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GATA1 Mutations in Red Cell Disorders

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Summary

GATA1 is an essential regulator of erythroid cell gene expression and maturation. In its absence, erythroid progenitors are arrested in differentiation and undergo apoptosis. Much has been learned about GATA1 function through animal models, which include genetic knockouts as well as ones with decreased levels of expression. However, even greater insights have come from the finding that a number of rare red cell disorders, including Diamond Blackfan Anemia, are associated with *GATA1* mutations. These mutations affect the amino-terminal zinc finger (N-ZF) and the amino-terminus of the protein, and in both cases can alter the DNA binding activity, which is primarily conferred by the third functional domain, the carboxyl-terminal zinc finger (C-ZF). Here we discuss the role of GATA1 in erythropoiesis with an emphasis on the mutations found in human patients with red cell disorders.

GATA1 Is Required for Erythropoiesis

The GATA family is composed of six zinc-finger transcription factors that bind the consensus DNA sequence (T/A)GATA(A/G). GATA½/3 predominantly function in hematopoietic system, while GATA4/5/6 are critical for non-hematopoietic tissues. GATA1 was originally identified as a trans-acting factor of globin and other erythroid-specific genes, and subsequently found to be expressed in megakaryocytes, mast cells, basophils and eosinophils but not at substantial levels in other hematopoietic lineages[1–9]. The murine *Gata1* gene has two promoters, the testis-specific promoter/first exon (IT) located ≈ 8 kb 5′ of the erythroid promoter and first exon (IE), erythroid promoter, which is predominantly used in hematopoietic cells[10]. Although, *Gata1* mRNA is detectable in mouse Sertoli cells of the testis, its function in this tissue remains uncertain[10–13]. Transcripts from the testis promoter have been reported to increase during the differentiation of erythroid precursors purified from the spleen of mice treated with phenylhydrazine (PHZ) or infected with the anemia-inducing strain of the Friend virus (FVA cells)[14]. Disruption of erythroid promoter, which doesn't diminish expression from the testis promoter, leads to embryonic lethality at E12.5 as a result of a major defect in primitive erythropoiesis[15].

During human hematopoiesis, GATA1 is expressed in hematopoietic stem and progenitor cells (HSPCs), and progressively increases in expression in $CD34^+/CD38^-$ primitive cells

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and $CD34^+/CD38^+$ committed progenitors[16,17]. With respect to the erythroid lineage, its expression is initially increased during specification, but is downregulated at the late stages; it is similarly downregulated late in megakaryocytic maturation[18]. An important animal study of loss of function of *Gata1* was reported by Costantini and colleagues[19]. Using chimeric mice, the investigators found that GATA1 is required for maturation of hemoglobinized red blood cells during the fetal stages and in adults. Notably, no other transcription factors could compensate effectively for the erythropoietic deficit of GATA1-null ES cells[19]. The *Gata1* germ line knockout mouse model was generated and characterized by the Orkin laboratory in 1996. In this study, they found that *Gata1* hemizygous deficient male embryos died between embryonic days 10.5 and 11.5 (E10.5-E11.5) from profound anemia[20]. They further demonstrated that erythropoiesis is arrested at the proerythroblast stage in the absence of GATA1 and that yolk sacs yielded pale CFU-E colonies with dying cells as well as large pale BFU-E colonies that included an abundance of megakaryocyte-like cells[20]. Subsequent studies demonstrated that *Gata1* deficient cells failed to contribute to maturation of red blood cells by arresting erythropoiesis at the proerythroblast stage through apoptosis of the cells[21].

Other studies have investigated the effect of decreasing GATA1 expression. For example, replacement of upstream sequences including a DNase I hypersensitive (HS) region with a neomycin-resistance cassette impaired erythropoiesis through a modest (4 to 5-fold) decrease of GATA1 expression, suggesting that erythroid differentiation is dose-dependent with respect to GATA1[22]. A second study of cells that express 5% of the normal level of GATA1, referred to GATA1.05 mice, also showed that, although this low level of GATA1 maintained the viability of definitive erythroid cells, there was aberrant expression of erythroid-specific transcription factors and heme biosynthetic enzymes. Moreover, the GATA1.05 ES cells were found to differentiated into blast-like cells[23].

Collectively, these studies with various animal models of a GATA1 deficiency showed that GATA1 is essential for both primitive and definitive erythropoiesis (Table 1). Although mice that express GATA2 or GATA3 from the GATA1 locus are rescued from embryonic lethality due to the absence of *Gata1*, the mice develop anemia after birth, indicating that there are functional differences among the family members[24].

