Human Molecular Genetics, 2002, Vol. 11, No. 2 147–152

Different substitutions at residue D218 of the X-linked transcription factor GATA1 lead to altered clinical severity of macrothrombocytopenia and anemia and are associated with variable skewed X inactivation

Kathleen Freson[1], Gert Matthijs[2], Chantal Thys[1], Paul Mariën[3,4], Marc F. Hoylaerts[1], Jos Vermylen[1] and Chris Van Geet[1,3,*]

[1]Center for Molecular and Vascular Biology, [2]Center for Human Genetics and [3]Department of Pediatrics, University of Leuven, Leuven, Belgium and [4]St Augustinus Hospital, Wilrijk, Belgium

Received September 18, 2001; Revised and Accepted November 15, 2001

GATA1 is the X-linked transcriptional activator required for megakaryocyte and erythrocyte differentiation. Missense mutations in the N-terminal zinc finger (Nf) of GATA1 result in abnormal hematopoiesis, as documented in four families: the mutation V205M leads to both severe macrothrombocytopenia and dyserythropoietic anemia, D218G to macrothrombocytopenia and mild dyserythropoiesis without anemia, G208S to macrothrombocytopenia and R216Q to macrothrombocytopenia with β-thalassemia. The three first GATA1 mutants display a disturbed binding to their essential transcription cofactor FOG1, whereas the fourth mutant shows an abnormal direct DNA binding. In this study, we describe a new family with deep macrothrombocytopenia, marked anemia and early mortality, if untreated, due to a different GATA1 mutation (D218Y) in the same residue 218 also implicated in the above mentioned milder phenotype. Zinc finger interaction studies revealed a stronger loss of affinity of D218Y–GATA1 than of D218G–GATA1 for FOG1 and a disturbed GATA1 self-association. Comparison of the phenotypic characteristics of patients from both families revealed that platelet and erythrocyte morphology as well as expression levels of the platelet GATA1-target gene products were more profoundly disturbed for the hemizygote D218Y mutation. The D218Y allele (as opposed to the D218G allele) was not expressed in the platelets of a female carrier while her leukocytes showed a skewed X-inactivation pattern. We conclude that the nature of the amino acid substitution at position 218 of the Nf of GATA1 is of crucial importance in determining the severity of the phenotype in X-linked macrothrombocytopenia patients and possibly also in inducing skewed X inactivation.

INTRODUCTION

GATA transcription factors contain highly conserved C4-type zinc fingers which mediate sequence-specific binding to a consensus motif (A/T)GATA(A/G) (1,2). GATA1, the founding member of the GATA transcription factor family, is expressed at high levels in erythroid, megakaryocytic, mast and eosinophilic cells (3,4). Virtually all characterized erythroid-specific genes (i.e. α- and β-*globin*) and megakaryocytic-specific genes (i.e. *GPIIb*, *GPIbα*, *GPIbβ*, *GPIX*, *PF4* and *mpl*) contain GATA motifs in critical *cis*-regulatory elements (4,5). The essential role of GATA1 in erythroid and megakaryocyte differentiation has been illustrated in two mouse models. Homozygous disruption of GATA1 causes embryonic lethality due to a severe erythroid defect (6,7). In contrast, mice with a mutation in the upstream region of *GATA1* (GATA1 knockdown mice) show a milder erythroid defect but suffer from severe thrombocytopenia due to absent GATA1 expression in the megakaryocytic lineage (4).

GATA1 contains two zinc fingers; the C-finger, sufficient for sequence-specific direct DNA binding, and the N-finger (Nf), which plays a role in the stabilization of GATA1 binding to DNA (8). The Nf can regulate transcription by binding to a GATA1-interacting nuclear zinc-finger protein FOG1, friend-of-GATA1. FOG1 is coexpressed with GATA1 during embryonic and hematopoietic development (9). FOG1 knockout mice die during mid-embryonic development with severe anemia and show a specific ablation of the megakaryocytic lineage (10). Recently, it was shown that the Nf can bind GATC sites in isolation and stabilizes binding to double GATA sites (11). The Nf also seems implicated in GATA1 self-association by interacting with the C-finger. Although there is a clear involvement in transcription regulation, the exact biological role of this self-association in gene activation is not certain (12,13).

