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

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Supplementary Materials

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Supplementary Table 1: Targeted sequencing panel used to screen molecular profile of the ELAM02 cohort[1]

Gene	RefSeq	Coverage
ASXL1	NM_015338.5	Exons 11–12
BCOR	NM_001123385.1	Exons 2–15
BCORL1	NM_021946.4	Exons 1–12
CBL	NM_005188.3	Exons 8–9
CEBPA	NM_004364.3	Exon 1
DNMT3A	NM_022552.4	Exons 2–23
ETV6	NM_001987.4	Exons 1–8
EZH2	NM_004456.4	Exons 2–20
FLT3	NM_004119.2	Exon 20
GATA1	NM_002049.3	Exon 2
GATA2	NM_032638.4	Exons 2-6
IDH1	NM_005896.3	Exon 4
IDH2	NM_002168.3	Exon 4
JAK2	NM_004972.3	Exons 12, 14, 16
KIT	NM_000222.2	Exons 8–13, 17
KRAS	NM_033360.2	Exons 2–3
MPL	NM_005373.2	Exon 10
NIPBL	NM_133433.3	Exons 2–47
NPM1	NM_002520.6	Exon 11
NRAS	NM_002524.3	Exons 2–3
PHF6	NM_001015877.1	Exons 2–10
PTEN	NM_000314.4	Exons 5–7
PTPN11	NM_002834.3	Exons 3, 13
RAD21	NM_006265.2	Exons 2–14
RUNX1	NM_001754.4	Exons 1–9
SETBP1	NM_015559.2	Exon 4
SF3B1	NM_012433.3	Exons 13–18
SMC1A	NM_006306.3	Exons 1–25
SMC3	NM_005445.3	Exons 1–29
SRSF2	NM_003016.4	Exon 1
STAG2	NM_001042749.2	Exons 3–35

TET2	NM_001127208.2	Exons 3–11
TP53	NM_001126112.2	Exons 2–11
U2AF1	NM_006758.2	Exons 2, 6
WT1	NM_024426.3	Exons 7, 9
ZRSR2	NM_005089.3	Exons 1–11

