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Human Phenotypes Associated with *GATA-1* Mutations

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Abstract

GATA-1 is one of the six members of the GATA gene family, a group of related transcription factors discovered in the 1980s. In the past few decades, the crucial role of GATA-1 in normal human hematopoiesis has been delineated. As would be expected, mutations in *GATA-1* have subsequently been found to have important clinical significance, and are directly linked to deregulated formation of certain blood cell lineages. This paper reviews the functional consequences of *GATA-1* mutations by linking specific errors in the gene, or its downstream protein products, to documented human diseases. These five human diseases are: X-linked thrombocytopenia (XLT), X-linked thrombocytopenia with thalassemia (XLTT), congenital erythroietic porphyria (CEP), transient myeloproliferative disorder (TMD) and acute megarakaryoblastic leukemia (AMKL) associated with Trisomy 21, and, lastly, a particular subtype of anemia associated with the production of GATA-1s, a shortened, mutant isoform of the wildtype GATA-1. The different phenotypic expressions associated with *GATA-1* mutations illustrate the transcription factor's integral function in overall body homeostasis. Furthermore, these direct genotype-phenotype correlations reinforce the importance of unraveling the human genome, as such connections may lead to important therapeutic or preventive therapies.

Keywords

X-linked thrombocytopenia; X-linked thrombocytopenia with thalassemia; congenital erythroietic porphyria; gray platelet syndrome; acute megakaryoblastic leukemia; Trisomy 21; GATA-1s

1. Introduction

The *GATA* genes are a family of X-linked transcription factors discovered in the late 1980s. Like many genes isolated and mapped in the early days of human genomic exploration, the defining function of this group was initially unclear. Today, the role and mode of action of the *GATA* transcription factors is more precisely elaborated and it is clear that these related genes have important clinical significance, and are implicated in a variety of human disease pathogenesis.

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GATA-1 is an important member of the *GATA* family, playing an integral role in the development of several hematopoietic cell lines, including the erythroid, megakaryocyte (MK), eosinophil, and mast cell lineages (Pevny et al., 1991,1995; Fujiwara et al., 1996; Shivdasani et al., 1997; Hirassawa et al., 2002; Migliaccio et al., 2002). At present, several inherited and one somatic mutation in *GATA-1* have been functionally linked to a number of syndromes. Inherited missense mutations in the *GATA-1* gene cause a group of blood disorders characterized by various cytopenias, while somatic mutations seem to be exclusively associated with cases of Trisomy 21 and lead to AMKL in affected patients (Crispino, 2005).

This paper synthesizes the current base of knowledge on *GATA-1* mutations and their phenotypic consequences in humans. It reviews five rare syndromes caused by defects in *GATA-1* gene expression or a malformed protein product. These disorders are: X-linked thrombocytopenia (XLT), X-linked thrombocytopenia with thalassemia (XLTT), congenital erythropoietic porphyria (CEP), transient myeloproliferative disorder (TMD) and acute megarakaryoblastic leukemia (AMKL) associated with Trisomy 21, and anemia associated with the production of GATA-1s.

Table 1 summarizes the mutations and clinical manifestations associated with these disorders. Inherited *GATA-1* mutations appear to be rare, however their exact prevalence is not known. Despite a seemingly small number of confirmed cases, the mutations may be more common than previously thought, particularly in patients with mild, unexplained thrombocytopenia or gray platelet syndrome (GPS) since birth (Tubman et al., 2007). Thus clinicians should remain conscious of this group of disorders when evaluating a clinical picture and laboratory data consistent with a *GATA-1* mutation.

