Chapter 4
Etiology of Leukemia in Children with Down Syndrome

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Abstract Down syndrome (DS) or trisomy 21 is the most common congenital genetic abnormality in the United States, and affected individuals have a unique predisposition to develop acute leukemias early in life. It is estimated that children with DS have a 40- and 150-fold increased risk of developing acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML), respectively. The increase in leukemia risk is likely caused by endogenous alterations of genetic factors, including imbalances in chromosome 21-localized genes and altered biochemical pathways in DS cells. The hallmark features of DS-AML include the early development of a precursor disorder known as transient abnormal myelopoiesis (TAM), which clinically resembles AML but is transient in nature, and the presence of *GATA1* (Xp11.23) mutations, which are detectable in the majority of TAM and DS-AML cases. On the other hand, DS-ALL leukemogenesis is linked to alterations in the *CRLF2* gene and associated mutations affecting pathways involving either the *JAK2* or *RAS* genes. In this chapter we review current concepts of mechanisms leading to mutagenesis and leukemia in DS.

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J.M. Mejía-Aranguré (ed.), *Etiology of Acute Leukemias in Children*,
DOI 10.1007/978-3-319-05798-9_4

Keywords Down syndrome • Acute lymphoblastic leukemia • Acute myeloid leukemia • Myeloproliferative disorders • GATA1 transcription factor

Introduction

Acute leukemia is the most common type of cancer in children. Although the etiology of acute leukemias remains largely unknown, there is supporting evidence in the literature suggesting that leukemogenesis is a multi-step process in which various genetic hits may be involved. Remarkable relationships exist between chromosome 21 and predisposition to leukemia, leukemogenesis, and response to therapy. Hallmark features of pediatric acute leukemias frequently involve quantitative and/or qualitative changes involving chromosome 21.

Down syndrome (DS; trisomy 21) is a disorder characterized by the constitutional presence of an extra copy of chromosome 21, and such individuals carry a significantly higher predisposition to develop leukemia, especially early in life. A progressively better understanding of the processes involved in malignant transformation in DS cells is providing additional opportunities to answer fundamental questions that still remain in relation to leukemogenesis and response to cancer therapy in patients without DS.

DS is the most common birth defect in the United States and is one of the most studied genetic conditions (Parker et al. 2010). John Langdon Down first described this disorder in 1866 in a group of children displaying common phenotypic features and cognitive impairments (Down 1866). It was only in 1959 that the presence of an extra copy of chromosome 21 was detected as constitutionally present in patients with DS (Lejeune et al. 1959). The first description of leukemia occurring in a child with DS was published in 1930 (Cannon 1930). Since then, it has become evident that individuals with DS have a striking predisposition to develop acute leukemia early in life and that the elevated risk can extend for several decades (Scholl et al. 1982; Hasle et al. 2000). Interestingly, the increased risk of malignancy seems to be limited to the development of leukemias only, since solid tumors occur significantly less frequently in children and adults with DS in comparison with individuals without DS (Xavier et al. 2009). In terms of leukemia risk, it is estimated that children with DS have a 40- and 150-fold increased risk of developing acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML), respectively, in comparison with children without DS (Fong and Brodeur 1987; Hasle 2001), making them a significant proportion of patients enrolled in clinical trials. In fact, children with DS represent approximately 2 % and 15 % of the pool of pediatric patients with ALL and AML, respectively (Zeller et al. 2005; Ragab et al. 1991). The increased risks of both types of acute leukemia in the same individual seem to be independent (Hellebostad et al. 2005). Interestingly, there is a 500-fold increased risk of children with DS developing the rare subtype of AML, acute megakaryocytic leukemia (AMkL; French-American-British [FAB] classification M7) (Zipursky et al. 1994). AMkL cases correspond to less than 2 % of adult patients with AML

and up to 10 % of pediatric AML cases (Athale et al. 2001). In contrast, AMkL is the most common subtype of AML in patients with DS (Zeller et al. 2005; Al-Ahmari et al. 2006; Rao et al. 2006; Kudo et al. 2007; Reinhardt et al. 2005; O'Brien et al. 2008; Ravindranath et al. 1992; Gamis et al. 2003). This unique increase in leukemia risk is likely the result of endogenous genetic factors including chromosome 21-localized genes and altered biochemical pathways in DS cells that may drive leukemogenesis.

Down Syndrome–Acute Lymphoblastic Leukemia

Essentially there are no clinical distinctions between patients with DS-ALL and those without it. The peak incidence of ALL in children without DS is between 2 and 5 years, with the majority of cases actually being diagnosed in children aged 2–3 years. Thereafter the incidence steadily decreases, being much less common among children older than 10 years (Howlader et al. 2013a, b). The age of presentation for children with DS-ALL is similar (Robison et al. 1984; Pui et al. 1993; Chessells et al. 2001; Whitlock et al. 2005) or slightly older (Ragab et al. 1991; Dordelmann et al. 1998). Strikingly, ALL has been unreported among children with DS younger than 1 year. No cases of infant DS-ALL were registered among 653 DS cases treated in various collaborative group clinical trials (Ponti di Legno Study Group) between 1995 and 2004 (Buitenkamp et al. 2014). Similarly, no cases of infant DS-ALL were present in other large treatment cohorts (Whitlock et al. 2005; Lundin et al. 2014; Arico et al. 2008). The reasons for this apparent protection against infant ALL in DS remain unknown.

Features including gender, race, initial white blood cell (WBC) count, lymphadenopathy, and hepatosplenomegaly are not significantly different between DS and children without DS with ALL at presentation (Ragab et al. 1991; Pui et al. 1993; Chessells et al. 2001; Dordelmann et al. 1998). However, DS children with ALL have a lower frequency of central nervous system involvement at presentation and less commonly present with an anterior mediastinal mass (Pui et al. 1993; Bassal et al. 2005), although these findings have not been consistent among different DS-ALL cohorts (Zeller et al. 2005; Pui et al. 1993; Chessells et al. 2001; Dordelmann et al. 1998).

There are some noticeable differences in regard to common pediatric prognostic features that may be secondary to different pathogenesis processes. ALL of the T-cell phenotype is a very aggressive malignancy derived from T-cell progenitor cells, accounting for about 15 % of the pediatric leukemia cases in children without DS (Pizzo and Poplack 2011), and historically has an inferior outcome in comparison with acute leukemias of the B-cell phenotype. For reasons that remain unknown, T-cell ALL occurs rarely among DS children. In fact, several cohorts of patients with DS-ALL covering a large time span reported no cases of T-cell ALL at all (Zeller et al. 2005). Lower frequencies of common cytogenetic abnormalities are also seen among children with DS-ALL. They have a lower incidence of the hyper-

diploid karyotype, the *ETV6-RUNX1* t(12;21) fusion protein (Zeller et al. 2005; Pui et al. 1993; Lundin et al. 2014), or other genetic alterations such as t(9;22) (q34;q11) (*BCR/ABL* fusion gene), *MLL* rearrangements, and t(1;19) (*TCF3-PBX1* fusion gene) (Pui et al. 1993; Chessells 2001; Forestier et al. 2008).

Another important differentiation is that DS children with ALL have an inferior outcome and a greater incidence of treatment-related mortality compared with children without DS with ALL. Early reports showed that despite having similar age and WBC count at diagnosis, patients with DS-ALL had significantly lower remission rates, higher mortality rates during induction, and decreased long-term overall survival (Robison et al. 1984; Kalwinsky et al. 1990; Levitt et al. 1990). These differences have since been confirmed by multiple different trials (Ragab et al. 1991; Pui et al. 1993; Chessells et al. 2001; Whitlock et al. 2005; Dordelmann et al. 1998; Bassal et al. 2005; Rajantie and Siimes 2003). Intensification of therapy may be beneficial in improving event-free survival (EFS), although patients with DS-ALL continue to face excessive treatment-related morbidity and mortality (Ragab et al. 1991; Buitenkamp et al. 2014; Patrick et al. 2014), with higher rates of severe mucositis and infections, owing to more severe and prolonged myelosuppression (Buitenkamp et al. 2014; Rabin et al. 2012). Systemic toxicity may be intrinsically related to the constitutional presence of an extra copy of chromosome 21, with subsequent differences in pharmacokinetics of drugs or pharmacodynamic effects in the tissues (Garre et al. 1987; Buitenkamp et al. 2010). For instance, DS children poorly tolerated treatment with the antifolate agent, methotrexate. The reduced folate carrier gene is localized to chromosome 21 (*SLC19A1*, 21q22), and its increased expression in various DS tissues may result in increased intracellular methotrexate transport and consequent increased cellular toxicity. These unique distinctions suggest that besides the linkage to leukemogenesis, trisomy 21 is also linked to metabolism of chemotherapy drugs, toxicity, and response to therapy (Xavier et al. 2009).