GATA1 Functional Domains and Partners

GATA1 contains three functional domains: the N-terminal transcription activation domain (N-TAD), an amino-terminal zinc finger (N-ZF) domain and a carboxyl-terminal zinc finger domain (C-ZF) (Figure 1). The two zinc fingers have been found to have different DNA binding specificities[25]; the C-ZF provides the primary DNA binding activity, as mutant isoforms containing only the N-ZF is deficient for binding [26,27]. However, the N-ZF domain contributes to the strength of binding to some sites, such as the GATApal element, which contains one complete [(A/T)GATA(A/G)] and one partial (GAT) canonical motif[28]. Furthermore, the two zinc fingers can form a composite binding domain improving the DNA-binding specificity from single C-ZF, and the covalent linkage of them is required for influencing one another[29]. Zinc fingers of GATA1 have been shown to partially rescue

erythroid and megakaryocytic differentiation[30,31], which indicates they may have other functions in addition to mediating DNA binding.

Of note, both N- and C-terminal domains of GATA1 have been shown to be important for regulation of expression of its target genes. For example, the Orkin laboratory showed that N-terminus is required for trans-activation by structure-function analysis[32]. Work by other groups demonstrated that both the N-terminus and C-terminus are important for GATA1 trans-activation of downstream target genes, and deletion of either of those transactivation regions led to deceased expression of downstream targets without affecting DNA binding or self-association activities[33]. Previous studies revealed that the N-terminus is required for proper chromatin occupancy of GATA1s in the G1ME model of erythron-megakaryocytic differentiation[34,35]. Genes that were not bound by GATA1s included prominent erythroid regulatory genes such as *KLF1*[34]. More recently, our group has found that loss of the N-terminus alters chromatin accessibility and methylation of H3K27, resulting in impaired expression of erythroid genes[36]. Although a prior study reported that the N-terminus associated with Rb and regulates erythropoiesis[37], the protein partners that bind the N-terminus to mediate its function remain largely undefined.

Previous studies have shown that GATA1 is a pioneer transcription factor that is sufficient to reprogram other hematopoietic cells to an erythroid fate[38,39]. Moreover, there is growing evidence that GATA1 temporally and spatially regulates downstream genes by interacting with other transcriptional partners. For example, GATA1 self-association (i.e. dimerization) was shown to be essential for its transcription regulation activity, and the N-ZF-C-ZF contact has been reported to be important for GATA1 intermolecular dimerization[40,41]. Furthermore, the Orkin laboratory demonstrated that there is a physical interaction between GATA1 and SP1, and EKLF in the absence of DNA[42]. SP1 was found to recognize GC and/or GT/CACC motifs and recruit GATA1 to promoters by interacting with the C-ZF of GATA1[42]. The function of the GATA1/SP1 or EKLF interactions has been confirmed in *Drosophila* S2 cells with erythroid promoter driven reporters[43].

Another key cofactor of GATA1 is Friend of GATA1 (FOG1), which was first identified through the yeast two-hybrid system as a binding partner of GATA1[44]. The sixth zinc finger of FOG1, a protein with nine zinc fingers, interacts with the N-ZF of GATA1[45]. Mutations within the GATA1 N-ZF that abolish the interaction between GATA1 and FOG1, but not the interaction between GATA1 and DNA, such as E203V, V205G and H222R, fail to induce differentiation of erythroid cells[46]. Restoring the interaction by creating second site mutations in FOG1 which can bind the GATA1 V205G mutant, rescued erythropoiesis, confirming that the interaction is critical for red cells[46]. As discussed below, further evidence of the requirement for the interaction between GATA1 and FOG1 in erythropoiesis was provided by the identification of FOG1 non-interacting *GATA1* mutations in patients with dyserythropoietic anemia[47].

A surprising discovery was the observation that a GATA1 mutant (V205), which failed to bind FOG1, displayed a striking redistribution on chromatin. Instead of being primary bound to erythroid and megakaryocytic genes, the V205G variant bound to a unique set of genes that are associated with mast cells[48]. Expression of FOG1 in mast cells led to displacement

of GATA1 from mast cell genes and reduced their expression. Overall these studies demonstrate that FOG1 can alter GATA1 chromatin binding and thus impact lineage decisions.

The N-ZF can also interact with LMO2 (LIM2 domain) in a complex that includes FOG1 and TAL1/E2A/LMO2/LDB1. This complex can co-occupy E-box/(T/A)GATA(A/G) sites to facilitate GATA1-mediated activation of transcription[49,50]. Additional studies by Blobel and colleagues revealed that this complex is a positive regulator of gene expression, and that specifically the inclusion of TAL1 biases the activity towards transcriptional activation[51]. FOG1 can activate expression of the p45 NF-E2 promoter[52], and also activate expression of some genes. Of note, FOG1 also acts a transcriptional repressor through its interaction with the co-repressor CtBP2[52,53]. More recent studies reveal that GATA1 can associate with epigenetic factors such as PRC2 and NuRD to control gene expression[50,54–56]. PRC2 has been implicated in suppression of a number of GATA1 targets, including *c-Kit*, *Gata2*, and *Myb* 50,55,56

Another partner of GATA1 is the Ets family member PU.1 (Spi-1), a master transcription regulator for myeloid lineage development. The C-ZF of GATA1 interacts with the Ets domain of PU.1, and the GATA1/PU.1 interaction has been shown critical for erythroid differentiation by impeding the activity of PU.1 through blocking its interaction with c-Jun[57]. The N-terminus of PU.1 has been also shown to repress the function of GATA1 by reducing the binding of GATA1 to DNA[58]. Moreover, GATA1 can inhibit the ability of PU.1 to transactivation of myeloid target promoters, and overexpression of PU.1 represses GATA1-mediated transactivation and block terminal differentiation of erythroid cells[59–61].

