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Germline pathogenic variants in transcription factors predisposing to pediatric acute myeloid leukemia: results from the French ELAM02 trial

Genetic predisposition to hematologic malignancies represents a heterogeneous group of rare disorders that have been included as distinct entities in the revised World Health Organization 2016 classification of myeloid neoplasms and acute leukemia.[1] The diagnosis of patients with these disorders is of clinical relevance, as they can benefit from specific clinical care, adapted surveillance and genetic counseling.[2-4] Clinical features as well as personal or family history may help to ascertain genetic predisposition to hematologic malignancies. However, up to 40% of patients diagnosed with a cancer predisposition syndrome or inherited bone marrow failure do not have any of these features.[5] The very broad variations in the type of hematologic malignancies, age at onset and penetrance also contribute to a lack of clinical recognition. Consequently, many patients present with a diagnosis of an apparently sporadic hematologic malignancy and it is assumed that cases with a genetic predisposition are currently underestimated.

The wide use of high-throughput sequencing technologies has revolutionized the genetic characterization of malignant diseases. Leukemogenesis is now usually considered as a multistep process involving many gene classes and pathways. Notably, some relevant genes, including transcription factors (*CEBPA, ETV6, GATA2, RUNX1* and *TP53*), are targets of both somatic and germline pathogenic variants or more rarely deletions.[6,7] Sequencing gene panels used clinically for the genetic characterization of hematologic malignancies usually include transcription factor genes, and may represent a gateway to the identification of germline variants.[7,8] High-throughput sequencing offers the possibility to estimate variant allele frequencies (VAF) and a broad view of tumor architecture. Specifically, pathogenic variants with low VAF are assumed to be somatically acquired while pathogenic variants with a VAF of ≥50% may indicate either somatic or germline origin requiring the study of non-tumor samples. Additionally, several studies have pointed out somatic mechanisms, including variant amplification (chromosome doubling, uniparental disomies) or double mutations that are associated with transformation in patients with a germline disorder.[3,9]

Here we aimed at retrospectively identifying syndromes with a genetic predisposition due to germline pathogenic variants or deletions in transcription factors (*CEBPA, ETV6, GATA2, RUNX1* and *TP53*) using a practical, molecular-based algorithm in pediatric patients with acute myeloid leukemia (AML) from the French ELAM02 cohort.

The randomized, prospective, multicenter, phase III ELAM02 trial (clinicaltrials.gov identifier: NCT00149162) was conducted from March, 2005 to December, 2011 in pediatric patients (aged 0-18 years old) with AML.[10] Children with secondary AML and AML with an underlying known predisposition disorder (Down syndrome, chromosomal breakage diseases, Kostman and Schwachman-Diamond diseases) were excluded. Of note, two patients have been previously reported: one with a germline *GATA2* pathogenic variant and the other one with a germline *RUNX1* deletion.[9,11] Patients with available amplicon-based high-throughput sequencing data and information from single nucleotide polymorphism (SNP)-array analyses at the time of diagnosis of AML were selected for the present study (385 patients out of the 438 patients included in the ELAM02 trial). The molecular profiling using a 36-gene panel with a high depth of coverage (>1500×) at AML diagnosis was reported previously[10] (*Online Supplementary Table S1*). *CEBPA* mutations were systematically sought by Sanger sequencing because of insufficient coverage by the high-throughput sequencing. Single nucleotide variants were selected based on the absence of a description in public databases of human polymorphisms, and their functional effect was assessed by *in silico* predictions as previous-