The "Two-Hit" Model of DS-ALL Leukemogenesis

The exact mechanisms by which an additional copy of chromosome 21 predisposes to leukemia remain unknown. Carcinogenesis is a complex process that results in essential alterations in cell physiology, usually driven by mutations, genomic instability, epigenetic events, etc. DNA can be modified spontaneously in nature or after environmental exposure to mutagenic factors, such as viruses, radiation, or chemicals. Mutagenesis in certain types of cancer is greatly influenced by environmental factors, such as tobacco exposure and lung cancer (Doll and Peto 1978), or virus oncogenicity in bladder cancer (Parada et al. 1982).

In terms of leukemogenesis, it has been proposed that pediatric ALL results from at least two independent and sequential genetic mutations or events (Greaves 1988). The "two-hit" model of leukemogenesis was postulated by Mel Greaves: a preleukemic clone would arise in utero during the expansion of the B-cell precursor

compartment ("first hit"), creating a "preleukemia state," and a second mutation ("second hit") would potentially occur after birth, likely resulting from environmental exposures such as infections, or even inherited susceptibility (Greaves 2002). This model is well accepted for ALL cases characterized by the presence of chromosomal translocations that result in functional leukemia fusion genes (e.g., *ETV6-RUNX1* fusion genes) (Greaves 2002).

Trisomy 21 Taking this model into account for DS-ALL, it is possible that the "first hit" is actually the presence of an extra copy of chromosome 21. A trisomic state would lead to gene dosage imbalances that promote changes in physiological cell processes or lead to deleterious mutations. In fact, some studies using DS mouse models have suggested that the presence of trisomic genes can induce the development of heart defects (Liu et al. 2011) and promote cognitive behavior changes. Liver and marrow of DS human fetuses collected at early gestational ages display expansion of the erythroid and megakaryocytic (Yu et al. 2010) compartments and changes in lymphopoiesis without the presence of additional mutations, suggesting that the abnormal fetal hematopoiesis is likely driven by the presence of an extra copy of chromosome 21 (Chou et al. 2008; Tunstall-Pedoe et al. 2008; Roberts et al. 2013) (Fig. 4.1).

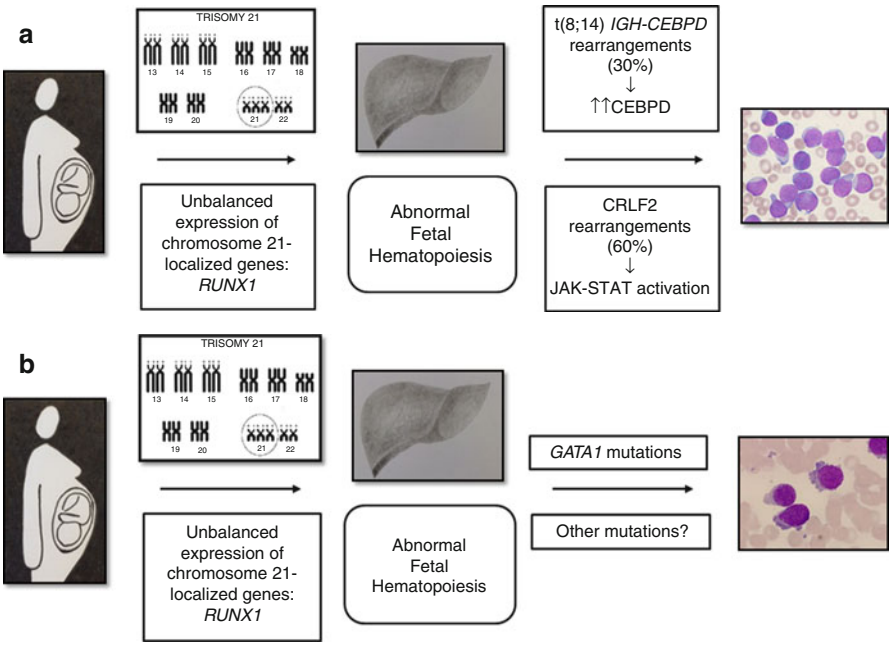

Fig. 4.1 Gene dosage imbalances caused by the presence of an extra copy of chromosome 21 in Down syndrome babies would lead to abnormal fetal hematopoiesis. Consequently, altered physiological cell processes would promote gene rearrangements and deleterious mutations, leading to the development of acute lymphoblastic leukemia (**a**) or acute myeloid leukemia (**b**)

Underlining the importance of chromosome 21 in the etiology of leukemias is the fact that somatic quantitative or qualitative changes in chromosome 21 are commonly found in non-DS B-precursor ALL patients. For instance, the t(12;21) (p13;q22) chromosomal translocation that leads to the *ETV6-RUNX1* fusion gene is present in about 20–30 % of the non-DS-ALL pediatric cases (Pui et al. 2008). Among these patients a large proportion exhibit secondary aberrations, with the most frequent being the presence of an extra copy of chromosome 21 (Loncarevic et al. 1999; Ma et al. 2001). High hyperdiploid karyotype (defined as 51–65 chromosomes per cell), which occurs in 20–25 % of the non-DS B-precursor ALL cases (Paulsson and Johansson 2009), almost uniformly have three to four copies of the chromosome 21.

Of note, children with DS-ALL may have similar cytogenetic abnormalities such as t(12;21) (p13;q22) or high hyperdiploid karyotype, although these changes are found in a much smaller proportion of cases (Zeller et al. 2005; Pui et al. 1993; Dordelmann et al. 1998; Buitenkamp et al. 2014; Arico et al. 2008; Bassal et al. 2005; Chessells 2001; Forestier et al. 2008; Lanza et al. 1997; Maloney et al. 2010). Interestingly, array comparative genome hybridization analyses of DS-ALL samples without *ETV6-RUNX1* fusion showed cytogenetic changes similar to those found in non-DS *ETV6-RUNX1* positive ALL samples (Lo et al. 2008). Other established genetic alterations such as t(9;22) (q34;q11) (*BCR/ABL* fusion gene), *MLL* rearrangements, and t(1;19) (*TCF3-PBX1* fusion gene) are also found in DS-ALL, but at a lower frequency (Pui et al. 1993; Chessells 2001; Forestier et al. 2008; Kalwinsky et al. 1990). This suggests that constitutional trisomy 21 may promote an apparent protection against common recurrent genetic abnormalities in ALL that involve chromosomal translocations, with consequent reduction of DS children presenting with infant leukemia (*MLL*-rearranged ALL), Ph+ ALL (*BCR-ABL*), and T-cell ALL (multiple different translocations involved) (Zeller et al. 2005; Buitenkamp et al. 2014). These differences stress the heterogeneity of childhood leukemia and the complexity of leukemogenesis in different groups of patients.

Chromosome 21-Localized Genes Several chromosome 21-localized genes can potentially play a role in leukemogenesis in an unbalanced state. *RUNX1* (alternative names *AML1*; core-binding factor, runt domain α-subunit 2, CBFA2) is the gene more frequently implicated in leukemia. *RUNX1* is part of the *RUNX* gene family (*RUNX2* and *RUNX3*) of transcription factors (TFs) that bind DNA via a Runt domain and a β-subunit encoded by the *CBFB* (core-binding factor, β-subunit, CBFB) gene. *RUNX1* plays key regulatory roles during hematopoiesis via regulation of various hematopoietic genes (Cohen 2009) which, when altered, result in leukemia. *RUNX1* is frequently found translocated in patients with AML (*AML1-ETO*, *AML1-MDS1-EAI1*, *AML1-FOG2*) (Helbling et al. 2004; McNeil et al. 1999; Chan et al. 2005) and ALL (*ETV6-RUNX1*) (Hong et al. 2008), and the chimeric proteins that result from the fusion induce leukemogenesis.