Studies of post-translational modifications (PTM) provide a different angle to better understand GATA1 function. CBP/p300 physically interacts with GATA1 to stimulate GATA1's transcriptional activity, and preventing the interaction between these proteins blocked erythroid differentiation[62]. Mechanistically, it has been reported that p300 acetylates GATA1 and enhances the GATA1-DNA interaction, which can stimulate GATA1-dependent transcription[63]. Phosphorylation of GATA1 also has been shown to increase the DNA-binding affinity of GATA1, and this is correlated with erythroid induction of K562 cells[64]. Finally, a recent study revealed that the deubiquitinase USP7 binds to GATA1 and catalyzes the removal of polyubiquitylation chains conjugated on K48, which results in stabilizing GATA1 during human terminal erythroid differentiation[65].

GATA1 Dysregulation in Human Red Blood Cell Disorders

GATA1 is mutated in a number of human red blood cell disorders (Figure 1 and Table 2). These mutations include missense mutations in the N-ZF and the N-terminus. Alterations in regulatory elements that affect GATA1 occupancy are also seen in rare cases of red cell disorders.

Cis element substitutions that attenuate the interaction of GATA1 with DNA

Genetic mutations in cis regulatory elements, which affect the ability of GATA1 to bind DNA and properly regulate downstream gene expression can result in human blood

disorders. For example, a single change at −175 (T to C) in the human γ-globin promoter, which disrupts the interaction between GATA1 and the GATA motif, is associated with increased fetal hemoglobin in nondeletion hereditary persistence of fetal hemoglobin (HPFH)[66]. Congenital erythropoietic porphyria (CEP), also known as Günther disease, is another disorder associated with cis-element mutations; a −70T->C substitution in the uroporphyrinogen III synthase (URO-synthase) locus was found in a CEP patient[67]. This change alters the binding of GATA1 and impairs erythroid-specific transcription/heme biosynthesis.

Another example includes the cis-element of 5-aminolevulinate synthase (ALAS2), which encodes an enzyme necessary for heme biosynthesis. Mutations in a GATA1 binding enhancer (int-1-GATA) of *ALAS2* was identified in patients with sideroblastic anemia[68,69]. Further investigation showed that deletion of the int-1-GATA site disrupts the recruitment of the GATA1/TAL1/LMO2/LDB1 complex and fails to fully activate *ALAS2* transcription, leading to a failure of developing mature red blood cells and embryonic lethality of mutant males at E12.5[70,71]. A fourth example can be found in the *PKLR* gene, which encodes pyruvate kinase, an enzyme that catalyzes the final step of glycolysis. Pyruvate kinase deficiency (PKD) is one of the most common causes of hereditary non-spherocytic hemolytic anemia. A mutation in a GATA-motif in the *PKLR* promoter results in substantial decrease in gene expression and results in the anemia[72,73].

Genome-wide association studies (GWAS) have identified 75 genetic loci associated with erythroid phenotypes, with many of the SNPs postulated to influence gene regulation[74]. The Sankaran laboratory leveraged a massively parallel reporter assay (MPRA) to explore the association of GWAS variants with human erythroid phenotypes, leading to the identification of 32 massively parallel reporter assay functional variants (MFVs) representing 23 of the original 75 GWAS hits[75]. Further functional investigation of three MFVs confirmed endogenous enhancer regulatory activity that affected expression of nearby target genes, which included SMIM1, RBM38, and CD164[75]. In a subsequent study, this group integrated data from chromatin accessibility studies and variants associated with erythroid traits. The study led to the discovery that genetic variants affect differentiation at various stages. Moreover, the work revealed that the GATA1 complex temporally and stage-specifically regulates accessibility regulatory elements and associated gene expression[76].

Mutations in the N-ZF of GATA1

Residues that are critical for the interaction between GATA1 and FOG1 were initially identified through a yeast-two hybrid assay[46]. These include E203, V205, and H222, all contained within the N-ZF. Additional mutations in the four cysteine residues were identified; these likely affect the folding of the entire finger leading to impaired FOG1 binding.