2. GATA-1

GATA-1, the first member of the *GATA* transcription factor family to be discovered (Evan et al., 1988), is located on chromosome Xp11.23 (Zon et al., 1990). Table 2 summarizes the chronology of several main observations reported about GATA-1. GATA-1 has a high level of expression in hematopoietic cells, namely red blood cells (RBCs), MKs, mast cells, and eosinophilic cells (Martin et al., 1990; Weiss et al., 1997; Hirassawa et al., 2002; Migliaccio et al., 2003). Its expression profile serves as an obvious clue to its function, as *GATA-1* is required for erythropoiesis and megakaryocytopoiesis (Pevny et al., 1991; Weiss et al., 1994; Pevny et al., 1995; Shivdasani et al., 1997; Hong et al., 2005). With the help of its cofactor, friend of GATA-1 *(FOG-1), GATA-1* coordinates hematopoietic cell differentiation by activating lineage-specific genes that favor the mature forms, and repressing genes associated with the undifferentiated states (Tsang et al, 1997; Hong et al., 2005). A complete knock out of the *GATA-1* gene in mouse models proves to be embryonic lethal, due to severe anemia (Fujiwara et al., 1996; Ohneda and Yamamoto, 2002), aptly illustrating its functional significance.

Structurally, GATA-1 is made up of a single polypeptide chain containing two zinc finger DNA binding domains, a carboxy terminal finger and an amino terminal finger. The carboxyl terminal finger (C-f) uses sequence specific DNA adherence to attach GATA-1 to its target genes (Tsai et al., 1989). The amino terminal finger (N-f) is a region of highly conserved amino acid sequences (Chang et al., 2002). N-f is required for GATA-1 interaction with its cofactor, FOG-1, a member of a family of zinc-finger proteins that modulate GATA activity (Balduini et al., 2004). Additionally, N-f influences GATA-1 affinity to bind DNA, specifically at complex or palindromic sites. These two crucial functions of the N-f finger, interaction with FOG-1 and modulation of DNA binding at palindromic sites, take place in two distinct locales. The amino acids involved in FOG-1 interaction are simple sites facing away from the DNA binding sites, while residues affecting DNA-binding affinity are highly conserved sites that

face the DNA (Yu et al., 2002). Recent studies have proposed that not only are local DNA and protein interactions important, but that long-range interactions and chromatin remodeling may affect *GATA-1* gene expression (Grass et al., 2006). These findings may prove to be important especially for the FOG-1 related mutations described below (XLT and GATA-1s) as these researchers demonstrated that FOG-1 facilitates GATA-1 chromatin occupancy (Grass et al., 2006).

The locations of the *GATA-1* mutations appear to have critical phenotypic consequences. For example, the crucial N-f domain harbors all known *GATA-1* mutations causing the GATA-1 related cytopenias. As would be predicted from N-f function, these mutations alter the ability of GATA-1 to bind specific DNA sequences and/or to interact with FOG-1. The correspondence between the *GATA-1* mutations and disease characteristics in the cases described to date (Kacena et al., 2006) could prove to be valuable for predicting lineage involvement and severity of cytopenia, and therefore might guide patient management.

In Trisomy 21, acquired somatic mutations lead to the production of a shorter GATA-1 isoform, termed GATA-1s, associated with TMD and AMKL (Munchau and Crispino, 2006). This same GATA-1s isoform was recently identified in one family without Trisomy 21. Here, exclusive production of the shortened isoform causes cytopenias more severe than those caused by *GATA-1* missense mutations. While there is some overlap of symptoms with Trisomy 21-TMD patients, there is no malignant state and no progression to AMKL to date in the family with inherited GATA1s (Hollanda et al., 2006).

3. XLT

3.1. GATA-1 Mutations Causing XLT

There are currently five documented families with an inherited form of X-linked thrombocytopenia without features of thalassemia due to missense mutations in GATA-1: V205M (Nichols et al., 2000), G208R (Del Vecchio et al., 2005), G208S (Mehaffey et al., 2001), D218G (Freson et al., 2001), and D218Y (Freson et al., 2002). In all cases, there is a single amino acid substitution on the N-f portion of GATA-1 that decreases GATA affinity for its essential cofactor, FOG-1 (Nichols et al., 2000; Freson et al., 2001; Mehaffey et al., 2001; Freson et al., 2002; Yu et al., 2002; Balduini et al., 2004; Del Vecchio et al., 2005).

3.2 Clinical Presentation of XLT

X-linked thrombocytopenia affects both the quantity and quality of the MK lineage. In addition to significant reduction in platelet number, the platelets are abnormally sized and contain few alpha granules. These defective platelets are less effective at clotting, and patients with XLT have a resultant propensity for bleeding. Of note, MK number is increased, presumably in response to negative feedback communicated through the decreased platelet number (Zhu et al., 1997).

