

# **GATA1 and cooperating mutations in Myeloid Leukaemia of Down Syndrome**

**Catherine Garnett, David Cruz Hernandez, Paresh Vyas**

## **Abstract**

Myeloid leukaemia of Down Syndrome (ML-DS) is an acute megakaryoblastic/erythroid leukaemia uniquely found in children with Down Syndrome (constitutive Trisomy 21). It has a unique clinical course, being preceded by a pre-leukaemic condition known as Transient Abnormal Myelopoiesis (TAM), and provides an excellent model in which to study multistep leukaemogenesis. Both TAM and ML-DS blasts carry acquired N-terminal truncating mutations in the erythro-megakaryocytic transcription factor *GATA1*. These result in exclusive production of a shorter isoform (*GATA1s*). The majority of TAM cases resolve spontaneously without the need for treatment, however around 10% acquire additional cooperating mutations and transform to leukaemia, with differentiation block and clinically significant cytopenias. Transformation is driven by the acquisition of additional mutation(s), which co-operate with *GATA1s* to perturb normal haematopoiesis.

## **Introduction**

Despite not being cancer prone in general, children with Down Syndrome due to an extra copy of chromosome 21 have an increased risk of developing acute myeloid or lymphoblastic leukaemia (~150 fold and 33 fold, respectively) (1). In this review we focus on Myeloid Leukaemia of Down Syndrome (ML-DS). This is an acute megakaryoblastic/erythroid leukaemia, which usually presents within the first four years of life and has a median onset at just over one year of age. Unlike adult acute myeloid leukaemia (AML), which is often genetically more complex, ML-DS comprises three, temporarily separable, stages of disease development: (i) constitutive trisomy 21 leading to abnormal fetal haematopoiesis (2); (ii) an acquired mutation in the key haematopoietic transcription factor *GATA1* during fetal life resulting in a preleukaemic condition known as Transient Abnormal Myelopoiesis or TAM (3–5); (iii) additional somatic mutation(s) in postnatal life transforming preleukaemic TAM clone(s) to ML-DS (6–8) (Figure 1).

The unique natural history and relative genetic simplicity of ML-DS makes it an excellent model to study both *GATA1* function and the cellular and molecular processes underlying multistep leukaemogenesis. Here, we discuss recent advances of the role of *GATA1* and cooperating mutations in the evolution of ML-DS.

## **Down Syndrome-Associated Myeloid Disease**

Following the first report of somatic N-terminal truncating *GATA1* mutations in ML-DS (9) multiple reports confirmed these findings and showed that these mutations were also found in TAM. In neonates that developed TAM and later progressed to ML-DS, the TAM and ML-DS clones often had the same *GATA1* mutation showing that ML-DS directly evolves from TAM (3, 5, 10–12). Around 25% of TAM and ML-DS cases had multiple *GATA1* mutant clones. Remarkably, a

prospective population-based study identified *GATA1* mutations in nearly 1 in 3 Down Syndrome neonates at birth (4).

### **Transient Abnormal Myelopoiesis (TAM)**

TAM has a variable clinical presentation (13–15). It is usually diagnosed at birth, however the diagnosis may be delayed and made at any stage within the first 8 weeks of life. The median age of diagnosis is 3-7 days. TAM is classically asymptomatic. However, it can present with jaundice, hepatomegaly, splenomegaly, skin rash and pleural/pericardial effusions. Laboratory tests may show leucocytosis, circulating blast cells and thrombocytopenia. Liver fibrosis, resulting from blast cell infiltration can lead to fulminant liver failure in a small number of cases. Consideration of treatment with low dose cytarabine is recommended for those patients at high risk of early death (high white cell count, severe liver disease, presence of effusions and coagulopathies) (13, 14, 16–19).

TAM has also been reported in fetal life, associated with hydrops fetalis or features mentioned above (20). TAM blasts are typically megakaryoblastic and often co-express myeloid (CD33/CD13), megakaryocyte (CD42, CD61) and markers of immature cells (CD34, CD117) (21).

