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CASE REPORT OPEN ACCESS

A Distinctive Type of Mosaic Variegated Aneuploidy: Case Report and Review of the Literature

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ABSTRACT

Mosaic variegated aneuploidy (MVA) is an autosomal recessive disorder characterized by mosaic aneuploidies, predominantly trisomies, involving multiple different chromosomes and tissues. The proportion of aneuploid cells varies, and most patients present with intrauterine growth delay, microcephaly, and a broad spectrum of congenital abnormalities. We report a patient with a distinctive type of MVA discovered in bone marrow (BM) when she was 3-month-old due to neutropenia and hypocellular bone marrow. She was followed up for more than 20 years, and different trisomic cells were repeatedly discovered in different tissues, whereas her clinical picture has never been severe. The main sign remained intermittent neutropenia, not cyclic and often not too severe, occasionally with anemia and thrombocytopenia. Retromicrognathia was the only dysmorphic sign. Unlike other patients with MVA, the trisomies in all tissues involved almost invariably chromosomes 18 and 19. Therefore, the peculiarities of our patient were the clinical and the atypical cytogenetic pictures. Nevertheless, we looked for mutations in the seven causative genes of the known types of MVA, but the results were negative. Then, we analyzed the entire exome to find out other possible causing mutations, but also this attempt failed to discover a possible cause of this distinctive form of MVA.

1 | Introduction

Mosaic variegated aneuploidy (MVA) is a rare autosomal recessive disorder characterized by mosaic aneuploidies, predominantly trisomies, and monosomies, involving multiple different chromosomes and tissues. The proportion of aneuploid cells varies and affected individuals typically present with severe intrauterine growth delay, microcephaly, and a broad spectrum of congenital abnormalities and severe pathological signs (García-Castillo et al. 2008). MVA is genetically heterogeneous. At present, seven forms of MVA have been described, all caused by mutations in genes involved in mitotic spindle and microtubule stabilization, as well as in chromosome segregation. They are listed and better presented in the Discussion section.

Annalisa Frattini and Giovanni Micheloni contributed equally to the work.

Here, we report a patient with a probable diagnosis of MVA discovered in bone marrow (BM) when she was 2 months old due to neutropenia and hypocellular BM. She was followed up for more than 20 years, and a few different trisomic cells were repeatedly discovered in different tissues, whereas her clinical picture has never been severe.

2 | Case Report

The patient is a female born to unrelated parents after pregnancy without any complications. Severe neutropenia was discovered in the first weeks of life. Blood counts at 2 months of age were RBC 2.3×10^{12}/L, Hb 8 g/L, and WBC 4.4×10^{9}/L with 8% neutrophils, 86% lymphocytes, 5% monocytes, 0 eosinophils, and 1% basophils; At 3 months of age, they were RBC 2.2×10^{12}/L, Hb 7.1 g/L, and WBC 3.8×10^{9}/L with 13% neutrophils, 81% lymphocytes, 6% monocytes, 0 eosinophils, 0 basophils, and platelets 242×10^{9}/L. The BM was hypocellular, with a hypoplastic myeloid line and slight signs of dyserythropoiesis. The child was transfused, and the hematological picture improved in the following months and years. Growth and development were and remained in the years within normal limits (or percentiles). The search of antineutrophil antibodies was negative, as were the diepoxybutane (DEB) test and the search of mutations of the Shwachman-Bodian-Diamond gene (*SBDS*): these analyses excluded an autoimmune cause of the neutropenia, Fanconi Anemia (FA), and Shwachman-Diamond syndrome. The only clear-cut dysmorphologic sign present was retromicrognathia.

The patient grew without clinical problems, milestones were normal, cognitive development was normal, the menarche took place at 10 years of age. At 12 years of age, her height was m 1.50 (50 percentile), and her weight was 45 kg (63 percentile).

The patient was followed up until present, to monitor the neutropenia revealed at neonatal time. The neutropenia was intermittent, not cyclic, and never very severe, sometimes accompanied by anemia, but never by thrombocytopenia. BM was often severely hypoplastic, but variable in time. For example, at 7 years of age, her blood counts were RBC 4.09×10^{12}/L, Hb 13.1 g/L, and WBC 3.92×10^{9}/L with 34% neutrophils, 61% lymphocytes, 5% monocytes, 0 eosinophils, 0 basophils, and platelets 252×10^{9}/L; at 21 years of age, they were RBC 3.69×10^{12}/L, Hb 13.2 g/L, and WBC 3.63×10^{9}/L with 59% neutrophils, 39% lymphocytes, 1.5% monocytes, 0 eosinophils, 0 basophils, and platelets 151×10^{9}/L. The hematological picture, in general, never was as severe as in the first months of life.

