

Molecular Genetic Characterization of Acute Myeloid Leukemia With Trisomy 4 as the Sole Chromosome Abnormality

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Abstract. *Background/Aim:* The aim of the study was to determine the genetic and molecular consequences of trisomy 4, a recurrent but rare chromosomal abnormality in acute myeloid leukemia (AML). *Materials and Methods:* Interphase fluorescence *in situ* hybridization, reverse transcriptase-quantitative polymerase chain reaction for 28 chromosomal gene translocations/fusion genes, and targeted sequencing analyses were performed on five AMLs with trisomy 4 as the sole chromosomal anomaly. *Results:* An *NPM1* frameshift mutation was found in all leukemic bone marrows, *DNMT3A*, *FLT3*, and *IDH1* mutations were found in three, *KIT* and *NRAS* mutations in two, whereas *IDH2 (R140Q)*, *RUNX1*, and *WT1* mutations were found in only one patient each. The three patients with a *DNMT3A (R882H)* mutation have died. In contrast, the two patients whose leukemic cells were without this mutation, are alive 55 and 31 months after diagnosis, respectively. *Conclusion:* The results suggest a possible association between trisomy 4 and additional mutations that may influence prognosis.

Acute myeloid leukemia (AML) is a heterogenous group of malignancies with different clinical phenotypes and responses to therapy (1, 2). AMLs can be classified according to distinct cytogenetic and genetic abnormalities of leukemic cells at

diagnosis, but also by phenotypic and epigenetic differences that act as a guide to risk assessment and choice of treatment (1, 2). AML cases with a single trisomy constitute a heterogenous subgroup with regard to clinical, morphological, and immunophenotypic features, as well as the mutational profiles (3).

Trisomy 4 is a recurrent, but rare chromosomal abnormality in AML. It may be the sole aberration or one of several chromosomal changes (4). Trisomy 4 was first described by Mecucci *et al.* in 1986, and a year later by Sandberg *et al.* (5), as the sole chromosomal abnormality in AML (6). In the Mitelman Database of Chromosome Aberrations and Gene Fusions in Cancer, 83 (48 female and 35 male patients) out of 18029 AML-cases had trisomy 4 as the sole chromosomal abnormality (<http://cgap.nci.nih.gov/Chromosomes/Mitelman>, database last updated on February 20, 2017). Most cases are morphologically classified as M1, M2, or M4 using the French-American-British (FAB) classification.

Because trisomy 4 is rare, few studies have addressed its clinical impact. As a consequence, the prognosis of AML with solitary trisomy 4 remains unclear (7, 8). However, a recent study based on 87 AML cases with trisomy 4 concluded that the prognostic significance of the aberration depended on the patient's age, and despite an initial good response to treatment, patients with trisomy 4 were prone to relapse (4).

Recently, we reported the genetic, molecular and clinical features of a pediatric AML case with trisomy 4 and an *FLT3-ITD* mutation (9). Fluorescence *in situ* hybridization assay demonstrated that part of the *RUNX1* probe had moved to chromosome band 6q25 indicating a cryptic t(6;21)(q25;q22) translocation. Molecular analysis of the translocation showed fusion of *RUNX1* with an intergenic sequence from 6q25, resulting in a putative *RUNX1* truncated protein that would contain the Runt homology domain responsible for both heterodimerization with CBFβ and DNA binding (9). The findings prompted us to investigate additional cases of myeloid

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Key Words: Acute myeloid leukemia, trisomy 4, targeted sequencing, mutations, *NPM1*.

Table I. Clinicopathological and hematological findings of AML patients with trisomy 4 as the sole chromosome abnormality.

