Leukemia Research 70 (2018) 74–78

Contents lists available at ScienceDirect

Leukemia Research

journal homepage: www.elsevier.com/locate/leukres

Research paper

Constitutional mutations of the *CHEK2* gene are a risk factor for MDS, but not for *de novo* AML

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ARTICLE INFO

Keywords:
MDS
AML
Congenital *CHEK2* mutations

ABSTRACT

CHEK2 plays a key role in cellular response to DNA damage, and also in regulation of mitosis and maintenance of chromosomal stability. In patients newly diagnosed with myelodysplastic syndrome (MDS, n = 107) or acute myeloid leukemia (AML, n = 117) congenital *CHEK2* mutations (c.444 + 1G > A, c.1100delC, del5395, p.I157 T) were tested by PCR and sequencing analysis. The karyotype of bone marrow cells of each patient was assessed at disease diagnosis using classical cytogenetic methods and fluorescence *in situ* hybridization. The *CHEK2* mutations were strongly associated with the risk of MDS ($p < 0.0001$) but not with the risk of *de novo* AML ($p = 0.798$). In *CHEK2*-positive MDS patients, two times higher frequency of aberrant karyotypes than in *CHEK2*-negative patients was found (71% *vs.* 37%, $p = 0.015$). In *CHEK2*-positive patients with cytogenetic abnormalities, subtypes of MDS: refractory anemia with excess blasts-1 or 2, associated with unfavorable disease prognosis, were diagnosed two times more often than in *CHEK2*-negative cases with aberrations (78% *vs.* 44%).

In conclusion, the congenital *CHEK2* inactivation is strongly associated with the risk of MDS and with a poorer prognosis of the disease. However, the chromosomal instability in AML is not correlated with the hereditary dysfunction of *CHEK2*.

1. Introduction

The C*HEK2* is a tumor suppressor gene (locus 22q12.1), that encodes the cell cycle G2 checkpoint kinase, which plays a key role in cell response to DNA damage (DDR) induced by replication stress and double strand DNA breaks. After *ATM* (*ataxia telangiectasia mutated gene*) - mediated activation, *CHEK2* can phosphorylate several substrates involved in cell cycle regulation, DNA repair (*e.g. BRCA1-breast cancer susceptibility gene 1*), *TP53* signaling, as well as induction and regulation of apoptosis [1,2]. In 2010, Stolz et al. [3,4] revealed another important role of *CHEK2* in regulation of mitosis, that is required for proper and timely formation of spindles, as well as accurate attachment of kinetochores and subsequent proper chromosome segregation.

In the present study we investigated the impact of constitutional *CHEK2* mutations on the risk of myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML), which pathogenesis is strongly associated with chromosomal instability (CIN).

Previously, some authors have investigated the relationship of somatic *CHEK2* mutations with MDS or AML and have found them infrequent or not significantly associated with these diseases [5–7]. Collado et al. [8] analyzed the c.1100delC germline mutation and did not find it nor in Spanish AML and MDS patients, neither in the control group.

The relatively high, 5.8%, cumulative frequency of four germline *CHEK2* mutations, *i.e.* missense c.470 T > C (p.I157 T) and three premature protein-truncating: c.444 + 1G > A (p.E149Kfs), del5395 and c.1100delC (p.T367Mfs) in the general Polish population allows for a reliable estimation of cancer risk in patient groups [9]. In the present study we also analyzed a correlation between inherited *CHEK2* mutations and patients age, karyotype of bone marrow cells at MDS or AML diagnosis, as well as family history of cancer.

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https://doi.org/10.1016/j.leukres.2018.05.013
Received 16 November 2017; Received in revised form 20 May 2018; Accepted 31 May 2018
Available online 03 June 2018
0145-2126/

Table 1
The association between constitutional *CHEK2* mutations and the risk of MDS or AML.

