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Letter to the Editor

Prevalence of germline predisposition gene mutations in pediatric acute myeloid leukemia: Genetic background of pediatric AML

Dear Editor,

Acute myeloid leukemia (AML) is a heterogeneous hematopoietic stem cell disorder of which development is either acquired and/or inherited. In adults, AML has been regarded as an acquired disease [1], while infantile leukemia and familial leukemia elucidated a role of genetic predisposition to leukemia. The importance of germline predisposition in myeloid malignancy is being more and more emphasized due to its clinical significance; different clinical management, genetic counseling for family members and hematopoietic stem cell donor selection. The family members are recommended to take genetic counseling and may benefit from the early and regular surveillance [2,3]. Recent studies demonstrated the prevalence of genetic susceptibility genes in pediatric cancers [4] and inherited bone marrow failure [5]. However, prevalence of germline predisposition genes in AML has not been reported both in adults and children. In this study, we investigated prevalence of genetic predisposition gene mutations in pediatric AML and compared the mutational profile of somatic mutation with that of adult AML in Korea.

A total of 17 pediatric patients who visited the department of pediatrics in Seoul National University Children's Hospital and were diagnosed with AML from 2013 to 2015 were enrolled in this study. The median age of the patients was 7 years old (range 0–17) and M:F ratio was 0.9. Family history of cancer was present in 5 (29.4%) patients: one leukemia and four other cancers (Tables 1 and 2). Seventeen bone marrow (BM) samples at initial diagnosis and 16 paired specimen of remission (less than 5% blasts in BM without evidence of residual cells by immunohistochemical stain and cytogenetic results, Supplementary Methods) and 1 saliva sample were collected. Chromosome analysis by conventional G-banding technique and fluorescent in situ hybridization (FISH) were performed to detect abnormalities related to AML and/or myelodysplastic syndrome (MDS) (Supplementary Methods). Targeted multi-gene sequencing was performed using 356 or 507 gene panel which includes WHO 2016 genetic predisposition genes and known leukemia-related genes (Supplementary Table S1). Variants that existed both in initial and remission BM or saliva specimen with variant allele frequency (VAF) of 30-70% were sorted out as potential germline variants. A total of 95 genes were analyzed for germline mutation analysis (Supplementary Table S2). Determination of pathogenicity was assessed according to ACMG 2015 guideline [6]. Meanwhile, variants that were present only in initial diagnosis BM and absent in remission samples or saliva were deemed as potential somatic variants. Then, mutations which were previously reported in ClinVar, International Cancer Genome Consortium (ICGC) and Catalogue of Somatic Mutations in Cancer (COSMIC) were classified as a somatic variants (Supplementary Figure S1). This study was approved by the institutional review board (IRB) of Seoul National University Hospital (IRB 1508-075-695), and the requirement for obtaining informed consent was waived.

G-banding analysis revealed that 16 out of 17 (94.1%) patients had abnormal karyotype and only 1 patient carried normal karyotype. Compared to reported frequency of 54.9% and 76.1% cytogenetic aberrations in Caucasian adult and pediatric AML respectively [7], our data showed much higher percentage of abnormal karyotype. Based on both G-banding and FISH results, 10 (58.8%) patients showed gene fusions: 6 (35.3%) *KMT2A* rearrangement, 3 (17.6%) *RUNX1-RUNX1T1* fusions and one (5.9%) *FUS-ERG* fusion. Complex karyotype ($\geq$3 cytogenetic abnormalities) were most common (n = 6, 37.5%), followed by karyotype with two numerical and/or structural abnormalities (n = 5, 23.5%) (Fig. 1, Table 2).