Recently, different missense mutations in the Nf of GATA1 were found in four families, leading to a variable degree of macrothrombocytopenia with or without abnormalities in the

*To whom correspondence should be addressed at: Center for Molecular and Vascular Biology, UZ-Gasthuisberg, Herestraat 49, 3000 Leuven, Belgium. Tel: +32 16 345775; Fax: +32 16 345990; Email: christel.vangeet@uz.kuleuven.ac.be

erythrocyte lineage. Two related patients with X-linked dyserythropoietic anemia and macrothrombocytopenia carry a V205M mutation, which leads to a reduced interaction of the Nf of GATA1 with FOG1 (14). We originally described a family with mutation D218G in the same Nf of GATA1, showing isolated X-linked macrothrombocytopenia and some features of dyserythropoiesis but without marked anemia (15). Another family with macrothrombocytopenia was described carrying mutation G208S, and finally a family with both macrothrombocytopenia and β-thalassemia carried mutation R216Q (16,17). The V205M, D218G and G208S mutations reduce the interaction of GATA1 with FOG1, although to a different degree, whereas the R216Q mutation results in a reduced interaction with a GATA DNA recognition site (11,14–17).

In the present study, we describe a new family with a different missense mutation at position D218. We compare the clinical severity in both our families and show that the phenotype not only depends on the position of the GATA1 mutation but also on the nature of the substituted amino acid. The GATA1 self-association was checked for both D218–GATA1 mutants. We determined the degree of skewed X inactivation in female carriers, because some X-linked genetic disorders in constitutional or in some specific lineages are known to give rise to skewing of X inactivation (18). Finally, we also studied the expression of the mutant GATA1 in the platelets of obligate carriers from both families.

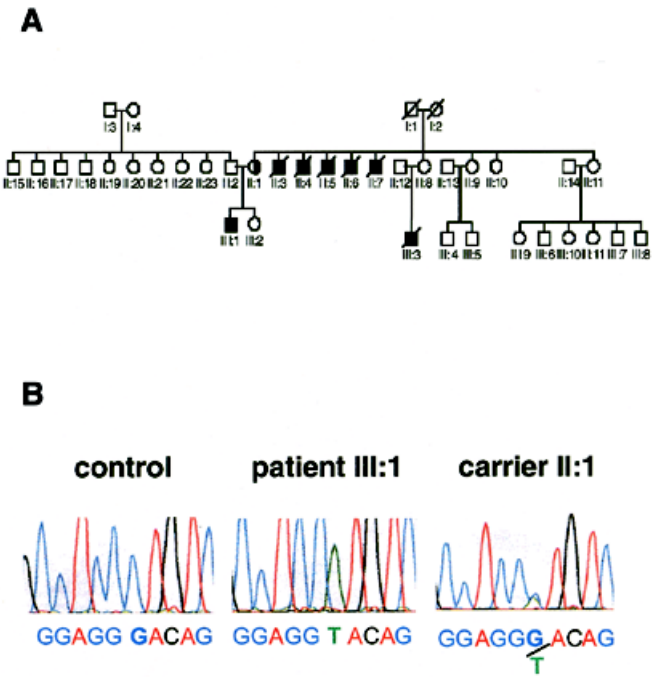

Figure 1. Family presentation and molecular analysis. (**A**) A pedigree of the family of patient III:1. Patients with the X-linked thrombocytopenia and anemia are represented by closed symbols, female carriers by half-closed circles, deceased members by a slash. (**B**) Sequencing electropherogram of a control, the patient III:1 and his mother II:1 (carrier), respectively, hemizygous and heterozygous for the base pair substitution G→T at nucleotide position 652 of the Nf of GATA1 in leukocyte genomic DNA.

RESULTS

Family description and mutation analysis

We describe a new family characterized by pronounced X-linked dyserythropoietic anemia and deep macrothrombocytopenia, in which six affected boys died before the age of 2 years (Fig. 1A). The only affected member still alive (patient III:1) is a 1-year-old boy with deep transfusion-dependent anemia and macrothrombocytopenia. The hematological data of this patient and his mother (obligate carrier) are listed in Table 1. The peripheral blood smear of patient III:1 shows a small number of normal to giant platelets, dysmorphic erythrocytes (anisocytosis and poikilocytosis) and the presence of some normoblasts. Electron microscopy of his platelets displays the typical paucity of α granules and the presence of clusters composed of smooth endoplasmic reticulum (15). Bone marrow examination by light and electron microscopy reveals strong dyserythropoiesis with dysmorphic erythroblasts (multinucleated and with clumping of the heterochromatin) and dysplastic megakaryoblasts. The mother (II:1) is not anemic, has no dysmorphic erythrocytes and has a normal platelet number with normal ultrastructure on electron microscopy. A very small number of enlarged platelets is however present.

The pedigree structure and the typical hematopoietic abnormalities were suggestive for a mutation in the X-linked gene *GATA1* (14,15). Therefore, we sequenced the *GATA1* cDNA and found a missense mutation (G→T) at nucleotide position 652 in patient III:1. The mutation was confirmed in genomic DNA from leukocytes. The mother is an obligate carrier and heterozygous for this mutation (Fig. 1B). The clinically non-affected males (III:6, III:7 and III:8) did not have this mutation. The mutation (652 G→T) is located immediately adjacent to our previously reported missense mutation (653 A→G) and results in a different amino acid substitution at the same position 218 (D218Y versus D218G) of the Nf of GATA1.

