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ASXL2 mutated myelodysplastic syndrome in a novel germline *G6b* variant

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ABSTRACT

The 2016 revised World Health Organization classification identified myeloid neoplasms with germline predisposition as a new diagnostic category. Germline *loss-of-function* mutations in *G6b* (G6b-B, C6orf25 or MPIG6B) are associated with congenital macro-thrombocytopenia with focal myelofibrosis, a rare autosomal recessive disease. It is unclear whether germline *G6b* variants increase the risk of developing a myeloid neoplasm. Here we describe an adult with Myelodysplastic syndromes and a homozygous germline *G6b* mutation who achieved hematopoietic reconstitution by hematopoietic stem cell transplantation. As far as we know, this is the first report of adult Myelodysplastic syndromes with germline *G6b* homozygous variant in the literatures.

1. Introduction

The 2016 revised World Health Organization (WHO) classification identified myeloid neoplasms (MNs) with germline predisposition as a new diagnostic category [1]. More than a dozen of germline genes predisposing to MNs are described. Germline *loss-of-function* (LOF) mutations in *G6b* (*G6b-B, C6orf25* or *MPIG6B*) are associated with congenital macro-thrombocytopenia with focal myelofibrosis, a rare autosomal recessive disease [2–6]. However, it is unclear whether germline *G6b* variants increase the risk of developing a MN. Here we describe an adult with myelodysplastic syndrome (MDS) and a homozygous germline *G6b* mutation.

2. CASE presentation

We report a 43-year-old male with MDS with a novel germline *G6b* variant whose parents were cousins (**Fig. 1A**). At age 4 years he was found to have splenomegaly and thrombocytopenia and underwent splenectomy after which his platelet concentration normalized. Thereafter he had occasional epistaxis. No subsequent CBC was done. At age 42 years he developed fatigue symptoms with a hemoglobin concentration of 77 g/L, WBC of 3.13 × 10E+9/L and a platelet concentration of 32 × 10E+9/L. Bone marrow biopsy showed normal cellularity with scattered megakaryocytes and grade-2 reticulin fibrosis. There were no cytogenetic abnormalities. Received cyclosporine, testosterone undecanoate, prednisone, eltrombopag and erythropoietin with no hematological improvement and required intermittent RBC-transfusions.

On referral to us the hemoglobin concentration was 68 g/L, the WBC concentration, 3.5 × 10E+9/L and the platelet concentration, 17 × 10E+9/L. A blood smear showed anisopoikilocytosis with dacrocytes, giant platelets and nucleated erythroid cells. The percentage of blasts of bone marrow and peripheral blood smears were 8% and 7%, respectively. In multi-parameter flow cytometry bone marrow cells expressed CD34, CD33, CD13, HLA-DR. and CD38. CD41-immune stained bone marrow studies showed 71% of megakaryocytes were micro-megakaryocytes and megakaryocytes with separate nuclei. There was normal bone marrow cellularity with grade-2 fibrosis. Megakaryocytes were small with hypo-lobated nuclei (**Fig. 2A-H**). Only one metaphase was detected, revealing 46, XY,? der(8)t(8;13)(p23;q12). In RNA-seq no fusion variants were detected. *ASXL2* pathogenic variant (c.1840C > T, p. R614*) was detected by targeted next generation sequencing (NGS)

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with a variable allele frequency (VAF) of 8.6%. Whole exon sequencing uncovered a novel homozygous pathogenic variant in G6b gene (c.420T > A, p. Tyr140 *). We used Swiss-PdbViewer to predict the complete G6b protein with the mutation. The wild type (WT) template used was G6b precursor downloaded from AlphaFold Protein Structure Database (AF-O95866-F1-model_v1). Compared with G6B-WT, G6B-Tyr140* lost the transmembrane domain (TMD), immune receptor tyrosine-based inhibition motif (ITIM) and immunoreceptor tyrosine-based switch-motif (ITSM) (**Fig. 1B**). G6b staining showing the loss of G6b expression on the surface of megakaryocytes (**Fig. 2I-J**). A heterozygous variant of *G6b* (c.420t > A*)* was detected in his father, mother, sister and son (**Fig. 1C**). No hematological abnormality was found in these persons except the father with a platelet concentration of 94 × 10E+9/L. The propositus was diagnosed as MDS with excess blasts 2 (MDS-EB2) and received a hematopoietic stem cell transplant (HSCT) from an unrelated donor. By day 28 posttransplant the hemoglobin concentration was 72 g/L, the WBC, 5.76 × 10E+9 L, neutrophil concentration, 3.6 × 10E+9/L and platelet concentration, 110 × 10E+9/L.

