Comprehensive analysis on phenotype and genetic basis of Chinese Fanconi anemia patients: dismal outcomes call for nationwide studies

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Abstract

Background: Fanconi anemia (FA) is the most common inherited bone marrow failure (BMF) syndrome with 22 related genes identified. The *ALDH2* rs671 variant has been proved related to accelerate the progression of BMF in FA patients. The phenotype and genetic basis of Chinese FA patients have not been investigated yet.

Methods: We analyzed the 22 FA-related genes of 63 BMF patients suspected to be FA. Clinical manifestations, morphological and cytogenetic features, *ALDH2* genotypes, treatment, and outcomes of the definite cases were retrospectively studied.

Results: A total of 24 patients were confirmed the diagnosis of FA with the median age of BMF onset was 4.5-year-old. The number of patients manifested as congenital malformations and growth retardation were 21/24 and 14/24, respectively. BM dysplasia and cytogenetic abnormalities were found in 15/23 and 10/22 patients. All the patients with abnormal karyotypes also manifested as BM dysplasia or had evident blasts. Thirty-nine different mutations were identified involving seven genes and including twenty-one novel mutations. *FANCA* mutations contributed to 58.33% of cases. Ten patients harboring *ALDH2*-G/A genotype have a significantly younger age of BMF onset ($p=0.024$). Within the 22 patients adhering to continuous follow-up, 18 patients underwent hematopoietic stem cell transplantations (HSCTs). During the 33.5 months of follow-up, 8/22 patients died, seven of which were HSCT-related, and one patient who did not receive HSCT died from severe infection.

Conclusion: The phenotypic and genetic spectrum of Chinese FA patients is broad. Bone marrow dysplasia and cytogenetic abnormalities are prevalent and highly consistent. The overall outcome of HSCTs is disappointing. Nationwide multicenter studies are needed for the rarity and adverse outcome of this disease.

Introduction

Fanconi anemia (FA) is a rare genetic disease highly heterogeneous in clinical manifestations and genetics. Clinical features primarily include congenital malformations, progressive bone marrow failure (BMF), and predisposition to hematopoietic and solid malignancies [1,2]. The most common congenital abnormalities include skin pigmentation, café au lait spots, short stature, and hypoplastic of radii and/or thumbs [2]. The time of BMF onset is variable but usually at pre-school age, with the cumulative incidence of 90% by the age of 40 [3]. The malignancy risk in FA patients is mounting, especially the risks of myelodysplastic syndrome/acute myeloid leukemia (MDS/AML), which are several hundred folds higher than those of the general population [3-6].

Twenty-two genes have been identified related to FA (Table S1) to date. Products of the 22 genes participate in FA-BRCA pathway, which is responsible for correcting interstrand crosslinks (ICLs) and other DNA damage events induced by genotoxic agents. Endogenous aldehyde is a genotoxic agent and is detoxicated by aldehyde dehydrogenases (ALDHs) *in vivo* [7]. Previous studies have suggested aldehydes are highly toxic in FA deficient cells and could speed up the development of BMF and leukemia in FA deficient mice models.[8-10] The mitochondrial ALDH2 isoform is the most efficient acetaldehyde-detoxifying enzyme in humans [11]. Inactivating *ALDH2* variant (rs671 c.1510G>A/p.E504L) is highly prevalent in east Asia and can abolish ALDH2 activity by a dominant-negative effect [12]. *ALDH2*-A/A and *ALDH2*-G/A genotypes have been proved related to accelerated progression of BMF and malignant transformation in FA patients [13,14].

Although the genetic basis, pathological mechanisms, and epidemiology of FA have been extensively studied, few researches focus on Chinese patients [15]. In the present study, we report 24 Chinese FA patients aiming to depict their genetic basis and clinical characteristics.

Subjects And Methods

Patient enrollment

We retrospectively analyzed 63 BMF patients who were suspected to be inherited BMF in Hebei Yanda Lu Daopei Hospital from May 2012 to Dec. 2017. Detailed disease histories and examination files were retrieved from the electronic medical record system of our institute. BMF is considered with one or more lineages decreased in whole blood cell counts and reduced hematopoiesis with routine Wright-Giemsa staining bone marrow morphological analysis and Hematoxylin-Eosin staining pathological analysis.