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Gender/age at diagnosis	F/62	F/54	M/53	M/75	M/67
Status	Dead/1 week after diagnosis	Dead/15 months after diagnosis	Alive/55 months	Alive/31 months	Dead/8 months after diagnosis.
Diagnosis	AML - with dysplastic changes	AML, FAB M2	AML, FAB M1	AML, FAB M4	AML, FAB M2
Co-morbidity	Myocardial infarction ×2	Asthma, fibromyalgia, smoking, COPD, hypothyreosis.	Asthma, depression, smoking, hyperlipidemia, COPD	Hypertension, hyperlipidemia, tumor ani; squamous cell hyperplasia	Bipolar disease (Lithium, lamictal), hypertension
Blasts (bone marrow)	40%, Auer rods seen	72%	75%, Auer rods seen	75%, Auer rods seen	93%
Clinical characteristics	Fatigue, fever	Fatigue, fever, hepatomegaly	Oral apthae	No specific symptoms	Fatigue, malaise, swelling left triceps surae.
Peripheral blood	Hb 8.9 g/dL, WBC 62.8×10 ⁹ /L, PLT 91×10 ⁹ /L	Hb 10.3 g/dL, WBC 44×10 ⁹ /L, PLT 197×10 ⁹ /L	Hb 9.8 g/dL, WBC 8.34×10 ⁹ /L, PLT 107×10 ⁹ /L	Hb 12.8 g/dL, WBC 1.24×10 ⁹ /L, PLT 108×10 ⁹ /L	Hb 11.8 g/dL, WBC 59×10 ⁹ /L, PLT 145×10 ⁹ /L
Treatment	1 Induction course; cerubidine + cytarabine (3+7)	1 Induction course; cerubidine + cytarabine (3+7) 3 consolidation courses; high dose cytarabine. A 4th consolidation course was not given due to neurological symptoms. At relapse: cytarabine, amsacrine, etoposide (MAE). 1 induction course: M5A5E5. 2 consolidation courses: M3A3E3.	1 Induction course; cerubidine + cytarabine (3+7) 3 consolidation courses; high dose cytarabine. Allogenic stem cell transplantation, unrelated donor, myeloablative conditioning regimen.	1 Induction course; cerubidine + cytarabine (3+7), elderly. 1st consolidation course; mitoxantrone day 1-3, cytarabine day 1-5 + CNS prophylaxis with "triple" intrathecal. 2nd consolidation course; amsacrine day 1-3, etoposide day 1-3, cytarabine day 1-5 and "triple" intrathecal.	1 Induction + 1st consolidation course; cerubidine + cytosar (3+7) 2nd consolidation course; mitoxantrone day 1-3 + cytarabine day 1-4. Allogenic stem cell transplantation, sibling donor, reduced intensity conditioning regimen At relapse: No further leukemia specific treatment given, palliative support.
Complete remission	Dead before achieving CR	CR following induction; relapse after 11 months of CR	CR following induction; still in remission	CR following induction; still in remission	CR following induction; relapse after 7 months of CR

AML: Acute myeloid leukemia; COPD: chronic obstructive pulmonary disease; Hb: hemoglobin; PLT: platelet count; WBC: white blood cell count; CR: complete remission.

malignancies with trisomy 4 as the sole karyotypic anomaly, for submicroscopic or molecular aberrations.

Materials and Methods

Patients and samples. Permission to perform the study was granted by the Regional Committee for Medical and Health Research Ethics of South-East Norway, and written informed consent was obtained from the patients.

Searching our archive of 5700 cytogenetically analyzed leukemia samples (from 2009 until 2017) five additional cases of AML with trisomy 4 as the sole anomaly were found (Table I).

Methods. Interphase fluorescence *in situ* hybridization (FISH) was performed on these samples with the CytoCell multiprobe AML/MDS panel (CytoCell, <http://www.cytoCell.co.uk/>) looking for del(5q), *PML/RARA*, del(17p) (*TP53*), *RUNX1/RUNX1T1*, trisomy 8, *KMT2A (MLL)* splitting, -7/del(7q), *CBFB/MYH11*, and del(20q). The HemaVision 28Q reverse transcription-quantitative polymerase chain

Table II. *Mutational profile after exome sequencing of AML patients with trisomy 4 as the sole chromosome abnormality.*

Genes	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
<i>NPM1</i>	W288fs*12	W288fs*12	W288fs*12	W288fs*12	W288fs*12
<i>DNMT3A</i>	R882H	R882H			R882H
<i>NRAS</i>	G12D		G12S		
<i>FLT3</i>	D835Y		D835Y	E598DYVDFREYE	
<i>KIT</i>		D816V			D816V
<i>IDH1</i>		R132H		R132H	R132L
<i>IDH2</i>			R140Q		
<i>RUNX1</i>			E223G		
<i>WT1</i>			D464N		
<i>CUX1</i>				S480G	
<i>KRAS</i>			T58I		

reaction (RT-qPCR–KIT) for 28 chromosome translocations/ fusion genes (DNA Diagnostics, Risskov, Denmark) was used to detect the most common AML-specific fusion transcripts.

The Illumina's TruSight Myeloid Sequencing Panel (Illumina, San Diego, CA, USA) was used to identify somatic mutations in the samples. According to the company's information, the TruSight Myeloid Sequencing Panel covers 15 full genes (exons only) and 39 additional genes that are oncogenic hotspots. Alignment and variant calling were performed against GRCh37/hg19 with MiSeq Reporter Software v.2.6.3.2 (Illumina). SNV's and indels <60 bp were annotated by VariantStudio v.2.2.

Results

Neither interphase FISH analyses found any aberrations nor HemaVision 28Q RT-qPCR–KIT analysis detected any of the most common AML-specific fusion transcripts.