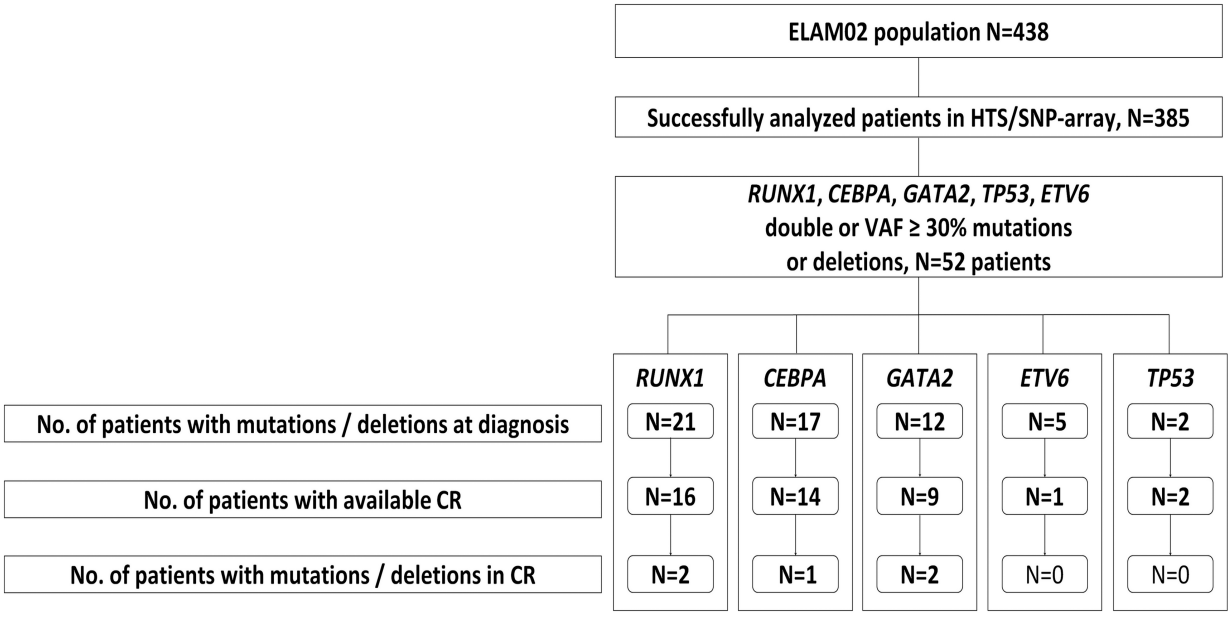

Figure 1. Study workflow. HTS: high-throughput sequencing; SNP: single nucleotide polymorphism; VAF: variant allele frequency; CR: complete remission.

Table 1. Characteristics of patients with identified germline mutations or deletions.

Patient ID	Sex	Age at Dx	FAB subtype	WBC count ($x10^9$/L)	BM blast count	Cytogenetics	Germline deletion/mutation	ACMG-AMP clinical significance (rules applied[¥])	Somatic mutations at AML diagnosis	Ref.
Patient 1	F	3	M1	13.4	50	46,XX[30]	*CEBPA* NM_004364.3: c.65_125del (p.Pro22Leufs*118)	Pathogenic	*CEBPA* NM_004364.3: c.934_936dup (p.Gln312dup) *WT1* NM_024426.3: c. 1357_1361delins AGTAG (p.Cys453_Lys454delinsSerArg) (VAF=26%)	
Patient 2	F	13	M5	4	77	45,XX,-7,t(11;19)(q23;p13.3)[3]/47,idem,+15,+20[21]	*GATA2* NM_032638.4:c. 1114G>A (p.Ala372Thr) (VAF=50%)	Likely pathogenic	None	10
Patient 3	M	16	M0	105	NA	45,XY,-7 [5]	*GATA2* NM_032638.4:c.1008del (p.Lys336Asnfs*51) (VAF=48%)	Pathogenic	*JAK2* NM_004972.3:c. 1849G>T (p.Val617Phe) (VAF=44%) *SETBP1* NM_015559.2:c.2602G>T (p.Asp868Tyr) (VAF=42%)	
Patient 4	F	9	M6	3	18	47,XX,+8[14]/49,XX,+8,+add (9)(q22),+21[9]	*RUNX1* NM_001754.4: c.601C>T (p.Arg201*) (VAF=48%)	Pathogenic	*KRAS* NM_NM_033360.2: c.38G>A (p.Gly13Asp) (VAF=19%)	
Patient 5	F	14	M6	1.7	21	48.XXXc.+21[22]/47,XXXc[3]	*RUNX1* NM_001754.4 deletion (size:1.8 Mb, CN: 1.0, mean log_2 ratio: -0.54)	Pathogenic	None	9