Categorization of hematopoietic cells, blast percentage, hematopoietic grade, dysplasia, and diagnosis of MDS were according to the 2008 edition of the World Health Organization Classification of Tumors of Hematopoietic and Lymphoid Tissues.[16] All patients enrolled were confirmed BMF and should meet at least two following inclusive criteria: 1) growth retardation; 2) congenital physical malformations; 3) early onset of BMF (≤ 6 years old); 4) chronic onset of BMF with a progressive course (disease course > 6 months); 5) suggestive family history (consanguinity or family history of cancer or hematological disorders); 6) positive for chromosome breakage test (Table S2). Other inherited syndromes manifested as BMF and malformations such as dyskeratosis congenita, Diamond-Blackfan anemia, and Neurofibromatosis-Noonan syndrome diagnosed based on syndromic presentations combined with genetic tests were excluded. The follow-up duration was defined as the time from referral to the last follow-up or loss of follow-up/death.

Written informed consents were obtained from the patients or their statutory guardians and all tested family members in accordance with the Declaration of Helsinki. The study was approved by the ethics committee of the Hebei Yanda Lu Daopei hospital.

Nucleic acid extraction

Peripheral blood (PB), bone marrow (BM), or cryopreserved DNA samples of the patients and their parents were obtained. Genomic DNA was extracted from PB/BM nucleated cells using silica gel column method.

High throughput sequencing, variant calling, and *ALDH2* genotyping

We carried out Sanger sequencing on the entire coding exons and flank regions of *FANCA*, *FANCC*, and *FANCG* in patients suspected to be inherited BMF from Apr. 2012 to May 2016. Targeted high-throughput sequencing (THS) has been applied since May 2016, *FANCD2* and *BRCA2* were added in the panel. Whole genomic sequencing (WGS) was carried out using cryopreserved samples for the enrolled cases where the panel test could not find the pathogenic mutations, and all the 22 FA genes were analyzed.

The THS process has been described previously [17]. For the WGS, libraries were constructed with NEBNext Ultra II DNA Library Prep Kit for Illumina (New England Biolabs, US), followed by sequencing on Illumina HiSeq X Ten platform (Illumina, US) using HiSeq X Ten Reagent Kit v2.5 (Illumina, US) running on paired-end 150bp mode.

Reads yielded by the two kinds of sequencing were all aligned to the human reference genome (hg19) with the Burrow-Wheeler Aligner (BWA) mem. Variants were called according to Genome Analysis Toolkit (GATK) best practices using bam files. Final confident variants were annotated using annovar and oncotator. Variants with minimal allele frequency (MAF) $\geq 1\%$ in general population were filtered out according to 1000 Genomes, EXAC, and gnomAD databases. The pathogenicity of the germline missense mutations was assessed by *in silico* prediction algorithms, and the putative causal variants were classified according to the standards and guidelines recommended by the American College of Medical Genetics and Genomics (ACMG) [18]. Only pathogenic, likely pathogenic, or uncertain significance variants were considered causative in the present study. The process of CNV analysis based on WGS has been described elsewhere [17].

ALDH2 genotyping was based on WGS data or Sanger sequencing with primers 5'-TGCTATGATGTGTTTGGAGCC-3' (forward) and 5'-ATTTAGGGTCTCTGCTGGGCG-3' (reverse).

Validation by Sanger sequencing

Polymerase chain reaction (PCR) and Sanger sequencing performed on the ABI 3500xL Genetic Analyzer (Thermo Fisher, US) were adopted to confirm all the mutations reported in this study. Single nucleotide variants (SNVs) and small insertions/deletions (InDels) were validated by PCR and Sanger sequencing using pedigree's samples when accessible. For the validation of CNVs, the

breakpoints were confirmed by Sanger sequencing using patients' DNA, and the parental origins were verified through PCR and agarose gel electrophoresis.