In vitro effects of the GATA1 mutation

Although their mutations are located in the same residue of GATA1, the differences in clinical severity between the patients in our previously reported family carrying the D218G mutation and the patients of the present family carrying the D218Y mutation are striking. Nf GATA1 interaction studies and expression studies of the GATA1-target gene products in platelets were therefore undertaken.

In vitro binding studies with either the FOG1 fingers 5–9 or FOG1 finger 1 were performed with the different GATA1 Nfs (Fig. 2A). D218Y–GATA1 had a weaker affinity for FOG1 than D218G–GATA1; its affinity was similar to that of the V205M mutant. Furthermore, the interaction of the different GATA1 Nfs with the complete wild-type GATA1 protein was studied. The binding of the D218 GATA1 mutants was decreased compared to that of the wild-type or of the V205M GATA1 Nf. We infer that the interaction with FOG1 not only depends on the position of the mutation as had been suggested by Fox *et al.* (19) and Crispino *et al.* (20), but also on the nature of the amino acid substituted. We also conclude that both D218 GATA1 mutations hamper self-association of the GATA1 protein.

Table 1. Hematological profiles of patient III:1 and obligate carrier II:1 with the D218Y–GATA1 mutation and obligate carrier IV:4 with the D218G–GATA1 mutation

	Hgb (g/dl) (10.5–16)	Hct (%) (35–45)	RBCs ($\times 10^6$/µl) (3.9–5.2)	RDW (%) (11.5–14.5)	LDH (U/l) (150–460)	Plts ($\times 10^3$/µl) (150–400)	ESR (mm/h)
III:1	9.7	33.5	3.42	17.2	812	12	2
II:1	14.9	43	4.73	13.6	310	255	–
IV:4	12.6	39	4.31	13.3	–	297	–

Hgb, hemoglobin; Hct, hematocrit; RBCs, red blood cells; RDW, red blood cell distribution width; LDH, lactate dehydrogenase; Plts, platelets; ESR, erythrocyte sedimentation rate. [Carrier IV:4 is described by Freson *et al.* (15).]
The normal values (in parentheses) are also indicated.

Direct binding of the *GATA1* mutants to DNA, studied as previously described by Freson *et al.* (15), was not disturbed (Fig. 2B).

In vivo effect of the GATA1 mutation

To approach the *in vivo* effects of the two D218 mutants, we studied the GATA1-dependent gene regulation in the patients' platelets of both families. Because we could not obtain enough mRNA from the platelets of patient III:1, only expression at the protein level was studied. Expression levels of GATA1-target gene products (GPIbα and GPIbβ) and of a non-directly GATA1-regulated gene product (Gsα) were analyzed. Western blot analysis showed undetectable levels of GPIbα and GPIbβ in the platelets of patient III:1 (with mutation D218Y), in contrast to the reduced but detectable levels of GPIbα in the patient with the D218G mutation (Fig. 2B) (15). The Gsα protein was studied as a marker for incomplete platelet maturation since it is upregulated during megakaryocyte differentiation and has no known GATA1 regulatory element in its promoter (21). Compared to the slightly weaker expression in the patient with mutation D218G, the Gsα expression is only 50% in the platelets of patient III:1, suggestive of incomplete platelet maturation. Similar to what was observed in the carriers of the D218G–GATA1 mutation, no abnormalities were found in the platelets of the D218Y carrier.

Study of the X-inactivation pattern and analysis of abnormal *GATA1* message in platelets of female carriers

The X-inactivation pattern in an obligate carrier from both families was analyzed. Carrier IV:4 with the D218G–GATA1 mutation (15) is informative for the androgen receptor (AR) polymorphism and showed slightly skewed X-chromosome inactivation (Fig. 3A). PCR fragments derived from one of the two alleles did not selectively disappear in either *Hha*I or *Cfo*I digested samples. In contrast, carrier II:1 with the D218Y–GATA1 mutation was informative for the monoamine oxidase A (MAOA) polymorphism and showed clear skewed X inactivation (Fig. 3A). After digestion with either *Hpa*II or *Sma*I, fragments amplified from one of the two alleles almost completely disappear.

The expression of the two D218–GATA1 mutations was also studied in the platelets from both carriers using RT–PCR and sequencing. Carrier IV:4 from the D218G–GATA1 family was heterozygous for the mutation at the mRNA level in her platelets (Fig. 3B). In the case of carrier II:1 of the D218Y–GATA1 family, the abnormal GATA1 allele was not detected at the mRNA level (Fig. 3B).

DISCUSSION

We showed that the severity of the phenotype of X-linked macrothrombocytopenia and dyserythropoiesis with or without anemia varies with the kind of missense mutation at a particular position and correlates with the degree of disturbed interaction of the mutant GATA1 with FOG1.