In keeping with previously published reports, data from the prospective multicentre Oxford Imperial Down Syndrome cohort study found that 10-15% of neonates with Down Syndrome were diagnosed with TAM in the context of *GATA1* mutation(s), circulating blasts of more than 10% and/or other clinical features. An additional 10-15% of neonates with Down Syndrome were found to have acquired *GATA1* mutations but no clinical or haematological features of TAM (blasts <10%) and were classified as having silent TAM (22).

All babies with TAM require regular clinical follow up and monitoring of their blood counts and films over the first 6 months. The majority of cases of both TAM and silent TAM resolve spontaneously without treatment and the children never develop ML-DS (14, 17, 22).

### **Myeloid Leukaemia of Down Syndrome (ML-DS)**

Approximately 10-20% of neonates with TAM will go on to develop ML-DS (4, 14, 17). ML-DS is characterised molecularly by both the persistence of a *GATA1s* mutation (present in preceding TAM sample(s) but not necessarily the largest clone) and acquisition of additional co-operating mutation(s) (discussed in detail below).

The median age of diagnosis of ML-DS is 12-18 months and cases have not been reported beyond five years of age. ML-DS often presents with progressive pancytopenia and a relatively low circulating blast count. Bone marrow aspirate may give few cells due to marrow fibrosis. Blast cells are morphologically and immunophenotypically similar to TAM blasts and carry *GATA1* mutations plus additional somatic variants. ML-DS patients usually respond well to chemotherapy, with most cases of treatment failure related to drug toxicity (23–25). Overall survival is better than for non-DS

AML at 87-93%, with recent trials focussing on offering reduced intensity therapy (24, 26). However treatment options for the ~10% patients who relapse or fail to enter remission are limited (24, 27, 28).

## **GATA1: an essential haematopoietic transcription factor**

### **The GATA Family of transcription factors**

The founding member of the GATA family, GATA1, was first identified as a protein binding the 3' enhancer of the beta-globin gene (29) and cloned in 1989 from mouse erythroid cells (30). It is now known to be expressed in megakaryocytes, erythroid cells, mast cells, eosinophils and basophils, as well as Sertoli cells of the testis (31). Five further GATA family members (GATA 2-6) were subsequently identified (reviewed in (32, 33)). These share two highly conserved zinc finger domains: a C-terminal zinc finger which is required for recognition and binding of the (A/T)GATA(A/G) consensus sequence (34) and an N-terminal zinc finger which is involved in stabilisation and recruitment of co-factors (35). All GATA family proteins also possess an N-terminal transactivation domain, however this shows considerable variation between factors (36, 37). Historically, the GATA factors have been divided into two subfamilies: GATA1, GATA2 and GATA3 which are expressed mainly in haematopoietic cells, but also endothelial cells and the nervous system (38) and GATA4, GATA5 and GATA6 which are expressed in mesoderm and endoderm-derived tissues including heart, liver and gonad (37).

### **GATA1 in normal haematopoiesis**

It is well established that full length GATA1 is essential for normal erythropoiesis. Embryonic stem cells deficient in *Gata1* are able to contribute to all tissues in chimeric mice, with the exception of mature erythroid cells (39) while *Gata1*-null mice die at E10.5-E11.5 from fetal anaemia (40). "GATA1 low" mutant mice, which express around 20% of wild type GATA1, have a milder phenotype. In the majority of cases, this is still embryonic lethal, with death between E13.5-E14.5 due to ineffective erythropoiesis. However, a small number of *Gata1*-low mice survive to birth and those that do recover from their anaemia and are viable to adulthood (41). GATA1 is also critical for normal megakaryocyte development. Deletion of an upstream enhancer in the *Gata1* locus results in loss of megakaryocytic *Gata1* expression, a block in terminal megakaryopoiesis and marked thrombocytopenia (42). Immature GATA1-deficient megakaryocyte progenitors accumulate in spleen and bone marrow and show abnormal differentiation and proplatelet formation (43). Important roles for GATA1 in eosinophil (44), mast cell (45) and dendritic cell (46) differentiation have also been established.