Results

Demography and clinical characteristics

A total of 24 patients (seven females and seventeen males) from non-related families were finally diagnosed as FA, including one who has already been reported (Case 9) [17]. The median referral age of this cohort was seven years old, and the median age of BMF onset was 4.5 years old (range, 1-15 years old). There were 23 Han Chinese and one Uyghur Chinese, and the geographical distribution spread nationwide though half of the patients came from the south or southwest of China. All patients were referred to our institute because of severe cytopenia except a thirty-year-old boy (Case 11) who was initially diagnosed as MDS for the myeloid dysplasia and increased myeloblasts indicated by BM morphology. Five patients had an indicative family history, among which, two patients had family members died from anemia (Case 4, Case 17), two patients were from consanguineous families (Case 18, Case 24), and one patient was an in vitro fertilize baby whose paternal grandmother died from pancreatic cancer (Table 1).

Fourteen (58.33%) patients were growth-retarded, and 21 (85.7%) patients manifested as congenital malformations. Congenital abnormalities in our cohort included skin pigmentation (13/24), café au lait spots (6/24), spin and limbs deformation (12/24), craniofacial malformations (8/24), genitourinary system malformations (7/24), cardiovascular system defects (2/24), nervous system diseases (2/24), and endocrine system defects (2/24) (Table 1).

Thoroughly evaluation of the hematologic phenotype is crucial to FA patients since BM dysplasia or pathological cytogenetics relate to disease progression and adverse hematopoietic stem cell transplantations (HSCT) outcomes [5,14]. Twenty-three patients' morphologic test results and 22 patients' cytogenetics test results before pre-HSCT conditioning regimen and/or chemotherapy were available. BM dysplasia was found in 15/23 (65.22%) patients, including one AML with the myeloblast count of 41% (Case 6) and one myelodysplasia with the blast count of 6% (Case 11). Karyotypes were described according to the International System for Human Cytogenetic Nomenclature 2013 [15]; at least 20 metaphases were analyzed for each assay. Cytogenetic abnormalities were found in 10/22 (45.45%) patients with clonality found in six patients, and half of the abnormal karyotypes involved chromosome 7 (-7, 7q-, or der(7)t(1;7)) (Table 2). The cytogenetic result of Case 6 who was diagnosed as AML was 46, XX, der(7)t(1;7)(q21;q36) [20], which was confirmed to be non-constitutional by matched peripheral blood, and the karyotype of patient Case 11 was highly complex (Table 2). All the patients with abnormal karyotypes also manifested as dysplasia on bone marrow smear or had evident blasts, suggesting the initiation of clonal evolution in the hematopoietic system.

Characteristics of mutations

A total of 44 mutations were identified involving seven different FA genes and composed of 16 missense mutations, ten large deletions, eight nonsense mutations, seven frameshift mutations, two splicing mutations, and one deep intron mutation (Figure 1, Table 3). All the large deletions were found within the *FANCA* gene. 21 (47.73%) mutations identified in our cohort were novel and the majority of mutations were private except *FANCA* c.367C>T and *FANCA* c.3627-607_3765+268del, which were shared by two patients separately. (Figure 2, Table 3). We did not find *FANCA* c.2546delC in our cohort, which accounts for over 30% *FANCA* mutations in Japanese and Korean patients [24,25].

Among the 24 patients, 17 patients carried compound heterozygous mutations, three patients carried homozygous mutations, three patients harbored monoallelic *FANCB* mutations who were all males, and one patient with a heterozygous *FANCE* mutation were identified. Biallelic *FANCA* mutations caused 58.33% (14/24) of the cases, followed by monoallelic *FANCB* mutations which constituted 12.5% (3/24) of the cases; both *FANCD2* and *FANCE* mutations made up to 8.33% (2/24) of the cases, and *FANCC*, *SLX4*, and *ERCC4* mutations caused one case each (Table 3). We did not find any case attributed to *FANCG* mutations, which is the second most prevalent responsible gene in East Asian according to Japanese and Korean studies [24,25]. Despite the limited size of this cohort, we identified three *FANCB* mutations, making it rank one of the most common causative genes in line with the Japanese study [24]. There were three homozygous mutations, *FANCA* c.1867C>T, *FANCC* c.545C>A, and *ERCC4* c.257G>A; the

latter two mutations were carried by patients both came from consanguineous families, and the *FANCC* c.545C>A was carried by the only Uyghur patient in our cohort.