GATA1-related thrombocytopenia typically presents in infancy as a bleeding disorder. Excessive hemorrhage and/or bruising can occur either spontaneously or after trauma or surgery (Mehaffey et al., 2001).

Anemia is the other major clinical problem, with symptoms varying widely amongst patients with different mutations. The anemia may be minimal with only mild dyserythropoiesis observed in the bone marrow (Freson et al 2001). Or, at the opposite end of the spectrum, GATA-1 mutations may cause severe fetal hydrops requiring *in utero* transfusions to maintain the fetus (Nichols et al 2000). After birth, patients may be RBC transfusion-dependent (Freson et al 2002).

3.3 Genotype-Phenotype Correlations

All GATA-1 mutations leading to XLT appear to solely affect GATA-1:FOG-1 interaction on N-f, and leave its ability to bind complex DNA sites intact. Thus the clinical consequences of these mutations result from reduced co-factor affinity, and not decreased stability of the GATA interaction with RBC and MK target genes at complex sites. All of these mutations occur in a narrow range of residues on the N-f face that binds FOG-1 and faces away from the DNA. Yet despite the proximity of these errors, marked phenotypic variation is conferred by the specific missense mutation present on the highly conserved FOG-1 binding face of N-f.

The V205M mutation reduces binding to FOG-1 zinc fingers 1, 6 and 9 and causes severe macrothrombocytopenia and dyserythropoietic anemia. As aforementioned, the V205M error occurs in a highly conserved region and prevents GATA-1 interaction with FOG-1; however it does not affect GATA-1 interaction with palindromic DNA sites. The V205M mutation decreases the ability of GATA-1 to promote the maturation and differentiation of the erythroid lineage. The two known affected individuals had life threatening fetal anemia requiring *in utero* RBC transfusions. These male half-siblings continued to have severe thrombocytopenia and anemia throughout life, and both had cryptorchidism. Blood smears revealed markedly decreased platelet number, poikilocytosis (abnormally shaped RBCs), and anisocytosis (abnormally sized RBCs). Bone marrow (BM) aspirates showed a hypercellular state with many large, multinucleated, erythroid precursors reflecting dyserythropoiesis (unregulated RBC maturation). Interestingly the mother of the two boys had a mild, chronic, thrombocytopenia presumably due to X-chromosome inactivation that preferentially silenced the wildtype allele more frequently than the mutant one (Nichols et al., 2000).

Like V205M, the G208R mutation is also associated with severe macrothrombocytopenia and dyserythropoietic anemia. The proband had anemia and thrombocytopenia at birth, with a normal leukocyte count and differential. He required exchange transfusions through his first five years of life, and his symptoms included easy bruising, petechiae, and, rarely, mucosal hemorrhage, specifically nosebleeds. The bleeding disorders decreased with age, despite the persistence of severe thrombocytopenia and a slight macrocytic anemia. This patient also had bilateral cryptochordism. Histological studies revealed abnormally shaped RBCs (poikilocytosis), and nucleated RBCs in the serum. Platelets were severely dysmorphic and heterogeneous (Del Vecchio et al., 2005).

A different mutation affecting the same residue, G208S, was found in a family where four men in two generations were afflicted with macrothrombocytopenia, profound bleeding, and mild dyserythropoiesis without anemia. The mutation impairs, but does not totally inhibit GATA-1 interaction with FOG-1, primarily affecting contact with zinc finger 9 of FOG-1. Affected patients had chronic, severe nosebleeds that required packing and transfusions, petechiae, easy bruising, and prolonged bleeding after mild trauma. Peripheral blood smears showed large, heterogeneous platelets and a BM aspirate from one symptomatic family was hypercellular with large MKs containing displaced nuclei (Mehaffey et al., 2001).