The *GATA1* gene is located on the X chromosome and comprises 6 exons (Figure 4). In normal human haematopoiesis, the *GATA1* locus is alternatively spliced to produce two mRNA transcripts. One of the transcripts contains exons 1-6 and can potentially be translated into two protein

isoforms, a full-length 413 amino-acid protein (GATA1fl) and a truncated 330 amino-acid isoform referred as GATA1 short or GATA1s. GATA1s results from translation from an alternative ATG codon located in exon 3 and lacks the 83 amino acids comprising the N-terminal transactivation domain (Figure 2) (47, 48). The second transcript lacks exon 2 but retains exon 1, and exon 3 to 6. This short transcript can only be translated into the GATA1s isoform. Both GATA1 isoforms contain two zinc finger domains, however only GATA1fl contains the classically defined transactivation domain. As yet, the role of GATA1s in normal haematopoiesis remains unknown.

### **GATA1 mutations in TAM and ML-DS**

The somatic *GATA1* mutations reported in TAM and ML-DS occur at the 5' end of the gene, resulting in exclusive production of GATA1s in TAM and ML-DS blasts (3, 9, 12, 49). These mutations prevent the synthesis of full length GATA-1 protein by impeding translation from the first ATG codon in exon 2. Instead only the alternative ATG codon in exon 3 is used to translate the N-terminal-truncated GATA1s protein. Mutations in the *GATA1* locus that exclusively give rise to GATA1s are not commonly found in non-Down Syndrome childhood leukaemias (9, 12), although they have been reported in cases of mosaic Down Syndrome (50) and in 9% of non-DS AMKL with an acquired T21 (12, 51). The majority of these mutations are deletions, insertions and point mutations and are clustered in exon 2 or the beginning of exon 3 (3, 10, 11, 14, 49). These mutations are present in disease but not remission. The same *GATA1* mutations are found in TAM and ML-DS blasts, supporting a clonal relationship between the two conditions (3, 14). In some patients, multiple *GATA1s* clones can be detected (3). However there does not appear to be a prognostic association between the number of *GATA1s* clones or type of *GATA1* mutations (5).

So how does exclusive production of GATA1s perturb haematopoiesis? There is growing evidence that the GATA1 N-terminus is important for terminal erythroid and megakaryocytic differentiation, particularly during fetal haematopoiesis. Early transfection experiments suggested that the GATA1 N-terminus acts as a transactivation domain, binding co-factors to augment GATA1 activity (48). Indeed, GATA1 has been shown to form a tricomplex with the retinoblastoma protein (RB1) and E2F2, stalling cell proliferation and driving terminal erythroid differentiation (52). This interaction was abrogated in GATA1s, suggesting an important role for the N-terminus in RB1/E2F binding (52, 53). Furthermore, internal deletions in the RB1 binding motif of GATA1 have been reported in TAM and ML-DS patients (54). The megakaryocytic transcription factor murine RUNX1 has also been shown by co-immunoprecipitation to physically interact with murine GATA1. In one study it was proposed that these interactions depended on Gata1 N- and C-terminals but not the zinc finger domain (55). However, this was queried in a later report showing physical interaction of all investigated patient-specific GATA1 mutants with RUNX1 (56).

Transgenic mouse studies looking at erythroid differentiation indicated that the short isoform may be able to compensate for loss of GATA1 full length, but only when overexpressed several fold above