In contrast to position 218, residue 205 was considered at least *in vitro* to be important for GATA1:FOG1 zinc fingers interaction. It is now obvious that residue 218 also is crucial for this interaction *in vitro* and *in vivo*; moreover, the degree of disturbance upon mutation depends on the amino acid residue it is mutated to.

Biochemical studies have demonstrated that the zinc finger domains of GATA1 are also involved in the GATA1 self-association (12). Recently, it became clear that self-association is mediated by the Nf of GATA1 making intermolecular contact with the C-finger (13). A deletion mutant of the GATA1 Nf affects this association and interferes with its ability to activate transcription in transfection studies, especially from multi-GATA1 site promoters. Mackay *et al.* (13) also speculated on the exact biological role of the self-association; it would increase the local concentration of GATA1 and therefore extend its potency as a transcriptional activator. Since many erythroid promoters contain multiple GATA sites, it could be involved in the ordered assembly of higher order complexes at such promoters. The possible contribution of a disturbed GATA1 self-association by the D218 mutation to the clinical phenotype is not clear. However, our work shows for the first time that a missense mutation in the Nf can lead to a disturbed GATA1 self-association.

We were also interested in the consequences of the GATA1 mutation for the carriers from both families. *GATA1* is an X-linked gene and a molecular defect can be involved in preferential cell selection and this results in skewed X inactivation in peripheral blood leukocytes. Such observations were found in other X-linked disorders as in X-linked agammaglobulinemia, incontinentia pigmenti type 2, Wiskott–Aldrich syndrome and dyskeratosis congenita (22–25). The carriers of both the D218G and the D218Y mutation had a normal platelet count but sporadically had a large-sized platelet in the peripheral blood smear. We analyzed isolated platelets from both carriers for the presence of mutant *GATA1* mRNA. Carrier