At 17 years of age, she underwent a septoturbinoplasty, and at 20 years of age the avulsion of molar teeth 2.8, 4.8, and 3.8.

3 | Materials And Methods

3.1 | Cytogenetics

Chromosome analyses were performed repeatedly during the follow-up with routine methods on BM direct preparations and after 24–48 h cultures, on peripheral blood (PB) unstimulated and PHA-stimulated cultures, on fibroblast cultures established from a skin biopsy, and on an EBV-generated lymphoblastoid cell line established from B lymphocytes isolated from of PB. The analyses were performed by means of QFQ-banding technique.

After trisomies 18 and 19 were recognized as recurrent in BM and PB cells, fluorescent in situ hybridization (FISH) was carried out, according to standard techniques, on nuclei and mitoses with probes able to recognize these numerical anomalies: the centromere-specific alphoid D18Z1 probe for chromosome 18, and the bac probe RP11-81M8 for chromosome 19 (provided as courtesy by N. Archidiacono and M. Rocchi, University of Bari, Italy).

Fibroblasts were obtained from a skin biopsy and cultured with routine methods in RPMI-1640 medium supplemented with 10% FCS. Chromosome preparations were made at various passages of culture when a sufficient number of cells were in mitosis. Also, in these analyses, the QFQ-banding technique was applied, and FISH was performed with the probes already mentioned.

3.2 | NGS Analysis

To identify the possible gene variants causing MVA, NGS was used. In particular, whole exome sequencing (WES) analysis has been performed on the "trio" composed of the proband and her parents. Briefly, DNA was extracted by fresh peripheral blood in EDTA by Flexigene kit (Qiagen Gmbh, Hilden, Deutschland). Exome was performed by IGA Technology Services srl (Udine, Italy) with the use of SureSelect Human All Exon V7' kit (Agilent, Santa Clara, CA) as enrichment kit. Sequence runs were performed on HiSeq2500 (Illumina, San Diego, CA), pair-end, with 125 cycles per read.

Raw FASTQs were analyzed by the enGenome "eVAI" software tool (enGenome, Pavia, Italy—https://evai.engenome.com/) on hg38 map.

All samples used for cytogenetic and molecular analyses were obtained and analyzed in accordance with the declaration of Helsinki, after written consent.

4 | Results

4.1 | Cytogenetics

The severe neutropenia present in the first months of life led to perform chromosome analysis on BM when the patient was less than 2 months old, and the karyotype was normal (Table 1). The chromosome analysis performed on PB stimulated cultures, at 6 months of age, showed a normal karyotype, while analyses of BM samples drawn at 4–6 months of age, showed trisomy 19 in four mitoses out of 19 and in 17 mitoses out of 21, respectively (Table 1). Cytogenetic analyses on BM and PB were repeatedly performed in the subsequent years and the results are shown in Tables 1 and 2. Trisomy 19 was recurrently found, although the proportion of abnormal cells was never as high as at 6 months of age (Table 1). Clonal trisomy 18, alone or together with trisomy 19, was found both in PB unstimulated and PHA-stimulated cultures as well as in

TABLE 1 | Karyotype and FISH results with probes specific for chromosomes 18 and 19 on BM mitoses and nuclei.

Age	Karyotype
2 mths	46,XX[20]
4 mths	47,XX,+19[4]/46,XX[15]
6 mths	47,XX,+19[17]/46,XX[4]
2 yrs	47,XX,+19[9]/48,XX,+18. + 19[3]/46,XX[8]
3 yrs	47,XX,+19[2]/48,XX,+18. + 19[1]/46,XX[27]
4 yrs	47,XX,+19[1]/46,XX[19]
6 yrs	47,XX,+19[1]/46,XX[7][a]
7 yrs	46,XX[20]
12 yrs	46;XX[20]
13 yrs	46,XX[20]
14 yrs	47,XX + 2[1]/46,XX[29]
15 yrs	46,XX[20]

Abbreviations: mths, months; yrs, years.
[a]FISH: ish18 (D18Z1 × 2) [26].nuc ish 18 (D18Z1 × 2) [546]/(D18Z1 × 3) [21].ish 19 (RP11-81 M8 × 2) [51]/(RP11-81 M8 × 3) [7]. nuc ish 19 (RP11-81 M8 × 2) [600]/(RP11-81 M8 × 3) [31].

BM (Tables 1 and 2). Very few other trisomies in single cells were detected (Tables 1 and 2).

Fibroblast cultures established from skin biopsies obtained at 2 and 5 years of age were analyzed, and the cytogenetic results are reported in Table 3, together with the chromosome analysis performed on the lymphoblastoid cell line established from PB at 5 years of age.

