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The genetic landscape of acute myeloid leukaemia in the South African public sector

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Background: Acute myeloid leukaemia (AML) is a heterogeneous group of myeloid neoplasms for which two international classification systems exist: the 2022 World Health Organization (WHO) and international consensus classification of myeloid neoplasms (ICC), with an emphasis on molecular abnormalities.

Aim: To determine the molecular-genetic profile of AML in the South African public sector.

Setting: The Charlotte Maxeke Johannesburg Academic Hospital, Somatic Cell Genetics Unit, National Health Laboratory Service, South Africa.

Methods: All newly diagnosed AML cases analysed with next generation sequencing (NGS) between January 2019 and December 2022 were retrospectively reviewed. Clinical and laboratory data were obtained from the laboratory information system.

Results: In total, 194 AML cases were tested by NGS (162 classifiable), with a median age of 42 years for adults and 7 years for the paediatric cohort. There were 21 cases of AML with mutated *TP53* (ICC), 5 of which were unclassifiable with the WHO classification system. In *t*(8;21)(q22;q22.1), *KIT* and *FLT3-ITD* mutations were present in 43% and 20% of cases respectively; *FLT3-ITD* in 50% of acute promyelocytic leukaemia (APL) and ~20% of AML with *NPM1* were triple mutated (*NPM1, DNMT3A, FLT3-ITD*).

Conclusion: This study revealed a high proportion of exon 17 *KIT* mutations in *t*(8;21), *FLT3-ITD* mutations in APL and triple mutated AML with mutated *NPM1*, all of which are likely to be driving the poor outcomes seen in these AML subgroups in our setting.

Contribution: This is the first nationwide description of the molecular-genetic landscape of AML in the South African public sector.

Keywords: acute myeloid leukaemia; South Africa; next generation sequencing; European LeukaemiaNet; international consensus classification; World Health Organization.

Introduction

Acute myeloid leukaemia (AML) refers to a heterogeneous group of myeloid neoplasms characterised by the accumulation of myeloid blasts and/or blast equivalents within the peripheral blood, bone marrow or extramedullary sites.[1] Acute myeloid leukaemia is more common in adults as compared to the paediatric population, with a median age of 65–70 years in high-income countries.[2,3] Factors likely to result in a higher prevalence of AML in adults include the progressive accumulation of genetic defects associated with ageing, more frequent antecedent myeloid neoplasms, previous chemotherapy and toxin exposure and longer latency periods.[1,4,5]

The heterogeneity of AML is evident in the spectrum of morphology, diverse genetic profiles and varied chemotherapeutic responses.[1] Historical classification systems relied heavily upon the morphologic features and immunophenotypic profile, which were inadequate to risk-stratify the majority of AML cases.[6] While conventional cytogenetics (CCY), fluorescence-*in-situ*-hybridisation

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(FISH) and polymerase chain reaction (PCR) techniques enabled the identification of specific subgroups of AML characterised by recurrent genetic abnormalities, there remained a significant number of cytogenetically normal AML (CN-AML) cases which could not be further classified.[7] With advancement in technologies, next generation sequencing (NGS) heralded a new era in cancer and AML diagnostics. This high throughput, massively parallel sequencing technology, allows for the detection of both fusion genes and DNA sequence variants.[8] Next generation sequencing has led to the development of molecular-cytogenetic classifications of AML, with increasing inclusion of genetic aberrations that better define risk categories, direct therapeutic approaches and identify individuals who may benefit from targeted therapies.[1,7]

There is limited literature on the genetic profile of AML in South Africa, with older studies assessing AML according to the historical cytogenetic and FISH-based classification systems, while others focused on specific subgroups such as CN-AML, acute promyelocytic leukaemia (APL) or AML with mutated nucleophosmin 1 (*NPM1*) and FMS-like tyrosine kinase 3 – internal tandem duplication (*FLT3-ITD*).[9,10,11,12,13,14,15] Two separate studies in South Africa found AML patients to have a younger median age of 38.5 years and 41 years, respectively.[10,13] This finding may be partly reflective of the young age structure in South Africa, which is characterised by a peak around the mid-thirties, or may be influenced by other genetic or environmental determinants.[10,16] To date, there are no comprehensive studies that characterise the AML molecular profile in South African patients. The study aimed to determine the molecular-genetic profile of AML in the South African public sector using a commercially available targeted NGS gene panel. From this data, the comparability of the current classification systems, the 2022 World Health Organization (WHO) and International Consensus Classification of Myeloid Neoplasms (ICC) was evaluated.[1,17]

Methods

Study design and patients

In this study, we retrospectively reviewed the NGS results of all newly diagnosed AML patients referred for NGS testing between January 2019 and December 2022 to the Somatic Cell Genetics Unit in the Department of Molecular Medicine and Haematology, National Health Laboratory Service (NHLS) and Charlotte Maxeke Johannesburg Academic Hospital (CMJAH), Gauteng, South Africa. Adult patients were defined as ≥ 18 years of age. Deoxyribonucleic acid (DNA) variants and ribonucleic acid (RNA) fusions obtained from NGS were reviewed together with data obtained from the laboratory information system (LIS) including patient demographics (age, sex), bone marrow aspirate and trephine biopsy reports, CCY, FISH, PCR for *FLT3-ITD* and *NPM1* mutation detection (fragment analysis) and flow cytometry (FACS Calibur instrument [BD Biosciences, San Jose, California, United States]) in order to sub-classify each case of AML according to the WHO and ICC. Conventional cytogenetics, FISH and PCR testing were performed at the referral sites, with results available on the LIS.