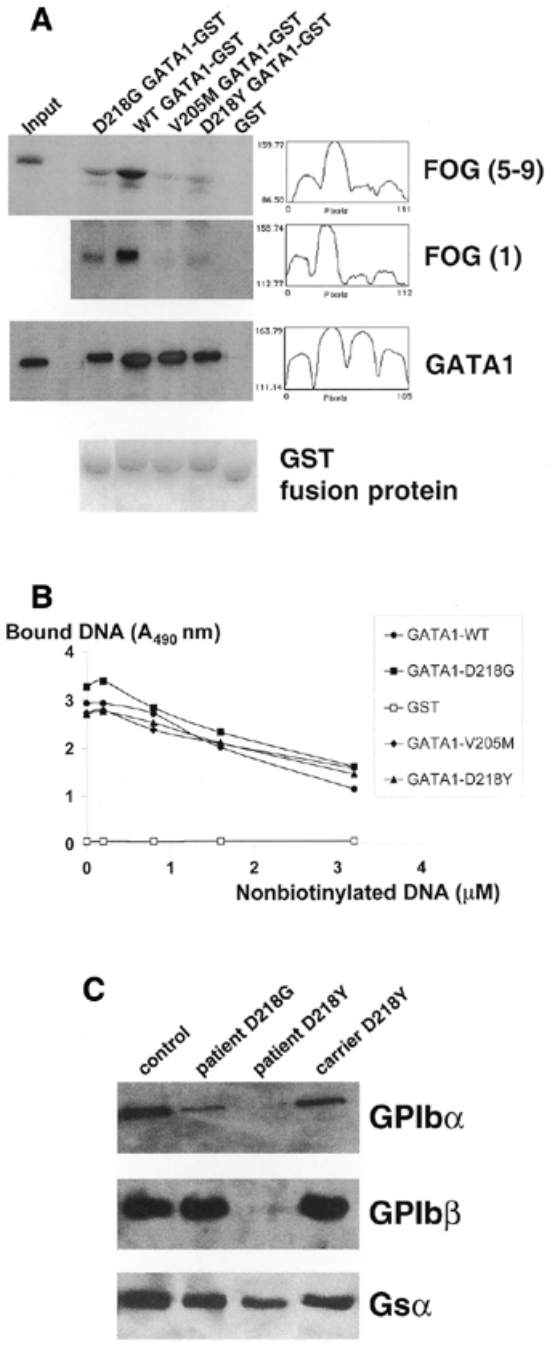

Figure 2. *In vitro* and *in vivo* characteristics of the two D218 GATA1 mutants. (**A**) GST pull-down assay to study the GATA1 Nf:FOG1 interaction and the GATA1 Nf:complete GATA1 self-association. *In vitro* transcription/translated FOG1 fingers (fingers 5–9 and finger 1) or complete GATA1 were incubated with the different GST fusion proteins or with GST immobilized on glutathion agarose beads. The autoradiogram shows the amount of retained ^{35}S-labeled FOG1 or ^{35}S-labeled GATA1. Densitometry plots are incorporated to quantify the amounts of retained FOG or GATA1. The lowest panel shows the Coomassie blue staining of the gel confirming equal GST fusion protein sample loading. (**B**) DNA-binding competition assay (representative for three experiments). The amount of biotinylated DNA (100 μM) bound by GST/GATA1 fusion proteins was plotted against the concentration of non-labeled competitor DNA. Control GST-bound beads gave no DNA binding. (**C**) Platelet glycoprotein and Gsα expression analysis in platelets of a patient with the D218G–GATA1 mutation, patient III:1 with the D218Y–GATA1 mutation and carrier II:1 versus control platelets. Each lane contains a similar amount of total platelet proteins.

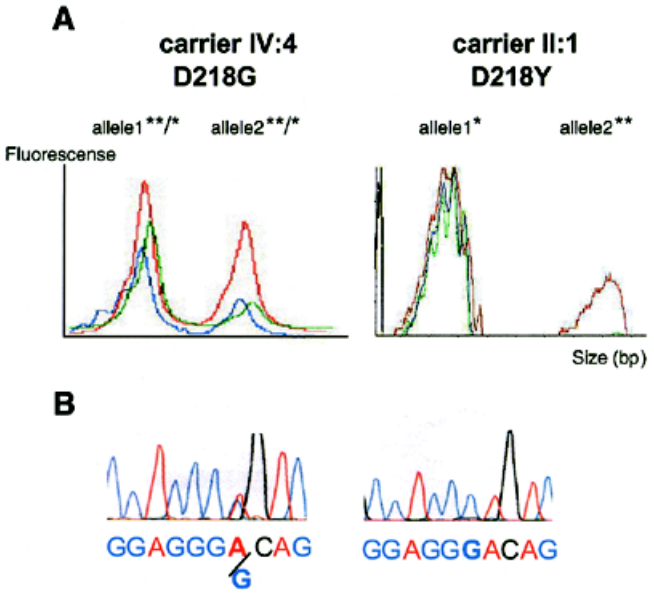

Figure 3. X-chromosome analysis and *GATA1* mutation detection in carriers. (**A**) Analysis of the pattern of X-chromosome inactivation in carrier IV:4 (mutation D218G) and carrier II:1 (mutation D218Y). Genomic DNA was extracted from leukocytes and amplified by PCR with FITC-labeled primers flanking the AR gene locus (for carrier IV:4) or the MAOA gene locus (for carrier II:1). These individuals were only informative for these respective polymorphisms. Before PCR analysis, DNA was digested with different methylation-sensitive enzymes *Hpa*II (green) or *Cfo*I (blue) in the case of carrier IV:4 and with *Hpa*II (green) or *Sma*I (blue) in the case of carrier II:1. For the MAOA polymorphism, digestion with a non-methylation-specific enzyme *Msp*I (black) is used as a digestion control reaction. Digested and non-digested (red) PCR fragments were sized and quantified on the A.L.F. DNA sequencer. Both alleles are represented by a peak and their activation or methylation pattern is indicated [methylated or inactive (*) versus unmethylated or active (**)]. (**B**) Sequence analysis revealed both A and G at position 653 (D218G) in the *GATA1* cDNA from the platelets of carrier IV:4. For carrier II:1, only one signal was detected at the site of the mutation (position 652) in the platelet *GATA1* cDNA. This is in contrast to the sequence of the leukocyte genomic DNA, where two signals were found (G and T in Fig. 1B).

IV:4 (D218G) is heterozygous in her mRNA for the GATA1 mutation whereas in platelets from carrier II:1 with the more severe D218Y mutation, no mutant *GATA1* mRNA could be detected although she is heterozygous at the genomic level. Although carrier IV:4 has platelets with abnormal *GATA1* message, only a few large-sized platelets were detected and the mean platelet volume was within the range of controls, however, at the upper limit. Why are there not two distinct platelet populations in this carrier? A possible explanation is that patients with a *GATA1* mutation also have some normal-sized platelets, so at least part of the megakaryocytes with mutant *GATA1* seem to still undergo a normal maturation. Carrier II:1 of the severe D218Y mutation has a normal peripheral blood count, agreeing with no mutant GATA1 expression in her platelets. The absence of mutant *GATA1* message could be due to the loss of large platelets during the centrifugation step for platelet isolation or to the lack of sensitivity of our RT–PCR analysis, which may not detect a subset of abnormal platelets expressing mutant *GATA1*. More likely, the absent or at least strongly reduced expression of the mutant *GATA1* allele in the platelets of carrier II:1 is related to the observed nearly complete skewing of the X inactivation in leukocytes. This

suggests that the severe D218Y mutation impedes embryonic cell growth, whereas clones derived from a single stem cell or stem cell precursors with a normal *GATA1* have a proliferative advantage. However, stochastic inactivation could also account for the observed skewing in this carrier (18). Skewing can be complete in random individuals (18). Nevertheless, in these two carriers, the X-inactivation patterns correlate with the clinical observations. Unfortunately, we did not have access to more samples.

