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Classical meets malignant hematology: a case of acquired $\epsilon\gamma\delta\beta$ -thalassemia in clonal hematopoiesis

Running title: Acquired beta-thalassemia in clonal hematopoiesis

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Authors' contributions

APP MT JHK MS SW performed research, APP MT JHK SW GH CH performed data analysis, APP WK TH GH CH wrote the manuscript, MM WK TH CH supervised the study.

Authors' disclosures

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Data sharing

Original data are available upon request in accordance with applying data protection rules.

Hemoglobinopathies including thalassemias are among the most frequent genetic disorders worldwide. Primarily, these entities result from germline variants in the globin gene clusters and their *cis*-acting regulatory elements ¹, and thus the WHO classifies thalassemias as inherited diseases ². Non-inherited disorders of globin chain synthesis mimicking the phenotype of thalassemias have also been described and are referred to as acquired thalassemias. These forms mainly affect the alpha-globin genes and are observed at much lower frequencies ³. Acquired alpha-thalassemias are associated mainly with myelodysplastic neoplasms (MDS) and are caused either by somatic deletions of the alpha-globin gene cluster on chromosome 16p13.3 limited to the hematological clone or, more commonly, by inactivating somatic variants of the trans-acting regulatory factor, ATRX, leading to down-regulation of alpha-globin gene expression ³. In contrast, acquired somatic genetic variants causing reduced beta-globin production and leading to a beta-thalassemic phenotype are a rarity, and only a few, single cases have been described so far ⁴⁻⁷.

Here, we describe a patient presenting with thalassemic erythrocyte parameter changes, i.e. microcytic, hypochromic anemia and a rather high erythrocyte count. Further evaluation including hemoglobin capillary electrophoresis and molecular genetic analyses exhibited clonal hematopoiesis with large deletions in the short and long arm of one chromosome 11 restricted to the neoplastic clone. The loss in the chromosomal band 11p15.4 harboring the complete beta-globin gene cluster resulted in an acquired $\epsilon\gamma\delta\beta$ -thalassemia which we documented at the molecular level.

Our laboratory received a peripheral blood sample from a 34 year old woman with microcytic, hypochromic anemia for further investigation after exclusion of iron deficiency. Blood count measurement at our laboratory confirmed a mild microcytic, hypochromic anemia (hemoglobin 11.0 g/dL, MCV 72.3 fl, MCH 22.0 pg, MCHC 30.5 g/dL, RDW 19.2 %, RBC count $4.99 \times 10^{12}/L$). The white blood cell count was normal ($7.97 \times 10^9/L$), and the thrombocyte count was slightly increased ($513 \times 10^9/L$). A peripheral blood smear showed polychromatic erythrocytes with basophilic stippling and target cells (Figure 1), and a normal leukocyte morphology and differential blood count (57% segmented neutrophils, 30% lymphocytes, 7% monocytes, and 6% eosinophils).

Further hemoglobinopathy work-up using capillary electrophoresis exhibited normal hemoglobin fractions with HbA 96.8%, HbA2 2.7% and HbF 0.5%; no hemoglobin variants were detected. Molecular genetics applying gap-PCR, Multiplex-Ligation-dependent Probe Amplification (MLPA) and targeted Next-Generation-Sequencing (NGS) of *HBA1*, *HBA2* and *HBB* revealed no aberration of the alpha-globin gene locus, but a loss of the complete beta-globin gene cluster with an allele frequency of about 50% in peripheral blood DNA (Figure 2).

To delimit the precise breakpoints of the deletions and to identify potential further genomic structural variants, whole genome sequencing (WGS) of DNA from peripheral blood leukocytes showed large deletions of 3.8 Mbp at chromosome 11p15.5-4 (GRCh37/hg19; chr11_2207001::5984000) including the beta-globin cluster, of 2.9 Mbp at 11p14.3-1 (chr11_24706001::27629000), and of 6.2 Mbp at chr11q22.3-q23.2 (chr11_108103001::114299000) involving the *ATM* gene (Figure 3A). Single nucleotide variants (SNVs) clinically significant in development of hematologic neoplasms were not detected.

