L, M, P, and S. FA-A was the most common subtype, accounting for 51.4 % (54/105), followed by FA-D2 and FA-P (Fig. 2C). These data differ from those previously reported in Japan and Korea [33,34]. New mutation sites were identified among the 13 subtypes (Figs. 3-5 and Table S3). The incidence of malformation and family history was highest for FA-A patients (Fig. 2D). Although FA-J patients showed a higher incidence, the difference was not significant because of only one FA-J case. Next, we evaluated patients with various mutation types to determine the proportion with malformations and a family history. FA-A patients accounted for a higher proportion with a family history and malformations, a finding that might be linked to the large sample size (Fig. 2E). Regarding the FA-B, FA-D1, FA-I and FA-J patients, a higher incidence of family history than malformation was found (Fig. 2E). Furthermore, we analysed the occurrence of malformations in different genotypes and found that various malformations occurred in FA-A patients, and circulatory system malformations mainly occurred in FA-D2/G/I/P patients (Fig. 2F).

3.4. FANCA mutation

Similar to previous reports [23–25], a broad mutational spectrum was observed for Chinese FA-A patients. In 54 FA-A patients from 51 unrelated families, we detected 106 mutant alleles, including 13 homozygous mutations and 91 heterozygous mutations. The mutation types mainly included missense, nonsense, splicing, insertion, and deletion mutations (Fig. 3 and Table S3). Among the missense mutations, we identified a new most common mutation site—exon 30c.2941 T > G (3 times). The most common splicing mutations were exon 34c.3348 + 1G > A (4 times) and exon 28c.2778 + 1G > A (3 times).

Among the 15 children treated with oral drugs, one with onset at birth progressed to AML-M5 at 20 years and died at 21 years. The mutant alleles of this child were FANCA exon 32c.3163C > T and exon 25c. 2222 + 1G > T (Table S3-S4). The other 14 children did not require a blood transfusion. The longest follow-up period occurred for two children, siblings, who developed the disease at the age of 7 years and his sister at the age of 5 years. The mutated allele was an exon 32c. 3188G > A homozygous nonsense mutation. The siblings were only treated with oral corylone and prednisone. These results suggest that patients with FANCA mutation at exon 32 may have a slow clinical progression but are prone to develop myeloid leukemia later.

Amongst the patients with homozygous FANCA mutations, two children had homozygous exon 29 mutations (Table S3). One of the