A total of 18 germline variants were detected in 11 patients: six (33.3%) variants of Fanconi anemia genes (*FANCA*, *FANCD2*, *FANCI*, *PALB2* and *SLX4*), 4 (22.2%) variants of 3 genes related to telomere biology disorder (*CTC1*, *RTEL1*, and *WRAP53*), 2 (11.1%) variants of germline myeloid neoplasm-associated genes (*DDX41* and *RUNX1*), 2 (11.1%) variants of 1 gene of severe congenital neutropenia (*VPS13B*) and 4 (22.2%) other cancer-related genes (*ATM*, *BRCA1*, *MLH1* and *MSH6*). Mean number of mutated germline predisposition genes per

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Table 1
Clinical characteristics of 17 pediatric AML patients.

Characteristics	Total (N = 17)
Age, years[a]	7 (0–17)
< 1 year, n (%)	1 (5.9)
Sex, n (%)	
Male	8 (47.1)
Female	9 (52.9)
Organomegaly, n (%)[b]	
Hepatomegaly	8 (47.1)
Splenomegaly	6 (35.3)
Extramedullary involvement	
Skin	1 (5.9)
Family history, n (%)	
Leukemia	1 (5.9)
Other cancers	4 (23.5)
Hematologic parameters[a]	
Hb (g/l)	8.3 (6.0–10.6)
WBC ($\times 10^9$/l)	7.29 (1.55–209.49)
Platelet ($\times 10^9$/l)	62 (23–291)
BM blast (%)[c]	73.5 (21.2–94.6)
FAB classification, n (%)	
FM1	1 (5.9)
M2	4 (23.5)
M3	1 (5.9)
M4	7 (41.2)
M5	2 (11.8)
M6	0 (0.0)
M7	2 (11.8)

[a] Values presented as the median (range).
[b] Six patients had hepatosplenomegaly.

one patient was 0.6. Evaluating pathogenicity based on ACMG 2015 guideline, 3 (16.7%) variants in 2 patients, that is, *DDX41* mutation (c.1547A > G, p.Y516C), *SLX4* mutation (c.5071_5073del, p.1691_1691del) and *WRAP53* mutation (c.1565delC, p.A522Gfs*26), were classified as likely pathogenic (LP). Fifteen (83.3%) variants in 9 patients were assessed as variant of unknown significance (VUS) (Fig. 1, Table 2). It was an interesting finding that *DDX41* mutation, of which presentation has been reported to range from 44 to 88 years old was detected in a 16-year-old patient [8]. Consequently, prevalence of germline predisposition gene mutations in pediatric AML patients was estimated to be 11.8%. When including VUS, the frequency was 58.8%. Zhang et al. reported that prevalence of germline mutations is 8.5% in pediatric solid tumor and 4.4% in leukemia, using whole genome study [4]. Our study revealed higher prevalence (11.8%) of cancer susceptibility gene mutations in pediatric AML using 95 multi-gene panel. Compared to the gene panel of Zhang et al.'s [4], the panel of the present study additionally included congenital neutropenia-related genes (*CSF3R*, *CXCR4*, *USB1*, *VPS13B*, *GFI1*, *AP3B1*, *SLC37A4* and *RMRP*), more Fanconi anemia gene (*SLX4*), telomere biology genes (*TERC* and *CTC1*) and predisposition genes related to myeloid neoplasm (*DDX41*, *ANKRD26*, *SRP72* and *SAMD9*). We suppose that prevalence of germline predisposition gene mutation is high in pediatric AML than other solid tumor or other kinds of leukemia.