3. Discussion

Congenital mega-thrombocytopenia with germline *G6b* mutation is a rare autosomal recessive disease. Only 19 persons from 9 affected families are reported including 17 of Arab descent [2,3,6], 1 of European descent [5] and 1 of Chinese descent [4]. All affected persons were from consanguineous families. The male: female ratio is about 2:1. Most persons presented with bleeding and thrombocytopenia within 5 years of birth, but they may be diagnosed after the age of 40 [2,5]. There is almost complete penetrance of homozygous LOF mutations. Most affected persons have macro-thrombocytopenia and focal myelofibrosis with variable degrees of anemia, leukocytosis and splenomegaly and a mild to moderate bleeding diathesis. Splenomegaly and bone marrow fibrosis may worsen over time and contribute to worsening anemia [5, 6]. The main clinical manifestations at onset in our patients were splenomegaly and thrombocytopenia, and bone marrow biopsy revealed grade-2 reticulin fibrosis, which is consistent with the typical clinical features of the disease with germline *G6b* mutation. Although the interval from onset to genetic diagnosis was up to 39 years in our patient, the advent of NGS has profoundly improved the early diagnosis of genetic diseases. Patients from consanguineous families with splenomegaly and thrombocytopenia should be noted for screening germline *G6b* mutations.

The types of *G6b* variants that have been reported include c.61_61+1dup, c.147insT, c.149dup, c.324C > A, c.392delC, c.469 *G* > A, and c.523C > T [2–6]. We identified a novel *G6b* truncation mutation (c.420T > A, p. Tyr140 *) in a Chinese family. The Tyr140* variant transforms the 140th amino acid into a stop codon resulting in early termination of protein translation. Truncated *G6b* loses the immune receptor tyrosine-based inhibition motif, which interacts with phosphatases SHP-1 and SHP-2 and affects megakaryocyte development, platelet production and activation [3,7,8]. *in vitro* studies showed the p. C108 * variant protein was unstable. Different from the truncated type, expression of wild-type human G6b enhances differentiation of K562 cells into megakaryocytes and erythrocytes [2]. *G6b* gene knockout leads to severe macro-thrombocytopenia, bone marrow fibrosis and platelet function abnormalities in mice [9].

As far as we know, this is the first report of adult MDS with germline *G6b* homozygous variant in the literatures. MDSs typically develop in persons with acquired somatic mutations. However, some occur on the background of a predisposing germline mutation. Typically there is early age onset and familial aggregation. The 2016 revised WHO classification identified MNs with germline predisposition as a new diagnostic category [1]. According to the clinical characteristics, predisposition syndromes was broadly assembled into 3 groups: MNs alone (*CEBPA, DDX41*), MSs with preexisting platelet disorders (*RUNX1, ANKRD26, ETV6*), and associated other organ dysfunctions (*GATA2,*