The discrepancy between clinical presentations of the two mutations affecting residue 208 is note worthy. The G208R mutation replaces the smallest amino acid glycine with the much larger, positively charged arginine. In G208S, serine, a polar amino acid that is much smaller than arginine, replaces glycine. It is speculated that the substitution of glycine for the very different arginine, has a greater affect on the destabilization of the FOG-1:GATA-1 interaction than the more conservative G208S switch. The G208S mutation reduces FOG-1 binding in primarily zinc finger 9 alone, thus maintaining a stronger residual interaction than the V205M mutation affecting zinc fingers 1, 6, and 9. This could explain the milder phenotype and lack of anemia associated with the G208S versus the G208R mutation (Mehaffey et al., 2001).

The D218G mutation decreases N-f binding to FOG-1 at zinc fingers 1 and 5–9. Despite its binding effect on five zinc fingers, this amino acid switch causes a phenotype with mild clinical severity, similar to that caused by the G208S mutation. Thirteen males spanning four generations of one family have macrothrombocytopenia and mild dyserythropoiesis, not associated with anemia. Clinical symptoms include mucocutaneous bleeding either spontaneously or after mild injury, hematuria, and easy bruising. Blood smears show huge, irregularly shaped platelet cells, but patients have a normal number and normal sized RBCs, albeit with some shape abnormalities (Freson et al., 2001). Recent studies by White et al. (White et al., 2007; White 2007) demonstrated that the platelets in these patients bound very few von Willebrand factor multimers, supporting the idea that the mutant platelets were macrothrombocytes because they were unable to detach properly from each other.

There is a second known mutation affecting residue 218, in which aspartic acid is replaced by tyrosine (D218Y) (Freson et al., 2002). Interestingly, in this family the phenotype is much more pronounced, with severe macrothrombocytopenia, dyserythropoietic anemia, and early mortality. In the family studied, six boys spanning two generations died before the age of two. One affected member was still alive at age one in 2002, but was completely dependent on transfusions. Peripheral blood smears showed a decreased number of normal to giant sized platelets, anisocytosis, poikilocytosis, and some normoblasts. BM examination revealed multinucleate erythroblasts, and dysplastic MKs. Like the D218G mutation, FOG-1 binding is reduced at zinc fingers 1, and 5–9. However, *in vitro* testing proves that the aspartic acid to tyrosine substitution hinders the transcription factor:co-factor interaction more than does a glycine substitution at this same codon (Freson et al., 2002). Unlike residues 205 and 208, and despite the clinical differences, both mutations at residue 218 hamper self-association of the GATA-1 protein. Self-association presumably increases local GATA-1 concentrations, thereby increasing its potency as a transcription activator (Freson et al., 2002).

In summary, the V205M, G208R, and D218Y mutations seem to result in the most severe hematologic presentations, while the G208S and D218G mutations result in a milder clinical expression (Table 1). All five mutations hinder GATA-1:FOG-1 interaction.

4. XLTT

4.1. GATA-1 Mutation causing XLTT

As in XLT, the inherited disorder XLTT is caused by a missense mutation, but here the mutation affects the DNA binding face. *In vitro* studies show that the substitution at codon 216 of glutamine for arginine (R216Q) reduces GATA-1 binding to palindromic DNA sites, but completely conserves the FOG-1 interaction. Codon 216 lies on the side of N-f that does not contact FOG-1, but rather faces the DNA. This portion of N-f is known to stabilize GATA-1 binding with palindromic, but not simple, recognition sites in DNA. Thus as predicted, R216Q shows comparable affinity with single GATA sites, but decreased affinity to palindromic sites versus the wildtype. Interestingly, the mutated GATA-1 can still direct erythroid maturation, but not as well as the wild-type form (Yu et al., 2002).

4.2. Clinical Presentation of XLTT

XLTT shares some features in common with XLT: MK number is also increased, MK maturation is defective, mature MKs are dysmorphic, and platelets are abnormal in size and shape, with a reduction in α-granules. However, distinguishing characteristics of XLTT are milder thrombocytopenia and anemia, and unbalancedα:β hemoglobin chain production resembling mild β-thalassemia (Balduni et al., 2004). There is an imbalance in chain production, so rather than the sole formation of α2:β2 hemoglobin molecules, α3:β1 and α4 molecules form and precipitate. RBCs with precipitated abnormal hemoglobin molecules are

frankly deformed. The misshapen cells are sequestered and destroyed in the spleen, a process which leads to splenomegaly. This is a hemolytic anemia because the RBCs are subsequently lysed and destroyed by the spleen.