Twelve out of 17 (70.6%) patients carried somatic mutations. Nine out of 12 (75.0%) patients harbored germline mutations as well. A total of 26 somatic variants in 19 genes were detected: 14 (53.8%) variants of 7 signaling genes (*FLT3*, *KIT*, *KRAS*, *ATR, BCR*, *MAP3K1* and *ALPK2*), 2 (7.7%) variants of chromatin-modifying genes (*BRD4* and *NCOA3*), 3 (11.5%) variants of transcription factors (*CREBBP*, *GATA1* and *ZNF93*), 1 (3.8%) variant of cohesin complex gene (*RAD21*), 2 (7.7%) variants of spliceosome-complex genes (*SF1* and *TRA2B*) and 4 (14.8%) variants of other genes (*DDX54*, *ZNF676* and *MUC16*). *FLT3* mutations were the most common (n = 4, 15.4%) followed by *KRAS* (n = 3, 11.5%) (Fig. 1, Table 2). Mean number of somatic mutations per one patient was 1.5, which is lower than that of Korean adult AML that is reported to be about 2.0 by whole exome sequencing [9]. Meanwhile, mean number of coding variants per individual with pediatric AML has been reported to be approximately 3 by targeted sequencing of 39 cancer-related genes [10]. In short, the number of somatic mutations was relatively lower in our data, compared to adult AML as wells as pediatric AML. Considering higher percentage (94.1%) of cytogenetic aberrations in the present study, genetic alteration occurring at chromosome level might be more important in the development mechanism of pediatric AML than variants at single nucleotide level. Among 19 somatically mutated genes of pediatric AML patients, 31.6% of them overlapped with those of Korean adult AML (*FLT3*, *KRAS*, *KIT*, *RAD21, ATR* and *CREBBP*), while 14 genes (*BCR*, *ALPK2*, *BRD4*, *DDX54*, *GATA1*, *MAP3K1*, *MUC16*, *NCOA3*, *SF1*, *TRA2B*, *WRAP53*, *ZNF93*, and *ZNF676*) were identified only in pediatric AML (Fig. 2). Meanwhile, mutations that are commonly found in adult AML such as *NPM1*, DNA methylation modifying genes (*IDH1*, *IDH2*, *TET2* and *DNMT3A*) and tumor suppressor genes (*TP53* and *WT1*) were not present in pediatric AML patients [9,10]. Interestingly, one variant of histone modifying gene (*NCOA3*) was detected in 7-year-old M3 patient (Fig. 2). Somatic mutation of histone modifying gene might be involved in leukemogenesis of pediatric AML, although it is rare.

Our study has a limitation of absence of germline specimen which was replaced by BM specimen at the time of remission. In Korea, access to patients' families is difficult due to cultural emotion that people attribute abnormal children to maternal cause. However, as to our best knowledge, this is the first study to elucidate the prevalence of germline predisposition gene mutations in pediatric AML.

In conclusion, prevalence of germline predisposition gene mutations in Korean pediatric AML was estimated to be approximately 11.8%, which suggests that work-up for germline mutation is necessary in pediatric AML.

Conflicts of interest

No relevant conflicts of interest to declare.

Table 2
Cytogenetic and molecular profiles of 17 pediatric AML patients.