Next generation sequencing

Nucleic acid extraction and quality assessment

Peripheral blood or bone marrow aspirate samples were referred for NGS testing from several provinces across South Africa, including Mpumalanga, the Western Cape, Free State, Eastern Cape and Gauteng, in either BD Vacutainer® EDTA tubes or in PAXgene® Blood RNA tubes (BD Sciences, Franklin Lakes, New Jersey, United States). Total nucleic acid (RNA and DNA) was extracted and purified using the Chemagic™ 360 Instrument (PerkinElmer, Waltham, MA, United States) according to the manufacturer's instructions, with minor modifications. The PAXgene tubes were subjected to centrifugation, the pellet was washed with wash buffer and then lysed.

Library preparation and ion torrent sequencing

The DNA and RNA concentrations were quantified with the Qubit 2.0 fluorometer (Thermo Fisher Scientific, Waltham, Massachusetts, United States) using the high sensitivity dsDNA and RNA assays (Thermo Fisher Scientific, Waltham, Massachusetts, United States), respectively. RNA was reverse-transcribed to cDNA using the SuperScript™ IV VILO™ Master Mix (Thermo Fisher Scientific) and placed in a thermal cycler with the following cycling conditions: 25°C for 10 min; 50°C for 10 min and 85°C for 5 min. An optimised DNA and cDNA/RNA amount of 30 ng and 50 ng, respectively, was used for NGS library preparation.

Libraries for eight paired DNA and matching RNA samples were prepared separately using the Oncomine™ Myeloid Chef-Ready Assay kit (Thermo Fisher Scientific, Waltham, Massachusetts, United States) on the Ion Chef™ System (Thermo Fisher Scientific, Waltham, Massachusetts, United States) according to the manufacturer's protocol.

The normalised DNA and RNA libraries were then combined in a ratio of 80:20 and a final pooled concentration of 33 pM was used for templating. Templating was performed on the Ion 510™ & 520™ & 530™ Kit (Thermo Fisher Scientific, Waltham, Massachusetts, United States) on the Ion Chef™ System (Thermo Fisher Scientific, Waltham, Massachusetts, United States) according to the manufacturer's protocol. The combined libraries were loaded onto a 530™- Chip using the Ion Chef™ and sequenced on the Ion Gene Studio S5™ according to the manufacturer's instructions.

Targeted NGS of DNA and RNA was performed using an Ion Torrent Oncomine™ Myeloid Research Assay (version 5.12 to 5.14) (Thermo Fisher Scientific, Waltham, Massachusetts, United States).

Ion Reporter™ Software

Sequencing data were processed with the Ion Torrent Browser™, variants analysed using the software Ion Reporter™ pipeline Software (IRS) and GRCh37/hg19 reference genome and reported with the Oncomine Knowledgebase Reporter™ (Thermo Fisher Scientific, Waltham, Massachusetts, United

States). The annotated variants were evaluated and the significant and clinically relevant variants were reported. The limit of detection was 5% variant allele frequency (VAF) for DNA variants. The cut-off quality values included a uniformity of ≥90%, a maximum of 40% polyclonals, maximum low-quality reads of 20% and a mean depth >875 per sample. Samples that did not meet these criteria were re-sequenced. Variant calling was confirmed using the Catalogue of Somatic Mutations in Cancer (COSMIC), ClinVar (National Center for Biotechnology Information: https://www.ncbi.nlm.nih.gov/clinvar) and Varsome (Saphetor, Lausanne, Switzerland) databases and classified according to the joint consensus of the Association for Molecular Pathology, American Society of Clinical Oncology and the College of American Pathologists.[18]

Statistical analysis

Statistical analysis was performed using Prism software, version 5 (GraphPad Software, San Diego, California, United States). Skewed numerical data were reported as a median (interquartile range [IQR]) and categorical data as a frequency (percentage). The two-sample Wilcoxon rank-sum (Mann–Whitney) and Kruskal–Wallis tests were used to compare skewed numerical and categorical data. Correlation between variables of interest (DNA variants, AML subgroups and patient age) was assessed using Spearman's correlation. A two-sided *p*-value of 0.05 was accepted as statistically significant.

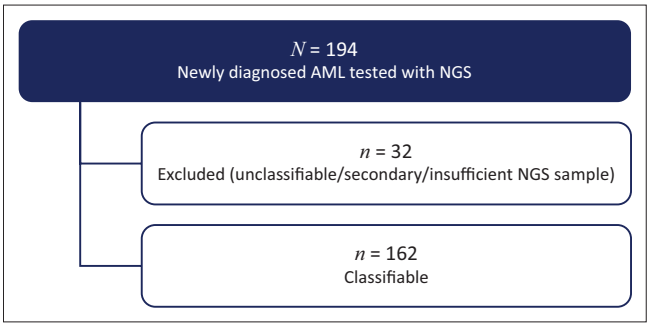
Ethical considerations

Ethical clearance for this study was obtained from the University of the Witwatersrand Human Ethics Committee (protocol number M12111144). Laboratory results were retrospectively obtained from the LIS (TrakCare, Intersystems, Cambridge, Massachusetts, United States), and patient confidentiality was maintained through de-identification and the use of a password-protected excel spreadsheet.