Supplementary Table 2: Mutations at diagnosis in the patient cohort

Patient ID	**Mutation at diagnostic**
Patient 1	*CEBPA* NM_004364.3: c.65_125del (p.Pro22Leufs*118)[¥], c.934_936dup (p.Gln312dup)[¥]
Patient 2	*GATA2* NM_032638.4: c.1114G>A (p.Ala372Thr) (VAF 50%)
Patient 3	*GATA2* NM_032638.4: c.1008del (p.Lys336Asnfs*51) (VAF 41%)
Patient 4	*RUNX1* NM_001754.4: c.601C>T (p.Arg201*) (VAF 55%)
Patient 5	*RUNX1* NM_001754.4: deletion (1.8Mb, CN:1.0, mean log2 ratio: -0.54)
Patient 6	*CEBPA* NM_004364.3: c.68dup (p.His24Alafs*84)[¥], c.937_939dup (p.Lys313dup)[¥]
Patient 7	*CEBPA* NM_004364.3: c.69del (p.His24Thrfs*136)[¥], c.930_931insAAG (p.Thr310_Gln311insLys)[¥]
Patient 8	*CEBPA* NM_004364.3: c.905_928dup (p.Lys302_Glu309dup) (homozygous mutation)[¥]
Patient 9	*CEBPA* NM_004364.3: c.317_318del (p.Phe106*)[¥], c.916_945dup (p.Arg306_Leu315dup)[¥]
Patient 10	*CEBPA* NM_004364.3: c.112_118del (p.Gly38Argfs*120)[¥], c.937_939dup (p.Lys313dup)[¥]
Patient 11	*CEBPA* NM_004364.3: c.146del (p.Pro49Argfs*111)[¥], c.911_928dup (p.Lys304_Glu309dup)[¥] // *GATA2* NM_032638.4: c.989G>T (p.Arg330Leu) (VAF 43%)
Patient 12	*CEBPA* NM_004364.3: c.162_163insTT (p.Ile55Leufs*106)[¥], c.935_937dup (p.Lys313dup)[¥]
Patient 13	*CEBPA* NM_004364.3: c.196_197insT (p.Ala66Valfs*42)[¥], c.926_928dupAGA (p.Glu309_Thr310insLys)[¥]
Patient 14	*CEBPA* NM_004364.3: c.196_197insTA (p.Ala66Valfs*95)[¥], c.937_939dup (p.Lys313dup)[¥]
Patient 15	*CEBPA* NM_004364.3: c.247del (p.Gln83Serfs*77)[¥], c.937_939dup (p.Lys313dup)[¥]
Patient 16	*CEBPA* NM_004364.3: c.247del (p.Gln83Serfs*77)[¥], c.937_939dup (p.Lys313dup)[¥]
Patient 17	*CEBPA* NM_004364.3: c.262C>T (p.Gln88*)[¥], c.949_950insACC (p.Glu316_Leu317insHis)[¥]
Patient 18	*CEBPA* NM_004364.3: c.273dup (p.Lys92Glnfs*16)[¥], c.926_927insTCA (p.Glu309delinsAspGln)[¥]
Patient 19	*CEBPA* NM_004364.3: c.296del (p.Gly99Alafs*61)[¥], c.921_938del (p.Asn307_Gln312del)[¥] // *GATA2* NM_032638.4: c.952G>A (p.Ala318Thr) (VAF 43%)
Patient 20	*CEBPA* NM_004364.3: c.46dup (p.Ser16Lysfs*92)[¥], c.899_931dup (p.Arg300_Thr310dup)[¥] // *GATA2* NM_032638.4: c.972G>C (p.Lys324Asn) (VAF 44%)
Patient 21	*CEBPA* NM_004364.3: c.56dup (p.Gln20Alafs*88)[¥], c.909_910insTTC (p.Ala303_Lys304insPhe)[¥] // *GATA2* NM_032638.4: c.1010G>T (p.Arg337Leu) (VAF 36%), c.1013G>A (p.Arg338Lys) (VAF 36%)
Patient 22	*ETV6* NM_001987.4: deletion (128kb, CN=1.0, mean log2 ratio: -0.43)
Patient 23	*ETV6* NM_001987.4: deletion (543kb, CN=1.0, mean log2 ratio: -0.44)
Patient 24	*ETV6* NM_001987.4: c.1127T>A (p.Leu376Gln) (VAF 39%)
Patient 25	*ETV6* NM_001987.4: c.1170del (p.Tyr391Metfs*14) (VAF 37%)
Patient 26	*ETV6* NM_001987.4: c.1252A>G (p.Arg418Gly) (VAF 45%)
Patient 27	*GATA2* NM_032638.4: c.95_98dup (p.Tyr33*) (VAF 37%)