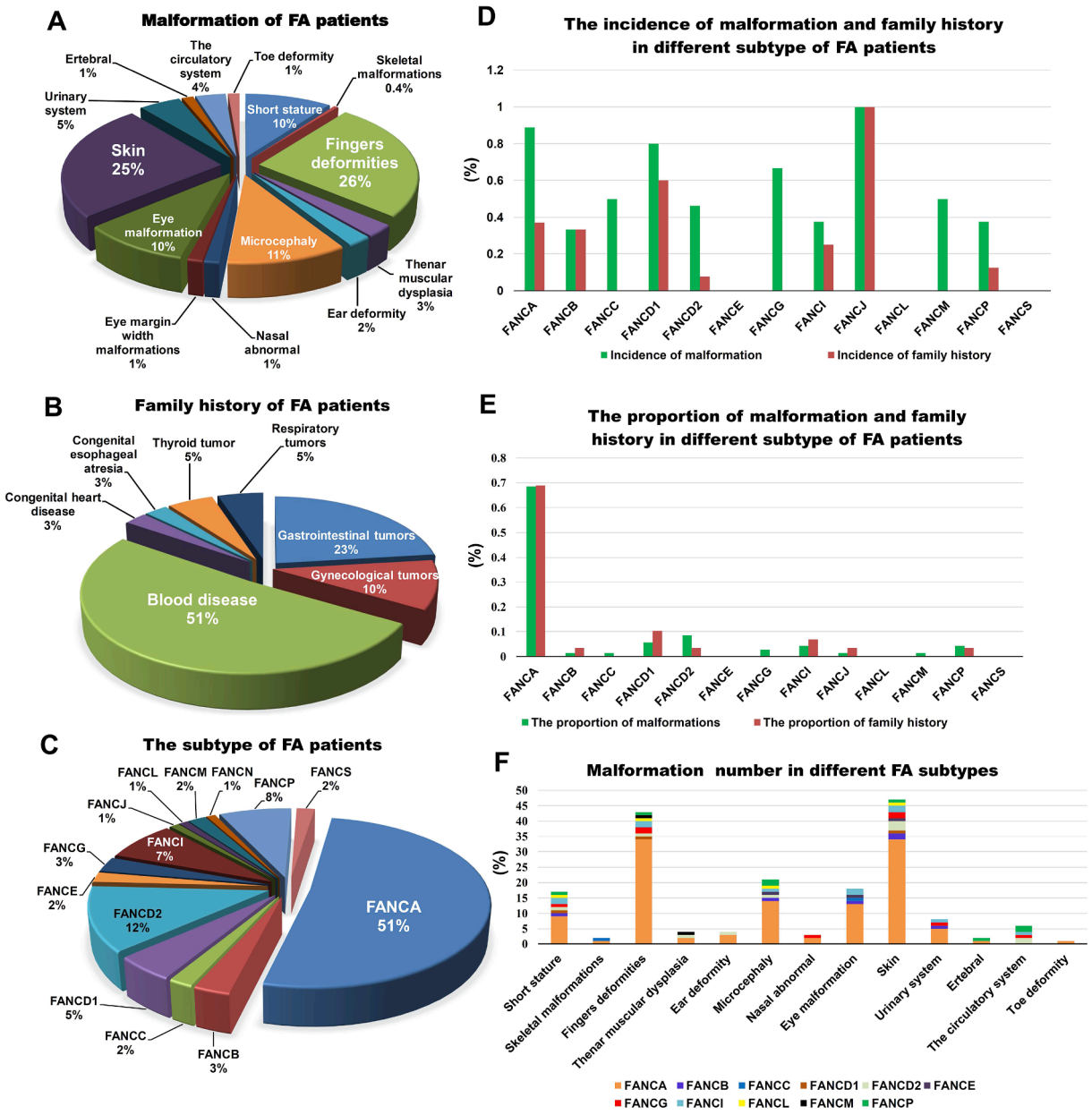

Fig. 2. Genetic mutation types and clinical characteristics of FA patients. (**A-C**) Description of malformation, family history and FA subtypes in FA patients. (**D-F**) Analysis of the relationship amongst malformation, family history and genotype in FA patients.

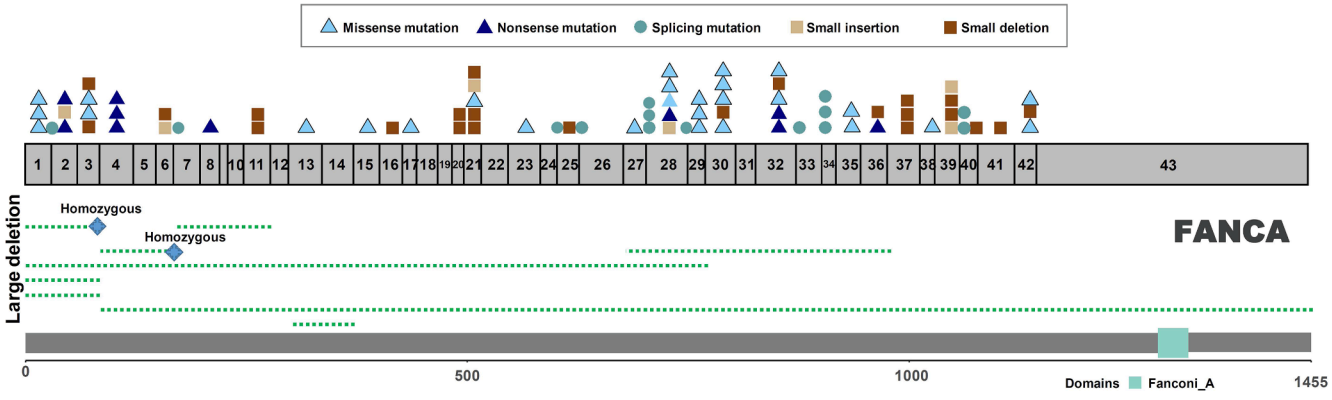

Fig. 3. Mutation sites and types of the FANCA gene.