Results

Characteristics of the acute myeloid leukaemia cohort

The cohort included 194 cases of newly diagnosed AML that were tested by NGS during the specified time period. Twenty-one cases could not be classified because of the absence of CCY, bone marrow aspirate and/or trephine biopsy analyses. A further 10 cases were excluded as they had transformed from underlying myeloproliferative neoplasms (8 cases) or aplastic anaemia (2 cases), and 1 case of APL lacked DNA NGS data (illustrated in Figure 1). Of the remaining 162 cases, there were 16 cases of AML without RNA NGS data; however, these could be classified based on the presence of AML defining genetic abnormalities identified on FISH/or CCY. All cases were reclassified according to the 5th edition of the WHO Classification System,[1] and the proportion of each AML subgroup is shown in Table 1, with AML myelodysplasia-related (AML-MR) and AML with *RUNX1::RUNX1T1* fusion; *t*(8;21)(q22;q22.1) the dominant subtypes, comprising half of the cases. There was a previous history of cytotoxic therapy in a single case of AML-MR and two cases of AML with *KMT2A* rearrangement.



AML, acute myeloid leukaemia; NGS, next generation sequencing.

FIGURE 1: Flow diagram showing cases of newly diagnosed acute myeloid leukaemia included in the study.

TABLE 1: The proportion of each acute myeloid leukaemia subgroup according to the 5th edition of the World Health Organization Classification System (*N* = 162).

AML subgroup	*n*	%
AML with defining genetic abnormalities	93	57.4
AML with *RUNX1::RUNX1T1* fusion; *t*(8;21)(q22;q22.1)	40	24.7
AML with *NPM1* mutation	15	9.3
AML with *PML::RARA* fusion	12	7.4
AML with *KMT2A* rearrangement	12	7.4
AML with *CBFB::MYH11* fusion; inv(16)/*t*(16;16)(p13.1;q22)	5	3.1
AML with *CEBPA* mutation	5	3.1
AML with *RBM15::MRTFA* fusion; *t*(1;22)(p13.3;q13.1)	2	1.2
AML with *DEK::NUP214* fusion; *t*(6;9)(p23;q34.1)	1	0.6
AML with *MECOM* rearrangement; inv(3)/*t*(3;3)(q21.3;q26.2)	1	0.6
AML myelodysplasia-related	42	26.0
AML defined by differentiation	20	12.3
ZMYND11::MBTD1, *t*(10;17)(p15;q21)	1	0.6
NUP98::HOXA13, *t*(7;11)(p15;p15)	1	0.6
KAT6A::CREBBP; *t*(8;16)(p11;p13)	1	0.6
PICALM::MLLT10; *t*(10;11)(p12.3;q14.2)	1	0.6
Other	16	9.9
AML associated with Down Syndrome (germline predisposition)	5	3.1
AML with eosinophilia and tyrosine kinase gene fusions	2	1.2
AML with *PCM1::JAK2* fusion	1	0.6
AML with *PDGFRA* rearrangement	1	0.6

AML, acute myeloid leukaemia.

According to the 2022 ICC, AML with mutated *TP53* (mono-/bi-allelic) constitutes a separate entity,[17] with 21 of cases classifiable according to the ICC, fulfilling the criteria for this entity (15 AML-MR, 5 AML-unclassifiable, 1 AML defined by differentiation). In addition, there was a single case of AML with *RUNX1::RUNX1T1* fusion*; t*(8;21)(q22;q22.1)) with a non-germline *TP53* mutation.

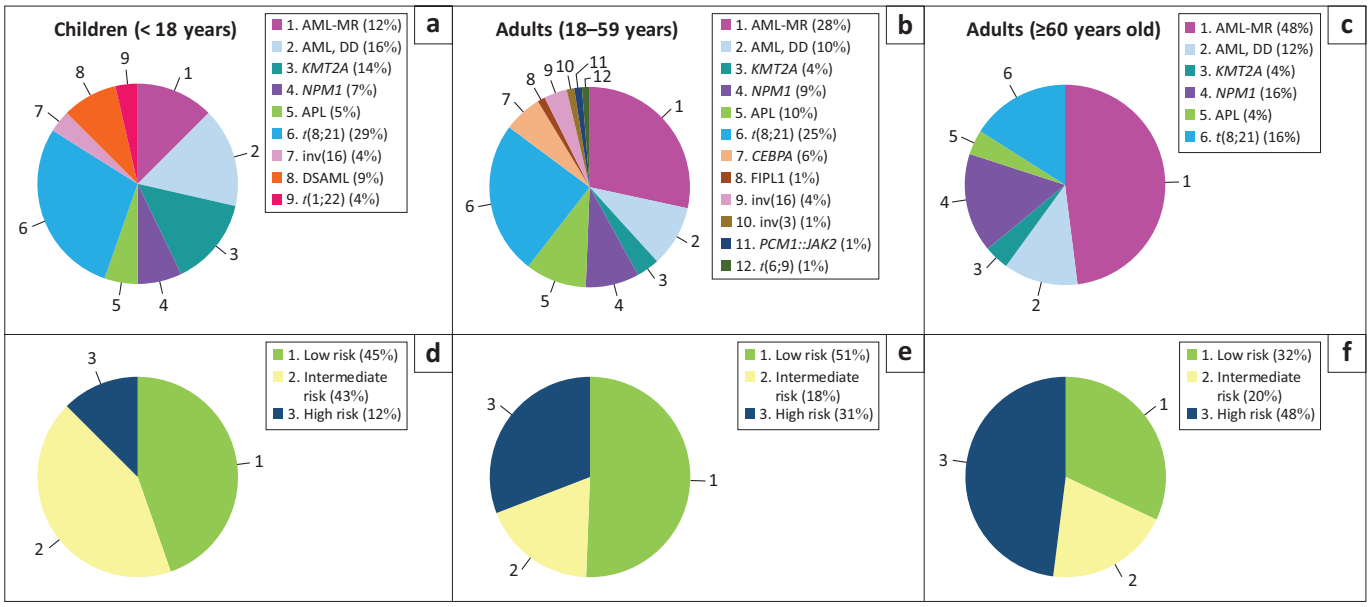
The median age (IQR) of the entire adult (defined as ≥18 years) and paediatric cohort was 42 (31–57) years and 7 (3–13) years, respectively, with the highest median age seen in AML-MR (these values were unchanged when excluding AML with germline predisposition and cases transformed from underlying myeloproliferative neoplasms and aplastic anaemia). Table 2 summarises the patient demographic and cytogenetic data from the major AML subgroups. Conventional cytogenetics was available for approximately

TABLE 2: Demographic and cytogenetic data from the major subgroups of acute myeloid leukaemia.