the endogenous level (57). This contrasted earlier in vitro experiments suggesting that the N-terminal domain was dispensable for megakaryocytic or erythroid differentiation (58–60). An important role for the N-terminus in control of fetal liver megakaryocyte proliferation was demonstrated using a *Gata1<sup>fl</sup>/Gata1<sup>s</sup>* rescue assay of primary GATA1 deficient fetal megakaryocyte progenitors (61) and cultured megakaryocytes (62). Exclusive expression of *Gata1<sup>s</sup>* in a transgenic knock-in mouse model resulted in a predominantly embryonic phenotype, with transient hyperproliferation and abnormal maturation of megakaryocyte progenitors and impaired erythropoiesis leading in a few cases to fetal loss (63). Similar mutations in *GATA1* have been reported in a small number of cases of Diamond Blackfan Anaemia lacking mutations in genes encoding ribosomal subunits (64) and in a family with a germline *GATA1* variations leading to the exclusive production of *GATA1<sup>s</sup>*, who were found to have a moderate-severe macrocytic anaemia, neutropenia and mild impairment in platelet morphology and function, although no defect in absolute platelet count (65). Significantly however, in the absence of trisomy 21 neither humans nor mice with *Gata1<sup>s</sup>* mutations develop TAM or ML-DS (63, 65).

At a molecular level, exclusive production of *Gata1<sup>s</sup>* has been shown to perturb gene expression profiles, with downregulation of genes important for terminal erythroid differentiation and upregulation of megakaryocytic pathways (63, 66–68). Studies utilizing ChIP-seq, Cleavage Under Targets and Release using Nuclease-seq (CUT&RUN-seq) and Assay for Transposase Accessible Chromatin using sequencing (ATAC-seq) suggest that *Gata1* and *Gata1<sup>s</sup>* show relatively similar occupancy of *Gata1* binding sites (66, 67). However, genes assigned to sites bound less by *Gata1<sup>s</sup>* than *Gata1<sup>fl</sup>* appear enriched for erythroid differentiation (66, 68). A recent study comparing *Gata1<sup>fl</sup>* and *Gata1<sup>s</sup>* murine fetal erythroblasts, revealed gain of *Gata1<sup>s</sup>* binding and chromatin accessibility at 329 sites and lost at 1844 sites compared to WT, with differential sites largely found in gene bodies and intergenic regions. This included enhanced binding at some megakaryocytic genes (*Fli1* and *Mef2c*) and reduced binding at erythroid genes (*Lmo2* and *Zfp1*). *Gata2* and *Runx1* were expressed at moderately increased levels in *Gata1<sup>s</sup>* mutant cells with associated increased accessibility, suggesting failure of appropriate repression by *Gata1<sup>s</sup>*. This was accompanied by a loss of the repressive histone mark H3K27me3 at the *Gata2* locus, leading the authors to suggest a role for reduced recruitment of Polycomb Repressor Complex 2 (PRC2) to key loci in *Gata1<sup>s</sup>* mutant cells, although no direct interaction between *Gata1* and Ezh2 or Suz12 was found (67).

Despite the significant amount of work that has been it still remains unclear why *GATA1<sup>s</sup>* mutations are so common in T21 cells and how *GATA1<sup>s</sup>* cooperates with T21 either in a cell autonomous or non-cell autonomous manner to cause disease.

T21 itself results in abnormal fetal haematopoiesis. In DS fetal liver, the immunophenotypic haematopoietic stem cell (HSC) compartment and megakaryocyte-erythroid progenitor (MEP) population are increased and there is a decrease in B-lymphoid development compared to disomic

controls (2). The underlying molecular mechanisms driving these changes have been challenging to study in vivo - in part because orthologs of human chromosome 21 map to segments of three different mouse chromosomes (Mmu16, 17 and 10). A number of elegant DS mouse models have been generated (reviewed in (69)). Although none of these models fully recapitulate the haematopoietic abnormalities seen in human T21, they have provided valuable insights into potential contributors to ML-DS pathogenesis (53, 70–72). Ts65Dn mice (trisomic for 104 genes on Mmu16) display a myeloproliferative disorder in adult life (73), which has been associated with overexpression of the human chromosome 21-encoded ETS transcription factor *ERG* (74). Overexpression of *ERG* in a mouse model caused expansion of fetal MEPs and double *ERG/Gata1s* transgenic mice developed a TAM-like condition with increased fetal MEPs, megakaryocyte progenitors and hepatic fibrosis, which progressed to a progenitor myeloid leukaemia (72). *DYRK1A* has also been implicated as a mediator of abnormal megakaryopoiesis in ML-DS in a mouse model of ML-DS-like leukaemia, generated by overexpression of constitutively active MPL in bone marrow cells from *Gata1s/Ts1Rhr* mice (trisomic for 33 human chromosome orthologues) by retroviral transduction (71). Roles for mir-125b (75) and the IGF2 signalling pathway (53) have also been postulated.