Patient number	Cytogenetics (initial)	FHx	Mutation category	Gene	Position	Nucleotide change	Protein change	Variant type
1	46,XY,der(7)r(7;?)(p22q36;?),-13, +mar[17]/46,XY[3]	Yes	Germline	*RTEL1*	62309666	c.335C > G	p.P112R	missense
			Somatic	*CREBBP*	3779211	c.5723delC	p.P1908fs	fs
			Somatic	*SF1*	64534503	c.1824_1826del	p.608_609del	in-frame del
			Somatic	*ZNF676*	22363617	c.902G > T	p.R301I	missense
2	51,XX, +6, +15, +19,der(20)t(1;20)(q25;q13.3), +21, +22[2]/51,idem,del(3)(q13.2q21)[6]/ 46,XX[3]	No	Germline	*FANCA*	89836366	c.2383A > G	p.R795G	missense
			Somatic	*TRA2B*	185637260	c.445_447del	p.149_149del	in-frame del
3	46,XX,t(8;21)(q22;q22)[17]/45,idem,-X [5]/46,XX[1]+	Yes	Germline	*PALB2*	23625404	c.3122A > C	p.K1041T	missense
			Somatic	*KRAS*	25398281	c.38G > A	p.G13D	missense
			Somatic	*BCR*	23653975	c.3142_3143insCCGG	p.S1048fs	fs
4	46,XX,t(16;21)(p11.2;q22)[8]/47,sl, +10[9]/48,sdl, +12[3]	No	Germline	*DDX41*[b]	176939499	c.1547A > G	p.Y516C	missense
			Somatic	*KRAS*	25398284	c.35G > A	p.G12D	missense
5	46,XX,16qh+[c] [19]	No	Somatic	*ALPK2*	56246440	c.1568delA	p.K523fs	fs
6	46,XY,add(7)(q32)[7]/45,idem,-Y[18] nuc ish(ETOX3,AML1X2)(ETO con AML1X1)[195/200]	No	Somatic	*FLT3*	28592641	c.2504A > T	p.D835V	missense
			Somatic	*KIT*	55589771	c.1253_1254insCTTCCT	p.Y418delinsYFL	in-frame ins
			Somatic	*WRAP53*	7606722	c.1565delC	p.A522fs	fs
			Somatic	*DDX54*	113601892	c.1916_1918del	p.639_640del	in-frame del
7	46,XX,t(11;19)(q23;p13.3)[20]	No	N/A	N/A	N/A	N/A	N/A	N/A
8	46,XX,t(9;11)(p22;q23)[9]/47,idem, +8[10]/46,XX[1]	No	Germline	*FANCI*	89843605	c.2698_2699insGGCAAT	p.R900delinsRQW	in-frame ins
			Somatic	*FLT3*	28592623	c.2522A > T	p.N841I	missense
			Somatic	*BRD4*	15375544	c.883A > C	p.T295P	missense
9	47,XX, +8,del(12)(p?12p13)[11]/46,XX[9] nuc ish(MLLX2)(5′MLL sep 3′MLLX1)[51/ 200]	Yes	N/A	N/A	N/A	N/A	N/A	N/A
10	45,X,-Y,t(8;21)(q22;q22)[20]	No	Germline	*MLH1*	37042521	c.283T > G	p.S95A	missense
			Germline	*VPS13B*	100454723	c.3305G > A	p.S1102N	missense
			Germline	*SLX4*[b]	3633178	c.5071_5073del	p.1691_1691del	in-frame del
			Germline	*WRAP53*[b]	7606722	c.1565delC	p.A522fs	fs
			Somatic	*KIT*	55599320	c.2446G > C	p.D816H	missense
11	46,XX,t(11;19)(q23;p13.1)[20]	No	Germline	*CTC1*	8137847	c.1744C > T	p.P582S	missense
			Somatic	*FLT3*	28608262	c.1793_1794ins CTACGTTGATTTCAGAG AATATGA	p.E598delinsDYVDFREY-E	in-frame ins
12	46,XY,t(11;19)(q23;p13.3),21pstk+ [14]/46,XY,21pstk+ [6]	No	Germline	*BRCA1*	41219631	c.4927A > C	p.K1643Q	missense
13	46,XY,?ins(1;14)(q32;q31q13),del(9) (q13q22)[19]/46,XY[2]	No	Germline	*FANCD2*	10116340	c.2842A > G	p.T948A	missense
			Germline	*VPS13B*	100168893	c.2130G > C	p.Q710H	missense
			Germline	*RUNX1*	36259286	c.124G > C	p.G42R	missense
14	46,XY,add(4)(p16),add(7)(p13)[17]/ 46,XY[3]	Yes	Germline	*WRAP53*	7606402	c.1360G > A	p.V454M	missense
			Somatic	*NCOA3*	46279834	c.3757_3759del	p.1253_1253del	in-frame del
			Somatic	*MAP3K1*	56177848	c.2821_2823del	p.941_941del	in-frame del
15	46,XX,t(9;11)(p22;q23)[13]/46,XX[9]	Yes	Somatic	*FLT3*	28592640	c.2505T > G	p.D835E	missense
			Somatic	*ATR*	142274740	c.2320delA	p.I774fs	fs
			Somatic	*BCR*	23653975	c.3142_3143insCCGG	p.S1048fs	fs
			Somatic	*ZNF93*	20045067	c.1303G > A	p.V435I	missense
16	46,XY,add(11)(q23)[2]/49,idem, +6, +8, +22[7]/46,XY[11]	No	N/A	N/A	N/A	N/A	N/A	N/A