AML subgroup	AML with defining genetic abnormalities																										AML, DD (*n* = 20)			
Variable	AML-MR (*n* = 42)				*RUNX1::RUNX1T1* (*n* = 40)				*NPM1* (*n* = 15)				*PML::RARA* (*n* = 12)				*KMT2A* (*n* = 12)				*CBFB::MYH11* (*n* = 5)				*CEBPA* (*n* = 5)							
	n	%	Median	IQR	*n*	%	Median	IQR	*n*	%	Median	IQR	*n*	%	Median	IQR	*n*	%	Median	IQR	*n*	%	Median	IQR	*n*	%	Median	IQR	*n*	%	Median	IQR
Demographics																														
Sex																														
Male	26	62	-	-	27	68	-	-	9	60	-	-	4	33	-	-	7	58	-	-	2	40	-	-	3	60	-	-	8	40	-	-
Female	16	38	-	-	13	32	-	-	6	40	-	-	8	67	-	-	5	42	-	-	3	60	-	-	2	40	-	-	12	60	-	-
Age (years)	-	-	50	35–61	-	-	20	12–33	-	-	36	22–62	-	-	29	19–34	-	-	13	6–30	-	-	27	7–40	-	-	41	21–49	-	-	25	9–49
Cytogenetics																														
Available†	29/42	69	-	-	24/40	60	-	-	11/15	73	-	-	7/12	58	-	-	9/12	75	-	-	3/5	60	-	-	3/5	60	-	-	20/20	100	-	-
Normal karyotype	8	28	-	-	0	0	-	-	11	100	-	-	0	0	-	-	0	0	-	-	0	0	-	-	1	33	-	-	9	45	-	-
-Y	0	0	-	-	5	21	-	-	0	0	-	-	0	0	-	-	0	0	-	-	0	0	-	-	0	0	-	-	0	0	-	-
-X	1	3	-	-	4	17	-	-	0	0	-	-	0	0	-	-	0	0	-	-	0	0	-	-	0	0	-	-	0	0	-	-
Complex karyotype	17	59	-	-	0	0	-	-	0	0	-	-	0	0	-	-	0	0	-	-	0	0	-	-	0	0	-	-	0	0	-	-
Monosomy 5	1	3	-	-	0	0	-	-	0	0	-	-	0	0	-	-	0	0	-	-	0	0	-	-	0	0	-	-	0	0	-	-
Deletion (5q)	11	38	-	-	0	0	-	-	0	0	-	-	0	0	-	-	0	0	-	-	0	0	-	-	0	0	-	-	0	0	-	-
Monosomy 7	7	24	-	-	1	4	-	-	0	0	-	-	0	0	-	-	0	0	-	-	0	0	-	-	0	0	-	-	0	0	-	-
Deletion (7q)	4	14	-	-	0	0	-	-	0	0	-	-	0	0	-	-	0	0	-	-	0	0	-	-	0	0	-	-	0	0	-	-
Trisomy 8	7	24	-	-	0	0	-	-	0	0	-	-	0	0	-	-	0	0	-	-	1	33	-	-	0	0	-	-	0	0	-	-
Inversion 9	0	0	-	-	0	0	-	-	0	0	-	-	0	0	-	-	0	0	-	-	1	33	-	-	0	0	-	-	0	0	-	-
Deletion (9q)	1	3	-	-	3	13	-	-	0	0	-	-	0	0	-	-	0	0	-	-	0	0	-	-	2	67	-	-	0	0	-	-
Trisomy 1	0	0	-	-	0	0	-	-	0	0	-	-	0	0	-	-	0	0	-	-	0	0	-	-	0	0	-	-	0	0	-	-
Inversion 12	0	0	-	-	0	0	-	-	0	0	-	-	1	13	-	-	0	0	-	-	0	0	-	-	0	0	-	-	0	0	-	-
Deletion (12p)	2	7	-	-	0	0	-	-	0	0	-	-	0	0	-	-	0	0	-	-	0	0	-	-	0	0	-	-	0	0	-	-
Deletion (17p)	6	21	-	-	0	0	-	-	0	0	-	-	0	0	-	-	0	0	-	-	0	0	-	-	0	0	-	-	0	0	-	-
Trisomy 21	3	10	-	-	0	0	-	-	0	0	-	-	0	0	-	-	2	22	-	-	0	0	-	-	1	33	-	-	0	0	-	-

AML, acute myeloid leukaemia; AML, DD, acute myeloid leukaemia defined by differentiation; AML-MR, acute myeloid leukaemia, myelodysplasia-related; *CBFB::MYH11*, acute myeloid leukaemia with *CBFB::MYH11* fusion; *CEBPA*, acute myeloid leukaemia with *CEBPA* mutation; *KMT2A*, acute myeloid leukaemia with *KMT2A* rearrangement; *NPM1*, acute myeloid leukaemia with *NPM1* mutation; *PML::RARA*, acute myeloid leukaemia with *PML::RARA* fusion; *RUNX1::RUNX1T1*, acute myeloid leukaemia with *RUNX1::RUNX1T1*; IQR, interquartile range.