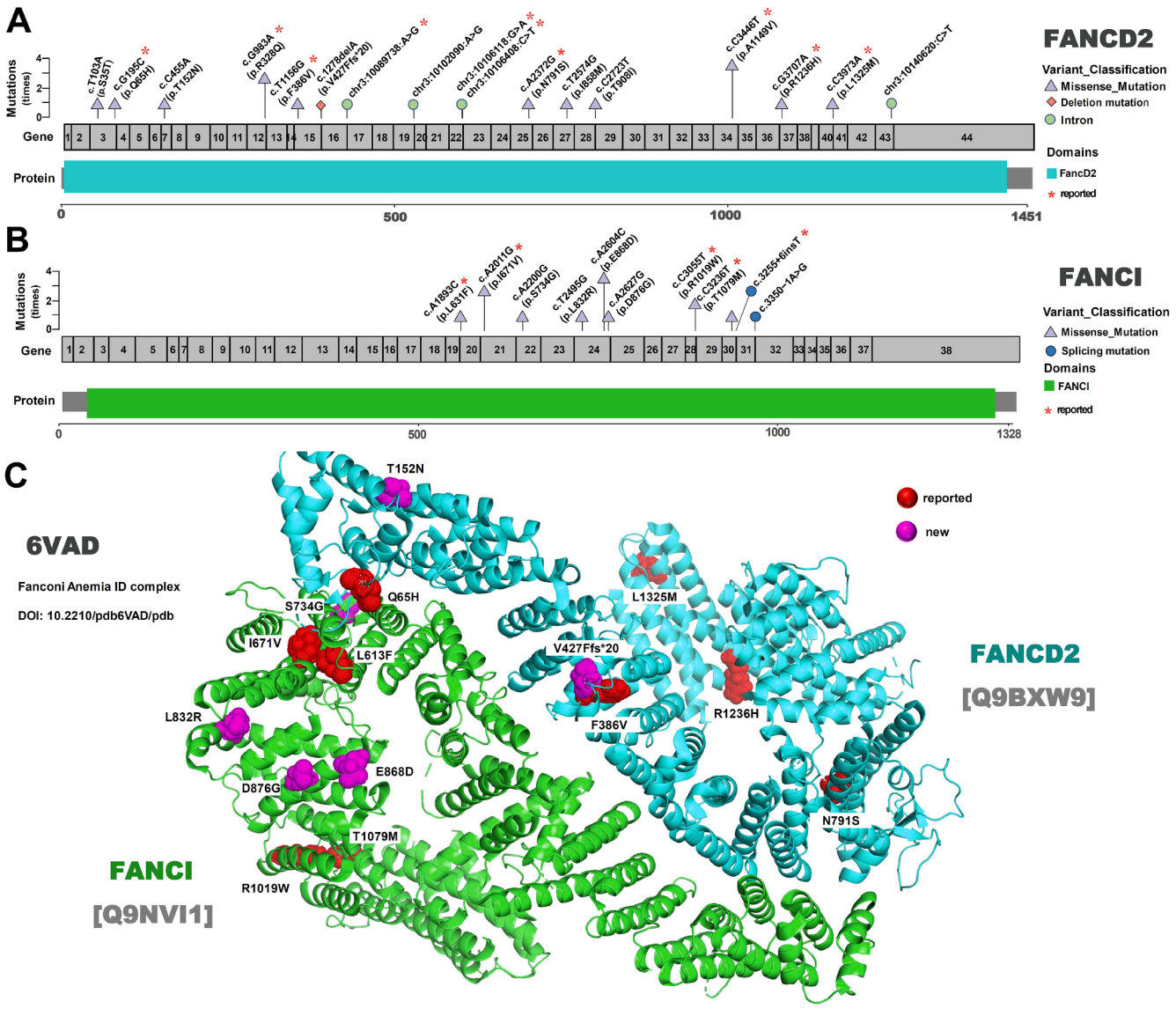

Fig. 4. Mutation features of FANCD2/FANCI. (**A**) Mutation sites of FANCD2. (**B**) Mutation sites of FANCI. (**C**) Amino acid mutation locations of FANCD2/FANCI proteins. Red indicates reported sites, and purple indicates new mutation sites.