Nonsense/missense or deletion mutations involving *RUNX1*—that lead to a gene "haploinsufficient state"—have been found to be causative of an autosomal dominant disorder known as familiar platelet disorder with associated myeloid

malignancy (FPD/AML). FPD/AML is characterized by platelet dysfunction, altered megakaryopoiesis, and elevated risk of developing AML (Song et al. 1999). In this condition the simple inactivation of one allele is sufficient to predispose to leukemia (Song et al. 1999). In mouse models, *RUNX1* haploinsufficiency has been shown to alter hematopoiesis (Mukouyama et al. 2000). Similarly, Preudhomme et al. (2000) found an elevated incidence of missense mutations or deletions in the Runt domain, likely resulting in nonfunctional AML1 protein, in multiple different cases of hematological disorders characterized by abnormalities of *RUNX1*, including acquired trisomy 21 and tetrasomy 21 (Mukouyama et al. 2000; Roumier et al. 2003). On the other hand, overexpression of *RUNX1* in a cell model (NIH3T3 cells) induced neoplastic transformation (Kurokawa et al. 1996), suggesting that a higher *RUNX1* gene dosage can induce leukemia per se. *RUNX1* gene amplification either via multiple copies of chromosome 21 or via high-level amplification (intrachromosomal amplification or extra chromosomes) has been reported in pediatric ALL. These cases usually have corresponding increases in AML1 transcripts that are equivalent to the number of the amplified *RUNX1* gene (Busson-Le Coniat et al. 2001). Interestingly, patients with B-precursor ALL and intrachromosomal amplification of chromosomal 21 (iAMP21) have a very poor prognosis. Genomic characterization of cases harboring iAMP21 showed recurrent abnormalities in other genes such as *IKZF1*, *CDKN2A/B*, *PAX5*, *ETV6*, and *RB1*, likely secondary to chromosome 21 rearrangements (Rand et al. 2011).

Subsequent Genetic Changes Once perturbed hematopoiesis is established, multiple additional genetic abnormalities can take place ("second hit"?). Specific cytogenetic changes have been observed in DS-ALL. Otherwise rare in non-DS-ALL (<3 %), up to 30 % of the patients with DS-ALL have translocations involving chromosomes 8 and 14 [t(8;14) (q11;q32)] (*IGH-CEBPD* fusion gene) (Forestier et al. 2008; Moore et al. 2003; Lundin et al. 2009). CCAAT/enhancer-binding protein δ (CEBPD) TF is part of the CEBP family of TFs composed of at least six multifunctional basic leucine zipper (bZIP) members, which play important roles in cellular differentiation, particularly hematopoietic tissues, hepatocytes, and adipocytes. The regulation of these genes is extremely complex and involves hormones, cytokines, nutrients, toxins, etc. (Ramji and Foka 2002), and all members of this family have been implicated in leukemias or solid tumors (Ramji and Foka 2002; Nerlov 2007). The fusion of *IGH* (immunoglobulin G heavy-chain locus; IGHG1) to *CEBPD* leads to activation of *CEBPD* and overexpression of the gene by mechanisms that remain unclear.

More recently, genomic abnormalities of cytokine receptor-like factor 2 (CRLF2) have been detected in approximately 60 % of DS-ALL cases and seem to be a unique feature of DS-ALL because of the rarity of non-DS cases with analogous abnormalities (Mullighan et al. 2009). Similar to the *IGH-CEBPD* scenario, *CRLF2* (Xp22/Yp11) rearrangements can result from either (1) an intrachromosomal deletion of PAR1 (pseudoautosomal region 1) leading to *P2RY8-CRLF2* fusion or (2) a translocation with the IGH locus at 14q32 [(X;14)(p22;q32)/t(Y;14)(p11;q32)] (Mullighan et al. 2009). Both aberrations lead to overexpression of CEBPD. Prior

to the CEBPD findings, Malinge et al. had detected a novel *JAK2* (9p24) mutation in a sample from a child with B-precursor DS-ALL that involved a 5-amino-acid deletion within the JH2 pseudokinase domain (JAK2DeltaIREED) (Malinge et al. 2007). *JAK2* is a tyrosine kinase that phosphorylates cytoplasmic targets essential for signaling of hematopoietic and growth factor receptors (Kralovics et al. 2005). Interestingly, expression of JAK2DeltaIREED in Ba/F3 cells led to constitutive activation of the JAK-STAT pathway and growth factor-independent cell proliferation (Malinge et al. 2007). Subsequently, the presence of *JAK2* mutations was found in a large proportion of patients with DS-ALL (Bercovich et al. 2008; Kearney 2009). *CRLF2* alterations were found to be associated with activating *JAK2* mutations and constitutive JAK-STAT activation, which likely contribute to DS-ALL leukemogenesis (Mullighan et al. 2009; Russell et al. 2009; Hertzberg et al. 2010). Interestingly, in patients not displaying abnormalities in the *JAK2* gene, driver mutations in *RAS* (*KRAS* and *NRAS*) were found in a high proportion of cases (Nikolaev et al. 2014). Additional analysis revealed that both *RAS* and *JAK2* drove subclonal expansions primarily initiated by *CRLF2* rearrangements, and/or mutations in chromatin remodelers and lymphocyte differentiation factors, providing new insights in the understanding of DS leukemogenesis (Nikolaev et al. 2014). Another way of altering expression of *CRLF2* is through gain of chromosome X, a common abnormality among patients with DS-ALL (38 % in DS-ALL cases versus 20 % of non-DS-ALL cases) (Zeller et al. 2005; Forestier et al. 2008; Baker et al. 2003). All DS cases displaying extra copies of chromosome X also had overexpression of CRLF2 (Mullighan et al. 2009; Hertzberg et al. 2010), suggesting that CRLF2 alterations are indeed important in DS-ALL generation.

The Environment The "two-hit" model of leukemias suggests that the postnatal genetic changes needed for leukemia development may be caused by an abnormal immune response to environmental factors, such as delayed infections (Greaves 2002). Lack of exposure to infections early in life would lead to poor immune system modulation and potentially result in leukemia (Greaves 1997, 2002). Among children with DS-ALL, the Children's Oncology Group (COG) found a negative association between acute leukemia and any infection in the first 2 years of life, supporting the idea that early infection may be protective against leukemia in DS children as well (Canfield et al. 2004). Conversely, a study conducted in Mexico City showed a nonsignificant association between early infections and DS-ALL (Flores-Lujano et al. 2009). This study also did not find breastfeeding to be protective of leukemia in DS (Flores-Lujano et al. 2009). Preconception, in utero, and postnatal medical test irradiation exposure was also studied by COG, and no positive association was found with DS-ALL (Linabery et al. 2006). Preconception vitamin supplementation was found to be protective against DS-ALL (Ross et al. 2005), as well as certain maternal conditions such as vaginal bleeding (Ognjanovic et al. 2009), while maternal exposure to professional pest exterminators, pesticides, and any chemicals was positively associated with DS-ALL (Alderton et al. 2006). Larger epidemiology studies are necessary to confirm or exclude environmental factors in the etiology of DS-ALL.

Down Syndrome: Acute Myeloid Leukemia (DS-AML)

Myeloid Proliferation Related to Down Syndrome

AMkL is the most common FAB subtype (M7) of patients with DS-AML, with a frequency ranging from 40 to 100 % of the DS-AML cases in different clinical trials (Zeller et al. 2005; Al-Ahmari et al. 2006; Rao et al. 2006; Kudo et al. 2007; Reinhardt et al. 2005; O'Brien et al. 2008; Ravindranath et al. 1992; Gamis et al. 2003). Zipursky et al. (1994) estimated that DS children have a 500-fold increased risk of developing AMkL compared with children without DS, once more highlighting the unique relationship between trisomy 21 and leukemogenesis for a specific leukemia phenotype (Zipursky et al. 1994). In contrast, AMkL is estimated to represent approximately 10 % of pediatric AML cases and 2 % of adult AML cases (Athale et al. 2001; Tallman et al. 2000).

The differences between DS and non-DS cases are not only restricted to differences in subtype of myeloid leukemia. Multiple pediatric oncology cooperative group clinical trials have reported that patients with DS-AML have remarkably high EFS rates (~80–100 %) when treated with cytarabine/anthracycline-based chemotherapy (Zeller et al. 2005; Al-Ahmari et al. 2006; Rao et al. 2006; Kudo et al. 2007; O'Brien et al. 2008; Ravindranath et al. 1992; Creutzig et al. 2005). In contrast, AMkL in children without DS is associated with a relatively poor prognosis, with EFS of less than 40 % (O'Brien et al. 2013).

Interestingly, up to 10 % of newborns with DS will present with a condition known as transient abnormal myelopoiesis (TAM). This disorder, previously called "transient leukemia," is characterized by circulating blast cells in the peripheral blood with AMkL morphology and immunophenotype. TAM resolves spontaneously without chemotherapy in a high proportion of patients (Zipursky 2003). However, a subset of patients with high-risk features (e.g., hyperleukocytosis, hepatic failure) requires therapy and has a guarded prognosis (Massey et al. 2006). TAM is considered a precursor of DS-AML, as approximately 30 % of patients with DS-TAM will subsequently develop AML or, more commonly, AMkL following clinical resolution of TAM (Zipursky 2003). Hence, patients with DS-TAM represent a subgroup of individuals with one of the highest predicted predispositions to develop acute leukemia.