†, n/N.



AML, DD, acute myeloid leukaemia, defined by differentiation; AML-MR, acute myeloid leukaemia, myelodysplasia-related; APL, acute myeloid leukaemia with *PML::RARA* fusion; *CEBPA*, acute myeloid leukaemia with *CEBPA* mutation; DSAML, acute myeloid leukaemia associated with Down Syndrome; inv(16), acute myeloid leukaemia with *CBFB::MYH11* fusion; *KMT2A*, acute myeloid leukaemia with *KMT2A* rearrangement; *NPM1*, acute myeloid leukaemia with *NPM1* mutation; *PCM1-JAK2*, acute myeloid leukaemia with *PCM1-JAK2* fusion; *PDGFRA*, acute myeloid leukaemia with *PDGFRA* rearrangement; *t*(1;22), acute myeloid leukaemia with *RBM15::MRTFA* fusion; *t*(6;9), acute myeloid leukaemia with *DEK::NUP214* fusion; *t*(8;21), acute myeloid leukaemia with *RUNX1::RUNX1T1* fusion.

FIGURE 2: Acute myeloid leukaemia subgroups and European LeukaemiaNet (ELN) risk stratification by age category.

71% (115/162) of all AML cases. Cases that would have been classified as CN-AML in the absence of NGS constituted 31% (38/123). Acute myeloid leukaemia with mutated *NPM1* comprised approximately 40% (15/38) of the latter and all cases of AML with mutated *NPM1* that had a cytogenetic result had a normal karyotype (Table 2).

The proportion of each AML subgroup differed according to the age categories (Figure 2). In older adults (≥ 60 years), AML-MR comprised the majority of cases, reflected in the high proportion of European LeukaemiaNet (ELN) high risk AML, while in younger adults (16–59 years), approximately half of the cases constituted low risk AML.[7] The frequency of APL was notably low (10% or less) across all age categories, while AML with mutated *NPM1* increased with age.

Correlation between patient demographics and acute myeloid leukaemia subgroup

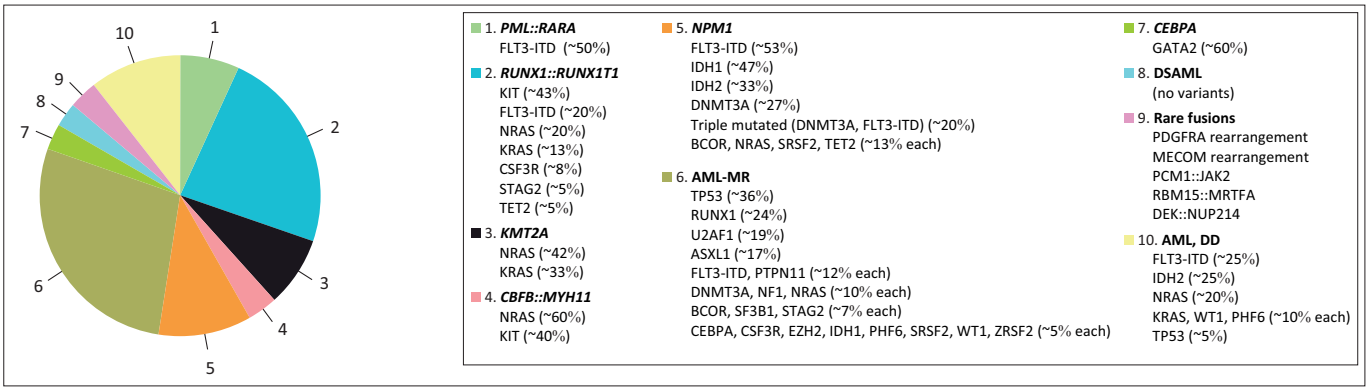
Statistically significant but weak correlations were identified between infants (defined as <2 years) and AML associated with Down Syndrome ($r_s = 0.235$, $p = 0.001$), and between late adolescence (defined as 15–19 years) and AML with *RUNX1::RUNX1T1* fusion ($r_s = 0.205$, $p = 0.004$). Very weak correlations were demonstrated between those of advanced age (defined as ≥ 60 years) and AML-MR ($r_s = 0.194$, $p = 0.0061$), and between adults aged 18–59 years with AML with *CEBPA* mutation ($r_s = 0.156$, $p = 0.028$). Furthermore, there was a marginal correlation between the female sex and AML with *PML::RARA* fusion ($r_s = 0.124$, $p = 0.082$), while AML with *RUNX1::RUNX1T1* fusion and AML transformed from underlying myeloproliferative neoplasms/aplastic anaemia were marginally correlated with the male sex ($r_s = 0.136$, $p = 0.056$ and $r_s = 0.120$, $p = 0.092$, respectively).

Frequency of DNA variants in each acute myeloid leukaemia subgroup

The frequency of the different DNA variants in each AML subgroup is illustrated in Figure 3, with AML with mutated *NPM1* and AML-MR having the highest number of DNA variants (median approximately 2.3–2.4) while AML with *PML::RARA* demonstrated the lowest (0.5).