WGS findings were in line with chromosome banding analysis from peripheral blood showing a derivative chromosome 11 with the expected deletions in the p- and q-arm: 46,XX,der(11)del(11)(p15p14)del(11)(q22q23)[16]/46,XX[8]. The aberrant clone was found in PHA stimulated cultures as well as in cultures stimulating the myeloid lineage. Interestingly, the aberrant karyotype was detected in only 16 out of 24 metaphases and additional interphase-FISH analyses with probes for the *NUP98* gene (11p15.4) and the *ATM* gene (11q22.3) confirmed the deletions in 85 of 100 and 80 of 100 cells, respectively, indicating that the deletions were not of germline origin but acquired in the hematopoietic cells. In a next step, we thus investigated DNA derived from oral mucosa of the patient as a germline surrogate by WGS to have a direct comparison with blood cell WGS results. The deletions on chromosome 11 were absent in the oral mucosa DNA of the patient (Figure 3B). In summary, we confirmed the somatic origin restricted to a hematopoietic clone of the deletion affecting the beta-globin cluster at a molecular level. The patient has given informed consent to publish the findings, and the study has been approved by the institutional review board.

In this case report, we describe an acquired thalassemic condition in clonal hematopoiesis resulting in a beta-thalassemic phenotype and document it at the molecular level. In contrast to acquired forms of alpha-thalassemia that are an established observation in MDS, acquired beta-thalassemia are extremely rare and described in only a few cases. So far, acquired ($\gamma\delta$) β -thalassemias have been described in MDS^{4,5,7}, acute myeloid leukemia⁸, juvenile myelomonocytic leukemia⁶ and common variable immunodeficiency⁹. In the present patient, bone marrow investigations would be necessary to differentiate between clonal cytopenia of unknown significance (CCUS) and a hematologic neoplasm, such as MDS, as final diagnosis. However, clonal hematopoiesis harboring a somatic 11p deletion as the pathophysiological cause of the acquired beta-thalassemia could be reliably demonstrated. Previous studies have shown that genomic gains and losses are a frequent phenomenon in clonal hematopoiesis^{10,11}. This includes recurrent chromosome 11 aberrations, and loss of *ATM* due to chromosomal deletions as in the present case. Interestingly, *ATM* mutations and chromosome 11q deletions are recurrent aberrations in MDS^{12,13}, while deletions in the short arm of chromosome 11 are rather rare in clonal hematopoiesis^{10,11}.

An increase in HbA2 above 3.6% with corresponding microcytic, hypochromic anemia with erythrocytosis is the hallmark phenotypical finding of inherited, heterozygous beta-thalassemia. Interestingly, none of the previously described cases of acquired beta-thalassemia exhibited an HbA2 increase suggesting the presence of deleterious aberrations of both the beta- and delta-globin genes. However, most of those patients presented with an increase in HbF pointing to intact gamma-globin genes⁵⁻⁸. Thus, these cases most likely represent delta-beta-thalassemias caused by deletions in the beta-globin gene cluster affecting the delta- and beta-globin gene, but leaving the gamma-globin genes intact. Despite the phenotypic observations in those studies, the genetic correlates remain hypothetical as precise molecular experiments were lacking in most studies. In our case, we observed a normal distribution of the hemoglobin fractions without an increase in HbA2 or HbF. High resolution molecular genetic analyses documented on the sequence level that the absence of an HbA2 and HbF increase was due to the loss of the complete beta-globin gene cluster in the neoplastic clone. The deletion includes the locus control region of the beta-

globin gene cluster in addition to the epsilon-, gamma-, delta and beta-globin genes and is thus the documentation of an acquired form of $\epsilon\gamma\delta\beta$ -thalassemia at the molecular level.

Alpha to non-alpha globin ratio imbalance represents the pathophysiological source of the typical thalassemic phenotype of microcytic, hypochromic anemia¹. Despite its common genetic basis of variants disturbing regular globin gene transcription, the origin of those alterations vary and either represent germline variants that are inherited from generation to generation or are somatic mutations acquired in a hematopoietic cell of an individual. In most cases, thalassemias originate from inherited germline variants and are classified as disorders from the field of classical, non-malignant hematology. However, clinicians also need to consider acquired thalassemic syndromes in hematological malignancies, especially in cases of discrepancy between tentative diagnosis and corresponding blood count changes. For example, macrocytic anemia is a typical finding in MDS, and thus, additional conditions like iron deficiency or acquired thalassemia need to be ruled out when MDS is accompanied with microcytic hypochromic erythrocyte parameters. In those cases, molecular genetic analysis of the neoplastic cell clone is key to a precise diagnosis, which may elude from standard tests like hemoglobin separation techniques used to screen for hereditary hemoglobinopathies.