children with exon 29c.2851C > T mutation had disease onset at the age of 4 years. The other child with exon 29c.2852 + 1G > T mutation died of bleeding infection during two years of follow-up, suggesting that the FA-A children with exon 29 splicing mutation may have more severe disease than those with missense mutation.

3.5. *FANCD2 and FANCI mutation*

In 13 FA-D2 patients from 12 unrelated families, we observed 22 mutant alleles, including 19 missense mutations, of which ten mutations were previously reported (marked by * in Fig. 4A). The common mutations were exon 34c.3446C > T (4 times). After analysing the 8 FA-I children from 8 families with complex heterozygous mutations, 11 mutation sites concentrated between exons 20 and 31 were detected (Fig. 4B). The most common mutation was exon 24c.2604A > C. Within the protein structure of the FANCI/FANCD2 complex (6VAD), most of these mutations were located at the α helix and β corners, and few were located in the random curl region (Fig. 4C).

The median age of onset of FA-D2 patients was four years (range: 0–18 years), and the incidence of malformation was 46 % (6/12). Of the nine surviving children, 3 had received haematopoietic stem cell transplantation, 5 had received oral drug treatment, and one was stable under observation. The mutation sites of the dead patient were *FANCD2* exon 34c.3446C > T and exon 16c.1413 + A > G. The onset age of the patient was 1.4 years, and no malformation or family history was found. In FA-D2 patients, the most extended follow-up was 11 years for a girl who had an onset age of 4.6 years, with deformity of short stature, six fingers of the right hand, oral androgen and prednisone treatment for eight years; after drug withdrawal for two years, the station is stable. The child had a younger brother with congenital thrombocytopenia, deformity of thenar dysplasia and coffee spot malformation. Her brother was untreated, and his blood was relatively stable. The mutated genes of the siblings were Chr3 10117949C > T and Chr310115671G > A [15], suggesting that the clinical symptoms of the two children with the mutated genes were relatively stable.

The incidence of malformation in FA-I children was 37.5 % (3/8), and the children with malformation were FA-I-1, FA-I-2, and FA-I-3. The FA-I-1 case was a two-year-old boy with mutations of exon 21c.2011A > G and exon 24c.2604A > C, malformation of six fingers on the right hand, and café au lait spots. The patient's blood count was normal after transplantation, and the condition was stable. The FA-I-2 case was a 1.7-year-old boy with mutations of exon 24c.2495 T > G and exon 31c.3350-