(*continued on next page*)

Table 2 (*continued*)

Patient number	Cytogenetics (initial)	FHx	Mutation category	Gene	Position	Nucleotide change	Protein change	Variant type
17	47,XY,9qh-, +21[14]/46,XY,9qh-[6]	No	Germline	*MSH6*	48023107	c.532C > T	p.R178C	missense
			Germline	*ATM*	108142088	c.3032C > G	p.T1011R	missense
			Germline	*PALB2*	23641346	c.2129C > T	p.T710M	missense
			Somatic	*KRAS*	25398284	c.35G > A	p.G12D	missense
			Somatic	*RAD21*	117878960	c.9C > G	p.Y3X	stop
			Somatic	*GATA1*	48649655	c.139delT	p.S47fs	fs
			Somatic	*MUC16*	8999554	c.40621G > C	p.D13541H	missense

Patient number	MAF[a]	SIFT	PolyPhen-2 HDIV	PolyPhen-2 HVAR	MT	MA	FATHMM	CADD
1	0.0006	T	D	D	D	M	D	23.8
	0.0038	.	.	.	.	.	.	.
	0.0078	.	.	.	.	.	.	.
	.	T	B	B	D	L	T	1.86
2	4.177E-05	T	B	B	N	M	D	13.38
	0.0010	.	.	.	.	.	.	.
3	1.73E-05	D	D	B	D	M	T	25.8
	.	D	P	P	D	M	T	28.8
	.	.	.	.	.	.	.	.
4	.	D	D	D	D	H	T	27.1
	0.0001	D	P	B	D	M	T	25.3
5	.	.	.	.	.	.	.	.
6	.	D	D	D	D	N	D	33
	.	.	.	.	.	.	.	.
	0.0010	.	.	.	.	.	.	.
	.	.	.	.	.	.	.	.
7	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
8	0.0009	.	.	.	.	.	.	.
	.	D	P	P	D	L	D	32
	.	D	D	D	D	M	T	23.4
9	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
10	0.0001	T	B	B	D	N	D	7.565
	0.0001	T	D	D	D	L	T	27.6
	.	.	.	.	.	.	.	.
	0.0011	.	.	.	.	.	.	.
	.	D	P	B	D	L	D	25.2
11	.	T	B	B	N	N	D	0.001
	.	.	.	.	.	.	.	.
12	0.0006	D	D	D	D	L	D	23.6
13	0.0001	D	B	B	N	M	T	23.3
	.	D	B	B	D	L	T	11.03
	0.0005	T	D	D	D	L	D	24.8
14	0.0002	T	P	B	N	M	T	12.14
	0.0003	.	.	.	.	.	.	.
	.	.	.	.	.	.	.	.
15	.	D	D	D	D	N	D	24.2
	0.0084	.	.	.	.	.	.	.
	.	.	.	.	.	.	.	.
	.	T	B	B	N	N	T	0.006
16	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

(*continued on next page*)

Table 2 (*continued*)

Patient number	MAF[a]	SIFT	PolyPhen-2 HDIV	PolyPhen-2 HVAR	MT	MA	FATHMM	CADD
17	.	T	D	B	D	L	T	27.7
	.	D	B	B	N	M	T	16.66
	8.637E-05	D	D	D	N	M	T	24.2
	0.0001	D	P	B	D	M	T	25.3
	.	.	.	.	A	.	.	39
	.	.	.	.	.	.	.	.
	4.66E-005	T	D	D	N	L	T	12.63

Abbreviations: A, disease-causing automatic (MT); B, benign (Polyphen-2); CADD, combined annotation dependent depletion; Chr, chromosome; D, damaging (Polyphen-2); D, deleterious (SIFT, FATHMM); D, disease-causing (MT); del, deletion; FHx, family history; FATHMM, Functional Annotation Through Hidden Markov Model; fs, frameshift indel; H, predicted functional, high (MA); ins, insertion; L, predicted non-functional, low (MA); LP, likely pathogenic; M, predicted functional, medium (MA); MA, Mutation Assessor; MAF, minor allele frequency; MT, Mutation Taster; N, predicted non-functional, neutral (MA); N, polymorphism (MT); N/A, not applicable; P, probably or possibly damaging (Polyphen-2); SIFT, Sorting Tolerant From Intolerant; T, tolerated (SIFT, FATHMM); VUS, variant of unknown significance.