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Figure legends

Figure 1. Peripheral blood smear

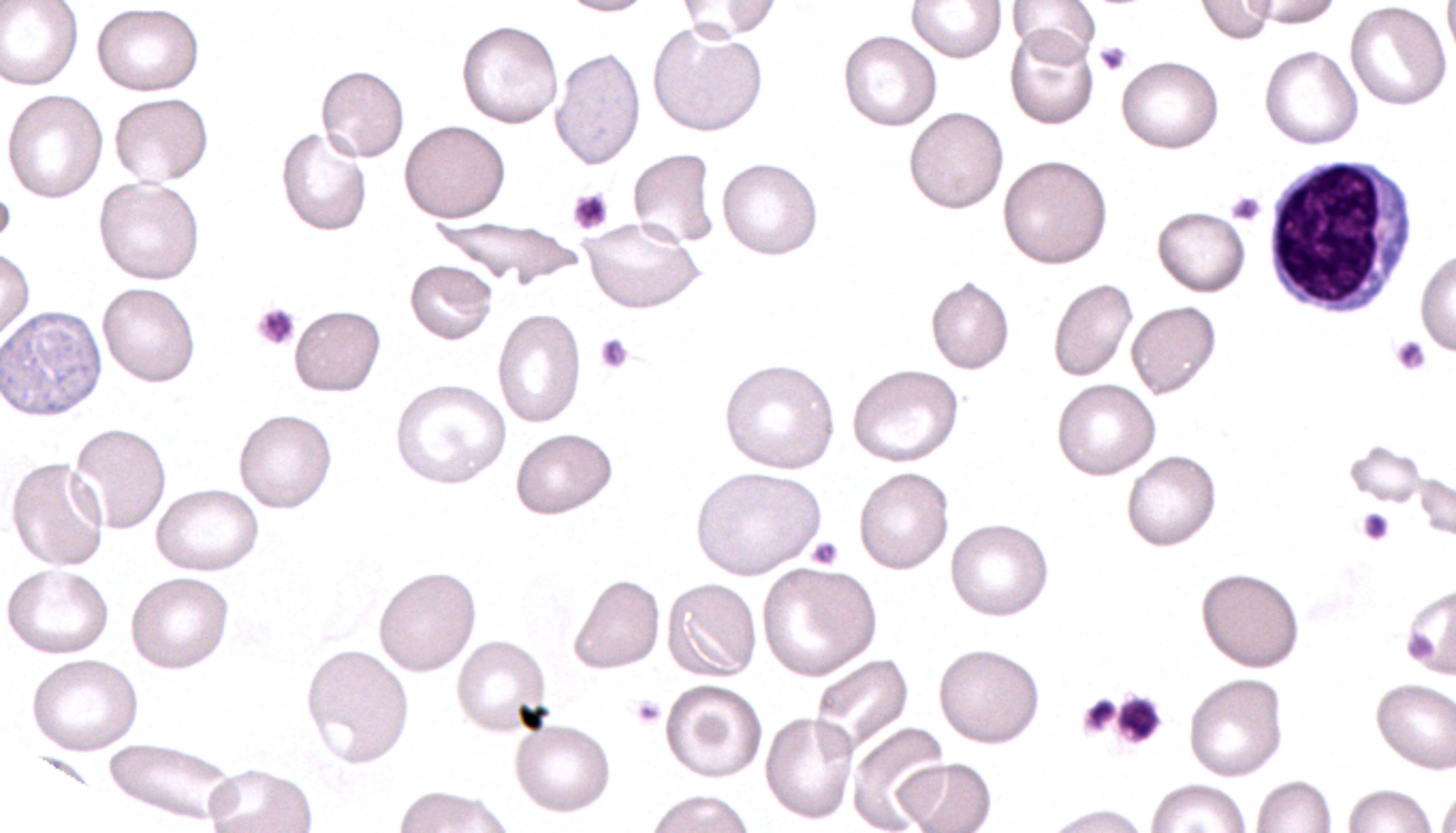
Peripheral blood smear showing microcytic and hypochrome erythrocytes, polychromasia, basophilic stippling and target cells.