Prior to the diagnosis of AML, DS patients may develop signs of myelodysplasia, characterized by progressive anemia and thrombocytopenia, dysplastic erythroid cells, and megakaryocytes in the bone marrow. The myelodysplastic phase frequently precedes the development of AML (Zipursky 2003). Both myelodysplastic syndrome (MDS) and AML are often referred as the "myeloid leukemia associated with DS" (ML-DS). TAM and ML-DS are now considered separately from the other subtypes of AML by the World Health Organization classification and are designated as Myeloid Proliferation related to DS (MP-DS) (Swerdlow et al. 2008).

GATA1 *Gene and DS-Acute Myeloid Leukemogenesis*

The GATA1 Gene. The *GATA1* gene (GATA-binding protein 1; Xp11.23) encodes a zinc finger DNA-binding transcriptional factor expressed in erythroid, megakaryocyte, mast, and eosinophil lineages, which detains critical roles during normal hematopoiesis. The GATA1 N-terminal region has transactivation activity and its C-terminal domain binds DNA or other factors (Calligaris et al. 1995). GATA1 protein forms essential activating or repressing complexes with other partner proteins, such as FOG1 (friend of GATA1), CBP (CREB-binding protein), and Med1 (mediator complex subunit 1), to control and promote differentiation of erythroid and megakaryocytic cells (Crispino et al. 1999; Blobel et al. 1998; Stumpf et al. 2006; Crispino 2005). Enforced expression of GATA1 in primitive myeloid cell lines or hematopoietic stem cells induced megakaryocytic/erythroid differentiation, and loss of self-renewal activity (Visvader et al. 1995; Iwasaki et al. 2003; Yamaguchi et al. 1998; Ferreira et al. 2007). On the other hand, inactivation of *GATA1* in a mouse model caused death of male mice during gestation from severe anemia resulting from erythroid development arrest and nonlethal anemia in female mice that exhibited a heterozygous state due to random inactivation of the X chromosome (Fujiwara et al. 1996).

There are two GATA1 isoforms that result from alternative translation initiation sites (Calligaris et al. 1995). The *GATA1* gene encodes a 1.8-kb mRNA that can be translated in a 47-kDa protein or a shorter 40-kDa protein, known as GATA1s. GATA1s is translated from a downstream initiation site and lacks the N-terminal transactivation domain. GATA1 and GATA1s share identical binding activity but differ in their transactivation capacity (Calligaris et al. 1995). The two isoforms have been shown to be present in mouse embryo tissues (Calligaris et al. 1995), and have been associated with diseases. Nonsense mutations leading to truncated GATA1 proteins have been found not only in mammals but also in a set of "bloodless" zebrafish mutants characterized by a severe reduction in blood cell progenitors and circulating blood cells (Lyons et al. 2002). Loss of GATA1 has also been shown to alter erythropoiesis into myelopoiesis (Galloway et al. 2005). In humans, germline GATA1 mutations have been associated with hematopoietic disorders. Patients with X-linked thrombocytopenia (Nichols et al. 2000; Freson et al. 2001), X-linked thrombocytopenia with β-thalassemia (Yu et al. 2002) or X-linked anemia with or without neutropenia and/or platelet abnormalities (Hollanda et al. 2006), and X-linked gray platelet syndrome (Tubman et al. 2007) show various degrees of anemia, thrombocytopenia, and dyserythropoiesis that result from abnormal interactions between GATA1 and partner proteins, depending on the location of the *GATA1* mutation (Ciovacco et al. 2008). Germline mutations leading to the formation of GATA1s have also been described (Hollanda et al. 2006). Those patients presented with anemia, neutropenia, or platelet disorders; however, no leukemia cases have been described, suggesting that, although altered, GATA1s can sustain erythropoiesis.

In 2002, Wechsler et al. (2002) analyzed several samples from individuals with AML for the presence of *GATA1* mutations. Mutations were detected uniformly and exclusively only in DS-AMkL samples. Each of the mutations altered the reading frame and introduced a premature stop codon in the N-terminal transactivation domain, leading to GATA1s production (Wechsler et al. 2002). Subsequent studies showed the uniform presence of acquired *GATA1* mutations in nearly all TAM and DS-AMkL cases (Hitzler et al. 2003; Mundschau et al. 2003; Rainis et al. 2003). The exclusive detection of somatic mutations in the X-linked chromosome gene *GATA1* in DS-AMkL cases is a unique association between a gene mutation in a homogeneous subgroup of leukemia patients, which is linked to altered hematopoiesis and the downstream development of leukemia.

The "Mutator Phenotype". There is no obvious relationship linking a X-linked chromosome gene mutation with chromosome 21, yet one must exist to account for the finding of GATA1 mutations only in the DS population (including individuals with mosaicism of chromosome 21), suggesting the possibility that trisomy 21 induces a "mutator phenotype." It has been well described that trisomy 21 alters fetal liver hematopoiesis, promoting expansion of erythroid and megakaryocytic compartments (Chou et al. 2008; Tunstall-Pedoe et al. 2008; Roberts et al. 2013; Hoeller et al. 2014). There is also supporting evidence that *GATA1* mutations arise during fetal development, as *GATA1* mutations have been retrospectively detected in Guthrie newborn screening cards from patients with DS-AMkL (Ahmed et al. 2004) and have been detected in DS fetal livers as early as 21 weeks of gestational age (Taub et al. 2004). However, the exact mechanism of mutagenesis in DS is not completely understood.

Multiple studies have demonstrated evidence of DNA repair defects in DS cells. DS lymphocytes showed lower baseline DNA repair, and exhibited increased sensitivity to phytohemagglutinin stimulation, N-methyl-N′-nitro-N-nitrosoguanidine, and γ-irradiation, indicating an increased sensitivity to DNA oxidation, methylation, and strand breaks (Agarwal et al. 1970; Ankathil et al. 1997; Morawiec et al. 2008; Lavin et al. 1989). While more than one DNA repair pathway might be affected by the DS phenotype, base excision repair (BER) deficiency is a compelling candidate because it repairs these types of DNA damage.

By analyzing all published studies in which sequence data on *GATA1* mutations was available, Cabelof et al. (2009) began to elucidate possible mechanisms by which these sequence alterations arise. Mutational analysis revealed a predominance of small insertion/deletion, duplication, and base substitution mutations including G:C>T:A, G:C>A:T, and A:T>G:C. This mutational spectrum suggests that oxidative stress and aberrant folate metabolism secondary to genes on chromosome 21 (e.g., superoxide dismutase [SOD] and cystathionine-β-synthase [CBS]) may be linked to the generation of *GATA1* mutations. Both CBS and SOD transcripts are significantly overexpressed in DS-AMkL blasts compared with non-DS-AML (median 12- and 4-fold, respectively) (Taub et al. 1999). CBS overexpression has been associated with a functional folate deficiency (Li et al. 2005) and may result in increased uracil incorporation into DNA, thus providing another mechanism for

generation of mutations in DS. As the rate-limiting enzyme in the BER pathway, loss of β-pol (DNA polymerase β) could result in increased susceptibility to the mutagenic effects of unrepaired endogenous damage caused by high levels of uracil incorporation.

The relationship between two key BER gene products involved in the repair of uracil in DNA, uracil DNA glycosylase (UDG) and β-pol, and DS phenotype was evaluated in DS tissues (Cabelof et al. 2009). UDG is a monofunctional glycosylase that excises uracil from DNA to initiate BER. Loss of UDG in *Escherichia coli* and in mouse models induces mutations characterized predominantly by the G:C>A:T transition, similar to what was observed in DS (Fix and Glickman 1987). DS samples exhibited 75 % lower *UDG* expression than the non-DS (Cabelof et al. 2009). Hence, DS may predispose to mutagenesis through a uracil intermediate as a result of reduced *UDG* expression. Interestingly, DS samples (TAM and AMkL together) showed a 90 % reduction in *β-pol* expression compared with non-DS-AMkL samples. This finding is striking, as 50 % reduction in *β-pol* expression predisposed mice to develop cancer (Cabelof et al. 2006). Germline *β-pol* polymorphisms, leading to slower catalytic rates, cause increased double-strand breaks, chromosomal aberrations, and cellular transformation (Yamtich et al. 2012). Furthermore, DNA repair capacity evaluated in DS and non-DS patient samples provided evidence that the BER pathway was compromised in DS tissues (Cabelof et al. 2009), suggesting that inability to repair DNA damage may also play critical roles in the unique susceptibility of DS children to develop leukemia.