Targeted sequencing showed that all five examined AML samples carried mutations in addition to trisomy 4 (Table II). Samples from patients 1, 2, 4, and 5 harbored four mutations whereas patient 3 had seven mutations. *NPM1* frameshift mutation was found in all patients, whereas *KIT* and *NRAS* mutations were found in two patients. *DNMT3A*, *FLT3*, and *IDH1* mutations were found in three patients, whereas *IDH2* (R140Q), *RUNX1*, *CUX1*, *KRAS*, and *WT1* mutations were found in one patient each. Patients 1, 2, and 5, whose AMLs showed a *DNMT3A* (R882H) mutation, have died. In contrast, patients 3 and 4, whose leukemic cells did not carry this mutation, are alive 55 and 31 months after diagnosis, respectively. The leukemic cells from the deceased patients 2 and 5 also harbored the *KIT* D816V mutation, whereas patients 3 and 4 without this mutation are alive 55 and 31 months after diagnosis. Patient 1, also deceased, did not have the acquired *KIT* D816V mutation. This patient succumbed suddenly probably due to a cardiac event rather than AML.

Discussion

The genetic and molecular consequences of trisomy 4 are, in general, unknown (3, 4, 7, 8). Possible mechanisms involve

global gene expression alterations because of gene dosage effects, including duplication of rearranged or mutated genes on chromosome 4. No studies have as yet specifically examined general expression patterns in AML with trisomy 4. Instead, analyses have focused on *KIT* at 4q12, which encodes a receptor tyrosine kinase related to *FLT3*. Ferrari *et al.* (10) reported overexpression of *KIT* in one case with trisomy 4 as compared to AML without this abnormality, suggesting a possible pathogenetic role. Beghini *et al.* (11) showed that trisomy 4 resulted in duplication of a mutated *KIT* allele in two out of six AML cases with trisomy 4 as the sole change. Similarly, Schnittger *et al.* (12) reported *KIT* mutations in two cases with trisomy 4. On the other hand, Bains *et al.* (7) studied 13 AML cases with isolated trisomy 4 finding a *KIT* mutation in only one of them, and concluded that trisomy 4 was not generally associated with *KIT* mutations. Instead, *FLT3* and *NPM1* mutations were found in 50% and 40% of the cases, respectively.

In the present study, targeted sequencing showed concurrent multiple mutations in all patients (Table II). An *NPM1* frameshift mutation was found in all leukemic bone marrows. *NPM1* mutations are common in AML and are found in 20-30% of the cases (13). In addition, mutations were found in genes which are involved in the regulation of DNA methylation (*DNMT3A*, *IDH1* and *IDH2*) and genes involved in cell signaling (*FLT3*, *NRAS* and *KIT*). *RUNX1*, *CUX1*, *KRAS*, and *WT1* mutations were found in one patient each.

The three patients with a *DNMT3A* (R882H) mutation have died. In contrast, the two patients whose leukemic cells did not carry a *DNMT3A* mutation, are alive 55 and 31 months after diagnosis, respectively.

DNMT3A mutations are the most frequent recurrent gene mutations in AML after *NPM1* and *FLT3* mutations (14). The prognostic significance of *DNMT3A* mutations is thought to be adverse. *DNMT3A* mutations arise early in AML evolution and may persist during remission (1, 14). There is, however, no clear information today as to whether

any particular relationship exists between trisomy 4 and mutations of *DNMT3A*, let alone whether any such combination impacts prognosis.

It is unclear whether the poor outcome for patients 1, 2, and 5 was influenced by additional mutation(s) in *DNMT3A*, *KIT*, *IDH1* or a combination of these changes. The results, nevertheless, suggest a possible association between trisomy 4 and additional mutations that may affect prognosis. Novel techniques, such as targeted sequencing used in this study, may help identify the critical genes and understand their role in leukemogenesis and their influence on prognosis. Such investigations in large groups of patients are needed to collect quality data for this purpose.

Conflicts of Interest

The Authors declare that they have no potential conflicts of interest.

Authors' Contributions

ST made hematological evaluations, treated patients, designed the research, and wrote the manuscript. LG interpreted the cytogenetics and FISH data. SH interpreted the cytogenetics and FISH data and wrote the manuscript. GET made hematologic evaluations, treated patients and wrote the manuscript. SS made hematopathologic evaluations and interpreted the targeted sequencing data. BR performed targeted sequencing and interpreted the data. HTTT made hematologic evaluations and treated patients. IP designed and supervised the research, interpreted the data and wrote the manuscript.

Acknowledgements

This work was supported by grants from the Norwegian Radium Hospital Foundation and the Department of Haematology, Oslo University Hospital.