Remarkably, Nichols and colleagues discovered that *GATA1* was mutated in a family with a rare form of dyserythropoietic anemia and thrombocytopenia[77]. The affected individuals harbored the V205M mutation, which like V205G, fails to interact with FOG1. Subsequent work by other groups identified additional N-ZF mutations that alter FOG1 binding and lead to a spectrum of related erythroid and platelet disorder[78]. Distinct mutations in GATA1 that

alter DNA binding, were discovered in affected members of other families with congenital erythroid disorders[78]. For example, the R216Q missense mutation in the GATA1 N-ZF was identified in a patient with X-linked thrombocytopenia with thalassemia; this change was associated with comparable affinity to single GATA motifs but decreased affinity to palindromic sites[79].

GATA1 mutations that lead to expression of the GATA1s isoform

In 1995, Calligaris and colleagues were the first to report that a ~40 kDa protein could be recognized by GATA1 specific antibodies[80]. This protein, determined to be a shorter isoform of GATA1 that begins at methionine at position 84 at the end of the N-TAD domain, is referred to as GATA1s. Genetic studies in 2002 identified mutations within exon 2 of GATA1 that lead to loss of the full-length protein but allow for expression of GATA1s: these mutations were initially found in leukemic blasts from patients with acute megakaryoblastic leukemia who had Down syndrome (DS-AMKL)[81]. The same mutations are also seen in the transient leukemia of Down syndrome, transient abnormal myelopoiesis[82].

Germline mutations that lead to the favored/exclusive production of the GATA1s isoform have also been identified in a small subset of patients with Diamond Blackfan Anemia (DBA) who lack ribosomal gene mutations (Table 2) (discussed below under *GATA1* mutations in DBA). The mutation has also been reported in a family with impaired erythropoiesis[83].

An Intronic GATA1 mutation

An intronic mutation in *GATA1* (chrX:48,652,176 C>T in hg19) was recently found in two patients with dyserythropoietic anemia by the Sankaran laboratory[84]. This mutation is located 24 nucleotides upstream of the canonical splice acceptor site in the fifth intron of *GATA1*, and its alteration results in reduced splicing and a five-amino acid insertion at the C-terminus of the C-ZF. Of note, the mutant protein displayed no observable transactivation activity, suggesting a mechanism by which this allele impairs erythropoiesis.

GATA1 Mutations in DBA

DBA is a rare congenital and inherited bone marrow disorder characterized by erythroid hypoplasia and minimal defects in other hematopoietic cells. Congenital abnormalities and a predisposition to cancer accompany the erythroid defect. DBA was first characterized by Hugh Josephs in 1936[85], and then described by pediatricians Louis Diamond and Kenneth Blackfan in 1938[86]. Most DBA patients are diagnosed in their first year after birth and have a lifelong impairment in erythropoiesis. Cells within colony-forming unit-erythroid (CFU-E) and burst-forming unit-erythroid (BFU-E) from DBA patients rapidly undergo apoptosis upon erythropoietin deprivation[87].

After the first genetic mutation, *RPS19,* was found in DBA patients in 1999[88], much attention has been drawn to studies of ribosome protein (RP) encoding genes. About 70% DBA patients have loss-of-function mutations of ribosomal protein encoding genes, which suggests that a malfunctioning translational machinery is a major contributor to the pathogenesis of DBA[89–91] (Figure 2). Alterations in ribosome biogenesis are known to

trigger activation of the P53 pathway and cell-cycle arrest[92–95], and the increased P53 activity likely contributes to the impaired erythropoiesis.

Mutations in *RPS19* alter ribosome biogenesis through impairing 18S rRNA synthesis and 40S subunits assembly, which subsequently promotes apoptosis[96]. Indeed, by comparing transcriptomes of three populations, $CD34^{+}CD71^{-}CD45RA^{-}$, $CD34^{+}CD71^{hi}CD45RA^{-}$ and $CD34^{+}CD71^{low}CD45RA^{+}$, from DBA patients versus control donors, Gazda and colleagues found that apoptotic genes were strongly upregulated in diseased erythroid cells harboring the *RPS19* mutation[97].

In 2012 Sankaran and colleagues reported that two siblings and a third individual with DBA who lack ribosomal gene mutations carried a *GATA1* mutation[98]. Subsequent studies identified additional cases of DBA patients with *GATA1* mutations[99–101]. In several cases, the patients were found to harbor a *GATA1* mutation (c.220G>C) that affects splicing and leads to a strong reduction in expression of full length GATA1, favoring expression of GATA1s[98,101]. An ATG to ACG mutation in the first translation initiation codon of *GATA1* has also been found in DBA patients; this change also favors GATA1s expression, as it results in a near complete loss of full length GATA1 but persistent expression of GATA1s[99,100]. Together these findings in patients suggest that the disruption of the levels of GATA1 versus GATA1s contribute to the pathogenesis of DBA. In follow up studies, Sankaran and colleagues investigated the relationship between DBA cases with *GATA1* mutations and those with ribosomal gene mutations[99]. They proposed that that ribosomal gene mutations result in anemia by reducing the production of full-length GATA1. Reduced translation of GATA1 was indeed observed upon knockdown of ribosome proteins; this was a selective effect in that other erythroid regulators such as EPOR, TAL1, transferrin receptor (CD71) and STAT5A were not significantly downregulated. Mechanistically, they found that the secondary structure of the GATA1 5'-UTR, which can affect the efficiency of translation, led to reduced translation under conditions of limiting ribosomes. Expression of a GATA1 cDNA with modified 5'-UTR, in which the ribosome levels sensitive structures were removed, was able to rescue erythroid differentiation of DBA $CD34^{+}$ cells by two- to fourfold[99].