The generation of GATA1s as an end result of the mutations may provide a selective growth advantage allowing for the survival of preleukemic clones, which may ultimately lead to the development of TAM and AMkL in DS. In fact, the induction of GATA1s expression in mice led to hyperproliferation of a unique, previously unrecognized yolk sac and fetal liver progenitor, which the authors proposed to account for the transient nature of TAM and the restriction of DS-AMkL to the first years of life (Li et al. 2005). *GATA1* knockdown in a DS-AMkL cell model resulting in lower GATA1s protein levels promoted cell differentiation towards the megakaryocytic lineage, repressed cell proliferation, and increased basal apoptosis and susceptibility to various chemotherapy drugs, accompanied by downregulation of Bcl-2 and altered expression of genes related to cell death, proliferation, and differentiation (Xavier et al. 2011).

Another important aspect is the fetal liver environment. It is possible that the initial genetic hits that drive leukemogenesis depend on interactions with local stroma. Miyauchi and Kawaguchi (2014) showed that fetal liver stromal cells, but not fetal bone marrow, supported the growth of TAM blast progenitors, mainly through humoral factors. They found high concentrations of hematopoietic growth factors in culture supernatants of the fetal liver stromal cells, suggesting that a unique hematopoietic microenvironment may be the key to sustain the growth of leukemia cells.

Footsteps to Leukemia. *GATA1* mutations and GATA1s represent early or initiating "genetic hits" in a multi-step process of leukemogenesis. Whole-genome and/or whole-exome sequencing of samples from individuals with DS with TAM showed

only the exclusive presence of GATA1 mutations (Yoshida et al. 2013). The natural history of patients with TAM is the spontaneous clinical regression in the majority of cases with support of care alone (Zipursky 2003). The mechanisms behind TAM involution remain unknown. However, a proportion of DS children will, after a period of latency that can last a few years, develop MDS/AMkL that will require treatment with multi-drug chemotherapy. What drives the full development of leukemia is not completely understood, and the presence of a mutated GATA1 protein is unlikely the only driving force in leukemogenesis. This has been shown in studies using DS mouse models in which the introduction of GATA1s resulted in increased megakaryopoiesis, abnormalities in the liver and bone marrow, or anemia, but did not result in leukemia (Alford et al. 2010; Carmichael et al. 2009).

In addition, genomic profile performed on samples from patients with DS-AMkL have revealed mutations in other target genes, including genes involved in epigenetic regulation, common signaling pathways, and multiple cohesion components, in addition to the presence of GATA1 mutations (Yoshida et al. 2013). *KIT*, *FLT3*, *JAK2 JAK3*, and *MPL* gene mutations have been identified DS TAM or AMkL samples (De Vita et al. 2007; Norton et al. 2007). More recently, and using DS TAM/AMkL exome sequencing and genome-wide single nucleotide polymorphism (SNP) microarray, Nikolaev et al. found that 40 % of TAM cases and all AMkL cases showed mutations/deletions other than GATA1 in genes proven as transformation drivers in non-DS leukemia (*EZH2*, *APC*, *FLT3*, *JAK1*, *PARK2-PACRG*, *EXT1*, *DLEC1*, *SMC3*). Two clonal expansions with different GATA1 mutations were found in a TAM sample, one clone with an additional driver mutation and a second clone that gave rise to AMkL after accumulation mutations in seven other genes (Nikolaev et al. 2013). These findings suggested that *GATA1* mutations alone are sufficient for clonal expansion, and that the presence of additional mutations at the TAM stage do not predict AMkL progression. The authors postulated that leukemia progression requires a "third-hit driver," putative driver mutations resulting in aberrant activation of WNT, JAK-STAT, or MAPK-PI3K pathways and consequent overexpression of MYC (Nikolaev et al. 2013). The presence of multiple subclones with varying leukemia-initiating potential and self-renewal capacity was also suggested in a xenograft model of TAM: during serial transplantation of TAM-derived cells, divergent subclones with another *GATA1* mutation and various copy number alterations emerged (Saida et al. 2013). Epigenetic changes can also contribute to leukemogenesis in DS. Early genome-wide DNA methylation changes were detected in DS fetal liver mononuclear cells prior to the presence of GATA1 mutations. These changes were characterized by loss of DNA methylation at genes associated with development disorders. Gain of methylation was detected in DS TAM/AMkL samples, affecting different sets of genes involved in hematopoiesis and the regulation of cell growth and proliferation (Malinge et al. 2013).

In summary, the mechanism of leukemogenesis in DS children is probably multifactorial and involves chromosome 21-localized genes as well as genes localized to other chromosomes. Studying leukemia in DS children is a paradigm to further improve our understanding of the role of genetic disorders associated with a

predisposition to develop cancer and the role of specific genes associated with cancer predisposition. Future work identifying the mechanisms underlying *GATA1* mutagenesis and leukemogenesis in DS will shed important light on why DS children have a significantly higher risk of developing acute leukemia in comparison with children without DS.

References

Agarwal SS, Blumberg BS, Gerstley BJ, London WT, Sutnick AI, Loeb LA. DNA polymerase activity as an index of lymphocyte stimulation: studies in Down's syndrome. J Clin Invest. 1970;49(1):161–9.

Ahmed M, Sternberg A, Hall G, et al. Natural history of GATA1 mutations in Down syndrome. Blood. 2004;103(7):2480–9.

Al-Ahmari A, Shah N, Sung L, Zipursky A, Hitzler J. Long-term results of an ultra low-dose cytarabine-based regimen for the treatment of acute megakaryoblastic leukaemia in children with Down syndrome. Br J Haematol. 2006;133(6):646–8.

Alderton LE, Spector LG, Blair CK, et al. Child and maternal household chemical exposure and the risk of acute leukemia in children with Down's syndrome: a report from the Children's Oncology Group. Am J Epidemiol. 2006;164(3):212–21.

Alford KA, Slender A, Vanes L, et al. Perturbed hematopoiesis in the Tc1 mouse model of Down syndrome. Blood. 2010;115(14):2928–37.

Ankathil R, Kusumakumary P, Nair MK. Increased levels of mutagen-induced chromosome breakage in Down syndrome children with malignancy. Cancer Genet Cytogenet. 1997; 99(2):126–8.

Arico M, Ziino O, Valsecchi MG, et al. Acute lymphoblastic leukemia and Down syndrome: presenting features and treatment outcome in the experience of the Italian Association of Pediatric Hematology and Oncology (AIEOP). Cancer. 2008;113(3):515–21.

Athale UH, Razzouk BI, Raimondi SC, et al. Biology and outcome of childhood acute megakaryoblastic leukemia: a single institution's experience. Blood. 2001;97(12):3727–32.

Baker JM, Coppes MJ, Roland B. A case of Down syndrome with acute lymphoblastic leukemia and isochromosome Xp. Cancer Genet Cytogenet. 2003;147(1):75–7.

Bassal M, La MK, Whitlock JA, et al. Lymphoblast biology and outcome among children with Down syndrome and ALL treated on CCG-1952. Pediatr Blood Cancer. 2005;44(1):21–8.

Bercovich D, Ganmore I, Scott LM, et al. Mutations of JAK2 in acute lymphoblastic leukaemias associated with Down's syndrome. Lancet. 2008;372(9648):1484–92.

Blobel GA, Nakajima T, Eckner R, Montminy M, Orkin SH. CREB-binding protein cooperates with transcription factor GATA-1 and is required for erythroid differentiation. Proc Natl Acad Sci U S A. 1998;95(5):2061–6.

Buitenkamp TD, Mathot RA, de Haas V, Pieters R, Zwaan CM. Methotrexate-induced side effects are not due to differences in pharmacokinetics in children with Down syndrome and acute lymphoblastic leukemia. Haematologica. 2010;95(7):1106–13.

Buitenkamp TD, Izraeli S, Zimmermann M, et al. Acute lymphoblastic leukemia in children with Down syndrome: a retrospective analysis from the Ponte di Legno study group. Blood. 2014;123(1):70–7.

Busson-Le Coniat M, Nguyen Khac F, Daniel MT, Bernard OA, Berger R. Chromosome 21 abnormalities with AML1 amplification in acute lymphoblastic leukemia. Genes Chromosomes Cancer. 2001;32(3):244–9.

Cabelof DC, Ikeno Y, Nyska A, et al. Haploinsufficiency in DNA polymerase beta increases cancer risk with age and alters mortality rate. Cancer Res. 2006;66(15):7460–5.