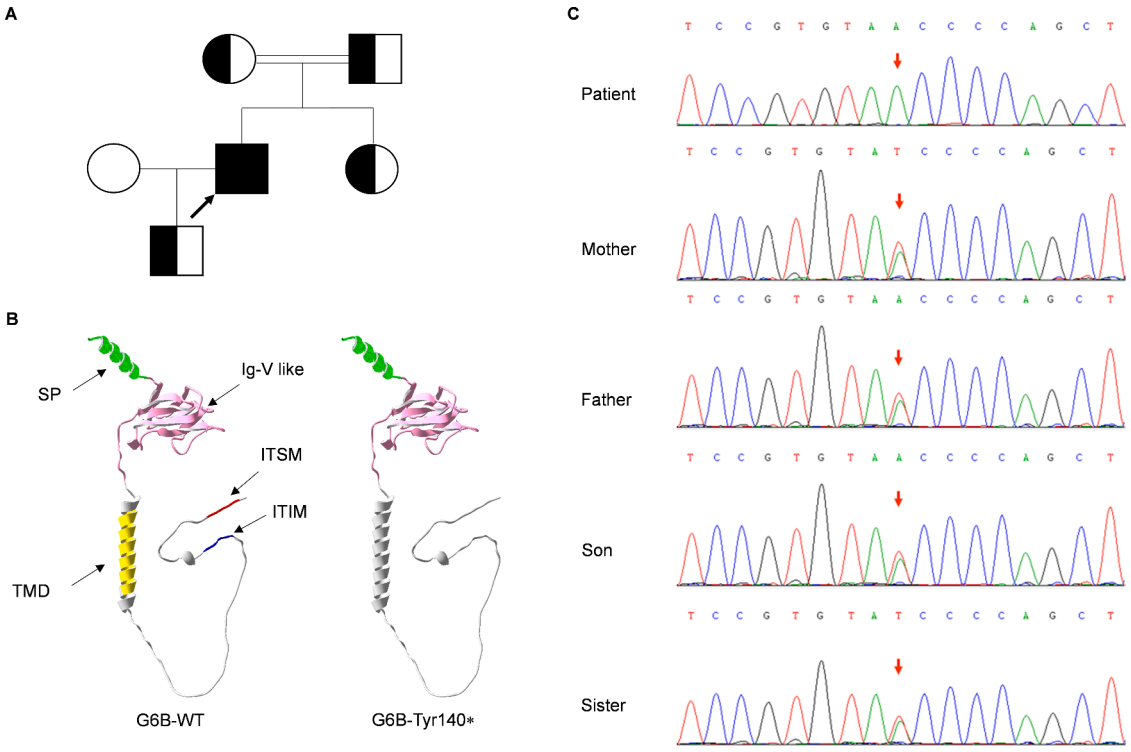

Fig. 1. (A) Family pedigrees. Black arrows point to the probands. Double line indicates a first cousin relationship, black fills represent variant alleles and blanks represent normal alleles. (B) Tertiary structure of wild type G6b protein (G6B-WT, left) and p.Tyr140* mutant G6b model (G6B-Tyr140*, right). Compared with G6B-WT, G6b-Tyr140* lost the transmembrane domain (TMD), immunoreceptor tyrosine-based inhibition motif (ITIM) and immunoreceptor tyrosine-based switch-motif (ITSM). SP, Signal peptide; Ig-V like, Ig-V like domain. (C) Chromatograms showing G6b-c.420T > A mutation and genotypes of all family members.

SRP72) [1]. Because there are a few cases it is difficult to determine whether germline *G6b* mutation predisposes to a MN like MDS. There is only a 10-month-old with a family history of hematologic cancers [4]. Disease progression in many predisposition syndromes occurs *via* acquisition of additional cooperating mutations such as *GATA2* and *SDBS*. *ASXL2* is an epigenetic regulator involved in polycomb repressive complex regulation. *ASXL2* plays a key role in inducing leukemogenesis, particularly in AML with t(8;21), as a cooperating mutation [10]. Although we detected a pathogenic variation of *ASXL2* (c.1840C> T, p. R614*) the VAF was only 8.6%. In addition, we detected one metaphase of 46, XY,? der(8)t(8;13)(p23;q12), but no fusion variants were detected by RNA-seq. Therefore, we speculate that this chromosomal translocation did not form a transcript.

Common treatment options for disease with germline *G6b* mutation include RBC-transfusions, corticosteroids, intravenous immunoglobulin, splenectomy and a hematopoietic cell transplant [3,5,6]. Corticosteroids