A previous study in yeast revealed that a ribosome deficiency can affect expression of a specific subset of genes[102]. However, how the changes in the ribosome machinery in DBA selectively impairs erythropoiesis has been unclear. Recent work by the Sankaran laboratory revealed that transcripts with shorter 5'-UTR, which are predicted to have less complex secondary structure and fewer upstream start codons (uAUGs), are more sensitive to ribosome protein haploinsufficiency[17]. Significantly longer 5′ UTR lengths and more complex 5′-UTR structures were found in most hematopoietic master regulators compared to the group of transcripts showing sensitivity to ribosomal protein haploinsufficiency[17]. Intriguingly, although GATA1 has a relative short and unstructured 5'-UTR, it exhibits unique features among hematopoietic transcriptional regulators, which may give GATA1 mRNA a higher translation efficiency but make it more sensitive to RP haploinsufficiency in DBA patients[17].

Another recent paper provided another piece of evidence in support of transcript-specific defects in translation of GATA1. This study showed that ribonuclease inhibitor 1 (RNH1)-deficient murine embryos died between E8.5 and E10 due to impaired maturation of erythroid progenitors. The authors found that RNH1 interacts with the 40S subunit of ribosomes and facilitates polysome formation on *Gata1* mRNA to confer transcript-specific translation[103].

Heme toxicity is thought to be another contributor to pathogenesis of DBA. In 2004, Abkowitz and colleagues identified Feline Leukemia Virus subgroup C Receptor (FLVCR), which exports cytoplasmic free heme, and reported it is required for erythroid cell development by protecting them from heme toxicity[104]. This study also showed that FLVCR was highly expressed on cell surface of CFU-E and decreased as erythropoiesis proceeds. FLVCR has two isoforms, FLVCR1a and FLVCR1b, and they function as heme transporters on cell surface membrane and mitochondrial membrane separately. Both FLVCR1a and FLVCR1b are required for heme homeostasis and for expansion of committed erythroid progenitors[105]. Later studies provided new insights into GATA1globin-heme feedback loop in the pathogenesis of DBA. Two recent papers suggest that decrease of GATA1 full length protein resulting from RP haploinsufficiency and deficiency of HSP70 can disturb the balance of globin-heme and leads to the accumulation of free cytoplasmic heme in erythroid progenitors, which can cause increase of P53-dependent apoptosis of DBA erythroid cells[106–108]. Of note, unlike in the presence of RP mutations in DBA patients, accumulation of heme upon only FLVCR1 depletion causes P53-independent apoptosis[107].

Finally, work by Bodine and colleagues suggest yet another model for pathogenesis of DBA[109]. By culturing $CD34^+$ cells from patients, they found that both RP and *GATA1* mutant cells showed reduced proliferation and delayed differentiation. Interestingly, by RNA-seq they discovered that RP mutant cases had prominent upregulation of genes involved in heme biosynthesis while *GATA1* mutant cases were associated with enrichment of genes in the translational apparatus. Thus, in this alternative model, dysregulation of translation appears to be a theme shared by these two groups of patients.

Animal models of GATA1s display a transient anemia with some parallels to DBA

A mouse model of *Gata1s* mutations was created by the Orkin laboratory[110]. *Gata1s* mutant animals display anemia in mid-gestation which improves with age. This fetal anemia is accompanied by a prominent transient expansion of megakaryocytes that bears some similarity to the transient leukemia seen in children with DS. Although the erythroid phenotype was initially reported to revert to normal by birth, ongoing studies reveal that there is a persistent underlying defect in erythropoiesis (TL and JDC unpublished observations). Mechanistically, the short isoform was found to be differentially occupy chromatin of a subset of target genes in *Gata1s* fetal liver erythroid[36]. Moreover, the loss of the N-TAD was associated with altered chromatin accessibility and reduced H3K27me3 modifications along the *Gata2* and *Runx1* loci, explaining their aberrant increased expression in *Gata1s* proerythroblasts. These observations further suggest that the N-TAD may be involved in the recruitment of other epigenetic factors to regulate the accessibility of regulatory elements of GATA1 downstream target genes. Of note, reducing the levels of

GATA2 substantially rescued erythropoiesis in the *Gata1s* embryos indicating that the impaired downregulation by GATA1s is a key pathogenic event. Future studies will clarify the extent to which aberrant erythropoiesis in *Gata1s* mice is relevant to human DBA.