Rigorous criteria were adopted in the process of criminal variant identification (Table 3, Table S3). Majority of the patients were assigned with compelling mutations with two exceptions. All mutations were classified as pathogenic or likely pathogenic according to the guideline of ACMG except *FANCA* c.2611C>G, which was harbored by Case 1. Albeit the ambiguous pathogenicity, it was rare in the general population and was the only possible responsible mutation found in this patient after an exhaustive search for variants in FA-related genes. Hence, we considered it to be the causative mutation. Case 21 carried compound heterozygous *FANCE* c.1111C>T mutation and *FANCE* c.1317-237C>G mutation. The c.1111C>T mutation was considered pathogenic, but the c.1317-237C>G mutation is an intron variant and classified as uncertain significance, therefore it was excluded in statistics.

ADLH2 rs671 genotype

14/24 (58.33%) patients in our cohort carried *ALDH2*-G/A genotype, and the other patients were all *ALDH2*-G/G genotype. There was no *ALDH2*-A/A genotype identified (Table 2). The age of BMF onset of *ALDH2*-G/A patients was significantly younger than that of the *ALDH2*-G/G patients ($p = 0.024$, t-test).

Treatment and outcome

Within the 24 patients, continuous medical records of 22 patients can be retrieved except Case 9 and Case 24, who only came to us once and were excluded in this section. All the 22 patients were eligible for HSCT for they were all transfusion-dependent, and HSCT was performed on 18 patients (81.81%). The numbers of patients accepted HSCT from HLA-matched unrelated donors (MUD), HLA-unmatched unrelated donors (UUD), HLA-haploidentical related (sibling or parental) donors (HRD), and HLA-matched related donors (MRD) were four, five, seven, and one, respectively. Another patient accepted HLA-unmatched unrelated cord blood (UUC) HSCT. The other four patients who did not undergo HSCT accepted androgen, cytokine, and/or intermittent transfusion support. All the patients with abnormal karyotype underwent HSCTs. In the HSCT subgroup, 11/18 (61.11%) were *ALDH2*-G/A genotype. The median follow-up duration was 33.5 months ranged from one month to 84 months. By the end of the study, eight patients (36.36%) have been dead. Seven of them were HSCT-related, mainly severe acute graft-versus-host disease (aGVHD) and/or infections, accounting for 38.89% of the subgroup. One patient who did not receive HSCT died from severe infection (Table 3).

Discussion

The 24 patients displayed a wide range of clinical phenotype and genetic variation spectrum that all physiological systems were involved (Table 1), and the responsible mutations were detected in seven different genes (Figure 1, Table 3). In keeping with other studies, bone marrow dysplasia and abnormal karyotypes were prevailing (65.22% and 45.45%, respectively) and highly consistent [14,30], denoting the risk of hematologic malignant transformation, especially the ones with aberration in chromosome 7, which is the most prevalent cytogenetic abnormality in pediatric MDS and indicates an adverse long-term outcome even after HSCTs in MDS/AML patients [31]. *ALDH2*-G/A and *ALDH4*-A/A genotypes are confirmed to be associated with more severe hematologic phenotype and more adverse outcomes of FA in Asian patients [13,14]. The same tendency was observed in our cohort, despite there was no patient of *ALDH2*-AA genotype.

All patients in our cohort presented with a more severe hematologic manifestation and the proportion of patients who received HSCTs was higher than that of most studies [3-6,14,32]. Although BMF is the typical and most prevalent feature, our data may not reflect the actual behavior of FA since all the patients were referred to our institute seeking for HSCTs. Studies suggest the high HSCT-related mortality in FA patients, of which infection and aGVHD were the two leading causes [5,32]. In our cohort, 38.89% of HSCT patients died from HSCT-related acute complications. Studies also suggest the overall dismal outcome that ten years cumulative risk of death was over 22% and the overall survival after 30 years of diagnosis dropped to below 40%; besides, the long-term survival of HSCT patients and non-HSCT patients were comparable [5,28,30,32], partly because HSCT in the context of

FA is explicitly challenging. Therefore, even with the optimized pre-HSCT conditioning regimens like the reduced intensity and the combination of fludarabine, meticulousness is needed in decision-making. Whether HSCT is the best treatment strategy depends much on the severity of cytopenia and the hematologic adverse events of a given patient and the type of donor he/she could get.