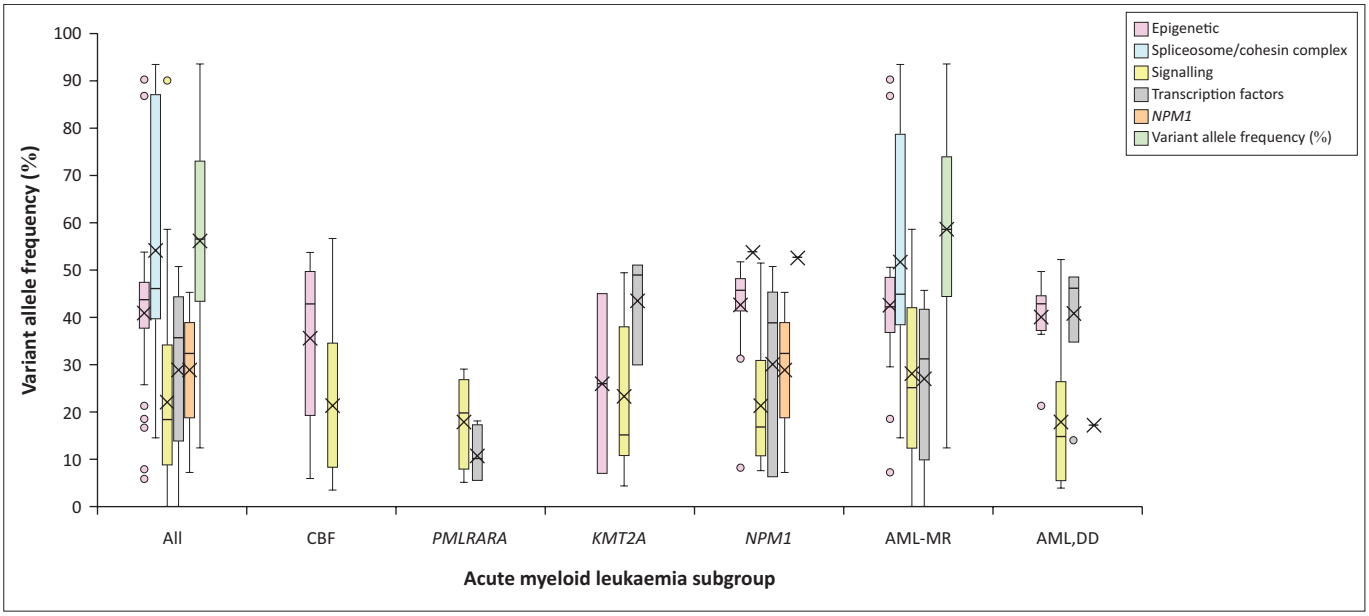
DNA variants were identified in roughly two thirds of core binding factor leukaemias (*t*[8;21]; inversion 16) and *KMT2A* positive cases, and approximately half of APL cases. DNA variants involved in signalling pathways predominated and were present at a VAF of around 20% in these subgroups (Figure 4). In contrast, AML-MR had a predominance of DNA variants involving tumour suppressor and epigenetic/spliceosome genes, with all of the category defining DNA variants demonstrating a VAF of >20%. Acute myeloid leukaemia with *NPM1* was characterised by the presence of DNA variants in all cases (in addition to mutated *NPM1*), predominantly involving genes of the signalling and epigenetic/spliceosome pathways. The epigenetic/spliceosome functional groups were found to have the highest VAF in both AML-MR and AML with *NPM1*, ranging between 40% and 50%.

Further analysis revealed correlations between AML with *RUNX1::RUNX1T1* and *KIT* mutations ($r_s = 0.562$, $p < 0.001$), AML with mutated *CEBPA* (biallelic) and *GATA2* mutations ($r_s = 0.535$, $p = 0.01$)(*GATA2* zinc finger 1 [ZF1][$n = 1$]; zinc finger 2 [ZF2][$n = 2$]), and between *DNMT3A* and *IDH1* mutations ($r_s = 0.308$, $p = < 0.001$). No DNA variants were identified in cases of AML associated with Down Syndrome or in AML with the *RBM15::MRTFA* fusion.



AML, DD, acute myeloid leukaemia, defined by differentiation; AML-MR, acute myeloid leukaemia, myelodysplasia-related; *DEK::NUP214*, AML with *DEK::NUP214* fusion; DSAML, acute myeloid leukaemia associated with Down Syndrome; *CBFB::MYH11*, acute myeloid leukaemia with *CBFB::MYH11* fusion; *CEBPA*, acute myeloid leukaemia with *CEBPA* mutation; *KMT2A*, acute myeloid leukaemia with *KMT2A* rearrangement; *MECOM*, AML with *MECOM* rearrangement; *NPM1*, acute myeloid leukaemia with *NPM1* mutation; *PCM1::JAK2*, *PCM1::JAK2* fusion; *PDGFRA*, AML with *PDGFRA* rearrangement; *PML::RARA*, acute myeloid leukaemia with *PML::RARA* fusion; *RBM15::MRTFA*, AML with *RBM15::MRTFA* fusion; *RUNX1::RUNX1T1*, acute myeloid leukaemia with *RUNX1::RUNX1T1* fusion.

FIGURE 3: The frequency of DNA variants in each acute myeloid leukaemia subgroup, n = 163.



AML, DD, acute myeloid leukaemia, defined by differentiation; AML-MR, acute myeloid leukaemia, myelodysplasia-related; CBF, core binding factor leukaemia; *KMT2A*, acute myeloid leukaemia with *KMT2A* rearrangement; *NPM1*, acute myeloid leukaemia with *NPM1* mutation; *PMLRARA*, acute myeloid leukaemia with *PML::RARA* fusion.

FIGURE 4: The median variant allele frequency of DNA variants grouped according to their mutated gene functional category in the main subgroups of acute myeloid leukaemia.