In conclusion, our data provide further evidence that there indeed is a link between the severity of clinical features associated with *GATA1* mutations and the extent of reduction in FOG1:GATA1 association. The clinical and biochemical differences between the two D218 *GATA1* mutants are reflected in the variable loss of expression of GATA1-dependent genes in the patients' platelets. Even in the asymptomatic carriers, a functional difference between the two D218–GATA1 mutants remains since it results in, respectively, a completely skewed X inactivation in leukocytes for D218Y and in a random X inactivation for D218G.

MATERIALS AND METHODS

RNA isolation and cDNA synthesis

Total RNA was extracted from platelets using TRIzol reagent (Gibco BRL) according to the manufacturer's protocol. Approximately 1 µg RNA was used for oligo(dT)-primed first-strand cDNA synthesis using M-MLV reverse transcriptase (Gibco BRL).

Genetic analysis of *GATA1*

The following primer set was used to generate a full-size *GATA1* cDNA fragment: GATA1-F1 (CCCAGAATTCTCCCCAGAGGCTCCATGGAG) and GATA1-R1 (GGCCCTCGAGTGTGCCCTCATGAGCTGAGC). PCR was performed by adding 4 µl cDNA aliquots to the reaction mixture containing 25 pmol of each primer, 10 mM Tris–HCl buffer, pH 8.7, 1 mM $MgCl_2$, 50 mM KCl, 0.01% gelatine, 1% DMSO, 200 mM dNTP, 1 U Ampli-Taq polymerase (Perkin Elmer Cetus). PCR was performed in a PTC-100™ programmable thermal controller (MJ Research Inc., Biozym) using the following conditions: 95°C for 5 min followed by 30 cycles of 95°C for 30 s, 60°C for 1 min and 72°C for 1.5 min, and final extension at 72°C for 10 min. Primers GATA1-F1, GATA1-F2 (GGTGTACCCATTGCTCAACTGTATG), GATA1-R1 and GATA1-R2 (GTTTACTGACAATCAGGCGCTTC) were used for sequencing PCR generated cDNA fragments from patients using BigDye terminator chemistry (Perkin-Elmer Cetus) on an ABI310 (Perkin-Elmer) sequencer. The resulting sequences were aligned and compared with the published sequence in GenBank (accession no. XM 010214). PCR and sequencing of genomic DNA from leukocytes were performed with primers GATA1-F2 and GATA1-R2 using the same conditions as described above.

GST pull-down assay with GATA1 peptides

GATA1 Nf, GATA1 Nf D218G, GATA1 Nf D218Y and GATA1 Nf V205M (residues 197–251)–GST fusion proteins, coupled to glutathione–agarose beads, were prepared as described earlier by Freson *et al.* (15). Experiments with either *in vitro* translated S^{35}-labeled FOG1 fragments [consisting of finger 1 (241–291) or fingers 5–9 (587–997)] or S^{35}-labeled complete GATA1 were carried out as described previously (15–17). Primer sequences are available on request.

GST fusion protein/DNA-binding assay

DNA-binding studies were performed using a single GATA recognition site (the mouse α-globin GATA site (GATCTCCGGCAACTGATAAGGATTCCCTG) with a biotinylated 5′ end) and the assay as described by Freson *et al.* (15).

Platelet immunoblot analysis

Proteins were extracted from platelets of patients, a carrier, and a normal individual using a modified lysis buffer [1% igepal CA-630 (Sigma Chemical Co.), 2 mmol/l Na_3VO_4, 1 mmol/l EDTA, 1 mmol/l phenylmethylsulfonyl fluoride, 2 mmol/l DTE, 1% aprotinin and 2 mmol/l NaF] by four cycles of freezing (–80°C) and thawing (37°C). Lysates were cleared of insoluble debris by centrifugation at 14 000 *g* for 20 min at 4°C. Protein expression was assessed by western blot analysis. Platelet protein fractions were mixed with Laemmli sample buffer (5% SDS reducing buffer), resolved by SDS–PAGE on 7% (for GPIbα), 12.5% (for GPIbβ) or 10% (for Gsα) acrylamide gels, and transferred to Hybond ECL-nitro-cellulose membrane (Amersham Pharmacia Biotech). The blots were blocked for 1 h at room temperature in Tris-buffered saline with tween (TBS-T; 0.1% Tween-20) supplemented with 5 % non-fat dry milk. Incubation with primary (overnight at 4°C) and secondary antibody (2–3 h at room temperature) was performed in TBS-T with 5% non-fat milk. The primary antibodies were produced in our laboratory and used at 50 µg/ml. Blots were revealed with a monoclonal anti-Gsα antibody (α3), a monoclonal anti-GPIbα antibody (G27C9) or a polyclonal anti-GPIbβ antibody. The secondary antibody was conjugated with HRP and staining was performed with the western blotting ECL detection reagent (Amersham Pharmacia Biotech).