Cabelof DC, Patel HV, Chen Q, et al. Mutational spectrum at GATA1 provides insights into mutagenesis and leukemogenesis in Down syndrome. Blood. 2009;114(13):2753–63.
Calligaris R, Bottardi S, Cogoi S, Apezteguia I, Santoro C. Alternative translation initiation site usage results in two functionally distinct forms of the GATA-1 transcription factor. Proc Natl Acad Sci U S A. 1995;92(25):11598–602.
Canfield KN, Spector LG, Robison LL, et al. Childhood and maternal infections and risk of acute leukaemia in children with Down syndrome: a report from the Children's Oncology Group. Br J Cancer. 2004;91(11):1866–72.
Cannon HE. Acute lymphatic leukemia: report of a case in an eleventh month Mongolian idiot. New Orleans Med Surg J. 1930;82:872–3.
Carmichael CL, Majewski IJ, Alexander WS, et al. Hematopoietic defects in the Ts1Cje mouse model of Down syndrome. Blood. 2009;113(9):1929–37.
Chan EM, Comer EM, Brown FC, et al. AML1-FOG2 fusion protein in myelodysplasia. Blood. 2005;105(11):4523–6.
Chessells JM. Pitfalls in the diagnosis of childhood leukaemia. Br J Haematol. 2001; 114(3):506–11.
Chessells JM, Harrison G, Richards SM, et al. Down's syndrome and acute lymphoblastic leukaemia: clinical features and response to treatment. Arch Dis Child. 2001;85(4):321–5.
Chou ST, Opalinska JB, Yao Y, et al. Trisomy 21 enhances human fetal erythro-megakaryocytic development. Blood. 2008;112(12):4503–6.
Ciovacco WA, Raskind WH, Kacena MA. Human phenotypes associated with GATA-1 mutations. Gene. 2008;427(1–2):1–6.
Cohen Jr MM. Perspectives on RUNX genes: an update. Am J Med Genet A. 2009; 149A(12):2629–46.
Creutzig U, Reinhardt D, Diekamp S, Dworzak M, Stary J, Zimmermann M. AML patients with Down syndrome have a high cure rate with AML-BFM therapy with reduced dose intensity. Leukemia. 2005;19(8):1355–60.
Crispino JD. GATA1 in normal and malignant hematopoiesis. Semin Cell Dev Biol. 2005;16(1):137–47.
Crispino JD, Lodish MB, MacKay JP, Orkin SH. Use of altered specificity mutants to probe a specific protein-protein interaction in differentiation: the GATA-1:FOG complex. Mol Cell. 1999;3(2):219–28.
De Vita S, Mulligan C, McElwaine S, et al. Loss-of-function JAK3 mutations in TMD and AMKL of Down syndrome. Br J Haematol. 2007;137(4):337–41.
Doll R, Peto R. Cigarette smoking and bronchial carcinoma: dose and time relationships among regular smokers and lifelong non-smokers. J Epidemiol Community Health. 1978;32(4): 303–13.
Dordelmann M, Schrappe M, Reiter A, et al. Down's syndrome in childhood acute lymphoblastic leukemia: clinical characteristics and treatment outcome in four consecutive BFM trials. Berlin-Frankfurt-Munster Group. Leukemia. 1998;12(5):645–51.
Down JHL. Observations on an ethnic classification of idiots. Clin Lect Rep: Lond Hosp. 1866;3:259–62.
Ferreira R, Wai A, Shimizu R, et al. Dynamic regulation of Gata factor levels is more important than their identity. Blood. 2007;109(12):5481–90.
Fix DF, Glickman BW. Asymmetric cytosine deamination revealed by spontaneous mutational specificity in an Ung- strain of Escherichia coli. Mol Gen Genet. 1987;209(1):78–82.
Flores-Lujano J, Perez-Saldivar ML, Fuentes-Panana EM, et al. Breastfeeding and early infection in the aetiology of childhood leukaemia in Down syndrome. Br J Cancer. 2009;101(5):860–4.
Fong CT, Brodeur GM. Down's syndrome and leukemia: epidemiology, genetics, cytogenetics and mechanisms of leukemogenesis. Cancer Genet Cytogenet. 1987;28(1):55–76.
Forestier E, Izraeli S, Beverloo B, et al. Cytogenetic features of acute lymphoblastic and myeloid leukemias in pediatric patients with Down syndrome: an iBFM-SG study. Blood. 2008;111(3):1575–83.

Freson K, Devriendt K, Matthijs G, et al. Platelet characteristics in patients with X-linked macrothrombocytopenia because of a novel GATA1 mutation. Blood. 2001;98(1):85–92.
Fujiwara Y, Browne CP, Cunniff K, Goff SC, Orkin SH. Arrested development of embryonic red cell precursors in mouse embryos lacking transcription factor GATA-1. Proc Natl Acad Sci U S A. 1996;93(22):12355–8.
Galloway JL, Wingert RA, Thisse C, Thisse B, Zon LI. Loss of gata1 but not gata2 converts erythropoiesis to myelopoiesis in zebrafish embryos. Dev Cell. 2005;8(1):109–16.
Gamis AS, Woods WG, Alonzo TA, et al. Increased age at diagnosis has a significantly negative effect on outcome in children with Down syndrome and acute myeloid leukemia: a report from the Children's Cancer Group Study 2891. J Clin Oncol. 2003;21(18):3415–22.
Garre ML, Relling MV, Kalwinsky D, et al. Pharmacokinetics and toxicity of methotrexate in children with Down syndrome and acute lymphocytic leukemia. J Pediatr. 1987; 111(4):606–12.
Greaves MF. Speculations on the cause of childhood acute lymphoblastic leukemia. Leukemia. 1988;2(2):120–5.
Greaves MF. Aetiology of acute leukaemia. Lancet. 1997;349(9048):344–9.
Greaves M. Childhood leukaemia. BMJ. 2002;324(7332):283–7.
Hasle H. Pattern of malignant disorders in individuals with Down's syndrome. Lancet Oncol. 2001;2(7):429–36.
Hasle H, Clemmensen IH, Mikkelsen M. Risks of leukaemia and solid tumours in individuals with Down's syndrome. Lancet. 2000;355(9199):165–9.
Helbling D, Mueller BU, Timchenko NA, et al. The leukemic fusion gene AML1-MDS1-EVI1 suppresses CEBPA in acute myeloid leukemia by activation of Calreticulin. Proc Natl Acad Sci U S A. 2004;101(36):13312–7.
Hellebostad M, Carpenter E, Hasle H, Mitchell C, Vyas P. GATA1 mutation analysis demonstrates two distinct primary leukemias in a child with down syndrome; implications for leukemogenesis. J Pediatr Hematol Oncol. 2005;27(7):408–9.
Hertzberg L, Vendramini E, Ganmore I, et al. Down syndrome acute lymphoblastic leukemia, a highly heterogeneous disease in which aberrant expression of CRLF2 is associated with mutated JAK2: a report from the International BFM Study Group. Blood. 2010; 115(5):1006–17.
Hitzler JK, Cheung J, Li Y, Scherer SW, Zipursky A. GATA1 mutations in transient leukemia and acute megakaryoblastic leukemia of Down syndrome. Blood. 2003;101(11):4301–4.
Hoeller S, Bihl MP, Tzankov A, et al. Morphologic and GATA1 sequencing analysis of hematopoiesis in fetuses with trisomy 21. Hum Pathol. 2014;45(5):1003–9.
Hollanda LM, Lima CS, Cunha AF, et al. An inherited mutation leading to production of only the short isoform of GATA-1 is associated with impaired erythropoiesis. Nat Genet. 2006;38(7):807–12.
Hong D, Gupta R, Ancliff P, et al. Initiating and cancer-propagating cells in TEL-AML1-associated childhood leukemia. Science. 2008;319(5861):336–9.
Howlader N, Noone A, Krapcho M, et al. Childhood cancer. SEER cancer statistics review, 1075–2010. Bethersda: National Cancer Institute; 2013a. http://seer.cancer.gob/csr/1975_2010, based on Nov 2012 SEER data submission, posted to the SEER web site, Apr, Section 28.
Howlader N, Noone A, Krapcho M et al. Childhood cancer by the International Classification of Childhood Cancer. SEER Cancer Statistics Review, 1075–2010. Bethesda: National Cancer Institute; 2013b. http://seer.cancer.gov/csr/1975_2010, based on Nov 2012 SEER data submission, posted to the SEER web site, Apr, Section 29
Iwasaki H, Mizuno S, Wells RA, Cantor AB, Watanabe S, Akashi K. GATA-1 converts lymphoid and myelomonocytic progenitors into the megakaryocyte/erythrocyte lineages. Immunity. 2003;19(3):451–62.
Kalwinsky DK, Raimondi SC, Bunin NJ, et al. Clinical and biological characteristics of acute lymphocytic leukemia in children with Down syndrome. Am J Med Genet Suppl. 1990;7:267–71.

Kearney L, Gonzalez De Castro D, Yeung J, et al. Specific JAK2 mutation (JAK2R683) and multiple gene deletions in Down syndrome acute lymphoblastic leukemia. Blood. 2009; 113(3):646–8.

Kralovics R, Passamonti F, Buser AS, et al. A gain-of-function mutation of JAK2 in myeloproliferative disorders. N Engl J Med. 2005;352(17):1779–90.