The tables report not only the clonal anomalies, but also those found in single cells, which is not usual, but may be accepted in this particular case either because found in single cells in different subsequent analyses (*ISCN (2020): An International System for Human Cytogenomic Nomenclature. 2020. Cytogenetic and Genome Research* 2020) or due to the uncommon cytogenetic features of our patient.

Tables 1–3 include also the results obtained by FISH on mitoses (when available) and nuclei.

4.2 | NGS Analysis

WES analysis performed by the eVAI software did not reveal any possible candidate gene. The analysis has been carried out

TABLE 2 | Karyotype and FISH results with probes specific for chromosomes 18 and 19 on mitoses and nuclei from unstimulated and PHA-stimulated cultures.

Age	Culture type	Karyotype
2 yrs. 3 mths	PB-PHA	47,XX,+19[27]/48,XX,+18,+19[2]/46,XX[74]
2 yrs. 4 mths	PB-PHA	47,XX,+19[19]/46,XX[31]
3 yrs	PB	47,XX,+19[1]/46,XX[2]
	PB-PHA	47,XX,+19[9]/46,XX[41]
5 yrs	PB-PHA	47,XX,+19[15]/47,XX,+8[1]/46,XX[84][a]
10 yrs	PB-PHA	47,XX,+19[1]/48,XX,+18,+19[1]/46,XX[48]
11 yrs	PB-PHA	47,XX,+8[2]/47,XX,+9[2]/47,XX,+18[4]/46,XX[42]
12 yrs	PB-PHA	47,XX,+18[2]/47,XX. + 19[1]/48,XX,+18,+19[2]/46,XX[46]

Abbreviations: mths, months; PB, unstimulated PB cultures; PB-PHA, PHA-stimulated PB cultures; yrs, years.
[a]FISH: ish 18 (D18Z1 × 2) [24].nuc ish 18 (D18Z1 × 2) [500]/(D18Z1 × 3) [13].ish 19 (RP11-81 M8 × 2) [163]/(RP11-81 M8 × 3) [38]. nuc ish (RP11-81 M8 × 2) [600]/(RP11-81 M8 × 3) [55].

TABLE 3 | Karyotype and FISH results with probes specific for chromosomes 18 and 19 on mitoses and nuclei from skin fibroblast cultures and EBV-generated lymphoblastoid cell line established from B lymphocytes of PB.

Age	Material	Karyotype
2 yrs. 5 mths	SF	47,XX,+18[2]/46,XX[150]
5 yrs	SF	47,XX,+18[1]/47,XX,+19[2]/46,XX[24][a]
5 yrs	LL	47,XX,+X[2]/45,X,-X[2]/45,XX,-10[1]/47,XX,+12 [1]/47,XX,+15[1]/47,XX,+17[1],45,XX,-17[1]/47,XX, +18[2],47,XX,+19[1]/46,XX[80][b]

Abbreviations: LL, lymphoblastoid cell line; mths, months; SF, skin fibroblasts; yrs, years.
[a]FISH: ish 18 (D18Z1 × 2) [20]/(D18Z1 × 3) [1].nuc ish 18 (D18Z1 × 2) [363]/(D18Z1 × 3) [23].ish 19 (RP11-81 M8 × 2) [6].nuc ish 19 (RP11-81 M8 × 2) [386]/(D18Z1 × 3) [20].
[b]FISH: ish 18 (D18Z1 × 2) [560]/(D18Z1 × 3) [23].nuc ish 18 (D18Z1 × 2) [600]/(D18Z1 × 3) [15].ish 19 (RP11-81 M8 × 2) [542]/(RP11-81 M8 × 3) [2]. nuc ish 19 (RP11-81 M8 × 2) [600]/(RP1-181 M8 × 3) [20].

both by inheritance approach on the trio and by phenotype approach. We verified possible causative mutations by analyzing the genes already known to be MVA causative and genes associated with MVA as reported in OMIM (https:www.omim.org) and MalaCards databases (https://www.malacards.org/) (Data S1).

5 | Discussion

The patient here reported was referred to our analyses due to the presence of severe neutropenia in her first months of life. Cytogenetic analysis revealed the presence of single cells or small cell clones with numerical chromosome anomalies in BM and PB. In almost all the cases, the chromosomal anomalies were trisomies, only in a few cells monosomies, and they were found during a follow-up of 23 years in all the different tissues examined (Tables 1–3). The presence of these chromosomal anomalies suggested a possible diagnosis of MVA, first described by Warburton in 1991 (Warburton et al. 1991), usually characterized by severe intrauterine growth retardation and microcephaly, eye anomalies, mild dysmorphism, variable developmental delay, and a broad spectrum of additional congenital abnormalities. The first MVA form identified was shown to be due to biallelic mutations of the *BUB1B* gene (Hanks et al. 2004). This gene encodes a kinase involved in spindle checkpoint function ensuring proper chromosome segregation.