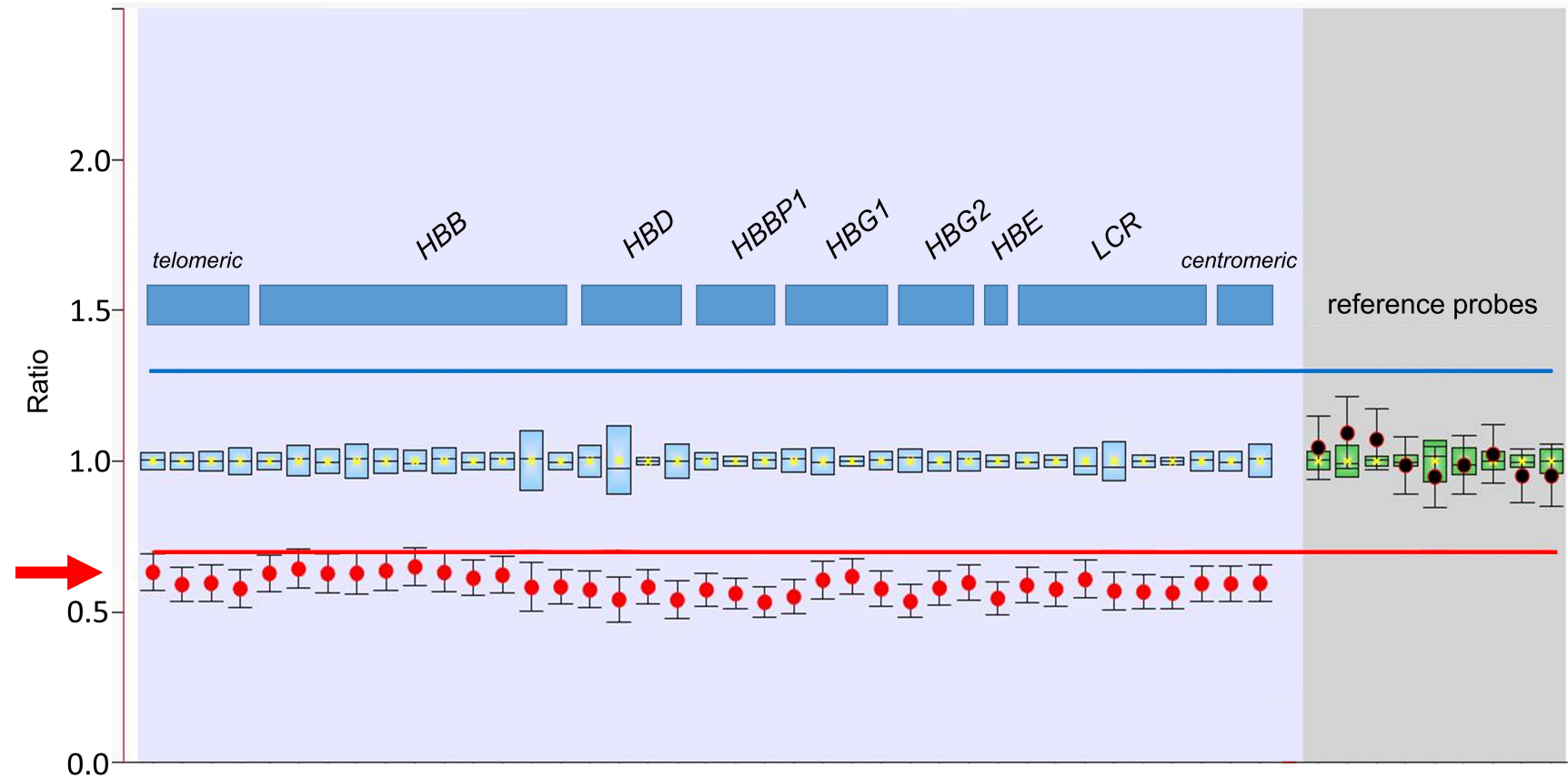
Figure 2. Multiplex-Ligation-dependent Probe Amplification

MLPA revealing a loss of the complete beta-globin gene cluster with an allele frequency of about 50% (red arrow).