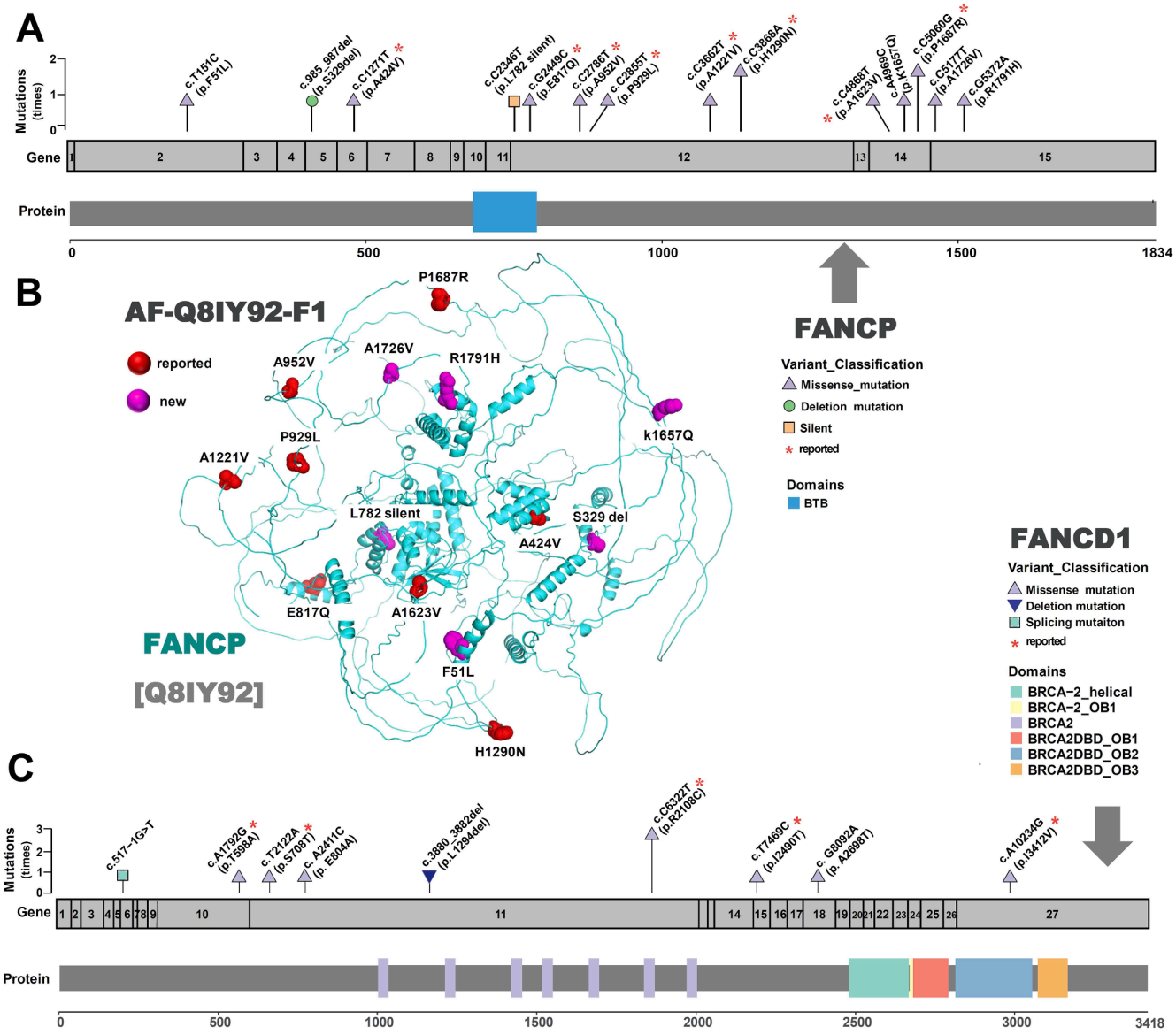

Fig. 5. Mutation features of FANCP/FANCD1. (**A**) Mutation sites of FANCP. (**B**) Amino acid mutation locations in FANCP protein. Red indicates reported sites, and purple indicates new mutation sites. (**C**) Mutation sites of FANCD1.

1A > G. The deformities were short stature, absence of right thumb, microcephaly, wide eye distance, and short penis. The patient's blood count remained stable for five years without treatment. The FA-I-3case was a ten-year-old girl with mutations of exon 24c.2604A > C and exon 30c.3255 + 6insT (splicing), accompanied by an RTEL1 exon 3c.208C > T heterozygous mutation, malformation of short stature, wide eye distance, café au lait spots, and patent ductus arteriosus, with a family history of gastric cancer in the grandfather. The patient died of severe infection at 16 years.