[¥]The interpretation of the pathogenicity of the identified germline alterations was based on the American College of Medical Genetics and Genomics and Association for Molecular Pathology guidelines and the adapted guidelines from the ClinGen Myeloid Malignancy Variant Curation Expert Panel for the *RUNX1* gene.[12,13] Chromosomal locations are annotated according to human reference assembly GRCh37/hg19. Rules applied to classify the variants are detailed in *Online Supplementary Table S4.* Dx: diagnosis; FAB: French-American-British classification; WBC: white blood cells; BM: bone marrow; ACMG/AMP: American College of Medical Genetics and Genomics/Association for Molecular Pathology; AML: acute myeloid leukemia; F: female; M: male; CN: copy number state. NA: not available.

ly described.[10] Cytoscan HD array (ThermoFisher) was used, according to the manufacturer's instructions, to screen for gene deletions. The data were analyzed using Chromosome Analysis Suite (ChAS) 3.1 software (ThermoFisher). Since germline specimen collection was not planned by the ELAM02 protocol, blood samples taken at the time of complete remission were used to assess the germline status of variants and deletions by direct Sanger sequencing and SNP-array, respectively. Complete remission was defined as cytological remission associated with a level of *WT1* expression below 2% in the bone marrow and below 0.1% in peripheral blood or a core-binding factor transcript expression level below 0.01% in core-binding factor AML. The pathogenic status of the suspected germline alterations was established as per American College of Medical Genetics and Genomics and Association for Molecular Pathology (ACMG-AMP) guidelines.[12,13] The study was approved by the Ethics Committee of Saint-Antoine Paris University Hospital (Paris, France) and was conducted in accordance with the Declaration of Helsinki.

Investigations were restricted to patients with deletions, double pathogenic variants or unique pathogenic variants with a high VAF (a threshold of ≥30% was chosen to avoid being too restrictive) in one of the following genes: *CEBPA, ETV6, GATA2, RUNX1* and *TP53* at AML diagnosis. Of note, as the *cis-trans* allelic configuration for identified variants was not assessed for this study, the term double mutation was preferred, when appropriate.

Fifty-two patients (13%) out of the 385 analyzed met at least one of these criteria (45 with pathogenic variants and 7 with targeted deletions) (*Online Supplementary Table S2*). Among these patients, 17 had *CEBPA* double pathogenic variants, including one patient with a homozygous pathogenic variant. *RUNX1* pathogenic variants were found in 16 patients (4 with double mutations and 12 with a VAF ≥30%). *GATA2*, *ETV6* and *TP53* pathogenic variants with a VAF ≥30% were identified in 12, three, and two patients respectively. Targeted deletions encompassing *RUNX1* or *ETV6* were found in five and two patients, respectively (size range: 50 kb to 1.8 Mb) (Figure 1). Three of the 52 patients did not reach complete remission, including one patient who died during induction.

Thirty-eight patients had available DNA samples at the time of complete remission. Direct Sanger sequencing and SNP-array analysis of these samples identified five patients with still detectable alterations comprising four with pathogenic/likely pathogenic variants (*CEBPA*, n=1; *GATA2*, n=2; *RUNX1*, n=1) and one with a pathogenic deletion (*RUNX1*; size: 1.8 Mb) (Table 1, *Online Supplementary Table S3, Online Supplementary Figure S1*).

Patient n. 1 was a 3-year old girl who carried a frameshift *CEBPA* pathogenic variant (NM_004364:c.65_125del (p.Pro22Leufs*118)) (Figure