CHEK2 mutation MDS cases (n = 107) AML cases (n = 117)	Mutations N (%)	Controls (n = 312) [a]			Controls (n = 5496) [b]		
		OR	95% CI	p-value	OR	95% CI	p-value
Protein truncating							
c.444 + 1G > A							
MDS cases [c, d]	5 (4.7%)	7.6	1.3-57.5	0.013	12.2	3.9-34.9	0.0001
AML cases	2 (1.7%)	2.7	0.3-27.0	0.300	4.3	0.7-19.3	0.088
Controls [a]	2 (0.6%)	1.0					
Controls [b]	22 (0.4%)	1.0					
del5395							
MDS cases [e]	4 (3.7%)	12.1	1.3-287.1	0.016	8.8	2.5-27.5	0.002
AML cases	0						
Controls [a]	1 (0.3%)	1.0					
Controls [b]	24 (0.4%)	1.0					
Any truncating mutation							
MDS cases [c,d,e]	9 (8.4%)	9.4	2.3-45.0	0.0004	8.6	3.9-18.6	< 0.0001
Controls [a]	3 (1.0%)	1.0					
Controls [b]	58 (1.0%)	1.0					
Missense p.I157 T							
MDS cases [d, e]	11 (10.3%)	2.6	1.1-6.5	0.028	2.3	1.1-4.4	0.020
AML cases	6 (5.1%)	1.2	0.4-3.6	0.610	1.1	0.4-2.5	0.661
Controls [a]	13 (4.2%)	1.0					
Controls [b]	264 (4.8%)	1.0					
Total *CHEK2* mutations							
MDS cases [c,d,e]	20 (18.7%)	4.2	2.0-9.1	< 0.0001	3.7	2.2-6.2	< 0.0001
MDS cases with any mutation [c,d,e]	17 (15.9%)	3.5	1.6-7.6	0.001	3.1	1.7-5.3	0.0002
all AML cases	8 (6.8%)	1.4	0.5-3.5	0.485	1.2	0.5-2.5	0.554
de novo AML cases	6 (6.0%)	1.2	0.4-3.1	0.798	1.03	0.4-2.4	0.831
Controls [a]	16 (5.1%)	1.0					
Controls [b]	321 (5.8%)	1.0					

[a] Control group consisting of healthy persons from the Wrocław, Toruń and Bydgoszcz regions of Poland.
[b] Control group of the general Polish population, published by Cybulski et al. [9].
[c] One MDS patient with homozygous c.444 + 1G > A.
[d, e] Three MDS patients with the double mutation: two with p.I157 T and c.444 + 1G > A [d], one with p.I157 T and del5395 [e].

2. Methods

2.1. Patients

The analysis included 224 consecutive patients, newly diagnosed with MDS (n = 107) or AML (n = 117; 100 *de novo* and 17 *post* MDS), according to World Health Organization criteria [10] at three Polish hematology centers (Wrocław, Toruń and Bydgoszcz). The median age at diagnosis of MDS was 66 years (range 26–87), *de novo* AML - 56 years (range 18–87), and AML *post* MDS - 65 years (range 35–89). At the time of diagnosis, samples of peripheral blood (PB), buccal swabs (BS) and bone marrow (BM) cells were collected from all tested patients.

Fifty-two (49%) MDS patients and forty-eight (39%) AML patients reported at least one first- or second-degree relative with a cancer, mainly of lung, breast, ovary, stomach and colon. In families of 7 MDS patients, leukemia occurred (lack of detailed data). In family of one patient, two MDS cases occurred: in the tested patient at the age of 61 and in his mother at the age of 70. In families of 3 AML patients, second case of leukemia occurred (lack of detailed data), and in 3 other families, single cases of Hodgkin's lymphoma. In one family two AML cases occurred: in the tested patient at the age of 19 and in his grandfather at the age of 58.

The control group formed by us included 312 persons without any malignancies, who originated from Wrocław, Bydgoszcz and Toruń regions. The data on the general Polish population (the sample of 5496 unselected people) reported by Cybulski et al. [9] were used as the second control group. Informed consent was obtained from all patients and control healthy persons. The study was approved by the Ethics Committee of the Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University in Toruń, Poland.

2.2. Molecular methods

Molecular analyses were performed in DNA from PB, extracted using the DNA extraction kit QIAmp® DNA Blood Mini (QIAGEN, Germany). The constitutional character of a mutation was verified by analysis of DNA from BS, extracted using Swab-Extract DNA Purification Kit (EURx, Poland). The p.I157 T and c.444 + 1G > A mutations were examined by restriction fragment length polymorphism PCR method with specific primers and restriction enzymes, BtsI and Hpy188III, respectively [11]. The c.1100delC was tested by an allele specific PCR assay [11]. The large deletion of exons 9 and 10 (del5395) was detected by multiplex PCR with two primers pairs flanking breakpoints in intron 8 and 10 [9].

Mutation-positive cases were confirmed by sequencing analysis using ABI-PRISM 3130 Genetic Analyzer (Applied Biosystems, USA).

2.3. Cytogenetic methods

The karyotype of heparinized BM cells of each patient was assessed at disease diagnosis using classical GTG-banding techniques and fluorescence *in situ* hybridization (FISH). FISH analyses were performed using whole chromosome painting probes (WCP): WCP 1 (add 1), WCP X and WCP 16 [t(X;16)], WCP 14 [der(14)], WCP 21 [der(21)], and probes: Del(5q) Deletion, Del(20q) Deletion, D13S319Plus (+13), IGH/CCND1 Plus Translocation - Dual Fusion [t(11;14)], Chromosomes 7 and 8 Alpha Satellite, Chromosomes X Alpha and Y Alpha Satellite, AML1 (RUNX1) Breakapart (-21) (Cytocell, Germany), according to the manufacturer's instructions.

Chromosomal aberrations were classified according to the International System for Human Cytogenetic Nomenclature (ISCN)