The cumulative incidence of leukemia and solid tumors in the middle age of FA patients was reported to be ~20% and ~30%, respectively [4-6,33]. In our cohort, no patient developed hematologic or solid malignancies during the follow-up up to date except the ones initially diagnosed as AML (Case 6) and MDS (Case 11), but the longest follow-up in our cohort was only seven years, which may not be long enough for the malignant phenotype emerge.

Although this study is limited by its cohort size, it is still informative and enriches the knowledge on Chinese FA patients which was nearly barren. Here we thoroughly investigated the clinical manifestations, morphologic and cytogenetic changes, genetic basis, and outcomes of 24 Chinese FA patients. Our data displayed a broad phenotypic and genetic variant spectrum of Chinese FA patients, the disappointing outcomes which need improving, and highlighted the urgency of nationwide multicenter studies to reveal the mask of Chinese FA patients and optimize the clinical management.

Declarations

Acknowledgments

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Ethics approval and consent to participate

The study was approved by the ethics committee of the Hebei Yanda Lu Daopei hospital. The patients provided written informed consent for genetic analysis.

Consent for publication

Written informed consents were obtained from the patients or their statutory guardians and all tested family members for publication of clinical details.

Availability of data and materials

Sequencing data in fastq format generated in this study are available from the corresponding author on reasonable request.

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Competing interests

The authors declare that no competing interests in this study.

Author's contributions

DN reviewed the medical history of the patients, analyzed sequencing data, and wrote the manuscript, JZ, FW, WZ, XM, and LL performed the sequencing process, analyzed the data, and wrote the manuscript, XC and YZ analyzed the morphology and karyotype results, PC designed the bioinformatic analysis process. MX followed up the patients, TW and PW carried out the morphologic and cytogenetic study, WT, MW, and KC analyzed the clinical data and supervised the study, HL designed and supervised the study.

Abbreviations

ACMG: American College of Medical Genetics and Genomics; AGE: agarose gel electrophoresis; aGVHD: acute graft-versus-host disease; ALDHs: aldehyde dehydrogenases; AML: acute myeloid leukemia; BM: bone marrow; BMF: bone marrow failure; BWA: Burrow-Wheeler Aligner; CNVs: copy number variants; FA: Fanconi anemia; HLA: human leukocyte antigen; HRD: HLA-haploidentical related donors; HSCT: hematopoietic stem cell transplantations; ICLs: interstrand crosslinks; InDels: insertions/deletions; MAF: minimal allele frequency; MDS: myelodysplastic syndrome; MRD: HLA-matched related donors; MUD: HLA-matched unrelated donors; PB: peripheral blood; PCR: polymerase chain reaction; SNVs: single nucleotide variants; THS: targeted high-throughput sequencing; UUC: HLA-unmatched unrelated cord blood; UUD: HLA-unmatched unrelated donors; WGS: whole genomic sequencing