Discussion

This study provides the first nationwide description of the genetic landscape of AML in the South African public sector. Previous studies in our setting were limited by the available genetic data or focused on specific subgroups of AML, and thus the molecular-genetic characteristics of AML have not been fully described.[9,10,11,12,13,14,15] Through the introduction of NGS into the routine diagnostic work-up of haematological neoplasms, we have been able to comprehensively classify and risk stratify AML in South Africa.

Our study revealed several similarities and differences in the proportion of the different AML subgroups as compared with international and local studies. In childhood APL, the findings were similar to the international literature (~9% of childhood AML), while in children and adults, the proportion of APL was notably lower than that reported in two local studies from the Gauteng province (less than a third of that reported) and internationally in adults (~19% of adolescent/adult AML).[10,12,19,20,21,22] In contrast, the proportion of *t*(8;21) in each age category was nearly twice as high as that reported both locally and internationally (~12% of childhood, ~6% of adolescent/young adult, ~7% of adult AML),[12,20,21,22] and in keeping with the literature, the *t*(8;21) translocation was shown to be correlated with late adolescence.[1] Given that this was a nationwide study, these differences are likely to reflect a combination of factors that could include: (1) genetic diversity in the population groups across South Africa, (2) different institutional testing practices, for APL this may include the use of fragment analysis PCR for FLT3-ITD mutations in place of NGS, or (3) patients with APL may have demised prior to testing because of higher rates of high-risk APL and early death in other provinces.

A challenge with the updated WHO and ICC AML classification systems is the divergent approach to *TP53* and *RUNX1* mutations. The ICC categorises *TP53* mutated AML (mono-/bi-allelic) as a poor prognostic entity,[23] while the WHO does not accommodate *TP53* mutated AML in a high-risk category, unless present with myelodysplasia-related cytogenetic abnormalities or gene mutations, termed AML myelodysplasia-related.[1,17] Although *TP53* mutations are strongly associated with a complex karyotype (mutated in ~70% of cases),[24] ~20% of *TP53* mutated AML in our cohort lacked CCY and did not qualify as high risk according to the WHO (AML, unclassifiable). In such situations, where definitive WHO classification is not possible, it may be prudent to provide the diagnostic labels from both classification systems to better risk-stratify these patients, and flag them as potential high-risk AML. Furthermore, the presence of a complex karyotype in an AML should direct testing for *TP53* mutations. In regard to *RUNX1* mutations, the ICC incorporates these into AML with myelodysplasia-related gene mutations while the WHO does not assign these cases to a specific category.[1,17] The impact was negligible in our cohort, a single cytogenetically normal case, which fulfilled the ICC criteria for AML with myelodysplasia-related gene mutations based on the *RUNX1* mutation alone, while classified as AML defined by differentiation on WHO classification.[1,23] The most significant impact on the ELN risk stratification was in the identification of high-risk DNA variants for which alternative testing was not available, high-risk fusions which were cryptic on CCY or for which FISH probes were not routinely available and in cases of CN-AML. This was most notable in AML-MR, where in the absence of NGS a striking 40% of cases would not have fulfilled criteria for AML-MR and would not have been appropriately risk-stratified, with the potential to impact both the treatment approach and patient outcomes. Ultimately, consensus between the WHO and ICC classification of AML may have to be a consideration to allow for a standardised approach to comprehensively manage AML in future.