All five families identified with XLTT to date were found to have an R216Q substitution (Raskind et al., 2000; Yu et al., 2002; Balduni et al., 2004; Hughan et al., 2005; Tubman et al., 2007; Raskind unpublished observation). Although most affected patients are male, females carrying this mutation may manifest mild-moderate symptoms and abnormal laboratory values, related to the proportion of cells containing the mutant *GATA-1* allele on the active X chromosome (Raskind et al., 2000; Balduini et al., 2004; Raskind unpublished observation). Platelet aggregation is normal in affected individuals, but bleeding time is prolonged (Raskind et al., 2000; Balduini et al., 2004; Raskind unpublished observation).

Of importance, the family identified by Tubman et al. (2007), was originally thought to have GPS. GPS is a term used to describe a heterogeneous group of rare disorders of unknown etiology characterized by the abnormal appearance of platelets under light microscopy caused by a reduction in intracellular granules. Both X-linked recessive and autosomal dominant transmission patterns have been documented (Mori et al. 1984). Recently a four-generation family with GPS was found to have an R216Q mutation in *GATA-1*. The symptomatic individuals were all male, consistent with an X-linked recessive pattern of inheritance. The R216Q missense mutation segregated with the phenotype in all affected males and carrier females. The clinical similarities between XLTT and X-GPS, and the identical histological appearance of platelets and MKs, including microcytosis (evidence of subtle thalassemia trait), suggest that X-linked GPS and XLTT are in fact one disorder (Tubman et al., 2007). Indeed, it was recently suggested that X-linked GPS should be renamed XLTT to avoid confusion (Balduini et al 2007). However, controversy surrounding the naming persists (Neufeld et al 2007). Many fields would adopt XLTT as the name for the syndrome described by Tubman and colleagues (2007), as the molecular etiology is known. Thus it would seem that GPS should be reserved for disorders that have a gray appearance to platelets but do not have a *GATA-1* mutation.

5. CEP

5.1 GATA-1 Mutation causing CEP

CEP is a rare photomutilating disorder that results from a buildup of uroporyphyrin I, a pigment that is an important component of heme. Most cases are autosomal recessive and caused by mutations in uroporphyrinogen III synthase (U3S), an enzyme involved in heme synthesis. However, one family with CEP was found to lack a mutation in U3S. The sex-linked pattern of inheritance of the CEP, along with the associated thrombocytopenia, and the fact that U3S is a known GATA-1 target, led investigators to evaluate the GATA-1 region of the affected individual. A mutation in codon 216 was found in the child and on one allele of his mother, replacing arginine with tryptophan (R216W). Apparently this mutation in the highly conserved, N-terminal zinc finger of GATA-1 disrupts the expression of the UROS gene in developing RBCs, leading to the resultant phenotypic expression (Phillips et al., 2007).

5.2 Clinical Presentation of CEP

Whether caused by mutations in U3S or GATA-1, the presentation of CEP includes photosensitive bullous dermatosis, hirsutism, and fluorescence of various body fluids 21 due to U3S buildup. The photosensitive bullous dermatosis is a painful, photomutilating condition often leading to skin lesions (Phillips, et al. 2007). Fluorocytes, RBCs that fluoresce when exposed to light of an appropriate frequency, may be present, as well as urine that fluoresces

under a Wood's lamp, and fluorescent teeth and BM (Hindmarsh, 1986). Also present is anemia and splenomegaly (Phillips et al. 2007).

The subject studied by Phillips et al., was a 3-year old boy with CEP and a hypochromic, microcytic anemia from birth. RBC morphology and globin chain labeling studies revealed a β-thalassemia, with an absence of beta chains and an elevation in fetal Hb chains (HbF). The increased HbF level could mean that GATA-1 plays a role in globin chain switching. This was the first documented association of CEP with thalassemia and thrombocytopenia (Phillips, et al. 2007).