Conclusions

GATA1 was originally identified as a key regulator of erythroid cell development. In the absence of GATA1, mouse embryos die in mid-gestation from severe anemia. This essential function of *Gata1* in mice supported the belief that *GATA1* mutations would not be found in humans. However, studies over the past 20 years have shown that *GATA1* mutations, although rare, can indeed be found in patients with a spectrum of red cell disorders, including congenital dyserythropoietic anemia and DBA. Molecular studies revealed that all three functional domains of GATA1 affect chromatin occupancy and its ability to properly regulate gene expression.

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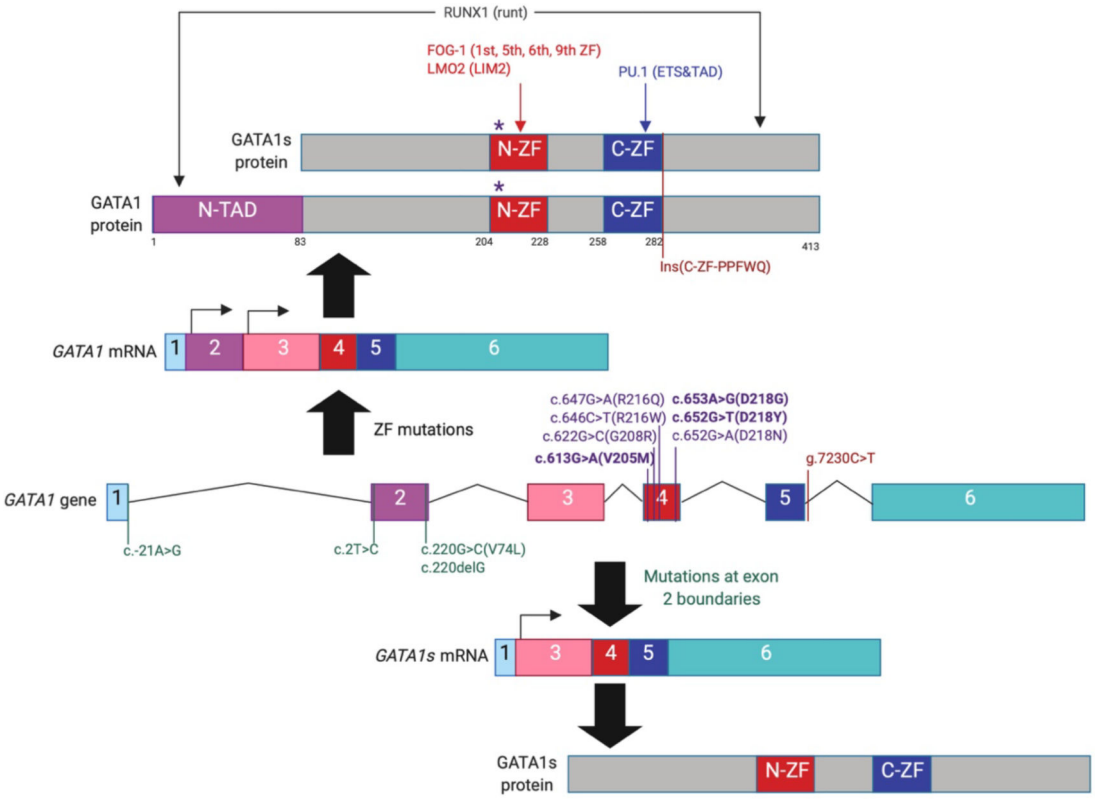

Figure 1. Schematic of the *GATA1* gene and known mutations in patients with congenital erythroid disorders.

GATA1 is composed of six exons which encode two transcripts that produce the full-length isoform (upper) or the N-terminally truncated GATA1s isoform (lower). The locations of *GATA1* mutations found in patients with congenital erythroid disorders are indicated along *GATA1* gene. Mutations at the boundaries of exon 2 (green), mutations within the sequences that encode the N-terminal zinc finger (purple), and those within intron 5 (red) are depicted. The mutations at the boundaries of exon 2 cause splicing defects that result in the exclusive generation of GATA1s, while mutations within exon 4 create variants that attenuate the GATA1-FOG1 interaction or impair DNA binding. The g.7230C>T alteration causes a five-amino acid (PPFWQ) insertion at the C-terminus of the C-terminal zinc finger. GATA1 cofactors and their interacting domains are shown on top. N-TAD, amino-terminal trans-activation domain; N-ZF, amino-terminal zinc finger; C-ZF, carboxyl-terminal zinc finger.