Additional insights have also been gleaned using induced pluripotent stem cells (iPSCs) from patients with T21 and *GATA1s* mutations. iPSCs from patients with TAM mutations were shown to have enhanced megakaryocytic and myeloid potential but impaired erythroid differentiation compared to *GATA1* wild type controls (68). A separate iPSC study found that T21 led to enhanced production of early haematopoietic progenitors and upregulation of *GATA1s*, with altered dosage of Chr21-encoded *RUNX1*, *ERG* and *ETS2* proposed to play a contributory role (76).

The reality may also be more complex than simple dosage adjustment in individual T21-associated genes. Letourneau et al. studied gene expression in fetal fibroblasts from a pair of monozygotic twins – one with T21 and one without. Global transcriptional differences were observed, with domains of genes along all chromosomes either up or down regulated (77). Equally, Malinge et al. reported genome-wide changes in methylation in T21 cells, with marked hypomethylation at genes associated with cardiovascular, neurological and developmental disorders. By contrast, TAM and ML-DS were characterised by gain of methylation (78).

### **Transformation to ML-DS: Cooperating mutations**

The advent of sensitive Next Generation Sequencing technologies has allowed detailed exploration of the mutational landscape of TAM and ML-DS. ML-DS is characterised by both the persistence of a *GATA1s* mutation (present in preceding TAM sample(s) but not necessarily in the largest clone) and acquisition of additional mutation(s), most commonly in genes encoding components of the

cohesin complex (*RAD21*, *STAG1/2*, *SMC1/3*, *CTCF*), epigenetic regulators (e.g. *KANSL1*, *EZH2*, and *SUZ12*,) and signalling molecules (e.g. *JAK* family, *MPL*, *KIT*) (6–8).

In a recent large-scale analysis of 111 TAM and 141 ML-DS samples (summarised in Figure 3), we found that, in addition to a *GATA1*s variant (present in all samples), 1.6 additional somatic variants were present in ML-DS samples, compared to 0.4 variants per sample in TAM (8). We then explored the functional consequences of some of these mutations, demonstrating that: (1) although occasional *JAK* and *MPL* variants are detected in both TAM and ML-DS samples, there are clear functional differences. In TAM these variants are largely non-functional while in ML-DS they lead to activation of downstream signalling pathways and are predicted to be oncogenic; (2) a novel gain of function hotspot mutation identified in the myeloid receptor *CSF2RB* gene was oncogenic, promoting proliferation of megakaryocytic and erythroid cells and blocking terminal maturation in *in vitro* and *in vivo* models; and (3) using a multiplex CRISPR-Cas9 loss-of-function screen in a novel fetal liver based murine TAM model, loss of function of 18/22 recurrently mutated ML-DS genes resulted in a megakaryocytic leukaemia closely resembling ML-DS (8). Interestingly, despite being detected in up to 60% of ML-DS patients, cohesin gene mutations were the one group under-represented in this loss-of-function screen – perhaps suggesting the importance of a human trisomic background to disease pathogenesis cohesin mutant cells.

### **The role of cohesin mutations in transformation to ML-DS**

We and others have shown that genes encoding cohesin complex components are amongst the most commonly mutated genes at transformation from TAM to ML-DS (6–8) and are found in nearly 50% of ML-DS patients (8). These are usually mutually exclusive, heterozygous (excepting *STAG2* and *SMC1A* mutations which are on the X Chromosome) and have a mutational profile suggestive of loss of function. Mutant cells do not display increase aneuploidy or gross chromosomal changes, suggesting the mutations may perturb haematopoiesis through effects on transcription.

