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Ruling out of Fanconi Anemia in children with bone marrow failure, important but not always easy - a case report

Nino Oniashvili [1], Ohad Frenkel [2], Jacques Mardoukh [1], Yehudit Birger [1]
Einat Zindany [2], Yael A. Glickman [2], Hannah Tamary [3]

1 Schneider Children's Medical Center, Petah Tikva, Israel; 2 Applied Spectral Imaging, Carlsbad, CA, USA; 3 Sheba Medical Center, Ramat Gan, Israel

Background

- Fanconi Anemia (FA)[1] is a rare genetic disorder characterized by bone marrow failure (BMF) and increased risk of malignancy[2].
- Excluding diagnosis of FA prior to hematopoietic stem cell transplantation (HSCT), a definitive treatment for BMF, is crucial as patients with FA cannot tolerate standard-dose conditioning chemotherapy.
- Based on the fact that cells from FA patients are hypersensitive towards DNA cross-linking agents such as mitomycin C (MMC), diepoxybutane (DEB), or cisplatinum, a "chromosomal breakage test" is routinely utilized to confirm or rule-out Fanconi Anemia[3].

Material and Methods

- Chromosome breakage analysis, assessed using HiBand chromosome analysis and karyotyping software (Applied Spectral Imaging) (Figure 1) was part of a comprehensive diagnostic approach for Fanconi Anemia which also included whole-exome sequencing (WES).
- According to the standard adopted in our laboratory, Fanconi Anemia is confirmed when peripheral blood lymphocytes exhibit a 3 to 10 times higher frequency of chromosomal aberrations upon exposure to DNA cross-linking agents like DEB or MMC compared to normal controls[4].
- Family segregation analysis of the FANCA variants was used to confirm inheritance pattern.
- FANCD2 monoubiquitination functional assay, performed in Prof. Jean Soulier's laboratory at Saint-Louis Hospital in Paris, was used to evaluate the integrity of the FA core complex.