X-chromosome inactivation analysis

The *AR* X-inactivation assay has been described elsewhere (26,27). In brief, treatment of genomic leukocyte DNA with the methylation-sensitive enzymes *Hpa*II and *Cfo*I results in digestion of alleles residing on the active X chromosome. Subsequent PCR with primers flanking the restriction sites and a polymorphic CAG repeat results in amplification of alleles on the inactive X only. In females heterozygous for the AR polymorphism, the two amplification products can be compared directly. We have used this technique and analyzed the PCR products on an A.L.F. DNA sequencer (Amersham Pharmacia Biotech) to determine the X inactivation pattern quantitatively.

Similarly, a VNTR polymorphism at the 5′ end of the *MAOA* gene coupled to a differentially methylated CpG island was used (28). For that purpose we used methylation-sensitive enzymes *Hpa*II and *Sma*I. Digestion with a non-methylation-sensitive enzyme (*Msp*I) served as a control.

ACKNOWLEDGEMENTS

We are indebted to E.Schollen for assistance with the X-inactivation studies. C.V.G. is holder of a fundamental clinical research mandate of the FWO Vlaanderen. Financial support by the FWO Vlaanderen (project G.0306.98) is greatly appreciated.

REFERENCES

1. Tsai,S.F., Martin,D.I., Zon,L.I., D'Andrea,A.D., Wong,G.G. and Orkin,S.H. (1989) Cloning of cDNA for the major DNA-binding protein of the erythroid lineage through expression in mammalian cells. *Nature*, **339**, 446–451.
2. Omichinski,J.G., Clore,G.M., Schaad,O., Felsenfeld,G., Trainor,C., Appella,E., Stahl,S.J. and Gronenborn,A.M. (1993) NMR structure of a specific DNA complex of Zn-containing DNA binding domain of GATA1. *Science*, **261**, 438–446.
3. Evans,T. and Felsenfeld,G. (1989) The erythroid-specific transcription factor eryf1: a new finger protein. *Cell*, **58**, 877–885.
4. Shivdasani,R.A., Fujiwara,Y., McDevitt,M.A. and Orkin,S.H. (1997) A lineage-selective knockout establishes the critical role of transcription factor GATA-1 in megakaryocyte growth and platelet development. *EMBO J.*, **16**, 3965–3973.
5. Orkin,S.H. (1992) GATA-binding transcription factors in hematopoietic cells. *Blood*, **80**, 575–581.
6. Pevny,L., Simon,M.C., Robertson,E., Klein,W.H., Tsai,S.F., D'Agati,V., Orkin,S.H. and Costantini,F. (1991) Erythroid differentiation in chimaeric mice blocked by a targeted mutation in the gene for transcription factor GATA-1. *Nature*, **349**, 257–260.
7. Pevny,L., Lin,C.S., D'agati,V., Simon,M.C., Orkin,S.H. and Constantini,F. (1995) Development of hematopoietic cells lacking transcription factor GATA-1. *Development*, **121**, 163–172.
8. Pedone,P.V., Omichinski,J.G., Nony,P., Trainor,C., Gronenborn,A.M., Clore,G.M. and Felsenfeld,G. (1997) The N-terminal fingers of chicken GATA-2 and GATA-3 are independent sequence-specific DNA binding domains. *EMBO J.*, **16**, 2874–2882.
9. Tsang,A.P., Visvader, J.E., Turner,C.A., Fujiwara,Y., Yu,C., Weiss,M.J., Crossley,M. and Orkin,S.H. (1997) FOG, a multitype zinc finger protein, acts as a cofactor for transcription factor GATA-1 in erythroid and megakaryocytic differentiation. *Cell*, **90**, 109–119.
10. Tsang,A.P., Fujiwara,Y., Hom,D.B. and Orkin,S.H. (1998) Failure of megakaryopoiesis and arrested erythropoiesis in mice lacking the GATA-1 transcriptional cofactor FOG. *Genes Dev.*, **12**, 1176–1188.
11. Newton,A., Mackay J. and Crossley,M. (2001) The N-terminal zinc finger of the erythroid transcription factor GATA-1 binds GATC motifs in DNA. *J. Biol. Chem.*, **276**, 35794–35801.
12. Crossley,M., Merika,M. and Orkin,S.H. (1995) Self-association of the erythroid transcription factor GATA-1 mediated by its zinc finger domains. *Mol. Cell. Biol.*, **15**, 2448–2456.