Cohesin is a highly conserved multi-protein complex comprising 4 main subunits: *SMC1*, *SMC3*, *RAD21* and *STAG1/2*. These form a ring-like structure around DNA, ensuring cohesion of newly replicated sister chromatids and DNA repair in S-Phase (79). This role of cohesin is essential and ensures that genomic information is faithfully passed on through cell division. In higher eukaryotes, cohesin is a major component of chromatin in both non-cycling and post mitotic cells, suggesting an important function outside of the cell cycle (80, 81). In particular, there is growing evidence for cohesin's role in regulation of long-range chromosomal interactions influencing gene expression, in association with *CTCF* and other transcriptional regulators (82).

It is now widely accepted that the eukaryote genome is not linear, but folded into a three dimensional structure within the nucleus. This compaction provides another level at which gene expression can be controlled as distant cis-acting regulatory elements are brought into close

proximity with each other (83). Chromosome conformation capture technologies have revealed that chromosomes can be divided into structural domains termed Topologically Associating Domains (TADs). TADs contain preferentially interacting regulatory elements and are separated by boundary elements, which may act to prevent inappropriate interactions between enhancers and promoters of neighbouring genes (84),(85). The highly conserved zinc-finger DNA binding protein CTCF is enriched at TAD boundaries and depletion of CTCF has been shown to alter genomic looping and disrupt the TAD structure, suggesting an important regulatory role (86).

Cohesin co-localises with CTCF extensively throughout the mammalian genome (87) where it is postulated to regulate interactions between multiple loci within these architectural compartments (88). Depleting cells of CTCF reduces the proportion of cohesin found at CTCF sites, but not the total amount of chromatin-bound cohesin, suggesting that CTCF may influence cohesin distribution rather than cohesin loading (86). One suggested model is that, once loaded into chromatin, cohesin extrudes a DNA loop, bringing distant cis-acting elements close together. CTCF-binding sites then act as a barrier, halting loop extrusion and joining the boundaries of a chromatin domain (89).

Postmitotic murine cells depleted of *RAD21* show transcriptional dysregulation and loss of chromatin contacts at multiple loci when *RAD21* levels are reduced to below 50% of wild type (90). Similarly, when HEK293T cells were depleted of cohesin through proteolytic cleavage of Rad21, a global loss of short-range enhancer-promoter interactions was seen within TADs (91). Interestingly however, a number of recent papers have suggested that although TAD or sub-TAD structures may be affected by loss of cohesin and/or CTCF, gene expression is often only subtly altered (86). In one recent study, fusion of TADs the *Sox9-Kcnj2* locus was achieved only following removal of all CTCF sites within the TADS and at the boundary and did not result in any major changes in gene expression, suggesting that (at this locus at least) TADs may be dispensable for gene regulation. By contrast, inverting or inserting new boundary elements resulted in aberrant gene expression and phenotype (92).

Cohesin also associates with sites independent of CTCF, occupying enhancer and core promoter sites bound by the Mediator complex, which in turn associates and recruits RNA Pol II (93). A number of transcription factors, including Gata1, have been shown to directly interact with the mediator complex (94). Cohesin is one of the last protein complexes to remain bound to condensing chromatin at mitosis. Therefore, it has been suggested that cohesin may facilitate re-association of transcription factors upon exit from mitosis helping to re-establish and maintain patterns of gene expression through progression of the cell cycle (95).

Even in the absence of GATA1s, mutations in cohesin complex genes have been shown to contribute to perturbed haematopoiesis. Recurrent acquired mutations in cohesin complex genes have been identified at high frequency in solid tumours (96). In haematological cancer, mutations



are found in ~10% of adult AML and myelodysplastic syndrome (MDS) (97–100). Analysis of cohesin-mutated leukaemias based on variant allele frequency (VAF) suggests that these cohesin mutations are present in the dominant clone (97, 98, 100) and may be relatively early events, although insufficient to drive leukaemogenesis in the absence of additional co-operating mutations. Indeed, in patients with the inherited multisystem disorders Cornelia de Lange syndrome and Roberts Syndrome who have germline mutations in cohesin genes no significant increase in solid tumours, leukaemia or blood count derangement have been described (101, 102), although recently case reports of acute lymphoblastic leukaemia (103) and acute megakaryoblastic leukaemia (104) in patients with Cornelia de Lange syndrome have been published.