6. *GATA-1* Mutations and Trisomy 21

6.1. GATA-1 Mutations Associated with Trisomy 21

A recent review by Vyas and Crispino (2007) nicely summarizes the current understanding of the consequences of *GATA-1* mutations with respect to Trisomy 21. It has been shown that two hematopoietic disorders in children with Trisomy 21 are associated with spontaneous *GATA-1* mutations (Wechsler et al., 2002). Transient myeloproliferative disorder (TMD) is a preleukemic condition afflicting up to 10% of infants with Trisomy 21. TMD usually resolves spontaneously, but it markedly increases the individual's risk for Trisomy 21-AMKL (M7 subtype). Unlike the cytopenias caused by germline *GATA-1* mutations, this condition is caused by a somatic mutation in exon 2 of *GATA-1*, and is present in the premalignant cells of virtually all cases of TMD and the more severe disease, Trisomy 21-AMKL, in both males and females (Wechsler et al., 2002; Hitzler and Zipursky, 2005).

Normal hematopoietic cells express both full length GATA-1 and a shorter isoform, termed GATA-1s. The shorter isoform retains both C-f and N-f zinc fingers, but it lacks the N-terminal transcription activation domain present on the full length protein. The specific function of each isoform is still not well understood (Mundschau and Crispino, 2006).

In almost every known case of AMKL associated with Trisomy 21, GATA-1 is mutated, resulting in the sole production of GATA-1s. Various acquired mutations lead to the premature arrest of protein translation and reinitiation of synthesis at a downstream site. All known mutations occur in exon 2, the first coding exon, and are either nonsense mutations introducing a stop codon before codon 84, or splice mutations that interfere with splicing of exon 2 to the rest of the mRNA, effectively removing the first translational start codon. In both cases, GATA-1 is now translated from the alternative start codon 84, producing the truncated GATA-1s lacking an N-terminus (Ahmed, et. al., 2004).

While it remains unclear why GATA-1 truncating mutations occur at such an extraordinarily high rate in individuals with Trisomy 21, research has logically focused on interactions of GATA-1 with genes located on chromosome 21 (Cantor, 2005). One potential candidate gene currently under investigation is Runx-1, also known as AML-1, Cbfa2, or PEBP2∝B. Runx-1 is a core-binding transcription factor necessary for hematopoiesis, expressed by MKs, and previously found to have a high mutation rate in leukemias and myelodyspastic syndromes (Speck and Gilliland, 2002). Runx-1 knockout mice are thrombocytopenic due to a halt in MK differentiation, and constitutional Runx-1 haploinsufficiency in humans leads to thrombocytopenia and the aforementioned increased rates of myeloid leukemia (Ichikawa et al., 2004, Song et al., 1999). GATA-1 and Runx-1 physical interaction has been documented by two research groups, with one mapping the interaction to the amino and carboxyl terminals, and the other localizing it to the zinc finger (Waltzer et al., 2003, Elagib et al., 2003). So, although the role of Runx-1 remains to be refined, its location on chromosome 21, the clinical consequences of its mutation in mouse models and humans, and its proven interaction with

GATA-1 make it a promising target for further research into why patients with Trisomy 21 are predisposed to GATA-1 mutations.

6.2. Clinical Presentation of Trisomy 21 Associated with GATA-1 Mutations

Acquired somatic mutations in *GATA-1*, in combination with Trisomy 21, cause TMD and AMKL with uncontrolled MK proliferation. Unlike the aforementioned inherited, X-linked GATA mutations, these somatic errors afflict females at the same rate as males. Synergy between Trisomy 21 and GATA-1s cause dramatic MK proliferation (Mundschau and Crispino, 2006), with suppression of other hematopoietic lineages due to crowding out by MKs.

TMD is estimated to be present in 10–20% of infants with Trisomy 21, suggesting the mutation may occur *in utero*. There is marked phenotypic variation. Some infants are asymptomatic with circulating blast cells found only incidentally. Other patients with Trisomy 21-TMD progress to AMKL. Rarely, death may result due to MK infiltration of the liver and massive hepatic fibrosis. The exact number of Trisomy 21-TMD cases is most likely underestimated because blood smears are not done, or because the subtle changes associated with milder cases can be easily overlooked. Usually TMD spontaneously resolves, but in approximately 30% of individuals with Trisomy 21-TMD, AMKL results by direct sequential progression from TMD, or sometimes after months or years of symptom free remission (Ahmed, et al., 2004).