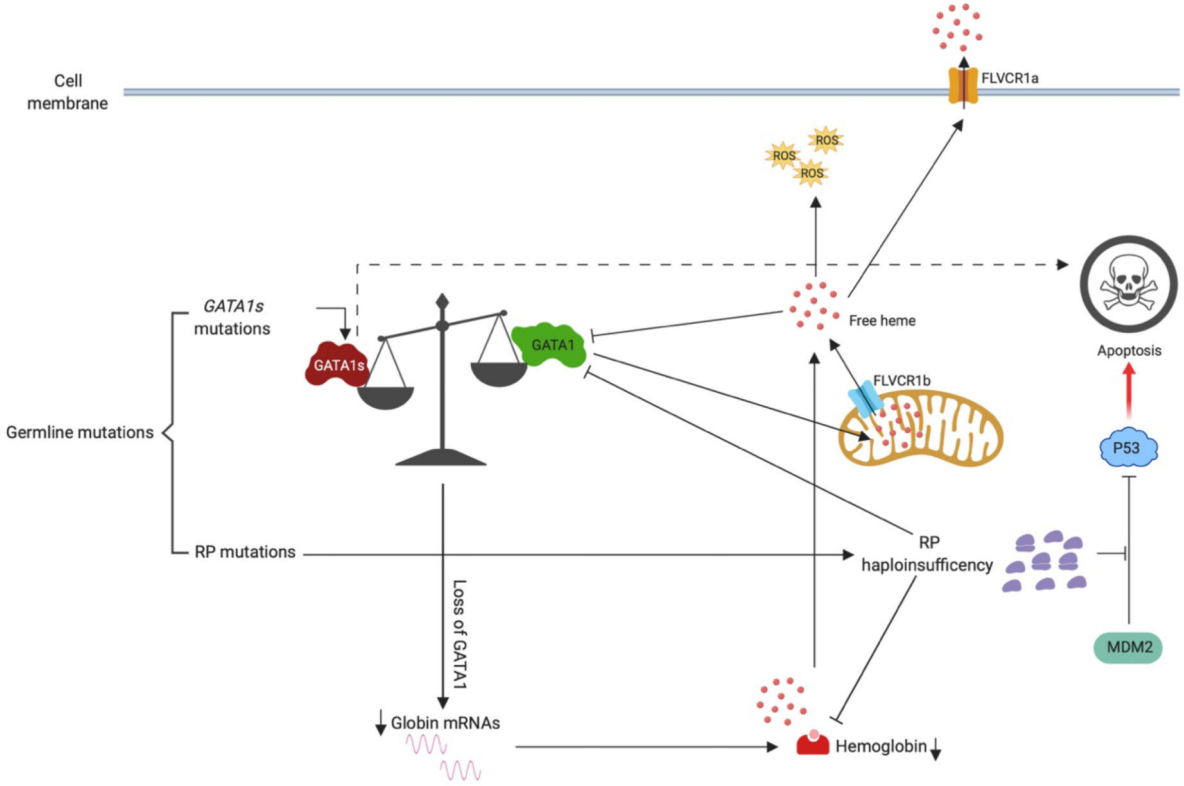

Figure 2. Mechanisms that lead to Diamond-Blackfan anemia.

The majority of germline mutations found in DBA patients reside in ribosome protein (RP) coding genes. The resulting RP haploinsufficiency leads to inefficient translation of a number of erythroid genes, such as globin and GATA1, resulting in impaired erythropoiesis. Moreover, free ribosomal subunits, such as RPL5 and RPL23, block MDM2-mediated P53 ubiquitination and degradation, leading an increase of P53-dependent apoptosis of the erythroid progenitors. In other patients, *GATA1* gene mutations result in loss of the full-length protein but allow for expression of the shorter isoform (*GATA1s* mutations), which also impairs erythropoiesis. Finally, an altered globin-heme balance has been shown to lead to the accumulation of free heme in cytoplasm, which downregulates *GATA1* at both the mRNA and protein level. FLVCR1a and FLVCR1b are heme transporters on cell membrane and mitochondrial membrane respectively.

Table 1:

Animal models of *Gata1* deficiencies

Genotype	Hematopoietic phenotype	Reference
Gata1$^{\Delta IE}$ (erythroid first exon (IE) deleted)	*Gata1*$^{\Delta IE}$ mRNA level is significantly down-regulated in the megakaryocyte lineage but is expressed in the erythroid lineage. These *Gata1*$^{\Delta IE}$ mRNA fail to produce full-length GATA1 protein, but instead generate GATA1 lacking the N-terminal domain inefficiently. Hemizygous male embryos die by E12.5 showing severe anemia. Conditional knockout male mice (3–4 weeks) suffer from severe thrombocytopenia and anemia. Nucleated erythrocytes can be found on the peripheral blood smear. Splenomegaly with apparent destruction of the splenic architecture, due to expansion of the red pulp, can be observed. Markedly increased CD41^{+}CD61^{+} megakaryocytes and accumulation of CD71^{hi}Ter119^{low} in the bone marrow can be detected by flow cytometric analysis.	111
Gata1$^{\Delta N}$ (Δ 3–63 AA) and *Gata1*$^{\Delta e2}$ (Δ 1–83 AA)	Adult hemizygotes have normal RBC, PLT, normal spleen structure and normal numbers of megakaryocytes in spleen. 10–15% of the mutant mice were lost in utero. Fetal liver pallor and reduced cellularity (by ~50%) can be observed in E12.5 embryos from hemizygous and homozygous compared to the wild type. At E12.5 more CD41^{+} cells can be detected by flow cytometric analysis, and megakaryocytes are hyperproliferative in liquid medium containing thrombopoietin. Erythroid colony-forming units (CFU-Es) in *Gata1*$^{\Delta N}$ fetal liver are reduced by ~50%. At E14.5 and thereafter, Gata1$^{\Delta N}$ fetal liver had normal cellularity and comparable Ter119^{+} cells as wild type.	110
Gata1^{-} (Start codon Disrupted)	Male embryos die between E10.5-E11.5 of gestation because of severe anemia. Female heterozygotes are born pale and recover after birth. Embryonic erythroid cells are arrested at an early proerythroblast-like stage of their development and die thereafter by apoptosis. Abundant acetylcholinesterase positive cells can be found in methylcellulose culture supplemented with Epo and kit ligand.	20
Gata1^{low} or Gata1$^{neo\Delta HS}$ (Disrupt DNase I-hypersensitive region by replacing ~8 kb of upstream sequences, including the IT and HS regions, with a PGK-NeoR cassette flanked by loxP sites)	Yolk sac erythropoiesis and fetal erythropoiesis are disturbed. Embryos are pale or dead by E13.5–14.5 and definitive erythroid cells are largely arrested at the proerythroblast stage of development. CFU-E and BFU-E are morphologically abnormal in colony forming assay with fetal liver cells. About 5% of hemizygous males survive fetal anemia into adult life. Abnormal nucleated erythroid precursors and erythrocytes can be found in peripheral blood smears of newborns, but anemia phenotypes at birth disappear by 4–5 weeks after birth. Mutant mice also loss of megakaryocyte GATA1 expression, resulting in markedly reduced PLT associated with deregulated megakaryocyte proliferation and severely impaired maturation. Female mice heterozygous have normal platelet numbers, but with increased megakaryocytes in hematopoietic tissues.	22,112
Gata1$^{.05}$ (Introduce a neo cassette into the intergenic region between an important regulatory region of the erythroid promoter and the mRNA cap site)	The expression of GATA1 mRNA in the mutant male embryos is less than 5% of the level present in wild-type embryos (E9.5). The yolk sacs of mutant males are pale and almost no blood vessels are present. Embryos die in utero due to impaired primitive erythropoiesis. There are decreased number of erythroid cells and CFU-E in mutant fetal livers.	15,113

Table 2.

GATA1 mutations identified in patients with congenital red blood cell disorders

Pathogenic mutations		Annotation of mutation	Red blood cell disorders	Reference
Genomic change	**Predicted protein change**			
c.613G>A	p.Val205Met (V205M)	Mutation in N-ZF of GATA1, strongly reduced FOG1 binding	Dyserythropoietic anemia	47
c.622G>C	p.Gly208Arg (G208R)	Mutation in N-ZF of GATA1	Dyserythropoietic anemia	114,115
c.647G>A	p.Arg216Gln (R216Q)	Mutation in N-ZF of GATA1	β-Thalassemia, dyserythropoietic anemia	79,116–118
c.646C>T	p.Arg216Trp (R216W)	Mutation in N-ZF of GATA1	β-Thalassemia, microcytic anemia, congenital erythropoietic porphyria	119
c.653A>G	p.Asp218Gly (D218G)	Mutation in N-ZF of GATA1, reduced FOG1 binding	Dysmorphic red blood cells	120
c.652G>T	p.Asp218Tyr (D218Y)	Mutation in N-ZF of GATA1, strongly reduced FOG1 binding	Dyserythropoietic anemia	121
c.652G>A	p.Asp218Asn (D218N)	Mutation in N-ZF of GATA1	Dyserythropoiesis	122
g.7230C>T		Five–amino acid insertion at the C-terminus of the C-ZF	Dyserythropoietic anemia	84
c.220G>C		Splicing defect; Loss of GATA1, increased GATA1s	Macrocytic anemia	83
c.220G>C		Splicing defect; Loss of GATA1, favoring GATA1s production	Diamond–Blackfan Anemia	98,101
c.220delG		Splicing defect; Loss of GATA1, favoring GATA1s production	Diamond–Blackfan Anemia	98
c.-21A>G		Splicing defect; Loss of GATA1, GATA1s only	Dyserythropoietic Anemia	123
c.2T>C		Translation defect; favoring GATA1s production	Diamond–Blackfan Anemia	99,100