Insights into the impact of cohesin gene mutations on haematopoiesis have been gained from animal models. RNAi-mediated depletion of *rad21* in zebrafish resulted in loss of expression of *runx1* and a failure in mature blood cell differentiation. Microinjection of *runx1* mRNA partially rescued the phenotype (105). Mice homozygous for loss of function mutations in in core cohesin components in haematopoietic cells are embryonic lethal, while haematopoietic heterozygous knockdown of *Rad21*, *Smc3* and *Stag1* results in increased self-renewal, expansion of the immunophenotypic multipotent myeloid progenitor compartment and impaired differentiation (106–108). To varying degrees in the different models, cohesin depletion resulted in changed chromatin accessibility. In an *Smc3* conditional knockout, Viny *et al.* reported a significant decrease in a large subset of genes in cohesin haploinsufficient bone marrow cells compared to wild type, with reduced chromatin accessibility in the -30kb to +10kb region surrounding the transcriptional start site of downregulated genes (106). Similarly, Mullenders *et al.* described increased accessibility of erythroid and myeloid genes in shRNA-mediated cohesin depleted haematopoietic stem and progenitor cells which correlated with changes in gene expression (107). Finally, in cohesin mutant AML cell lines and human CD34+ cells, Mazumdar *et al.* described a global decrease in chromatin accessibility but increased accessibility at key sites enriched for GATA2, RUNX1 and ERG transcription factor binding. Depletion of these transcription factors reverted the cohesin-mutant phenotype (109). Interestingly, a recent study revealed that while deletion of *Stag2* in HSPCs resulted in impaired differentiation, increased self renewal and decreased chromatin accessibility, this role was distinct from its shared role with *Stag1* in chromatid alignment (110)

Taken together, it seems possible that these cohesin mutations (in the context of megakaryocyte proliferation and differentiation delay driven by T21 and Gata1s) may be acting at multiple loci to alter chromatin structure and impact gene regulation, ultimately leading to cellular transformation and leukaemogenesis. This would fit with a model of tumourigenesis where culmination of multiple subtle perturbations in a cell or population of cells together result in significant proliferation and differentiation block.

## Conclusions and Future Directions

Children with DS have a markedly increased susceptibility to acute myeloid leukaemia (ML-DS). This is preceded by a neonatal preleukaemic clonal disorder, termed TAM, which may present clinically or be silent. TAM is unique to DS and characterized by the presence of fetally acquired N terminal truncating mutations in the *GATA1* gene. The majority of TAM cases resolve spontaneously within the first few weeks or months of life as the *GATA1* mutant clone is lost. However, in ~20% of survivors, the *GATA1* mutant clone persists, acquiring one or more additional transforming mutations leading to ML-DS. Despite advances in our understanding of how *GATA1* and cooperating mutations may act together in T21 to cause leukaemia, many questions remain unanswered: Why are *GATA1* mutations so common in the context of T21? How important is the T21 fetal microenvironment in this process? How do T21 and *GATA1*s interact to cause disease? Why do mutations in the genes encoding the cohesin complex occur at a higher frequency in ML-DS than adult AML? What is the combined role of these mutations in cellular transformation? Equally, with respect to normal hematopoiesis: Why do we need two isoforms of *GATA1*? Does alternative splicing of *GATA1* pre-mRNA to produce both *GATA1fl* and *GATA1s* transcripts provide lineage specific mechanisms of individual isoform usage? It is hoped that greater insight into these processes will not only translate to therapeutic advances for patients with TAM and ML-DS, but also our understanding of the molecular and cellular events underlying leukaemogenesis.

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