AMKL is a biologically heterogeneous form of acute myeloid leukemia. Compared to the general population, children with Trisomy 21 have a 500-fold increased risk of AMKL. However, Trisomy 21-AMKL has a much better prognosis than AMKL not accompanied by Trisomy 21. Trisomy 21-AMKL almost always manifest before age 4, and in most cases there is a documented history of TMD with the MKs in both diseases proving to be ultrastructurally, morphologically, and immunologically similar (Ahmed, et al., 2004). BM biopsy reveals the presence of overproduction of blast cells with a greater than 30% frequency of blast cells confirming a diagnosis of acute leukemia. BM aspirates may also demonstrate myelofibrosis. In a study conducted by Athale et al. in 2001, two findings were identified as highly diagnostic for AMKL: leukemic cells isolated from the BM had distinct morphologic features, such as surface blebs, cell clumping, and binucleation, and the presence of multifocal punctate cytoplasmic alpha naphthyl acetate esterase cytochemical staining that is inhibited by sodium fluoride. As aforementioned Trisomy 21-AMKL has a much better outcome than AMKL not associated with Trisomy 21 or *GATA-1* mutations, and the same study found an estimated 83% two year event free survival rate for Trisomy 21-AMKL, versus 14% and 20% for *de novo* and secondary AMKL not associated with Trisomy 21 or spontaneous *GATA-1* mutations (Athale et al., 2001).

7. Germline GATA-1s

7.1. GATA-1s in Normal Individuals

Hollanda et al. (2006) described a family with a germline *GATA-1* splice site mutation like the somatic mutation seen in those with Trisomy 21-AMKL. Here too, the result is the exclusive production of the amino terminal truncated GATA-1s protein. While these individuals share some clinical and laboratory features with Trisomy 21-AMKL patients, none has yet developed TMD or other malignant phenotypes. Data on the individuals with exclusive production of GATA-1s in the absence of Trisomy 21 support the assumption that although a normal isoform, GATA-1s production alone will not sustain normal erythropoiesis (Hollanda et al., 2006).

7.2 GATA-1s Clinical Presentation in the absence of Trisomy 21

Seven affected males from two generations of a family were studied. These individuals exhibit a unique phenotype that includes macrocytic anemia and variable neutropenia, but normal

platelet counts. This is the first known GATA-1 mutation to influence neutrophil production. Generation of the neutrophil lineage appears to be selectively affected (directly or indirectly) by loss of the GATA-1 amino terminus (Hollanda et al., 2006).

Affected individuals may be predisposed to infection, anemia, and platelet disorders, but they do not develop leukemia as do patients with concomitant Trisomy 21. At the time of the study, one affected individual had severe anemia present from infancy, two males had mild, asymptomatic anemia, two subjects were in remission following allogeneic BM transplants to treat severe anemia and neutropenia, and two others required monthly erythrocyte transfusions.

8. Conclusion

The GATA gene family is highly conserved across evolution, testifying to their importance in genetic transcription. Mutations in *GATA-1* can lead to highly unregulated, misdirected, and ineffective hematopoiesis. It is now becoming clear that the location of the mutation has critical consequences on the spectrum and severity of the individual phenotype, with seemingly subtle differences in *GATA-1* mutations producing markedly different functional outcomes.