Five patients had FANCI without malformation, including FA-I-4, 5, 6, 7, and 8. The FA-I-4 case was female, with an onset at three years, no malformation or family history, and mutations of exon 22c.2200A > G and exon 30c.3255 + 5insT(splicing). The child also carried heterozygous mutations of CD46 exon 5c.574G > C, MYBL2 exon 7c.1238A > G, and RPL31 exon 2 c.83A > G. The child died at eight years of follow-up, and the specific cause of death was unknown. The FA-I-5case was male, with an onset age of 0.4 years. The mutation sites were the same as those of FANCI-3, but no DC-related gene was carried. He had an older brother with transient cytopenia who recovered after six months. The present onset was six years, and the blood count was stable. The other three children (FA-I-6, FA-I-7, and FA-I-8) were stable by oral medication. The above data demonstrated that the two FA-I children who died had splicing mutations near exon 30c.3255, suggesting that the clinical prognosis of splicing mutations at this site is poor. Whether the poor prognosis is related to carrying DC, DBA and other genes requires further analysis by expanding the sample size.

3.6. *FANCP and FANCD1 mutation*

Eight FA-P patients from 8 families were identified, and 14 mutant alleles were found, all of which were complex heterozygous mutations and eight were previously reported (marked * in Fig. 5A). *FANCP* gene mutation sites were mainly concentrated in exons 12, 14 and 15 (Fig. 5A). In the predicted protein structure of FANCP (AF-Q8IY92-F1), these mutation sites were located at the α helix, β corners and other regions, which are different from the FANCD2/FANCI protein complex (Fig. 5B).

Amongst the 8 FA-P patients, 3 had malformations, with an incidence rate of 37.5 %. One child developed AML-M7, and another child developed MDS-RAEB (refractory anemia with excess blasts) (Table S4).

Five FA-D1 patients were identified in 5 families. One child had an exon 10c.1792A > G homozygous mutation, and the others all had complex heterozygous mutations (Fig. 5C). Two FA-D1 patients had malformations (FA-D1-1, FA-D1-2). The FA-D1-1 patient with mutations of exon 7c.517-1G > T splicing and exon 11c.3881_3882del had café au lait spots and progressed to leukemia at the onset. All the other FA-D1 patient mutations were missense, suggesting that splicing and del mutations were more harmful in FA-D1 patients. The FA-D1-2 patient was a 1-year-old boy born with mutations of exon 11c.2411A > C and exon 18c.8092 G > A, characterized by short stature and six fingers of the left hand, spherical erythrocytosis and intermittent haemolysis, and the patient's condition was stable at follow-up at 6 years. In addition, another FA-D1 child was exon 10c.1792A > G homozygous mutation. The child's sister developed pancytopenia when she was 10 years, and her blood count recovered after traditional Chinese medicine treatment. The child was treated with oral Chinese medicine only, and the blood count was stable at 12 years of follow-up. Thus, the clinical symptoms of homozygous mutation in FANCD1 exon 10c.1792A > G are likely mild.

3.7. FA-B/C/E/G/J/L/M/S mutation

Three FA-B patients (FA-B-1, 2, and 3) from 3 families were identified; they were all male and had malformations. The onset age of the FA-B-1 case was 1 year, with mutation exon 3C.869 T > G. The patient had ventricular septal defect malformation and was transfusion-dependent at the time of diagnosis; unfortunately, he was lost to follow-up. The age of onset of the FA-B-2 case was 3 years with mutation of ChrX 14,781,120C > T. The patient had short stature, café au lait spots, and congenital hypospadias and died of infection at 10 years. The onset age of the FA-B-3 case was 7 years, with splicing mutation of exon 9c.2165 + 10A > T. The patient had microcephaly, microphthalmia, dark skin and café au lait spots. The child's grandmother had breast cancer, her grandmother had lung cancer, and the child developed leukemia 3 years after the onset. Chromosome changes of + 8 and del (3) (p13p21) were observed (Table S4). Thus, the overall clinical manifestations of children with FANCB mutations are severe, and the prognosis is poor.