Patient 28	*GATA2* NM_032638.4: c.1009C>T (p.Arg337*) (VAF 40%) // *RUNX1* NM_001754.4: c.1017_1022dup (p.Ile339_Gly340dup) (VAF 38%)
Patient 29	*GATA2* NM_032638.4: c.1083_1085dup (p.Arg362dup) (VAF 35%)
Patient 30	*GATA2* NM_032638.4: c.1083_1085dup (p.Arg362dup) (VAF 42%)
Patient 31	*GATA2* NM_032638.4: c.1114G>A (p.Ala372Thr) (VAF 47%)
Patient 32	*GATA2* NM_032638.4: c.1187G>A (p.Arg396Gln) (VAF 50%)
Patient 33	*RUNX1* NM_001754.4: deletion (115kb, CN:1.0, mean log2 ratio: -0.42)
Patient 34	*RUNX1* NM_001754.4: deletion (160kb, CN=1.0, mean log2 ratio: -0.56)
Patient 35	*RUNX1* NM_001754.4: deletion (332kb, CN=1.0, mean log2 ratio: -0.37)
Patient 36	*RUNX1* NM_001754.4: deletion (50kb, CN=1.0, mean log2 ratio: -0.48)
Patient 37	*RUNX1* NM_001754.4: c.279_280dup (p.Ser94Thrfs*29) (VAF 40%)
Patient 38	*RUNX1* NM_001754.4: c.283C>T (p.Pro95Ser) (VAF 34%)
Patient 39	*RUNX1* NM_001754.4: c.422C>A (p.Ser141*) (VAF 33%), c.493G>A (p.Gly165Ser) (VAF 33%)
Patient 40	*RUNX1* NM_001754.4: c.427_428insGGCTCGGCTG (p.Glu143Glyfs*4) (VAF 36%)
Patient 41	*RUNX1* NM_001754.4: c.453G>T (p.Met151Ile) (VAF 44%), c.697C>T (p.Arg233Cys) (VAF 46%)
Patient 42	*RUNX1* NM_001754.4: c.456_458dup (p.Lys152dup) (VAF 38%)
Patient 43	*RUNX1* NM_001754.4: c.492_493insCCTAACCA (p.Gly165Profs*14) (VAF 33%)
Patient 44	*RUNX1* NM_001754.4: c.602G>A (p.Arg201Gln) (VAF 42%)
Patient 45	*RUNX1* NM_001754.4: c.602G>A (p.Arg201Gln) (VAF 43%)
Patient 46	*RUNX1* NM_001754.4: c.610C>T (p.Arg204*) (VAF 42%), c.619C>T (p.Arg207Trp) (VAF 45%)
Patient 47	*RUNX1* NM_001754.4: c.665_666dup (p.Glu223Profs*15) (VAF 38%), c.1352_1353insGATAATTAGTA (p.Asp451fs) (VAF 43%)
Patient 48	*RUNX1* NM_001754.4: c.908C>G (p.Ser303*) (VAF 37%)
Patient 49	*RUNX1* NM_001754.4: c.1170_1180del (p.Ala391Profs*205) (VAF 38%)
Patient 50	*RUNX1* NM_001754.4: c.1184C>G (p.Pro395Arg) (VAF 56%)
Patient 51	*TP53* NM_001126112.2: c.637C>T (p.Arg213*)(VAF 85%)
Patient 52	*TP53* NM_001126112.2: c.917G>A (p.Arg306Gln) (VAF 48%)

Supplementary Table 2 legend

¥: variant identified by Sanger sequencing.

CN: Copy number state

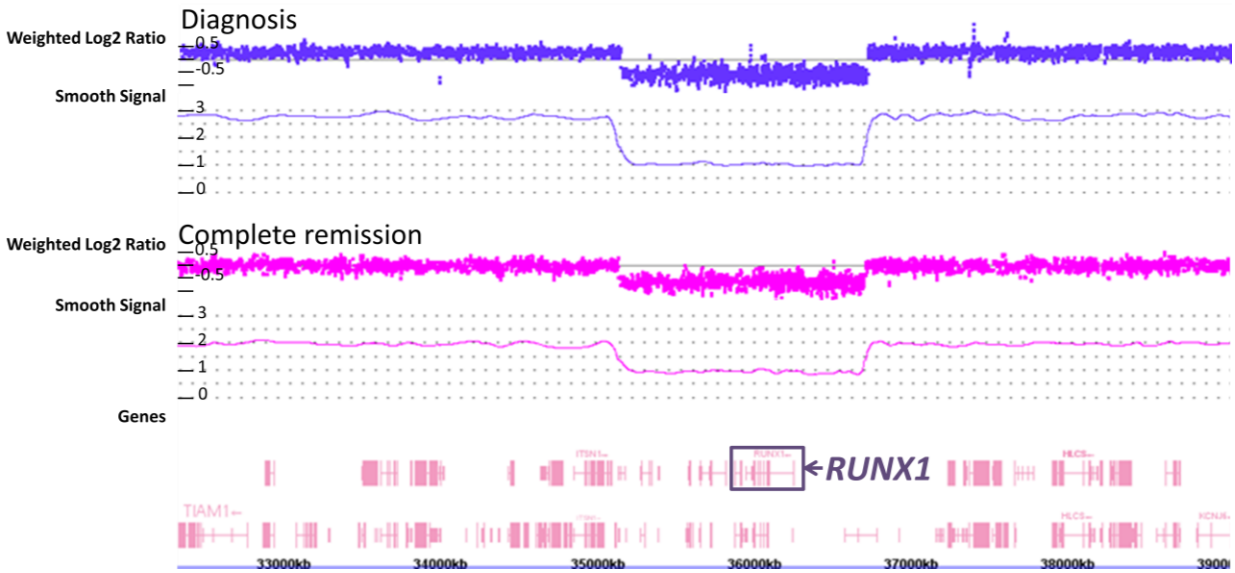
Supplementary Table 3: Identified variants at diagnosis and at time of complete remission