The DNA variant landscape in the various AML subgroups was found to be comparable to that described in the literature. In APL, *FLT3-ITD* mutations were the only DNA variants identified and occurred at a similar frequency (~46%) to international studies (~30% – 43%).[25,26] Although *FLT3-ITD* positive APL is linked with a poorer prognosis, with studies citing poor relapse free survival (RFS)(hazard ratio of 2.4; 95% CI: 1–4.5; $p = 0.01$),[25] decreased post-consolidation complete remission (CR), poor overall survival (OS) (hazard ratio of 3.25 95% CI: 1.14–9.25; $p = 0.027$),[26] and reduced post-relapse survival,[27] the introduction of Arsenic trioxide (ATO) into the management of APL has largely mitigated this negative impact.[28] In a previous study, we showed that ~11% of patients with APL in Johannesburg relapsed after having achieved CR, a finding that may be explained by the high percentage of APL cases with *FLT3-ITD* mutations, the unavailability of ATO therapy and potential differences in therapeutic practices.[22] These results, together with international recommendations, strongly advocate for the inclusion of ATO therapy as part of the standard therapeutic approach to APL in the South African public sector.[29,30,31] In AML with *RUNX1::RUNX1T1* fusion; *t*(8;21)(q22;q22.1), *KIT* mutations made up the majority of the DNA variants (~43%), involving exon 17 in approximately 88% of cases (D816 in 53%, D820 in 6%, N822 in 29%), with the remainder involving exon 8 (D419 in 12%). This was followed by *RAS* (~30%) and *FLT3-ITD* (~20%) mutations. These mutations were generally present at frequencies comparable to the literature ([*KIT*~12% – 43%][32,33,34,35]; [*RAS*~8% – 36%][18,36]; [*FLT3*~1.4% – 13%][18,36]). Although *t*(8;21) is associated with high rates of CR (~98%) when treated with intensive consolidation therapy,[20,37] the long-term treatment outcomes are not universally good. Inferior outcomes have been demonstrated in adults with *t*(8;21) and *KIT* mutations (especially exon 17) with shorter disease free survival (DFS),[38] RFS,[32] event free survival (EFS),[32,33,34] OS,[32,33,34,38,39] and higher cumulative incidence of relapse (CIR).[36] *FLT3-ITD* mutations which are mutually exclusive with *KIT* mutations in *t*(8;21)[40] have also been linked with reduced EFS, RFS and high CIR.[36,41] In our previous study from Johannesburg, there was a high rate of relapse (~21%) in those patients with *t*(8;21) achieving CR.[22] Given that a high proportion of cases in our current study had either *KIT* or *FLT3-ITD* mutations (~60%) and the association with poorer outcomes, these DNA variants are predicted to be the likely culprits for this high rate of disease relapse. Interestingly, the ELN guidelines maintain *t*(8;21) with *KIT* or *FLT3* mutations in a favourable risk category, with upfront use of Midostaurin™ for *FLT3* positive cases, and escalation of treatment, based on therapy response (as evidenced by results of measurable residual disease [MRD] assessments).[7] In the absence of available Midostaurin™ therapy, it would remain to be seen whether close monitoring of MRD and treatment escalation would change the narrative in our setting. As seen elsewhere, AML with *NPM1* associates with CN-AML (~40% – 74%),[42,43,44,45,46,47,48,49] showing a similar frequency of the co-mutations *FLT3-ITD* (~53% vs ~25% – 55%), *DNMT3A* (~27% versus ~33% – 60%), a higher frequency of *IDH1* (~47% versus ~15%) and a distinct absence of *FLT3-TKD* (~8.2% – 15%).[42,44,47,50,51,52,53,54,55] While *NPM1* with *FLT3-ITD* falls into an intermediate risk category in adults,[7] the presence of triple mutated cases (*NPM1*, *FLT3-ITD*, *DNMT3A*) has been shown to confer the worst prognostic outcomes.[56] Triple mutated cases comprised nearly a quarter of AML with *NPM1*, resulting in one of the highest mutation burdens, and may explain the poor outcomes of this entity in our setting.[22] Our data analysis confirmed the well-known correlation between AML with mutated *CEBPA* (biallelic mutations) and *GATA2* ZF1 and ZF2 domain mutations. Acute myeloid leukaemia with mutated *CEBPA* (mono-allelic bZIP region or biallelic mutations) carries a favourable risk profile, which is significantly better (OS and RFS) in *GATA2* mutated patients as compared with their wild-type counterparts,[57] with *GATA2* ZF1 demonstrating an even better prognosis than ZF2 domain mutations.[58] It remains to be seen whether the presence or absence of *GATA2* mutations (ZF1 and ZF2) translates into similar clinical outcomes in our patients.

What is evident from this study is that AML does not represent a single disease entity but rather a genetically heterogeneous group of leukaemias. In core binding factor leukaemia, *KMT2A* and APL, the hallmark chromosomal rearrangements are known to effect a differentiation block, with the concurrent DNA variants seen in our study almost exclusively activating signalling pathways. Epigenetic variants, when present, demonstrated a high VAF, suggesting these to be early events and potentially reflecting preceding clonal haematopoiesis of indeterminate potential (CHIP)/ clonal haematopoiesis of oncogenic potential (CHOP) in these subgroups.[59] In contrast, AML-MR was typified by a stepwise acquisition of large structural and numerical chromosomal abnormalities and DNA variants, with high VAF driver mutations involving tumour suppressor genes, the spliceosome/cohesion complex and epigenetic modification. Added to the above, there is a complex interplay between the genetic variants, subclones and the microenvironment in AML.[60] This molecular heterogeneity highlights the importance of approaching each subgroup of AML individually in both prognostication and treatment, with a need to tailor the treatment approach to both the specific and combination of variants seen.

Limitations

This is a retrospective analysis that did not collect intervention and outcome data. The absence of cytogenetic bone marrow aspirate and/or trephine biopsy analyses for some of the cases did not allow these cases to be classified. Owing to diagnostic capacity, it is unclear if this subset is representative of the South African population as not all cases of AML were referred for NGS analysis because of differing institutional referral practices. Prospective studies to analyse and ascertain the impact of molecular variants on prognosis and survival are needed. In addition, the use of more comprehensive NGS panels may identify variants and fusions not tested for with this NGS panel, allowing for further genetic profiling of local AML.

Conclusion

This is the first nationwide description of the molecular genetic landscape of AML in the South African public sector. This revealed a high proportion of exon 17 *KIT* mutations in *t*(8;21), *FLT3-ITD* mutations in APL and triple mutated AML with mutated *NPM1*, all of which are likely to be driving the poor outcomes seen in these AML subgroups in our setting.

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Competing interests

The co-author Dewaldt Engelbrecht is currently working at Thermo Fisher Scientific, South Africa (t/a LTC Tech), 200 Smit Street, Fairland, Johannesburg, South Africa, but was not employed with them at the time of the study. The remaining authors state that they have no conflict of interests.

Authors' contributions

K.E.H. conceptualised the project, was involved in the data collection, data analysis and drafting of the original transcript. A.H.W. was part of the initial conceptualisation, methodology (processing of the samples, performing the NGS), manuscript review and editing. I.K. assisted with the drafting of the initial manuscript. P.W., T.W., J.V. were part of the formal analysis, and manuscript editing and review. M.M., D.E., P.P., H.v.Z. were part of the methodology (processed the samples and performed the next generation sequencing), manuscript review and editing. Z.C., J.J., J.K., J.M., V.M., J.O. and J.P. referred samples from their respective centres for next-generation sequencing and contributed to the editing and review of the final transcript.

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Data availability

The next generation sequencing data will not be available on a public repository, but are available on reasonable request from the corresponding author, K.E.H.

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