[a] The highest value among 1000 Genomes, ESP6500 and Exome Aggregation Consortium.

[b] Likely pathogenic variants according to ACMG 2015 guideline.

[c] Normal variant.

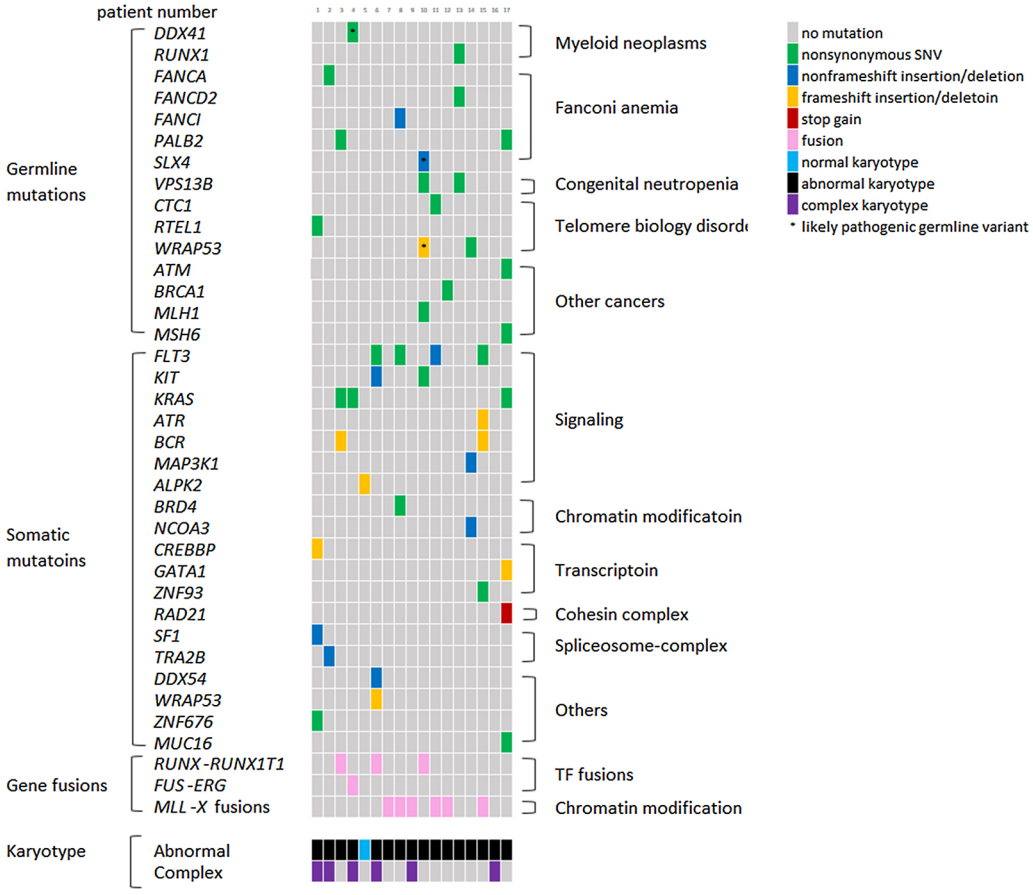

Fig. 1. Germline, somatic mutations and cytogenetics of 17 pediatric AML patients.

