GATA1 pathogenic variants disrupt MYH10 silencing during megakaryopoiesis

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INTRODUCTION

GATA1 is an essential transcription factor for erythrocyte and megakaryocyte (MK) differentiation. It belongs to the GATA family of zinc finger (ZF) transcription factors, which recognize the (A/T)GATA(A/G) DNA pattern. The GATA1 protein contains two highly conserved ZFs, which are separated by a short linker. The C-terminal ZF binds with high affinity and specificity to (AT)GATA(A/G) motifs throughout the genome, while the N-terminal ZF can bind protein cofactors, such as P400. Previous studies have shown that GATA1 is required for both polyploidisation and MK differentiation. Bone marrow from GATA1 variant carriers shows a moderate to marked increase in MK numbers, which are also small and hypoplastoid.

OBJECTIVE

To extensively phenotype two pedigrees displaying different variants in the GATA1 gene and determine how GATA1 act to control polyploidisation and megakaryopoiesis.

MATERIALS & METHODS

146 unrelated propos with constitutional thrombocytopenia were screened on a multigene panel. Two novel GATA1 variants were identified in three deoxytwin boys. We described the genetic correlate and investigated the effect of GATA1 variant on transcription assays using MK lysate luciferase constructs. Platelet aggregation was assessed by measuring light transmission through the stirred platelet-rich plasma (PRP) suspensions and platelet granules using electron microscopy. Circulating CD43+ prothrombin and isolated and cultivated in the presence of TPO and SCF to induce MK differentiation. Mkcs were analyzed via flow cytometry for CD41 and CD42a markers as well as for DNA ratio using Hoechst-33342. WT-type GATA1-DNA was inserted into a pC2-DN1: expression vector. The MYH10 regulatory region (introns 8 and the 3′ UTR) were cloned into a pGL3 vector. Site-directed mutagenesis was used to substitute the GATA1 binding sites with an AAAA sequence in the pGL3 and to generate GATA1 variants in pC2-DN1.

RESULTS

Identification of two novel GATA1 variants

The clinical profile associated with the p.L268M variant localized in the C terminal Zinc finger was unusual in the patient displayed bleeding and severe platelet aggregation defects without early-onset thrombocytopenia.

Platelet count and megacorpuscular volume of erythrocyte (MCV)

Peripheral blood and bone marrow smears (WBC staining)

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1. Identification of two novel GATA1 variants

2. Cases description

3. In vitro MK differentiation

4. MYH10 expression in platelet

5. Identification MYH10 regulatory regions

6. Functional analysis of the MYH10 regulatory regions

CONCLUSION

• The study supports the notion that impaired GATA1 silencing via an intronic regulatory element is the most likely cause of GATA1-related thrombocytopenia.

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