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Abbreviations

AMKL
: acute megakaryoblastic leukemia

BM
: bone marrow

CBC
: complete blood count

CEP
: congenital erythropoietic porphyria

C-f
: carboxyl terminal finger

Hb-f
: fetal hemoglobin

FOG-1
: friend of GATA-1

GPS
: gray platelet syndrome

MK
: megakaryocyte

N-f
: amino terminal finger

RBC
: red blood cell

TMD
transient myeloproliferative disease

U3S
uroporphyrinogen III

XLT
X-linked thrombocytopenia

XLTT
X-linked thrombocytopenia with thalassemia

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TABLE 1

GENOTYPIC-PHENOTYPIC CORRELATIONS OF GATA-1 MUTATIONS

	MUTATION	NUMBER OF CASES CURRENTLY IDENTIFIED	CLINICAL MANIFESTATION	REFERENCE
XLT	**i.** V205M **ii.** G208R **iii.** G208S **iv.** D218G **v.** D218Y	**i.** 1 family with 2 affected individuals **ii.** 1 family with 1 affected individual **iii.** 1 family with 4 affected individuals **iv.** 1 family with 13 affected individuals **v.** 1 family with 7 affected individuals	**i.** Severe macro-thrombocytopenia and dyserythropoietic anemia **ii.** Severe macro-thrombocytopenia and dyserythropoietic anemia **iii.** Macrothrombocytopenia and mild dyserythropoiesis without anemia **iv.** Macrothrombocytopenia and mild dyserythropoiesis without anemia **v.** Severe macrothrombocytopenia, dyserythropoietic anemia, and early mortality	**i.** Nichols et al.., 2000 **ii.** Del Vecchio et al., 2005 **iii.** Mehaffey et al.., 2001 **iv.** Freson et al., 2001 **v.** Freson et al.., 2002
XLTT	R216Q	4 families with at least 9 affected individuals[#]	Macrothrombocytopenia, mild, hemolytic anemia with β decreased β chain production resembling mild β-thalassemia, splenomegaly	Raskind. et al., 2000; Yu. et al.,2002; Balduini. et al., 2004 Hughan et al., 2005[#] Raskind unpublished.
XLTT/X-GPS[*]	R216Q	1 known case caused by R216Q, total cases of all categories, unknown	Variable, mild bleeding diathesis, thrombocytopenia and large, agranular, functionally abnormal platelets.	Tubman et al., 2007
CEP	R216W	1 known case caused by R216W, total cases of all categories, unknown	Photosensitive bullous dermatosis, hypochromic, microcytic anemia, and thrombocytopenia, splenomegaly, hirsutism	Phillips et al., 2007
TMD and DS-AMKL	Somatic, splice mutation in exon 2 of *GATA-1*, leading to the production of GATA-1s	TMD: 10% of all DS cases DS-AMKL: 30% of TMD cases progress	Hypercellular bone marrow with anemia, thrombocytopenia, and decreased leukocytes predisposing to bleeding disorders and infection	Ahmed et al., 2004, Wechsler et al., 2002; Hitzler and Zipursky, 2005
Macrocytic anemia with variable neutropenia	Inherited *GATA-1* splice mutation	1 family with 7 affected individuals	Macrocytic anemia, variable neutropenia, trilineage dysplasia in the bone marrow. infection, anemia, and platelet disorders	Hollanda et al., 2006

[#] A single affected male is described in this report with reference to other family members

[*] Naming is currently in debate although this appears to be another family with XLTT and not a separate syndrome.

TABLE 2

TIMELINE OF SEVERAL KEY *GATA-1* OBSERVATIONS (NOT LISTED IN TABLE 1)

YEAR	OBSERVATION/FINDING	REFERENCES
1988	*GATA-1* first identified as an erythroid nuclear protein (named *Eryf1*)	Evans et al., 1988
1989	Cloning of *GATA-1*and structural evidence (C-f)	Tsai et al., 1989
1990	Expression of *GATA-1* in MK and mast cells	Martin et al., 1990
1990	*GATA-1* location identified on the × chromosome	Zon et al., 1990
1991	*GATA-1* mutations block erythroid differentiation	Pevny et al., 1991
1994	*GATA-1* might be essential only in later primitive erythroid precursors	Weiss et al., 1994
1995	*GATA-1* is predominant GATA factor for erythroid maturation	Pevny et al., 1995
1996	*GATA-1* mutation study shows that GATA-1 plays an essential role in terminal erythroid maturation	Fujiwara et al., 1996
1997	Megakaryocyte growth and platelet development (lineage-selective *GATA-1* knockdown)	Shivdasani et al., 1997
2002	Structural evidence (N-f) of *GATA-1*	Chang et al., 2002
2002	*GATA-1* in eosinophil development	Hirassawa et al., 2002
2003	*GATA-1* is a regulator of mast cell differentiation	Migliaccio et al., 2003
2006	Chromatin remodeling affects *GATA-1* expression	Grass et al., 2006