Kudo K, Kojima S, Tabuchi K, et al. Prospective study of a pirarubicin, intermediate-dose cytarabine, and etoposide regimen in children with Down syndrome and acute myeloid leukemia: the Japanese Childhood AML Cooperative Study Group. J Clin Oncol. 2007;25(34):5442–7.

Kurokawa M, Tanaka T, Tanaka K, et al. Overexpression of the AML1 proto-oncoprotein in NIH3T3 cells leads to neoplastic transformation depending on the DNA-binding and transactivational potencies. Oncogene. 1996;12(4):883–92.

Lanza C, Volpe G, Basso G, et al. The common TEL/AML1 rearrangement does not represent a frequent event in acute lymphoblastic leukaemia occurring in children with Down syndrome. Leukemia. 1997;11(6):820–1.

Lavin MF, Bates P, Le Poidevin P, Chen PC. Normal inhibition of DNA synthesis following gamma-irradiation of radiosensitive cell lines from patients with Down's syndrome and Alzheimer's disease. Mutat Res. 1989;218(1):41–7.

Lejeune LG, Gautier M, Turpin R. Les chromosomes humains en culture de tissues. C R Acad Bulg Sci. 1959;248:602–3.

Levitt GA, Stiller CA, Chessells JM. Prognosis of Down's syndrome with acute leukaemia. Arch Dis Child. 1990;65(2):212–6.

Li Z, Godinho FJ, Klusmann JH, Garriga-Canut M, Yu C, Orkin SH. Developmental stage-selective effect of somatically mutated leukemogenic transcription factor GATA1. Nat Genet. 2005;37(6):613–9.

Linabery AM, Olshan AF, Gamis AS, et al. Exposure to medical test irradiation and acute leukemia among children with Down syndrome: a report from the Children's Oncology Group. Pediatrics. 2006;118(5):e1499–508.

Liu C, Morishima M, Yu T, et al. Genetic analysis of Down syndrome-associated heart defects in mice. Hum Genet. 2011;130(5):623–32.

Lo KC, Chalker J, Strehl S, et al. Array comparative genome hybridization analysis of acute lymphoblastic leukaemia and acute megakaryoblastic leukaemia in patients with Down syndrome. Br J Haematol. 2008;142(6):934–45.

Loncarevic IF, Roitzheim B, Ritterbach J, et al. Trisomy 21 is a recurrent secondary aberration in childhood acute lymphoblastic leukemia with TEL/AML1 gene fusion. Genes Chromosomes Cancer. 1999;24(3):272–7.

Lundin C, Heldrup J, Ahlgren T, Olofsson T, Johansson B. B-cell precursor t(8;14)(q11;q32)-positive acute lymphoblastic leukemia in children is strongly associated with Down syndrome or with a concomitant Philadelphia chromosome. Eur J Haematol. 2009;82(1):46–53.

Lundin C, Forestier E, Klarskov Andersen M, et al. Clinical and genetic features of pediatric acute lymphoblastic leukemia in Down syndrome in the Nordic countries. J Hematol Oncol. 2014;7(1):32.

Lyons SE, Lawson ND, Lei L, Bennett PE, Weinstein BM, Liu PP. A nonsense mutation in zebrafish gata1 causes the bloodless phenotype in Vlad tepes. Proc Natl Acad Sci U S A. 2002;99(8):5454–9.

Ma SK, Wan TS, Cheuk AT, et al. Characterization of additional genetic events in childhood acute lymphoblastic leukemia with TEL/AML1 gene fusion: a molecular cytogenetics study. Leukemia. 2001;15(9):1442–7.

Malinge S, Ben-Abdelali R, Settegrana C, et al. Novel activating JAK2 mutation in a patient with Down syndrome and B-cell precursor acute lymphoblastic leukemia. Blood. 2007;109(5):2202–4.

Malinge S, Chlon T, Dore LC, et al. Development of acute megakaryoblastic leukemia in Down syndrome is associated with sequential epigenetic changes. Blood. 2013;122(14):e33–43.

Maloney KW, Carroll WL, Carroll AJ, et al. Down syndrome childhood acute lymphoblastic leukemia has a unique spectrum of sentinel cytogenetic lesions that influences treatment outcome: a report from the Children's Oncology Group. Blood. 2010;116(7):1045–50.

Massey GV, Zipursky A, Chang MN, et al. A prospective study of the natural history of transient leukemia (TL) in neonates with Down syndrome (DS): children's Oncology Group (COG) study POG-9481. Blood. 2006;107(12):4606–13.

McNeil S, Zeng C, Harrington KS, et al. The t(8;21) chromosomal translocation in acute myelogenous leukemia modifies intranuclear targeting of the AML1/CBFalpha2 transcription factor. Proc Natl Acad Sci U S A. 1999;96(26):14882–7.

Miyauchi J, Kawaguchi H. Fetal liver stromal cells support blast growth in transient abnormal myelopoiesis in Down syndrome through GM-CSF. J Cell Biochem. 2014;115(6):1176–86.

Moore S, Suttle J, Bain S, Story C, Rice M. Acute lymphoblastic leukemia characterized by t(8;14)(q11.2;q32). Cancer Genet Cytogenet. 2003;141(1):1–4.

Morawiec Z, Janik K, Kowalski M, et al. DNA damage and repair in children with Down's syndrome. Mutat Res. 2008;637(1–2):118–23.

Mukouyama Y, Chiba N, Hara T, et al. The AML1 transcription factor functions to develop and maintain hematogenic precursor cells in the embryonic aorta-gonad-mesonephros region. Dev Biol. 2000;220(1):27–36.

Mullighan CG, Collins-Underwood JR, Phillips LA, et al. Rearrangement of CRLF2 in B-progenitor- and Down syndrome-associated acute lymphoblastic leukemia. Nat Genet. 2009;41(11):1243–6.

Mundschau G, Gurbuxani S, Gamis AS, Greene ME, Arceci RJ, Crispino JD. Mutagenesis of GATA1 is an initiating event in Down syndrome leukemogenesis. Blood. 2003;101(11):4298–300.

Nerlov C. The C/EBP family of transcription factors: a paradigm for interaction between gene expression and proliferation control. Trends Cell Biol. 2007;17(7):318–24.

Nichols KE, Crispino JD, Poncz M, et al. Familial dyserythropoietic anaemia and thrombocytopenia due to an inherited mutation in GATA1. Nat Genet. 2000;24(3):266–70.

Nikolaev SI, Santoni F, Vannier A, et al. Exome sequencing identifies putative drivers of progression of transient myeloproliferative disorder to AMKL in infants with Down syndrome. Blood. 2013;122(4):554–61.

Nikolaev SI, Garieri M, Santoni F, et al. Frequent cases of RAS-mutated Down syndrome acute lymphoblastic leukaemia lack JAK2 mutations. Nat Commun. 2014;5:4654.

Norton A, Fisher C, Liu H, et al. Analysis of JAK3, JAK2, and C-MPL mutations in transient myeloproliferative disorder and myeloid leukemia of Down syndrome blasts in children with Down syndrome. Blood. 2007;110(3):1077–9.

O'Brien MM, Taub JW, Chang MN, et al. Cardiomyopathy in children with Down syndrome treated for acute myeloid leukemia: a report from the Children's Oncology Group Study POG 9421. J Clin Oncol. 2008;26(3):414–20.

O'Brien MM, Cao X, Pounds S, et al. Prognostic features in acute megakaryoblastic leukemia in children without Down syndrome: a report from the AML02 multicenter trial and the Children's Oncology Group Study POG 9421. Leukemia. 2013;27(3):731–4.

Ognjanovic S, Puumala S, Spector LG, et al. Maternal health conditions during pregnancy and acute leukemia in children with down syndrome: a Children's Oncology Group study. Pediatr Blood Cancer. 2009;52(5):602–8.

Parada LF, Tabin CJ, Shih C, Weinberg RA. Human EJ bladder carcinoma oncogene is homologue of Harvey sarcoma virus ras gene. Nature. 1982;297(5866):474–8.

Parker SE, Mai CT, Canfield MA, et al. Updated national birth prevalence estimates for selected birth defects in the United States, 2004–2006. Birth Defects Res A Clin Mol Teratol. 2010;88(12):1008–16.

Patrick K, Wade R, Goulden N, et al. Outcome of Down syndrome associated acute lymphoblastic leukaemia treated on a contemporary protocol. Br J Haematol. 2014;165(4):552–5.