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Tables

Table 1 Clinical features of the 24 FA patients

Case No.	Gender	Age of referral (years)	Age of clinical BMF onset (years)	Congenital malformations	Growth retardation	Family history
1	F	16	15	None	No	Negative
2	M	12	2	S, C, M	Yes	Negative
3	M	7	2	S, C, G	Yes	Negative
4	M	10	5	S, G	Yes	One sibling manifested as polydactyly and died from anemia
5	M	11	5	C, G, M,	Yes	IVF and paternal grandmother died from pancreatic cancer
6	F	11	10	C, H	Yes	Negative
7	M	7	5	S, M	No	Negative
8	M	17	10	S, M	No	Negative
9	M	7	7	S, C, M, H	No	Negative
10	M	5	4	S, M	No	Negative
11	M	13	13	E	Yes	Negative
12	M	9	6	S	Yes	Negative
13	F	7	7	M	Yes	Negative
14	M	7	1	S, M	Yes	Negative
15	M	5	2	S	No	Negative
16	M	4	1	S, M	No	Negative
17	M	14	5	C, G	Yes	Two family members died from anemia
18*	M	6	4	S, C, M	Yes	2nd degree consanguinity
19	M	4	3	None	No	Negative
20	F	9	4	S, M	No	Negative
21	F	7	4	S, G, N	Yes	Negative
22	M	9	7	None	No	Negative
23	F	6	3	S, C, G	Yes	Negative
24	F	6	4	S, M, H, E, G	Yes	2nd degree consanguinity

* Case 18 is a Uyghur Chinese.

F, female; M, male; S, skin and annex; C, craniofacial anomalies; M, musculoskeletal system; G, genitourinary system; H, cardiovascular system; E, endocrine system; N, nervous system. IVF, in vitro fertilized.

Skin and annex abnormalities include skin pigmentation, café au lait spots, excess hair; craniofacial anomalies include microcephalus, ptosis, hypertelorism, hypotelorism, flat nose bridge; malformations in musculoskeletal system include polydactyly, deformity of thumbs, absence of thumbs, hypoplasia of thenar eminence, and scoliosis; genitourinary system malformations include kidney malformation, hydronephrosis, indirect inguinal hernia, cryptorchidism, ovary absence, and uterine malformation/absence; cardiovascular system defects include patent ductus arteriosus and ventricular septal defect; nervous system abnormalities include encephalatrophy and moyamoya disease; endocrine system defects include hypothyroidism, primary adrenocortical insufficiency, and obesity.

Table 2 Bone marrow morphology, karyotype, chromosome breakage tests, and *ALDH2* genotypes of the 24 FA patients.

BM morphology	BM karyotype	Chromosome breakage test	<i>ALDH2</i> genotype
Dysplasia	47,XX,+8[1]/46,XX[20]	Negative	G/G
Dysplasia	NA	Positive	G/A
Dysplasia	47,XY,+15[1]/46,XY[20]	Positive	G/A
Hypoplasia	normal	Positive	G/A
NA	NA	Positive	G/G
AML	46,XX,der(7)t(1;7)(q21;q36)[20]	Positive	G/G
Hypoplasia	Normal	Positive	G/A
Dysplasia	46,XY,-7,+21[5]/46,XY[16]	Positive	G/G
Hypoplasia	Normal	Positive	G/G
Hypoplasia	Normal	Positive	G/A
MDS	Complex	Positive	G/G
Hypoplasia	Normal	Positive	G/A
Dysplasia	Normal	Positive	G/G
Dysplasia	Normal	Positive	G/G
Dysplasia	Normal	Positive	G/A
Hypoplasia	Normal	Negative	G/A
Dysplasia	46,XY,del(7)(p13)[13]/46,XY[7]	Positive	G/A
Hypoplasia	Normal	Positive	G/A
Dysplasia	Normal	Positive	G/A
Dysplasia	46,XX,t(1;5)(p36.1;q13)[1]/46,XX[19]	Positive	G/G
Dysplasia	46,XX,del(14)(q24)[1]/46,XX[20]	Positive	G/G
Dysplasia	45,XY,-7[19]/46,XY[3]	Negative	G/A
Dysplasia	46,XX,del(7)(q22)[8]/46,XX,del(5)(p11)/46,XX[19]	Positive	G/A
Hypoplasia	Normal	Positive	G/A

* Case 6 is diagnosed as acute myeloid leukemia. The myeloblasts count 41% of the nucleated cells according to morphologic test of bone marrow smears.