Patient	Gene mutation	Variant status at diagnosis	Variant status at time of complete remission
Patient 1	*CEBPA* NM_004364.3:c.65_125del (p.Pro22Leufs*118)	Present (Sanger sequencing)	Present (Sanger sequencing)
	CEBPA NM_004364.3:c.934_936dup (p.Gln312dup)	Present, VAF: 41%	Not found
	WT1 NM_024426.3:c. 1357_1361delinsAGTAG (p.Cys453_Lys454delinsSerArg)	Present, VAF: 26%	Not found
Patient 2	*GATA2* NM_032638.4:c. 1114G>A (p.Ala372Thr)	Present, VAF: 50%	Present, VAF: 49%
Patient 3	*GATA2* NM_032638.4:c.1008del (p.Lys336Asnfs*51)	Present, VAF: 48%	Present, VAF: 53%
	JAK2 NM_004972.3:c. 1849G>T (p.Val617Phe)	Present, VAF: 44%	Not found
	SETBP1 NM_015559.2:c.2602G>T (p.Asp868Tyr)	Present, VAF: 42%	Not found
Patient 4	RUNX1 NM_001754.4:c.601C>T (p.Arg201*)	Present, VAF: 48%	Present, VAF: 49%
	KRAS NM_NM_033360.2:c.38G>A (p.Gly13Asp)	Present, VAF: 19%	Not found
Patient 5	*RUNX1* NM_001754.4 deletion	Present, CN:1, mean log2 ratio: -0.54 (SNP-array)	Present, CN:1, mean log2 ratio: -0.61 (SNP-array)

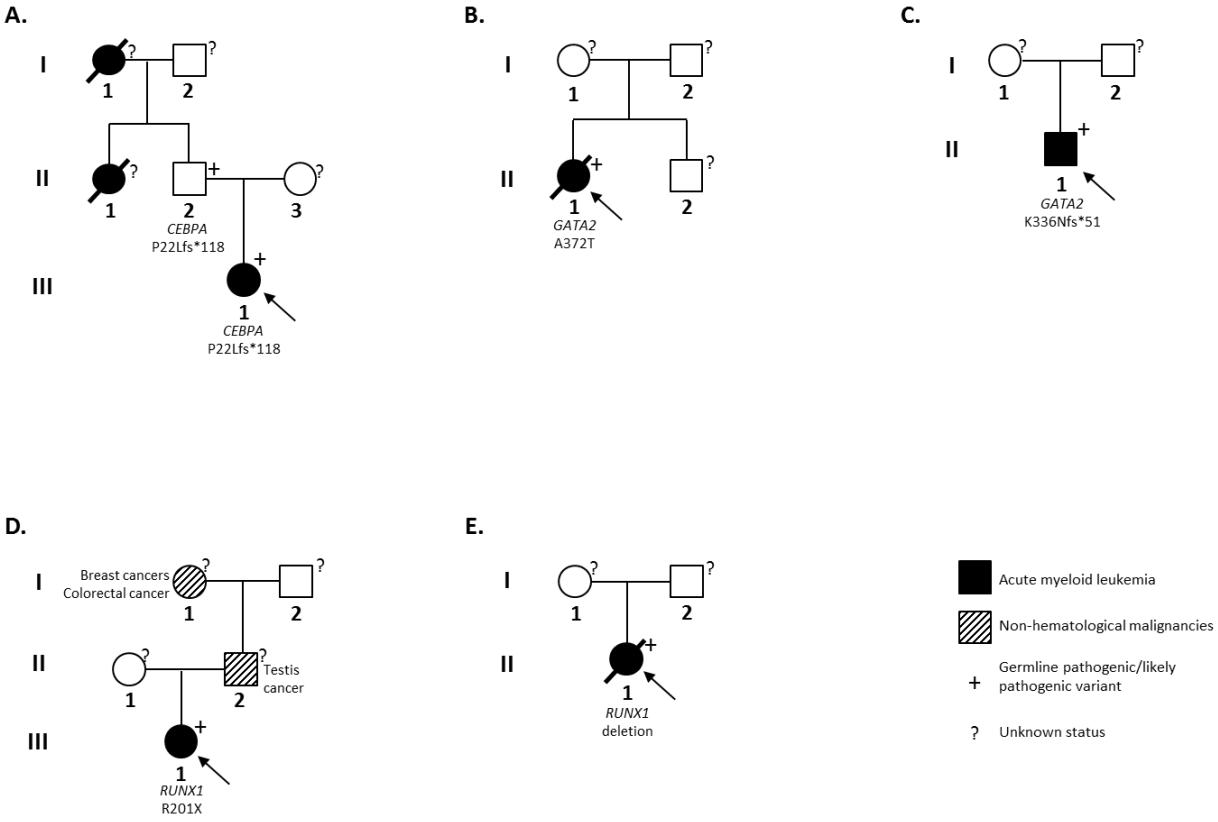
Supplementary Table 4: Rules applied to classify the variants as per American College of Medical Genetics and Genomics and Association for Molecular Pathology (ACMG-AMP) guidelines[2]