Exon 15c.1677 is a common mutation site of FNACC. Two FA-C patients (FA-C-1,2) from 2 families were identified. The FA-C-1 case was a 4.6-year-old boy with mutations of exon 8c.782 T > G and exon 15c.1677 + 77C > T. He was treated with Chinese medicine, and his condition was stable after 4 years of follow-up. The FA-C-2 case was a 5-month-old girl with mutations of exon 15c.1677 + 5G > A and exon 11c.1000C > T, and a spontaneous SF3B1 exon 13c.1732A > G mutation. The child died at the age of 9 years of infection, suggesting that SF3B1 mutation is a factor contributing to the poor prognosis of FA patients.

Two FA-E patients (FA-E-1, FA-E-2) with homozygous mutation of exon 5c.1111C > T from two families were identified. Although the two patients had the same mutation site, the clinical phenotypes were different. The FA-E-1 patient was an 8-year-old boy with microcephaly, microphthalmia and café au lait macules who died at 15 years old of leukemia. The FA-E-2 case was a 3-year-old girl without malformation or a family history. Umbilical cord blood transplantation was performed 2 years after disease onset, and the disease was stable 5 years after transplantation. The different phenotypes of patients with the same mutated allele suggest other copathogenic factors exist in the pathogenesis of Fanconi anemia.

Three FA-G patients from 3 families were identified (FA-G-1, FA-G-2, and FA-G-3). The FA-G-1 case was a 2-year-old boy with mutations of exon 5c.552dupG and exon 12c.1434-1G > C, with short stature, polydactyl malformation of the right thumb, coffee spot, no family history, oral stanzolol and prednisone treatment; he died of disease progression at the age of 7 years. The FA-G-2 case was a 4-year-old boy with mutations of exon 3c.212 T > C and exon 2 c.175_175 + 1GG > AT. He had multiple finger deformities of the right hand, café au lait macules, right kidney absence and patent ductus arteriosus, and the blood was stable after 2 years of follow-up. The FA-G-3case was a 3-year-old girl with mutations of exon 3c.119C > G and exon 2 c.172G > A, no malformation, and no family history. Allogeneic haematopoietic stem cell transplantation was performed 3 years after the onset of the disease. Three years after transplantation, the blood cell count was normal. The mutation sites of the FA-G-2 and FA-G-3cases were similar, and the condition was stable, suggesting that the condition of children with FANCG exon 3 and exon 2 mutations is relatively stable.

One case of FANCJ was a 7-year-old boy with no malformation or family history. The mutation sites were exon 17c.2440C > T (reported) and exon 5c.430G > A. At five years of onset, a related semicompatible haematopoietic stem cell transplant was performed. Seven years after the transplant, his condition was stable. One case of FANCL was a 5-year-old girl with microcephaly, short stature, multiple fingers on the right thumb, dark skin, no family history, and mutations of exon 12c.1000 T > C and exon 8c.691C > T (previously reported). After follow-up for four years, the patient's condition was stable.

Two children with FANCM (FA-M−1 and FA-M−2) from 2 families were identified. The FA-M−1 case was a 7-year-old boy with no malformation or family history. The mutation sites were Chr14-45645251 exon 14c.3294 T > G and Chr14-45658156 exon 20c.4931G > A. Sibling transplantation was performed three years after the onset of the disease, and the blood cell count was stable four years after transplantation. The FA-M−2 case was a 7-year-old boy with mutations of Chr14 44,714,456 A > G and Chr14 44,727,906 G > A. He had a malformation of the right thumb and right thenar muscular dysplasia. He developed myeloid leukemia at 14 years (Table S4) and then died because of stem cell transplantation failure.

One case of the FA-N subtype was identified, a 2-year-old girl with no malformation or family history. The mutation sites were exon 13c.3379 T > C and exon 4c.925A > G, and the patient was lost to follow-up. Two cases of FA-S (FA-S-1,2) from two families were identified with no malformation or family history. The FA-S-1 case was an 8.6-year-old girl with mutations of exon 10c.2566 T > C (previously reported) and exon 10c.1310A > C. The child was in good condition after genetic semicompatibility transplantation at the age of 10.2 years. The FA-S-2 case was a 3.5-year-old girl with mutations of exon 21c.5406 + 33A > T and exon 13c.4484 + 14A > G. The patient's station was stable by treatment with cyclosporine and Chinese medicine at 6.5 years.