Paulsson K, Johansson B. High hyperdiploid childhood acute lymphoblastic leukemia. Genes Chromosomes Cancer. 2009;48(8):637–60.
Pizzo P, De Poplack. Principles and practice of pediatric oncology. 6th ed. Philadelphia: Lippincott Williams & Wilkins; 2011.
Preudhomme C, Warot-Loze D, Roumier C, et al. High incidence of biallelic point mutations in the Runt domain of the AML1/PEBP2 alpha B gene in Mo acute myeloid leukemia and in myeloid malignancies with acquired trisomy 21. Blood. 2000;96(8):2862–9.
Pui CH, Raimondi SC, Borowitz MJ, et al. Immunophenotypes and karyotypes of leukemic cells in children with Down syndrome and acute lymphoblastic leukemia. J Clin Oncol. 1993;11(7):1361–7.
Pui CH, Robison LL, Look AT. Acute lymphoblastic leukaemia. Lancet. 2008;371(9617): 1030–43.
Rabin KR, Smith J, Kozinetz CA. Myelosuppression and infectious complications in children with Down syndrome and acute lymphoblastic leukemia. Pediatr Blood Cancer. 2012;58(4):633–5.
Ragab AH, Abdel-Mageed A, Shuster JJ, et al. Clinical characteristics and treatment outcome of children with acute lymphocytic leukemia and Down's syndrome. A Pediatric Oncology Group study. Cancer. 1991;67(4):1057–63.
Rainis L, Bercovich D, Strehl S, et al. Mutations in exon 2 of GATA1 are early events in megakaryocytic malignancies associated with trisomy 21. Blood. 2003;102(3):981–6.
Rajantie J, Siimes MA. Long-term prognosis of children with Down's syndrome and leukaemia: a 34-year nation-wide experience. J Intellect Disabil Res. 2003;47(Pt 8):617–21.
Ramji DP, Foka P. CCAAT/enhancer-binding proteins: structure, function and regulation. Biochem J. 2002;365(Pt 3):561–75.
Rand V, Parker H, Russell LJ, et al. Genomic characterization implicates iAMP21 as a likely primary genetic event in childhood B-cell precursor acute lymphoblastic leukemia. Blood. 2011;117(25):6848–55.
Rao A, Hills RK, Stiller C, et al. Treatment for myeloid leukaemia of Down syndrome: population-based experience in the UK and results from the Medical Research Council AML 10 and AML 12 trials. Br J Haematol. 2006;132(5):576–83.
Ravindranath Y, Abella E, Krischer JP, et al. Acute myeloid leukemia (AML) in Down's syndrome is highly responsive to chemotherapy: experience on Pediatric Oncology Group AML Study 8498. Blood. 1992;80(9):2210–4.
Reinhardt D, Diekamp S, Langebrake C, et al. Acute megakaryoblastic leukemia in children and adolescents, excluding Down's syndrome: improved outcome with intensified induction treatment. Leukemia. 2005;19(8):1495–6.
Roberts I, O'Connor D, Roy A, Cowan G, Vyas P. The impact of trisomy 21 on foetal haematopoiesis. Blood Cells Mol Dis. 2013;51(4):277–81.
Robison LL, Nesbit Jr ME, Sather HN, et al. Down syndrome and acute leukemia in children: a 10-year retrospective survey from Childrens Cancer Study Group. J Pediatr. 1984;105(2): 235–42.
Ross JA, Blair CK, Olshan AF, et al. Periconceptional vitamin use and leukemia risk in children with Down syndrome: a Children's Oncology Group study. Cancer. 2005;104(2):405–10.
Roumier C, Fenaux P, Lafage M, Imbert M, Eclache V, Preudhomme C. New mechanisms of AML1 gene alteration in hematological malignancies. Leukemia. 2003;17(1):9–16.
Russell LJ, Capasso M, Vater I, et al. Deregulated expression of cytokine receptor gene, CRLF2, is involved in lymphoid transformation in B-cell precursor acute lymphoblastic leukemia. Blood. 2009;114(13):2688–98.
Saida S, Watanabe K, Sato-Otsubo A, et al. Clonal selection in xenografted TAM recapitulates the evolutionary process of myeloid leukemia in Down syndrome. Blood. 2013;121(21):4377–87.
Scholl T, Stein Z, Hansen H. Leukemia and other cancers, anomalies and infections as causes of death in Down's syndrome in the United States during 1976. Dev Med Child Neurol. 1982;24(6):817–29.

Song WJ, Sullivan MG, Legare RD, et al. Haploinsufficiency of CBFA2 causes familial thrombocytopenia with propensity to develop acute myelogenous leukaemia. Nat Genet. 1999;23(2):166–75.

Stumpf M, Waskow C, Krotschel M, et al. The mediator complex functions as a coactivator for GATA-1 in erythropoiesis via subunit Med1/TRAP220. Proc Natl Acad Sci U S A. 2006;103(49):18504–9.

Swerdlow S, Camps E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J, Vardiman JW. WHO classification of tumors of haematopoietic and lymphoid tissues. 4th ed. Lyon: IARC; 2008.

Tallman MS, Neuberg D, Bennett JM, et al. Acute megakaryocytic leukemia: the Eastern Cooperative Oncology Group experience. Blood. 2000;96(7):2405–11.

Taub JW, Huang X, Matherly LH, et al. Expression of chromosome 21-localized genes in acute myeloid leukemia: differences between Down syndrome and non-Down syndrome blast cells and relationship to in vitro sensitivity to cytosine arabinoside and daunorubicin. Blood. 1999;94(4):1393–400.

Taub JW, Mundschau G, Ge Y, et al. Prenatal origin of GATA1 mutations may be an initiating step in the development of megakaryocytic leukemia in Down syndrome. Blood. 2004; 104(5):1588–9.

Tubman VN, Levine JE, Campagna DR, et al. X-linked gray platelet syndrome due to a GATA1 Arg216Gln mutation. Blood. 2007;109(8):3297–9.

Tunstall-Pedoe O, Roy A, Karadimitris A, et al. Abnormalities in the myeloid progenitor compartment in Down syndrome fetal liver precede acquisition of GATA1 mutations. Blood. 2008;112(12):4507–11.

Visvader JE, Crossley M, Hill J, Orkin SH, Adams JM. The C-terminal zinc finger of GATA-1 or GATA-2 is sufficient to induce megakaryocytic differentiation of an early myeloid cell line. Mol Cell Biol. 1995;15(2):634–41.

Wechsler J, Greene M, McDevitt MA, et al. Acquired mutations in GATA1 in the megakaryoblastic leukemia of Down syndrome. Nat Genet. 2002;32(1):148–52.

Whitlock JA, Sather HN, Gaynon P, et al. Clinical characteristics and outcome of children with Down syndrome and acute lymphoblastic leukemia: a Children's Cancer Group study. Blood. 2005;106(13):4043–9.

Xavier AC, Ge Y, Taub JW. Down syndrome and malignancies: a unique clinical relationship: a paper from the 2008 William Beaumont Hospital Symposium on Molecular Pathology. J Mol Diagn. 2009;11(5):371–80.

Xavier AC, Edwards H, Dombkowski AA, et al. A unique role of GATA1s in Down syndrome acute megakaryocytic leukemia biology and therapy. PLoS One. 2011;6(11):e27486.

Yamaguchi Y, Zon LI, Ackerman SJ, Yamamoto M, Suda T. Forced GATA-1 expression in the murine myeloid cell line M1: induction of c-Mpl expression and megakaryocytic/erythroid differentiation. Blood. 1998;91(2):450–7.

Yamtich J, Nemec AA, Keh A, Sweasy JB. A germline polymorphism of DNA polymerase beta induces genomic instability and cellular transformation. PLoS Genet. 2012;8(11):e1003052.

Yoshida K, Toki T, Okuno Y, et al. The landscape of somatic mutations in Down syndrome-related myeloid disorders. Nat Genet. 2013;45(11):1293–9.

Yu C, Niakan KK, Matsushita M, Stamatoyannopoulos G, Orkin SH, Raskind WH. X-linked thrombocytopenia with thalassemia from a mutation in the amino finger of GATA-1 affecting DNA binding rather than FOG-1 interaction. Blood. 2002;100(6):2040–5.

Yu T, Liu C, Belichenko P, et al. Effects of individual segmental trisomies of human chromosome 21 syntenic regions on hippocampal long-term potentiation and cognitive behaviors in mice. Brain Res. 2010;1366:162–71.

Zeller B, Gustafsson G, Forestier E, et al. Acute leukaemia in children with Down syndrome: a population-based Nordic study. Br J Haematol. 2005;128(6):797–804.

Zipursky A. Transient leukaemia – a benign form of leukaemia in newborn infants with trisomy 21. Br J Haematol. 2003;120(6):930–8.

Zipursky A, Thorner P, De Harven E, Christensen H, Doyle J. Myelodysplasia and acute megakaryoblastic leukemia in Down's syndrome. Leuk Res. 1994;18(3):163–71.