** Case 11 is diagnosed as myelodysplastic syndrome. His bone marrow morphology shows dysplasia was observed in his granulocytic lineage and megakaryocytic lineage with the myeloblasts count 6% of the nucleated cells. The result of his karyotype is:46,XY,dup(1)(q21q23),add(2)(p11.2),add(3)(q27),der(5)t(1;5)(q21;q35),add(20)(p12)[17]/45,XY,der(1)(?:1q42->1q21::1p36.3->1q32::1q21->1q44::?),add(2)(p11.2),add(3)(q27),add(4)(p16),der(5)t(1;5)(q21;q35),-18,add(20)(p12),ace[2]/46,XY[1]

Chromosome breakage tests were induced by mitomycin C.

NA, not available; AML, acute myeloid leukemia; MDS, myelodysplastic syndrome.

Table 3 Variant details

* Case 5, Case 18, and Case 24 carries homozygous variants.

NA, not available.

Table 4 Treatment and outcomes of the 24 FA patients.

Case No.	Gene	Mutation 1 (maternal)			Mutation 2 (paternal)		
		Genomic location	cDNA/Protein	Ref./Com.	Genomic location	cDNA/Protein	Ref./Com.
1	<i>FANCA</i>	chr16:89831465	c.2611C>G/p.L871V	NA	chr16:89808940-89809954	c.3627-607_3765+268del	16
2	<i>FANCA</i>	chr16:89833593	c.2557C>T/p.R853X	21	chr16:89877396	c.367C>T/p.Q123X	NA
3	<i>FANCA</i>	chr16:89815145-89815146	c.3270_3271delCT/p.C1090RfsX25	Novel	chr16:89868906-89875410	c.792+761_c.523-635del	Novel
4	<i>FANCA</i>	chr16:89877396	c.367C>T/p.Q123X	NA	chr16:89818822	c.2982-192A>G	23
5*	<i>FANCA</i>	chr16:89842183	c.1867C>T/p.Q623X	Novel	chr16:89842183	c.1867C>T/p.Q623X	Novel
6	<i>FANCA</i>	chr16:89804935-89806139	c.3935-178_4368+74del	Novel	chr16:89819567-89839134	c.c.2014+545_2982-937del	Novel
7	<i>FANCA</i>	chr16:89811185-89815741	c.3239+397_3626+202del	Novel	chr16:89858887	c.1074_1075delGT/p.Y359PfsX49	NA
8	<i>FANCA</i>	chr16:89826812-89919023	<i>FANCA</i> c.2852+1545_5167del	Novel	chr16:89825071	c.2894_2895delCT/p.P965RfsX9	Novel
9	<i>FANCA</i>	chr16:89780001-89822000	<i>VPS9D1</i> c.432-877_89822000del	16	chr16:89808940-89809954	c.3627-607_3765+268del	16
10	<i>FANCA</i>	chr16:89823177-89825446	c.2853-333_2981+1808del	Novel	chr16:89809270	c.3703C>T/p.Q1235X	Novel
11	<i>FANCA</i>	chr16:89818619	c.2990_2993delGTTA/p.S997MfsX28	NA	chr16:89862229	c.987_990delTCAC/p.H330AfsX4	24,27
12	<i>FANCA</i>	chr16:89816286	c.3091C>T/p.Q1031X	NA	chr16:89792569-89821767	<i>ZNF276</i> c.1007-1118_89821767del	Novel
13	<i>FANCA</i>	chr16:89806417	c.3918dupT/p.Q1307SfsX6	25	chr16:89831438	c.2638C>G/p.R880G	NA
14	<i>FANCA</i>	chr16:89858941	c.1021C>T/p.Q341X	Novel	chr16:89811412	c.3581C>T/p.P1194L	25
15	<i>FANCB</i>	chrX:14868651	c.1472T>A/p.V491E	Novel	—	—	—
16	<i>FANCB</i>	chrX:14875979	c.1197+5insA	Novel	—	—	—
17	<i>FANCB</i>	chrX:14877390	c.1018C>A/p.Q340K	Novel	—	—	—
18*	<i>FANCC</i>	chr9:97912346	c.545C>A/p.S182Y	Novel	chr9:97912346	c.545C>A/p.S182Y	Novel
19	<i>FANCD2</i>	chr3:10084828	c.983G>A/p.R328Q	NA	chr3:10114634	c.2574T>G/p.I858M	Novel
20	<i>FANCD2</i>	chr3:10132005	c.3713T>A/p.M1238K	NA	chr3:10089599	c.1279-2A>T	Novel
21	<i>FANCE</i>	—	—	NA	chr6:35426215	c.1111C>T/p.R371W	22,23,24
22	<i>FANCE</i>	chr6:35423547	c.272C>T/p.P91L	Novel	chr6:35427467-35427470	c.1246_1249delCAAA/p.T417SfsX7	NA
23	<i>SLX4</i>	chr16:3645671	c.1948C>T/p.L650F	NA	chr16:3633419	c.4832A>G/p.E1611G	NA
24*	<i>ERCC4</i>	chr16:14015937	c.257G>A/p.R86H	NA	chr16:14015937	c.257G>A/p.R86H	NA