Patient	ACMG-AMP clinical significance	Rules applied	Comments
Patient 1	Pathogenic	PVS1, PM1, PM2	**PVS1:** Frameshift variant in the N-terminal region resulting in the loss of *CEBPA* function[3] **PM1:** Mutation located in the N-terminal region, a critical region of well-established germline mutations[4] **PM2:** Absent from controls in the Genome Aggregation Database
Patient 2	Likely pathogenic	PM1, PM2, PM6, PP3, PP4, PP5	**PM1:** Mutation located in the zinc finger 2 domain **PM2:** Absent from controls in the Genome Aggregation Database **PM6:** The variant is assumed de novo (parentage not confirmed) **PP3:** Mutation predicted deleterious according to the following computational tools: MAPP (77%), PhD-SNP (88%), PolyPhen-1 (74%), PolyPhen-2 (81%), SIFT (79%) and SNAP (81%)[5] **PP4:** The patient displayed features compatible with *GATA2*-related phenotype: monosomy 7, AML, monocytopenia, B-cell and NK cell deficiencies, and viral infections (labial HSV and H1N1 influenza) **PP5:** The same variant has previously been described as pathogenic in other patients[6,7]
Patient 3	Pathogenic	PVS1, PM2, PM6	**PVS1:** Frameshift mutation located between the two zinc finger domains, resulting in the loss of the second zinc finger domain, which is a known mechanism of *GATA2* disorders[8,9] **PM2:** Absent from controls in the Genome Aggregation Database **PM6:** The variant is assumed de novo (parentage not confirmed)
Patient 4	Pathogenic	PVS1, PS1_strong, PS4_supporting[10]	**PVS1:** Nonsense variant located in the Runt Homology Domain, resulting in the loss of DNA-binding activity which is a known mechanism of familial platelet disorder with predisposition to myeloid malignancy[11,12] **PS1_strong:** This amino acid change has been previously reviewed and established as pathogenic by the ClinGen Myeloid Malignancy Variant Curation Expert Panel **PS4_supporting:** The patient displays AML, which is part of the *RUNX1*-related phenotype
Patient 5	Pathogenic	As per *RUNX1* PVS1 decision tree for CNVs[10]	Full gene deletion

Supplementary Figure 1: *RUNX1* deletion in patient 5 at diagnosis and at time of complete remission



Supplementary Figure 2: Family pedigrees of patients (1 to 5) with germline pathogenic/likely pathogenic variants (represented in A to E, respectively)



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