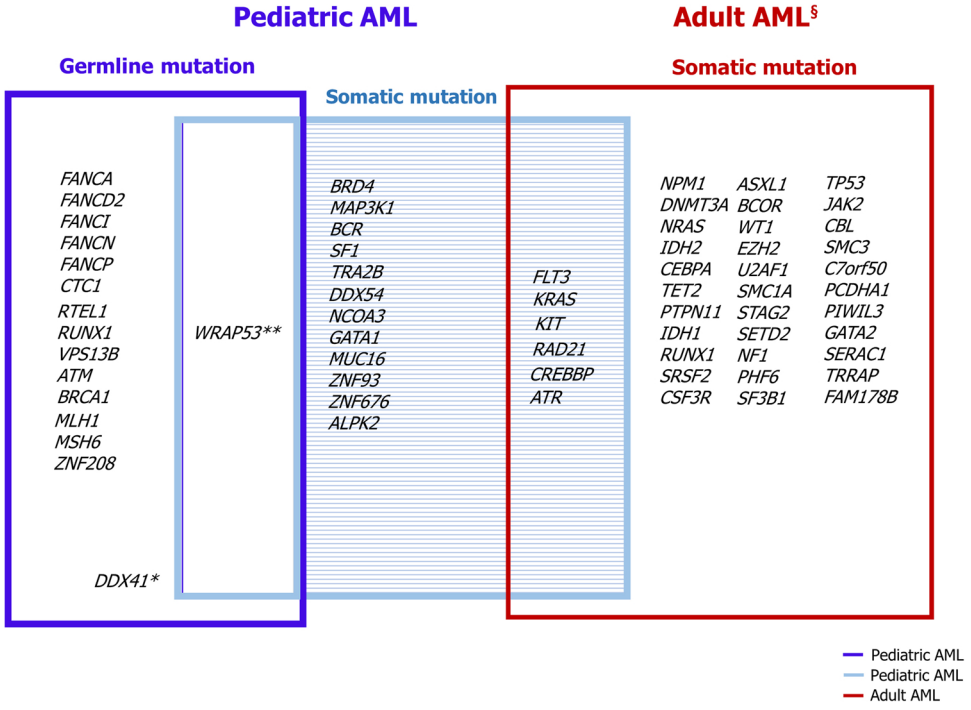

Fig. 2. Germline and somatic mutations detected in 17 pediatric AML patients and comparisons with known adult-onset germline predisposition genes and Korean adult AML somatic mutations. *Late-onset germline predisposition gene [8]. **_WRAP53_ mutations were detected as both germline and somatic variants in different pediatric patients in our data. §Somatic mutations of Korean adult AML [9]. _Abbreviation_: AML, acute myeloid leukemia.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.leukres.2019.106210.

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Dajeong Jeong[a], Dong Soon Lee[a,c,*], Namhee Kim[a], Seongmin Choi[b], Kwangsoo Kim[b], Sung-Min Kim[c], Kyongok Im[c], Hee Sue Park[d], Jiwon Yun[a], Kyu Min Lim[c], Kyoung Soo Park[e], Hyoung Jin Kang[f], Yong-Oon Ahn[c], Sang Mee Hwang[g]

[a] *Department of Laboratory Medicine, Seoul National University Hospital, Seoul, Korea*
[b] *Division of Clinical Bioinformatics Biomedical Research Institute, Seoul National University Hospital, Seoul, Republic of Korea*
[c] *Cancer Research Institute, Seoul National University College of Medicine, Seoul, Republic of Korea*
[d] *Department of Laboratory Medicine, Chungbuk National University Hospital, Cheongju, Korea*
[e] *Department of Internal Medicine, Seoul National University College of Medicine, Seoul National University Hospital, Seoul, Korea*
[f] *Department of Pediatrics, Cancer Research Institute, Seoul National University College of Medicine, Seoul, Korea*
[g] *Department of Laboratory Medicine, Seoul National University Bundang Hospital, Seongnam, Korea*

E-mail address: soonlee@snu.ac.kr (D.S. Lee).

* Corresponding author.