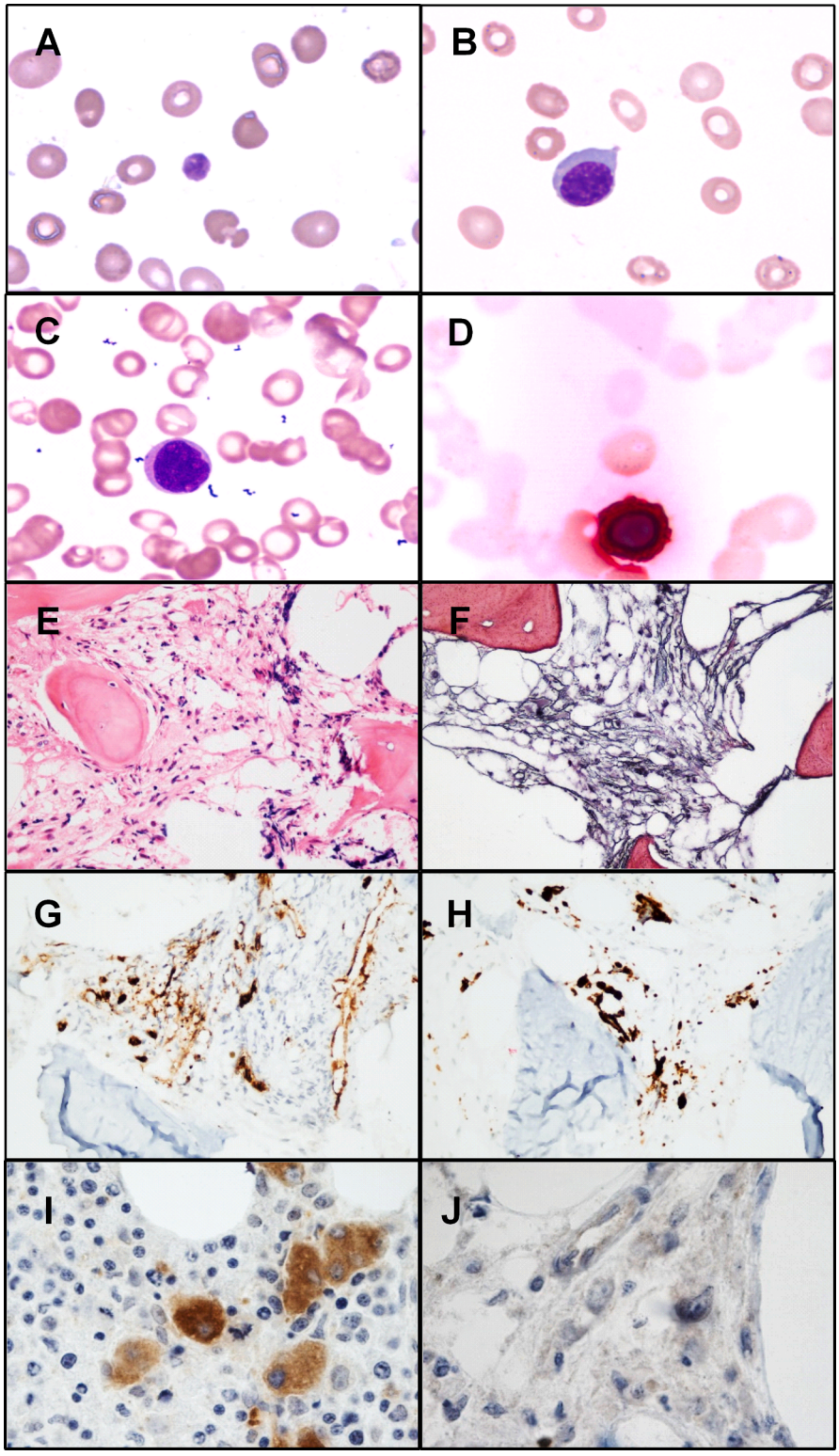

Fig. 2. Histologic features of blood and bone marrow. Wright–Giemsa-stained peripheral blood smear showing giant platelets (A, 1000 x) and leukoerythroblastosis with nucleated red blood cells (B, 1000 x) and myeloblast (C, 1000 x). CD41-immune stained bone marrow films showing micro-megakaryocytes (D, 1000 x). H&E-stained histologic sections of a bone marrow biopsy showing a normal cellularity and megakaryocytes with scattered distribution (E, 400 x). Silver staining highlights the marked reticulin fibrosis (grade 2) (F, 400 x). CD34 staining showing increased myeloblasts (G, 400 x). CD42b staining showing atypical megakaryocytes with small size and hypo-lobated nuclei (H, 400 x). G6b staining showing the expression of G6b on the surface of megakaryocytes in the positive control (I, 1000 x). G6b staining showing the loss of G6b expression on the surface of megakaryocytes in patients (J, 1000 x).

and splenectomy are transiently effective in some cases, and transplants can cure occasional patients [3,5,6]. Our patient underwent multiple regimens during the course of disease, including splenectomy, and eventually he achieved hematopoietic reconstitution by allogeneic HSCT in advanced MDS stage.

In conclusion we describe an adult with MDS and a homozygous germline *G6b* mutation. More data are needed to determine a causal relationship.

Informed consent

This study was approved by Ethics Committee of Blood Disease Hospital, Chinese Academy of Medical Sciences compliant with principles of the Declaration of Helsinki. Patients gave written informed consent.

Declaration of Competing Interest

RPG is a consultant to BeiGene Ltd., Fusion Pharma LLC, LaJolla NanoMedical Inc., Mingsight Parmaceuticals Inc. CStone Pharmaceuticals, NexImmune Inc. and Prolacta Bioscience; advisor to Antengene Biotech LLC, Medical Director, FFF Enterprises Inc.; partner, AZAC Inc.; Board of Directors, Russian Foundation for Cancer Research Support; and Scientific Advisory Board: StemRad Ltd.

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