Therapeutics	Donor & HLA matching	Pre-HSCT conditioning regimen	Outcomes
HSCT	UUD 7/10	Bu+CTX+Flu+Alemtuzumab	Alive
HSCT	UUD 8/10	Bu+CTX+Flu+Alemtuzumab	Dead (aGVHD, infections)
HSCT	UUD 9/10	Bu+CTX+Flu+ATG+Me-CCNU	Alive
HSCT	MUD 10/10	Bu+Flu+CTX+ATG	Alive
HSCT	HRD 8/10	Bu+CTX+Flu+ATG+Me-CCNU	Alive
HSCT	HRD 6/10	Decitabine+Ara-C+Bu+Flu+ATG+Me-CCNU	Dead (aGVHD, drug-induced encephalopathy)
	—	—	Alive
Androgen and transfusion			
HSCT	HRD 7/10	Decitabine+Ara-C+Bu+Flu+ATG+Me-CCNU	Dead (aGVHD, MODS)
Lost follow-up	—	—	—
HSCT	MRD 10/12	Bu+Flu+CTX+ATG	Dead (aGVHD, septic shock)
HSCT	HRD 5/10	Decitabine+Ara-C+Bu+Flu+ATG+Me-CCNU	Dead (aGVHD, septic shock)
HSCT	UUC 5/8	Bu+Flu+CTX+ATG	Dead (aGVHD, pulmonary infection, CMV infection)
Androgen, cytokine, transfusion	—	—	Alive
	—	—	Alive
Androgen and cytokine			
HSCT	HRD 7/10	Bu+Flu+CTX+ATG	Dead (aGVHD, TMA, pulmonary infection)
HSCT	MUD 10/10	Bu+Flu+CTX+ATG	Alive
HSCT	HRD 7/10	Bu+Flu+CTX+ATG	Alive
	—	—	Dead (pulmonary infection, septic shock)
Androgen and transfusion			
HSCT	MUD 10/10	Bu+Flu+CTX+ATG	Alive
HSCT	UUD 8/10	Bu+Flu+CTX+ATG	Alive
HSCT	UUD 9/10	TBI+CTX+Flu+ATG	Alive
HSCT	HRD 6/10	Bu+Flu+CTX+ATG	Alive
HSCT	MUD 10/10	Bu+Flu+CTX+ATG	Alive
Lost follow-up	—	—	—

HSCT, hematologic stem cell transplantation; UUD, HLA-unmatched unrelated donor; MUD, HLA-matched unrelated donor; HRD, HLA-haploidentical related donor; MRD, HLA-matched related donors; UUC, HLA-unmatched unrelated cord blood; Bu, Busulfan; CTX, cyclophosphamide; Flu, Fludarabine; ATG, antithymocyte globulin; Me-CCNU Semustine; TBI; total body irradiation; aGVHD, acute graft-versus-host disease; CMV, cytomegalovirus.

Figures

Figure 1

Distribution and composition of the 44 mutations. a. mutation distribution. b. mutation composition.

Figure 2

Locations, frequencies, and types of mutations in FANCA, FANCB, FANCC, FANCD2, FANCE, SLX4, and ERCC4 genes. Exons represent by colored rectangles; mutation types are represented by colored patterns; large deletions are represented by the black horizontal bars.

Supplementary Files

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