3.8. Pediatric patients with MDS and AML

Seven patients presented with MDS bone marrow at admission, and 7 children developed leukemia (Table S4). In the early stage of MDS, chromosomal abnormalities were mostly concentrated on chromosomes 1, 3, and 7. When chromosomes 1, 3, 7, and 8 (+8) occurred, children's diseases were mainly at the AML or MDS-RAEB-T stage. In children with leukemia, the most common chromosomal karyotype change was + 8. In KLP children, the chromosomal dup1 change occurred in the MDS state, and + 8 occurred in leukemia progression. Additionally, +8 occurred in patients with FANCP converted to MDS-RAEB and + 8 occurred in patients with AML-M5 progression. Amongst children without + 8 abnormalities, one was AML-M7, and the other was AML-M5, suggesting that the occurrence of + 8 was the main cytogenetic abnormal event for the progression of leukemia in FA children.

4. Discussion

Twenty-three subtypes of FA have been identified, and the most common type of FA in Chinese patients was FA-A, followed by FA-D2 and FA-P in our study, a finding that is different from previously reported data from Korea and Japan [33,34]. The most common deformities in FA-A patients are finger deformities and café au lait spots, while circulatory system deformities are more common in FA-D2/G/I/P subtypes. This finding is similar to that in a previous report [32], likely because biallelic FANCA mutations do not challenge embryonic

development in humans [25]. Thus, mutations in different FA genes can lead to predisposition to deformities.

Mutation c.3788_3790delTCT in FANCA is the most common mutation in Spain, Brazil, and other countries [25]. In our study, we identified a new most common mutation site— exon 30c.2941 T > G (3 times). FANCD2 mutations reported in Japan include c.3561-2A > C and c.3072_3073insa [34]. The most common mutation of FACND2 in our study was the exon 34c.3446C > T mutation. The main FANCI mutation was exon 24c.2604A > C. *FANCP* gene mutation sites are mainly concentrated in exons 12, 14 and 15. In addition, FANCP patients in Japan mostly have deletion mutations [34]. However, most of our FA-P patients had missense mutations.

Homozygous nonsense mutations of exon 32c.3188G > A and exon 29c.2851C > T in FANCA are likely benign, but the homozygous splicing mutation in exon 29c.2852 + 1G > T is harmful. Ivana Radulovic reported that the homozygous nonsense mutation of exon 5c.469A > T in FANCD1 is related to severe Fanconi anemia [35]. We did not find an exon 5 mutation in the *FANCD1* gene. We found that mutations of exon 7c.517-1G > T splicing and exon 11c.3881_3882del were harmful, while children with homozygous mutations of exon 10c.1792A > G had mild clinical symptoms.

Cases of an initial diagnosis of MDS or poor disease progression tend to occur in FA-B/C/E/G/J/L/M/S subtypes. Genotype and mutation analysis results of these cases found that the clinical prognosis of patients with FA carrying SF3B1 mutations was poor. Interestingly, the clinical phenotypes of the two FA-E patients with the same mutation were significantly different, suggesting other potential factors for the phenotype of the patients with FA. Analysis of patients with disease progression to MDS and leukemia revealed chromosome 1, 7, and 3 abnormalities early in the disease, such as chromosomal clonogenesis abnormalities previously reported in patients with FA with progression to AML [36–38]. However, chromosome + 8 gradually appears in the progression of the disease to leukemia or MDS-RAEB-T, suggesting a process of clonal evolution in patients with FA at different stages of the disease.

Taken together, we conducted a comprehensive investigation of the clinical characteristics and FA-related gene mutation features of pediatric patients with FA in a large Chinese rare disease cohort. We proposed a set of mutation sites related to clinical prognosis and the cytogenetic abnormalities of FA progression. Our study provides new evidence for a deeper understanding of FA in the Asian population and will be useful for FA management in the future.

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CRediT authorship contribution statement

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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.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cca.2022.11.030.

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