Afterward, MVA was found to be genetically heterogeneous, as it may be caused by mutations of different genes involved in mitotic spindle, microtubule stabilization, and chromosome segregation. Nowadays, seven forms of MVA have been described: MVA1 due to mutations in the *BUB1B* gene, MVA2 caused by *CEP57* mutations (Snape et al. 2011), MVA3 due to *TRIP13* mutations (Yost et al. 2017), MVA4 due to *CENATAC* mutations (de Wolf et al. 2021), MVA5 and MVA6 due to *SLF2* and *SMC5*, respectively (Grange et al. 2022), and MVA7, with systemic inflammation and tumor predisposition, due to *MAD1L1* mutations (Villarroya-Beltri et al. 2022). The clinical picture of affected patients is variable, although several symptoms are present in most MVA cases. Noteworthy, MVA1 is reported in a consistent number of patients: a review by Callier and coworker collected 28 cases, while the other MVA types are all reported in a few patients, from 1 to 12 (Snape et al. 2011; Yost et al. 2017; de Wolf et al. 2021; Santos-Simarro et al. 2021; Grange et al. 2022; Villarroya-Beltri et al. 2022; Pinson et al. 2014).

The variegated mosaicism of the reported patients affected by different forms of MVA typically shows different chromosomes involved in numerical anomalies (Callier et al. 2005; Grange et al. 2022). In particular, the majority of patients with MVA show high numbers of cells in different tissues with aneuploidies involving different chromosomes (Callier et al. 2005); some patients show predominant trisomy of a few chromosomes (Callier et al. 2005); some patients diagnosed as MVA show beyond the variable trisomic clones other cytogenetic characteristics, predominantly breaks and rearrangements, as dicentric chromosomes or other structural changes, as well as high-degree hyperdiploidy (Grange et al. 2022). These different cytogenetic features are not associated specifically to the different causative MVA gene.

The patient reported here should be included in the second of these cytogenetic groups, as trisomies 19 and 18 were largely predominant in all the tissues examined (Tables 1–3). The proportion of abnormal cells in different subsequent analyses was highly variable in BM. They were from 0% to 80%, with the higher percentage in the first 2 years of life (Table 1), in cells from PB-stimulated cultures 4%–38% (Table 2), in skin fibroblasts 1%–11% (Table 3). Obviously, in these evaluations, we must take into account the number of mitoses that were possible to score in the different cytogenetic analyses. Worthy of note, the number of patients reported in this group is small, less than 10 in the review by Callier and coworkers (Callier et al. 2005). Most patients of the literature were reported before the discovery of the causal gene mutations, and, in addition, it remains unclear how mutations in the causative genes of the different MVA forms may give rise to numerical anomalies of some specific chromosomes, and not of all chromosomes, as it seems to be the case of the MVA1 caused by *BUB1B* mutations.

The second peculiar feature of our patient in comparison with other MVA-reported cases is the less severe clinical picture. The severe most common signs present in the reported cases of MVA, although variable, were not found in our patient, who showed only retromicrognathia as dysmorphism, and no sign of microcephaly and growth retardation. By contrast, consistent features were intermittent neutropenia and anemia, which last not always present and often not severe. Hematologic symptoms were present only in a few cases of MVA from the literature: risk of leukemia in some patients with MVA1 (Hanks et al. 2004), anemia in MVA5 (Grange et al. 2022), variable hematological signs in MV6 (Grange et al. 2022).

With the aim to identify the genetic cause of MVA in our patient, we looked first for mutations in the seven genes already identified as etiology of the various MVA types, then we performed exome analysis in the proband and her parents. We did not find mutations, either as biallelic or monoallelic changes, in the known MVA causative genes. Moreover, exome analysis failed to identify any variations in other putative candidate genes suggesting that further genes are responsible for MVA.

Author Contributions

A.F. performed molecular analysis. G.Mi. and R.R. performed cytogenetic analysis. G.Mo. performed cell cultures. R.V. performed FISH analysis. A.F., A.M., F.O., and R.V. performed WES analysis. M.B.A., J.B., E.C., and P.S. were responsible of the clinical management of the patient and provided clinical data. R.V., F.A., G.P., and F.P. designed the research and analyzed the data. D.M.C. and C.F.L. performed statistical analysis. R.V. and F.P. coordinated the work. F.P., G.P., and R.V. wrote the paper.

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Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.