13. Mackay,J.P., Kowalski,K., Fox,A.H., Czolij,R., King,G.F. and Crossley,M. (1998) Involvement of the N-finger in the self-association of GATA-1. *J. Biol. Chem.*, **273**, 30560–30567.
14. Nichols,K.E., Crispino,J.D., Poncz,M., White,J.G., Orkin,S.H., Maris,J.M. and Weiss,M.J. (2000) Familial dyserythropoietic anaemia and thrombocytopenia due to an inherited mutation in GATA1. *Nat. Genet.*, **24**, 266–270.
15. Freson,K., Devriendt,K., Matthijs,G., Van Hoof,A., De Vos,R., Thys,C., Minner,K., Hoylaerts,M.F., Vermylen,J. and Van Geet,C. (2001) Platelet characteristics in patients with X-linked macrothrombocytopenia due to a novel GATA-1 mutation. *Blood*, **98**, 85–92.
16. Mehaffey,M.G., Newton,A.L., Gandhi,M.J., Crossley,M. and Drachman,J.G. (2001) X-linked thrombocytopenia caused by a novel mutation of GATA-1. *Blood*, **98**, 2681–2688.
17. Yu,C., Raskind,W.H., Niakan,K.K., Matsushita,M., Stamatoyannopoulos,G. and Orkin,S.H. (2000) X-linked thrombocytopenia with thalassemia due to a mutation affecting DNA-binding contribution of the N-finger of transcription factor GATA-1. *Blood*, **96**, 495.
18. Belmont,J.W. (1996) Genetic control of X-inactivation and processes leading to X-inactivation skewing. *Am. J. Hum. Genet.*, **58**, 1101–1108.
19. Fox,A.H., Kowalski,K., King,G.F., Mackay,J.P. and Crossley,M. (1998) Key residues characteristic of GATA N-fingers are recognized by FOG. *J. Biol. Chem.*, **273**, 33595–33603.
20. Crispino,J.D., Lodish,M.B., Mackay,J.P. and Orkin,S.H. (1999) Use of altered specificity mutants to probe a specific protein–protein interaction in differentiation: the GATA-1:FOG complex. *Mol. Cell*, **3**, 219–228.
21. Van der Vuust,H., Hendriks,M., Lapetina,E.G., van Willigen,G. and Akkerman,J.W.N. (1998) Maturation of megakaryoblastic cells is accompanied by upregulation of Gsα-L subtype and increased cAMP accumulation. *Thromb. Haemost.*, **79**, 1014–1020.
22. Fearon,E.R., Winkelstein,J.A., Civin,C.I., Pardoll,D.M. and Vogelstein,B. (1987) Carrier detection in X-linked agammglobulinemia by analysis of X-chromosome inactivation. *New Engl. J. Med.*, **316**, 427–431
23. Parrish,J.E., Schuerle,A.E., Lewis,R.A., Levy,M.L. and Nelson,D.L. (1996) Selection against mutant alleles is a consistent feature in incontinentia pigmenti type 2. *Hum. Mol. Genet.*, **5**, 1777–1783.
24. Parolini,O., Ressman,G., Haas,O.A., Pawlowsky,J., Gadner,H., Knapp,W. and Holter,W. (1998) X-linked Wiskott–Aldrich syndrome in a girl. *New Engl. J. Med.*, **338**, 291–295.
25. Devriendt,K., Matthijs,G., Legius,E., Schollen,E., Blockmans,D., Van Geet,C., Degreef,H., Cassiman,J.-J. and Fryns,J.-P. (1997) Skewed X-chromosome inactivation in female carrriers of dyskeratosis congenita. *Am. J. Hum. Genet.*, **60**, 581–587.
26. Allen,R.C., Zoghbi,H.Y., Moseley,A.B., Rosenblatt,H.M. and Belmont,J.M. (1992) Methylation of *Hpa*II and *Hpa*I sites near the polymorphic CAG repeat in the human androgen-receptor gene correlates with X-chromosome inactivation. *Am. J. Hum. Genet.*, **51**, 1229–1239.
27. Plenge,R.M., Hendrich,B.D., Schwartz,C., Arena,J.F., Naumova,A., Sapienza,C., Winter,R.M. and Willard,H.F. (1997) A promoter mutation in the XIST gene in two unrelated families with skewed X-chromosome inactivation. *Nat. Genet.*, **17**, 353–356.
28. Hendriks,R.W., Chen,Z.Y., Hinds,H., Schuurman,R.K. and Craig,I.W. (1992) An X chromosome inactivation assay based on differential methylation of a CpG island coupled to a VNTR polymorphism at the 5′ end of the monoamine oxidase A gene. *Hum. Mol. Genet.*, **3**, 187–194.