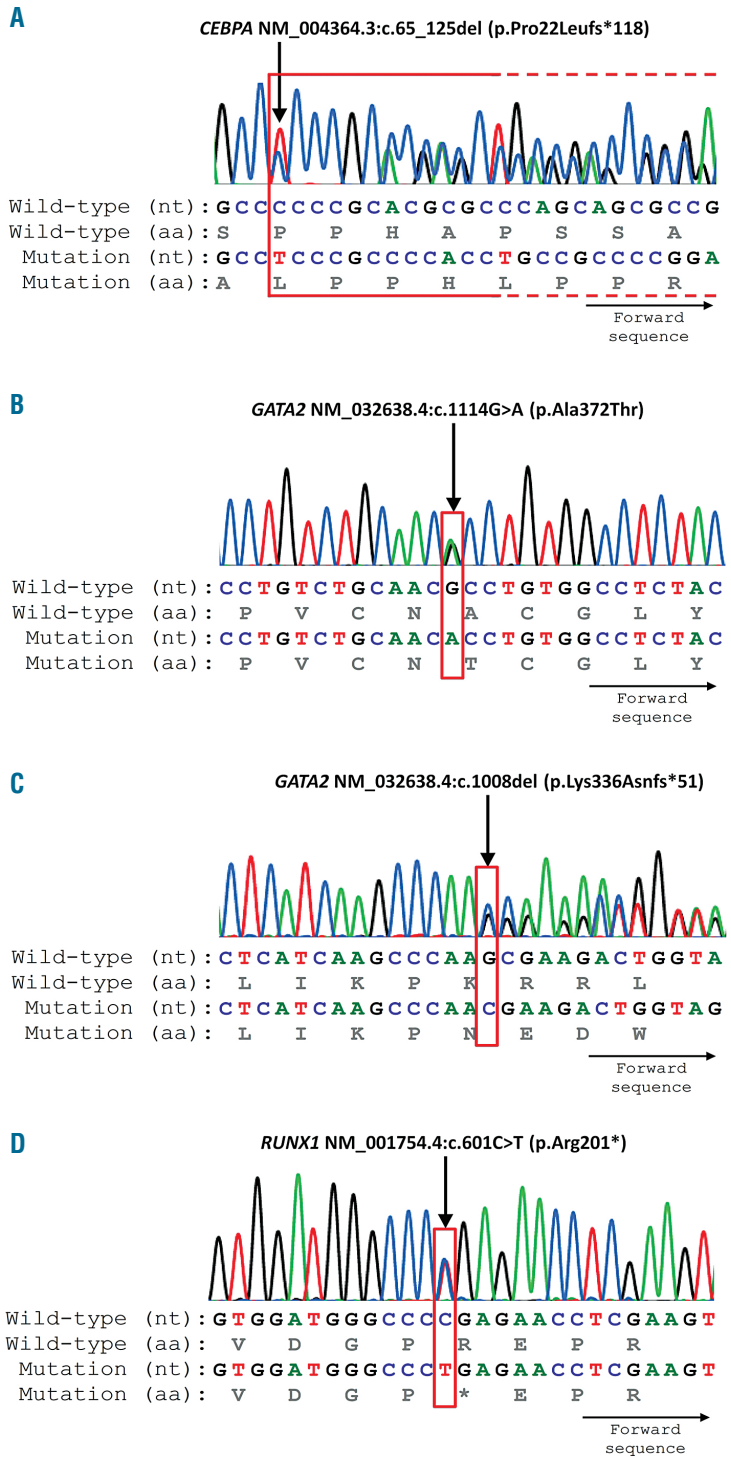

Figure 2. Variants confirmed by Sanger sequencing in samples taken at complete remission. nt: nucleotide; aa: amino acid.

2A) in the N-terminal part of the protein. The patient's family history revealed leukemia in her paternal grandmother at the age of 30 years and childhood leukemia in her paternal aunt (*Online Supplementary Figure S2*). Most of the described germline *CEBPA* mutations include N-terminal frameshift upstream of the p30 start codon and are supposed to be highly penetrant.[3] Interestingly, the *CEBPA* variant in our patient was subsequently found to be inherited from the proband's father who had no history of leukemia. Along with the family history, the detection of the *CEBPA* variant in the proband's complete remission sample and in her father is consistent with a germline mutation.

Patient n. 2 was a 13-year old girl with a *GATA2* variant, likely pathogenic, involving a highly conserved zinc finger domain of the protein (NM_032638:c.1114G>A (p.Ala372Thr)) (Figure 2B). This patient had no family history of malignancies (*Online Supplementary Figure S2*). When achieving complete remission, the patient developed a progressive decrease of neutrophils, monocytes, B cells, and natural killer cells associated with numerous infections. The *GATA2* variant found in this patient was previously reported in another patient with recurrent episodes of pancytopenia, fever and infections.[14] The

clinical features and persistence of the *GATA2* variant in complete remission finally led to the diagnosis of a germline *GATA2*-mutated disorder (reported by Pasquet *et al*.[11]).