References

- Saultz JN and Garzon R: Acute myeloid leukemia: A concise review. *J Clin Med* 5, 2016. PMID: 26959069. DOI: 10.3390/jcm5030033
- Dohner H, Estey E, Grimwade D, Amadori S, Appelbaum FR, Buchner T, Dombret H, Ebert BL, Fenaux P, Larson RA, Levine RL, Lo-Coco F, Naoe T, Niederwieser D, Ossenkoppele GJ, Sanz M, Sierra J, Tallman MS, Tien HF, Wei AH, Lowenberg B and Bloomfield CD: Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. *Blood* 129: 424-447, 2017. PMID: 27895058. DOI: 10.1182/blood-2016-08-733196
- Lazarevic VL, Rosso A, Juliusson G, Antunovic P, Derolf AR, Deneberg S, Mollgard L, Uggla B, Wennstrom L, Wahlin A, Hoglund M, Lehmann S and Johansson B: Incidence and prognostic significance of isolated trisomies in adult acute myeloid leukemia: A population-based study from the Swedish AML registry. *Eur J Haematol* 98: 493-500, 2017. PMID: 28152233. DOI: 10.1111/ejh.12861
- Chilton L, Hills RK, Burnett AK and Harrison CJ: The prognostic significance of trisomy 4 in acute myeloid leukaemia is dependent on age and additional abnormalities. *Leukemia* 30: 2264-2267, 2016. PMID: 27451976. DOI: 10.1038/leu.2016.200
- Sandberg AA, Morgan R, Sait SN, Berger R, Flandrin G, Schrier S and Hecht F: Trisomy 4: an entity within acute nonlymphocytic leukemia. *Cancer Genet Cytogenet* 26: 117-125, 1987. PMID: 3470127.
- Mecucci C, Van Orshoven A, Tricot G, Michaux JL, Delannoy A and Van den Berghe H: Trisomy 4 identifies a subset of acute nonlymphocytic leukemias. *Blood* 67: 1328-1332, 1986. PMID: 3697508.
- Bains A, Lu G, Yao H, Luthra R, Medeiros LJ and Sargent RL: Molecular and clinicopathologic characterization of AML with isolated trisomy 4. *Am J Clin Pathol* 137: 387-394, 2012. PMID: 22338050. DOI: 10.1309/AJCP7ZC9YQERSKGX
- Gupta V, Minden MD, Yi QL, Brandwein J and Chun K: Prognostic significance of trisomy 4 as the sole cytogenetic abnormality in acute myeloid leukemia. *Leuk Res* 27: 983-991, 2003. PMID: 12859991.
- Panagopoulos I, Torkildsen S, Gorunova L, Ulvmoen A, Tierens A, Zeller B and Heim S: RUNX1 truncation resulting from a cryptic and novel t(6;21)(q25;q22) chromosome translocation in acute myeloid leukemia: A case report. *Oncol Rep* 36: 2481-2488, 2016. PMID: 27667292. DOI: 10.3892/or.2016.5119
- Ferrari S, Grande A, Zucchini P, Manfredini R, Tagliafico E, Rossi E, Temperani P, Torelli G, Emilia G and Torelli U: Overexpression of c-kit in a leukemic cell population carrying a trisomy 4 and its relationship with the proliferative capacity. *Leuk Lymphoma* 9: 495-501, 1993. PMID: 7687917. DOI: 10.3109/10428199309145756
- Beghini A, Ripamonti CB, Cairoli R, Cazzaniga G, Colapietro P, Elice F, Nadali G, Grillo G, Haas OA, Biondi A, Morra E and Larizza L: KIT activating mutations: Incidence in adult and pediatric acute myeloid leukemia, and identification of an internal tandem duplication. *Haematologica* 89: 920-925, 2004. PMID: 15339674.
- Schnittger S, Kohl TM, Haferlach T, Kern W, Hiddemann W, Spiekermann K and Schoch C: KIT-D816 mutations in AML1-ETO-positive AML are associated with impaired event-free and overall survival. *Blood* 107: 1791-1799, 2006. PMID: 16254134. DOI: 10.1182/blood-2005-04-1466.
- Heath EM, Chan SM, Minden MD, Murphy T, Shlush LI and Schimmer AD: Biological and clinical consequences of NPM1 mutations in AML. *Leukemia* 31: 798-807, 2017. PMID: 28111462. DOI: 10.1038/leu.2017.30
- Medinger M, Lengerke C and Passweg J: Novel prognostic and therapeutic mutations in acute myeloid leukemia. *Cancer Genomics Proteomics* 13: 317-329, 2016. PMID: 27566651.

Received March 2, 2019

Revised March 28, 2019

Accepted April 1, 2019