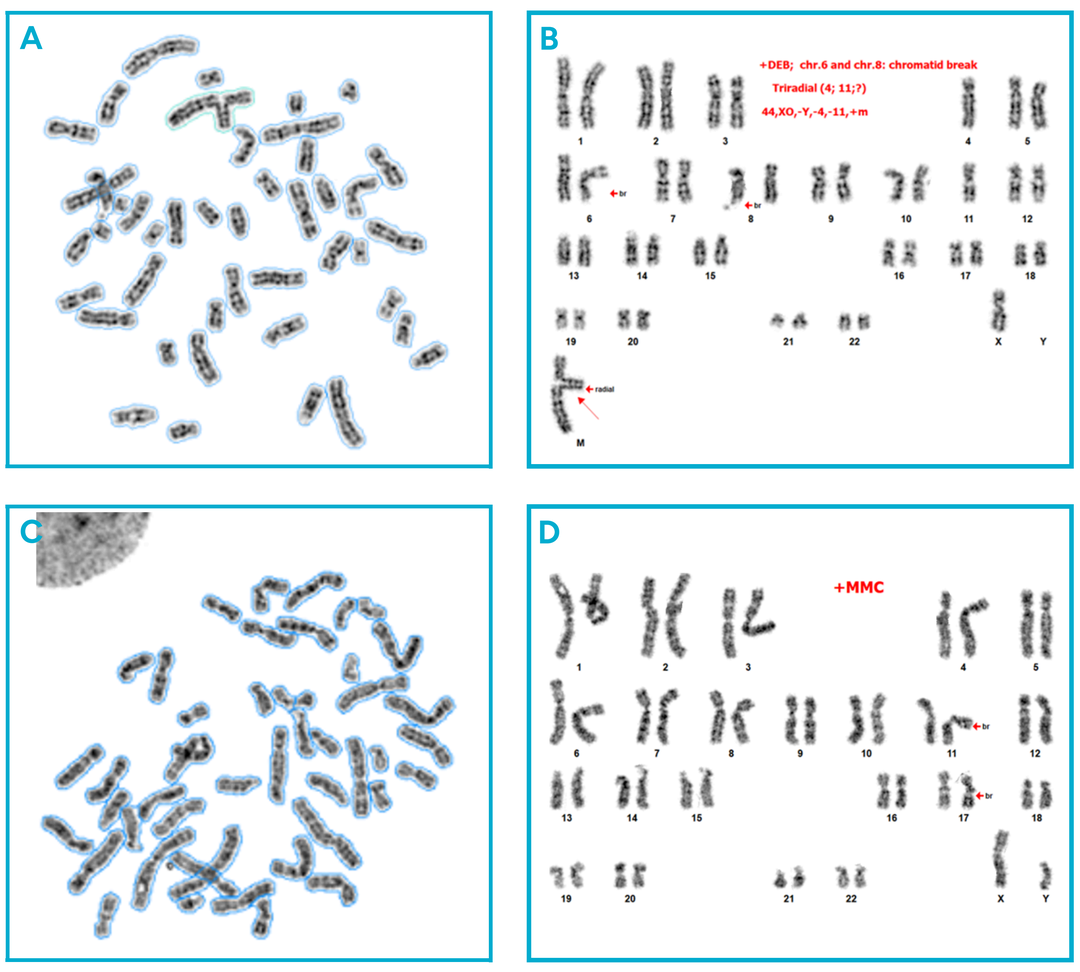

Figure 1
Representative examples of chromosomal breaks in metaphase and corresponding karyotype tables with DEB (A, B) and MMC (C, D). Chromatid gaps, chromatid breaks and triradial figure are highlighted in red.

	Clastogen	Final Concentration	0 break	1 break	2 breaks	3 breaks	>3 breaks	Radials 1>1	Total number of breaks	Total number of cells
Patient	MMC 1	40ng/ml	44	8					Radial+ 17	52
	DEB	100ng/ml	71	7	1			1		79
Control	MMC 1	40ng/ml	26	6					11	32
	DEB	100ng/ml	33	5						38

Table 1
Chromosome breakage analysis results for the patient reporting 9 breaks +1 Radial detected within 79 screened metaphases after culture with DEB and 8 breaks within 52 after culture with MMC. No increased sensitivity was detected compared to the control (5 breaks within 38 screened metaphases after culture with DEB and 6 break within 32 after culture with MMC).

Results

- This reports the case of a twelve-year-old patient who was incidentally found to have thrombocytopenia (58,000/mm³), macrocytosis (MCV 103 fL) and elevated fetal hemoglobin levels (13.9%) at age 8.
- Bone marrow biopsy demonstrated hypocellular marrow (20-50%), consistent with bone marrow failure.
- His mother was found to be a carrier of a known founder FANCA mutation[5], but no other family members carried the same genotype.
- Based on the analysis of chromosomal break using DNA cross-linking agents, MMC and DEB, compared to the control variant, no increased sensitivity to chromosomal abnormalities was observed, taking into account chromatid, chromosomal breaks and gaps (Table 1 and Table 2).
- Whole genome sequencing revealed that, in addition to the founder mutation, the patient carried an additional FANCA sequence variant that was classified as a variant of uncertain significance (VUS).
- The FANCD2 monoubiquitination functional assay demonstrated normal FANCD2 activation, indicating intact function of the Fanconi Anemia core complex, including FANCA.
- In addition, repeat chromosomal breakage testing revealed increased number of chromosome breakages, but not exceeding the threshold.

Conclusions

- Given these findings, Fanconi Anemia was ruled out for this patient.
- It is known that the detection of 1 triradial chromosome in >100 analyzed metaphases (in our case as a result of DEB exposure) does not consider the cytogenetic test for chromosomal aberrations positive for the diagnosis of Fanconi Anemia.
- However, because there are many false-negative results, we believe that in such cases the test needs to be validated or the sensitivity algorithm needs to be revised.
- Further investigations are ongoing to elucidate the etiology of the patient's bone marrow failure, including whole-genome sequencing (WGS) and RNA sequencing (RNA-seq).

	Clastogen	Counted Chromosomes	Analyzed Chromosomes	Number of Breaks	Total Chromosomes
Patient	MMC A	5	17	4	22
	MMC B	11	19	4	30
	MMC Total	**16**	**36**	**8**	**52**
	DEB A	16	34	7	50
	DEB B	13	16	2 + Triradial*	29
	DEB Total	**29**	**50**	**9 + Triradial***	**79**
	Total/Patient	**45**	**86**	**17 + Triradial***	**131**
Control	MMC A	10	30	6	40
	MMC B				
	MMC Total	**10**	**30**	**6**	**40**
	DEB A	20	26	5	
	DEB B				
	DEB Total	**20**	**26**	**5**	**46**
	Total/Control	**30**	**56**	**11**	**86**

Table 2
Chromosome breakage test algorithm for the patient reporting 9+Triradial breaks with DEB and 8 with MMC compared to control reporting 5 breaks with DEB and 6 with MMC. One Triradial chromosomal breakage is counted as 2 breaks (*).

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Conflicts of interest

NO, JM, YB and HT have no conflict of interest.
OF, EZ and YAG are employees of Applied Spectral Imaging (ASI).