Patient n. 3 was a 16-year old boy who carried a frameshift *GATA2* pathogenic variant (NM_032638:c.1008del (p.Lys336Asnfs*51)) (Figure 2C) which was located between the two zinc finger domains of the GATA2 protein. This patient had no particular personal or family history (*Online Supplementary Figure S2*). Of note, absence of family history is common in germline *GATA2*-mutated children/adolescents with hematologic malignancies,[15] suggesting a *de novo* mutation in this patient.

Patient n. 4 was a 9-year old girl with a pathogenic variant in the highly conserved homology domain (RHD) of the *RUNX1* protein (NM_001754.4:c.601C>T (p.Arg201*)) (Figure 2D). Pathogenic variants involving this codon have been reported several times as somatic or germline.[16] The family history of this patient identified solid tumors (testicular cancer in her father, 2 breast cancers and colorectal cancer in her paternal grandmother) but neither thrombocytopenia nor hematologic malignancies (*Online Supplementary Figure S2*).

Patient n. 5 was followed up for a thrombocytopenia since the age of 2 years. At 14 years old, she developed AML shortly after a phase of refractory anemia with excess blasts (*Online Supplementary Figure S2*). Cytogenetics revealed a constitutional triple X syndrome and an acquired trisomy 21. SNP-array analysis revealed a complete deletion of the *RUNX1* locus (*Online Supplementary Figure S1*). This case was previously reported by Preudhomme *et al*.[9]

This study emphasizes the complexity of identifying inherited syndromes without any extra-hematologic features. In such cases, high-throughput sequencing appears to be an informative tool for identifying syndromes predisposing to hematologic malignancies.[5,7,8] When using amplicon-based high-throughput sequencing, copy number analysis (i.e. comparative genome hybridization/SNP-array) should also be used since large deletions could be missed with the sequencing approach. Current capture-based high-throughput sequencing techniques are good alternatives to detect both mutations and copy-number alterations with specific pipelines. Assessment of both the inherited nature of variants and their pathological significance remains a challenge in practice. Cultured skin fibroblasts are recommended to ascertain the germline status of a specific variant. Particular attention should be paid to epidemiological data, functional studies and expert recommendations. Recent guidelines on the interpretation of variants written by the ACMG-AMP working group may help to refine the clinical significance of variants.[12,13,17,18]

We restricted our study to transcription factor alterations reported to be involved in predisposition syndromes. Other well-known targets, such as *ANKRD26, DDX41* (which is mostly present in adult patients) and *SAMD9/SAMD9L* were not studied here. Consequently, we were not able to evaluate the incidence of germline disorders in pediatric AML with known predisposing gene mutations.[19]

The identification of germline pathogenic variants has a strong impact on patients' management. The downstream genetic counseling requires cautious consideration of potential, related donors, excluding mutation carriers and searching for a matched, unrelated donor if necessary.

Genetic counseling should be adapted taking into account the characteristics of the predisposition syndrome and its penetrance. A few guidelines are being produced to help with genetic counseling in daily practice.[2,17,20,21] Further efforts are needed to provide a more formal passage from suspicion to genetic counseling relying on a multidisciplinary approach integrating the expertise of clinicians, hematologists and geneticists.

In conclusion, this study highlights the complexity of identifying predisposition syndromes to myeloid malignancies in the absence of extra-hematologic symptoms. Although focusing on transcription factor genes, predisposition syndromes to pediatric AML were not rare in this cohort (5/38 patients, 13%) and should be sought. In this setting, we show here the performance of high-throughput sequencing and a SNP-array. Non-mosaic deletions smaller than 5 Mb should foster further investigations to identify germline pathogenic variants. Interestingly, in our series of patients, none of the selected pathogenic or likely pathogenic variants with allele frequencies comprised between 30% and 40% remained in complete remission, corroborating the previously suggested[7] threshold of 40% as that for seeking an underlying predisposition syndrome in daily practice.

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Contributions: GL was the principal investigator of the ELAM02 trial. AP, GL, TL, VG, WAC, SD, MP, PS, CD, PS and CHD enrolled patients in the study. HL and AP created the patient database. HL centralized all the AML samples in the ELAM02 national tumor bank. LF, AMR and ND performed the genomic analysis. LF performed the research and wrote the manuscript. CP revised the manuscript which was approved by all authors.

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