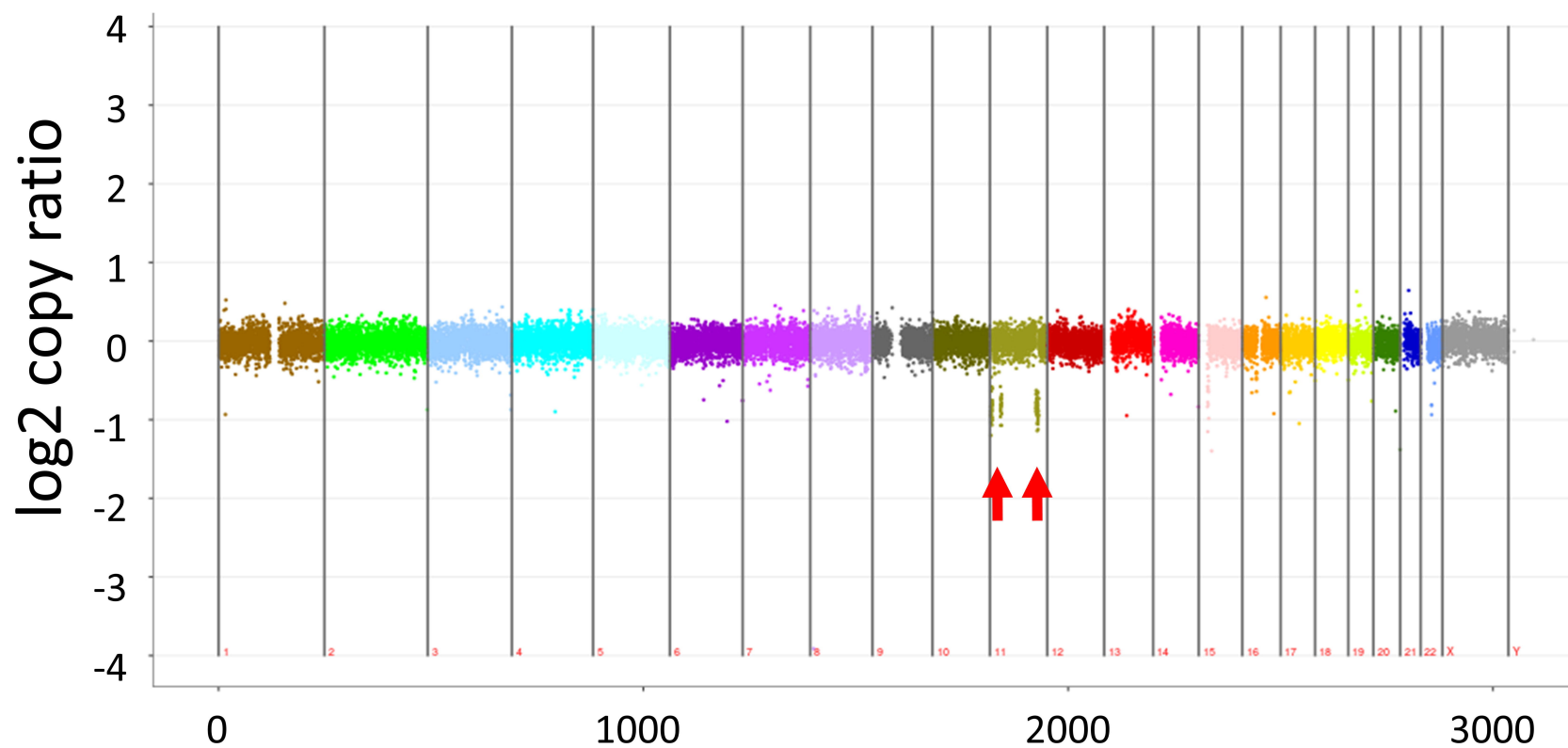
Figure 3. Copy number variation analysis of the entire genome by next generation sequencing

CNV analysis of the entire genome by NGS indicating the presence of deletions on chromosome 11 (red arrows) in peripheral blood DNA (panel A) and their absence in oral mucosa DNA (panel B).





A



B

